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(54) Title: A MODIFIED RAAV CAPSID PROTEIN FOR GENE THERAPY

(57) Abstract: The invention relates to recombinant adeno-associated virus (rAAV) virions for gene therapy, wherein the rAAV virions comprise a novel capsid protein. In particular, the invention relates to the use of such virions in gene therapy for the treatment of an arthritic disease, such as for example rheumatoid arthritis, or symptoms thereof, preferably by intraarticular administration.



WO 2019/141765 A1

A modified rAAV capsid protein for gene therapy

Field of the invention

The invention relates to the field of recombinant adeno-associated virus (rAAV) based gene therapy, in particular to the use of a mutant capsid rAAV in the treatment or prevention of an arthritic disease.

Background of the invention

Recombinant adeno-associated virus (rAAV) vectors have demonstrated excellent safety and efficacy profiles for the delivery of genes in humans *in vivo*. Therefore, rAAV vectors have been extensively used for *in vivo* gene therapy and have been shown safe and effective in pre-clinical models as well as in clinical trials. rAAV vectors have been successful in a number of gene therapy clinical trials for a range of diseases including haemophilia B, haemophilia A, cystic fibrosis, alpha-1 anti-trypsin deficiency, spinal muscular atrophy (SMA), Parkinson disease, Duchenne muscular dystrophy and Leber's congenital amaurosis (Selot *et al.*, Current Pharmaceutical Biotechnology, 2013, 14, 1072-1082). Alipogene tiparvovec (Glybera®, uniQure) has been granted marketing authorization in Europe as a gene therapy for the treatment of lipoprotein lipase deficiency (LPLD). Subsequently, gene therapy drug approval was granted for herpes-virus based Talimogene laherparepvec for the treatment of skin cancer (T-Vec, Imlygic®, Amgen) and for *ex vivo* stem cell retroviral-based gene therapy Strimvelis for the treatment of ADA-SCID (GSK).

rAAV vector-based gene therapy has also been applied in rheumatoid arthritis (RA), which is a chronic inflammatory disease that affects ~1% of the population. The pathology of RA extends throughout the synovial joint. The localized nature of the joint makes *in vivo* gene therapy very attractive. Therapies providing anti-inflammatory proteins aimed at shifting the balance in RA towards an anti-inflammatory state have been applied.

Much work has focused on the development of AAV capsid proteins with desired properties. Such properties can include higher transduction efficiency, tissue/organ tropism, de-targeting of non-desired tissues/organs, or avoidance of pre-existing neutralizing antibodies.

There is, however, still a need in the art to further improve rAAV gene therapy vectors. In particular, there is a need to improve the use of rAAV gene therapy vectors in arthritic disease and, more precisely, to improve the efficiency of delivering genetic material to the targeted tissue, such as the synovial joint or specific cell types within the synovial joint, preferably to fibroblast-like synoviocytes (FLS).

Summary of the invention

In a first aspect, the present invention relates to a recombinant adeno-associated virus (rAAV) virion comprising a modified capsid protein for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease, wherein the modified capsid protein comprises in the C-terminal part of the protein an amino acid sequence

Z, residues of which are exposed on the surface of the capsid protein. Preferably, amino acid sequence Z:

- a. comprises or consists of a sequence of amino acid residues of the formula I:

5 $y - G - Q - x - G - (x)_3 - R - (x)_3 - y - A - Q - A - A$

wherein x represents a single amino acid residue and wherein y represents 0, 1 or 2 amino acid residues; and

- b. is present at a location corresponding to a position 100 – 200, preferably 120
10 – 180, more preferably 130 – 170, more preferably 140 – 160 amino acid residues from the C terminus of a wild-type AAV capsid protein.

Preferably, amino acid residues of the formula I are exposed on the surface of the capsid protein. In a preferred embodiment, sequence Z is comprised in the modified capsid protein at a location represented by the formula II:

15 $EEEIxxxxPVATExxGxxxxNxQy - Z - (x)_nLPGMVWQxRDVYLQGPIWAKIPHTDG$

wherein Z, x and y are as defined above; and wherein n is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

In a preferred embodiment, the present invention relates to an rAAV virion comprising a
20 modified capsid protein for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease, wherein the capsid protein comprises an amino acid sequence selected from the group consisting of: i) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 1 and wherein amino acids at positions 588 – 602 of SEQ ID NO: 1 have at least 80% sequence identity with SEQ ID
25 NO: 11; ii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 2 and wherein amino acids at positions 585 – 599 of SEQ ID NO: 2 have at least 80% sequence identity with SEQ ID NO: 10; iii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 3 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 3 have at least 80% sequence identity with SEQ ID
30 NO: 9; iv) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 4 and wherein amino acids at positions 586 – 600 of SEQ ID NO: 4 have at least 80% sequence identity with SEQ ID NO: 8; v) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 5 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 5 have at least 80% sequence identity with SEQ ID NO:
35 9; vi) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 6 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 6 have at least 80% sequence identity with SEQ ID NO: 8; and vii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 7 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 7 have at least 80% sequence identity with SEQ ID
40 NO: 12; wherein the modified capsid protein provides for an at least two-fold increase in expression

in comparison to an unmodified capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions, wherein preferably the unmodified capsid protein has the amino acid sequence having SEQ ID NO: 19 or has the same serotype as the modified capsid protein.

5 In a preferred embodiment, the modified capsid protein provides for an at least two-fold increase in expression in human FLS cells in comparison to the unmodified capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions, wherein preferably the unmodified capsid protein has the amino acid sequence having SEQ ID NO: 19 or has the same serotype as the modified capsid protein.

10 Alternatively, or in combination with any one of the preceding embodiments, in a preferred embodiment of the present invention, the capsid protein comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO:1 – 7.

Alternatively, or in combination with any one of the preceding embodiments, in a preferred embodiment of the present invention, the rAAV virion comprises a nucleotide sequence comprising
15 at least one AAV inverted terminal repeat (ITR) sequence. Preferably, the virion further comprises a nucleotide sequence encoding a gene product of interest. Even more preferably, the nucleotide sequence encoding a gene product of interest is located between two AAV ITR sequences.

In a preferred embodiment, the gene product of interest treats, prevents or suppresses symptoms associated with an arthritic disease, wherein preferably the gene product of interest
20 is selected from the group consisting of interleukins, immune-modulators, antibodies, shRNA, miRNA, guide RNA, lncRNA, growth factors, proteases, nucleotidases/nucleosidases, peptides, protease inhibitors, inhibitors, enzymes and combinations thereof, and wherein more preferably the gene product of interest is at least one of CD39, CD73 and IFN- β .

Alternatively, or in combination with any one of the preceding embodiments, in a preferred
25 embodiment of the present invention, the rAAV virion comprises at least one of: (i) a polynucleotide comprising a sequence encoding at least one guide RNA; wherein the or each guide RNA is substantially complementary to a target polynucleotide sequence(s) in a genome; and (ii) a polynucleotide comprising a sequence encoding a nuclease; wherein the nuclease forms a ribonuclease complex with the guide RNA, and wherein the ribonuclease complex makes site-
30 specific double-stranded DNA breaks (DSDB) in the genome.

In another aspect, the present invention relates to an rAAV composition for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease, wherein the rAAV composition comprises an rAAV virion according to the invention and a pharmaceutically acceptable carrier. In an embodiment, the rAAV composition further
35 comprises an empty capsid in a ratio of empty capsid to rAAV virion of at least 1:1, at least 5:1 or at least 10:1.

In another aspect, the present invention relates to an rAAV composition and an immunosuppressant for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease, wherein the rAAV composition is an rAAV
40 composition according to the invention and preferably wherein the treatment or prevention

comprises the administration of the rAAV composition and the administration of the immunosuppressant to an individual.

Alternatively, or in combination with any one of the preceding embodiments, in a preferred embodiment of the present invention, the arthritic disease is selected from the group consisting of
5 rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis, arthritis, juvenile idiopathic arthritis, blunt trauma, joint replacement and Still's disease.

Alternatively, or in combination with any one of the preceding embodiments, in a preferred embodiment of the present invention, the rAAV virion or the rAAV composition is administered
10 systemically and / or locally. In a preferred embodiment, at least one of the rAAV composition and the immunosuppressant is administered locally. Preferably, the local administration is intraarticular administration.

In a further aspect, the present invention relates to a method for treating, preventing, or suppressing symptoms associated with an arthritic disease, wherein the method comprises the step
15 of intraarticular administration of a medicament comprising an effective amount of an rAAV virion or an rAAV composition according to the invention.

Detailed description of the invention

The inventors have discovered that a recombinant adeno-associated virus (rAAV) virion
20 comprising a modified capsid protein is surprisingly efficient at transducing cells, and in particular efficient at transducing cells of the synovial joint. As fibroblast-like synoviocytes (FLS) typically are the primary target cell in the joint in the treatment of arthritic diseases, such as for example rheumatoid arthritis, the aim of the present invention is to provide for capsid proteins which are improved in one or more of the following properties: i) higher expression levels in the synovial tissue,
25 in particular in FLS; ii) improved synovial tissue tropism, in particular improved tropism for FLS; and/or iii) improved de-targeting to non-desired tissues/organs upon rAAV administration as compared to capsid proteins known in the art. In particular, these properties of the rAAV virion comprising a modified capsid protein of the invention are improved as compared to unmodified capsid proteins, preferably the wild-type capsid protein of the same serotype as the modified capsid
30 protein and/or AAV5 capsid proteins. It has been previously established that AAV5 capsid gives rise to the highest FLS expression levels when compared with other AAV serotypes (Adriaansen et al. (2005) Ann Rheum Dis 64:1677-1684; Apparailly et al. (2005) Hum. Gene Ther. 16:426-434). As it is the capsid that confers the tissue/cell tropism properties, the modified capsids described in this invention have the property of enhanced FLS transduction potential preferably when compared
35 with unmodified AAV5. In particular, it is preferred that the capsid proteins of the present invention provide higher expression levels in synovial tissue, in particular in FLS, preferably upon intraarticular administration, as compared to unmodified capsid proteins (that is, the same capsid protein without the modification that is to be tested, preferably of the same serotype as the modified capsid proteins), preferably wild-type unmodified capsid proteins (preferably of the same serotype
40 as the modified capsid proteins), more preferably unmodified AAV5 or wtAAV5 capsid proteins.

Hence, in a first aspect, the invention pertains to an rAAV virion comprising a modified capsid protein. The rAAV virion as defined herein is particularly useful for use in gene therapy.

As used herein, "gene therapy" is the insertion of nucleic acid sequences (such as for example a transgene (also referred to as a nucleotide sequence encoding a gene product of interest) as defined herein below) into an individual's cells and/or tissues to treat or prevent a disease or disorder or to treat or prevent the symptoms of a disease or disorder.

AAV can infect both dividing and quiescent cells and infection occurs by interaction of the capsid proteins with a cell-membrane receptor, followed by endocytosis of the AAV virion. AAV belongs to the genus Dependovirus, which in turn belongs to the subfamily of the Parvovirinae, also referred to as parvoviruses, which are capable of infecting vertebrates. Parvovirinae belong to a family of small DNA animal viruses, *i.e.* the Parvoviridae family. As may be deduced from the name of their genus, members of the Dependovirus are unique in that they usually require coinfection with a helper virus such as adenovirus or herpes virus for productive infection in cell culture. The genus Dependovirus includes AAV, which normally infects humans, and related viruses that infect other warm-blooded animals (*e.g.*, bovine, canine, equine, and ovine adeno-associated viruses). Further information on parvoviruses and other members of the Parvoviridae is described in Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication," Chapter 69 in Fields Virology (3d Ed. 1996). For convenience, the present invention is further exemplified and described herein by reference to AAV. It is however understood that the invention is not limited to AAV but may equally be applied to other parvoviruses.

The genomic organization of all known AAV serotypes is very similar. The genome of AAV is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides (nt) in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VP1, -2 and -3) form the capsid or protein shell with the help of the assembly-activating protein (AAP) (for some serotypes), which is encoded in an alternative open reading frame overlapping with that of VP2/VP3. The terminal nucleotides are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. The size of the terminal nucleotides is serotype-dependent. For example, in the case of AAV2, of the terminal 145 nt, 125 nt are self-complementary and the remaining 20 nt remain single-stranded. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild-type AAV (wtAAV) infection in mammalian cells the Rep proteins (*i.e.* Rep78 and Rep52) are expressed from mRNAs transcribed by the p5 promoter and the p19 promoter, respectively. Both Rep proteins have a function in the replication of the viral genome. A splicing event in the Rep ORF results in the expression of actually four Rep proteins (*i.e.* Rep78, Rep68, Rep52 and Rep40). However, it has been shown that the Rep78 and Rep52 proteins, encoded by the unspliced mRNAs, in mammalian cells are sufficient for AAV vector production. Production of wtAAV or rAAV in mammalian cells moreover relies on a combination of alternate usage of two splice acceptor sites and the suboptimal utilization of an ACG initiation codon

for VP2, which ensures proper expression of all three capsid proteins in an approximate 1:1:10 ratio (VP1:VP2:VP3).

An "rAAV virion" (also referred to as an "rAAV vector" or an "rAAV transgene vector" herein) as used herein means an AAV capsid comprising a non-native nucleic acid sequence. Such a sequence in rAAV is generally flanked by ITR sequences, preferably from wtAAV, and preferably encodes a gene product of interest, such as for example a transgene or homology arms. Said differently, an rAAV virion means an rAAV genome, comprising (i) a nucleotide sequence encoding a gene product of interest and (ii) at least one AAV ITR sequence, encapsidated by capsid proteins. An rAAV genome may have one or preferably all wtAAV genes deleted, but may still comprise functional ITR nucleic acid sequences. Preferably, the rAAV virion does not comprise any nucleotide sequences encoding viral proteins, such as the *rep* (replication) or *cap* (capsid) genes of AAV. Thus, an rAAV virion is distinguished from a wtAAV virion, since all or a part of the viral genome has been replaced with a nucleotide sequence encoding a gene product of interest, which is a non-native nucleic acid with respect to the AAV nucleic acid sequence as further defined herein.

In a preferred embodiment, an rAAV virion comprising a modified capsid protein of the invention is for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease. The medical use (*e.g.* gene therapy for treatment or prevention of (symptoms associated with) arthritic disease) herein described is formulated as an rAAV virion according to the invention for use as a medicament for prevention or treatment of the disease(s) and/or disorder(s) defined herein, but could equally be formulated as (i) a method of prevention or treatment of the disease(s) and/or disorder(s) defined herein or symptoms thereof comprising administering a sufficient or an effective amount of an rAAV virion according to the invention to a subject in need thereof, as (ii) an rAAV virion according to the invention for use in the preparation of a medicament to prevent or treat the disease(s) and/or disorder(s) defined herein, or as (iii) use of an rAAV virion according to the invention for the prevention or treatment of the disease(s) and/or disorder(s) defined herein. Such medical uses are all envisaged by the present invention. Preferably, the modified capsid protein comprises in the C-terminal part of the protein an amino acid sequence Z, residues of which are exposed on the surface of the capsid protein.

As used herein, the terms "treat", "treatment", or "treating" refer to application or administration of an rAAV virion of the invention to a subject who has an arthritic disease, wherein the object is to cure, partially or completely reverse, alleviate, ameliorate, inhibit, delay, suppress, slow down or stop the progression or severity of an arthritic disease, or of the symptoms associated with the arthritic disease. The term "treating" includes reducing or alleviating at least one adverse effect or symptom of the arthritic disease. Treatment is generally "effective" if one or more symptoms or clinical markers are reduced. Alternatively, treatment is "effective" if the progression of the arthritic disease is reduced or halted. That is, "treatment" includes not just the improvement of symptoms or markers, but also a cessation or at least slowing of progress or worsening of symptoms that would be expected in absence of treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of one or more symptom(s), diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression,

amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. The term "treatment" of an arthritic disease also includes providing relief from the symptoms or side-effects of the arthritic disease (including palliative treatment). As used herein, the term "prevent", "prevention", or "preventative" (also referred to as prophylactic) refer to
5 application or administration of an rAAV virion according to the invention to a subject who has a predisposition toward an arthritic disease, with the purpose to delay or prevent onset of, alleviate, ameliorate, relieve, inhibit progression of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a future arthritic disease. Thus, an rAAV virion according to the invention may be administered to a subject who does not exhibit signs of an arthritic disease and/or
10 to a subject who exhibits only early signs of an arthritic disease, preferably for the purpose of decreasing the risk of developing pathology associated with the arthritic disease.

The term "cure" or "curing" as used herein means to completely alleviate one or more, preferably all of the symptoms or features of an arthritic disease. The term "delay" or "delaying" as used herein means to delay onset of and/or inhibit progression of and/or reduce severity of one or
15 more of the symptoms or features of the arthritic disease.

In a preferred embodiment, the modified capsid protein of the invention provides for an at least two-fold increase in expression in comparison to an unmodified capsid protein, when tested under the same conditions. Preferably, the unmodified capsid protein, is a capsid protein of the same serotype as the modified capsid protein, but without the modification that is to be tested. More
20 preferably, the unmodified capsid protein is a wild-type (wt) capsid protein of the same serotype as the modified capsid protein, wherein the wt capsid protein preferably has an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19. Alternatively, it is preferred that the unmodified capsid protein has an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19. Most preferably, the unmodified capsid protein has the amino acid sequence as
25 shown in SEQ ID NO: 19. The preferred unmodified capsid proteins may depend on the tissue that is to be targeted by the rAAV virion. For example, an rAAV with AAV5 capsid proteins appears to be a virion of choice for FLS cells (Apparailly et al. (2005) Human Gene Therapy 16(4):426-434; Adriaansen et al. (2005) Ann. Rheum. Dis. 64(12):1677-1684) and therefore – irrespective of the original serotype of the rAAV mutant virions – the rAAV control virion preferably comprises AAV5 capsid proteins, more preferably wild-type AAV5 (wtAAV5) capsid protein, more preferably the AAV5 capsid protein has the amino acid sequence shown in SEQ ID NO:19, even more preferably the rAAV control virion is an rAAV5 virion. An rAAV control virion is an rAAV virion comprising unmodified capsid proteins as defined herein instead of modified capsid proteins. In a preferred
35 embodiment, the rAAV virion (comprising modified capsid proteins) provides for higher expression upon *in vitro* transduction in Fibroblast Like Synoviocytes from rheumatoid arthritis patients (RA-FLS) and/or HEK 293, preferably HEK293T, cells as compared to the same rAAV virion with unmodified capsid proteins as defined herein instead of modified capsid proteins, using a method as described in the Examples. In other words, apart from the capsid proteins, the rAAV virion and the rAAV control virion preferably are identical. Preferably, transduction efficiency is detected in an
40 *in vitro* transduction assay: by measuring expression levels of a reporter gene encoded by the

transgene, such as GFP, YFP and/or Luciferase. In a preferred embodiment, the test to determine the expression is an *in vitro* transduction assay as described in Example 2/3. Briefly, RA-FLS (isolated as described in van de Sande MG et al., (2011) Ann Rheum Dis 70: 423-427) are plated at 2500 cells/well or HEK293T cells (human embryonic kidney cells) are plated at 40,000 cells/well in a 96-well plate (DMEM-GlutaMAX-I (Gibco, ref. 31966-021), 10% FBS (heat inactivated (HI) Bovine Serum Gold, Gibco ref. A15-151), 100 µg/ml Penicillin/100 µg/ml Streptomycin (Sigma-Aldrich, ref.P0781; 37°C / 5% CO₂). After 24 hours, supernatant is removed and replaced by medium (DMEM-glutaMAX-I (Gibco, ref. 31966-021), 0.001% pluronic F68 (Sigma, ref. p5559)) containing the rAAV mutant virions or the rAAV control virions – all expressing yellow fluorescent protein (yFP) and/or luciferase under control of a cytomegalovirus (CMV) promoter– at a multiplicity of infection (MOI) of 10,000, 20,000 and 100,000. Crude lysates (*i.e.* non-purified supernatants of cells transfected with all plasmids needed for rAAV production and containing reporter-expressing virions) or purified AAV (preferably based on iodixanol purification or cesium chloride (CsCl) density gradient purification) can be used. Four hours after transduction, medium (DMEM-GlutaMAX-I, 10% FBS 100 U/ml penicillin, 100 µg/ml streptomycin) containing doxorubicin (Sigma, ref. D1515; final concentration 0.4 µM), FBS (final concentration 1%), is added. After 48h hours (HEK293T) or 4-6 days (RA-FLS), cells are assayed for the percentage of cells expressing YFP or luciferase by fluorescence microscopy or FLOW cytometry. Preferably, the *in vitro* transduction assay is performed multiple times with FLS isolated from different patients, such as for example FLS isolated from 2, 3, 4, 5, 6, 7, 8, 9, 10 or more patients.

A "serotype" is traditionally defined on the basis of a lack of cross-reactivity between antibodies to one virus as compared to another virus. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (*e.g.*, due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes). Under the traditional definition, a serotype means that the virus of interest has been tested against serum specific for all existing and characterized serotypes for neutralizing activity and no antibodies have been found that neutralize the virus of interest. As more naturally occurring virus isolates are discovered and capsid mutants generated, there may or may not be serological differences with any of the currently existing serotypes. Thus, in cases where the new AAV has no serological difference, this new AAV would be a subgroup or variant of the corresponding serotype. In many cases, serology testing for neutralizing activity has yet to be performed on mutant viruses with capsid sequence modifications to determine if they are of another serotype according to the traditional definition of serotype. Accordingly, for the sake of convenience and to avoid repetition, the term "serotype" broadly refers to both serologically distinct viruses (*e.g.*, AAV) as well as viruses (*e.g.*, AAV) that are not serologically distinct that may be within a subgroup or a variant of a given serotype.

"Transduction" refers to the transfer of a transgene into a recipient host cell by a viral vector. Transduction of a target cell by an rAAV virion of the invention leads to transfer of the transgene contained in that rAAV virion into the transduced cell. "Host cell" or "target cell" refers to the cell into which the DNA delivery takes place, such as the synoviocytes or synovial cells of an individual, or such as isolated FLS cells from patients or HEK293T cells in case of the *in vitro* transduction assay.

AAV vectors are able to transduce both dividing and non-dividing cells. In a cell comprising a gene product of interest, such as for example GFP, the gene product of interest has been introduced/transferred/transduced by rAAV "transduction" of the cell. A cell into which the transgene has been introduced is referred to as a "transduced" cell.

5 The recipient host cell wherein the transgene is transduced preferably is a cell that is affected by the disease that is to be treated, such as for example synovial cells, more specifically FLS, macrophages, monocytes, neutrophils, osteoblasts, osteoclasts, chondrocytes, T-lymphocytes, dendritic cells, plasma cells, mast cells, B lymphocytes in case of an arthritic disease. The "synovium" or "synovial tissue" or "synovial cells" as used herein refers to the cellular lining covering
10 the non-cartilaginous surfaces of the synovial joints, as further described in Tak (2000, Examination of the synovium and synovial fluid. In: Firestein GS, Panyani GS, Wollheim FA editors. Rheumatoid Arthritis. New York: Oxford Univ. Press, Inc. 55-68) and incorporated herein by reference. The synovium consists of the intimal lining layer (or synovial lining layer) and the synovial sublining (subsynovium), which merges with the joint capsule. The intimal lining layer comprises intimal
15 macrophages (or macrophage-like synoviocytes or type A synoviocytes) and FLS (or type B synoviocytes). "Synovium" may therefore be replaced by, or is synonymous with, "synovial tissue". A synovial cell can include any cell present in the synovium including FLS and macrophage-like synoviocytes. A synoviocyte cell may also be a neutrophil, T, B cells and/or connective tissue cells, which may all be present in the synovium.

