



(51) International Patent Classification:

A61K 48/00 (2006.01) C12N 15/113 (2010.01)

C12N 15/11 (2006.01) A61K 35/76 (2015.01)

C12N 9/22 (2006.01) C12N 15/86 (2006.01)

(21) International Application Number:

PCT/US2022/037490

(22) International Filing Date:

18 July 2022 (18.07.2022)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

PCT/US21/42100 16 July 2021 (16.07.2021) US

63/222,972 17 July 2021 (17.07.2021) US

63/300,822 19 January 2022 (19.01.2022) US

63/326,571 01 April 2022 (01.04.2022) US

(71) Applicant: ORTHOBIO THERAPEUTICS, INC.

[US/US]; P.O. Box 4532, Edwards, CO 81632 (US).

(72) Inventor; and

(71) Applicant: GENTSCH, George [GB/GB]; 12 Lents Way, Cambridge CB4 1UA (GB).

(72) Inventors: MILLETT, Peter J.; 975 Pilgrim Drive, Edwards, CO 81632 (US). RUSSELL, Iain Alasdair; 11 Headington Drive, Cambridge CB1 9HE (GB). ALLEN, Matthew J.; 33 High Street, Swaffham Bulbeck, Cambs CB25 0HP (GB).

(74) Agent: ANTCZAK, Andrew J. et al.; Morgan Lewis & Bockius LLP, 1701 Market Street, Philadelphia, PA 19103 (US).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, 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,

(54) Title: GENE EDITING TO IMPROVE JOINT FUNCTION

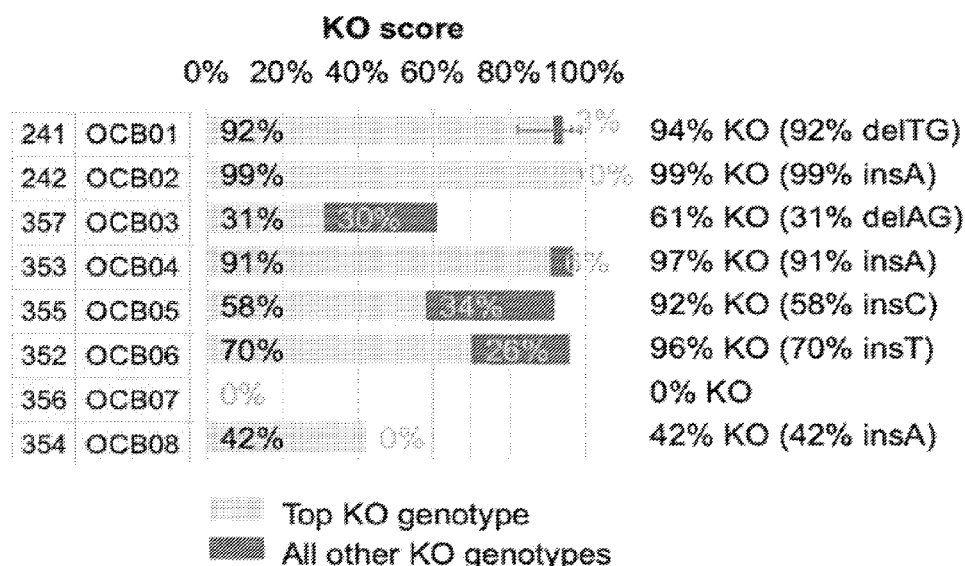


FIG. 33C

(57) **Abstract:** Treatment of osteoarthritis, degenerative joint disease, and other joint dysfunction is complex and there are few long term options for either symptomatic relief or restoring joint function. The present disclosure provides compositions and methods for treating joint disorders that are characterized by an inflammatory component. In some aspects, the compositions and methods are to prevent the progression of osteoarthritis and other arthritis and to treat osteoarthritis and other arthritis in a mammalian joint.





RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

- (84) Designated States** (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (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).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:

23 February 2023 (23.02.2023)

GENE EDITING TO IMPROVE JOINT FUNCTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/222,972, filed July 17, 2021, U.S. Provisional Patent Application No. 63/300,822, filed January 19, 2022, U.S. Provisional Patent Application No. 63/326,571, filed April 1, 2022, and International Application No. WO 2022/016121, filed July 16, 2021, the contents of which are hereby incorporated by reference, in their entireties, for all purposes.

FIELD

[0002] Compositions and Methods for treating synovial joint dysfunction are described herein. In addition, methods for gene-editing synovial cells and/or synoviocytes, chondrocytes, synovial macrophages, and synovial fibroblasts, and uses of gene-edited synovial cells and/or synoviocytes, chondrocytes, synovial macrophages, and synovial fibroblasts, in the treatment of diseases such as osteoarthritis are disclosed herein.

BACKGROUND

[0003] Treatment of osteoarthritis, degenerative joint disease, and other joint dysfunction is complex and there are few long term options for either symptomatic relief or restoring joint function. Osteoarthritis (OA) is the leading cause of disability due to pain. Neogi, *Osteoarthritis Cartilage* **2013**; 21:1145-53. All mammal species are affected: working animals, domestic pets, and their owners all suffer OA-related discomfort, pain, and disability, depending on the degree of disease progression.

[0004] OA is a complex disease characterized by a progressive course of disability. Systemic inflammation is associated with OA and with OA disease progression. Inflammation is driven by increased levels of pro-inflammatory cytokines. New methods and compositions to treat this disease are acutely needed. Disclosed herein are compositions and methods useful for treating OA as well as other inflammatory joint disorders.

SUMMARY

[0005] The present disclosure provides compositions and methods for treating joint disorders that are characterized by an inflammatory component. In some aspects, the

compositions and methods are to prevent the progression of osteoarthritis and other arthritides and to treat osteoarthritis and other arthritides in a mammalian joint. According to exemplary embodiments, at least a portion of the joint synovial cells and/or synoviocytes, chondrocytes, synovial macrophages, or synovial fibroblasts are gene-edited to reduce the expression of inflammatory cytokines. In some aspects, at least a portion of the joint synovial cells and/or synoviocytes, chondrocytes, synovial macrophages, or synovial fibroblasts, are gene-edited to reduce the expression of IL-1 α , IL-1 β , or both IL-1 α , IL-1 β .

[0006] In some embodiments, the gene-editing causes expression of one or more cytokine and/or growth factor genes to be silenced or reduced in at least a portion of the cells comprising a mammalian joint. In some aspects, the cells are synovial cells. In some aspects, the cells are synovial fibroblasts. In some aspects, the cells are synoviocytes. In some aspects, the cells are chondrocytes. In some aspects, the cells are synovial macrophages.

[0007] In some embodiments, the one or more cytokine and/or growth factor genes is/are selected from the group comprising IL-1 α , and IL-1 β .

[0008] In some embodiments, the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more cytokine and/or growth factor genes.

[0009] In some embodiments, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[0010] In some embodiments, the gene-editing comprises a CRISPR method.

[0011] In some embodiments, the CRISPR method is a CRISPR-Cas9 method.

[0012] In some embodiments, the gene-editing comprises a TALE method.

[0013] In some embodiments, the gene-editing comprises a zinc finger method.

[0014] In some embodiments, the gene-editing causes expression of one or more cytokine and/or growth factor genes to be silenced or reduced in at least a portion of the cells comprising the joint. In some embodiments, the portion of cells edited are synoviocytes. In an aspect, the portion of cells edited are synovial fibroblasts. In some embodiments, the portion of cells edited are synoviocytes. In some embodiments, the portion of cells edited are chondrocytes. In some embodiments, the portion of cells edited are synovial macrophages.

[0015] In some embodiments, an adeno-associated virus (AAV) delivery system is used to deliver the gene-editing system. In some embodiments, the AAV delivery system is injected into a joint.

[0016] Some aspects of the present disclosure provide a pharmaceutical composition for the treatment or prevention of a joint disease or condition comprising a gene-editing system and a pharmaceutically acceptable carrier. In an aspect, the gene-editing system comprises one or more nucleic acids targeting one or more genetic locus selected from the group consisting of IL-1 α , IL-1 β , TNF- α , IL-6, IL-8, and IL-18.

[0017] In some embodiments, the gene-editing system comprises a composition for the treatment or prevention of a joint disease or condition, comprising an RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease and at least one guide RNA or a nucleic acid encoding at least one guide RNA targeting an IL-1 α , or IL-1 β gene, wherein the guide RNA specifically binds a target sequence that is adjacent to a protospacer adjacent motif (PAM) sequence for the Cas9 protein.

[0018] In some embodiments, at least one guide RNA targets a human IL-1 α and comprises a crRNA sequence having at least 85%, 90%, 95%, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710.

[0019] In some embodiments, at least one guide RNA targets a human IL-1 β gene and comprises a crRNA sequence having at least 85%, 90%, 95%, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740.

[0020] In some embodiments, at least one guide RNA targets a canine IL-1 α gene and comprises a crRNA sequence having at least 85%, 90%, 95%, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770.

[0021] In some embodiments, at least one guide RNA targets a canine IL-1 β gene and comprises a crRNA sequence having at least 85%, 90%, 95%, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800.

[0022] In some embodiments, the gene-editing system comprises one or more lipid nanoparticles (LNP) collectively comprising an RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease and at least one guide RNA or the nucleic acid encoding the at least one guide RNA.

[0023] In some embodiments, the LNP comprise a first plurality of LNP comprising a first nucleic acid, encapsulating the nucleic acid encoding the RNA-guided nuclease; and a second

plurality of LNP comprising a second nucleic acid, encapsulating the nucleic acid encoding the at least one guide RNA.

[0024] In some embodiments, the LNP comprise a first plurality of LNP, encapsulating the RNA-guided nuclease and a second plurality of LNP comprising a second nucleic acid, encapsulating the nucleic acid encoding the at least one guide RNA.

[0025] In some embodiments, the LNP comprise a single nucleic acid, wherein the single nucleic acid encodes the RNA-guided nuclease and the at least one guide RNA.

[0026] In some embodiments, the gene-editing system comprises one or more liposomes collectively comprising the RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease and at least one guide RNA or the nucleic acid encoding the at least one guide RNA.

[0027] In some embodiments, the nucleic acid encoding the RNA-guided nuclease and/or the nucleic acid encoding the at least one guide RNA are present in a naked state.

[0028] In some embodiments, the RNA-guided nuclease in the gene-editing system is a Cas9 protein. In some embodiments, the Cas9 protein is spCas9. In some embodiments, the Cas9 protein is espCas9. In other embodiments, the Cas9 protein is saCas9.

[0029] An embodiment provides a method of treating canine lameness, the method comprising administering a gene-editing composition, wherein the composition causes expression of IL-1 α and IL-1 β to be silenced or reduced in a portion of a lame joint's synoviocytes, chondrocytes, synovial macrophages, or synovial fibroblasts.

[0030] An embodiment provides a method for treating a joint disease or condition in a subject in need thereof. In some embodiments, the joint disease or condition is arthritis. In some embodiments, the joint disease or condition is osteoarthritis.

[0031] In some embodiments, the gene-editing composition is formulated for parenteral administration. In some embodiments, the gene-editing composition is formulated for intra-articular injection within a joint of a subject.

[0032] In some embodiments, the above method further comprises one or more features recited in any of the methods and compositions described herein.

DESCRIPTION OF THE DRAWINGS

[0033] The presently disclosed embodiments will be further explained with reference to the attached drawings. The drawings shown are not necessarily to scale, with emphasis instead generally being placed upon illustrating the principles of the presently disclosed embodiments.

[0034] Fig. 1A illustrates an agarose gel electrophoresis analysis of 100 ng mouse DNA (gBlocks, Integrated DNA Technologies) designed against the *Mus musculus Il1a* and *Il1b* genes, cleaved by 0.5 µg *Spy*Cas9 (TrueCut™ Cas9 protein v2, ThermoFisher Scientific) and 200 ng Phosphorothioate-modified single guide (sg)RNAs targeted against the *Il1a* gene (#43-46) and *Il1b* gene (#47-50) *in vitro*.

[0035] Fig. 1B illustrates an agarose gel electrophoresis analysis of 100 ng mouse DNA (gBlocks, Integrated DNA Technologies) designed against the *Mus musculus Il1a* and *Il1b* genes, cleaved by 0.5 µg *Sau*Cas9 (GeneSnipper™ Cas9, BioVision) and 200 ng Phosphorothioate-modified guide sgRNAs against the *Il1a* (#51-53) and *Il1b* (#54-56) genes.

[0036] Figs. 2A, 2B, 2C, and 2D collectively illustrate graphs displaying editing efficiencies of *Spy*Cas9 and *Sau*Cas9 used with a range of guide RNA's in J774.2 ("J") and NIH3T3 ("N") cells; Fig. 2A: *in vivo* cleavage of *Il1a*, edited with 4 x sgRNAs (*Spy* Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); Fig. 2B: *in vivo* cleavage of *Il1b*, edited with 4 x sgRNAs (*Spy* Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); Fig. 2C: *in vivo* cleavage of *Il1a*, edited with 3 x sgRNAs (*Sau* Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); Fig. 2D: *in vivo* cleavage of *Il1b*, edited with 3 x sgRNAs (*Sau*Cas9) in two separate pools (Pool 1 and 2), across two cell lines, NIH 3T3 ("N"), and J774.2 ("J"); editing efficiencies determined using deconvolution of Sanger sequencing traces (ICE tool, Synthego) of each pool.

[0037] Fig. 3 illustrates GFP expression measured using the IVIS system. Flux values were based on a region of interest centred on the animal's injected knee joint. Data are presented as mean (SD) for four specimens per group.

[0038] Fig. 4 illustrates the design of a study as described in Example 5 of the present disclosure.

[0039] Fig. 5 illustrates the in-life outcome measurements obtained in a study as described in Example 5 of the present disclosure.

[0040] Fig. 6 illustrates the change in body weight of mice treated with an intra-articular (IA) injection of PBS, AAV-6 with a scrambled vector, AAV-6 with CRISPR-Cas guides 1 and 2, AAV-5 with a scrambled vector, or AAV-5 with CRISPR-Cas guides 1 and 2 in a study as described in Example 5 of the present disclosure.

[0041] Figs. 7A and 7B collectively illustrate (A) change in knee caliper measurements from baseline of mouse joints over time, and (B) mean difference in ankle caliper measurements with AUC in mice treated with an intra-articular (IA) injection of PBS, AAV-6 with a scrambled vector, AAV-6 with CRISPR-Cas guides 1 and 2, AAV-5 with a scrambled vector, or AAV-5 with CRISPR-Cas guides 1 and 2 in a study as described in Example 5 of the present disclosure.

[0042] Figs. 8A and 8B collectively illustrate (A) change in von Frey measurements, and (B) mean absolute threshold in von Frey measurements obtained from mice treated with an intra-articular (IA) injection of PBS, AAV-6 with a scrambled vector, AAV-6 with CRISPR-Cas guides 1 and 2, AAV-5 with a scrambled vector, or AAV-5 with CRISPR-Cas guides 1 and 2 in a study as described in Example 5 of the present disclosure.

[0043] Figs. 9 illustrate results of a qPCR assay for IL-1 β expression in synovial fluid obtained from mice treated with an intra-articular (IA) injection of PBS, AAV-6 with a scrambled vector, AAV-6 with CRISPR-Cas guides 1 and 2, AAV-5 with a scrambled vector, or AAV-5 with CRISPR-Cas guides 1 and 2 in a study as described in Example 5 of the present disclosure.

[0044] Figs. 10A, 10B, 10C, and 10D collectively illustrate immunohistochemistry for murine IL-1 β in synovial tissue of MSU injected animals (A, B) pre-treated with PBS, and (C, D) treated with CRISPR. Figures 10B and 10D show isotype controls for each of Figures 10A and 10C, respectively.

[0045] Figures 11A, 11B, and 11C collectively illustrate an alignment between the mouse, human, equine, feline, and canine IL-1 alpha genes.

[0046] Figures 12A, 12B, 12C, and 12D collectively illustrate an alignment between the mouse, human, equine, feline, and canine IL-1 beta genes.

[0047] Figures 13A, 13B, 13C, and 13D collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the human IL-1 alpha gene.

[0048] Figures 14A, 14B, 14C, 14D, and 14E collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the human IL-1 beta gene.

[0049] Figures 15A, 15B, and 15C collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the canine IL-1 alpha gene.

[0050] Figures 16A and 16B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the canine IL-1 beta gene.

[0051] Figures 17A, 17B, 17C, and 17D collectively illustrate the results of cell-based and in-silico gene editing analysis of crRNA sequences targeting the human IL-1 alpha gene (Fig. 7A), human IL-1 beta gene (Fig. 7B), canine IL-1 alpha gene (Fig. 7C), and canine IL-1 beta gene (Fig. 7D), as described in Example 8. ° CRISPR cut position within the translation frame of amino acids (AA). * Optimized score from Doench, Fusi et al. (2016). This score is optimized for 20bp guides with NGG. Score spans from 0 to 100. Higher is better. **

Specificity score from Hsu et al. (2013). Score spans from 0 to 100. Higher is better. ***

This score is based on experiments in U2OS. A high precision score (>0.4) implies that DNA repair outcomes are uniform and enriched for just a handful of unique genotypes. **** This score is based on experiments in U2OS. A high (>80%) frameshift frequency will tend to knock a protein-coding gene out of frame. The typical genomic frameshift frequency is above 66% because 1-bp insertions and 1-2 bp deletions are particularly common repair outcomes.

^ Combined score = (Off-target score + Precision score*100 + Frameshift)/3. † Pipe symbol '|' indicates CRISPR cut site. Curly braces '{}' indicate insertion. Hyphen '-' indicates deletion. \$ Potential off-target sites. Scoring according to Hsu et al. (2013). The on-target site has a score of 100.

[0052] Figures 18A, 18B, 18C, and 18D collectively illustrate canine IL-1 alpha (Figures 18A and 18B) and canine IL-1 beta (Figures 18C and 18D) release from non-edited (control) and double IL-1 α /IL-1 β KO (edited) canine chondrocytes 6 hours (Figures 18A and 18C) and 24 hours (Figures 18B and 18D) after exposure to PBS or LPS, as described in Example 9.

[0053] Figures 19A, 19B, 19C, and 19D collectively illustrate human IL-1 alpha (Figures 19A and 19B) and canine IL-1 beta (Figures 19C and 19D) release from non-edited (control) and double IL-1 α /IL-1 β KO (edited) canine chondrocytes 6 hours (Figures 19A and 19C) and 24 hours (Figures 19B and 19D) after exposure to PBS or LPS, as described in Example 9.

[0054] Figure 20 illustrates the results of a tissue-specific splicing and expression analysis of human IL1A (IL-1a) gene.

[0055] Figure 21 illustrates the results of a tissue-specific splicing and expression analysis of human IL1B (IL-1b) gene.

[0056] Figure 22 illustrates the results of an *in silico* analysis of human IL-1a- and IL-1b-targeted gRNA targeting domains. On-target score (*see* Doench et al.) is optimized for 20-bp gRNA with NGG protospacer adjacent motif (PAM). Score spans from 0 to 1. Precision score is based on experiments in U2OS cells. A high precision score (>0.4) implies that DNA repair outcomes are uniform and enriched for just a handful of unique genotypes. Frameshift percentage is based on experiments in U2OS cells. A high (>80%) frameshift frequency will tend to knock a protein-coding gene out of frame. The typical genomic frameshift frequency is above 66% because 1-bp insertions and 1-2 bp deletions are particularly common repair outcomes. Off-target score from CRISPOR assess the number of matches in the genome with a given number of mismatches. Mismatches in Seed sequence have a more deleterious effect.

[0057] Figures 23A, 23B, 23C, and 23D collectively illustrate results of splicing and functional analyses on canine IL1A (IL-1a) and IL1B (IL-1b) genes. The reference canine genome assembly (CanFam3.1) was used for these analyses. gRNA designs may be tailored to the individual breed, as necessary.

[0058] Figure 24 illustrates the results of an *in silico* analysis of canine IL-1a- and IL-1b-targeted gRNA targeting domains.

[0059] Figures 25A, 25B, and 25C collectively illustrate knockdown efficacy of selected gRNA targeting domains in human chondrocytes (Fig. 25A), canine chondrocytes (Fig. 25B) and synoviocytes (Fig. 25C). Genomic DNA was collected from pooled cells at 8-10 days post-administration prior to sequencing analysis.

[0060] Figure 26 illustrates the results of an *in silico* analysis of the off-target effects for lead candidate gRNA targeting domains in canine cells.

[0061] Figures 27A and 27B collectively illustrate the efficacy of enhanced-specificity Cas9 (espCas9) to abrogate the off-target editing of sgRNA 242 in canine cells. Fig. 27A shows the effects with canonical spCas9, resulting in strong on-target editing but high off-target activity also. Fig. 27B shows that espCas9 maintain the high on-target efficiency without off-target effects.

[0062] Figures 28A and 28B collectively illustrate editing activity for sgRNA targeting targeting IL-1a and IL-1b in human (A) and canine (B) chondrocytes.

[0063] Figure 29 illustrates results of co-administrating lead candidate sgRNAs in canine cells either simultaneously or sequentially.

[0064] Figures 30A and 30 B illustrate sequence alignments of IL1A (A) and IL1B (B) genomic sequences from human, equine, murine, and canine for potential cross-species targets with a single sgRNA.

[0065] Figures 31A, 31B, and 31C collectively illustrate CRISPR-mediated editing of canine IL-1A. Specifically, Figure 31A shows mean KO scores determined by inference of CRISPR edits from Sanger traces in canine chondrocytes (n=3+), fibroblast-like synoviocytes (n=3) and monocytes DH82 (n=2). Stacked bar graph shows the top and all remaining KO genotypes of sgRNAs assembled with wild-type Cas9 in light and dark, respectively. Figure 31B shows the amino acid sequence of wild-type and truncated canine IL1A prior to (top) and after (bottom) CRISPR-mediated genome editing. Wild-type IL1A includes a propeptide, which is cleaved off to make functional (mature) protein (highlighted). Figure 31C illustrates the predicted three-dimensional structure of wild-type and truncated IL1A, as predicted by AlphaFold2. N marks the N-terminus, the canonical translational start.

[0066] Figures 32A, 32B, and 32C collectively illustrate design and testing of eight canine IL1A-targeting sgRNAs: sg239/OCA01, sg360/OCA02, sg240/OCA03, sg359/OCA04, sg358/OCA05, sg251/OCA07, sg361/OCA06, and sg252/OCA08. Figure 32A shows the target sequence edited by the guide (equivalent to the crRNA sequence in the guide, except that the guide sequence includes uracil in place of thymidine). Figure 32B shows scores for predicted on-target, off-target, and frameshift effects for each of the gRNA, as described in Example 16. Figure 32C shows results of in vitro editing assays analyzed by sequencing of edited targets.

[0067] Figures 33A, 33B, 33C, and 33D collectively illustrate design and testing of eight canine IL1B-targeting sgRNAs: sg241/OCB01 (SEQ ID NO: XX), sg242/OCB02 (SEQ ID NO: XX), sg352/OCB06 (SEQ ID NO: XX), sg353/OCB04 (SEQ ID NO: XX), sg354/OCB08 (SEQ ID NO: XX), sg355/OCB05 (SEQ ID NO: XX), sg356/OCB07 (SEQ ID NO: XX), and sg357/OCB03 (SEQ ID NO: XX). Figure 33A shows the target sequence

edited by the guide (equivalent to the crRNA sequence in the guide, except that the guide sequence includes uracil in place of thymidine). Figure 32B shows scores for predicted on-target, off-target, and frameshift effects for each of the gRNA, as described in Example 17. Figure 32C shows results of in vitro editing assays analyzed by sequencing of edited targets. Figure 33D shows results of anti-IL-1B ELISA assays on the supernatant of canine monocytes targeted by the editing constructs.

[0068] Figures 34A and 34B collectively illustrate performance of IL1B-targeting sgRNA #241 (OCB01) and #242 (OCB02), as measured by mean IL1B KO scores inferred from Sanger-sequencing CRISPR-mediated genomic edits in chondrocytes, synoviocytes and monocytes DH82 (Figure 34A) and ELISA results for IL1B in either control or two IL1A/B double KO monocytes generated from different combinations of sgRNAs (Figure 34B; Sanger sequence-inferred KO scores for each combination at left).

[0069] Figures 35A and 35B collectively illustrate the impact of electroporation on canine monocytes with either OCB02/sg242 (KO1), or OCB01/sg241 (KO2), as measured by supernatant ELISA at either (A) 6 and (B) 24 hours following challenge with lipopolysaccharide (LPS).

[0070] Figure 36 illustrates a comparison of editing efficiencies for 5 potential gRNAs targeting canine IL-1 beta in monocytes, as measured by Abcam IL-1 beta ELISA kit.

[0071] Figures 37A and 37B collectively illustrate the effect of different Cas9 mutants with enhanced specificity, including (A) a comparison of on-target editing efficiency with the OCB02 gRNA plus either standard Cas9 or enhanced specificity/fidelity Cas9 variants and (B) a comparison of the on-target editing efficiency of OCB01 with the ARCas9 variant or wild-type Cas9.

[0072] Figures 38A and 38B collectively illustrate the in vitro performance of Cas9 mutants with enhanced specificity. Figure 38A illustrates editing efficiency for on-targets and off-targets, where percentages represent the KO scores and editing efficiencies, respectively. Figure 38B shows predicted off-target editing sites for OCB01.

[0073] Figures 39A and 39B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the human IL-1 alpha gene.

[0074] Figures 40A and 40B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the human IL-1 beta gene.

[0075] Figures 41A and 41B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the canine IL-1 alpha gene.

[0076] Figures 42A and 42B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the canine IL-1 beta gene.

[0077] Figures 43A and 43B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the equine IL-1 alpha gene.

[0078] Figures 44A and 44B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the equine IL-1 beta gene.

[0079] Figures 45A and 45B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the feline IL-1 alpha gene.

[0080] Figures 46A and 46B collectively illustrate example CRISPR/Cas9 crRNA sequences designed for editing the feline IL-1 beta gene.

[0081] While the above-identified drawing sets forth presently disclosed embodiments, other embodiments are also contemplated, as noted in the discussion. This disclosure presents illustrative embodiments by way of representation and not limitation. Numerous other modifications and embodiments can be devised by those skilled in the art which fall within the scope and spirit of the principles of the presently disclosed embodiments.

DETAILED DESCRIPTION

[0082] As described herein, embodiments of the present disclosure provide compositions and methods for improving joint function and treating joint disease. In particular embodiments, compositions and methods are provided to gene-edit synovial fibroblasts, synoviocytes, chondrocytes, or synovial macrophages to reduce expression of inflammatory cytokines, for example, IL-1 α , IL-1 β , TNF- α , IL-6, IL-8, IL-18, one or more matrix metalloproteinases (MMPs), or one or more component of the NLRP3 inflammasome. Embodiments are used for treating osteoarthritis and other inflammatory joint diseases. Embodiments are further useful for treating canine lameness due to osteoarthritis. Embodiments are further useful for treating equine lameness due to joint disease. Embodiments are also useful for treating post-traumatic

arthritis, gout, pseudogout, and other inflammation-mediated or immune-mediated joint diseases.

Definitions

[0083] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this disclosure belongs. All patents and publications referred to herein are incorporated by reference in their entireties.

[0084] The term “*in vivo*” refers to an event that takes place in a subject’s body.

[0085] The term “*in vitro*” refers to an event that takes places outside of a subject’s body. In vitro assays encompass cell-based assays in which cells alive or dead are employed and may also encompass a cell-free assay in which no intact cells are employed.

[0086] The term “*ex vivo*” refers to an event which involves treating or performing a procedure on a cell, tissue and/or organ which has been removed from a subject’s body. Aptly, the cell, tissue and/or organ may be returned to the subject’s body in a method of surgery or treatment.

[0087] The term “IL-1” (also referred to herein as “IL1”) refers to the pro-inflammatory cytokine known as interleukin-1, and includes all forms of IL-1, including IL1- α and IL-1 β , human and mammalian forms, conservative amino acid substitutions, glycoforms, biosimilars, and variants thereof. IL-1 α and IL-1 β bind to the same receptor molecule, which is called type I IL-1 receptor (IL-1R1). There is a third ligand of this receptor: Interleukin 1 receptor antagonist (IL-1Ra), which does not activate downstream signaling; therefore, acting as an inhibitor of IL-1 α and IL-1 β signaling by competing with them for binding sites of the receptor. *See, e.g., Dinarello, Blood* **117**: 3720–32 (2011) and Weber *et al., Science Signaling* **3**(105): cm1, doi:10.1126/scisignal.3105cm1. IL-1 is described, *e.g., in Dinarello, Cytokine Growth Factor Rev.* **8**:253-65 (1997), the disclosures of which are incorporated by reference herein. For example, the term IL-1 encompasses human, recombinant forms of IL-1.

Table 1. Example amino acid sequences.

Identifier	Sequence (One-Letter Amino Acid Symbols)				
SEQ ID NO:1 recombinant human IL-1 α (rhIL-1 α)	10 MAKVPMDFED 60 SVLSISSETS 110 ANDSEEEIIK 160 YLTAAALHNL 210 PVLLKEMPEI 260 WVCLAGGPPS	20 LKNCYSENEE 70 KTSKLTFKES 120 PRSAPFSFLS 170 DEAVKFDMDGA 220 PKTITGSETN 270 ITDFQILENQ	30 DSSSIDHLSL 80 MVVATNGKV 130 NVKYNFMRII 180 YKSSKDDAKI 230 LLFFWETHGT A	40 NQKSFYHVSY 90 LKKRRLSLSQ 140 KYEFILNDAL 190 TVILRISKTD 240 KNYFTSVAHP NLF	50 GPLHEGCMDQ 100 SITDDDLLEAI 150 NQSIIRANDQ 200 LYVTAQDEDQ 250 NLFIATKQDY
SEQ ID NO:2 recombinant human IL-1 β (rhIL-1 β)	10 MAEVPDLASE 60 RISDHHYSKG 110 EPIFFDTWDN 160 DMEQQVVFMS 210 VDPKNYPKKK 260 GGTKGGQDIT	20 MMAYYSGNED 70 FRQAASVVVA 120 EAYVHDAPVR 170 SFVQGEESND 220 MEKRFVFNKI DFTMQFVSS	30 DLFFEADGPK 80 MDKLRKMLVP 130 SLNCTLRDSQ 180 KIPVALGLKE 230 EINNKEFES	40 QMKCSFQDLD 90 CPQTFQENDL 140 QKSLVMSGPY 190 KNLYLSCVLK 240 AQFPNWIYST	50 LCPLDGGIQL 100 STFFPFIFEE 150 ELKALHLQGG 200 DDKPTLQLES 250 SQAENMPVFL
SEQ ID NO:3 recombinant mouse IL-1 α (rmIL-1 α)	10 MAKVPMDFED 60 FVSLRTSETS 110 QSITHDLEET 160 DKHYLSTTWL 210 EDQPVLLKEL 260 EQSRVHLARG	20 LKNCYSENEE 70 KMSNFTFKES 120 IQPRSAPTY 170 NDLQQEVKFD 220 PETPKLITGS LPSMTDFQIS	30 YSSAIDHLSL 80 RVTVSATSSN 130 QSDLRKMLK 180 MYAYSSGGDD 230 ETDLIFFWKS	40 NQKSFYDASY 90 GKILKRRRLS 140 LVRQKFVMMND 190 SKYPVTLKIS 240 INSKNYFTSA	50 GSLHETCTDQ 100 FSETFTEDDL 150 SLNQTIYQDV 200 DSQLFVSAQG 250 AYPELFIATK
SEQ ID NO:4 recombinant mouse IL-1 β (rmIL-1 β)	10 MATVPELNCE 60 ISQQHINKSF 110 PILCDSWDDD 160 QNINQQVIFS 210 SVDPKQYPPK 260 LGNNSGQDII	20 MPPFDSDEED 70 RQAVSLIVAV 120 DNLLVCDVPI 170 MSFVQGEPSN 220 KMEKRFVFNK DFTMESVSS	30 LFFEVDGPQK 80 EKLWQLPVSF 130 RQLHYRLRDE 180 DKIPVALGLK 230 IEVKSKEVEFE	40 MKGCFQTFDL 90 PWTFQDEDMS 140 QQKSLVLSDP 190 GKNLYLSCVM 240 SAEFPNWIYS	50 GCPDESIQLQ 100 TFFSFIFBEE 150 YELKALHLNG 200 KDGTPTLQLE 250 TSQAEHKPVF
SEQ ID NO:5 recombinant human IL-1 receptor antagonist (rhIL-1Ra)	10 MEICRGLRSH 60 RNNQLVAGYL 110 TRLQLEAVNI 160 EADQPVSLTN	20 LITLLFLFLH 70 QGPNVNLEEK 120 TDLSENKQD 170 MPDEGVMVTK	30 SETICRPSGR 80 IDVVPIDPHA 130 KRFAFIRSDS FYFQEDE	40 KSSKMQAARI 90 LFLGIHGGKM 140 GPTTSFESAA	50 WDVNQKTFYL 100 CLSCVKSGDE 150 CPGWFLCTAM
SEQ ID NO:6 recombinant mouse IL-1 receptor antagonist (rmIL-1Ra)	10 MEICWGPYSH 60 LRNNQLIAGY 110 DIKLQLEEVN 160 LEADRPVSLT	20 LISLLLILLF 70 LQGPNIKLEE 120 ITDLSKNKEE 170 NTPEEPLIVT	30 HSEAACRPSG 80 KIDMVPIDLI 130 DKRFTFIRSE KFYFQEDQ	40 KRPMQAFRI 90 SVFLGIHGGK 140 KGPTTSFESA	50 IWDTNQKTFY 100 LCLSCAKSGD 150 ACPGWFLCTT

[0088] The term “NLRP3 inflammasome” refers to the multiprotein complex responsible for the activation of some inflammatory responses. The NLRP3 inflammasome promotes the production of functional pro-inflammatory cytokines, for example, IL-1 β and IL-18. Core components of the NLRP3 inflammasome are NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD), and caspase-1, as described by Lee *et al.*, *Lipids Health Dis.* **16**:271 (2017) and Gros Lambert and Py, *J. Inflamm. Res.* **11**:359-374 (2018).

[0089] The terms “matrix metalloproteinase” and “MMP” are defined to be any one of the members of the matrix metalloproteinase family of zinc-endoropeptidases, for example, as characterized by Fanjul-Fernandez *et al.*, *Biochem. Biophys. Acta* **1803**:3-19 (2010). In the art, family members are frequently referred to as archetypical MMPs, gelatinases, matrilysins, and/or furin-activatable MMPs. As used herein, the “matrix metalloproteinase” and “MMP” encompass the entire MMP family, including, but not limited to MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-18, MMP-19, MMP-20, MMP-21, MMP-23, MMP-25, MMP-26, MMP-27 and MMP-28.

[0090] The terms “co-administration,” “co-administering,” “administered in combination with,” “administering in combination with,” “simultaneous,” and “concurrent,” as used herein, encompass administration of two or more active pharmaceutical ingredients (in a preferred embodiment of the present disclosure, for example, at least one anti-inflammatory compound in combination with a viral vector functionally engineered to deliver a gene-editing nucleic acid as described herein) to a subject so that both active pharmaceutical ingredients and/or their metabolites are present in the subject at the same time. Co-administration includes simultaneous administration in separate compositions, administration at different times in separate compositions, or administration in a composition in which two or more active pharmaceutical ingredients are present. Simultaneous administration in separate compositions and administration in a composition in which both agents are present are preferred.

[0091] The term “effective amount” or “therapeutically effective amount” refers to that amount of a composition or combination of compositions as described herein that is sufficient to effect the intended application including, but not limited to, disease treatment. A therapeutically effective amount may vary depending upon the intended application (*in vitro* or *in vivo*), or the subject and disease condition being treated (*e.g.*, the weight, age and gender

of the subject), the severity of the disease condition, or the manner of administration. The term also applies to a dose that will induce a particular response in target cells (*e.g.*, the reduction of platelet adhesion and/or cell migration). The specific dose will vary depending on the particular compositions chosen, the dosing regimen to be followed, whether the composition is administered in combination with other compositions or compounds, timing of administration, the tissue to which it is administered, and the physical delivery system in which the composition is carried.

[0092] The terms “treatment”, “treating”, “treat”, and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. For example, a composition, method, or system of the present disclosure may be administered as a prophylactic treatment to a subject that has a predisposition for a given condition (*e.g.*, arthritis). “Treatment”, as used herein, covers any treatment of a disease in a mammal, particularly in a human, canine, feline, or equine, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development or progression; and (c) relieving the disease, *i.e.*, causing regression of the disease and/or relieving one or more disease symptoms. “Treatment” is also meant to encompass delivery of an agent in order to provide for a pharmacologic effect, even in the absence of a disease or condition. For example, “treatment” encompasses delivery of a composition that can elicit an immune response or confer immunity in the absence of a disease condition, *e.g.*, in the case of a vaccine. It is understood that compositions and methods of the present disclosure are applicable to treat all mammals, including, but not limited to human, canine, feline, equine, and bovine subjects.

[0093] The term “heterologous” when used with reference to portions of a nucleic acid or protein indicates that the nucleic acid or protein comprises two or more subsequences that are not found in the same relationship to each other in nature. For instance, the nucleic acid is typically recombinantly produced, having two or more sequences from unrelated genes arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a coding region from another source, or coding regions from different sources. Similarly, a

heterologous protein indicates that the protein comprises two or more subsequences that are not found in the same relationship to each other in nature (*e.g.*, a fusion protein).

[0094] The terms “polynucleotide,” “nucleotide,” and “nucleic acid” are used interchangeably herein to refer to all forms of nucleic acid, oligonucleotides, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Polynucleotides include genomic DNA, cDNA and antisense DNA, and spliced or unspliced mRNA, rRNA, tRNA, lncRNA, RNA antagomirs, and inhibitory DNA or RNA (RNAi, *e.g.*, small or short hairpin (sh)RNA, microRNA (miRNA), aptamers, small or short interfering (si)RNA, trans-splicing RNA, or antisense RNA). Polynucleotides also include non-coding RNA, which include for example, but are not limited to, RNAi, miRNAs, lncRNAs, RNA antagomirs, aptamers, and any other non-coding RNAs known to those of skill in the art. Polynucleotides include naturally occurring, synthetic, and intentionally altered or modified polynucleotides as well as analogues and derivatives. The term “polynucleotide” also refers to a polymeric form of nucleotides of any length, including deoxyribonucleotides or ribonucleotides, or analogs thereof, and is synonymous with nucleic acid sequence. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment as described herein encompassing a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form. Polynucleotides can be single, double, or triplex, linear or circular, and can be of any length. In discussing polynucleotides, a sequence or structure of a particular polynucleotide may be described herein according to the convention of providing the sequence in the 5’ to 3’ direction.

[0095] The term “gene” or “nucleotide sequence encoding a polypeptide” refers to the segment of DNA involved in producing a polypeptide chain. The DNA segment may include regions preceding and following the coding region (leader and trailer) involved in the transcription/translation of the gene product and the regulation of the transcription/translation, as well as intervening sequences (introns) between individual coding

segments (exons). For example, a gene includes a polynucleotide containing at least one open reading frame capable of encoding a particular protein or polypeptide after being transcribed and translated.

[0096] The term “homologous” in terms of a nucleotide sequence includes a nucleotide (nucleic acid) sequence that is either identical or substantially similar to a known reference sequence. In one embodiment, the term “homologous nucleotide sequence” is used to characterize a sequence having nucleic acid sequence that is at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to a known reference sequence.

[0097] “Heterologous” means derived from a genotypically distinct entity from the rest of the entity to which it is being compared to. For example, a polynucleotide introduced by genetic engineering techniques into a plasmid or vector derived from a different species is a heterologous polynucleotide. A promoter removed from its native coding sequence and operatively linked to a coding sequence it is not naturally found linked to a heterologous promoter. Although the term “heterologous” is not always used herein in reference to polynucleotides, reference to a polynucleotide even in the absence of the modifier “heterologous” is intended to include heterologous polynucleotides in spite of the omission.

[0098] The terms “sequence identity,” “percent identity,” and “sequence percent identity” (or synonyms thereof, *e.g.*, “99% identical”) in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software are known in the art that can be used to obtain alignments of amino acid or nucleotide sequences. Suitable programs to determine percent sequence identity include for example the BLAST suite of programs available from the U.S. Government’s National Center for Biotechnology Information BLAST web site. Comparisons between two sequences can be carried using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. ALIGN, ALIGN-2 (Genentech, South San Francisco,

California) or MegAlign, available from DNASTAR, are additional publicly available software programs that can be used to align sequences. ClustalW and ClustalX may be used to produce alignments, Larkin *et al.*, *Bioinformatics* 23:2947-2948 (2007); Goujon *et al.*, *Nucleic Acids Research*, 38 Suppl:W 695-9 (2010); and, McWilliam *et al.*, *Nucleic Acids Research* 41(Web Server issue):W 597-600 (2013). One skilled in the art can determine appropriate parameters for maximal alignment by particular alignment software. In certain embodiments, the default parameters of the alignment software are used.

[0099] As used herein, the term “variant” encompasses but is not limited to antibodies or fusion proteins which comprise an amino acid sequence which differs from the amino acid sequence of a reference antibody by way of one or more substitutions, deletions and/or additions at certain positions within or adjacent to the amino acid sequence of the reference antibody. The variant may comprise one or more conservative substitutions in its amino acid sequence as compared to the amino acid sequence of a reference antibody. Conservative substitutions may involve, *e.g.*, the substitution of similarly charged or uncharged amino acids. The variant retains the ability to specifically bind to the antigen of the reference antibody. The term variant also includes pegylated antibodies or proteins.

[00100] “Joint disease” is defined as measurable abnormalities in the cells or tissues of the joint that could lead to illness, for example, metabolic and molecular derangements triggering anatomical and/or physiological changes in the joint. Including, but not limited to, radiographic detection of joint space narrowing, subchondral sclerosis, subchondral cysts, and osteophyte formation.

[00101] “Joint illness” is defined in human subjects as symptoms that drive the subject to seek medical intervention, for example, subject reported pain, stiffness, swelling, or immobility. For non-human mammals, “joint illness” is defined, for example, as lameness, observable changes in gait, weight bearing, allodynia, or exploratory behavior.

[00102] As used herein, a sgRNA (single guide RNA) is a RNA, preferably a synthetic RNA, composed of a targeting sequence and scaffold. It is used to guide Cas9 to a specific genomic locus in genome engineering experiments. The sgRNA can be administered or formulated, *e.g.*, as a synthetic RNA, or as a nucleic acid comprising a sequence encoding the gRNA, which is then expressed in the target cells. As would be evident to one of ordinary skill in the art, various tools may be used to design and/or optimize the sequence of a sgRNA, for example to increase the specificity and/or precision of genomic editing. In general,

candidate sgRNAs may be designed by identifying a sequence within the target region that has a high predicted on-target efficiency and low off-target efficiency based on any of the available web-based tools. Candidate sgRNAs may be further assessed by manual inspection and/or experimental screening. Examples of web-based tools include, without limitation, CRISPR seek, CRISPR Design Tool, Cas-OFFinder, E-CRISP, ChopChop, CasOT, CRISPR direct, CRISPOR, BREAKING-CAS, CrispRGold, and CCTop. See, e.g., Safari, et al. *Current Pharma. Biotechnol.* (2017) 18(13), which is incorporated by reference herein in its entirety for all purposes. Such tools are also described, for example, in PCT Publication No. WO2014093701A1 and Liu, et al., “Computational approached for effective CRISPR guide RNA design and evaluation”, *Comput Struct Biotechnol J.*, 2020; 18: 35–44, each of which is incorporated by reference herein in its entirety for all purposes.

[00103] As used herein, “Cas9” refers to CRISPR Associated Protein; the Cas9 nuclease is the active enzyme for the Type II CRISPR system. “nCas9” refers to a Cas9 that has one of the two nuclease domains inactivated, i.e., either the RuvC or HNH domain. nCas9 is capable of cleaving only one strand of target DNA (a “nickase”). The term “Cas9” refers to an RNA-guided double-stranded DNA-binding nuclease protein or nickase protein, or a variant thereof. Herein, “Cas9” refers to both naturally-occurring and recombinant Cas9s. Wild-type Cas9 nuclease has two functional domains, e.g., RuvC and HNH, that cut different DNA strands. Cas9 enzymes described herein can comprise a HNH or HNH-like nuclease domain and/or a RuvC or RuvC-like nuclease domain. Cas9 can induce double-strand breaks in genomic DNA (target locus) when both functional domains are active. The Cas9 enzyme can comprise one or more catalytic domains of a Cas9 protein derived from bacteria belonging to the group consisting of *Corynebacter*, *Sutterella*, *Legionella*, *Treponema*, *Filifactor*, *Eubacterium*, *Streptococcus*, *Lactobacillus*, *Mycoplasma*, *Bacteroides*, *Flaviivola*, *Flavobacterium*, *Sphaerochaeta*, *Azospirillum*, *Gluconacetobacter*, *Neisseria*, *Roseburia*, *Parvibaculum*, *Staphylococcus*, *Nitratifactor*, and *Campylobacter*. In some embodiments, the two catalytic domains are derived from different bacteria species.

[00104] As used herein, “PAM” refers to a Protospacer Adjacent Motif and is necessary for Cas9 to bind target DNA, and immediately follows the target sequence. The Cas9 can be administered or formulated, e.g., as a protein (e.g., a recombinant protein), or as a nucleic acid comprising a sequence encoding the Cas9 protein, which is then expressed in the target cells. Naturally occurring Cas9 molecules recognize specific PAM sequences (e.g.,

the PAM recognition sequences for *S. pyogenes*, *S. thermophilus*, *S. mutans*, *S. aureus* and *N. meningitidis*). In an embodiment, a Cas9 molecule has the same PAM specificities as a naturally occurring Cas9 molecule. In other embodiments, a Cas9 molecule has a PAM specificity not associated with a naturally occurring Cas9 molecule. In other embodiments, a Cas9 molecule's PAM specificity is not associated with the naturally occurring Cas9 molecule to which it has the closest sequence homology. For example, a naturally occurring Cas9 molecule can be altered such that the PAM sequence recognition is altered to decrease off target sites, improve specificity, or eliminate a PAM recognition requirement. In an embodiment, a Cas9 molecule may be altered (e.g., to lengthen a PAM recognition sequence, improve Cas9 specificity to high level of identity, to decrease off target sites, and/or increase specificity). In an embodiment, the length of the PAM recognition sequence is at least 4, 5, 6, 7, 8, 9, 10 or 15 amino acids in length. In some embodiments, a Cas9 molecule may be altered to ablate PAM recognition.

[00105] An “expression cassette” is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular polynucleotide sequence in a host cell. An expression cassette or vector may be part of a plasmid, viral genome, or nucleic acid fragment. Typically, an expression cassette or vector includes a polynucleotide to be transcribed, operably linked to a promoter.

[00106] The term “promoter” is used herein to refer to an array of nucleic acid control sequences that direct transcription of a nucleic acid. As used herein, a promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. Other elements that may be present in an expression vector include those that enhance transcription (e.g., enhancers) and terminate transcription (e.g., terminators), as well as those that confer certain binding affinity or antigenicity to the recombinant protein produced from the expression vector.

[00107] The term “operably linked” refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate

transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[00108] An “isolated” plasmid, nucleic acid, vector, virus, virion, host cell, or other substance refers to a preparation of the substance devoid of at least some of the other components present where the substance or a similar substance naturally occurs or from which it is initially prepared. Thus, for example, an isolated substance may be prepared by using a purification technique to enrich it from a source mixture. Enrichment can be measured on an absolute basis, such as weight per volume of solution, or it can be measured in relation to a second, potentially interfering substance present in the source mixture. Increasing enrichments of the embodiments of this disclosure are increasingly more isolated. An isolated plasmid, nucleic acid, vector, virus, host cell, or other substance is in some embodiments purified, *e.g.*, from about 80% to about 90% pure, at least about 90% pure, at least about 95% pure, at least about 98% pure, or at least about 99%, or more, pure.

[00109] An “AAV vector” as used herein refers to an AAV vector nucleic acid sequence encoding for various nucleic acid sequences, including in some embodiments a variant or chimeric capsid polypeptide (*i.e.*, the AAV vector comprises a nucleic acid sequence encoding for a variant or chimeric capsid polypeptide). AAV vectors can also comprise a heterologous nucleic acid sequence not of AAV origin as part of the nucleic acid insert. This heterologous nucleic acid sequence typically comprises a sequence of interest for the genetic transformation of a cell. In general, the heterologous nucleic acid sequence is flanked by at least one, and generally by two AAV inverted terminal repeat sequences (ITRs). In certain embodiments, a Cas sequence, a guide RNA sequence, and any other genetic element (*e.g.*, a promoter sequence, a PAM sequence, and the like) may be on the same AAV vector or on two or more different AAV vectors when administered to a subject. In certain embodiments, a Cas sequence, a guide RNA sequence, and any other genetic element (*e.g.*, a promoter sequence, a PAM sequence, and the like) may be on two or more different AAV vectors when administered to a subject, and the AAV may be the same serotype, or the AAV may be two or more different serotypes (*e.g.*, AAV5 and AAV6).

[00110] An “AAV virion” or “AAV virus” or “AAV viral particle” or “AAV vector particle” refers to a viral particle composed of at least one AAV capsid polypeptide and an encapsidated polynucleotide AAV transfer vector. If the particle comprises a heterologous

nucleic acid (*i.e.* a polynucleotide other than a wild-type AAV genome, such as a transgene to be delivered to a cell), it can be referred to as an “AAV vector particle” or simply an “AAV vector”. Thus, production of AAV virion or AAV particle necessarily includes production of AAV vector as such a vector is contained within an AAV virion or AAV particle.

[00111] “Carrier” or “vehicle” as used herein refer to carrier materials suitable for drug administration. Carriers and vehicles useful herein include any such materials known in the art, e.g., any liquid, gel, solvent, liquid diluent, solubilizer, surfactant, or the like, which is nontoxic and which does not interact with other components of the composition in a deleterious manner.

[00112] The phrase “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

[00113] The terms “pharmaceutically acceptable carrier” or “pharmaceutically acceptable excipient” are intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and inert ingredients. The use of such pharmaceutically acceptable carriers or pharmaceutically acceptable excipients for active pharmaceutical ingredients is well known in the art. Except insofar as any conventional pharmaceutically acceptable carrier or pharmaceutically acceptable excipient is incompatible with the active pharmaceutical ingredient, its use in the therapeutic compositions of the disclosure is contemplated. Additional active pharmaceutical ingredients, such as other drugs, can also be incorporated into the described compositions and methods.

[00114] The term “pharmaceutically acceptable excipient” is intended to include vehicles and carriers capable of being co-administered with a compound to facilitate the performance of its intended function. The use of such media for pharmaceutically active substances is well known in the art. Examples of such vehicles and carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. Any other conventional carrier suitable for use with the multi-binding compounds also falls within the scope of the present disclosure.

[00115] As used herein, the term “a”, “an”, or “the” generally is construed to cover both the singular and the plural forms.

[00116] The terms “about” and “approximately” mean within a statistically meaningful range of a value. Such a range can be within an order of magnitude, preferably within 50%, more preferably within 20%, more preferably still within 10%, and even more preferably within 5% of a given value or range. The allowable variation encompassed by the terms “about” or “approximately” depends on the particular system under study, and can be readily appreciated by one of ordinary skill in the art. Moreover, as used herein, the terms “about” and “approximately” mean that compositions, amounts, formulations, parameters, shapes and other quantities and characteristics are not and need not be exact, but may be approximate and/or larger or smaller, as desired, reflecting tolerances, conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art. In general, a dimension, size, formulation, parameter, shape or other quantity or characteristic is “about” or “approximate” whether or not expressly stated to be such. It is noted that embodiments of very different sizes, shapes and dimensions may employ the described arrangements.

[00117] The term “substantially” as used herein can refer to a majority of, or mostly, as in at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, 99.99%, or at least about 99.999% or more.

[00118] The transitional terms “comprising,” “consisting essentially of,” and “consisting of,” when used in the appended claims, in original and amended form, define the claim scope with respect to what unrecited additional claim elements or steps, if any, are excluded from the scope of the claim(s). The term “comprising” is intended to be inclusive or open-ended and does not exclude any additional, unrecited element, method, step or material. The term “consisting of” excludes any element, step or material other than those specified in the claim and, in the latter instance, impurities ordinary associated with the specified material(s). The term “consisting essentially of” limits the scope of a claim to the specified elements, steps or material(s) and those that do not materially affect the basic and novel characteristic(s) of the claimed methods and compositions. All compositions, methods, and kits described herein that embody the present disclosure can, in alternate embodiments, be more specifically defined by any of the transitional terms “comprising,” “consisting essentially of,” and “consisting of.”

[00119] A subject treated by any of the methods or compositions described herein can be of any age and can be an adult, infant or child. In some cases, the subject is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 years old, or within a range therein (e.g., without limitation, between 2 and 20 years old, between 20 and 40 years old, or between 40 and 90 years old). The subject can be a human or non-human subject. A particular class of subjects that can benefit from the compositions and methods of the present disclosure include subjects over the age of 40, 50, or 60 years. Another class of subjects that can benefit from the compositions and methods of the present disclosure are subjects that have arthritis (e.g., osteoarthritis).

[00120] Any of the compositions disclosed herein can be administered to a non-human subject, such as a laboratory or farm animal. Non-limiting examples of a non-human subject include laboratory or research animals, pets, wild or domestic animals, farm animals, etc., e.g., a dog, a goat, a guinea pig, a hamster, a mouse, a pig, a non-human primate (e.g., a gorilla, an ape, an orangutan, a lemur, a baboon, etc.), a rat, a sheep, a horse, a cow, or the like.

[00121] CRISPR/Cas Systems – Minimum Requirements

[00122] In one aspect, clustered regularly interspaced short palindromic repeats and CRISPR-associated RNA-guided nuclease-related methods, components and compositions of the disclosure (hereafter, CRISPR/Cas systems) minimally require at least one isolated or non-naturally-occurring protein component (e.g., a Cas protein) and at least one isolated or non-naturally-occurring nucleic acid component (e.g., a guide RNA (gRNA)) to effectuate augmentation of a nucleic acid sequence (e.g., genomic DNA).

[00123] In some embodiments, a CRISPR/Cas system effectuates the alteration of a targeted gene or locus in a eukaryotic cell by effecting an alteration of the sequence at a target position (e.g., by creating an insertion or deletion (collectively, an indel) resulting in loss-of-function of (i.e., knocking out) the affected gene or allele; e.g., a nucleotide substitution resulting in a truncation, nonsense mutation, or other type of loss-of-function of an encoded gene product of, for example, the encoded IL1A or IL1B mRNA or protein; a

deletion of one or more nucleotides resulting in a truncation, nonsense mutation, or other type of loss-of-function of, for example, an encoded IL1A or IL1B gene product; e.g., loss-of-function of the encoded mRNA or protein by a single nucleotide, double nucleotide, or other frame-shifting deletion, or a deletion resulting in a premature stop codon; or an insertion resulting in a truncation, nonsense mutation, or other type of loss-of-function of an encoded gene product, such as, for example, the encoded IL1A or IL1B mRNA or protein; e.g., a single nucleotide, double nucleotide, or other frame-shifting insertions, or an insertion resulting in a premature stop codon. In some embodiments, a CRISPR/Cas system of the present disclosure provides for the alteration (e.g., knocking out) of a gene associated with inflammatory joint diseases (e.g., rheumatoid arthritis or osteoarthritis) by altering the sequence at a target position, e.g., creating an indel that results in nonsense-mediated decay of an encoded gene product, e.g., an encoded transcript.

[00124] In one aspect, CRISPR/Cas systems effectuate changes to the sequence of a nucleic acid through nuclease activity. For example, in the case of genomic DNA, the nuclease—guided by a protein-associated exogenous nucleic acid that locates a target position within a targeted gene or locus by sequence complementarity with the target genomic sequence (e.g., CRISPR RNA (crRNA) or a complementary component of a synthetic single guide RNA (sgRNA))—cleaves the genomic DNA upon recognition of particular, nuclease-specific motif called the protospacer adjacent motif (PAM). See generally, Collias, D., & Beisel, C. L. (2021). *Nature Communications*, 12(1), 1-12.

[00125] Nuclease activity (i.e., cleavage) induces a double-strand break (DSB) in the case of genomic DNA. Endogenous cellular mechanisms of DSB repair, namely non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), and homologous recombination, result in erroneous repair at a given target position with some calculable frequency as a result of interference from said components of the CRISPR/Cas system, thereby introducing substitutions or indels into the genomic DNA. See generally Scully, R., et al. (2019). *Nature Reviews Molecular Cell Biology*, 20(11), 698-714. At some frequency, these indels and/or substitutions may result in frameshifts, nonsense mutations (i.e., early stop codons) or truncations that impact the availability of gene products, such as mRNA and/or protein. In certain embodiments, the CRISPR/Cas system may induce a homology-directed repair (HDR) mechanism leading to insertions of non-random sequences

at a target position through the use of templates (e.g., an HDR template) provided to the cell as part of the system along with the nuclease and gRNA. See Bloh, K., & Rivera-Torres, N. (2021). *International Journal of Molecular Sciences*, 22(8), 3834.

[00126] In general, the minimum requirements of the CRISPR/Cas system will be dependent upon the nuclease (i.e., Cas protein) provided therewith. To this extent, these bacterially-derived nucleases have been functionally divided into Types I, III, and V, which all fall into Class 1 and Types II, IV, and VI that are grouped into Class 2.

[00127] Class 1 CRISPR/Cas systems:

[00128] The exact components, compositions, and methods for effectuating a change in a targeted nucleic acid sequence using a Class 1 CRISPR/Cas system will vary, but should minimally include: a nuclease (selected from at least Types I, and III), at least one guide RNA selected from 1) sgRNA or 2) a combination of crRNA and tracrRNA. These CRISPR/Cas systems have been categorized together as Class 1 CRISPR/Cas systems due to their similarities in requirements and mode of action within a eukaryotic cell. To this end, compositions, components, and methods among Class 1 constituents may be considered functionally interchangeable, and the following details, provided merely for exemplary purposes, do not represent an exhaustive list of class members:

[00129] Cas3 (see Table 1) is the prototypical Type I DNA nuclease that functions as the effector protein as part of a larger complex (the Cascade complex comprising Cse1, Cse2, etc.), that is capable of genome editing. See generally He, L., et al. (2020). *Genes*, 11(2), 208. Unlike other CRISPR/Cas systems, Type I systems localize to the DNA target without the Cas3 nuclease via the Cascade complex, which then recruits Cas3 to cleave DNA upon binding and locating the 3' PAM. The Cascade complex is also responsible for processing crRNAs such that they can be used to guide it to the target position. Because of this functionality, Cascade has the ability to process multiple arrayed crRNAs from a single molecule. See Luo, M. (2015). *Nucleic Acids Research*, 43(1), 674-681. As such, Type I system may be used to edit multiple targeted genes or loci from a single molecule.

[00130] Because the natural Cas3 substrate is ssDNA, its function in genomic editing is thought to be as a nickase; however, when targeted in tandem, the resulting edit is a result of blunt end cuts to opposing strands to approximate a blunt-cutting endonuclease, such as

Cas9. See Pickar-Oliver, A., & Gersbach, C. A. (2019). *Nature Reviews Molecular Cell Biology*, 20(8), 490-507.

[00131] Like Type I nucleases, the Type III system relies upon an complex of proteins to effect nucleic acid cleavage. Particularly, Cas10 possesses the nuclease activity to cleave ssDNA in prokaryotes. See Tamulaitis, G. *Trends in Microbiology*, 25(1), 49-61.

Interestingly, this CRISPR/Cas system, native to archaea, exhibits dual specificity and targets both ssDNA and ssRNA. Aside from this change, the system functions much like Type I in that the crRNA targets an effector complex (similar to Cascade) in a sequence-dependent manner. Similarly, the effector complex processes crRNAs prior to association. The dual nature of this nuclease makes its applications to genomic editing potentially more powerful, as both genomic DNA and, in some cases, mRNAs with the same sequence may be targeted to silence particular targeted genes.

[00132] Class 2 CRISPR/Cas systems:

[00133] The exact components, compositions, and methods for effectuating a change in a targeted nucleic acid sequence using a Class 2 CRISPR/Cas system will vary, but should minimally include: a nuclease (selected from at least Types II, and V), at least one guide RNA selected from 1) sgRNA or 2) a combination of crRNA and tracrRNA. These CRISPR/Cas systems have been categorized together as Class 2 CRISPR/Cas systems due to their similarities in requirements and mode of action within a eukaryotic cell. To this end, compositions, components, and methods among Class 2 constituents may be considered functionally interchangeable, and the following details, provided merely for exemplary purposes, do not represent an exhaustive list of class members:

[00134] Type II nucleases are the best-characterized CRISPR/Cas systems, particularly the canonical genomic editing nuclease Cas9 (see Table 1). Multiple Cas9 proteins, derived from various bacterial species, have been isolated. The primary distinction between these nucleases is the PAM, a required recognition site within the targeted dsDNA. After association with a gRNA molecule, the crRNA (or targeting domain of a sgRNA) orients the nuclease at the proper position, but the protein's recognition of the PAM is what induces a cleavage event near that site, resulting in a blunt DSB.

[00135] In addition to the naturally-derived Cas9 proteins, several engineered variants have similarly been reported. These range from Cas9 with enhanced specific (i.e., less off-target activity), such as espCas9. Others have been catalytically modified via point mutations in the RuvC (e.g., D10A) and HNH (e.g., H840A) domains such that they induce only single-strand breaks (i.e., Cas9 nickases). See Frock, R. et al. (2015). *Nature Biotechnology*, 33(2), 179-186. These variants, collectively referred to herein as enhanced specificity Cas9 variants (spCas9), have also been shown to be less error-prone in editing. Such mitigation of off-target effects becomes paramount when selecting for a desired insertion (i.e., a knock in mutation, in which a desired nucleotide sequence is introduced into a target nucleic acid molecule) rather than a deletion. Indeed, less off-target effects may aid in the preferred DNA repair mechanism (HDR, in most instances for knock in mutations). See generally Naeem, M., et al. (2020). *Cells*, 9(7), 1608.

[00136] Additional exemplary further engineered variants of canonical Cas proteins (e.g., mutants, chimeras, and include the following (which are hereby incorporated by reference): WO2015035162A2, WO2019126716A1, WO2019126774A1, WO2014093694A1, and WO2014150624A1.

[00137] For the avoidance of doubt, spCas9 collectively refers to any one of the group consisting of espCas9 (also referred to herein as ESCas9 or esCas9), HFCas9, PECas9, arCas9.

[00138] Like the canonical Cas9 systems, Type V nucleases only require a synthetic sgRNA with a targeting domain complementary to a genomic sequence to carry out genomic editing. These nucleases contain a RuvC domain but lack the HNH domain of Type II nucleases. Further, Cas12, for example, leaves a staggered cut in the dsDNA substrate distal to the PAM, as compared to Cas9's blunt cut next to the PAM. Both Cas12a, also known as Cpf1, and Cas12b, also known as C2c1 (see Table 1), act as part of larger complex of two gRNA-associated nucleases that) acts on dsDNA as quaternary structure nicking each strand simultaneously. See Zetsche B, , et al. *Cell*. 2015;163(3):759-771.; see also Liu L, Chen P, Wang M, et al. *Mol Cell*. 2017;65(2):310-322. Additionally, Cas12b (C2c1) is a highly accurate nuclease with little tolerance for mismatches. See Yang H, et al. *Cell*. 2016;167(7):1814-1828.e12.

Table 1. Exemplary list of Cas nucleases and their requirements

Nuclease (Species)	PAM (5' → 3')	Type of end generated (nucleic acid target)	Spacer length (nt)
Cas9 (<i>S. pyogenes</i>)	NGG	Blunt (dsDNA)	20
Cas9 (<i>S. aureus</i>)	NNGRRT	Blunt (dsDNA)	20
Cas9 (<i>C. jejuni</i>)	NNNNRYAC	Blunt (dsDNA)	22
Cas 9 (<i>S. thermophilus</i>)	NNAGAAW	Blunt (dsDNA)	20
Cas 9 (<i>N. meningitidis</i>)	NNNNGATT	Blunt (dsDNA)	24
Cas 9 (<i>F. novicida</i>)	NGG	Blunt (dsDNA)	21
Cas12a (<i>L. bacterium</i>)	TTTV	5' staggered (dsDNA/ssDNA)	23-25
Cas12a (<i>Acidaminococcus sp.</i>)	TTTN	5' staggered (dsDNA/ssDNA)	24
Cas3 (<i>E. coli</i>)	CTT/CCT/CAT/CTC	None/blunt (ssDNA)	32

See generally Wang, J., Zhang, C., & Feng, B. (2020). Journal of Cellular and Molecular Medicine, 24(6), 3256-3270, where N=any nucleotide; R=any purine (A or G); Y=any pyrimidine (C or T); W=A or T ; V=A, C or G.

[00139] In one aspect, the CRISPR/Cas system of the present disclosure comprises at least one Cas protein derived from one or more of the following selected bacterial genera: Corynebacterium, Sutterella, Legionella, Treponema, Filifactor, Eubacterium, Streptococcus, Lactobacillus, Mycoplasma, Bacteroides, Flavobacterium, Spirochaeta, Azospirillum, Gluconacetobacter, Neisseria, Roseburia, Parvibaculum, Nitratifactor, Campylobacter, Pseudomonas, Streptomyces, Staphylococcus, Francisella, Acidaminococcus, Lachnospiraceae, Leptotrichia, and Prevotella. In some embodiments, the Cas protein is derived from Deltaproteobacteria or Planctomycetes bacterial species.

[00140] Some aspects of the present disclosure provide strategies, methods, compositions, and treatment modalities for altering a targeted sequence within a gene locus (e.g., altering the sequence of a wild type and/or of a mutant in a cell or in a patient having or experiencing effects of rheumatoid arthritis, osteoarthritis, or other inflammatory diseases of

the joint or cells therein) by insertion or deletion of one or more nucleotides mediated by an RNA-guided nuclease and one or more guide RNAs (gRNAs), resulting in loss of function of the targeted gene product. Such an alteration is alternatively referred to as “knocking out” the gene of interest (i.e., generation of a “knock out”).

[00141] In certain embodiments, any region of the IL1A gene (e.g., 5' untranslated region [UTR], exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, intronic regions, intron/exon junctions, the 3' UTR, or polyadenylation signal) is targeted by an RNA-guided nuclease to alter the gene. In certain embodiments, any region of the IL1B gene (e.g., 5' untranslated region [UTR], exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, intronic regions, intron/exon junctions, the 3' UTR, or polyadenylation signal) is targeted by an RNA-guided nuclease to alter the gene. In certain embodiments, the targeted gene is selected from inflammatory effectors (e.g., IL1A, IL1B, IL1R1, IL1R2, IL6, IL18, TNF, TGFB1. In some embodiments, any region of the targeted gene (e.g., a promoter region, a 5' untranslated region, a 3' untranslated region, an exon, an intron, or an exon/intron border) is targeted by an RNA-guided nuclease to alter the gene. In some embodiments, a non-coding region of the targeted gene (e.g., an enhancer region, a promoter region, an intron, 5' UTR, 3' UTR, polyadenylation signal) is targeted to alter the gene.

[00142] CRISPR guide RNAs:

[00143] In one aspect, the CRISPR/Cas system of the present disclosure further provides a gRNA molecule (e.g., an isolated or non-naturally occurring RNA molecule) that interacts with the Cas protein. In certain embodiments, the gRNA is an sgRNA, in which the targeting (i.e. complementary) domain, comprising a nucleotide sequence which is complementary with a target domain from a targeted gene, is incorporated into a single RNA molecule with the protein-interacting domain. In certain embodiments, the targeting domain is a crRNA that is provided to a eukaryotic cells with tracrRNA, which acts as a scaffold through interactions with both the crRNA and the nuclease. In some embodiments, the system is further, optionally, comprised of an oligonucleotide—an HDR template with homology to either side of the target position. See Bloh, K., & Rivera-Torres, N, at 3836.

[00144] In an embodiment, the targeting domain of the gRNA molecule is configured to orient an associated nuclease such that a cleavage event, (e.g., a double strand break or a

single strand break) occurs sufficiently close to a target position, in the targeted gene or locus, thereby facilitating an alteration in the nucleic acid sequence. In some embodiments, the targeting domain is 20 nucleotides in length. In some embodiments, the targeting domain is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[00145] In some embodiments, the targeting domain orients the nuclease such that a cleavage event occurs within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, or 200 nucleotides of a target position. The double-strand or single-strand break, may be positioned upstream or downstream of a target position, and either within or upstream a functional domain cluster within the targeted gene.

[00146] In certain embodiments, a second gRNA molecule, comprising a second targeting domain orients a second associated nuclease such that a cleavage event occurs sufficiently close to a target position, in the targeted gene or locus, thereby facilitating an alteration in the nucleic acid sequence. In an embodiment, the second gRNA molecule targets the same targeted gene or locus as the first gRNA molecule. In other embodiments, the second gRNA molecule targets a different targeted gene or locus as the first gRNA molecule. In some embodiments, the second targeting domain is 20 nucleotides in length. In some embodiments, the second targeting domain is 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[00147] In some embodiments, the second targeting domain orients the nuclease such that a cleavage event occurs within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, or 200 nucleotides of a target position. The double-strand or single-strand break, may be positioned upstream or downstream of a target position, and either within or upstream a functional domain cluster within the targeted gene.

[00148] In an embodiment, the targeting domains of the first and second gRNA molecules are configured such that a cleavage event is positioned, independently for each of the gRNA molecules, within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, or 200 nucleotides of the respective target position. In an embodiment, the first and second gRNA molecules alter the targeted nucleic acid sequences simultaneously. In an embodiment, the first and second gRNA molecules alter the targeted nucleic acid sequences sequentially.

[00149] In an embodiment, a single-strand break is accompanied by a second single-strand break, positioned by the targeting domains of a first and second gRNA molecule, respectively. For example, the targeting domains may orient the associated nucleases such that a cleavage event, (e.g., the two single-strand breaks), are positioned within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, or 200 nucleotides of a target position. In an embodiment, the targeting domain of a first and second gRNA molecules are configured to orient associated nucleases such that, for example, two single-strand breaks occurs at the same target position, or within 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 nucleotides of one another, on opposing strands of genomic DNA, thereby essentially approximating a double strand break.

[00150] In an embodiment, a nucleic acid encodes a targeting domain of a first gRNA molecule and a targeting domain of a second gRNA molecule selected from sequences in Fig. 5. In an embodiment, a nucleic acid encodes a targeting domain of a first gRNA molecule and a targeting domain of a second gRNA molecule selected from sequences in Fig. 7. In an embodiment, a nucleic acid encodes a first sgRNA molecule. In an embodiment a nucleic acid encodes a second sgRNA molecule. In an embodiment, a nucleic acid encodes a third sgRNA molecule. In an embodiment, a nucleic acid encodes a fourth sgRNA molecule.

[00151] In an embodiment, a nucleic acid encodes a crRNA sequence of a first gRNA molecule and a crRNA sequence of a second gRNA molecule selected from sequences in Fig. 5. In an embodiment, a nucleic acid encodes a crRNA sequence of a first gRNA molecule and a crRNA sequence of a second gRNA molecule selected from sequences in Fig. 7. In an embodiment, a nucleic acid encodes a first gRNA molecule comprised of a crRNA sequence and a tracrRNA. In an embodiment, a nucleic acid encodes a second gRNA molecule comprised of a crRNA sequence and a tracrRNA. In an embodiment, a nucleic acid encodes a third gRNA molecule comprised of a crRNA sequence and a tracrRNA. In an embodiment, a nucleic acid encodes a fourth gRNA molecule comprised of a crRNA sequence and a tracrRNA.

[00152] In certain embodiments, a nucleic acid encodes a first gRNA molecule and a second gRNA molecule, comprising a chimeric gRNA molecule. In other embodiments, a nucleic acid encodes a first gRNA molecule, a second gRNA molecule, and a third gRNA molecule, comprising a chimeric gRNA molecule. In some embodiments, a nucleic acid

encodes a first gRNA molecule, a second gRNA molecule, a third gRNA molecule, and a fourth gRNA molecule, comprising a chimeric gRNA molecule.

[00153] In certain embodiments, a nucleic acid may comprise (a) a sequence encoding a first gRNA molecule, comprising a targeting domain that is complementary with a target position in the targeted gene or locus, (b) a sequence encoding a second gRNA molecule, comprising a targeting domain that is complementary with a target position in the second targeted gene or locus, and (c) a sequence encoding an RNA-guided nuclease (e.g., Cas9 or other Cas protein). Optionally, (d) and (e) are sequences encoding a third and fourth gRNA molecule, respectively. In some embodiments, the second targeted gene or locus is the same as the first targeted gene or locus. In other embodiments, the second targeted gene or locus is different from the first targeted gene or locus. In some embodiments, (a), (b), and (c) are encoded within the same nucleic acid molecule (i.e., the same vector, the same viral vector, the same adeno-associated virus (AAV) vector). In some embodiments, (a) and (b) are encoded within the same nucleic acid molecule. In some embodiments, (a), (b) and (d) are encoded within the same nucleic acid molecule. In some embodiments, (a), (b) and (e) are encoded within the same nucleic acid molecule. In some embodiments, (a), (b), (d) and (e) are encoded within the same nucleic acid molecule. In some embodiments, (a), (b), and (c) are encoded within separate nucleic acid molecules. When more than two gRNAs are used, any combination of (a), (b), (c), (d) and (e) may be encoded within a single or separate nucleic acid molecules.

[00154] In an embodiment, the nucleic acid molecule is an AAV vector. Exemplary AAV vectors that may be used with any CRISPR/Cas system of the present disclosure include an AAV1 vector, an AAV2 vector, an AAV3 vector, an AAV4 vector, an AAV5 vector, an AAV6 vector, an AAV7 vector, an AAV8 vector, an AAV9 vector, an AAV10 vector.

[00155] In some embodiments, (a), (b), and (c) are encoded within the same vector. In some embodiments, (a) and (b) are encoded within the same vector. In some embodiments, (a), (b) and (d) are encoded within the same vector. In some embodiments, (a), (b) and (e) are encoded within the same vector. In some embodiments, (a), (b), (d) and (e) are encoded within the same vector. In some embodiments, (a), (b), and (c) are encoded within separate

vectors. When more than two gRNAs are used, any combination of (a), (b), (c), (d) and (e) may be encoded within a single or separate vectors.

[00156] In one aspect, the nucleic acid molecules (i.e., those encoding (a), (b), (c), (d) or (e)) are delivered to a target cell (i.e., any combination of the encoded RNA-guided nuclease of (c) and at least one encoded gRNA molecule of (a), (b), (d), or (e) contact a target cell). In some embodiments, said nucleic acid molecules are delivered to a target cell *in vivo*. In other embodiments, said nucleic acid molecules are delivered to a target cell *ex vivo*. In some embodiments, said nucleic acid molecules are delivered to a target cell *in vitro*. In certain embodiments, said nucleic acid molecules are delivered to a target cell as DNA. In other embodiments, said nucleic acid molecules are delivered to a target cell as RNA (e.g., mRNA). In some embodiments, the products of said nucleic acid molecules are delivered as an assembled ribonucleoprotein (RNP).

[00157] In an embodiment, contacting a target cell comprises delivering said encoded RNA-guided nuclease of (c), as a protein or mRNA with at least one said nucleic acid molecules selected from (a), (b), (d), and (e).

[00158] In an embodiment, contacting a target cell comprises delivering said encoded RNA-guided nuclease of (c), as DNA with at least one said nucleic acid molecules selected from (a), (b), (d), and (e).

[00159] In certain embodiments, CRISPR components are delivered are delivered to a target cell via nanoparticles. Exemplary nanoparticles that may be used with all CRISPR/Cas systems disclosed herein include, at least, lipid nanoparticles or liposomes, hydrogel nanoparticles, metalorganic nanoparticles, gold nanoparticles, and magnetic nanoparticles. See generally Xu, C. F., et al. (2021). *Advanced Drug Delivery Reviews*, 168, 3-29.

[00160] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein may be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[00161] The present disclosure provides compositions useful for treating joint disorders with an inflammatory component. In some aspects, the compositions are useful to prevent the progression of osteoarthritis and to treat osteoarthritis in a mammalian joint.

[00162] In some aspects, the pharmaceutical composition comprises a gene-editing system, wherein the gene-editing system causes expression the at least one genetic locus related to joint function to be silenced or reduced in at least a portion of the cells comprising the joint.

[00163] In an aspect, the pharmaceutical composition comprises a gene-editing system, wherein the gene-editing system targets one or more of IL-1 α , and IL-1 β . In some aspects, the pharmaceutical composition comprises a gene-editing system, wherein the gene-editing system targets one or more of TNF- α , IL-6, IL-8, IL-18, a matrix metalloproteinase (MMP), or components of the NLRP3 inflammasome.

[00164] In some aspects, the pharmaceutical composition comprises a gene-editing system, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at the at least one locus related to joint function. In some embodiments, the gene-editing system reduces the gene expression of the targeted locus or targeted loci. In some embodiments, the at least one locus related to joint tissue is silenced or reduced in at least a portion of the cells comprising the joint.

[00165] In some aspects, the cells comprising the joint are synoviocytes. In some aspects, the cells are synovial macrophages. In some aspects, the cells are synovial fibroblasts. In some aspects at least a portion of the synoviocytes are edited. In some aspects, the cells comprising the joint are chondrocytes.

[00166] In an aspect, the pharmaceutical composition targets the one or more cytokine and/or growth factor genes is/are selected from the group comprising IL-1 α , IL-1 β , TNF- α , IL-6, IL-8, IL-18, a matrix metalloproteinase (MMP), or a component of the NLRP3 inflammasome. In some embodiments, the component of the NLRP3 inflammasome comprises NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD), caspase-1, and combinations thereof.

[00167] Pharmaceutical compositions are also provided, wherein the gene-editing causes expression of one or more cytokine and/or growth factor genes to be enhanced in at least a portion of the cells comprising the joint, the cytokine and/or growth factor gene(s)

being selected from the group comprising IL-1Ra, TIMP-1, TIMP-2, TIMP-3, TIMP-4, and combinations thereof.

[00168] In some embodiments, the pharmaceutical composition provides for gene-editing, wherein the gene-editing comprises the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at said one or more cytokine and/or growth factor genes. In some embodiments, the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[00169] In an aspect, the gene-editing comprises a CRISPR method. In yet other aspects, the CRISPR method is a CRISPR-Cas9 method. In some aspects, the Cas9 is mutated to enhance function.

Animal Models of Osteoarthritis

[00170] Several animal models for osteoarthritis are known to the art. Exemplary nonlimiting animal models are summarized; however, it is understood that various models may be used. Many different species of animals are used to mimic OA, for example, studies have been conducted on mice, rats, rabbits, guinea pigs, dogs, pigs, horses, and even other animals. *See, e.g., Kuyinu et al., J Orthop Surg Res. 11:19 (2016) (hereinafter “Kuyinu, 2016”).*

[00171] It is understood that the various methods for inducing OA may be used in any mammal. In the mouse, spontaneous, chemically induced, surgically induced, and non-invasive induction are commonly used. *E.g., Kuyinu, 2016; Bapat et al., Clin Transl Med. 7:36 (2018) (hereinafter “Bapat, 2018”); and Poulet, Curr Rheumatol Rep 18:40 (2016).* In the horse, osteochondral fragment-exercise model, chemical induction, traumatic induction, and induction through overuse are commonly used. In sheep, surgical induction is most common; in the guinea pig, surgical induction, chemical induction, and spontaneous (Durkin Hartley) methods are frequently used. *E.g. Bapat, 2018.*

[00172] The destabilized medial meniscus (DMM) is frequently used in mice to model posttraumatic osteoarthritis, *e.g. Culley et al., Methods Mol Biol. 1226:143-73 (2015).* The DMM model mimics clinical meniscal injury, a known predisposing factor for the development of human OA, and permits the study of structural and biological changes over the course of the disease. Mice are an attractive model organism, because mouse strains with defined genetic backgrounds may be used. Additionally, knock-out or other genetically

manipulated mouse strains may be used to evaluate the importance of various molecular pathways in the response to various OA treatment modalities and regimens. For example, STR/ort mice have features that make the strain particularly susceptible to developing OA, including, increased levels of the inflammatory cytokine IL1 β , Bapat *et al.*, *Clin Transl Med.* 7:36 (2018). These mice commonly develop OA in knee, ankle, elbow, and temporo-mandibular joints, Jaeger *et al.*, *Osteoarthritis Cartilage* 16:607–614 (2008). Other useful mutant strains of mice are known to the skilled artisan, for example, Col9a1(–/–) mice, Allen *et al.*, *Arthritis Rheum*, 60:2684–2693 (2009).

[00173] Another commonly used surgical model for OA is anterior cruciate ligament transection (ACLT) model. Little and Hunter, *Nat Rev Rheumatol.*, 9(8):485–497 (2013). The subject's ACL is surgically transected causing joint destabilization. The anterior drawer test with the joint flexed is used to confirm that transection of the ligament has occurred. In some cases, other ligaments such as the posterior cruciate ligament, medial collateral ligament, lateral collateral ligament, and/or either meniscus may be transected. As with the DMM model, a variety of mouse strains may be used to investigate various molecular pathways.

[00174] Depending on the technical objective, animals of varying size may be selected for use. Rodents are useful because of the short time needed for skeletal maturity and consequently shorter time to develop OA following surgical or other technique to induce OA. Larger animals are particularly useful to evaluate therapeutic interventions. The anatomy in larger animals is very similar to humans; for example, in dogs the cartilage thickness is less than about half the thickness of humans; this striking similarity is exemplary of why such cartilage degeneration and osteochondral defects studies are much more useful in large animal models. *E.g.* McCoy, *Vet. Pathol.*, 52:803-18 (2015); and, Pelletier *et al.*, *Therapy*, 7:621–34(2010).

