(12) STANDARD PATENT (11) Application No. **AU 2017351914 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Composition for alleviating or treating pain

(51) International Patent Classification(s)
A61K 48/00 (2006.01)

(21) Application No: **2017351914** (22) Date of Filing: **2017.10.31**

(87) WIPO No: **WO18/080277**

(30) Priority Data

(31) Number	(32) Date	(33) Country
10-2016-0143519	**2016.10.31**	**KR**

(43) Publication Date: **2018.05.03**
(44) Accepted Journal Date: **2020.09.10**

(71) Applicant(s)
Kolon Life Science, Inc.

(72) Inventor(s)
Kim, Sujeong;Choi, Heonsik;Kwon, Yejin;Kim, Minjung;Kim, Minju;Kim, Daewook;Park, Jangjoon;Cho, Jongho;Lee, Soondong;Kim, Joonsung;Sim, Yeomoon

(74) Agent / Attorney
FB Rice Pty Ltd, Level 14 90 Collins Street, Melbourne, VIC, 3000, AU

(56) Related Art
WO 2017/052160 A1
CN 101586096 A
DENES, B. et al., 'Durable Multicomponent Vaccine Suppression of Diabetes Autoimmunity', Molecular Therapy. 2009, Vol. 17, Supplement 1, page S67, Abstract 170
LIU, X. et al., Diabetes Metabolism Research and Reviews. Published online March 2016, Vol. 32, No. 6, pages 522-533
RAINOV, N.G. et al., 'Targeted biological therapies for pain', Expert Opinion on Biological Therapy. 2011, Vol. 11, No. 10, pages 1315-1326

(12) 특허협력조약에 의하여 공개된 국제출원

(19) 세계지식재산권기구
국제사무국

(43) 국제공개일
2018 년 5 월 3 일 (03.05.2018)

WIPO | PCT

(10) 국제공개번호
WO 2018/080277 A1

(51) 국제특허분류:
A61K 48/00 (2006.01)

(21) 국제출원번호: PCT/KR2017/012136

(22) 국제출원일: 2017 년 10 월 31 일 (31.10.2017)

(25) 출원언어: 한국어

(26) 공개언어: 한국어

(30) 우선권정보:
10-2016-0143519 2016 년 10 월 31 일 (31.10.2016) KR

(71) 출원인: **코오롱생명과학 주식회사 (KOLON LIFE SCIENCE, INC.)** [KR/KR]; 13837 경기도 과천시 코오롱로 13, Gyeonggi-do (KR).

(72) 발명자: **김수정 (KIM, Sujeong)**; 07062 서울시 동작구 여의대방로10길 100, 101동 306호, Seoul (KR). **최헌식 (CHOI, Heonsik)**; 07030 서울시 동작구 사당로 2길 76, 101동 204호, Seoul (KR). **권예진 (KWON, Yejin)**; 07216 서울시 영등포구 당산로42길 16, 505동 1501호, Seoul (KR). **김민정 (KIM, Minjung)**; 02034 서울시 중랑구 숙선옹주로 18-11, 402호, Seoul (KR). **김민주 (KIM, Minju)**; 03382 서울시 은평구 은평로21가길 6, 202호, Seoul (KR). **김대욱 (KIM, Daewook)**; 17111 경기도 용인시 기흥구 서천동로91번길 8, 202호, Gyeonggi-do (KR). **박장준 (PARK, Jangjoon)**; 07253 서울시 영등포구 국회대로54길 65, 804호, Seoul (KR). **조종호 (CHO, Jongho)**; 01673 서울시 노원구 한글비석로 530, 1201동 103호, Seoul (KR). **이순동 (LEE, Soondong)**; 14247 경기도 광명시 하안로 318, 1101동 404호, Gyeonggi-do (KR). **김준성 (KIM, Joonsung)**; 16418 경기도 수원시 장안구 화산로187번길 19, 110동 301호, Gyeonggi-do (KR). **심여문 (SIM, Yeomoon)**; 05301 서울시 강동구 명일로27길 25-1, 201호, Seoul (KR).

(74) 대리인: **제일특허법인 (FIRSTLAW P.C.)**; 06775 서울시 서초구 마방로 60, Seoul (KR).

(81) 지정국 (별도의 표시가 없는 한, 가능한 모든 종류의 국내 권리의 보호를 위하여): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) 지정국 (별도의 표시가 없는 한, 가능한 모든 종류의 역내 권리의 보호를 위하여): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), 유라시아 (AM, AZ, BY, KG, KZ, RU, TJ, TM), 유럽 (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

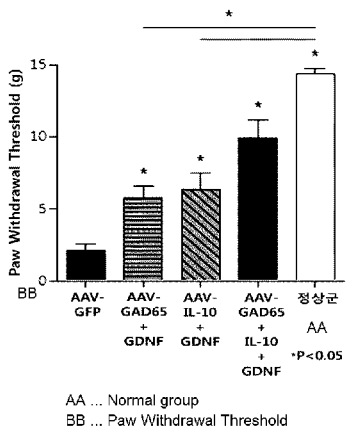
공개:

— 국제조사보고서와 함께 (조약 제21조(3))

— 명세서의 서열목록 부분과 함께 (규칙 5.2(a))

(54) **Title:** COMPOSITION FOR ALLEVIATING OR TREATING PAIN

(54) **발명의 명칭:** 통증 완화 또는 치료용 조성물



AA ... Normal group
BB ... Paw Withdrawal Threshold

(57) **Abstract:** The present invention relates to a composition for alleviating or treating pain. A pharmaceutical composition of the present invention contains two or more selected from the group consisting of GAD, IL-10, and a gene encoding GDNF. The pharmaceutical composition of the present invention exhibits an excellent analgesic effect at a dosage lower than that of individual administration since genes are coadministered, and thus conventional side effects and toxicity can be reduced. Therefore, the pharmaceutical composition of the present invention can be useful in alleviating or treating pain.

(57) **요약서:** 본 발명은 통증의 완화 또는 치료용 조성물에 관한 것이다. 본 발명의 약학 조성물은 GAD, IL-10, 및 GDNF를 암호화하는 유전자로 구성되는 군으로부터 선택되는 2종 이상을 포함한다. 본 발명의 약학 조성물은 유전자를 병용 투여함으로써 단독 투여하는 경우보다 적은 투여량으로도 우수한 진통 효과를 나타내므로 종래 부작용 및 독성을 낮출 수 있다. 따라서, 본 발명의 약학 조성물은 통증의 완화 또는 치료에 유용하게 사용될 수 있다.

WO 2018/080277 A1

Description

Title of Invention

COMPOSITION FOR ALLEVIATING OR TREATING PAIN

Technical Field

The present invention relates to a composition for alleviating or treating pain and a method for alleviating or treating pain using the same.

Background Art

Pain means an experience of actual or potential tissue damage or unpleasant sensations and feelings associated with such damage. Pain protects parts of the body that have been damaged during the healing of the damaged tissues from the damaged situation and provides motivation to avoid similar experiences in the future. Most pain is alleviated slowly when the causal stimulus is removed, but sometimes pain persists even though the tissues have been healed as the stimulus has disappeared and the damage has clearly healed, or pain occurs in a state without any irritation, damage or disease.

For the treatment of pain, mainly, narcotic analgesics such as morphine, which is an opioid alkaloid, or non-narcotic analgesics such as non-steroidal anti-inflammatory

drugs (NSAIDs) having the ingredient of acetylsalicylic acid, ibuprofen, or acetaminophen are widely used.

Narcotic analgesics have the advantage of showing the dose-response and high efficacy, but they can lead to nervous system side effects and if used for a long period, they can lead to the resistance and physical dependence, and pain may worsen.

If aspirin, a non-narcotic analgesic having acetylsalicylic acid as a main ingredient, is used for an analgesic purpose, it should be administered at a high dose of at least 500 mg. However, aspirin is a non-steroidal anti-inflammatory analgesic that blocks the enzyme (COX-1) that promotes the production of prostaglandins, which protect the stomach, thereby preventing gastric mucosa formation. Therefore, the stomach may be easily damaged by gastric acid and gastrointestinal bleeding may occur. In addition, ibuprofen is also a non-steroidal anti-inflammatory analgesic, which can cause gastric disturbances. Also, in the case of analgesics having acetaminophen as a main ingredient, such as Tylenol, acetaminophen is mostly metabolized in the liver, and liver damage may be induced.

Even if the above analgesics are effective at an early stage, they often become ineffective due to resistance when used for a long period. Specifically, in the case of neuropathic pain, there is a problem that the pain is non-responsive to the maximum dose of a nonsteroidal anti-inflammatory agent, and thus, it is administered at a high dose for a short period.

Recently, new therapeutic agents for neuropathic pain have been developed, but still have side effects. For example, sodium channel blockers are mostly in the form of small molecules and show low selectivity for isoform proteins. In addition, they show side effects such as cardiac toxicity and movement disorder.

Therefore, there is an imperative need to develop a new analgesic for neuropathic pain which is excellent in analgesic efficacy while reducing side effects.

Disclosure of Invention

Technical Problem

Accordingly, the present inventors have endeavored to develop a new analgesic for neuropathic pain exhibiting an excellent analgesic efficacy even at a low dosage. As a result, the present inventors have found that when a combination of two or more of glutamate decarboxylase, an anti-inflammatory cytokine, and a glial cell-derived neurotrophic factor is used, pain can be significantly alleviated or treated as compared with an individual use, and have completed the present invention.

Solution to Problem

The present invention provides a pharmaceutical composition for alleviating or treating pain comprising two or more selected from the group consisting of a gene encoding glutamate decarboxylase (GAD), a gene encoding interleukin-10 (IL-10), and a gene encoding a glial cell-derived neurotrophic factor (GDNF).

In addition, the present invention provides a method for alleviating or treating pain, comprising administering the pharmaceutical composition according to the present invention.

Advantageous Effects of Invention

A pharmaceutical composition of the present invention comprises two or more selected from the group consisting of genes encoding GAD, IL-10, and GDNF. Therefore, the pharmaceutical composition of the present invention exhibits an excellent analgesic efficacy at a dosage lower than that of individual administration since genes are co-administered, and thus conventional side effects and toxicity can be reduced. Therefore, the pharmaceutical composition of the present invention can be useful in alleviating or treating pain.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

Brief Description of Drawings

Fig. 1 shows the schematic diagram of the plasmids pAAV-GAD65 and pAAV-GAD65-modi used for the construction of the recombinant adeno-associated virus:

(a) shows the schematic diagram of pAAV-GAD65, and (b) shows the schematic diagram of pAAV-GAD65-modi.

Fig. 2 shows the schematic diagram of the plasmid pAAV-IL-10 used for the construction of the recombinant adeno-associated virus.

Fig. 3 shows the schematic diagram of the plasmid pAAV-GDNF used for the construction of the recombinant adeno-associated virus.

Fig. 4 is a schematic diagram showing the pAAV-GDNF-IL-10 plasmid.

Fig. 5 shows the expression of each introduced gene by the pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid:

(a) shows the expression of GAD65 by pAAV-GAD65 plasmid; (b) shows the expression of IL-10 by pAAV-IL-10 plasmid; and (c) shows the expression of GDNF by the pAAV-GDNF plasmid.

Fig. 6 shows the expression of GDNF gene and IL-10 gene by the pAAV-GDNF-IL-10 plasmid:

(a) shows the expression of IL-10 by pAAV-GDNF-IL-10 plasmid; and (b) shows the expression of GDNF by pAAV-GDNF-IL-10 plasmid.

Fig. 7 shows the results of Western blot showing expression of each protein after treatment of 293T or HeLa cells with each recombinant adeno-associated virus after construction of the recombinant adeno-associated viruses into which GAD65 gene, IL-10 gene and GDNF gene were introduced, respectively:

(a) shows the expression of GAD65 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65; (b) shows the expression of GAD65 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65-modi; (c) shows the expression of IL-10 after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-IL-10; and (d) shows the

expression of GDNF after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GDNF.

Fig. 8 shows the levels of GABA expression measured by ELISA after treatment of 293T or HeLa cells with the recombinant adeno-associated virus AAV-GAD65 or AAV-GAD65-modi:

(a) is a graph showing the level of GABA expression after 293T or HeLa cells were treated with the recombinant adeno-associated virus AAV-GAD65; and (b) is a graph showing the level of GABA expression after 293T or HeLa cells were treated with the recombinant adeno-associated virus AAV-GAD65-modi.

Fig. 9 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of AAV-GAD65, AAV-IL-10, or AAV-GDNF virus and co-administration of AAV-GAD65 and AAV-GDNF viruses, or AAV-IL-10 and AAV-GDNF viruses.

Fig. 10 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of AAV-GAD65, AAV-IL-10, or AAV-GDNF virus and co-administration of AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Fig. 11 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of AAV-GAD65 and AAV-GDNF viruses, or AAV-IL-10 and AAV-GDNF viruses and co-administration of all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Fig. 12 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid and co-administration of pAAV-GAD65 and pAAV-GDNF plasmids, or pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 13 is a graph showing the results of comparing the pain alleviating efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid and co-administration of all of the pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 14 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of pAAV-GAD65 and pAAV-GDNF plasmids, or pAAV-IL-10 and pAAV-GDNF plasmids and co-administration of all of the pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids.

Fig. 15 is a graph showing the results of comparing the pain alleviating efficacies between co-administration of AAV-GAD65-modi and AAV-GDNF-IL-10 viruses and co-administration of all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses.

Best Mode for Carrying out the Invention

Hereinafter, the present invention will be described in detail.

The present invention provides a pharmaceutical composition for alleviating or treating pain comprising two or more selected from the group consisting of a gene

encoding glutamate decarboxylase (GAD), a gene encoding interleukin-10 (IL-10), and a gene encoding a glial cell-derived neurotrophic factor (GDNF).

In one embodiment, the combination of two or more may be GAD and IL-10, GAD and GDNF, IL-10 and GDNF, or GAD, IL-10 and GDNF.

Two or more genes selected from the group consisting of a gene encoding GAD, a gene encoding IL-10, and a gene encoding GDNF may be in a form of being contained in a carrier. Herein, the carrier may be a viral vector, or a non-viral vector such as a plasmid, a liposome, etc. In addition, the genes may be in a form in which some of the genes are contained in a viral vector and the remaining genes are contained in a non-viral vector.

In one embodiment, the genes may be in a form in which GAD is contained in a viral vector, and IL-10 is contained in a non-viral vector. In addition, the genes may be in a form in which GAD is contained in a viral vector, and GDNF is contained in a non-viral vector. Further, the genes may be in a form in which IL-10 is contained in a viral vector, and GDNF is contained in a non-viral vector. In addition, the genes may be in a form in which GAD is contained in a viral vector, and IL-10 and GDNF are contained in a non-viral vector. In addition, the genes may be in a form in which GAD and IL-10 are contained in a viral vector, and GDNF is contained in a non-viral vector. In addition, the genes may be in a form in which GAD and GDNF are contained in a viral vector, and IL-10 is contained a non-viral vector. In addition, the genes may be in a form in which IL-10 is contained in a viral vector, and GAD and GDNF are

contained in a non-viral vector. In addition, the genes may be in a form in which IL-10 and GDNF are contained in a viral vector, and GAD is contained in a non-viral vector. Also, the genes may be in a form in which GDNF is contained in a viral vector, and GAD and IL-10 are contained in a non-viral vector.

In addition, the gene may be in a form of being operably contained in a vector. Specifically, the gene may be in a form of being operably contained in a viral vector or a non-viral vector.

The viral vector may be at least one selected from the group consisting of adenovirus, adeno-associated virus (AAV), herpes simplex virus, lentivirus, retrovirus, cytomegalovirus, baculovirus, poxvirus, etc. Specifically, the viral vector may be adeno-associated virus.

In one embodiment, the gene encoding GAD may be operably contained in a carrier 1 (e.g., a first vector), and the gene encoding IL-10 may be operably contained in a carrier 2 (e.g., a second vector), and the gene encoding GDNF may be operably contained in a carrier 3 (e.g., a third vector). In addition, one carrier may contain two or more genes.

The non-viral vector may be at least one selected from the group consisting of a plasmid, a liposome, a cationic polymer, a micelle, an emulsion, and solid lipid nanoparticles.

The term "plasmid" as used herein refers to a circular DNA fragment existing separately outside the chromosome of bacteria. Plasmids have no genes essential for

the survival of bacteria, but contain genes essential for resistance to certain antibiotics and for interbacterial gene exchange. In addition, plasmids can grow independently of chromosomes and contain selectable markers.

The term "liposome" as used herein refers to a small vesicle produced by forming a bilayer due to the hydrophilic portion and the hydrophobic portion when a molecule having both a hydrophobic portion and a hydrophilic portion in a molecule, such as a phospholipid, is suspended in an aqueous solution. Liposome is isolated from the outer membrane by a membrane composed of a lipid bilayer, and liposomes containing DNA, mRNA, etc., can be used as mediators of genetic information.

The term "cationic polymer" as used herein refers to a cationic lipid or a polymer compound which is a substance that forms a complex by an ionic bond with anionic DNA and delivers the DNA into a cell.

The term "micelle" as used herein refers to a thermodynamically stable colloidal aggregate formed from the molecules consisting of a polar group and a nonpolar hydrophobic group, such as surfactants and lipid molecules, through association by a van der Waals force or the like in a solution. In addition, micelles containing DNA, mRNA and the like can be used as mediators of genetic information.

The term "emulsion" as used herein means that, when two solutions of different phases are mixed, one liquid forms fine particles and is dispersed in another liquid. DNA, mRNA and the like may be contained in the center of the emulsion particle to be used as mediators of genetic information.

As used herein, the term "solid lipid nanoparticle" refers to a preparation of a form in which a drug is contained in a nano-sized microparticle made of a solid lipid instead of a liquid lipid.

A carrier 1 (e.g., a first vector) comprising any one gene selected from the group consisting of GAD, IL-10, and GDNF, and a carrier 2 (e.g., a second vector) comprising any one gene selected from the remaining gene group not included in the carrier 1 according to the present invention may have a virus titer-based mixing ratio per unit volume of 1: 1 to 100 or 1 to 100: 1. Specifically, the virus titer-based mixing ratio per unit volume of the carrier 1 and the carrier 2 may be 1: 1 to 10 or 1 to 10: 1.

A carrier 1 (e.g., a first vector) comprising a gene encoding GAD, a carrier 2 (e.g., a second vector) comprising a gene encoding IL-10 and a carrier 3 (e.g., a third vector) comprising a gene encoding GDNF according to the present invention may have a virus titer-based mixing ratio per unit volume of 1: 0.1 to 10: 0.1 to 10.

As used herein, the term "operably" means that an introduced gene is linked to a regulatory sequence in such a way that expression can take place in a host cell. The regulatory sequence is a DNA sequence that regulates the expression of the gene, and may include other regulatory elements such as promoters and enhancers or polyadenylation. In addition, the regulatory sequence provides a site for binding of a transcription factor that controls the expression of the introduced gene, and can influence the complex structure with the transcription factor to determine the function of the transcription factor.

The term "GAD" as used herein refers to an enzyme that decarboxylates glutamate to produce GABA (gamma-aminobutyric acid). The GAD may be GAD65 or GAD67. Specifically, GAD65 may be derived from a human, a rat, a dog, a cat, or a horse, but is not limited thereto. The gene encoding GAD may be the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, 4, 32, 34, or 36.

In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1 may be the DNA sequence represented by SEQ ID NO: 2 or 3, and may be the mRNA sequence shown in NCBI Reference Sequence: NM_000818.2. In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 4 may be the nucleotide sequence which was codon-optimized to be suitable for the DNA sequence represented by SEQ ID NO: 5 or the gene encoding the amino acid sequence represented by SEQ ID NO: 4, which may be the mRNA sequence shown in NCBI Reference Sequence: NM_000817.2. In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 32 may be the DNA sequence represented by SEQ ID NO: 33. The nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 34 may be the DNA sequence represented by SEQ ID NO: 35, and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 36 may be the DNA sequence represented by SEQ ID NO: 37.

In addition, the gene encoding GAD may be a nucleotide sequence encoding a GAD variant which can retain GAD activity and produce GABA. The GAD variant includes all sequences which retain GAD's characteristics of producing GABA. Although not limited to any one sequence, the nucleotide sequence encoding the GAD variant may be, preferably, a nucleotide sequence encoding an amino acid sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, or 90% or more, and to the GAD's amino acid sequence described above, and most preferably, may be a nucleotide sequence encoding an amino acid sequence having a sequence homology of 95% or more.

In addition, the nucleotide sequence encoding the GAD variant may be a nucleotide sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, or 90% or more to the GAD nucleotide sequence described above, and most preferably, may be a nucleotide sequence having a sequence homology of 95% or more.

The "% of sequence homology" is determined by comparing the comparison regions in a state in which two sequences are optimally aligned. In addition, some of the nucleotide sequences in the comparison regions may include additions or deletions (i.e., gaps) relative to the reference sequence (without addition or deletion) for the optimal alignment of the two sequences.

The term "IL-10" as used herein refers to an anti-inflammatory cytokine belonging to the class II cytokine (Renauld, Nat Rev Immunol, 2003). The IL-10 is in a

form of a homodimer consisting of two subunits each of which has the length of 178 amino acids. It is also known as the cytokine synthesis inhibitory factor (CSIF) in humans. IL-10 serves the function of inhibiting the activity of NK (natural killer) cells in the immune response, and forms a complex with an IL-10 receptor to be involved in signal transduction. IL-10 may be a protein derived from a human, a rat, a dog, a cat, or a horse, but is not limited thereto. The gene encoding IL-10 may be the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6, 9, 38, 40, or 42.

Specifically, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6 may be the DNA sequence represented by SEQ ID NO: 7 or 8, and may be the mRNA sequence shown in NCBI Reference Sequence: NM_012854.2. In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 9 may be the DNA sequence represented by SEQ ID NO: 10 or 14, and may be the mRNA sequence shown in NCBI Reference Sequence: NM_000572.2. The nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 38 may be the DNA sequence represented by SEQ ID NO: 39. In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 40 may be the DNA sequence represented by SEQ ID NO: 41, and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 42 may be the DNA represented by SEQ ID NO: 43.

In addition, the gene encoding IL-10 may be a nucleotide sequence encoding an IL-10 variant that retains the activity of IL-10. The nucleotide sequence encoding the IL-10 variant may be a nucleotide sequence encoding an amino acid sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, 90% or more to the IL-10 amino acid sequence shown above, and most preferably, may be a nucleotide sequence encoding an amino acid sequence having a sequence homology of 95% or more.

The nucleotide sequence encoding the IL-10 variant may be a nucleotide sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, 90% or more to the IL-10 nucleotide sequence shown above, and most preferably, may be a nucleotide sequence having a sequence homology of 95% or more.

The term "GDNF" as used herein refers to a protein constituting the GDNF ligand family. The GDNF ligand family consists of GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN). In addition, GDNF is a protein that promotes the survival of many kinds of neurons and transmits signals through the GFRα1 receptor. GDNF may be a protein derived from a human, a rat, a dog, a cat, or a horse, but is not limited thereto. The gene encoding GDNF may be the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 11, 44, 46, or 48.

Specifically, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 11 may be the DNA sequence represented by SEQ ID NO: 12 or 13, and may be the mRNA sequence shown in NCBI Reference Sequence:

NM_199231.2. In addition, the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 44 may be the DNA sequence represented by SEQ ID NO: 45. The nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 46 may be the DNA sequence represented by SEQ ID NO: 47, and the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 48 may be the DNA sequence represented by SEQ ID NO: 49.

In addition, the gene encoding GDNF may be a nucleotide sequence encoding a GDNF variant which retains the GDNF activity. The nucleotide sequence encoding the GDNF variant may be a nucleotide sequence encoding an amino acid sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, or 90% or more to the GDNF's amino acid sequence shown above, and most preferably, may be a nucleotide sequence encoding an amino acid sequence having a sequence homology of 95% or more.