20 "Fibroblast-like synoviocytes" (FLS) are cells of mesenchymal origin that display many characteristics that are in common with fibroblasts, such as expression of specific proteins, such as for example several types of collagens. However, FLS also secrete proteins that are normally absent in other fibroblast lineages, such as for example lubricin. In addition, FLS express molecules that are important for the mediation of cell adhesion, such as cadherin-11, VCAM-1, several
25 integrins and their receptors. Specific for FLS is the expression of CD55 and this protein is therefore typically used to identify FLS in the synovium by immunohistochemistry. FLS represent a specialized cell type located inside joints in the synovium, whose cells play a crucial role in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis (RA). The term "rheumatoid synovium" or "rheumatoid synovial cells" or "rheumatoid synovial tissue" refers to the
30 inflamed synovium of the joints of an individual suffering from RA. The rheumatoid synovium is characterized by intimal lining hyperplasia and by accumulation of FLS, T-cells, plasma cells, macrophages, B-cells, natural killer cells and dendritic cells in the synovial sublining. These accumulated cells are comprised in the definition of rheumatoid synovial cells. During the progression of RA, the synovial tissue becomes a place where constant inflammatory processes
35 take place, which can eventually lead to cartilage damage and joint destruction and deformation. FLS that are present in the synovium during RA have been reported to display an altered phenotype compared to the FLS present in normal tissues. For example, the FLS in rheumatoid synovium lose "contact inhibition", *i.e.*, they lose the property to arrest their growth when more cells come into contact with each other. In addition, they lose the dependency to grow on adhesive surfaces. As a
40 result, the number of FLS in the diseased synovium increases. The inflammation is further

enhanced by the production of several pro-inflammatory signaling molecules, particularly interleukins IL-6 and IL-8, prostanoids and matrix metalloproteinases (MMPs).

Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, an rAAV virion comprising a modified capsid protein according to the invention provides for an at least two-fold increase in expression of the transgene in human FLS in comparison to an rAAV virion comprising an unmodified capsid protein as defined herein, preferably to the unmodified capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions, wherein preferably the unmodified capsid protein has the same serotype as the modified capsid protein or has the amino acid sequence shown in SEQ ID NO: 19.

More preferably, the rAAV virion of the invention results in increased expression levels of the transgene upon *in vitro* transduction as described above by at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 25-, 30-, 35-, 40-, 50-fold increased expression levels in human FLS cells as compared to an rAAV control virion.

Also preferred, or in addition to the above, the rAAV virion provides for increased expression of the transgene upon *in vivo* administration to the air pouch synovium (APS) mouse model (adapted from Edwards et al (1981) J Pathol 134: 147-156 as described in Example 4) as compared to an rAAV control virion, preferably as compared to an rAAV virion comprising wtAAV5 capsid proteins, provided that the rAAV is otherwise identical (apart from its capsid protein(s)). Preferably, expression of the transgene is at least 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 15-, 20-, 25-, 30-, 35-fold increased using an rAAV comprising the mutant capsid proteins of the method of the invention. An exemplary method is provided in the Examples.

Also preferred, or in addition to the above, the rAAV virion comprising a modified capsid protein provides for similar or lower neutralizing antibody (nAb) titers as compared with the same rAAV virion comprising unmodified, preferably wild-type, AAV capsid protein of the same serotype. WtAAV5 capsids are known to have an attractive nAb profile, and therefore, similar or lower nAb titers of rAAV comprising a modified capsid protein according to the present invention as compared to wtAAV5 is preferred.

Alternatively, the *in vitro* transduction assay could be performed similarly as described above, but in a cell type/cell line different from FLS, depending on the cell type that is to be targeted, such as for example, in cells selected from the group consisting of primary hepatocytes, liver cell lines, e.g. HuH, HepG2, HepA1-6, heart cells, skeletal muscle cells, lung cells such as the cell line A549, CNS cells, eye cells, gastrointestinal tract cells, bone marrow cells and blood cells, such as the cell line THP-1. This also may require a different AAV serotype as preferred control, depending on the tropism of the wild-type capsid proteins. In general, a control vector preferably comprises wild-type capsid proteins that naturally target to the tissue of choice. As the skilled person will appreciate, this may also depend on mode of administration: locally or systemically. For example AAV2, which has been the most extensively examined AAV, presents tropism towards skeletal muscle cells, neurons, vascular smooth muscle cells and hepatocytes; AAV6 presents tropism towards airway epithelial cells; AAV7 presents tropism towards skeletal muscle cells; AAV8 presents tropism

towards hepatocytes; AAV1 and AAV5 present tropism towards vascular endothelial cells. Upon systemic administration, AAV 1-3 and 5-9 have tropism towards the liver, with high protein levels observed with AAV9, 8, 7, 6, 1 and to a lesser extent 5 and 2; heart is transduced by AAV4, 6, 7, 8 and 9; thoracic expression is seen for AAV4 and 6 (Zincarelli et al (2008) Molecular Therapy 16(6): 1073-1080).

Without wishing to be bound by any theory, we believe that the increased expression achieved by the rAAV virions comprising a modified capsid protein of the invention as compared to rAAV control virions is caused by an improved transduction of the rAAV into the cell, possibly by altered tropism, resulting in (i) an increased number of cells within the cell population being transduced, and/or (ii) an increased level of expression per cell, for example, due to better virion uptake and/or intracellular processing..

Another advantage of the rAAV virion with a modified capsid protein according to the invention could preferably be other improvements such as possible avoidance of pre-existing neutralizing antibodies.

In a preferred embodiment of the present invention, the modified capsid protein comprises an amino acid sequence Z, preferably amino acid sequence Z is comprised in the C-terminal part of the protein. Preferably, sequence Z is 12 – 18 amino acid residues in length (herein also referred to as “loop region” and as “insert”). In a preferred embodiment, sequence Z is located in the C-terminal part of the capsid protein, preferably at a location corresponding to a position at 100 – 200, preferably 120 – 180, more preferably 130 – 170, more preferably 140 – 160, most preferably about 150 amino acids from the C-terminus of the wild-type capsid protein, such as for example shown in SEQ ID NO's: 13 - 19. Residues of amino acid sequence Z are preferably exposed on the surface of the capsid protein, such as for example at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 residues are exposed on the surface of the capsid protein (a so-called “loop”). In a preferred embodiment, the sequence Z is 14 – 18 amino acid residues in length, more preferably, 15, 16, 17 or 18 residues in length, most preferably, 15, 17 or 18 amino acid residues in length. Sequence Z may replace some amino acid residues as compared to the unmodified, such as the wild-type, capsid protein sequence. Preferably, the insert replaces 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues, more preferably 6 or 7 amino acid residues of the same sequence but without the insert, preferably of the unmodified, more preferably of the wild-type sequence. Apart from the sequence Z/insert, thus in the framework, the capsid protein may comprise further modifications, such as amino acid substitutions (for example conservative amino acid substitutions) or the framework capsid protein may be as the wild-type amino acid sequence. The framework AAV wherein the insert is comprised can be of any serotype, such as for example AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAVrh10 or AAVDJ. Preferably, the framework AAV wherein the sequence Z is comprised is selected from the group consisting of AAV1, AAV2, AAV7, AAV9, AAVrh10, AAVDJ, more preferably from the unmodified capsid proteins having an amino acid sequence as shown in any one of SEQ ID NO: 13 – 19. The insert according to the invention is preferably comprised in the C-terminal part of the capsid protein, preferably at a location corresponding to 100 – 200, preferably 120 – 180, more preferably 130 – 170, more preferably 140

– 160, most preferably approximately 150 amino acid residues from the C-terminus of the wild-type capsid protein, such as for example shown in SEQ ID NO's: 13 – 19, wherein the location of the insert is represented by formula II:

5 EEEIxxxxPVATExxGxxxxNxQy – Z – (x)_nLPGMWVQxRDVYLQGPWAKIPHTDG

wherein x represents a single amino acid residue, wherein y represents 0, 1 or 2 amino acid residue(s) (which thus may be absent), and wherein n is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, preferably 8, 9 or 10 or wherein the location of the insert is represented by a sequence having at
10 least 90, 93, 95, 96, 97, 98 or 99% sequence identity with formula II. Preferably, the last three amino acid residues preceding the N-terminal end of sequence Z of the invention are NLQ, NHQ or NFQ. Preferably, y represents 0 or 2 amino acid residues. In some cases, y represents 2 amino acid residues, thus two additional amino acid residues, preferably two serine residues, may be present
15 between the NxQ motif and the insert of the invention. This is preferably the case where the NxQ motif is NFQ, for example if the AAV capsid is an AAV1 capsid sequence as represented in SEQ ID NO:1. The skilled person will be able to determine these motifs and this region where the insert is located, also if it harbors some variations, such as amino acid substitutions or deletions, which are also encompassed in the scope of the present invention.

In a preferred embodiment, based on the alignments shown in Figures 4 and 5, the insert
20 (sequence Z) comprises or consists of a sequence of the formula: x₁-G-Q-x₂-G-x₃-x₄-x₅-R-x₆-x₇-x₈-x₉-x₁₀-x₁₁-x₁₂-x₁₃-x₁₄-x₁₅, wherein x₁ is Q or none, x₂ is S or R, x₃ is N or C, x₄ is D, Y or E, x₅ is C, V, S or A, x₆ is G, S or V, x₇ is none, A, V or R, x₈ is D, N or E, x₉ is C or A, x₁₀ is F or Q, x₁₁ is none, C or A, x₁₂ is none or A, x₁₃ is none or Q, x₁₄ is none or A and x₁₅ is none or A. Alternatively, the insert (sequence Z) comprises or consists of a sequence of the formula: y₁-G-Q-y₂-G-y₃-y₄-y₅-R-y₆-
25 y₇-y₈-y₉-y₁₀-A-y₁₁-y₁₂-y₁₃, wherein y₁ is Q or none, y₂ is S or R, y₃ is N or C, y₄ is D, Y or E, y₅ is C, V, S or A, y₆ is G, S or V, y₇ is none or D, y₈ is none or C, y₉ is A, V, R or F, y₁₀ is N, D, E or C, y₁₁ is none or Q, y₁₂ is none or A, y₁₃ is none or A. In yet another alternative, in the most preferred embodiment, based on the alignments shown in Figures 6 and 7, the insert (sequence Z) comprises or consists of a sequence of the general formula I:

30 y – G – Q – x – G – (x)₃ – R – (x)₃ – y – A – Q – A – A

wherein x represents a single amino acid residue and wherein y represents 0, 1 or 2 amino acid residues (which thus may be absent). Preferably, (i) if at the N-terminus y represents 0 amino acids,
35 then the other y within formula I represents 0 amino acid residues or (ii) if at the N-terminus y represents 1 amino acid residue, then the other y within formula I represents 2 amino acid residues. More preferably, the insert (sequence Z) comprises or consists of a sequence of the more specific formula:

40 z₀ – G – Q – z₁ – G – z₂ – z₃ – z₄ – R – z₅ – z₆ – z₇ – z₈ – z₉ – A – Q – A – A

wherein z_0 is none or Q, z_1 is R or S, z_2 is C or N, z_3 is D, E or Y, z_4 is C, A, S or V, z_5 is G, V or S, z_6 is d or none, z_7 is C or none, z_8 is F, R, V or A, z_9 is C, D, N or E. More preferably, if z_0 is none, then also z_6 and z_7 both represent none.

5 More preferably, sequence Z/insert comprises or consists of an amino acid sequence that has at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99%, most preferably 100% sequence identity with any one of the amino acid sequences selected from the group consisting of SEQ ID NOs: 8 – 12. It is preferred that sequence Z/insert comprises or consists of an amino acid sequence represented by any one of the formulae above and that has at least 80, 85, 87, 90, 91, 92, 93, 94,
10 95, 96, 97, 98 or 99%, most preferably 100% sequence identity with any one of the amino acid sequences selected from the group consisting of SEQ ID NOs: 8 – 12.

In mammalian cells, expression of the three AAV capsid proteins (VP1, VP2 and VP3) in the correct stoichiometry relies on a combination of alternate usage of two splice acceptor sites and the suboptimal utilization of a ACG initiation codon for VP2 that is not accurately reproduced by
15 insect cells. Correct stoichiometry is important for infectivity of the AAV particles. For production of the three AAV capsid proteins in insect cells in the correct stoichiometry, it is common in the art to use a construct that is transcribed into a single polycistronic messenger that is able to express all three VP proteins without requiring splicing. In order to achieve this, the VP1 protein could be under control of a suboptimal translation initiation codon instead of ATG. Examples of such a suboptimal
20 translation initiation codon are ACG, TTG, CTG and GTG (Urabe et al. (2002) Human Gene Therapy 13: 1935-1943; US 20030148506; US 20040197895; WO 2007/046703). Alternatively, in the production of rAAV in insect cells a nucleic acid cassette can be used for expressing VP1, VP2 and VP3 proteins, where these proteins are encoded by a nucleic acid sequence comprising overlapping open reading frames (ORFs) as described in European patent No. 2,061,891 B1, where
25 a VP expression cassette is disclosed that comprises an intron comprising a promoter prior to the VP2 ACG initiation codon. The modified capsid protein of the invention is defined with respect to the protein sequence of the VP1 capsid protein. However, since sequence Z/insert is located in the C-terminal part of the VP1 protein, it is included in the invention that also VP2 and VP3 proteins harbor the sequence Z/insert and thus are modified (irrespective of the method of production of
30 rAAV, such as for example in insect cells or in mammalian cells).

Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the modified capsid protein according to the invention comprises or consists of an amino acid sequence selected from the group consisting of: i) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably
35 having 100% sequence identity with an amino acid sequence having SEQ ID NO: 1 and wherein amino acids at positions 588 – 602 of SEQ ID NO: 1 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 11, ii) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO:
40 2 and wherein amino acids at positions 585 – 599 of SEQ ID NO: 2 have at least 80, 85, 87, 90, 91,

92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 10, iii) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO: 3 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 3 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 9, iv) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO: 4 and wherein amino acids at positions 586 – 600 of SEQ ID NO: 4 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 8, v) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO: 5 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 5 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 9, vi) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO: 6 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 6 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 8, and vii) an amino acid sequence having at least 70, 75, 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with an amino acid sequence having SEQ ID NO: 7 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 7 have at least 80, 85, 87, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, most preferably having 100% sequence identity with SEQ ID NO: 12. Preferably, the framework AAV capsid protein wherein the insert is comprised, has the amino acid sequence of a wild-type AAV capsid, such as for example of AAV5, AAV1, AAV2, AAV7, AAV9, AAVrh10 or AAVDJ or an amino acid sequence comprising conservative amino acid substitutions. More preferably, the framework AAV capsid protein wherein the insert is comprised, has the amino acid sequence of a wtAAV5 capsid or an amino acid sequence comprising conservative amino acid substitutions.

"Sequence identity" is herein defined as a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In a preferred embodiment, sequence identity is calculated based on the full length of two given SEQ ID NO or on part thereof. Part thereof preferably means at least 10, 20, 30, 40, 50, 60, 70, 80, 90%, or 100% of both SEQ ID NO. In the art, "identity" also means the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case may be, as determined by the match between strings of such sequences. Unless otherwise indicated herein, identity or similarity with a given SEQ ID NO means identity or similarity based on the full length of said sequence (*i.e.* over its whole length or as a whole).

"Similarity" between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (Computational Molecular Biology, Lesk, A. M., ed., Oxford

University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heine, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48:1073 (1988)).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include *e.g.*, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12 (1): 387 (1984)), BestFit, BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Mol. Biol. 215:403-410 (1990)). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S. et al., J. Mol. Biol. 215:403-410 (1990)). The well-known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970); Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992); Gap Penalty: 12; and Gap Length Penalty: 4. Such a program is publicly available as the "Ogap" program from Genetics Computer Group, located in Madison, WI. The aforementioned parameters are the default parameters for amino acid comparisons (along with no penalty for end gaps).

Preferred parameters for nucleic acid comparison include the following Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970); Comparison matrix: matches=+10, mismatch=0; Gap Penalty: 50; Gap Length Penalty: 3. Available as the Gap program from Genetics Computer Group, located in Madison, Wis (www.biology.wustl.edu/gcg/gap). Given above are the default parameters for nucleic acid comparisons.

Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called "conservative" amino acid substitutions, as will be clear to the skilled person. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids

are as follows: Ala to Ser; Arg to Lys; Asn to Gln or His; Asp to Glu; Cys to Ser or Ala; Gln to Asn; Glu to Asp; Gly to Pro; His to Asn or Gln; Ile to Leu or Val; Leu to Ile or Val; Lys to Arg; Gln or Glu; Met to Leu or Ile; Phe to Met, Leu or Tyr; Ser to Thr; Thr to Ser; Trp to Tyr; Tyr to Trp or Phe; and, Val to Ile or Leu.

5 Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the capsid protein comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO:1 – 7, more preferably from the group consisting of SEQ ID NO: 1, 2, 3, 4, 6 and 7, even more preferably from the group consisting of SEQ ID NO: 3, 4 and 6, still more preferably from the group consisting of SEQ ID NO: 4 and 6, most preferably
10 SEQ ID NO:4.

 Functional ITR sequences are necessary for the replication, rescue and packaging of rAAV virions. The ITR sequences may be wild-type sequences or may have at least 80%, 85%, 90%, 95%, or 100% sequence identity with wild-type sequences or may be altered by, for example, insertion, mutation, deletion or substitution of nucleotides, as long as they remain functional. In this
15 context, functionality refers to the ability to directly package the genome into the capsid shell and then allow for expression in the host cell to be transduced or target cell. Typically, the ITRs of the wild-type AAV genome are retained in the rAAV-vector. The ITRs can be cloned from the AAV viral genome or excised from a vector comprising the AAV ITRs. The ITR nucleotide sequences can be either ligated at either end to a transgene as defined herein using standard molecular biology
20 techniques, or the wild-type AAV sequence between the ITRs can be replaced with the desired nucleotide sequence. The rAAV-vector preferably comprises at least the nucleotide sequences of the ITR regions of one of the AAV serotypes, or nucleotide sequences substantially identical thereto, and at least one nucleotide sequence encoding a therapeutic protein (under control of a suitable regulatory element) inserted between the two ITRs. The majority of currently used rAAV-
25 vectors use the ITR sequences from AAV serotype 2. Most preferred ITRs present in an rAAV-vector are of the AAV2 serotype. Other preferred ITRs are of the AAV1, AAV3, AAV5 or AAV6 serotype (Grimm et al. (2006) J Virol 80(1):426-439). An rAAV genome can comprise single-stranded or double-stranded (self-complementary) DNA. The single-stranded nucleic acid molecule is either sense or antisense strand, as both polarities are equally capable of packaging into AAV
30 capsids. Single-stranded rAAV-vectors may utilize the wild-type AAV serotype 2 (AAV2) ITR sequences, and double-stranded (self-complementary) rAAV-vectors may utilize a modified version of the ITRs. Alternatively, in an embodiment, a double-stranded vector comprises one ITR, which ITR is from AAV4. The rAAV-vector may further comprise a marker or reporter gene, such as a gene for example encoding an antibiotic resistance gene, a fluorescent protein (*e.g.*, *gfp*) or a gene
35 encoding a chemically, enzymatically or otherwise detectable and/or selectable product (*e.g.*, *lacZ*, alkaline phosphatase (AP), SEAP, Luc, Neo, Bla, etc.) known in the art.

 The rAAV-vector, including any possible combination of AAV serotype capsid and AAV genome ITRs, is produced using methods known in the art, for example using a mammalian rAAV production system or an insect cell rAAV production system. Methods known in the art are for
40 example described in Pan et al. (J. of Virology (1999) 73: 3410-3417), Clark et al. (Human Gene

Therapy (1999) 10: 1031-1039), Wang et al. (Methods Mol. Biol. (2011) 807: 361-404), Grimm (Methods (2002) 28(2): 146-157), and the insect cell system based on Urabe et al (Human Gene Therapy (2002) 13:1935-1943), Kohlbrenner et al (Molecular Therapy (2005) 12(6):1217-1225), International Patent publication WO 2007/046703, International Patent publication WO 5 2007/148971, International Patent publication WO 2009/014445, International Patent publication WO 2009/104964, International Patent publication WO 2009/154452, International Patent publication WO 2011/112089, International Patent publication WO 2013/036118, US patent No. 6,723,551 B, which are incorporated herein by reference. In short, the methods generally may involve (a) the introduction of the rAAV genome construct into a host cell, (b) the introduction of an AAV helper construct into the host cell, wherein the helper construct comprises the viral functions 10 missing from the wild-type rAAV genome and (c) introducing a helper virus construct into the host cell. All functions for rAAV vector replication and packaging need to be present, to achieve replication and packaging of the rAAV genome into rAAV vectors. The introduction into the host cell can be carried out using standard molecular biology techniques and can be simultaneously or 15 sequentially. Finally, the host cells are cultured to produce rAAV vectors which are then purified using standard techniques such as CsCl gradients (Xiao et al. 1996, J. Virol. 70: 8098-8108) or Iodixanol purification. The purified rAAV vector is then typically ready for use in the methods. High titers of more than 10^{12} particles per ml and high purity (free of detectable helper and wild-type viruses) can be achieved (see for example Clark et al. *supra* and Flotte et al. 1995, Gene Ther. 2: 20 29-37). The total size of the transgene inserted into the rAAV vector between the ITR regions is generally smaller than 5 kilobases (kb) in size.

In the context of the present invention a capsid protein shell may be of a different serotype than the rAAV-genome, comprising (i) a nucleotide sequence encoding a gene product of interested and (ii) at least one AAV ITR sequence. An rAAV-genome of the invention may thus be 25 encapsidated by a capsid protein shell of the present invention, *i.e.* the icosahedral capsid, which comprises capsid proteins (VP1, VP2, and/or VP3) according to the present invention, *e.g.*, mutants of AAV capsid proteins according to the invention, whereas the ITRs sequences contained in that rAAV-vector may be any of the AAV serotypes described above, including for example AAV2 or AAV5. In an embodiment, the rAAV genome or ITRs present in the rAAV virion are derived from 30 AAV serotype 2 or AAV serotype 5 or AAV serotype 8. The complete genome of AAV5 and other AAV serotypes has been sequenced (Chiorini et al. 1999, J. of Virology Vol. 73, No.2, p1309-1319) and the nucleotide sequence of AAV5 is available in GenBank (Accession No. AF085716). The ITR nucleotide sequences of AAV2 and AAV5 are thus readily available to a skilled person. The complete genome of AAV2 is available in NCBI (NCBI Reference Sequence NC_001401.2). They 35 can be either cloned or made by chemical synthesis as known in the art, using for example an oligonucleotide synthesizer as supplied *e.g.*, by Applied Biosystems Inc. (Fosters, CA, USA) or by standard molecular biology techniques.

Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the rAAV vector comprises a nucleotide sequence encoding a gene 40 product of interest.