Gene-Editing Processes

Overview: Compositions to gene-edit Synovial Cells

[00175] Embodiments of the present disclosure are directed to methods for gene-editing synovial cells (synoviocytes), the methods comprising one or more steps of gene-editing at least a portion of the synoviocytes in a joint to treat osteoarthritis or other joint disorder. As used herein, “gene-editing,” “gene editing,” and “genome editing” refer to a type of genetic modification in which DNA is permanently modified in the genome of a cell, *e.g.*, DNA is inserted, deleted, modified or replaced within the cell's genome. In some embodiments, gene-

editing causes the expression of a DNA sequence to be silenced (sometimes referred to as a gene knockout) or inhibited/reduced (sometimes referred to as a gene knockdown). In other embodiments, gene-editing causes the expression of a DNA sequence to be enhanced (*e.g.*, by causing over-expression). In accordance with embodiments of the present disclosure, gene-editing technology is used to reduce the expression or silence pro-inflammatory genes and/or to enhance the expression of regenerative genes.

Interleukins

[00176] According to additional embodiments, gene-editing methods of the present disclosure may be used to increase the expression of certain interleukins, such as one or more of IL-1 α , IL-1 β , IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-18, and TNF- α . Certain interleukins have been demonstrated to augment inflammatory responses in joint tissue and are linked to disease progression.

Expression Constructs

[00177] Expression constructs encoding one or both of guide RNAs and/or Cas9 editing enzymes can be administered in any effective carrier, *e.g.*, any formulation or composition capable of effectively delivering the component gene to cells *in vivo*. Approaches include, for example, electroporation and/or insertion of the gene in viral vectors, including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered naked or with the help of, for example, cationic liposomes (lipofectamine) or derivatized (*e.g.*, antibody conjugated), polylysine conjugates, gramicidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or CaPO₄ precipitation carried out *in vivo*.

[00178] A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use of a viral vector containing nucleic acid, *e.g.*, a cDNA. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells that have taken up viral vector nucleic acid.

[00179] Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo*, particularly into humans. These vectors provide efficient delivery of genes into cells. In some instances, the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. In

other instances, particularly for adeno-associated virus vectors, stable integration into the host DNA may be a rare event, resulting into episomal expression of the transgene and transient expression of the transgene.

[00180] The development of specialized cell lines (termed “packaging cells”) which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, *Blood* 76:271 (1990)). A replication defective retrovirus can be packaged into virions, which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells in vitro or in vivo with such viruses can be found in Ausubel, et al., eds., *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989), Sections 9.10-9.14, and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include Ψ Crip, Ψ Cre, Ψ 2 and Ψ Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, in vitro and/or in vivo (see, e.g., Eglitis, et al. (1985) *Science* 230:1395-1398; Danos and Mulligan (1988) *Proc. Natl. Acad. Sci. USA* 85:6460-6464; Wilson et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3014-3018; Armentano et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6141-6145; Huber et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8039-8043; Ferry et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:8377-8381; Chowdhury et al. (1991) *Science* 254:1802-1805; van Beusechem et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:7640-7644; Kay et al. (1992) *Human Gene Therapy* 3:641-647; Dai et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:10892-10895; Hwu et al. (1993) *J. Immunol.* 150:4104-4115; U.S. Pat. No. 4,868,116; U.S. Pat. No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573, each of which is incorporated by reference herein in its entirety for all purposes).

[00181] Another viral gene delivery system useful in the present methods utilizes adenovirus-derived vectors. The genome of an adenovirus can be manipulated, such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See, for example, Berkner et al., *BioTechniques* 6:616 (1988); Rosenfeld et al., *Science* 252:431-434 (1991); and Rosenfeld et al., *Cell*

68:143-155 (1992). Suitable adenoviral vectors may be derived from any strain of adenovirus (e.g., Ad2, Ad3, Ad5, or Ad7 etc.), including Adenovirus serotypes from other species (e.g., mouse, dog, human, etc.) that are known to those skilled in the art. The virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situ, where introduced DNA becomes integrated into the host genome (e.g., retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner et al., supra; Haj-Ahmand and Graham, J. Virol. 57:267 (1986).

[00182] Helper-dependent (HDA) vectors can also be produced with all adenoviral sequences deleted except the origin of DNA replication at each end of the viral DNA along with packaging signal at 5-prime end of the genome downstream of the left packaging signal. HDA vectors are constructed and propagated in the presence of a replication-competent helper adenovirus that provides the required early and late proteins necessary for replication.

[00183] Yet another viral vector system useful for delivery of nucleic acids is the adeno-associated virus (AAV). Adeno-associated virus is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle. (For a review see Muzyczka et al., Curr. Topics in Micro. and Immunol. 158:97-129 (1992). It is also one of the few viruses that may integrate its DNA into non-dividing cells, and exhibits a high frequency of stable integration (see for example Flotte et al., Am. J. Respir. Cell. Mol. Biol. 7:349-356 (1992); Samulski et al., J. Virol. 63:3822-3828 (1989); and McLaughlin et al., J. Virol. 62:1963-1973 (1989). Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate. Space for exogenous DNA is limited to about 4.5 kb. An AAV vector such as that described in Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985) can be used to introduce DNA into cells. A variety of nucleic acids have been introduced into different cell types using AAV vectors (see for example Hermonat et al., Proc. Natl. Acad. Sci. USA 81:6466-6470 (1984); Tratschin et al., Mol. Cell. Biol. 4:2072-2081 (1985); Wondisford et al., Mol. Endocrinol. 2:32-39 (1988); Tratschin et al., J. Virol. 51:611-619 (1984); and Flotte et al., J. Biol. Chem. 268:3781-3790 (1993). The identification of Staphylococcus aureus (SaCas9) and other

smaller Cas9 enzymes that can be packaged into adeno-associated viral (AAV) vectors that are highly stable and effective in vivo, easily produced, approved by FDA, and tested in multiple clinical trials, paves new avenues for therapeutic gene editing.

[00184] In some embodiments, nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex (e.g., Cas9 or gRNA) are entrapped in liposomes bearing positive charges on their surface (e.g., lipofectins), which can be tagged with antibodies against cell surface antigens of the target cells. These delivery vehicles can also be used to deliver Cas9 protein/gRNA complexes.

[00185] In clinical settings, the gene delivery systems for the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex can be introduced into a subject by any of a number of methods, each of which is familiar in the art. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, e.g., by intravenous injection, and specific transduction of the protein in the target cells will occur predominantly from specificity of transfection, provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex is more limited, with introduction into the subject being quite localized. For example, the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex can be introduced by intra-articular injection into a joint exhibiting joint disease (e.g., osteoarthritis). In some embodiments, the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex are administered during or after surgery; in some embodiments, a controlled-release hydrogel comprising the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex is administered at the conclusion of surgery before closure to prevent reduce or eliminate osteoarthritis by providing a steady dose of the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex over time.

[00186] A pharmaceutical preparation of the nucleic acids encoding a CRISPR IL-1 α or IL-1 β gene editing complex can consist essentially of the gene delivery system (e.g., viral vector(s)) in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Alternatively, where the complete gene delivery system can be produced intact from recombinant cells, e.g., adeno-associated viral vectors, the

pharmaceutical preparation can comprise one or more cells, which produce the gene delivery system.

[00187] Preferably, the CRISPR IL-1 α or IL-1 β editing complex is specific, i.e., induces genomic alterations preferentially at the target site (IL-1 α or IL-1 β), and does not induce alterations at other sites, or only rarely induces alterations at other sites. In certain embodiments, the CRISPR IL-1 α or IL-1 β editing complex has an editing efficiency of at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99%.

[00188] The sgRNAs for use in the CRISPR/Cas system for HR typically include a guide sequence (*e.g.*, crRNA) that is complementary to a target nucleic acid sequence (target gene locus) and a scaffold sequence (*e.g.*, tracrRNA) that interacts with a Cas nuclease (*e.g.*, Cas9 polypeptide) or a variant or fragment thereof. A single guide RNA (sgRNA) can include a crRNA and a tracrRNA.

[00189] Exemplary target sequences for inducing genomic alterations in the IL-1 α or IL-1 β gene by the CRISPR-Cas editing complex are provided in Tables 2 and 12. Exemplary guide RNAs for use with the compositions, methods, and systems of the present disclosure are provided in Tables 3 and 13.

Table 12. Exemplary Target *IL-1 α* and *IL-1 β* Gene Sequences

Identifier	Genome	Gene	Exon	Target Sequence 5'-3'	PAM
SEQ ID NO: 37	Homo sapiens	<i>IL-1α</i>	4	GCCATAGCTTACATGATAGA	AGG
SEQ ID NO: 38	Homo sapiens	<i>IL-1α</i>	4	TCCTTCTATCATGTAAGCTA	TGG
SEQ ID NO: 39	Homo sapiens	<i>IL-1α</i>	4	CCATGCAGCCTTCATGGAGT	GGG
SEQ ID NO: 40	Homo sapiens	<i>IL-1α</i>	4	TCCATGCAGCCTTCATGGAG	TGG
SEQ ID NO: 41	Homo sapiens	<i>IL-1α</i>	4	AGCTATGGCCCACTCCATGA	AGG
SEQ ID NO: 42	Homo sapiens	<i>IL-1α</i>	4	ATTGATCCATGCAGCCTTCA	TGG
SEQ ID NO: 43	Homo sapiens	<i>IL-1α</i>	4	CCCACTCCATGAAGGCTGCA	TGG
SEQ ID NO: 44	Homo sapiens	<i>IL-1α</i>	4	GCTCTCCTTGAAGGTAAGCT	TGG
SEQ ID NO: 45	Homo sapiens	<i>IL-1α</i>	4	TACCACCATGCTCTCCTTGA	AGG
SEQ ID NO: 46	Homo sapiens	<i>IL-1α</i>	4	GCTTACCTTCAAGGAGAGCA	TGG
SEQ ID NO: 47	Homo sapiens	<i>IL-1α</i>	4	TACCTTCAAGGAGAGCATGG	TGG
SEQ ID NO: 48	Homo sapiens	<i>IL-1α</i>	4	ATGGTGGTAGTAGCAACCAA	CGG
SEQ ID NO: 49	Homo sapiens	<i>IL-1α</i>	4	TGGTGGTAGTAGCAACCAAC	GGG
SEQ ID NO: 50	Homo sapiens	<i>IL-1α</i>	4	CTTCTTCAGAACCTTCCCGT	TGG
SEQ ID NO: 51	Homo sapiens	<i>IL-1α</i>	4	GGTAGTAGCAACCAACGGGA	AGG
SEQ ID NO: 52	Homo sapiens	<i>IL-1α</i>	4	GGAAGGTCTGAAGAAGAGA	CGG
SEQ ID NO: 53	Homo sapiens	<i>IL-1α</i>	4	CTCCAGGTCATCATCAGTGA	TGG
SEQ ID NO: 54	Homo sapiens	<i>IL-1α</i>	4	CATCACTGATGATGACCTGG	AGG
SEQ ID NO: 55	Homo sapiens	<i>IL-1α</i>	4	AGTCATTGGCGATGGCCTCC	AGG
SEQ ID NO: 56	Homo sapiens	<i>IL-1α</i>	4	TTCCTCTGAGTCATTGGCGA	TGG

SEQ ID NO: 57	Homo sapiens	<i>IL-1β</i>	4	TCCCATGTGTCTGAAGAAGAT	AGG
SEQ ID NO: 58	Homo sapiens	<i>IL-1β</i>	4	AACCTATCTTCTTCGACACA	TGG
SEQ ID NO: 59	Homo sapiens	<i>IL-1β</i>	4	ACCTATCTTCTTCGACACAT	GGG
SEQ ID NO: 60	Homo sapiens	<i>IL-1β</i>	4	CTTCGACACATGGGATAACG	AGG
SEQ ID NO: 61	Homo sapiens	<i>IL-1β</i>	4	GTGCAGTTCAGTGATCGTAC	AGG
SEQ ID NO: 62	Homo sapiens	<i>IL-1β</i>	4	GATCACTGAACTGCACGCTC	CGG
SEQ ID NO: 63	Homo sapiens	<i>IL-1β</i>	4	ATCACTGAACTGCACGCTCC	GGG
SEQ ID NO: 64	Homo sapiens	<i>IL-1β</i>	4	CAAAAAAGCTTGGTGATGTC	TGG
SEQ ID NO: 65	Homo sapiens	<i>IL-1β</i>	4	CCATATCCTGTCCCTGGAGG	TGG
SEQ ID NO: 66	Homo sapiens	<i>IL-1β</i>	4	CTGAAAGCTCTCCACCTCCA	GGG
SEQ ID NO: 67	Homo sapiens	<i>IL-1β</i>	4	GCTCCATATCCTGTCCCTGG	AGG
SEQ ID NO: 68	Homo sapiens	<i>IL-1β</i>	4	AGCTCTCCACCTCCAGGGAC	AGG
SEQ ID NO: 69	Homo sapiens	<i>IL-1β</i>	4	GTTGCTCCATATCCTGTCCC	TGG
SEQ ID NO: 70	Homo sapiens	<i>IL-1β</i>	4	GGACAGGATATGGAGCAACA	AGG
SEQ ID NO: 71	Canis familiaris	<i>IL-1α</i>	3	GTCACAGCTCATATCATAGA	AGG
SEQ ID NO: 72	Canis familiaris	<i>IL-1α</i>	3	ACATGCAGTCCTCATGAAGT	GGG
SEQ ID NO: 73	Canis familiaris	<i>IL-1α</i>	3	GACATGCAGTCCTCATGAAG	TGG
SEQ ID NO: 74	Canis familiaris	<i>IL-1α</i>	3	GAGCTGTGACCCACTTCATG	AGG
SEQ ID NO: 75	Canis familiaris	<i>IL-1α</i>	3	GGATGTCTTTGAGATTTTCAG	AGG
SEQ ID NO: 76	Canis familiaris	<i>IL-1α</i>	3	ATTTTCCTTGAAGGTAAGCT	GGG
SEQ ID NO: 77	Canis familiaris	<i>IL-1α</i>	3	GACATCCCAGCTTACCTTCA	AGG

SEQ ID NO: 78	Canis familiaris	<i>IL-1α</i>	3	CTTCAAGGAAAATGTGGTAG	TGG
SEQ ID NO: 79	Canis familiaris	<i>IL-1α</i>	3	GTGGTAGTGGTGGCAGCCAA	TGG
SEQ ID NO: 80	Canis familiaris	<i>IL-1α</i>	3	TGGTAGTGGTGGCAGCCAAT	GGG
SEQ ID NO: 81	Canis familiaris	<i>IL-1α</i>	3	CTTCTTTAGAATCTTCCCAT	TGG
SEQ ID NO: 82	Canis familiaris	<i>IL-1α</i>	3	GGAAGATTCTAAAGAAGAGA	CGG
SEQ ID NO: 83	Canis familiaris	<i>IL-1α</i>	3	AATGTCTTCCAGGTCATCAT	CGG
SEQ ID NO: 84	Canis familiaris	<i>IL-1α</i>	3	ATTCATCACCGATGATGACC	TGG
SEQ ID NO: 85	Canis familiaris	<i>IL-1α</i>	3	ATTGCCAATGACACAGAAGA	AGG
SEQ ID NO: 86	Canis familiaris	<i>IL-1β</i>	4	CCTCATCTACCAGAGAACTG	TGG
SEQ ID NO: 87	Canis familiaris	<i>IL-1β</i>	4	CCACAGTTCTCTGGTAGATG	AGG
SEQ ID NO: 88	Canis familiaris	<i>IL-1β</i>	4	CACAGTTCTCTGGTAGATGA	GGG
SEQ ID NO: 89	Canis familiaris	<i>IL-1β</i>	4	GCTGGTGGGAGACTTGCAAC	TGG
SEQ ID NO: 90	Canis familiaris	<i>IL-1β</i>	4	ACTCTTGTTACAGAGCTGGT	GGG
SEQ ID NO: 91	Canis familiaris	<i>IL-1β</i>	4	GACTCTTGTTACAGAGCTGG	TGG
SEQ ID NO: 92	Canis familiaris	<i>IL-1β</i>	4	TCAGACTCTTGTTACAGAGC	TGG
SEQ ID NO: 93	Canis familiaris	<i>IL-1β</i>	4	AGCTCTGTAACAAGAGTCTG	AGG
SEQ ID NO: 94	Canis familiaris	<i>IL-1β</i>	4	CGTGTCAGTCATTGTAGCTT	TGG
SEQ ID NO: 95	Canis familiaris	<i>IL-1β</i>	4	TCCTGGAGGACCTGTGGGCA	GGG
SEQ ID NO: 96	Canis familiaris	<i>IL-1β</i>	4	GCTGAAGAAGCCCTGCCCCAC	AGG
SEQ ID NO: 97	Canis familiaris	<i>IL-1β</i>	4	CATCCTCCTGGAGGACCTGT	GGG
SEQ ID NO: 98	Canis familiaris	<i>IL-1β</i>	4	TCATCCTCCTGGAGGACCTG	TGG

SEQ ID NO: 99	Canis familiaris	<i>IL-1β</i>	4	GCCCTGCCCACAGGTCCTCC	AGG
SEQ ID NO: 100	Canis familiaris	<i>IL-1β</i>	4	CTGCCCACAGGTCCTCCAGG	AGG
SEQ ID NO: 101	Canis familiaris	<i>IL-1β</i>	4	TCTTCAGGTCATCCTCCTGG	AGG
SEQ ID NO: 102	Canis familiaris	<i>IL-1β</i>	4	TGCTCTTCAGGTCATCCTCC	TGG
SEQ ID NO: 103	Canis familiaris	<i>IL-1β</i>	4	TGTAGCAAAAGATGCTCTTC	AGG
SEQ ID NO: 104	Canis familiaris	<i>IL-1β</i>	4	TTTTGCTACATCTTTGAAGA	AGG
SEQ ID NO: 105	Equus caballus	<i>IL-1α</i>	4	GTCATAGCTTGCATCATAGA	AGG
SEQ ID NO: 106	Equus caballus	<i>IL-1α</i>	4	CCATGCAGTCCTCAGGAAGT	GGG
SEQ ID NO: 107	Equus caballus	<i>IL-1α</i>	4	TCCATGCAGTCCTCAGGAAG	TGG
SEQ ID NO: 108	Equus caballus	<i>IL-1α</i>	4	AAGCTATGACCCACTTCCTG	AGG
SEQ ID NO: 109	Equus caballus	<i>IL-1α</i>	4	AATGTATCCATGCAGTCCTC	AGG
SEQ ID NO: 110	Equus caballus	<i>IL-1α</i>	4	CCCACTTCCTGAGGACTGCA	TGG
SEQ ID NO: 111	Equus caballus	<i>IL-1α</i>	4	GGATGTCCTTAGAGGTTTCAG	AGG
SEQ ID NO: 112	Equus caballus	<i>IL-1α</i>	4	GTTCAGCTTGGATGTCTTAG	AGG
SEQ ID NO: 113	Equus caballus	<i>IL-1α</i>	4	GCTCTCCTTGAAGTTCAGCT	TGG
SEQ ID NO: 114	Equus caballus	<i>IL-1α</i>	4	GACATCCAAGCTGAACTTCA	AGG
SEQ ID NO: 115	Equus caballus	<i>IL-1α</i>	4	GCTGAACTTCAAGGAGAGCG	TGG
SEQ ID NO: 116	Equus caballus	<i>IL-1α</i>	4	CTTCAAGGAGAGCGTGGTGC	TGG
SEQ ID NO: 117	Equus caballus	<i>IL-1α</i>	4	CAAGGAGAGCGTGGTGCTGG	TGG
SEQ ID NO: 118	Equus caballus	<i>IL-1α</i>	4	GTGGTGCTGGTGGCAGCCAA	CGG
SEQ ID NO: 119	Equus caballus	<i>IL-1α</i>	4	TGGTGCTGGTGGCAGCCAAC	GGG

SEQ ID NO: 120	Equus caballus	<i>IL-1α</i>	4	CTTCTTCAGAGTCTTCCCGT	TGG
SEQ ID NO: 121	Equus caballus	<i>IL-1α</i>	4	GGAAGACTCTGAAGAAGAGA	CGG
SEQ ID NO: 122	Equus caballus	<i>IL-1α</i>	4	AATGGCTTCCAGGTCATCAT	TGG
SEQ ID NO: 123	Equus caballus	<i>IL-1α</i>	4	GTTCATCACCAATGATGACC	TGG
SEQ ID NO: 124	Equus caballus	<i>IL-1α</i>	4	TTCTTCTGGATCATTGGCAA	TGG
SEQ ID NO: 125	Equus caballus	<i>IL-1β</i>	4	GGTGGTGGGAGATTGCAAC	TGG
SEQ ID NO: 126	Equus caballus	<i>IL-1β</i>	4	AGTCTTGTTGTAGAGGTGGT	GGG
SEQ ID NO: 127	Equus caballus	<i>IL-1β</i>	4	AAGTCTTGTTGTAGAGGTGG	TGG
SEQ ID NO: 128	Equus caballus	<i>IL-1β</i>	4	TGAAAGTCTTGTTGTAGAGG	TGG
SEQ ID NO: 129	Equus caballus	<i>IL-1β</i>	4	GTTTGAAAGTCTTGTTGTAG	AGG
SEQ ID NO: 130	Equus caballus	<i>IL-1β</i>	4	ACATGCCATGTCAATCATTG	TGG
SEQ ID NO: 131	Equus caballus	<i>IL-1β</i>	4	CATGTCAATCATTGTGGCTG	TGG
SEQ ID NO: 132	Mus musculus	<i>IL-1α</i>	4	GCCATAGCTTGCATCATAGA	AGG
SEQ ID NO: 133	Mus musculus	<i>IL-1α</i>	4	TCCTTCTATGATGCAAGCTA	TGG
SEQ ID NO: 134	Mus musculus	<i>IL-1α</i>	4	GGACATCTTGACGTTTCAG	AGG
SEQ ID NO: 135	Mus musculus	<i>IL-1α</i>	4	GATGTCCAACCTCACCTCA	AGG
SEQ ID NO: 136	Mus musculus	<i>IL-1α</i>	4	TGTCACCCGGCTCTCCTTGA	AGG
SEQ ID NO: 137	Mus musculus	<i>IL-1α</i>	4	CTTCACCTTCAAGGAGAGCC	GGG
SEQ ID NO: 138	Mus musculus	<i>IL-1α</i>	4	ACGTTGCTGATACTGTCACC	CGG
SEQ ID NO: 139	Mus musculus	<i>IL-1α</i>	4	GTATCAGCAACGTCAAGCAA	CGG
SEQ ID NO: 140	Mus musculus	<i>IL-1α</i>	4	TATCAGCAACGTCAAGCAAC	GGG

SEQ ID NO: 141	Mus musculus	<i>IL-1α</i>	4	GGAAGATTCTGAAGAAGAGA	CGG
SEQ ID NO: 142	Mus musculus	<i>IL-1α</i>	4	CTGCAGGTCATCTTCAGTGA	AGG
SEQ ID NO: 143	Mus musculus	<i>IL-1α</i>	4	ACCTTCCAGATCATGGGTTA	TGG
SEQ ID NO: 144	Mus musculus	<i>IL-1α</i>	4	CTCCTTACCTTCCAGATCAT	GGG
SEQ ID NO: 145	Mus musculus	<i>IL-1α</i>	4	TCCATAAACCCATGATCTGGA	AGG
SEQ ID NO: 146	Mus musculus	<i>IL-1α</i>	4	AACCCATGATCTGGAAGGTA	AGG
SEQ ID NO: 147	Mus musculus	<i>IL-1β</i>	4	GACAGCCCAGGTCAAAGGTT	TGG
SEQ ID NO: 148	Mus musculus	<i>IL-1β</i>	4	ATCAGGACAGCCCAGGTCAA	AGG
SEQ ID NO: 149	Mus musculus	<i>IL-1β</i>	4	TGCTTCCAAACCTTTGACCT	GGG
SEQ ID NO: 150	Mus musculus	<i>IL-1β</i>	4	TGCTCTCATCAGGACAGCCC	AGG
SEQ ID NO: 151	Mus musculus	<i>IL-1β</i>	4	TGAAGCTGGATGCTCTCATC	AGG
SEQ ID NO: 152	Mus musculus	<i>IL-1β</i>	4	GCTGCTGCGAGATTGGAAGC	TGG
SEQ ID NO: 153	Mus musculus	<i>IL-1β</i>	4	CATCAACAAGAGCTTCAGGC	AGG
SEQ ID NO: 154	Mus musculus	<i>IL-1β</i>	4	GCAGGCAGTATCACTCATTG	TGG
SEQ ID NO: 155	Mus musculus	<i>IL-1β</i>	4	AGTATCACTCATTGTGGCTG	TGG
SEQ ID NO: 156	Mus musculus	<i>IL-1β</i>	4	TTGTGGCTGTGGAGAAGCTG	TGG
SEQ ID NO: 157	Mus musculus	<i>IL-1β</i>	4	AAGGTCCACGGGAAAGACAC	AGG
SEQ ID NO: 158	Mus musculus	<i>IL-1β</i>	4	AGCTACCTGTGTCTTTCCCG	TGG
SEQ ID NO: 159	Mus musculus	<i>IL-1β</i>	4	CCTCATCCTGGAAGGTCCAC	GGG
SEQ ID NO: 160	Mus musculus	<i>IL-1β</i>	4	TCCTCATCCTGGAAGGTCCA	CGG
SEQ ID NO: 161	Mus musculus	<i>IL-1β</i>	4	GCTCATGTCCTCATCCTGGA	AGG

SEQ ID NO: 162	Mus musculus	<i>IL-1β</i>	4	CCCGTGGACCTTCCAGGATG	AGG
SEQ ID NO: 163	Mus musculus	<i>IL-1β</i>	4	AGGTGCTCATGTCCTCATCC	TGG
SEQ ID NO: 164	Mus musculus	<i>IL-1β</i>	4	TTCAAAGATGAAGGAAAAGA	AGG
SEQ ID NO: 165	Mus musculus	<i>IL-1β</i>	4	AGTACCTTCTTCAAAGATGA	AGG
SEQ ID NO: 167	Mus musculus	<i>IL-1β</i>	4	TTTCCTTCATCTTTGAAGA	AGG

Table 13. Exemplary crRNA sequences for CRISPR sgRNAs

Identifier	Genome	Gene	Exon	Target Sequence 5'-3'	PAM
SEQ ID NO: 168	Homo sapiens	<i>IL-1α</i>	4	GCCAUAGCUUACAUGAUAGA	AGG
SEQ ID NO: 169	Homo sapiens	<i>IL-1α</i>	4	UCCUUCUAUCAUGUAAGCUA	TGG
SEQ ID NO: 170	Homo sapiens	<i>IL-1α</i>	4	CCAUGCAGCCUUCAUGGAGU	GGG
SEQ ID NO: 171	Homo sapiens	<i>IL-1α</i>	4	UCCAUGCAGCCUUCAUGGAG	TGG
SEQ ID NO: 172	Homo sapiens	<i>IL-1α</i>	4	AGCUAUGGCCACUCCAUGA	AGG
SEQ ID NO: 173	Homo sapiens	<i>IL-1α</i>	4	AUUGAUCCAUGCAGCCUUCA	TGG
SEQ ID NO: 174	Homo sapiens	<i>IL-1α</i>	4	CCCACUCCAUGAAGGCUGCA	TGG
SEQ ID NO: 175	Homo sapiens	<i>IL-1α</i>	4	GCUCUCCUUGAAGGUAAGCU	TGG
SEQ ID NO: 176	Homo sapiens	<i>IL-1α</i>	4	UACCACCAUGCUCUCCUUGA	AGG
SEQ ID NO: 177	Homo sapiens	<i>IL-1α</i>	4	GCUUACCUUCAAGGAGAGCA	TGG
SEQ ID NO: 178	Homo sapiens	<i>IL-1α</i>	4	UACCUUCAAGGAGAGCAUGG	TGG
SEQ ID NO: 179	Homo sapiens	<i>IL-1α</i>	4	AUGGUGGUAGUAGCAACCAA	CGG
SEQ ID NO: 180	Homo sapiens	<i>IL-1α</i>	4	UGGUGGUAGUAGCAACCAAC	GGG
SEQ ID NO: 181	Homo sapiens	<i>IL-1α</i>	4	CUUCUUCAGAACCUUCCCGU	TGG
SEQ ID NO: 182	Homo sapiens	<i>IL-1α</i>	4	GGUAGUAGCAACCAACGGGA	AGG
SEQ ID NO: 183	Homo sapiens	<i>IL-1α</i>	4	GGAAGGUUCUGAAGAAGAGA	CGG
SEQ ID NO: 184	Homo sapiens	<i>IL-1α</i>	4	CUCCAGGUCAUCAUCAGUGA	TGG
SEQ ID NO: 185	Homo sapiens	<i>IL-1α</i>	4	CAUCACUGAUGAUGACCUGG	AGG
SEQ ID NO: 186	Homo sapiens	<i>IL-1α</i>	4	AGUCAUUGGCGAUGGCCUCC	AGG
SEQ ID NO: 187	Homo sapiens	<i>IL-1α</i>	4	UUCCUCUGAGUCAUUGGCGA	TGG

SEQ ID NO: 188	Homo sapiens	<i>IL-1β</i>	4	UCCCAUGUGUCGAAGAAGAU	AGG
SEQ ID NO: 189	Homo sapiens	<i>IL-1β</i>	4	AACCUAUCUUCUUCGACACA	TGG
SEQ ID NO: 190	Homo sapiens	<i>IL-1β</i>	4	ACCUAUCUUCUUCGACACAU	GGG
SEQ ID NO: 191	Homo sapiens	<i>IL-1β</i>	4	CUUCGACACAUGGGGAUAACG	AGG
SEQ ID NO: 192	Homo sapiens	<i>IL-1β</i>	4	GUGCAGUUCAGUGAUCGUAC	AGG
SEQ ID NO: 193	Homo sapiens	<i>IL-1β</i>	4	GAUCACUGAACUGCACGCUC	CGG
SEQ ID NO: 194	Homo sapiens	<i>IL-1β</i>	4	AUCACUGAACUGCACGCUCC	GGG
SEQ ID NO: 195	Homo sapiens	<i>IL-1β</i>	4	CAAAAAAGCUUGGUGAUGUC	TGG
SEQ ID NO: 196	Homo sapiens	<i>IL-1β</i>	4	CCAUAUCCUGUCCCUGGAGG	TGG
SEQ ID NO: 197	Homo sapiens	<i>IL-1β</i>	4	CUGAAAGCUCUCCACCUCCA	GGG
SEQ ID NO: 198	Homo sapiens	<i>IL-1β</i>	4	GCUCCAUAUCCUGUCCCUGG	AGG
SEQ ID NO: 199	Homo sapiens	<i>IL-1β</i>	4	AGCUCUCCACCUCCAGGGAC	AGG
SEQ ID NO: 200	Homo sapiens	<i>IL-1β</i>	4	GUUGCUGCAUAUCCUGUCCC	TGG
SEQ ID NO: 201	Homo sapiens	<i>IL-1β</i>	4	GGACAGGAUAUGGAGCAACA	AGG
SEQ ID NO: 202	Canis familiaris	<i>IL-1α</i>	3	GUCACAGCUCAUAUCAUAGA	AGG
SEQ ID NO: 203	Canis familiaris	<i>IL-1α</i>	3	ACAUGCAGUCCUCAUGAAGU	GGG
SEQ ID NO: 204	Canis familiaris	<i>IL-1α</i>	3	GACAUGCAGUCCUCAUGAAG	TGG
SEQ ID NO: 205	Canis familiaris	<i>IL-1α</i>	3	GAGCUGUGACCCACUUCAUG	AGG
SEQ ID NO: 206	Canis familiaris	<i>IL-1α</i>	3	GGAUGUCUUUGAGAUUUCAG	AGG
SEQ ID NO: 207	Canis familiaris	<i>IL-1α</i>	3	AUUUUCCUUGAAGGUAAGCU	GGG
SEQ ID NO: 208	Canis familiaris	<i>IL-1α</i>	3	GACAUCCCAGCUUACCUUCA	AGG

SEQ ID NO: 209	Canis familiaris	<i>IL-1α</i>	3	CUUCAAGGAAAAUGUGGUAG	TGG
SEQ ID NO: 210	Canis familiaris	<i>IL-1α</i>	3	GUGGUAGUGGUGGCAGCCAA	TGG
SEQ ID NO: 211	Canis familiaris	<i>IL-1α</i>	3	UGGUAGUGGUGGCAGCCAAU	GGG
SEQ ID NO: 212	Canis familiaris	<i>IL-1α</i>	3	CUUCUUUAGAAUCUCCCAU	TGG
SEQ ID NO: 213	Canis familiaris	<i>IL-1α</i>	3	GGAAGAUUCUAAAGAAGAGA	CGG
SEQ ID NO: 214	Canis familiaris	<i>IL-1α</i>	3	AAUGUCUCCAGGUCAUCAU	CGG
SEQ ID NO: 215	Canis familiaris	<i>IL-1α</i>	3	AUUCAUCACCGAUGAUGACC	TGG
SEQ ID NO: 216	Canis familiaris	<i>IL-1α</i>	3	AUUGCCAAUGACACAGAAGA	AGG
SEQ ID NO: 217	Canis familiaris	<i>IL-1β</i>	4	CCUCAUCUACCAGAGAACUG	TGG
SEQ ID NO: 218	Canis familiaris	<i>IL-1β</i>	4	CCACAGUUCUCUGGUAGAUG	AGG
SEQ ID NO: 219	Canis familiaris	<i>IL-1β</i>	4	CACAGUUCUCUGGUAGAUGA	GGG
SEQ ID NO: 220	Canis familiaris	<i>IL-1β</i>	4	GCUGGUGGGAGACUUGCAAC	TGG
SEQ ID NO: 221	Canis familiaris	<i>IL-1β</i>	4	ACUCUUGUUACAGAGCUGGU	GGG
SEQ ID NO: 222	Canis familiaris	<i>IL-1β</i>	4	GACUCUUGUUACAGAGCUGG	TGG
SEQ ID NO: 223	Canis familiaris	<i>IL-1β</i>	4	UCAGACUCUUGUUACAGAGC	TGG
SEQ ID NO: 224	Canis familiaris	<i>IL-1β</i>	4	AGCUCUGUAACAAGAGUCUG	AGG
SEQ ID NO: 225	Canis familiaris	<i>IL-1β</i>	4	CGUGUCAGUCAUUGUAGCUU	TGG
SEQ ID NO: 226	Canis familiaris	<i>IL-1β</i>	4	UCCUGGAGGACCUGUGGGCA	GGG
SEQ ID NO: 227	Canis familiaris	<i>IL-1β</i>	4	GCUGAAGAAGCCCUGCCCAC	AGG
SEQ ID NO: 228	Canis familiaris	<i>IL-1β</i>	4	CAUCCUCCUGGAGGACCUGU	GGG
SEQ ID NO: 229	Canis familiaris	<i>IL-1β</i>	4	UCAUCCUCCUGGAGGACCUG	TGG

SEQ ID NO: 230	Canis familiaris	<i>IL-1β</i>	4	GCCCUGCCCACAGGUCCUCC	AGG
SEQ ID NO: 231	Canis familiaris	<i>IL-1β</i>	4	CUGCCCACAGGUCCUCCAGG	AGG
SEQ ID NO: 232	Canis familiaris	<i>IL-1β</i>	4	UCUUCAGGUCAUCCUCCUGG	AGG
SEQ ID NO: 233	Canis familiaris	<i>IL-1β</i>	4	UGCUCUUCAGGUCAUCCUCC	TGG
SEQ ID NO: 234	Canis familiaris	<i>IL-1β</i>	4	UGUAGCAAAAGAUGCUCUUC	AGG
SEQ ID NO: 235	Canis familiaris	<i>IL-1β</i>	4	UUUUGCUACAUCUUUGAAGA	AGG
SEQ ID NO: 236	Equus caballus	<i>IL-1α</i>	4	GUCAUAGCUUGCAUCAUAGA	AGG
SEQ ID NO: 237	Equus caballus	<i>IL-1α</i>	4	CCAUGCAGUCCUCAGGAAGU	GGG
SEQ ID NO: 238	Equus caballus	<i>IL-1α</i>	4	UCCAUGCAGUCCUCAGGAAG	TGG
SEQ ID NO: 239	Equus caballus	<i>IL-1α</i>	4	AAGCUAUGACCCACUCCUG	AGG
SEQ ID NO: 240	Equus caballus	<i>IL-1α</i>	4	AAUGUAUCCAUGCAGUCCUC	AGG
SEQ ID NO: 241	Equus caballus	<i>IL-1α</i>	4	CCCACUCCUGAGGACUGCA	TGG
SEQ ID NO: 242	Equus caballus	<i>IL-1α</i>	4	GGAUGUCUUAGAGGUUUCAG	AGG
SEQ ID NO: 243	Equus caballus	<i>IL-1α</i>	4	GUUCAGCUUGGAUGUCUUAG	AGG
SEQ ID NO: 244	Equus caballus	<i>IL-1α</i>	4	GCUCUCCUUGAAGUUCAGCU	TGG
SEQ ID NO: 245	Equus caballus	<i>IL-1α</i>	4	GACAUCCAAGCUGAACUUCA	AGG
SEQ ID NO: 246	Equus caballus	<i>IL-1α</i>	4	GCUGAACUUCAAGGAGAGCG	TGG
SEQ ID NO: 247	Equus caballus	<i>IL-1α</i>	4	CUUCAAGGAGAGCGUGGUGC	TGG
SEQ ID NO: 248	Equus caballus	<i>IL-1α</i>	4	CAAGGAGAGCGUGGUGCUGG	TGG
SEQ ID NO: 249	Equus caballus	<i>IL-1α</i>	4	GUGGUGCUGGUGGCAGCCAA	CGG
SEQ ID NO: 250	Equus caballus	<i>IL-1α</i>	4	UGGUGCUGGUGGCAGCCAAC	GGG

SEQ ID NO: 251	Equus caballus	<i>IL-1α</i>	4	CUUCUUCAGAGUCUUCCCGU	TGG
SEQ ID NO: 252	Equus caballus	<i>IL-1α</i>	4	GGAAGACUCUGAAGAAGAGA	CGG
SEQ ID NO: 253	Equus caballus	<i>IL-1α</i>	4	AAUGGCUUCCAGGUCAUCAU	TGG
SEQ ID NO: 254	Equus caballus	<i>IL-1α</i>	4	GUUCAUCACCAAUGAUGACC	TGG
SEQ ID NO: 255	Equus caballus	<i>IL-1α</i>	4	UUCUUCUGGAUCAUUGGCAA	TGG
SEQ ID NO: 256	Equus caballus	<i>IL-1β</i>	4	GGUGGUGGGAGAUUUGCAAC	TGG
SEQ ID NO: 257	Equus caballus	<i>IL-1β</i>	4	AGUCUUGUUGUAGAGGUGGU	GGG
SEQ ID NO: 258	Equus caballus	<i>IL-1β</i>	4	AAGUCUUGUUGUAGAGGUGG	TGG
SEQ ID NO: 259	Equus caballus	<i>IL-1β</i>	4	UGAAAGUCUUGUUGUAGAGG	TGG
SEQ ID NO: 260	Equus caballus	<i>IL-1β</i>	4	GUUUGAAAGUCUUGUUGUAG	AGG
SEQ ID NO: 261	Equus caballus	<i>IL-1β</i>	4	ACAUGCCAUGUCAAUCAUUG	TGG
SEQ ID NO: 262	Equus caballus	<i>IL-1β</i>	4	CAUGUCAAUCAUUGUGGCUG	TGG
SEQ ID NO: 263	Mus musculus	<i>IL-1α</i>	4	GCCAUAGCUUGCAUCAUAGA	AGG
SEQ ID NO: 264	Mus musculus	<i>IL-1α</i>	4	UCCUUCUAUGAUGCAAGCUA	TGG
SEQ ID NO: 265	Mus musculus	<i>IL-1α</i>	4	GGACAUCUUUGACGUUUCAG	AGG
SEQ ID NO: 266	Mus musculus	<i>IL-1α</i>	4	GAUGUCCAACUUCACCUUCA	AGG
SEQ ID NO: 267	Mus musculus	<i>IL-1α</i>	4	UGUCACCCGGCUCUCCUUGA	AGG
SEQ ID NO: 268	Mus musculus	<i>IL-1α</i>	4	CUUCACCUUCAAGGAGAGCC	GGG
SEQ ID NO: 269	Mus musculus	<i>IL-1α</i>	4	ACGUUGCUGAUACUGUCACC	CGG
SEQ ID NO: 270	Mus musculus	<i>IL-1α</i>	4	GUAUCAGCAACGUCAAGCAA	CGG
SEQ ID NO: 271	Mus musculus	<i>IL-1α</i>	4	UAUCAGCAACGUCAAGCAAC	GGG

SEQ ID NO: 272	Mus musculus	<i>IL-1α</i>	4	GGAAGAUUCUGAAGAAGAGA	CGG
SEQ ID NO: 273	Mus musculus	<i>IL-1α</i>	4	CUGCAGGUCAUCUUCAGUGA	AGG
SEQ ID NO: 274	Mus musculus	<i>IL-1α</i>	4	ACCUUCCAGAUC AUGGGUUA	TGG
SEQ ID NO: 275	Mus musculus	<i>IL-1α</i>	4	CUCCUUACCUUCCAGAUCAU	GGG
SEQ ID NO: 276	Mus musculus	<i>IL-1α</i>	4	UCCAUAACCCAUGAUCUGGA	AGG
SEQ ID NO: 277	Mus musculus	<i>IL-1α</i>	4	AACCCAUGAUCUGGAAGGUA	AGG
SEQ ID NO: 278	Mus musculus	<i>IL-1β</i>	4	GACAGCCCAGGUCAAAGGUU	TGG
SEQ ID NO: 279	Mus musculus	<i>IL-1β</i>	4	AUCAGGACAGCCCAGGUCAA	AGG
SEQ ID NO: 280	Mus musculus	<i>IL-1β</i>	4	UGC UCCAAACCUUUGACCU	GGG
SEQ ID NO: 281	Mus musculus	<i>IL-1β</i>	4	UGCUCUCAUCAGGACAGCCC	AGG
SEQ ID NO: 282	Mus musculus	<i>IL-1β</i>	4	UGAAGCUGGAUGCUCUCAUC	AGG
SEQ ID NO: 283	Mus musculus	<i>IL-1β</i>	4	GCUGCUGCGAGAUUUGAAGC	TGG
SEQ ID NO: 284	Mus musculus	<i>IL-1β</i>	4	CAUCAACAAGAGCUUCAGGC	AGG
SEQ ID NO: 285	Mus musculus	<i>IL-1β</i>	4	GCAGGCAGUAUCACUCAUUG	TGG
SEQ ID NO: 286	Mus musculus	<i>IL-1β</i>	4	AGUAUCACUCAUUGUGGCUG	TGG
SEQ ID NO: 287	Mus musculus	<i>IL-1β</i>	4	UUGUGGCUGUGGAGAAGCUG	TGG
SEQ ID NO: 288	Mus musculus	<i>IL-1β</i>	4	AAGGUCCACGGGAAAGACAC	AGG
SEQ ID NO: 289	Mus musculus	<i>IL-1β</i>	4	AGCUACCUGUGUCUUUCCCG	TGG
SEQ ID NO: 290	Mus musculus	<i>IL-1β</i>	4	CCUCAUCCUGGAAGGUCCAC	GGG
SEQ ID NO: 291	Mus musculus	<i>IL-1β</i>	4	UCCUCAUCCUGGAAGGUCCA	CGG
SEQ ID NO: 292	Mus musculus	<i>IL-1β</i>	4	GCUCAUGUCCUCAUCCUGGA	AGG

SEQ ID NO: 293	Mus musculus	<i>IL-1β</i>	4	CCCGUGGACCUUCCAGGAUG	AGG
SEQ ID NO: 294	Mus musculus	<i>IL-1β</i>	4	AGGUGCUCAUGUCCUCAUCC	TGG
SEQ ID NO: 295	Mus musculus	<i>IL-1β</i>	4	UUCAAAGAUGAAGGAAAAGA	AGG
SEQ ID NO: 296	Mus musculus	<i>IL-1β</i>	4	AGUACCUUCUCAAAGAUGA	AGG
SEQ ID NO: 297	Mus musculus	<i>IL-1β</i>	4	UUUCCUUCUUCUUGAAGA	AGG

[00190] In certain embodiments, the sequence of a guide RNA (e.g., a single guide RNA or sgRNA) may be modified to increase editing efficiency and/or reduce off-target effects. In certain embodiments, the sequence of a guide RNA may vary from the target sequence by about 1 base, about 2 bases, about 3 bases, about 4 bases, about 5 bases, about 5 bases, about 6 bases, about 7 bases, about 8 bases, about 9 bases, about 10 bases, about 15 bases, or greater than about 15 bases. In certain embodiments, the sequence of a guide RNA may vary from the target sequence by about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, about 15%, about 16%, about 17%, about 18%, about 19%, about 20%, or greater than about 20%. As used herein, variation from a target sequence may refer to the degree of complementarity.

[00191] In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 95% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 90% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 85% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 80% identical to a sequence as shown in any one of SEQ ID NO.:

21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 75% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 70% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 65% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 60% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 55% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 50% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 45% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 40% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present disclosure is at least about 35% identical to a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297.

[00192] In certain embodiments, a guide RNA used with a composition, method or system of the present has 1 base substitution in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 2 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 3 base substitutions

in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 4 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 4 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 6 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 7 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 8 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 9 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 10 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 11 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 12 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 13 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 14 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297. In certain embodiments, a guide RNA used with a composition, method or system of the present has 15 base substitutions in a sequence as shown in any one of SEQ ID NO.: 21-34 and SEQ ID NO.: 168-297.

[00193] In certain embodiments, a guide RNA of the present disclosure is designed to and/or capable of knocking down an expression of a target gene as shown in any one of SEQ ID NO.: 7-20 and SEQ ID NO.: 37-167. In certain embodiments, a guide RNA of the present

disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 1 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 2 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 3 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 4 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 5 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 6 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 7 of the human IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 α gene by binding to at least a portion of Exon 8 of the human IL-1 α gene.

[00194] In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 1 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 2 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 3 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 4 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 5 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 6 of the human IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is

designed to or capable of knocking down the human IL-1 β gene by binding to at least a portion of Exon 7 of the human IL-1 β gene.

[00195] In certain embodiments, a guide RNA of the present disclosure is designed to and/or capable of knocking down an expression of a target gene as shown in any one of SEQ ID NO.: 7-20 and SEQ ID NO.: 37-167. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 1 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 2 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 3 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 4 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 5 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 6 of the canis familiaris IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 α gene by binding to at least a portion of Exon 7 of the canis familiaris IL-1 α gene.

[00196] In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 β gene by binding to at least a portion of Exon 1 of the canis familiaris IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 β gene by binding to at least a portion of Exon 2 of the canis familiaris IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 β gene by binding to at least a portion of Exon 3 of the canis familiaris IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the canis familiaris IL-1 β gene by binding to at least a portion of Exon 4 of the canis familiaris IL-1 β gene.

least a portion of Exon 4 of the *canis familiaris* IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *canis familiaris* IL-1 β gene by binding to at least a portion of Exon 5 of the *canis familiaris* IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *canis familiaris* IL-1 β gene by binding to at least a portion of Exon 6 of the *canis familiaris* IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *canis familiaris* IL-1 β gene by binding to at least a portion of Exon 7 of the *canis familiaris* IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *canis familiaris* IL-1 β gene by binding to at least a portion of Exon 8 of the *canis familiaris* IL-1 β gene.

[00197] In certain embodiments, a guide RNA of the present disclosure is designed to and/or capable of knocking down an expression of a target gene as shown in any one of SEQ ID NO.: 7-20 and SEQ ID NO.: 37-167. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 1 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 2 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 3 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 4 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 5 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 6 of the *equus caballus* IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the *equus caballus* IL-1 α gene by binding to at least a portion of Exon 7 of the *equus caballus* IL-1 α gene.

[00198] In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 1 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 2 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 3 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 4 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 5 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 6 of the equus caballus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the equus caballus IL-1 β gene by binding to at least a portion of Exon 7 of the equus caballus IL-1 β gene.

[00199] In certain embodiments, a guide RNA of the present disclosure is designed to and/or capable of knocking down an expression of a target gene as shown in any one of SEQ ID NO.: 7-20 and SEQ ID NO.: 37-167. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 1 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 2 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 3 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 4 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 5 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is

designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 6 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 7 of the mus musculus IL-1 α gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 α gene by binding to at least a portion of Exon 8 of the mus musculus IL-1 α gene.

[00200] In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 1 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 2 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 3 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 4 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 5 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 6 of the mus musculus IL-1 β gene. In certain embodiments, a guide RNA of the present disclosure is designed to or capable of knocking down the mus musculus IL-1 β gene by binding to at least a portion of Exon 7 of the mus musculus IL-1 β gene.

[00201] In some instances, the sgRNA is introduced into a cell (*e.g.*, an in vitro cell such as a primary cell for ex vivo therapy, or an in vivo cell such as in a patient) with a recombinant expression vector comprising a nucleotide sequence encoding a Cas nuclease (*e.g.*, Cas9 polypeptide) or a variant or fragment thereof. In some embodiments, the sgRNA is complexed with a Cas nuclease (*e.g.*, a Cas9 polypeptide) or a variant or fragment thereof to form a ribonucleoprotein (RNP)-based delivery system for introduction into a cell (*e.g.*, an in vitro cell such as a primary cell for ex vivo therapy, or an in vivo cell such as in a patient). In

other instances, the sgRNA is introduced into a cell (*e.g.*, an in vitro cell such as a primary cell for ex vivo therapy, or an in vivo cell such as in a patient) with an mRNA encoding a Cas nuclease (*e.g.*, Cas9 polypeptide) or a variant or fragment thereof.

[00202] Any heterologous or foreign nucleic acid (*e.g.*, target locus-specific sgRNA and/or polynucleotide encoding a Cas9 polynucleotide) can be introduced into a cell using any method known to one skilled in the art. Such methods include, but are not limited to, electroporation, nucleofection, transfection, lipofection, transduction, microinjection, electroinjection, electrofusion, nanoparticle bombardment, transformation, conjugation, and the like.

[00203] The nucleic acid sequence of the sgRNA can be any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence (*e.g.*, target DNA sequence) to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence of the sgRNA and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman- Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (*e.g.* the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about 1 nucleotide, 2 nucleotides, 3 nucleotides, 4 nucleotides, 5 nucleotides, 6 nucleotides, 7 nucleotides, 8 nucleotides, 9 nucleotides, 10 nucleotides, 11 nucleotides, 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, 30 nucleotides, 35 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 75 nucleotides, or more nucleotides in length. In some instances, a guide sequence is about 20 nucleotides in length. In other instances, a guide sequence is about 15 nucleotides in length. In other instances, a guide sequence is about 25 nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of a

CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions.

[00204] The nucleic acid sequence of a sgRNA can be selected using any of the web-based software described above. Considerations for selecting a DNA-targeting RNA include the PAM sequence for the Cas nuclease (*e.g.*, Cas9 polypeptide) to be used, and strategies for minimizing off-target modifications. Tools, such as the CRISPR Design Tool, can provide sequences for preparing the sgRNA, for assessing target modification efficiency, and/or assessing cleavage at off-target sites. Another consideration for selecting the sequence of a sgRNA includes reducing the degree of secondary structure within the guide sequence. Secondary structure may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. Examples of suitable algorithms include mFold (Zuker and Stiegler, *Nucleic Acids Res*, 9 (1981), 133-148), UNAFold package (Markham et al, *Methods Mol Biol*, 2008, 453:3-31) and RNAfold from the ViennaRNA Package.

[00205] The sgRNA can be about 10 to about 500 nucleotides, *e.g.*, about 10 nucleotides, 15 nucleotides, 20 nucleotides, 25 nucleotides, 30 nucleotides, 35 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, 60 nucleotides, 65 nucleotides, 70 nucleotides, 75 nucleotides, 80 nucleotides, 85 nucleotides, 90 nucleotides, 95 nucleotides, 100 nucleotides, 105 nucleotides, 110 nucleotides, 120 nucleotides, 130 nucleotides, 140 nucleotides, 150 nucleotides, 160 nucleotides, 170 nucleotides, 180 nucleotides, 190 nucleotides, 200 nucleotides, 210 nucleotides, 220 nucleotides, 230 nucleotides, 240 nucleotides, 250 nucleotides, 260 nucleotides, 270 nucleotides, 280 nucleotides, 290 nucleotides, 300 nucleotides, 310 nucleotides, 320 nucleotides, 330 nucleotides, 340 nucleotides, 350

nucleotides, 360 nucleotides, 370 nucleotides, 380 nucleotides, 390 nucleotides, 400 nucleotides, 410 nucleotides, 420 nucleotides, 430 nucleotides, 440 nucleotides, 450 nucleotides, 460 nucleotides, 470 nucleotides, 480 nucleotides, 490 nucleotides, or about 500 nucleotides. In some embodiments, the sgRNA is about 20 to about 500 nucleotides, *e.g.*, 20 nucleotides, 25 nucleotides, 30 nucleotides, 35 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, 60 nucleotides, 65 nucleotides, 70 nucleotides, 75 nucleotides, 80 nucleotides, 85 nucleotides, 90 nucleotides, 95 nucleotides, 100 nucleotides, 105 nucleotides, 110 nucleotides, 115 nucleotides, 120 nucleotides, 125 nucleotides, 130 nucleotides, 135 nucleotides, 140 nucleotides, 145 nucleotides, 150 nucleotides, 155 nucleotides, 160 nucleotides, 165 nucleotides, 170 nucleotides, 175 nucleotides, 180 nucleotides, 185 nucleotides, 190 nucleotides, 195 nucleotides, 200 nucleotides, 205 nucleotides, 210 nucleotides, 215 nucleotides, 220 nucleotides, 225 nucleotides, 230 nucleotides, 235 nucleotides, 240 nucleotides, 245 nucleotides, 250 nucleotides, 255 nucleotides, 260 nucleotides, 265 nucleotides, 270 nucleotides, 275 nucleotides, 280 nucleotides, 285 nucleotides, 290 nucleotides, 295 nucleotides, 300 nucleotides, 305 nucleotides, 310 nucleotides, 315 nucleotides, 320 nucleotides, 325 nucleotides, 330 nucleotides, 335 nucleotides, 340 nucleotides, 345 nucleotides, 350 nucleotides, 355 nucleotides, 360 nucleotides, 365 nucleotides, 370 nucleotides, 375 nucleotides, 380 nucleotides, 385 nucleotides, 390 nucleotides, 395 nucleotides, 400 nucleotides, 405 nucleotides, 410 nucleotides, 415 nucleotides, 420 nucleotides, 425 nucleotides, 430 nucleotides, 435 nucleotides, 440 nucleotides, 445 nucleotides, 450 nucleotides, 455 nucleotides, 460 nucleotides, 465 nucleotides, 470 nucleotides, 475 nucleotides, 480 nucleotides, 485 nucleotides, 490 nucleotides, 495 nucleotides, or 500 nucleotides. In certain embodiments, the sgRNA is about 20 to about 100 nucleotides, *e.g.*, about 20 nucleotides, *e.g.*, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, 30 nucleotides, 31 nucleotides, 32 nucleotides, 33 nucleotides, 34 nucleotides, 35 nucleotides, 36 nucleotides, 37 nucleotides, 38 nucleotides, 39 nucleotides, 40 nucleotides, 41 nucleotides, 42 nucleotides, 43 nucleotides, 44 nucleotides, 45 nucleotides, 46 nucleotides, 47 nucleotides, 48 nucleotides, 49 nucleotides, 50 nucleotides, 51 nucleotides, 52 nucleotides, 53 nucleotides, 54 nucleotides, 55 nucleotides, 56 nucleotides, 57 nucleotides, 58 nucleotides, 59 nucleotides, 60 nucleotides, 61 nucleotides, 62 nucleotides, 63 nucleotides, 64 nucleotides, 65 nucleotides, 66 nucleotides, 67 nucleotides, 68

nucleotides, 69 nucleotides, 70 nucleotides, 71 nucleotides, 72 nucleotides, 73 nucleotides, 74 nucleotides, 75 nucleotides, 76 nucleotides, 77 nucleotides, 78 nucleotides, 79 nucleotides, 80 nucleotides, 81 nucleotides, 82 nucleotides, 83 nucleotides, 84 nucleotides, 85 nucleotides, 86 nucleotides, 87 nucleotides, 88 nucleotides, 89 nucleotides, 90 nucleotides, 91 nucleotides, 92 nucleotides, 93 nucleotides, 94 nucleotides, 95 nucleotides, 96 nucleotides, 97 nucleotides, 98 nucleotides, 99 nucleotides, or about 100 nucleotides.

[00206] The scaffold sequence can be about 10 to about 500 nucleotides, *e.g.*, about 10 nucleotides, 15 nucleotides, 20 nucleotides, 25 nucleotides, 30 nucleotides, 35 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, 60 nucleotides, 65 nucleotides, 70 nucleotides, 75 nucleotides, 80 nucleotides, 85 nucleotides, 90 nucleotides, 95 nucleotides, 100 nucleotides, 105 nucleotides, 110 nucleotides, 120 nucleotides, 130 nucleotides, 140 nucleotides, 150 nucleotides, 160 nucleotides, 170 nucleotides, 180 nucleotides, 190 nucleotides, 200 nucleotides, 210 nucleotides, 220 nucleotides, 230 nucleotides, 240 nucleotides, 250 nucleotides, 260 nucleotides, 270 nucleotides, 280 nucleotides, 290 nucleotides, 300 nucleotides, 310 nucleotides, 320 nucleotides, 330 nucleotides, 340 nucleotides, 350 nucleotides, 360 nucleotides, 370 nucleotides, 380 nucleotides, 390 nucleotides, 400 nucleotides, 410 nucleotides, 420 nucleotides, 430 nucleotides, 440 nucleotides, 450 nucleotides, 460 nucleotides, 470 nucleotides, 480 nucleotides, 490 nucleotides, or about 500 nucleotides. In some embodiments, the scaffold sequence is about 20 to about 500 nucleotides, *e.g.*, 20 nucleotides, 25 nucleotides, 30 nucleotides, 35 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, 60 nucleotides, 65 nucleotides, 70 nucleotides, 75 nucleotides, 80 nucleotides, 85 nucleotides, 90 nucleotides, 95 nucleotides, 100 nucleotides, 105 nucleotides, 110 nucleotides, 115 nucleotides, 120 nucleotides, 125 nucleotides, 130 nucleotides, 135 nucleotides, 140 nucleotides, 145 nucleotides, 150 nucleotides, 155 nucleotides, 160 nucleotides, 165 nucleotides, 170 nucleotides, 175 nucleotides, 180 nucleotides, 185 nucleotides, 190 nucleotides, 195 nucleotides, 200 nucleotides, 205 nucleotides, 210 nucleotides, 215 nucleotides, 220 nucleotides, 225 nucleotides, 230 nucleotides, 235 nucleotides, 240 nucleotides, 245 nucleotides, 250 nucleotides, 255 nucleotides, 260 nucleotides, 265 nucleotides, 270 nucleotides, 275 nucleotides, 280 nucleotides, 285 nucleotides, 290 nucleotides, 295 nucleotides, 300 nucleotides, 305 nucleotides, 310 nucleotides, 315 nucleotides, 320 nucleotides, 325 nucleotides, 330 nucleotides, 335 nucleotides, 340 nucleotides, 345

nucleotides, 350 nucleotides, 355 nucleotides, 360 nucleotides, 365 nucleotides, 370 nucleotides, 375 nucleotides, 380 nucleotides, 385 nucleotides, 390 nucleotides, 395 nucleotides, 400 nucleotides, 405 nucleotides, 410 nucleotides, 415 nucleotides, 420 nucleotides, 425 nucleotides, 430 nucleotides, 435 nucleotides, 440 nucleotides, 445 nucleotides, 450 nucleotides, 455 nucleotides, 460 nucleotides, 465 nucleotides, 470 nucleotides, 475 nucleotides, 480 nucleotides, 485 nucleotides, 490 nucleotides, 495 nucleotides, or 500 nucleotides. In certain embodiments, the scaffold sequence is about 20 to about 100 nucleotides, *e.g.*, about 20 nucleotides, *e.g.*, 20 nucleotides, 21 nucleotides, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, 29 nucleotides, 30 nucleotides, 31 nucleotides, 32 nucleotides, 33 nucleotides, 34 nucleotides, 35 nucleotides, 36 nucleotides, 37 nucleotides, 38 nucleotides, 39 nucleotides, 40 nucleotides, 41 nucleotides, 42 nucleotides, 43 nucleotides, 44 nucleotides, 45 nucleotides, 46 nucleotides, 47 nucleotides, 48 nucleotides, 49 nucleotides, 50 nucleotides, 51 nucleotides, 52 nucleotides, 53 nucleotides, 54 nucleotides, 55 nucleotides, 56 nucleotides, 57 nucleotides, 58 nucleotides, 59 nucleotides, 60 nucleotides, 61 nucleotides, 62 nucleotides, 63 nucleotides, 64 nucleotides, 65 nucleotides, 66 nucleotides, 67 nucleotides, 68 nucleotides, 69 nucleotides, 70 nucleotides, 71 nucleotides, 72 nucleotides, 73 nucleotides, 74 nucleotides, 75 nucleotides, 76 nucleotides, 77 nucleotides, 78 nucleotides, 79 nucleotides, 80 nucleotides, 81 nucleotides, 82 nucleotides, 83 nucleotides, 84 nucleotides, 85 nucleotides, 86 nucleotides, 87 nucleotides, 88 nucleotides, 89 nucleotides, 90 nucleotides, 91 nucleotides, 92 nucleotides, 93 nucleotides, 94 nucleotides, 95 nucleotides, 96 nucleotides, 97 nucleotides, 98 nucleotides, 99 nucleotides, or about 100 nucleotides.

[00207] The nucleotides of the sgRNA can include a modification in the ribose (*e.g.*, sugar) group, phosphate group, nucleobase, or any combination thereof. In some embodiments, the modification in the ribose group comprises a modification at the 2' position of the ribose.

[00208] In some embodiments, the nucleotide includes a 2'fluoro-arabino nucleic acid, tricycle-DNA (tc-DNA), peptide nucleic acid, cyclohexene nucleic acid (CeNA), locked nucleic acid (LNA), ethylene-bridged nucleic acid (ENA), a phosphodiamidate morpholino, or a combination thereof.

[00209] Modified nucleotides or nucleotide analogues can include sugar- and/or backbone-ribonucleotides (*i.e.*, include modifications to the phosphate-sugar backbone). For example,

the phosphodiester linkages of a native or natural RNA may be to include at least one of a nitrogen or sulfur heteroatom. In some backbone- ribonucleotides the phosphoester group connecting to adjacent ribonucleotides may be replaced by a group, *e.g.*, of phosphothioate group. In some sugar- ribonucleotides, the 2' moiety is a group selected from H, OR, R, halo, SH, SR, H₂, HR, R₂ or ON, wherein R is C₁-C₆ alkyl, alkenyl or alkynyl and halo is F, Cl, Br, or I.

[00210] In some embodiments, the nucleotide contains a sugar modification. Non-limiting examples of sugar modifications include 2'-deoxy-2'-fluoro-oligoribonucleotide (2'-fluoro-2'-deoxycytidine-5'-triphosphate, 2'-fluoro-2'-deoxyuridine-5'-triphosphate), 2'-deoxy-2'-deamine oligoribonucleotide (2'-amino-2'-deoxycytidine-5'-triphosphate, 2'-amino-2'-deoxyuridine-5'-triphosphate), 2'-O-alkyl oligoribonucleotide, 2'-deoxy-2'-C-alkyl oligoribonucleotide (2'-O-methylcytidine-5'-triphosphate, 2'-methyluridine-5'-triphosphate), 2'-C-alkyl oligoribonucleotide, and isomers thereof (2'-aracytidine-5'-triphosphate, 2'-arauridine-5'-triphosphate), azidotriphosphate (2'-azido-2'-deoxycytidine-5'-triphosphate, 2'-azido-2'-deoxyuridine-5'-triphosphate), and combinations thereof.

[00211] In some embodiments, the sgRNA contains one or more 2'-fluoro, 2'-amino and/or 2'-thio modifications. In some instances, the modification is a 2'-fluoro-cytidine, 2'-fluoro-uridine, 2'-fluoro-adenosine, 2'-fluoro-guanosine, 2'-amino-cytidine, 2'-amino-uridine, 2'-amino-adenosine, 2'-amino-guanosine, 2,6-diaminopurine, 4-thio-uridine, 5-amino-allyl-uridine, 5-bromo-uridine, 5-iodo-uridine, 5-methyl-cytidine, ribo-thymidine, 2-aminopurine, 2'-amino-butyryl-pyrene-uridine, 5-fluoro-cytidine, and/or 5-fluoro-uridine.

[00212] There are more than 96 naturally occurring nucleoside modifications found on mammalian RNA. See, *e.g.*, Limbach et al., *Nucleic Acids Research*, 22(12):2183-2196 (1994). The preparation of nucleotides and nucleosides are well-known in the art and described in, *e.g.*, U.S. Patent Nos. 4,373,071, 4,458,066, 4,500,707, 4,668,777, 4,973,679, 5,047,524, 5,132,418, 5,153,319, 5,262,530, and 5,700,642. Numerous nucleosides and nucleotides that are suitable for use as described herein are commercially available. The nucleoside can be an analogue of a naturally occurring nucleoside. In some cases, the analogue is dihydrouridine, methyladenosine, methylcytidine, methyluridine, methylpseudouridine, thiouridine, deoxycytidine, and deoxyuridine.

[00213] In some cases, the sgRNA described herein includes a nucleobase- ribonucleotide, *i.e.*, a ribonucleotide containing at least one non-naturally occurring nucleobase instead of a naturally occurring nucleobase. Non-limiting examples of nucleobases which can be incorporated into nucleosides and nucleotides include m5C (5-methylcytidine), m5U (5-methyluridine), m6A (N6-methyladenosine), s2U (2-thiouridine), Um (2'-O-methyluridine), m1A (1-methyl adenosine), m2A (2-methyladenosine), Am (2-1-O-methyladenosine), ms2m6A (2-methylthio-N6-methyladenosine), i6A (N6-isopentenyl adenosine), ms2i6A (2-methylthio-N6isopentenyladenosine), io6A (N6-(cis-hydroxyisopentenyl) adenosine), ms2io6A (2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine), g6A (N6-glycylcarbamoyladenosine), t6A (N6-threonyl carbamoyladenosine), ms2t6A (2-methylthio-N6-threonyl carbamoyladenosine), m6t6A (N6-methyl-N6-threonylcarbamoyladenosine), hn6A (N6-hydroxynorvalylcarbamoyl adenosine), ms2hn6A (2-methylthio-N6-hydroxynorvalyl carbamoyladenosine), Ar(p) (2'-O-ribosyladenosine(phosphate)), I (inosine), mi l (1-methylinosine), m'l m (1,2'-O-dimethylinosine), m3C (3-methylcytidine), Cm (2T-o-methylcytidine), s2C (2-thiocytidine), ac4C (N4-acetylcytidine), f5C (5-fonnylcytidine), m5Cm (5,2-O-dimethylcytidine), ac4Cm (N4acetyl2TOMethylcytidine), k2C (lysidine), m1G (1-methylguanosine), m2G (N2-methylguanosine), m7G (7-methylguanosine), Gm (2'-O-methylguanosine), m22G (N2,N2-dimethylguanosine), m2Gm (N2,2'-O-dimethylguanosine), m22Gm (N2,N2,2'-O-trimethylguanosine), Gr(p) (2'-O-ribosylguanosine(phosphate)), yW (wybutosine), o2yW (peroxywybutosine), OHyW (hydroxywybutosine), OHyW* (under hydroxywybutosine), imG (wyosine), mimG (methylguanosine), Q (queuosine), oQ (epoxyqueuosine), galQ (galtactosyl-queuosine), manQ (mannosyl- queuosine), preQo (7-cyano-7-deazaguanosine), preQi (7-aminomethyl-7-deazaguanosine), G (archaeosine), D (dihydrouridine), m5Um (5,2'-O-dimethyluridine), s4U (4-thiouridine), m5s2U (5-methyl-2-thiouridine), s2Um (2-thio-2'-O-methyluridine), acp3U (3-(3-amino-3-carboxypropyl)uridine), ho5U (5-hydroxyuridine), mo5U (5-methoxyuridine), cmo5U (uridine 5-oxyacetic acid), mcmo5U (uridine 5-oxyacetic acid methyl ester), chm5U (5-(carboxyhydroxymethyl)uridine)), mchm5U (5-(carboxyhydroxymethyl)uridine methyl ester), mcm5U (5-methoxycarbonyl methyluridine), mcm5Um (S-methoxycarbonylmethyl-2-O-methyluridine), mcm5s2U (5-methoxycarbonylmethyl-2-thiouridine), nm5s2U (5-aminomethyl-2-thiouridine), mnm5U (5-methylaminomethyluridine), mnm5s2U (5-methylaminomethyl-2-thiouridine), mnm5se2U

(5-methylaminomethyl-2-selenouridine), ncm5U (5-carbamoylmethyl uridine), ncm5Um (5-carbamoylmethyl-2'-O-methyluridine), cmnm5U (5-carboxymethylaminomethyluridine), cnmm5Um (5-carboxymethylaminomethyl- 2-L-Omethyluridine), cmnm5s2U (5-carboxymethylaminomethyl-2-thiouridine), m62A (N6,N6-dimethyladenosine), Tm (2'-O-methylinosine), m4C (N4-methylcytidine), m4Cm (N4,2-O-dimethylcytidine), hm5C (5-hydroxymethylcytidine), m3U (3 -methyluridine), cm5U (5-carboxymethyluridine), m6Am (N6,T-O-dimethyladenosine), m62Am (N6,N6,0-2- trimethyladenosine), m2'7G (N2,7-dimethylguanosine), m2'2'7G (N2,N2,7- trimethylguanosine), m3Um (3,2T-O-dimethyluridine), m5D (5-methyldihydrouridine), f5Cm (5-formyl-2'-O-methylcytidine), m1Gm (1,2'-O-dimethylguanosine), m'Am (1,2-O- dimethyl adenosine)irinomethyluridine), tm5s2U (S-taurinomethyl-2-thiouridine)), imG-14 (4-demethyl guanosine), imG2 (isoguanosine), or ac6A (N6-acetyladenosine), hypoxanthine, inosine, 8-oxo-adenine, 7-substituted derivatives thereof, dihydrouracil, pseudouracil, 2- thiouracil, 4-thiouracil, 5-aminouracil, 5-(C1-C6)-alkyluracil, 5-methyluracil, 5-(C2-C6)- alkenyluracil, 5-(C2-C6)- alkynyluracil, 5-(hydroxymethyl)uracil, 5-chlorouracil, 5- fluorouracil, 5-bromouracil, 5 -hydroxy cytosine, 5-(C1-C6)-alkylcytosine, 5-methylcytosine, 5-(C2-C6)-alkenylcytosine, 5-(C2-C6)-alkynylcytosine, 5-chlorocytosine, 5-fluorocytosine, 5- bromocytosine, N²-dimethylguanine, 7-deazaguanine, 8-azaguanine, 7-deaza-7-substituted guanine, 7-deaza-7-(C2-C6)alkynylguanine, 7-deaza-8-substituted guanine, 8- hydroxyguanine, 6-thioguanine, 8-oxoguanine, 2-aminopurine, 2-amino-6-chloropurine, 2,4- diaminopurine, 2,6-diaminopurine, 8-azapurine, substituted 7-deazapurine, 7-deaza-7- substituted purine, 7-deaza-8-substituted purine, and combinations thereof.

[00214] The sgRNA can be synthesized by any method known by one of ordinary skill in the art. In some embodiments, the sgRNA is chemically synthesized. Modified sgRNAs can be synthesized using 2'-O-thionocarbamate-protected nucleoside phosphoramidites. Methods are described in, *e.g.*, Dellinger et al., *J.American Chemical Society*, 133, 11540-11556 (2011); Threlfall et al., *Organic & Biomolecular Chemistry*, 10, 746-754 (2012); and Dellinger et al, *J. American Chemical Society*, 125, 940-950 (2003). Modified sgRNAs are commercially available from, *e.g.*, TriLink BioTechnologies (San Diego, CA).

[00215] Additional detailed description of useful sgRNAs can be found in, *e.g.*, Hendel et al., *Nat Biotechnol*, 2015, 33(9): 985-989 and Dever et al., *Nature*, 2016, 539: 384-389, the disclosures are herein incorporated by reference in their entirety for all purposes.

[00216] A person having skill in the art will appreciate that a guide RNA as disclosed in the present disclosure may be used in combination with any Cas protein known in the art (e.g., any Cas type, from any suitable organism or bacterial species).

[00217] The Cas protein may be a type I, type II, type III, type IV, type V, or type VI Cas protein. The Cas protein may comprise one or more domains. Non-limiting examples of domains include, a guide nucleic acid recognition and/or binding domain, nuclease domains (e.g., DNase or RNase domains, RuvC, HNH), DNA binding domain, RNA binding domain, helicase domains, protein-protein interaction domains, and dimerization domains. The guide nucleic acid recognition and/or binding domain may interact with a guide nucleic acid. The nuclease domain may comprise catalytic activity for nucleic acid cleavage. The nuclease domain may lack catalytic activity to prevent nucleic acid cleavage. The Cas protein may be a chimeric Cas protein that is fused to other proteins or polypeptides. The Cas protein may be a chimera of various Cas proteins, for example, comprising domains from different Cas proteins.

[00218] Non-limiting examples of Cas proteins include c2c1, C2c2, c2c3, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5e (CasD), CasH, Cas6e, Cas6f, Cas7, Cas8a, Cas8a1, Cas8a2, Cas8b, Cas8c, Cas9 (Csn1 or Csx12), Cas10, Cas10d, Cas1O, Cas1Od, CasF, CasG, CasH, Cpf1, Csy1, Csy2, Csy3, Cse1 (CasA), Cse2 (CasB), Cse3 (CasE), Cse4 (CasC), Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx1O, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, and Cul966, and homologs or modified versions thereof.

[00219] The Cas protein may be from any suitable organism. Non-limiting examples include *Streptococcus pyogenes*, *Streptococcus thermophilus*, *Streptococcus* sp., *Staphylococcus aureus*, *Nocardiopsis dassonvillei*, *Streptomyces pristinae spiralis*, *Streptomyces viridochromogenes*, *Streptomyces viridochromogenes*, *Streptosporangium roseum*, *Streptosporangium roseum*, *Alicyclobacillus acidocaldarius*, *Bacillus pseudomycoides*, *Bacillus selenitireducens*, *Exiguobacterium sibiricum*, *Lactobacillus delbrueckii*, *Lactobacillus salivarius*, *Microscilla marina*, *Burkholderiales bacterium*, *Polaromonas naphthalenivorans*, *Polaromonas* sp., *Crocospaera watsonii*, *Cyanothece* sp., *Microcystis aeruginosa*, *Pseudomonas aeruginosa*, *Synechococcus* sp., *Acetohalobium arabaticum*, *Ammonifex degensii*, *Caldicelulosiruptor beccarii*, *Candidatus Desulforudis*, *Clostridium*

botulinum, *Clostridium difficile*, *Finnegoldia magna*, *Natranaerobius thermophilus*, *Pelotomaculum thermopropionicum*, *Acidithiobacillus caldus*, *Acidithiobacillus ferrooxidans*, *Allochromatium vinosum*, *Marinobacter* sp., *Nitrosococcus halophilus*, *Nitrosococcus watsoni*, *Pseudoalteromonas haloplanktis*, *Ktedonobacter racemifer*, *Methanohalobium evestigatum*, *Anabaena variabilis*, *Nodularia spumigena*, *Nostoc* sp., *Arthrospira maxima*, *Arthrospira platensis*, *Arthrospira* sp., *Lyngbya* sp., *Microcoleus chthonoplastes*, *Oscillatoria* sp., *Petrogalea mobilis*, *Thermosiphon africanus*, *Acaryochloris marina*, *Leptotrichia shahii*, and *Francisella novicida*. In some aspects, the organism is *Streptococcus pyogenes* (*S. pyogenes*). In some aspects, the organism is *Staphylococcus aureus* (*S. aureus*). In some aspects, the organism is *Streptococcus thermophilus* (*S. thermophilus*).

[00220] The Cas protein may be derived from a variety of bacterial species including, but not limited to, *Veillonella atypical*, *Fusobacterium nucleatum*, *Filifactor alocis*, *Solobacterium moorei*, *Coprococcus catus*, *Treponema denticola*, *Peptoniphilus duerdenii*, *Catenibacterium mitsuokai*, *Streptococcus mutans*, *Listeria innocua*, *Staphylococcus pseudintermedius*, *Acidaminococcus intestine*, *Olsenella uli*, *Oenococcus kitaharae*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Finnegoldia magna*, *Mycoplasma mobile*, *Mycoplasma gallisepticum*, *Mycoplasma ovipneumoniae*, *Mycoplasma canis*, *Mycoplasma synoviae*, *Eubacterium rectale*, *Streptococcus thermophilus*, *Eubacterium dolichum*, *Lactobacillus coryniformis* subsp. *Torquens*, *Ilyobacter polytropus*, *Ruminococcus albus*, *Akkermansia muciniphila*, *Acidothermus cellulolyticus*, *Bifidobacterium longum*, *Bifidobacterium dentium*, *Corynebacterium diphtheria*, *Elusimicrobium minutum*, *Nitratifactor salsuginis*, *Sphaerochaeta globus*, *Fibrobacter succinogenes* subsp. *Succinogenes*, *Bacteroides fragilis*, *Capnocytophaga ochracea*, *Rhodopseudomonas palustris*, *Prevotella micans*, *Prevotella ruminicola*, *Flavobacterium columnare*, *Aminomonas paucivorans*, *Rhodospirillum rubrum*, *Candidatus Puniceispirillum marinum*, *Verminephrobacter eiseniae*, *Ralstonia syzygii*, *Dinoroseobacter shibae*, *Azospirillum*, *Nitrobacter hamburgensis*, *Bradyrhizobium*, *Wolinella succinogenes*, *Campylobacter jejuni* subsp. *Jejuni*, *Helicobacter mustelae*, *Bacillus cereus*, *Acidovorax ebreus*, *Clostridium perfringens*, *Parvibaculum lavamentivorans*, *Roseburia intestinalis*, *Neisseria meningitidis*, *Pasteurella multocida* subsp. *Multocida*, *Sutterella wadsworthensis*, *proteobacterium*, *Legionella pneumophila*, *Parasutterella excrementihominis*, *Wolinella succinogenes*, and *Francisella novicida*. The term, “derived,” in this instance, is defined as modified from the

naturally-occurring variety of bacterial species to maintain a significant portion or significant homology to the naturally-occurring variety of bacterial species. A significant portion may be at least 10 consecutive nucleotides, at least 20 consecutive nucleotides, at least 30 consecutive nucleotides, at least 40 consecutive nucleotides, at least 50 consecutive nucleotides, at least 60 consecutive nucleotides, at least 70 consecutive nucleotides, at least 80 consecutive nucleotides, at least 90 consecutive nucleotides or at least 100 consecutive nucleotides. Significant homology may be at least 50% homologous, at least 60% homologous, at least 70% homologous, at least 80% homologous, at least 90% homologous, or at least 95% homologous. The derived species may be modified while retaining an activity of the naturally-occurring variety.

Gene Editing Methods

[00221] As discussed above, embodiments of the present disclosure provide compositions and methods to treat joint disorders, wherein a portion of the joint cells are genetically modified via gene-editing to treat a joint disorder. Embodiments of the present disclosure embrace genetic editing through nucleotide insertion (RNA or DNA), or recombinant protein insertion, into a population of synoviocytes for both promotion of the expression of one or more proteins and inhibition of the expression of one or more proteins, as well as combinations thereof. Embodiments of the present disclosure also provide methods for delivering gene-editing compositions to joint cells, and in particular delivering gene-editing compositions to synoviocytes. There are several gene-editing technologies that may be used to genetically modify joint cells, which are suitable for use in accordance with the present disclosure.

[00222] In some embodiments, a method of genetically modifying joint cells includes the step of stable incorporation of genes for production of one or more proteins. In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of retroviral transduction. In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of lentiviral transduction. Lentiviral transduction systems are known in the art and are described, e.g., in Levine, et al., Proc. Nat'l Acad. Sci. 2006, 103, 17372-77; Zufferey, et al., Nat. Biotechnol. 1997, 15, 871-75; Dull, et al., J. Virology 1998, 72, 8463-71, and U.S. Patent No. 6,627,442, the disclosures of each of which are incorporated by reference herein. In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of gamma-retroviral

transduction. Gamma-retroviral transduction systems are known in the art and are described, e.g., Cepko and Pear, *Cur. Prot. Mol. Biol.* 1996, 9.9.1-9.9.16, the disclosure of which is incorporated by reference herein. In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of transposon-mediated gene transfer. Transposon-mediated gene transfer systems are known in the art and include systems wherein the transposase is provided as DNA expression vector or as an expressible RNA or a protein such that long-term expression of the transposase does not occur in the transgenic cells, for example, a transposase provided as an mRNA (e.g., an mRNA comprising a cap and poly-A tail). Suitable transposon-mediated gene transfer systems, including the salmonid-type Tel-like transposase (SB or Sleeping Beauty transposase), such as SB10, SB11, and SB100x, and engineered enzymes with increased enzymatic activity, are described in, e.g., Hackett, *et al.*, *Mol. Therapy* **2010**, 18, 674-83 and U.S. Patent No. 6,489,458, the disclosures of each of which are incorporated by reference herein.