In addition, the nucleotide sequence encoding the GDNF variant may be a nucleotide sequence having a sequence homology of at least 60% or more, 70% or more, 80% or more, 90% or more to the GDNF nucleotide sequence shown above, and most preferably, may be a nucleotide sequence having a sequence homology of 95% or more.

GABA, the product of GAD gene, has the effect of blocking pain signal transduction, but excessive amounts can cause symptoms such as itching, dizziness,

drowsiness, etc., as well as the side effects such as increase in the heart rate or respiratory rate (Longo, Am Fam Physician, 2000).

IL-10 is known to be a cytokine which shows anti-inflammatory actions, but side effects such as flu symptoms and the like can occur (Friedrich, J Invest Dermatol, 2002).

Furthermore, it is known that the expression of GDNF exhibits analgesic efficacies on a variety of pains such as neuropathic pain and the like, but it has been reported in monkey experiments that administration in excess caused neuronal damage of brain (Hovland, Toxicol Pathol, 2007).

A pharmaceutical composition of the present invention can exhibit analgesic actions with a small amount of genes or carriers containing the same. The composition of the present invention consists of a vector containing a gene encoding GAD, a vector containing a gene encoding an anti-inflammatory cytokine in nervous tissues, and/or a vector containing a gene encoding GDNF. And by co-administering substances having different analgesic mechanisms, it is possible to achieve the same or better pain alleviation or treatment effects at a dosage lower than that of individual administration.

Particularly, according to the present invention, when two or more genes selected from the group consisting of genes encoding GAD65, IL-10, and GDNF are co-administered, a synergistic pain-alleviating effect takes place. Therefore, the pharmaceutical composition of the present invention can be useful for alleviating or treating pain.

According to one embodiment of the present invention, the first vector, the second vector, and/or the third vector may be an adeno-associated virus. The adeno-associated virus is not limited to a particular serotype, and preferably may be any one of AAV1 to AAV9.

The pain may be selected from the group consisting of nociceptive pain, psychogenic pain, inflammatory pain, pathological pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, or migraine. In a specific example, the pain may be lumbosacral radiculopathy (LSR).

The inflammatory pain refers to the pain associated with a tissue damage and infiltration of immune cells. In addition, the pathological pain means a disease state in which pain is caused by damage to a nerve tissue or its abnormal function. Also, the pathological pain may be dysfunctional pain, such as fibromyalgia, irritable bowel syndrome, or tension headache.

In addition, pain can include back pain which can be anatomically distinguished: neck pain, middle back pain, lower back pain, or tailbone pain. In addition, the pain may be at least one selected from the group consisting of neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, migraine, and the like. In a specific example, the pain may be lumbosacral radiculopathy.

Neuropathic pain can be caused by a damage or disease that affects the somatosensory system. Neuropathic pain can be an abnormal sensation called allodynia

and dysesthesia. In addition, the general characteristics of neuropathic pain include the sense of hot or cold, pins and needles, numbness, and itching. In contrast, nociceptive pain is often expressed as aching.

In addition, migraine is associated with a number of autonomic nervous system symptoms, and is a chronic disorder that causes headaches of normal to serious severities. Migraine is known to be associated with increased excitability of the cerebral cortex and abnormal regulation of pain neurons in the trigeminal nucleus of the brainstem (Noseda, Pain, 2013).

Specifically, a pharmaceutical composition of the present invention can be used for alleviating or treating neuropathic pain and chronic cancer pain.

As used herein, the term "alleviating or treating" means any action that improves or alters pain symptom in a beneficial way by administering the composition of the present invention.

The pharmaceutical composition of the present invention may further comprise a physiologically acceptable carrier. In addition, the pharmaceutical compositions of the present invention may further comprise suitable excipients and diluents conventionally used in the preparation of pharmaceutical compositions. In addition, the compositions of the present invention may be used by preparing them as oral formulations such as powders, granules, tablets, capsules, suspensions, emulsions, syrups, aerosols, etc., external formulations, suppositories, or injections by general methods. Specifically, the pharmaceutical composition may be in the form of an injection. As for the suitable

formulations known in the art, those listed in Remington's Pharmaceutical Science (1985) may be used.

In addition, the pharmaceutical composition may comprise a salt (sodium chloride), lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methylcellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil, etc., as carriers, excipients, and diluents. For the formulation of the pharmaceutical composition of the present invention, generally used diluents or excipients such as fillers, extenders, binders, humectants, disintegrators, surfactants, etc. may be utilized.

Formulations for parenteral administration may include sterile solutions, non-aqueous solvents, suspensions, emulsions, freeze-dried formulations, and suppositories. Propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable ester such as ethylolate, etc., may be used for non-aqueous solvents and suspensions. Witepsol, macrogol, Tween 61, cacao oil, laurin oil, glycerogelatin, etc. may be used for suppository bases.

The present invention also provides a method for alleviating or treating pain, comprising administering a pharmaceutical composition comprising two or more selected from the group consisting of genes encoding GAD, IL-10, and GDNF to a subject in need thereof.

The pain is as described above with regard to the pharmaceutical composition.

The subject may be a mammal including a human, or a cell and/or tissue isolated from a mammal including a human. The term "non-human animal" as used herein is intended to encompass all vertebrate animals, which include mammals and non-mammals such as primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc.

As for the administration route, dosage, and administration frequency, the pharmaceutical composition may be administered to a subject in various ways and amounts depending on the condition of a patient and the presence or absence of side effects, and the range of optimal administration methods, dosages and administration frequencies may be appropriately selected by those having ordinary skill in the art. In addition, the pharmaceutical composition may be administered in combination with another drug or a physiologically active substance which is known to show a therapeutic efficacy on a disorder to be treated. Also, the pharmaceutical composition may be prepared in the form of a combination formulation.

Specifically, the pharmaceutical composition of the present invention may be provided in the form of an injection. For example, subcutaneous injection, intramuscular injection, intravenous injection, epidural injection, or intrathecal injection, and the like may be included. Specifically, the pharmaceutical composition may be administered via epidural injection or intrathecal injection, and more specifically, it may be administered via transforaminal epidural injection or intrathecal injection.

As used herein, the term "transforaminal epidural injection" refers to a method of injecting a drug into the inside of an intervertebral foramen which is a space where nerves emerge from the spinal cord through the space between spinal bones, and into the space outside of the dura which surrounds the spinal cord and spinal nerves. In one embodiment, if the pharmaceutical composition of the present invention is made of viruses, the drug can be administered to the inside of the intervertebral foramen of a subject by conducting epidural injection therapy.

As used herein, the term "intrathecal injection" refers to a method of administration by injecting a drug to a space inside dura in the spinal canal. In one embodiment, if the pharmaceutical composition of the present invention is made of plasmids, the drug can be administered to the inside of the spinal canal of a subject by conducting intrathecal injection therapy.

Specifically, if the pharmaceutical composition is made of viral vectors, it can be administered in an amount of 1.0×10^6 to 1.0×10^{14} vg on an adult basis. In addition, when there are two types of viruses to be administered, each type of the viruses can be administered in an amount of 5.0×10^5 to 5.0×10^{13} vg. If there are three types of viruses to be administered, each type of the viruses can be administered in an amount of 3.0×10^5 to 3.0×10^{13} vg.

In addition, if the pharmaceutical composition is made of non-viral vectors, it can be administered in a concentration of 0.1 mg/ml to 10 mg/ml, on an adult basis. Also, if the pharmaceutical composition is made of plasmid vectors, the dosage may be 0.1 ml,

1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, 10 ml or more, including all values and ranges between them.

As for the administration frequency of a viral vector, it may be administered once or more, or 1 to 10 times. Also, it may be administered at the interval of 1 day to 1 month, or 1 month to 1 year in the case of repeated administration. In addition, if the pharmaceutical composition is made of non-viral vectors, it may be administered 1 or more, or 1 to 10 times. Also, it may be administered at the interval of 12 to 24 hours or 1 to 14 days in the case of repeatedly administration.

The present invention provides a use of the pharmaceutical composition of the present invention for alleviating or treating pain.

The present invention provides a use of the pharmaceutical composition of the present invention for preparing a therapeutic agent for alleviating or treating pain.

Modes for Carrying Out the Invention

Hereinafter, the present invention will be described in detail with reference to examples. However, the following examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

Example 1. Preparation and property analysis of recombinant adeno-associated virus

Adeno-associated viruses required for the present invention were constructed and produced on the basis of the AAV helper-free system (Agilent).

Example 1.1. Construction of pAAV-GAD65 plasmid

To construct the pAAV-GAD65 plasmid of Fig. 1, the CMV promoter region of pJDK-rGAD65 (Lee, Gene Ther, 2005) was amplified by PCR, and then the resultant was introduced into pGEM-T (Promega) to construct pGEM-T-CMV. The primer sequences used for CMV promoter amplification are as follows:

F-JDK (SEQ ID NO: 15): 5'-TTCGGCCGTCGAGGAGCTTGGCCCATTG-3'

R-JDK (SEQ ID NO: 16): 5'-GACGTCGACCTAGCTAGCGAATTCGGGGCCGCGGAG-3'.

As for the GAD65 gene, the gene represented by SEQ ID NO: 3 was designed by codon-optimization to be suitable for humans based on the human GAD65 (NCBI NM_000818.2) represented by the amino acid sequence of SEQ ID NO: 1, and referred to Bioneer for gene synthesis. The hGAD65 gene introduced into pGEM-T was treated with NheI and SalI to prepare a 1.7 Kb DNA fragment. Thereafter, it was subjected to ligation with the 3.7 Kb DNA fragment obtained by treating pGEM-T-CMV with NheI and SalI, to complete pGEM-T-CMV-hGAD65 construction.

SV40pA was amplified by conducting PCR using pCI (Invitrogen) as a template, and then the resultant was treated with ClaI and SalI to prepare a 222 bp DNA fragment. The DNA fragment was subjected to ligation with the 5.4 Kb DNA fragment prepared by cutting pGEM-T-CMV-hGAD65 with ClaI and SalI, to finally prepare pGEM-T-CMV-hGAD65-SV40pA. The primer sequences used for SV40pA amplification are as follows:

F-SV40pA (SEQ ID NO: 17): 5'-CCATCGATCAGACATGATAAGATACATTGATGAG-3'

R-SV40pA (SEQ ID NO: 18): 5'-GACGTCGACGCGGCCGCTACCACATTTGTAGAGGTTTTACTTG-3'.

To construct an adeno-associated virus vector, the ampicillin resistance gene in pAAV-MCS (Agilent) was replaced with the kanamycin resistance gene. The kanamycin resistance gene was amplified by PCR using pET-28 (a) (Novagen) as a template. The amplified 816 bp kanamycin resistance gene was subjected to ligation with pGEM-T to construct pGEM-T-Kan^r. The primer sequences used for kanamycin resistance gene amplification are as follows:

F-Kan (SEQ ID NO: 19): 5'-AGGCGCCATGAGCCATATTCAACGGGAA-3'

R-Kan (SEQ ID NO: 20): 5'-TTCATGATTAGAAAAACTCATCGAGCATC-3'.

To introduce the kanamycin resistance gene, SpeI and EcoRV sites were respectively generated by mutagenesis upstream and downstream of the ampicillin resistance gene in pAAV-MCS, and then the resultant was treated with SpeI and EcoRV again. The resultant was subjected to ligation with the DNA fragment obtained by cutting the previously constructed pGEM-T-Kan^r with NheI and EcoRV, to construct pAAV-MCS-Kan^r.

The constructed pAAV-MCS-Kan^r was treated with NotI and BamHI, and then subjected to ligation with the 2.7 Kb DNA fragment obtained by cutting pGEM-T-CMV-hGAD65-SV40pA with EagI and PvuI, to construct pssAAV-GAD65.

To introduce the GAD65 expression cassette into pVAX1 (Invitrogen), the BamHI site was generated by mutagenesis downstream of the bGHpA. Then, the resultant was cut with MluI and NheI to prepare DNA fragments. The LITR and CMV promoter regions were amplified by PCR using pssaAV-GAD65 as a template and cloned into pGEM-T easy (Promega). Thereafter, the resultant was cut with AscI and NheI, and subjected to ligation with the pVAX1 vector previously prepared, to construct pVAX1-LITR-CMV. Primer sequences used for LITR and CMV promoter region amplification are as follows:

F-ITR (SEQ ID NO: 21): 5'-ATGGCGCGCCCCTGGCCTTTTGCTGGCC-3',

R-JDK (SEQ ID NO: 16): 5'-GACGTCGACCTAGCTAGCGAATTCGGGGCCGCGGAG-3'.

pVAX1-LITR-CMV was cut with NotI and NheI again to prepare DNA fragments. PSSAAV-GAD65 was cut with EagI and NheI, and subjected to ligation with the DNA fragments previously prepared, to construct pVAX1-LITR-CMV-hGAD65-SV40pA.

The pVAX1-LITR-CMV-hGAD65-SV40pA was cut with HpaI and BamHI to prepare DNA fragments. In addition, psA-SV40pA-RITR, which had been prepared by amplifying through PCR using pssaAV-GAD65 as a template and cloning into pGEM-T easy, was treated with HpaI and BamHI, to prepare DNA fragments. The two DNA fragments were ligated to complete pVAX1-LITR-CMV-hGAD65-SV40pA-RITR (hereinafter abbreviated as "pAAV-GAD65"). Primer sequences used for SV40pA and RITR region amplification are as follows:

F-SV40pA (SEQ ID NO: 17): 5'-CCATCGATCAGACATGATAAGATACATTGATGAG-3'

R-ITR (SEQ ID NO: 22): 5'-ATGGATCCGCTAGTAAATACCGCATCAG-3'.

The schematic diagram of the pAAV-GAD65 plasmid is shown in Fig. 1.

The following procedure was carried out to construct modified pAAV-GAD65.

First, the vector was cut with Nhe1, and then an arbitrary random nucleotide sequence was inserted between the CMV promoter and GAD65 gene by an infusion method. The inserted nucleotide sequences are as follows:

Scramble stuffer (SEQ ID NO: 29):

5'-GTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCC-3'

Stuffer_scramble_F (SEQ ID NO: 30):

5'-CTAGGTCGACGGTATCGATAAGCTTGATATCGAATTCCTGCAGCCC-3'

Stuffer_scramble_R (SEQ ID NO: 31):

5'-CTAGGGGCTGCAGGAATTCGATATCAAGCTTATCGATACCGTCGAC-3'.

Next, the WPRE nucleotide sequence (Schambach, Gene Ther, 2006), from which the X-protein region which can provide an oncogenic effect was removed, was amplified by PCR, and inserted at the back of GAD65 gene using Pac1 and Hpa1 restriction enzymes. At the same time, some portion of SV40pA was removed to

construct a modified SV40pA. The primer sequences used for WPRE amplification are as follows:

WPRE_Pac1_F (SEQ ID NO: 25):

5'-GGTGGTTTAATTAAAATCAACCTCTGGATTACAAAATTTG-3'

WPRE_modi_Hpa1_R (SEQ ID NO: 26): 5'-GGTGGTGTTAACGACAACACCACGGAATTG-3'.

The finally modified plasmid was pVAX1-LITR-CMV-scramble stuffer-hGAD65-WPRE (modi)-SV40pA (modi)-RITR (hereinafter abbreviated as "pAAV-GAD65-modi"), and its schematic diagram is shown in Fig. 1.

Example 1.2. Construction of pAAV-IL-10 plasmid

The pAAV-IL-10 plasmid was constructed by the same method as in Example 1.1. As for the rat IL-10 gene, the gene represented by the nucleotide sequence of SEQ ID NO: 8 was designed by codon-optimization to be suitable for rats based on the human IL-10 (NCBI NM-012854) represented by the amino acid sequence of SEQ ID NO: 6, and referred to Bioneer for gene synthesis. The rIL-10 gene was amplified by conducting PCR using the rat IL-10 gene introduced into pGEM-T easy as a template, and then the resultant was treated with NheI and SalI to prepare a 0.5 Kb DNA fragment. In addition, PGEM-T-CMV was treated with NheI and SalI to prepare a 3.7 Kb DNA fragment. The two DNA fragments were ligated to prepare pGEM-T-CMV-rIL-10. The primer sequences used for rIL-10 amplification are as follows:

F-rIL-10 (SEQ ID NO: 23): 5'-CCGCTAGCGCCACCATGCCT-3'

R-rIL-10 (SEQ ID NO: 24): 5'-GACGTCGACGCCATCGATGGCTTAATTAATCAATTCTTC-3'.

As for the SV40pA, the gene was amplified by conducting PCR using pCI as a template and then treated with NotI and SalI to prepare a 222 bp DNA fragment. In addition, the pGEM-T-CMV-rIL-10 was treated with ClaI and SalI to prepare a 4.2 Kb DNA fragment. The two DNA fragments were ligated to construct pGEM-T-CMV-rIL-10-SV40pA. The primer sequences used for SV40pA amplification are as follows:

F-SV40pA (SEQ ID NO: 17): 5'-CCATCGATCAGACATGATAAGATACATTGATGAG-3'

R-SV40pA (SEQ ID NO: 18): 5'-GACGTCGACGCGGCCGCTACCACATTTGTAGAGGTTTTACTTG-3'.

pGEM-T-CMV-rIL-10-SV40pA was treated with EagI to prepare a 1.6 Kb DNA fragment. In addition, pAAV-MCS-Kan^r was treated with NotI and BamHI to prepare DNA fragments. Thereafter, the two DNA fragments were ligated to construct pssAAV-CMV-rIL-10-SV40pA (hereinafter abbreviated as "pAAV-IL-10"). The schematic diagram of the pAAV-IL-10 plasmid is shown in Fig. 2.

Example 1.3. Construction of pAAV-GDNF plasmid

As for the human GDNF gene, the gene represented by the SEQ ID NO: 13 was designed by codon-optimization to be suitable for humans based on human GDNF (NCBI NM_199231.2) represented by the amino acid sequence of SEQ ID NO: 11, and referred to Bioneer for gene synthesis. The hGDNF gene introduced into the pGEM-B1

plasmid was treated with NheI and PacI to prepare a DNA fragment of about 0.6 kb. The pGEM-T-CMV-rIL-10-SV40pA plasmid was treated with NheI and PacI to prepare a 2.8 kb fragment in which the rIL-10 gene was removed. The two DNA fragments were ligated to construct the pGEM-T-CMV-hGDNF-SV40pA plasmid.

Then, the completed pGEM-T-CMV-hGDNF-SV40pA plasmid was treated with EagI to prepare a 1.5 kb DNA fragment. In addition, pAAV-MCS-Kan^r was treated with NotI and BamHI to prepare a 1.8 kb DNA fragment. The two DNA fragments were ligated to construct pssAAV-CMV-hGDNF-SV40pA-Kan^r (hereinafter abbreviated as "pAAV-GDNF"). The schematic diagram of the pAAV-GDNF plasmid is shown in Fig. 3.

Example 1.4. Construction of pAAV-GDNF-IL-10 plasmid

The CAG promoter (cytomegalovirus enhancer, chicken β-actin promoter, and rabbit β-globin poly A signal) was subjected to PCR amplification using pAxCAwtit2 contained in the Adenovirus dual expression kit (Takara), and treated with ApaI and XbaI to improve expression, thereby removing about 80% of the chicken ß-actin region in the CAG promoter to produce a short CAG (sCAG) promoter (Fagoe, Gene Ther, 2014). As for the human IL-10 gene, the gene encoding the SEQ ID NO: 14 was designed by codon-optimization of the gene encoding SEQ ID NO: 9 to be suitable for humans, and referred to Bioneer for gene synthesis. Next, DNA fragment for bovine growth hormone (bGH) poly A was obtained by PCR amplification. The

pVAX1/sCAG-hIL-10-bGHpA was constructed using pVAX1 (Invitrogen) to contain the promoter and poly A and human IL-10 genes.

Next, pVAX1/CMV-hGDNF-SV40pA was prepared by the same method as in the Example 1.1., using the human GDNF gene of the Example 1.3. Thereafter, the SV40pA-hGDNF-CMV gene cassette was amplified by conducting PCR using pVAX1/CMV-hGDNF-SV40pA as a template, and a 1.5 kb DNA fragment was prepared. The primer sequences used for the gene cassette amplification are as follows:

SV40-CMV-sCAG-bGHpA-Infu-F (SEQ ID NO: 27):

5'-CCTGCGGCCGGTCGACTACCACATTTGTAGAGGTTTTACTTGC-3'

SV40-CMV-sCAG-bGHpA-Infu-R (SEQ ID NO: 28):

5'-AATAATCAATGTCGACTCGAGGAGCTTGGCCCATT-3'

Next, pVAX1/sCAG-hIL-10-bGHpA was treated with SalI to prepare a DNA fragment of about 3.9 kb, and the 1.5 kb DNA fragment described above was inserted into the 3.9 kb DNA fragment using an In-Fusion HD Cloning Kit (Clontech) to construct pVAX1/SV40pA-hGDNF-CMV-sCAG-hIL-10-bGHpA (hereinafter abbreviated as "pAAV-GDNF-IL-10"). The pAAV-GDNF-IL-10 plasmid is schematically shown in Fig. 4.

Experimental Example 1. Confirmation of expression of pAAV-GAD65, pAAV-IL-10, pAAV-GDNF and pAAV-GDNF-IL-10 plasmids

The pAAV-GAD65, pAAV-IL-10, pAAV-GDNF, or pAAV-GDNF-IL-10 plasmids prepared in the Examples 1.1. to 1.4 were respectively transfected into human embryonic kidney cell line 293T cells using jetPRIME (Polyplus). The transfected cells were cultured in a 37°C incubator for 48 hours. Thereafter, the cell culture medium or cultured cells were harvested. The cells were dissolved with a solvent, and the prepared samples were treated with each of the antibodies to GAD65 (Merck Millipore), IL-10 (Santa Cruz), and GDNF (R&D systems), and subjected to Western blotting.

Specifically, in the case of pAAV-GAD65, the human embryonic kidney cell line 293T cells were treated with 2 μg of pAAV-GAD65 plasmid and cultured for 48 hours. Thereafter, the cultured cells were dissolved and the expression of GAD65 in the cells was confirmed through Western blotting.

In the case of pAAV-IL-10 and pAAV-GDNF plasmids, the human embryonic kidney cell line 293T cells were treated with 1 μg of pAAV-IL-10 or pAAV-GDNF plasmid, and cultured for 48 hours. Thereafter, the culture medium was harvested and the expression of IL-10 or GDNF in the medium was confirmed through Western blotting.

In the case of the pAAV-GDNF-IL-10 plasmid, the human embryonic kidney cell line 293T cells were treated with 1 μg of pAAV-GDNF-IL-10 plasmid and cultured for 48 hours. Thereafter, the cultured cells were dissolved, and the expression of intracellular IL-10 and GDNF was confirmed through Western blotting.

As a result, it was confirmed that the transfected pAAV-GAD65, pAAV-IL-10, pAAV-GDNF or pAAV-GDNF-IL-10 plasmid was expressed (Figs. 5 and 6).

Example 2. Preparation of recombinant adeno-associated virus

The AAV-IL-10 virus used in the experiment was produced and purified by UNC vector core. The production method is as follows. The pVax-rIL-10, pHelper and pRC5 were transfected into human embryonic kidney cell line 293T cells. Thereafter, the resultant was subject to purification by column chromatography to secure AAV5-IL-10 virus. The titer of the produced virus was measured using qPCR.

The AAV-GAD65 and AAV-GDNF viruses were produced and purified by KRcrogen. The production method is as follows. The AAV-transgenes (pAAV-GAD65 and pAAV-GDNF plasmids) were respectively transfected into the human embryonic kidney cell line 293T cells using the calcium phosphate method along with pHelper and pRC. In the case of GAD65, pRC5 introduced with the capsid gene of serotype 5 was used. In the case of GDNF, pRC1 introduced with the capsid gene of AAV serotype 1 was used. The transfected cells were cultured in a 37°C incubator and the cells were harvested after 48 hours.