The terms "transgene" or "gene product of interest" are used interchangeably herein and refer to a non-native nucleic acid with respect to the AAV nucleic acid sequence. They are used to refer to a polynucleotide that can be introduced into a cell or organism. Gene products of interest include any polynucleotide, such as a gene that encodes a polypeptide or protein, a polynucleotide that is transcribed into an inhibitory polynucleotide, or a polynucleotide that is not transcribed (e.g., lacks an expression control element, such as a promoter that drives transcription). A gene product of interest of the invention may comprise at least two nucleotide sequences each being different or encoding different therapeutic molecules. The at least two different nucleotide sequences may be linked by an IRES (internal ribosome entry site) element, providing a bicistronic transcript under control of a single promoter. Suitable IRES elements are described in e.g., Hsieh et al. (1995, Biochem. Biophys. Res. Commun. 214:910-917). Furthermore, the at least two different nucleotide sequences encoding different (therapeutic) polypeptides or proteins may be linked by a viral 2A sequence to allow for efficient expression of both transgenes from a single promoter. Examples of 2A sequences include those from foot and mouth disease virus, equine rhinitis A virus, Thosa asigna virus and porcine teschovirus-1 (Kim et al., PLoS One (2011) 6(4): e18556). A gene product of interest is preferably inserted within the rAAV genome or between ITR sequences. A gene product of interest may also be an expression construct comprising an expression regulatory element such as a promoter or transcription regulatory sequence operably linked to a coding sequence and a 3' termination sequence. The gene product of interest can be a functional mutant allele that replaces or supplements a defective one. Gene therapy also includes insertion of transgenes that are inhibitory in nature, i.e., that inhibit, decrease or reduce expression, activity or function of an endogenous or exogenous gene or protein, such as an undesirable or aberrant (e.g., pathogenic) gene or protein. Such transgenes may be exogenous. An exogenous molecule or sequence is understood to be a molecule or sequence not normally occurring in the cell, tissue and/or individual to be treated. Both acquired and congenital diseases are amenable to gene therapy.

"Gene" or "coding sequence" refers to a DNA or RNA region which "encodes" a particular protein. A coding sequence is transcribed (DNA) and translated (RNA) into a polypeptide when placed under the control of an appropriate regulatory region, such as a promoter. A gene may comprise several operably linked fragments, such as a promoter, a 5' leader sequence, an intron, a coding sequence and a 3' non-translated sequence, comprising a polyadenylation site or a signal sequence. A chimeric or recombinant gene is a gene not normally found in nature, such as a gene in which for example the promoter is not associated in nature with part or all of the transcribed DNA region. "Expression of a gene" refers to the process wherein a gene is transcribed into an RNA and/or translated into an active protein.

As used herein, the term "promoter" or "transcription regulatory sequence" refers to a nucleic acid fragment that functions to control the transcription of one or more coding sequences, and is located upstream with respect to the direction of transcription of the transcription initiation site of the coding sequence, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including,

but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. A “constitutive” promoter is a promoter that is active in most tissues under most physiological and developmental conditions. An “inducible” promoter is a promoter that is physiologically or developmentally regulated, *e.g.*, by the application of a chemical inducer. A preferred inducible promoter is an NF- κ B responsive promoter which is inducible upon inflammation. A “tissue specific” promoter is preferentially active in specific types of tissues or cells. The selection of an appropriate promoter sequence generally depends upon the host cell selected for the expression of a DNA segment. Preferred promoter sequences within the rAAV and/or transgene of the invention are promoters which confer expression in cells of the rheumatoid synovium, such as in intimal macrophages and/or in FLS and/or other synovial cells such as, but not limited to, T-cells. Preferred promoters are for example the promoters of genes known to be expressed in synovial cells, such as the CMV promoter, the promoter of the IL-6 gene or the SV40 promoter, or an NF- κ B inducible promoter as earlier identified herein and others, as readily determined by a skilled person. Alternatively, a transgene is operably linked to a promoter that allows for efficient systemic expression. Suitable promoter sequences are CMV promoter, CBA (chicken beta-actin), or liver-specific promoters such as human alpha-1 anti-trypsin (hAAT) or TBG (thyroxine-binding globulin). Preferably, the promoter within the rAAV and/or transgene is not a steroid-inducible promoter. More preferably, the promoter within the rAAV and/or transgene is not a dexamethasone-inducible promoter.

As used herein, the term “operably linked” refers to a linkage of polynucleotide (or polypeptide) elements in a functional relationship. A nucleic acid is “operably linked” when it is placed into a functional relationship with another nucleic acid sequence. For instance, a transcription regulatory sequence is operably linked to a coding sequence if it affects the transcription of the coding sequence. “Operably linked” means that the DNA sequences being linked are typically contiguous and, where necessary to join two protein-encoding regions, contiguous and in reading frame.

A “gene product of interest” can be a “therapeutic polypeptide” or “therapeutic protein” which are to be understood herein as a polypeptide or protein that can have a beneficial effect on an individual, preferably said individual is a human, more preferably said human suffers from a disease. Such therapeutic polypeptide may be selected from, but is not limited to, the group consisting of an enzyme, a co-factor, a cytokine, an antibody, a growth factor, a hormone and an anti-inflammatory protein.

Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the nucleotide sequence encoding a gene product of interest is located between two AAV ITR sequences. Alternatively said, the nucleotide sequence encoding the gene product of interest is flanked by two AAV ITR sequences, *i.e.*, one ITR on either end of the nucleotide sequence encoding the gene product of interest.

Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the gene product of interest treats, prevents or suppresses symptoms associated

with an arthritic disease, wherein preferably the gene product of interest is selected from the group consisting of interleukins, immune-modulators, antibodies, shRNA, miRNA, growth factors, proteases, nucleotidases/nucleosidases, peptides, protease inhibitors, inhibitors, enzymes and combinations thereof, and wherein more preferably the gene product of interest is at least one of

5 CD39, CD73 and IFN- β . Examples of these are: interleukin1 (IL-1) inhibitor (e.g. anakinra, canakinumab, rilonacept), tumor necrosis factor alpha (TNF α) inhibitor (e.g. etanercept, infliximab, adalimumab, certilzumab pegol, golimumab), IL-1 receptor antagonist, soluble IL-1 receptor, IL-17 inhibitor (e.g. secukinumab, brodalumab, ixekizumab, IL-12/IL-23 inhibitor (ustekinumab, risankizumab, guselkumab, tildrakizumab), T-cell costimulation inhibitor (e.g. abatacept), B cell

10 depleting and inhibiting agents (e.g. rituximab, belimumab, ianalumab, tabalumab), IL-15 inhibitor (e.g. AMG-714), IL-22 inhibitor (e.g. Fezakinimab), inhibitor of GM-CSF (lenzilumab, namilumab) insulin-like growth factor (IGF-1), fibroblast-growth factor (FGF) (e.g. rhFGF-18/sprifermin), receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor (e.g. denosumab), complement 5a inhibitor (e.g. C5aR-151), bone morphogenetic protein family member (BMPs),

15 transforming growth factor-beta (TGF- β), growth differentiation factor family (GDFs), interleukin-18 inhibitor (e.g. Tadekinig alfa/ IL-18 binding protein), IL-2 inhibitors (e.g. basiliximab, daclizumab), soluble TNF α (sTNF α) receptor p55 or sTNF α receptor p75, sTNF α receptors fused with an IgG, inhibitors of TNF α receptor p55, inhibitors of sTNF α receptor p75, dominant negative I κ B-kinase (dn-IKK- β), interleukin-4 (IL-4), interleukin-10 (IL-10) (F8IL10/Dekavil), interleukin-13 (IL-13),

20 interferon beta (IFN- β), tissue inhibitor of MMP family (TIMPs), plasminogen-activator inhibitor (PAIs), serine protease inhibitors (serpins), signaling molecules/transcription factors (e.g. SMAD, Sox9, I κ B), extracellular matrix components (e.g. collagen, cartilage oligomeric matrix protein (COMP), proteoglycans, elastin), vasoactive intestinal peptide (VIP), Cluster of Differentiation 39 (CD39) and Cluster of Differentiation 73 (CD73), Superoxide dismutase (SOD), and combinations

25 thereof.

Functional genome editing systems for use in all embodiments of the invention are known to the person skilled in the art and include: Transcription Activator-Like Effector Nucleases (TALENs, Gaj et al. (2013) Trends Biotechnol. 31(7):397-405), zinc-finger nucleases (ZFNs, Gaj et al. (2013) *supra*), meganucleases such as I-SceI (Arnould et al. (2007) J Mol Biol 371(1):49-65;

30 Takeuchi et al. (2011) PNAS USA 108(32):13077-13082), RNA-guided endonuclease systems like CRISPR/Cas (Mali et al. (2013) Nat methods 10(10):957-963; Mali et al. (2013) Nat Biotechnol 31(9):833-838; Cong et al. (2013) Science 339(6121):819-823) and CRISPR/Cpf1 (Zetsche et al. (2015) Cell 163(3):759-771), triplex-forming molecules, synthetic polyamides and designer zinc-finger proteins (Uil et al. (2003) Nucleic Acids Res 31(21):6064-6078). Functional genome editing

35 systems employ nucleases which create site-specific double-strand breaks at desired locations in the genome. The induced double-strand breaks are repaired through nonhomologous end-joining or homologous recombination. As a result, targeted mutations are obtained. It is advantageous to replace a defective gene (causing a disease or disorder) with a normal allele at its natural location by any of these methods, because it does not require that the full coding sequences and regulatory

40 sequences are included in the rAAV virion when only a small portion of the gene needs to be altered.

Expression of the partially replaced gene is also thought to be more consistent with normal cell biology than full genes that are carried by virions. The preferred gene editing system is CRISPR (comprising CRISPR/Cpf1 and CRISPR-Cas), because it is quicker and cheaper than other methods. A major advantage is also that CRISPR can be easily repurposed to target different DNA sequences using the CRISPR single guide RNAs. Thus, alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the rAAV genome comprises at least one of: (i) a polynucleotide comprising a sequence encoding at least one guide RNA (gRNA); wherein the guide RNA is substantially complementary – preferably complementary – to a target polynucleotide sequence(s) in a genome; and (ii) a polynucleotide comprising a sequence encoding a nuclease; wherein the nuclease forms a ribonuclease complex with the guide RNA, and wherein the ribonuclease complex makes site-specific double-stranded DNA breaks in the genome.

In a second aspect, the present invention relates to an rAAV composition for use in treating, preventing or suppressing symptoms associated with an arthritic disease, wherein the rAAV composition comprises an rAAV virion of the invention and a pharmaceutically acceptable carrier, diluant, solubilizer, filler, preservative and/or excipient, preferably a pharmaceutically acceptable carrier. Such pharmaceutically acceptable carrier, diluents, solubilizer, filler, preservative and/or excipient may for instance be found in Remington: The Science and Practice of Pharmacy, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins, 2000. Any suitable pharmaceutically acceptable carrier, diluant, solubilizer, filler, preservative and/or excipient can be used in the present compositions (See *e.g.*, Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro (Editor) Mack Publishing Company, April 1997). Preferred pharmaceutical forms would be in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluids. Alternatively, a solid carrier may be used such as, for example, microcarrier beads.

Pharmaceutical compositions are typically sterile and stable under the conditions of manufacture and storage. Pharmaceutical compositions may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to accommodate high drug concentration. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. The parvoviral virion may be administered as a bolus or in a controlled release formulation, for example in a composition which includes a slow release polymer or other carriers that will protect the compound against rapid release, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may for example be used, such as ethylene vinyl acetate,

polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). As used herein, "pharmaceutically acceptable carrier" or "excipient" preferably includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible.

5 Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated.

10 It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete units suited as unitary dosages for subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of
15 the invention may be dictated by the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and by the limitations inherent in the art of compounding such an active compound for the treatment of a condition in individuals.

Supplementary active compounds can also be incorporated into the pharmaceutical compositions of the invention. Guidance on co-administration of additional therapeutics may for
20 example be found in the Compendium of Pharmaceutical and Specialties (CPS) of the Canadian Pharmacists Association.

In an embodiment, the rAAV composition further comprises empty particles (*i.e.*, capsid-only particles, thus not comprising an rAAV genome). Therefore, alternatively, or in combination with another embodiment, in a further embodiment of the present invention, the rAAV composition
25 of the invention further comprises an empty capsid in a ratio of empty capsid to rAAV virion of at least 1:1, more preferably at least 5:1, even more preferably at least 10:1. The rAAV composition can comprise the rAAV virion as defined above and an empty capsid, such as for example defined in WO 2016/055437, which is herein incorporated by reference, and as described in Aalbers et al. (2017) Hum. Gene Ther. 28(2):168-178. The empty capsid can be of the same serotype or of a
30 different serotype as compared to the rAAV-transgene vector of the composition of the invention. Preferably, the empty capsid is of the same serotype as the rAAV virion. Within such rAAV composition, the empty capsid and the capsid of the rAAV virion can comprise a modified capsid protein of the invention, preferably the same type of modified capsid proteins. However, also encompassed is an rAAV composition wherein the empty capsids have a different serotype or are
35 differently modified capsid proteins as compared to the modified capsid proteins of the rAAV virion. Further encompassed is an rAAV composition wherein the empty capsids have a mixture of serotypes, such as, but not limited to, a mixture of AAV2 and AAV5 capsids. The inventors report an increasing effect of transgene expression in joints after intraarticular administration of rAAV-
40 virions admixed with a significant amount of empty capsids. Preferably in the rAAV-virion and the empty capsid are present within the composition in a ratio of empty capsid to rAAV-virion of at least

1:1, 2:1, 3:1, 4:1, 5:1, 10:1, 15:1, 20:1, 50:1, 100:1, or 1000:1, preferably at least 5:1 (*i.e.* an amount of empty capsids that is at least 5 times the amount of rAAV-transgene vectors). Preferably, said composition comprises rAAV-virion and empty capsid in a ratio of empty capsid to rAAV-transgene vector of at most 10000:1, 5000:1, 4000:1, 3000:1, 2000:1, 1000:1, 500:1, 400:1, 300:1, 200:1, 5 100:1, 90:1, 80:1, 70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 15:1, 10:1 or 5:1, preferably at most 1000:1. Preferably said composition comprises rAAV-virion and empty capsids in a ratio of empty capsid to rAAV-virion of between 1:1 to 100:1, 2:1 to 100:1, 5:1 to 100:1, 1:1 to 20:1, 2:1 to 20:1 or preferably between 5:1 to 20:1.

10 Provided herein above is an embodiment wherein the rAAV-virion and the empty capsids are present in a single composition. Also encompassed within the present invention is an alternative embodiment wherein the rAAV-virion and the empty capsids are present in (at least two or more) separate, distinct compositions. In this alternative embodiment, the rAAV-virion and the empty capsids can be administered separately in time (*e.g.*, sequentially) and/or localization, wherein localization is to be understood as the site of administration. Furthermore, the rAAV-virion and the 15 empty capsids can be administered simultaneously, *e.g.*, at substantially the same timing, optionally at a separate location.

In a third aspect, the present invention relates to an rAAV composition and an immunosuppressant for use in treating or preventing an arthritic disease or for use in treating or preventing symptoms associated with an arthritic disease, wherein the rAAV composition is as 20 defined above and wherein the treatment or prevention comprises the administration of the rAAV composition and the administration of the immunosuppressant to an individual. WO 2016/055437, herein incorporated by reference, discloses an increasing effect of an immunosuppressant on AAV transgene expression when subjects were treated with both immunosuppressants and rAAV-virions. Furthermore, WO 2016/055437 discloses a surprising synergistic effect of the 25 immunosuppressant together with empty vectors on rAAV transgene expression. In one embodiment, the immunosuppressant is applied separately from the rAAV composition, separate meaning separate in location and/or time. In such an embodiment, the immunosuppressant and the rAAV composition may be present in separate and distinct compositions. The immunosuppressant, the rAAV-virion and optionally the empty vectors may even each be present in a separate, distinct 30 composition. In another embodiment, the immunosuppressant and the rAAV composition may be present in a single composition. In a further embodiment, the rAAV-virion and the immunosuppressant are present in a single composition, and preferably this composition is used in treatment together with a separate composition comprising the empty capsid. In an even further embodiment, the immunosuppressant and the empty capsid are present in a single composition, 35 and preferably this composition is used in treatment together with a separate composition comprising the rAAV-virion. Therefore, the invention also provides for a composition comprising an empty capsid and an immunosuppressant as defined herein, for a composition comprising an rAAV-virion and an immunosuppressant as defined herein, and for a composition comprising an rAAV composition and an immunosuppressant as defined herein.

Preferably, an immunosuppressant for use in the present invention is an innate immune cell inhibitor, preferably a macrophage inhibitor. An innate immune cell is defined herein as a neutrophil, macrophage, monocyte, eosinophil, basophil, or dendritic cell, that has the potential to participate in the inflammatory response to a foreign substance. An innate immune cell inhibitor is herein defined as an agent that results in a decrease in innate immune cell activity and/or innate immune cell number. A macrophage inhibitor is defined herein as an agent that results in a decrease in macrophage activity and/or macrophage number. A "macrophage" is understood herein as an innate immune cell that engulfs and digests cellular debris, foreign substances, microbes, and cancer cells in a process called phagocytosis. Preferably, the innate immune cell or macrophage inhibitor of the invention, results in a decrease of at least 1, 2, 5, 10, 15, 20, 25, 30, 35, 45, 55, 65, 75, 85, 95% or preferably of 100% of the number or activity of innate immune cells or macrophages as compared to the initial number or activity of innate immune cells or macrophages before treatment. Innate immune cell or macrophage activity and/or number can be detected by any suitable assay known by the person skilled in the art, such as, but not limited to, MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) colorimetric assay for testing macrophage cytotoxic activity *in vitro* as described by Ferrari et al. (Journal of Immunological Methods, 131 (1990) 165-172), by measurement of cytokine levels (e.g., CCL2, TNF), by histological and histochemical detection methods, for instance, by CD68 labeling or by *in vivo* magnetic resonance imaging (MRI) detection of superparamagnetic iron oxide (SPIO) uptake by macrophages, preferably after intravenous administration of SPIO as reviewed by Yi-Xiang J. Wang (Quant. Imaging Med Surg (2011)1:35-40). The detection can either be *in vitro* or *in vivo*. Preferably, *in vivo* detection is in an animal model, preferably a rat or murine model.

Preferably, the immunosuppressant is a glucocorticoid and/or a bisphosphonate, preferably a liposomal bisphosphonate. Particular non-limiting examples of glucocorticoids are cortisol, cortisone, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate and aldosterone. Preferably, the immunosuppressant is triamcinolone. Particular non-limiting examples of bisphosphonates are etidronate, clodronate, tiludronate, pamidronate, neridronate, olpadronate, alendronate, ibandronate, risedronate and zoledronate. Preferably, the bisphosphonate is a liposome-encapsulated bisphosphonate or liposomal bisphosphonate, preferably liposomal clodronate. Preferably, the glucocorticoid is not dexamethasone. It is to be understood that the inflammatory or macrophage inhibitor of the invention is not limited to a glucocorticoid and/or a bisphosphonate. For instance, the inflammatory or macrophage inhibitor of the invention can also be an inflammatory or macrophage-depleting antibody such as an anti-F4/80 antibody. Preferably, such antibody is a human or humanized antibody. Further relevant immunosuppressants to be used in the present invention are cytostatic drugs (e.g. alkylating agents and/or antimetabolites such as methotrexate), drugs that modify the purinergic signaling pathway (e.g. methotrexate, adenosine analogs, adenosine receptor antagonists or agonists), non-steroidal anti-inflammatory drugs (NSAIDS, e.g. ibuprofen, diclofenac, meloxicam, naproxen, acetylsalicylic acid), biologicals such as TNF blockers (e.g. infliximab, etanercept, adalimumab, certolizumab, golimumab), IL-6 blockers

(e.g. tocilizumab), IL-2 blockers (e.g. basiliximab, daclizumab), IL-1 β blockers (e.g. anakinra, rilonacept, canakinumab) IL-17 (secukinumab, brodalumab, ixekinumab), anti-IL-12/IL-23 (ustekinumab), a PDE4-inhibitor (apremilast) muromonab, abatacept, and/or rituximab, and/or other compounds such hydroxychloroquine, chloroquine, leflunomide, sulfasalazine, azathioprine, cyclophosphamide, cyclosporine, gold salt, mTOR inhibitors (e.g. rapamycin/sirolimus, everolimus) and penicillamine.

Preferably, the rAAV composition and/or composition comprising empty capsids and/or the composition comprising the immunosuppressant further comprises a pharmaceutically acceptable carrier, diluents, solubilizer, filler, preservative and/or excipient as defined elsewhere herein.

Preferably, gene therapy according to the present invention further comprises the administration of an immunosuppressant as defined herein, either present within the rAAV composition, or comprised within a separate, distinct composition, *i.e.* separate and distinct from the rAAV composition. At administration, the rAAV composition and/or empty capsids and/or immunosuppressant of the invention is delivered to an individual, a cell, tissue or organ of said individual, preferably an individual suffering from a condition or disease as defined herein. Preferably, the rAAV composition and the immunosuppressant are administered simultaneously. Simultaneous administration is to be understood herein as administration at more or less the same time, preferably no longer separated than 15 min, 30 min, 1 hour, 2 hours, 3 hours, 12 hours or 24 hours in time, preferably no longer separated than 15 min in time. In another embodiment, the rAAV composition and the immunosuppressant are administered sequentially, wherein preferably the immunosuppressant is administered prior to the rAAV composition. Preferably, the immunosuppressant is administered at least 1 hour, 3 hours, 12 hours, 24 hours, 2 days, 4 days or 1 week before administration of the rAAV composition. In case the rAAV-virions and the empty capsids are present in separate compositions, the immunosuppressant may be administered simultaneously or within at least 15 min, 1 hour, 2 hours, 3 hours, 1 day, 2 days or 1 week prior to the empty capsids and the empty capsids in turn are administered simultaneously or within at least 15 min, 1 hour, 2 hours, 3 hours, 1 day, 2 days or 3 days prior to the rAAV-virion.

Within the embodiments defined herein, the immunosuppressant may be administered repeatedly, *i.e.* prior to and/or simultaneously with the rAAV composition. As indicated herein above, preferably the rAAV composition comprises a significant amount of empty capsids. Furthermore, the invention encompasses the administration of both rAAV-transgene vectors and empty capsids in separate, distinct compositions, which may be administered simultaneously or sequentially in a method or use of the invention. If comprised in separate compositions, the rAAV-transgene vectors and empty capsids are preferably administered simultaneously. In a further embodiment, the empty capsids are administered at most 3 days, 2 days, 1 day, 24 hours, 12 hours, 3 hours, 2 hours, 1 hour, 30 min, 15 min or 5 min, preferably at most 24 hours, prior to rAAV-transgene vector administration. Furthermore, if comprised in separate compositions, the rAAV-transgene vectors and empty capsids are preferably administered at the same site.

The immunosuppressant dose depends on the type of immunosuppressant. Effective dosages are known by the skilled person. A preferred therapeutic effective dosage of triamcinolone

is indicated above. A preferred therapeutic effective dosage of liposomal clodronate is preferably a therapeutic effective dose as known by the skilled person, e.g. preferably 80-320 mg/dose intraarticular, more preferably 160 mg/dose intraarticular (Barrera et al. 2000, Arthritis & Rheumatism Vol 43(9), p1951-1959).