[00223] In some aspects, viral vectors or systems are used to introduce a gene-editing system into cells comprising a joint. In some aspects, the cells are synovial fibroblasts. In some aspects, the viral vectors are an AAV vector. In some aspects, the AAV vector comprises a serotype selected from the group consisting of: AAV1, AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV4, AAV5, AAV5(Y436+693+719F), AAV6, AAV6 (VP3 variant Y705F/Y731F/T492V), AAV-7m8, AAV8, AAV8(Y733F), AAV9, AAV9 (VP3 variant Y731F), AAV10(Y733F), AAV-ShH10, and AAV-DJ/8. In some aspects, the AAV vector comprises a serotype selected from the group consisting of: AAV1, AAV5, AAV6, AAV6 (Y705F/Y731F/T492V), AAV8, AAV9, and AAV9 (Y731F).

[00224] In some aspects, the viral vector is a lentivirus. In an aspect, the lentivirus is selected from the group consisting of: human immunodeficiency-1 (HIV-1), human immunodeficiency-2 (HIV-2), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), Jembrana Disease Virus (JDV), equine infectious anemia virus (EIAV), and caprine arthritis encephalitis virus (CAEV).

[00225] In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of stable incorporation of genes for production or inhibition (e.g., silencing) of one or more proteins. In an embodiment, a method of genetically

modifying a portion of a joint's synoviocytes includes the step of liposomal transfection. Liposomal transfection methods, such as methods that employ a 1:1 (w/w) liposome formulation of the cationic lipid *N*-[1-(2,3-dioleoyloxy)propyl]-*n,n,n*-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanolamine (DOPE) in filtered water, are known in the art and are described in Rose, *et al.*, *Biotechniques* **1991**, 10, 520-525 and Felgner, *et al.*, *Proc. Natl. Acad. Sci. USA*, **1987**, 84, 7413-7417 and in U.S. Patent Nos. 5,279,833; 5,908,635; 6,056,938; 6,110,490; 6,534,484; and 7,687,070, the disclosures of each of which are incorporated by reference herein. In an embodiment, a method of genetically modifying a portion of a joint's synoviocytes includes the step of transfection using methods described in U.S. Patent Nos. 5,766,902; 6,025,337; 6,410,517; 6,475,994; and 7,189,705; the disclosures of each of which are incorporated by reference herein.

[00226] According to an embodiment, the gene-editing process may comprise the use of a programmable nuclease that mediates the generation of a double-strand or single-strand break at one or more immune checkpoint genes. Such programmable nucleases enable precise genome editing by introducing breaks at specific genomic loci, *i.e.*, they rely on the recognition of a specific DNA sequence within the genome to target a nuclease domain to this location and mediate the generation of a double-strand break at the target sequence. A double-strand break in the DNA subsequently recruits endogenous repair machinery to the break site to mediate genome editing by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). Thus, the repair of the break can result in the introduction of insertion/deletion mutations that disrupt (*e.g.*, silence, repress, or enhance) the target gene product.

[00227] Major classes of nucleases that have been developed to enable site-specific genomic editing include zinc finger nucleases (ZFNs), transcription activator-like nucleases (TALENs), and CRISPR-associated nucleases (*e.g.*, CRISPR-Cas9). These nuclease systems can be broadly classified into two categories based on their mode of DNA recognition: ZFNs and TALENs achieve specific DNA binding via protein-DNA interactions, whereas CRISPR systems, such as Cas9, are targeted to specific DNA sequences by a short RNA guide molecule that base-pairs directly with the target DNA and by protein-DNA interactions. *See, e.g.*, Cox *et al.*, *Nature Medicine*, 2015, Vol. 21, No. 2.

[00228] Non-limiting examples of gene-editing methods that may be used in accordance with the methods of the present disclosure include CRISPR methods, TALE methods, and ZFN methods, which are described in more detail below.

CRISPR Methods

[00229] A pharmaceutical composition for the treatment or prevention of a joint disease or condition comprising a gene-editing system, wherein said gene-editing system targets at least one locus related to joint function, wherein the gene-editing at least a portion of a joint's synoviocytes by a CRISPR method (*e.g.*, CRISPR-Cas9, CRISPR-Cas13a, or CRISPR/Cpf1 (also known as CRISPR-Cas12a). According to particular embodiments, the use of a CRISPR method to gene-edit joint synoviocytes causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the joint's synoviocytes.

[00230] CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." A method of using a CRISPR system for gene editing is also referred to herein as a CRISPR method. There are three types of CRISPR systems which incorporate RNAs and Cas proteins, and which may be used in accordance with the present disclosure: Types II, V, and VI. The Type II CRISPR (exemplified by Cas9) is one of the most well-characterized systems.

[00231] CRISPR technology was adapted from the natural defense mechanisms of bacteria and archaea (the domain of single-celled microorganisms). These organisms use CRISPR-derived RNA and various Cas proteins, including Cas9, to foil attacks by viruses and other foreign bodies by chopping up and destroying the DNA, or RNA, of a foreign invader. A CRISPR is a specialized region of DNA with two distinct characteristics: the presence of nucleotide repeats and spacers. Repeated sequences of nucleotides are distributed throughout a CRISPR region with short segments of foreign DNA (spacers) interspersed among the repeated sequences. In the type II CRISPR-Cas system, spacers are integrated within the CRISPR genomic loci and transcribed and processed into short CRISPR RNA (crRNA). These crRNAs anneal to trans-activating crRNAs (tracrRNAs) and direct sequence-specific cleavage and silencing of pathogenic DNA by Cas proteins. Target recognition by the Cas9 protein requires a "seed" sequence within the crRNA and a conserved dinucleotide-containing protospacer adjacent motif (PAM) sequence upstream of the crRNA-binding region. The CRISPR-Cas system can thereby be retargeted to cleave virtually any DNA sequence by redesigning the crRNA. The crRNA and tracrRNA in the native system can be simplified into a single guide RNA (sgRNA) of approximately 100 nucleotides for use in

genetic engineering. The CRISPR-Cas system is directly portable to human cells by co-delivery of plasmids expressing the Cas9 endo-nuclease and the necessary crRNA and tracrRNA (or sgRNA) components. Different variants of Cas proteins may be used to reduce targeting limitations (*e.g.*, orthologs of Cas9, such as Cpf1).

[00232] *CRISPR-Cas Mediated Homologous Recombination*

[00233] The CRISPR-Cas system for homologous recombination (HR) includes a Cas nuclease (*e.g.*, Cas9 nuclease) or a variant or fragment thereof, a DNA-targeting RNA (*e.g.*, single guide RNA (sgRNA)) containing a guide sequence that targets the Cas nuclease to the target genomic DNA and a scaffold sequence that interacts with the Cas nuclease, and a donor template. The CRISPR-Cas system can be utilized to create a double-strand break at a desired target gene locus in the genome of a cell, and harness the cell's endogenous mechanisms to repair the induced break by homology-directed repair (HDR).

[00234] The CRISPR-Cas9 nuclease can facilitate locus-specific chromosomal integration of exogenous DNA delivered by AAV vectors. Typically, the size of the exogenous DNA (*e.g.*, transgene, expression cassette, and the like) that can be integrated is limited by the DNA packaging capacity of an AAV vector which is about 4.0 kb. With the inclusion of two homology arms that are necessary for homologous recombination, a single AAV vector can only deliver less than about 3.7 kb of exogenous DNA. The method described herein allows for the delivery of exogenous DNA that is 4 kb or longer by splitting the nucleotide sequence between two different AAV vectors. The donor templates are designed for sequential homologous recombination events that can integrate and fuse the two parts of the nucleotide sequence.

[00235] Homologous recombination of the present disclosure can be performed using an engineered nuclease system for genome editing such as, but not limited to, CRISPR-Cas nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), engineered mega-nucleases. In one aspect, a CRISPR-Cas-based nuclease system is used. Detailed descriptions of useful nuclease system can be found, *e.g.*, in Gaj et al., *Trends Biotechnol.*, 2013, Jul: 31(7):397-405.

[00236] Any suitable CRISPR/Cas system may be used for the methods and compositions disclosed herein. The CRISPR/Cas system may be referred to using a variety of naming systems. Exemplary naming systems are provided in Makarova, K. S. et al, "An updated

evolutionary classification of CRISPR-Cas systems,” *Nat Rev Microbiol* (2015) 13:722-736 and Shmakov, S. et al, “Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems,” *Mol Cell* (2015) 60:1-13. The CRISPR/Cas system may be a type I, a type II, a type III, a type IV, a type V, a type VI system, or any other suitable CRISPR/Cas system. The CRISPR/Cas system as used herein may be a Class 1, Class 2, or any other suitably classified CRISPR/Cas system. The Class 1 CRISPR/Cas system may use a complex of multiple Cas proteins to effect regulation. The Class 1 CRISPR/Cas system may comprise, for example, type I (e.g., I, IA, IB, IC, ID, IE, IF, IU), type III (e.g., III, IIIA, IIIB, IIIC, IIID), and type IV (e.g., IV, IVA, IVB) CRISPR/Cas type. The Class 2 CRISPR/Cas system may use a single large Cas protein to effect regulation. The Class 2 CRISPR/Cas systems may comprise, for example, type II (e.g., II, IIA, IIB) and type V CRISPR/Cas type. CRISPR systems may be complementary to each other, and/or can lend functional units in trans to facilitate CRISPR locus targeting.

[00237] In some embodiments, a nucleotide sequence encoding the Cas nuclease is present in a recombinant expression vector. In certain instances, the recombinant expression vector is a viral construct, *e.g.*, a recombinant adeno-associated virus construct, a recombinant adenoviral construct, a recombinant lentiviral construct, etc. For example, viral vectors can be based on vaccinia virus, poliovirus, adenovirus, adeno-associated virus, SV40, herpes simplex virus, human immunodeficiency virus, and the like. A retroviral vector can be based on Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, mammary tumor virus, and the like. Useful expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example for eukaryotic host cells: pXTI, pSG5, pSVK3, pBPV, pMSG, and pSVLSV40. However, any other vector may be used if it is compatible with the host cell. For example, useful expression vectors containing a nucleotide sequence encoding a Cas9 enzyme are commercially available from, *e.g.*, Addgene, Life Technologies, Sigma- Aldrich, and Origene.

[00238] Host cells are necessary for generating infectious AAV vectors as well as for generating AAV virions based on the disclosed AAV vectors. Various host cells are known

in the art and find use in the methods of the present disclosure. Any host cells described herein or known in the art can be employed with the compositions and methods described herein.

[00239] In some embodiments, the host cell for use in generating infectious virions can be selected from any biological organism, including prokaryotic (*e.g.*, bacterial) cells, and eukaryotic cells, including, insect cells, yeast cells and mammalian cells. A variety of cells, *e.g.*, mammalian cells, including, *e.g.*, murine cells, and primate cells (*e.g.*, human cells) can be used. Particularly desirable host cells are selected from among any mammalian species, including, without limitation, cells such as A549, WEHI, 3T3, 10T1/2, BHK, MDCK, COS 1, COS 7, BSC 1, BSC 40, BMT 10, VERO, WI38, HeLa, CHO, 293, Vero, NIH 3T3, PC12, Huh-7 Saos, C2C12, RAT1, Sf9, L cells, HT1080, human embryonic kidney (HEK), human embryonic stem cells, human adult tissue stem cells, pluripotent stem cells, induced pluripotent stem cells, reprogrammed stem cells, organoid stem cells, bone marrow stem cells, HLHepG2, HepG2 and primary fibroblast, hepatocyte and myoblast cells derived from mammals including human, monkey, mouse, rat, rabbit, and hamster. The requirement for the cell used is it is capable of infection or transfection by an AAV vector. In some embodiments, the host cell is one that has rep and cap stably transfected in the cell.

[00240] In some embodiments, the preparation of a host cell according to the disclosure involves techniques such as assembly of selected DNA sequences. This assembly may be accomplished utilizing conventional techniques. Such techniques include cDNA and genomic cloning, which are well known and are described in Sambrook *et al.*, cited above, use of overlapping oligonucleotide sequences of the adenovirus and AAV genomes, combined with polymerase chain reaction, synthetic methods, and any other suitable methods for providing the desired nucleotide sequence.

[00241] In addition to the AAV vector, the host cell can contain sequences to drive expression of the AAV capsid polypeptide (in the host cell and rep (replication) sequences of the same serotype as the serotype of the AAV Inverted Terminal Repeats (ITRs) found in the AAV vector, or a cross-complementing serotype. The AAV capsid and rep (replication) sequences may be independently obtained from an AAV source and may be introduced into the host cell in any manner known to one of skill in the art or as described herein. Additionally, when pseudotyping an AAV vector in an AAV8 capsid for example, the

sequences encoding each of the essential rep (replication) proteins may be supplied by AAV8, or the sequences encoding the rep (replication) proteins may be supplied by different AAV serotypes (*e.g.*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, and/or AAV9).

[00242] In some embodiments, the host cell stably contains the capsid protein under the control of a suitable promoter. In some embodiments, the capsid protein is supplied to the host cell *in trans*. When delivered to the host cell *in trans*, the capsid protein may be delivered via a plasmid containing the sequences necessary to direct expression of the selected capsid protein in the host cell. In some embodiments, when delivered to the host cell *in trans*, the vector encoding the capsid protein also carries other sequences required for packaging the AAV, *e.g.*, the rep (replication) sequences.

[00243] In some embodiments, the host cell stably contains the rep (replication) sequences under the control of a suitable promoter. In another embodiment, the rep (replication) proteins are supplied to the host cell *in trans*. When delivered to the host cell *in trans*, the rep (replication) proteins may be delivered via a plasmid containing the sequences necessary to direct expression of the selected rep (replication) proteins in the host cell. In some embodiments, when delivered to the host cell *in trans*, the vector encoding the capsid protein (also carries other sequences required for packaging the AAV vector, *e.g.*, the rep (replication) sequences).

[00244] In some embodiments, the rep (replication) and capsid sequences may be transfected into the host cell on a single nucleic acid molecule and exist stably in the cell as an unintegrated episome. In another embodiment, the rep (replication) and capsid sequences are stably integrated into the chromosome of the cell. Another embodiment has the rep (replication) and capsid sequences transiently expressed in the host cell. For example, a useful nucleic acid molecule for such transfection comprises, from 5' to 3', a promoter, an optional spacer interposed between the promoter and the start site of the rep (replication) gene sequence, an AAV rep (replication) gene sequence, and an AAV capsid gene sequence.

[00245] Although the molecule(s) providing rep (replication) and capsid can exist in the host cell transiently (*i.e.*, through transfection), in some embodiments, one or both of the rep (replication) and capsid proteins and the promoter(s) controlling their expression be stably expressed in the host cell, *e.g.*, as an episome or by integration into the chromosome of the host cell. The methods employed for constructing embodiments of the disclosure are

conventional genetic engineering or recombinant engineering techniques such as those described in the references above.

[00246] A variety of methods of generating AAV virions are known in the art and can be used to generate AAV virions comprising the AAV vectors described herein. Generally, the methods involved inserting or transducing an AAV vector of the disclosure into a host cell capable of packaging the AAV vector into and AAV virion. Exemplary methods are described and referenced below; however, any method known to one of skill in the art can be employed to generate the AAV virions of the disclosure.

[00247] An AAV vector comprising a heterologous nucleic acid (*e.g.*, a donor template) and used to generate an AAV virion can be constructed using methods that are well known in the art. *See, e.g.*, Koerber *et al.* (2009) *Mol. Ther.*, 17:2088; Koerber *et al.* (2008) *Mol Ther.*, 16: 1703-1709; as well as U.S. Pat. Nos. 7,439,065, 6,951,758, and 6,491,907. For example, the heterologous sequence(s) can be directly inserted into an AAV genome with the major AAV open reading frames (“ORFs”) excised therefrom. Other portions of the AAV genome can also be deleted, so long as a sufficient portion of the ITRs remain to allow for replication and packaging functions. Such constructs can be designed using techniques well known in the art. *See, e.g.*, U.S. Pat. Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published Jan. 23, 1992) and WO 93/03769 (published Mar. 4, 1993); Lebkowski *et al.* (1988) *Molec. Cell. Biol.* 8:3988-3996; Vincent *et al.* (1990) *Vaccines 90* (Cold Spring Harbor Laboratory Press); Carter, B. J. (1992) *Current Opinion in Biotechnology* 3:533-539; Muzyczka, N. (1992) *Curr. Topics Microbiol. Immunol.* 158:97-129; Kotin, R. M. (1994) *Human Gene Therapy* 5:793-801; Shelling and Smith (1994) *Gene Therapy* 1:165-169; and Zhou *et al.* (1994) *J. Exp. Med.* 179:1867-1875.

[00248] In order to produce AAV virions, an AAV vector is introduced into a suitable host cell using known techniques, such as by transfection. A number of transfection techniques are generally known in the art. *See, e.g.*, Graham *et al.* (1973) *Virology*, 52:456, Sambrook *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, Davis *et al.* (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu *et al.* (1981) *Gene* 13:197. Particularly suitable transfection methods include calcium phosphate co-precipitation (Graham *et al.* (1973) *Virol.* 52:456-467), direct micro-injection into cultured cells (Capecchi, M. R. (1980) *Cell* 22:479-488), electroporation (Shigekawa *et al.* (1988)

BioTechniques 6:742-751), liposome mediated gene transfer (Mannino *et al.* (1988) *BioTechniques* 6:682-690), lipid-mediated transduction (Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7417), and nucleic acid delivery using high-velocity microprojectiles (Klein *et al.* (1987) *Nature* 327:70-73).

[00249] Depending on the expression system used, any of a number of transcription and translation control elements, including promoter, transcription enhancers, transcription terminators, and the like, may be used in the expression vector. Useful promoters can be derived from viruses, or any organism, *e.g.*, prokaryotic or eukaryotic organisms. Suitable promoters include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter, adenovirus major late promoter (Ad MLP), a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter (such as the CMV immediate early promoter region; CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6), an enhanced U6 promoter, and a human HI promoter (HI), etc.

[00250] In some embodiments, polynucleotide encoding a Cas nuclease can be used in the present disclosure. Such a polynucleotide (*e.g.*, mRNA) can be commercially obtained from, for example, TriLink BioTechnologies, GE Dharmacon, ThermoFisher, and the like.

[00251] In certain embodiments, a Cas nuclease (*e.g.*, Cas9 polypeptide) can be used in the present disclosure. Detailed description of useful Cas9 polypeptides can be found in, *e.g.*, Hendel *et al.*, *Nat Biotechnol*, 2015, 33(9): 985-989 and Dever *et al.*, *Nature*, 2016, 539: 384-389, the disclosures are herein incorporated by reference in their entirety for all purposes.

[00252] In some embodiments, a Cas nuclease (*e.g.*, Cas9 polypeptide) is complexed with a sgRNA to form a Cas ribonucleoprotein (*e.g.*, Cas9 ribonucleoprotein). The molar ratio of Cas nuclease to sgRNA can be any range that facilitates sequential homologous recombination of the targeting AAV vectors and target genetic locus. In some embodiments, the molar ratio of Cas9 polypeptide to sgRNA is about 1:5; 1:4; 1:3; 1:2.5; 1:2; or 1:1. In other embodiments, the molar ratio of Cas9 polypeptide to sgRNA is about 1:2 to about 1:3. In certain embodiments, the molar ratio of Cas9 polypeptide to sgRNA is about 1:2.5.

[00253] The Cas nuclease and variants or fragments thereof can be introduced into a cell (*e.g.*, a cell isolated from a subject, or an *in vivo* cell such as in a subject) as a Cas polypeptide or a variant or fragment thereof, an mRNA encoding a Cas polypeptide or a

variant or fragment thereof, a recombinant expression vector comprising a nucleotide sequence encoding a Cas polypeptide or a variant or fragment thereof, or a Cas ribonucleoprotein. One skilled in the art would recognize that any method of delivering an exogenous polynucleotide, polypeptide, or a ribonucleoprotein can be used. Non-limiting examples of such methods include electroporation, nucleofection, transfection, lipofection, transduction, microinjection, electroinjection, electrofusion, nanoparticle bombardment, transformation, conjugation, and the like.

[00254] In one aspect, the present disclosure provides for the use of nanoparticles as a means of delivering CRISPR components to a subject in need thereof. In some embodiments, the nanoparticles are selected from, lipid nanoparticles (LNPs) or liposomes, hydrogel nanoparticles, metalorganic nanoparticles, gold nanoparticles, and magnetic nanoparticles, See, e.g. Xu, C. F., et al. (2021). *Advanced Drug Delivery Reviews*, 168, 3-29; see also Buschmann et al. (2021). *Vaccines* 9:65; Kenjo, E., et al. (2021). *Nature Communications*, 12(1), 7101.

[00255] In some embodiments, CRISPR components are delivered by a nanoparticle. Without wishing to be bound by any particular theory, in certain embodiments, nucleic acids, when present in the nanoparticle, are resistant in aqueous solution to degradation with a nuclease. Lipid nanoparticles comprising nucleic acids and their method of preparation is disclosed in at least WO2017/019935, WO2017/049074, WO2017/201346, WO2017/218704, WO2018/006052, WO2018/013525, WO2018/089540, WO2018/119115, WO2018/126084, WO2018/157009, WO2018/170336, WO2018/222890, WO2019/046809, WO2019/089828, WO2020/061284, WO2020/061317, WO2020/081938, WO2020/097511, WO2020/097520, WO2020/097540, WO2020/097548, WO2020/214946, WO2020/219941, WO2020/232276, WO2020/227615, WO2020/061295, WO2021/007278, WO2021/016430, WO2021/021988, EP Patent No. EP 2 972 360, US20200155691, US20200237671, U.S. Patent Nos. 8,058,069, 8,492,359, 8,822,668, 9,364,435, 9,404,127, 9,504,651, 9,593,077, 9,738,593, 9,868,691, 9,868,692, 9,950,068, 10,138,213, 10,166,298, 10,221,127, 10,238,754, 10,266,485, 10,383,952, 10,730,924, 10,766,852, 11,141,378 and 11,246,933, which are incorporated herein by reference in their entirety for all purposes.

Lipid Nanoparticle Compositions

[00256] In some embodiments, the largest dimension of a nanoparticle composition is 1 micrometer or shorter (e.g., 1 micrometer, 900 nm, 800 nm, 700 nm, 600 nm, 500 nm, 400 nm, 300 nm, 200 nm, 175 nm, 150 nm, 125 nm, 100 nm, 75 nm, 50 nm, or shorter), e.g., when measured by dynamic light scattering (DLS), transmission electron microscopy, scanning electron microscopy, or another method. Nanoparticle compositions include, for example, lipid nanoparticles (LNPs), liposomes, lipid vesicles, and lipoplexes. In some embodiments, nanoparticle compositions are vesicles including one or more lipid bilayers. In certain embodiments, a nanoparticle composition includes two or more concentric bilayers separated by aqueous compartments. Lipid bilayers may be functionalized and/or crosslinked to one another. Lipid bilayers may include one or more ligands, proteins, or channels.

[00257] In various embodiments, lipid nanoparticles described herein have a mean diameter of from about 30 nm to about 150 nm, from about 40 nm to about 150 nm, from about 50 nm to about 150 nm, from about 60 nm to about 130 nm, from about 70 nm to about 110 nm, from about 70 nm to about 100 nm, from about 80 nm to about 100 nm, from about 90 nm to about 100 nm, from about 70 nm to about 90 nm, from about 80 nm to about 90 nm, from about 70 nm to about 80 nm, or about 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm, and are substantially non-toxic.

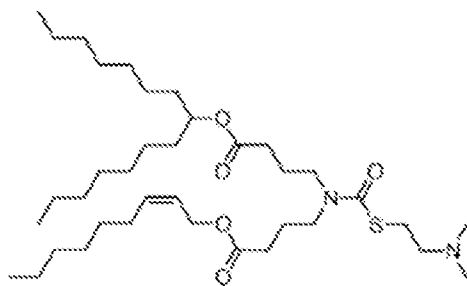
[00258] In certain embodiments, the lipid nanoparticles described herein comprise one or more components, including a lipid component, and (optionally) a structural component. The lipid component comprises lipids selected from ionizable and/or cationic lipids (i.e., lipids that may have a positive or partial positive charge at physiological pH), neutral lipids (e.g., phospholipids, or sphingolipids), and polymer-conjugated lipids (e.g., PEGylated lipids). In some embodiments, the lipid component comprises a single ionizable lipid. In other embodiments, the lipid component comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 ionizable lipids. In some embodiments, the lipid component comprises a single neutral lipid. In other embodiments, the lipid component comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 neutral lipids. In some embodiments, the lipid component comprises a single polymer-conjugated lipid. In other embodiments, the lipid component comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 polymer-conjugated lipids. In some embodiments, the structural component comprises a single structural lipid. In other embodiments, the structural component

comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, or 20 structural lipids. In some embodiments, the lipid component comprises at least one cationic lipid, at least one neutral lipid, and at least one polymer-conjugated lipid. The present disclosure contemplates that the lipid component may comprise any combination of the foregoing constituents.

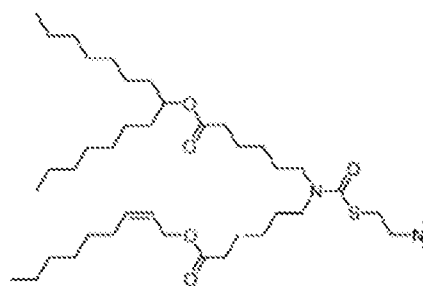
Ionizable/Cationic Lipids

[00259] In some embodiments, the lipid component comprises an ionizable lipid. In some embodiments, the ionizable lipid is anionic. In other embodiments, the ionizable lipid is a cationic lipid. In some embodiments, the lipid component comprises cationic lipids including, but not limited to, a cationic lipid selected from the group consisting of 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z-,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), (2S)-2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z-,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), a lipid including a cyclic amine group, and mixtures thereof.

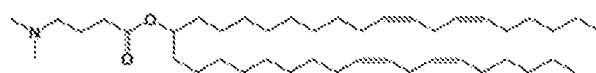
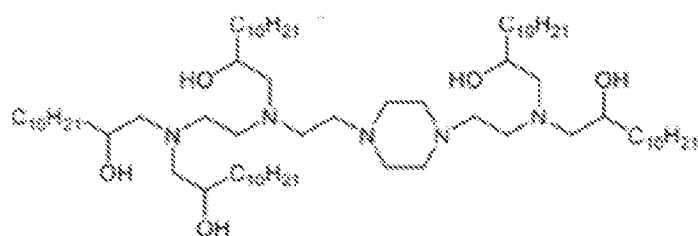
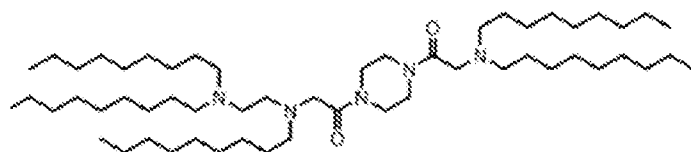
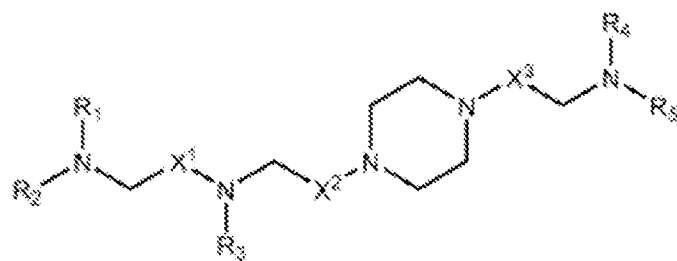
[00260] Non-exhaustive and non-limiting examples of cationic lipids include:



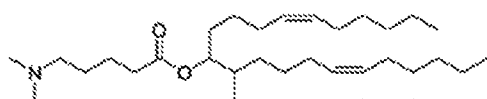
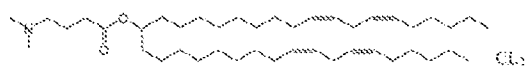
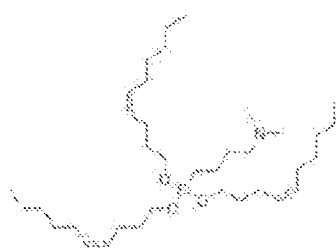
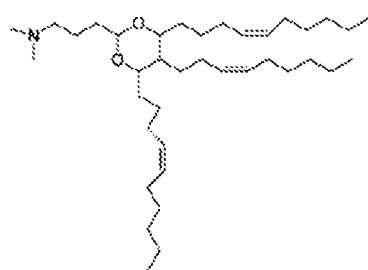
AFX-0081

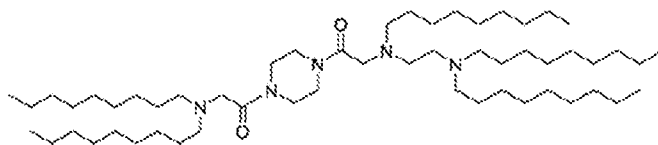


AFX-0095

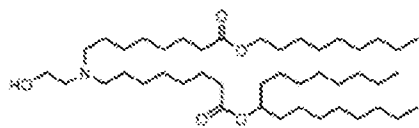


MC3

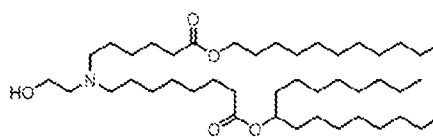
Genevant CL₁CL₂CL₄



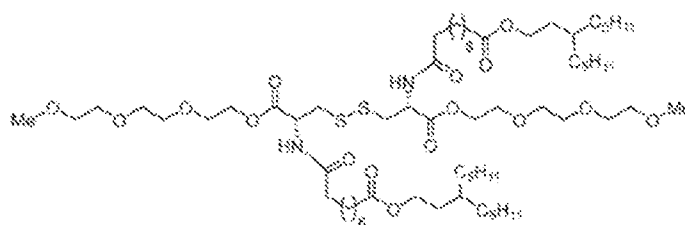
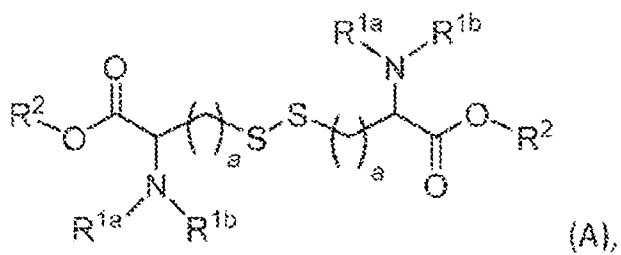
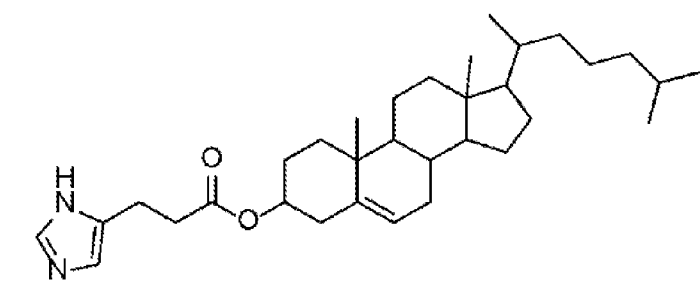
Compound (A),

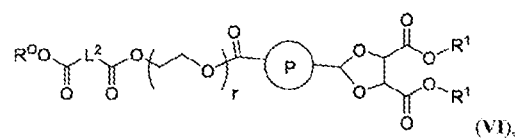
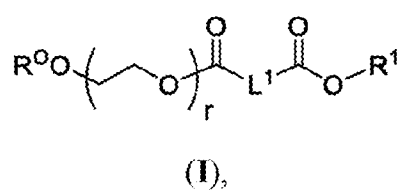
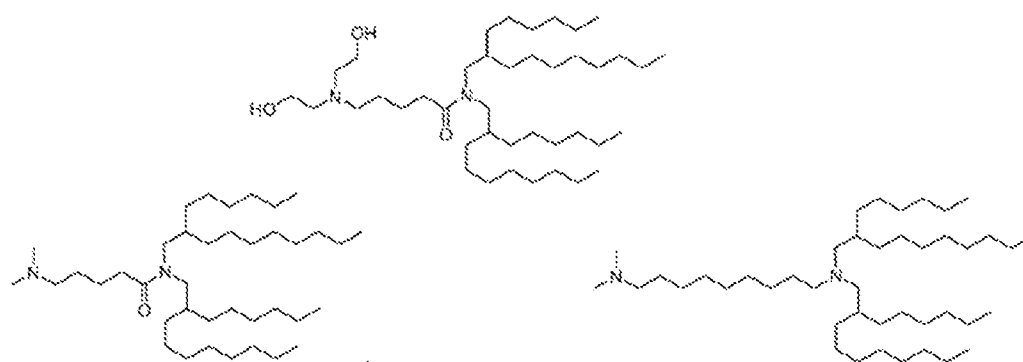
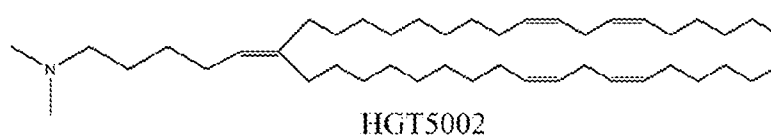
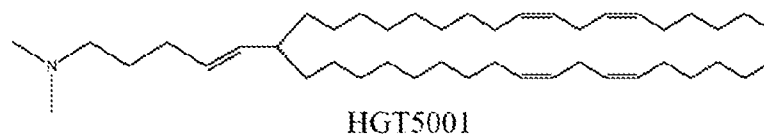
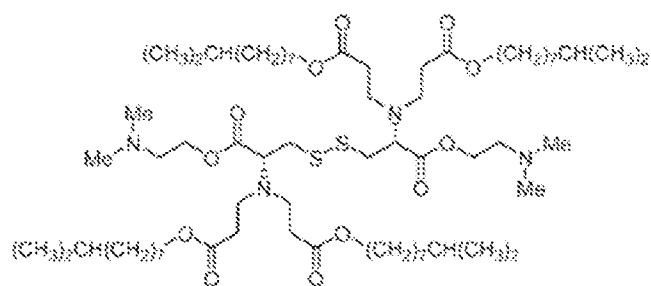


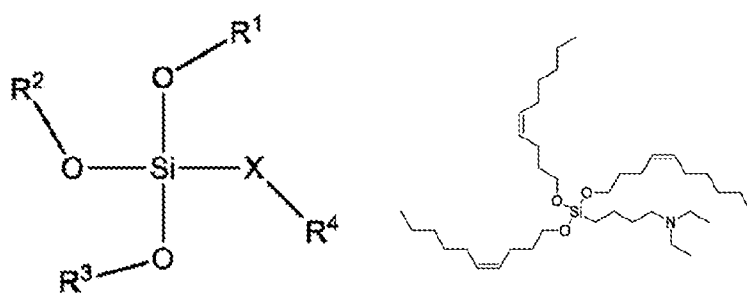
Moderna Lipid 5

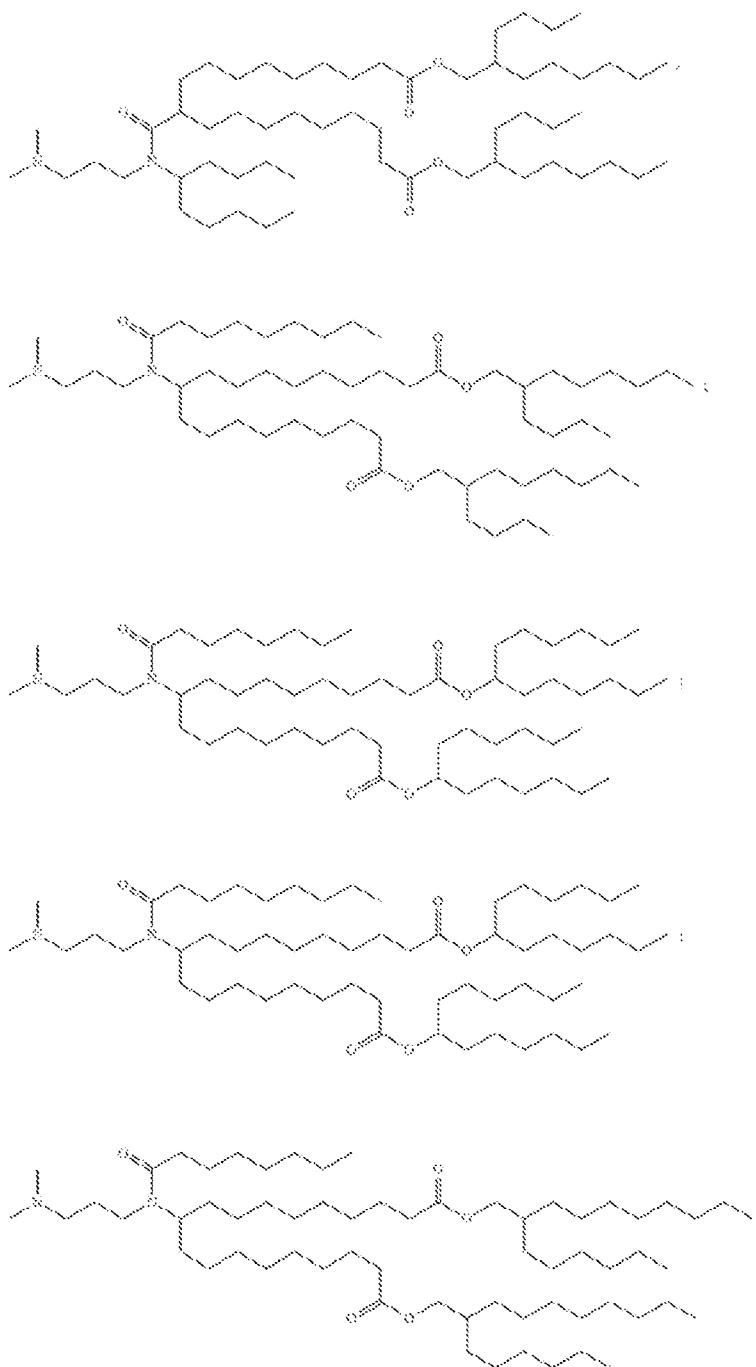


Moderna Lipid H









Neutral Lipids/Phospholipids

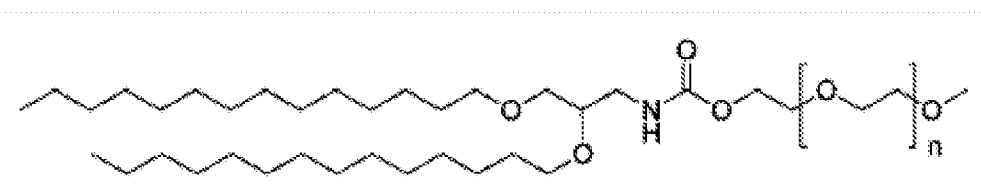
[00261] In some embodiments, the lipid component further comprises neutral lipids including, but not limited to, a phospholipid selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-

dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin (SM), and mixtures thereof.

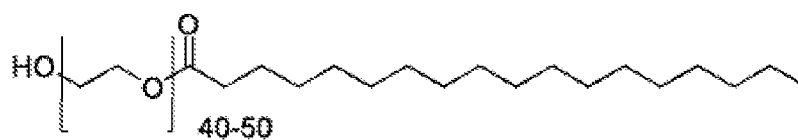
Polymer-conjugated Lipids

[00262] In some embodiments, the lipid component further comprises polymer-conjugated lipids, including, but not limited to, a PEGylated lipid selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DMA or a PEG-DSPE lipid.

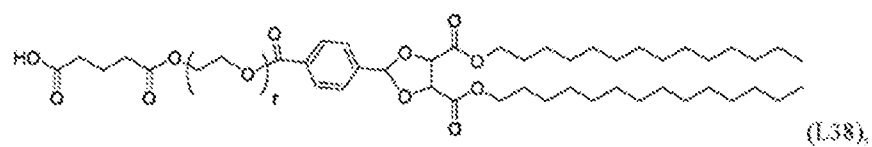
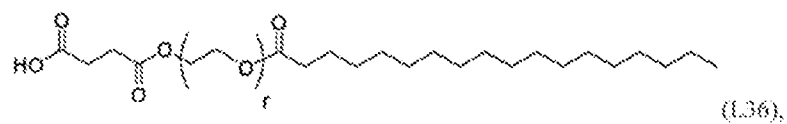
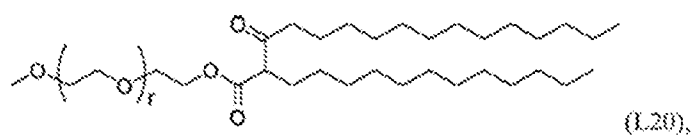
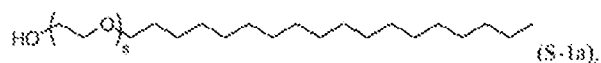
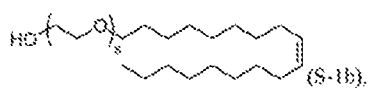
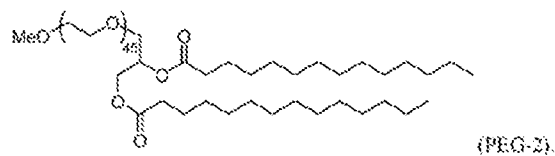
[00263] Non-exhaustive and non-limiting examples of PEG lipids include:

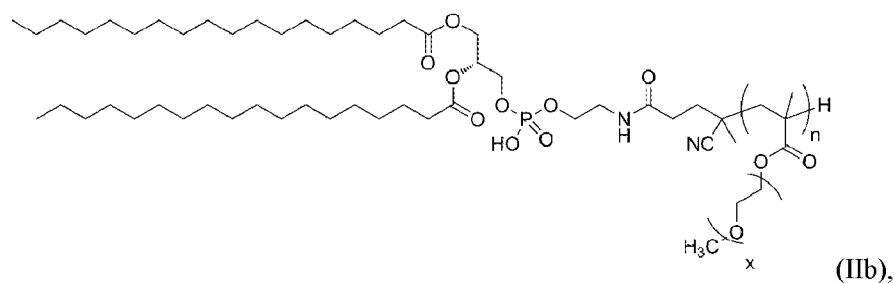
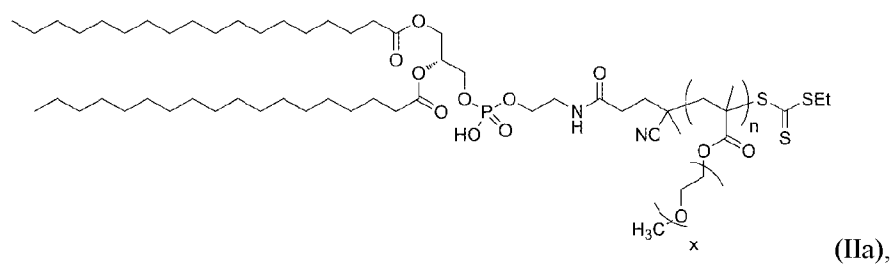
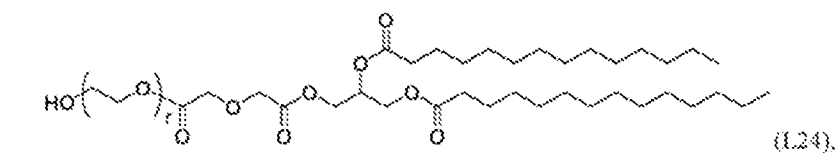


PEG-C-DMA



PEG Stearate (Compound I)



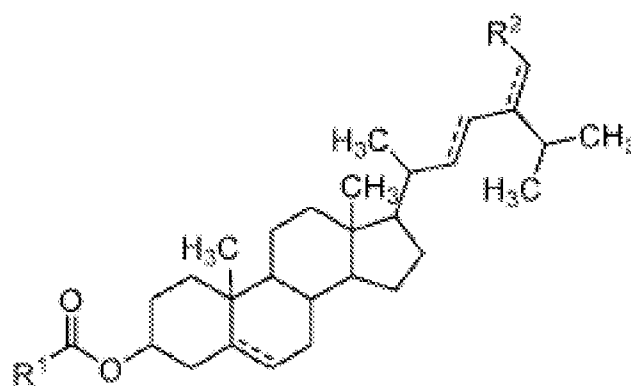
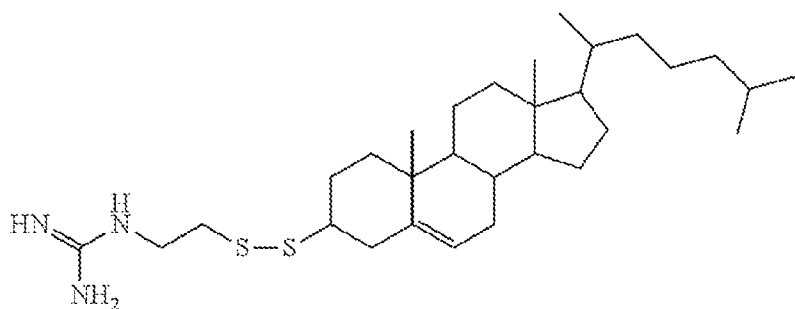


Structural Lipids/Sterols

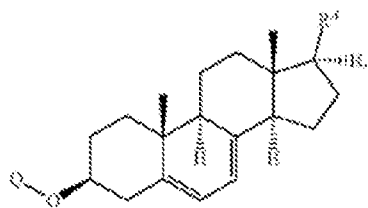
[00264] In some embodiments, the LNP further comprises a structural component. *See generally* Patel, S., et al. (2020). *Nature Communications*, 11(1), 1-13. In some embodiments, the structural component comprises a sterol including, but not limited to, a sterol selected from the group consisting of cholesterol, fecosterol, stigmasterol, stigmasterol, sitosterol, β -sitosterol, lupeol, betulin, ursolic acid, oleanolic acid, campesterol, fucosterol, brassicasterol, ergosterol, 9, 11-dehydroergosterol, tomatidine, tomatine, α -tocopherol, and mixtures thereof. In other embodiments, the structural lipid includes cholesterol and a corticosteroid (e.g., prednisolone, dexamethasone, prednisone, and hydrocortisone), or a combination thereof.

[00265] Non-exhaustive and non-limiting examples of structural lipids include:

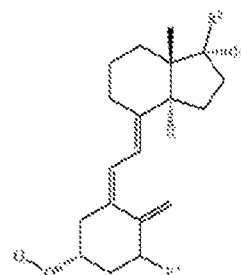
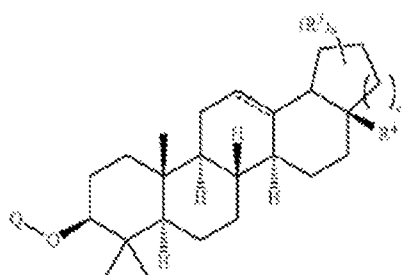
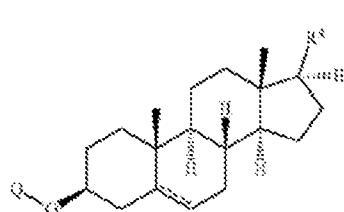
(HGT4002)



(A2)



(A1)



Formulations

[00266] Nanoparticle compositions may include a lipid component and one or more additional components, such as a therapeutic and/or prophylactic. A nanoparticle composition

may be designed for one or more specific applications or targets. The elements of a nanoparticle composition may be selected based on a particular application or target, and/or based on the efficacy, toxicity, expense, ease of use, availability, or other feature of one or more elements. Similarly, the particular formulation of a nanoparticle composition may be selected for a particular application or target according to, for example, the efficacy and toxicity of particular combinations of elements.

[00267] The lipid component of a nanoparticle composition may include, for example, a cationic lipid, a phospholipid (such as an unsaturated lipid, e.g., DOPE or DSPC), a PEG lipid, and a structural lipid. The elements of the lipid component may be provided in specific fractions.

[00268] In some embodiments, the lipid component of a nanoparticle composition includes an ionizable lipid, a phospholipid, a PEG lipid, and a structural lipid. In certain embodiments, the lipid component of the nanoparticle composition includes about 30 mol % to about 60 mol % ionizable lipid, about 0 mol % to about 30 mol % phospholipid, about 0 mol % to about 10 mol % of PEG lipid, and about 17.5 mol % to about 50 mol % structural lipid, provided that the total mol % does not exceed 100%. In some embodiments, the lipid component of the nanoparticle composition includes about 35 mol % to about 55 mol % compound of ionizable lipid, about 5 mol % to about 25 mol % phospholipid, about 0 mol % to about 10 mol % of PEG lipid, and about 30 mol % to about 40 mol % structural lipid. In a particular embodiment, the lipid component includes about 50 mol % said compound, about 10 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In another embodiment, the lipid component includes about 40 mol % said compound, about 20 mol % phospholipid, about 38.5 mol % structural lipid, and about 1.5 mol % of PEG lipid. In some embodiments, the phospholipid may be DOPE or DSPC. In other embodiments, the PEG lipid may be PEG-DMG and/or the structural lipid may be cholesterol.

[00269] In some embodiments, the ionizable lipids comprise between about 20 and about 60 mol % of the lipid component. In other embodiments, the ionizable lipids comprise between about 35 and about 55 mol % of the lipid component. In various embodiments, the ionizable lipids comprise about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, or 60 mol % of the lipid component.

[00270] In some embodiments, the neutral lipids comprise between about 0 and about 30 mol % of the lipid component. In other embodiments, the neutral lipids comprise between about 5 and about 25 mol % of the lipid component. In various embodiments, the neutral lipids comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 mol % of the lipid component.

[00271] In some embodiments, the polymer-conjugated lipids comprise between about 0 and about 15 mol % of the lipid component. In other embodiments, the polymer-conjugated lipids comprise between about 0.5 and about 10 mol % of the lipid component. In various embodiments, the polymer-conjugated lipids comprise about 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, or 15 mol % of the lipid component.

[00272] In some embodiments, the structural component comprises about 17.5 mol % to about 50 mol % of the lipid component. In other embodiments, the structural component comprises about 30 to about 40 mol % of the lipid component. In various embodiments, the structural component comprises about 17.5, 20, 22.5, 25, 27.5, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 mol % of the lipid component.

[00273] The structural component may alternatively be expressed as a ratio relative to the lipid component. In some embodiments, the structural component is in a ratio of about 1:1 with the lipid component (sterol:lipids). In other embodiments, the structural component is in a ratio of about 1:5 with the lipid component (sterol:lipids). In various embodiments, the structural component is in a ratio of about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:15, 1:20, or 1:25 with the lipid component (sterol:lipids).

[00274] Nanoparticle compositions may be designed for one or more specific applications or targets. For example, a nanoparticle composition may be designed to deliver a therapeutic and/or prophylactic such as an RNA to a particular cell, tissue, organ, or system or group thereof in a mammal's body. Physiochemical properties of nanoparticle compositions may be altered in order to increase selectivity for particular bodily targets. For instance, particle sizes may be adjusted based on the fenestration sizes of different organs. The therapeutic and/or prophylactic included in a nanoparticle composition may also be selected based on the desired delivery target or targets. For example, a therapeutic and/or prophylactic may be selected for a particular indication, condition, disease, or disorder and/or for delivery to a

particular cell, tissue, organ, or system or group thereof (e.g., localized or specific delivery). In certain embodiments, a nanoparticle composition may include an mRNA encoding a polypeptide of interest capable of being translated within a cell to produce the polypeptide of interest. Such a composition may be designed to be specifically delivered to a particular organ. In some embodiments, a composition may be designed to be specifically delivered to a mammalian joint.

[00275] The amount of a therapeutic and/or prophylactic in a nanoparticle composition may depend on the size, composition, desired target and/or application, or other properties of the nanoparticle composition as well as on the properties of the therapeutic and/or prophylactic. For example, the amount of an RNA useful in a nanoparticle composition may depend on the size, sequence, and other characteristics of the RNA. The relative amounts of a therapeutic and/or prophylactic and other elements (e.g., lipids) in a nanoparticle composition may also vary. In some embodiments, the wt/wt ratio of the lipid component to a therapeutic and/or prophylactic in a nanoparticle composition may be from about 5:1 to about 60:1, such as 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, and 60:1. For example, the wt/wt ratio of the lipid component to a therapeutic and/or prophylactic may be from about 10:1 to about 40:1. In certain embodiments, the wt/wt ratio is about 20:1. The amount of a therapeutic and/or prophylactic in a nanoparticle composition may, for example, be measured using absorption spectroscopy (e.g., ultraviolet-visible spectroscopy).

[00276] In some embodiments, the therapeutic and/or prophylactic comprises a nucleic acid component. In some embodiments, the nucleic acid component comprises RNA including, but not limited to, RNA selected from the group consisting of messenger RNA (mRNA), CRISPR RNA (crRNA), tracrRNA, single-guide RNA (sgRNA), short interfering RNA (siRNA), antisense oligonucleotides (ASO), and mixtures thereof. In other embodiments, the nucleic acid component comprises DNA including, but not limited to, DNA selected from the group consisting of linear DNA, plasmid DNA, antisense oligonucleotide, and mixtures thereof.

[00277] In some embodiments, a nanoparticle composition includes one or more RNAs, and the one or more RNAs, lipids, and amounts thereof may be selected to provide a specific N:P ratio. The N:P ratio of the composition refers to the molar ratio of nitrogen atoms in one or

more lipids to the number of phosphate groups in an RNA. In general, a lower N:P ratio is preferred. The one or more RNA, lipids, and amounts thereof may be selected to provide an N:P ratio from about 2:1 to about 30:1, such as 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 12:1, 14:1, 16:1, 18:1, 20:1, 22:1, 24:1, 26:1, 28:1, or 30:1. In certain embodiments, the N:P ratio may be from about 2:1 to about 8:1. In other embodiments, the N:P ratio is from about 5:1 to about 8:1. For example, the N:P ratio may be about 5.0:1, about 5.5:1, about 5.67:1, about 6.0:1, about 6.5:1, or about 7.0:1. For example, the N:P ratio may be about 5.67:1.

[00278] In some embodiments, the nucleic acid component is comprised of a modified nucleic acid. For example, an RNA may be a modified RNA. That is, an RNA may include one or more nucleobases, nucleosides, nucleotides, or linkers that are non-naturally occurring. A “modified” species may also be referred to herein as an “altered” species. Species may be modified or altered chemically, structurally, or functionally. For example, a modified nucleobase species may include one or more substitutions that are not naturally occurring.

[00279] In certain embodiments, the present disclosure comprises methods for treating a joint disease or disorder. In other embodiments, the present disclosure comprises methods for treating osteoarthritis. In some embodiments, the present disclosure comprises methods for treating joint inflammation, the method comprising administering a therapeutically effective amount of a CRISPR-Cas composition encapsulated within or associated with a lipid nanoparticle (LNP), wherein the composition comprises one or more non-naturally occurring polynucleotides encoding a Cas protein and at least one sgRNA. In some embodiments, LNPs are administered intra-articularly.

[00280] In certain embodiments, the present disclosure comprises methods for treating fibrosis or scarring. In some embodiments, the fibrosis and/or scarring is postoperative and/or post-surgical fibrosis and/or scarring. In some embodiments, the fibrosis and/or scarring is post-ligament reconstruction. In some embodiments, the fibrosis and/or scarring is post-anterior cruciate ligament (ACL) reconstruction. In some embodiments, the fibrosis and/or scarring is post-autograft anterior cruciate ligament (ACL) reconstruction. In some embodiments, the fibrosis and/or scarring is post-allograft anterior cruciate ligament (ACL) reconstruction. In some embodiments, the fibrosis and/or scarring is due to knee arthrofibrosis. In some embodiments, the fibrosis and/or scarring is due to intra-articular

fibrous nodules. In some embodiments, the fibrosis and/or scarring is post-total knee arthroplasty (TKA). In some embodiments, the fibrosis and/or scarring is due to knee arthrofibrosis following TKA. In some embodiments, the fibrosis and/or scarring is post-microdiscectomy. In some embodiments, the fibrosis and/or scarring is due to epidural fibrosis post-microdiscectomy. In some embodiments, the present disclosure comprises methods for treating fibrosis or scarring, the method comprising administering a therapeutically effective amount of a CRISPR-Cas composition encapsulated within or associated with a lipid nanoparticle (LNP), wherein the composition comprises one or more non-naturally occurring polynucleotides encoding a Cas protein and at least one sgRNA. In some embodiments, LNPs are administered locally. In other embodiments, LNPs are administered intra-articularly. . In some embodiments, the pharmaceutical composition is administered during a surgery and/or after a surgery.

[00281] In certain embodiments, the present disclosure comprises methods for treating low back pain. In other embodiments, the present disclosure comprises methods for treating discogenic disorders. In some embodiments, the present disclosure comprises methods for treating localized nociception, inflammation, or morphological changes associated with back or spine conditions or disorders in a subject in need thereof, the method comprising administering a therapeutically effective amount of a CRISPR-Cas composition encapsulated within or associated with a lipid nanoparticle (LNP), wherein the composition comprises one or more non-naturally occurring polynucleotides encoding a Cas protein and at least one sgRNA. In some embodiments, LNPs are administered intradiscally. In other embodiments, LNPs are administered epidurally.

[00282] As used herein, a “lipid component” is that component of a nanoparticle composition that includes one or more lipids. For example, the lipid component may include one or more cationic/ionizable, PEGylated, structural, or other lipids, such as phospholipids.

[00283] As used herein, the term “delivering” means providing an entity to a destination. For example, delivering a therapeutic and/or prophylactic to a subject may involve administering a nanoparticle composition including the therapeutic and/or prophylactic to the subject (e.g., by an intravenous, intramuscular, intradermal, subcutaneous, intraarticular, or intradiscal route). Administration of a nanoparticle composition to a mammal or mammalian cell may involve contacting one or more cells with the nanoparticle composition.

[00284] As used herein, “naturally occurring” means existing in nature without artificial aid.

[00285] As used herein, a “PEG lipid” or “PEGylated lipid” refers to a lipid comprising a polyethylene glycol component. These lipids may also be referred to a PEG-modified lipids.

[00286] As used herein, a “phospholipid” is a lipid that includes a phosphate moiety and one or more carbon chains, such as unsaturated fatty acid chains. A phospholipid may include one or more multiple (e.g., double or triple) bonds (e.g., one or more unsaturations). Particular phospholipids may facilitate fusion to a membrane. For example, a cationic phospholipid may interact with one or more negatively charged phospholipids of a membrane (e.g., a cellular or intracellular membrane). Fusion of a phospholipid to a membrane may allow one or more elements of a lipid-containing composition to pass through the membrane permitting, e.g., delivery of the one or more elements to a cell.

Physical Properties

[00287] The characteristics of a nanoparticle composition may depend on the components thereof. For example, a nanoparticle composition including cholesterol as a structural lipid may have different characteristics than a nanoparticle composition that includes a different structural lipid. Similarly, the characteristics of a nanoparticle composition may depend on the absolute or relative amounts of its components. For instance, a nanoparticle composition including a higher molar fraction of a phospholipid may have different characteristics than a nanoparticle composition including a lower molar fraction of a phospholipid. Characteristics may also vary depending on the method and conditions of preparation of the nanoparticle composition.

[00288] Nanoparticle compositions may be characterized by a variety of methods. For example, microscopy (e.g., transmission electron microscopy or scanning electron microscopy) may be used to examine the morphology and size distribution of a nanoparticle composition. Dynamic light scattering or potentiometry (e.g., potentiometric titrations) may be used to measure zeta potentials. Dynamic light scattering may also be utilized to determine particle sizes. Instruments such as the Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) may also be used to measure multiple characteristics of a nanoparticle composition, such as particle size, polydispersity index, and zeta potential.

[00289] The mean size of a nanoparticle composition may be between 10 nm and 1 micrometer, e.g., measured by dynamic light scattering (DLS). For example, the mean size may be from about 40 nm to about 150 nm, such as about 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm. In some embodiments, the mean size of a nanoparticle composition may be from about 50 nm to about 100 nm, from about 50 nm to about 90 nm, from about 50 nm to about 80 nm, from about 50 nm to about 70 nm, from about 50 nm to about 60 nm, from about 60 nm to about 100 nm, from about 60 nm to about 90 nm, from about 60 nm to about 80 nm, from about 60 nm to about 70 nm, from about 70 nm to about 100 nm, from about 70 nm to about 90 nm, from about 70 nm to about 80 nm, from about 80 nm to about 100 nm, from about 80 nm to about 90 nm, or from about 90 nm to about 100 nm. In certain embodiments, the mean size of a nanoparticle composition may be from about 70 nm to about 100 nm. In a particular embodiment, the mean size may be about 80 nm. In other embodiments, the mean size may be about 100 nm.

[00290] A nanoparticle composition may be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of a nanoparticle composition, e.g., the particle size distribution of the nanoparticle compositions. A small (e.g., less than 0.3) polydispersity index generally indicates a narrow particle size distribution. A nanoparticle composition may have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some embodiments, the polydispersity index of a nanoparticle composition may be from about 0.10 to about 0.20.

[00291] The zeta potential of a nanoparticle composition may be used to indicate the electrokinetic potential of the composition. For example, the zeta potential may describe the surface charge of a nanoparticle composition. Nanoparticle compositions with relatively low charges, positive or negative, are generally desirable, as more highly charged species may interact undesirably with cells, tissues, and other elements in the body. In some embodiments, the zeta potential of a nanoparticle composition may be from about -10 mV to about +20 mV, from about -10 mV to about +15 mV, from about -10 mV to about +10 mV, from about -10 mV to about +5 mV, from about -10 mV to about 0 mV, from about -10 mV to about -5 mV, from about -5 mV to about +20 mV, from about -5 mV to about +15 mV, from about -5 mV

to about +10 mV, from about -5 mV to about +5 mV, from about -5 mV to about 0 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

[00292] The efficiency of encapsulation of a therapeutic and/or prophylactic describes the amount of therapeutic and/or prophylactic that is encapsulated or otherwise associated with a nanoparticle composition after preparation, relative to the initial amount provided. The encapsulation efficiency is desirably high (e.g., close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of therapeutic and/or prophylactic in a solution containing the nanoparticle composition before and after breaking up the nanoparticle composition with one or more organic solvents or detergents. Fluorescence may be used to measure the amount of free therapeutic and/or prophylactic (e.g., RNA) in a solution. For the nanoparticle compositions described herein, the encapsulation efficiency of a therapeutic and/or prophylactic may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In certain embodiments, the encapsulation efficiency may be at least 90%.

[00293] A nanoparticle composition may optionally comprise one or more coatings. For example, a nanoparticle composition may be formulated in a capsule, film, or tablet having a coating. A capsule, film, or tablet including a composition described herein may have any useful size, tensile strength, hardness, or density.

[00294] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing synoviocytes via a CRISPR method include IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, and TNF- α .

[00295] Non-limiting examples of genes that may be enhanced by permanently gene-editing synoviocytes via a CRISPR method include IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, and TNF- α .

[00296] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a CRISPR method, and which may be used in accordance with embodiments of the present disclosure, are described in U.S. Patent Nos. 8,697,359; 8,993,233; 8,795,965; 8,771,945; 8,889,356; 8,865,406; 8,999,641; 8,945,839; 8,932,814;

8,871,445; 8,906,616; and 8,895,308, which are incorporated by reference herein. Resources for carrying out CRISPR methods, such as plasmids for expressing CRISPR-Cas9 and CRISPR-Cpf1, are commercially available from companies such as GenScript.

[00297] In an embodiment, genetic modifications of at least a portion of a joint's synoviocytes, as described herein, may be performed using the CRISPR-Cpf1 system as described in U.S. Patent No. US 9,790,490, the disclosure of which is incorporated by reference herein.

In an embodiment, genetic modifications of at least a portion of a joint's synoviocytes, as described herein, may be performed using a CRISPR-Cas system comprising single vector systems as described in U.S. Patent No. 9,907,863, the disclosure of which is incorporated by reference herein. TALE Methods

[00298] A pharmaceutical composition for the treatment or prevention of a joint disease or condition comprising a gene-editing system, wherein said gene-editing system targets at least one locus related to joint function, wherein the method further comprises gene-editing at least a portion of joint synoviocytes by a TALE method. According to particular embodiments, the use of a TALE method to target at least one locus related to joint function, wherein the gene-editing at least a portion of a joint's synoviocytes. Alternatively, the use of a TALE method during to target at least one locus related to joint function, wherein the gene-editing at least a portion of a joint's synoviocytes to cause expression of at least one locus related to joint function genes to be enhanced in at least a portion of the joint synoviocytes.

[00299] TALE stands for "Transcription Activator-Like Effector" proteins, which include TALENs ("Transcription Activator-Like Effector Nucleases"). A method of using a TALE system for gene editing may also be referred to herein as a TALE method. TALEs are naturally occurring proteins from the plant pathogenic bacteria genus *Xanthomonas*, and contain DNA-binding domains composed of a series of 33–35-amino-acid repeat domains that each recognizes a single base pair. TALE specificity is determined by two hypervariable amino acids that are known as the repeat-variable di-residues (RVDs). Modular TALE repeats are linked together to recognize contiguous DNA sequences. A specific RVD in the DNA-binding domain recognizes a base in the target locus, providing a structural feature to assemble predictable DNA-binding domains. The DNA binding domains of a TALE are fused to the catalytic domain of a type IIS FokI endonuclease to make a targetable TALE nuclease. To induce site-specific mutation, two individual TALEN arms, separated by a 14-

20 base pair spacer region, bring FokI monomers in close proximity to dimerize and produce a targeted double-strand break.

[00300] Several large, systematic studies utilizing various assembly methods have indicated that TALE repeats can be combined to recognize virtually any user-defined sequence. Custom-designed TALE arrays are also commercially available through Collectis Bioresearch (Paris, France), Transposagen Biopharmaceuticals (Lexington, KY, USA), and Life Technologies (Grand Island, NY, USA). TALE and TALEN methods suitable for use in the present disclosure are described in U.S. Patent Application Publication Nos. US 2011/0201118 A1; US 2013/0117869 A1; US 2013/0315884 A1; US 2015/0203871 A1 and US 2016/0120906 A1, the disclosures of which are incorporated by reference herein.

[00301] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing synoviocytes via a TALE method include IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, and TNF- α .

[00302] Non-limiting examples of genes that may be enhanced by permanently gene-editing synoviocytes via a TALE method include IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, and TNF- α .

[00303] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a TALE method, and which may be used in accordance with embodiments of the present disclosure, are described in U.S. Patent No. 8,586,526, which is incorporated by reference herein.

Zinc Finger Methods

[00304] A pharmaceutical composition for the treatment or prevention of a joint disease or condition comprising a gene-editing system, wherein said gene-editing system targets at least one locus related to joint function, wherein the method further comprises gene-editing at least a portion of joint synoviocytes by a zinc finger or zinc finger nuclease method. According to particular embodiments, the use of a zinc finger method to target at least one locus related to joint function, wherein the gene-editing at least a portion of a joint's synoviocytes.

Alternatively, the use of a zinc finger method during to target at least one locus related to joint function, wherein the gene-editing at least a portion of a joint's synoviocytes to cause expression of at least one locus related to joint function genes to be enhanced in at least a portion of the joint synoviocytes.

[00305] An individual zinc finger contains approximately 30 amino acids in a conserved $\beta\beta\alpha$ configuration. Several amino acids on the surface of the α -helix typically contact 3 bp in the

major groove of DNA, with varying levels of selectivity. Zinc fingers have two protein domains. The first domain is the DNA binding domain, which includes eukaryotic transcription factors and contain the zinc finger. The second domain is the nuclease domain, which includes the FokI restriction enzyme and is responsible for the catalytic cleavage of DNA.

[00306] The DNA-binding domains of individual ZFNs typically contain between three and six individual zinc finger repeats and can each recognize between 9 and 18 base pairs. If the zinc finger domains are specific for their intended target site then even a pair of 3-finger ZFNs that recognize a total of 18 base pairs can, in theory, target a single locus in a mammalian genome. One method to generate new zinc-finger arrays is to combine smaller zinc-finger “modules” of known specificity. The most common modular assembly process involves combining three separate zinc fingers that can each recognize a 3 base pair DNA sequence to generate a 3-finger array that can recognize a 9 base pair target site.

Alternatively, selection-based approaches, such as oligomerized pool engineering (OPEN) can be used to select for new zinc-finger arrays from randomized libraries that take into consideration context-dependent interactions between neighboring fingers. Engineered zinc fingers are available commercially; Sangamo Biosciences (Richmond, CA, USA) has developed a propriety platform (CompoZr®) for zinc-finger construction in partnership with Sigma-Aldrich (St. Louis, MO, USA).

[00307] Non-limiting examples of genes that may be silenced or inhibited by permanently gene-editing synoviocytes via a zinc finger method include IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, TNF- α , IL-6, IL-8, IL-18, a matrix metalloproteinase (MMP), or a component of the NLRP3 inflammasome. In some embodiments, the component of the NLRP3 inflammasome comprises NLRP3, ASC (apoptosis-associated speck-like protein containing a CARD), caspase-1, and combinations thereof.

[00308] Non-limiting examples of genes that may be enhanced by permanently gene-editing synoviocytes via a zinc finger method include group comprising IL-1Ra, TIMP-1, TIMP-2, TIMP-3, TIMP-4, and combinations thereof. In an aspect, the disclosure provides compositions for up-regulation of anti-inflammatory cytokines.

[00309] Examples of systems, methods, and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used in accordance with embodiments of the present disclosure, are described in U.S. Patent Nos. 6,534,261,

6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, and 6,479,626, which are incorporated by reference herein.

[00310] In some aspects, cells may be gene-edited ex vivo, wherein the gene-editing targets one or more anti-inflammatory cytokine locus. In some aspects, the cells are non-synovial cells. In some aspects, the cells are mesenchymal stem cells. In some aspect, the cells are macrophages. In some aspects, the present disclosure provides for a pharmaceutical composition for the treatment or prevention of a joint disease or condition comprising a population of gene-edited cells, wherein said gene-edited cells are edited by a gene-editing system targeting at least one locus related to joint function. In an aspect, the population of gene-edited cells are injected into a synovial joint.

[00311] Other examples of systems, methods, and compositions for altering the expression of a target gene sequence by a zinc finger method, which may be used in accordance with embodiments of the present disclosure, are described in Beane, *et al.*, *Mol. Therapy*, **2015**, 23 1380-1390, the disclosure of which is incorporated by reference herein.

[00312] Example Embodiments

[00313] In some embodiments, the present disclosure provides a pharmaceutical composition for the treatment or prevention of a joint disease or condition, the composition including a therapeutically effective amount of one or more nucleic acids encoding a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene-editing system. The system includes a CRISPR Associated Protein 9 (Cas9) protein, and at least one guide RNA targeting an IL-1 α or IL-1 β gene, wherein the target sequence is adjacent to a protospacer adjacent motif (PAM) sequence for the Cas9 protein.

[00314] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 2 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 2 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 2 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 2 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1

mismatch with the target sequence in exon 2 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 2 of the human IL-1 α gene.

[00315] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 3 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 3 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 3 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 3 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 3 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 3 of the human IL-1 α gene.

[00316] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 4 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 4 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 4 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 4 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 4 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 4 of the human IL-1 α gene.

[00317] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 5 of the human IL-1 α gene. In some

embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 5 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 5 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 5 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 5 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 5 of the human IL-1 α gene.

[00318] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 6 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 6 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 6 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 6 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 6 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 6 of the human IL-1 α gene.

[00319] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 7 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 7 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 7 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 7 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 7 of the human IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 7 of the human

IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 7 of the human IL-1 α gene.

[00320] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 3 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 7 of the IL-1 α gene.

[00321] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 7 of the IL-1 α gene.

[00322] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 7 of the IL-1 α gene.

[00323] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 or exon 7 of the IL-1 α gene.

[00324] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 6, or exon 7 of the IL-1 α gene.

[00325] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 6, or exon 7 of the IL-1 α gene.

[00326] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00327] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 7 of

the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00328] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00329] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a

target sequence in exon 2, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 5, exon 6, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00330] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710.

[00331] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence is SEQ ID NO:301.

[00332] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:309. In

some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence is SEQ ID NO:309.

[00333] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740.

[00334] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 2 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 2 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 2 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 2 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 2 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 2 of the human IL-1 β gene.

[00335] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5

mismatches with the target sequence in exon 3 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 3 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 3 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 3 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 3 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 3 of the human IL-1 β gene.

[00336] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 4 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 4 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 4 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 4 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 4 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 4 of the human IL-1 β gene.

[00337] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 5 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 5 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 5 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 5 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 5 of the human

IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 5 of the human IL-1 β gene.

[00338] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 6 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 6 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 6 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 6 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 6 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 6 of the human IL-1 β gene.

[00339] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 7 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 7 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 7 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 7 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 7 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 7 of the human IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 7 of the human IL-1 β gene.

[00340] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 3 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA

targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 7 of the IL-1 β gene.

[00341] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 7 of the IL-1 β gene.

[00342] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 7 of the IL-1 β gene.

[00343] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 or exon 7 of the IL-1 β gene.

[00344] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 6, or exon 7 of the IL-1 β gene.

[00345] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 6 of the IL-1 β gene.

gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 6, or exon 7 of the IL-1 β gene.

[00346] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00347] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β

gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00348] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00349] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 5, exon 6, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence that is

complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00350] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence is SEQ ID NO:462.

[00351] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence is SEQ ID NO:391.

[00352] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence is SEQ ID NO:393.

[00353] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence is SEQ ID NO:388.

[00354] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence is SEQ ID NO:389.

[00355] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770.

[00356] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence is SEQ ID NO:552.

[00357] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:554. In some

embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence is SEQ ID NO:554.

[00358] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence is SEQ ID NO:578.

[00359] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence is SEQ ID NO:579.

[00360] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800.

[00361] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:498. In

some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence is SEQ ID NO:498.

[00362] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence is SEQ ID NO:506.

[00363] In some embodiments, the pharmaceutical composition includes one or more viral vectors, as described herein, collectively comprising the one or more nucleic acids. In some embodiments, the one or more viral vectors include a recombinant virus selected from a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, and a herpes simplex virus-1. In some embodiments, the one of more viral vectors include a recombinant adeno-associated virus (AAV). In some embodiments, the recombinant AAV is of serotype 5 (AAV5). In some embodiments, the recombinant AAV is of serotype 6 (AAV6).

[00364] In some embodiments, the one of more viral vectors include a first viral vector comprising a first nucleic acid, in the one or more nucleic acids, encoding the Cas9 protein, and a second viral vector comprising a second nucleic acid, in the one or more nucleic acids, encoding the at least one guide RNA. In some embodiments, the one of more viral vectors comprise a viral vector comprising a single nucleic acid, wherein the single nucleic acid encodes the Cas9 protein and the at least one guide RNA.

[00365] In some embodiments, the composition includes one or more liposomes collectively comprising the one or more nucleic acids. In some embodiments, the one or more nucleic acids are present in a naked state.

[00366] In some embodiments, the Cas9 protein is an *S. pyogenes* Cas9 polypeptide. In some embodiments, the Cas9 protein is an *S. aureus* Cas9 polypeptide.

[00367] In some embodiments, the composition is formulated for parenteral administration. In some embodiments, the composition is formulated for intra-articular injection within a joint of a subject.

[00368] In another aspect, the disclosure provides a method for the treatment or prevention of a joint disease or condition in a subject in need thereof. The method includes administering, to a joint of the subject, a pharmaceutical composition comprising a pharmaceutically effective amount of a composition comprising one or more nucleic acids encoding a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene-editing system. The system includes a CRISPR Associated Protein 9 (Cas9) protein, and at least one guide RNA targeting an IL-1 α or IL-1 β gene, wherein the target sequence is adjacent to a protospacer adjacent motif (PAM) sequence for the Cas9 protein.

[00369] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710.

[00370] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:301. In some embodiments, the crRNA sequence is SEQ ID NO:301.

[00371] In some embodiments, the at least one guide RNA targets a human IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:309. In some embodiments, the crRNA sequence is SEQ ID NO:309.

[00372] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740.

[00373] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:462. In some embodiments, the crRNA sequence is SEQ ID NO:462.

[00374] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:391. In

some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:391. In some embodiments, the crRNA sequence is SEQ ID NO:391.

[00375] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:393. In some embodiments, the crRNA sequence is SEQ ID NO:393.

[00376] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:388. In some embodiments, the crRNA sequence is SEQ ID NO:388.

[00377] In some embodiments, the at least one guide RNA targets a human IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:389. In some embodiments, the crRNA sequence is SEQ ID NO:389.

[00378] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence

has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770.

[00379] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:552. In some embodiments, the crRNA sequence is SEQ ID NO:552.

[00380] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:554. In some embodiments, the crRNA sequence is SEQ ID NO:554.

[00381] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:578. In some embodiments, the crRNA sequence is SEQ ID NO:578.

[00382] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:579. In some

embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:579. In some embodiments, the crRNA sequence is SEQ ID NO:579.

[00383] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 2 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 2 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 2 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 2 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 2 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 2 of the canine IL-1 α gene.

[00384] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 3 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 3 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 3 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 3 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 3 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 3 of the canine IL-1 α gene.

[00385] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 4 of the canine IL-1 α gene. In some embodiments, the

crRNA sequence forms no more than 4 mismatches with the target sequence in exon 4 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 4 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 4 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 4 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 4 of the canine IL-1 α gene.

[00386] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 5 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 5 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 5 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 5 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 5 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 5 of the canine IL-1 α gene.

[00387] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 6 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 6 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 6 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 6 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 6 of the canine IL-1 α gene.

In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 6 of the canine IL-1 α gene.

[00388] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 7 of the IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 7 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 7 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 7 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 7 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 7 of the canine IL-1 α gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 7 of the canine IL-1 α gene.

[00389] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 3 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 7 of the IL-1 α gene.

[00390] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target

sequence in exon 3 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 7 of the IL-1 α gene.

[00391] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 7 of the IL-1 α gene.

[00392] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 or exon 7 of the IL-1 α gene.

[00393] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 4 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide

RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 6, or exon 7 of the IL-1 α gene.

[00394] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 6, or exon 7 of the IL-1 α gene.

[00395] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA

sequence that is complementary to a target sequence in exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00396] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 5 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00397] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00398] In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 5, exon 6, or exon 6 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 6, or exon 7 of the IL-1 α gene. In some embodiments, the at least one guide RNA targets a canine IL-1 α gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 α gene.

[00399] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 80% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 85% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 90% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence has at least 95% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800. In some embodiments, the crRNA sequence is selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800.

[00400] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:498. In

some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:498. In some embodiments, the crRNA sequence is SEQ ID NO:498.

[00401] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence having at least 75% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 80% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 85% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 90% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence has at least 95% identity to SEQ ID NO:506. In some embodiments, the crRNA sequence is SEQ ID NO:506.

[00402] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 2 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 2 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 2 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 2 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 2 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 2 of the canine IL-1 β gene.

[00403] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 3 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 3 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 3 of the canine IL-1 β gene. In some

embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 3 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 3 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 3 of the canine IL-1 β gene.

[00404] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 4 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 4 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 4 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 4 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 4 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 4 of the canine IL-1 β gene.

[00405] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 5 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 5 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 5 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 5 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 5 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 5 of the canine IL-1 β gene.

[00406] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 of the

IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 6 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 6 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 6 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 6 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 6 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 6 of the canine IL-1 β gene.

[00407] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 7 of the IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 5 mismatches with the target sequence in exon 7 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 4 mismatches with the target sequence in exon 7 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 3 mismatches with the target sequence in exon 7 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 2 mismatches with the target sequence in exon 7 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no more than 1 mismatch with the target sequence in exon 7 of the canine IL-1 β gene. In some embodiments, the crRNA sequence forms no mismatches with the target sequence in exon 7 of the canine IL-1 β gene.

[00408] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 3 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide

RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2 or exon 7 of the IL-1 β gene.

[00409] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3 or exon 7 of the IL-1 β gene.

[00410] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4 or exon 7 of the IL-1 β gene.

[00411] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5 or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 6 or exon 7 of the IL-1 β gene.

[00412] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 4 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 5 of the IL-1 β gene. In some embodiments, the at least

one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 6, or exon 7 of the IL-1 β gene.

[00413] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 6, or exon 7 of the IL-1 β gene.

[00414] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00415] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 5 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00416] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target

sequence in exon 3, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00417] In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 5, exon 6, or exon 6 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 6, or exon 7 of the IL-1 β gene. In some embodiments, the at least one guide RNA targets a canine IL-1 β gene, and includes a crRNA sequence that is complementary to a target sequence in exon 2, exon 3, exon 4, exon 5, exon 6, or exon 7 of the IL-1 β gene.

[00418] Generally, the crRNA sequences described herein may include one or more nucleotide substitutions, e.g., relative to the reverse complement of the target sequence. Guidance for making nucleotide substitutions can be found, for example, in Jiang et al. and Doudna (Jiang and Doudna, *Annu. Rev. Biophys.*, 46:505–29 (2017)), the content of which is incorporated herein by reference, in its entirety, for all purposes. Specifically, Jiang and Doudna considers molecular structures generated for many different confirmations of the

CRISPR/Cas9 system, ranging from apo Cas9 protein (Figure 3) to Cas9-sgRNA complexes bound to the target strand of an invaded double-stranded DNA molecule (Figures 5 and 7), to arrive at a detailed molecule model of CRISPR/Cas9 binding and cleavage in Figure 6. From these molecular models, the person of ordinary skill in the art would know which nucleotide positions in the crRNA sequence would be more tolerant of mismatches with the target sequence.

[00419] For instance, Jiang teaches that the PAM-proximal 10-12 nucleotides, also known as the ‘seed region’ of the crRNA targeting sequence, are most critical for robust CRISPR/Cas9 binding. Specifically, Jiang discloses that mismatches in the seed region “severely impair or completely abrogate target DNA binding and cleavage, whereas close homology in the seed region often leads to off-target binding events even with many mismatches elsewhere,” i.e., in the PAM-distal 8-10 nucleotides. Jiang at 512. Similarly, Jiang teaches that “[p]erfect complementarity between the seed region of sgRNA and target DNA is necessary for Cas9-mediated DNA targeting and cleavage, whereas imperfect base pairing at the nonseed region is much more tolerated for target binding specificity. Id., citations omitted.