Thereafter, only the bands containing viruses were isolated and purified through the ultrahigh speed centrifugation method according to the cesium concentration gradient, to secure AAV5-GAD65, and AAV1-GDNF viruses. The titers of the produced viruses were measured using qPCR.

The AAV-GDNF-IL-10 virus was produced and purified by Cdmogen. The production method is as follows. The AAV-transgene (pAAV-GDNF-IL-10 plasmid) was transfected into the human embryonic kidney cell line 293T cells using the calcium phosphate method along with pHelper and pRC5. The transfected cells were cultured in a 37°C incubator and the cells were harvested after 48 hours.

Thereafter, only the bands containing viruses were isolated and purified through ultrahigh speed centrifugation according to the cesium concentration gradient, to secure AAV5-GDNF-IL-10 virus. The titer of the produced virus was measured using qPCR.

The AAV-GAD65-modi virus was produced and purified by Cdmogen. The production method is as follows. The AAV-transgene (pAAV-hGAD65-modi plasmid) was transfected into human embryonic kidney cell line 293T cells using the calcium phosphate method along with pHelper and pRC5. The transfected cells were cultured in a 37°C incubator and the cells were harvested after 48 hours.

Thereafter, only the bands containing viruses were isolated and purified by ultra-high-speed centrifugation according to the cesium concentration gradient to secure the AAV5-GAD65-modi virus. The titer of the produced virus was measured using qPCR.

Experimental Example 2. Property analysis of recombinant adeno-associated virus

In order to examine the protein expression of recombinant adeno-associated virus delivered into a cell, human embryonic kidney cell line 293T or HeLa cells were

treated with the AAV-GAD65, AAV-GAD65-modi, AAV-IL-10 or AAV-GDNF virus obtained above, and protein expression was examined by Western blotting. Specifically, 293T or HeLa cells were seeded at 5 x 10^5 cells/well in a 6-well plate. And on the next day, the cells were respectively treated with 3 types of viruses at 10,000 vg/well, and then cultured in a 37°C incubator. After 48 hours, the cells were harvested and were dissolved with a solvent and the culture medium was concentrated using the amicon (Merck Millipore). Then, the prepared samples were respectively treated with the antibodies to GAD65 (Cell signaling), IL-10 (Santa Cruz) and GDNF (R&D systems), and subjected to Western blotting.

As a result, it was confirmed that each target protein was expressed in the cell lysate of human embryonic kidney cell line 293T or HeLa cell line treated with AAV-GAD65, AAV-GAD65-modi, AAV-IL-10 or AAV-GDNF virus (Fig. 7). Therefore, it was confirmed that there was no abnormality in the structures and properties of the recombinant adeno-associated viruses used in the experiment.

Also, in order to confirm that GABA is produced by AAV-GAD65 or AAV-GAD65-modi virus, the culture medium of the cells treated with the AAV-GAD65 or AAV-GAD65-modi virus was harvested and subjected to GABA ELISA (LDN) analysis. For each experimental group, two identical samples were prepared separately to conduct the analysis, and the bar graph shows the value for each sample.

As a result, it was confirmed that GABA was secreted to the culture medium by GAD65 introduced into cells by AAV-GAD65 or AAV-GAD65-modi virus (Fig. 8).

Experimental Example 3. Comparison of analgesic efficacies between individual administration of AAV-GAD65, AAV-IL-10 or AAV-GDNF virus and co-administration of AAV-GAD65 and AAV-GDNF, or AAV-IL-10 and AAV-GDNF viruses

Experimental Example 3.1. Preparation of administration sample

The viruses prepared in Example 2 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. AAV-GAD65, AAV-IL-10, AAV-GDNF, and AAV-GFP viruses were diluted in PBS to obtain the titers shown in Table 1. The AAV-GFP virus was administered in the same amount as other recombinant adeno-associated viruses. The GFP is a protein having no analgesic efficacy. The viruses required were mixed according to the contents indicated in Table 1, and administered in an amount of 9.0×10^8 vg/5 μl per animal (vg: virus genome).

[Table 1]

Samples	Virus types and contents			
	AAV-GAD65	AAV-IL-10	AAV-GDNF	AAV-GFP
AAV-GFP	-	-	-	9.0×10^8 vg/5 μl
AAV-GAD65	9.0×10^8	-	-	-

	vg/5 μl			
AAV-IL-10	-	9.0×10^8 vg/5 μl	-	-
AAV-GDNF	-	-	9.0×10^8 vg/5 μl	-
AAV-GAD65 + AAV-GDNF	4.5×10^8 vg/5 μl	-	4.5×10^8 vg/5 μl	-
AAV-IL-10 + AAV-GDNF	-	4.5×10^8 vg/2.5 μl	4.5×10^8 vg/2.5 μl	-

Experimental Example 3.2. Construction of neuropathic pain-induced rats and administration of samples

150 to 200 g male SD-rats were subjected to inhalation anesthesia. And then the upper part of the calf was incised and both ends of the common peroneal nerve and tibial nerve were tied and knots were made at the interval of 0.5 to 1 cm by 7-0 suture. The regions between the knots of the two nerve bundles were cut with a scissor and the incision site was sutured. Thereafter, the rats were recovered to be awakened from the anesthesia and returned to the cage. Two weeks later, the *von Frey* filament test was

conducted to examine pain induction, and then the samples prepared in Example 2.1 were respectively administered (Decosterd, Pain, 2000).

The samples were administered by the transforaminal epidural injection method at a location adjacent to the dorsal root ganglion (DRG). The pain-induced rat was subjected to inhalation anesthesia, and the vertebrae were exposed by linearly incising the back of the rat at the levels of lumbar spines L3 to L5. At the side of the exposed space, the L4 transverse process, one of the spinal projections, was made visible. The rat was laid down sideways such that its lateral side is visible from above and the L4 intervertebral foramen was visible.

Thereafter, a needle attached to the catheter was inserted into the prepared sample, and a Hamilton syringe was connected to the opposite end of the catheter and pulled to the marking line of 5 μl to inject the sample into the catheter. The Hamilton syringe was removed from the catheter and then the catheter was secured by holding the point 1 cm away from the tip of the needle using Halsted-Mosquito. Then, while holding and pulling the L4 spine upward with tweezers, the tip of the needle secured by Halstead Mosquito was taken around the L4 intervertebral foramen with the other hand. The tip of the needle was inserted into the bent region inside the intervertebral foramen whose space was secured. Then, the needle which was being held was released.

After confirming that the needle was fixed, a 1 ml syringe was connected to the catheter connected to the opposite side of the needle. By gently pressing the piston of the syringe, the sample was slowly injected around the dorsal root ganglion of the rat.

Thereafter, the incision site was sutured. 4 weeks after the sample administration, pain responses were observed using *von Frey* filament test.

Experimental Example 3.3. Pain observation using *von Frey* filament test

Pain was observed using the *von Frey* filament test. The method is to calculate the threshold value according to a predetermined pattern of the pain response with a total of eight filaments of 0.4, 0.6, 1, 2, 4, 6, 8 and 15 g.

Pain generating regions were searched by changing the position from the beginning portion of the outermost toe to the heel of the sole where pain was generated. Since the rats suddenly took off the soles and shrank or licked their soles with their mouths when pain was generated, the pain generating regions could be found. If there were reactions three times or more when the surrounding area was pricked five times with the filament of each step, it was regarded as a pain response. And the test was proceeded by replacing the filament with that of the next step. In this way, the pattern of each step was recorded.

The pain patterns were recorded based on the pattern table established by S.R. Chaplan, and the threshold values were calculated using the pain patterns (Chaplan, J Neurosci Methods, 1994). As for the behavioral analysis, the animal groups were blinded at a specified time and at least 3 researchers observed, and the results of recorded patterns were statistically processed, to analyze the pain results.

As a result, it was found that the pain-alleviating effect was higher when AAV-GAD65 and AAV-GDNF, or AAV-IL-10 and AAV-GDNF viruses were combined and co-administered as compared to individual administration of AAV-GAD65, AAV-IL-10 or AAV-GDNF virus (Fig. 9).

Experimental Example 4. Comparison of analgesic efficacies between individual administration of AAV-GAD65, AAV-IL-10 or AAV-GDNF virus and co-administration of AAV-GAD65, AAV-IL-10, and AAV-GDNF viruses

The viruses prepared in Example 2 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. AAV-GAD65, AAV-IL-10, AAV-GDNF, and AAV-GFP viruses were diluted in PBS to obtain the titers shown in Table 2. The AAV-GFP virus was administered in the same amount as other recombinant adeno-associated viruses. The GFP is a protein whose analgesic efficacy has not been reported. The viruses were mixed according to the contents shown in Table 2, and administered in an amount of 9.0×10^8 vg/5 μl per animal.

[Table 2]

Samples	Virus types and contents			
	AAV-GAD65	AAV-IL-10	AAV-GDNF	AAV-GFP

AAV-GFP	-	-	-	9.0×10^8 vg/5 μl
AAV-GAD65	9.0×10^8 vg/5 μl	-	-	-
AAV-IL-10	-	9.0×10^8 vg/5 μl	-	-
AAV-GDNF	-	-	9.0×10^8 vg/5 μl	-
AAV-GAD65 + AAV-IL-10 + AAV-GDNF	3.0×10^8 vg/5 μl	3.0×10^8 vg/5 μl	3.0×10^8 vg/5 μl	-

To the pain animal model produced by the same method as in Experimental Example 3.2., samples prepared according to the virus contents shown in Table 2 were administered. Thereafter, the *von Frey* filament test was conducted by the same method as in Experimental Example 3.3. to observe the pain responses.

As a result, it was found that the pain-alleviating effect was higher when all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses were co-administered as

compared to individual administration of AAV-GAD65, AAV-IL-10 or AAV-GDNF virus (Fig. 10).

Experimental Example 5. Comparison of analgesic efficacies between co-administration of AAV-GAD65 and AAV-GDNF, or AAV-IL-10 and AAV-GDNF viruses and co-administration of AAV-GAD65, AAV-IL-10, and AAV-GDNF viruses

The adeno-associated viruses prepared in Example 2 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. AAV-GAD65, AAV-IL-10, AAV-GDNF, and AAV-GFP viruses were diluted in PBS to obtain the titers shown in Table 3. The AAV-GFP was administered in the same amount as other recombinant adeno-associated viruses. The GFP is a protein which has no analgesic efficacy. The viruses required were mixed according to the contents shown in Table 3, and administered in an amount of 9.0×10^8 vg/5 μl per animal.

[Table 3]

Samples	Virus types and contents			
	AAV-GAD65	AAV-IL-10	AAV-GDNF	AAV-GFP
AAV-GFP	-	-	-	9.0×10^8 vg/5 μl

AAV-GAD65 + AAV-GDNF	4.5×10^8 vg/5 μl	-	4.5×10^8 vg/5 μl	-
AAV-IL-10 + AAV-GDNF	-	4.5×10^8 vg/5 μl	4.5×10^8 vg/5 μl	-
AAV-GAD65 + AAV-IL-10 + AAV-GDNF	3.0×10^8 vg/5 μl	3.0×10^8 vg/5 μl	3.0×10^8 vg/5 μl	-

To the pain animal model produced by the same method as in Experimental Example 3.2., samples prepared according to the virus contents shown in Table 3 were administered. Thereafter, the *von Frey* filament test was conducted by the same method as in Experimental Example 3.3. to observe the pain responses.

As a result, it was found that the pain-alleviating effect was higher when all of the AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses were co-administered as compared to co-administration of AAV-GAD65 and AAV-GDNF, or AAV-IL-10 and AAV-GDNF viruses (Fig. 11).

Experimental Example 6. Comparison of analgesic efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid and co-administration of pAAV-GAD65 and pAAV-GDNF, or pAAV-IL-10 and pAAV-GDNF plasmids

Experimental Example 6.1. Preparation of administration samples

The plasmids prepared in Example 1 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. The pAAV-GAD65, pAAV-IL-10, pAAV-GDNF, and pVAX1 plasmids were diluted in the Tris-EDTA buffer to obtain the concentrations shown in Table 4. The pVAX1 plasmid was administered in the same amount as the other plasmids. The pVAX1 plasmid has not been reported to have an analgesic efficacy. The required plasmids were mixed according to the contents shown in Table 4, and administered in an amount of 30 μg/50 μl per animal.

[Table 4]

Samples	Plasma types and contents			
	pAAV-GAD65	pAAV-IL-10	pAAV-GDNF	pVAX1
pVAX1	-	-	-	30 μg/50 μl
pAAV-GAD65	30 μg/50 μl	-	-	-
pAAV-IL-10	-	30 μg/50 μl	-	-
pAAV-GDNF	-	-	30 μg/50 μl	-

pAAV-GAD65 + pAAV-GDNF	15 μg/50 μl	-	15 μg/50 μl	-
pAAV-IL-10 + pAAV-GDNF	-	15 μg/50 μl	15 μg/50 μl	-

Experimental Example 6.2. Construction of neuropathic pain animal model and sample administration

150 to 200 g male SD-rats were subjected to inhalation anesthesia. And then the upper part of the calf was incised and both ends of the common peroneal nerve and tibial nerve were tied and knots were made at the interval of 0.5 to 1 cm by 7-0 suture. The regions between the knots of the two nerve bundles were cut with a scissor and the incision site was sutured. Thereafter, the rats were recovered to be awakened from the anesthesia and returned to the cage. Two weeks later, the *von Frey* filament test was conducted to examine pain induction, and then the samples prepared in Example 6.1 were administered (Decosterd, Pain, 2000).

The samples were administered by the intrathecal injection method. The pain-induced rat was subjected to inhalation anesthesia, and the spinous process was exposed by linearly incising the back of the rat at the region of lumbar spines L5. A 50 ml tube was placed under the rat to widen the space between L5 and L6, and a needle

of 27 G x 13 mm size was inserted. Thereafter, a 1 mL syringe filled with 50 μl of the sample was connected to the needle. The sample was slowly injected by slightly pressing the piston of the syringe. Thereafter, the incision was sutured and this step was finished. One day after the substance administration, the pain responses were observed using the *von Frey* filament test.

Experimental Example 6.3. Pain observation using *von Frey* filament test

The *von Frey* filament test was carried out by the same method as in Experimental Example 3.3. As a result, it was found that the pain-alleviating effect was higher when pAAV-GAD65 and pAAV-GDNF, or pAAV-IL-10 and pAAV-GDNF plasmids were co-administered as compared to individual administration of pAAV-GAD65, pAAV-IL-10 or pAAV-GDNF plasmid (Fig. 12).

Experimental Example 7. Comparison of analgesic efficacies between individual administration of pAAV-GAD65, pAAV-IL-10, or pAAV-GDNF plasmid and co-administration of pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids

The plasmids prepared in Example 1 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. The pAAV-GAD65, pAAV-IL-10, pAAV-GDNF, and pVAX1 plasmids were diluted in the Tris-EDTA buffer to

obtain the concentrations shown in Table 5. The pVAX1 plasmid was administered in the same amount as the other plasmids. The pVAX1 plasmid has not been reported to have an analgesic efficacy. The required plasmids were mixed according to the contents shown in Table 5, and administered in an amount of 30 μg/50 μl per animal.

[Table 5]

Samples	Plasma types and contents			
	pAAV-GAD65	pAAV-IL-10	pAAV-GDNF	pVAX1
pAAV-VAX1	-	-	-	30 μg/50 μl
pAAV-GAD65	30 μg/50 μl	-	-	-
pAAV-IL-10	-	30 μg/50 μl	-	-
pAAV-GDNF	-	-	30 μg/50 μl	-
pAAV-GAD65 + pAAV-IL-10 + pAAV-GDNF	10 μg/50 μl	10 μg/50 μl	10 μg/50 μl	-

To the pain animal model produced by the same method as in Experimental Example 6.2., samples prepared according to the virus contents shown in Table 5 were

administered. Thereafter, the *von Frey* filament test was conducted by the same method as in Experimental Example 3.3. to observe the pain responses.

As a result, it was found that the pain-alleviating effect was higher when all of the pAAV-GAD65, pAAV5-IL-10, and pAAV5-GDNF plasmids were co-administered as compared to individual administration of pAAV-GAD65, pAAV-IL-10 or pAAV-GDNF plasmid (Fig. 13).

Experimental Example 8. Comparison of the analgesic efficacies between co-administration of pAAV-GAD65 and pAAV-GDNF, or pAAV-IL-10 and pAAV-GDNF plasmids and co-administration of pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids

The plasmids prepared in Example 1 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. The pAAV-GAD65, pAAV-IL-10, pAAV-GDNF, and pVAX1 plasmids were diluted in the Tris-EDTA buffer to obtain the concentrations shown in Table 6. The pVAX1 plasmid was administered in the same concentration and amount as the other plasmids. The pVAX1 plasmid has not been reported to have an analgesic efficacy. The required plasmids were mixed according to the contents shown in Table 6, and administered in an amount of 30 μg/50 μl per animal.

[Table 6]

Samples	Plasma types and contents			
	pAAV-GAD65	pAAV-IL-10	pAAV-GDNF	pVAX1
pVAX1	-	-	-	30 μg/50 μl
pAAV-GAD65 + pAAV-GDNF	15 μg/50 μl	-	15 μg/50 μl	-
pAAV-IL-10 + pAAV-GDNF	-	15 μg/50 μl	15 μg/50 μl	-
pAAV-GAD65 + pAAV-IL-10 + pAAV-GDNF	10 μg/50 μl	10 μg/50 μl	10 μg/50 μl	-

To the pain animal model produced by the same method as in Experimental Example 6.2., samples prepared according to the plasma contents shown in Table 6 were administered. Thereafter, the *von Frey* filament test was conducted by the same method as in Experimental Example 6.3. to observe the pain responses.

As a result, it was found that the pain-alleviating effect was higher when all of the pAAV-GAD65, pAAV-IL-10 and pAAV-GDNF plasmids were co-administered as compared to co-administration of pAAV-GAD65 and pAAV-GDNF or pAAV-IL-10 and pAAV-GDNF plasmids (Fig. 14).

Experimental Example 9. Comparison of analgesic efficacies of co-administration of AAV-GAD65-modi and AAV-GDNF-IL-10 viruses and co-administration of AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses

The adeno-associated viruses prepared in Example 2 were used for the test. 30 minutes prior to the animal administration experiment, the reagents stored at -80°C were thawed at room temperature and prepared by mixing by a vortexer. AAV-GAD65-modi, AAV-GDNF-IL-10, AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses were diluted in PBS to obtain the titers shown in Table 7. The viruses required were mixed according to the contents shown in Table 7, and administered in an amount of 1.0×10^9 vg/5 μl or 1.5×10^9 vg/5 μl per animal.

[Table 7]

Samples	Virus types and contents				
	AAV-GAD65-modi	AAV-GDNF-IL-10	AAV-GAD65	AAV-IL-10	AAV-GDNF
Control	-	-	-	-	-

AAV-GAD65-modi + AAV-GDNF-IL-10	5.0×10^8 vg/5 μl	5.0×10^8 vg/5 μl	-	-	-
AAV-GAD65 + AAV-IL-10 + AAV-GDNF	-	-	5.0×10^8 vg/5 μl	5.0×10^8 vg/5 μl	5.0×10^8 vg/5 μl

To the pain animal model produced by the same method as in Experimental Example 3.2., samples prepared according to the virus contents shown in Table 7 were administered. Thereafter, the *von Frey* filament test was conducted by the same method as in Experimental Example 3.3. to observe the pain responses.

As a result, it was found that the pain-alleviating effect was higher when AAV-GAD65-modi and AAV-GDNF-IL-10 viruses were co-administered as compared to co-administration of all of AAV-GAD65, AAV-IL-10 and AAV-GDNF viruses (Fig. 15).

Claims:

1. A method of alleviating or treating pain, comprising administering a pharmaceutical composition comprising a gene encoding glutamate decarboxylase (GAD) and a gene encoding a glial cell-derived neurotrophic factor (GDNF); a gene encoding interleukin-10 (IL-10) and a gene encoding GDNF; or a gene encoding GAD, a gene encoding IL-10, and a gene encoding GDNF, wherein the gene is in a form of being operably contained in a vector.

2. The method of claim 1, wherein the vector is at least one viral vector selected from the group consisting of adenovirus, adeno-associated virus, herpes simplex virus, lentivirus, retrovirus, and poxvirus.

3. The method of claim 1, wherein the vector is at least one non-viral vector selected from the group consisting of a plasmid, a liposome, a cationic polymer, a micelle, an emulsion, and solid lipid nanoparticles.

4. The method of any of claims 1-3, wherein the GAD is GAD65 or GAD67.

5. The method of any of claims 1-4, wherein the gene encoding GAD is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 1, SEQ ID NO: 4, SEQ ID NO: 32, SEQ ID NO: 34 or SEQ ID NO: 36.

6. The method of any of claims 1-5, wherein the gene encoding GAD is the nucleotide sequence represented by SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 33, SEQ ID NO: 35 or SEQ ID NO: 37.

7. The method of any of claims 1-6, wherein the gene encoding IL-10 is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 6, SEQ ID NO: 9, SEQ ID NO: 38, SEQ ID NO: 40 or SEQ ID NO: 42.

8. The method of any of claims 1-7, wherein the gene encoding IL-10 is the nucleotide sequence represented by SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 14, SEQ ID NO: 39, SEQ ID NO: 41 or SEQ ID NO: 43.

9. The method of any of claims 1-8, wherein the gene encoding GDNF is the nucleotide sequence encoding the amino acid sequence represented by SEQ ID NO: 11, SEQ ID NO: 44, SEQ ID NO: 46, or SEQ ID NO: 48.

10. The method of any of claims 1-9, wherein the gene encoding GDNF is the nucleotide sequence represented by SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 45, SEQ ID NO: 47 or SEQ ID NO: 49.

2017351914 07 Aug 2020

11. The method of any of claims 1-10, wherein the pain is nociceptive pain, psychogenic pain, inflammatory pain, pathological pain, neuropathic pain, cancer pain, postoperative pain, trigeminal neuralgia pain, idiopathic pain, diabetic neuropathic pain, or migraine.

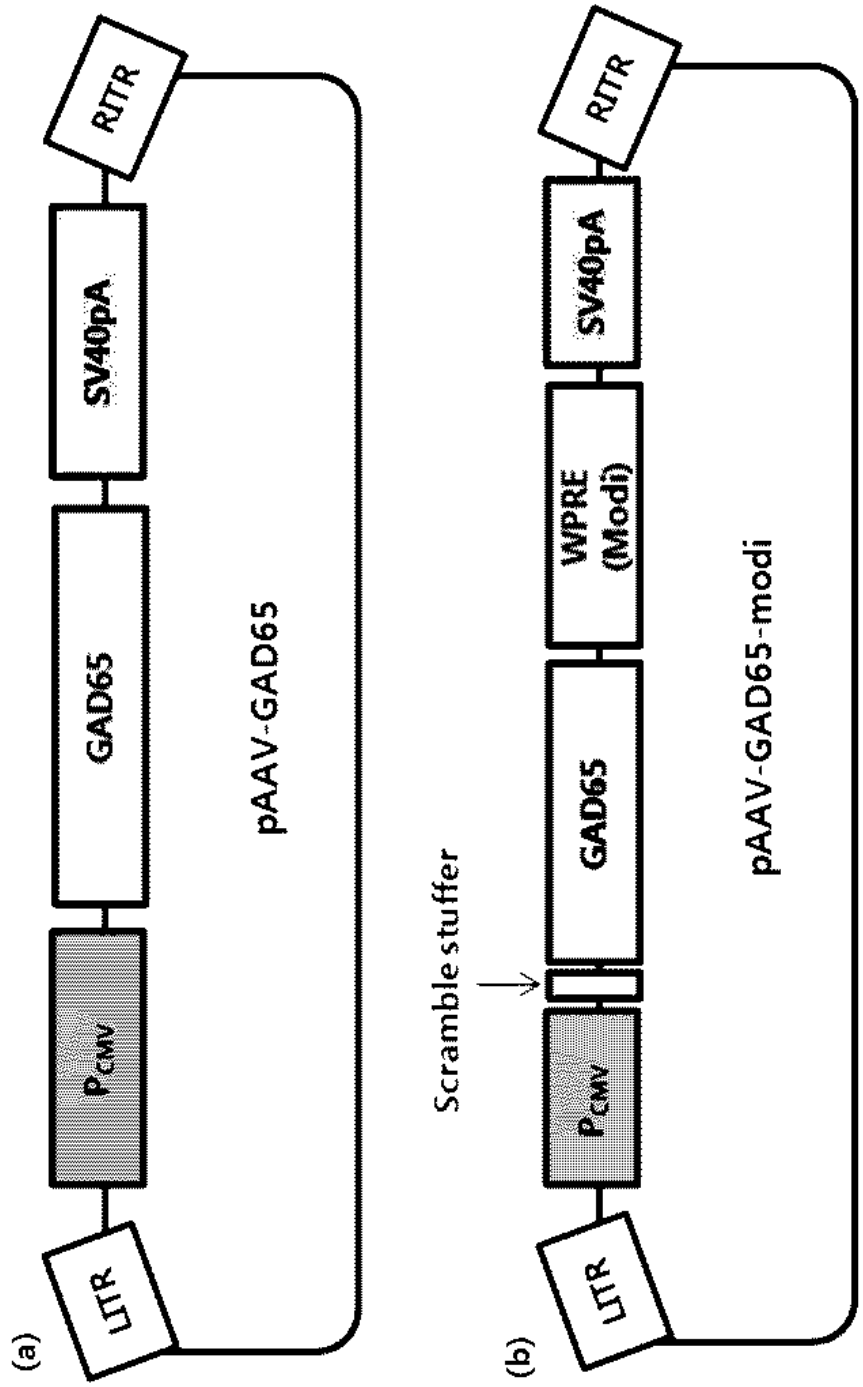
12. The method of any of claims 1-11, wherein the pharmaceutical composition further comprises a physiologically acceptable carrier.