5 Generally, a joint disorder is termed an arthropathy, and when involving inflammation of one or more joints the disorder is termed an arthritis. Most joint disorders involve arthritis, however, joint damage caused by external physical trauma is typically not termed arthritis. The term "arthritis disease" as used herein, also referred to as "arthritis", is herein defined as a form of joint disorder that involves inflammation of one or more joints. Currently, it is estimated that there are over a
10 hundred different forms of arthritis. The arthritic disease is herein understood as referring to "joint pain" or "joint disease". In a preferred embodiment, the arthritic disease is selected from the group consisting of Adult-onset Still's disease, ankylosing spondylitis, arthritis, back pain, Behçet's disease, blunt trauma, bursitis, calcium pyrophosphate deposition disease (CPPD), carpal tunnel syndrome, chondromalacia patella, chronic fatigue syndrome, complex regional pain syndrome,
15 cryopyrin-associated periodic syndromes (CAPS), degenerative disc disease, developmental-dysplasia of hip, Ehlers-Danlos, familial mediterranean fever, fibromyalgia, fifth disease, giant cell arteritis, gout, hemochromatosis, infectious arthritis, inflammatory arthritis, inflammatory bowel disease, joint replacement, juvenile arthritis, juvenile dermatomyositis (JD), juvenile idiopathic arthritis (JIA), juvenile rheumatoid arthritis, juvenile scleroderma, Kawasaki disease, lupus, lupus in
20 children & teens, Lyme disease, mixed connective tissue disease, myositis (inc. polymyositis, dermatomyositis), osteoarthritis (OA), osteoporosis, pagets, palindromic rheumatism, patellofemoral pain syndrome, pediatric rheumatic diseases, pediatric SLE, polymyalgia rheumatica, pseudogout, psoriatic arthritis, Raynaud's phenomenon, reactive arthritis, reflex sympathetic dystrophy, Reiter's syndrome, rheumatic fever, rheumatism, rheumatoid arthritis,
25 scleroderma, septic arthritis, Sjögren's disease, spinal stenosis, spondyloarthritis, Still's disease, systemic juvenile idiopathic arthritis, systemic lupus erythematosus, systemic lupus erythematosus in children & teens, systemic sclerosis, temporal arteritis, tendinitis, vasculitis and Wegener's granulomatosis. In a further preferred embodiment the arthritic disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis,
30 arthritis, juvenile idiopathic arthritis, blunt trauma, joint replacement and Still's disease. In a more preferred embodiment, the arthritic disease is a joint disorder that involves inflammation of one or more joints. Preferably, the arthritic disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis
35 (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis, arthritis, juvenile idiopathic arthritis and Still's disease.

 Alternatively, or in combination with another embodiment, in a further preferred embodiment of the present invention, the rAAV virion or the rAAV composition is administered systemically and/or locally. An rAAV composition and/or empty capsids and/or an immunosuppressant of the
40 invention may be directly or indirectly administrated using suitable means known in the art. Methods

and uses of the invention include delivery and administration of the rAAV composition and/or empty vector and/or immunosuppressant systemically, regionally or locally, or by any route, for example, by injection, infusion, orally (e.g., ingestion or inhalation), or topically (e.g., transdermally). Exemplary administration and delivery routes include intravenous (i.v.), intraarticular, intraperitoneal (i.p.), intraarterial, intramuscular, parenteral, subcutaneous, intrapleural, topical, dermal, intradermal, transdermal, parenterally, e.g. transmucosal, intracranial, intraspinal, oral (alimentary), mucosal, respiration, intranasal, intubation, intrapulmonary, intrapulmonary instillation, buccal, sublingual, intravascular, intrathecal, intracavity, iontophoretic, intraocular, ophthalmic, optical, intraglandular, intraorgan, intralymphatic. Improvements in means for providing an individual or a cell, tissue, organ of said individual with an rAAV composition and/or empty capsids and/or an immunosuppressant of the invention, are anticipated considering the progress that has already been achieved thus far. Such future improvements may of course be incorporated to achieve the mentioned effect of the invention. When administering an rAAV composition and/or empty capsids and/or an immunosuppressant of the invention, it is preferred that such combination and/or composition is dissolved in a solution that is compatible with the delivery method. For intravenous, subcutaneous, intramuscular, intrathecal, intraarticular and/or intraventricular administration it is preferred that the solution is a physiological salt solution. In case an immunosuppressant is present within the rAAV composition of the invention, the immunosuppressant is administered at the same site as the rAAV composition, *i.e.* preferably locally as indicated above. In the embodiment wherein the immunosuppressant is comprised within a separate composition distinct from the rAAV composition, the immunosuppressant may be administered systemically, preferably intramuscularly or intravenously. The rAAV composition may also be administered locally, preferably at a site of the body comprising substantive numbers of macrophages as defined herein, and the immunosuppressant is administered systemically, preferably intramuscularly or intravenously. Also encompassed in the invention is an embodiment wherein the immunosuppressant and the rAAV composition, even though present in distinct compositions, are administered at the same site, preferably locally, more preferably intraarticularly. As further indicated herein, administration of such distinct compositions may be either simultaneously or sequentially. In a preferred embodiment of the present invention, at least one of the rAAV composition and the immunosuppressant is administered locally. More preferably, the local administration is intraarticular administration. "Intraarticular injection" (also known as "joint injection" or "intraarticular injection") is herein defined as injection or infusion into the joint. Intraarticular injection is typically used for administration of an anti-inflammatory agent into a joint affected by inflammation.

In a further aspect, the present invention relates to an rAAV composition comprising an rAAV virion of the invention and a pharmaceutically acceptable carrier, diluant, solubilizer, filler, preservative and/or excipient, preferably a pharmaceutically acceptable carrier as defined herein. In a preferred embodiment, the composition further comprises empty capsids as herein defined and/or an immunosuppressant as herein defined.

In a further aspect, the present invention relates to a method for treating, preventing, or suppressing symptoms associated with an arthritic disease, wherein the method comprises the step of intraarticular administration of a medicament comprising an effective amount of an rAAV virion as defined in any one of claims 1 – 8 or of an rAAV composition as defined above.

5 A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of a nucleic acid, nucleic acid construct, rAAV virion or pharmaceutical composition may vary according to factors such as the disease state, age, sex, and weight of the subject to be treated, and the ability of the nucleic acid, nucleic acid construct, rAAV virion or pharmaceutical composition to elicit a
10 desired response in the subject. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also typically one in which any toxic or detrimental effects of the nucleic acid, nucleic acid construct, rAAV virion or pharmaceutical composition are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the
15 desired prophylactic result, such as preventing or inhibiting various conditions. A prophylactic dose may be used in subjects prior to or at an earlier stage of disease, and a prophylactically effective amount may be more or less than a therapeutically effective amount in some cases. The dosage to be administered may depend to a large extent on the condition and size of the subject being treated as well as the therapeutic formulation, frequency of treatment and the route of administration.
20 Regimens for continuing therapy, including dose, formulation, and frequency may be guided by the initial response and clinical judgment.

The term "subject" or "patient" is used interchangeably herein and refers to an animal, including the human species, that is treatable with the compositions and/or rAAV of the present invention. Accordingly, the term "subject" or "patient" includes, but is not limited to, human, non-
25 human primate such as chimpanzees, and other apes and monkey species, or any mammal such as dog, cat, horse, sheep, pig, cow etc. In a preferred embodiment of the present invention, the subject treated with an rAAV according to the present invention is a mammal, more preferably a human, dog, cat or horse, most preferably a human.

In this document and in its claims, the verb "to comprise" and its conjugations are used in
30 their non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

35 The word "approximately" or "about" when used in association with a numerical value (approximately 10, about 10) preferably means that the value may be the given value of 10 more or less 10% of the value.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Description of the figures

5 The present invention will be discussed in more detail below, with reference to the attached drawings:

Figure 1: Screening of capsid serotypes on HEK293T and FLS cells. Crude lysate containing 7 mutant capsid serotypes (plus AAV5) expressing yellow fluorescent protein (YFP) were used to transduce HEK293T cells or 3 different FLS cell lines (each from a different RA patient).
10 After 72 hours (HEK293T) or 6 days (FLS), cells were assayed for percentage of cells expressing YFP by FLOW cytometry. Panel A shows % of HEK 293T cells expressing YFP; Panel B shows the % of YFP-expressing cells in 3 different FLS cell lines; Panel C shows the mean fluorescent intensity (MFI) in HEK293T cells; Panel D shows MFI in 3 different FLS cell lines (all cells); Panel E shows MFI in 3 different FLS cell lines (only positive population). The sample legend is depicted in Table
15 2.

Figure 2: Capsid mutants show increased luciferase expression vs wt-AAV5 in FLS cells. Purified AAV (4 mutant serotypes or AAV5) expressing YFP-Luc fusion protein were used to transduce three different FLS cell lines from different RA patients: BB5498 (FLS 1), BB5540 (FLS 2) and BB7144 (FLS 3) using two MOIs (20000 or 100000 rAAV particles per cell). After 4 days,
20 cells were lysed and luciferase expression was measured. Data is presented as absolute luciferase expression levels (RLU; white bars) or fold increase over AAV5 (black bars). Panel A shows FLS 1 at MOI 20K; Panel B shows FLS 1 at MOI 100K; Panel C shows FLS 2 at MOI 20K; Panel D shows FLS 2 at MOI 100K; Panel E shows FLS 3 at MOI 20K; and Panel F shows FLS 3 at MOI 100K. Open bars show luciferase (RLU) and filled bars show "fold increase" over AAV5. In a different
25 experiment, three additional FLS cell lines from RA patients were transduced with AAV (7 mutant serotypes or AAV5) expressing luciferase: BB4308 (FLS 4), BX 1592 (FLS 5), BB4426 (FLS 6) using 2 MOIs (10K or 100K rAAV particles per cell). Panel G shows FLS 4 at MOI 10K; Panel H shows FLS 4 at MOI 100K; Panel I shows FLS 5 at MOI 10K; Panel J shows FLS 5 at MOI 100K; Panel K shows FLS 6 at MOI 10K; and Panel L shows FLS 3 at MOI 100K.

30 Transduction efficacy of the 7 mutant serotypes or AAV5 (MOI 100K) was also evaluated in HEK293T cells (Panel M). Open bars show luciferase expression (RLU) and filled bars show "fold increase" over AAV5.

Figure 3A: Capsid mutants exhibit increased gene expression *in vivo*. Two capsid mutants (AAV9-A2 and AAV7-A6) were compared with wtAAV5 using the air pouch synovium model.
35 Luciferase-expressing vector was administered on day 0 following air pouch formation and luciferase expression was measured by live animal imaging (IVIS) on day 3 following transduction. Data shown is the luminescence (photon/second/square centimeter m²/steradian) in air pouch in mean+SEM.

Figure 3B: In a second experiment, 5 selected capsid mutants (AAV1-P4, AAV7-A6, AAV9-A2, AAVrh10-A2, AAVrh10-A6) and wtAAV5 were injected into the knee joints of mice. A luciferase
40

expressing vector was injected on day 0 and expression was measured by live imaging (IVIS) at indicated time points after administration. Data shown is the luminescence (photon/second/square centimeter m2/steradian)(left panel) in mean+SEM. **P<0.05, ***P<0.01, ****P<0.00001 vs. wtAAV5 at day 14. Figure 3C: Fold increase vs. wtAAV5.

5 Figure 4: CLUSTAL format alignment by MAFFT FFT-NS-I (v7.215). Below the alignment is a key denoting a conserved residue (*);and a non-conservative mutation ().

Figure 5: CLUSTAL multiple sequence alignment by MUSCLE (3.8). Below the alignment is a key denoting a conserved residue (*); a conservative mutation (:); a semi-conservative mutation (.); and a non-conservative mutation ().

10 Figure 6: CLUSTAL format alignment of inserts P4, A2, A6, P2 and QR-P2 (SEQ ID NO's: 8 – 12) by MAFFT FFT-NS-I (v7.215). Below the alignment is a key denoting a conserved residue (*); and a non-conservative mutation ().

15 Figure 7: CLUSTAL multiple sequence alignment of inserts P4, A2, A6, P2 and QR-P2 (SEQ ID NO's: 8-12) by MUSCLE (3.8). Below the alignment is a key denoting a conserved residue (*); and a non-conservative mutation ().

Sequence listing

Table 1 provides an explanation of the sequence references in correlation with the SEQ ID No's.

Table 1: Explanation of sequence references

SEQ ID NO:	serotype	Modified capsid/insert/wild-type
1	AAV1	Modified capsid
2	AAV2	Modified capsid
3	AAV7	Modified capsid
4	AAV9	Modified capsid
5	AAVrh10	Modified capsid
6	AAVrh10	Modified capsid
7	AAV DJ-QR	Modified capsid
8	Insert A2	Insert
9	Insert A6	Insert
10	Insert P2	Insert
11	Insert P4	Insert
12	Insert QR-P2	Insert
13	AAV1	Wild-type capsid
14	AAV2	Wild-type capsid
15	AAV7	Wild-type capsid
16	AAV9	Wild-type capsid
17	AAVrh10	Wild-type capsid
18	AAV DJ-QR	Synthetic capsid
19	AAV5	Wild-type capsid

Examples**Example 1***Initial screening of capsid library*1.1. Materials and methods

5 96-well plates spotted (and subsequently dried) with crude lysate containing AAV from 91 different AAV capsid serotypes were obtained from Dirk Grimm and Kathleen Börner at the University of Heidelberg. Each vector encoded a YFP transgene driven by a CMV promoter. As FLS are the primary target cells in the joint, an AAV capsid mutant library was screened for serotypes that show increased expression in human FLS isolated from joints of rheumatoid arthritis patients (RA-FLS) 10 (as described in van de Sande MG et al., (2011) Ann Rheum Dis 70: 423-427). RA-FLS were plated (2500/well, 37°C / 5% CO₂) directly onto the spotted plates (DMEM-GlutaMAX-I (Gibco, ref.31966-021), 10% FBS (heat inactivated (HI) Bovine Serum Gold, Gibco, ref A15-151), 10 mM HEPES (Gibco, ref.15630-056), 50 µg/ml gentamycin (Gibco, ref.15710-049), 100U/ml penicillin/100µg/ml streptomycin (Sigma-Aldrich, ref.P0781) and all wells were visualized for YFP expression by 15 fluorescence microscopy after 6 days.

1.2. Results

Transduction efficacy of capsid mutants vs. WT-AAV5 in FLS from RA patients.

In screening of the 91 capsid mutants, while the overall expression levels were low, the present 20 inventors identified 7 different serotypes that showed higher expression than wtAAV5: AAV9-A2, AAV7-A6, AAV1-P4, AAVDJ-QR-P2, AAVrh10-A6, AAVrh10-A2 and AAV2-P2 (amino acid sequences SEQ ID NO: 1 – 7; wtAAV5 SEQ ID NO: 19).

Crude lysates of all 7 vectors were used in an *in vitro* transduction assay in 3 different patient FLS cell lines and in HEK293T cells (example 2).

25

Table 2: Sample legend for figure 1

Sample	Capsid serotype	Insert/modified sequence	Insert	Position in VP1	SEQ ID NO:
5	5	none	none	-	19
61	AAV1	GQSGNDVRSANAQAA	P4	588 – 602	1
33	AAV9	GQRGNYSRGVDAQAA	A2	586 – 600	4
34	AAVrh10	GQRGNYSRGVDAQAA	A2	588 – 602	6
50	AAV2	QQQSGCDCRGDCFCA (QAA)	P2	585 – 599	2
88	AAV-DJ-QR	QQQSGCDCRGDCFCA(QAA)	QR-P2	587 – 601	7
43	AAV7	GQRGNEARVREAQAA	A6	587 – 601	3
46	AAVrh10	GQRGNEARVREAQAA	A6	588 – 602	5

Example 2*Expression of crude lysates of 7 selected mutants*2.1. Materials and methods*AAV production*

5 Details on the production of the crude AAV lysates can be found in Grosse et al. (J. Virol, 2017, doi: 10.1128/JVI.01198-17).

Aliquots of crude lysate for each of the selected 7 capsid mutants (plus wtAAV5 as a control) were used to transduce cells (HEK293T or 3 different FLS cell lines isolated from RA patients) and YFP expression was measured by flow cytometry 3 (HEK293T) -5 days (FLS) following transduction. In detail, HEK293T were seeded in a 96-well plate (Greiner Bio-One, ref.655180) at 45000 cells per well. RA-FLS were seeded in a 96-well plate at 2500 cells per well. After overnight incubation, the cell supernatants were replaced with 40 μ l DMEM-glutaMAX-I (Gibco 31966-021) containing 0.001% pluronic F68 solution (Sigma P5556). The virus lysates were added *in duplo*, 10 μ l per well. After 4 hours, doxorubicin (final concentration 0.4 μ M) (Sigma D1515) in DMEM-glutaMAX-I containing FBS (heat inactivated (HI) Bovine Serum Gold, Gibco, ref A15-151), final concentration 1%) was added to the wells (50 μ l per well). The day after, the medium of FLS was removed and DMEM-glutaMAX-I (10% FBS (heat inactivated (HI) Bovine Serum Gold, Gibco, ref A15-151), 10 mM HEPES (Gibco, ref.15630), 50 μ g/ml gentamycin (Gibco ref. 15710-049), 100 U/ml penicillin/100 μ g/ml streptomycin (Sigma-Aldrich, ref. P0781)) was added (200 μ l per well). The medium of HEK293T cells was not changed. Three (HEK293T cells) or 6 days (FLS) after transduction cells were trypsinized using 0.5% Trypsin/EDTA (Gibco ref.15400-054) in PBS (Gibco, ref. 10010) and analyzed for YFP expression by FLOW cytometry (FACSCanto II, BD Biosciences). Both percentage of expressing cells and mean fluorescence intensity (MFI) for all cells was determined.

25

2.2. Results

Crude lysates of all 7 vectors were used in an *in vitro* transduction assay in 3 different patient FLS cell lines and in HEK293T cells. Cells were assayed for the percentage of cells expressing YFP by fluorescence microscopy (data not shown) or FLOW cytometry (Figure 1 panels A – E). While there was some variability between cell types, all mutant capsids gave higher expression in both, FLS and HEK293T cells than AAV5-WT (Figure 1). Table 2 provides the sample legend for Figure 1. Based on these results, four capsid mutants were selected for further investigation (see example 3).

35 **Example 3***In vitro testing of capsid variants in HEK293T and FLS*3.1 materials and methods

3.1.1 Four of the mutant capsid proteins, AAV9-A2, AAV7-A6, AAV1-P4, and AAVDJ-QR-P2, were further investigated. Purified vector (Iodixanol gradient) expressing a YFP-Luciferase fusion protein

40

(to allow for visualization (YFP) as well as quantification by luciferase assay) was generated. Three different primary FLS lines isolated from rheumatoid arthritis patients (as described in van de Sande MG et al., (2011) Ann Rheum Dis 70: 423-427) were transduced with each serotype at 2 vector doses (MOI 20,000 or 100,000) and after 4 days, cells were harvested and gene expression was

5 quantified by luciferase assay (Promega Luciferase assay Kit).

In detail, RA-FLS were plated at 2500 cell/well in a 96-well plate (Greiner Bio-One, ref.655207) in medium (DMEM-GlutaMAX (Gibco ref.31966-021), 10% FBS (heat inactivated (HI) Bovine Serum Gold, ref A15-151), 10 mM HEPES (Gibco ref. 15630-056), 50 µg/ml gentamycin (Gibco, ref 15710-049), 100u/ml penicillin/100µg/ml streptomycin (Sigma-Aldrich Merck ref. P0781). After 48h,

10 medium was removed and virus (in DMEM-Glutamax containing 0.001% Pluronic-68 (Sigma, ref. p5556)) was added at an MOI of 20,000 or 100,000. After 4h, medium containing Doxorubicin (Sigma, ref.D1515, final concentration 0.4 µM) and FBS (final concentration 1%) was added.

24h later, medium was replaced with DMEM-GlutaMAX, (10% FBS, 10 mM HEPES, 50 µg/ml gentamycin, 100u/ml penicillin, 100µg/ml streptomycin). Four days post-transduction, cells were

15 washed 1x with 100 µl PBS (Gibco, ref. 10010) and luciferase activity was determined using the ONE Glo™ luciferase assay system (Promega, ref.E6110): 100 µl Lysis buffer was added and cells were placed on a shaker for 10', 900 rpm at RT. Subsequently, 20 µl lysate was transferred to a white 96-well plate, 80 µl substrate (was added for 3' (dark) and luciferase activity was determined on a luminometer (1 sec/well, synergy HT, Biotek).

3.1.2. In a similar experiment, three additional FLS cell lines isolated from rheumatoid arthritis patients were transduced with AAV5 and 7 capsid mutants from a different AAV preparation than described in 3.1.1 (AAV9-A2, AAV1-P4, AAV7-A6, AAVDJ-QR-P2, AAVrh10-A6, AAVrh10-A2, AAV2-P2) containing a luciferase gene (MOI 10,000 and 100,000). The number of empty particles differed between the AAV preparations. To exclude a possible effect on transduction efficacy, empty

25 capsid correction was done by adding AAV5 empty particles to equalize the percentage of empty particles per preparation.

3.1.3. The 7 capsid mutants from the same preparation as described in 3.1.2 were also tested in HEK293T cells. In detail, HEK293T were seeded in a 96-well plate (Greiner Bio-One, ref.655180) at 50000 cells per well. After overnight incubation, the cell supernatants were replaced with DMEM-glutaMAX-I (Gibco 31966-021) containing 0.001% pluronic F68 solution (Sigma P5556). The different vectors were added *in duplo*, at an MOI of 100,000. In this protocol, empty capsid correction was done as described for 3.1.2. After 4 hours, doxorubicin (final concentration 0.4 µM) (Sigma D1515) in DMEM-glutaMAX-I-containing FBS (heat inactivated (HI) Bovine Serum Gold, Gibco, ref A15-151), final concentration 1%, was added to the wells. Three days after transduction,

30 cells were harvested and gene expression was quantified by luciferase assay (Promega Luciferase assay Kit) on a luminometer (BMG Labtech Fluostar Omega).

3.2. Results

3.2.1 *In vitro* transductions of three different FLS cell lines were performed using

40 recombinant AAV comprising one of the 4 mutant capsids (as well as AAV5 as control, made in the

identical manner) following the protocol described in 3.1.1. All 4 serotypes showed increased expression levels when compared with AAV5, ranging from 2-fold to 35-fold increases, depending on the serotype and cell line used (Figure 2A-F).

3.2.2 In another series of experiments, *in vitro* transduction efficacy of 7 mutant capsids (as well as AAV5 control, made in the identical manner) was assessed in 3 FLS cell lines. All 7 serotypes showed increased luciferase expression levels when compared with AAV5, ranging from 6-fold to 55-fold increases depending on the serotype and cell line used (Figure 2G-L)

3.2.3 A similar experiment was performed in HEK293T cells. Transduction with all 7 serotypes resulted in enhanced luciferase expression compared with wtAAV5, ranging from 2-fold to 12-fold increases (Figure 2M).

Example 4

In vivo study in the air pouch synovium model

4.1. Materials and methods

Animals

Female Balb/c mice (8–10 weeks old and weighing 20–25 g; (Harlan, Boxmeer, the Netherlands)) were housed in individual ventilated cages at the animal facility of the Academic Medical Center, Amsterdam. Food and water were available *ad libitum*. All animal experiments were performed according to the guidelines of the Animal Research Ethics Committee of the University of Amsterdam.

Air pouch synovium (APS) model

Two serotypes, AAV9-A2 and AAV7-A6, were compared against wtAAV5. The air pouch synovium model was adapted from Edwards et al (1981; J Pathol 134: 147-156). At day 0, 3 ml of air was injected subcutaneously into the dorsal skin of 7-9 week-old female Balb/cOlaHsd mice (Harlan) (day 0). Immediately following the formation of the air pouch, 1 ml of air was removed and 1 ml of AAV (2e10 vector genomes/mouse in PBS (Gibco, ref.10010 containing 0.001% pluronic F68 (Sigma, ref.p5556) was added directly into the air pouch. Three days following transduction, gene expression was measured by *in vivo* animal imaging.

Imaging of luciferase expression

Luciferase expression was measured at day 3. It was initially planned to continue monitoring expression for up to 3 months following vector administration, however, a parvovirus infection of the animal facility resulted in the premature termination of all ongoing experiments. D-luciferin potassium-salt substrate (Caliper Life Sciences, Hopkinton, MA, USA) was injected intraperitoneally (150 mg/kg of body weight, in a volume of approximately 200 µl). Photon counts were acquired 10 min after substrate administration for 5 min using a cooled charge-coupled device (CCD) camera system (Photon Imager, Biospace Lab, Paris, France) and image processing and signal intensity quantification and analysis were performed using M3 Vision (Biospace Lab). The number of photons

emitted per second per square centimeter per steradian was calculated as a measure of luciferase activity.