[00420] Accordingly, in some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure include one or more nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides. In some embodiments, a crRNA sequence includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides. In some embodiments, a crRNA sequence includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides. In some embodiments, a crRNA sequence includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides. In some embodiments, a crRNA sequence includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides. In some embodiments, a crRNA sequence includes five nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the PAM-distal 8-10 nucleotides.

[00421] Accordingly, in some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure include one or more nucleotide substitutions,

e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes five nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 8 positions of the crRNA sequence.

[00422] Similarly, in some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure include one or more nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence. In some embodiments, a crRNA sequence includes five nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within the first 10 positions of the crRNA sequence.

[00423] Further, Jiang and Doudna postulates that base pairing of PAM-distal nucleotides at positions 14-17 of the crRNA targeting sequence are important for cleavage activity, following binding to the target sequence.

[00424] Accordingly, in some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure include one or more nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the

crRNA sequence. In some embodiments, a crRNA sequence includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the crRNA sequence. In some embodiments, a crRNA sequence includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the crRNA sequence. In some embodiments, a crRNA sequence includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the crRNA sequence. In some embodiments, a crRNA sequence includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the crRNA sequence. In some embodiments, a crRNA sequence includes five nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8-10 of the crRNA sequence.

[00425] Similarly, in some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes one or more nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8 of the crRNA sequence. In some embodiments, a crRNA sequence includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8 of the crRNA sequence. In some embodiments, a crRNA. In some embodiments, a crRNA sequence includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8 of the crRNA sequence. In some embodiments, a crRNA. In some embodiments, a crRNA sequence includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8 of the crRNA sequence. In some embodiments, a crRNA. In some embodiments, a crRNA sequence includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, within nucleotide positions 1-3 and 8 of the crRNA sequence. In some embodiments, a crRNA.

[00426] In yet other embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure include one or more nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined through experimentation. In some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes one nucleotide substitution, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined

through experimentation. In some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes two nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined through experimentation. In some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes three nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined through experimentation. In some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes four nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined through experimentation. In some embodiments, a crRNA sequence used in the compositions and/or methods of the disclosure includes five nucleotide substitutions, e.g., relative to any of SEQ ID NOs: 298-590, throughout the entire sequence of the crRNA, e.g., as determined through experimentation.

[00427] In some embodiments, the joint disease or condition is arthritis. In some embodiments, the arthritis is osteoarthritis.

[00428] In some embodiments, the administering includes intra-articular injection of the pharmaceutical composition into the joint of the subject. In some embodiments, the pharmaceutical composition is administered during surgery. In some embodiments, the pharmaceutical composition is administered after surgery. In some embodiments, the pharmaceutical composition is a controlled release pharmaceutical composition.

[00429] In some embodiments, the pharmaceutical composition includes one or more viral vectors, as described herein, collectively comprising the one or more nucleic acids. In some embodiments, the one or more viral vectors include a recombinant virus selected from a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, and a herpes simplex virus-1. In some embodiments, the one or more viral vectors include a recombinant adeno-associated virus (AAV). In some embodiments, the recombinant AAV is of serotype 5 (AAV5). In some embodiments, the recombinant AAV is of serotype 6 (AAV6).

[00430] In some embodiments, the one or more viral vectors include a first viral vector comprising a first nucleic acid, in the one or more nucleic acids, encoding the Cas9 protein, and a second viral vector comprising a second nucleic acid, in the one or more nucleic acids,

encoding the at least one guide RNA. In some embodiments, the one or more viral vectors comprise a viral vector comprising a single nucleic acid, wherein the single nucleic acid encodes the Cas9 protein and the at least one guide RNA.

[00431] In some embodiments, the composition includes one or more liposomes collectively comprising the one or more nucleic acids. In some embodiments, the one or more nucleic acids are present in a naked state.

[00432] In some embodiments, the Cas9 protein is an *S. pyogenes* Cas9 polypeptide. In some embodiments, the Cas9 protein is an *S. aureus* Cas9 polypeptide.

Methods of Treating Osteoarthritis and Other Diseases

[00433] The compositions and methods described herein can be used in a method for treating diseases. In an embodiment, they are for use in treating inflammatory joint disorders. They may also be used in treating other disorders as described herein and in the following paragraphs. In an aspect, the compositions and methods are used to treat osteoarthritis (OA).

[00434] In some embodiments, the present disclosure provides a method for the treatment or prevention of a joint disease or condition the method comprising introducing a gene-editing system, wherein the gene-editing system targets at least one locus related to joint function. In some embodiments, the joint disease is osteoarthritis. In an aspect, the method is used to treat a canine with osteoarthritis. In another aspect, the method is used to treat a mammal with degenerative joint disease. In some aspects, the method is used to treat a canine or an equine with a joint disease. In some aspects, the method is used to treat osteoarthritis, post-traumatic arthritis, post-infectious arthritis, rheumatoid arthritis, gout, pseudogout, auto-immune mediated arthritides, inflammatory arthritides, inflammation-mediated and immune-mediated diseases of joints.

[00435] In some embodiments, the method further comprises gene-editing a portion of a the joint synoviocytes to reduce or silence the expression of one or more of IL-1 α , IL-1 β , IL-4, IL-9, IL-10, IL-13, and TNF- α . In an aspect, the method further comprises gene-editing a portion of a the joint synoviocytes to reduce or silence the expression of one or more of IL-1 α , IL-1 β .

[00436] In an aspect, the method further comprises gene-editing, wherein the gene-editing comprises one or more methods selected from a CRISPR method, a TALE method, a zinc finger method, and a combination thereof.

[00437] In some aspects, the method further comprises delivering the gene-editing using an AAV vector, a lentiviral vector, or a retroviral vector. In a preferred embodiment, the method further comprises delivering the gene-editing using AAV1, AAV1(Y705+731F+T492V), AAV2(Y444+500+730F+T491V), AAV3(Y705+731F), AAV5, AAV5(Y436+693+719F), AAV6, AAV6 (VP3 variant Y705F/Y731F/T492V), AAV-7m8, AAV8, AAV8(Y733F), AAV9, AAV9 (VP3 variant Y731F), AAV10(Y733F), and AAV-ShH10. In some aspects, the AAV vector comprises a serotype selected from the group consisting of: AAV1, AAV5, AAV6, AAV6 (Y705F/Y731F/T492V), AAV8, AAV9, and AAV9 (Y731F).

Pharmaceutical Compositions and Methods of Administration

[00438] The methods described herein include the use of pharmaceutical compositions comprising CRISPR gene (e.g., IL-1 α and/or IL-1 β) editing complexes as an active ingredient.

[00439] Depending on the method/route of administration, pharmaceutical dosage forms come in several types. These include many kinds of liquid, solid, and semisolid dosage forms. Common pharmaceutical dosage forms include pill, tablet, or capsule, drink or syrup, and natural or herbal form such as plant or food of sorts, among many others. Notably, the route of administration (ROA) for drug delivery is dependent on the dosage form of the substance in question. A liquid pharmaceutical dosage form is the liquid form of a dose of a chemical compound used as a drug or medication intended for administration or consumption.

[00440] In one embodiment, a composition of the present disclosure can be delivered to a subject subcutaneously (e.g., intra-articular injection), dermally (e.g., transdermally via patch), and/or via implant. Exemplary pharmaceutical dosage forms include, e.g., pills, osmotic delivery systems, elixirs, emulsions, hydrogels, suspensions, syrups, capsules, tablets, orally dissolving tablets (ODTs), gel capsules, thin films, adhesive topical patches, lollipops, lozenges, chewing gum, dry powder inhalers (DPIs), vaporizers, nebulizers, metered dose inhalers (MDIs), ointments, transdermal patches, intradermal implants, subcutaneous implants, and transdermal implants.

[00441] As used herein, “dermal delivery” or “dermal administration” can refer to a route of administration wherein the pharmaceutical dosage form is taken to, or through, the dermis (i.e., layer of skin between the epidermis (with which it makes up the cutis) and subcutaneous tissues). “Subcutaneous delivery” can refer to a route of administration wherein the pharmaceutical dosage form is to or beneath the subcutaneous tissue layer.

[00442] Methods of formulating suitable pharmaceutical compositions are known in the art, see, e.g., Remington: The Science and Practice of Pharmacy, 21st ed., 2005; and the books in the series Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs (Dekker, N.Y.). For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfate; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00443] Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[00444] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00445] Therapeutic compounds that are or include nucleic acids can be administered by any method suitable for administration of nucleic acid agents, such as a DNA vaccine. These methods include gene guns, bio injectors, and skin patches as well as needle-free methods such as the micro-particle DNA vaccine technology disclosed in U.S. Pat. No. 6,194,389, and the mammalian transdermal needle-free vaccination with powder-form vaccine as disclosed in U.S. Pat. No. 6,168,587. Additionally, intranasal delivery is possible, as described in, inter alia, Hamajima et al., Clin. Immunol. Immunopathol., 88(2), 205-10 (1998). Liposomes (e.g., as described in U.S. Pat. No. 6,472,375) and microencapsulation can also be used. Biodegradable targetable microparticle delivery systems can also be used (e.g., as described in U.S. Pat. No. 6,471,996).

[00446] Therapeutic compounds can be prepared with carriers that will protect the therapeutic compounds against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as collagen, ethylene vinyl acetate, polyanhydrides (e.g., poly[1,3-bis(carboxyphenoxy)propane-co-sebacic-acid] (PCPP-SA) matrix, fatty acid dimer-sebacic acid (FAD-SA) copolymer, poly(lactide-co-glycolide)), polyglycolic acid, collagen, polyorthoesters, polyethyleneglycol-coated liposomes, and polylactic acid. Such formulations can be prepared using standard techniques, or obtained commercially, e.g., from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to selected cells with monoclonal antibodies to cellular antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811. Semisolid, gelling, soft-gel, or other formulations (including controlled release) can be used,

e.g., when administration to a surgical site is desired. Methods of making such formulations are known in the art and can include the use of biodegradable, biocompatible polymers. See, e.g., Sawyer et al., Yale J Biol Med. 2006 December; 79(3-4): 141-152.

[00447] The pharmaceutical compositions can be included in a container, kit, pack, or dispenser together with instructions for administration.

EXAMPLES

[00448] The embodiments encompassed herein are now described with reference to the following examples. These examples are provided for the purpose of illustration only and the disclosure encompassed herein should in no way be construed as being limited to these examples, but rather should be construed to encompass any and all variations which become evident as a result of the teachings provided herein.

EXAMPLE 1. Reducing IL-1 expression by CRISPR Gene-engineering in a Mouse Model of Osteoarthritis.

[00449] Sixty C57B mice are selected and distributed into four groups of fifteen mice each. The DMM surgical method is used to induce OA in each of the mice. Once the mice have developed OA, the mice are treated as follows:

Group 1: Direct injection into the OA joint a CRISPR AAV vector engineered to target IL-1 α and IL-1 β , and silence or reduce the expression of IL-1 protein.

Group 2: Direct injection into the OA joint a CRISPR AAV vector engineered with a “nonsense” payload that will not affect an IL-1 production; a negative control.

Group 3: Direct injection into the OA joint a CRISPR AAV vector engineered to target IL-1Ra, and silence or reduce the expression of IL-1Ra protein.

Group 4: Direct injection into the OA joint sterile buffered saline; a control for the injection process.

[00450] The mice are monitored before and after treatment to assess effects on their locomotion, and exploratory activities. Mechanical sensitivity and changes to the gait are also monitored. Allodynia and hind limb grip force may also be monitored.

[00451] After about eight weeks, the animals are sacrificed and the OA joint tissue assessed for gross histopathology, and IL-1 expression by IHC. Biomarkers of inflammation are also assessed, for example, MMP-3 expression in the OA joint.

[00452] Group 1 mice, treated with a CRISPR AAV vector engineered to target IL-1 α and IL-1 β , and silence or reduce the expression of IL-1 protein, will show reduced levels of IL-1 by IHC, tissue regeneration by histopathology, and lower levels of inflammation biomarkers than any of the three other Groups. Group 3 mice will show relatively higher levels of inflammation biomarker than any of the other three groups.

EXAMPLE 2. Assessing Guide Cutting Efficiency Against Mouse IL1A and IL1B

[00453] *In vitro cleavage assay*

[00454] CRISPR guide RNA's (Phosphorothionate-modified sgRNA, Table 3) were designed against Exon 4 of *Il1a* and Exon 4 of *Il1b* (Il1a-201 ENSMUST00000028882.1 and Il1b-201 ENSMUST00000028881.13; see Table 2 for target sequences on Exon 4 of *Il1a* and Exon 4 of *Il1b*). C57BL/6 mouse genomic DNA was used to amplify Exon 4 of *Il1a* and *Il1b* by PCR (Phusion High-Fidelity DNA polymerase, NEB cat#M0530S) Il1a primer fwd: CATTGGGAGGATGCTTAGGA (SEQ ID NO:620), Il1a primer rev: GGCTGCTTTCTCTCCAACAG (SEQ ID NO:621), Il1b primer fwd: AGGAAGCCTGTGTCTGGTTG (SEQ ID NO:622), Il1b primer rev: TGGCATCGTGAGATAAGCTG (SEQ ID NO:623). Amplicons were PCR purified (QiaQuick PCR purification kit cat#28106). Guide cutting efficiency was determined using an in vitro cleavage assay using 100 ng purified PCR product, 200 ng modified guide RNA (Sigma Aldrich) and 0.5 μ g TrueCut *Spy* Cas9 protein V2 (Invitrogen A36498) or 0.5 μ g Gene Snipper NLS *Sau* Cas9 (BioVision Cat#M1281-50-1). The two types of Cas9, *S. pyogenes* Cas9 and *S. aureus* Cas9, were compared for their editing capabilities. A 2% agarose gel was used for a qualitative readout of the cleavage assay.

[00455] *Editing Cell Lines*

[00456] CRISPR guide RNA's (Phosphorothionate-modified sgRNA, Table 2) were designed against Exon 4 of *Il1a* and Exon 4 of *Il1b* (Il1a-201 ENSMUST00000028882.1 and Il1b-201 ENSMUST00000028881.13). Guide RNA cutting efficiency was determined in a pool of J774.2 and NIH3T3 cells using Sanger sequencing and Synthego ICE (see, e.g., Inference of CRISPR Edits from Sanger Trace Data, Hsiao T, Maures T, Waite K, Yang J et al. biorxiv. 2018, which is incorporated by reference herein for all purposes), or TIDE (see, e.g., Easy quantitative assessment of genome editing by sequence trace decomposition, Brinkman E, Chen T, Amendola M and Van Steensel B. Nucleic Acids res 2014, which is

incorporated by reference herein for all purposes) web tools to calculate percent editing. The experiment also compared the efficiency of *S. pyogenes* Cas9 and *S. aureus* Cas9. The cells were electroporated (Amaxa 4D Nucleofector unit, Lonza) with 5 µg TrueCut *Spy* Cas9 protein V2 (Invitrogen A36498) or 5 µg EnGen *Sau* Cas9 protein (NEB M0654T) with 100 pmol modified guide RNA (Sigma Aldrich). SF nucleofector solution and programme CM139 was used for J774.2 cells and SG nucleofector solution and programme EN158 was used for NIH3T3 cells. A cell pellet was taken 3 days' post electroporation and gDNA was extracted from each pool (Qiagen, DNeasy blood and tissue kit, 69506). Exon 4 of *Il1a* or *Il1b* was amplified in the appropriate pool by PCR (Phusion High-Fidelity DNA polymerase, NEB, cat#M0530S). *Il1a* primer fwd: TGGTTTCAGGAAAACCCAAG (SEQ ID NO:624), *Il1a* primer rev: GCAGTATGGCCAAGAAAGGA (SEQ ID NO:625), *Il1b* primer fwd: AGGAAGCCTGTGTCTGGTTG (SEQ ID NO:622), *Il1b* primer rev: CTGGGCAAGAACATTGGATT (SEQ ID NO:626). Amplicons were subjected to Sanger sequencing, and analyzed using either the Synthego ICE or TIDE web tools to determine the absence of wild type sequence in each clone and the presence of indels resulting in a frameshift in the cDNA sequence.

Table 2. Target *Il1a* and *Il1b* Sequences

Identifier	Guide ID	Gene	Exon	Cas9	Target Sequence 5'-3'	PAM
SEQ ID NO: 7	sg43	<i>Il1a</i>	4	<i>S.pyogenes</i>	GTATCAGCAACGTCAAGCAA	CGG
SEQ ID NO: 8	sg44	<i>Il1a</i>	4	<i>S.pyogenes</i>	CTGCAGGTCATCTTCAGTGA	AGG
SEQ ID NO: 9	sg45	<i>Il1a</i>	4	<i>S.pyogenes</i>	TATCAGCAACGTCAAGCAAC	GGG
SEQ ID NO: 10	sg46	<i>Il1a</i>	4	<i>S.pyogenes</i>	GCCATAGCTTGCATCATAGA	AGG
SEQ ID NO: 11	sg47	<i>Il1b</i>	4	<i>S.pyogenes</i>	CATCAACAAGAGCTTCAGGC	AGG
SEQ ID NO: 12	sg48	<i>Il1b</i>	4	<i>S.pyogenes</i>	TGCTCTCATCAGGACAGCCC	AGG
SEQ ID NO: 13	sg49	<i>Il1b</i>	4	<i>S.pyogenes</i>	GCTCATGTCCTCATCCTGGA	AGG
SEQ ID NO: 14	sg50	<i>Il1b</i>	4	<i>S.pyogenes</i>	CCTCATCCTGGAAGGTCCAC	GGG
SEQ ID NO: 15	sg51	<i>Il1a</i>	4	<i>S.aureus</i>	TTACTCCTTACCTTCCAGATC	ATGGGT
SEQ ID NO: 16	sg52	<i>Il1a</i>	4	<i>S.aureus</i>	GAAACTCAGCCGTCTCTTCTT	CAGAAT
SEQ ID NO: 17	sg53	<i>Il1a</i>	4	<i>S.aureus</i>	CAACTTCACCTTCAAGGAGAG	CCGGGT
SEQ ID NO: 18	sg54	<i>Il1b</i>	4	<i>S.aureus</i>	GTGTCTTTCCCGTGGACCTTC	CAGGAT
SEQ ID NO: 19	sg55	<i>Il1b</i>	4	<i>S.aureus</i>	CACAGCTTCTCCACAGCCACA	AGTAGT
SEQ ID NO: 20	sg56	<i>Il1b</i>	4	<i>S.aureus</i>	GTGCTGCTGCGAGATTTGAAG	CTGGAT

Table 3. CRISPR Guide RNA's.

Identifier	Guide ID	Gene	Exon	Cas9	cRNA Sequence 5'-3'	PAM
SEQ ID NO: 21	sg43	<i>Ill1a</i>	4	<i>S.pyogenes</i>	GUAUCAGCAACGUCAAGCAA	CGG
SEQ ID NO: 22	sg44	<i>Ill1a</i>	4	<i>S.pyogenes</i>	CUGCAGGUCAUCUUCAGUGA	AGG
SEQ ID NO: 23	sg45	<i>Ill1a</i>	4	<i>S.pyogenes</i>	UAUCAGCAACGUCAAGCAAC	GGG
SEQ ID NO: 24	sg46	<i>Ill1a</i>	4	<i>S.pyogenes</i>	GCCAUAGCUUGCAUCAUAGA	AGG
SEQ ID NO: 25	sg47	<i>Ill1b</i>	4	<i>S.pyogenes</i>	CAUCAACAAGAGCUUCAGGC	AGG
SEQ ID NO: 26	sg48	<i>Ill1b</i>	4	<i>S.pyogenes</i>	UGCUCUCAUCAGGACAGCCC	AGG
SEQ ID NO: 27	sg49	<i>Ill1b</i>	4	<i>S.pyogenes</i>	GCUCAUGUCCUCAUCCUGGA	AGG
SEQ ID NO: 28	sg50	<i>Ill1b</i>	4	<i>S.pyogenes</i>	CCUCAUCCUGGAAGGUCCAC	GGG
SEQ ID NO: 29	sg51	<i>Ill1a</i>	4	<i>S.aureus</i>	UUACUCCUUAACCUUCCAGAUC	ATGGGT
SEQ ID NO: 30	sg52	<i>Ill1a</i>	4	<i>S.aureus</i>	GAAACUCAGCCGUCUCUUCUU	CAGAAT
SEQ ID NO: 31	sg53	<i>Ill1a</i>	4	<i>S.aureus</i>	CAACUUCACCUUCAAGGAGAG	CCGGGT
SEQ ID NO: 32	sg54	<i>Ill1b</i>	4	<i>S.aureus</i>	GUGUCUUUCCCGUGGACCUUC	CAGGAT
SEQ ID NO: 33	sg55	<i>Ill1b</i>	4	<i>S.aureus</i>	CACAGCUUCUCCACAGCCACA	AGTAGT
SEQ ID NO: 34	sg56	<i>Ill1b</i>	4	<i>S.aureus</i>	GUGCUGCUGCGAGAUUUGAAG	CTGGAT

[00457] Each cRNA (see, e.g., Table 3) was synthesized as a single guide RNA consisting of the cRNA sequences above fused to the tracrRNA sequences below (see, e.g., SEQ ID Nos: 35-36). In certain embodiments, an A<gtU flip is used to increase guide RNA activity.

[00458] Sau Cas9:

GUUAUAGUACUCUGGAAACAGAAUCUACUAAAACAAGGCAAAAUGCCGUGUU
UAUCUCGUCAACUUGUUGGCGAGAUUUUU (SEQ ID NO: 35)

[00459] *Spy* Cas9:

GUUAUAGAGCUAGAAAUAGCAAGUUAUUAAAGGCUAGUCCGUUAUCAACUU
GAAAAAGUGGCACCGAGUCGGUGCUUUUUU (SEQ ID NO: 36)

[00460] *In Vitro Cleavage Assay*

[00461] Fig. 1A illustrates agarose gel electrophoresis analysis of 100 ng mouse DNA, cleaved by 0.5 µg *Spy* Cas9 and 200 ng modified guide RNA's 43-46 for *Il1a* gene and 47-50 for *Il1b*. DNA is cut at a specific site by the Cas9 using the guide RNA to create a predictable band pattern on the agarose gel compared to the uncut control (without wishing to be bound by any particular theory, the agarose gel electrophoresis for sg8* appears to show a failed synthesis).

[00462] Fig. 1B illustrates agarose gel electrophoresis analysis of 100 ng mouse DNA, cleaved by 0.5 µg *Sau* Cas9 and 200 ng modified guide RNA's 51-53 for *Il1a* gene and 54-56 for *Il1b*. DNA is cut at a specific site by the Cas9 using the guide RNA to create a predictable band pattern on the agarose gel compared to the uncut control.

[00463] *Editing Cell Lines*

[00464] Genomic DNA was extracted from the edited pools and the *Il1a* or *Il1b* exon 4 was PCR amplified in the appropriate pools. The PCR products were sent for sanger sequencing and then deconvoluted using TIDE or Synthego ICE software. Synthego ICE was used to deconvolute the *Spy* Cas9 pools. The software can determine the patterns of editing in each pool based on the guide RNA sequence and PAM site. It can distinguish between editing which has caused an in frame deletion that could lead to a truncated functional protein, and editing which has causes a frameshift mutation which will lead to a true knockout. The *Sau*Cas9 pools were analysed with TIDE because Synthego ICE software cannot deconvolute *Sau*Cas9 editing. TIDE analysis works in a similar way to ICE by determining patterns of editing in a pool based on the guide RNA and PAM site. However, rather than giving a true knockout score, it gives an editing efficiency score, which cannot distinguish between in frame and frameshift editing patterns. Therefore, editing efficiency scores may over represent the guide RNA's ability to knockout a protein. *Spy*Cas9 is the standard protein used in CRISPR gene editing. However, it is 4101bp compared to *Sau* Cas9 which is 3156bp. Due to the size limitations of packaging some viruses, such as AAV, it was decided to compare the editing capabilities of *Sau*Cas9 and *Spy*Cas9 to see whether the smaller *Sau* Cas9 could be used in the vector being designed for this project.

[00465] Figs. 2A – 2D illustrate graphs displaying editing efficiencies of *Spy* Cas9 (Figs. 2A and 2B) and *Sau*Cas9 (Figs. 2C and 2D) used with a range of guide RNA's in J774.2 ("J") and NIH3T3 ("N") cells. Editing efficiencies were determined using Synthego ICE or TIDE sanger deconvolution software. Fig. 2A: knock out efficiency of *Il1a* using guide RNA 43-46 with *Spy*Cas9 in J774.2 and NIH3T3. Synthego ICE was used to deconvolute the sanger sequence trace and determine knock out efficiency. Fig. 2B: knock out efficiency of *Il1b* using guide RNA 47-50 with *Spy*Cas9 in J774.2 and NIH3T3; without wishing to be bound by any particular theory, the data for sgRNA8 appears to show a failed synthesis. Synthego ICE was used to deconvolute the sanger sequence trace and determine knock out efficiency. Fig. 2C: knock out efficiency of *Il1a* using guide RNA 51-53 with saCas9 in J774.2 and NIH3T3. TIDE was used to deconvolute the sanger sequence trace and determine the editing efficiency. Fig. 2D: knock out efficiency of *Il1b* using guide RNA 54-56 with *Sau* Cas9 in J774.2 and NIH3T3. TIDE was used to deconvolute the sanger sequence trace and determine the editing efficiency.

EXAMPLE 3. Reducing IL-1 β expression by CRISPR Gene-engineering in a Mouse Uric Acid Model.

[00466] *Time Course Experiment to determine Optimal Pre-Treatment Time*

[00467] A pilot experiment is performed to determine optimal pre-treatment time of mice with virus prior to challenging the mice with uric acid. Mice are injected with GFP-labeled AAV5 vector into the knee joint. Viral load is then quantified by PCR and location of viral infection is quantified by histology at 3, 5, and 7 days after infection. A treatment time that yields robust expression of virus inside the joint is selected as the optimal lead time for injecting viral vectors into the mice for the experiments to determine the reduction of IL-1b in a mouse uric acid model by a CRISPR AAV vector engineered to target IL-1b and silence or reduce expression of IL-1b.

[00468] *Experiment to confirm CRISPR AAV (AAV-spCas9) knock down of IL-1b expression and treatment effect in uric acid model*

[00469] Mice are selected and distributed into three groups:

Group 1: mice injected with a CRISPR AAV vector (AAV-spCas9) engineered to target IL-1b, and silence or reduce expression of IL-1 protein,

Group 2: mice injected with “scrambled” guide RNA / Cas9 (AAV-spCas9), a CRISPR AAV vector engineered with a payload that will not affect IL-1 production, and

Group 3: mice injected with saline.

[00470] The mice are then challenged with uric acid after an optimal pre-treatment time. Within 24 hours of injection with uric acid, the animals are sacrificed and the joint tissue is analyzed for cytokine expression (e.g., assessed for IL-1 expression by IHC). The joint tissue may also be assessed for gross histopathology and for expression of biomarkers of inflammation.

[00471] Group 1 mice treated with a CRISPR AAV vector engineered to target IL-1b, and silence or reduce the expression of IL-1 protein, will show reduced levels of IL-1 by IHC and lower levels of inflammation biomarkers than any of the two other groups.

EXAMPLE 4. Time Course Study of Intra-Articular Injection of AAV in Mice.

[00472] A study was conducted to evaluate the time course for injecting AAV into the joint of male C57BL/6 mice.

[00473] Materials & Methods

[00474] *Test Article Identification and Preparation* - The eGFP AAVPrime™ Purified Adeno-associated Viral Particles: GFP-tagged AAV5 GeneCopoeia™, catalogue No. AB201, lot No. GC08222K1902, 1.18×10^{13} Genome Copies/mL) and AAV6 (GeneCopoeia™, catalogue No. AB401, lot No. GC09242K1905, 5.47×10^{12} Genome Copies/mL) we supplied. AAV- particles were shipped on dry ice and were stored at -80°C immediately upon receipt. Just prior to dosing, the AAV- particles were reconstituted in phosphate buffered saline (PBS without calcium and magnesium: Corning, lot No. 11419005) for IA dosing at 10 μL per knee. See the study protocol (Appendix A) for additional details of test article preparation, storage, and handling.

[00475] *Test System Identification* - Male C57BL/6 mice (N = 30) that were 8 to 10 weeks old were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice weighed approximately 24 to 29 grams (mean of 26 g) at enrollment on study day 0. The animals were identified by a distinct mark at the base of the tail delineating group and animal number.

After randomization, all cages were labeled with protocol number, group numbers, and animal numbers with appropriate color-coding (Appendix A).

[00476] *Environment & Husbandry* - Upon arrival, the animals were housed 3 to 5 per cage in polycarbonate cages with wood chip bedding and suspended food and water bottles. The mice were housed either in shoebox cages (static airflow, approximately 70 in² floor space) with filter tops or in individually ventilated pie cages (passive airflow, approximately 70-75 in² floor space). Animal care including room, cage, and equipment sanitation conformed to the guidelines cited in the Guide for the Care and Use of Laboratory Animals (8th Edition). National Research Council, National Academy of Sciences, Washington, DC, 2011, which is incorporated by reference herein in its entirety for all purposes.

[00477] The animals were acclimated for 4 days prior to being paced in the study. An attending veterinarian was on site or on call during the live phase of the study. No concurrent medications were given.

[00478] During the acclimation and study periods, the animals were housed in a laboratory environment with temperatures ranging 19 °C to 25 °C and relative humidity of 30% to 70%. Automatic timers provided 12 hours of light and 12 hours of dark. The animals were allowed access ad libitum to Envigo Teklad 8640 diet and fresh municipal tap water.

[00479] *Experimental Design* - On study day 0, the mice were randomized by body weight into treatment groups. Following randomization, the animals were dosed by intra-articular (IA) injection as indicated in Table 4. Animal body weights were measured as described in section 8.5.1. The mice were euthanized for necropsy and tissue collection at 3 time points (days 3, 5, and 7) as described below in the section titled ‘Necropsy Specimens’.

Table 4. Group and Treatment Information

Group	N	Treatment	Dose Level (particles)	Dose Vol.	Dose Conc. (particles/ml)	Dose Route	Regimen
1	30	GFP-tagged AAV5	5×10 ⁹	10 µL	5×10 ¹¹ /mL	IA (right knee)	1× (Day 0)
		GFP-tagged AAV6	5×10 ⁹	10 µL	5×10 ¹¹ /mL	IA (left knee)	1× (Day 0)

[00480] *Observations, Measurements, and Specimens*

[00481] **Body Weight Measurements** - The mice were weighed for randomization on study day 0 and again on days 1, 3, 5, and 7. Body weight measurements can be found in Table 6.

[00482] Necropsy Specimens - The mice were necropsied on study days 3, 5, and 7 as indicated in Table 5.

Table 5. Necropsy Schedule

Group	Animal No.	Time-point(s)
1	1-10	Day 3
1	11-20	Day 5
1	21-30	Day 7

[00483] At necropsy, the mice were bled to exsanguination via cardiac puncture followed by cervical dislocation. Right and left knees were harvested from all animals. The skin and muscle were removed from the joints while keeping the joint capsule intact. Joints were flash-frozen separately in 15-mL conical tubes labeled with only mouse number, day of collection, and right or left leg. Knee joints were stored frozen at - 80 °C for shipment.

[00484] Animal Disposition - Animal carcasses were disposed of according to BBP SOPs.

[00485] Specimen and Raw Data Storage - Specimens (right and left knee joints), study data, and reports were delivered during or at the completion of the study.

[00486] Statement of Effect of Deviations on the Quality and Integrity of the Study - There were no deviations from the study protocol.

[00487] Results / Conclusions

[00488] On study day 0, male C57BL/6 mice received IA injections of GFP-tagged AAV5 (5×10^9 particles, 10 μ L) into right knees and IA injections of GFP-tagged AAV6 (5×10^9 particles, 10 μ L) into left knees. The animals were weighed on study days 0, 1, 3, 5, and 7. Necropsies were performed on study day 3 (animals 1-10), day 5 (animals 11-20), and day 7 (animals 21-30), and right and left knee joints were collected for shipment. The live portion of this study was completed successfully including animal weighing, dosing, and biological sample collection. All animals survived to study termination.

[00489] References

[00490] Guide for the Care and Use of Laboratory Animals (8th Edition). National Research Council, National Academy of Sciences, Washington, DC, 2011, which is incorporated by reference herein in its entirety for all purposes.

Table 6. Body Weight and Dose Calculation Data (MTC-UCM-1)

Treatment Group	Day 0				Day 1		Day 3	
	Body Wt.				Body Wt.		Body Wt.	
	(g)				(g)		(% Δ Baseline)	
Group 1								
C57Bl/6								
1A, 1x (D0)								
1	26.68	0.01	0.01	0.01	26.53	0.01	-0.6%	-1.7%
2	27.63	0.01	0.01	0.01	27.55	0.01	-0.4%	-4.0%
3	28.64	0.01	0.01	0.01	27.97	0.01	-2.3%	-0.2%
4	28.13	0.01	0.01	0.01	27.60	0.01	-1.9%	-1.5%
5	26.18	0.01	0.01	0.01	26.07	0.01	-0.4%	-1.2%
6	26.35	0.01	0.01	0.01	26.02	0.01	-1.4%	-0.3%
7	28.13	0.01	0.01	0.01	29.11	0.01	0.1%	-1.0%
8	24.22	0.01	0.01	0.01	23.90	0.01	-1.3%	-2.9%
9	25.40	0.01	0.01	0.01	24.97	0.01	-1.7%	-3.6%
10	24.85	0.01	0.01	0.01	24.21	0.01	-2.6%	-4.0%
11	27.76	0.01	0.01	0.01	27.26	0.01	-1.8%	0.9%
12	25.23	0.01	0.01	0.01	24.77	0.01	-1.8%	-1.3%
13	24.92	0.01	0.01	0.01	24.45	0.01	-1.9%	-1.3%
14	24.33	0.01	0.01	0.01	24.19	0.01	-0.6%	-1.9%
15	23.82	0.01	0.01	0.01	23.81	0.01	0.0%	-2.4%
16	24.83	0.01	0.01	0.01	24.39	0.01	-1.8%	-2.7%
17	25.94	0.01	0.01	0.01	25.93	0.01	0.0%	1.3%
18	27.21	0.01	0.01	0.01	27.44	0.01	0.8%	1.4%
19	25.61	0.01	0.01	0.01	25.17	0.01	-1.7%	-1.5%
20	27.81	0.01	0.01	0.01	27.26	0.01	-2.0%	-2.9%
21	26.63	0.01	0.01	0.01	26.63	0.01	0.0%	0.3%
22	26.96	0.01	0.01	0.01	27.40	0.01	1.8%	-4.3%
23	27.69	0.01	0.01	0.01	27.21	0.01	-1.7%	-1.7%
24	25.90	0.01	0.01	0.01	25.71	0.01	-0.7%	-1.7%
25	24.03	0.01	0.01	0.01	24.11	0.01	0.3%	-2.6%
26	27.60	0.01	0.01	0.01	26.67	0.01	-3.4%	-2.2%
27	27.87	0.01	0.01	0.01	27.59	0.01	-1.0%	-2.3%
28	24.43	0.01	0.01	0.01	24.25	0.01	-0.7%	-1.2%
29	26.75	0.01	0.01	0.01	26.02	0.01	-2.7%	-1.1%
30	25.93	0.01	0.01	0.01	25.99	0.01	0.2%	-0.5%
Mean	26.28				26.01		-1.0%	-1.6%
SE	0.27				0.27		0.2%	0.3%

Table 6 (continued). Body Weight and Dose Calculation Data (MTC-UCM-1)

Treatment Group	Day 5					Day 7		Change in Body weight from Baseline (g)
	Body Wt. (g)		Body Wt. % Δ Baseline		Body Wt. (g)	Body Wt. % Δ Baseline		
Group 1 C57Bl/6 1A, 1x (100)	1						-0.46	
	2						-1.11	
	3						-0.07	
	4						-0.50	
	5						-0.31	
	6						-0.08	
	7						-0.30	
	8						-0.71	
	9						-0.91	
	10						-0.99	
	11						0.86	
	12						-0.19	
	13						0.02	
	14						-0.65	
	15						-0.11	
	16						-0.57	
	17						0.21	
	18						0.67	
	19						0.03	
	20						-0.10	
	21						0.07	
	22					26.70	0.3%	-1.21
	23					25.75	-4.5%	-0.36
	24					27.33	-1.3%	0.10
	25					26.00	0.4%	0.07
	26					24.10	0.3%	-0.42
	27					27.18	-1.5%	-0.57
	28					27.30	-2.0%	0.33
	29					24.76	1.4%	-0.27
	30					26.48	-1.0%	-0.04
					25.73	-0.8%		
Mean SE	25.90		-0.6%		26.15	-0.8%	-0.25	
	0.33		0.4%		0.34	0.5%	0.09	

[00491] PROTOCOL**[00492] Test System**

Number of animals: 33 (30 + 3 extra)

Species/Strain or Breed: C57BL/6

Vendor: Jackson

Age/Wt at Arrival: 8-10 weeks old (~20 grams)

Gender: Male

Age/Wt Range at Study Initiation: At least 9 weeks by study initiation

Acclimation: Will be acclimated for at least 3 days after arrival at BBP

Housing: 3-5 animals/cage

Study Calendar

Mon	Tue	Wed	Thu	Fri	Sat	Sun
Week 1 <i>Day -4</i>	Week 1 <i>Day -3</i>	Week 1 <i>Day -2</i>	Week 1 <i>Day -1</i>	Week 1 <i>Day 0</i>	Week 1 <i>Day 1</i>	Week 1 <i>Day 2</i>
Distribute animals on arrival into groups for acclimation				Weigh & Randomize. IA Injections	Weigh	
Week 2 <i>Day 3</i>	Week 2 <i>Day 4</i>	Week 2 <i>Day 5</i>	Week 2 <i>Day 6</i>	Week 2 <i>Day 7</i>	Week 2 <i>Day 8</i>	Week 2 <i>Day 9</i>
Weigh, Necropsy Animals 1-10		Weigh, Necropsy Animals 11-20		Weigh, Necropsy Animals 21-30		

[00493] Materials

Name	Supplier	Cat #*
Isoflurane	VetOne	502017
Syringes & Needles	BD	As needed
Serum Separator Tubes (if needed)	Greiner Bio-One	#450472 (via Fisher)
Li Hep Mini-Collect (if needed)	Greiner Bio-One	#450480 (via Fisher)
EDTA Mini-Collect (if needed)	Greiner Bio-One	#450477 (via Fisher)
K3EDTA (if needed)	Covidien	#8881311149 (via Fisher)
K2EDTA Vacutainer (if needed)	BD	#367856 (via Fisher)
Na Hep Vacutainer (if needed)	BD	#367871 (via Fisher)
Li Hep Vacutainer (if needed)	BD	#367960 (via Fisher)

[00494] Test Article and Vehicle Information

[00495] *Unformulated Test Article Storage Conditions* - GFP-tagged AAV5 (Group 1): - 80°C; GFP-tagged AAV6 (Group 1): - 80 °C.

[00496] *Vehicle Information* - GFP-tagged AAV5 (Group 1): PBS (w/o Ca & Mg); GFP-tagged AAV6 (Group 1): PBS (w/o Ca & Mg).

[00497] *Test Article Formulation Instructions & Calculations* - GFP-tagged AAV5 (Group 1): Dilute stock to appropriate concentration using PBS; GFP-tagged AAV6 (Group 1): Dilute stock to appropriate concentration using PBS.

[00498] *Dosing Formulations and Vehicle Storage & Stability* - GFP-tagged AAV5 (Group 1): Dilute just prior to injecting; GFP-tagged AAV6 (Group 1): Dilute just prior to injecting.

[00499] *Disposition of Test Articles Following Dosing* - GFP-tagged AAV5 (Group 1): Discard formulations, retain stock solution for future studies; GFP-tagged AAV6 (Group 1): Discard formulations, retain stock solution for future studies.

[00500] Live Phase Deliverables

Live Phase Data Collection			
Type	Study Day	Grp (An)	Details
Body Weight	Day 0, 1, 3, 5, 7	All (Remaining)	

[00501] Necropsy Information

Sacrifice Schedule: Group 1 **An 1-10**: Day 3
Group 1 **An 11-20**: Day 5
Group 1 **An 21-30**: Day 7

Method of Euthanasia: Bleed by cardiac puncture to exsanguinate followed by cervical dislocation.

Time Points: Not Timed

Necropsy Tissue Sample Collection:				
Type	Gr/An	Details	Storage Condition	Disposition
Right Injected Knee	All	Remove skin and muscle keeping joint capsule intact	Flash Freeze (15 ml conical vial*)	Ship
Left Injected Knee	All	Remove skin and muscle keeping joint capsule intact	Flash Freeze (15 ml conical vial*)	Ship

* Label tubes with only mouse number, day of collection, and left or right leg. Samples will be tested without reference to whether they are AAV-2 or AAV-5 injected. Key to be provided only after PCR completion.

[00502] Sample Analysis

[00503] *Tissue Specimens* - Hind limbs from AAV-injected mice were snap-frozen and shipped. On arrival, specimens were transferred to the – 80 °C freezer for storage.

[00504] *GFP Expression in Target Tissues* - Hind limbs (paired) were thawed at room temperature and imaged in an IVIS bioluminescence imaging system (Lumina III; Perkin Elmer). GFP fluorescence was quantified using excitation at 488 nm and measuring emission at 510 nm. A total of 4 mice were evaluated at each time point (3 days, 5 days and 7 days). Tissues from the remaining 6 animals at each time point were retained for subsequent confirmation of viral burden using real-time PCR.

[00505] *Results* - As can be seen in Figure 3, there was high-level expression of GFP within injected knee joints at 3 days post-injection. Viral loads decreased slightly at 5 days, then rose again to 7 days. With the limited sample size in this pilot study there was no significant difference between the behaviours of AAV-5 and AAV-6.

[00506] *Discussion* - The data from this study support the use of either AAV-5 or AAV-6 for intra-articular delivery of CRISPR-Cas9 into the mouse knee joint. The levels of both viral serotypes increased from 5 to 7 day, leaving open the possibility that they may have increased further if the follow-up had been extended to 2 or maybe 3 weeks. Additional work would be needed to confirm this, but the data thus far would suggest that there should be an interval of at least one week before the injection of the vector and challenge with intra-articular monoiodoacetate (MIA) crystals.

[00507] *Background & Rationale* - The monoiodoacetate (MIA)-induced OA model is used in this work for two reasons. First, natural (spontaneous) OA is extremely uncommon in mice, whereas the injection of MIA results in an induced model of OA that is relatively fast in onset, predictable and that provides good clinical correlation to the disease phenotype seen in human OA patients, including intra-articular inflammation, pain and cartilage degeneration. Second, in contrast with surgical models such as destabilization of the medial meniscus (DMM) and transection of the anterior cruciate ligament (ACLT), the MIA model does not involve surgical incision of the joint capsule, making it much more relevant to the capsules of human patients with OA.

[00508] Injection of MIA crystals in rodents reproduces OA-like lesions and functional impairment that can be analyzed and quantified by techniques such as behavioral testing and objective lameness assessment. MIA is an inhibitor of glyceraldehyde-3-phosphatase and the

resulting alterations in cellular glycolysis eventual cause the death of cells within the joint, including chondrocytes. Chondrocyte death manifests as cartilage degeneration and alterations in proteoglycan staining. Mice injected with MIA usually exhibit pain-like behavior within 72 hours, and cartilage loss by around 4 weeks post-injection. Increases in IL-1 expression have been documented within 2-3 days of injection in rats and in mice.

[00509] *Study Design* - Mice are injected unilaterally with either MIA or the saline vehicle control (one joint per animal). Within each group, half of the animals are pre-treated with the AAV-CRISPR-Cas9 vector targeting the mouse IL-1 beta gene, and the other half are injected with an AAV-CRISPR-Cas9 scrambled control. Animals from both groups will be taken off study at one of two time points: an early time point of 48 hours, to allow for assessment of the impact of therapy on the levels of IL-1 within the synovial fluid, and a late time point of 4 weeks to allow for assessment of the impact of therapy on cartilage breakdown and histological evidence of osteoarthritis.

[00510] *Methods*

[00511] **Experimental Animals** - A total of 80 mice are used in this study.

The experimental procedures are reviewed and approved by the local IACUC. Mice are housed in micro-isolator cages, fed a standard laboratory animal diet, and allowed access to water ad libitum.

[00512] **MIA Model & Anti-IL1 Therapy** - Mice are acclimated for a period of 7 days ahead of the study. On the first day of the study, mice are anaesthetized with an inhaled mixture of isoflurane in oxygen. Once a surgical plane of anesthesia has been confirmed, the right hind limb is clipped and the skin scrubbed with a surgical antiseptic. 40 mice (**Treated**) receive an intra-articular injection of the AAV-CRISPR-Cas9 vector targeting IL-1, and the remaining 40 animals (**Control**) are injected intra-articularly with the AAV-CRISPR-Cas9 scrambled control. Seven days later, half of the animals in each group are injected in the same joint with MIA and half with the saline vehicle. This leads to the establishment of four study groups:

Group 1: **Treated-MIA** (20 mice)

Group 2: **Control-MIA** (20 mice)

Group 3: **Treated-Vehicle** (20 mice)

Group 4: **Control-Vehicle** (20 mice)

[00513] Ten mice per group are euthanised 48 hours after the MIA challenge in order to document IL-1 levels in the knee joint. The remaining animals will be housed for 4 weeks in order to evaluate the effects of therapy on pain behavior (behavioral testing, including von Frey testing), lameness (limb use), joint swelling (caliper measurement) and joint pathology (histopathology).

[00514] Euthanasia & Tissue Collection - Mice are killed by exsanguination, followed by cervical dislocation. Joints are opened and either flushed for IL-1 measurement (48-hour group) or immersion fixed in 10% formalin for decalcified histopathology (4-week group).

EXAMPLE 5. Efficacy of AAV-6 and AAV-5 mediated CRISPR Treatment in MSU-crystal Induced Joint Arthritis in Mice

[00515] *Introduction and Objectives*

[00516] The objective of these studies is to identify compounds/proteins that inhibit the inflammation induced by monosodium urate (MSU) crystal induced release of interleukin 1J3 (IL-1J3). This is a simple prescreen that identifies anti-inflammatory activity of various types of anti-inflammatory agents, especially IL-1 pathway blockers like interleukin receptor antagonists or antibodies that block IL-1 or IL1R1 (Torres R, et al. Hyperalgesia, synovitis and multiple biomarkers of inflammation are suppressed by interleukin 1 inhibition in a novel animal model of gouty arthritis. *Ann Rheum Dis.* 2009; 68(10):1602-1608, which is incorporated by reference herein in its entirety for all purposes). Gout is the most common form of inflammatory arthritis and is increasing in prevalence worldwide (Roddy E and Doherty M. *Epidemiology of Gout. Arthritis Research & Therapy.* 2010; 12(6):223, which is incorporated by reference herein in its entirety for all purposes). Gouty arthritis is characterized by increased serum urate concentration and deposits of monosodium urate crystals (MSU) in and around the joints, leading to swollen joints and severe pain (Sabina EP, Chandel S, and Rasool MK. Inhibition of monosodium urate crystal-induced inflammation by withaferin A. *J Pharm Pharmaceut Sci.* 2008; 11(4):46-55, which is incorporated by reference herein in its entirety for all purposes). Current treatments include nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, or colchicine. For some patients these treatments may not be effective in treating gout or have adverse side effects (Sabina, 2008; Getting SJ, et al. Activation of melanocortin type 3 receptor as a molecular mechanism for

adrenocorticotrophic hormone efficacy in gouty arthritis. *Arthritis & Rheumatism*. 2002; 46(10):2765-2775, which is incorporated by reference herein in its entirety for all purposes). The MSU-induced inflammation model provides a good, simple screening tool for identifying compounds that may have activity in the more complex disease process, such as systemic arthritis and more complex IL-1 driven diseases.

[00517] A study was conducted to evaluate the efficacy of adeno-associated virus (AAV)-mediated CRISPR therapy in monosodium urate (MSU) crystal induced inflammation in mice. On study day 0, male C57BL/6 mice were dosed into the right knee with a single (1x) intra-articular (IA) injection of placebo control (diluent, phosphate buffered saline [PBS]), a mixture of two variants of AAV-6 (one carrying Guide RNA 1 and the other carrying Guide RNA 2, 5×10^9 virus genome [vg] copies per mL), a mixture of two variants of AAV-5 (Guide 1 + Guide 2, 5×10^9 vg/mL), the scrambled AAV-6 control (carrying non-targeting guide RNA, 1×10^{10} vg/mL), or the scrambled AAV-5 control (1×10^{10} vg/mL). On study day 7, the mice were given injections into right knee (same joint as treatment) with MSU crystals (25 mg/mL: 250 μ g in 10 μ L PBS). The mice were euthanized for necropsy approximately 6 hours post-MSU injection on study day 7. Efficacy evaluation was based on animal body weights, von Frey testing, and knee caliper measurements.

[00518] Mice treated IA (1x on day 0) with AAV-6 (Guide 1 + 2: 5×10^9 vg/guide per knee) showed a statistically significant reduction in referred pain, as measured by von Frey testing, 6 hours after MSU injection on day 7 as compared to mice injected IA with AAV-5 scramble vector ($p = 0.025$) with results being nearly significant as compared to the AAV-6 scramble vector and PBS control groups ($p = 0.051$ and $p = 0.075$, respectively). Area under the curve (AUC) calculations for von Frey assessments did not differ statistically across groups. Animal body weight gain and knee swelling did not differ statistically across groups (Table 7). All animals survived to study termination.

Table 7. Summary of Data

Group	Treatment	von Frey Absolute Threshold AUC (day -1 to 7) – Right Foot	Knee Caliper Change from Baseline AUC (day -1 to 7) – Right Knee
1	AAV-6 Scramble Vector (1×10^{10} particles/knee), IA, 1x (d0)	5.92 (0.39)	0.02 (0.01)
2	AAV-6 Guide 1 + 2 (5×10^9 of each guide/knee), IA, 1x (d0)	6.01 (0.36)	0.01 (0.01)
3	PBS, IA (d0)	6.12 (0.33)	0.02 (0.01)

4	AAV-5 Scramble Vector (1×10^{10} particles/knee), IA, 1x (d0)	5.09 (0.31)	0.02 (0.01)
5	AAV-5 Guide 1 + 2 (5×10^9 of each guide/knee), IA, 1x (d0)	5.73 (0.39)	0.02 (0.01)

Values represent group means and standard errors (SE)

PBS = Phosphate buffered saline control, AAV = adeno-associated virus, AUC = Area under the curve

* $p < 0.05$ ANOVA (Tukey's post-hoc) vs. AAV-6 Guide 1

† $p < 0.05$ ANOVA (Tukey's post-hoc) vs. PBS

‡ $p < 0.05$ ANOVA (Tukey's post-hoc) vs. AAV-5 Scramble Vector

§ $p < 0.05$ ANOVA (Tukey's post-hoc) vs. AAV-5 Guide 1

[00519] Summary of Clinical Outcomes - No significant differences were observed between groups over time. No clinical evidence that virus injection provoked a response greater than that seen in the vehicle group. No clinical evidence that virus injection altered the effects of MSU on joint swelling. Specific role of IL-1 in MSU-induced inflammation is unclear, so lack of clinical effect may not be unexpected.

[00520] Summary of qPCR - qPCR data confirm that CRISPR editing with AAV-6 or AAV-5 is effective in restoring IL-1 beta mRNA expression to normal levels. Statistical significance is hard to demonstrate given the sample size. Confirmation of this effect can be obtained through IHC analysis of synovial tissues.

[00521] *Regulatory Compliance*

[00522] This study was conducted in accordance with the test facility standard operating procedures (SOPs), the World Health Organization Quality Practices in Basic Biomedical Research guidelines, and in compliance with all state and federal regulations, including USDA Animal Welfare Act 9 CFR Parts 1-3. Federal Register 39129, July 22, 1993.

[00523] *Institutional Animal Care and Use*

[00524] This study was conducted in accordance with The Guide for the Care & Use of Laboratory Animals (8th Edition). No acceptable alternative test systems were identified for the animals used in this study.

[00525] *Materials and Methods*

[00526] Test Article Identification and Preparation

[00527] AAV vectors were pre-formulated as a viral particle suspension ($>5 \times 10^{12}$ virus genome [vg] copies per mL) in frozen aliquots. The aliquots were stored at -80°C and

reconstituted in diluent (sterile filtered PBS [Corning, lot No. 01420007]) immediately before use. Standard biosafety level 2 (BSL-2) handling was used by personnel handling the AAV vectors prior to injection. The AAV scramble controls were prepared in sterile PBS to form working stocks containing 1×10^{12} vg/mL for IA injection at 10 μ L/knee to deliver 1×10^{10} vg of the scramble control into the knee joint. The active AAV vectors were prepared by mixing equal parts of each of the two active AAV-5 or AAV-6 constructs with sterile PBS to form working stocks containing 5×10^{11} vg/mL for each of the two guides. The active AAV formulations were injected IA at 10 μ L/knee to deliver 5×10^9 vg of each of the two guides into the knee joint. See the study protocol (Appendix B) for further details of test article preparation, storage, and handling.

[00528] The AAV vectors were identified as follows:

AAVPrime™ Adeno-Associated Virus - Serotype6 (AAV-6) Particles for sgRNA (GeneCopoeia™, catalog No. AA06-MCP001682-AD01-2-200-a, lot No. GC03182K2001)
AAVPrime™ Adeno-Associated Virus - Serotype6 (AAV-6) Particles for sgRNA (GeneCopoeia™, catalog No. AA06-MCP001682-AD01-2-200-b, lot No. GC03202K2001)
AAVPrime™ Adeno-Associated Virus - Serotype6 (AAV-6) Particles for sgRNA (GeneCopoeia™, catalog No. AA06-CCPCTR01-AD01-200, lot No. GC03112K2002)
AAVPrime™ Adeno-Associated Virus – Serotype5 (AAV-5) Particles for sgRNA (GeneCopoeia™, catalogue No. AA05-MCP001682-AD01-2-200A, lot No. GC-03182K2002)
AAVPrime™ Adeno-Associated Virus – Serotype5 (AAV-5) Particles for sgRNA (GeneCopoeia™, catalogue No. AA05-MCP001682-AD01-2-200B, lot No. GC03182K2003)
AAVPrime™ Adeno-Associated Virus – Serotype5 (AAV-5) Particles for sgRNA (GeneCopoeia™, catalogue No. AA05-CCPCTR01-AD01-200, lot No. GC03032K2003)

[00529] Monosodium urate (MSU) crystals were obtained from Invivogen (catalogue No. Tlrl-25-MSU, lot No. MSU-42-01). MSU crystals were prepared at 25 mg/mL in PBS (without Ca or Mg: Corning, catalogue No. 21-031-CV, lot No. 31719003) in a plastic tube, vortexed for approximately 1 minute, sonicated for approximately 15 to 20 minutes, and vortexed before pipetting and use.

[00530] *Test System*

Number of animals:	75 (70 + 5 extra)
Species/Strain or Breed:	C57BL/6
Vendor:	Jackson
Age/Wt at Arrival:	8-10 weeks old (~20 grams)
Gender:	Male
Age/Wt Range at Study Initiation:	At least 9 weeks by study initiation
Acclimation:	Will be acclimated for at least 3 days after arrival at BBP
Housing:	3-5 animals/cage

[00531] Male C57BL/6 mice (N = 70 + 4 extra) that were 8 to 10 weeks of age were obtained from The Jackson Laboratory (Bar Harbor, ME). The mice weighed approximately 20 to 29 grams (mean of approx. 25 g) at enrollment on study day -1.

[00532] Animals were identified by color-coded dots at the base of the tail delineating animal number. After enrollment, all cages were labeled with protocol number, group number, and animal numbers.

[00533] *Environment and Husbandry*

[00534] Upon arrival, the animals were housed 3 to 5 per cage in polycarbonate cages with corncob bedding and suspended food and water bottles. The mice were housed in individually ventilated pie cages (passive airflow, approximately 0.045-0.048 m² floor space). Animal care including room, cage, and equipment sanitation conformed to the guidelines cited in the Guide for the Care and Use of Laboratory Animals (Guide, 2011) and the applicable BBP SOPs.

[00535] The animals were acclimated for 9 days prior to being paced in the study. An attending veterinarian was on site or on call during the live phase of the study. No concurrent medications were given.

[00536] During the acclimation and study periods, the animals were housed in a laboratory environment with temperatures ranging 19°C to 25°C and relative humidity of 30% to 70%. Automatic timers provided 12 hours of light and 12 hours of dark. The animals were allowed access ad libitum to Envigo Teklad 8640 diet fresh municipal tap water.

[00537] *Study Design*

[00538] On study day -1, the animals were randomized by body weight into treatment groups, knees were shaved, and baseline knee caliper measurements were taken. On study day 0, the animals were dosed with treatments (IA into the right knee) as indicated in Table 8. On study day 7, the animals were given IA injections of MSU crystals (a total of 10 µL, 250 µg of MSU) into right knees (same knee as treatments). Body weight measurements were taken as described. Referred pain was measured by von Frey testing at 5 time points as described. Caliper measurements of right knees were taken at 5 time points as described. The animals were euthanized for necropsy following the final behavioral testing on day 7, as described.

Table 8. Group and Treatment Information

Grp	N	MSU	Treatment	Dose Level (particles/knee)	Dose Vol.	Dose Conc. (particles/mL)	Dose Route	Regimen
1	14	Yes	AAV-6 Scramble Vector	1×10^{10}	10 µL	1×10^{12}	IA (right knee)	1x (Day 0)
2	14	Yes	AAV-6 Guide 1 + 2	5×10^9 (of each guide)	10 µL	1×10^{12}	IA (right knee)	1x (Day 0)
3	14	Yes	PBS	~	10 µL	~	IA (right knee)	1x (Day 0)
4	14	Yes	AAV-5 Scramble Vector	1×10^{10}	10 µL	1×10^{12}	IA (right knee)	1x (Day 0)
5	14	Yes	AAV-5 Guide 1 + 2	5×10^9 (of each guide)	10 µL	1×10^{12}	IA (right knee)	1x (Day 0)

[00539] Disease Induction

[00540] MSU crystals were prepared at a concentration of 25mg/mL in sterile PBS. Crystals did solubilize, and injection preparation was carefully mixed prior to use. 10uL of MSU crystal solution was injected into the right knee joint.

Unformulated MSU Storage Conditions: Room temperature, protected from light
Vehicle: PBS
Dose Route: IA into the right knee
Dose Volume: 10uL per knee
MSU Formulation Instructions & Calculations: 0.01ml/knee * 75 animals = 0.72ml of 25mg/ml MSU needed, minimum
MSU Storage & Stability: Stored in glass. Prepared just prior to dosing. Vortexed before injecting
MSU Disposition Following Dosing: Formulations discarded after dosing. Unformulated stock retained.

[00541] Body Weight Measurements and Live Phase Sampling

[00542] The mice were weighed on study day -1 (pre-injection) for randomization, and body weights were measured again on study days 2 and 6. Animal body weight measurements can be found in Table 9.

Table 9. Body Weight and Dose Calculation Data (MGA-UCM-2)

Treatment Group	Day 1					Day 2					Day 6				
	Body Wt. (g)	Dose Vol. (0.1 ml)	Body Wt. (g)	Body Wt. % Δ from Baseline	Body Wt. (g)	Body Wt. % Δ from Baseline	Body Wt. (g)	Body Wt. % Δ from Baseline	Body Wt. (g)	Body Wt. % Δ from Baseline	Body Wt. (g)	Body Wt. % Δ from Baseline	Body Wt. (g)	Body Wt. % Δ from Baseline	Change in Body Weight Day 1 to 6
Group 1 ANOVA (Tukey's post-hoc) vs. Group 2 ANOVA (Tukey's post-hoc) vs. Group 3 ANOVA (Tukey's post-hoc) vs. Group 4 ANOVA (Tukey's post-hoc) vs. Group 5	1	25.99	0.01	24.19	-6.9%	-6.9%	24.67	-5.1%	24.67	-5.1%	24.67	-5.1%	24.67	-5.1%	-1
	2	25.08	0.01	24.64	-1.4%	-1.4%	25.37	1.3%	25.37	1.3%	25.37	1.3%	25.37	1.3%	0
	3	23.58	0.01	24.61	4.7%	4.7%	24.95	6.2%	24.95	6.2%	24.95	6.2%	24.95	6.2%	1
	4	23.92	0.01	23.14	-3.3%	-3.3%	23.81	-4.6%	23.81	-4.6%	23.81	-4.6%	23.81	-4.6%	0
	5	23.70	0.01	23.14	-3.7%	-3.7%	23.38	-4.7%	23.38	-4.7%	23.38	-4.7%	23.38	-4.7%	0
	6	23.84	0.01	23.97	0.6%	0.6%	24.51	4.0%	24.51	4.0%	24.51	4.0%	24.51	4.0%	1
	7	27.44	0.01	28.18	2.8%	2.8%	27.98	1.7%	27.98	1.7%	27.98	1.7%	27.98	1.7%	0
	8	23.79	0.01	22.83	-4.0%	-4.0%	23.89	3.5%	23.89	3.5%	23.89	3.5%	23.89	3.5%	0
	9	23.88	0.01	24.34	2.0%	2.0%	25.71	7.7%	25.71	7.7%	25.71	7.7%	25.71	7.7%	1
	10	27.46	0.01	27.97	1.9%	1.9%	28.66	4.4%	28.66	4.4%	28.66	4.4%	28.66	4.4%	1
	11	26.50	0.01	26.37	-0.5%	-0.5%	26.88	1.4%	26.88	1.4%	26.88	1.4%	26.88	1.4%	0
	12	26.51	0.01	26.48	-0.1%	-0.1%	27.76	4.5%	27.76	4.5%	27.76	4.5%	27.76	4.5%	1
	13	22.88	0.01	23.38	2.2%	2.2%	23.77	4.3%	23.77	4.3%	23.77	4.3%	23.77	4.3%	1
	14	24.77	0.01	24.68	-0.4%	-0.4%	24.98	0.9%	24.98	0.9%	24.98	0.9%	24.98	0.9%	0
Total															
Group 2 ANOVA (Tukey's post-hoc) vs. Group 3 ANOVA (Tukey's post-hoc) vs. Group 4 ANOVA (Tukey's post-hoc) vs. Group 5	1	23.56	0.01	22.47	-4.6%	-4.6%	22.92	-1.6%	22.92	-1.6%	22.92	-1.6%	22.92	-1.6%	0
	2	28.49	0.01	29.03	1.9%	1.9%	29.18	2.1%	29.18	2.1%	29.18	2.1%	29.18	2.1%	1
	3	24.65	0.01	24.65	0.0%	0.0%	25.06	1.7%	25.06	1.7%	25.06	1.7%	25.06	1.7%	0
	4	23.83	0.01	23.29	-2.3%	-2.3%	24.05	1.8%	24.05	1.8%	24.05	1.8%	24.05	1.8%	0
	5	22.78	0.01	23.19	1.8%	1.8%	24.14	6.0%	24.14	6.0%	24.14	6.0%	24.14	6.0%	1
	6	27.32	0.01	27.60	1.0%	1.0%	28.54	4.8%	28.54	4.8%	28.54	4.8%	28.54	4.8%	1
	7	26.83	0.01	27.49	2.5%	2.5%	28.69	6.4%	28.69	6.4%	28.69	6.4%	28.69	6.4%	1
	8	26.96	0.01	26.84	-0.4%	-0.4%	28.70	6.5%	28.70	6.5%	28.70	6.5%	28.70	6.5%	1
	9	22.89	0.01	22.86	-0.1%	-0.1%	23.66	3.4%	23.66	3.4%	23.66	3.4%	23.66	3.4%	0
	10	22.45	0.01	23.12	3.0%	3.0%	23.86	6.3%	23.86	6.3%	23.86	6.3%	23.86	6.3%	1
	11	24.53	0.01	24.53	0.0%	0.0%	25.42	3.7%	25.42	3.7%	25.42	3.7%	25.42	3.7%	0
	12	25.80	0.01	26.11	1.2%	1.2%	26.56	2.9%	26.56	2.9%	26.56	2.9%	26.56	2.9%	0
	13	25.38	0.01	25.59	0.8%	0.8%	26.14	3.3%	26.14	3.3%	26.14	3.3%	26.14	3.3%	0
	14	23.45	0.01	23.85	1.7%	1.7%	24.14	2.9%	24.14	2.9%	24.14	2.9%	24.14	2.9%	0
Total															
Group 3 ANOVA (Tukey's post-hoc) vs. Group 4 ANOVA (Tukey's post-hoc) vs. Group 5	1	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	2	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	3	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	4	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	5	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	6	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	7	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	8	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	9	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	10	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	11	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	12	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	13	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
	14	24.85	0.01	25.05	0.8%	0.8%	25.7	3.57%	25.7	3.57%	25.7	3.57%	25.7	3.57%	0.9
Total															

Table 9 (continued). Body Weight and Dose Calculation Data (MGA-UCM-2

Treatment Group						
Group 1 PBS FA, 1x (100)	Day -1 Body Wt. (g)	Day 0 Dose Vol. 10 µl (100)	Day 1 Body Wt. (g)	Day 1 Body Wt. % Δ Baseline	Day 6 Body Wt. (g)	Day 6 Body Wt. % Δ Baseline
1	22.14	0.00	24.19	1.0%	26.18	2.4%
2	25.39	0.00	25.11	-0.9%	26.33	3.1%
3	24.72	0.00	25.07	1.2%	25.41	2.8%
4	26.53	0.00	29.04	1.0%	26.62	5.8%
5	23.50	0.00	25.09	1.0%	26.16	1.6%
6	28.25	0.00	25.30	0.0%	26.06	3.1%
7	28.45	0.00	25.81	1.0%	26.42	3.1%
8	28.80	0.00	26.48	2.6%	26.57	4.6%
9	24.77	0.00	25.73	2.7%	26.24	5.9%
10	19.83	0.00	20.15	1.6%	21.23	7.1%
11	25.66	0.00	26.39	2.7%	27.14	5.8%
12	24.77	0.00	24.58	1.5%	25.02	5.1%
13	20.95	0.00	20.91	-0.2%	21.27	5.6%
14	24.74	0.00	24.88	0.6%	24.92	0.7%
Mean	24.79		25.22	1.77%	25.8	4.36%
SE	8.62		0.63	0.36%	0.6	0.31%
ANOVA (Tukey's post-hoc) vs. Group 4						1.1
ANOVA (Tukey's post-hoc) vs. Group 5						0.1 >0.9999 0.983
Group 4 AAV-8 Nucleoside Vector (1:10 ⁶ 10 particles / horse) FA, 1x (100)	Day -1 Body Wt. (g)	Day 0 Dose Vol. 10 µl (100)	Day 1 Body Wt. (g)	Day 1 Body Wt. % Δ Baseline	Day 6 Body Wt. (g)	Day 6 Body Wt. % Δ Baseline
1	25.60	0.00	25.68	1.7%	26.51	6.6%
2	26.90	0.00	29.66	3.6%	30.49	6.9%
3	28.37	0.00	25.41	0.2%	25.52	0.6%
4	23.46	0.00	23.63	2.6%	24.73	5.4%
5	24.86	0.00	26.77	1.6%	26.16	4.4%
6	26.93	0.00	27.27	1.2%	27.68	3.3%
7	24.79	0.00	25.07	0.7%	25.63	2.6%
8	24.52	0.00	24.69	0.7%	25.38	3.8%
9	24.66	0.00	27.89	9.9%	27.21	10.1%
10	24.62	0.00	26.66	5.8%	26.11	6.1%
11	23.73	0.00	24.66	1.6%	25.89	6.6%
12	23.43	0.00	24.64	2.6%	24.56	6.2%
13	22.63	0.00	22.60	-0.1%	23.04	2.9%
14	25.59	0.00	25.66	0.3%	25.62	1.3%
Extra	26.78	0.00	28.38	1.7%	26.78	0.0%
Mean	24.91		25.46	2.23%	26.8	4.27%
SE	0.42		0.47	0.72%	0.5	0.74%
ANOVA (Tukey's post-hoc) vs. Group 5						1.1 8.2 0.073
Group 5 AAV-2 Nucleoside 1 + 2 (5x10 ⁶ 9 of each particle / horse) FA, 1x (100)	Day -1 Body Wt. (g)	Day 0 Dose Vol. 10 µl (100)	Day 1 Body Wt. (g)	Day 1 Body Wt. % Δ Baseline	Day 6 Body Wt. (g)	Day 6 Body Wt. % Δ Baseline
1	23.37	0.00	26.47	3.5%	26.21	2.5%
2	23.56	0.00	23.77	-1.3%	24.14	2.1%
3	24.28	0.00	24.42	0.6%	24.71	1.8%
4	23.09	0.00	23.31	1.0%	24.18	4.7%
5	24.52	0.00	23.34	-3.7%	24.97	1.8%
6	25.90	0.00	26.60	2.7%	27.25	5.2%
7	23.92	0.00	26.68	6.6%	26.28	1.4%
8	23.32	0.00	25.48	6.6%	26.49	4.6%
9	25.99	0.00	27.51	5.8%	27.39	5.4%
10	25.50	0.00	27.63	9.7%	25.62	4.8%
11	24.89	0.00	25.54	3.4%	25.86	4.7%
12	24.15	0.00	24.97	3.4%	26.61	7.7%
13	22.60	0.00	21.99	-1.7%	22.90	1.7%
14	23.96	0.00	24.72	3.5%	24.93	4.6%
Extra	24.78	0.00	25.38	2.6%	26.23	5.8%
Mean	24.80		25.29	1.96%	25.7	3.95%
SE	8.35		0.46	0.49%	0.4	0.30%

[00543] Von Frey Methods

[00544] Von Frey analysis was performed on right hind paws at 5 time points: baseline (day -1), 6 hours post-dose (day 0), 24 hours post-dose (day 1), pre-MSU injection (0 h, day 7), and 6 hours post-MSU injection (day 7). The groups were blinded to the researcher during von Frey testing.

[00545] The von Frey method evaluates mechanical allodynia (pain due to a stimulus that does not normally provoke pain) based on the response of animals to the application of calibrated filaments (Bioseb, Vitrolles, France) to the foot. The filaments are identified by a number representing $\log_{10}$ of the force in milligrams $\times 10$. The animals are habituated to the testing rack three times (45 to 60 minutes) prior to baseline evaluation. When testing, the von Frey hair is placed on the surface of the hind paw and pushed smoothly until the hair has a significant bend in it; the hair is pressed against the paw for six seconds. Responses are recorded as either a 0 (no response) or a 1 (response). A response is defined as lifting the hind paw away from the hair, jerking the leg away, walking away from the hair, etc. The starting hair is 3.22, if the animal responds the tester moves down to 2.83, if there is no response to the 3.22 hair then the tester moves up to 3.61; the tester continues to test hairs based on the response and moves up or down, as appropriate. The hair increments are as follows: 1.65, 2.36, 2.44, 2.83, 3.22, 3.61, 3.84, 4.08, 4.17. Each paw is tested 5 times, moving up and down between hairs until the final filament is reached. Data is entered into a spreadsheet and used to translate the response rate into a paw withdraw threshold. Results of testing are converted to an absolute threshold (50% response rate) in grams, using the formula $10(x + yz)/10000$, where x equals the log unit value of the final tested filament, y equals the tabular value for the response pattern from Dixon's up-and-down method for small samples (Dixon, 1965), and z equals the average interval between filament values. Testing is done on the hind portions of the hind paw as the heel tends to give a more reliable and sensitive response. The testers monitor the animals for hyper-responding or freezing, in which case the animals are left alone until calm. Von Frey data can be found in Table 10.

Table 10. von Frey Data (MGA-UCM-2)

Treatment Group											Day 147	
	Day -1		Day 0/15		Day 1		Day 7		Day 225			
	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)		AUC
Group 1 <i>Salivary Secretion Volume (µL) 10 partitions / hour</i> <i>1.0, 3.0 (200)</i>	1	3.72500	0.53	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.72500	0.53	6.05
	2	3.96000	0.93	3.72500	0.53	3.72500	0.53	4.12500	1.33	3.41500	0.26	7.09
	3	3.72500	0.53	3.62500	0.11	3.62500	0.11	3.96000	0.93	3.41500	0.26	3.68
	4	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.96000	0.93	2.63500	0.04	7.42
	5	3.72500	0.53	3.72500	0.53	3.62500	0.11	3.72500	0.53	3.41500	0.26	2.91
	6	3.96000	0.93	3.72500	0.53	3.96000	0.93	3.96000	0.93	3.02500	0.11	7.04
	7	4.12500	1.33	3.72500	0.53	3.41500	0.26	3.72500	0.53	3.41500	0.26	3.93
	8	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.41500	0.26	7.44
	9	3.72500	0.53	3.72500	0.53	3.96000	0.93	3.72500	0.53	3.41500	0.26	5.63
	10	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.96000	0.93	2.63500	0.04	7.42
	11	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.96000	0.93	3.02500	0.11	6.34
	12	4.12500	1.33	3.72500	0.53	3.72500	0.53	3.96000	0.93	3.41500	0.26	6.04
	13	3.96000	0.93	3.72500	0.53	3.72500	0.53	3.96000	0.93	2.63500	0.04	5.75
	14	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.72500	0.53	6.20
Extra	4.12500	1.33	3.41500	0.26	3.41500	0.26	4.12500	1.33	3.02500	0.11	6.15	
Group 2 <i>ANOVA (Tukey's post-hoc) vs. Group 1</i> <i>ANOVA (Tukey's post-hoc) vs. Group 2</i> <i>ANOVA (Tukey's post-hoc) vs. Group 3</i> <i>ANOVA (Tukey's post-hoc) vs. Group 4</i> <i>ANOVA (Tukey's post-hoc) vs. Group 5</i>	Mean	0.86	0.66	0.64	0.81	0.23	5.92					
	SE	0.07	0.07	0.08	0.06	0.04	0.39					
	ANOVA (Tukey's post-hoc) vs. Group 1	0.979	0.917	1.000	0.974	0.051	1.000					
	ANOVA (Tukey's post-hoc) vs. Group 2	0.998	>0.9999	>0.9999	0.987	>0.9999	0.994					
	ANOVA (Tukey's post-hoc) vs. Group 3	0.999	0.943	0.493	0.956	0.987	0.478					
ANOVA (Tukey's post-hoc) vs. Group 4	0.984	0.873	0.995	0.998	0.599	0.496						
ANOVA (Tukey's post-hoc) vs. Group 5												

Table 10 (continued). von Frey Data (MGA-UCM-2)

Treatment Group	Group 2														Day 15					
	Day -1		Day 0.25		Day 1		Day 7		Day 7.25		Day 14.75									
	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)										
A. N. & C. 10000 1 vs 2 (6000 vs 10000 golden / kernel) 3.0, 1.0, (300)	1	3.96000	0.91	3.41500	0.26	3.72500	0.53	4.12500	1.33	3.41500	0.26	6.82								
	2	3.96000	0.91	3.02500	0.11	3.96000	0.91	3.72500	0.53	3.41500	0.26	5.45								
	3	3.72500	0.53	3.72500	0.53	3.41500	0.26	3.96000	0.91	3.72500	0.53	4.66								
	4	3.72500	0.53	3.96000	0.91	3.96000	0.91	3.96000	0.91	3.41500	0.26	7.20								
	5	3.96000	0.91	3.96000	0.91	3.96000	0.91	3.72500	0.53	3.72500	0.53	6.29								
	6	3.96000	0.91	3.96000	0.91	3.60000	0.91	4.12500	1.33	3.72500	0.53	8.79								
	7	4.12500	1.33	3.72500	0.53	3.72500	0.53	3.96000	0.91	3.96000	0.91	6.12								
	8	3.72500	0.53	3.41500	0.26	3.02500	0.11	3.96000	0.91	3.96000	0.91	3.91								
	9	4.12500	1.33	3.41500	0.26	3.96000	0.91	3.72500	0.53	3.72500	0.53	5.90								
	10	3.96000	0.91	3.96000	0.91	3.02500	0.11	3.96000	0.91	3.41500	0.26	4.72								
	11	3.96000	0.91	3.72500	0.53	3.72500	0.53	3.72500	0.53	3.72500	0.53	4.62								
	12	3.96000	0.91	3.96000	0.91	3.41500	0.26	3.96000	0.91	3.41500	0.26	5.24								
	13	3.96000	0.91	3.72500	0.53	3.96000	0.91	3.96000	0.91	3.62500	0.11	7.84								
	14	4.12500	1.33	3.72500	0.53	3.96000	0.91	3.96000	0.91	3.72500	0.53	7.36								
Extra														3.72500	0.53	3.72500	0.53	3.41500	0.26	4.73
Means														0.92	0.58	0.62	0.86	0.46	6.01	
SE														0.87	0.08	0.09	0.07	0.86	0.36	
ANOVA (Tukey's post-hoc) vs. Group 3														0.886	0.941	>0.9999	0.998	0.075	1.000	
ANOVA (Tukey's post-hoc) vs. Group 4														0.924	>0.9999	0.639	0.690	0.025	0.769	
ANOVA (Tukey's post-hoc) vs. Group 5														0.828	1.000	0.999	0.999	0.855	0.981	

Table 10 (continued). von Frey Data (MGA-UCM-2)

Treatment Group	Group 3	Day -1		Day 0.25		Day 1		Day 7		Day 2.25		Day 4.75
		Absolute Threshold		Absolute Threshold		Absolute Threshold		Absolute Threshold		Absolute Threshold		
		Log units	(g)	Log units	(g)	Log units	(g)	Log units	(g)	Log units	(g)	
PBS EA, 1x (D6)	1	3.96000	0.43	3.41500	0.26	3.96000	0.93	4.12500	1.33	3.72500	0.53	8.34
	2	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.96000	0.93	2.63500	0.04	7.42
	3	3.96000	0.93	3.72500	0.53	3.96000	0.93	3.96000	0.93	3.41500	0.26	7.06
	4	3.72500	0.53	3.96000	0.93	3.96000	0.93	3.72500	0.53	2.63500	0.04	5.99
	5	3.72500	0.53	3.96000	0.93	3.72500	0.53	3.96000	0.93	2.63500	0.04	5.89
	6	3.96000	0.93	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.41500	0.26	8.25
	7	3.96000	0.93	3.72500	0.53	3.72500	0.53	3.96000	0.93	3.72500	0.53	5.81
	8	4.12500	1.33	3.72500	0.53	3.41500	0.26	3.72500	0.53	3.41500	0.26	3.93
	9	3.72500	0.53	3.41500	0.26	3.72500	0.53	3.96000	0.93	3.41500	0.26	5.27
	10	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.96000	0.93	3.72500	0.53	6.39
	11	3.72500	0.53	3.72500	0.53	3.72500	0.53	3.72500	0.53	2.63500	0.04	4.52
	12	3.96000	0.93	3.96000	0.93	3.72500	0.53	3.72500	1.33	3.02500	0.13	7.48
	13	3.96000	0.93	3.72500	0.53	3.72500	0.53	3.72500	1.33	3.02500	0.13	7.07
	14	3.96000	0.93	3.72500	0.53	3.41500	0.26	3.96000	0.93	3.41500	0.26	4.58
ANOVA (Tukey's post-hoc) vs. G500g 4 ANOVA (Tukey's post-hoc) vs. G500g 5	Mean	6.83		6.66		6.63		6.89		6.23		6.12
	SE	0.06		0.07		0.06		0.08		0.05		0.33
		>0.9999		0.982		0.400		0.548		0.985		0.259
		0.999		0.901		0.998		0.993		0.660		0.937

Table 10 (continued). von Frey Data (MGA-UCM-2)

Treatment Group	Day -1		Day 0.25		Day 1		Day 3		Day 7.25		Day 17
	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	
Group 4 AAV-Z Scramble Vector (Exon 10 deletion / control) 1A, 3A (20%)	1	3.72500	0.53	3.96000	0.91	3.72500	0.53	3.96000	0.91	3.41500	5.92
	2	3.96000	0.91	3.72500	0.53	3.41500	0.26	3.72500	0.53	2.63500	3.64
	3	3.72500	0.53	3.02500	0.11	3.72500	0.53	3.96000	0.91	3.41500	5.11
	4	3.72500	0.53	3.72500	0.53	3.72500	0.53	3.72500	0.53	2.63500	4.32
	5	4.12500	1.33	3.96000	0.91	3.72500	0.53	3.96000	0.91	3.41500	6.42
	6	3.96000	0.91	3.96000	0.91	3.72500	0.53	3.72500	0.53	3.41500	4.97
	7	3.96000	0.91	3.96000	0.91	3.72500	0.53	3.96000	0.91	2.63500	6.13
	8	3.96000	0.91	3.96000	0.91	3.02500	0.11	3.72500	0.53	3.02500	3.51
	9	3.96000	0.91	3.72500	0.53	3.96000	0.91	3.96000	0.91	2.63500	7.83
	10	3.96000	0.91	3.72500	0.53	3.62500	0.31	3.72500	0.53	3.41500	3.15
	11	4.12500	1.33	3.02500	0.11	3.72500	0.53	3.96000	0.91	3.72500	5.65
	12	3.72500	0.53	3.41500	0.26	3.72500	0.53	3.96000	0.91	3.02500	5.25
	13	3.72500	0.53	3.72500	0.53	3.72500	0.53	3.72500	0.53	3.72500	4.38
	14	3.96000	0.91	3.72500	0.53	3.72500	0.53	3.96000	0.91	2.63500	5.75
Extra		3.96000	0.91	3.96000	0.91	3.72500	0.53	3.72500	0.53	3.41500	4.97
ANOVA (Tukey's post-hoc) vs. Group 5	Mean	0.84	0.59	0.48	0.75	0.29	0.85	0.31	0.85	0.41	5.99
	SE	0.07	0.08	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.31
		0.999	0.999	0.776	0.869	0.411	0.869	0.411	0.411	0.713	0.713

Table 10 (continued). von Frey Data (MGA-UCM-2)

Treatment Group												
	Day -1		Day 0/25		Day 1		Day 7		Day 7/25		Day 1-7	
	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	(g)	Absolute Threshold Log units	AFC
AAV-2 Guide 1 + 2 (50% of each guide: knee 1%, 1x (200))	1	3.72500	0.53	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.41500	0.26	7.20
	2	3.72500	0.53	3.41500	0.26	3.96000	0.31	3.96000	0.31	3.72500	0.53	6.59
	3	4.17500	1.33	3.96000	0.31	3.72500	0.53	3.96000	0.31	3.96000	0.31	6.50
	4	3.72500	0.53	3.96000	0.31	3.96000	0.31	3.72500	0.53	3.41500	0.26	5.21
	5	3.72500	0.53	3.96000	0.31	3.96000	0.31	4.17500	1.33	3.96000	0.31	8.60
	6	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.72500	0.53	2.63500	0.04	3.98
	7	3.72500	0.53	3.72500	0.53	3.72500	0.53	4.12500	1.33	3.96000	0.31	8.83
	8	3.96000	0.31	3.41500	0.26	3.41500	0.26	3.96000	0.31	3.72500	0.53	4.62
	9	3.96000	0.31	3.72500	0.53	3.96000	0.31	3.96000	0.31	3.41500	0.26	7.06
	10	3.96000	0.31	3.96000	0.31	3.72500	0.53	3.72500	0.53	3.41500	0.26	4.16
	11	3.72500	0.53	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.41500	0.26	4.46
	12	3.96000	0.31	3.41500	0.26	3.96000	0.31	3.72500	0.53	2.63500	0.04	5.57
	13	4.12500	1.33	3.96000	0.31	3.41500	0.26	3.96000	0.31	3.72500	0.53	5.94
	14	3.96000	0.31	3.41500	0.26	3.72500	0.53	3.72500	0.53	3.96000	0.31	4.29
Mean	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.96000	0.31	3.96000	5.73
SE	0.01	0.08	0.01	0.08	0.01	0.08	0.01	0.08	0.01	0.08	0.01	0.38

[00546] Caliper Methods

[00547] Caliper measurements of right knees were taken at 5 time points: baseline (day -1), 6 hours post-dose (day 0), 24 hours post-dose (day 1), pre-MSU injection (0 h, day 7), and 6 hours post- MSU injection (day 7). Knee caliper measurements were made using a spring-loaded micrometer caliper (Mitutoyo). Knee caliper measurements can be found in Table 11.