13. The method of any of claims 1-12, wherein the pharmaceutical composition is an injection.

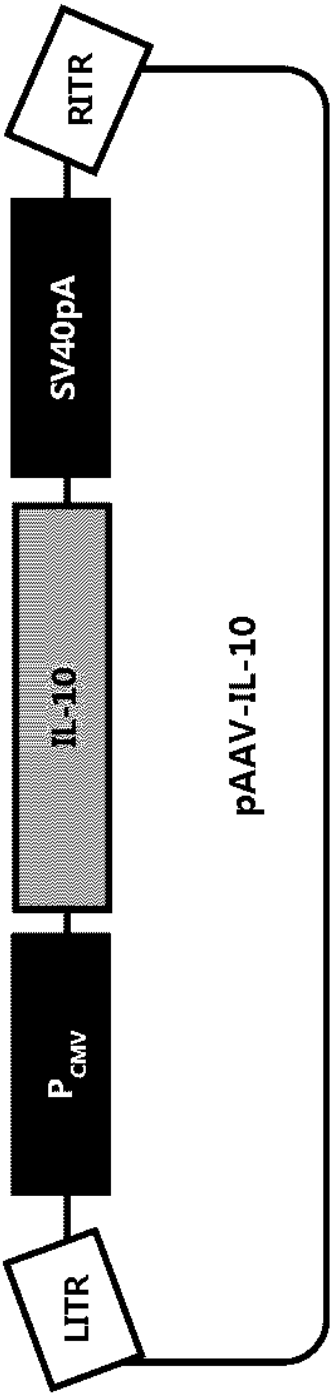
14. The method of any of claims 1-13, wherein the administering is administration via epidural injection or intrathecal injection.

15. Use of a gene encoding glutamate decarboxylase (GAD) and a gene encoding a glial cell-derived neurotrophic factor (GDNF); a gene encoding interleukin-10 (IL-10) and a gene encoding GDNF; or a gene encoding GAD, a gene encoding IL-10, and a gene encoding GDNF, in the manufacture of a medicament for alleviating or treating pain.

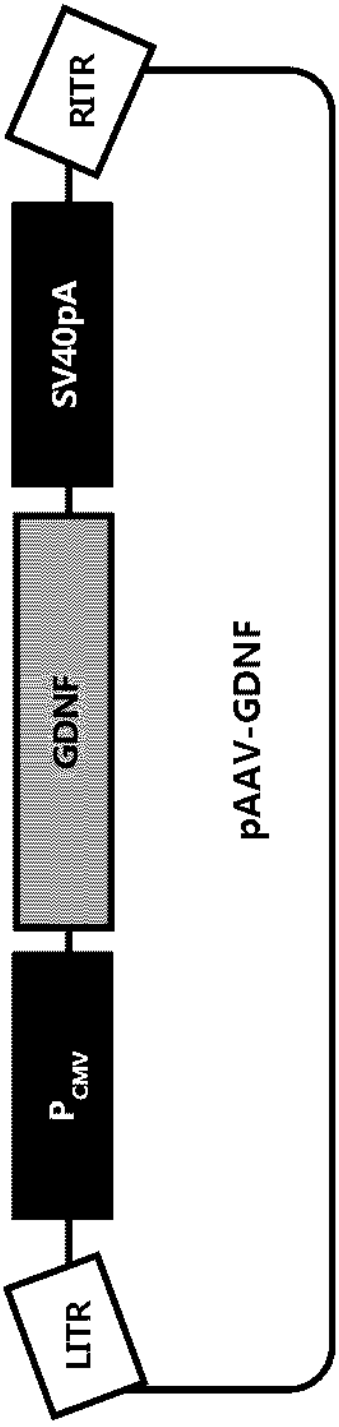
[Fig.1]


(a)
LITR
P_{CMV}
GAD65
SV40pA
RITR
pAAV-GAD65
(b)
LITR
P_{CMV}
Scramble stuffer
GAD65
WPRE (Modi)
SV40pA
RITR
pAAV-GAD65-modi

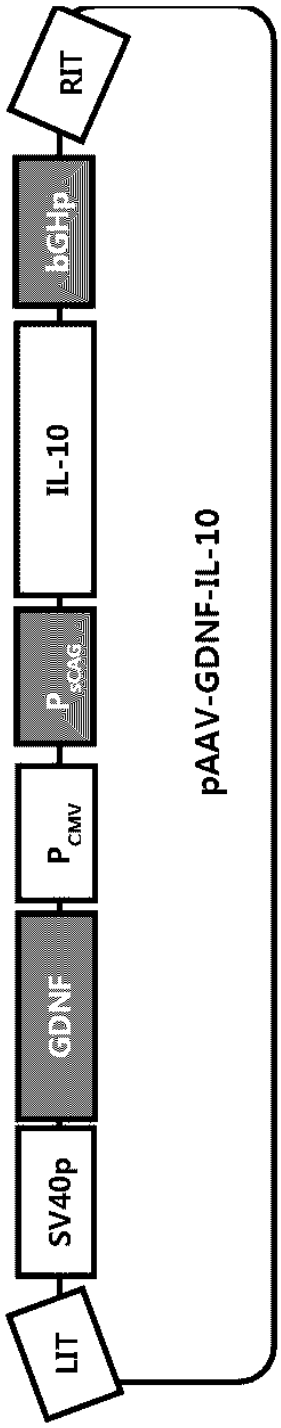
[Fig. 2]



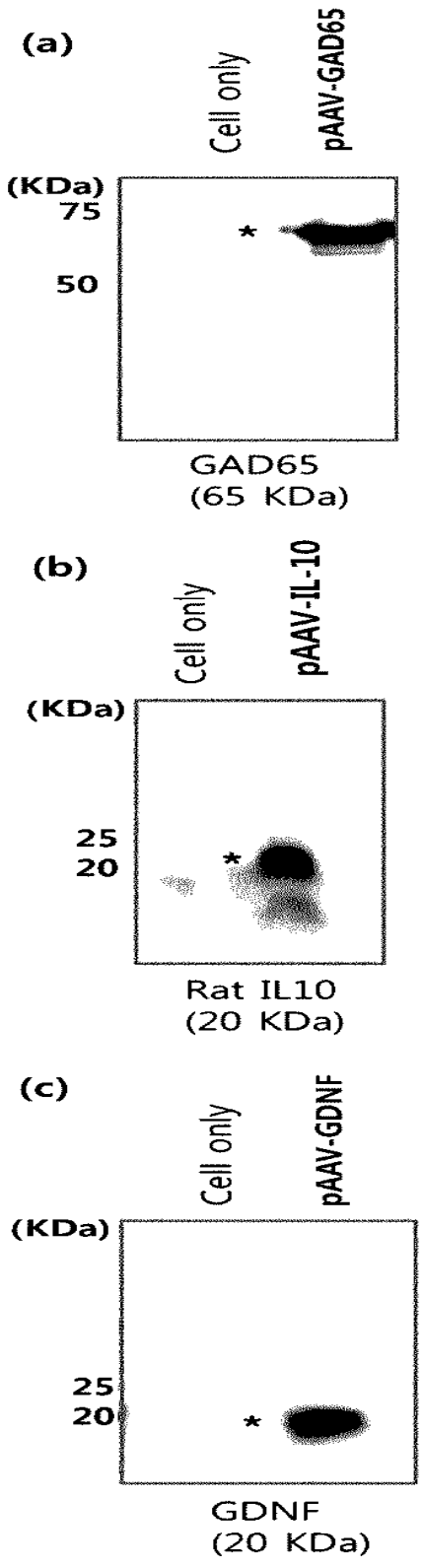
[Fig. 3]



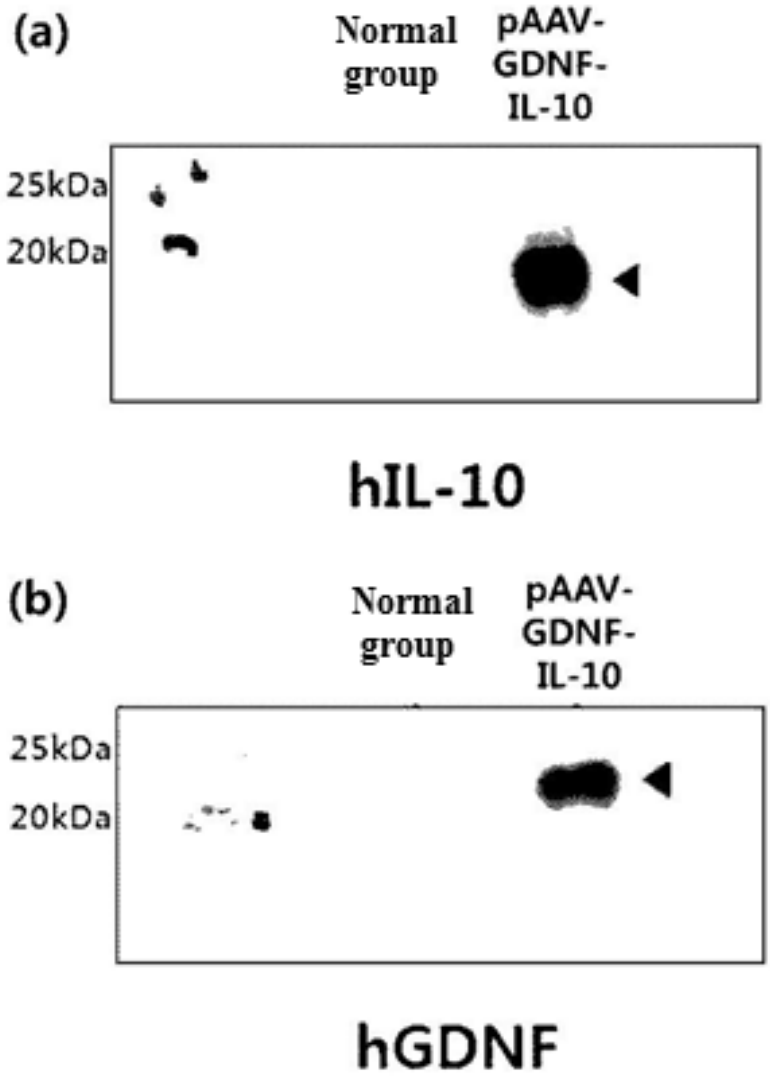
[Fig. 4]



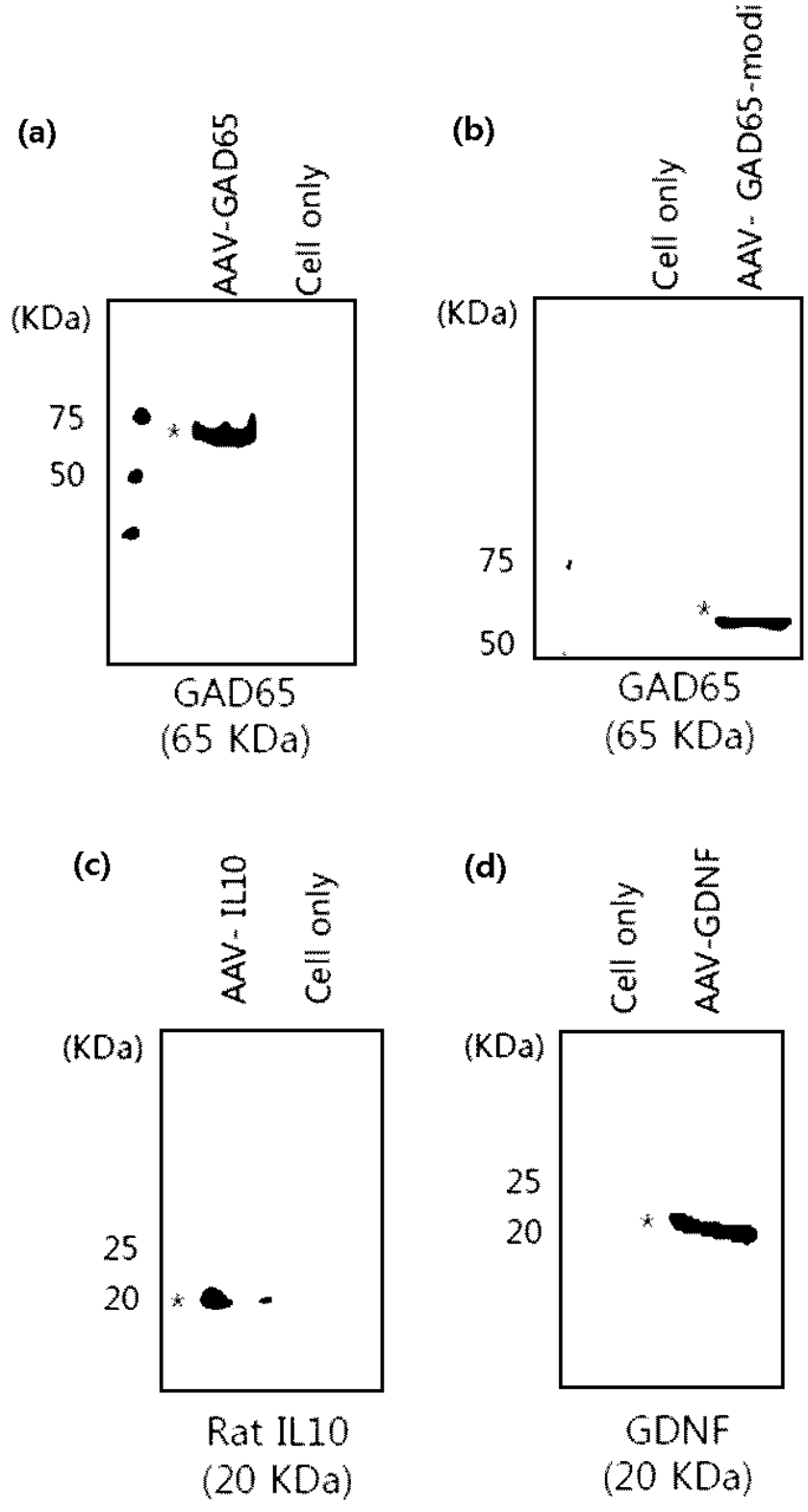
[Fig. 5]


(a)
Cell only
pAAV-GAD65
(KDa)
75
50
*
GAD65
(65 KDa)
(b)
Cell only
pAAV-IL-10
(KDa)
25
20
*
Rat IL10
(20 KDa)
(c)
Cell only
pAAV-GDNF
(KDa)
25
20
*
GDNF
(20 KDa)

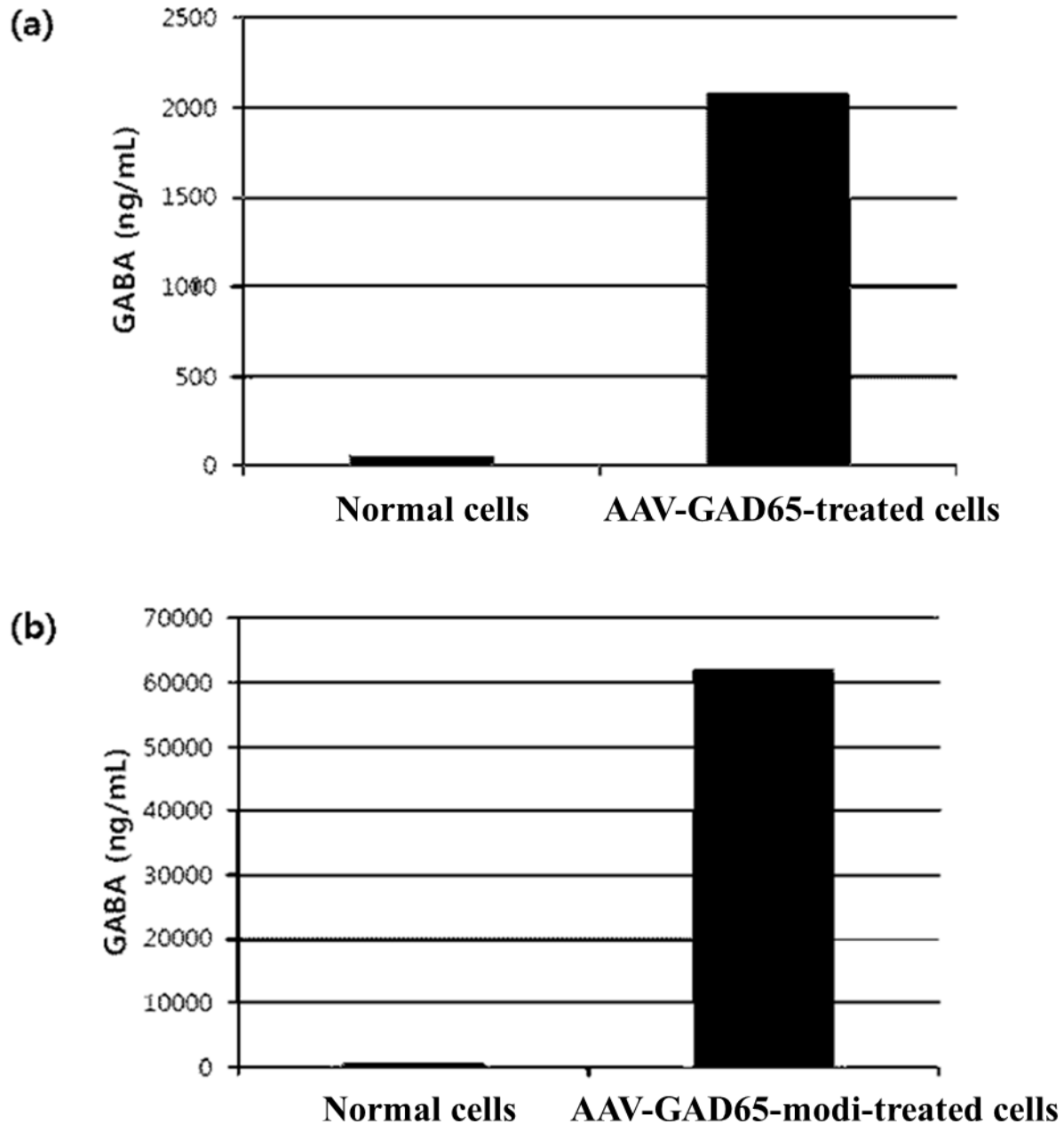
[Fig. 6]


(a)
Normal group
pAAV-GDNF-IL-10
25kDa
20kDa
hIL-10
(b)
Normal group
pAAV-GDNF-IL-10
25kDa
20kDa
hGDNF

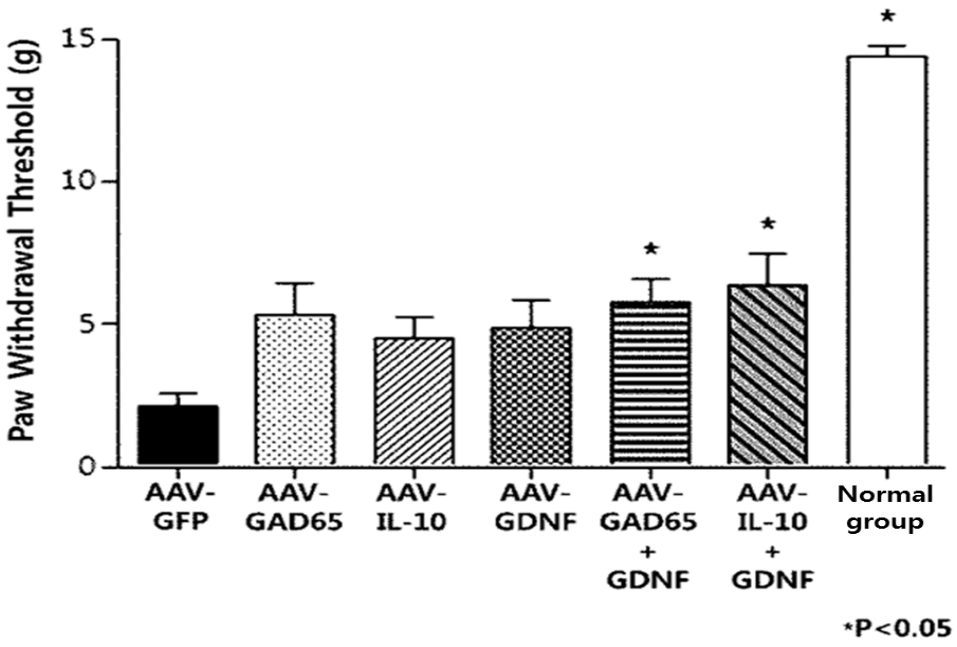
[Fig. 7]


(a)
AAV-GAD65
Cell only
(KDa)
75
50
GAD65
(65 KDa)
(b)
Cell only
AAV- GAD65-modi
(KDa)
75
50
GAD65
(65 KDa)
(c)
AAV- IL10
Cell only
(KDa)
25
20
Rat IL10
(20 KDa)
(d)
Cell only
AAV-GDNF
(KDa)
25
20
GDNF
(20 KDa)