General animal conditions and ethics statement

5 Air pouch formation, vector administration and *in vivo* imaging were performed under isoflurane anaesthesia (3% isoflurane and oxygen). At the end of the experiments, animals were sacrificed by cardiac puncture under isoflurane anaesthesia, followed by cervical dislocation. The studies were reviewed and approved by the animal care and use committee of the University of Amsterdam and carried out in strict accordance with the recommendations in the Dutch Law on Animal Welfare
10 (Dutch: "Wet op Dierproeven"). Animals were maintained under pathogen-free conditions in the animal facility of the University of Amsterdam.

4.2. Results

Based on these promising results, a preliminary *in vivo* study was performed using the air
15 pouch synovium (APS) model, where two serotypes, AAV9-A2 and AAV7-A6, were compared against wtAAV5. Due to an unfortunate infection in the animal facility that necessitated the premature termination of this study, we were only able to obtain data from a single time point, day 3 post vector administration. At this time point it was clear that the capsid mutants were giving rise to increased gene expression when compared with AAV5, with AAV7-A6 showing ~6-fold increased
20 expression and AAV9-A2 ~22-fold (Figure 3A).

Example 5:

In vivo study: intra-articular injections in healthy animals

25 5.1. Material and methods

Animals

Male DBA1/J mice (12 weeks old, Envigo) were housed in individual ventilated cages at the animal facility of the Academic Medical Center, Amsterdam. Food and water were available *ad libitum*. All
30 animal experiments were performed after approval of the Central Commission Animal Experiments (CCD) and the Animal Research Ethics Committee of the University of Amsterdam, the Netherlands.

Expression study

35 Five rAAV comprising capsid mutants, i.e., AAV9-A2, AAV1-P4, AAV7-A6, AAVrh10-A6 and AAVrh10-A2, were compared against wtAAV5. As capsid load may affect expression (Aalbers CJ et al., Hum Gene Ther 2017;28 (2):168-178), rAAV preparations were corrected for the capsid load by adding wtAAV5 empty particles. Healthy mice (n=9 per group) received intra-articular injections of AAV vector carrying the luciferase gene in both knees (7.5×10^9 viral genomes per knee). Gene
40 expression was determined by *in vivo* imaging at several time points after vector administration.

Imaging of luciferase expression

Luciferase expression was determined at indicated time points (Fig 3B). At each time point, D-luciferin potassium-salt substrate (Caliper Life Sciences, Hopkinton, MA, USA) was injected intraperitoneally (150 mg/kg of body weight, in a volume of approximately 200 µl). Photon counts were acquired 15 min after substrate administration for 5 min using a cooled charge-coupled device (CCD) camera system (Photon Imager, Biospace Lab, Paris, France). Image processing and signal intensity quantification and analysis were performed using M3 Vision (Biospace Lab). The number of photons emitted per second per square centimeter per steradian was calculated as a measure of luciferase activity.

General animal conditions and ethics statement

Vector administration and *in vivo* imaging were performed under isoflurane anaesthesia (4% isoflurane and oxygen). The studies were carried out in strict accordance with the recommendations in the Dutch Law on Animal Welfare (Dutch: "Wet op Dierproeven"). Animals were maintained under pathogen-free conditions in the animal facility of the University of Amsterdam.

5.2. Results

At the first time point, day 3, AAV-mediated expression in the knee is detected in all groups and increases in time (Figure 3B). All capsid mutants except AAV1-P4 show increased expression vs. wtAAV5 with AAV9-A2 showing the highest expression (~5 fold increased vs. wtAAV5 at day 14) (Figure 3C). Expression levels at day 14 from high to low: AAV9-A2 > AAVrh10-A2 > AAVrh10-A6 > AAV7-A6 > wtAAV5 > AAV1-P4 . On day 7, AAVrh10-A2, AAV9-A2 and AAVrh10-A6 show significantly increased expression vs. wtAAV5 (**P<0.05, ***P<0.01, ****P<0.00001 vs. wtAAV5 at day 14. (Figure 3B).

Example 6:

Determination of neutralizing antibody titers against capsid mutants in human sera

6.1 Material and methods

HEK293T cells were plated in DMEM containing 9% FBS, 0.9% penicillin/streptomycin in 96-well clear-bottomed plates. Cells were allowed to rest for 24 hours (at 37°C, 5% CO₂) before transduction. Human serum samples (obtained from the French blood institute) were diluted as follows: neat undiluted serum – 1:4 – 1:16 – 1:64 – 1:256 – 1:1,024 (neat serum means 1 volume of virus for 1 volume of serum). A pooled mouse plasma sample (from 10 DBA/1 mice, taken 42 days after intra-articular injection of an AAV5-vector) was serially diluted in FBS as follows: 1:10 – 1:50 – 1:250 – 1:6,250 – 1:31,250. A solution of human Intravenous Immunoglobulin (IVig, Sanquin, lot 15D30H4560A) was serially semi-log diluted from 1:10 down to 1:10,000. Samples and controls were incubated together with the appropriate capsid mutant or wtAAV5 vector for 30' ± 5 min. at 35-38°C at an MOI of 2,500 (as determined previously). After 48 ± 2 hours, luciferase reagent was

added and luminescence emission was measured with the VictorX microplate reader. Transduction inhibition titers were determined as the highest dilution of serum still associated to a detectable neutralizing activity, i.e. a neutralizing activity >50%.

5 6.2 Results

As presented in Table 3, 70%-85% of the samples did not contain neutralizing antibodies against wtAAV5 or the 7 capsid mutants. Most of the samples shared reactivity against the seven capsid mutants, thus a serum sample having reactivity against the wild type AAV5 capsid also reacted against other capsids. In terms of the level of response, they were also comparable between
10 capsid mutants. The number of samples that did not react (ND = not detected) is indicated for each capsid mutant. These data are only given as information as it is very difficult to compare titers with different vectors. Regarding the pooled mouse serum sample from intra-articular injected joints, it only reacted against the WT AAV5 capsid that was used to immunize the animals, whereas no response was observed against mutant capsids (Table 3). All capsid
15 mutants and WT AAV5 were neutralized by IVIg (titers >100) (data not shown).

Table 3: For each serum sample as well as the pooled mouse plasma, the inhibitory titer is reported and corresponds to the highest dilution still associated to a detectable neutralizing activity. Titers > 8 are considered as seropositive. Positive signals are highlighted in bold/italic. ND = Not Detectable

	AAV5	AAV9A2	AAV-DJ-QR-P2	AAVrh10-A2	AAV1-P4	AAV2-P2	AAV7-A6	AAVrh10-A6
sample 1	256	256	256	256	>1024	256	>1024	>1024
sample 2	4	ND	ND	ND	ND	ND	ND	ND
sample 3	ND	ND	ND	ND	ND	ND	ND	ND
sample 4	ND	ND	ND	ND	ND	ND	ND	ND
sample 5	ND	ND	ND	ND	ND	ND	ND	ND
sample 6	ND	ND	ND	ND	ND	ND	ND	ND
sample 7	64	64	64	256	256	64	256	256
sample 8	16	16	16	64	16	64	64	64
sample 9	ND	ND	ND	ND	ND	ND	ND	ND
sample 10	4	4	16	4	64	64	64	64
sample 11	ND	ND	ND	ND	ND	ND	ND	ND
sample 12	ND	ND	ND	ND	ND	1	ND	ND
sample 13	ND	ND	ND	ND	ND	ND	ND	ND
sample 14	ND	1	ND	ND	4	1	ND	1
sample 15	ND	ND	ND	ND	ND	ND	ND	ND
sample 16	1	1	4	4	4	16	1	1

P6057598pct

39

sample 17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
sample 18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
sample 19	ND	ND	ND	ND	4	ND	ND	ND	ND	ND	ND	ND
sample 20	4	256	64	4	ND	256	16	16	16	16	16	16
% negative samples	85	80	75	85	80	70	75	75	75	75	75	75
mouse plasma	256	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

The present invention has been described above with reference to a number of exemplary embodiments as shown in the drawings. Modifications and alternative implementations of some parts or elements are possible, and are included in the scope of protection as defined in the appended claims

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

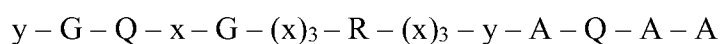
Claims

1. A method for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein the symptom is joint pain or the inflammation of one or more arthritic joints, the method comprising administering to a subject a recombinant adeno-associated virus (rAAV) virion, wherein the rAAV comprises a modified capsid protein and a nucleotide sequence encoding a gene product of interest that treats, prevents or suppresses a symptom associated with the arthritic disease;

wherein the modified capsid protein comprises in the C-terminal part of the protein an amino acid sequence Z, residues of which are exposed on the surface of the capsid protein, and

wherein the amino acid sequence Z:

a. comprises or consists of a sequence of amino acid residues of the formula I:



wherein x represents a single amino acid residue and wherein y represents 0, 1 or 2 amino acid residues; and

b. is present at a location corresponding to a position 100 – 200 amino acid residues from the C terminus of a wild-type AAV capsid protein.

2. The method according to claim 1, wherein b. is present at a location corresponding to a position 120 – 180 amino acid residues from the C terminus of a wild-type AAV capsid protein.

3. The method according to claim 2, wherein b. is present at a location corresponding to a position 130 – 170 amino acid residues from the C terminus of a wild-type AAV capsid protein.

4. The method according to claim 2, wherein b. is present at a location corresponding to a position 140 – 160 amino acid residues from the C terminus of a wild-type AAV capsid protein.

5. The method according to any one of claims 1 to 4, wherein the sequence Z is comprised in the modified capsid protein at a location represented by the formula II:



c. wherein Z, x and y are as defined in any one of claims 1 to 4; and

d. wherein n is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

6. The method according to any one of claims 1 to 4, wherein the capsid protein comprises an amino acid sequence selected from the group consisting of:

i) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 1 and wherein amino acids at positions 588 – 602 of SEQ ID NO: 1 have at least 80% sequence identity with SEQ ID NO: 11,

ii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 2 and wherein amino acids at positions 585 – 599 of SEQ ID NO: 2 have at least 80% sequence identity with SEQ ID NO: 10,

iii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 3 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 3 have at least 80% sequence identity with SEQ ID NO: 9,

iv) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 4 and wherein amino acids at positions 586 – 600 of SEQ ID NO: 4 have at least 80% sequence identity with SEQ ID NO: 8,

v) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 5 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 5 have at least 80% sequence identity with SEQ ID NO: 9,

vi) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 6 and wherein amino acids at positions 588 - 602 of SEQ ID NO: 6 have at least 80% sequence identity with SEQ ID NO: 8, and

vii) an amino acid sequence having at least 70% sequence identity with an amino acid sequence having SEQ ID NO: 7 and wherein amino acids at positions 587 – 601 of SEQ ID NO: 7 have at least 80% sequence identity with SEQ ID NO: 12,

wherein the modified capsid protein provides for an at least two-fold increase in expression in a cell in comparison to an unmodified capsid protein with an amino acid sequence selected from the group consisting of SEQ ID NO: 13 – 19, when tested under the same conditions.

7. The method according to claim 6, wherein the cell is a human FLS cell.

8. The method according to claim 7, wherein the unmodified capsid protein has the amino acid sequence SEQ ID NO: 19 or has the same serotype as the modified capsid protein.

9. The method according to any one of claims 1 to 4 and 6 to 8, wherein the capsid protein comprises or consists of an amino acid sequence selected from the group consisting of SEQ ID NO:1 – 7.

10. The method according to any one of claims 1 to 9, wherein the rAAV virion comprises a nucleotide sequence comprising at least one AAV inverted terminal repeat (ITR) sequence.

11. The method according to claim 10, wherein the nucleotide sequence encoding a gene product of interest is located between two AAV ITR sequences.

12. The method according to any one of claims 1 to 11, wherein the gene product of interest is selected from the group consisting of interleukins, immune-modulators, antibodies, shRNA, miRNA, guide RNA, growth factors, proteases, nucleotidases/nucleosidases, peptides, protease inhibitors, inhibitors, enzymes and combinations thereof.

13. The method according to claim 12, wherein the gene product of interest is at least one of CD39, CD73 and IFN- β .

14. The method according to any one of claims 1 to 11, wherein the gene product of interest is a tumor necrosis factor alpha (TNF α) inhibitor.

15. The method according to claim 14, wherein the TNF α inhibitor is selected from the group consisting of etanercept, infliximab, adalimumab, certilzumab pegol and golimumab.
16. The method according to claim 15, wherein the TNF α inhibitor is etanercept.
17. The method according to any one of claims 1 to 16, wherein the rAAV virion comprises at least one of:
- (i) a polynucleotide comprising a sequence encoding at least one guide RNA; wherein the or each guide RNA is substantially complementary to a target polynucleotide sequence(s) in a genome; and
 - (ii) a polynucleotide comprising a sequence encoding a nuclease; wherein the nuclease forms a ribonuclease complex with the guide RNA, and wherein the ribonuclease complex makes site-specific double-stranded DNA breaks (DSDB) in the genome.
18. A method for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein the symptom is joint pain or the inflammation of one or more arthritic joints, the method comprising administering to a subject an rAAV composition, wherein the rAAV composition comprises the rAAV virion as defined in any one of claims 1 to 17 and a pharmaceutically acceptable carrier.
19. The method according to claim 18, wherein the rAAV composition further comprises an empty capsid in a ratio of empty capsid to rAAV virion of at least 1:1.
20. The method according to claim 18 or claim 19, further comprising the administration of an immunosuppressant to the subject.
21. The method according to claim 20, wherein at least one of the rAAV composition and the immunosuppressant is administered locally.

22. The method according to claim 21, wherein the local administration is intraarticular administration.

23. The method according to any one of claims 1 to 22, wherein the arthritic disease is selected from the group consisting of rheumatoid arthritis (RA), juvenile rheumatoid arthritis, osteoarthritis (OA), gout, pseudogout, spondyloarthritis (SpA), psoriatic arthritis, ankylosing spondylitis, septic arthritis, arthritis, juvenile idiopathic arthritis, joint replacement and Still's disease.

24. The method according to any one of claims 1 to 23, wherein the rAAV virion or the rAAV composition is administered to the subject systemically and/or locally.

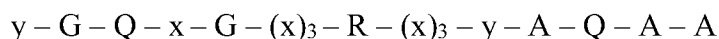
25. Use of a recombinant adeno-associated virus (rAAV) virion in the manufacture of a medicament for treating or preventing an arthritic disease or for treating or preventing a symptom associated with an arthritic disease, wherein the rAAV virion comprises a modified capsid protein and a nucleotide sequence encoding a gene product of interest that treats, prevents or suppresses a symptom associated with the arthritic disease,

wherein the symptom is joint pain or the inflammation of one or more arthritic joints,

wherein the modified capsid protein comprises in the C-terminal part of the protein an amino acid sequence Z, residues of which are exposed on the surface of the capsid protein, and

wherein the amino acid sequence Z:

a. comprises or consists of a sequence of amino acid residues of the formula I:



wherein x represents a single amino acid residue and wherein y represents 0, 1 or 2 amino acid residues; and

b. is present at a location corresponding to a position 100 – 200 amino acid residues from the C terminus of a wild-type AAV capsid protein.

26. The use according to claim 25, wherein the rAAV virion is formulated for administration in an rAAV composition comprising an empty capsid in a ratio of empty capsid to rAAV virion of at least 1:1.
27. The use according to claim 26 or claim 27, wherein the rAAV virion comprises a nucleotide sequence comprising at least one AAV inverted terminal repeat (ITR) sequence.
28. The use according to claim 27, wherein the nucleotide sequence encoding a gene product of interest is located between two AAV ITR sequences.
29. The use according to any one of claims 25 to 28, wherein the gene product of interest is a tumor necrosis factor alpha (TNF α) inhibitor.
30. The use according to claim 29, wherein the TNF α inhibitor is selected from the group consisting of etanercept, infliximab, adalimumab, certilzumab pegol and golimumab.
31. The use according to claim 30, wherein the TNF α inhibitor is etanercept.