Table 11. Knee Caliper Data (MGA-UCM-2)

Treatment Group	Day 1				Day 7				Day 25				Day 75				Δ from Baseline MLC
	Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)				
	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)	Right	Δ from Baseline (H-I)			
ANOVAs (Turkey's post-hoc) vs. Group 1 1/3, 1/2, 2/3	1	3.63	0.06	3.63	0.02	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06
	2	3.58	0.06	3.58	0.04	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06
	3	3.67	0.06	3.67	0.06	3.67	0.06	3.67	0.06	3.67	0.06	3.67	0.06	3.67	0.06	3.67	0.06
	4	3.65	0.06	3.65	0.05	3.65	0.06	3.65	0.06	3.65	0.06	3.65	0.06	3.65	0.06	3.65	0.06
	5	3.58	0.06	3.58	0.04	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06	3.58	0.06
	6	3.66	0.06	3.66	0.04	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06
	7	3.69	0.06	3.69	0.05	3.69	0.06	3.69	0.06	3.69	0.06	3.69	0.06	3.69	0.06	3.69	0.06
	8	3.73	0.06	3.73	0.04	3.73	0.06	3.73	0.06	3.73	0.06	3.73	0.06	3.73	0.06	3.73	0.06
	9	3.76	0.06	3.76	0.05	3.76	0.06	3.76	0.06	3.76	0.06	3.76	0.06	3.76	0.06	3.76	0.06
	10	3.66	0.06	3.66	0.03	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06	3.66	0.06
	11	3.63	0.06	3.63	0.04	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06
	12	3.63	0.06	3.63	0.05	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06	3.63	0.06
	13	3.72	0.06	3.72	0.06	3.72	0.06	3.72	0.06	3.72	0.06	3.72	0.06	3.72	0.06	3.72	0.06
	14	3.78	0.06	3.78	0.05	3.78	0.06	3.78	0.06	3.78	0.06	3.78	0.06	3.78	0.06	3.78	0.06
	Mean	3.64	0.06	3.64	0.05	3.64	0.06	3.64	0.06	3.64	0.06	3.64	0.06	3.64	0.06	3.64	0.06
ANOVAs (Turkey's post-hoc) vs. Group 2 ANOVAs (Turkey's post-hoc) vs. Group 3 ANOVAs (Turkey's post-hoc) vs. Group 4 ANOVAs (Turkey's post-hoc) vs. Group 5	Mean	3.70	0.06	3.70	0.03	3.70	0.06	3.70	0.06	3.70	0.06	3.70	0.06	3.70	0.06	3.70	0.06
	SE	0.03		0.03		0.03		0.03		0.03		0.03		0.03		0.03	
	ANOVAs (Turkey's post-hoc) vs. Group 2																
	ANOVAs (Turkey's post-hoc) vs. Group 3																
	ANOVAs (Turkey's post-hoc) vs. Group 4																
ANOVAs (Turkey's post-hoc) vs. Group 5																	

Table 11 (continued). Knee Caliper Data (MGA-UCM-2)

Treatment Group	Treatments																
	Day 1				Day 4-25				Day 7				Day 25				A Priori Biasing ALC
	Caliper Measurement (mm)		A Priori Biasing (D-1)		Caliper Measurement (mm)		A Priori Biasing (D-1)		Caliper Measurement (mm)		A Priori Biasing (D-1)		Caliper Measurement (mm)		A Priori Biasing (D-1)		
Group 2 N=16 160° vs. 120°	1	3.48	3.69	3.48	3.69	3.48	3.69	3.48	3.69	3.48	3.69	3.48	3.69	3.48	3.69	3.48	3.69
	2	4.04	4.10	4.04	4.10	4.04	4.10	4.04	4.10	4.04	4.10	4.04	4.10	4.04	4.10	4.04	4.10
	3	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60
	4	3.62	3.69	3.62	3.69	3.62	3.69	3.62	3.69	3.62	3.69	3.62	3.69	3.62	3.69	3.62	3.69
	5	3.54	3.10	3.54	3.10	3.54	3.10	3.54	3.10	3.54	3.10	3.54	3.10	3.54	3.10	3.54	3.10
	6	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60
	7	3.88	3.69	3.88	3.69	3.88	3.69	3.88	3.69	3.88	3.69	3.88	3.69	3.88	3.69	3.88	3.69
	8	3.75	3.10	3.75	3.10	3.75	3.10	3.75	3.10	3.75	3.10	3.75	3.10	3.75	3.10	3.75	3.10
	9	3.78	3.60	3.78	3.60	3.78	3.60	3.78	3.60	3.78	3.60	3.78	3.60	3.78	3.60	3.78	3.60
	10	3.64	3.69	3.64	3.69	3.64	3.69	3.64	3.69	3.64	3.69	3.64	3.69	3.64	3.69	3.64	3.69
	11	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10
	12	3.75	3.60	3.75	3.60	3.75	3.60	3.75	3.60	3.75	3.60	3.75	3.60	3.75	3.60	3.75	3.60
	13	3.68	3.69	3.68	3.69	3.68	3.69	3.68	3.69	3.68	3.69	3.68	3.69	3.68	3.69	3.68	3.69
	14	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10	3.65	3.10
Extra	3.67	3.60	3.64	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	3.67	3.60	
Group 3 N=16 160° vs. 120°	Mean	3.78	4.04	3.71	4.04	3.78	4.04	3.78	4.04	3.78	4.04	3.78	4.04	3.78	4.04	3.78	4.04
	SE																
	ANOVA (Tukey's post-hoc) vs. Group 1																
	ANOVA (Tukey's post-hoc) vs. Group 4																
	ANOVA (Tukey's post-hoc) vs. Group 5																

Table 11 (continued). Knee Caliper Data (MGA-UCM-2)

Treatment Group	Group 6																Day 25	Day 28	Day 32	Day 35	Day 37	Day 40	Day 43	Day 46	Day 49	Day 52	Day 55	Day 58	Day 61	Day 64	Day 67	Day 70	Day 73	Day 76	Day 79	Day 82	Day 85	Day 88	Day 91	Day 94	Day 97	Day 100	Day 103	Day 106	Day 109	Day 112	Day 115	Day 118	Day 121	Day 124	Day 127	Day 130	Day 133	Day 136	Day 139	Day 142	Day 145	Day 148	Day 151	Day 154	Day 157	Day 160	Day 163	Day 166	Day 169	Day 172	Day 175	Day 178	Day 181	Day 184	Day 187	Day 190	Day 193	Day 196	Day 199	Day 202	Day 205	Day 208	Day 211	Day 214	Day 217	Day 220	Day 223	Day 226	Day 229	Day 232	Day 235	Day 238	Day 241	Day 244	Day 247	Day 250	Day 253	Day 256	Day 259	Day 262	Day 265	Day 268	Day 271	Day 274	Day 277	Day 280	Day 283	Day 286	Day 289	Day 292	Day 295	Day 298	Day 301	Day 304	Day 307	Day 310	Day 313	Day 316	Day 319	Day 322	Day 325	Day 328	Day 331	Day 334	Day 337	Day 340	Day 343	Day 346	Day 349	Day 352	Day 355	Day 358	Day 361	Day 364	Day 367	Day 370	Day 373	Day 376	Day 379	Day 382	Day 385	Day 388	Day 391	Day 394	Day 397	Day 400	Day 403	Day 406	Day 409	Day 412	Day 415	Day 418	Day 421	Day 424	Day 427	Day 430	Day 433	Day 436	Day 439	Day 442	Day 445	Day 448	Day 451	Day 454	Day 457	Day 460	Day 463	Day 466	Day 469	Day 472	Day 475	Day 478	Day 481	Day 484	Day 487	Day 490	Day 493	Day 496	Day 499	Day 502	Day 505	Day 508	Day 511	Day 514	Day 517	Day 520	Day 523	Day 526	Day 529	Day 532	Day 535	Day 538	Day 541	Day 544	Day 547	Day 550	Day 553	Day 556	Day 559	Day 562	Day 565	Day 568	Day 571	Day 574	Day 577	Day 580	Day 583	Day 586	Day 589	Day 592	Day 595	Day 598	Day 601	Day 604	Day 607	Day 610	Day 613	Day 616	Day 619	Day 622	Day 625	Day 628	Day 631	Day 634	Day 637	Day 640	Day 643	Day 646	Day 649	Day 652	Day 655	Day 658	Day 661	Day 664	Day 667	Day 670	Day 673	Day 676	Day 679	Day 682	Day 685	Day 688	Day 691	Day 694	Day 697	Day 700	Day 703	Day 706	Day 709	Day 712	Day 715	Day 718	Day 721	Day 724	Day 727	Day 730	Day 733	Day 736	Day 739	Day 742	Day 745	Day 748	Day 751	Day 754	Day 757	Day 760	Day 763	Day 766	Day 769	Day 772	Day 775	Day 778	Day 781	Day 784	Day 787	Day 790	Day 793	Day 796	Day 799	Day 802	Day 805	Day 808	Day 811	Day 814	Day 817	Day 820	Day 823	Day 826	Day 829	Day 832	Day 835	Day 838	Day 841	Day 844	Day 847	Day 850	Day 853	Day 856	Day 859	Day 862	Day 865	Day 868	Day 871	Day 874	Day 877	Day 880	Day 883	Day 886	Day 889	Day 892	Day 895	Day 898	Day 901	Day 904	Day 907	Day 910	Day 913	Day 916	Day 919	Day 922	Day 925	Day 928	Day 931	Day 934	Day 937	Day 940	Day 943	Day 946	Day 949	Day 952	Day 955	Day 958	Day 961	Day 964	Day 967	Day 970	Day 973	Day 976	Day 979	Day 982	Day 985	Day 988	Day 991	Day 994	Day 997	Day 1000	Day 1003	Day 1006	Day 1009	Day 1012	Day 1015	Day 1018	Day 1021	Day 1024	Day 1027	Day 1030	Day 1033	Day 1036	Day 1039	Day 1042	Day 1045	Day 1048	Day 1051	Day 1054	Day 1057	Day 1060	Day 1063	Day 1066	Day 1069	Day 1072	Day 1075	Day 1078	Day 1081	Day 1084	Day 1087	Day 1090	Day 1093	Day 1096	Day 1099	Day 1102	Day 1105	Day 1108	Day 1111	Day 1114	Day 1117	Day 1120	Day 1123	Day 1126	Day 1129	Day 1132	Day 1135	Day 1138	Day 1141	Day 1144	Day 1147	Day 1150	Day 1153	Day 1156	Day 1159	Day 1162	Day 1165	Day 1168	Day 1171	Day 1174	Day 1177	Day 1180	Day 1183	Day 1186	Day 1189	Day 1192	Day 1195	Day 1198	Day 1201	Day 1204	Day 1207	Day 1210	Day 1213	Day 1216	Day 1219	Day 1222	Day 1225	Day 1228	Day 1231	Day 1234	Day 1237	Day 1240	Day 1243	Day 1246	Day 1249	Day 1252	Day 1255	Day 1258	Day 1261	Day 1264	Day 1267	Day 1270	Day 1273	Day 1276	Day 1279	Day 1282	Day 1285	Day 1288	Day 1291	Day 1294	Day 1297	Day 1300	Day 1303	Day 1306	Day 1309	Day 1312	Day 1315	Day 1318	Day 1321	Day 1324	Day 1327	Day 1330	Day 1333	Day 1336	Day 1339	Day 1342	Day 1345	Day 1348	Day 1351	Day 1354	Day 1357	Day 1360	Day 1363	Day 1366	Day 1369	Day 1372	Day 1375	Day 1378	Day 1381	Day 1384	Day 1387	Day 1390	Day 1393	Day 1396	Day 1399	Day 1402	Day 1405	Day 1408	Day 1411	Day 1414	Day 1417	Day 1420	Day 1423	Day 1426	Day 1429	Day 1432	Day 1435	Day 1438	Day 1441	Day 1444	Day 1447	Day 1450	Day 1453	Day 1456	Day 1459	Day 1462	Day 1465	Day 1468	Day 1471	Day 1474	Day 1477	Day 1480	Day 1483	Day 1486	Day 1489	Day 1492	Day 1495	Day 1498	Day 1501	Day 1504	Day 1507	Day 1510	Day 1513	Day 1516	Day 1519	Day 1522	Day 1525	Day 1528	Day 1531	Day 1534	Day 1537	Day 1540	Day 1543	Day 1546	Day 1549	Day 1552	Day 1555	Day 1558	Day 1561	Day 1564	Day 1567	Day 1570	Day 1573	Day 1576	Day 1579	Day 1582	Day 1585	Day 1588	Day 1591	Day 1594	Day 1597	Day 1600	Day 1603	Day 1606	Day 1609	Day 1612	Day 1615	Day 1618	Day 1621	Day 1624	Day 1627	Day 1630	Day 1633	Day 1636	Day 1639	Day 1642	Day 1645	Day 1648	Day 1651	Day 1654	Day 1657	Day 1660	Day 1663	Day 1666	Day 1669	Day 1672	Day 1675	Day 1678	Day 1681	Day 1684	Day 1687	Day 1690	Day 1693	Day 1696	Day 1699	Day 1702	Day 1705	Day 1708	Day 1711	Day 1714	Day 1717	Day 1720	Day 1723	Day 1726	Day 1729	Day 1732	Day 1735	Day 1738	Day 1741	Day 1744	Day 1747	Day 1750	Day 1753	Day 1756	Day 1759	Day 1762	Day 1765	Day 1768	Day 1771	Day 1774	Day 1777	Day 1780	Day 1783	Day 1786	Day 1789	Day 1792	Day 1795	Day 1798	Day 1801	Day 1804	Day 1807	Day 1810	Day 1813	Day 1816	Day 1819	Day 1822	Day 1825	Day 1828	Day 1831	Day 1834	Day 1837	Day 1840	Day 1843	Day 1846	Day 1849	Day 1852	Day 1855	Day 1858	Day 1861	Day 1864	Day 1867	Day 1870	Day 1873	Day 1876	Day 1879	Day 1882	Day 1885	Day 1888	Day 1891	Day 1894	Day 1897	Day 1900	Day 1903	Day 1906	Day 1909	Day 1912	Day 1915	Day 1918	Day 1921	Day 1924	Day 1927	Day 1930	Day 1933	Day 1936	Day 1939	Day 1942	Day 1945	Day 1948	Day 1951	Day 1954	Day 1957	Day 1960	Day 1963	Day 1966	Day 1969	Day 1972	Day 1975	Day 1978	Day 1981	Day 1984	Day 1987	Day 1990	Day 1993	Day 1996	Day 1999	Day 2002	Day 2005	Day 2008	Day 2011	Day 2014	Day 2017	Day 2020	Day 2023	Day 2026	Day 2029	Day 2032	Day 2035	Day 2038	Day 2041	Day 2044	Day 2047	Day 2050	Day 2053	Day 2056	Day 2059	Day 2062	Day 2065	Day 2068	Day 2071	Day 2074	Day 2077	Day 2080	Day 2083	Day 2086	Day 2089	Day 2092	Day 2095	Day 2098	Day 2101	Day 2104	Day 2107	Day 2110	Day 2113	Day 2116	Day 2119	Day 2122	Day 2125	Day 2128	Day 2131	Day 2134	Day 2137	Day 2140	Day 2143	Day 2146	Day 2149	Day 2152	Day 2155	Day 2158	Day 2161	Day 2164	Day 2167	Day 2170	Day 2173	Day 2176	Day 2179	Day 2182	Day 2185	Day 2188	Day 2191	Day 2194	Day 2197	Day 2200	Day 2203	Day 2206	Day 2209	Day 2212	Day 2215	Day 2218	Day 2221	Day 2224	Day 2227	Day 2230	Day 2233	Day 2236	Day 2239	Day 2242	Day 2245	Day 2248	Day 2251	Day 2254	Day 2257	Day 2260	Day 2263	Day 2266	Day 2269	Day 2272	Day 2275	Day 2278	Day 2281	Day 2284	Day 2287	Day 2290	Day 2293	Day 2296	Day 2299	Day 2302	Day 2305	Day 2308	Day 2311	Day 2314	Day 2317	Day 2320	Day 2323	Day 2326	Day 2329	Day 2332	Day 2335	Day 2338	Day 2341	Day 2344	Day 2347	Day 2350	Day 2353	Day 2356	Day 2359	Day 2362	Day 2365	Day 2368	Day 2371	Day 2374	Day 2377	Day 2380	Day 2383	Day 2386	Day 2389	Day 2392	Day 2395	Day 2398	Day 2401	Day 2404	Day 2407	Day 2410	Day 2413	Day 2416	Day 2419	Day 2422	Day 2425	Day 2428	Day 2431	Day 2434	Day 2437	Day 2440	Day 2443	Day 2446	Day 2449	Day 2452	Day 2455	Day 2458	Day 2461	Day 2464	Day 2467	Day 2470	Day 2473	Day 2476	Day 2479	Day 2482	Day 2485	Day 2488	Day 2491	Day 2494	Day 2497	Day 2500	Day 2503	Day 2506	Day 2509	Day 2512	Day 2515	Day 2518	Day 2521	Day 2524	Day 2527	Day 2530	Day 2533	Day 2536	Day 2539	Day 2542	Day 2545	Day 2548	Day 2551	Day 2554	Day 2557	Day 2560	Day 2563	Day 2566	Day 2569	Day 2572	Day 2575	Day 2578	Day 2581	Day 2584	Day 2587	Day 2590	Day 2593	Day 2596	Day 2599	Day 2602	Day 2605	Day 2608	Day 2611	Day 2614	Day 2617	Day 2620	Day 2623	Day 2626	Day 2629	Day 2632	Day 2635	Day 2638	Day 2641	Day 2644	Day 2647	Day 2650	Day 2653	Day 2656	Day 2659	Day 2662	Day 2665	Day 2668	Day 2671	Day 2674	Day 2677	Day 2680	Day 2683	Day 2686	Day 2689	Day 2692	Day 2695	Day 2698	Day 2701	Day 2704	Day 2707	Day 2710	Day 2713	Day 2716	Day 2719	Day 2722	Day 2725	Day 2728	Day 2731	Day 2734	Day 2737	Day 2740	Day 2743	Day 274
-----------------	---------	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	--------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	---------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	---------

Table 11 (continued). Knee Caliper Data (MGA-UCM-2)

Treatment Group	Day 1				Day 8.25				Day 1				Day 7.25				Day 14.75 A Base Baseline AKC	
	Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)		Caliper Measurement (mm)					
	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)	Right	A Base Baseline (D-T)				
PRN 1A, Ex 1089																		
1	3.85	0.00	3.86	0.04	3.85	0.00	3.85	0.00	3.85	0.00	3.86	0.01	3.86	0.01	3.86	0.01	-0.06	
2	3.78	0.00	3.78	0.00	3.78	0.00	3.78	0.00	3.78	0.00	3.79	0.01	3.79	0.01	3.79	0.01	0.05	
3	3.68	0.00	3.72	0.04	3.68	0.00	3.68	0.00	3.68	0.00	3.68	0.00	3.73	0.05	3.73	0.05	0.06	
4	3.72	0.00	3.77	0.05	3.72	0.00	3.72	0.00	3.72	0.00	3.74	0.02	3.76	0.02	3.76	0.02	0.12	
5	3.62	0.00	3.62	0.00	3.62	0.00	3.62	0.00	3.62	0.00	3.62	0.00	3.70	0.08	3.70	0.08	0.01	
6	3.68	0.00	3.68	0.00	3.68	0.00	3.68	0.00	3.68	0.00	3.66	-0.02	3.74	0.06	3.74	0.06	-0.06	
7	3.78	0.00	3.78	0.00	3.78	0.00	3.78	0.00	3.78	0.00	3.86	0.08	3.86	0.08	3.86	0.08	0.01	
8	3.72	0.00	3.72	0.00	3.72	0.00	3.72	0.00	3.72	0.00	3.73	0.01	3.80	0.08	3.80	0.08	0.08	
9	3.65	0.00	3.69	0.04	3.65	0.00	3.65	0.00	3.65	0.00	3.63	-0.02	3.71	0.06	3.71	0.06	0.02	
10	3.58	0.00	3.58	0.00	3.58	0.00	3.58	0.00	3.58	0.00	3.59	0.01	3.66	0.03	3.66	0.03	0.06	
11	3.65	0.00	3.65	0.00	3.65	0.00	3.65	0.00	3.65	0.00	3.65	0.00	3.68	0.03	3.68	0.03	0.06	
12	3.79	0.00	3.79	0.00	3.79	0.00	3.79	0.00	3.79	0.00	3.77	-0.02	3.82	0.03	3.82	0.03	-0.06	
13	3.56	0.00	3.56	0.00	3.56	0.00	3.56	0.00	3.56	0.00	3.61	0.05	3.61	0.05	3.61	0.05	0.03	
14	3.75	0.00	3.75	0.00	3.75	0.00	3.75	0.00	3.75	0.00	3.72	-0.03	3.75	0.02	3.75	0.02	0.02	
Mean	3.70	0.00	3.72	0.02	3.70	0.00	3.70	0.00	3.70	0.00	3.75	0.03	3.75	0.03	3.75	0.03	0.02	
SE	0.02	0.00	0.02	0.01	0.02	0.00	0.02	0.00	0.02	0.00	0.02	0.01	0.02	0.01	0.02	0.01	0.01	
ANOVA (Tukey's post-hoc vs. Group 4																		>0.0000
ANOVA (Tukey's post-hoc vs. Group 5																		>0.0000

Table 11 (continued). Knee Caliper Data (MGA-UCM-2)

Treatment Group	Day 1										Day 7										Day 25										Day 47																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
	Day 4					Day 13					Day 31					Day 63					Day 91					Day 119					Day 147																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																											
	Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)			Caliper Measurement (mm)		A from Baseline (D-1)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
AAV-4 Gene-delivered Vectors (1x10 ¹⁰ particles / knee) 1x, 1x, 100x	1	3.67	0.00	3.70	0.03	3.67	0.00	3.68	0.01	3.70	0.03	3.70	0.00	3.71	0.01	3.73	0.03	3.73	0.00	3.75	0.02	3.76	0.01	3.78	0.02	3.79	0.01	3.81	0.02	3.82	0.01	3.84	0.02	3.85	0.01	3.87	0.02	3.88	0.01	3.90	0.02	3.91	0.01	3.93	0.02	3.94	0.01	3.96	0.02	3.97	0.01	3.99	0.02	4.00	0.01	4.02	0.02	4.03	0.01	4.05	0.02	4.06	0.01	4.08	0.02	4.09	0.01	4.11	0.02	4.12	0.01	4.14	0.02	4.15	0.01	4.17	0.02	4.18	0.01	4.20	0.02	4.21	0.01	4.23	0.02	4.24	0.01	4.26	0.02	4.27	0.01	4.29	0.02	4.30	0.01	4.32	0.02	4.33	0.01	4.35	0.02	4.36	0.01	4.38	0.02	4.39	0.01	4.41	0.02	4.42	0.01	4.44	0.02	4.45	0.01	4.47	0.02	4.48	0.01	4.50	0.02	4.51	0.01	4.53	0.02	4.54	0.01	4.56	0.02	4.57	0.01	4.59	0.02	4.60	0.01	4.62	0.02	4.63	0.01	4.65	0.02	4.66	0.01	4.68	0.02	4.69	0.01	4.71	0.02	4.72	0.01	4.74	0.02	4.75	0.01	4.77	0.02	4.78	0.01	4.80	0.02	4.81	0.01	4.83	0.02	4.84	0.01	4.86	0.02	4.87	0.01	4.89	0.02	4.90	0.01	4.92	0.02	4.93	0.01	4.95	0.02	4.96	0.01	4.98	0.02	4.99	0.01	5.01	0.02	5.02	0.01	5.04	0.02	5.05	0.01	5.07	0.02	5.08	0.01	5.10	0.02	5.11	0.01	5.13	0.02	5.14	0.01	5.16	0.02	5.17	0.01	5.19	0.02	5.20	0.01	5.22	0.02	5.23	0.01	5.25	0.02	5.26	0.01	5.28	0.02	5.29	0.01	5.31	0.02	5.32	0.01	5.34	0.02	5.35	0.01	5.37	0.02	5.38	0.01	5.40	0.02	5.41	0.01	5.43	0.02	5.44	0.01	5.46	0.02	5.47	0.01	5.49	0.02	5.50	0.01	5.52	0.02	5.53	0.01	5.55	0.02	5.56	0.01	5.58	0.02	5.59	0.01	5.61	0.02	5.62	0.01	5.64	0.02	5.65	0.01	5.67	0.02	5.68	0.01	5.70	0.02	5.71	0.01	5.73	0.02	5.74	0.01	5.76	0.02	5.77	0.01	5.79	0.02	5.80	0.01	5.82	0.02	5.83	0.01	5.85	0.02	5.86	0.01	5.88	0.02	5.89	0.01	5.91	0.02	5.92	0.01	5.94	0.02	5.95	0.01	5.97	0.02	5.98	0.01	6.00	0.02	6.01	0.01	6.03	0.02	6.04	0.01	6.06	0.02	6.07	0.01	6.09	0.02	6.10	0.01	6.12	0.02	6.13	0.01	6.15	0.02	6.16	0.01	6.18	0.02	6.19	0.01	6.21	0.02	6.22	0.01	6.24	0.02	6.25	0.01	6.27	0.02	6.28	0.01	6.30	0.02	6.31	0.01	6.33	0.02	6.34	0.01	6.36	0.02	6.37	0.01	6.39	0.02	6.40	0.01	6.42	0.02	6.43	0.01	6.45	0.02	6.46	0.01	6.48	0.02	6.49	0.01	6.51	0.02	6.52	0.01	6.54	0.02	6.55	0.01	6.57	0.02	6.58	0.01	6.60	0.02	6.61	0.01	6.63	0.02	6.64	0.01	6.66	0.02	6.67	0.01	6.69	0.02	6.70	0.01	6.72	0.02	6.73	0.01	6.75	0.02	6.76	0.01	6.78	0.02	6.79	0.01	6.81	0.02	6.82	0.01	6.84	0.02	6.85	0.01	6.87	0.02	6.88	0.01	6.90	0.02	6.91	0.01	6.93	0.02	6.94	0.01	6.96	0.02	6.97	0.01	6.99	0.02	7.00	0.01	7.02	0.02	7.03	0.01	7.05	0.02	7.06	0.01	7.08	0.02	7.09	0.01	7.11	0.02	7.12	0.01	7.14	0.02	7.15	0.01	7.17	0.02	7.18	0.01	7.20	0.02	7.21	0.01	7.23	0.02	7.24	0.01	7.26	0.02	7.27	0.01	7.29	0.02	7.30	0.01	7.32	0.02	7.33	0.01	7.35	0.02	7.36	0.01	7.38	0.02	7.39	0.01	7.41	0.02	7.42	0.01	7.44	0.02	7.45	0.01	7.47	0.02	7.48	0.01	7.50	0.02	7.51	0.01	7.53	0.02	7.54	0.01	7.56	0.02	7.57	0.01	7.59	0.02	7.60	0.01	7.62	0.02	7.63	0.01	7.65	0.02	7.66	0.01	7.68	0.02	7.69	0.01	7.71	0.02	7.72	0.01	7.74	0.02	7.75	0.01	7.77	0.02	7.78	0.01	7.80	0.02	7.81	0.01	7.83	0.02	7.84	0.01	7.86	0.02	7.87	0.01	7.89	0.02	7.90	0.01	7.92	0.02	7.93	0.01	7.95	0.02	7.96	0.01	7.98	0.02	7.99	0.01	8.01	0.02	8.02	0.01	8.04	0.02	8.05	0.01	8.07	0.02	8.08	0.01	8.10	0.02	8.11	0.01	8.13	0.02	8.14	0.01	8.16	0.02	8.17	0.01	8.19	0.02	8.20	0.01	8.22	0.02	8.23	0.01	8.25	0.02	8.26	0.01	8.28	0.02	8.29	0.01	8.31	0.02	8.32	0.01	8.34	0.02	8.35	0.01	8.37	0.02	8.38	0.01	8.40	0.02	8.41	0.01	8.43	0.02	8.44	0.01	8.46	0.02	8.47	0.01	8.49	0.02	8.50	0.01	8.52	0.02	8.53	0.01	8.55	0.02	8.56	0.01	8.58	0.02	8.59	0.01	8.61	0.02	8.62	0.01	8.64	0.02	8.65	0.01	8.67	0.02	8.68	0.01	8.70	0.02	8.71	0.01	8.73	0.02	8.74	0.01	8.76	0.02	8.77	0.01	8.79	0.02	8.80	0.01	8.82	0.02	8.83	0.01	8.85	0.02	8.86	0.01	8.88	0.02	8.89	0.01	8.91	0.02	8.92	0.01	8.94	0.02	8.95	0.01	8.97	0.02	8.98	0.01	9.00	0.02	9.01	0.01	9.03	0.02	9.04	0.01	9.06	0.02	9.07	0.01	9.09	0.02	9.10	0.01	9.12	0.02	9.13	0.01	9.15	0.02	9.16	0.01	9.18	0.02	9.19	0.01	9.21	0.02	9.22	0.01	9.24	0.02	9.25	0.01	9.27	0.02	9.28	0.01	9.30	0.02	9.31	0.01	9.33	0.02	9.34	0.01	9.36	0.02	9.37	0.01	9.39	0.02	9.40	0.01	9.42	0.02	9.43	0.01	9.45	0.02	9.46	0.01	9.48	0.02	9.49	0.01	9.51	0.02	9.52	0.01	9.54	0.02	9.55	0.01	9.57	0.02	9.58	0.01	9.60	0.02	9.61	0.01	9.63	0.02	9.64	0.01	9.66	0.02	9.67	0.01	9.69	0.02	9.70	0.01	9.72	0.02	9.73	0.01	9.75	0.02	9.76	0.01	9.78	0.02	9.79	0.01	9.81	0.02	9.82	0.01	9.84	0.02	9.85	0.01	9.87	0.02	9.88	0.01	9.90	0.02	9.91	0.01	9.93	0.02	9.94	0.01	9.96	0.02	9.97	0.01	9.99	0.02	10.00	0.01	10.02	0.02	10.03	0.01	10.05	0.02	10.06	0.01	10.08	0.02	10.09	0.01	10.11	0.02	10.12	0.01	10.14	0.02	10.15	0.01	10.17	0.02	10.18	0.01	10.20	0.02	10.21	0.01	10.23	0.02	10.24	0.01	10.26	0.02	10.27	0.01	10.29	0.02	10.30	0.01	10.32	0.02	10.33	0.01	10.35	0.02	10.36	0.01	10.38	0.02	10.39	0.01	10.41	0.02	10.42	0.01	10.44	0.02	10.45	0.01	10.47	0.02	10.48	0.01	10.50	0.02	10.51	0.01	10.53	0.02	10.54	0.01	10.56	0.02	10.57	0.01	10.59	0.02	10.60	0.01	10.62	0.02	10.63	0.01	10.65	0.02	10.66	0.01	10.68	0.02	10.69	0.01	10.71	0.02	10.72	0.01	10.74	0.02	10.75	0.01	10.77	0.02	10.78	0.01	10.80	0.02	10.81	0.01	10.83	0.02	10.84	0.01	10.86	0.02	10.87	0.01	10.89	0.02	10.90	0.01	10.92	0.02	10.93	0.01	10.95	0.02	10.96	0.01	10.98	0.02	10.99	0.01	11.01	0.02	11.02	0.01	11.04	0.02	11.05	0.01	11.07	0.02	11.08	0.01	11.10	0.02	11.11	0.01	11.13	0.02	11.14	0.01	11.16	0.02	11.17	0.01	11.19	0.02	11.20	0.01	11.22	0.02	11.23	0.01	11.25	0.02	11.26	0.01	11.28	0.02	11.29	0.01	11.31	0.02	11.32	0.01	11.34	0.02	11.35	0.01	11.37	0.02	11.38	0.01	11.40	0.02	11.41	0.01	11.43	0.02	11.44	0.01	11.46	0.02	11.47	0.01	11.49	0.02	11.50	0.01	11.52	0.02	11.53	0.01	11.55	0.02	11.56	0.01	11.58	0.02	11.59	0.01	11.61	0.02	11.62	0.01	11.64	0.02	11.65	0.01	11.67	0.02	11.68	0.01	11.70	0.02	11.71	0.01	11.73	0.02	11.74	0.01	11.76	0.02	11.77	0.01	11.79	0.02	11.80	0.01	11.82	0.02	11.83	0.01	11.85	0.02	11.86	0.01	11.88	0.02	11.89	0.01	11.91	0.02	11.92	0.01	11.94	0.02	11.95	0.01	11.97	0.02	11.98	0.01	12.00	0.02	12.01	0.01	12.03	0.02	12.04	0.01	12.06	0.02	12.07	0.01	12.09	0.02	12.10	0.01	12.12	0.02	12.13	0.01	12.15	0.02	12.16	0.01	12.18	0.02	12.19	0.01	12.21	0.02	12.22	0.01	12.24	0.02	12.25	0.01	12.27	0.02	12.28	0.01	12.30	0.02	12.31	0.01	12.33	0.02	12.34	0.01	12.36	0.02	12.37	0.01	12.39	0.02	12.40	0.01	12.42	0.02	12.43	0.01	12.45	0.02	12.46	0.01	12.48	0.02	12.49	0.01	12.51	0.02	12.52	0.01	12.54	0.02	12.55	0.01	12.57	0.02	12.58	0.01	12.60	0.02	12.61	0.01	12.63	0.02	12.64	0.01	12.66	0.02	12.67	0.01	12.69	0.02	12.70	0.01	12.72	0.02	12.73	0.01	12.75	0.02	12.76	0.01	12.78	0.02	12.79	0.01	12.81	0.02	12.82	0.01	12.84	0.02	12.85	0.01	12.87	0.02	12.88	0.01	12.90	0.02	12.91	0.01	12.93	0.02	12.94	0.01	12.96	0.02	12.97	0.01	12.99	0.02	13.00	0.01	13.02	0.02	13.03	0.01	13.05	0.02	13.06	0.01	13.08	0.02	13.09	0.01	13.11	0.02	13.12	0.01	13.14	0.02	13.15	0.01	13.17	0.02	13.18	0.01	13.20	0.02	13.21	0.01	13.23	0.02	13.24	0.01	13.26	0.02	13.27	0.01	13.29	0.02	13.30	0.01	13.32	0.02	13.33	0.01	13.35	0.02	13.36	0.01	13.38	0.02	13

[00548] Moribund or Found Dead Animals

[00549] If animals were found dead no samples were taken. For animals needing to be euthanized, regardless of reason, samples were taken as they would at necropsy (see Necropsy Information section). Animals on health assessment may be given SC fluids as well as hydrogel and food on the bottom of the cage.

[00550] Study Group Designations

Grp	N	MSU	Treatment	Dose Level (particles/knee)	Dose Vol.	Dose Conc. (particles/ml)	Dose Route	Regimen
1	14	Yes	AAV-6 Scramble Vector	1x10 ¹⁰	10µl	1x10 ¹²	IA (right knee)	1x (Day 0)
2	14	Yes	AAV-6 Guide 1 + 2	5x10 ⁹ (of each guide)	10µl	1x10 ¹²	IA (right knee)	1x (Day 0)
3	14	Yes	PBS	--	10µl	--	IA (right knee)	1x (Day 0)
4	14	Yes	AAV-5 Scramble Vector	1x10 ¹⁰	10µl	1x10 ¹²	IA (right knee)	1x (Day 0)
5	14	Yes	AAV-5 Guide 1 + 2	5x10 ⁹ (of each guide)	10µl	1x10 ¹²	IA (right knee)	1x (Day 0)

[00551] Study Calendar

Mon	Tue	Wed	Thu	Fri	Sat	Sun
5/4/2020 Day -10	5/5/2020 Day -9	5/6/2020 Day -8	5/7/2020 Day -7	5/8/2020 Day -6	5/9/2020 Day -5	5/10/2020 Day -4
Distribute animals on arrival into groups for acclimation				Acclimate to VF		
5/11/2020 Day -3	5/12/2020 Day -2	5/13/2020 Day -1	5/14/2020 Day 0	5/15/2020 Day 1	5/16/2020 Day 2	5/17/2020 Day 3
Acclimate to VF	Acclimate to VF	Weigh & Randomize, Baseline Caliper, Baseline Behavior. Shave Knees	Timed Dose, Timed Caliper, Timed Behavior	Timed Caliper, Timed Behavior	Weigh	
5/18/2020 Day 4	5/19/2020 Day 5	5/20/2020 Day 6	5/21/2020 Day 7	5/22/2020 Day 8	5/23/2020 Day 9	5/24/2020 Day 10
		Weigh, Shave Knees?	Timed MSU, Timed Caliper, Timed Behavior, Timed Necropsy			

[00552] *Materials*

Name	Supplier	Cat #*
MSU crystals	Invivogen	Tlrl-msu-25
Vehicle (PBS)	Corning	21-031-CV
Gene	SgRNA	AA06-CCPCTROI—AD01-200
Gene	SgRNA	AA06-MCP001682-AD01-2-200A
Gene	SgRNA	AA06-MCP001682-AD01-2-200B
Gene	SgRNA	AA05-CCPCTROI-AD01-200
Gene	SgRNA	AA05-MCP001682-AD01-2-200-9
Gene	SgRNA	AA05-MCP001682-AD01-2-200-6
Isoflurane	VetOne	502017
Syringes & Needles	BD	As needed
Serum Separator Tubes (if needed)	Greiner Bio-One	#450472 (via Fisher)
Li Hep Mini-Collect (if needed)	Greiner Bio-One	#450480 (via Fisher)
EDTA Mini-Collect (if needed)	Greiner Bio-One	#450477 (via Fisher)
K3EDTA (if needed)	Covidien	#8881311149 (via Fisher)

[00553] *Test Article and Vehicle Information*

Grp	Cpmd	N	ml/animal	Days of Dosing	Doses Per Day	Compound Needed (ml)	25% Overage	TOTAL Needed (ml)
1	AAV-6 Scramble Vector	14	0.01	1	1	0.14	0.04	0.18
2	AAV-6 Guide 1+2	14	0.01	1	1	0.14	0.04	0.18
3	PBS	14	0.01	1	1	0.14	0.04	0.18
4	AAV-5 Scramble Vector	14	0.01	1	1	0.14	0.04	0.18
5	AAV-5 Guide 1+2	14	0.01	1	1	0.14	0.04	0.18

[00554] Unformulated Test Article Storage Conditions:

AAV-6 Scrambled Vector (Group 1): -80C
AAV-6 Guide 1+2 (Group 2): -80C
PBS (Group 3): 4C
AAV-5 Scrambled Vector (Group 4): -80C
AAV-5 Guide 1+2 (Group 5): -80C

[00555] Vehicle Information:

AAV-6 Scrambled Vector (Group 1): Sterile PBS (w/o Ca & Mg)
AAV-6 Guide 1+2 (Group 2): Sterile PBS (w/o Ca & Mg)
PBS (Group 3): Sterile PBS (w/o Ca & Mg)
AAV-5 Scrambled Vector (Group 4): Sterile PBS (w/o Ca & Mg)
AAV-5 Guide 1+2 (Group 5): Sterile PBS (w/o Ca & Mg)

[00556] Test Article Formulation Instructions & Calculations:

AAV-6 Scrambled Vector (Group 1): Dilute stock to appropriate concentration using sterile PBS (See AAV Preparation section)
AAV-6 Guide 1+2 (Group 2): Dilute stock to appropriate concentration using sterile PBS (See AAV Preparation section)
PBS (Group 3): Use sterile PBS (See AAV Preparation section)
AAV-5 Scrambled Vector (Group 4): Dilute stock to appropriate concentration using sterile PBS (See AAV Preparation section)
AAV-5 Guide 1+2 (Group 5): Dilute stock to appropriate concentration using sterile PBS (See AAV Preparation section)

[00557] Dosing Formulations and Vehicle Storage & Stability:

AAV-6 Scrambled Vector (Group 1): Dilute just prior to injecting
AAV-6 Guide 1+2 (Group 2): Dilute just prior to injecting
AAV-5 Scrambled Vector (Group 4): Dilute just prior to injecting
AAV-5 Guide 1+2 (Group 5): Dilute just prior to injecting

[00558] Disposition of Test Articles Following Dosing:

AAV-6 Scrambled Vector (Group 1): Discard formulations, retain stock solution at -80C for future studies
AAV-6 Guide 1+2 (Group 2): Discard formulations, retain stock solution at -80C for future studies
AAV-5 Scrambled Vector (Group 4): Discard formulations, retain stock solution at -80C for future studies
AAV-5 Guide 1+2 (Group 5): Discard formulations, retain stock solution at -80C for future studies

[00559] *Live Phase*

Live Phase Data Collection			
Type	Study Day	Grp (An)	Details
Body Weight	Day -1, 2, 6	All	
Knee Caliper	Day -1 (Baseline) Day 0 (6hr) Day 1 (24 hour)	All	Right Knee Change from baseline

	Day 7 (0hr, 6 hour)		
Von Frey	Day -1 (Baseline) Day 0 (6hr) Day 1 (24 hour) Day 7 (0hr, 6 hour)	All	Right foot only

[00560] *Necropsy Information*

[00561] The animals were necropsied after the final behavioral testing on study day 7 (approx. 6 h post-MSU). At necropsy, the animals were bled by cardiac puncture to exsanguination and euthanized by cervical dislocation for tissue collection. Whole blood was processed for serum (≥ 200 μ L/mouse), which was stored frozen at -80°C for shipment to the study sponsor. Right (injected) and left (normal) knees from all animals were collected (skin, muscle, and feet were removed while keeping the knee joint intact). The joints were flash-frozen straight in 15-mL conical tubes for shipment to the sponsor.

<i>Sacrifice Schedule:</i>	Study Day 7 (6 hours post MSU): ALL ANIMALS
<i>Method of Euthanasia:</i>	Bled by cardiac puncture to exsanguinate followed by cervical dislocation.
<i>Time Points:</i>	After final behavior time point (6 hours post MSU)

Necropsy Sample Collection:						
Type	Process For	Anti-Coag	Gr/An	Final Volume	Storage Condition	Disposition
Cardiac Puncture Whole Blood	Serum	N/A	All	$\geq 200\mu\text{l}$	-80C, Epps	Ship to Sponsor

Necropsy Tissue Sample Collection:				
Type	Gr/An	Details	Storage Condition	Disposition
Right (Injected) Knee	All	Remove skin & muscle keeping knee joint intact, remove foot	Flash freeze straight, place in 15ml conical	Ship to Sponsor
Left (Normal) Knee	All	Remove skin & muscle keeping	Flash freeze straight, place in 15ml conical	Ship to Sponsor

		knee joint intact, remove foot		
--	--	-----------------------------------	--	--

[00562] *Statistical Analysis*

[00563] Data were entered into Microsoft Excel and means and standard errors (SE) for each group were determined. The groups were compared using a one-way analysis of variance (ANOVA) or a repeated measures (RM) ANOVA with a Tukey's post-hoc analysis. ANOVA were performed using Prism v8.0.2 software (GraphPad). Unless indicated, BBP performs statistical analysis on raw (untransformed) data only. Statistical tests make certain assumptions regarding the data's normality and homogeneity of variance, and further analysis may be required if testing resulted in violations of these assumptions. Significance for all tests was set at $p < 0.050$ with p values rounded to the third decimal place.

[00564] *AAV Preparation*

[00565] Standard Operating Procedure for Preparing Virus for Injection

[00566] Create a working stock that contains 5×10^{11} vg per ml for each construct (Guide 1, Guide 2). Note that for equivalence, the scramble control group needs to be injected with a total of 1×10^{10} copies of the scrambled vector. Diluent (PBS) will be used in the vehicle control group.

[00567] Materials

AA06-CCPCTR01-AD01-200: Scramble control AAV-6 particles, 100 microliter solution containing 5×10^{12} vg per ml.
AA06-MCP001682-AD01-2-200-Guide 1: AAVPrime™ particles, AAV-6 containing Guide 1, 100 microliter solution containing 5×10^{12} vg per ml.
AA06-MCP001682-AD01-2-200-Guide 2: AAVPrime™ particles, AAV-6 containing Guide 2, 100 microliter solution containing 5×10^{12} vg per ml.
AA05-CCPCTR01-AD01-200: Scramble control AAV-5 particles, 100 microliter solution containing $>5 \times 10^{12}$ vg per ml.
AA05-MCP001682-AD01-2-200-Guide 1: AAVPrime™ particles, AAV-5 containing Guide 1, 100 microliter solution containing $>5 \times 10^{12}$ vg per ml.
AA05-MCP001682-AD01-2-200-Guide 2: AAVPrime™ particles, AAV-5 containing Guide 2, 100 microliter solution containing $>5 \times 10^{12}$ vg per ml.

[00568] Procedure

[00569] Group 1: AAV-6 Scramble Control - Aliquot 400 microliters of sterile-filtered Ca- and Mg-free PBS into a sterile Eppendorf tube. Add 100 microliters (equivalent to 5×10^{11} vg) of the stock AA06-CCPCTR01-AD01-200. This results in a 0.5 ml volume of working stock containing 1×10^{12} vg/ml of the AAV-5 scramble control. Injection of 10 microliters of this solution into the knee joint delivers 1×10^{10} vg of the AAV-6 scramble control.

[00570] Group 2: Active AAV-6 Guide 1+2 - Aliquot 800 microliters of sterile-filtered Ca- and Mg-free PBS into a sterile Eppendorf tube. Add 100 microliters (equivalent to 5×10^{11} vg) of each of the two active AAV-6 constructs – this means 100 microliters (equivalent to 5×10^{11} vg) of AA06-MCP001682-AD01-2-200-a and 100 microliters (equivalent to 5×10^{11} vg) of the stock AA06-MCP001682-AD01-2-200-b. This results in a 1 ml volume of working stock containing 5×10^{11} vg/ml for each of the two guides. Injection of 10 microliters of this solution into the knee joint delivers 5×10^9 vg for each of the two AAV-6 guides.

[00571] Group 3: PBS - The sterile-filtered Ca- and Mg-free PBS used to dilute the virus stock served as the vehicle control for this study. It was dosed at 10 microliters per knee joint.

[00572] Group 4: AAV-5 Scramble Control - Aliquot 400 microliters of sterile-filtered Ca- and Mg-free PBS into a sterile Eppendorf tube. Add 100 microliters (equivalent to 5×10^{11} vg) of the stock AA05-CCPCTR01-AD01-200. This results in a 0.5 ml volume of working stock containing 1×10^{12} vg/ml of the AAV-5 scramble control. Injection of 10 microliters of this solution into the knee joint delivered 1×10^{10} vg of the AAV-5 scramble control.

[00573] Group 5: Active AAV-6 Guide 1+2 - Aliquot 800 microliters of sterile-filtered Ca- and Mg-free PBS into a sterile Eppendorf tube. Add 100 microliters (equivalent to 5×10^{11} vg) of each of the two active AAV-6 constructs – this means 100 microliters (equivalent to 5×10^{11} vg) of AA05-MCP001682-AD01-2-200-a and 100 microliters (equivalent to 5×10^{11} vg) of the stock AA05-MCP001682-AD01-2-200-b. This results in a 1 ml volume of working stock containing 5×10^{11} vg/ml for each of the two guides. Injection of 10 microliters of this solution into the knee joint delivered 5×10^9 vg for each of the two AAV-6 guides.

[00574] *qPCR*

[00575] Snap-frozen synovial tissues resected en bloc (including distal femur and proximal tibia) and placed in RLT buffer. Homogenised using Cyrolys Evolution tissue homogenizer (“HARD” programme cycle). RNA extracted using RNeasy or RNeasy Plus kits followed by QIAshredder (from QIAGEN). RNA quantified using Nanonstring. cDNA reverse transcribed and qPCR performed using mouse-specific primers for IL-1 beta, beta-actin and RPL13.

[00576] *Results*

[00577] As shown in Fig. 6, PBS control mice had mean body weight gain of 4.3% (1.1 g) over the course of the study. Body weight gain did not differ statistically across groups (Table 7, Table 9).

[00578] As shown in Fig. 7A, knee caliper measurements in all groups peaked 6 hours post-dose on study day 0, returned to baseline by 24 hours post-dose, and then peaked again 6 hours post-MSU on study day 7. Knee caliper change from baseline did not differ statistically across groups over time (Table 11). As shown in Fig. 7B, knee caliper change AUC for days -1 through 7 did not differ statistically across groups (Table 7, Table 11).

[00579] As shown in Fig. 8A, von Frey absolute thresholds decreased slightly in all groups following IA dosing on study day 0 and then trended toward baseline on study day 7 before decreasing sharply following IA injection of MSU. There were no statistical differences over time between the AAV scramble vector control groups and the PBS control group. Mice treated with AAV-6 (Guide 1 + 2) had von Frey absolute thresholds that were increased 6 hours post-MSU on day 7 as compared to the control groups; the increase in von Frey absolute thresholds at this time point nearly reached statistical significance as compared to the AAV-6 scramble vector and PBS control groups ($p = 0.051$ and $p = 0.075$, respectively) and was statistically significant as compared to the AAV-5 scramble vector control group ($p = 0.025$). Mice treated with AAV-5 (Guide 1 + 2) had von Frey absolute thresholds that did not differ statistically from the control groups over time (Table 10). As shown in Fig. 8B, von Frey absolute threshold AUC for days -1 through 7 did not differ statistically across groups (Table 7, Table 10).

[00580] As shown in Fig. 10, immunohistochemistry data for murine IL-1 β in synovial tissue showed reduced IL-1 β expression in CRISPR-treated animals. (A) In MSU injected

animals pre-treated with PBS there is robust expression of IL-1 β (brown staining). This effect is not seen in CRISPR-treated animals (panel C). The absence of IL-1 β (brown staining) in CRISPR-treated animals resembles negative antibody controls (panels B & D). All images are 10x original magnification.

[00581] *Discussion & Conclusions*

[00582] Mice treated IA (1x on day 0) with AAV-6 (Guide 1 + 2: 5×10^9 vg/guide per knee) showed a statistically significant reduction in referred pain, as measured by von Frey testing, 6 hours after MSU injection on day 7 as compared to mice injected IA with AAV-5 scramble vector ($p = 0.025$) with results being nearly significant as compared to the AAV-6 scramble vector and PBS control groups ($p = 0.051$ and $p = 0.075$, respectively). AUC calculations for von Frey assessments did not differ statistically across groups. Animal body weight gain and knee swelling did not differ statistically across groups. All animals survived to study termination.

EXAMPLE 6. Guide RNA Design

[00583] Guide RNAs targeting human IL-1 α and IL-1 β (Table 14) were designed according to the following procedure:

1. Identify appropriate genome assembly and gene model (Tools: Ensemble, UCSC Genome Browser);
2. Identify key functional domains to map out targeting window (Tools: Ensemble; Literature);
3. Generate list of all possible guide RNAs across key exons (Tools: Ensemble, UCSC, InDelphi);
4. Rank guides based on ML-predicted frameshifting score and exclude poor performers;
5. Exclude guides <5bp from intron:exon boundaries and with homopolynucleotide tracts of 6 x T's or greater;
6. Determine on-target (Doench 2016) and off-target (Hsu 2013) metrics for each guide (Tools: UCSC, Deskgen);

7. Filter out guides with poor on- and off-target scores to generate final list; and
8. Rank based on frameshift index.

[00584] Guide RNAs targeting cat, dog, or horse IL-1 α and IL-1 β (Table 15) were designed according to the following procedure:

1. Identify appropriate genome assembly and gene model (Tools: Ensemble, UCSC Genome Browser)
2. Identify key functional domains to map out targeting window (Tools: Ensemble; Literature)
3. Retrieve coding sequence from appropriate exons and relevant flanking intronic sequences (Tools: Ensemble, APE)
4. Generate list of all possible guide RNAs across key exons (Tools: Ensemble, InDelphi)
5. Rank guides based on ML-predicted frameshifting score and exclude poor performers
6. Exclude guides <5bp from intron:exon boundaries and with homopolynucleotide tracts of 6 x T's or greater
7. Determine off-target metrics for each guide (Tools: Cas Off-Finder, Excel)
8. Filter out guides with poor off-target scores to generate final list
9. Rank based on frameshift index.

Gene transcript information for all species considered are included in Table 16.

Table 14

Gene symbol	SEQ ID NO:	Species	Chromosome	Genome	Frameshift (%)	Doench 2016	Hsu 2013	gRNA	PAM
IL1B	627	Human	chr2	hg38	80.1	55	76	AGCTGGATGCCGCCATCCAG	AGG
IL1B	628	Human	chr2	hg38	78.5	44	71	ACCACTACAGCAAGGGCTTC	AGG
IL1B	629	Human	chr2	hg38	75.4	63	92	CATGGCCACAACAACGTGACG	CGG
IL1B	630	Human	chr2	hg38	74	60	97	GGTGGTCGGAGATTCGTAGC	TGG
IL1B	631	Human	chr2	hg38	87.6	55	61	CTACAGCAAGGGCTTCAGGC	AGG
IL1B	632	Human	chr2	hg38	85.6	61	58	GACCTCTGCCCCCTCTGGATGG	CGG
IL1B	633	Human	chr2	hg38	85.2	35	62	CTCTCCGCAAGTGCTCCTTCC	AGG
IL1B	634	Human	chr2	hg38	78.5	50	62	CATTCTCCTGGAAGGTCTGT	GGG
IL1A	635	Human	chr2	hg38	92.1	70	87	ATGGTGGTAGTAGCAACCAA	CGG
IL1A	636	Human	chr2	hg38	88.3	51	88	GCCATAGCTTACATGATAGA	AGG
IL1A	637	Human	chr2	hg38	87	68	88	CAGAGACAGATGATCAATGG	AGG
IL1A	638	Human	chr2	hg38	87	50	74	ATTCAGAGACAGATGATCAA	TGG
IL1A	639	Human	chr2	hg38	83.1	53	91	TGGTGGTAGTAGCAACCAAC	GGG
IL1A	640	Human	chr2	hg38	85.7	60	67	CTCCAGGTCATCATCAGTGA	TGG

Table 15

Gene symbol	SEQ ID NO:	Species	Chromosome	Exon	Genome	Frameshift (%)	CasOffFinder total	gRNA	PAM
IL1A	641	Horse	chr15	4	EquCab3.0	80.9	21	TGGTGTGGTGGCAGCCAAC	GGG
IL1A	642	Horse	chr15	4	EquCab3.0	79.2	14	CTTCTTCAGAGTCTTCCCGT	TGG
IL1A	643	Horse	chr15	3	EquCab3.0	74.9	37	TTACCTGAGTCAGAGAGAGA	TGG
IL1A	644	Horse	chr15	4	EquCab3.0	74.6	11	AAGCTATGACCCACTTCCTG	AGG
IL1A	645	Horse	chr15	4	EquCab3.0	71.5	14	CCATGCAGTCTCAGGAAAGT	GGG
IL1A	646	Dog	chr17	3	canFam4	75.7	7	ACATGCAGTCTCTCATGAAGT	GGG
IL1A	647	Dog	chr17	3	canFam4	73.8	10	GACATCCCAGCTTACCTTCA	AGG
IL1A	648	Dog	chr17	3	canFam4	72.6	10	GAGCTGTGACCCACTTCATG	AGG
IL1A	649	Dog	chr17	3	canFam4	79.2	14	CATTTTCCTTGAAGGTAAGC	TGG
IL1A	650	Dog	chr17	3	canFam4	87	16	TGGTAGTGGTGGCAGCCAAT	GGG
IL1A	651	Dog	chr17	3	canFam4	76.8	18	ATTTTCCTTGAAGGTAAGCT	GGG
IL1A	652	Dog	chr17	3	canFam4	79.1	23	CTTCTTTAGAAATCTTCCCAT	TGG
IL1A	653	Dog	chr17	3	canFam4	71.4	23	GGATGTCTTTGAGATTTCAG	AGG
IL1A	654	Dog	chr17	3	canFam4	88.5	28	GTGGTAGTGGTGGCAGCCAA	TGG
IL1A	655	Dog	chr17	3	canFam4	78.7	31	CACACACATTTTCCCTTGA	AGG
IL1A	656	Cat	chrA3	3	Fe9.0	73.3	12	GGTAAGCTGGGTGTCTTAG	AGG
IL1A	657	Cat	chrA3	3	Fe9.0	74.3	13	ATTCCTCACTGCTGATGACC	TGG
IL1A	658	Cat	chrA3	3	Fe9.0	86.7	15	TGGTGATGGTGGCAGCCAAT	GGG
IL1A	659	Cat	chrA3	3	Fe9.0	79.3	20	CTTCCAGGTCTATCAGCAGTG	AGG
IL1B	660	Horse	chr15	4	EquCab3.0	87.6	27	TGAAAGTCTTGTGTAAAAGT	TGG
IL1B	661	Horse	chr15	4	EquCab3.0	79.6	15	GACCTCAGCTCCATGGGCGA	TGG
IL1B	662	Horse	chr15	4	EquCab3.0	78.5	13	CTGGATGCCCCCATCGCCCA	TGG

Gene symbol	SEQ ID NO:	Species	Chromosome	Exon	Genome	Frameshift (%)	CasOffFinder total	gRNA	PAM
IL1B	663	Horse	chr15	4	EquCab3.0	75.4	17	CCCCATCGCCCATGGAGCTG	AGG
IL1B	664	Horse	chr15	4	EquCab3.0	75.2	12	AAGTCTTGTGTAAAGTTGG	TGG
IL1B	665	Dog	chr17	3	canFam4	82.1	2	CGTGTCACTCATTGTAGCTT	TGG
IL1B	666	Dog	chr17	3	canFam4	74.2	11	CCACAGTTCTCTGGTAGATG	AGG
IL1B	667	Dog	chr17	3	canFam4	80	13	AGACCTGAACACAGTTCTC	TGG
IL1B	668	Dog	chr17	3	canFam4	80	14	CCTCATCTACCCAGAGAACTG	TGG
IL1B	669	Dog	chr17	3	canFam4	80	15	TCAGACTCTTGTACAGAGC	TGG
IL1B	670	Cat	chrA3	2	Fe9.0	82.4	8	GTAGTAAGCCATCATTTCAC	TGG
IL1B	671	Cat	chrA3	4	Fe9.0	82	4	GAGTCTTAGGCATGCCGTGT	CGG
IL1B	672	Cat	chrA3	4	Fe9.0	81.8	14	AACTTGAGCCACAGTTTTC	TGG
IL1B	673	Cat	chrA3	2	Fe9.0	80.7	17	ATCATTTCACCTGGTGAGTTC	AGG
IL1B	674	Cat	chrA3	4	Fe9.0	80.4	18	ACTCTTGTTCGGGCTGGT	GGG
IL1B	675	Cat	chrA3	4	Fe9.0	80.1	14	GACTCTTGTTCGGGCTGG	TGG
IL1B	676	Cat	chrA3	4	Fe9.0	76.7	24	CCTCATCTCCACAGAAACTG	TGG
IL1B	677	Cat	chrA3	3	Fe9.0	76	23	TGAGAAATGACCTGTTCTTTG	AGG
IL1B	678	Cat	chrA3	4	Fe9.0	75.7	5	TAAGACTCTTGTTCGGGC	TGG
IL1B	679	Cat	chrA3	4	Fe9.0	74.3	18	AACCTGAGCCACAGTTTCT	GGG

Table 16

Gene	Species	Ensemble	Transcript	Transcript ID	Location
IL1A	Human	ENSG00000115008	IL1A-201	ENST00000263339.4	<u>Chromosome 2: 112,773,925-112,784,493</u>
IL1A	Mouse	ENSMUSG000000027399	Il1a-201	ENSMUST000000028882.1	<u>Chromosome 2: 129,299,610-129,309,972</u>
IL1A	Cat	ENSFCAG000000008095	IL1A-201	ENSFCAT000000008097.5	<u>Chromosome A3: 105,113,193-105,121,209</u>
IL1A	Dog	ENSCAFG000000007245	IL1A-201	ENSCAFT0000000045928.3	<u>Chromosome 17: 36,970,573-36,978,736</u>
IL1A	Horse	ENSECAG000000023727	IL1A-202	ENSECAT000000025409.2	<u>Primary_assembly 15: 16,360,700-16,369,812</u>
IL1B	Human	ENSG00000125538	IL1B-201	ENST00000263341.7	<u>Chromosome 2: 112,829,751-112,836,779</u>
IL1B	Mouse	ENSMUSG000000027398	Il1b-201	ENSMUST000000028881.13	<u>Chromosome 2: 129,364,570-129,371,139</u>
IL1B	Cat	ENSFCAG000000005899	IL1B-201	ENSFCAT000000005904.5	<u>Chromosome A3: 105,049,334-105,056,407</u>
IL1B	Dog	ENSCAFG000000007249	IL1B-201	ENSCAFT000000011613.4	<u>Chromosome 17: 37,019,329-37,024,176</u>
IL1B	Horse	ENSECAG000000000051	IL1B-202	ENSECAT000000061518.2	<u>Primary_assembly 15: 16,418,738-16,444,697</u>

EXAMPLE 7. Immunohistochemistry for Murine IL-1 β

[00585] Immunohistochemistry was performed on Murine synovial tissue to detect IL-1 β according to the following protocol:

[00586] *Reagents & Preparation*

1. 10X PBS with 0.5% (v/v) Tween-20
2. Sterile PBS
3. IHC buffer
4. Primary antibody (goat anti-mouse IL-1; AF-401-NA, R&D Systems, Inc.)
reconstituted to a final concentration of 0.2 mg/ml in sterile PBS.
 - a. Short-term storage at +4C
 - b. Long-term storage at -20 to -70C
5. Reconstitute the control antibody (normal goat IgG; AB-108-C, R&D Systems Inc.) at a final concentration of 1 mg/ml in sterile PBS.
 - a. Short-term storage at +4C
 - b. Long-term storage at -20 to -70C
6. Secondary antibody (HRP conjugated donkey anti-goat IgG; ab6885; Abcam)
comes as reconstituted product
 - a. Short-term storage at +4C
 - b. Long-term storage at -20 to -70C
7. Peroxidase block (BLOXALL reagent, Vector Laboratories)
8. Normal horse serum, diluted to 2.5% (v/v) (Impress Polymer Kit; Vector Laboratories)
9. DAB chromogen

[00587] *Method*

[00588] Antigen retrieval was performed for 1 hour (manual or automated, user preference), and then samples were transferred to PBS for short-term storage. Peroxidase block was performed for 10 minutes at room temperature, and samples were subsequently washed in IHC buffer for 5 mins. Samples were blocked with control horse serum for 60 minutes at room temperature, and exposed to primary antibody (1:100 or 1:200 diluted in 1 x PBS-Tween) for 2 hours at room temperature. Samples were washed in IHC buffer twice for 5 mins (each wash). Samples were then exposed to secondary antibody (1:500 diluted in 1 x PBS-Tween) for 1 hour, and washed in IHC buffer twice for 5 mins (each wash). Detection was performed with DAB chromogen for 30 seconds. Counterstaining was performed with

Mayer's haematoxylin (6 minutes), and samples were returned through graded alcohol series to xylene. DPX mountant was applied, and a coverslip was attached.

[00589] As shown in Fig. 10, immunohistochemistry data for murine IL-1 β in synovial tissue showed reduced IL-1 β expression in CRISPR-treated animals. (A) In MSU injected animals pre-treated with PBS there is robust expression of IL-1 β (brown staining). This effect is not seen in CRISPR-treated animals (panel C). The absence of IL-1 β (brown staining) in CRISPR-treated animals resembles negative antibody controls (panels B & D). All images are 10x original magnification. (A) and (B) are adjacent sections taken from the same joint in the same animal, with (A) showing tissue labeled specifically for IL-1 beta, and (B) showing tissue labeled with the negative (isotope) control antibody. Differences in staining reflect demonstrable IL-1 beta expression in MSU injected animals pre-treated with PBS in this animal (e.g., a positive control animal pre-treated with PBS, then challenged with MSU crystal). (C) and (D) are similarly adjacent sections, but from an animal that was pre-treated with the CRISPR editing virus prior to MSU injection. (C) There is no obvious staining for IL-1 beta in the section treated with IL-1 antibody, and (D) the same negative pattern is seen in the section treated with the negative (isotope) control antibody. Without wishing to be bound by any particular theory, this confirms that there is no detectable IL-1 beta expression in the synovium of CRISPR-treated animals.

[00590] EXAMPLE 8. Design and validation of CRISPR/Cas9 RNA guides against canine and human interleukin-1 alpha (IL-1 α) and interleukin-1 beta (IL-1 β).

[00591] Potential crRNA sequences were identified for various exons of the human and canine interleukin-1 alpha (IL-1 α) and interleukin-1 beta (IL-1 β) genes. Figures 13A-13D show a ranked list of crRNA sequences identified from exons 2-7 of the human IL-1 α gene. Figures 14A-14E show a ranked list of crRNA sequences identified from exons 2-7 of the human IL-1 β gene. Figures 15A-15C show crRNA sequences identified from exons 3-5 of the canine IL-1 β gene. Figures 16A-16B show a crRNA sequences identified from exons 3-5 of the canine IL-1 α gene.

[00592] Publicly accessible genomes (human, hg38; dog, CanFam3.1), collapsed gene models (merged Ensembl/Havana), tissue-specific exon expression (gtexportal.org) and various gRNA models were then used to select two to five individual crRNA sequences per

gene, targeting canine and human interleukin-1 alpha (IL-1 α) and interleukin-1 beta (IL-1 β).

The following gRNA design rules were applied:

1. The gRNA target region was limited to the first 5-50% of the coding sequence (CDS).
2. Single gRNAs were ranked according to maximal on-target editing using Azimuth 2.0 model (10.1038/nbt.3437) and minimal off-target editing using Cutting Frequencing Determination (CFD) (10.1038/nbt.3437) and the specificity score from Hsu et al. (10.1038/nbt.2647).
3. Highly ranked sgRNA with high frameshift frequencies (>75%) and uniform DNA repair outcomes (>0.48) as predicted by inDelphi (10.1038/s41586-018-0686-x) were selected for in vitro synthesis.

[00593] Using this selection criteria, crRNA guide sequences targeting different exons of the respective target genes were selected for further investigation. Specifically, as shown in Figure 17A, sg235 (SEQ ID NO:301) and sg236 (SEQ ID NO:309) target exons 3 and 4 of the human IL-1 α gene were selected. Likewise, as shown in Figure 17B, sg237 (SEQ ID NO:462), sg238 (SEQ ID NO:391), sg248 (SEQ ID NO:393), sg249 (SEQ ID NO:388), and sg250 (SEQ ID NO:389) targeting exons 3, 4, and 5 of the human IL-1 β gene were selected. As shown in Figure 17C, sg239 (SEQ ID NO:552), sg240 (SEQ ID NO:554), sg251 (SEQ ID NO:578), and sg252 (SEQ ID NO:579) targeting exons 3, 4, and 5 of the canine IL-1 α gene were selected. Likewise, as shown in Figure 17D, sg241 (SEQ ID NO:498) and sg242 (SEQ ID NO:506) targeting exons 3 and 4 of the canine IL-1 β gene were selected.

[00594] Single guide RNAs (sgRNAs), fusing the selected crRNA guide sequences to a scaffold sequence were then synthesised (Synthego) with scaffold modifications designed to increase their stability and decrease their cellular immunogenicity. Primers for genotyping were designed to be at least 200 bp from the target site and generate PCR amplicons <1.5kb and synthesized (Merck).

[00595] The following quantities were used for single electroporation-based transfection using the 4D-nucleofector (Lonza, Catalog AAF-1002B and AAF-1002X) and nucleocuvette strips. 80 pmol synthesised sgRNA were pre-complexed with 4 μ g Cas9 nuclease at room temperature for at least 10 min. 300-400K dissociated cells were washed with PBS before resuspending them in 20 μ l supplemented P3 nucleofection solution and adding the Cas9

RNP complex. These cells were then transferred into a nucleocuvette well and electroporated using the pulse code ER-100. Directly after electroporation, the nucleocuvette was placed into the 37°C / 5% CO₂ incubator for 10 min for the cells to recover from the electrical voltage. Afterwards, 80 µl growth medium was added to the nucleocuvette well and cells transferred into 6-well dishes with prewarmed growth medium.

[00596] Between two- and eleven-days post-electroporation, genomic DNA was extracted from 50-200K cells using DNeasy Blood & Tissue kit (Qiagen, Catalog 69506). Single gRNA target (and off-target) regions were amplified by PCR.

[00597] PCR products were size-verified by gel electrophoresis, purified using QIAquick PCR purification kit (Qiagen, Catalog 28106) and submitted for Sanger sequencing at Source BioScience. Sanger traces (ab1) were deconvoluted using ICE version 1.2 (found online at the URL github.com/synthego-open/ice) to infer CRISPR edits. In addition, machine-learning predictions of gene editing using the selected probes was generated using inDelphi. In addition, the predicted off-target sites were analysed through direct sequencing to verify whether gRNA facilitates off-target editing.

[00598] Results of the empirical experiments and machine-learning prediction of gene editing using the selected guide sequences is shown in Figure 17.

[00599] EXAMPLE 9. - Effect of selected CRISPR/Cas9 RNA guides on canine and human interleukin-1 alpha (IL-1 α) and interleukin-1 beta (IL-1 β) release.

[00600] The gRNA with the highest knockout (KO) scores from Example 8 (i.e., the highest frameshift frequency) were used to generate double IL-1 α /IL-1 β knock out (KO) cells. Specifically, human chondrocytes were edited to achieve >99% IL-1 α KO using crRNA sequence CAGAGACAGAUGAUCAAUGG (SEQ ID NO:301) and 67% IL-1 β KO using crRNA sequence GUGCAGUUCAGUGAUCGUAC (SEQ ID NO:389). Canine chondrocytes were edited to achieve 97% IL-1 α KO using crRNA sequence GACAUCCCAGCUUACCUUCA (SEQ ID NO:554) and 99% IL-1 β KO using crRNA sequence ACUCUUGUUACAGAGCUGGU (SEQ ID NO:506).

[00601] Canine chondrocytes (Catalog Cn402K-05), human chondrocytes (Catalog 402-05a) and human fibroblast-like synovial cells (Catalog 408-05a) were purchased as frozen stocks (5 x 10⁵ cells) from Cell Applications, Inc., San Diego, CA. Chondrocytes were cultured in growth medium consisting of DMEM/Ham's F12 (Gibco, Catalog 21331-020) supplemented

with 20% (v/v) untreated FBS (Gibco, Catalog 10270-106) and 1x GlutaMAX (Gibco, Catalog 35050-038). Synovial cells were cultured in growth medium consisting of DMEM (Gibco, Catalog 11960-044), 10% non-treated FBS (Gibco, Catalog 10270-106) and 1x GlutaMAX (Gibco, Catalog 35050-038). Cells were confirmed as being negative for Mycoplasma spp. and subjected to STR profiling prior to use. For electroporation and subculture, cells were dissociated using 0.25% trypsin (Gibco, Catalog 25200056). Trypsin was quenched with 9 volumes of growth medium and cells were spun at 1,000g to remove the supernatant.

[00602] Induction of IL-1 by LPS. Interleukin-1 release was induced by challenging sub-confluent monolayers of cells (edited or wild-type non-edited) with lipopolysaccharide (LPS). In brief, non-edited (control) and double IL-1 α /IL-1 β KO (edited) human or canine chondrocytes were seeded at density of approximately 5×10^4 cells per well in 24-well plates. After 24-48 hours, the medium was replaced with fresh, serum-free medium containing either LPS (50 μ g/ml) or PBS vehicle and the plates returned to the incubator. Plates were harvested after 6 and 24 hours for the determination of IL-1 release. Media were snap-frozen in liquid nitrogen and stored at -20°C until they were assayed.

[00603] Measurement of IL-1 alpha and IL-1 beta release. The concentration of IL-1 alpha and IL-1 beta in culture medium was measured with species-specific commercial assays, following the manufacturer's instructions. Prior to measurement, frozen media were thawed and then centrifuged (1,500 g for 2 mins) in order to remove cellular debris. Aliquots of medium were measured in duplicate and the concentration of IL-1 determined from a standard curve of recombinant human or canine IL-1 alpha or beta, as appropriate. The results of IL-1 alpha release in canine cells are shown in Figures 18A (6 hours) and 18B (24 hours). The results of IL-1 beta release in canine cells are shown in Figures 18C (6 hours) and 18D (24 hours). The results of IL-1 alpha release in human cells are shown in Figures 19A (6 hours) and 19B (24 hours). The results of IL-1 beta release in human cells are shown in Figures 19C (6 hours) and 19D (24 hours).

[00604] P3 primary cell nucleofection reagents and nucleocuvette strips (Catalog V4XP-3032) were purchased from Lonza (Slough, UK). Cas9 nuclease (Catalog A36499) was purchased from Thermo Fisher Scientific. Lipopolysaccharide (LPS) from E. coli O55:B5 (Catalog L6529) was purchased from Merck. ELISA kits for human IL-1 alpha (Catalog

ab214025) and human IL-1 beta (Catalog ab100560), canine IL-1 alpha (Catalog A4270) and canine IL-1 beta (Catalog ab273170) were purchased from Abcam (Cambridge, UK).

[00605] EXAMPLE 10. Increased specificity of CRISPR/Cas9 mediated gene editing.

[00606] The analysis of gene editing specificity reported in Example 8 was repeated using an enhanced Specificity CRISPR associated protein 9. The eSpCas9 includes three specificity enhancing mutations: K848A, K1003A, and R1060A, as described in Slaymaker et al., Science, 351:84-88 (2016). The eSpCas9 was expressed in *E. coli* and purified to homogeneity. The construct has a molecular weight of 161 kDa and contains N-terminal Flag-tags and a C-terminal hexa-His-tag. The sequence of the eSpCas9 is:

MDYKDHGDYKDHDIDYKDDDDKMAPKKRKVGIIHGVPAAADKKYSIGLDIGTNSV
 GWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT
 RRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEK
 YPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLV
 QTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGL
 TPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDI
 LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLEPKYKEIFFDQSKNGYAGYI
 DGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
 LRRQEDFYFPFLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEETITPWNFEE
 VVDKGASASQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRK
 PAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTY
 HDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLK
 RRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQK
 AQVSGQGDSLHEHIANLAGSPAIIKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 QTTQKGQKNSRERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
 VDQELDINRLSDYDVDHIVPQSFLADDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMK
 NYWRQLLNAKLITQRKFDNLTKAERGGSELKAGFIKRQLVETRQITKHVAQILDS
 RMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAV
 GTALIKKYPALSEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEIT
 LANGEIRKAPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKKEVQTGGFSKE
 SILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELL

GITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQK
 GNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRV
 ILADANLDKVL SAYNKH RD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS
 TKEVL DATLIHQ SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKAAALEHHHHH
 H (SEQ ID NO:680).

[00607] Briefly, the same sgRNAs used in Example 8, and shown in Figure 17, were complexed with the eSpCas9. As shown in Figure 17A, sg235 (SEQ ID NO:301) and sg236 (SEQ ID NO:309) target exons 3 and 4 of the human IL-1 α gene were used. Likewise, as shown in Figure 17B, sg237 (SEQ ID NO:462), sg238 (SEQ ID NO:391), sg248 (SEQ ID NO:393), sg249 (SEQ ID NO:388), and sg250 (SEQ ID NO:389) targeting exons 3, 4, and 5 of the human IL-1 β gene were used. As shown in Figure 17C, sg239 (SEQ ID NO:552), sg240 (SEQ ID NO:554), sg251 (SEQ ID NO:578), and sg252 (SEQ ID NO:579) targeting exons 3, 4, and 5 of the canine IL-1 α gene were used. Likewise, as shown in Figure 17D, sg241 (SEQ ID NO:498) and sg242 (SEQ ID NO:506) targeting exons 3 and 4 of the canine IL-1 β gene were used.

[00608] Single guide RNAs (sgRNAs), fusing the selected crRNA guide sequences to a scaffold sequence were then synthesised (Synthego) with scaffold modifications designed to increase their stability and decrease their cellular immunogenicity. Primers for genotyping were designed to be at least 200 bp from the target site and generate PCR amplicons <1.5kb and synthesized (Merck).

[00609] The following quantities were used for single electroporation-based transfection using the 4D-nucleofector (Lonza, Catalog AAF-1002B and AAF-1002X) and nucleocuvette strips. 80 pmol synthesised sgRNA were pre-complexed with eSpCas9 nuclease at room temperature for at least 10 min. 300-400K dissociated cells were washed with PBS before resuspending them in 20 μ l supplemented P3 nucleofection solution and adding the Cas9 RNP complex. These cells were then transferred into a nucleocuvette well and electroporated using the pulse code ER-100. Directly after electroporation, the nucleocuvette was placed into the 37°C / 5% CO₂ incubator for 10 min for the cells to recover from the electrical voltage. Afterwards, 80 μ l growth medium was added to the nucleocuvette well and cells transferred into 6-well dishes with prewarmed growth medium.

[00610] Between two- and eleven-days post-electroporation, genomic DNA was extracted from 50-200K cells using DNeasy Blood & Tissue kit (Qiagen, Catalog 69506). Single gRNA target (and off-target) regions were amplified by PCR.

[00611] PCR products were size-verified by gel electrophoresis, purified using QIAquick PCR purification kit (Qiagen, Catalog 28106) and submitted for Sanger sequencing at Source BioScience. Sanger traces (ab1) were deconvoluted using ICE version 1.2 (found online at the URL github.com/synthego-open/ice) to infer CRISPR edits. In addition, machine-learning predictions of gene editing using the selected probes was generated using inDelphi. In addition, the predicted off-target sites were analysed through direct sequencing to verify whether gRNA facilitates off-target editing.

[00612] As compared to the Cas9 editing reported in Example 8, use of eSpCas9 reduces off-target editing without losing on-target activity. For example, the off-target editing by sgRNA #242 (targeting canine IL-1B) of three loci, having 2, 3, and 3 mismatches, respectively, were evaluated by amplifying and then sequencing the loci reported in Table 18. As shown in Table 18, the first off-target loci experienced no editing in the experiment described in Example 8, and was not tested here. The second off-target loci experienced almost complete off-target editing (98-99%) in the experiment described in Example 8, but experienced no editing when eSpCas9 was used. The third off-target loci experienced some editing (0-25%) in the experiment described in Example 8, but again experienced no editing when eSpCas9 was used. Further, as shown in Table 17, the “enhanced on-target score,” corresponding to editing using eSpCas9 as described in this example, for each sgRNA tested was as high, if not higher, than the “on-target score,” corresponding to the editing described in Example 8.