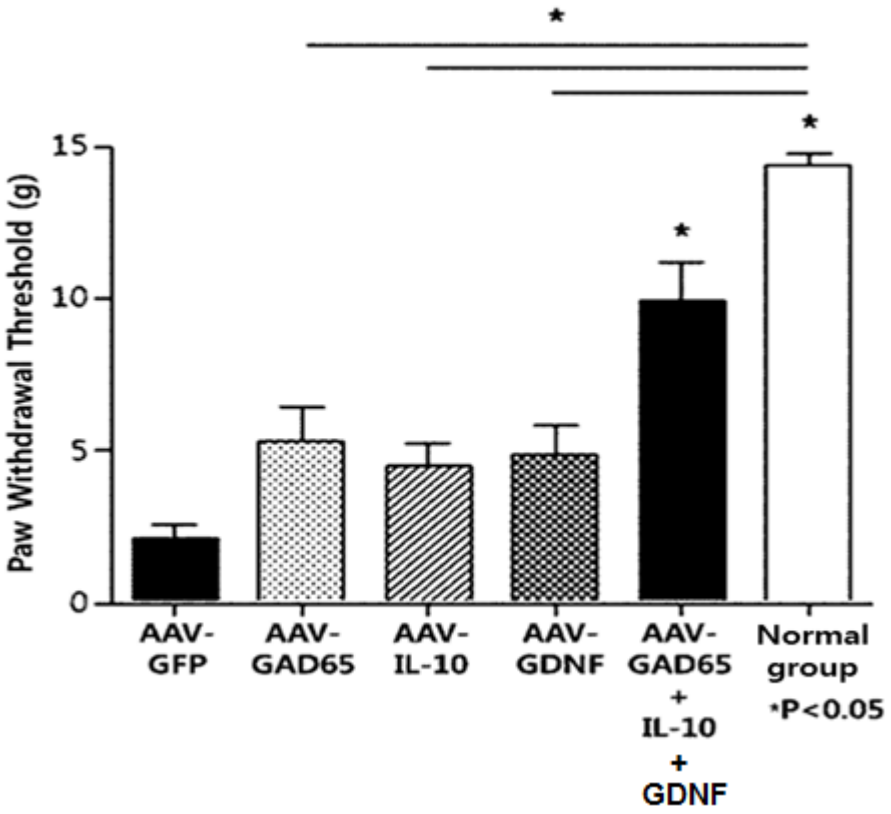
[Fig. 8]


(a)
2500
2000
1500
1000
500
0
GABA (ng/mL)
Normal cells
AAV-GAD65-treated cells
(b)
70000
60000
50000
40000
30000
20000
10000
0
GABA (ng/mL)
Normal cells
AAV-GAD65-modi-treated cells

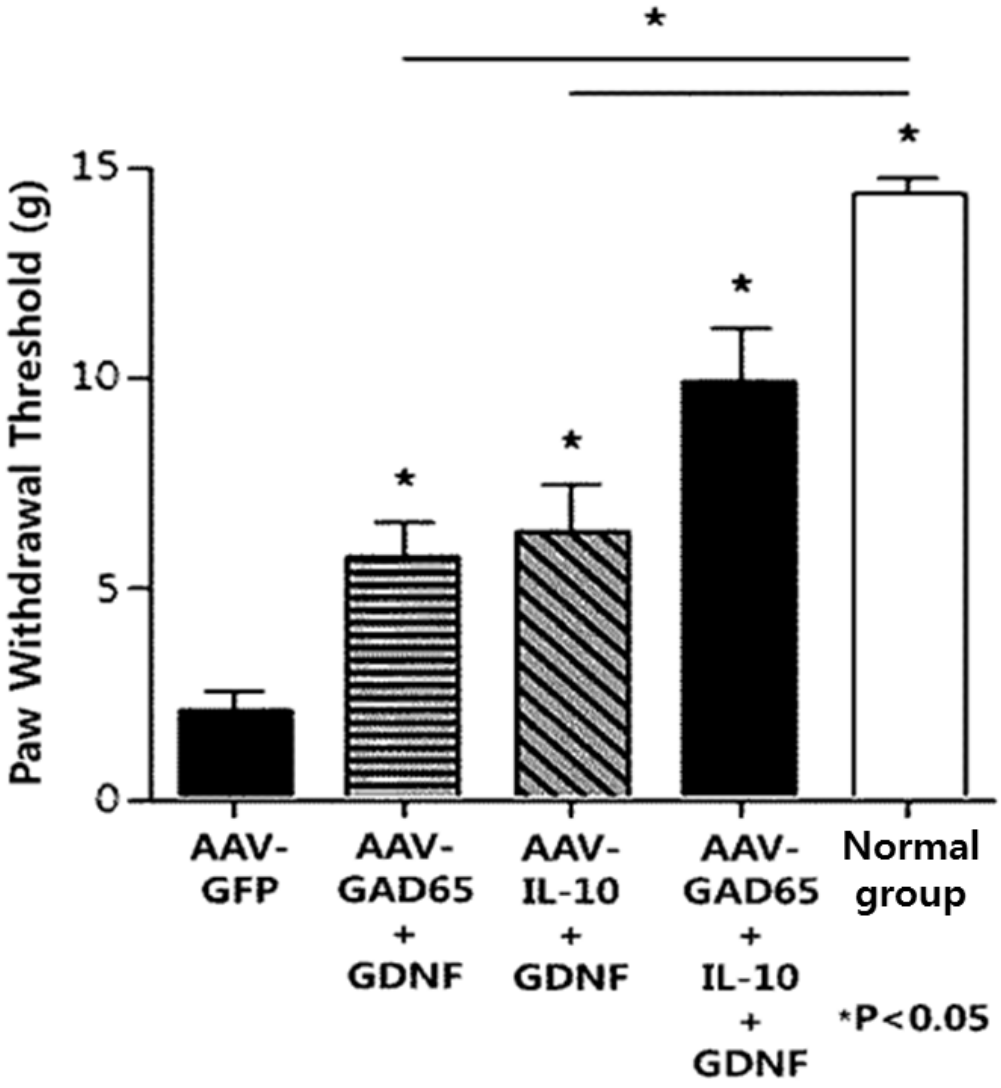
[Fig. 9]



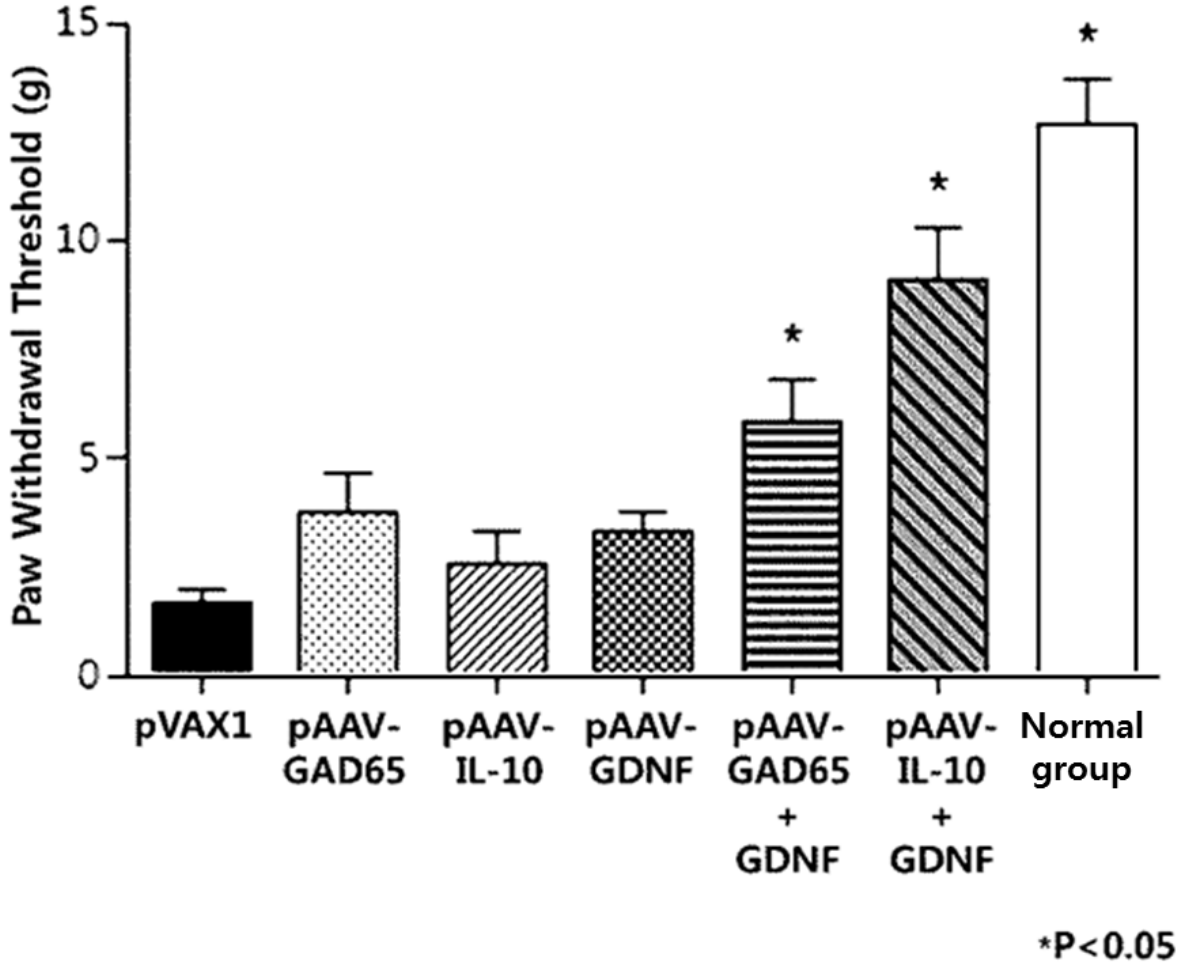
[Fig. 10]



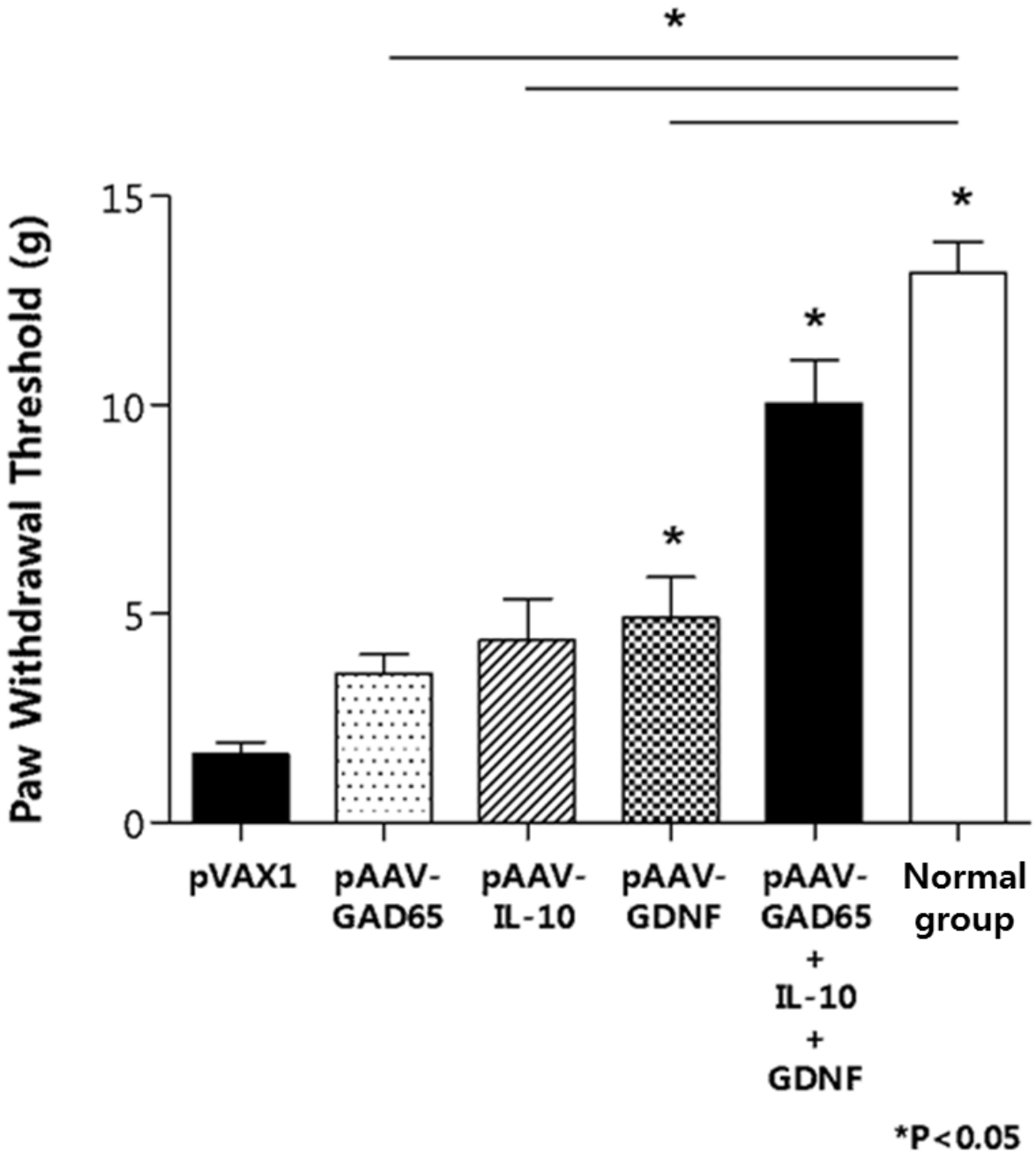
[Fig. 11]


Paw Withdrawal Threshold (g)
15
10
5
0
*
*
*
*
*
AAV-GFP
AAV-GAD65 + GDNF
AAV-IL-10 + GDNF
AAV-GAD65 + IL-10 + GDNF
Normal group
*P<0.05

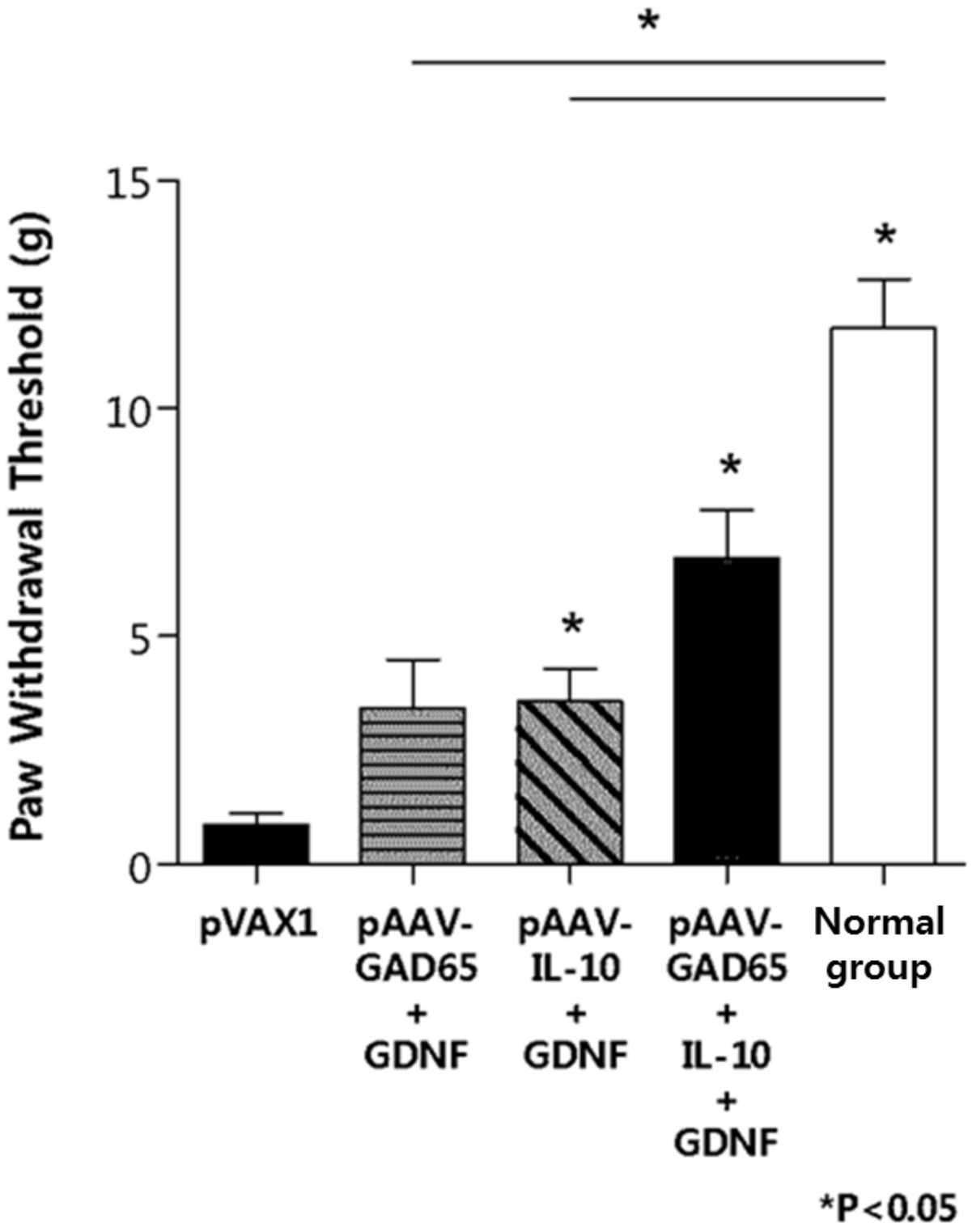
[Fig. 12]


Paw Withdrawal Threshold (g)
15
10
5
0
*
*
*
*
pVAX1
pAAV-GAD65
pAAV-IL-10
pAAV-GDNF
pAAV-GAD65 + GDNF
pAAV-IL-10 + GDNF
Normal group
*P<0.05

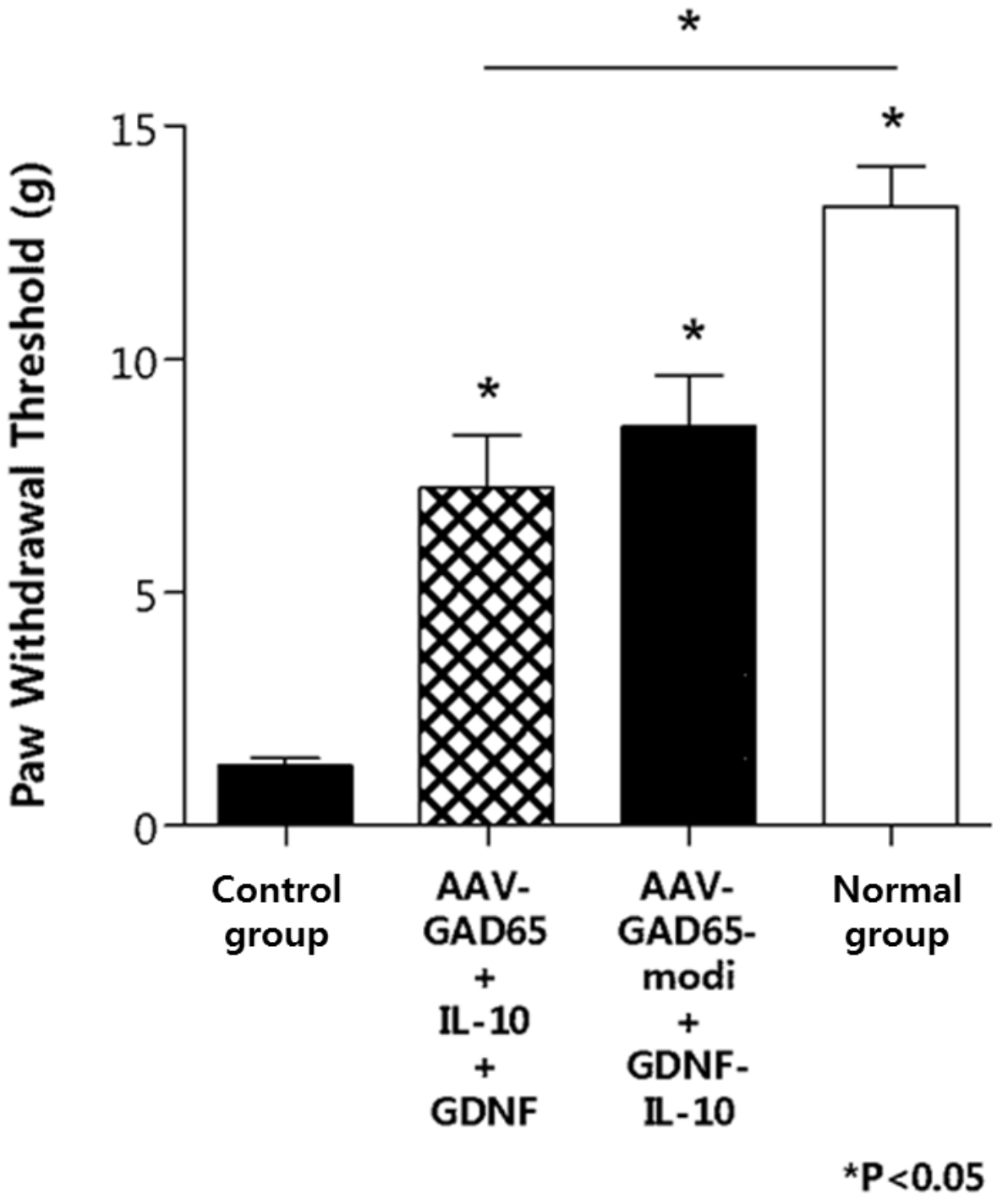
[Fig. 13]


*
*
*
*
*
15
10
5
0
Paw Withdrawal Threshold (g)
pVAX1
pAAV-GAD65
pAAV-IL-10
pAAV-GDNF
pAAV-GAD65 + IL-10 + GDNF
Normal group
*P<0.05

[Fig. 14]


*
*
*
*
*
15
10
5
0
Paw Withdrawal Threshold (g)
pVAX1
pAAV-GAD65 + GDNF
pAAV-IL-10 + GDNF
pAAV-GAD65 + IL-10 + GDNF
Normal group
*P<0.05

[Fig. 15]


*
*
*
*
15
10
5
0
Paw Withdrawal Threshold (g)
Control group
AAV-GAD65 + IL-10 + GDNF
AAV-GAD65-modi + GDNF-IL-10
Normal group
*P<0.05

<110> KOLON LIFE SCIENCE, INC.