Fig. 1A

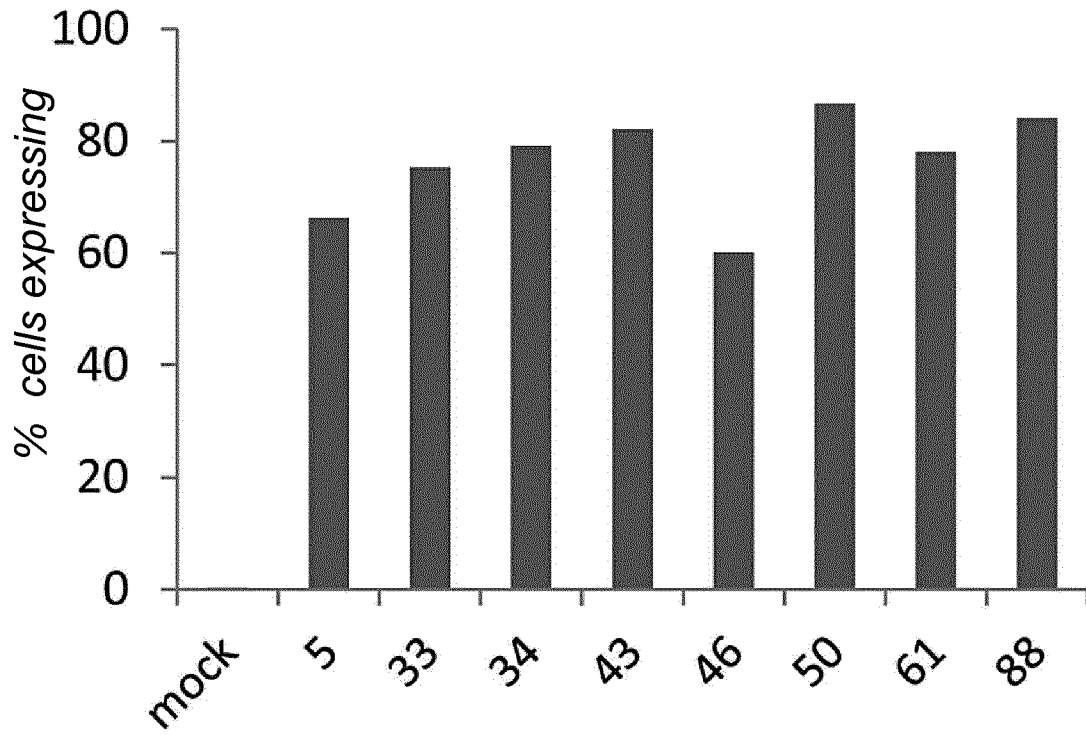


Fig. 1B

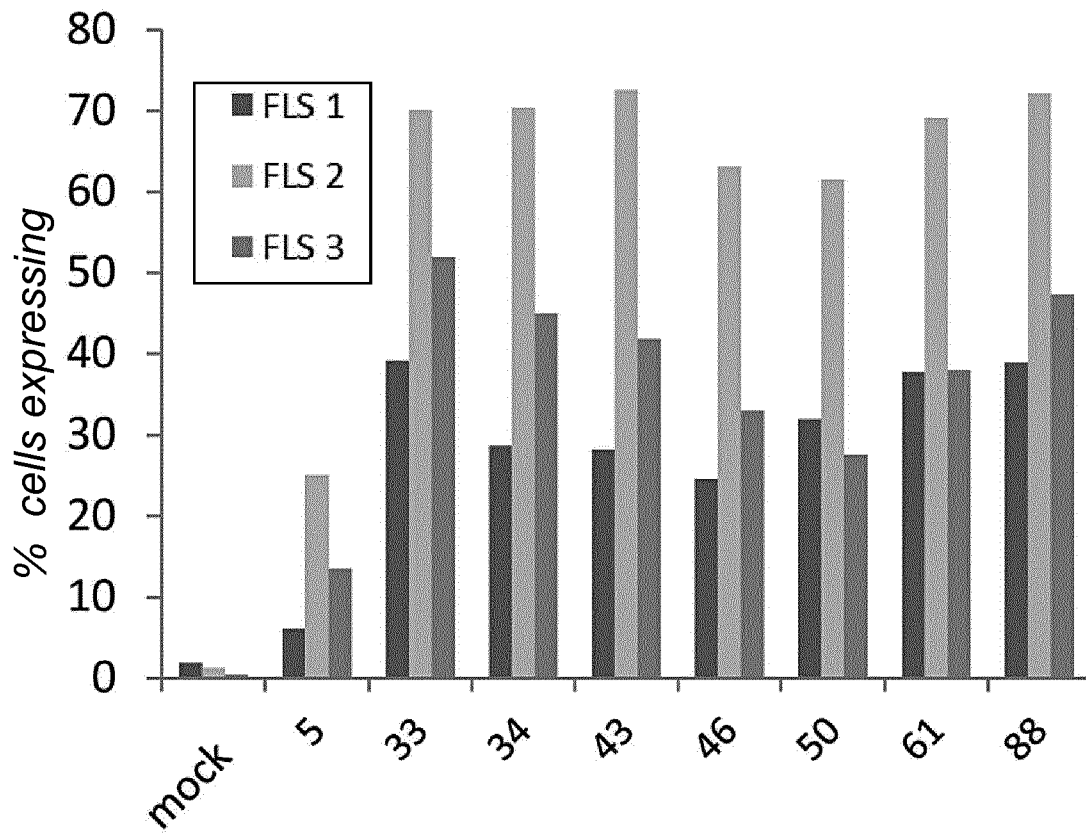


Fig. 1C

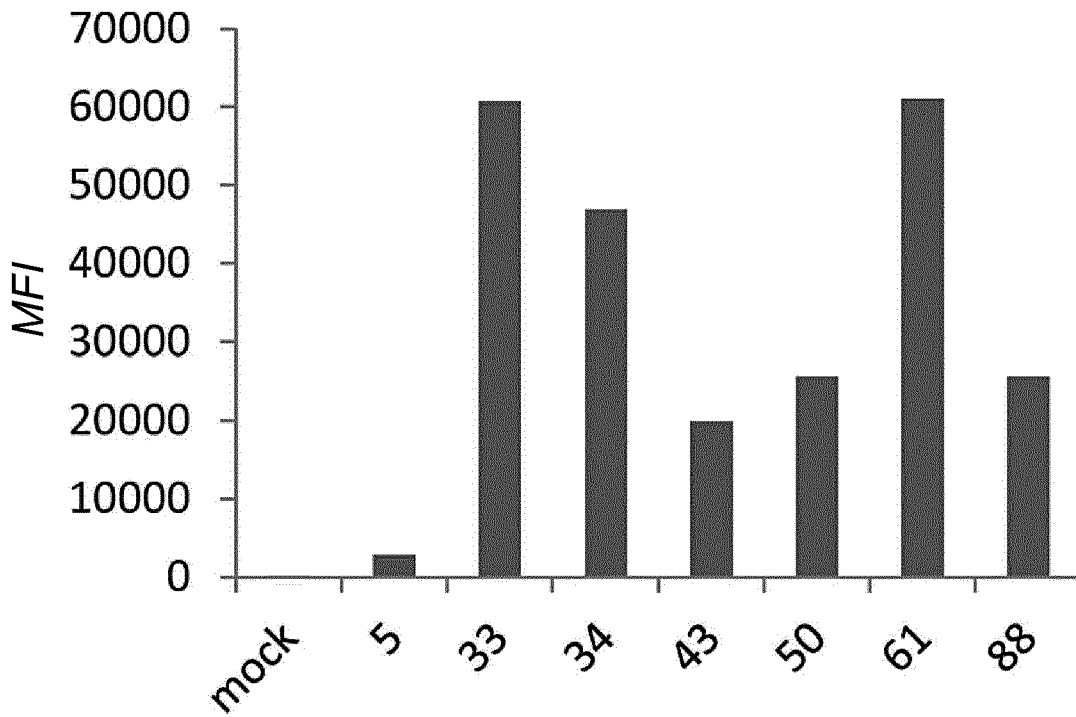


Fig. 1D

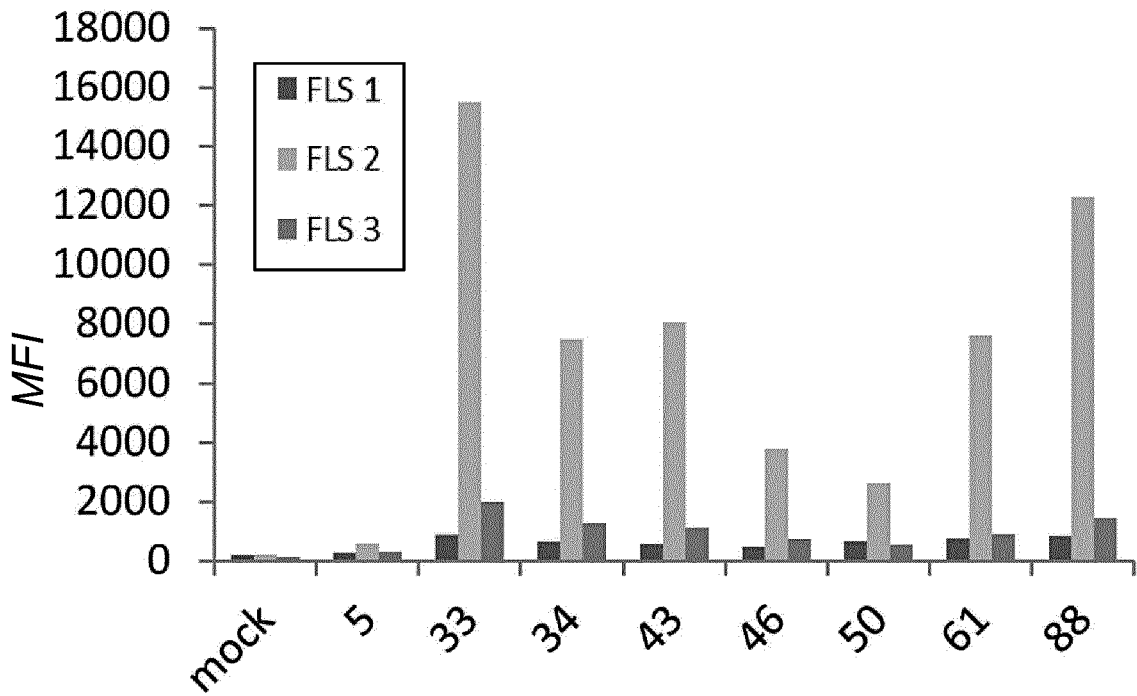


Fig. 1E

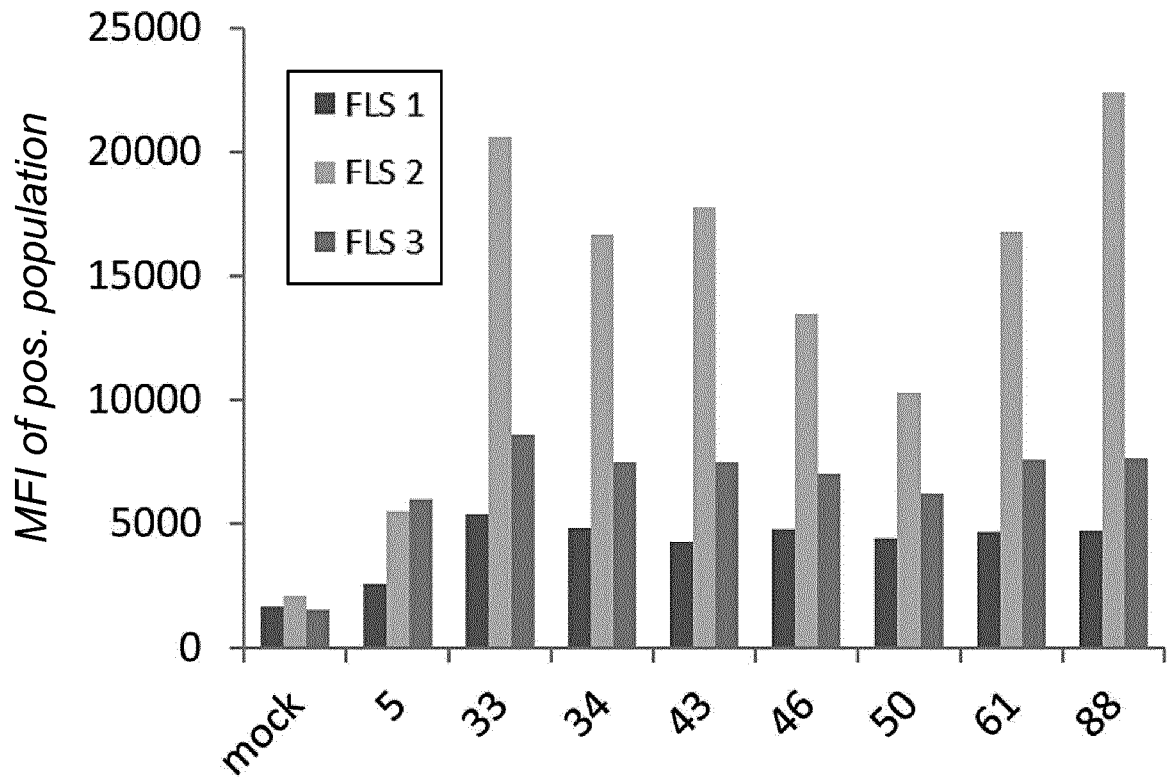


Fig. 2B

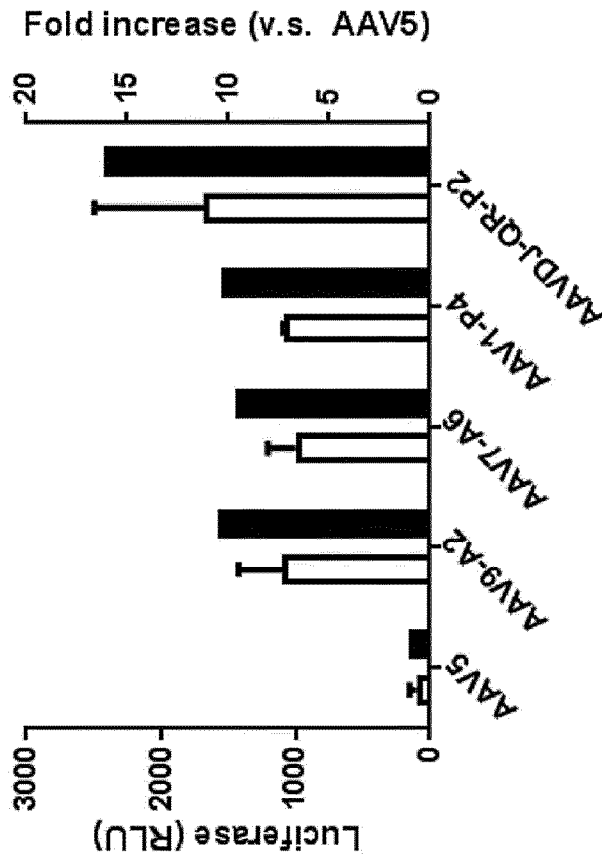
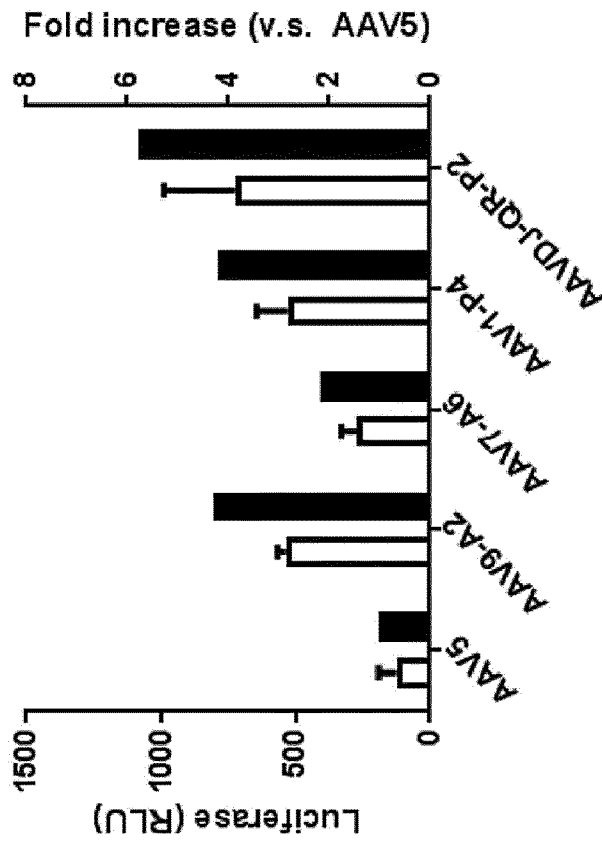


Fig. 2A



5/14

Fig. 2D

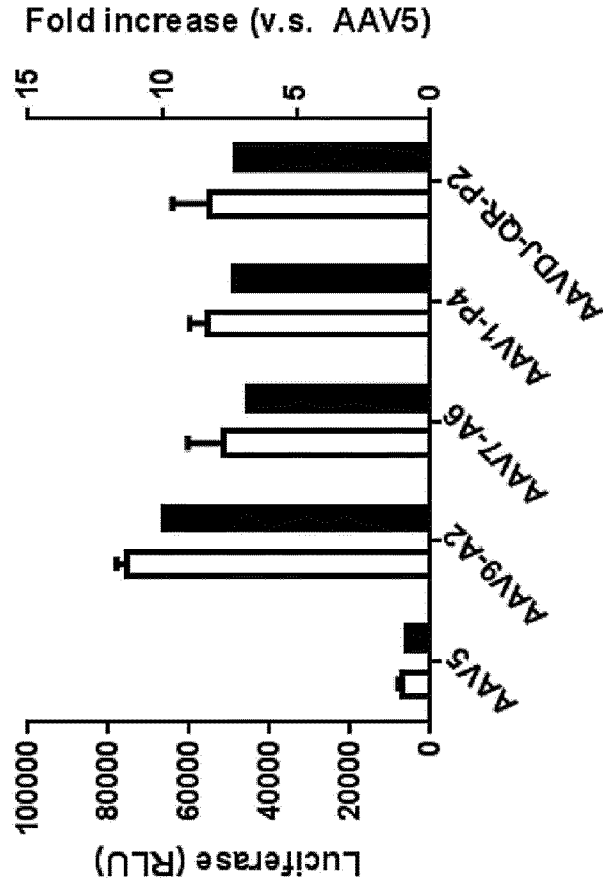


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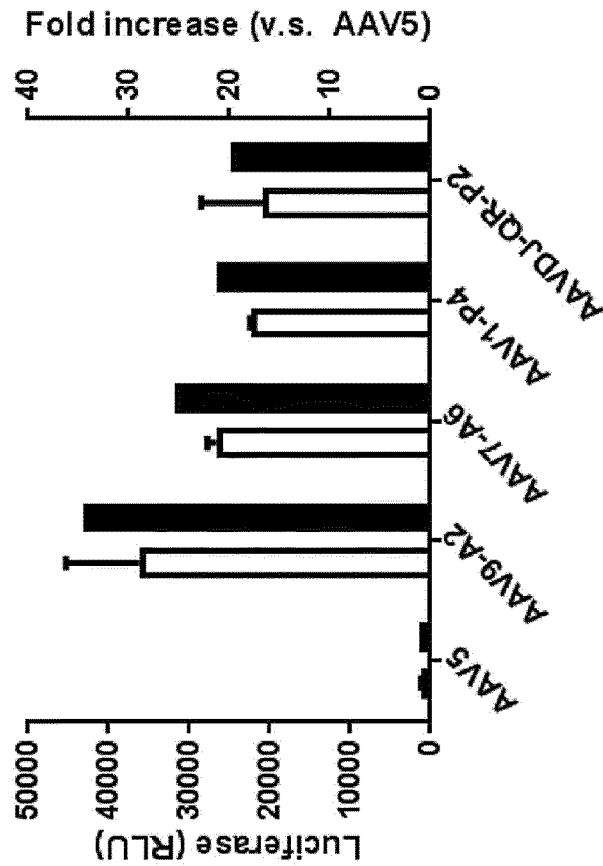


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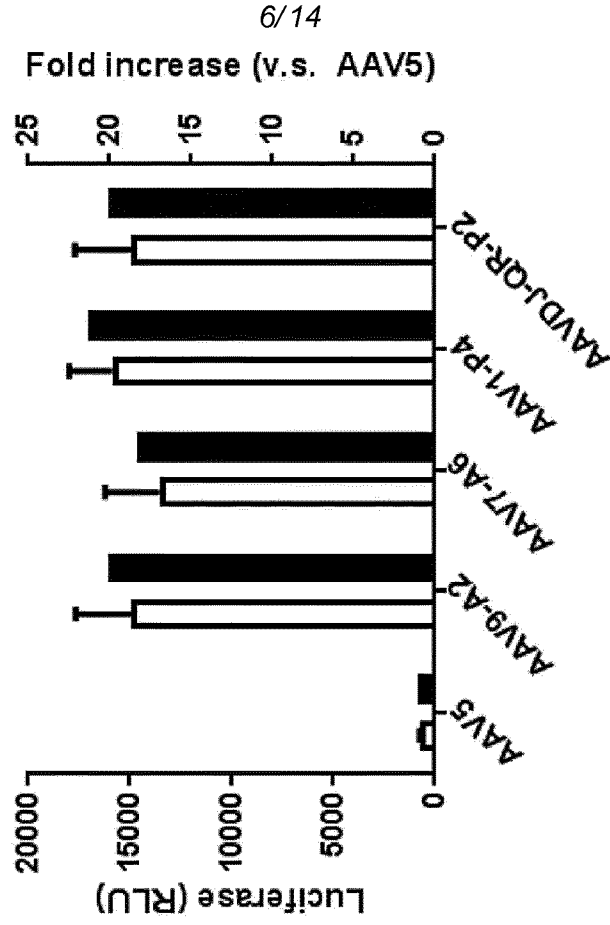


Fig. 2E

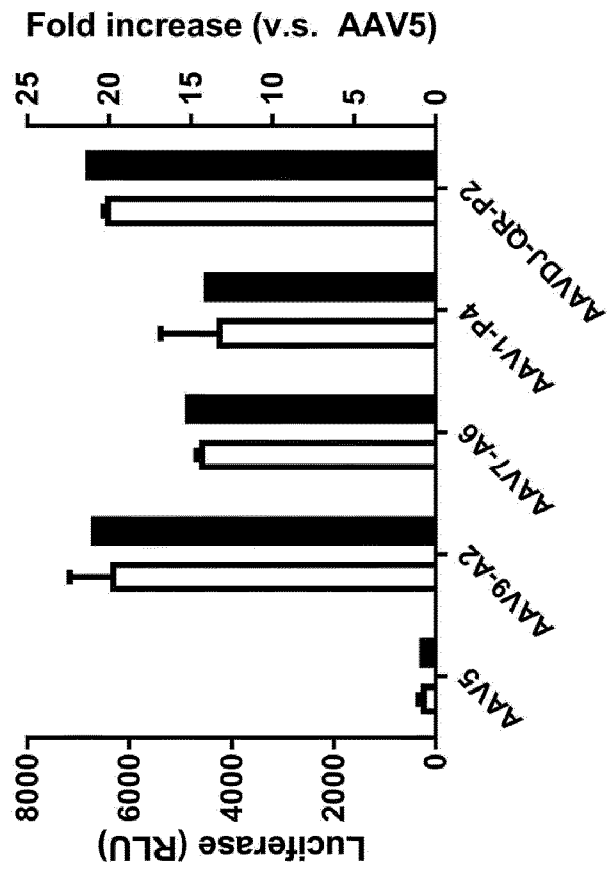


Fig. 2H

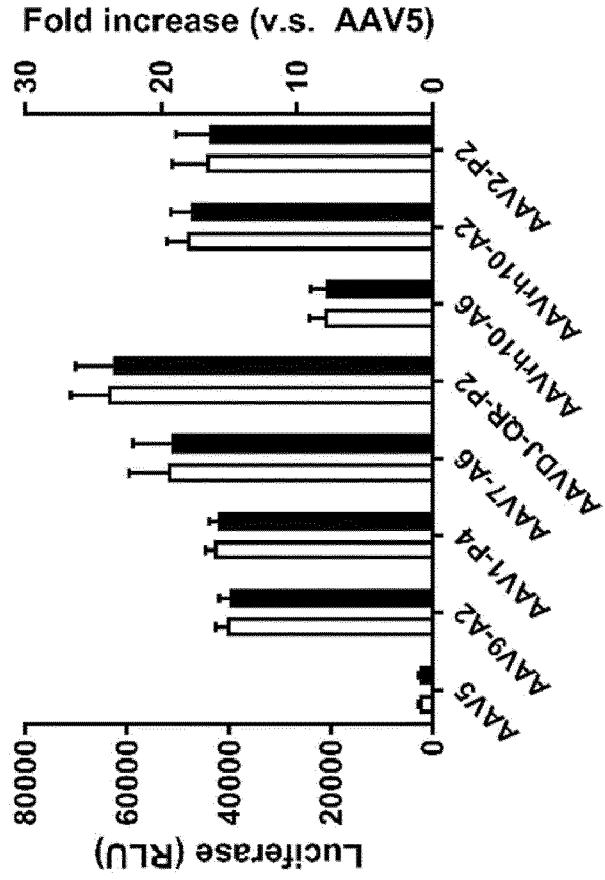


Fig. 2G

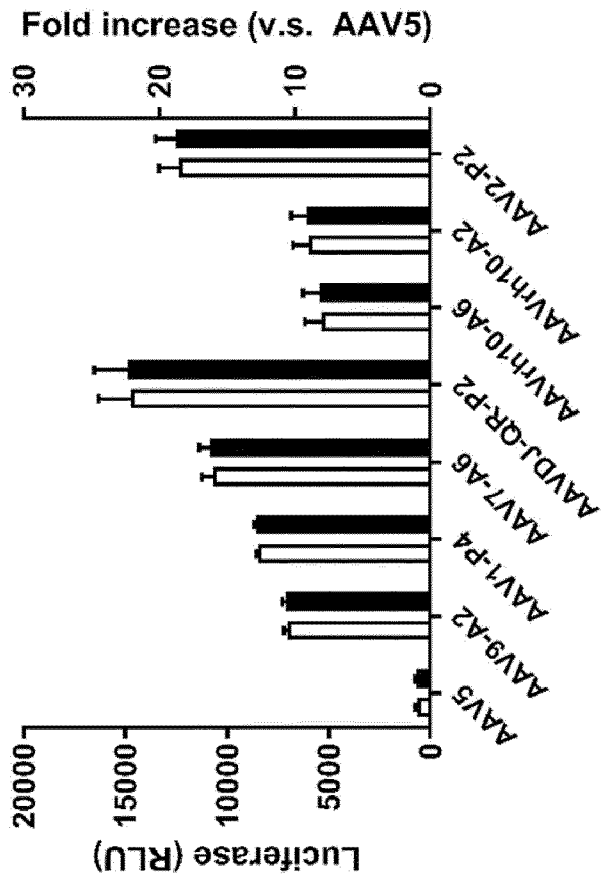


Fig. 2J

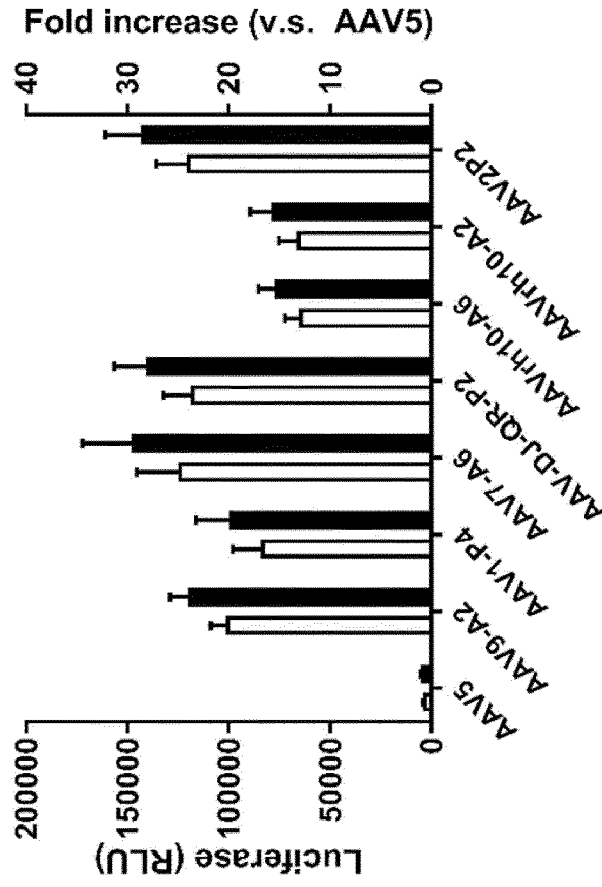
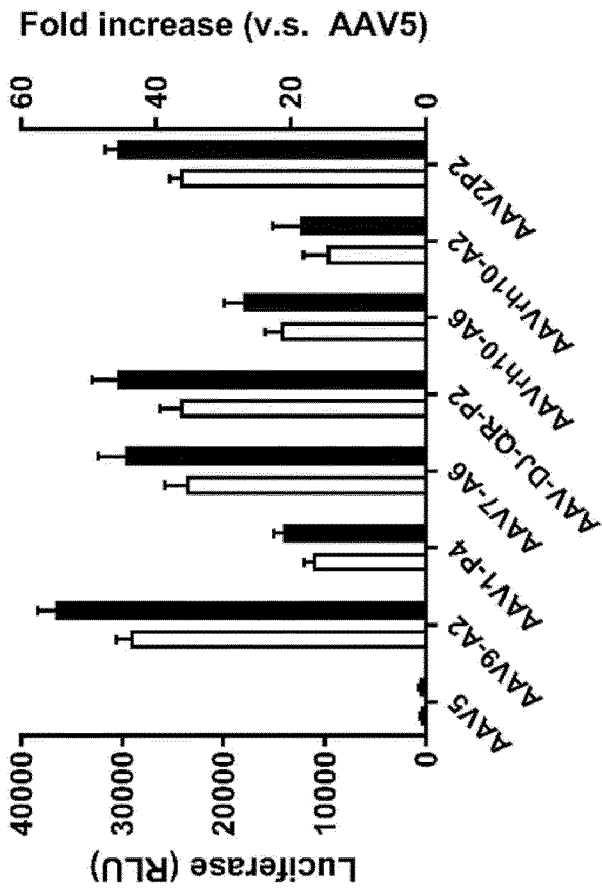