Table 17

#	Target		On-target metrics		Precision score**	Frameshift %***	Enhanced on-target score****	Off-target metrics	
	Exon #	Strand	On-target score*					Off-target score^	
sg235	3	-	69.5		0.57	93.7	72.4	67	
sg236	4	-	53.9		0.65	93.9	66.7	76.9	
Human IL1B (IL1B-201; GRCh38)									
#	Target		On-target metrics		Precision score**	Frameshift %***	Enhanced on-target score****	Off-target metrics	
	Exon #	Strand	On-target score*					Off-target score^	
sg237	3	+	60.9		0.57	80	64.7	66.7	
sg238	4	-	58.3		0.6	86.3	65.7	49.3	
sg248	5	+	61.7		0.65	89.9	69.6	86.5	
sg249	5	+	68.2		0.52	75.3	65.9	93.8	
sg250	5	-	64.4		0.48	83.9	65.2	91	
Canine IL1A (IL1A-201; CanFam3.1)									
#	Target		On-target metrics		Precision score**	Frameshift %***	Enhanced on-target score****	Off-target metrics	
	Exon #	Strand	On-target score*					Off-target score^	
sg239	3	+	49.5		0.55	78.3	58.1	57.7	
sg240	4	+	43.8		0.61	87.2	59.0	41.8	
sg251	5	+	72.1		0.49	77.6	67.7	85.1	
sg252	5	-	68.4		0.48	90.4	68.8	66.6	

Canine IL1B (IL1B-201; CanFam3.1)						
	Target	On-target metrics		Off-target metrics		
#	Exon #	Strand	On-target score*	Precision score**	Frameshift %***	Enhanced on-target score****
sg241	3	+	66.1	0.53	91.2	69.1
sg242	4	-	68.9	0.64	84	71.5
						51.5
						62

Table 18

Sequence	PAM	Score	Gene	Chromosome	Strand	Position	Mismatches	CRISPR Edits with Cas9	CRISPR Edits with enhanced specificity Cas9
ACTCTTGTTACAGAGCTGGT	GGG	100	ENSCAFG000000007249	chr17	1	37022194	0		
ACTTTTGTTTCAGAGCTGGT	CAG	6.16161972		chr33	-1	20234937	2	0%	
CCTCAATGCTACAGAGCTGGT	GGG	2.76564774		chr1	-1	47541563	3	98-99%	0%
GTGCTTGTTACAGAGCTGGT	GGG	2.32143742		chr26	-1	32323843	3	0-25%	0%

[00613] EXAMPLE 11. – Selection of gRNAs targeting IL1a and IL1b in human and canine patients

[00614] Given that the ultimate goal of CRISPR target design is fabrication of nucleotide sequences that will hybridize to genomic DNA sequences resulting in the most robust knockout of a targeted gene as part of the CRISPR/Cas system, the process begins with assessment of splicing at the target loci. Human IL-1a (hIL-1a) exhibits almost exclusively canonical splicing (i.e., no major variants) across various tissue types with the mature mRNA including exons 2-7, making each of these a potential CRISPR gRNA target (Fig. 20). Additional functional analysis (*see* Michlits, et al. (2020). *Nature Methods*, 17(7), 708-716) of the hIL-1a gene demonstrated that all functional domains cluster within Exons 5-7. In order to avoid a truncation that retains residual post-editing functionality, hIL-1a CRISPR targets were limited to those upstream of the functional domain cluster (exons 2-4). In so doing, a resultant frameshift or premature stop codon (i.e., missense mutation) at the editing site will impact all functional domains.

[00615] Similar analysis of human IL-1b (hIL-1b) found a stronger overall expression pattern and more variation in splicing as compared to hIL-1a (Fig. 21). However, as no tissue exhibited a variant omitting exons 2-7, each of these remained viable CRISPR targets. Application of the same functional analysis tools for hIL-1b found that functional domains cluster in exons 5-7, leaving exons 2-4 as viable CRISPR targets.

[00616] Having established the human gene targets, emphasis then shifted to gRNA targeting domain design. Generated CRISPR targeting domains were first tested *in silico* through at least, four separate algorithms, yielding scores assessing cutting activity (On-Target score; *see* Doench et al. (2016). *Nature Biotechnology*, 34(2), 184-191), reproducibility of the particular mutation via double-strand break repair mechanisms (Precision score; inDelphi), likelihood of creating a frameshift mutation (Frameshift score; inDelphi), and specificity of gRNA binding (Off-Target score; CRISPOR) (Fig. 22). Cutoffs were set for On-Target score at >0.30 and for Off-Target scores of 0 for 0 or 1 mismatch (first two columns).

[00617] The same design process was then repeated for the orthologous canine gene targets. Splicing and functional analyses of canine IL-1a (cIL-1 α) shows 6 exons (exons 2-7) incorporated in the mature mRNA, of which exons 6 and 7 contain functional domains (Fig. 23A). This leaves exons 2-5 as viable CRISPR targets. The mature mRNA of canine IL-1b

(cIL-1b) contains exons 2-7, with the core function domains clustered in exons 5-7 (Fig. 23B). As such, exons 2-4 are potentially viable CRISPR targets. Generated candidate CRISPR targeting domains were then analyzed *in silico*; those exceeding the minimum cutoff scores are shown in Fig. 24.

[00618] EXAMPLE 12. Characterizing the ablation of IL1a and IL1b in primary human and canine cells

[00619] Algorithm-validated gRNA targeting domains were then tested in primary cells. Briefly, plasmid DNA encoding a sgRNA with the selected targeting domain was introduced into the primary cells with an encoded Cas9 plasmid via electroporation. Pooled cell populations then underwent DNA extraction and sequencing to assess editing efficiency.

[00620] The results, shown in Fig. 25, demonstrate a wide range of editing efficiencies in both human and canine cells. Indeed, for each gene target, at least one targeting domain demonstrates effective editing (between 89% and 99%) with reproducibility between different cell types (in canine).

[00621] However, in addition to this robust editing of the gene target, sgRNA 242, which targets cIL-1b, also exhibited high levels of off-target editing, as anticipated by the *in silico* analysis (Fig. 26). To rectify these off-target effects, the experiments were repeated with an engineered, enhanced-specificity Cas9 (eSpCas9). This engineered Cas9 completely abrogated the previously-observed off-target effects while still maintain maximal editing efficiency at the target site (Fig. 27). These data demonstrate that the lead candidate sgRNA targeting domain for each gene target can safely (i.e., without off-target effects) and reliably generate genetic knockouts through creation of a primary missense mutation within the targeted locus in human (Fig. 28A) and canine (Fig. 28B) chondrocytes.

[00622] EXAMPLE 13. Co-administration of IL1a- and IL1b-targeted gRNAs in primary human and canine cells

[00623] Having observed robust and reproducible efficacy for each individual gRNA targeting domain, the lead candidates were next assessed in the context of co-administration in order to generate double knockouts. To do this, each sgRNA was administered to canine synoviocytes as described in Example 2 either simultaneously or sequentially (in either order). The results show that sgRNA 242 is highly effective at knocking out cIL-1b under all

conditions (Fig. 29). Conversely, sgRNA 240, targeting cIL-1 α , demonstrated optimal efficacy when it edits first with both simultaneous and secondary administration reducing efficacy by roughly 30%. However, such significant reduction likely remains beyond the threshold needed to impact functionality at the organismal level.

[00624] EXAMPLE 14. Extension to additional species

[00625] Interleukin 1 is a highly conserved gene in terms of both sequence and function among mammals (*see* Dinarello, C. A. (1991). *Blood* 77 (8): 1627–1652). As a result, the gRNA targeting domains that have been generated and characterized for specific species may result in efficient editing of the IL-1 locus of additional species. An alignment to discover conserved IL1A (Fig. 30A) and IL1B (Fig. 30B) gene target positions among humans, horses, dogs, and mice finds relatively few mismatched base pairs across all species at particular target positions. For example, sgRNA 239, targeting cIL-1 α , is predicted to also edit mouse IL-1a. Given the reported flexibility within the CRISPR/Cas system to tolerate imperfect sequence alignments under certain conditions (*see generally* Zischewski, J., et al. (2017). *Biotechnology Advances*, 35(1), 95-104), cross-species reactivity for particular targeting domains is wholly anticipated.

[00626] EXAMPLE 15. Assessing impact of knockout on the cIL-1 α gene

[00627] Having observed CRISPR-mediated knock out of the IL-1a gene in canine chondrocytes using guides sg239, sg240, sg251, and sg252 (Example 12 and Figure 28B), it was tested whether these guides could mediate effective and consistent IL-1A editing in a range of canine cells that are believed to mediate inflammatory effects in joint disease, namely chondrocytes, monocytes, and synoviocytes. Briefly, sgRNA was precomplexed with wild-type *SpCas9* and electroporated into primary canine chondrocytes (experimental n=3), monocytes (experimental n=2), and synoviocytes (experimental n=3) with an encoded Cas9 plasmid via electroporation. Pooled cell populations then underwent DNA extraction and sequencing to assess editing efficiency.

[00628] As shown in Figure 31A, efficient editing was achieved across all three cell types when using sg252 (average efficiency = 81%), sg240 (average efficiency = 90%), and sg239 (average efficiency = 79%). In contrast, use of sg251 resulted in an average editing efficiency of only 25% across all three cell types, despite having a higher predicted on-target

editing score than each of sg239, sg240, and sg252 (as shown in Table 17). Next, the homogeneity of observed editing was evaluated. As shown in Figure 31A, editing with sg252 resulted in nearly homogenous incorporation of a single guanine nucleotide (96% of edits). In contrast, editing with sg239 and sg240 was less homogenous. Specifically, 54% of all edits using sg240 resulted in incorporation of a thymidine nucleotide, while 58% of all edits using sg239 resulted in incorporation of an adenine nucleotide.

[00629] Each of the most common edits caused by sg239, sg240, sg251, and sg252 is a single nucleotide addition. As shown in Figure 31B, these single nucleotide additions cause a frameshift at amino acid position 61 that introduces a premature stop codon at amino acid position 91. Protein folding modeling using AlphaFold2 was performed to evaluate whether the truncated protein caused by the editing would retain any IL-1A structure. Figure 31C shows the predicted structure for the wild-type IL-1A (top) and truncated polypeptide (bottom). As can be seen, the truncated protein is unstructured, suggesting that it does not retain any biological functions of the wild-type IL-1A. Taken together, these results suggest that editing of the IL-1 α gene disrupts all IL-1 α activity.

[00630] Finally, the most efficient guide (sgRNA240) was used to generate two double IL1A/B KO lines of canine monocytes DH82 achieving at least 96% IL1A and 99% IL1B knockout scores.

[00631] EXAMPLE 16. Comparative analyses of guide RNAs targeting the cIL-1 α gene

[00632] To identify additional sgRNA targeting IL-1A with low off-target editing effects, four additional putative target sequences were identified based on in silico prediction of off-target score. Figure 32 shows the target sequence for four previously generated anti-IL-1A sgRNAs: sg239 (renamed as OCA01), sg240 (renamed as OCA03), sg251 (renamed as OCA07), and sg252 (renamed as OCA08), as well as the target sequence for four newly generated sgRNA having low predicted off-target effects: sg358 (renamed as OCA05), sg359 (renamed as OCA04), sg360 (renamed as OCA02), and sg361 (renamed as OCA06). Briefly, putative target sequences were evaluated by multiple models for each of on-target editing efficiency, off-target editing effects, and frameshift edits. Scores for each putative target sequence are shown in Figure 32B.

[00633] Off-target editing effects were predicted by averaging scores generated by the MIT model and the CFD model. The MIT algorithm, also known as Hsu-Zhang score. Hsu, P. D.

et al. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nature Biotechnology* 31, 827–832 (2013). This model is based on a positional penalty matrix (1x20) generated from 15 EMX1 sgRNA libraries with mismatches against target at every position. The CFD algorithm (Cutting Frequency Determination) is based on threat matrix (12x20) considering both position and mismatch type and PAM integrity (27,897 'CD33' sgRNAs + 10,618 negative control sgRNAs). Doench, J. G. et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology* 34, 184–191 (2016).

[00634] On-target editing efficiencies were predicted by averaging scores generated by the Azimuth model, the DeepSpCas9 model, and the CrisprScan model. The Azimuth model is a boosted regression tree model, trained with 881 sgRNAs (MOLM13/NB4/TF1 cells + unpublished additional data) delivered by lentivirus. Doench, J. G. et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology* 34, 184–191 (2016). DeepSpCas9 is a deep learning model trained using editing data from 12,832 sgRNA. Kim, H. K. et al. SpCas9 activity prediction by DeepSpCas9, a deep learning–based model with high generalization performance. *Science Advances* 5, (2019). CrisprScan is a linear regression model, trained using editing data from 1000 sgRNAs injected into zebrafish embryos targeting >100 genes. Moreno-Mateos, M. A. et al. CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo. *Nature Methods* 12, 982–988 (2015).

[00635] The putative guides' potential to generate frameshift mutations were predicted by averaging scores generated by the Lindel model and the InDelphi model. Lindel is a machine learning model trained using profile data of 1.16 million independent mutational events triggered by CRISPR/Cas9-mediated cleavage and non-homologous end joining-mediated double strand break repair of 6872 synthetic target sequences, introduced into a human cell line via lentiviral infection. Chen, W. et al. Massively parallel profiling and predictive modeling of the outcomes of CRISPR/Cas9-mediated double-strand break repair. *Nucleic Acids Research* 47, 7989–8003 (2019). InDelphi is machine learning model trained with indels generated by 1872 sgRNAs. Shen, M. W. et al. Predictable and precise template-free CRISPR editing of pathogenic variants. *Nature* 563, 646–651 (2018).

[00636] The editing efficiency of these guides was then tested in canine monocytes DH82. Briefly, sgRNA was precomplexed with wild-type *SpCas9* and electroporated into primary

canine monocytes DH82. Pooled cell populations then underwent DNA extraction and sequencing to assess editing efficiency. As shown in Figure 32C, OCA01-OCA05 and OCA08 mediated efficient knock-out editing. Further, OCA05 and OCA08 did so with consistent single nucleotide addition edits.

[00637] EXAMPLE 17. Comparative analyses of guide RNAs targeting the cIL-1 β gene

[00638] To identify additional sgRNA targeting IL-1B with low off-target editing effects, four additional putative target sequences were identified based on in silico prediction of off-target score. Figure 33A shows the target sequence for two previously generated anti-IL-1B sgRNAs: sg241 (renamed as OCB01) and sg242 (renamed as OCB02), as well as the target sequence for six newly generated sgRNA having low predicted off-target effects: sg352 (renamed OCB06), sg353 (renamed as OCB04), sg354 (renamed as OCB08), sg355 (renamed as OCB05), sg356 (renamed as OCB07), and sg357 (renamed as OCB03). Briefly, putative target sequences were evaluated by multiple models for each of on-target editing efficiency, off-target editing effects, and frameshift edits, as described in Example 16. Scores for each putative target sequence are shown in Figure 33B.

[00639] The editing efficiency of these guides was then tested in canine monocytes DH82. Briefly, sgRNA was precomplexed with wild-type *SpCas9* and electroporated into primary canine monocytes DH82. Pooled cell populations then underwent DNA extraction and sequencing to assess editing efficiency. As shown in Figure 33C, OCB01-02 and OCB04-06 mediated efficient knock-out editing. Further, OCB01, OCB02, and OCB04 did so with consistent edits. The low editing efficiencies of sgRNAs OCB3 (61%) and OCB07 (0%) were predicted in silico by low on-target scores (Figure 33B; 39 and 32 points, respectively). However, the poor performance of OCB08 could not be explained by the in silico metrics.

[00640] Finally, these genetic effects were further confirmed by performing anti-IL-1B ELISA assays on the supernatant of edited cells. As shown in Figure 33D, each of the sgRNA that showed efficient editing by sequence analysis also resulted in significant reduction in IL-1B secretion by cells targeted by the guides. Taken together, these results confirm editing in canine monocytes by the designed guides.

[00641] EXAMPLE 18. Interrogation of guide RNAs targeting the cIL-1 β gene

[00642] Top sgRNA candidates were further assessed for their impact on the cIL-1 β gene and protein. First, in a repeat of the *in vitro* sequencing assay, extremely consistent results are observed (Fig. 34A). Indeed, a single edit occurs in the edited cells in over 90% of cells, suggesting strong reproducibility when taken together with previous results. Next, both cIL-1 β -targeting candidates were assessed in the context of a double knockout with an sgRNA targeting cIL-1 α . Not only all sgRNAs retain strong knockout efficiency (Fig. 34B, left panel), but these edits result in disruption of protein function. Supernatant ELISA results show that secretion of cIL-1 β protein is significantly abrogated, with reductions of more than 95% compared to control. Taken together, these results suggest that the lead sgRNA candidates retain potency, even when combined.

[00643] EXAMPLE 19. Assessment of cIL-1 β -targeting sgRNA in primary monocytes

[00644] Monocytes are among the most important cells in cellular immunity (and autoimmunity), given their ability to differentiate into numerous different cell fates. As such, these cells are among the prime targets of therapies directed to joint diseases. Therefore, understanding the impact of gene editing on these particular cells becomes pivotal.

[00645] Several sgRNA candidates targeting cIL-1 β were introduced to canine monocytes via electroporation. The edited cells were then subsequently challenged with LPS for either 6 or 24 hours, at which time supernatants were analyzed via ELISA. Results show that negligible amount of IL-1 β are detected in LPS-challenged monocytes that had been edited with either OCB01 or OCB02 (Figs. 35A, B). Conversely, while control cells respond robustly at both time points.

[00646] The same assay was then repeated for a broader panel of sgRNAs. Again, strong inhibition of IL-1 β secretion at 24 hours is observed across the board (Fig. 36). In no instance was average inhibition less than 80% of the unedited control.

[00647] EXAMPLE 20. Impact of mutant Cas9 proteins with enhanced specificity

[00648] It was then investigated whether OCB02 caused off-targeting editing by sequencing two genomic loci in the canine monocytes edited as described in Example 17 with high sequence identity to the target loci (Off-target #2 and Off-target #3). As shown in Figure 38, when complexed with wild-type Cas9, OCB02 caused high levels of off targeting editing at both loci.

[00649] To determine whether this off-target editing could be avoided by using Cas9 variants known to have enhanced specificity, canine monocytes were edited with either OCB01 or OCB02 precomplexed with wild-type Cas9, ES-Cas9 (Enhanced Specificity Cas9, described in Slaymaker, I. M. et al. Rationally engineered Cas9 nucleases with improved specificity. *Science* (1979) 351, (2016)), PE-Cas9 (PUredit® Cas9; Merck), HF-Cas9 (TrueCut® HiFi Cas9; Invitrogen), or AR-Cas9 (Alt-R® HiFi Cas9; IDT). The on-target editing efficiency of these complexes was evaluated by sequencing the IL1B gene in the edited cells. As shown in Figure 38, all of the high efficiency Cas9 variants mediated efficient on-targeting editing (IL1B knockout) when paired with the OCB02 sgRNA. However, only some of the high fidelity Cas9 variants mediated efficient on-targeting editing (IL1B knockout) when paired with the OCB01 sgRNA. The effect of the high fidelity Cas9 proteins on off-targeting editing with the OCB02 sgRNA were then evaluated by sequencing off-target sites #2 and #3 in edited cells. As shown in Figure 38, each of the high fidelity Cas9 proteins eliminated off-target editing at these loci.

[00650] The effect of the high-fidelity Cas9 variants on IL-1B expression/secretion was then tested, as described above. As expected from the sequencing results, editing using each of the enhanced specificity Cas9 proteins resulted in efficient inhibition of IL1B secretion 24 hours following LPS challenge (Figure 37A). Similar results were shown for OCB01-mediated editing using AR-Cas9, as expected from the sequencing data (Figure 37B).

[00651] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the disclosure, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the embodiments of the disclosure that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the disclosure pertains.

[00652] EXAMPLE 21. Identification of example sgRNA targeting human, canine, equine, or feline IL-1A or IL-1 β genes.

[00653] To identify additional sgRNA targeting IL-1A or IL-1B in silico predictions of on-target editing efficiency, off-target editing effects, and frameshift editing probability were

generated for putative target human, canine, equine, and feline sequences as described in Example 16, except that for human guide sequences the on-target scores that were averaged also included a score generated using the Elevation model. Elevation is an end-to-end machine learning model trained by GUIDE-Seq and other aggregated data. See, Listgarten, J. et al. Prediction of off-target activities for the end-to-end design of CRISPR guide RNAs. *Nature Biomedical Engineering* 2, 38–47 (2018); and Tsai, S. Q. et al. GUIDE-seq enables genome-wide profiling of off-target cleavage by CRISPR-Cas nucleases. *Nature Biotechnology* 33, 187–197 (2015). An overall score for each putative crRNA sequence was then calculated as the average of the on-target, off-target, and frameshift score. The highest scoring crRNA sequences for each gene and species were selected. Scores for each putative target sequence are shown in Figures 39A-46B.

[00654] The genomic location of the exons reported in Figures 39A-46B is provided in Tables 17-24, below.

Table 17 - Human IL1A

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	ENSE00001325622	hg38	chr2	reverse	112,784,493	112,784,443
2	Yes	ENSE00001128612	hg38	chr2	reverse	112,783,778	112,783,724
3	Yes	ENSE00001145252	hg38	chr2	reverse	112,782,764	112,782,716
4	Yes	ENSE00000856832	hg38	chr2	reverse	112,781,826	112,781,604
5	Yes	ENSE00000773893	hg38	chr2	reverse	112,779,666	112,779,496
6	Yes	ENSE00000773892	hg38	chr2	reverse	112,778,111	112,777,987
7	Yes	ENSE00001128606	hg38	chr2	reverse	112,775,267	112,773,925

Table 18 - Human IL1B

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	ENSE00001000625	hg38	chr2	reverse	112,836,779	112,836,708
2	Yes	ENSE00003508205	hg38	chr2	reverse	112,836,244	112,836,183
3	Yes	ENSE00003647827	hg38	chr2	reverse	112,835,617	112,835,566
4	Yes	ENSE00003506405	hg38	chr2	reverse	112,833,575	112,833,374
5	Yes	ENSE00003582426	hg38	chr2	reverse	112,832,826	112,832,662
6	Yes	ENSE00003615183	hg38	chr2	reverse	112,831,422	112,831,292
7	Yes	ENSE00000856835	hg38	chr2	reverse	112,830,573	112,829,751

Table 19 - Canine IL1A

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	ENSCAFE00000309751	canFam3	chr17	reverse	36,979,435	36,979,356
2	Yes	ENSCAFE00000078619	canFam3	chr17	reverse	36,978,720	36,978,666
3	Yes	ENSCAFE00000078621	canFam3	chr17	reverse	36,977,683	36,977,635
4	Yes	ENSCAFE00000078623	canFam3	chr17	reverse	36,977,242	36,977,032
5	Yes	ENSCAFE00000078624	canFam3	chr17	reverse	36,975,266	36,975,093
6	Yes	ENSCAFE00000078626	canFam3	chr17	reverse	36,973,248	36,973,130
7	Yes	ENSCAFE00000078627	canFam3	chr17	reverse	36,970,929	36,970,573

Table 20 - Canine IL1B

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	NA	canFam3	chr17	reverse	NA	NA
2	Yes	ENSCAFE00000409713	canFam3	chr17	reverse	37,024,176	37,023,944
3	Yes	ENSCAFE00000241094	canFam3	chr17	reverse	37,023,405	37,023,354
4	Yes	ENSCAFE00000078700	canFam3	chr17	reverse	37,022,260	37,022,065
5	Yes	ENSCAFE00000078702	canFam3	chr17	reverse	37,021,958	37,021,797
6	Yes	ENSCAFE00000241077	canFam3	chr17	reverse	37,020,557	37,020,427
7	Yes	ENSCAFE00000309007	canFam3	chr17	reverse	37,019,681	37,019,329

**Table 21 -
Equine IL1A**

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	ENSECAE00000193601	equCab3	chr15	reverse	16,369,812	16,369,762
2	Yes	ENSECAE00000193613	equCab3	chr15	reverse	16,369,119	16,369,065
3	Yes	ENSECAE00000193626	equCab3	chr15	reverse	16,368,070	16,368,022
4	Yes	ENSECAE00000193646	equCab3	chr15	reverse	16,367,110	16,366,888
5	Yes	ENSECAE00000193669	equCab3	chr15	reverse	16,365,310	16,365,137
6	Yes	ENSECAE00000193696	equCab3	chr15	reverse	16,364,119	16,363,998
7	Yes	ENSECAE00000193723	equCab3	chr15	reverse	16,361,752	16,360,700

**Table 22 -
Equine IL1B**

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	ENSECAE00000001011	equCab3	chr15	reverse	16,444,697	16,444,653
2	Yes	ENSECAE00000000363	equCab3	chr15	reverse	16,423,448	16,423,390
3	Yes	ENSECAE00000269159	equCab3	chr15	reverse	16,422,859	16,422,808
4	Yes	ENSECAE00000000420	equCab3	chr15	reverse	16,422,550	16,422,346
5	Yes	ENSECAE00000000449	equCab3	chr15	reverse	16,421,794	16,421,633
6	Yes	ENSECAE00000000487	equCab3	chr15	reverse	16,420,363	16,420,233
7	Yes	ENSECAE00000308144	equCab3	chr15	reverse	16,419,536	16,418,738

**Table 23 -
Feline IL1A**

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	No	NA	felCat9	chrA3	forward	NA	NA
2	Yes	ENSFCAE00000082073	felCat9	chrA3	forward	105,113,193	105,113,247
3	Yes	ENSFCAE00000473731	felCat9	chrA3	forward	105,114,215	105,114,263
4	Yes	ENSFCAE00000345284	felCat9	chrA3	forward	105,115,053	105,115,275
5	Yes	ENSFCAE00000082076	felCat9	chrA3	forward	105,117,059	105,117,232
6	Yes	ENSFCAE00000082077	felCat9	chrA3	forward	105,118,594	105,118,715
7	Yes	ENSFCAE00000293842	felCat9	chrA3	forward	105,120,921	105,121,209

**Table 24 -
Feline IL1B**

<i>Exon #</i>	<i>Coding</i>	<i>Ensembl ID</i>	<i>UCSC Genome Assembly ID</i>	<i>Chromosome</i>	<i>Strand</i>	<i>Start</i>	<i>End</i>
1	Yes?	ENSFCAE00000421394	felCat9	chrA3	forward	105,049,334	105,050,213
2	Yes	ENSFCAE00000060114	felCat9	chrA3	forward	105,050,633	105,050,694
3	Yes	ENSFCAE00000060119	felCat9	chrA3	forward	105,051,232	105,051,280
4	Yes	ENSFCAE00000060121	felCat9	chrA3	forward	105,052,908	105,053,112
5	Yes	ENSFCAE00000060125	felCat9	chrA3	forward	105,053,655	105,053,816
6	Yes	ENSFCAE00000060131	felCat9	chrA3	forward	105,055,063	105,055,193
7	Yes	ENSFCAE00000398371	felCat9	chrA3	forward	105,056,165	105,056,407

[00655] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the disclosure described herein.

[00656] It is to be understood that the methods described herein are not limited to the particular methodology, protocols, subjects, and sequencing techniques described herein and as such can vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the methods and compositions described herein, which will be limited only by the appended claims. While some embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein can be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00657] Several aspects are described with reference to example applications for illustration. Unless otherwise indicated, any embodiment can be combined with any other embodiment. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. A skilled artisan, however, will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[00658] While some embodiments have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. It is not intended that the disclosure be limited by the specific examples provided within the specification. While the disclosure has been described with reference to the aforementioned specification, the descriptions and illustrations of the embodiments herein are not meant to be construed in a limiting sense. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure.

[00659] Furthermore, it shall be understood that all aspects of the disclosure are not limited to the specific depictions, configurations or relative proportions set forth herein which depend upon a variety of conditions and variables. It should be understood that various alternatives to

the embodiments of the disclosure described herein can be employed in practicing the disclosure. It is therefore contemplated that the disclosure shall also cover any such alternatives, modifications, variations or equivalents. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00660] All publications, patents, and patent applications herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

CLAIMS

1. A composition for the treatment or prevention of a joint disease or condition, comprising:
 - (i) an RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease; and
 - (ii) at least one guide RNA or a nucleic acid encoding at least one guide RNAtargeting an IL-1 α or IL-1 β gene, wherein the guide RNA specifically binds a target sequence that is adjacent to a protospacer adjacent motif (PAM) sequence for the Cas9 protein.
2. The composition of claim 1, wherein the at least one guide RNA targets a human IL-1 α gene.
3. The composition of claim 2, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the human IL-1 α gene.
4. The composition of claim 2, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 168-187, 298-387, and 681-710.
5. The composition of claim 1, wherein the at least one guide RNA targets a human IL-1 β gene.
6. The composition of claim 5, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the human IL-1 β gene.
7. The composition of claim 5, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 188-201, 388-496, and 711-740.
8. The composition of claim 1, wherein the at least one guide RNA targets a canine IL-1 α gene.

9. The composition of claim 8, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the canine IL-1 α gene.
10. The composition of claim 8, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 202-216, 552-590, and 741-770.
11. The composition of claim 1, wherein the at least one guide RNA targets a canine IL-1 β gene.
12. The composition of claim 11, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the canine IL-1 β gene.
13. The composition of claim 11, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 217-235, 497-551, and 771-800.
14. The composition of claim 1, wherein the at least one guide RNA targets an equine IL-1 α gene.
15. The composition of claim 14, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the equine IL-1 α gene.
16. The pharmaceutical composition of claim 14, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 236-255 and 801-830.
17. The composition of claim 1, wherein the at least one guide RNA targets an equine IL-1 β gene.

18. The composition of claim 17, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the equine IL-1 β gene.
19. The pharmaceutical composition of claim 17, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 256-262 and 831-860.
20. The composition of claim 1, wherein the at least one guide RNA targets a feline IL-1 α gene.
21. The composition of claim 20, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the feline IL-1 α gene.
22. The pharmaceutical composition of claim 20, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 861-890.
23. The composition of claim 1, wherein the at least one guide RNA targets a feline IL-1 β gene.
24. The composition of claim 23, wherein the at least one guide targets exon 2, exon 3, exon 4, exon 5, or exon 6 of the feline IL-1 β gene.
25. The composition of claim 23, wherein the at least one guide RNA comprises a crRNA sequence having at least 85% identity, at least 90% identity, at least 95% identity, or 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 891-920.
26. The composition of any one of claims 1-25, wherein the RNA-guided nuclease is a CRISPR associated protein 9 (Cas9) protein.

27. The composition of claim 26, wherein the Cas9 protein is an *S. pyogenes* Cas9 protein.
28. The composition of claim 26, wherein the Cas9 protein is an *S. aureus* Cas9 protein.
29. The composition of any one of claims 26-28, wherein the Cas9 protein is an enhanced specificity Cas9 variant protein.
30. The composition of any one of claims 1-29, wherein the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease is the RNA-guided nuclease.
31. The composition of any one of claims 1-29, wherein the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease is DNA encoding the RNA-guided nuclease.
32. The composition of any one of claims 1-29, wherein the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease is mRNA encoding the RNA-guided nuclease.
33. The composition of any one of claims 1-32, wherein the at least one guide RNA or a nucleic acid encoding at least one guide RNA is the at least one guide RNA.
34. The composition of any one of claims 1-32, wherein the at least one guide RNA or a nucleic acid encoding at least one guide RNA is DNA encoding the at least one guide RNA.
35. The composition of any one of claims 1-34, comprises a nucleic acid encoding both the RNA-guided nuclease and the at least one guide RNA.
36. The composition of any one of claims 1-35, wherein the at least one guide RNA is a single guide RNA (sgRNA).
37. The composition of any one of claims 1-36, wherein the composition comprises one or more lipid nanoparticles (LNP) collectively encapsulating:

(i) the RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease;
and

(ii) the at least one guide RNA or the nucleic acid encoding the at least one guide RNA.

38. The composition of claim 37, wherein the one or more LNP comprises:

a first plurality of LNP encapsulating the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease; and

a second plurality of LNP encapsulating the at least one guide RNA or a nucleic acid encoding at least one guide RNA.

39. The composition of claim 37 or 38, wherein the one or more LNP comprises a component selected from the group consisting of 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10), N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoley1-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoley1-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z-,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), (2S)-2-({8-[(3.beta.)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z-,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), a lipid including a cyclic amine group, and a mixture thereof.

40. The composition of any one of claims 37-39, wherein the LNP comprises a component selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-

O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoeoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin (SM), and a mixture thereof.

41. The composition of any one of claims 37-40, wherein the LNP comprises a component selected from the group consisting of PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, PEG-modified dialkylglycerols, and mixtures thereof. For example, a PEG lipid may be PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, PEG-DMA, a PEG-DSPE lipid, and a mixture thereof.

42. The composition of any one of claims 37-41, wherein the LNP comprises a component selected from the group consisting of a cholesterol, fecosterol, stigmasterol, stigmastanol, sitosterol, β -sitosterol, lupeol, betulin, ursolic acid, oleanolic acid, campesterol, fucosterol, brassicasterol, ergosterol, 9, 11-dehydroergosterol, tomatidine, tomatine, α -tocopherol, and a mixture thereof.

43. The composition of any one of claims 1-36, wherein the composition comprises one or more liposomes collectively encapsulating:

- (i) the RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease; and
- (ii) the at least one guide RNA or the nucleic acid encoding the at least one guide RNA.

44. The composition of claim 43, wherein the one or more liposomes comprises:

a first plurality of liposomes encapsulating the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease; and

a second plurality of liposomes encapsulating the at least one guide RNA or a nucleic acid encoding at least one guide RNA.

45. The composition of any one of claims 1-36, wherein the composition comprises one or more recombinant viruses collectively encapsulating:

(i) the RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease; and

(ii) the at least one guide RNA or the nucleic acid encoding the at least one guide RNA.

46. The composition of claim 45, wherein the one or more recombinant viruses comprises:

a first plurality of recombinant viruses encapsulating the RNA-guided nuclease or a nucleic acid encoding an RNA-guided nuclease; and

a second plurality of recombinant viruses encapsulating the at least one guide RNA or a nucleic acid encoding at least one guide RNA.

47. The composition of claim 45 or 46, wherein the one of more recombinant viruses is selected from the group consisting of a retrovirus, an adenovirus, an adeno-associated virus, a lentivirus, and a herpes simplex virus-1.

48. The composition of claim 45 or 46, wherein the one of more recombinant viruses comprises a recombinant adeno-associated virus (AAV).

49. The composition of claim 48, wherein the recombinant AAV is of serotype 5 (AAV5).

50. The composition of claim 48, wherein the recombinant AAV is of serotype 6 (AAV6).

51. The composition of any one of claims 1-36, wherein the RNA-guided nuclease or the nucleic acid encoding the RNA-guided nuclease and the at least one guide RNA or the nucleic acid encoding the at least one guide RNA are not encapsulated.
52. The composition of any one of claims 1-51, wherein the composition is formulated for parenteral administration.
53. The composition of any one of claims 1-51, wherein the composition is formulated for intra-articular injection within a joint of a subject.
54. A method for treating a joint disease or condition, the method comprising:
administering, to a subject in need thereof, a composition of any one of claims 1-53.
55. The method of claim 54, wherein the joint disease or condition is arthritis.
56. The method of claim 55, wherein the arthritis is osteoarthritis.
57. The method of claim 54, wherein the joint disease or disorder is post-traumatic arthritis, gout, or pseudogout.
58. The method of any one of claims 54-57, wherein the subject is a human.
59. The method of any one of claims 54-57, wherein the subject is a canine, equine, or feline.
60. The method of claim 59, wherein the joint disease or disorder is canine, equine, or feline lameness.
61. The method of any one of claims 54-60, wherein the administering comprises intra-articular injection of the composition into the joint of the subject.
62. The method of any one of claims 54-61, wherein the composition is administered during surgery.

63. The method of any one of claims 54-62, wherein the composition is administered after surgery.
64. The method of any one of claims 54-63, wherein the composition is a controlled release pharmaceutical composition.
65. A composition according to any one of claims 1-53 for use in treating a joint disease or disorder.
66. The composition of claim 65, wherein the joint disease or disorder is arthritis.
67. The composition of claim 66, wherein the arthritis is osteoarthritis.
68. The composition of claim 65, wherein the joint disease or disorder is post-traumatic arthritis, gout, or pseudogout.
69. The composition of claim 65, wherein the joint disease or disorder is canine, equine, or feline lameness.
70. Use of a composition according to any one of claims 1-53 in the manufacture of a medicament for treating a joint disease or disorder.
71. The use of claim 70, wherein the joint disease or disorder is arthritis.
72. The use of claim 71, wherein the arthritis is osteoarthritis.
73. The use of claim 70, wherein the joint disease or disorder is post-traumatic arthritis, gout, or pseudogout.
74. The use of claim 70, wherein the joint disease or disorder is canine, equine, or feline lameness.

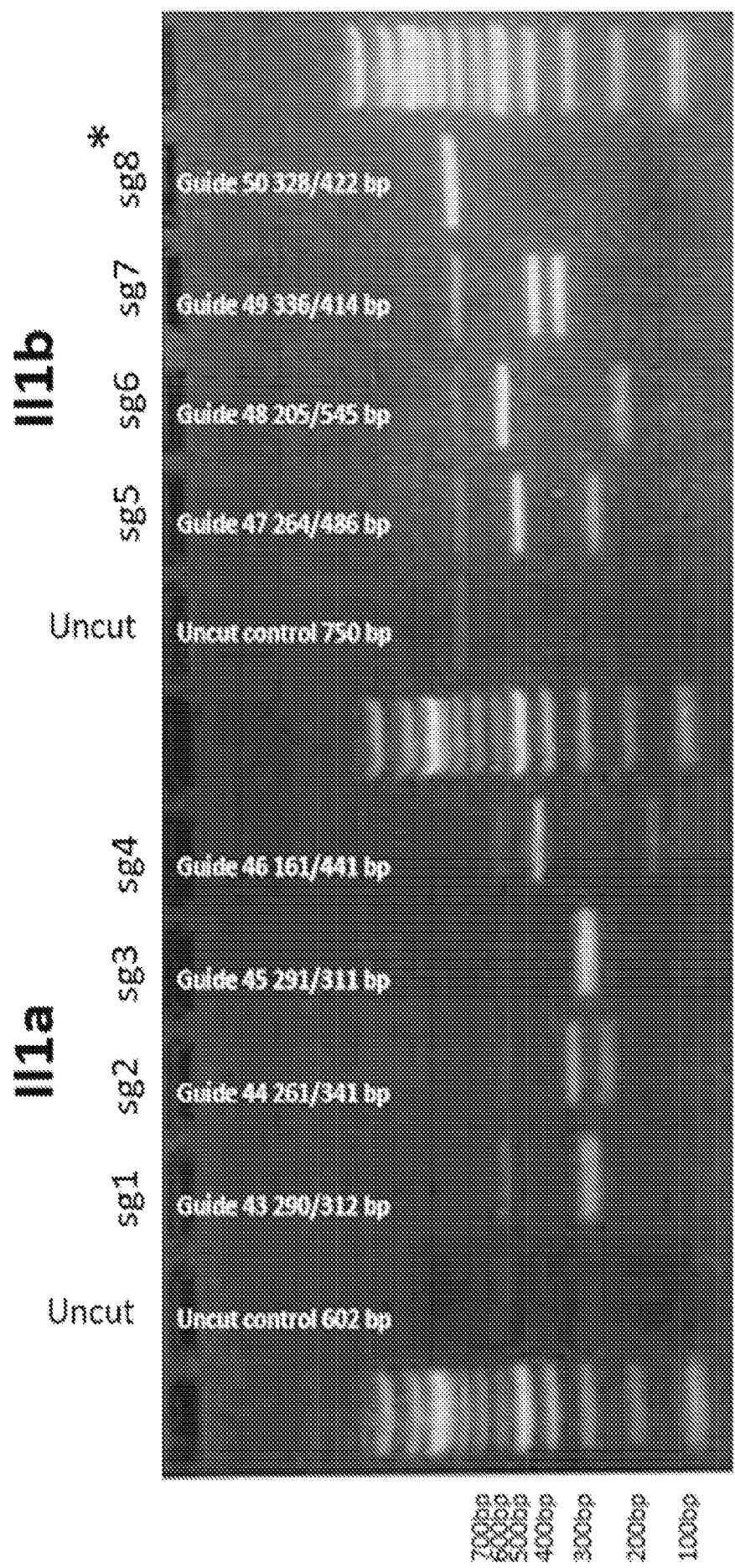


FIG. 1A

II1b

Uncut

sg6

sg5

sg4

II1a

Uncut

sg1

sg2

sg3

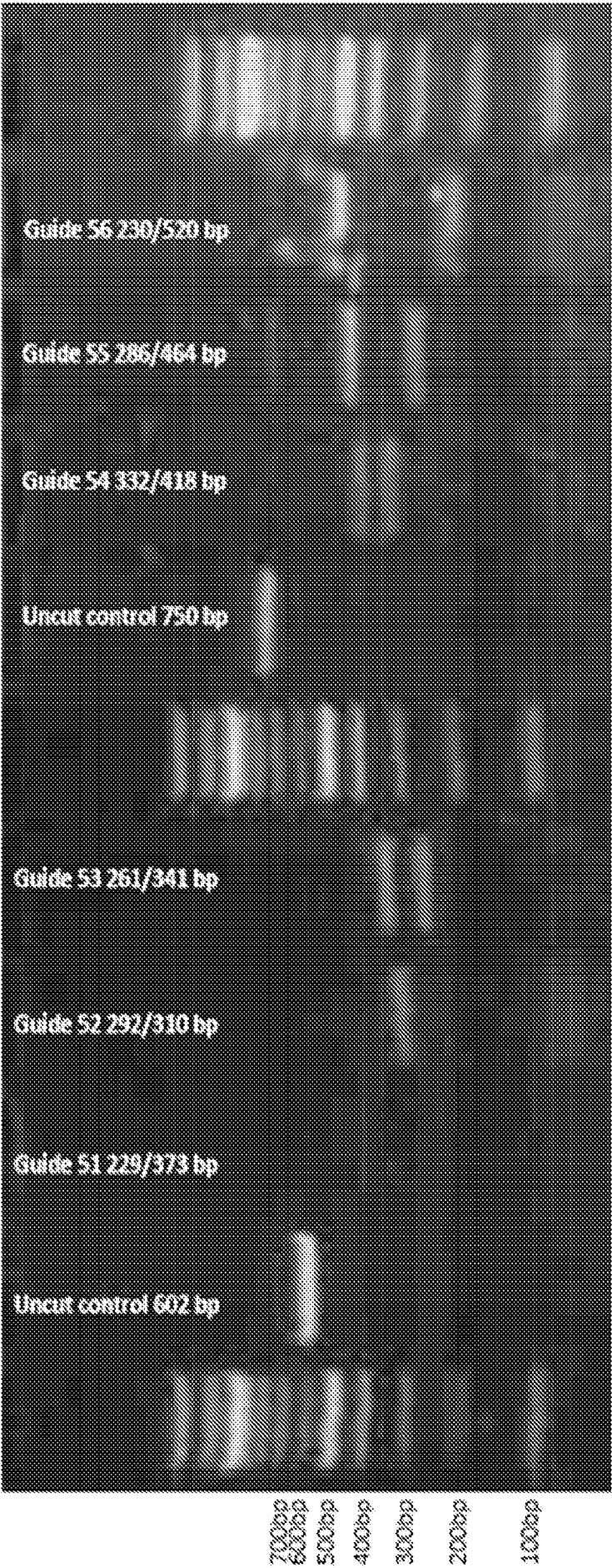


FIG. 1B

II1a

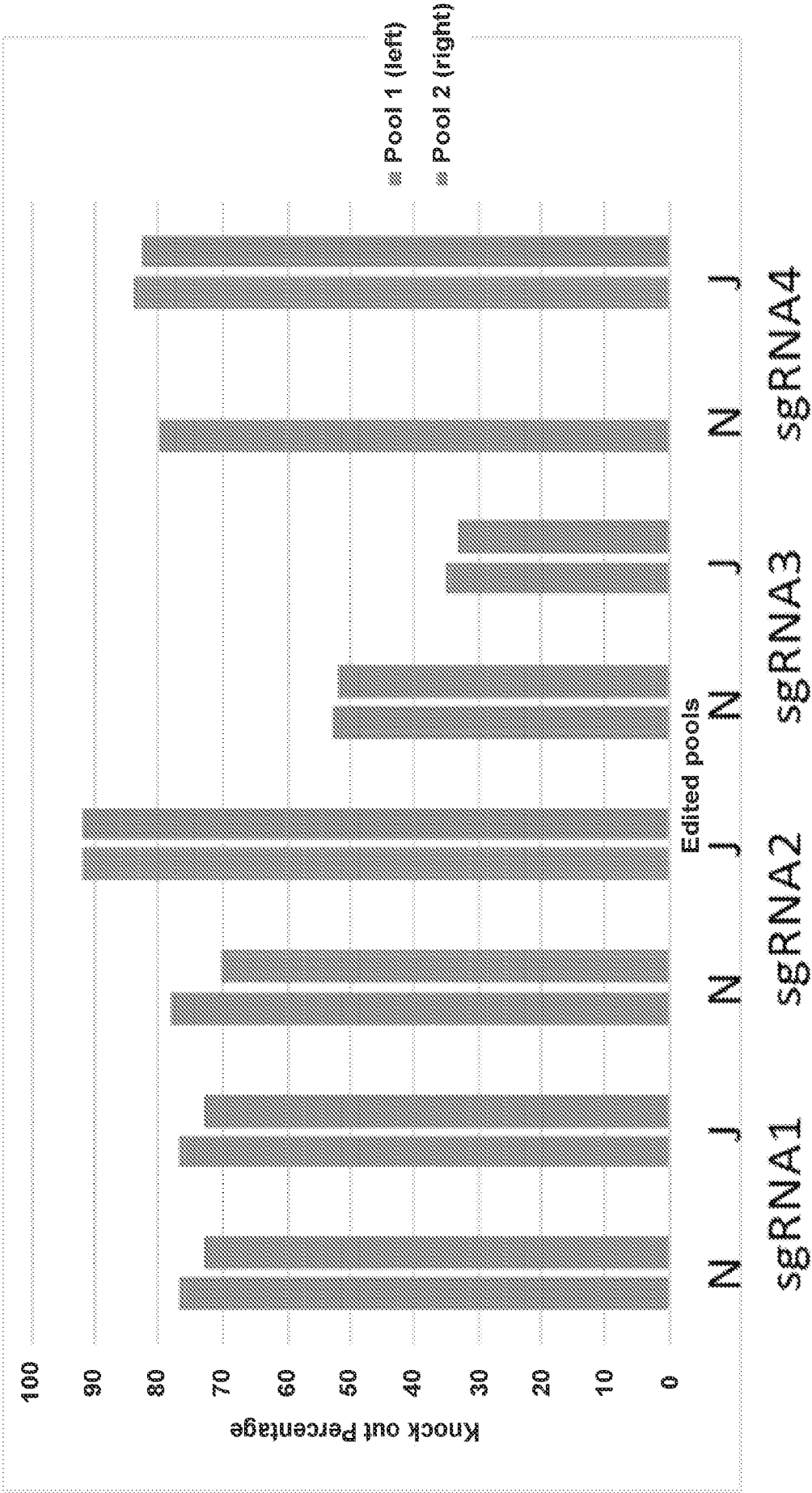


FIG. 2A

111b

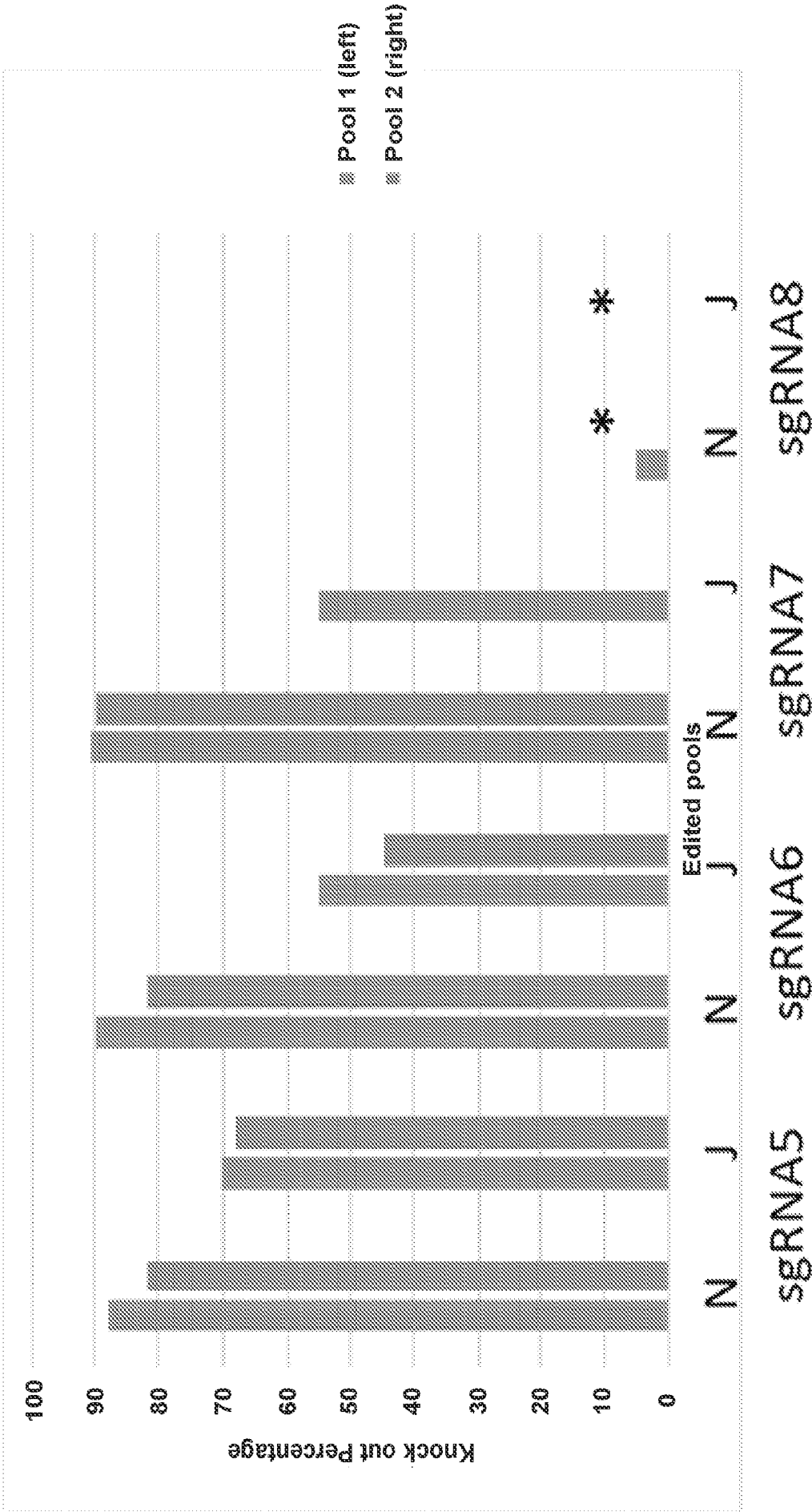


FIG. 2B

II1a

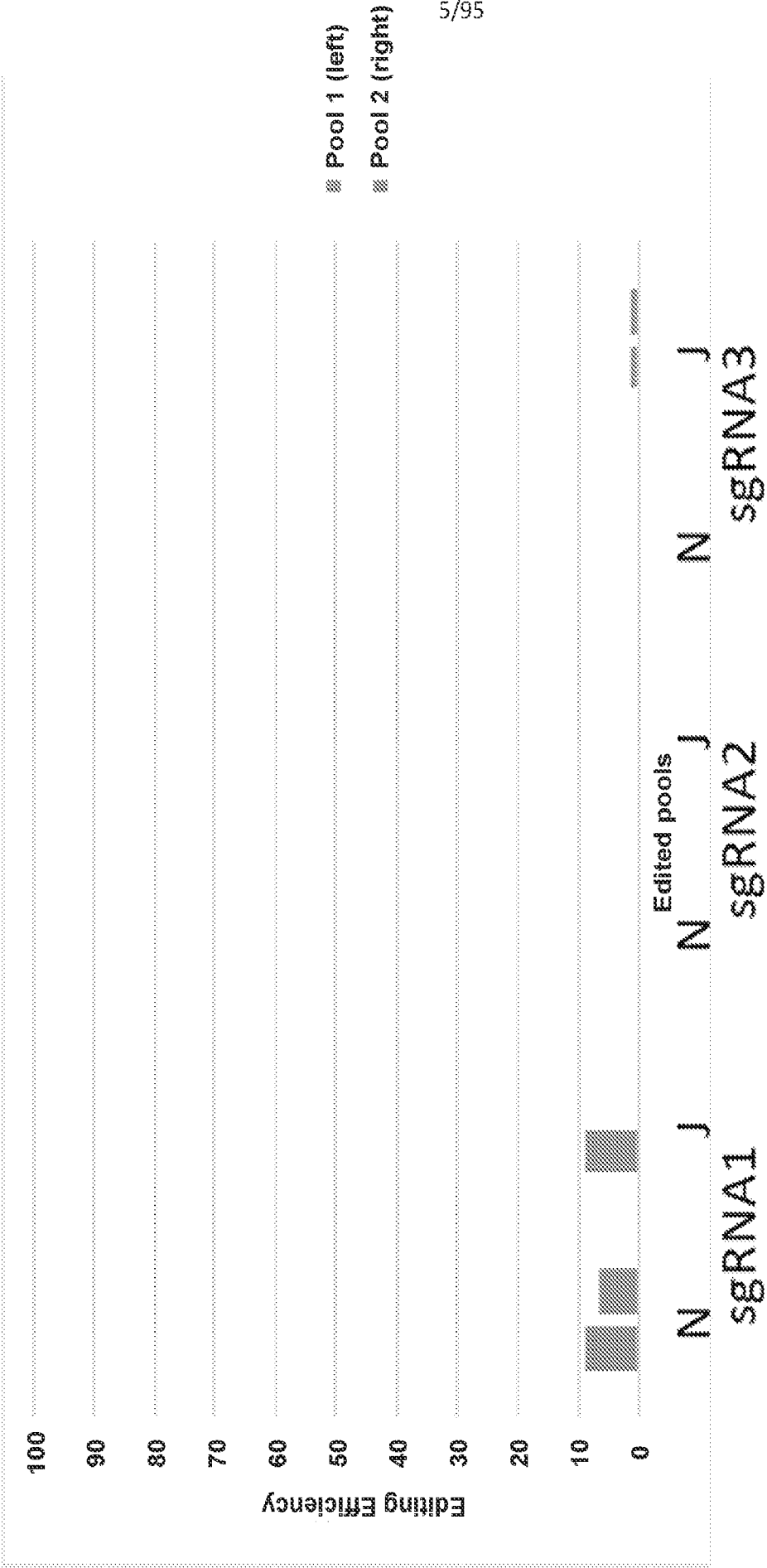


FIG. 2C

II1b

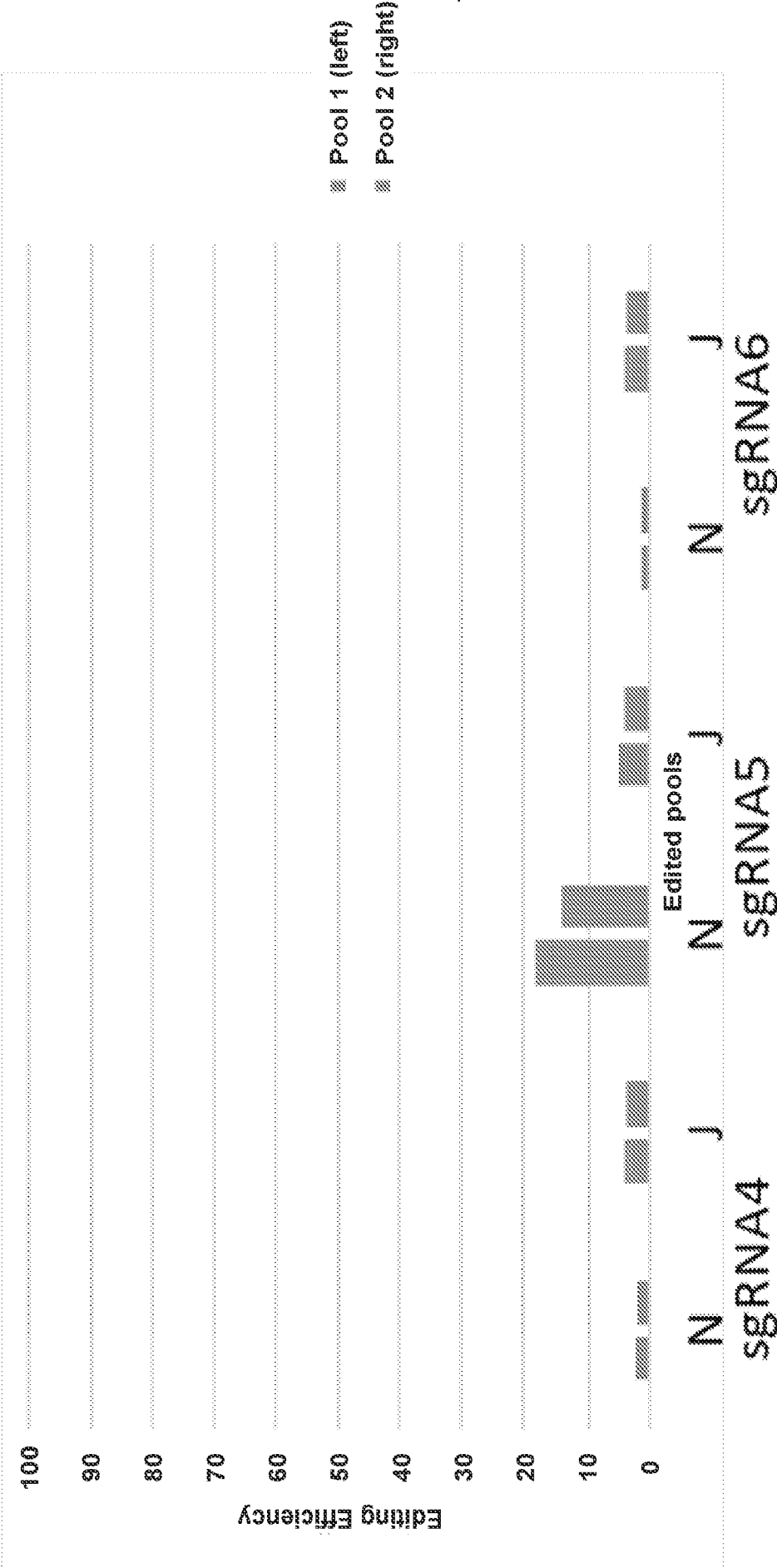


FIG. 2D

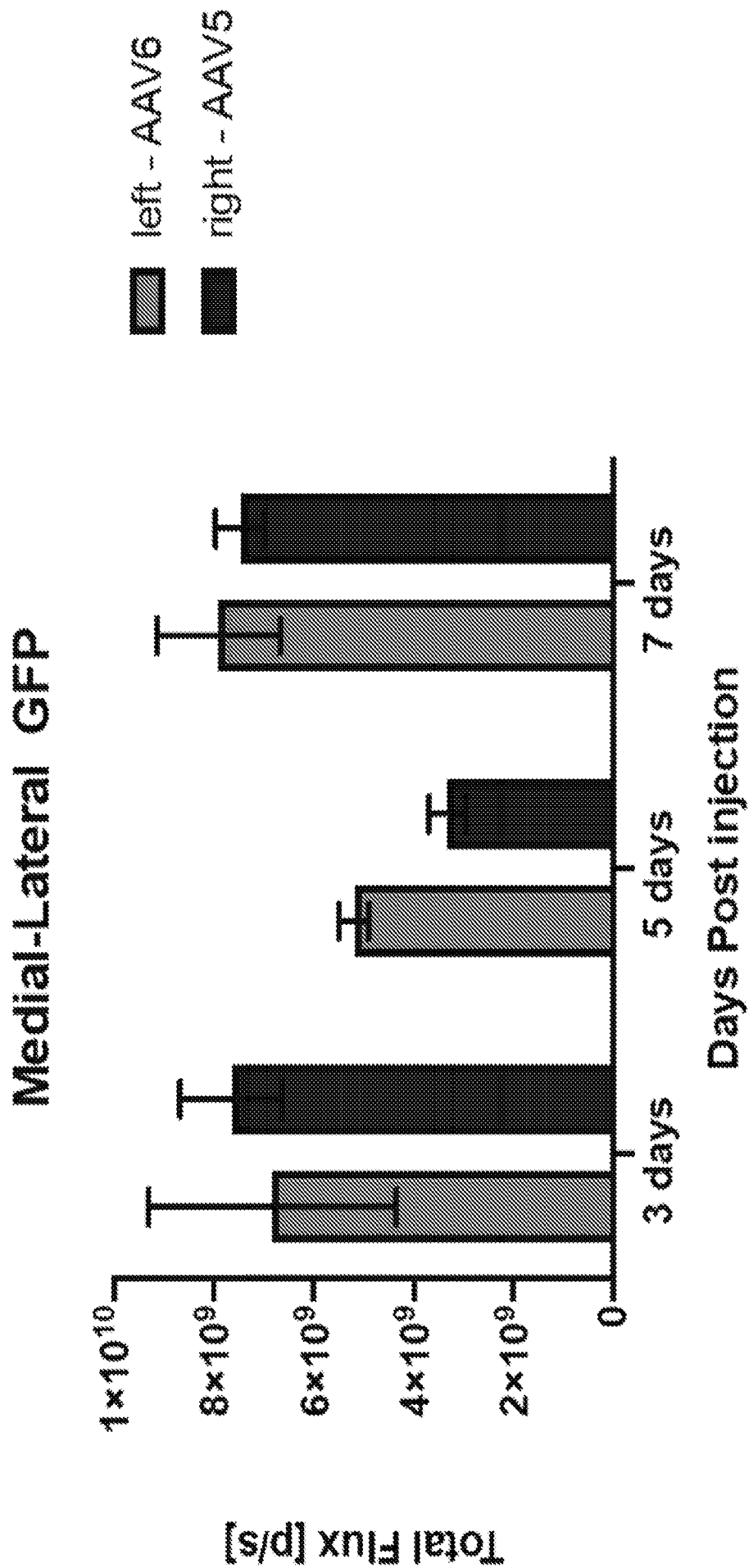
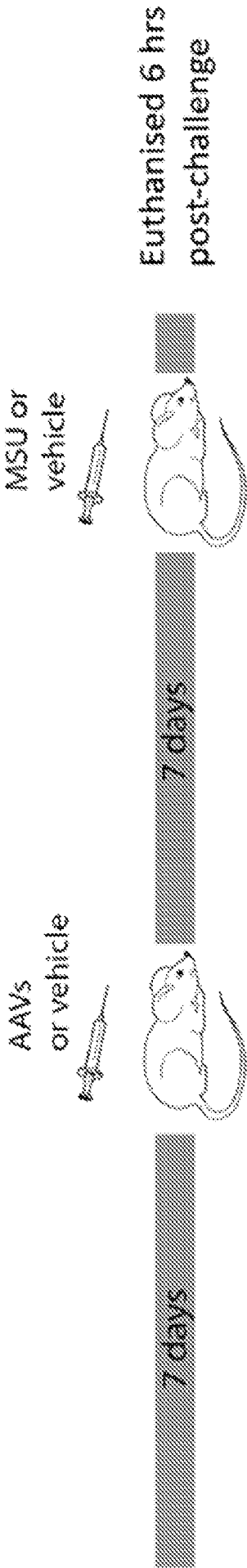


FIG. 3



Group	Treatment	Challenge
AAV-6 Active	AAV-6 with both guides	MSU
AAV-6 Scramble	AAV-6 with scramble guide	MSU
Untreated	Saline	MSU
AAV-5 Active	AAV-5 with both guides	MSU
AAV-5 Scramble	AAV-5 with scramble guide	MSU
Normal*	None	None

* represents left knees from untreated group

FIG. 4

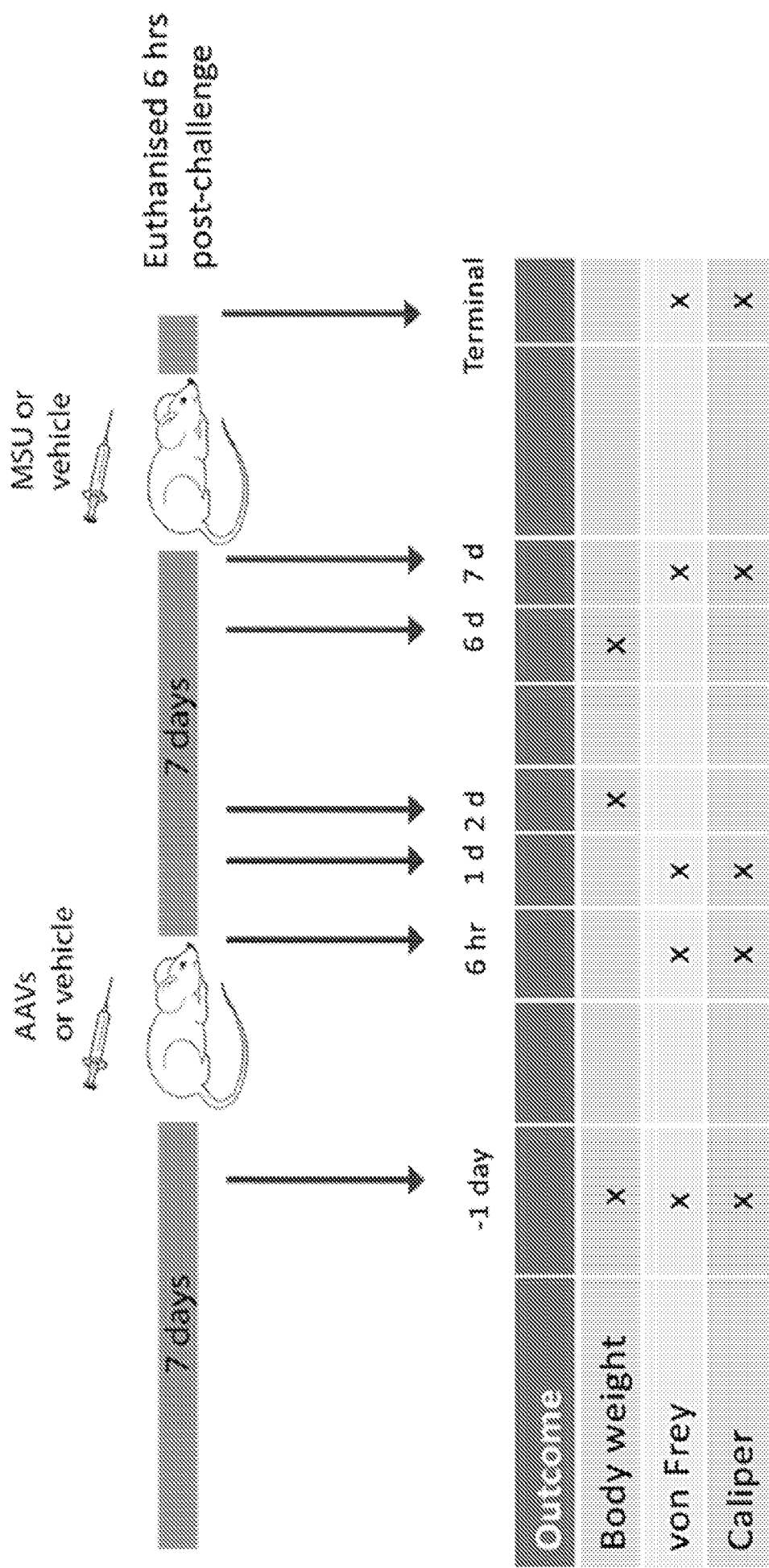
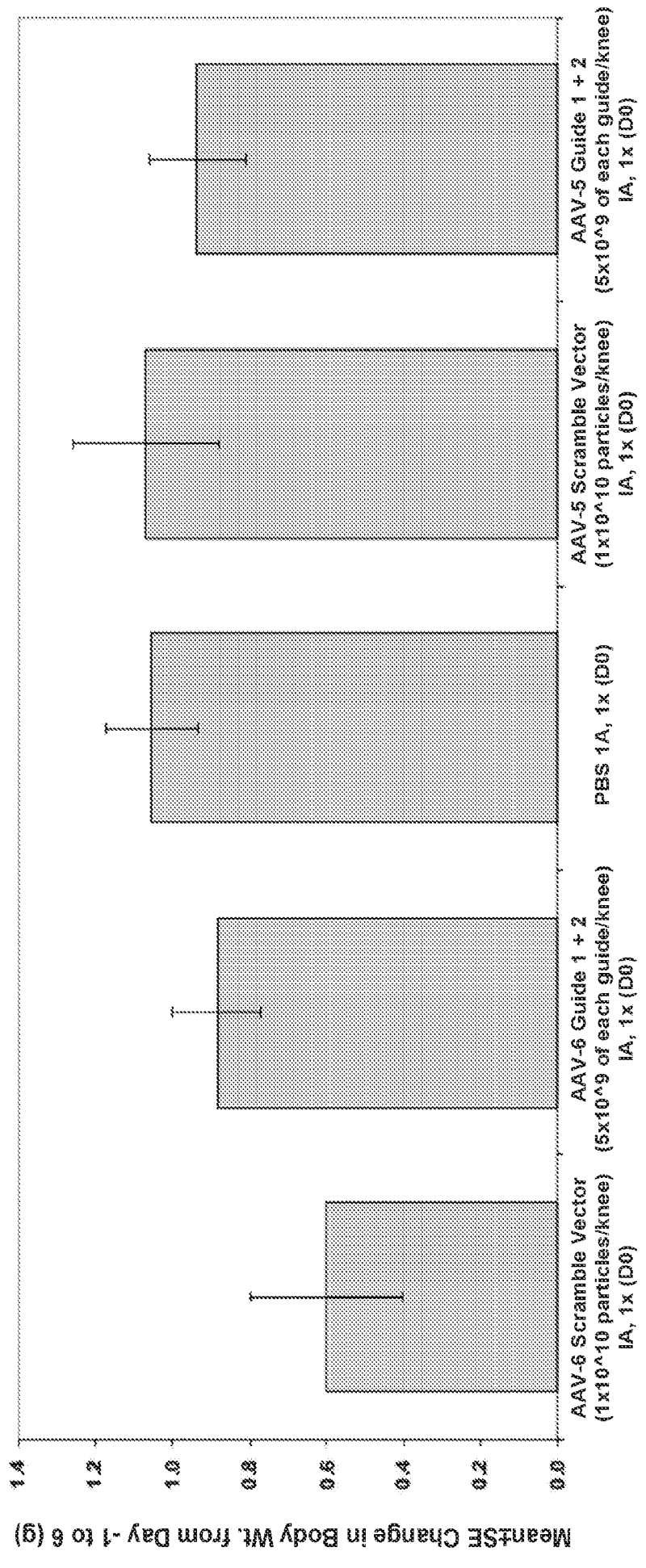