<120> COMPOSITION FOR ALLEVIATING OR TREATING PAIN

<130> PCB706093KLS/PCT

<150> KR 2016/0143519
<151> 2016-10-31

<160> 49

<170> KopatentIn 2.0

<210> 1
<211> 585
<212> PRT
<213> Homo sapiens

<400> 1

```
Met Ala Ser Pro Gly Ser Gly Phe Trp Ser Phe Gly Ser Glu Asp Gly
  1               5                  10                  15

Ser Gly Asp Ser Glu Asn Pro Gly Thr Ala Arg Ala Trp Cys Gln Val
             20                  25                  30

Ala Gln Lys Phe Thr Gly Gly Ile Gly Asn Lys Leu Cys Ala Leu Leu
         35                  40                  45

Tyr Gly Asp Ala Glu Lys Pro Ala Glu Ser Gly Gly Ser Gln Pro Pro
     50                  55                  60

Arg Ala Ala Ala Arg Lys Ala Ala Cys Ala Cys Asp Gln Lys Pro Cys
 65                  70                  75                  80

Ser Cys Ser Lys Val Asp Val Asn Tyr Ala Phe Leu His Ala Thr Asp
                 85                  90                  95

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

Asp Val Met Asn Ile Leu Leu Gln Tyr Val Val Lys Ser Phe Asp Arg
        115                 120                 125

Ser Thr Lys Val Ile Asp Phe His Tyr Pro Asn Glu Leu Leu Gln Glu
    130                 135                 140

Tyr Asn Trp Glu Leu Ala Asp Gln Pro Gln Asn Leu Glu Glu Ile Leu
145                 150                 155                 160

Met His Cys Gln Thr Thr Leu Lys Tyr Ala Ile Lys Thr Gly His Pro
                165                 170                 175
```

```
Arg Tyr Phe Asn Gln Leu Ser Thr Gly Leu Asp Met Val Gly Leu Ala
            180                 185                 190

Ala Asp Trp Leu Thr Ser Thr Ala Asn Thr Asn Met Phe Thr Tyr Glu
        195                 200                 205

Ile Ala Pro Val Phe Val Leu Leu Glu Tyr Val Thr Leu Lys Lys Met
    210                 215                 220

Arg Glu Ile Ile Gly Trp Pro Gly Gly Ser Gly Asp Gly Ile Phe Ser
225                 230                 235                 240

Pro Gly Gly Ala Ile Ser Asn Met Tyr Ala Met Met Ile Ala Arg Phe
                245                 250                 255

Lys Met Phe Pro Glu Val Lys Glu Lys Gly Met Ala Ala Leu Pro Arg
            260                 265                 270

Leu Ile Ala Phe Thr Ser Glu His Ser His Phe Ser Leu Lys Lys Gly
        275                 280                 285

Ala Ala Ala Leu Gly Ile Gly Thr Asp Ser Val Ile Leu Ile Lys Cys
    290                 295                 300

Asp Glu Arg Gly Lys Met Ile Pro Ser Asp Leu Glu Arg Arg Ile Leu
305                 310                 315                 320

Glu Ala Lys Gln Lys Gly Phe Val Pro Phe Leu Val Ser Ala Thr Ala
                325                 330                 335

Gly Thr Thr Val Tyr Gly Ala Phe Asp Pro Leu Leu Ala Val Ala Asp
            340                 345                 350

Ile Cys Lys Lys Tyr Lys Ile Trp Met His Val Asp Ala Ala Trp Gly
        355                 360                 365

Gly Gly Leu Leu Met Ser Arg Lys His Lys Trp Lys Leu Ser Gly Val
    370                 375                 380

Glu Arg Ala Asn Ser Val Thr Trp Asn Pro His Lys Met Met Gly Val
385                 390                 395                 400

Pro Leu Gln Cys Ser Ala Leu Leu Val Arg Glu Glu Gly Leu Met Gln
                405                 410                 415

Asn Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His
            420                 425                 430

Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg
        435                 440                 445

His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr
```

```
    450                 455                 460

Thr Gly Phe Glu Ala His Val Asp Lys Cys Leu Glu Leu Ala Glu Tyr
465                 470                 475                 480

Leu Tyr Asn Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp
                485                 490                 495

Gly Lys Pro Gln His Thr Asn Val Cys Phe Trp Tyr Ile Pro Pro Ser
            500                 505                 510

Leu Arg Thr Leu Glu Asp Asn Glu Glu Arg Met Ser Arg Leu Ser Lys
        515                 520                 525

Val Ala Pro Val Ile Lys Ala Arg Met Met Glu Tyr Gly Thr Thr Met
    530                 535                 540

Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val
545                 550                 555                 560

Ile Ser Asn Pro Ala Ala Thr His Gln Asp Ile Asp Phe Leu Ile Glu
                565                 570                 575

Glu Ile Glu Arg Leu Gly Gln Asp Leu
            580                 585
```

<210> 2
<211> 2824
<212> DNA
<213> Homo sapiens

<400> 2

```
gcggccgccc gcacttcccg cctctggctc gcccgaggac gcgctggcac gcctcccacc         60

ccctcactct gactccagct ggcgtgcatg gtctgcctcg catcctcacg actcagctcc        120

ctccctctct cgtgtttttt tcctccgccg ccccctcatt catccccact gggctccctt        180

tccctcaaat gctctggggc tctccgcgct ttcctgagtc cgggctccga ggacccttag        240

gtagtcccgg tctcttttaa agctccccgg cttccaaagg gttgccacgt ccctaaaccc        300

tgtctccagc tcgcatacac acacgcacag acacgcacgt tttctgttcc tgcgtgacac        360

ccgccctcgc cgctcggccc cgccggtccc cgcgcggtgc cctcctcccg ccacacgggc        420

acgcacgcgc gcgcagggcc aagcccgagg cagctcgccc gcagctcgca ctcgcaggcg        480

acctgctcca gtctccaaag ccgatggcat ctccgggctc tggcttttgg tctttcgggt        540

cggaagatgg ctctggggat tccgagaatc ccggcacagc gcgagcctgg tgccaagtgg        600
```

ctcagaagtt cacgggcggc atcggaaaca aactgtgcgc cctgctctac ggagacgccg 660

agaagccggc ggagagcggc gggagccaac ccccgcgggc cgccgcccgg aaggccgcct 720

gcgcctgcga ccagaagccc tgcagctgct ccaaagtgga tgtcaactac gcgtttctcc 780

atgcaacaga cctgctgccg gcgtgtgatg gagaaaggcc cactttggcg tttctgcaag 840

atgttatgaa cattttactt cagtatgtgg tgaaaagttt cgatagatca accaaagtga 900

ttgatttcca ttatcctaat gagcttctcc aagaatataa ttgggaattg gcagaccaac 960

cacaaaattt ggaggaaatt ttgatgcatt gccaaacaac tctaaaatat gcaattaaaa 1020

cagggcatcc tagatacttc aatcaacttt ctactggttt ggatatggtt ggattagcag 1080

cagactggct gacatcaaca gcaaatacta acatgttcac ctatgaaatt gctccagtat 1140

ttgtgctttt ggaatatgtc acactaaaga aaatgagaga aatcattggc tggccagggg 1200

gctctggcga tgggatattt tctcccggtg gcgccatatc taacatgtat gccatgatga 1260

tcgcacgctt taagatgttc ccagaagtca aggagaaagg aatggctgct cttcccaggc 1320

tcattgcctt cacgtctgaa catagtcatt tttctctcaa gaagggagct gcagccttag 1380

ggattggaac agacagcgtg attctgatta aatgtgatga gagagggaaa atgattccat 1440

ctgatcttga aagaaggatt cttgaagcca aacagaaagg gtttgttcct ttcctcgtga 1500

gtgccacagc tggaaccacc gtgtacggag catttgaccc cctcttagct gtcgctgaca 1560

tttgcaaaaa gtataagatc tggatgcatg tggatgcagc ttggggtggg ggattactga 1620

tgtcccgaaa acacaagtgg aaactgagtg gcgtggagag ggccaactct gtgacgtgga 1680

atccacacaa gatgatggga gtccctttgc agtgctctgc tctcctggtt agagaagagg 1740

gattgatgca gaattgcaac caaatgcatg cctcctacct ctttcagcaa gataaacatt 1800

atgacctgtc ctatgacact ggagacaagg ccttacagtg cggacgccac gttgatgttt 1860

ttaaactatg gctgatgtgg agggcaaagg ggactaccgg gtttgaagcg catgttgata 1920

aatgtttgga gttggcagag tatttataca acatcataaa aaaccgagaa ggatatgaga 1980

tggtgtttga tgggaagcct cagcacacaa atgtctgctt ctggtacatt cctccaagct 2040

tgcgtactct ggaagacaat gaagagagaa tgagtcgcct ctcgaaggtg gctccagtga 2100

ttaaagccag aatgatggag tatggaacca caatggtcag ctaccaaccc ttgggagaca 2160

aggtcaattt cttccgcatg gtcatctcaa acccagcggc aactcaccaa gacattgact 2220

tcctgattga agaaatagaa cgccttggac aagatttata ataaccttgc tcaccaagct 2280

gttccacttc tctagagaac atgccctcag ctaagccccc tactgagaaa cttcctttga 2340

gaattgtgcg acttcacaaa atgcaaggtg aacaccactt tgtctctgag aacagacgtt 2400

accaattatg gagtgtcacc agctgccaaa atcgtaggtg ttggctctgc tggtcactgg 2460

agtagttgct actcttcaga atatggacaa agaaggcaca ggtgtaaata tagtagcagg 2520

atgaggaacc tcaaactggg tatcattttg cacgtgctct tctgttctca aatgctaaat 2580

gcaaacactg tgtatttatt agttaggtgt gccaaactac cgttcccaaa ttggtgtttc 2640

tgaatgacat caacattccc ccaacattac tccattacta aagacagaaa aaaataaaaa 2700

cataaaatat acaaacatgt ggcaacctgt tcttcctacc aaatataaac ttgtgtatga 2760

tccaagtatt ttatctgtgt tgtctctcta aacccaaata aatgtgtaaa tgtggacaca 2820

tctc 2824

<210> 3
<211> 1758
<212> DNA
<213> Artificial Sequence

<220>
<223> Optimized human GAD65

<400> 3
atggcatctc cgggctccgg cttttggtcc ttcgggtcgg aagatggctc aggggattcc 60

gagaatcccg gcacagcgcg ggcctggtgt caagtggctc agaagttcac gggcggcatc 120

ggaaacaaac tgtgtgccct gctctacggc gacgccgaga agcccgcaga gagcggcggg 180

agccaacccc cgcgggccgc cgcccggaag gccgcctgcg cctgtgacca gaagccctgc 240

tcatgcagca aggtagatgt caactacgcg tttctccatg ccacagatct gctgccggct 300

tgcgacggtg aaaggcccac tttggccttt ctgcaggatg ttatgaacat tctgctgcag 360

tacgtggtga aaagtttcga ccggtcaacc aaagtgatcg actttcacta tcctaatgaa 420

cttctccagg agtacaattg ggagctggct gaccagccac agaacctgga ggaaatcttg 480

atgcattgcc aaactactct aaaatatgca attaaaacag gccatcctag atacttcaac 540

cagctttcta ccggtttgga tatggtgggg ctggcagccg actggctgac atccaccgca 600

aataccaaca tgttcaccta tgagatcgct cctgtcttcg tgcttttgga atacgtcacc 660

ctaaagaaga tgcgtgaaat cattggctgg ccaggaggct ctggtgatgg tatattttct 720

cccggcggcg cgatctctaa catgtatgcc atgatgatcg cacgctttaa gatgttccca 780

gaagtcaagg agaaaggaat ggctgctctt cccaggctca ttgccttcac gagtgaacac 840

agtcactttt ccctcaagaa gggggctgcc gccttaggga tcggaacaga cagcgtgatt 900

ctgataaagt gcgacgagag agggaaaatg attccatctg atcttgagag aaggattctt 960

gaagccaaac agaaagggtt tgtccctttc ctcgtgagtg ccacagctgg aaccaccgtg 1020

tacggcgcat ttgaccccct cttagctgtc gcggatatat gtaagaagta taagatctgg 1080

atgcacgtgg atgctgcttg gggtggggga ttactgatgt ccaggaaaca caagtggaaa 1140

ctgtctggcg tggagcgcgc caacagcgtg acgtggaatc cacacaaaat gatgggagtc 1200

cctttgcagt gctctgctct cctggttcga gaagagggac tgatgcagaa ttgcaaccaa 1260

atgcatgcct cctacctctt tcagcaggat aaacattatg acctgtctta cgacactggt 1320

gacaaggccc tgcagtgtgg gcgccacgtt gatgtattca agctatggct gatgtggagg 1380

gcaaagggga ctaccggttt tgaagcccat gttgacaaat gtctggagtt ggcagagtat 1440

ttatacaata tcataaaaaa ccgagaagga tatgagatgg tgtttgatgg caagcctcag 1500

cacacaaatg tctgcttctg gtacatccct cccagcctac gtactctgga ggacaacgaa 1560

gagagaatga gtcgcctctc gaaggtggct ccagtgatta aagccagaat gatggagtat 1620

ggaaccacaa tggtcagcta ccaacccttg ggggacaagg taaatttctt ccgcatggtc 1680

atctcaaacc cagcggcaac tcaccaagac attgatttcc tgattgaaga gatcgagcgg 1740

ctcggccagg atctgtga 1758

<210> 4
<211> 594
<212> PRT
<213> Homo sapiens

<400> 4
Met Ala Ser Ser Thr Pro Ser Ser Ser Ala Thr Ser Ser Asn Ala Gly

```
  1               5                  10                  15

Ala Asp Pro Asn Thr Thr Asn Leu Arg Pro Thr Thr Tyr Asp Thr Trp
             20                  25                  30

Cys Gly Val Ala His Gly Cys Thr Arg Lys Leu Gly Leu Lys Ile Cys
         35                  40                  45

Gly Phe Leu Gln Arg Thr Asn Ser Leu Glu Glu Lys Ser Arg Leu Val
     50                  55                  60

Ser Ala Phe Lys Glu Arg Gln Ser Ser Lys Asn Leu Leu Ser Cys Glu
 65                  70                  75                  80

Asn Ser Asp Arg Asp Ala Arg Phe Arg Arg Thr Glu Thr Asp Phe Ser
                 85                  90                  95

Asn Leu Phe Ala Arg Asp Leu Leu Pro Ala Lys Asn Gly Glu Glu Gln
            100                 105                 110

Thr Val Gln Phe Leu Leu Glu Val Val Asp Ile Leu Leu Asn Tyr Val
        115                 120                 125

Arg Lys Thr Phe Asp Arg Ser Thr Lys Val Leu Asp Phe His His Pro
    130                 135                 140

His Gln Leu Leu Glu Gly Met Glu Gly Phe Asn Leu Glu Leu Ser Asp
145                 150                 155                 160

His Pro Glu Ser Leu Glu Gln Ile Leu Val Asp Cys Arg Asp Thr Leu
                165                 170                 175

Lys Tyr Gly Val Arg Thr Gly His Pro Arg Phe Phe Asn Gln Leu Ser
            180                 185                 190

Thr Gly Leu Asp Ile Ile Gly Leu Ala Gly Glu Trp Leu Thr Ser Thr
        195                 200                 205

Ala Asn Thr Asn Met Phe Thr Tyr Glu Ile Ala Pro Val Phe Val Leu
    210                 215                 220

Met Glu Gln Ile Thr Leu Lys Lys Met Arg Glu Ile Val Gly Trp Ser
225                 230                 235                 240

Ser Lys Asp Gly Asp Gly Ile Phe Ser Pro Gly Gly Ala Ile Ser Asn
                245                 250                 255

Met Tyr Ser Ile Met Ala Ala Arg Tyr Lys Tyr Phe Pro Glu Val Lys
            260                 265                 270

Thr Lys Gly Met Ala Ala Val Pro Lys Leu Val Leu Phe Thr Ser Glu
        275                 280                 285
```

```
Gln Ser His Tyr Ser Ile Lys Lys Ala Gly Ala Ala Leu Gly Phe Gly
    290                 295                 300

Thr Asp Asn Val Ile Leu Ile Lys Cys Asn Glu Arg Gly Lys Ile Ile
305                 310                 315                 320

Pro Ala Asp Phe Glu Ala Lys Ile Leu Glu Ala Lys Gln Lys Gly Tyr
                325                 330                 335

Val Pro Phe Tyr Val Asn Ala Thr Ala Gly Thr Thr Val Tyr Gly Ala
            340                 345                 350

Phe Asp Pro Ile Gln Glu Ile Ala Asp Ile Cys Glu Lys Tyr Asn Leu
        355                 360                 365

Trp Leu His Val Asp Ala Ala Trp Gly Gly Gly Leu Leu Met Ser Arg
    370                 375                 380

Lys His Arg His Lys Leu Asn Gly Ile Glu Arg Ala Asn Ser Val Thr
385                 390                 395                 400

Trp Asn Pro His Lys Met Met Gly Val Leu Leu Gln Cys Ser Ala Ile
                405                 410                 415

Leu Val Lys Glu Lys Gly Ile Leu Gln Gly Cys Asn Gln Met Cys Ala
            420                 425                 430

Gly Tyr Leu Phe Gln Pro Asp Lys Gln Tyr Asp Val Ser Tyr Asp Thr
        435                 440                 445

Gly Asp Lys Ala Ile Gln Cys Gly Arg His Val Asp Ile Phe Lys Phe
    450                 455                 460

Trp Leu Met Trp Lys Ala Lys Gly Thr Val Gly Phe Glu Asn Gln Ile
465                 470                 475                 480

Asn Lys Cys Leu Glu Leu Ala Glu Tyr Leu Tyr Ala Lys Ile Lys Asn
                485                 490                 495

Arg Glu Glu Phe Glu Met Val Phe Asn Gly Glu Pro Glu His Thr Asn
            500                 505                 510

Val Cys Phe Trp Tyr Ile Pro Gln Ser Leu Arg Gly Val Pro Asp Ser
        515                 520                 525

Pro Gln Arg Arg Glu Lys Leu His Lys Val Ala Pro Lys Ile Lys Ala
    530                 535                 540

Leu Met Met Glu Ser Gly Thr Thr Met Val Gly Tyr Gln Pro Gln Gly
545                 550                 555                 560
```

Asp Lys Ala Asn Phe Phe Arg Met Val Ile Ser Asn Pro Ala Ala Thr
565 570 575

Gln Ser Asp Ile Asp Phe Leu Ile Glu Glu Ile Glu Arg Leu Gly Gln
580 585 590

Asp Leu

<210> 5
<211> 1784
<212> DNA
<213> Homo sapiens

<400> 5
atggcgtctc gaccccatct tcgtccgcaa cctcctcgaa cgcgggagcg gaccccaata 60

ccactaacct gcgccccaca acgtacgata cctggtgcgg cgtggcccat ggatgcacca 120

gaaaactggg gctcaagatc tgcggcttct tgcaaaggac caacagcctg gaagagaaga 180

gtcgccttgt gagtgccttc aaggagaggc aatcctccaa gaacctgctt tcctgtgaaa 240

acagcgaccg ggatgcccgc ttccggcgca cagagactga cttctctaat ctgtttgcta 300

gagatctgct tccggctaag aacggtgagg agcaaaccgt gcaattcctc ctggaagtgg 360

tggacatact cctcaactat gtccgcaaga catttgatcg ctccaccaag gtgctggact 420

ttcatcaccc acaccagttg ctggaaggca tggagggctt caacttggag ctctctgacc 480

accccgagtc cctggagcag atcctggttg actgcagaga caccttgaag tatggggttc 540

gcacaggtca tcctcgattt ttcaaccagc tctccactgg attggatatt attggcctag 600

ctggagaatg gctgacatca acggccaata ccaacatgtt tacatatgaa attgcaccag 660

tgtttgtcct catggaacaa ataacactta agaagatgag agagatagtt ggatggtcaa 720

gtaaagatgg tgatgggata ttttctcctg ggggcgccat atccaacatg tacagcatca 780

tggctgctcg ctacaagtac ttcccggaag ttaagacaaa gggcatggcg gctgtgccta 840

aactggtcct cttcacctca gaacagagtc actattccat aaagaaagct ggggctgcac 900

ttggctttgg aactgacaat gtgattttga taaagtgcaa tgaaaggggg aaaataattc 960

cagctgattt tgaggcaaaa attcttgaag ccaaacagaa gggatatgtt ccctttttatg 1020

tcaatgcaac tgctggcacg actgtttatg gagcttttga tccgatacaa gagattgcag 1080

atatatgtga gaaatataac ctttggttgc atgtcgatgc tgcctgggga ggtgggctgc 1140

tcatgtccag gaagcaccgc cataaactca acggcataga aagggccaac tcagtcacct 1200

ggaaccctca caagatgatg ggcgtgctgt tgcagtgctc tgccattctc gtcaaggaaa 1260

agggtatact ccaaggatgc aaccagatgt gtgcaggata cctcttccag ccagacaagc 1320

agtatgatgt ctcctacgac accggggaca aggcaattca gtgtggccgc cacgtggata 1380

tcttcaagtt ctggctgatg tggaaagcaa agggcacagt gggatttgaa aaccagatca 1440

acaaatgcct ggaactggct gaatacctct atgccaagat taaaaacaga gaagaatttg 1500

agatggtttt caatggcgag cctgagcaca caaacgtctg tttttggtat attccacaaa 1560

gcctcagggg tgtgccagac agccctcaac gacgggaaaa gctacacaag gtggctccaa 1620

aaatcaaagc cctgatgatg gagtcaggta cgaccatggt tggctaccag ccccaagggg 1680

acaaggccaa cttcttccgg atggtcatct ccaacccagc cgctacccag tctgacattg 1740

acttcctcat tgaggagata gaaagactgg gccaggatct gtaa 1784

<210> 6
<211> 178
<212> PRT
<213> rattus norvegicus

<400> 6

Met Pro Gly Ser Ala Leu Leu Cys Cys Leu Leu Leu Leu Ala Gly Val
1 5 10 15

Lys Thr Ser Lys Gly His Ser Ile Arg Gly Asp Asn Asn Cys Thr His
20 25 30

Phe Pro Val Ser Gln Thr His Met Leu Arg Glu Leu Arg Ala Ala Phe
35 40 45

Ser Gln Val Lys Thr Phe Phe Gln Lys Lys Asp Gln Leu Asp Asn Ile
50 55 60

Leu Leu Thr Asp Ser Leu Leu Gln Asp Phe Lys Gly Tyr Leu Gly Cys
65 70 75 80

Gln Ala Leu Ser Glu Met Ile Lys Phe Tyr Leu Val Glu Val Met Pro
85 90 95

Gln Ala Glu Asn His Gly Pro Glu Ile Lys Glu His Leu Asn Ser Leu
100 105 110

Gly Glu Lys Leu Lys Thr Leu Trp Ile Gln Leu Arg Arg Cys His Arg
115 120 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn
130 135 140

Asp Phe Asn Lys Leu Gln Asp Lys Gly Val Tyr Lys Ala Met Asn Glu
145 150 155 160

Phe Asp Ile Phe Ile Asn Cys Ile Glu Ala Tyr Val Thr Leu Lys Met
165 170 175

Lys Asn

<210> 7
<211> 682
<212> DNA
<213> rattus norvegicus

<400> 7
catgcctggc tcagcactgc tatgttgcct gctcttactg gctggagtga agaccagcaa 60

aggccattcc atccggggtg acaataactg cacccacttc ccagtcagcc agacccacat 120

gctccgagag ctgagggctg ccttcagtca agtgaagact ttctttcaaa agaaggacca 180

gctggacaac atactgctga cagattcctt actgcaggac tttaagggtt acttgggttg 240

ccaagccttg tcagaaatga tcaagtttta cctggtagaa gtgatgcccc aggcagagaa 300

ccatggccca gaaatcaagg agcatttgaa ttccctggga gagaagctga agaccctctg 360

gatacagctg cgacgctgtc atcgatttct cccctgtgag aataaaagca aggcagtgga 420

gcaggtgaag aatgatttta ataagctcca agacaaaggt gtctacaagg ccatgaatga 480

gtttgacatc ttcatcaact gcatagaagc ctacgtgaca ctcaaaatga aaaattgaac 540

cacccggcat ctactggact gcaggacata aatagagctt ctaaatctga tccagagatc 600

ttagctaacg ggagcaactc cttggaaaac ctcgtttgta cctctctcca aaatatttat 660

tacctctgat acctcagttc cc 682

<210> 8
<211> 537
<212> DNA
<213> Artificial Sequence

<220>
<223> Optimized rat IL-10

<400> 8
atgcctggct cagccctgct atgttgcctt ctcctgctgg cgggagtcaa gacaagcaag 60

ggccattcca tccggggaga taataactgc acccacttcc cagtctctca aacccacatg 120

ttgcgagagc tgagggctgc cttcagtcag gtgaagacgt tcttccagaa gaaggaccag 180

ctggacaaca ttctgctgac tgacagcctg ctgcaggatt tcaagggtta tttggggtgt 240

caagccctgt ctgaaatgat caagttttac ctggtagaag tgatgcccca ggcagagaat 300

catggccccg agatcaagga gcacctcaac tccctggggg agaagctgaa gaccctgtgg 360

attcagctga ggcgctgcca cagatttctc ccctgtgaaa acaagagcaa ggcagtggag 420

caggtgaaga acgattttaa taagctccag gacaagggcg tctacaaggc catgaacgag 480

ttcgacatct ttatcaactg catagaagct tacgttacac tcaagatgaa gaattga 537

<210> 9
<211> 178
<212> PRT
<213> Homo sapiens

<400> 9
Met His Ser Ser Ala Leu Leu Cys Cys Leu Val Leu Leu Thr Gly Val
  1   5   10   15

Arg Ala Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His
             20   25   30

Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe
         35   40   45

Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu
     50   55   60

Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys
 65   70   75   80

Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro
                 85   90   95

Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu
            100   105   110

Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg

115 120 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn
130 135 140

Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu
145 150 155 160

Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile
165 170 175

Arg Asn

<210> 10
<211> 1600
<212> DNA
<213> Homo sapiens

<400> 10
aaaccacaag acagacttgc aaaagaaggc atgcacagct cagcactgct ctgttgcctg 60

gtcctcctga ctggggtgag ggccagccca ggccagggca cccagtctga gaacagctgc 120

acccacttcc caggcaacct gcctaacatg cttcgagatc tccgagatgc cttcagcaga 180

gtgaagactt tctttcaaat gaaggatcag ctggacaact tgttgttaaa ggagtccttg 240

ctggaggact ttaagggtta cctgggttgc caagccttgt ctgagatgat ccagttttac 300

ctggaggagg tgatgcccca agctgagaac caagacccag acatcaaggc gcatgtgaac 360

tccctggggg agaacctgaa gaccctcagg ctgaggctac ggcgctgtca tcgatttctt 420

ccctgtgaaa acaagagcaa ggccgtggag caggtgaaga atgcctttaa taagctccaa 480

gagaaaggca tctacaaagc catgagtgag tttgacatct tcatcaacta catagaagcc 540

tacatgacaa tgaagatacg aaactgagac atcagggtgg cgactctata gactctagga 600

cataaattag aggtctccaa aatcggatct ggggctctgg gatagctgac ccagcccctt 660

gagaaacctt attgtacctc tcttatagaa tatttattac ctctgatacc tcaaccccca 720

tttctattta tttactgagc ttctctgtga acgatttaga aagaagccca atattataat 780

ttttttcaat atttattatt ttcacctgtt tttaagctgt ttccataggg tgacacacta 840

tggtatttga gtgttttaag ataaattata agttacataa gggaggaaaa aaaatgttct 900

ttggggagcc aacagaagct tccattccaa gcctgaccac gctttctagc tgttgagctg 960

ttttccctga cctccctcta atttatcttg tctctgggct tggggcttcc taactgctac 1020

aaatactctt aggaagagaa accagggagc ccctttgatg attaattcac cttccagtgt 1080

ctcggaggga ttcccctaac ctcattcccc aaccacttca ttcttgaaag ctgtggccag 1140

cttgttattt ataacaacct aaatttggtt ctaggccggg cgcggtggct cacgcctgta 1200

atcccagcac tttgggaggc tgaggcgggt ggatcacttg aggtcaggag ttcctaacca 1260

gcctggtcaa catggtgaaa ccccgtctct actaaaaata caaaaattag ccgggcatgg 1320

tggcgcgcac ctgtaatccc agctacttgg gaggctgagg caagagaatt gcttgaaccc 1380

aggagatgga agttgcagtg agctgatatc atgcccctgt actccagcct gggtgacaga 1440

gcaagactct gtctcaaaaa ataaaaataa aaataaattt ggttctaata gaactcagtt 1500

ttaactagaa tttattcaat tcctctggga atgttacatt gtttgtctgt cttcatagca 1560

gattttaatt ttgaataaat aaatgtatct tattcacatc 1600

<210> 11
<211> 185
<212> PRT
<213> Homo sapiens

<400> 11

```
Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu His Thr
  1               5                  10                  15

Ala Ser Ala Phe Pro Leu Pro Ala Ala Asn Met Pro Glu Asp Tyr Pro
             20                  25                  30

Asp Gln Phe Asp Asp Val Met Asp Phe Ile Gln Ala Thr Ile Lys Arg
         35                  40                  45

Leu Lys Arg Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu
     50                  55                  60

Arg Asn Arg Gln Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys
 65                  70                  75                  80

Gly Arg Arg Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala
                 85                  90                  95

Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu
            100                 105                 110

Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr
```

```
        115                 120                 125

Thr Tyr Asp Lys Ile Leu Lys Asn Leu Ser Arg Asn Arg Arg Leu Val
    130                 135                 140

Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp
145                 150                 155                 160

Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys
                165                 170                 175

His Ser Ala Lys Arg Cys Gly Cys Ile
            180                 185
```

<210> 12
<211> 558
<212> DNA
<213> Homo sapiens

<400> 12

```
atgaagttat gggatgtcgt ggctgtctgc ctggtgctgc tccacaccgc gtccgccttc         60

ccgctgcccg ccgcaaatat gccagaggat tatcctgatc agttcgatga tgtcatggat        120

tttattcaag ccaccattaa aagactgaaa aggtcaccag ataaacaaat ggcagtgctt        180

cctagaagag agcggaatcg gcaggctgca gctgccaacc cagagaattc cagaggaaaa        240

ggtcggagag gccagagggg caaaaaccgg ggttgtgtct taactgcaat acatttaaat        300

gtcactgact tgggtctggg ctatgaaacc aaggaggaac tgatttttag gtactgcagc        360

ggctcttgcg atgcagctga gacaacgtac gacaaaatat tgaaaaactt atccagaaat        420

agaaggctgg tgagtgacaa agtagggcag gcatgttgca gacccatcgc ctttgatgat        480

gacctgtcgt ttttagatga taacctggtt taccatattc taagaaagca ttccgctaaa        540

aggtgtggat gtatctga                                                      558
```

<210> 13
<211> 558
<212> DNA
<213> Artificial Sequence

<220>
<223> optimized human GDNF

<400> 13

atgaaacttt gggacgtggt ggctgtctgc ctggtgctcc tccacaccgc cagtgcgttt 60

ccgctgcccg ccgctaacat gccagaggat tatcctgatc agttcgatga tgttatggac 120

ttcattcaag ccacaatcaa gcggctgaaa cgatcaccag ataaacagat ggcagtgctt 180

cctcgccgcg agcgtaatcg gcaggctgca gcagccaatc ccgagaattc ccgaggaaaa 240

gggcgcaggg gtcagagggg caagaaccgg gggtgtgtcc tgactgcaat acatttaaac 300

gtgactgact tgggtctggg ctatgagacc aaggaagaac tcattttcag gtactgcagc 360

ggctcttgcg atgccgcgga aacaacgtac gacaaaatct tgaagaacct ctccagaaac 420

agaaggctgg tgagtgacaa ggtaggacag gcctgttgca gacccatcgc ctttgacgac 480

gatctgagct ttctggatga caatctggtt taccacatcc tacggaagca ttctgctaaa 540

agatgtggat gtatttga 558

<210> 14
<211> 537
<212> DNA
<213> Artificial Sequence

<220>
<223> optimized human IL-10

<400> 14
atgcacagct cagcactgct gtgctgcctg gtcctgctga caggggtgag ggcaagccca 60

ggccagggaa cccaatctga gaacagctgc acccacttcc ctggcaatct gcctaacatg 120

ctgcgcgacc tccgagatgc cttcagcaga gtgaagactt ttttccagat gaaggatcag 180

ctggacaacc tgctgctgaa ggagtccctc ctggaggact ttaagggcta cctgggatgc 240

caggccctgt ctgagatgat ccaattctac ctggaagaag ttatgcccca ggctgagaac 300

caggacccag acattaaggc ccatgtcaac tccctggggg aaaatctgaa gaccctcagg 360

ctgcggctac ggcgctgtca ccgttttctg ccctgtgaga ataagagcaa ggctgtggag 420

caggtgaaga acgccttcaa taagctccag gagaagggta tctacaaagc gatgagtgaa 480

tttgatatct tcattaatta tatagaagct tatatgacaa tgaaaatcag aaactga 537

<210> 15
<211> 28

<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for amplifying CMV promoter (F-JDK)

<400> 15
ttcggccgtc gaggagcttg gcccattg 28

<210> 16
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for amplifying CMV promoter (R-JDK)

<400> 16
gacgtcgacc tagctagcga attcggggcc gcggag 36

<210> 17
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for amplifying SV40pA promoter (F-SV40pA)

<400> 17
ccatcgatca gacatgataa gatacattga tgag 34

<210> 18
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for amplifying SV40pA promoter (R-SV40pA)

<400> 18
gacgtcgacg cggccgctac cacatttgta gaggttttac ttg 43

<210> 19
<211> 28

<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for amplifying Kanamycin resistant gene (F-Kan)

<400> 19
aggcgccatg agccatattc aacgggaa 28

<210> 20
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for amplifying Kanamycin resistant gene (R-Kan)

<400> 20
ttcatgatta gaaaaactca tcgagcatc 29

<210> 21
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for amplifying LITR and CMV (F-ITR)

<400> 21
atggcgcgcc cctggccttt tgctggcc 28

<210> 22
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for amplifying SV40pA and RITR (R-ITR)

<400> 22
atggatccgc tagtaaatac cgcatcag 28

<210> 23
<211> 20

<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for amplifying rIL-10 (F-rIL-10)

<400> 23
ccgctagcgc caccatgcct 20

<210> 24
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for amplifying rIL-10 (R-rIL-10)

<400> 24
gacgtcgacg ccatcgatgg cttaattaat caattcttc 39

<210> 25
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for WPRE_Pac1_F

<400> 25
ggtggtttaa ttaaaatcaa cctctggatt acaaaatttg 40

<210> 26
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for WPRE_modi_Hpa1_R

<400> 26
ggtggtgtta acgacaacac cacggaattg 30

<210> 27
<211> 43

<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for SV40-CMV-sCAG-bGHpA-Infu-F

<400> 27
cctgcggccg gtcgactacc acatttgtag aggttttact tgc 43

<210> 28
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for SV40-CMV-sCAG-bGHpA-Infu-R

<400> 28
aataatcaat gtcgactcga ggagcttggc ccatt 35

<210> 29
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> nucleotide sequence of Stuffer scramble

<400> 29
gtcgacggta tcgataagct tgatatcgaa ttcctgcagc cc 42

<210> 30
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Forward primer for Stuffer_scramble_F

<400> 30
ctaggtcgac ggtatcgata agcttgatat cgaattcctg cagccc 46

<210> 31
<211> 46

<212> DNA
<213> Artificial Sequence

<220>
<223> Reverse primer for Stuffer_scramble_R

<400> 31
ctaggggctg caggaattcg atatcaagct tatcgatacc gtcgac 46

<210> 32
<211> 585
<212> PRT
<213> Canis lupus

<400> 32
Met Ala Ser Pro Gly Ser Gly Phe Trp Ser Phe Gly Ser Glu Asp Gly
1 5 10 15

Ser Gly Asp Pro Glu Asn Pro Ser Thr Ala Arg Ala Trp Cys Gln Val
20 25 30

Ala Gln Lys Phe Thr Gly Gly Ile Gly Asn Lys Leu Cys Ala Leu Leu
35 40 45

Tyr Gly Asp Ala Glu Lys Pro Ala Glu Ser Gly Gly Ser Glu Pro Pro
50 55 60

Arg Ala Thr Ser Arg Lys Ala Ala Cys Ala Cys Asn Gln Lys Pro Cys
65 70 75 80

Ser Cys Pro Lys Ala Glu Val Asn Tyr Ala Phe Leu His Ala Thr Asp
85 90 95

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

Asp Val Met Asp Ile Leu Leu Gln Tyr Val Val Lys Ser Phe Asp Arg
115 120 125

Ser Thr Lys Val Ile Asp Phe His Tyr Pro Asn Glu Leu Leu Gln Glu
130 135 140

Tyr Asn Trp Glu Leu Ala Asp Gln Pro Gln Asn Leu Glu Glu Ile Leu
145 150 155 160

Met His Cys Gln Thr Thr Leu Lys Tyr Ala Ile Lys Thr Gly His Pro
165 170 175

Arg Tyr Phe Asn Gln Leu Ser Thr Gly Leu Asp Met Val Gly Leu Ala
180 185 190

```
Ala Asp Trp Leu Thr Ser Thr Ala Asn Thr Asn Met Phe Thr Tyr Glu
        195                 200                 205

Ile Ala Pro Val Phe Val Leu Leu Glu Tyr Val Thr Leu Lys Lys Met
    210                 215                 220

Arg Glu Ile Ile Gly Trp Pro Gly Gly Ser Gly Asp Gly Ile Phe Ser
225                 230                 235                 240

Pro Gly Gly Ala Ile Ser Asn Met Tyr Ala Met Leu Ile Ala Arg Phe
                245                 250                 255

Lys Met Phe Pro Glu Val Lys Glu Lys Gly Met Ala Ala Val Pro Arg
            260                 265                 270

Leu Ile Ala Phe Thr Ser Glu His Ser His Phe Ser Leu Lys Lys Gly
        275                 280                 285

Ala Ala Ala Leu Gly Ile Gly Thr Asp Ser Val Ile Leu Ile Lys Cys
    290                 295                 300

Asp Glu Arg Gly Lys Met Val Pro Ser Asp Leu Glu Arg Arg Ile Leu
305                 310                 315                 320

Glu Ala Lys Gln Lys Gly Phe Val Pro Phe Leu Val Ser Ala Thr Ala
                325                 330                 335

Gly Thr Thr Val Tyr Gly Ala Phe Asp Pro Leu Leu Ala Val Ala Asp
            340                 345                 350

Ile Cys Lys Lys Tyr Lys Ile Trp Met His Val Asp Ala Ala Trp Gly
        355                 360                 365

Gly Gly Leu Leu Met Ser Arg Lys His Lys Trp Lys Leu Ser Gly Val
    370                 375                 380

Glu Arg Ala Asn Ser Val Thr Trp Asn Pro His Lys Met Met Gly Val
385                 390                 395                 400

Pro Leu Gln Cys Ser Ala Leu Leu Val Arg Glu Glu Gly Leu Met Gln
                405                 410                 415

Ser Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His
            420                 425                 430

Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg
        435                 440                 445

His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr
    450                 455                 460
```

```
Thr Gly Phe Glu Ala His Ile Asp Lys Cys Leu Glu Leu Ala Glu Tyr
465                 470                 475                 480

Leu Tyr Ser Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp
                485                 490                 495

Gly Lys Pro Gln His Thr Asn Val Cys Phe Trp Tyr Val Pro Pro Ser
            500                 505                 510

Leu Arg Val Leu Glu Asp Asn Glu Glu Arg Met Asn Arg Leu Ser Lys
        515                 520                 525

Val Ala Pro Val Ile Lys Ala Arg Met Met Glu Tyr Gly Thr Thr Met
    530                 535                 540

Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val
545                 550                 555                 560

Ile Ser Asn Pro Ala Ala Thr His Gln Asp Ile Asp Phe Leu Ile Glu
                565                 570                 575

Glu Ile Glu Arg Leu Gly Gln Asp Leu
            580                 585
```

<210> 33
<211> 1758
<212> DNA
<213> Canis lupus

<400> 33

```
atggcatctc caggctctgg cttctggtcc ttcgggtctg aagatggctc cggggatccc         60

gagaacccca gcacagcgag agcctggtgt caggtggccc agaagttcac gggcggcatc        120

ggaaacaagc tgtgcgccct gctctacgga gatgccgaga agcccgcgga gagtggcggg        180

agcgagcccc cgcgcgccac ctccaggaag gccgcctgcg cttgtaatca gaagccttgc        240

agctgcccca aagcggaggt caactatgcg tttctacacg caacagacct gctgccagcc        300

tgtgatggag aaaggcccac gttggcgttt ctgcaagatg ttatggacat tttgcttcag        360

tatgttgtga aaagtttcga tagatcaacc aaagtgattg atttccatta ccctaatgag        420

ctccttcaag agtataactg ggaattggca gaccaaccac aaaatttgga ggaaattttg        480

atgcattgcc aaacgactct aaaatatgca attaaaacag ggcatcccag atatttcaat        540

cagctttcca ctggactgga tatggttgga ttagcagcag actggctgac atcaacagca        600

aacacaaaca tgttcaccta tgaaattgct ccagtatttg tgctcttgga atatgtcaca        660
```

ctaaagaaaa tgagagaaat cattggctgg ccgggaggct ctggcgatgg gatattttct 720

cctggtggcg ctatttctaa catgtatgcc atgctgatcg cacgctttaa gatgttccca 780

gaagtcaagg agaaaggaat ggctgcggtt cccaggctca ttgccttcac atctgagcat 840

agtcactttt ctctcaagaa gggagctgca gctttgggga ttggaacaga cagcgtgatt 900

ctgattaaat gtgatgagag ggggaaaatg gtcccatctg atcttgaaag aaggatcctt 960

gaagccaaac aaaaaggatt tgttcctttc cttgtgagcg ccacagctgg gaccaccgtg 1020

tatggagcat tcgaccccct cttagcagtt gctgacattt gtaaaaagta caagatctgg 1080

atgcatgtgg atgctgcttg gggtggggga ttactgatgt cccggaagca caaatggaag 1140

ctgagcggcg tggagagggc caactctgtg acatggaacc cacacaagat gatgggcgtc 1200

cctttacagt gctccgctct cctggttaga gaagagggat tgatgcagag ttgcaaccag 1260

atgcatgcct cctacctctt ccagcaagat aaacactatg acctgtccta tgatactggg 1320

gataaggcct tacagtgtgg acgccacgtt gatgttttta aattatggct aatgtggagg 1380

gcaaagggca ccactgggtt tgaagcacat attgataagt gcctggagct ggctgagtat 1440

ttatacagta tcataaaaaa ccgagaagga tacgaaatgg tgtttgatgg aaagcctcag 1500

cacacaaatg tctgcttctg gtacgtgcct ccaagtttgc gtgtcctgga agacaatgaa 1560

gagagaatga accgcctctc aaaggtggcc ccagtgatta aagcccgaat gatggagtat 1620

gggaccacaa tggtcagcta tcagcccttg ggagacaagg tcaatttctt ccgcatggtt 1680

atctcaaatc ccgcagcaac tcaccaagac atcgacttcc tgattgaaga aatagaacgc 1740

cttggacaag atttataa 1758

<210> 34
<211> 585
<212> PRT
<213> Felis catus

<400> 34
Met Ala Thr Pro Gly Ser Gly Phe Trp Ser Phe Gly Ser Glu Asp Gly
1 5 10 15

Ser Gly Asp Pro Glu Asn Pro Gly Thr Ala Arg Ala Trp Cys Gln Val
20 25 30

```
Ala Gln Lys Phe Thr Gly Gly Ile Gly Asn Lys Leu Cys Ala Leu Leu
         35                  40                  45

Tyr Gly Asp Ser Glu Lys Pro Ala Glu Ser Gly Gly Ser Gln Pro Ala
     50                  55                  60

Arg Ala Thr Ser Arg Lys Ala Thr Cys Ala Cys Asn Gln Lys Pro Cys
 65                  70                  75                  80

Ser Cys Pro Lys Ala Asp Val Asn Tyr Ala Phe Leu His Ala Thr Asp
                 85                  90                  95

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

Asp Val Met Gly Ile Leu Leu Gln Tyr Val Val Lys Ser Phe Asp Arg
        115                 120                 125

Ser Thr Lys Val Ile Asp Phe His Tyr Pro Asn Glu Leu Leu Gln Glu
    130                 135                 140

Tyr Asn Trp Glu Leu Ala Asp Gln Pro Gln Asn Leu Glu Glu Ile Leu
145                 150                 155                 160

Met His Cys Gln Thr Thr Leu Lys Tyr Ala Ile Lys Thr Gly His Pro
                165                 170                 175

Arg Tyr Phe Asn Gln Leu Ser Thr Gly Leu Asp Met Val Gly Leu Ala
            180                 185                 190

Ala Asp Trp Leu Thr Ser Thr Ala Asn Thr Asn Met Phe Thr Tyr Glu
        195                 200                 205

Ile Ala Pro Val Phe Val Leu Leu Glu Tyr Val Thr Leu Lys Lys Met
    210                 215                 220

Arg Glu Ile Ile Gly Trp Pro Gly Gly Ser Gly Asp Gly Ile Phe Ser
225                 230                 235                 240

Pro Gly Gly Ala Ile Ser Asn Met Tyr Ala Met Leu Ile Ala Arg Phe
                245                 250                 255

Lys Met Phe Pro Glu Val Lys Glu Lys Gly Met Ala Ala Val Pro Arg
            260                 265                 270

Leu Ile Ala Phe Thr Ser Glu His Ser His Phe Ser Leu Lys Lys Gly
        275                 280                 285

Ala Ala Ala Leu Gly Ile Gly Thr Asp Ser Val Ile Leu Ile Lys Cys
    290                 295                 300

Asp Glu Arg Gly Lys Met Ile Pro Ser Asp Leu Glu Arg Arg Ile Leu
```

```
305                 310                 315                 320

Glu Ala Lys Gln Lys Gly Phe Val Pro Phe Leu Val Ser Ala Thr Ala
                325                 330                 335

Gly Thr Thr Val Tyr Gly Ala Phe Asp Pro Leu Leu Ala Val Ala Asp
            340                 345                 350

Ile Cys Lys Lys Tyr Lys Ile Trp Met His Val Asp Ala Ala Trp Gly
        355                 360                 365

Gly Gly Leu Leu Met Ser Arg Lys His Lys Trp Lys Leu Ser Gly Val
    370                 375                 380

Glu Arg Ala Asn Ser Val Thr Trp Asn Pro His Lys Met Met Gly Val
385                 390                 395                 400

Pro Leu Gln Cys Ser Ala Leu Leu Val Arg Glu Glu Gly Leu Met Gln
                405                 410                 415

Ser Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His
            420                 425                 430

Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg
        435                 440                 445

His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr
    450                 455                 460

Thr Gly Phe Glu Ala His Ile Asp Lys Cys Leu Glu Leu Ala Glu Tyr
465                 470                 475                 480

Leu Tyr Asn Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp
                485                 490                 495

Gly Lys Pro Gln His Thr Asn Val Cys Phe Trp Tyr Val Pro Pro Ser
            500                 505                 510

Leu Arg Val Leu Glu Asp Asn Glu Glu Arg Met Ser Arg Leu Ser Lys
        515                 520                 525

Val Ala Pro Val Ile Lys Ala Arg Met Met Glu Tyr Gly Thr Thr Met
    530                 535                 540

Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val
545                 550                 555                 560

Ile Ser Asn Pro Ala Ala Thr His Gln Asp Ile Asp Phe Leu Ile Glu
                565                 570                 575

Glu Ile Glu Arg Leu Gly Gln Asp Leu
            580                 585
```