Fig. 2I



9/14

Fig. 2L

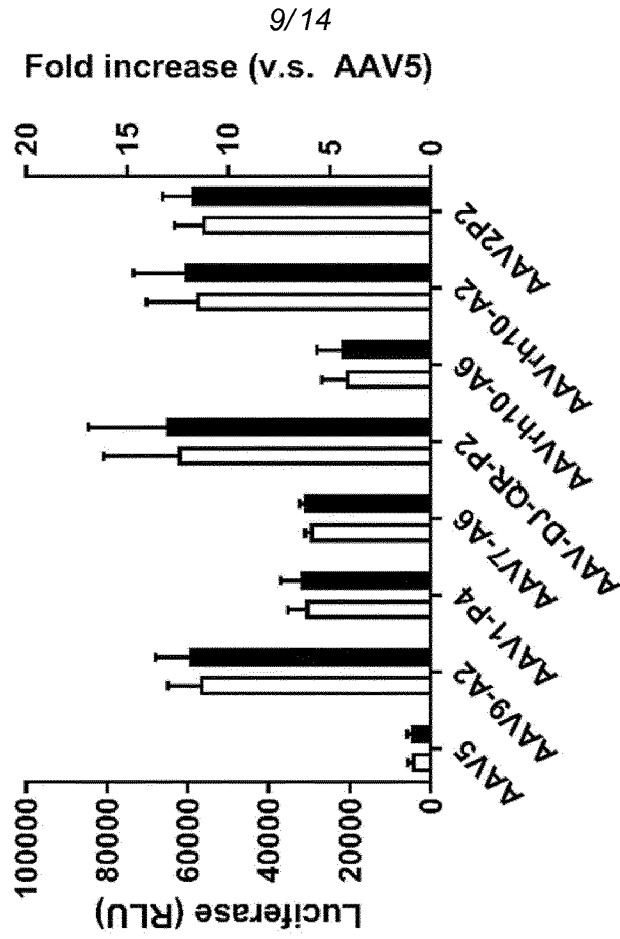


Fig. 2K

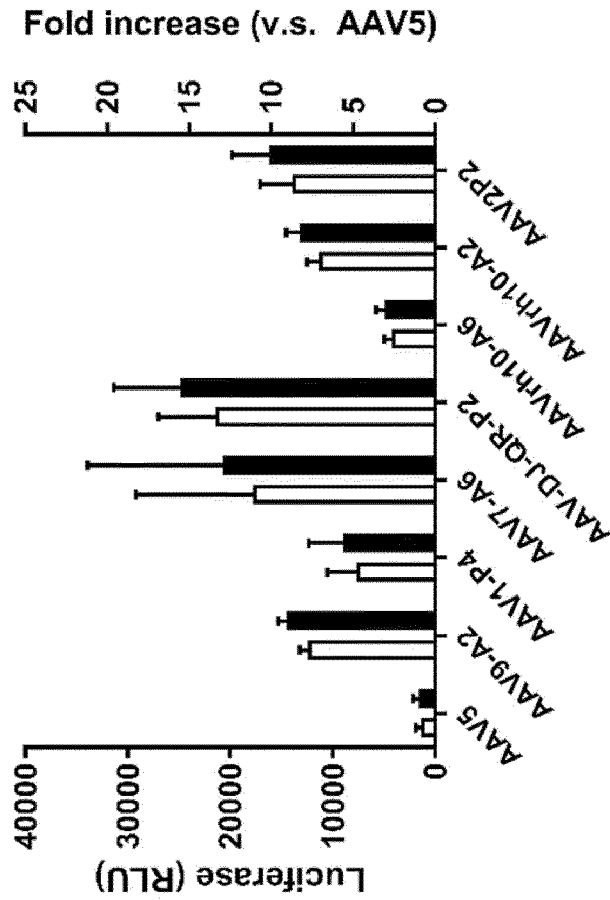


Fig. 2M

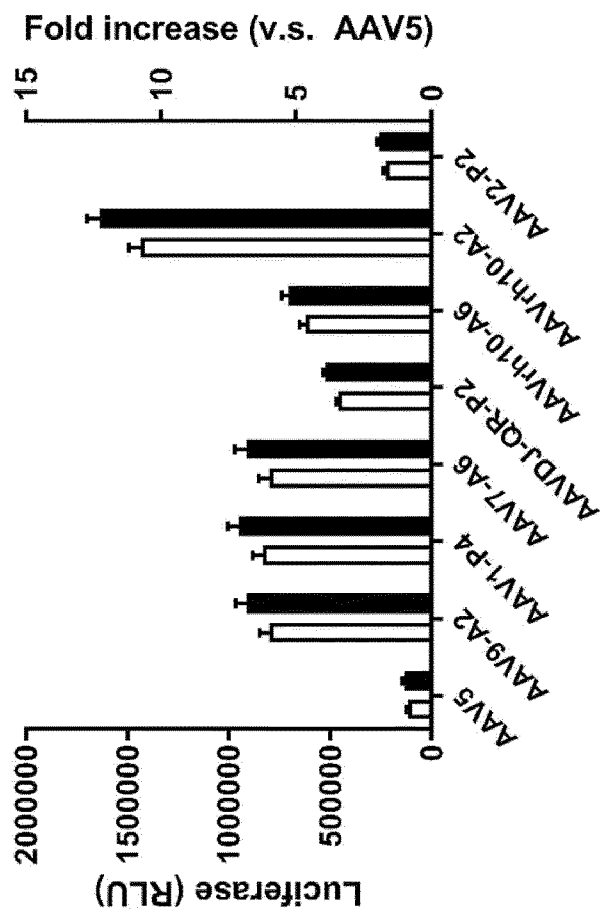


Fig. 3A

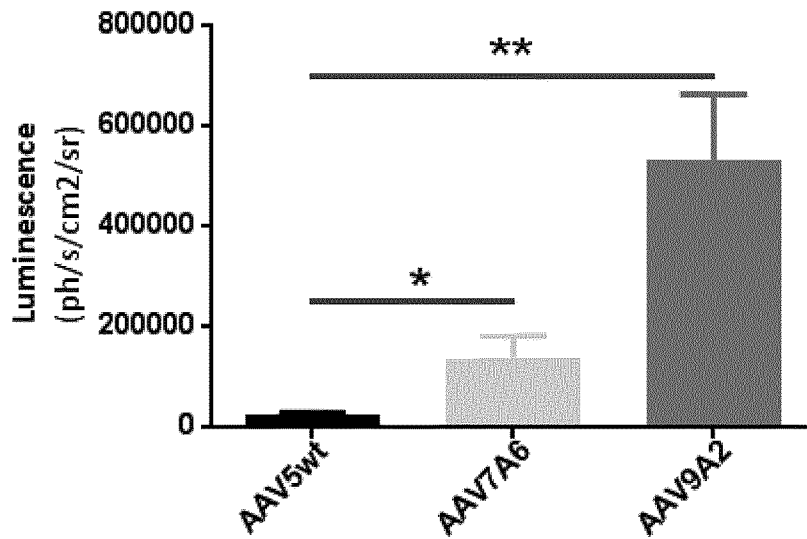


Fig. 3B

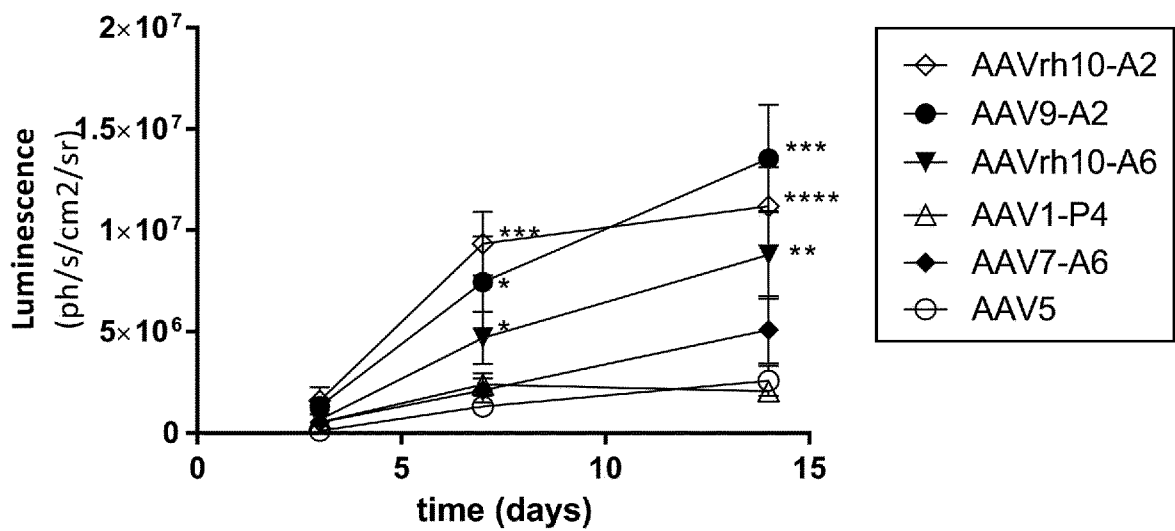


Fig. 3C

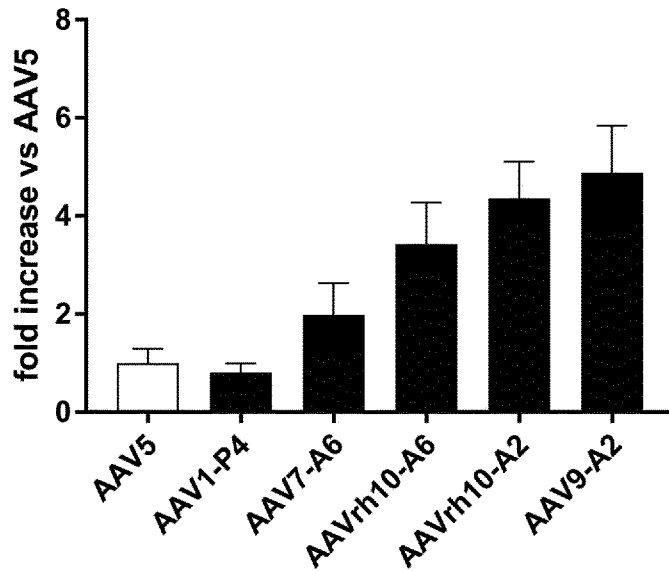


Fig. 4

CLUSTAL format alignment by MAFFT FFT-NS-i (v7.215)

```

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AAV9A2      -gqrgnysrg--vdaqaa
AAVrh10A2   -gqrgnysrg--vdaqaa
AAV7A6      -gqrgnearv--reaq--
AAV7A6+    -gqrgnearv--reaqaa
AAV10A6     -gqrgnearv--reaqaa
AAV2P2      qqqsgcdcrgcdcfca---
AAV-DJ-QR-P2 qqqrgcdcrgcdcfca---
AAV2P2+     qqqsgcdcrgcdcfcaqaa
AAV-DJ-QR-P2+ qqqrgcdcrgcdcfcaqaa
              ** * * *
    
```

Fig. 5

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

```

AAV2P2          QGQSGCDCRG-DCFCA---
AAV2P2+        QGQSGCDCRG-DCFCAQAA
AAV-DJ-QR-P2   QGQSGCDCRG-DCFCA---
AAV-DJ-QR-P2+  QGQSGCDCRG-DCFCAQAA
AAV1P4         -GQSGNDVRSANAQAA---
AAV9A2         -GQSGNYSRGVDAQAA---
AAVrh10A2      -GQSGNYSRGVDAQAA---
AAV7A6         -GQSGNEARVREAQ-----
AAV7A6+        -GQSGNEARVREAQAA---
AAV10A6        -GQSGNEARVREAQAA---
                ** *      *   :.
    
```

Fig. 6

CLUSTAL format alignment by MAFFT FFT-NS-i (v7.215)

```

insertP4       -ggsgndvrs--anaqaa
insertA2       -gqrgnysrg--vdaqaa
insertA6       -gqrgnearv--reaqaa
insertP2       qgqsgcdcrgdcfcaqaa
insertQR-P2    qgqrgcdcrgdcfcaqaa
                ** *      *   ****
    
```