FIG. 5



Change in Body Weight (Day -1 to 6)

n = 14/group

*p ≤ 0.05 ANOVA (Tukey's post-hoc) vs. AAV-6 Guide 1

†p ≤ 0.05 ANOVA (Tukey's post-hoc) vs. PBS

‡p ≤ 0.05 ANOVA (Tukey's post-hoc) vs. AAV-5 Scramble Vector

\$p ≤ 0.05 ANOVA (Tukey's post-hoc) vs. AAV-5 Guide 1

FIG. 6

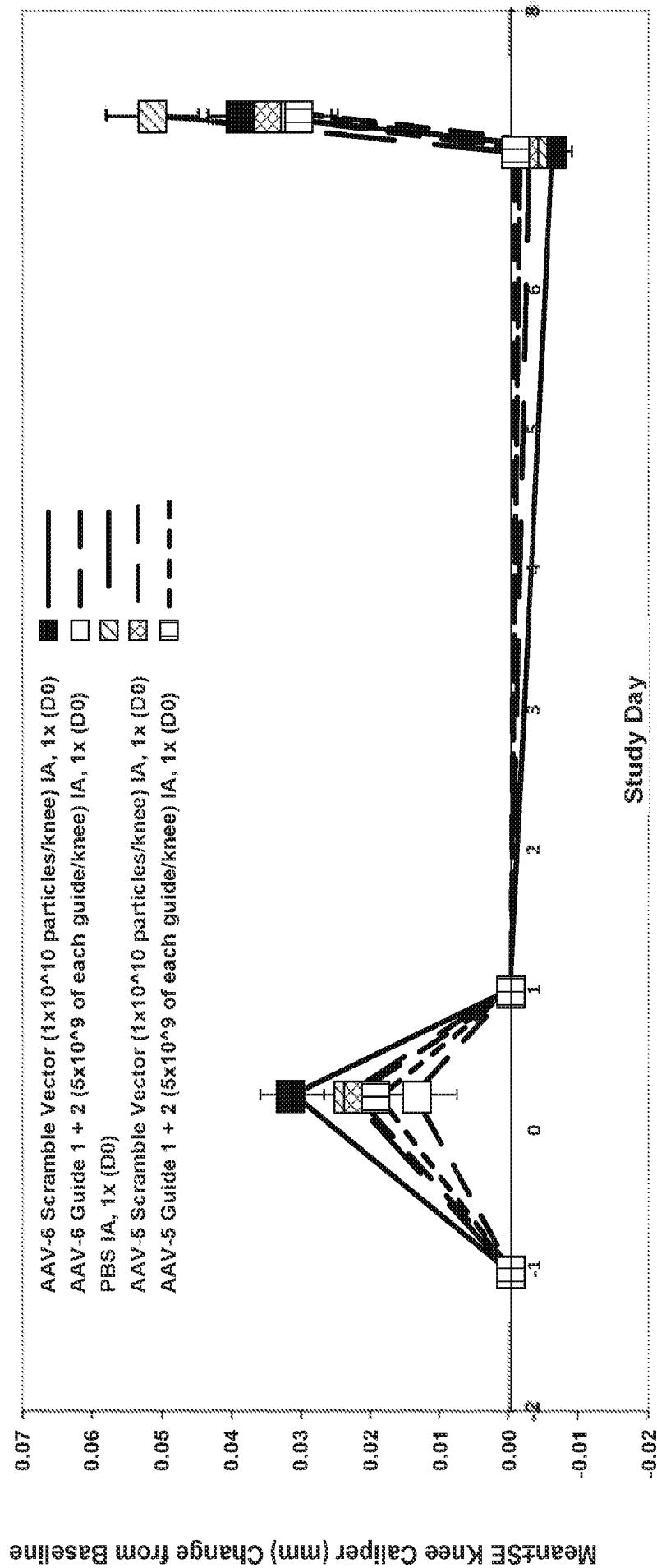


FIG. 7A

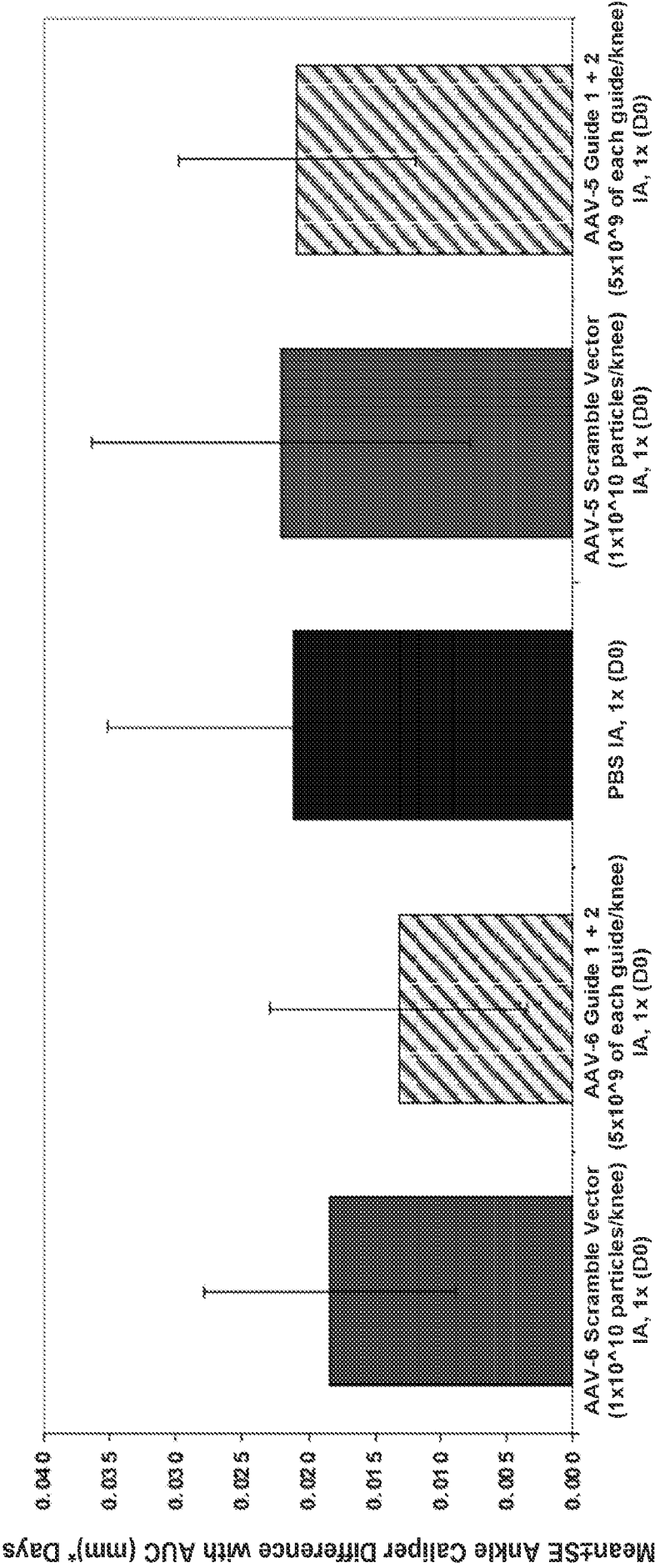


FIG. 7B

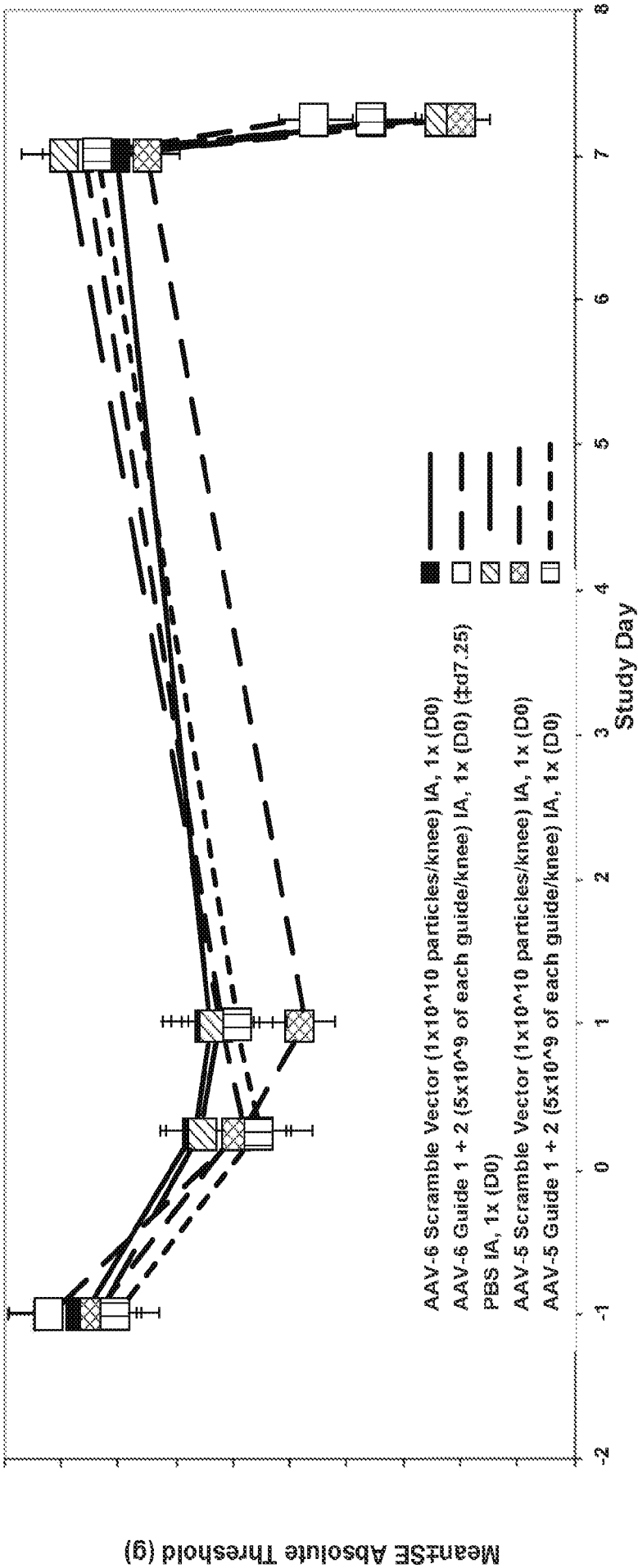


FIG. 8A

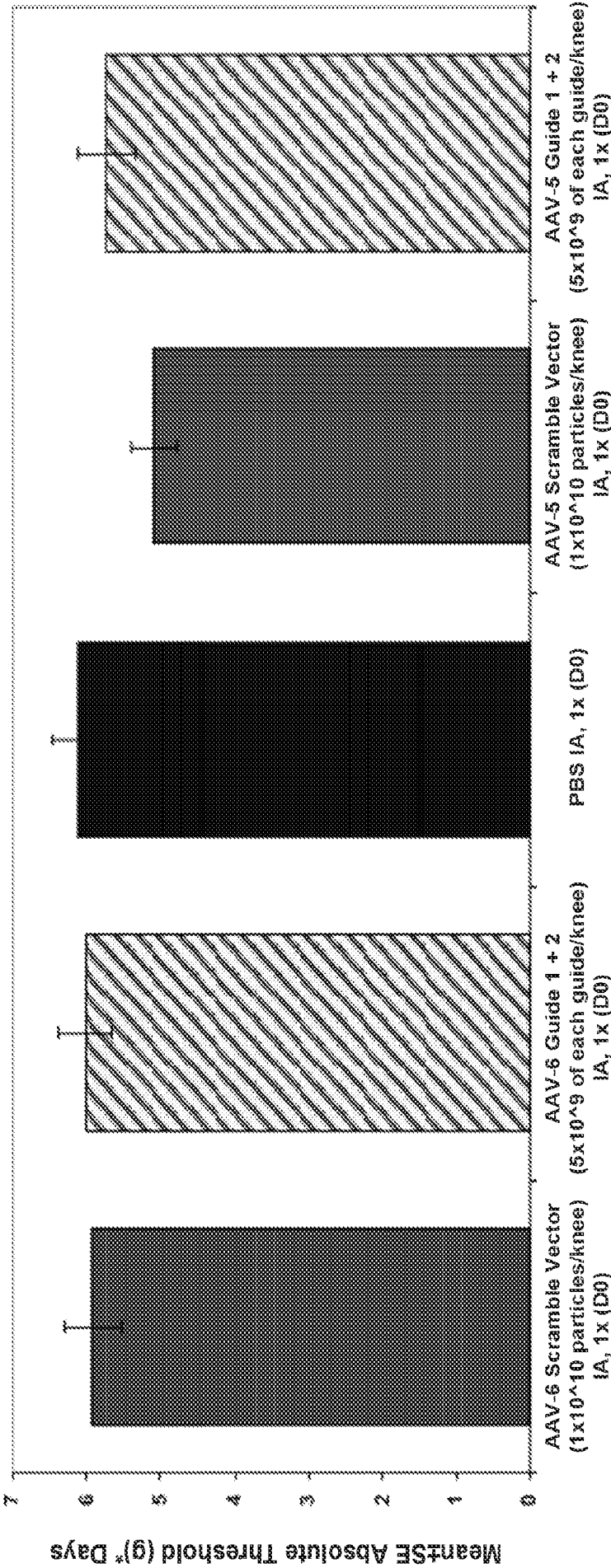


FIG. 8B

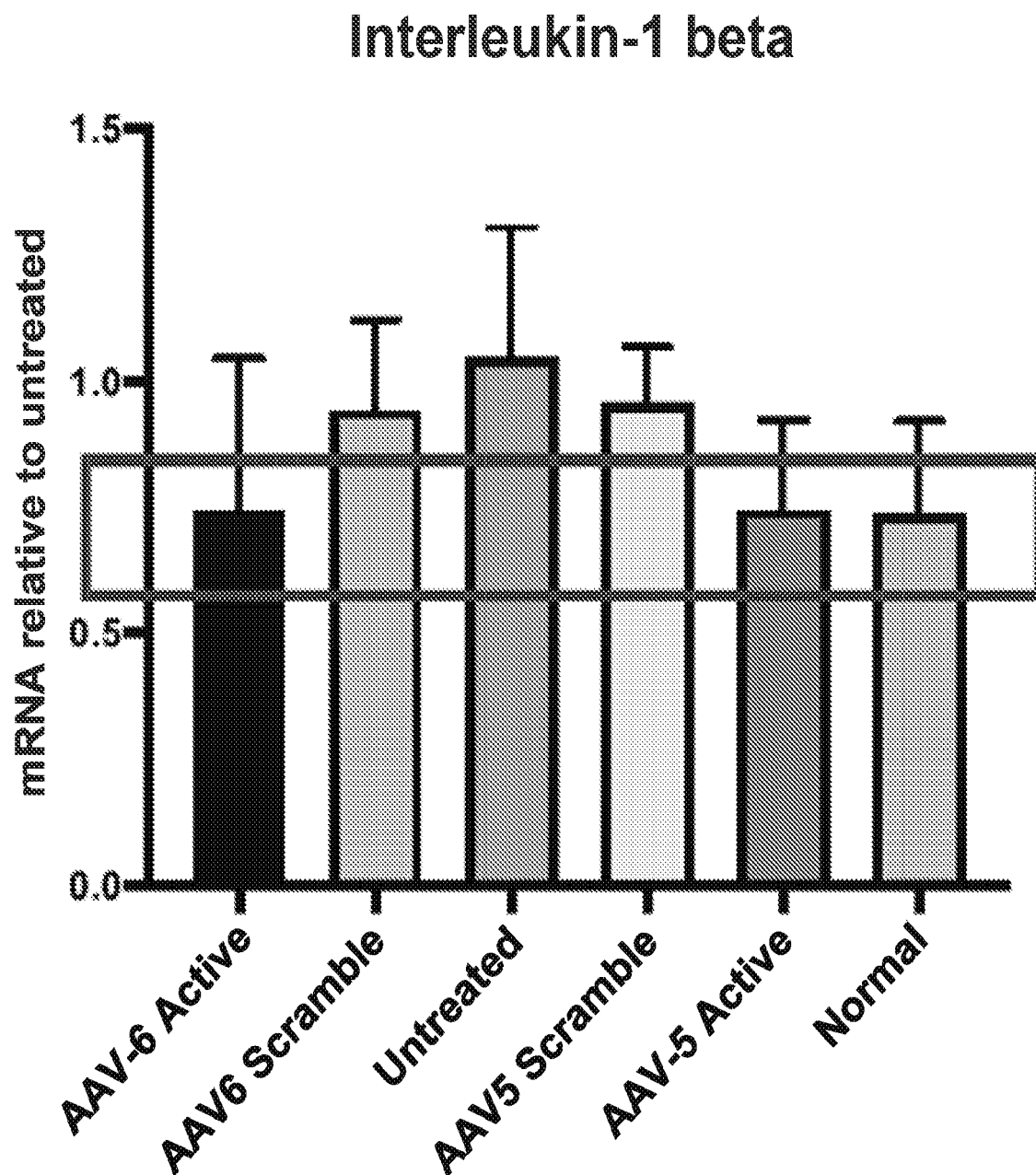


FIG. 9

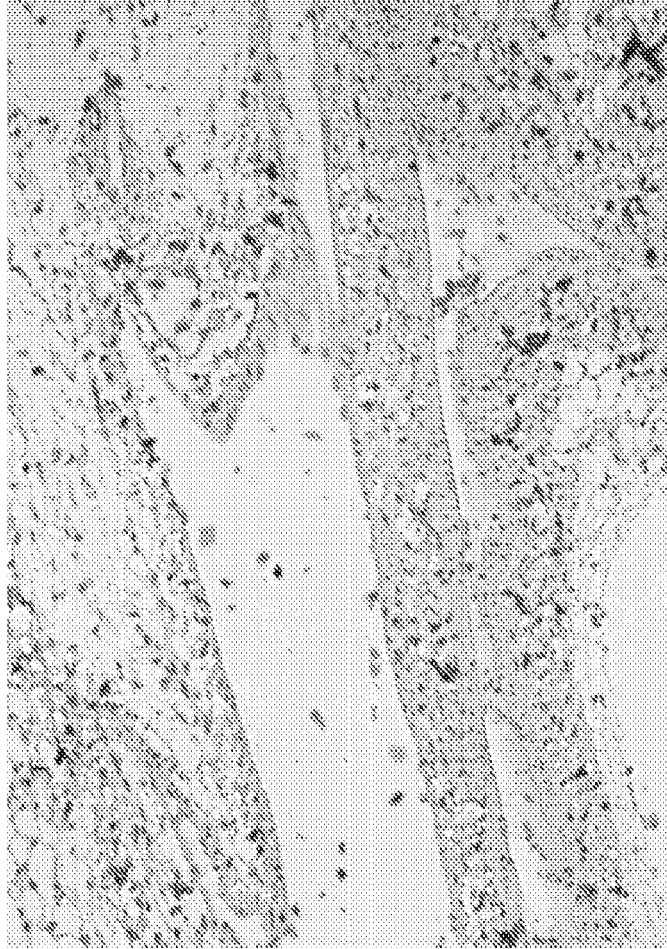


FIG. 10A

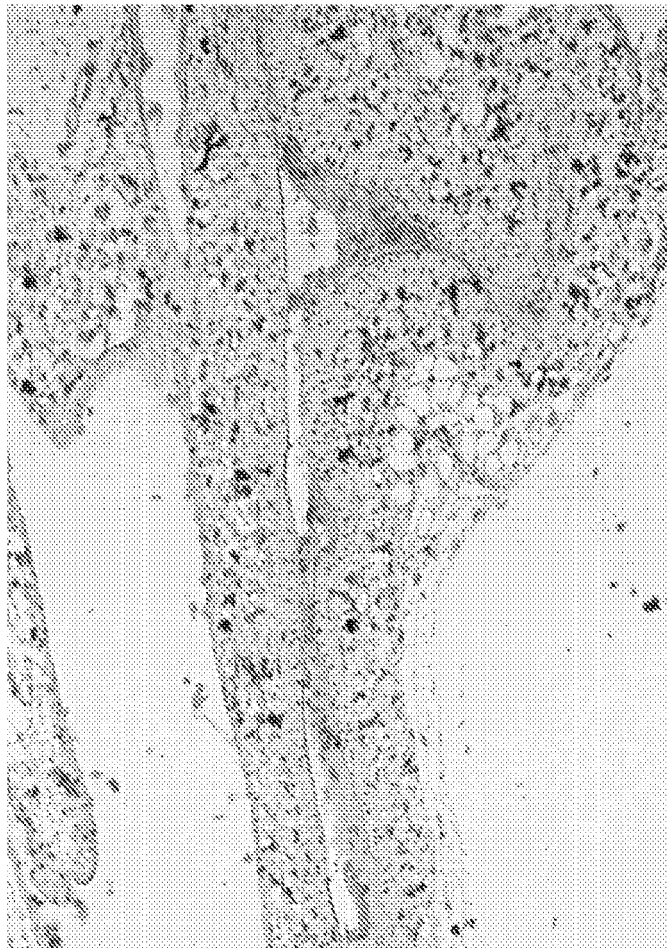


FIG. 10A

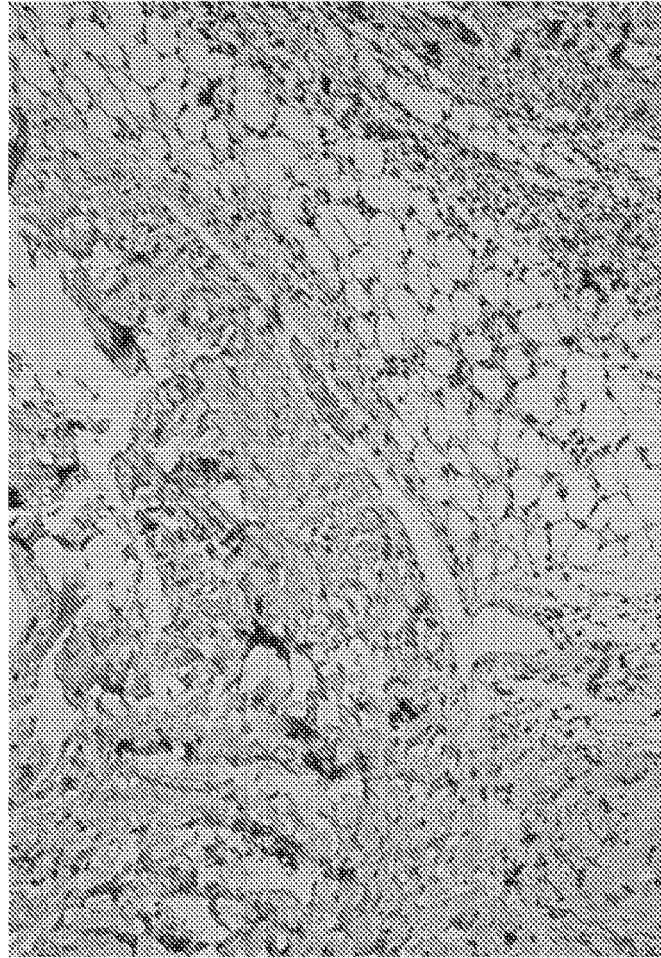


FIG. 10D

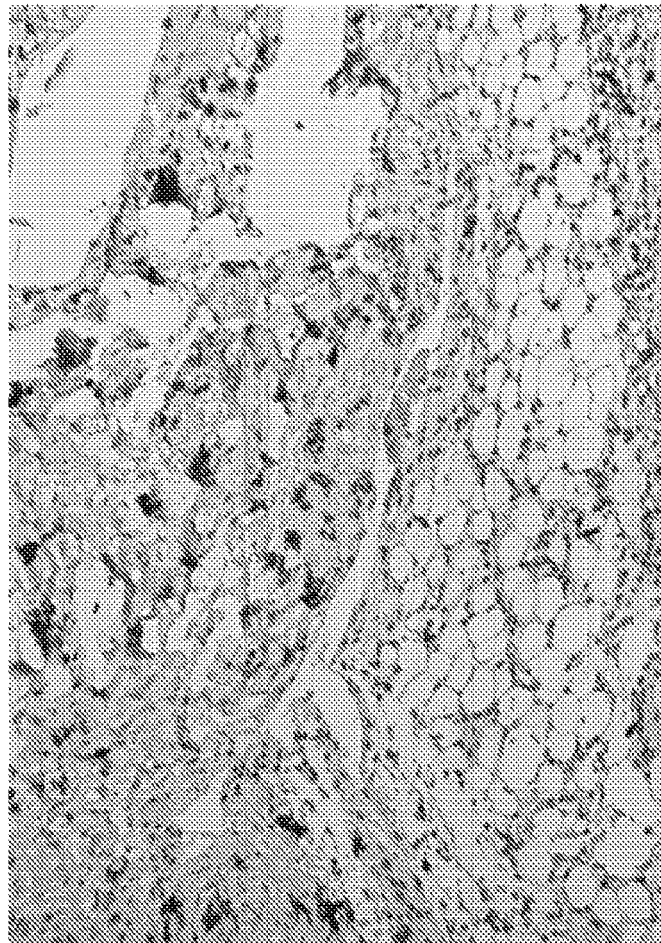


FIG. 10C

CLUSTAL O(1.2.4) multiple sequence alignment

```

IL1a-201_cds:protein_coding_Mouse  ATGGCCAAAGTTCTCTGACTTGTTTGAAGACCTAAAGAACTGTTACAGTGAAAACGGAAGAC
IL1a-201_cds:protein_coding_Human  ATGGCCAAAGTTCCAGACATGTTTGAAGACCTGAAGAACTGTTACAGTGAAAATGAAGAA
IL1a-202_cds:protein_coding_Horse  ATGGCGAAAGTCCCTGACCTCTTTGAAGACCTGAAGAACTGTTACAGTGAAAATGAAGAC
IL1a-201_cds:protein_coding_Cat    ATGGCCAAAGTTCCCTGACCTCTTTGAAGACCTGAAGAACTGTTACAGTGAAAATGAAGAA
IL1a-203_cds:protein_coding_Dog    ATGGCCAAAGTTCCCTGACCTCTTTGAAGACCTGAAGAACTGTTACAGTGAAAATGAAGAA
***** ** *** * ***** ***** ***** ***** *
TACAGTTCTGCCATTGACCATCTCTCTCTGAATCAGAAATCCTTCTATGATGCNAAGCTAT
IL1a-201_cds:protein_coding_Mouse  TACAGTTCTGCCATTGACCATCTCTCTCTGAATCAGAAATCCTTCTATGATGCNAAGCTAT
IL1a-201_cds:protein_coding_Human  GACAGTTCTCCATTGATCATCTGTCTCTGAATCAGAAATCCTTCTATGATGCNAAGCTAT
IL1a-202_cds:protein_coding_Horse  TACAGTTCTGAATGACCATCTCTCTCTGACTCAGAAATCCTTCTATGATGCNAAGCTAT
IL1a-201_cds:protein_coding_Cat    TACAGTTCTGAATGACCATCTCTCTCTGACTCAGAAATCCTTCTATGATGCNAAGCTAT
IL1a-203_cds:protein_coding_Dog    TACAGTTCTGAATGACCATCTCTCTCTGACTCAGAAATCCTTCTATGATGCNAAGCTAT
***** ***** ***** ***** ***** ***** ***** ***** *
GGCTCACTTCATGAGACTTGACAGATCAGTTTGTATCTCTGAGAACTCTGAAAACGTCA
IL1a-201_cds:protein_coding_Mouse  GGCTCACTTCATGAGACTTGACAGATCAGTTTGTATCTCTGAGAACTCTGAAAACGTCA
IL1a-201_cds:protein_coding_Human  GGCCCACTCCATGAAGCTGATGGATCAATCTGTGTCCTGAGTATCTCTGAAAACCTCT
IL1a-202_cds:protein_coding_Horse  GACCCACTTCCTGAGGACTGCATGGATACATTTATGTCCTGAGCACTCTGAAAACCTCT
IL1a-201_cds:protein_coding_Cat    GACCCACTTCATGAGGACTGTACAGATAAATTCATGTCTCCGAGTACTTCTGAAAACCTCT
IL1a-203_cds:protein_coding_Dog    GACCCACTTCATGAGGACTG-----CATGTCTCTGAGTACCTCTGAAATCTCA
* * **** * *** ** * **** * *** * ***** **
AAGATGTCCAACTTCACTTCAAGGAGAGCCGGGTGACAGTATCAGCAACGTCAAGCAAC
IL1a-201_cds:protein_coding_Mouse  AAGATGTCCAACTTCACTTCAAGGAGAGCCGGGTGACAGTATCAGCAACGTCAAGCAAC
IL1a-201_cds:protein_coding_Human  AAAACATCCAAGCTTACCTTCAAGGAGAGCATGGTGGTAGTAG-----CAACCAAC
IL1a-202_cds:protein_coding_Horse  AAGACATCCAAGCTGAACCTTCAAGGAGAGCGTGGTGGTGG-----CAGCCAAAC
IL1a-201_cds:protein_coding_Cat    AAGACACCCAGCTTACCTTCAAGAAAGATGTGGTGGTGG-----CAGCCAAAT
IL1a-203_cds:protein_coding_Dog    AAGACATCCAGCTTACCTTCAAGAAATGTGGTGGTGG-----CAGCCAAAT
** * ** * * * * **** * * * **** * * * **** *
GGCAAGATTCTGAAGAAGAGACGGCTGAGTTTCAGTGAAGACCTTCACTGAAGATGACCTG
IL1a-201_cds:protein_coding_Mouse  GGCAAGATTCTGAAGAAGAGACGGCTGAGTTTCAGTGAAGACCTTCACTGAAGATGACCTG
IL1a-201_cds:protein_coding_Human  GGGAAGGTTCTGAAGAAGAGACGGTTGAGTTTAAAGCCAAATCCATCACTGATGACCTG
IL1a-202_cds:protein_coding_Horse  GGGAAGACTCTGAAGAAGAGACGGTTGAGTTTAAATCAGTTTCACTACCAATGATGACCTG
IL1a-201_cds:protein_coding_Cat    GGGAAGATTCTGAAGAAGAGACGGTTGAGTTTAAATCAATTCCTCACTGCTGATGACCTG
IL1a-203_cds:protein_coding_Dog    GGGAAGATTCTGAAGAAGAGACGGTTGAGTTTAAAGTCAATTCATCACCGATGATGACCTG
***** *** ***** ***** ***** ***** * * * **** *

```

(Continued . . .)

FIG. 11A

IL1a-201_cds:protein_coding_Mouse	CAGTCCATAACCCATGATCTGGAA---GAGACCATCCAAACCCAGATCAGCACCTTACACC
IL1a-201_cds:protein_coding_Human	GAGGCCATGCGCAATGACTCAGAGAAGAAATCATCAAGCCTAGGTGAGCACCTTTTASG
IL1a-202_cds:protein_coding_Horse	GAAGCCATTGCCAATGATCCAGAAGAAGAAATCATCAAGCCCCGATCAGTACATTACAAC
IL1a-201_cds:protein_coding_Cat	GAAGCCATTGCCAATGATCCAGAAGAAGAAATCATCAAGCCCCGATCAGTACAGCACCCAAC
IL1a-203_cds:protein_coding_Dog	GAAGACATTGCCAATGACACAGAAGAAGTAATCATCAAGCCCCAGATCAGTAGCATACAAC
	* *** ** * ** ** ** * * * * * * * * * * * * *
IL1a-201_cds:protein_coding_Mouse	TACCAGAGTGATTGAGATACAAACTGATGAAGCTCGTCAGGCAGAAAGTTTGTCAATGAAT
IL1a-201_cds:protein_coding_Human	TTCTGTGAGCAATGTGAATACAACTTTATGAGGATCATCAAAATACGAATTCATCTCTGAAT
IL1a-202_cds:protein_coding_Horse	TTCCAGAGCAATACAAATACAACTTTATGAGGATCGTCAACCCAGTGTACTCTGAAT
IL1a-201_cds:protein_coding_Cat	TTCTATAGCAGTGAAAAATACAACTATCAGAAGATCATCAAAATCCCAATTCATCTCTGAAT
IL1a-203_cds:protein_coding_Dog	TTCCATAACAATGAAAAATACAACTATATAAGGATCATCAAAATCCCAATTCATCTCTGAAT
	* *
IL1a-201_cds:protein_coding_Mouse	GATTCCTCAACCAAACTATATATCAGGATGTGGACAAACACTATCTCAGCACCACTTGG
IL1a-201_cds:protein_coding_Human	GATGCTCTCAATCAAAGTATAATTCGAGCCAA-----TGATCAGTACCTCAGSGCTGCTGCA
IL1a-202_cds:protein_coding_Horse	CATGCTCTCAATCAAAGTATAATTCGAGCCAA-----TGATCAGTACCTCAGSGCTGCTGCA
IL1a-201_cds:protein_coding_Cat	GACAACTCTCAGTCAAGTGTAAATTCGAAAGCAGGGGGGAAATACCTCGCAGCCGCTGCA
IL1a-203_cds:protein_coding_Dog	GACAACTCTCAATCAAAGTATAATTCGACAAACACAGGAGGAAATACCTCATGACTGCTGCA
	** ***** **** * ** * * * * * * * * * * *
IL1a-201_cds:protein_coding_Mouse	TTAAATGACCTGCAACAGGAAGTAAATAATTGACATGTATGCCCTACTCGTCGGGAGGAGAC
IL1a-201_cds:protein_coding_Human	TTACATAATCTGGATGAAGCAGTGAATTTGACATGGTGCTTATAAGTATCAAAAGGAT
IL1a-202_cds:protein_coding_Horse	TTAAATAATCTGGACGACGAGTGAATTTGACATGGTGCTTATAAGTATCAAAAGGAT
IL1a-201_cds:protein_coding_Cat	TTACAGAAATCTGGATGATGAGTGAATTTGACATGGGGGCTTATACATCAA-----AAGAA
IL1a-203_cds:protein_coding_Dog	TTACAGAAATTTGGATGATGAGTGAATTTGACATGGGAGCTTACACATCA-----GAA
	*** *
IL1a-201_cds:protein_coding_Mouse	GACTCTAAATATCCTGTTACTCTAAAAATCTCAGATTCACAACTGTTGTTGAGCGCTCAA
IL1a-201_cds:protein_coding_Human	GATGCTAAATATACCGTGATTTAAGAAATCTCAAAAATCTCAAAATGTATGTGACTGCCCAA
IL1a-202_cds:protein_coding_Horse	GATTCCTCAACTTCTGTGACTCTAAGAAATCTCAAAAATCTCGACTGTTTGTGAGTGCCCAA
IL1a-201_cds:protein_coding_Cat	GATTCCTCAACTTCTGTGACTCTCAGAAATCTCAAAAATCTCGACTGTTTGTGAGTGCCCAA
IL1a-203_cds:protein_coding_Dog	GATTCCTCAACTTCTGTGACTCTAAGAAATCTCAAAAATCTCGACTGTTTGTGAGTGCCCAA
	** ** ** * * * * * * * * * * * * * * * * * * *

(Continued . . .)

FIG. 11B

```

IL1a-201_cds:protein_coding_Mouse  GGAGAAGACCGAGCCCGTGTGCTGAAGGAGTTGCCAGAAACACCAAAACATCATCACAGGT
IL1A-201_cds:protein_coding_Human  GATGAAGACCAACACGATGCTGCTGAAGGAGATGCCCTGAGATACCCAAAACCATCACAGGT
IL1A-202_cds:protein_coding_Horse  AATGAAGATGAACCCGTAATCTCTAAAGGAGATGCCCTGACACACCCAAAACATATCAAAG--
IL1A-201_cds:protein_coding_Cat    AATGAAGATGAGCCCTGTATTGCTTAAGGAGATGCCCTGAGACACCCAAAACCATCACAG--
IL1A-203_cds:protein_coding_Dog    AATGAAGATGAACCTGTATTGCTTAAGGAGATGCCCTGAGACACCCAAAACATATCACAG--
                                     *****  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
IL1a-201_cds:protein_coding_Mouse  AATGAAGACCGAGCCCTCATTTTCTTCTGGAAAMGTAATCMCTCTAAGAACTACTTCACATCA
IL1A-201_cds:protein_coding_Human  AGTGAGACCAACCTCCTCTTCTTCTGGAAACTCAGGCACCTAAGAACTATTTACATCA
IL1A-202_cds:protein_coding_Horse  -AATGAGACCAACCTCCTCTTCTTCTGGAAACGTCACGGCTCTAAGAACTACTTCAAATCG
IL1A-201_cds:protein_coding_Cat    -AATGAGACCAACCTCCTCTTCTTCTGGAAACGTCATGGCACTAAGAACTACTTCAAATCA
IL1A-203_cds:protein_coding_Dog    -AATGAGACCAACCTCCTTCTTTTGGGAGCGTCATGGCAGTAAGCACTACTTCAAATCA
                                     *****  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
IL1a-201_cds:protein_coding_Mouse  GCTGCTTATCCAGAGCTGTTTATTGGCCACCAAMGAACAAAGTGGGTGCACCTGGCACGG
IL1A-201_cds:protein_coding_Human  GTTGCCCATCCAAACTTGTATTATGGCCACAAAGCAAGACTACTGGGTGTGCTTGGCAGGG
IL1A-202_cds:protein_coding_Horse  GTTGCCCATCCAAAGTTGTTATTGGCCACAAAGCAGGGAAAACTGGTGCCACATGGCAAGG
IL1A-201_cds:protein_coding_Cat    GTTGCCCATCCAAAGTTGTTATTGGCCACACAGGAAGAACTGGTGCCACATGGCAAGA
IL1A-203_cds:protein_coding_Dog    GTTGCCCATCCCAAGTTGTTATTGGCCACACAGGAACGAAACTGGTGCCACATGGCAAGA
                                     *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
IL1a-201_cds:protein_coding_Mouse  GGACTGCCCTCTATGACAGACTTCCAGATATCATAA----- (SEQ ID NO: 591)
IL1A-201_cds:protein_coding_Human  GGGCCACCTCTATCACTGACTTTCAGATACTGGAACACGAGCGTAG (SEQ ID NO: 592)
IL1A-202_cds:protein_coding_Horse  GGGCAACCTCTATCACTGACTTTCAGATACTGGACAACCACTTTTGA (SEQ ID NO: 593)
IL1A-201_cds:protein_coding_Cat    GGACTACCTCTCTCACTGACTTTCAGATACTGGAAACCACTCTTGA (SEQ ID NO: 594)
IL1A-203_cds:protein_coding_Dog    GGGCAACCTCTATCACTGACTTTCGTTTACTGGAAACCACTTGA (SEQ ID NO: 595)
                                     **  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

```

FIG. 11C


```

IL1b-201_cds:protein_coding_Mouse      CTTTGACCTGGGCTGTCCT---GATGAGAGCATCCAGCTTCAATCTCGCAGCAGCAGCAT
IL1b-201_cds:protein_coding_Human      CCTGGACCTCTGCCCTCTG-----GATGGGGCCATCCAGCTACGAATCTCCGACCCACTA
IL1b-201_cds:protein_coding_Dog        CCTGAACCAACAGTTCTCTGTGATGAGGGGCATCCAGTTGCAAGTCTCCACCAAGCTCTG
IL1b-201_cds:protein_coding_Cat        CCTGAGCCACAGTTTCTCTGGAGATGAGGGGCATCCAGTTGCAAAATCTCCACCAAGCCCGA
IL1b-203_cds:protein_coding_Horse      CCTGGACCTCAGCTCCATGGCGATGGGGGCATCCAGCTTCAATTTCTCCCAACACTTTA
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
IL1b-201_cds:protein_coding_Mouse      CAACAAGAGCTTCAGGCAGGCAGTATCACTCATTTGTGGCTGTGGAGAAGCTGTGGCAGCT
IL1b-201_cds:protein_coding_Human      CAGCAAGGGCTTCAGGCAGGGCCGCTCAGTTGTTGTGGCCATGGACAAGCTGAGGAAGAT
IL1b-201_cds:protein_coding_Dog        TAACAAGAGTCTGAGGCATTTGCTGTCACTCAGTCAATGTAGCTTTGGAGAAGCTGAAGAAG---
IL1b-201_cds:protein_coding_Cat        CAACAAGAGTCTTAGGCATGCCGTGTCGGTCAATTTGTGGCCATGGAGAAACTGAAGAAGAT
IL1b-203_cds:protein_coding_Horse      CAACAAGACTTTCAGGCATGTCTGTCTCAATCAATTTGTGGCTATGGAGAAGCTGAAGAAGAT
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
IL1b-201_cds:protein_coding_Mouse      ACCTGTGTCTTTCCGTTGGACCTTCCAGGATGAGGACATGAGCACCTTCCTTTCCCTTCAT
IL1b-201_cds:protein_coding_Human      GCTGGTTCCCTGCCACAGACCTTCCAGGAGATGACCTGAGCACCTTCCTTTCCCTTCAT
IL1b-201_cds:protein_coding_Dog        -----CCCTGCCACAGTTCCTCCAGGAGGATGACCTGAGAGCATCTTTTGGCTACAT
IL1b-201_cds:protein_coding_Cat        ATCTTTTCCCTGTCTCAACCCCTCCAGGATGAGGACCTGAGAGCCCTCTTTTGTGTCAT
IL1b-203_cds:protein_coding_Horse      ACCCGTTCCCTGTCTCACAGGCCCTTCCAGGATGATGACTTGAGGAGCCCTCTTTTCTGTCTCAT
* * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
IL1b-201_cds:protein_coding_Mouse      CTTTGAAGAAGAGCCCATCCTCTGTGACTCATGGGATGATGATGAATACCTGCTGGTGTG
IL1b-201_cds:protein_coding_Human      CTTTGAAGAAGAAACCTATCTTCTTGACACATGGGATAACGAGGCTTATGTG-----CA
IL1b-201_cds:protein_coding_Dog        CTTTGAAGAAGAAACCTATCACTCTGCAAAACAGATGCGG---ATAATTTTATG-----AG
IL1b-201_cds:protein_coding_Cat        CTTTGAAGAAGAAACCCATCATCTGTGACACACGTGGATG---ACGGTTTTGTG-----TG
IL1b-203_cds:protein_coding_Horse      CTTTGAAGAAGAAACCCATCATCTGTGACAACTGGGATG---ATGATTATGTG-----TG
***** * * * * * * * * * * * * * * * * * * * * * * * * * * *
IL1b-201_cds:protein_coding_Mouse      TGACGTTCCCATTTAGACAACCTGCACCTACAGGCTCCGAGATGAACAACAAAAAGCCTCGT
IL1b-201_cds:protein_coding_Human      CGATGCACCTGTAGCATCACTGAACCTGCACGCTCCGGGACTCACAGCAAAAAGCTTGGT
IL1b-201_cds:protein_coding_Dog        TGATGCAGCCCATGCAATCGGTGGACTGCAAGTTACAGGACATAAGCCACAATACCTGGT
IL1b-201_cds:protein_coding_Cat        TGATGCGGCCATACAAATCACAGGACTACAGCTTCCGAGACATAAGCCAAAAGAGCCTGGT
IL1b-203_cds:protein_coding_Horse      TGATGCAGCTGTGCATTCAGTGAACCTGCAGACTCCGGGACATATACCATAAATCCCTGGT
** * * * * * * * * * * * * * * * * * * * * * * * * * * *
(Continued . . .)

```

FIG. 12B

(Continued . . .)

```

IL1b-201_cds:protein_coding_Mouse      CTGGTACATCAGCACCTCAAGCAGAGCACAAGCCTGTCTTCTCTGGGAAACAACAGTGG
IL1b-201_cds:protein_coding_Human      CTGGTACATCAGCACCTCTCAAGCAGAAAACATGCCGTCTTCTCTGGAGGACCAAAAGG
IL1b-201_cds:protein_coding_Dog        CTGGTACATCAGCACCTCTCAAGTCGAAGGAATGCCGTCTTCTCTAGGAAATACCAAGG
IL1b-201_cds:protein_coding_Cat        CTGGTACATCAGCACCTCTCAAGCAGAGAATAATGCCGTCTTCTCTAGGAAATACCAAGG
IL1b-203_cds:protein_coding_Horse      CTGGTACATCAGCACCTCTCAAGCAGAAAAAAGCCTGTCTTCTCTAGGAAATACCAAGG
*****
IL1b-201_cds:protein_coding_Mouse      T---CAGGACATAATTGACTTCACCATGGAAATCCGTGTCTTCCTAA (SEQ ID NO:596)
IL1b-201_cds:protein_coding_Human      CGGCCAGCATATAACTGACTTCACCATGCAATTTGTGTCTTCCTAA (SEQ ID NO:597)
IL1b-201_cds:protein_coding_Dog        TGGCCAGGATATAACTGACTTCACCATGGAAATCTCTCTCTAG---- (SEQ ID NO:598)
IL1b-201_cds:protein_coding_Cat        TGGTCAGGATATAACTGACTTCATCATGGAAGGGCTTCCTAA---- (SEQ ID NO:599)
IL1b-203_cds:protein_coding_Horse      CGGCCGGGACATAAATGACTTCATCATGGAATCACCCTCTGCCTAA (SEQ ID NO:600)
* *** ***** **
* *** ***** **
* *** ***** **

```

FIG. 12D

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1A	UCACCAGUGAAAUUGACAU	298	GGG	-	4	6	1	6
IL1A	AUGGUGGUAGUAGCAACCAA	299	CGG	-	3	14	2	4
IL1A	AGCCAAUGAUCAGUACCUCA	300	CGG	-	8	15	3	5
IL1A	CAGAGACAGAUCAUAUGG	301	AGG	+	6	18	4	3
IL1A	UGAGACUCCAGACCUACGCC	302	TGG	+	24	2	5	7
IL1A	UUCACCAGUGAAAUUUGACA	303	TGG	-	16	19	6	6
IL1A	UUUAGAAAUCAUCAAGCCU	304	AGG	-	21	16	7	5
IL1A	UAUGUAAUGCAGCAGCCGUG	305	AGG	+	5	35	8	5
IL1A	UGGUGGUAGUAGCAACCAAC	306	GGG	-	38	3	9	4
IL1A	GGCCAUCGCCAAUGACUCAG	307	AGG	-	11	34	10	4
IL1A	AUGUGAAAUACAACUUUAUG	308	AGG	-	37	10	11	5
IL1A	GCCAUAGCUUACAUGAUAGA	309	AGG	+	42	5	12	4
IL1A	AGCUAUGGCCCCACUCCAUAGA	310	AGG	-	23	26	13	4
IL1A	AUCUGAAAGUCAGUGAUAGA	311	GGG	+	41	9	14	7
IL1A	AGCCGUGAGGUACUGAUCAU	312	TGG	+	39	13	15	5
IL1A	AUUCAGAGACAGAUCAUCAA	313	TGG	+	47	7	16	3
IL1A	UGAAAGUCAGUGAUAGAGGG	314	TGG	+	7	48	17	7
IL1A	UGGCAAUAAACAAGUUUGGA	315	TGG	+	45	12	18	7
IL1A	GCACCCAUUGUCAAAUUCAC	316	TGG	+	27	31	19	6
IL1A	UUCUCUGAGUCAUUGGCCGA	317	TGG	+	30	29	20	4
IL1A	AUCGCCAUGACUCAGAGGA	318	AGG	-	13	54	21	4
IL1A	CUCUUCUUCUGGGAAACUCA	319	CGG	-	20	49	22	7
IL1A	AAGCUAAAAGGUGCUGACCU	320	AGG	+	10	59	23	5
IL1A	CUCGAAUUAUACUUUGAUUG	321	AGG	+	50	20	24	5
IL1A	UGGAAAACACAGGCGUAGGUC	322	TGG	-	70	1	25	7

FIG. 13A

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1A	CUUCUUCAGAACCUUCCCGU	323	TGG	+	34	37	26	4
IL1A	GUUGGUCUCACUACCUUGA	324	TGG	+	44	28	27	7
IL1A	UCCUUCUACUAGUAAAGCUA	325	TGG	-	55	17	28	4
IL1A	GAUACUGGAAACCCAGGCGU	326	AGG	-	12	63	29	7
IL1A	UACCUUCAAGGAGAGCAUGG	327	TGG	-	1	74	30	4
IL1A	AGACCAACCCAGUGGUCUGA	328	AGG	-	9	68	31	6
IL1A	UAUCUGAAAGUCAGUGUAG	329	AGG	+	35	43	32	7
IL1A	GGCAUAACAAGUUUGGAU	330	GGG	+	67	11	33	7
IL1A	CAUCACUGAUGAUGACCUUG	331	AGG	-	2	78	34	4
IL1A	GAGAUCCCAAAACCAUCAC	332	AGG	-	31	50	35	7
IL1A	UUUUGAGAUUCUUAGAUCA	333	CGG	+	61	22	36	6
IL1A	UUGCCACAAAGCAAGACUAC	334	TGG	-	51	33	37	7
IL1A	UGACCUUCAGCAGCACUGGU	335	TGG	+	25	60	38	6
IL1A	CUUUCAGAUACUGGAAACC	336	AGG	-	22	64	39	7
IL1A	CCAUGCAGCCUUCUAGGAGU	337	GGG	+	17	69	40	4
IL1A	GGUAAGCUUGGAUGUUUUG	338	AGG	+	81	8	41	4
IL1A	UUACAUAUUCUGGAUGAAGC	339	AGG	-	29	61	42	5
IL1A	GGCUGCUGCAUUAACAUAUC	340	TGG	-	86	4	43	5
IL1A	ACAUUGCUCAGGAGGCUAAA	341	AGG	+	68	23	44	5
IL1A	GUUACCUUCAAAGGAGAGCA	342	TGG	-	26	65	45	4
IL1A	UUUGUGGCAUAUAAACAAGUU	343	TGG	+	60	32	46	7
IL1A	CUCCAGGUCAUCAUCAGUGA	344	TGG	+	19	73	47	4
IL1A	CACCCAGUAGUCUUGCUUUG	345	TGG	+	49	44	48	7
IL1A	GAGUUUCCCAAGAGAGAGG	346	AGG	+	18	76	49	7
IL1A	AGUUGUAUUUCCACAUGGCU	347	AGG	+	56	38	50	5

FIG. 13B

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1A	GGUCAUCAUCAGUGAGGGAU	348	TGG	+	54	41	51	4
IL1A	UUCAAACAUGUCUGGAACUU	349	TGG	+	75	21	52	2
IL1A	GGUAGUAGCAACCAACGGGA	350	AGG	-	14	85	53	4
IL1A	UCCAUUGCAGCCUUCUAGGAG	351	TGG	+	33	66	54	4
IL1A	UCGAUUUAUACUUUGAUUGA	352	GGG	+	79	24	55	5
IL1A	GGAGGUUCUGAAGAGAGAGA	353	CGG	-	15	88	56	4
IL1A	UUCAGGUCUUCACAAACAUUC	354	TGG	+	77	27	57	2
IL1A	ACUACUGGGUGUGCUUGGCA	355	GGG	-	63	42	58	7
IL1A	AACAUCCCAAGCUUACCUUCA	356	AGG	-	76	30	59	4
IL1A	CGUGAGUUUCCCAAGAGAG	357	AGG	+	36	71	60	7
IL1A	UUUGAUUGAGGGCGUCAUUC	358	AGG	+	82	25	61	5
IL1A	AUCCAUCACUGAUGAUGACC	359	TGG	-	52	55	62	4
IL1A	UUCCAGAGAGAGAGGAGGU	360	TGG	+	28	80	63	7
IL1A	GCUCUCCUUGAAGGUAAAGCU	361	TGG	+	57	51	64	4
IL1A	ACUGGGUGUGCUUGGCAGGG	362	GGG	-	40	72	65	7
IL1A	GGGUGCUUUAUAGUCAUCA	363	AGG	-	32	83	66	6
IL1A	UGAUCUUCUGUCUCUGAUC	364	AGG	-	69	46	67	3
IL1A	CUGAAGACUGUUACAGGUA	365	AGG	-	62	58	68	2
IL1A	AAGACCUGAAGAACUGUUAC	366	AGG	-	84	36	69	2
IL1A	UACCACCAUGUCUCCUUGA	367	AGG	+	65	57	70	4
IL1A	GCAAGACUACUGGGUGUGCU	368	TGG	-	43	81	71	7
IL1A	AUUGAUCCAUGCAGGCCUUNCA	369	TGG	+	73	52	72	4
IL1A	CAAUGACUCAGAGGAAGGUA	370	AGG	-	64	62	73	4
IL1A	CUUACCUUCCUUGAGUCAU	371	TGG	+	59	67	74	4
IL1A	UAUCACUGACUUUCAGAUAC	372	TGG	-	74	53	75	7

FIG. 13C

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1A	CCCACUCCAUGAAGGCUGCA	373	TGG	-	46	82	76	4
IL1A	CUCACUACCCUGUGAUGGUUU	374	TGG	+	90	39	77	7
IL1A	UCACUACCCUGUGAUGGUUU	375	GGG	+	89	40	78	7
IL1A	CACUGGUUGGUUUCUUCU	376	GGG	+	83	47	79	6
IL1A	CUUACCUUGUAACAGUUCUUC	377	AGG	+	87	45	80	2
IL1A	AGUCAUUGGCGAUGGCCUCC	378	AGG	+	48	90	81	4
IL1A	UGCCACAAAGCAGACUACU	379	GGG	-	53	86	82	7
IL1A	GACUACUGGGUGUGCUUGGC	380	AGG	-	85	56	83	7
IL1A	CAACUGACCUUCAGCAGCAC	381	TGG	+	58	87	84	6
IL1A	CUACUGGGUGUGCUUGGCAG	382	GGG	-	72	75	85	7
IL1A	UACUGGGUGUGCUUGGCAGG	383	GGG	-	66	84	86	7
IL1A	GCACUGGUUGGUUUCUUCU	384	TGG	+	80	70	87	6
IL1A	GACCAACCCUUCUUCUUCU	385	GGG	-	78	77	88	7
IL1A	GUGAUGGUUUUGGGUAUCUC	386	AGG	+	71	89	89	7
IL1A	AGACCAACCCUUCUUCUUC	387	TGG	-	88	79	90	7

FIG. 13D

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1B	CUUCGACACAUGGGAAUAAAG	388	AGG	-	8	7	1	5
IL1B	GUCAGAUUCAGUGAUCGUAC	389	AGG	+	11	8	2	5
IL1B	CAUGGCCACAACAACUGACG	390	CGG	+	4	18	3	4
IL1B	GGUGGUCGAGAUUCGUAGC	391	TGG	+	32	1	4	4
IL1B	GGACUCACAGCAAAAAGCU	392	TGG	-	21	16	5	5
IL1B	ACCUAUCUUCUUCGACACAU	393	GGG	-	15	25	6	5
IL1B	AGGUCUGAUGUACCAAGUUG	394	GGG	+	9	32	7	7
IL1B	CUUAVCAUCUUUCAAACACGC	395	AGG	+	12	31	8	6
IL1B	UCGGACCAACACUACAGCAA	396	GGG	-	13	30	9	4
IL1B	GUAAUAAGCCAUCAUUUAC	397	TGG	+	42	5	10	2
IL1B	GAGGUCUGAUGUACCAAGU	398	GGG	+	31	19	11	7
IL1B	CUGAAGCUCUCCACCUCCA	399	GGG	-	14	37	12	5
IL1B	AACCUAUCUUCUUCGACACA	400	TGG	-	28	24	13	5
IL1B	UUUUUAUUACAGUGGCAAG	401	AGG	-	6	46	14	3
IL1B	UUCUCCAUGUCCUUUGUACA	402	AGG	-	30	23	15	6
IL1B	AAGUAAUGACAAAUAACCU	403	TGG	-	3	57	16	6
IL1B	AACAUGCCCGUCUCCUGGG	404	AGG	-	10	51	17	7
IL1B	GGACAGGAUAUGGAGCAACA	405	AGG	-	2	61	18	5
IL1B	UUAGGAAGACACAAAUUGCA	406	TGG	+	26	38	19	7
IL1B	GAGCUCAGGUACUUCUGCCA	407	TGG	+	29	36	20	2
IL1B	UUCUCCUUGUACAAAGGACA	408	TGG	+	53	13	21	6
IL1B	AUCACUGAACUGCAAGCUCC	409	GGG	-	56	10	22	5
IL1B	UGAAGGGAAAAGAGGUGCUC	410	AGG	+	50	17	23	4
IL1B	CAUCUUUCAACACGCAGGAC	411	AGG	+	66	2	24	6
IL1B	CUCCGACCACCACUACAGCA	412	AGG	-	20	49	25	4

FIG. 14A

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1B	UGAUAAGCCACUCUACAGC	413	TGG	-	67	3	26	6
IL1B	GCAGGCGGUGACUUGUUG	414	TGG	-	60	11	27	4
IL1B	UAAGCCACUCUACAGCUGG	415	AGG	-	18	54	28	6
IL1B	CUUACCUCCAGCUGUAGAGU	416	GGG	+	61	12	29	6
IL1B	GCUCCAUUCCUGUCCUGG	417	AGG	+	7	73	30	5
IL1B	UUUCCUUCAUCUUUGAAGA	418	AGG	-	54	28	31	4
IL1B	GCCUUUGCUGUAGUGGUGGU	419	CGG	+	23	60	32	4
IL1B	GCUGGAUGCCGCCAUCCAGA	420	GGG	+	19	64	33	4
IL1B	UUCUUGGAGGGACCAAGG	421	CGG	-	25	59	34	7
IL1B	ACUUACCUCCAGCUGUAGAG	422	TGG	+	43	41	35	6
IL1B	UUGUGGCCAUGGACAAGCUG	423	AGG	-	5	79	36	4
IL1B	GAAACAUGCCCGUCUCCU	424	GGG	-	64	22	37	7
IL1B	GGGCAUGUUUCUGCUUGAG	425	AGG	+	38	48	38	7
IL1B	UGGUGAAGUCAGUUUAUCC	426	TGG	+	68	20	39	7
IL1B	UCCCAUGUGUGGAGAAGAU	427	AGG	+	51	39	40	5
IL1B	UGAAGCCUUGCUGUAGUGG	428	TGG	+	22	68	41	4
IL1B	UGAGCUCGCCAGUGAAUAGA	429	TGG	-	58	33	42	2
IL1B	GAUCACUGAACUGCAGCUC	430	CGG	-	52	40	43	5
IL1B	AUCAUUUACUUGGCGAGCUC	431	AGG	+	84	9	44	2
IL1B	UUGGUCCUCCAGGAAGAC	432	GGG	+	49	45	45	7
IL1B	GACCUUGCCUCCUGGAUGG	433	CGG	-	17	77	46	4
IL1B	ACUACCUUCUCCAAAGAUGA	434	AGG	+	69	29	47	4
IL1B	GUGAAUUGAUGGCUUAUAC	435	AGG	-	94	4	48	2
IL1B	AGCUUUUUUGCUGUAGUCC	436	CGG	+	73	26	49	5
IL1B	ACAUGCCCGUCUCCUGGGA	437	GGG	-	24	76	50	7

FIG. 14B

Fig. 14C

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1B	AGGUGCUCAGGUCAUUCUCC	463	TGG	+	74	65	76	4
IL1B	AAAUUACCCAAAGAAGAAGA	464	TGG	-	44	96	77	7
IL1B	UUCAAAGAUGAAGGGAAGA	465	AGG	+	48	95	78	4
IL1B	CUGGACCUCUGCCUCUGGA	466	TGG	-	72	72	79	4
IL1B	GAUAGAAUCAAUACAAGC	467	TGG	-	55	90	80	7
IL1B	ACCACUACAGCAAGGCUUC	468	AGG	-	95	50	81	4
IL1B	AGUCUGCCAGUUCGCCAAC	469	TGG	-	91	55	82	7
IL1B	UCCUGGAAGGUCUGGGCA	470	GGG	+	65	81	83	4
IL1B	GUCUCCUGGGAGGACCAA	471	AGG	-	45	102	84	7
IL1B	UGAUGUACAGUUGGGGAAC	472	TGG	+	96	53	85	7
IL1B	GUCUACCUUUAUCUGUUUA	473	GGG	+	106	44	86	3
IL1B	ACCUGGSCUUGGGCCUCA	474	AGG	-	85	66	87	6
IL1B	UUUGGUCCUCCAGGAAGA	475	CGG	+	77	78	88	7
IL1B	GCCUGAAGCCUUGCUGUAG	476	TGG	+	99	56	89	4
IL1B	GCAGAGGUCCAGGUCCUGGA	477	AGG	+	47	109	90	4
IL1B	CCACCUCAGGACAGGAUA	478	TGG	-	101	58	91	5
IL1B	AGCUCUCCACCUCAGGGAC	479	AGG	-	75	87	92	5
IL1B	UCCUGGCCACAGACCUUCC	480	AGG	-	90	74	93	4
IL1B	GCAGUGCUCCUCCAGGACC	481	TGG	-	83	84	94	4
IL1B	CCAUUCCUGUCCUCCAGG	482	TGG	+	89	80	95	5
IL1B	GACAAAUACCUUGGCCUU	483	GGG	-	71	99	96	6
IL1B	CUCUGGAAGGUCUGUGGC	484	AGG	+	81	94	97	4
IL1B	CGGUCAGUUGUGGGCCA	485	TGG	-	70	105	98	4
IL1B	AGUUAUACCUGGCCGCCUU	486	TGG	+	92	89	99	7
IL1B	GGCCGCCUUUGGUCCUCC	487	AGG	+	78	103	100	7

FIG. 14D

Gene	crRNA sequence	SEQ ID NO:	PAM	Strand	On-target ranking	Off-target ranking	Combined ranking	Exon
IL1B	CAUCCAGAGGGCAGAGGUCC	488	AGG	+	88	93	101	4
IL1B	GAGGGCAGAGGUCCAGGUCC	489	TGG	+	97	86	102	4
IL1B	GGGAGGGACCAAGGCGGCC	490	AGG	-	79	106	103	7
IL1B	GACUUGUUCUUUGAAGCUGA	491	TGG	-	80	107	104	3
IL1B	CGUUUUUCCAUCUUCUUCUU	492	TGG	+	107	88	105	7
IL1B	GGACCGGGACCUCUGCCUC	493	TGG	-	93	104	106	4
IL1B	CUUCUUCUUUGGUAUUUU	494	TGG	+	108	91	107	7
IL1B	GUUUUUCCAUCUUCUUCUU	495	GGG	+	104	97	108	7
IL1B	UUCUUCUUUGGUAUUUUU	496	GGG	+	109	108	109	7

FIG. 14E

Gene	crRNA	SEQ ID NO:	PAM	Exon	Strand	On-target (Azimuth2)	Off-target	Precision (Delphi)	Frameshift (Delphi)	Combined (Off + Pre + Frames)
IL1b	GAAGUGAAGGUGACAGACCA	497	TGG	3	+	70.6	54.6	0.56	88.3	66.3
IL1b	UGAUGGCCUGGAAAUGUGA	498	AGG	3	+	66.1	51.5	0.53	91.2	65.2
IL1b	GGUCACACUUCACAUUCC	499	AGG	3	-	36.6	44.3	0.59	87.6	63.6
IL1b	GUCACACUUCACAUUCCA	500	GGG	3	-	62.4	48.6	0.57	83.2	62.9
IL1b	AAAGUGAAGGUGACAGACCAU	501	GGG	3	+	68.4	61.9	0.41	78.2	60.4
IL1b	GACCAUUCUUGAAGCUGA	502	TGG	3	+	55.9	58.4	0.48	60.4	55.6
IL1b	UUCUUUGAAGCUGAUGGCC	503	TGG	3	+	47.6	67	0.51	47.8	55.3
IL1b	GGCAUCAGCUUCAAGAAU	504	AGG	3	-	46.5	45.6	0.46	69.5	53.7
IL1b	CGUGCAGUCAUUGAGCUU	505	TGG	4	+	42.6	86.8	0.43	84.4	71.4
IL1b	ACUCUUGUACAGAGCUGGU	506	GGG	4	-	68.9	62	0.64	84	70
IL1b	CCUCAUCUACAGAGAACUG	507	TGG	4	-	76.7	60.6	0.58	90	69.5
IL1b	AGACUGAACACAGAUUCUC	508	TGG	4	+	54.5	62.1	0.55	89.3	68.8
IL1b	GCUGGUGGAGACUUGCAAC	509	TGG	4	-	48.7	74.9	0.53	78.2	68.7
IL1b	CACAGUUCUCUGGUAGAUGA	510	GGG	4	+	61.8	66.6	0.56	81	67.9
IL1b	AGGUCUUGGCAGCAGCACUG	511	TGG	4	-	69.3	49.5	0.57	91.5	66
IL1b	UCAGACUCUUGUACAGAGC	512	TGG	4	-	56.6	65.4	0.49	82.7	65.7
IL1b	ACCUCUGUAAACAACACUCUG	513	AGG	4	+	64.1	58.3	0.62	76.8	65.7
IL1b	GAGAACUGUGGUUCAGGUUCU	514	TGG	4	-	49.3	63.7	0.49	80.7	64.5
IL1b	GCAGCACUGUGGAGAGACCA	515	AGG	4	-	62.5	50.7	0.55	84	63.2
IL1b	CCACAGUUCUCUGGUAGAUG	516	AGG	4	+	56.7	66.1	0.43	78.1	62.4
IL1b	CUACCAGAGAACUGUGGUUC	517	AGG	4	-	35.7	68	0.47	69.9	61.6
IL1b	CAUCCUCCUGGAGGACCUUGU	518	GGG	4	-	57.1	59.3	0.45	78.1	60.8
IL1b	GACUCUUGUACAGAGCUGG	519	TGG	4	-	54.4	60.4	0.44	75.4	59.9
IL1b	UUUUGCUACAUCUUUGAAGA	520	AGG	4	+	48.9	49.2	0.55	73.5	59.2
IL1b	UGUAGCAAAAGAUCCUUCUUC	521	AGG	4	-	34.5	59.5	0.46	70.6	58.7

FIG. 15A

Gene	crRNA	SEQ ID NO:	PAM	Exon	Strand	On-target (Azimuth2)	Off-target	Precision (Delphi)	Frameshift (Delphi)	Combined (Off + Pre + Frames)
IL1b	UCCUGGAGGACCUGUGGGCA	522	GGG	4	-	47.8	43.8	0.45	78.7	55.8
IL1b	GCUGAAGAAGCCCGCCAC	523	AGG	4	+	54.5	52.3	0.41	71.3	54.9
IL1b	UGCUCUUCAGGCUAUCUCC	524	TGG	4	-	48.6	60.4	0.59	43.9	54.4
IL1b	CUCUGGAGGACCUGUGGGC	525	AGG	4	-	43	46.1	0.41	74.4	53.8
IL1b	CUGCCACAGGUCCUCCAGG	526	AGG	4	+	68.4	42.1	0.55	62.4	53.2
IL1b	UCAUCCUCCUGGAGGACCUG	527	TGG	4	-	55.5	44.9	0.45	66.6	52.2
IL1b	UCUUCAGGUCAUCCUCCUGG	528	AGG	4	-	70.1	54	0.45	55.5	51.5
IL1b	GCCUGCCCAACAGGUCCUCC	529	AGG	4	+	48.7	40.8	0.6	42.7	47.8
IL1b	UGAUGCAGCCCAUGCAAUCGG	530	TGG	5	+	67.5	89.8	0.6	87.7	79.2
IL1b	CUUGCAGUCCACCGAUUGCA	531	TGG	5	-	57.9	87.4	0.61	89.3	79.2
IL1b	AUCGGUGGACUGCAAGUUAAC	532	AGG	5	+	47.1	85.8	0.57	83	75.3
IL1b	GUGAACAAACAAGGUAACGG	533	GGG	5	+	74.7	81.2	0.59	82.9	74.4
IL1b	CUUCGGGCUCCUCCUCCUCAA	534	TGG	5	+	50.3	74.1	0.62	87.1	74.4
IL1b	UCGGGCUCCUCCUCCUCAAUG	535	GGG	5	+	74.9	80.8	0.56	84.6	73.8
IL1b	GGACAUAGGCCACAAUACC	536	TGG	5	+	58.7	66.4	0.64	82.1	70.8
IL1b	UAGACAGCACCCAGGUAUUUG	537	TGG	5	-	43.7	66.6	0.61	83.6	70.4
IL1b	GAGUGAUGCAGCCCAUGCAAU	538	CGG	5	+	57	77.1	0.49	85	70.4
IL1b	UUCGGGCUCCUCCUCCUCAAU	539	GGG	5	+	33.9	79.3	0.49	82.3	70.2
IL1b	UGUGAACAAACAAGGUAACG	540	GGG	5	+	73.2	76.5	0.53	78.7	69.4
IL1b	AUGUGAACAAACAAGGUAAC	541	GGG	5	+	42.5	58.5	0.62	85.1	68.5
IL1b	GGGMAAAUGUGAACAAACA	542	AGG	5	+	62.3	49.2	0.63	91.2	67.8
IL1b	GUCUAACUCAUAUGAGCUUC	543	GGG	5	+	36.8	79.3	0.43	80.4	67.6
IL1b	CAUAUGAGUUAGACAGCACC	544	AGG	5	-	57.8	76.9	0.56	68.9	67.3
IL1b	UGUUCACAUUUUCCCAUUG	545	AGG	5	-	58.9	55.1	0.57	87	66.4
IL1b	UGUCUAAACUCAUAUGAGCUU	546	CGG	5	+	49.5	73.7	0.4	82.3	65.3

FIG. 15B

Gene	crRNA	SEQ ID NO:	PAM	Exon	Strand	On-target (Axinuth2)	Off-target	Precision (Delphi)	Frameshift (Delphi)	Combined (Off + Pre + Frames)
IL1b	AGAUGAUAGGUDUCGAAAG	547	TGG	5	-	66.3	48.3	0.53	90.3	63.9
IL1b	GCAUCUGUUUGCAGAU	548	AGG	5	-	48.6	56.4	0.66	67.9	63.4
IL1b	UCACAUUUUCCCCAUUGAGG	549	TGG	5	-	60.6	57.1	0.46	77.6	60.2
IL1b	AAUGUGAACAAACAAGGUAA	550	CGG	5	+	58.8	48.2	0.48	78.3	58.2
IL1b	UAUCAUCUGCAAAACAGAUG	551	CGG	5	+	68.8	44.5	0.42	80.1	55.5

FIG. 15C

Gene	crRNA	SEQ ID NO:	PAM	Exon	Strand	On-target (Azimuth2)	Off-target	Precision (Delphi)	Frameshift (Delphi)	Combined (Off + Pre + Frames)
IL1a	UGACCAUCUCUCUGAAUC	552	AGG	3	+	49.5	57.7	0.55	78.3	63.7
IL1a	UUACCUGAUUCAGAGAGAGA	553	TGG	3	-	58.3	40.9	0.56	74.7	57.2
IL1a	GACAUCCCAGCUUACCUCA	554	AGG	4	+	43.8	41.8	0.61	87.2	63.3
IL1a	GUCACAGCUCUAUUCUAAGA	555	AGG	4	-	54.2	35.6	0.62	91.9	63.2
IL1a	AUGACACAGAAGAGGUAAG	556	AGG	4	+	66.2	30.5	0.63	96	63.2
IL1a	CACUACCACAUUUCUUGA	557	AGG	4	-	37.5	34.3	0.63	89.6	62.3
IL1a	AAUGUCUUCAGGUCUCAU	558	CGG	4	-	54.4	39.3	0.7	73.5	60.9
IL1a	UGGUAGUGGCGCAGCCAAU	559	GGG	4	+	55.8	39.3	0.52	90.4	60.6
IL1a	AUCAUAGAAGGAUUUCUUG	560	AGG	4	-	60.9	32.4	0.65	83.7	60.4
IL1a	GAUGUCUUUGAGAUUUCAG	561	AGG	4	-	70.3	33.6	0.61	83.9	59.5
IL1a	CAUUUCCUUGAAGGUAAGC	562	TGG	4	-	48.3	39.5	0.53	85.7	59.4
IL1a	AUUUCCUUGAAGGUAAGCU	563	GGG	4	-	61.3	38.6	0.55	84.5	59.4
IL1a	ACAUGCAGUCCUCAUGAAGU	564	GGG	4	-	64.4	39.6	0.54	83.2	58.9
IL1a	UGUCAUUGGCAUUGUCUCC	565	AGG	4	-	30.2	37.1	0.64	74.8	58.6
IL1a	GUGUAGUGGUGGCAGCCAA	566	TGG	4	+	56.3	34.9	0.48	88.9	57.3
IL1a	GAGCUGUGACCCACUUCUAG	567	AGG	4	+	71.5	42.7	0.47	79.7	56.5
IL1a	CUUACCUUCUUCUGUGUCAU	568	TGG	4	-	41.3	34.8	0.57	77.8	56.5
IL1a	UAGAAGGAUUUCUAUGAGGA	569	AGG	4	-	55.8	35.5	0.54	76.9	55.5
IL1a	GCUUACCUUCAAGGAAAUG	570	TGG	4	+	67.3	33.4	0.54	78.5	55.3
IL1a	GGAAGAUUCUAAAGAAGAGA	571	CGG	4	+	61	26.1	0.52	85.5	54.5
IL1a	AUUCAUACCCGAUGAUGACC	572	TGG	4	+	57.1	44	0.45	71.9	53.6
IL1a	CUUCUUUAGAAUUCUCCCAU	573	TGG	4	-	50.7	34	0.44	79.9	52.6
IL1a	GACAUGCAGUCCUCAUGAAG	574	TGG	4	-	58.5	40.5	0.44	72.8	52.4
IL1a	AUUGCCAAUGACACAGAAGA	575	AGG	4	+	61.9	32.1	0.6	64.6	52.2
IL1a	CUUCAGGAAAUGUGGUAG	576	TGG	4	+	58.6	35.1	0.59	37.5	43.9

FIG. 16A

Gene	crRNA	SEQ ID NO:	PAM	Exon	Strand	On-target (Azimuth2)	Off-target	Precision (Delphi)	Frameshift (Delphi)	Combined (Off + Pre + Frames)
IL1a	CAACGANAAGUGGUAGUGG	577	TGG	4	+	66.8	25.7	0.57	39.6	40.8
IL1a	AGUAAAGUUCGACAAACAGG	578	AGG	5	+	72.1	85.1	0.49	77.6	70.6
IL1a	UCUGUAAUGCAGCAGUCAUG	579	AGG	5	-	68.4	66.6	0.48	90.4	68.3
IL1a	UUACAGAAUUGGAUGAUGC	580	AGG	5	+	57	57.6	0.56	80.8	64.8
IL1a	GUCCGAACUAAUUCUUCAUUG	581	AGG	5	-	54.7	85.7	0.62	93.5	80.4
IL1a	AAGUUGUAUGCUACUGAUCU	582	GGG	5	-	51.5	73.5	0.6	92.6	75.4
IL1a	CAAGUAUAGUUCGACAAAC	583	AGG	5	+	44.3	91.5	0.6	87	79.5
IL1a	GUUGUCAUUCAGGAUAAUU	584	GGG	5	-	42	59	0.56	84.4	66.5
IL1a	AUGAAAAAUAACAUAUAUA	585	AGG	5	+	38	41.8	0.65	94.3	67
IL1a	GAAGUUGUAUGCUACUGAUC	586	TGG	5	-	37.5	81.1	0.52	85.4	72.8
IL1a	GGUUGUCAUUCAGGAUGAAU	587	TGG	5	-	34.6	64.7	0.47	77.9	63.2
IL1a	UUUGAUUGCAGGUUGUCAUUC	588	AGG	5	-	29.9	69.4	0.6	85.6	71.7
IL1a	AGUUGUAUUUUUCAUUGUUA	589	TGG	5	-	29.3	38.8	0.49	72.5	53.4
IL1a	GACUGCUGCAUUAACAGAAUU	590	TGG	5	+	27.7	63.5	0.54	86.1	67.9

FIG. 16B

Human IL1A (IL1A-001; GRCh38)									
#	crRNA sequence	Target		On-target score*	Off-target score**	Precision score***	Frameshift %****	Combined score^	
		Exon #	Strand						
sg235	CAGAGACAGAUCAUAUGG	3	-	69.5	67	0.57	93.7	72.6	
sg236	GCCAUAGCUUACAUGAUAGA	4	-	53.9	76.9	0.65	93.9	78.6	

Machine-Learning (inDelphi) Predictions	
Top genotype	Repair outcome†
insA (35.3%)	TACCTGATTCAGAGACAGATGATCAA (A) TGGAGGAACTGTCTTCTTCATTTCa
insT (56%)	ATGGAGTGGGCCATAGCTTACATGAT (T) AGAAGGATTTCTGTGAGGAAAGGAAAA

SEQ ID NO:601
SEQ ID NO:602

Empirical data: ICE, day 10 post-nucleofection			
KO score	Top genotype	inDelphi	
		rank	Repair outcome
99%	insA (99%)	1st	TACCTGATTCAGAGACAGATGATCAA (A) TGGAGGAACTGTCTTCTTCATTTCa
48%	insT (32%)	1st	ATGGAGTGGGCCATAGCTTACATGAT (T) AGAAGGATTTCTGTGAGGAAAGGAAAA

SEQ ID NO:603
SEQ ID NO:604

FIG. 17A

Human IL1B (IL1B-201; GRCh38)

#	crRNA sequence	Target		On-target score*	Off-target score**	Precision score***	Frameshift %***	Combined score^
		Exon #	Strand					
sg237	UGAUGGCCCUAAACAGAUCA	3	+	60.9	66.7	0.57	80	67.9
sg238	GGUGGUCGGAGAUUCGUAGC	4	-	58.3	49.3	0.6	86.3	65.2
sg248	ACCUAUCUUCUUCGACACAU	5	+	61.7	86.5	0.65	89.9	80.5
sg249	CUUCGACACAUUGGAUAACG	5	+	68.2	93.8	0.52	75.3	73.7
sg250	GUGCAGUUCAGUGAUCGUAC	5	-	64.4	91	0.48	83.9	74.3

Machine-Learning (inDelphi) Predictions

Top genotype	Repair outcome†
insA (33.3%)	CTTTGAAGCTGATGGCCCTAAACAGA (A) TGAAGGTAAGACTATGGGTTTAACTC
insT (51.5%)	TGCTGTAGTGGTGGTCGGAGATTGGT (T) AGCTGGATGCCGCCCATCCAGAGGGCA
insA (51.9%)	CATTTCAGAACCTATCTTCTTCGACA (A) CATGGGATAACGAGGCTTATGTGCAC
insA (33.4%)	ACCTATCTTCTTCGACACATGGGATA (A) ACGAGGCTTATGTGCACGATGCACCT
insT (18.3%)	TCCCGGAGCGTGCAGTTCAGTGATCG (T) TACAGGTGCATCGTGACACATAAGCCT

SEQ ID NO:605
SEQ ID NO:606
SEQ ID NO:607
SEQ ID NO:608
SEQ ID NO:609

Empirical data: ICE, day 10 (*day 3) post-nucleofection

KO score	Top genotype	inDelphi		Repair outcome	
		rank	rank		
66%	insA (40%)	1st	1st	CTTTGAAGCTGATGGCCCTAAACAGA (A) TGAAGGTAAGACTATGGGTTTAACTC	SEQ ID NO:605
0%	wild-type	NA	NA	TGCTGTAGTGGTGGTCGGAGATTGGT AGCTGGATGCCGCCCATCCAGAGGGCA	SEQ ID NO:606
3%*	CAdel (3%)*	2nd	2nd	CATTTCAGAACCTATCTTCTTCGACA --TGGGATAACGAGGCTTATGTGCAC	SEQ ID NO:607
33%*	insA (22%)*	1st	1st	ACCTATCTTCTTCGACACATGGGATA (A) ACGAGGCTTATGTGCACGATGCACCT	SEQ ID NO:608
81%*	Gdel (55%)*	8th	8th	TCCCGGAGCGTGCAGTTCAGTGATC- TACAGGTGCATCGTGACACATAAGCCT	SEQ ID NO:609

FIG. 17B

Canine IL1A (IL1A-201; CanFam3.1)

#	crRNA sequence	Target		On-target score*	Off-target score**	Precision score***	Frameshift %****	Combined score^
		Exon #	Strand					
sg239	UGACCAUCUCUCUCUGAAUC	3	+	49.5	57.7	0.55	78.3	63.7
sg240	GACAUCCCGAGCUUACCUUCA	4	+	43.8	41.8	0.61	87.2	63.3
sg251	AGUAUAGUUCGACAAACAGG	5	+	72.1	85.1	0.49	77.6	70.6
sg252	UCUGUAAUGCAGCAGUCAUG	5	-	68.4	66.6	0.48	90.4	68.3

Machine-Learning (inDelphi) Predictions		
Top genotype	Repair outcome†	
insA (35.2%)	TTCTGAAATTGACCATCTCTCTCTGA {A}ATCAGGTAAAGTAAATGACTGCAATTC	
insT (52.7%)	AATCTCAAAGACATCCGAGCTTACCT {T}TCAAGGAAAATGTGGTAGTGGTGGCA	
insC (30.6%)	CTCAATCAAAGTATAGTTCGACAAAC {C}AGGAGGAAAATTACCTCATGACTGCTG	
insC (20.4%)	CATCCAAATTCTGTAAATGCAGCAGTC {C}ATGAGGTAATTTCTCTCTCTGTTGTCG	

SEQ ID NO:610
SEQ ID NO:611
SEQ ID NO:612
SEQ ID NO:613

Empirical data: ICE, day 10 post-nucleofection			
KO score	Top genotype	inDelphi rank	Repair outcome
	90% insA (56%)	1st	TTCTGAAATTGACCATCTCTCTCTGA {A}ATCAGGTAAAGTAAATGACTGCAATTC
	97% insT (57%)	1st	AATCTCAAAGACATCCGAGCTTACCT {T}TCAAGGAAAATGTGGTAGTGGTGGCA

SEQ ID NO:614
SEQ ID NO:615

FIG. 17C

Canine IL1B (IL1B-201; CanFam3.1)

#	crRNA sequence	Target		On-target score*	Off-target score**	Precision score***	Frameshift %****	Combined score^
		Exon #	Strand					
sg241	UGAUGGCCCCUGGAAAUUGA	3	+	66.1	51.5	0.53	91.2	65.2
sg242	ACUCUUGUUACAGAGCUGGU	4	-	68.9	62	0.64	84	70

Machine-Learning (inDelphi) Predictions

Top genotype	Repair outcome†
insT (22.9%)	CTTTGAAGCTGATGCCCTGGAAATG {T}TGAAGGTGAGACCATGGGCTTAGCTC SEQ ID NO:616
insT (56.4%)	ATGCCTCAGACTCTTTGTTACAGAGCT {T}GGTGGGAGACTTGCAACTGGATGCC SEQ ID NO:617

Empirical data: ICE, day 10 post-nucleofection			
KO score	Top genotype	inDelphi rank	Repair outcome
	99% delTG (95%)	2nd	CTTTGAAGCTGATGCCCTGGAAATG ---AAGGTGAGACCATGGGCTTAGCTC SEQ ID NO:618
	99% insT (99%)	1st	ATGCCTCAGACTCTTTGTTACAGAGCT {T}GGTGGGAGACTTGCAACTGGATGCC SEQ ID NO:619

FIG. 17D

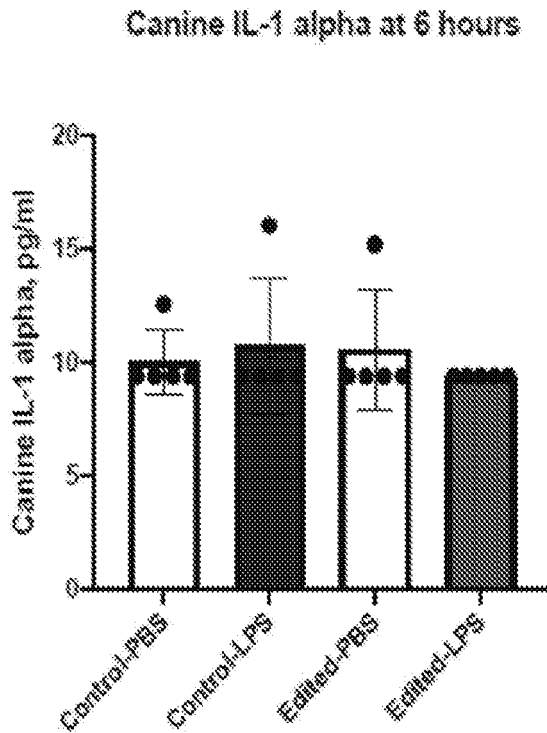


FIG. 18A

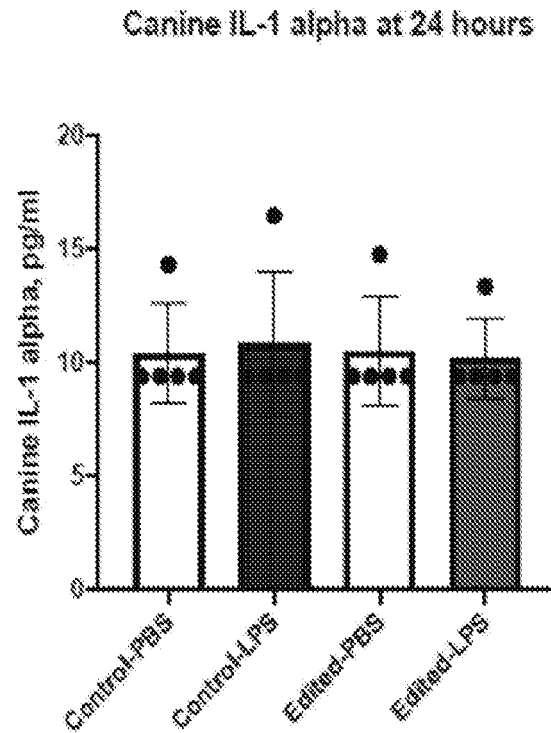


FIG. 18B

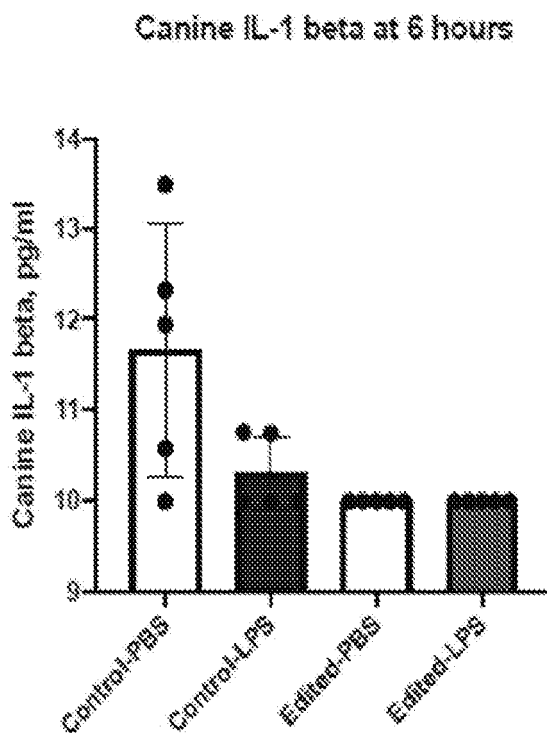


FIG. 18C

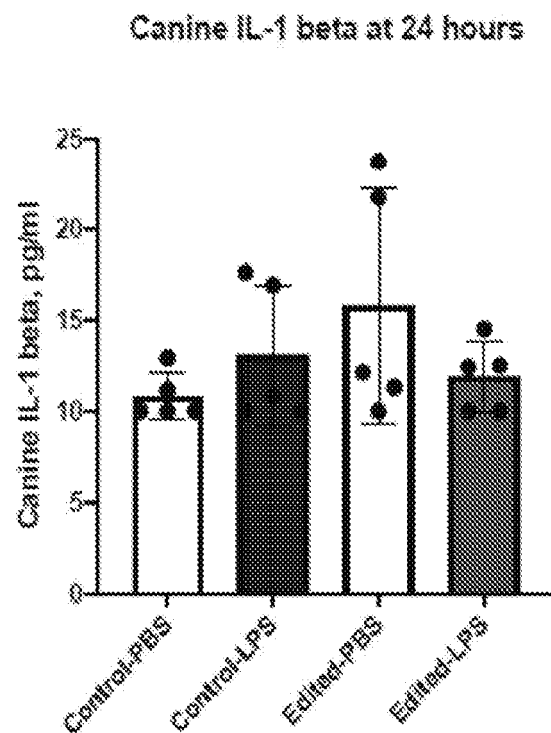


FIG. 18D

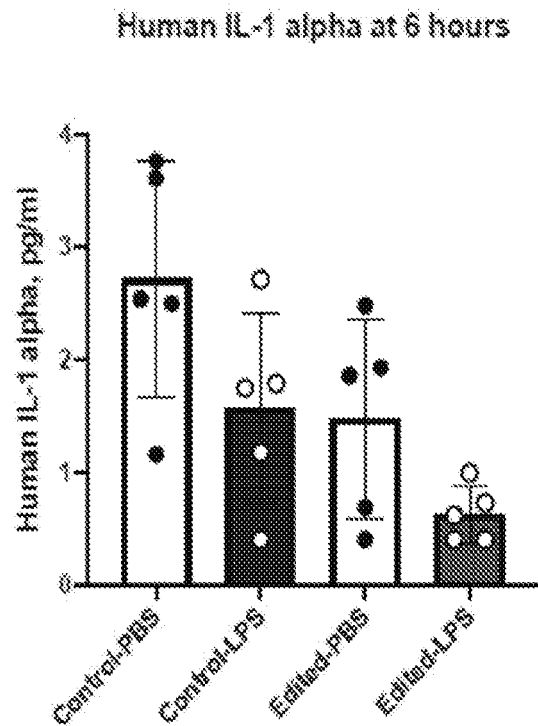


FIG. 19A

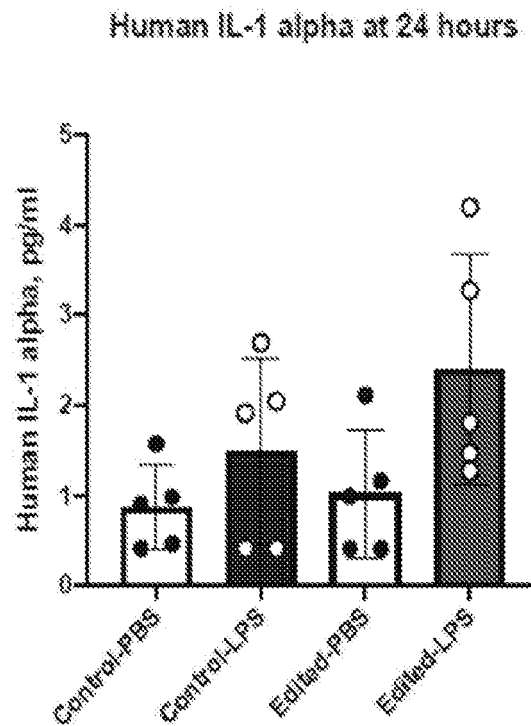


FIG. 19B

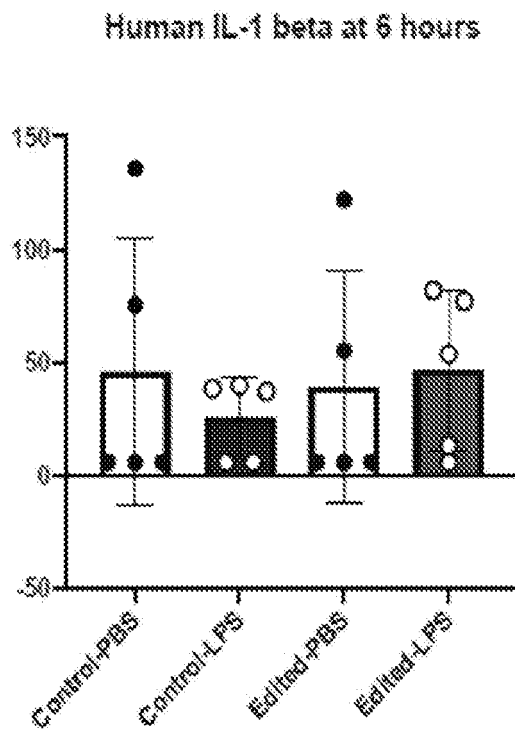


FIG. 19C

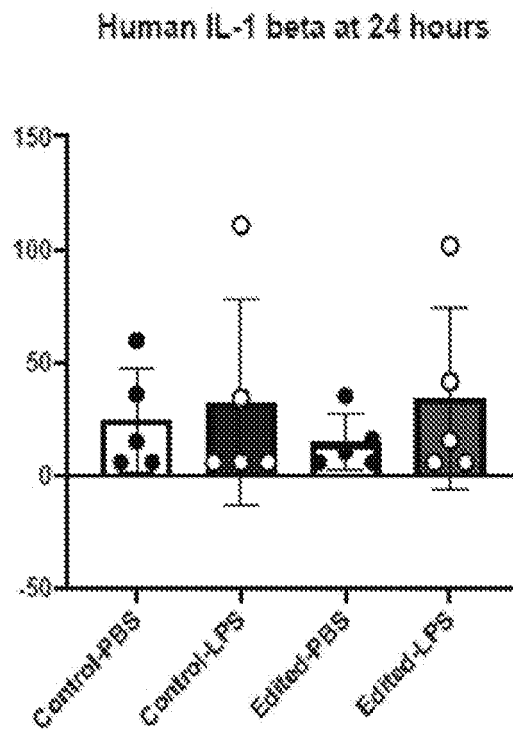


FIG. 19D

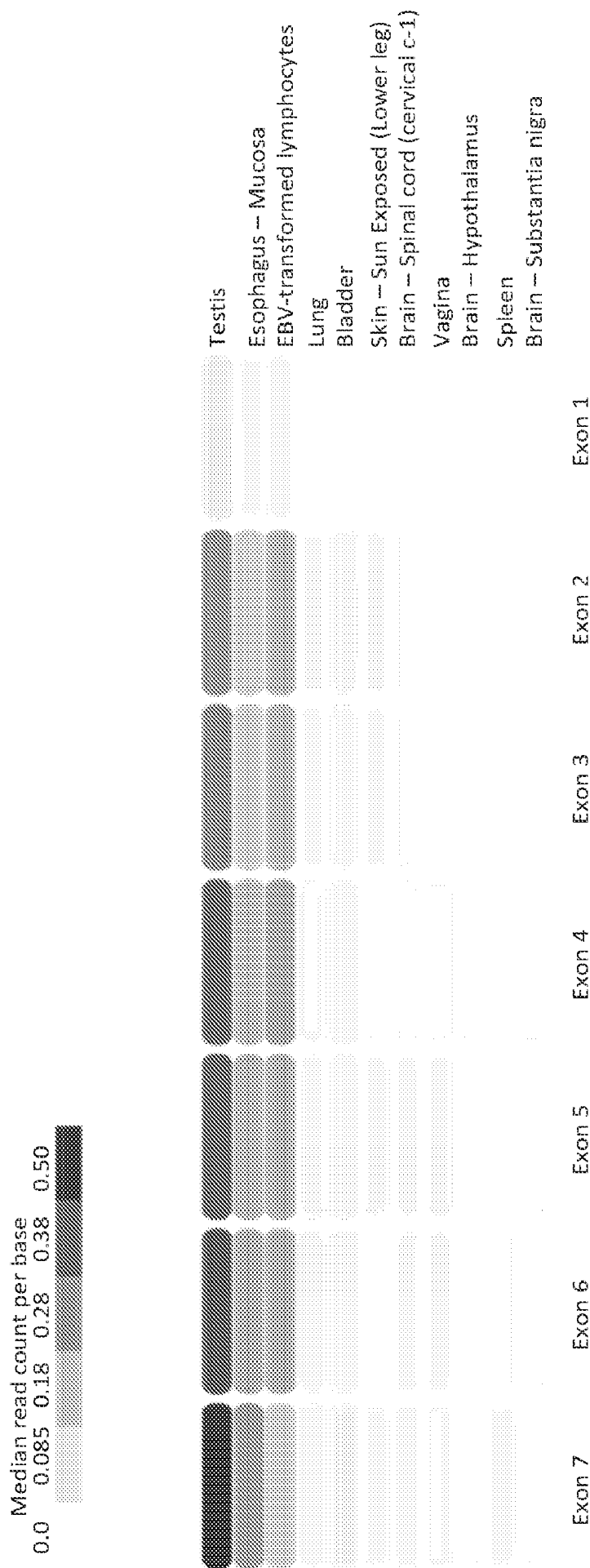


FIG. 20

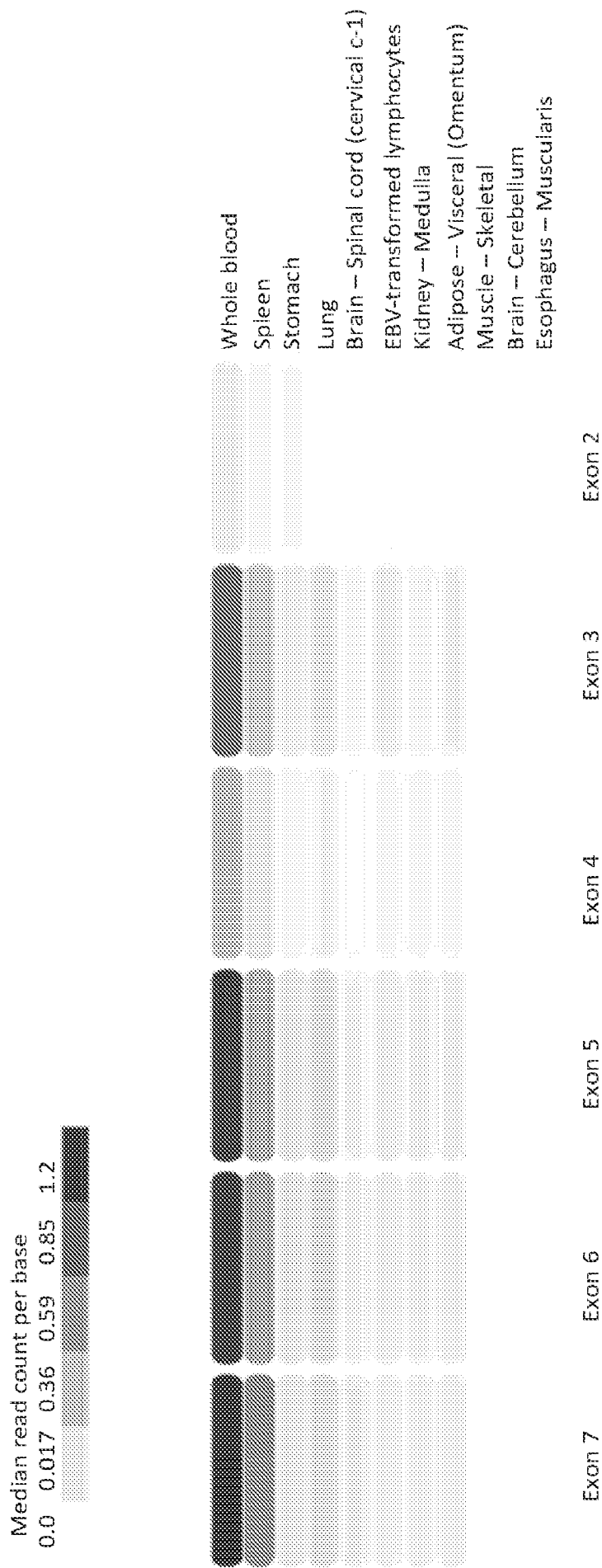


FIG. 21

Single guide RNA (sgRNA)		Target			On-target	InDelphi Predictions		Off-Targets
#	Target sequence	Exon #	Strand	Domain	AA	Score	Precision	Frameshift
0-1-2-3-4 mismatches (seed only)								
Human IL-1A (IL1A-001; GRCh38)								
235	CAGAGACAGATGATCAATGG	3	-	Interleukin-1 propeptide (1-109 aa)	25	0.68	0.57	94%
236	GCCATAGCTTACATGATAGA	4	-	Interleukin-1 propeptide (1-109 aa)	36	0.52	0.65	94%
Human IL-1B (IL1B-201; GRCh38)								
237	TGATGGCCCTAAACAGATGA	3	+	Interleukin-1 propeptide (1-103 aa)	32	0.55	0.57	80%
238	GGTGGTCGGAGATTCTGTAGC	4	-	Interleukin-1 propeptide (1-103 aa)	50	0.57	0.6	86%
248	ACCTATCTTCTTCGACACAT	5	+	Interleukin-1 propeptide (1-103 aa)	107	0.63	0.65	90%
249	CTTCGACACATGGGATAACG	5	+	Interleukin-1 propeptide (1-103 aa)	110	0.66	0.52	75%
250	GTGCAGTTCAGTGATCGTAC	5	-	Interleukin-1 propeptide (1-103 aa)	119/120	0.64	0.48	84%
259	CATGGCCACAACAACACTGACG	4	-	Interleukin-1 propeptide (1-103 aa)	66	0.67	0.46	79%
260	AGGTCCTGGAAGGAGCACTG	4	-	Interleukin-1 propeptide (1-103 aa)	33/34	0.79	0.64	92%
0-0-14-4-377 (0-0-4-3-12)								