<210> 35
<211> 1758
<212> DNA
<213> Felis catus

<400> 35
atggcaactc caggctcagg cttttggtcc ttcgggtctg aagatggctc cggggatccc 60

gagaaccccg gcacagcgag agcctggtgt caggtggccc agaagttcac gggcggcatc 120

ggaaacaagc tgtgcgccct gctctacggg gattcagaga agccggcaga gagtggaggg 180

agccagcccg cgcgggccac ctcccggaag gccacctgtg cctgtaacca gaagccttgc 240

agctgcccca aagcggatgt caactatgcg tttctacacg caacagacct gctgccagcc 300

tgtgatggag aaaggcccac tttggcgttt ctgcaagatg taatgggcat tttgcttcag 360

tatgtggtga aaagtttcga cagatcaacc aaagtgattg atttccatta ccctaatgag 420

ctcctgcaag agtataactg ggaattggca gaccaaccac aaaatttgga ggaaattttg 480

atgcattgcc aaacgactct aaaatatgca ataaaaacag ggcatcccag gtacttcaat 540

caactttcca cgggactgga tatggttgga ttagcagcag actggctgac atcaacagca 600

aacactaata tgttcaccta tgaaattgct ccagtatttg tgctcttgga atatgtcaca 660

ctgaaaaaaa tgagagaaat cattggctgg cctgggggct ccggcgatgg gatattttct 720

cctggtggcg ctatatctaa catgtatgcc atgctgattg cacgctttaa gatgttccca 780

gaagtcaagg agaaaggaat ggctgctgtt cccaggctca ttgccttcac atccgagcat 840

agtcattttt ctctcaagaa gggagctgca gctctgggga ttggaacaga cagcgtgatt 900

ctgattaaat gcgatgagag agggaaaatg atcccatctg atcttgaaag aaggatcctt 960

gaagccaaac agaaaggatt tgttcctttc cttgtgagtg ccacagctgg gaccactgtg 1020

tatggagcat ttgaccccct cttggcggtc gctgacattt gcaaaaagta caagatctgg 1080

atgcatgtgg atgcagcttg gggtggggga ttactgatgt cccggaaaca caagtggaaa 1140

ctgagcggcg tggagagggc caactctgtg acatggaacc cacacaagat gatgggcgtc 1200

cccttacagt gctctgctct cctggttaga gaagaggggt tgatgcagag ttgcaaccag 1260

atgcatgctt cctacctttt ccagcaagat aaacactacg acctgtccta cgacactgga 1320

gacaaggcct tacagtgtgg acgccatgtc gatgttttta aattatggct aatgtggagg 1380

gcaaagggca ccactgggtt tgaagcacat attgataagt gcttggagct ggcagaatat 1440

ttatacaata tcataaaaaa ccgagaagga tatgaaatgg tgtttgatgg aaagcctcag 1500

cacacaaatg tctgcttctg gtacgtgcct ccaagtttgc gagtcctgga agacaatgaa 1560

gagagaatga gccgcctctc aaaggtggcc ccagtgatta aagccagaat gatggagtat 1620

gggaccacaa tggtcagcta tcagcccttg ggagacaagg tcaatttctt ccgcatggtc 1680

atctcaaatc ccgcagcaac tcaccaagac attgacttcc tgattgaaga aatagaacgc 1740

cttggacaag atttataa 1758

<210> 36
<211> 585
<212> PRT
<213> Equus caballus

<400> 36

Met Ala Ser Pro Gly Ser Gly Phe Trp Ser Phe Gly Ser Glu Asp Gly
1 5 10 15

Ser Gly Asp Pro Glu Asn Pro Gly Thr Ala Arg Ala Trp Cys Gln Val
20 25 30

Ala Gln Lys Phe Thr Gly Gly Ile Gly Asn Lys Leu Cys Ala Leu Leu
35 40 45

Tyr Gly Asp Ala Glu Lys Ala Ala Glu Ser Gly Gly Ser Glu Pro Pro
50 55 60

Arg Ala Thr Ser Arg Lys Ala Ala Cys Ser Cys Asn Gln Lys Pro Cys
65 70 75 80

Ser Cys Ser Lys Ala Asp Val Asn Tyr Ala Phe Leu His Ala Thr Asp
85 90 95

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

Asp Val Met Asp Ile Leu Leu Gln Tyr Val Val Lys Ser Phe Asp Arg
115 120 125

Ser Thr Lys Val Ile Asp Phe His Tyr Pro Asn Glu Leu Leu Gln Glu
130 135 140

Tyr Asn Trp Glu Leu Ala Asp Gln Pro Gln Asn Leu Glu Glu Ile Leu
145 150 155 160

```
Met His Cys Gln Thr Thr Leu Lys Tyr Ala Ile Lys Thr Gly His Pro
                165                 170                 175

Arg Tyr Phe Asn Gln Leu Ser Thr Gly Leu Asp Met Val Gly Leu Ala
            180                 185                 190

Ala Asp Trp Leu Thr Ser Thr Ala Asn Thr Asn Met Phe Thr Tyr Glu
        195                 200                 205

Ile Ala Pro Val Phe Val Leu Leu Glu Tyr Val Thr Leu Lys Lys Met
    210                 215                 220

Arg Glu Ile Ile Gly Trp Pro Gly Gly Ser Gly Asp Gly Ile Phe Ser
225                 230                 235                 240

Pro Gly Gly Ala Ile Ser Asn Met Tyr Ala Met Leu Ile Ala Arg Phe
                245                 250                 255

Lys Met Phe Pro Glu Val Lys Glu Lys Gly Met Ala Ala Val Pro Arg
            260                 265                 270

Leu Ile Ala Phe Thr Ser Glu His Ser His Phe Ser Leu Lys Lys Gly
        275                 280                 285

Ala Ala Ala Leu Gly Ile Gly Thr Asp Ser Val Ile Leu Ile Arg Cys
    290                 295                 300

Asp Glu Arg Gly Lys Met Ile Pro Ser Asp Leu Glu Arg Arg Ile Leu
305                 310                 315                 320

Glu Ala Lys Gln Lys Gly Phe Val Pro Phe Leu Val Ser Ala Thr Ala
                325                 330                 335

Gly Thr Thr Val Tyr Gly Ala Phe Asp Pro Leu Leu Ala Val Ala Asp
            340                 345                 350

Ile Cys Lys Lys Tyr Lys Ile Trp Met His Val Asp Ala Ala Trp Gly
        355                 360                 365

Gly Gly Leu Leu Met Ser Arg Lys His Lys Trp Lys Leu Ser Gly Val
    370                 375                 380

Glu Arg Ala Asn Ser Val Thr Trp Asn Pro His Lys Met Met Gly Val
385                 390                 395                 400

Pro Leu Gln Cys Ser Ala Leu Leu Val Arg Glu Glu Gly Leu Met Gln
                405                 410                 415

Ser Cys Asn Gln Met His Ala Ser Tyr Leu Phe Gln Gln Asp Lys His
            420                 425                 430
```

```
Tyr Asp Leu Ser Tyr Asp Thr Gly Asp Lys Ala Leu Gln Cys Gly Arg
        435                 440                 445

His Val Asp Val Phe Lys Leu Trp Leu Met Trp Arg Ala Lys Gly Thr
    450                 455                 460

Thr Gly Phe Glu Ala His Ile Asp Lys Cys Leu Glu Leu Ala Glu Tyr
465                 470                 475                 480

Leu Tyr Asn Ile Ile Lys Asn Arg Glu Gly Tyr Glu Met Val Phe Asp
                485                 490                 495

Gly Lys Pro Gln His Thr Asn Val Cys Phe Trp Tyr Val Pro Pro Ser
            500                 505                 510

Leu Arg Val Leu Glu Asp Asn Glu Glu Arg Met Ser Arg Leu Ser Lys
        515                 520                 525

Val Ala Pro Val Ile Lys Ala Arg Met Met Glu Tyr Gly Thr Thr Met
    530                 535                 540

Val Ser Tyr Gln Pro Leu Gly Asp Lys Val Asn Phe Phe Arg Met Val
545                 550                 555                 560

Ile Ser Asn Pro Ala Ala Thr His Gln Asp Ile Asp Phe Leu Ile Glu
                565                 570                 575

Glu Ile Glu Arg Leu Gly Gln Asp Leu
            580                 585
```

<210> 37
<211> 1758
<212> DNA
<213> Equus caballus

<400> 37

```
atggcatctc ccggctccgg cttttggtcc tttgggtctg aagatggctc cggggatccc         60

gagaaccctg gcacagcgag agcctggtgt caggtggccc agaagttcac cggcggcatc        120

ggaaacaagc tatgcgccct gctctacgga gacgccgaga aggcggcgga gagcggcggg        180

agcgagcccc cgcgggccac ctcccggaag gccgcctgct cctgcaacca gaagccctgc        240

agctgctcca aagccgatgt caactatgcg tttctacacg caacagactt gctgccagct        300

tgtgacggag aaagacccac tttggcgttt ctgcaagatg ttatggacat tttgcttcag        360

tatgtggtga aaagtttcga tagatcaacc aaagtgattg acttccatta ccctaatgag        420

ctccttcaag agtataattg ggaattggca gaccaaccac aaaatctgga ggaaattttg        480
```

atgcattgcc aaacaacttt aaaatatgca attaaaacag ggcatcctag atatttcaat 540

caactttcca ctggactgga tatggttgga ttagcagcag actggctgac atcaacagca 600

aacaccaaca tgttcaccta tgaaattgct ccagtattcg tgcttttgga atatgtcaca 660

ttaaagaaaa tgagagaaat cattggctgg ccaggaggct ctggcgatgg aatattttct 720

cctggtggcg ccatatctaa catgtatgcc atgctgattg cacgctttaa gatgttccca 780

gaagtcaagg agaaaggaat ggccgctgtt cccaggctca ttgccttcac gtctgagcat 840

agtcattttt ctctcaagaa gggagctgca gccttgggga ttggaacaga cagcgtaatt 900

ctgattagat gtgatgagag ggggaaaatg atcccatcgg atcttgaaag aagaatcctt 960

gaagccaaac aaaaaggatt tgtccctttt cttgtgagtg ccacggctgg gaccaccgtg 1020

tatggagcat tcgatcccct cttagctgtc gctgacattt gcaaaaagta caagatctgg 1080

atgcatgtgg atgcagcttg gggcggggga ttactgatgt cccggaaaca caagtggaaa 1140

ctgagtggcg tggagagggc caactctgtg acatggaatc cacacaagat gatgggtgtc 1200

cctttgcagt gctctgctct cctggttaga gaagagggat tgatgcagag ttgcaaccag 1260

atgcatgcct cctacctctt tcagcaagat aaacactatg acctgtccta tgacactgga 1320

gacaaggcct tgcagtgcgg acgccacgtg gatgttttta agttatggct catgtggagg 1380

gcaaagggaa caactgggtt tgaagcacat attgataagt gtttggagtt ggcggagtat 1440

ttatacaata tcataaaaaa ccgagaagga tatgaaatgg tgtttgacgg aaagcctcag 1500

cacaccaatg tctgcttctg gtatgtacct ccgagtctgc gtgttctaga agacaatgaa 1560

gagagaatga gccgcctctc aaaggtggcc ccggtgatta aagccagaat gatggagtat 1620

gggaccacaa tggtcagcta ccagcccttg ggagacaagg tcaatttctt ccgcatggtc 1680

atctcaaatc ccgcagcaac tcaccaagac attgacttcc tgattgaaga aatagaacgc 1740

cttggacaag atttataa 1758

<210> 38
<211> 181
<212> PRT
<213> Canis lupus

<400> 38

```
Met His Gly Ser Ala Leu Leu Cys Cys Cys Leu Val Leu Leu Ala Gly
  1               5                  10                  15

Val Gly Ala Ser Arg His Gln Ser Thr Leu Leu Glu Asp Asp Cys Thr
             20                  25                  30

His Phe Pro Ala Ser Leu Pro His Met Leu Arg Glu Leu Arg Ala Ala
         35                  40                  45

Phe Gly Arg Val Lys Ile Phe Phe Gln Met Lys Asp Lys Leu Asp Asn
     50                  55                  60

Ile Leu Leu Thr Gly Ser Leu Leu Glu Asp Phe Lys Ser Tyr Leu Gly
 65                  70                  75                  80

Cys Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met
                 85                  90                  95

Pro Arg Ala Glu Asn His Asp Pro Asp Ile Lys Asn His Val Asn Ser
            100                 105                 110

Leu Gly Glu Lys Leu Lys Thr Leu Arg Leu Arg Leu Arg Leu Arg Arg
        115                 120                 125

Cys His Arg Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln
    130                 135                 140

Val Lys Ser Ala Phe Ser Lys Leu Gln Glu Lys Gly Val Tyr Lys Ala
145                 150                 155                 160

Met Ser Glu Phe Asp Ile Phe Ile Asn Tyr Ile Glu Thr Tyr Met Thr
                165                 170                 175

Met Arg Met Lys Ile
            180
```

<210> 39
<211> 546
<212> DNA
<213> Canis lupus

<400> 39

```
atgcatggct cagcactgct ctgttgctgc ctggtcctcc tggccggggt gggagccagc         60

cgacaccaga gcaccctact tgaggacgac tgcacccact tcccagccag cctgccccac        120

atgctccgag agctccgagc tgccttcggg agggtgaaga tcttctttca aatgaaggac        180

aagctggaca acatactgct gaccgggtcc ctgctggagg actttaagag ttacctgggt        240

tgccaagccc tgtcggagat gatccagttt tacttggagg aggtgatgcc ccgggctgag        300
```

aaccacgacc cagacatcaa gaaccacgtg aactccctgg gagagaagct caagaccctc 360

aggctgagac tgaggctgcg acgctgtcac cgatttcttc cctgtgagaa taagagcaag 420

gcggtggagc aggtgaagag cgcatttagt aagctccagg agaaaggtgt ctacaaagcc 480

atgagtgagt ttgacatctt catcaactac atagaaacct acatgacaat gaggatgaaa 540

atctga 546

<210> 40
<211> 178
<212> PRT
<213> Felis catus

<400> 40

```
Met His Ser Ser Ala Leu Leu Cys Phe Leu Val Phe Leu Ala Gly Val
  1               5                  10                  15

Gly Ala Ser Arg His Gln Ser Thr Leu Ser Glu Asp Asn Cys Thr His
             20                  25                  30

Phe Ser Val Ser Leu Pro His Met Leu Arg Glu Leu Arg Ala Ala Phe
         35                  40                  45

Gly Lys Val Lys Thr Phe Phe Gln Thr Lys Asp Glu Leu His Ser Ile
     50                  55                  60

Leu Leu Thr Arg Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys
 65                  70                  75                  80

Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro
                 85                  90                  95

Gln Ala Glu Asn Glu Asp Pro Asp Ile Lys Gln His Val Asn Ser Leu
            100                 105                 110

Gly Glu Lys Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg
        115                 120                 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Val Val Glu Gln Val Lys Ser
    130                 135                 140

Thr Phe Ser Lys Leu Gln Glu Lys Gly Val Tyr Lys Ala Met Gly Glu
145                 150                 155                 160

Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Met
                165                 170                 175

Lys Ile
```

<210> 41
<211> 537
<212> DNA
<213> Felis catus

<400> 41
atgcacagct cagcacttct gtgtttcctg gtcttcctgg ccggggtagg agccagccga 60

caccagagca ccctgtctga ggacaactgc acccacttct cagtcagcct gccccacatg 120

ctccgagagc tccgagctgc cttcggcaag gtgaagactt tctttcaaac caaggacgag 180

ctgcacagca tattgttgac caggtccttg ctggaggact ttaagggtta cctgggttgc 240

caagccttgt ccgagatgat ccagttttat ttggaggagg tgatgcccca ggctgagaac 300

gaggacccag acatcaaaca gcacgtgaac tccctgggag aaaagctgaa gaccctccgg 360

ctgagactgc ggcgctgtca tcgatttctg ccctgtgaaa acaagagcaa ggtggtggag 420

caggtgaaga gtacctttag taagctccaa gagaaaggtg tctacaaagc catgggtgag 480

tttgacatct tcatcaacta catagaagct tacatgacaa tgaagatgaa aatctga 537

<210> 42
<211> 178
<212> PRT
<213> Equus caballus

<400> 42
Met His Ser Ser Ala Leu Leu Cys Tyr Leu Val Phe Leu Ala Gly Val
1 5 10 15

Gly Ala Ser Arg Asp Arg Gly Thr Gln Ser Glu Asn Ser Cys Thr His
20 25 30

Phe Pro Thr Ser Leu Pro His Met Leu His Glu Leu Arg Ala Ala Phe
35 40 45

Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Met
50 55 60

Leu Leu Asn Gly Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys
65 70 75 80

Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro
85 90 95

```
Gln Ala Glu Asn His Gly Pro Asp Ile Lys Glu His Val Asn Ser Leu
            100                 105                 110

Gly Glu Lys Leu Lys Thr Leu Arg Val Arg Leu Arg Arg Cys His Arg
        115                 120                 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Ser
    130                 135                 140

Ala Phe Ser Lys Leu Gln Glu Lys Gly Val Tyr Lys Ala Met Ser Glu
145                 150                 155                 160

Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Thr Lys Met
                165                 170                 175

Lys Asn
```

<210> 43
<211> 537
<212> DNA
<213> Equus caballus

<400> 43

```
atgcacagct cagcactgct atgttacctg gtcttcctgg ccggggtggg agccagccga         60

gaccggggca cccagtctga gaacagctgc acccacttcc caaccagcct gccccacatg        120

ctccatgagc tccgagccgc cttcagcagg gtgaagactt tctttcaaat gaaggaccag        180

ctggacaaca tgttgttgaa cgggtccctg ctggaggact ttaagggtta cctgggttgc        240

caagccttgt cggagatgat ccagttttac ctggaggagg tgatgcccca ggctgagaac        300

cacggcccag acatcaagga gcacgtgaac tccctggggg aaaagctgaa gaccctccga        360

gtgaggctgc ggcgctgtca tcgatttctg ccctgtgaaa ataagagcaa ggcagtggag        420

caggtgaaga gtgccttcag taagctccaa gagaaaggtg tctacaaagc catgagtgag        480

tttgacatct tcatcaacta catagaagcc tatatgacaa cgaagatgaa aaactga           537
```

<210> 44
<211> 185
<212> PRT
<213> Canis lupus

<400> 44

```
Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu His Thr
  1               5                  10                  15
```

```
Ala Ser Ala Leu Pro Leu Pro Ala Ala Asn Val Pro Glu Asp Tyr Ser
            20                  25                  30

Asp Gln Phe Asp Asp Val Met Asp Phe Ile Gln Ala Thr Ile Arg Arg
        35                  40                  45

Leu Lys Arg Ser Pro Glu Lys Gln Met Ala Val Pro Ala Arg Arg Glu
    50                  55                  60

Arg Asn Arg Gln Ala Ala Ala Ala Gly Pro Glu His Ser Arg Gly Lys
65                  70                  75                  80

Gly Arg Arg Gly Pro Arg Gly Arg Asn Arg Gly Cys Val Leu Thr Ala
                85                  90                  95

Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu
            100                 105                 110

Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr
        115                 120                 125

Met Tyr Asp Lys Ile Leu Lys Asn Leu Ser Lys Ser Arg Arg Leu Ala
    130                 135                 140

Ser Asp Lys Ala Gly Gln Ala Cys Cys Arg Pro Ile Ala Tyr Asp Asp
145                 150                 155                 160

Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys
                165                 170                 175

His Ser Ala Lys Arg Cys Gly Cys Ile
            180                 185
```

<210> 45
<211> 558
<212> DNA
<213> Canis lupus

<400> 45

```
atgaagttat gggatgtcgt ggctgtctgc ctggtgctgc tccacaccgc gtccgccctc         60

ccgctgcccg ccgcaaacgt gccggaggac tattctgatc agtttgatga cgtcatggat        120

tttattcagg ccaccatcag aaggctgaaa aggtcacccg agaaacaaat ggccgtgcca        180

gcgagacgag agcggaatcg tcaggccgcg gccgccggcc cggaacattc caggggaag         240

gggcggcgag gcccgagggg cagaaaccgg ggttgtgtct tgactgcgat acatttaaac        300

gtcactgacc tgggcttggg ctacgaaacc aaggaggaac tgatttttag gtactgcagc        360
```

ggctcctgcg acgcggccga gaccatgtac gacaaaatat taaaaaactt atccaaaagt 420

agaaggctgg cgagtgacaa agcagggcag gcttgctgca gacccatcgc ctacgatgac 480

gacctgtcgt ttttagatga caacctggtt taccatattc taagaaagca ttccgctaaa 540

aggtgtggat gtatctga 558

<210> 46
<211> 211
<212> PRT
<213> Felis catus

<400> 46

```
Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu His Thr
  1               5                  10                  15

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

Ala Glu Asp Arg Ser Leu Gly Arg Arg Arg Ala Pro Phe Ala Leu Ser
         35                  40                  45

Ser Asp Ser Asn Met Pro Glu Asp Tyr Pro Asp Gln Phe Asp Asp Val
     50                  55                  60

Met Asp Phe Ile Gln Ala Thr Ile Arg Arg Leu Lys Arg Ser Pro Glu
 65                  70                  75                  80

Lys Gln Met Ala Leu Pro Pro Arg Arg Glu Arg Asn Arg Gln Ala Ala
                 85                  90                  95

Ala Ala Asn Pro Glu Asn Ser Arg Gly Lys Gly Arg Arg Gly Gln Arg
            100                 105                 110

Gly Arg Asn Arg Gly Cys Val Leu Thr Ala Ile His Leu Asn Val Thr
        115                 120                 125

Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu Glu Leu Ile Phe Arg Tyr
    130                 135                 140

Cys Ser Gly Ser Cys Asp Ala Ala Glu Thr Met Tyr Asp Lys Ile Leu
145                 150                 155                 160

Lys Asn Leu Ser Lys Asn Arg Arg Leu Val Ser Asp Lys Val Gly Gln
                165                 170                 175

Ala Cys Cys Arg Pro Ile Ala Tyr Asp Asp Asp Leu Ser Phe Leu Asp
            180                 185                 190
```

Asp Asn Leu Val Tyr His Ile Leu Arg Lys His Ser Ala Lys Arg Cys
195 200 205

Gly Cys Ile
210

<210> 47
<211> 636
<212> DNA
<213> Felis catus

<400> 47
atgaagttat gggatgtcgt ggctgtctgc ctggtgctgc tccacaccgc gtccgccttc 60

ccgctgcccg ccggtaagag gcctcccgag gcgcccgccg aagaccgctc cctcggccgc 120

cgccgcgcgc ccttcgcgct gagcagtgac tcaaatatgc cagaggatta tcctgatcag 180

tttgacgacg tcatggattt tattcaagct accatcagaa gactgaaaag gtcacccgag 240

aaacaaatgg ccttgccgcc tagaagagag cggaatcggc aggcggcggc cgccaacccg 300

gagaattcca gagggaaagg tcggcgaggc cagaggggca gaaatcgggg ttgtgtctta 360

actgcgatac atttgaacgt caccgacctg ggtttgggct acgaaaccaa ggaggaactg 420

atttttaggt actgcagcgg ctcctgtgat gcagctgaga caatgtacga caaaatatta 480

aaaaacttat ccaaaaacag aaggctggtg agtgacaaag tcgggcaggc atgttgcaga 540

cccatcgcct atgacgacga cctgtcgttt ttagatgaca acctggttta ccatattcta 600

agaaagcatt ccgctaaaag gtgtggatgt atctga 636

<210> 48
<211> 185
<212> PRT
<213> Equus caballus

<400> 48
Met Lys Leu Trp Asp Val Val Ala Val Cys Leu Val Leu Leu His Thr
1 5 10 15

Ala Ser Ala Phe Pro Leu Pro Ala Ala Asn Met Pro Glu Asp Tyr Pro
20 25 30

Asp Gln Phe Asp Asp Val Met Asp Phe Ile Gln Ala Thr Ile Lys Arg
35 40 45

Leu Lys Arg Ser Pro Asp Lys Gln Met Ala Val Leu Pro Arg Arg Glu

```
     50                  55                  60

Arg Asn Arg Gln Ala Ala Ala Ala Asn Pro Glu Asn Ser Arg Arg Lys
 65                  70                  75                  80

Gly Gln Arg Gly Gln Arg Gly Lys Asn Arg Gly Cys Val Leu Thr Ala
                 85                  90                  95

Ile His Leu Asn Val Thr Asp Leu Gly Leu Gly Tyr Glu Thr Lys Glu
            100                 105                 110

Glu Leu Ile Phe Arg Tyr Cys Ser Gly Ser Cys Glu Ala Ala Glu Thr
        115                 120                 125

Met Tyr Asp Lys Ile Leu Lys Asn Leu Ser Lys Asn Arg Arg Leu Val
    130                 135                 140

Ser Asp Lys Val Gly Gln Ala Cys Cys Arg Pro Ile Ala Phe Asp Asp
145                 150                 155                 160

Asp Leu Ser Phe Leu Asp Asp Asn Leu Val Tyr His Ile Leu Arg Lys
                165                 170                 175

His Ser Ala Lys Arg Cys Gly Cys Ile
            180                 185
```

<210> 49
<211> 558
<212> DNA
<213> Equus caballus

<400> 49

```
atgaagttat gggatgtcgt ggctgtctgc ctggtgctgc tccacaccgc gtccgccttc         60

ccgctgcccg ccgcaaatat gccagaggat tatcctgatc agtttgatga tgtcatggat        120

tttattcaag ccaccattaa aagactgaaa aggtcaccag ataaacaaat ggcagtgctt        180

cctagaagag agcggaatcg gcaggctgca gctgccaacc cggagaattc cagaaggaaa        240

ggtcagcgag gccagagggg caaaaaccgg ggttgtgtct taaccgcgat acatttaaat        300

gtcactgact tgggtttggg ctacgaaacc aaggaggaac tgatttttag gtactgcagt        360

ggctcctgcg aggcagccga gacaatgtac gacaaaatat taaaaaactt atccaaaaat        420

agaaggctgg tgagtgacaa agtagggcag gcatgttgca gacccatcgc cttcgatgac        480

gacctgtcat ttttagatga taacttggtt taccatattc taagaaagca ttccgctaaa        540

aggtgtggat gtatctga                                                      558
```