Fig. 7

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

```
insertP2      QGQSGCDCRGDCFCAQAA
insertQR-P2   QGQRGCDCRGDCFCAQAA
insertP4      -GQSGNDVRSAA--NAQAA
insertA2      -GQRGNYSRGV--DAQAA
insertA6      -GQRGNEARVR--EAQAA
              ** *      *      ****
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SEQUENCE LISTING

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 Universitt Heidelberg

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<130> P6057598PCT

<150> EP 18152133.7

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 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
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Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
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Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
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 690 695 700

Asn Pro Glu Val Gln Tyr Thr Ser Asn Tyr Ala Lys Ser Ala Asn Val
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 20 25 30

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 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
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Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala Asp
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Val Phe Met Val Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly Ser
 370 375 380

Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro Ser
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 485 490 495

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 500 505 510

Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His Lys Asp
 515 520 525

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 530 535 540

Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met Ile Thr
 545 550 555 560

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 580 585 590

Arg Gly Asp Cys Phe Cys Ala Gln Ala Ala Thr Ala Asp Val Asn Thr
 595 600 605

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 610 615 620

Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly His Phe His
 625 630 635 640

Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro Gln
645 650 655

Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asn Pro Ser Thr Thr Phe
660 665 670

Ser Ala Ala Lys Phe Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln
675 680 685

Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg
690 695 700

Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr Asn Lys Ser Val Asn
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Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asn Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
 85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
 115 120 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
 130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
 145 150 155 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
 165 170 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
 180 185 190

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

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
 210 215 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
 225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
 245 250 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
 260 265 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
 275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
 290 295 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
 305 310 315 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
 325 330 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
 340 345 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
 355 360 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
 370 375 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
 385 390 395 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
 405 410 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
 420 425 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
 435 440 445

Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser
 450 455 460

Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro
 465 470 475 480

Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln Asn
 485 490 495

Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn
 500 505 510

Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys
 515 520 525

Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly
 530 535 540

Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile
 545 550 555 560

Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser
 565 570 575

Tyr Gly Gln Val Ala Thr Asn His Gln Gly Gln Arg Gly Asn Tyr Ser
 580 585 590

Arg Gly Val Asp Ala Gln Ala Ala Gln Thr Gly Trp Val Gln Asn Gln
595 600 605

Gly Ile Leu Pro Gly Met Val Trp Gln Asp Arg Asp Val Tyr Leu Gln
610 615 620

Gly Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe His Pro
625 630 635 640

Ser Pro Leu Met Gly Gly Phe Gly Met Lys His Pro Pro Pro Gln Ile
645 650 655

Leu Ile Lys Asn Thr Pro Val Pro Ala Asp Pro Pro Thr Ala Phe Asn
660 665 670

Lys Asp Lys Leu Asn Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val
675 680 685

Ser Val Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys Arg Trp
690 695 700

Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr Tyr Lys Ser Asn Asn Val
705 710 715 720

Glu Phe Ala Val Asn Thr Glu Gly Val Tyr Ser Glu Pro Arg Pro Ile
725 730 735

Gly Thr Arg Tyr Leu Thr Arg Asn Leu
740 745

<210> 5

<211> 747

<212> PRT

<213> Artificial Sequence

<220>

<223> AAVrh10 with insert A6

<400> 5

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
 85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
 115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
 130 135 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
 145 150 155 160

Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
 165 170 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
 180 185 190

Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
 195 200 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
 210 215 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
 225 230 235 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
 245 250 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
 260 265 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
 275 280 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
 290 295 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
 305 310 315 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
 325 330 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
 340 345 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
 355 360 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
 370 375 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
 385 390 395 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
 405 410 415

Gln Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
 420 425 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
 435 440 445

Ser Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu
 450 455 460

Phe Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp
 465 470 475 480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser
 485 490 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
 500 505 510

Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
 515 520 525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met
 530 535 540

Phe Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val
545 550 555 560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
565 570 575

Glu Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gly Gln Arg Gly Asn
580 585 590

Tyr Ser Arg Gly Val Asp Ala Gln Ala Ala Ile Val Gly Ala Val Asn
595 600 605

Ser Gln Gly Ala Leu Pro Gly Met Val Trp Gln Asn Arg Asp Val Tyr
610 615 620

Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His Thr Asp Gly Asn Phe
625 630 635 640

His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu Lys His Pro Pro Pro
645 650 655

Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala Asp Pro Pro Thr Thr
660 665 670

Phe Ser Gln Ala Lys Leu Ala Ser Phe Ile Thr Gln Tyr Ser Thr Gly
675 680 685

Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln Lys Glu Asn Ser Lys
690 695 700

Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn Tyr Tyr Lys Ser Thr
705 710 715 720

Asn Val Asp Phe Ala Val Asn Thr Asp Gly Thr Tyr Ser Glu Pro Arg
725 730 735

Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
740 745

<210> 7

<211> 748

<212> PRT

<213> Artificial Sequence

<220>

<223> AAVDJ with insert QR-P2

<400> 7

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser

<220>

<223> insert A2

<400> 8

Gly	Gln	Arg	Gly	Asn	Tyr	Ser	Arg	Gly	Val	Asp	Ala	Gln	Ala	Ala
1				5					10					15

<210> 9

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> insert A6

<400> 9

Gly	Gln	Arg	Gly	Asn	Glu	Ala	Arg	Val	Arg	Glu	Ala	Gln	Ala	Ala
1				5					10					15

<210> 10

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> insert P2

<400> 10

Gln	Gly	Gln	Ser	Gly	Cys	Asp	Cys	Arg	Gly	Asp	Cys	Phe	Cys	Ala	Gln
1				5					10					15	

Ala Ala

<210> 11

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> insert P4

<400> 11

Gly	Gln	Ser	Gly	Asn	Asp	Val	Arg	Ser	Ala	Asn	Ala	Gln	Ala	Ala
1				5					10					15

<210> 12

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> insert QR-P2

<400> 12

Gln Gly Gln Arg Gly Cys Asp Cys Arg Gly Asp Cys Phe Cys Ala Gln
 1 5 10 15

Ala Ala

<210> 13
 <211> 736
 <212> PRT
 <213> adeno-associated virus 1
 <400> 13

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
 1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
 20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
 85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
 115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
 130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ser Gly Ile Gly
 145 150 155 160

Lys Thr Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
 165 170 175

Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Leu Gly Glu Pro Pro
 180 185 190

Ala Thr Pro Ala Ala Val Gly Pro Thr Thr Met Ala Ser Gly Gly Gly
195 200 205

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ala
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Ser Ala Ser Thr Gly Ala Ser Asn Asp Asn His
260 265 270

Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg Phe
275 280 285

His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn Asn
290 295 300

Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile Gln
305 310 315 320

Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn Asn
325 330 335

Leu Thr Ser Thr Val Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu Pro
340 345 350

Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro Ala
355 360 365

Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn Gly
370 375 380

Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe Pro
385 390 395 400

Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Thr Phe Ser Tyr Thr Phe
405 410 415

Glu Glu Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu Asp
420 425 430

Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Asn Arg
435 440 445

Thr Gln Asn Gln Ser Gly Ser Ala Gln Asn Lys Asp Leu Leu Phe Ser
 450 455 460

Arg Gly Ser Pro Ala Gly Met Ser Val Gln Pro Lys Asn Trp Leu Pro
 465 470 475 480

Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Lys Thr Asp Asn
 485 490 495

Asn Asn Ser Asn Phe Thr Trp Thr Gly Ala Ser Lys Tyr Asn Leu Asn
 500 505 510

Gly Arg Glu Ser Ile Ile Asn Pro Gly Thr Ala Met Ala Ser His Lys
 515 520 525

Asp Asp Glu Asp Lys Phe Phe Pro Met Ser Gly Val Met Ile Phe Gly
 530 535 540

Lys Glu Ser Ala Gly Ala Ser Asn Thr Ala Leu Asp Asn Val Met Ile
 545 550 555 560

Thr Asp Glu Glu Glu Ile Lys Ala Thr Asn Pro Val Ala Thr Glu Arg
 565 570 575

Phe Gly Thr Val Ala Val Asn Phe Gln Ser Ser Ser Thr Asp Pro Ala
 580 585 590

Thr Gly Asp Val His Ala Met Gly Ala Leu Pro Gly Met Val Trp Gln
 595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
 610 615 620

Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Leu
 625 630 635 640

Lys Asn Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
 645 650 655

Asn Pro Pro Ala Glu Phe Ser Ala Thr Lys Phe Ala Ser Phe Ile Thr
 660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
 675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Val Gln Tyr Thr Ser Asn
 690 695 700

Tyr Ala Lys Ser Ala Asn Val Asp Phe Thr Val Asp Asn Asn Gly Leu
 705 710 715 720

Tyr Thr Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Pro Leu
 725 730 735

<210> 14
 <211> 735
 <212> PRT
 <213> adeno-associated virus 2

<400> 14

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
 1 5 10 15

Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
 20 25 30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
 85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
 115 120 125

Leu Gly Leu Val Glu Glu Pro Val Lys Thr Ala Pro Gly Lys Lys Arg
 130 135 140

Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
 145 150 155 160

Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
 165 170 175

Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Leu Gly Gln Pro Pro
 180 185 190

Ala Ala Pro Ser Gly Leu Gly Thr Asn Thr Met Ala Thr Gly Ser Gly

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn
 210 215 220

Ala Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
 225 230 235 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
 245 250 255

Leu Tyr Lys Gln Ile Ser Ser Glu Thr Ala Gly Ser Thr Asn Asp Asn
 260 265 270

Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
 275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
 290 295 300

Asn Trp Gly Phe Arg Pro Lys Lys Leu Arg Phe Lys Leu Phe Asn Ile
 305 310 315 320

Gln Val Lys Glu Val Thr Thr Asn Asp Gly Val Thr Thr Ile Ala Asn
 325 330 335

Asn Leu Thr Ser Thr Ile Gln Val Phe Ser Asp Ser Glu Tyr Gln Leu
 340 345 350

Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro
 355 360 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn
 370 375 380

Gly Ser Gln Ser Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
 385 390 395 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr Ser
 405 410 415

Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
 420 425 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ala
 435 440 445

Arg Thr Gln Ser Asn Pro Gly Gly Thr Ala Gly Asn Arg Glu Leu Gln
 450 455 460

Phe Tyr Gln Gly Gly Pro Ser Thr Met Ala Glu Gln Ala Lys Asn Trp
 465 470 475 480

Leu Pro Gly Pro Cys Phe Arg Gln Gln Arg Val Ser Lys Thr Leu Asp
 485 490 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
 500 505 510

Leu Asn Gly Arg Asn Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
 515 520 525

His Lys Asp Asp Glu Asp Arg Phe Phe Pro Ser Ser Gly Val Leu Ile
 530 535 540

Phe Gly Lys Thr Gly Ala Thr Asn Lys Thr Thr Leu Glu Asn Val Leu
 545 550 555 560

Met Thr Asn Glu Glu Glu Ile Arg Pro Thr Asn Pro Val Ala Thr Glu
 565 570 575

Glu Tyr Gly Ile Val Ser Ser Asn Leu Gln Ala Ala Asn Thr Ala Ala
 580 585 590

Gln Thr Gln Val Val Asn Asn Gln Gly Ala Leu Pro Gly Met Val Trp
 595 600 605

Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
 610 615 620

His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
 625 630 635 640

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
 645 650 655

Ala Asn Pro Pro Glu Val Phe Thr Pro Ala Lys Phe Ala Ser Phe Ile
 660 665 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
 675 680 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
 690 695 700

Asn Phe Glu Lys Gln Thr Gly Val Asp Phe Ala Val Asp Ser Gln Gly
 705 710 715 720

Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 16
<211> 736
<212> PRT
<213> adeno-associated virus 9
<400> 16

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Ala Leu Lys Pro Gly Ala Pro Gln Pro
20 25 30

Lys Ala Asn Gln Gln His Gln Asp Asn Ala Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Gly Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Gln Ser Pro Gln Glu Pro Asp Ser Ser Ala Gly Ile Gly
145 150 155 160

Lys Ser Gly Ala Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln Thr
165 170 175

Gly Asp Thr Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
180 185 190

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

Ala Pro Val Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser Ser
210 215 220

Ser Gly Asn Trp His Cys Asp Ser Gln Trp Leu Gly Asp Arg Val Ile
225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
245 250 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
260 265 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
290 295 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn Ile
305 310 315 320

Gln Val Lys Glu Val Thr Asp Asn Asn Gly Val Lys Thr Ile Ala Asn
325 330 335

Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp Ser Asp Tyr Gln Leu
340 345 350

Pro Tyr Val Leu Gly Ser Ala His Glu Gly Cys Leu Pro Pro Phe Pro
355 360 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asp
370 375 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
385 390 395 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Ser Tyr Glu
405 410 415

Phe Glu Asn Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
420 425 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
435 440 445

Lys Thr Ile Asn Gly Ser Gly Gln Asn Gln Gln Thr Leu Lys Phe Ser
 450 455 460

Val Ala Gly Pro Ser Asn Met Ala Val Gln Gly Arg Asn Tyr Ile Pro
 465 470 475 480

Gly Pro Ser Tyr Arg Gln Gln Arg Val Ser Thr Thr Val Thr Gln Asn
 485 490 495

Asn Asn Ser Glu Phe Ala Trp Pro Gly Ala Ser Ser Trp Ala Leu Asn
 500 505 510

Gly Arg Asn Ser Leu Met Asn Pro Gly Pro Ala Met Ala Ser His Lys
 515 520 525

Glu Gly Glu Asp Arg Phe Phe Pro Leu Ser Gly Ser Leu Ile Phe Gly
 530 535 540

Lys Gln Gly Thr Gly Arg Asp Asn Val Asp Ala Asp Lys Val Met Ile
 545 550 555 560

Thr Asn Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr Glu Ser
 565 570 575

Tyr Gly Gln Val Ala Thr Asn His Gln Ser Ala Gln Ala Gln Ala Gln
 580 585 590

Thr Gly Trp Val Gln Asn Gln Gly Ile Leu Pro Gly Met Val Trp Gln
 595 600 605

Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro His
 610 615 620

Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly Met
 625 630 635 640

Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro Ala
 645 650 655

Asp Pro Pro Thr Ala Phe Asn Lys Asp Lys Leu Asn Ser Phe Ile Thr
 660 665 670

Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu Gln
 675 680 685

Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser Asn
 690 695 700

Tyr Tyr Lys Ser Asn Asn Val Glu Phe Ala Val Asn Thr Glu Gly Val
705 710 715 720

Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn Leu
725 730 735

<210> 17
<211> 738
<212> PRT
<213> adeno-associated virus rh10

<400> 17

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Asn Leu Ser
1 5 10 15

Glu Gly Ile Arg Glu Trp Trp Asp Leu Lys Pro Gly Ala Pro Lys Pro
20 25 30

Lys Ala Asn Gln Gln Lys Gln Asp Asp Gly Arg Gly Leu Val Leu Pro
35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
50 55 60

Val Asn Ala Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
65 70 75 80

Gln Gln Leu Lys Ala Gly Asp Asn Pro Tyr Leu Arg Tyr Asn His Ala
85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Gln Glu Asp Thr Ser Phe Gly Gly
100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro
115 120 125

Leu Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Gly Lys Lys Arg
130 135 140

Pro Val Glu Pro Ser Pro Gln Arg Ser Pro Asp Ser Ser Thr Gly Ile
145 150 155 160

Gly Lys Lys Gly Gln Gln Pro Ala Lys Lys Arg Leu Asn Phe Gly Gln
165 170 175

Thr Gly Asp Ser Glu Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro
180 185 190

Pro Ala Gly Pro Ser Gly Leu Gly Ser Gly Thr Met Ala Ala Gly Gly
 195 200 205

Gly Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Ser
 210 215 220

Ser Ser Gly Asn Trp His Cys Asp Ser Thr Trp Leu Gly Asp Arg Val
 225 230 235 240

Ile Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His
 245 250 255

Leu Tyr Lys Gln Ile Ser Asn Gly Thr Ser Gly Gly Ser Thr Asn Asp
 260 265 270

Asn Thr Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn
 275 280 285

Arg Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn
 290 295 300

Asn Asn Trp Gly Phe Arg Pro Lys Arg Leu Asn Phe Lys Leu Phe Asn
 305 310 315 320

Ile Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala
 325 330 335

Asn Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln
 340 345 350

Leu Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe
 355 360 365

Pro Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn
 370 375 380

Asn Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr
 385 390 395 400

Phe Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Glu Phe Ser Tyr
 405 410 415

Gln Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser
 420 425 430

Leu Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu
 435 440 445

Ser Arg Thr Gln Ser Thr Gly Gly Thr Ala Gly Thr Gln Gln Leu Leu
 450 455 460

Phe Ser Gln Ala Gly Pro Asn Asn Met Ser Ala Gln Ala Lys Asn Trp
 465 470 475 480

Leu Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Thr Thr Leu Ser
 485 490 495

Gln Asn Asn Asn Ser Asn Phe Ala Trp Thr Gly Ala Thr Lys Tyr His
 500 505 510

Leu Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Val Ala Met Ala Thr
 515 520 525

His Lys Asp Asp Glu Glu Arg Phe Phe Pro Ser Ser Gly Val Leu Met
 530 535 540

Phe Gly Lys Gln Gly Ala Gly Lys Asp Asn Val Asp Tyr Ser Ser Val
 545 550 555 560

Met Leu Thr Ser Glu Glu Glu Ile Lys Thr Thr Asn Pro Val Ala Thr
 565 570 575

Glu Gln Tyr Gly Val Val Ala Asp Asn Leu Gln Gln Gln Asn Ala Ala
 580 585 590

Pro Ile Val Gly Ala Val Asn Ser Gln Gly Ala Leu Pro Gly Met Val
 595 600 605

Trp Gln Asn Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile
 610 615 620

Pro His Thr Asp Gly Asn Phe His Pro Ser Pro Leu Met Gly Gly Phe
 625 630 635 640

Gly Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val
 645 650 655

Pro Ala Asp Pro Pro Thr Thr Phe Ser Gln Ala Lys Leu Ala Ser Phe
 660 665 670

Ile Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu
 675 680 685

Leu Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr
 690 695 700

Ser Asn Tyr Tyr Lys Ser Thr Asn Val Asp Phe Ala Val Asn Thr Asp
 705 710 715 720

Gly Thr Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg
 725 730 735

Asn Leu

<210> 18
 <211> 737
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> AAV DJ-QR

<400> 18

Met Ala Ala Asp Gly Tyr Leu Pro Asp Trp Leu Glu Asp Thr Leu Ser
 1 5 10 15

Glu Gly Ile Arg Gln Trp Trp Lys Leu Lys Pro Gly Pro Pro Pro Pro
 20 25 30

Lys Pro Ala Glu Arg His Lys Asp Asp Ser Arg Gly Leu Val Leu Pro
 35 40 45

Gly Tyr Lys Tyr Leu Gly Pro Phe Asn Gly Leu Asp Lys Gly Glu Pro
 50 55 60

Val Asn Glu Ala Asp Ala Ala Ala Leu Glu His Asp Lys Ala Tyr Asp
 65 70 75 80

Arg Gln Leu Asp Ser Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala
 85 90 95

Asp Ala Glu Phe Gln Glu Arg Leu Lys Glu Asp Thr Ser Phe Gly Gly
 100 105 110

Asn Leu Gly Arg Ala Val Phe Gln Ala Lys Lys Arg Leu Leu Glu Pro
 115 120 125

Leu Gly Leu Val Glu Glu Ala Ala Lys Thr Ala Pro Gly Lys Lys Arg
 130 135 140

Pro Val Glu His Ser Pro Val Glu Pro Asp Ser Ser Ser Gly Thr Gly
 145 150 155 160

Lys Ala Gly Gln Gln Pro Ala Arg Lys Arg Leu Asn Phe Gly Gln Thr
 165 170 175

Gly Asp Ala Asp Ser Val Pro Asp Pro Gln Pro Ile Gly Glu Pro Pro
 180 185 190

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

Ala Pro Met Ala Asp Asn Asn Glu Gly Ala Asp Gly Val Gly Asn Ser
 210 215 220

Ser Gly Asn Trp His Cys Asp Ser Thr Trp Met Gly Asp Arg Val Ile
 225 230 235 240

Thr Thr Ser Thr Arg Thr Trp Ala Leu Pro Thr Tyr Asn Asn His Leu
 245 250 255

Tyr Lys Gln Ile Ser Asn Ser Thr Ser Gly Gly Ser Ser Asn Asp Asn
 260 265 270

Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr Phe Asp Phe Asn Arg
 275 280 285

Phe His Cys His Phe Ser Pro Arg Asp Trp Gln Arg Leu Ile Asn Asn
 290 295 300

Asn Trp Gly Phe Arg Pro Lys Arg Leu Ser Phe Lys Leu Phe Asn Ile
 305 310 315 320

Gln Val Lys Glu Val Thr Gln Asn Glu Gly Thr Lys Thr Ile Ala Asn
 325 330 335

Asn Leu Thr Ser Thr Ile Gln Val Phe Thr Asp Ser Glu Tyr Gln Leu
 340 345 350

Pro Tyr Val Leu Gly Ser Ala His Gln Gly Cys Leu Pro Pro Phe Pro
 355 360 365

Ala Asp Val Phe Met Ile Pro Gln Tyr Gly Tyr Leu Thr Leu Asn Asn
 370 375 380

Gly Ser Gln Ala Val Gly Arg Ser Ser Phe Tyr Cys Leu Glu Tyr Phe
 385 390 395 400

Pro Ser Gln Met Leu Arg Thr Gly Asn Asn Phe Gln Phe Thr Tyr Thr
 405 410 415

Phe Glu Asp Val Pro Phe His Ser Ser Tyr Ala His Ser Gln Ser Leu
 420 425 430

Asp Arg Leu Met Asn Pro Leu Ile Asp Gln Tyr Leu Tyr Tyr Leu Ser
 435 440 445

Arg Thr Gln Thr Thr Gly Gly Thr Thr Asn Thr Gln Thr Leu Gly Phe
 450 455 460

Ser Gln Gly Gly Pro Asn Thr Met Ala Asn Gln Ala Lys Asn Trp Leu
 465 470 475 480

Pro Gly Pro Cys Tyr Arg Gln Gln Arg Val Ser Lys Thr Ser Ala Asp
 485 490 495

Asn Asn Asn Ser Glu Tyr Ser Trp Thr Gly Ala Thr Lys Tyr His Leu
 500 505 510

Asn Gly Arg Asp Ser Leu Val Asn Pro Gly Pro Ala Met Ala Ser His
 515 520 525

Lys Asp Asp Glu Glu Lys Phe Phe Pro Gln Ser Gly Val Leu Ile Phe
 530 535 540

Gly Lys Gln Gly Ser Glu Lys Thr Asn Val Asp Ile Glu Lys Val Met
 545 550 555 560

Ile Thr Asp Glu Glu Glu Ile Arg Thr Thr Asn Pro Val Ala Thr Glu
 565 570 575

Gln Tyr Gly Ser Val Ser Thr Asn Leu Gln Arg Gly Asn Arg Gln Ala
 580 585 590

Ala Thr Ala Asp Val Asn Thr Gln Gly Val Leu Pro Gly Met Val Trp
 595 600 605

Gln Asp Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
 610 615 620

His Thr Asp Gly His Phe His Pro Ser Pro Leu Met Gly Gly Phe Gly
 625 630 635 640

Leu Lys His Pro Pro Pro Gln Ile Leu Ile Lys Asn Thr Pro Val Pro
 645 650 655

Ala Asp Pro Pro Thr Thr Phe Asn Gln Ser Lys Leu Asn Ser Phe Ile
 660 665 670

Thr Gln Tyr Ser Thr Gly Gln Val Ser Val Glu Ile Glu Trp Glu Leu
 675 680 685

Gln Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln Tyr Thr Ser
690 695 700

Asn Tyr Tyr Lys Ser Thr Ser Val Asp Phe Ala Val Asn Thr Glu Gly
705 710 715 720

Val Tyr Ser Glu Pro Arg Pro Ile Gly Thr Arg Tyr Leu Thr Arg Asn
725 730 735

Leu

<210> 19
<211> 724
<212> PRT
<213> adeno-associated virus 5

<400> 19

Met Ser Phe Val Asp His Pro Pro Asp Trp Leu Glu Glu Val Gly Glu
1 5 10 15

Gly Leu Arg Glu Phe Leu Gly Leu Glu Ala Gly Pro Pro Lys Pro Lys
20 25 30

Pro Asn Gln Gln His Gln Asp Gln Ala Arg Gly Leu Val Leu Pro Gly
35 40 45

Tyr Asn Tyr Leu Gly Pro Gly Asn Gly Leu Asp Arg Gly Glu Pro Val
50 55 60

Asn Arg Ala Asp Glu Val Ala Arg Glu His Asp Ile Ser Tyr Asn Glu
65 70 75 80

Gln Leu Glu Ala Gly Asp Asn Pro Tyr Leu Lys Tyr Asn His Ala Asp
85 90 95

Ala Glu Phe Gln Glu Lys Leu Ala Asp Asp Thr Ser Phe Gly Gly Asn
100 105 110

Leu Gly Lys Ala Val Phe Gln Ala Lys Lys Arg Val Leu Glu Pro Phe
115 120 125

Gly Leu Val Glu Glu Gly Ala Lys Thr Ala Pro Thr Gly Lys Arg Ile
130 135 140

Asp Asp His Phe Pro Lys Arg Lys Lys Ala Arg Thr Glu Glu Asp Ser
145 150 155 160

Lys Pro Ser Thr Ser Ser Asp Ala Glu Ala Gly Pro Ser Gly Ser Gln
 165 170 175

Gln Leu Gln Ile Pro Ala Gln Pro Ala Ser Ser Leu Gly Ala Asp Thr
 180 185 190

Met Ser Ala Gly Gly Gly Gly Pro Leu Gly Asp Asn Asn Gln Gly Ala
 195 200 205

Asp Gly Val Gly Asn Ala Ser Gly Asp Trp His Cys Asp Ser Thr Trp
 210 215 220

Met Gly Asp Arg Val Val Thr Lys Ser Thr Arg Thr Trp Val Leu Pro
 225 230 235 240

Ser Tyr Asn Asn His Gln Tyr Arg Glu Ile Lys Ser Gly Ser Val Asp
 245 250 255

Gly Ser Asn Ala Asn Ala Tyr Phe Gly Tyr Ser Thr Pro Trp Gly Tyr
 260 265 270

Phe Asp Phe Asn Arg Phe His Ser His Trp Ser Pro Arg Asp Trp Gln
 275 280 285

Arg Leu Ile Asn Asn Tyr Trp Gly Phe Arg Pro Arg Ser Leu Arg Val
 290 295 300

Lys Ile Phe Asn Ile Gln Val Lys Glu Val Thr Val Gln Asp Ser Thr
 305 310 315 320

Thr Thr Ile Ala Asn Asn Leu Thr Ser Thr Val Gln Val Phe Thr Asp
 325 330 335

Asp Asp Tyr Gln Leu Pro Tyr Val Val Gly Asn Gly Thr Glu Gly Cys
 340 345 350

Leu Pro Ala Phe Pro Pro Gln Val Phe Thr Leu Pro Gln Tyr Gly Tyr
 355 360 365

Ala Thr Leu Asn Arg Asp Asn Thr Glu Asn Pro Thr Glu Arg Ser Ser
 370 375 380

Phe Phe Cys Leu Glu Tyr Phe Pro Ser Lys Met Leu Arg Thr Gly Asn
 385 390 395 400

Asn Phe Glu Phe Thr Tyr Asn Phe Glu Glu Val Pro Phe His Ser Ser
 405 410 415

Phe Ala Pro Ser Gln Asn Leu Phe Lys Leu Ala Asn Pro Leu Val Asp
 420 425 430

Gln Tyr Leu Tyr Arg Phe Val Ser Thr Asn Asn Thr Gly Gly Val Gln
 435 440 445

Phe Asn Lys Asn Leu Ala Gly Arg Tyr Ala Asn Thr Tyr Lys Asn Trp
 450 455 460

Phe Pro Gly Pro Met Gly Arg Thr Gln Gly Trp Asn Leu Gly Ser Gly
 465 470 475 480

Val Asn Arg Ala Ser Val Ser Ala Phe Ala Thr Thr Asn Arg Met Glu
 485 490 495

Leu Glu Gly Ala Ser Tyr Gln Val Pro Pro Gln Pro Asn Gly Met Thr
 500 505 510

Asn Asn Leu Gln Gly Ser Asn Thr Tyr Ala Leu Glu Asn Thr Met Ile
 515 520 525

Phe Asn Ser Gln Pro Ala Asn Pro Gly Thr Thr Ala Thr Tyr Leu Glu
 530 535 540

Gly Asn Met Leu Ile Thr Ser Glu Ser Glu Thr Gln Pro Val Asn Arg
 545 550 555 560

Val Ala Tyr Asn Val Gly Gly Gln Met Ala Thr Asn Asn Gln Ser Ser
 565 570 575

Thr Thr Ala Pro Ala Thr Gly Thr Tyr Asn Leu Gln Glu Ile Val Pro
 580 585 590

Gly Ser Val Trp Met Glu Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp
 595 600 605

Ala Lys Ile Pro Glu Thr Gly Ala His Phe His Pro Ser Pro Ala Met
 610 615 620

Gly Gly Phe Gly Leu Lys His Pro Pro Pro Met Met Leu Ile Lys Asn
 625 630 635 640

Thr Pro Val Pro Gly Asn Ile Thr Ser Phe Ser Asp Val Pro Val Ser
 645 650 655

Ser Phe Ile Thr Gln Tyr Ser Thr Gly Gln Val Thr Val Glu Met Glu
 660 665 670

Trp Glu Leu Lys Lys Glu Asn Ser Lys Arg Trp Asn Pro Glu Ile Gln
 675 680 685

Tyr Thr Asn Asn Tyr Asn Asp Pro Gln Phe Val Asp Phe Ala Pro Asp
 690 695 700

Ser Thr Gly Glu Tyr Arg Thr Thr Arg Pro Ile Gly Thr Arg Tyr Leu
 705 710 715 720

Thr Arg Pro Leu

<210> 20
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> insertAAV7A6

<400> 20

Gly Gln Arg Gly Asn Glu Ala Arg Val Arg Glu Ala Gln
 1 5 10

<210> 21
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> insertAAV2P2

<400> 21

Gln Gly Gln Ser Gly Cys Asp Cys Arg Gly Asp Cys Phe Cys Ala
 1 5 10 15

<210> 22
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> insertAAVDJQRP2

<400> 22

Gln Gly Gln Arg Gly Cys Asp Cys Arg Gly Asp Cys Phe Cys Ala
 1 5 10 15

<210> 23
 <211> 17
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> general formula I

<220>
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 <222> (1)..(1)
 <223> Xaa represents 0, 1 or 2 amino acid residues

<220>
 <221> MISC_FEATURE
 <222> (4)..(4)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (6)..(6)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (7)..(7)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (8)..(8)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (10)..(10)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (11)..(11)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (12)..(12)
 <223> Xaa represents a single amino acid residue

<220>
 <221> MISC_FEATURE
 <222> (13)..(13)
 <223> Xaa represents 0, 1 or 2 amino acid residues

<400> 23

Xaa Gly Gln Xaa Gly Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Ala Gln Ala
 1 5 10 15

Ala

<210> 24
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
<223> alternative formula for sequence Z

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> Xaa is Q or none

<220>
<221> MISC_FEATURE
<222> (4)..(4)
<223> Xaa is S or R

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> Xaa is N or C

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> Xaa is D, Y or E

<220>
<221> MISC_FEATURE
<222> (8)..(8)
<223> Xaa is C, V, S or A

<220>
<221> MISC_FEATURE
<222> (10)..(10)
<223> Xaa is G, S or V

<220>
<221> MISC_FEATURE
<222> (11)..(11)
<223> Xaa is none, A, V or R

<220>
<221> MISC_FEATURE
<222> (12)..(12)
<223> Xaa is D, N or E

<220>
<221> MISC_FEATURE
<222> (13)..(13)
<223> Xaa is C or A

<220>
<221> MISC_FEATURE
<222> (14)..(14)
<223> Xaa is F or Q

<220>
<221> MISC_FEATURE
<222> (15)..(15)
<223> Xaa is none, C or A

<220>
<221> MISC_FEATURE
<222> (16)..(16)
<223> Xaa is none or A

<220>
 <221> MISC_FEATURE
 <222> (17)..(17)
 <223> Xaa is none or Q

<220>
 <221> MISC_FEATURE
 <222> (18)..(18)
 <223> Xaa is none or A

<220>
 <221> MISC_FEATURE
 <222> (19)..(19)
 <223> Xaa is none or A

<400> 24

Xaa Gly Gln Xaa Gly Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1 5 10 15

Xaa Xaa Xaa

<210> 25
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> alternative formula for sequence Z

<220>
 <221> MISC_FEATURE
 <222> (1)..(1)
 <223> Xaa is Q or none

<220>
 <221> MISC_FEATURE
 <222> (4)..(4)
 <223> Xaa is S or R

<220>
 <221> MISC_FEATURE
 <222> (6)..(6)
 <223> Xaa is N or C

<220>
 <221> MISC_FEATURE
 <222> (7)..(7)
 <223> Xaa is D, Y or E

<220>
 <221> MISC_FEATURE
 <222> (8)..(8)
 <223> Xaa is C, V, S or A

<220>
 <221> MISC_FEATURE
 <222> (10)..(10)
 <223> Xaa is G, S or V

<220>
 <221> MISC_FEATURE
 <222> (6)..(6)
 <223> Xaa is C or N

<220>
 <221> MISC_FEATURE
 <222> (7)..(7)
 <223> Xaa is D, E or Y

<220>
 <221> MISC_FEATURE
 <222> (8)..(8)
 <223> Xaa is C, A, S or V

<220>
 <221> MISC_FEATURE
 <222> (10)..(10)
 <223> Xaa is G, V or S

<220>
 <221> MISC_FEATURE
 <222> (11)..(11)
 <223> Xaa is D or none

<220>
 <221> MISC_FEATURE
 <222> (12)..(12)
 <223> Xaa is C or none

<220>
 <221> MISC_FEATURE
 <222> (13)..(13)
 <223> Xaa is F, R, V or A

<220>
 <221> MISC_FEATURE
 <222> (14)..(14)
 <223> Xaa is C, D, N or E

<400> 26

Xaa Gly Gln Xaa Gly Xaa Xaa Xaa Arg Xaa Xaa Xaa Xaa Xaa Ala Gln
 1 5 10 15

Ala Ala

<210> 27
 <211> 52
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> formula II

<220>
 <221> MISC_FEATURE
 <222> (5)..(8)
 <223> Xaa represents a single amino acid residue

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<220>
<221> MISC_FEATURE
<222> (14)..(15)
<223> Xaa represents a single amino acid residue

<220>
<221> MISC_FEATURE
<222> (17)..(20)
<223> Xaa represents a single amino acid residue

<220>
<221> MISC_FEATURE
<222> (22)..(22)
<223> Xaa represents a single amino acid residue

<220>
<221> MISC_FEATURE
<222> (24)..(24)
<223> Xaa represents 0, 1 or 2 amino acid residue(s)

<220>
<221> MISC_FEATURE
<222> (25)..(25)
<223> Xaa represents sequence Z

<220>
<221> MISC_FEATURE
<222> (26)..(26)
<223> Xaa represents 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid
residues

<220>
<221> MISC_FEATURE
<222> (34)..(34)
<223> Xaa represents a single amino acid residue

<400> 27

Glu Glu Glu Ile Xaa Xaa Xaa Xaa Pro Val Ala Thr Glu Xaa Xaa Gly
1          5          10          15

Xaa Xaa Xaa Xaa Asn Xaa Gln Xaa Xaa Xaa Leu Pro Gly Met Val Trp
20          25          30

Gln Xaa Arg Asp Val Tyr Leu Gln Gly Pro Ile Trp Ala Lys Ile Pro
35          40          45

His Thr Asp Gly
50

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