FIG. 22

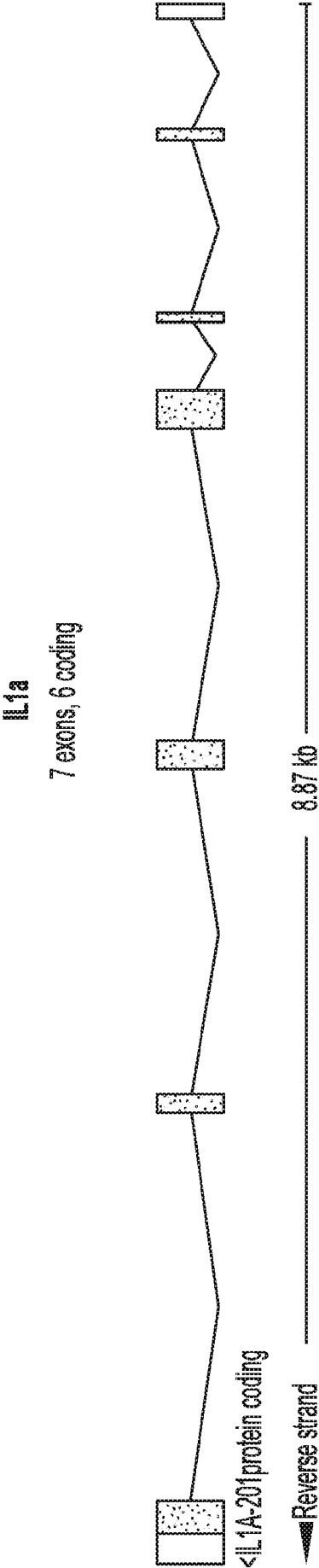


FIG. 23A

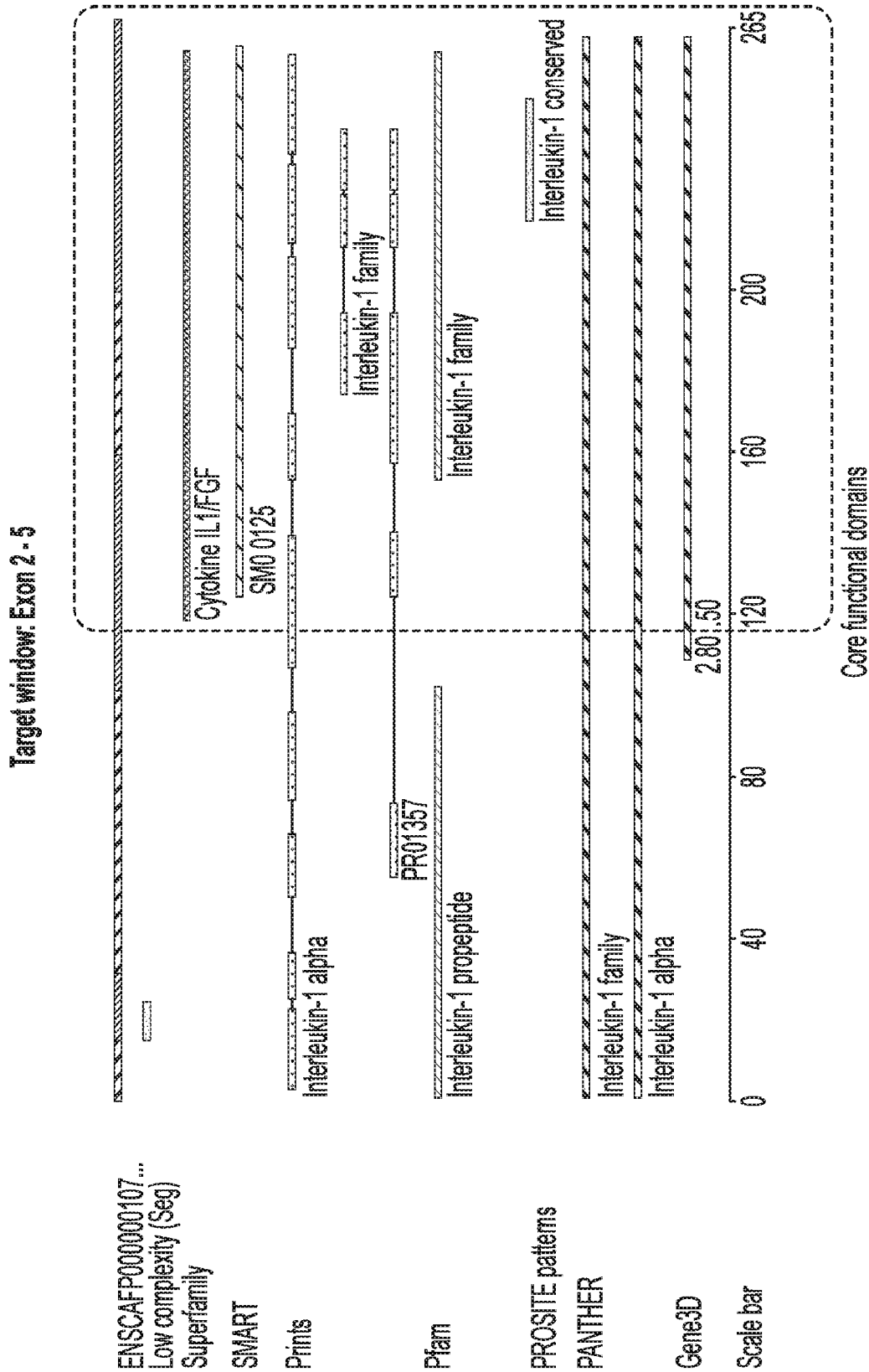


FIG. 23B

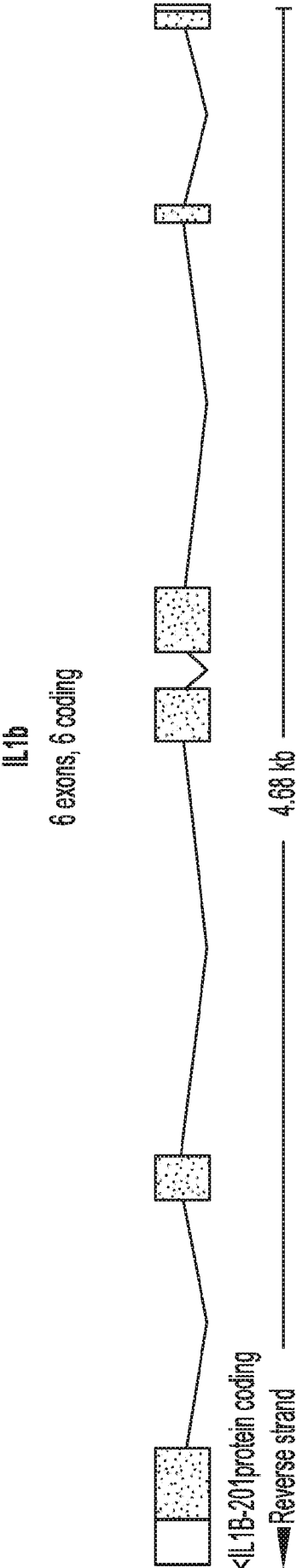


FIG. 23C

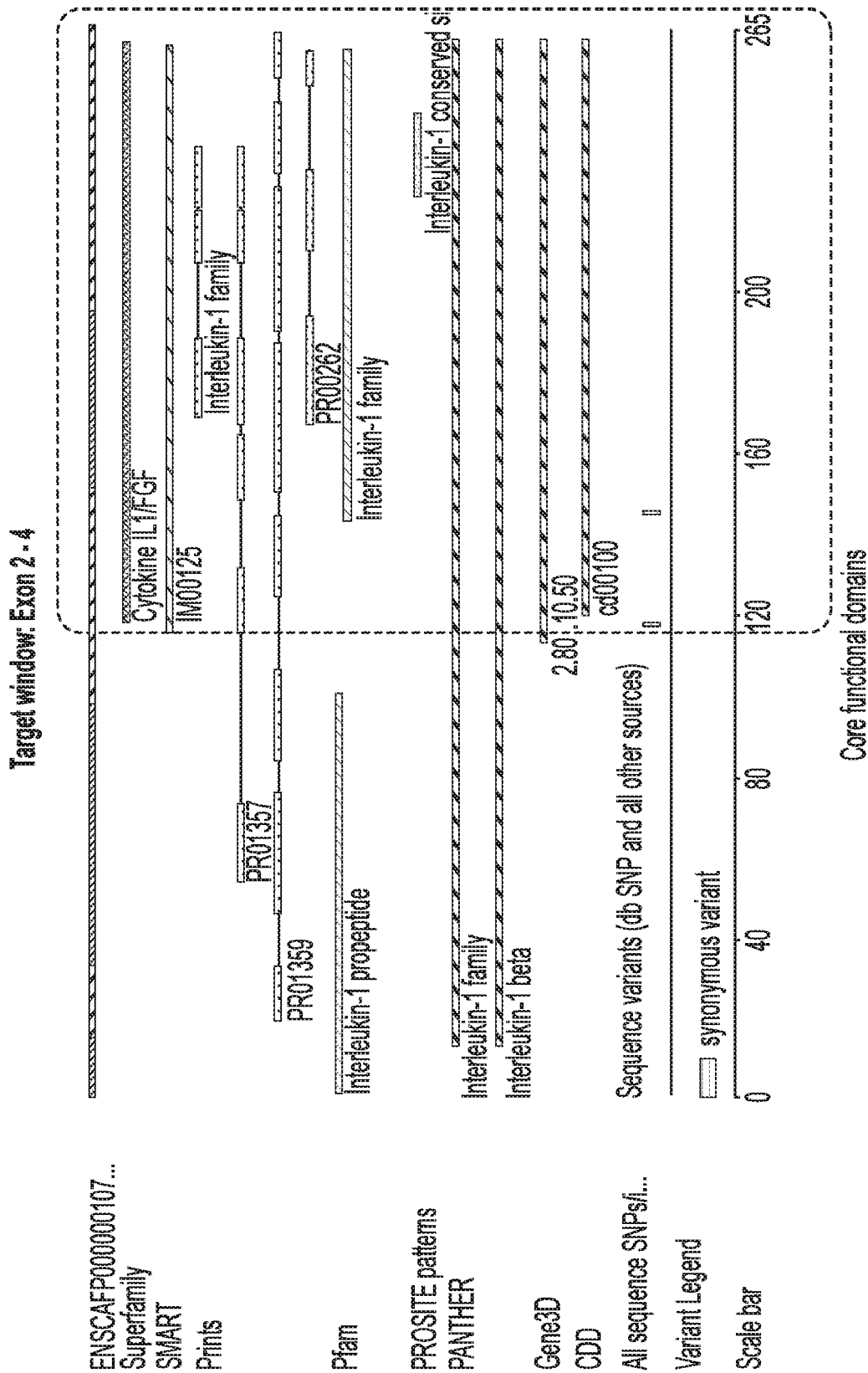


FIG. 23D

Single guide RNA (sgRNA)		Target			On-target	InDelphi Predictions		Off-Targets
#	Target sequence	Exon #	Strand	Domain	AA	Score	Precision	Frameshift
Canine IL-1A (IL1A-201; CanFam3.1)								
239	TGACCATCTCTCTCTGAATC	3	+	Interleukin-1 propeptide (1-103 aa)	31	0.50	0.55	78%
240	GACATCCCAGCTTACCTTCA	4	+	Interleukin-1 propeptide (1-103 aa)	63	0.44	0.61	87%
251	AGTATAGTTCGACAAACAGG	5	+	Cytokine IL1/FGF (122- 260 aa)	144	0.72	0.49	78%
252	TCTGTAATGCAGCAGTCATG	5	-	Cytokine IL1/FGF (122- 260 aa)	150	0.68	0.48	90%
Canine IL-1B (IL1B-201; CanFam3.1)								
241	TGATGGCCCTGGAAATGTGA	3	+	Interleukin-1 propeptide (1-103 aa)	32	0.66	0.53	91%
242	ACTCTTGTTACAGAGCTGGT	4	-	Interleukin-1 propeptide (1-103 aa)	56	0.69	0.64	84%
						0-1-2-3-4 mismatches (seed only)		

FIG. 24

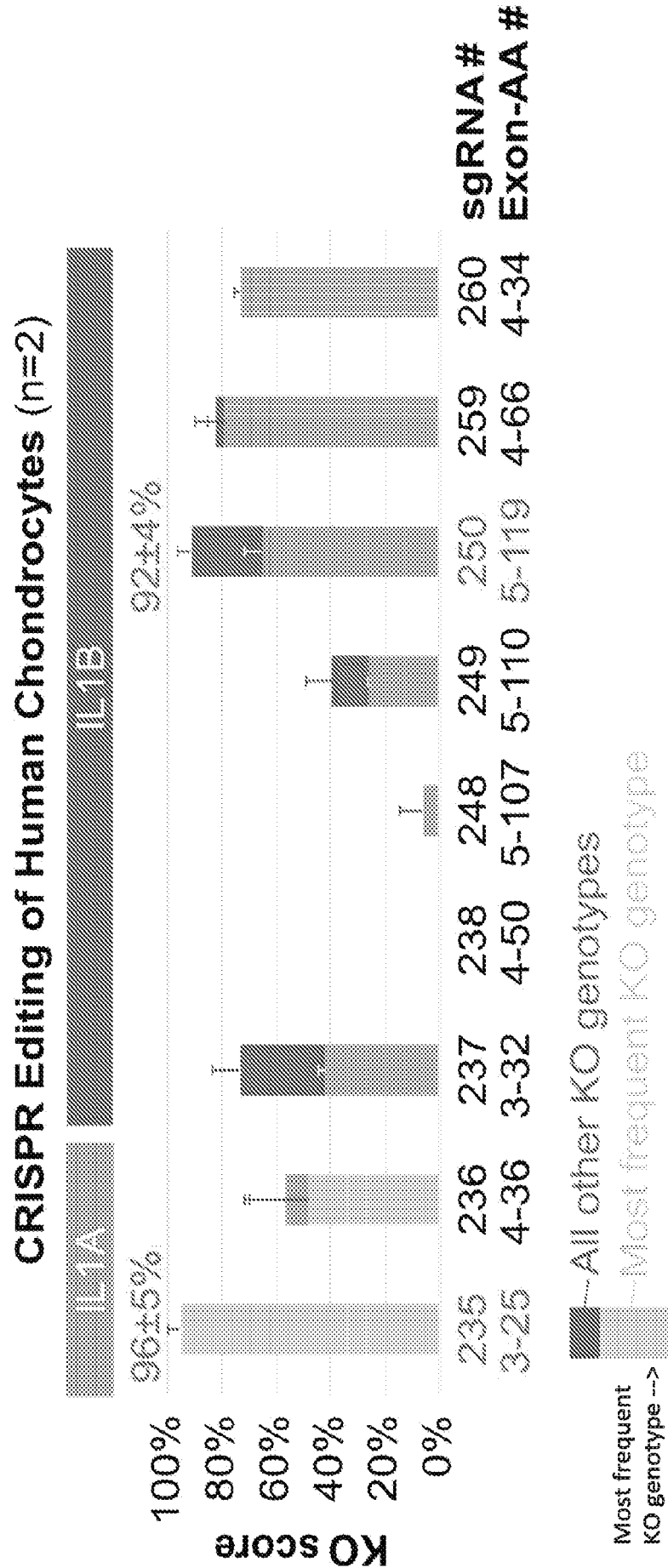


FIG. 25A

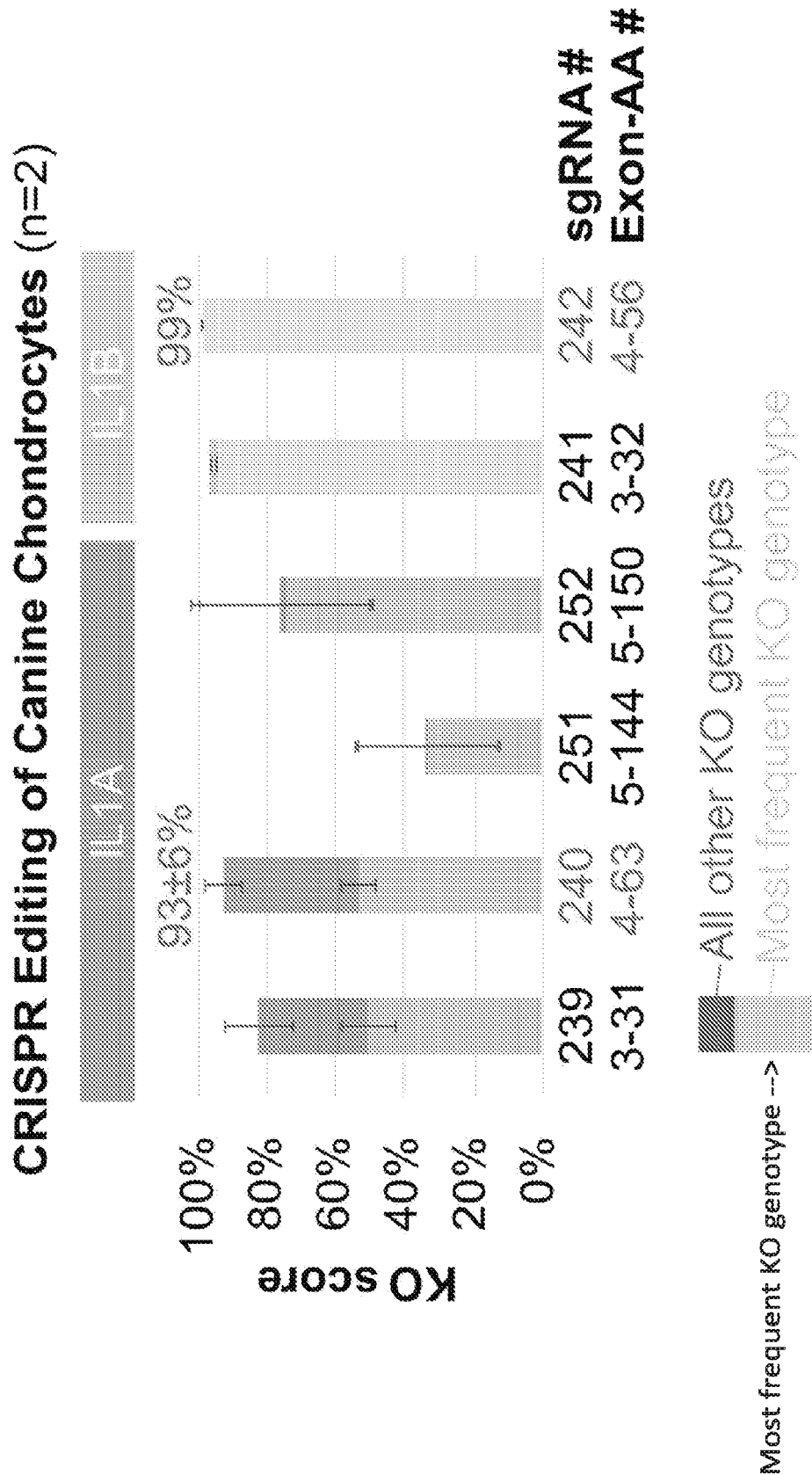


FIG. 25B

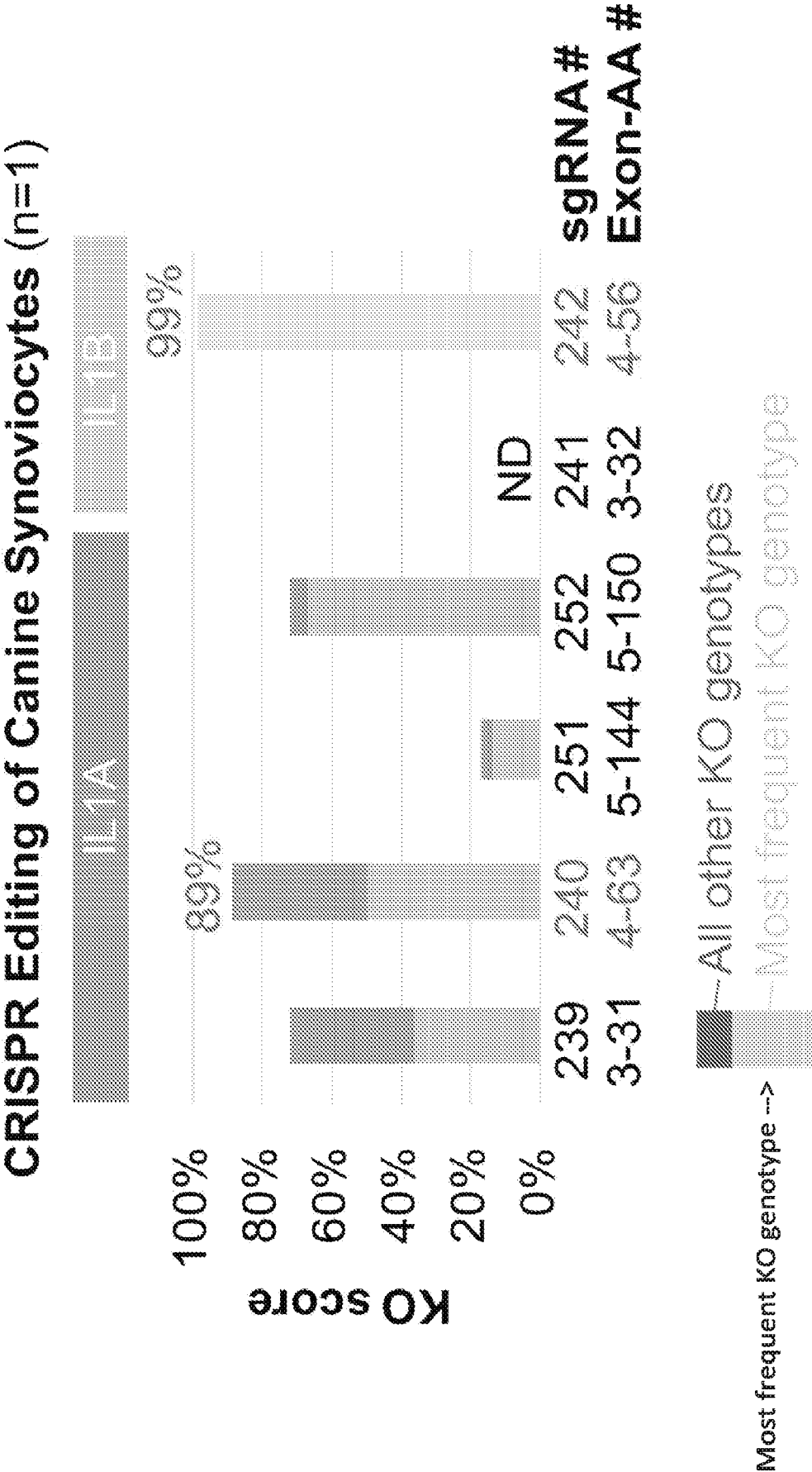


FIG. 25C

In silico predicted off-targets of sgRNA #240 in the dog *Canis familiaris* genome

Off-target	Sequence (Seed)	PAM	Score	Gene	Chromosome	Strand	Position	Mismatches	On-target	Editing efficiency
	GACATCCCAGCTTACCTTCA	AGG	100	IL1A	chr17	-1	36977452	0	TRUE	97%
#1	CACATCCCCTCCTTACCTTCA	CGG	1.67322207	lncRNA	chr27	1	34712401	3	FALSE	0%
#2	TAAATACCAAGCTTACCTTCA	CAG	1.54205034		chr35	-1	18871659	3	FALSE	0%
#3	GTGGACCCAGCTTACCTTCA	GAG	1.32551887		chr10	-1	2693301	4	FALSE	0%

In silico predicted off-targets of sgRNA #242 in the dog *Canis familiaris* genome

Off-target	Sequence (Seed)	PAM	Score	Gene	Chromosome	Strand	Position	Mismatches	On-target	Editing efficiency
	ACTCTTGTTACAGAGCTGGT	GGG	100	IL1B	chr17	1	37022194	0	TRUE	99%
#1	ACTTTTGTTACAGAGCTGGT	CAG	6.16161972		chr33	-1	20234937	2	FALSE	0%
#2	CCTCATGCTACAGAGCTGGT	GGG	2.76564774		chr1	-1	47541563	3	FALSE	99%
#3	GTGCTTGTTACAGAGCTGGT	GGG	2.32143742		chr26	-1	32323843	3	FALSE	25%

FIG. 26

	On- target	Off-target #2	Off-target #3
day 2	99%	99%	0%
day 10	99%	99%	6%

FIG. 27A

	On-target	Off-target #2	Off-target #3
Day 2	99%	0%	0%
Day 10	99%	0%	0%

FIG. 27B

CHONDROCYTES						
Single guide RNA (sgRNA)		Inference of CRISPR edits from Sanger traces (ICE), day 8-10 post-nucleofection				
#	Target sequence	Exon #	KO score	Top genotype	Predicted	Edit
Human IL-1A (IL1A-001; GRCh38)						
235	CAGAGACAGATGATCAATGG	3	96±5%	insA	35%	ATT CAGAGACAGATGATCAA (A) TGGAGGAAC
236	GCCATAGCTTACATGATAGA	4	57±13%	insT	56%	TGGGCCATAGCTTACATGAT (T) AGAAGGATTT
Human IL-1B (IL1B-201; GRCh38)						
237	TGATGGCCCTAAACAGATGA	3	74±11%	insA	42±3%	AGCTGATGGCCCTAAACAGA (A) TGAAGGTAAG
238	GGTGGTCGGAGATTCGTAGC	4	0%	wt	0%	AGTGGTGGTCGGAGATTCGT AGCTGGATGCCGC
248	ACCTATCTTCTCGACACAT	5	6±8%	delCA	6±8%	AGAACCTATCTTCTTCGACA --TGGGATAACGA
249	CTTCGACACATGGGATAACG	5	40±9%	insA	26±0%	CTTCTTCGACACATGGGATA (A) AGAAGGCTTA
250	GTGCAGTTCAGTGATCGTAC	5	92±4%	delG	66±6%	AGCCTGCAGTTCAGTGATC- TACAGGTGCATCG
259	CATGGCCACAACAACACTGACG	4	83±8%	delG	80±6%	GTCCATGGCCACAACAAC- AGCGGGCCTGCCT
260	AGGTCCTGGAAGGAGCACTG	4	74±2%	insA	74±2%	TCCAGGTCCTGGAAGGAGCA (A) CTGCGGAGAG

FIG. 28A

CHONDROCYTES					
Single guide RNA (sgRNA)		Inference of CRISPR edits from Sanger traces (ICE), day 8-10 post-nucleofection			
#	Target sequence	Exon #	KO score	Top genotype	Predicted Edit

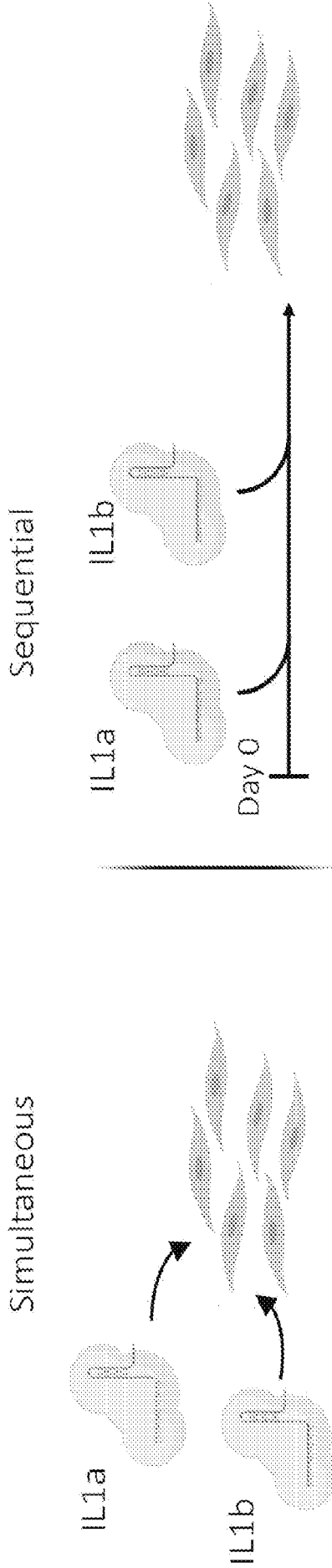
Canine IL-1A (IL1A-201; CanFam3.1)

239	TGACCATCTCTCTCTGAATC	3	83±10%	insA	51±8%	35%	AATTCACCATCTCTCTCTGA (A) ATCAGGTAAG
240	GACATCCCAGCTTACCTTCA	4	93±6%	insT/delT	54±5%	53%	AAAGACATCCCAGCTTACCT (T) TCAAGGAAAA
251	AGTATAGTTCGACAAACAGG	5	34±21%	insC	33±21%	31%	CAAAGTATAGTTCGACAAAC (C) AGGAGGAAAT
252	TCTGTAAATGCAGCAGTCATG	5	77±26%	insC	75±25%	20%	AATTCTGTAAATGCAGCAGTC (C) ATGAGGTAAT

Canine IL-1B (IL1B-201; CanFam3.1)

241	TGATGGCCCTGGAAATGTGA	3	99%	delTG	95%	17%	AGCTGATGGCCCTGGAAATG --AAGGTGAGACC
242	ACTCTTGTACAGAGCTGGT	4	99±0%	insT	99±0%	56%	CAGACTCTTGTACAGAGCT (T) GGTGGGAGAC

FIG. 28B



	IL1A KO score	IL1B KO score
Simultaneous KO: wild-type cells + sgRNA #240 + sgRNA #242	69%	99%
Sequential KO: canine IL1B KO cells + sgRNA #240	68%	99%
Sequential KO: canine IL1A KO cells + sgRNA #242	97%	99%

FIG. 29

FIG. 30A

FIG. 30B

CRISPR IL1A in canine cells

- Chondrocytes (n=3+)
- Monocytes DH82 (n=2)
- Synoviocytes (n=3)

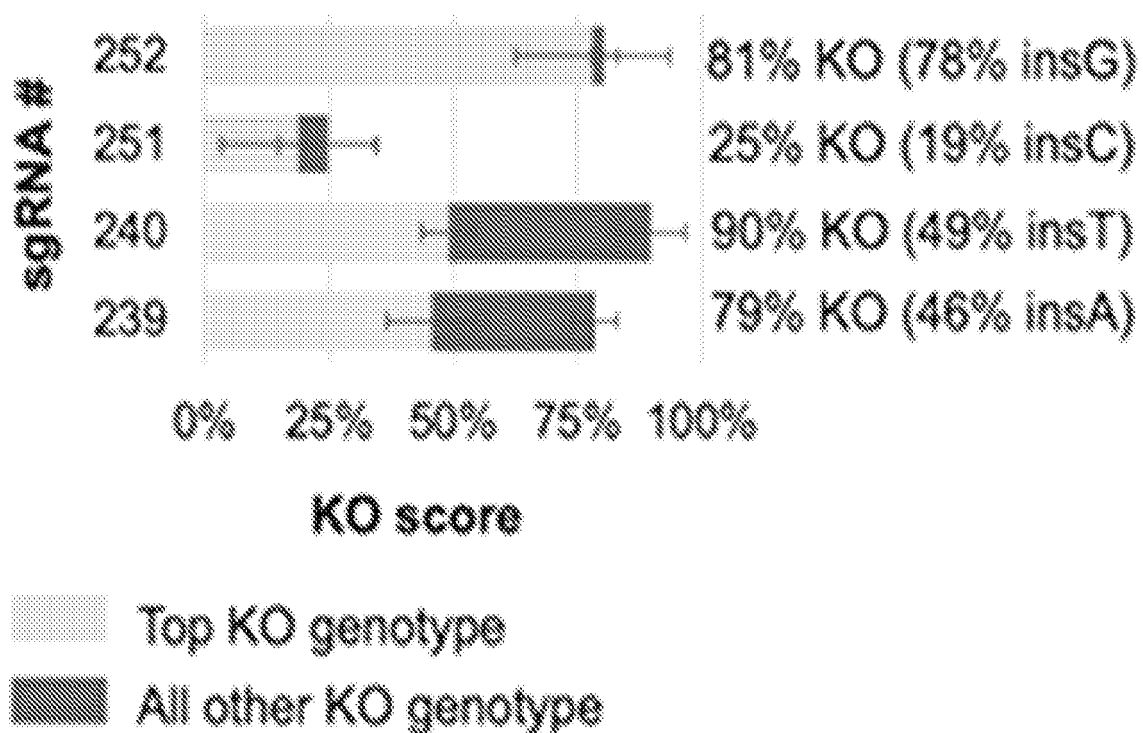


FIG. 31A

IL1A (265 aa, 30.6 kDa)

Propeptide	MAKVPDLFEDLKNCYSENEEYSSEIDHLSLNQKSPYDMS
	CDPLNEDCMSLSTSEISKTSQLTFKENVVVVAANGKILK
	KRRLSLSQFITDDDLIEDIANDTEEVIMKPRSVAYNPFHNN
	EKYNVIRI I I K S Q F I L N D N L N Q S I V R Q T C G N Y L M T A A L Q N
Mature IL1A	LDDAVKFDNGAYTSEDSKLPVTLRISKTRLFVSAQNEDE
	PVLLKEMPETPKTIRDETNLFFWEREGSKHYFKSVAQP
	KLFIATQERKLVEHARGQPSITDFRLLETQP*

Altered amino acid sequence before premature
stop codon (*) due to indel-induced frameshift

Truncated IL1B (90 aa, 10.3 kDa)

MAKVPDLFEDLKNCYSENEEYSSEIDHLSLNQKSPYDMS
CDPLNEDCMSLSTSEISKTSQLTFQKCGSGGSQWEDSK
EETVEFKSIHHR*

FIG. 31B

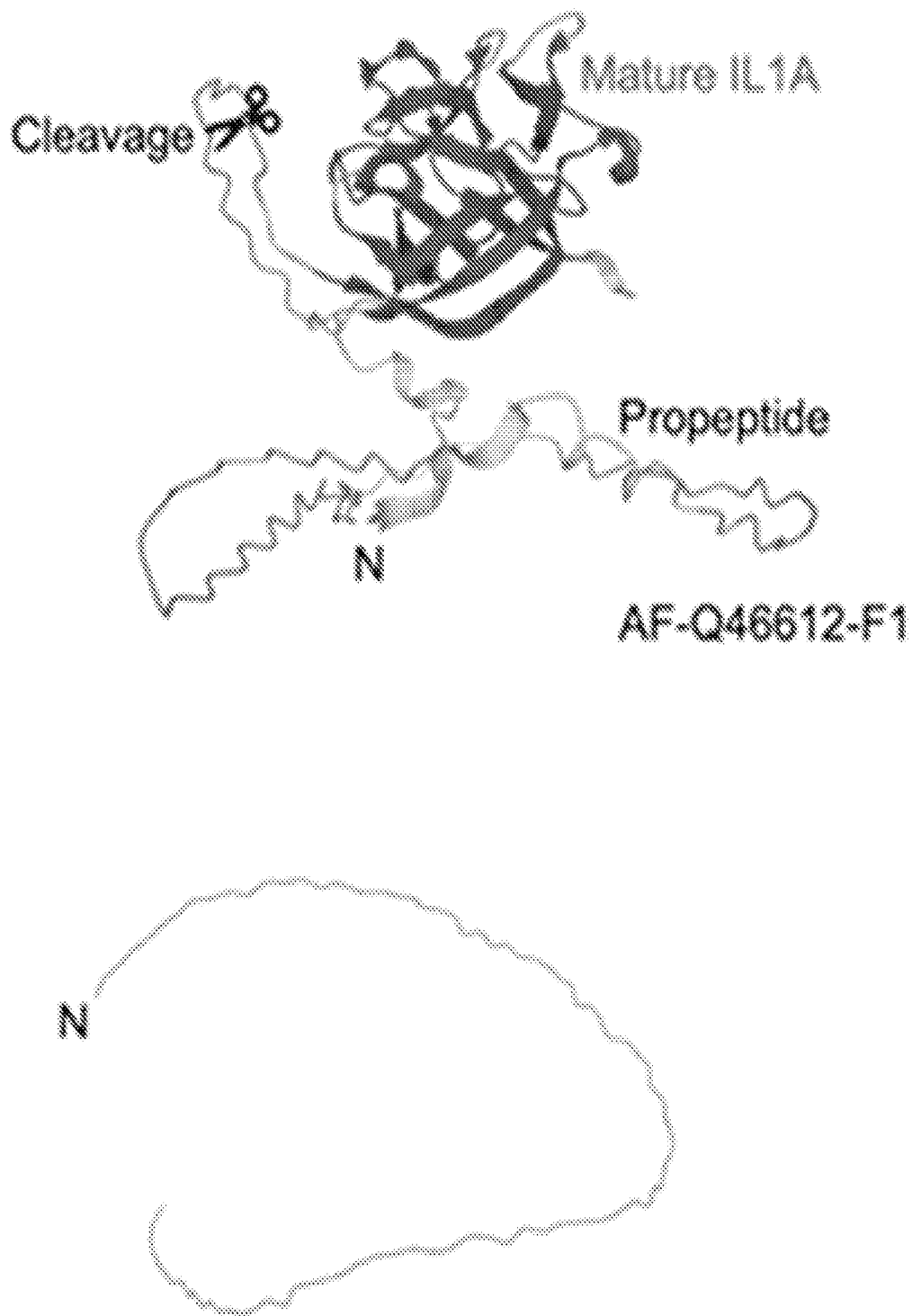


FIG. 31C

Old sg #	New Ref. #	Exon	Amino acid cut	Target sequence	GC %	PAM
239	OCA01	3	IL1A-203: DHLSL-N[31]	TGACCATCTCTCTCTGAATC	45	AGG
360	OCA02	4	IL1A-203: MSCDP-L[43]	ACATGCAGTCCTCATGAAGT	45	GGG
240	OCA03	4	IL1A-203: TSQLT-F[63]	GACATCCCAGCTTACCTTCA	50	AGG
358	OCA05	5	IL1A-203: LNDNL-N[137]	GTCGAACTATACTTTGATTG	35	AGG
251	OCA07	5	IL1A-203: SIVRQ-T[144]	AGTATAGTTCGACAAACAGG	40	AGG
359	OCA04	5	IL1A-203: IMKPR-S[109]	AAGTTGTATGCTACTGATCT	35	GGG
361	OCA06	5	IL1A-203: QSIVR-Q[143]	CAAAGTATAGTTCGACAAAC	35	AGG
252	OCA08	5	IL1A-203: GGNYL-M[150]	TCTGTAATGCAGCAGTCATG	45	AGG

FIG. 32A

New Ref. #	Exon	Protein O46612: amino acid context [cut position]	Off-target			On-target				Frameshift				
			MIT	CFD	OFF	OffTarget #	Azimuth	DeepSpCas9	CrisprScan	ON	Lindel	InDelphi	Frameshift	Precision
OCA01	3	DHLSL-N[31]	60	81	71	477	45	23	33	34	67	78	73	55
OCA02	4	MSCDP-L[43]	81	89	85	112	67	62	33	54	82	83	83	54
OCA03	4	TSQLT-F[63]	79	86	83	135	42	18	27	29	83	87	85	61
OCA04	5	IMKPR-S[109]	77	90	84	125	51	46	49	49	86	93	89	60
OCA05	5	LNDNL-N[137]	91	97	94	29	56	40	52	49	91	94	92	62
OCA06	5	QSIVR-Q[143]	94	93	94	66	43	11	58	37	82	87	85	60
OCA07	5	SIVRQ-T[144]	91	97	94	41	68	62	49	60	72	78	75	49
OCA08	5	GGNYL-M[150]	51	86	69	168	62	49	40	50	84	90	87	48

Fitness

FIG. 32B

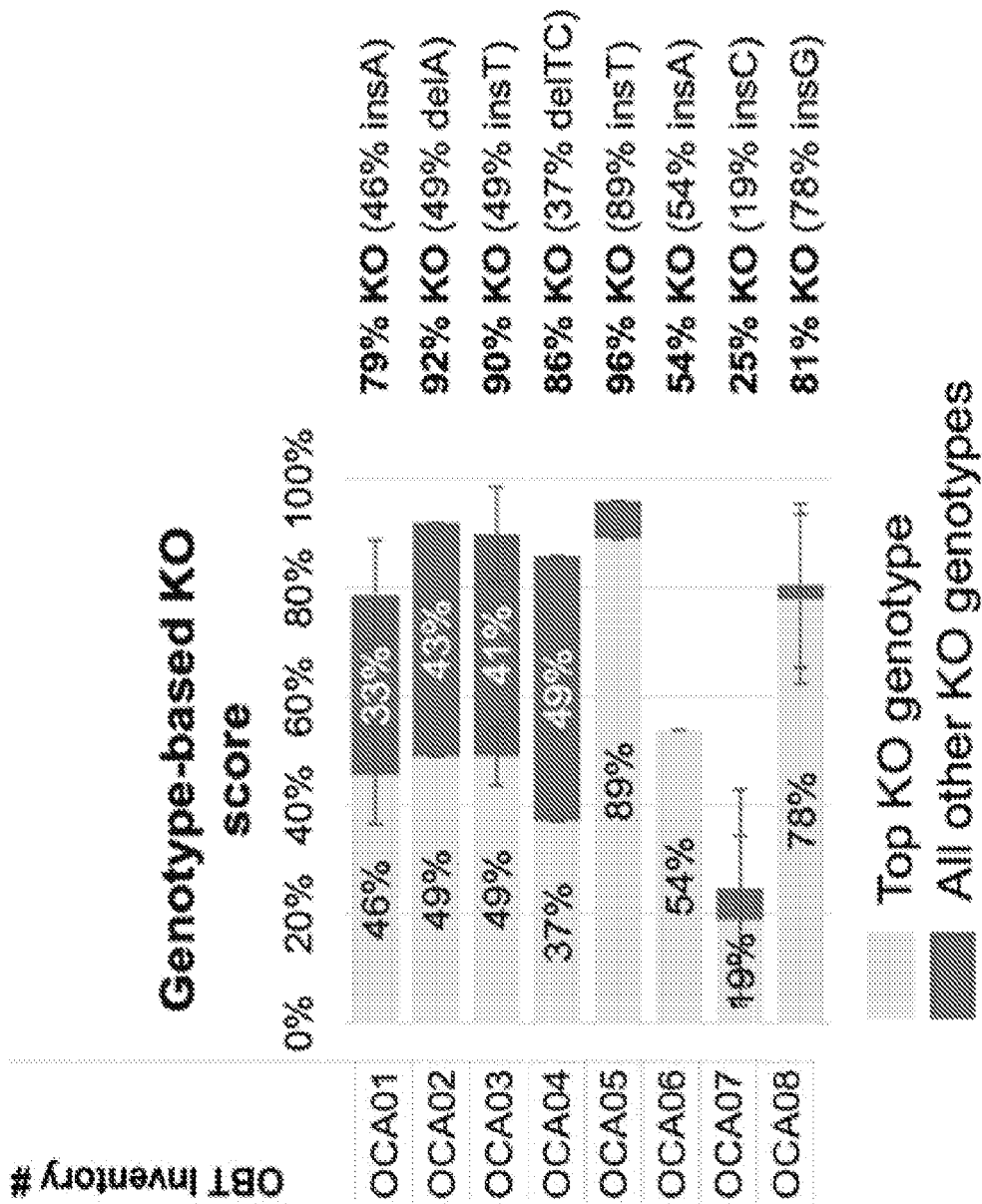


FIG. 32C

New Ref. #	Exon	Amino acid out	Target Sequence	GC %	PAM
					
353	5	MSDAAM-Q[117]	CTTGCAGTCCACCGATTGCA	55	TGG
356	5	ELRALH-L[144]	TTCGGGCTCTCCACCTCAAT	55	GGG
352	5	SDAAMQ-S[118]	TGATGCAGCCATGCAATCGG	55	TGG
354	5	LRALHL-N[145]	TCGGGCTCTCCACCTCAATG	60	GGG
355	5	MSDAAM-Q[117]	GAGTGATGCAGCCATGCAAT	50	CGG
357	4	LQVSHQ-L[57]	TCAGACTCTTGTTACAGAGC	45	TGG
242	4	QLQVSH-Q[56]	ACTCTTGTTACAGAGCTGGT	45	GGG
241	3	ADGPGN-V[32]	TGATGGCCCTGGAAATGTGA	50	AGG

FIG. 33A

Old sg #	New Ref. #	Exon	Uniprot Ref #:	Amino acid (AA)	context - cut @ AA [position]	Off-target				On-target				Frameshift			
						MIT	CFD	Off-target #	Azimuth	DeepSpCas9	CrisprScan	ON	Lindel	Indelphi	Frameshift	Precision	
241	OCB01	3	Q28292:	ADGPGN-V[32]		65	74	70	213	68	54	42	55	88	91	90	53
242	OCB02	4	Q28292:	QLQVSH-Q[56]		73	83	78	140	67	66	37	57	83	84	84	64
357	OCB03	4	Q28292:	LOVSHQ-L[57]		77	86	82	164	52	49	15	39	81	83	82	49
353	OCB04	5	Q28292:	MSDAAM-Q[117]		92	96	94	43	56	51	43	50	91	89	90	61
355	OCB05	5	Q28292:	MSDAAM-Q[117]		81	91	86	100	57	40	61	53	78	85	82	49
352	OCB06	5	Q28292:	SDAAMQ-S[118]		90	94	92	78	63	73	57	64	86	88	87	60
356	OCB07	5	Q28292:	ELRALH-L[144]		89	96	93	47	29	17	50	32	75	82	79	49
354	OCB08	5	Q28292:	LRALHL-N[145]		85	93	89	81	69	67	52	63	78	85	81	56

FIG. 33B

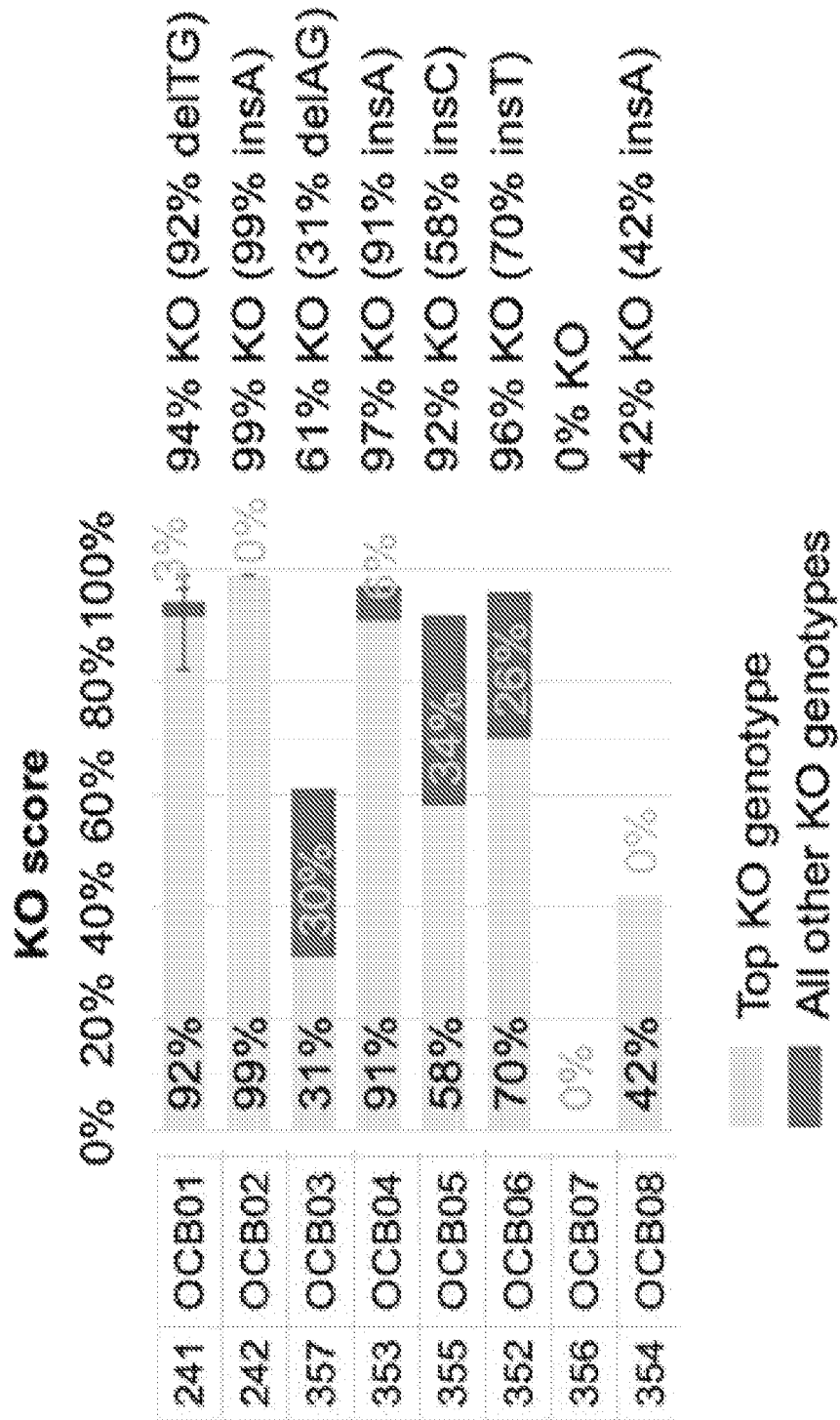
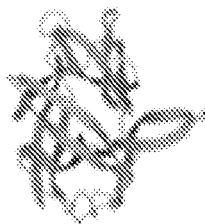


FIG. 33C



**Protein/Phenotype-based
KO score (ELISA)**

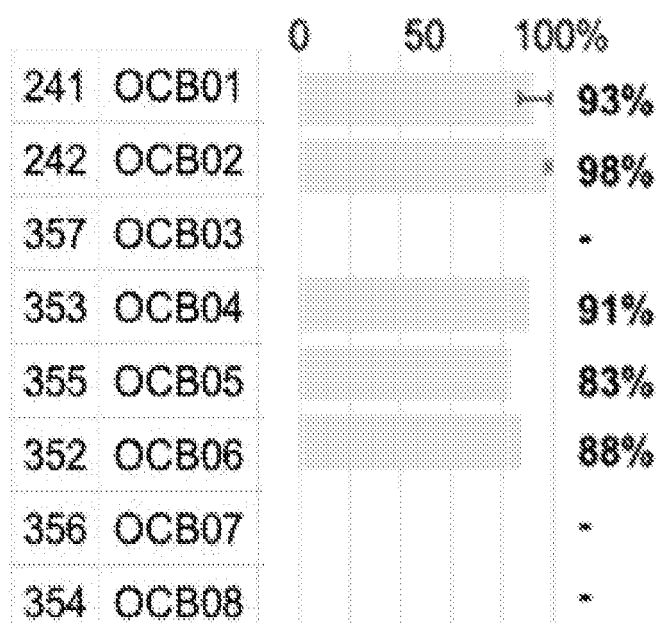


FIG. 33D

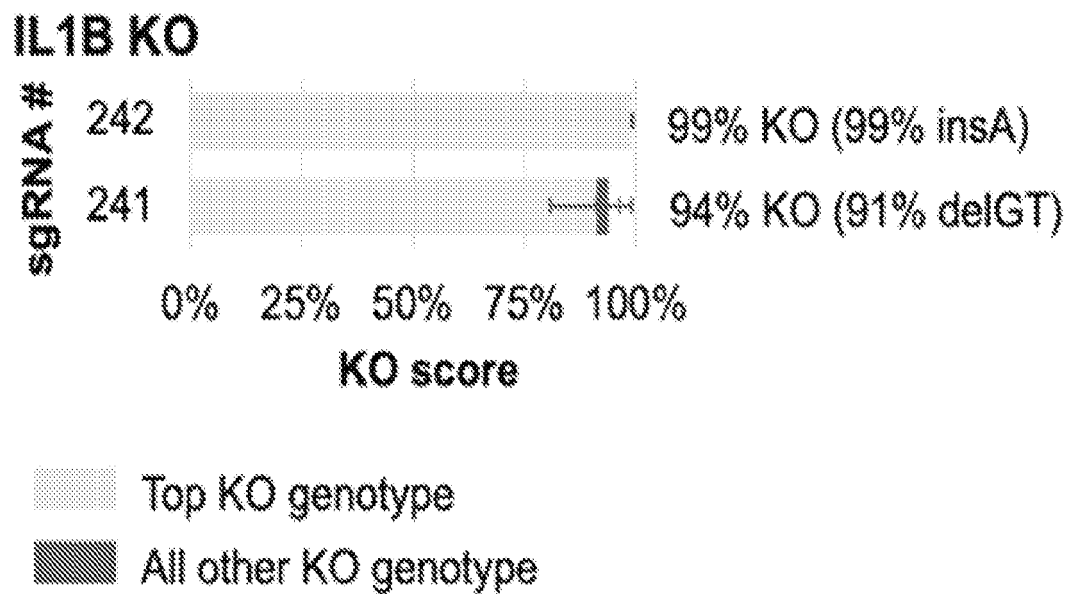


FIG. 34A

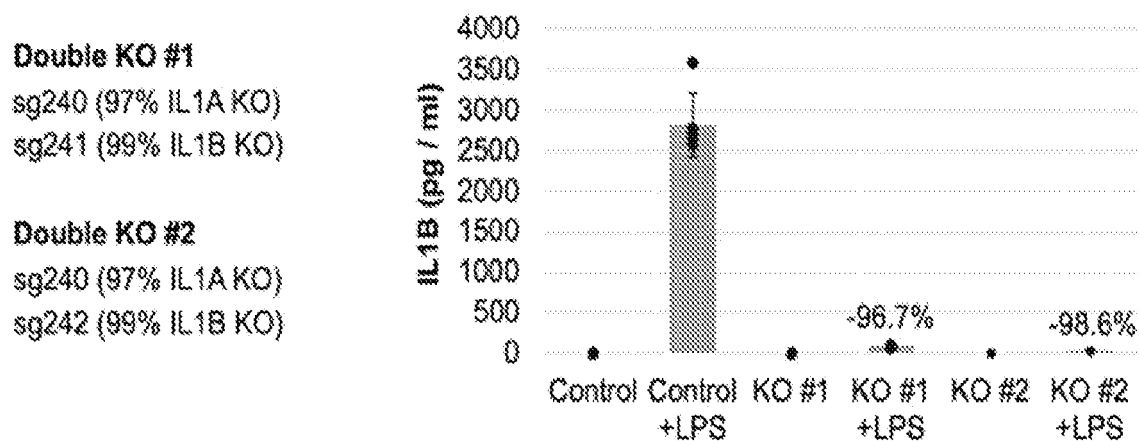


FIG. 34B

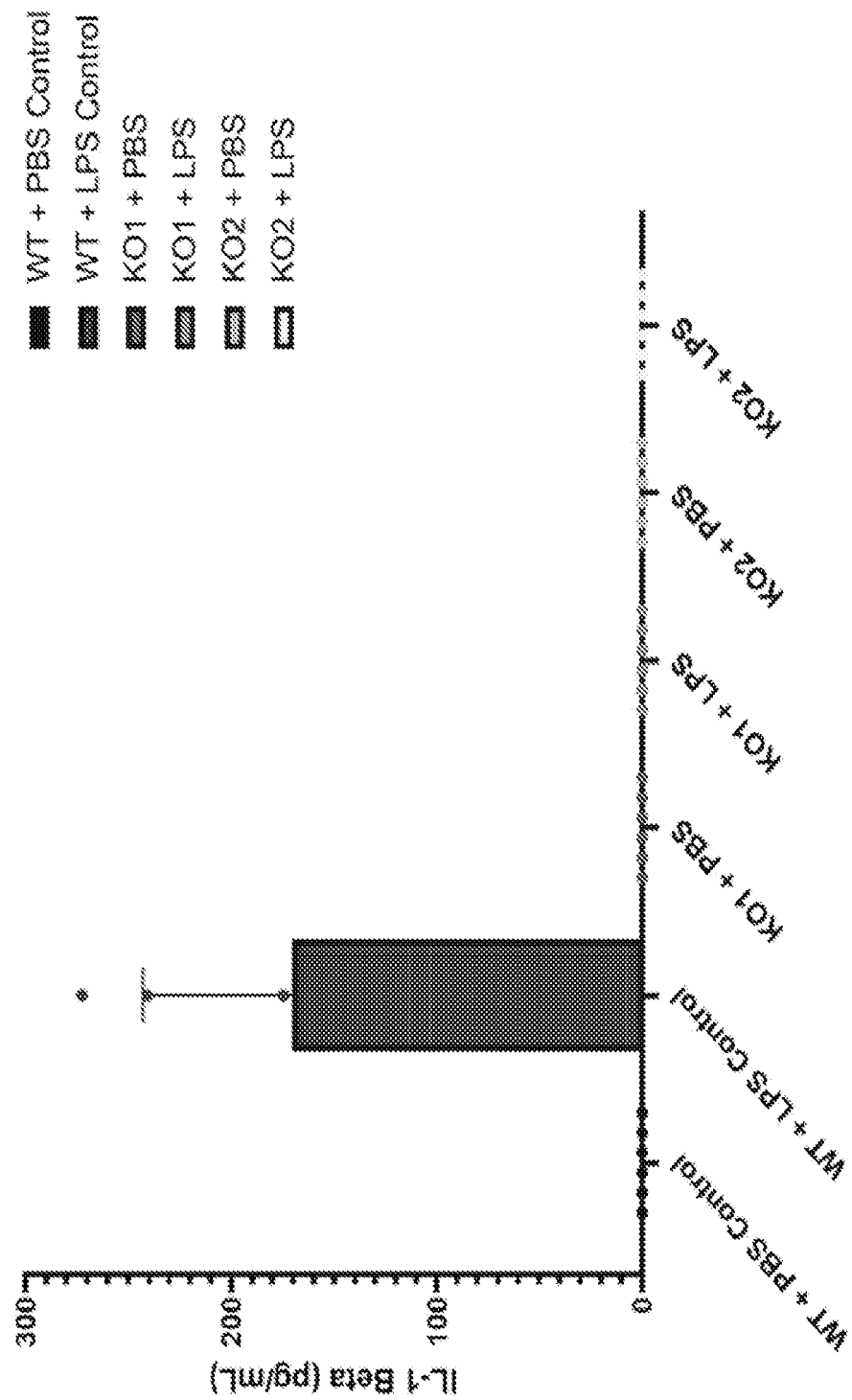


FIG. 35A

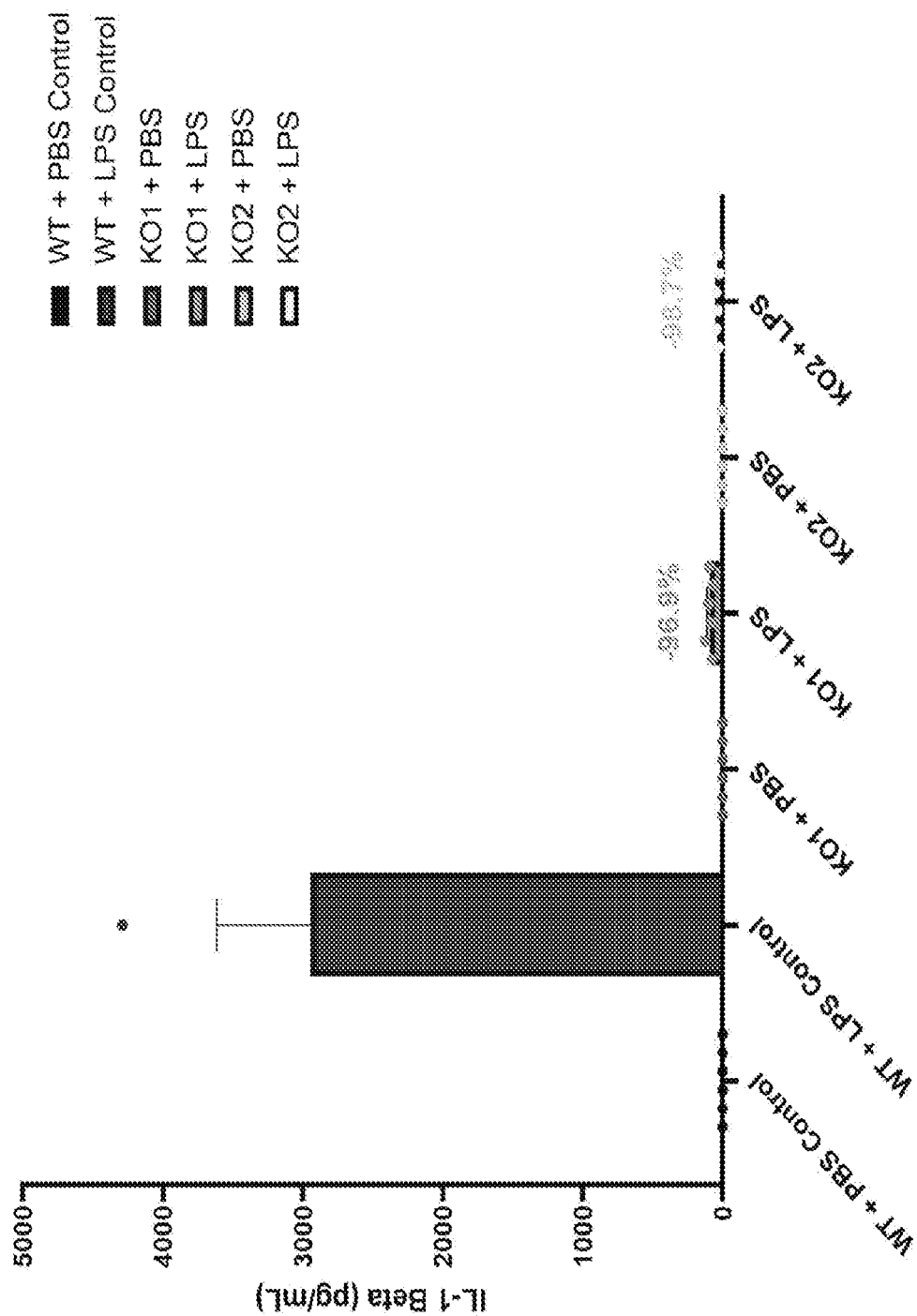


FIG. 35B

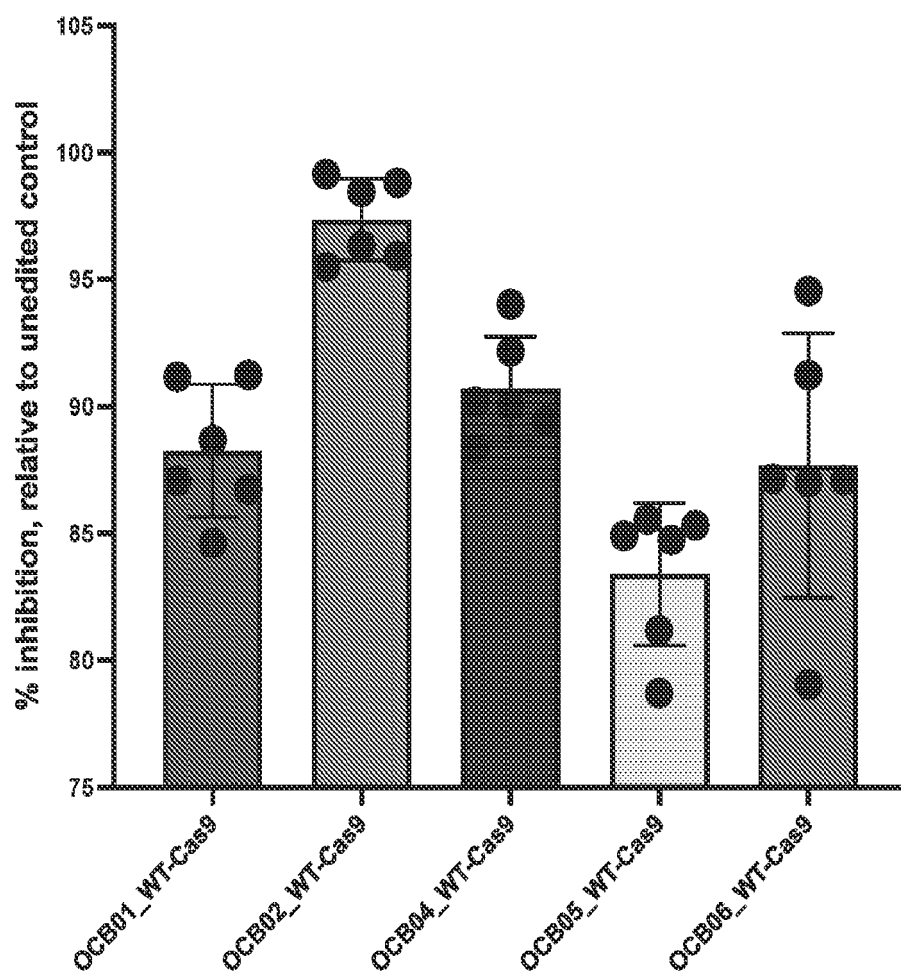


FIG. 36

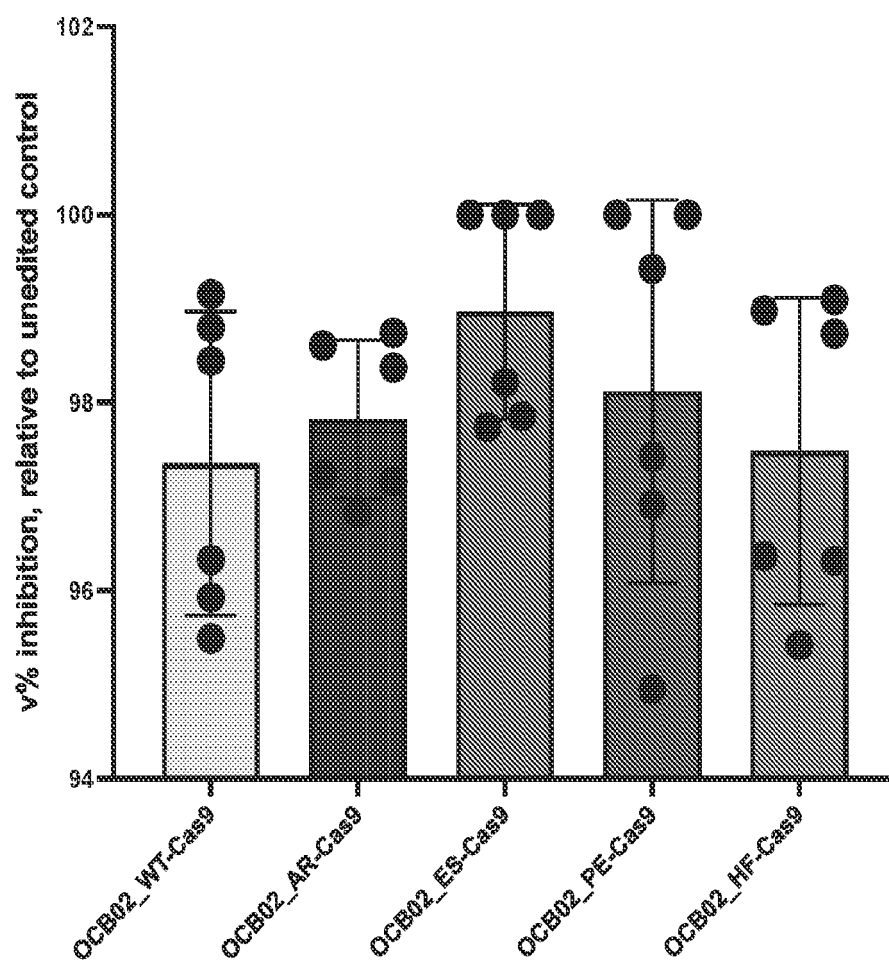


FIG. 37A

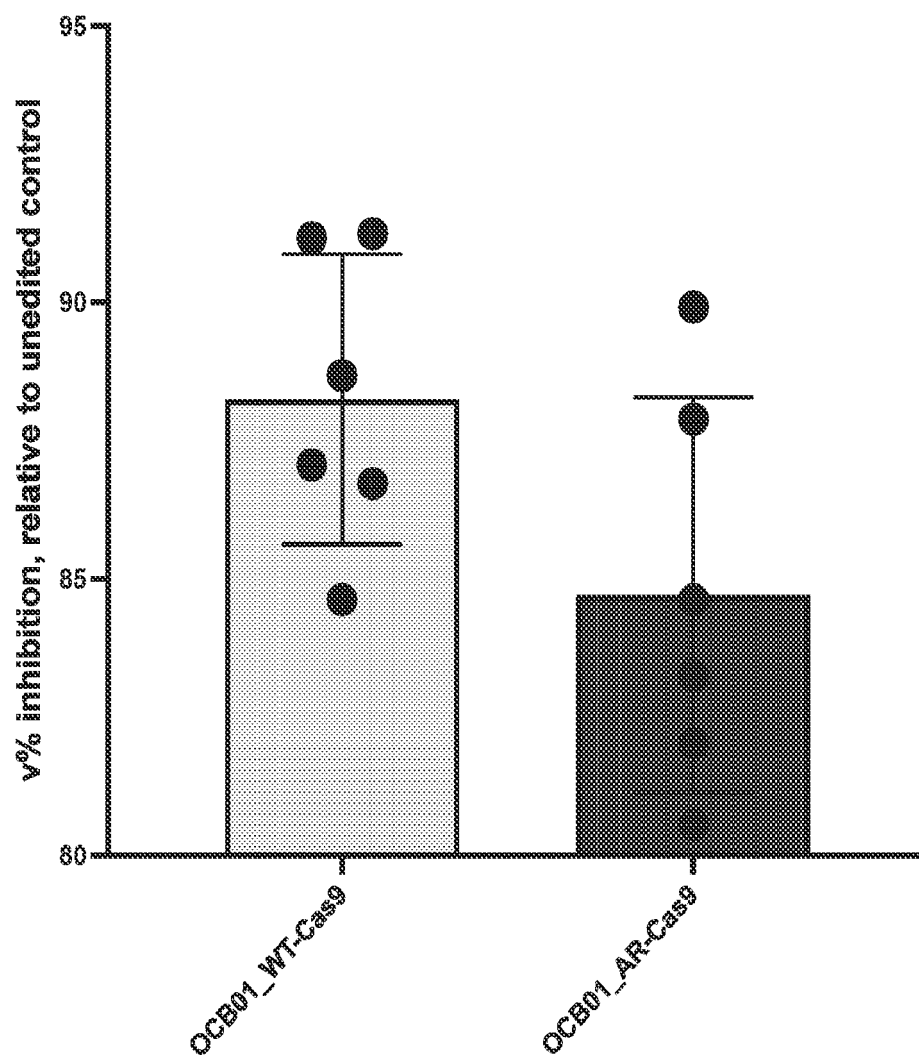


FIG. 37B

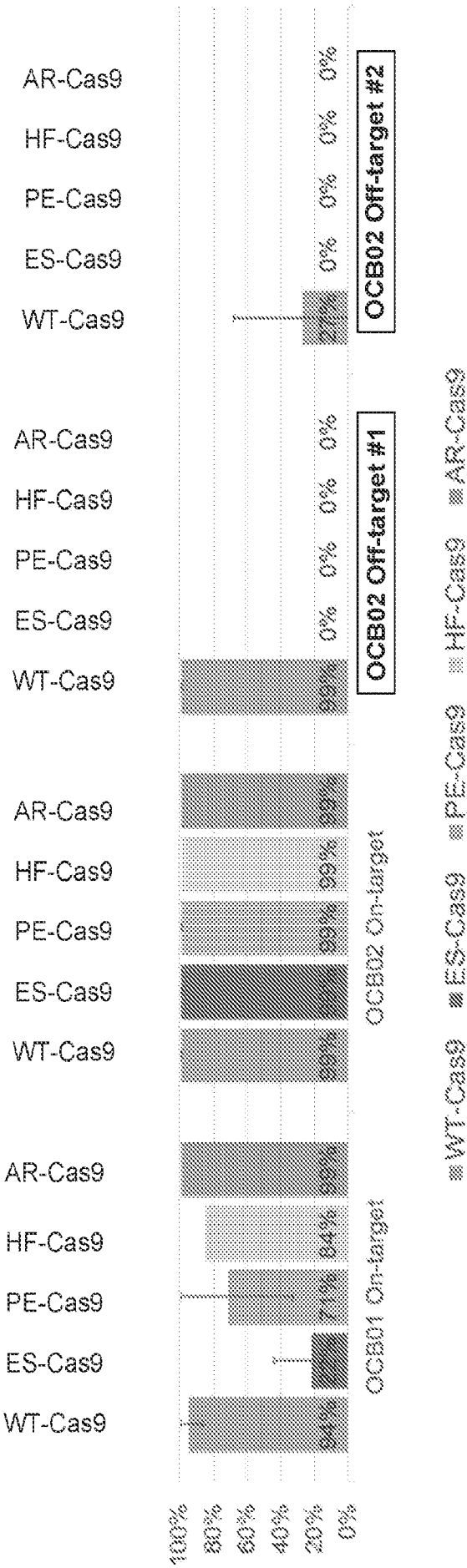


FIG. 38A

#	Mismatch (MM) Positions	# MM	MIT↓	CFD	Genomic Locus
	ACTCTGTGTFACAGAGCTGGINGG	0	100	100	exon: IL1B
1	C...A..C.....	3	2.5	43	intron: SYNJ2
2	GTC.....	3	2.3	45	intergenic: U6-ZWINT
3	G.....A.....A	2	1.4	6	intergenic: HTR2C-ENS
4	...T.....T.....A.	2	1.2	18	intergenic: ENSCAF000000027183
5	.AAI..C.....	4	0.9	29	intergenic: LSAMP-ENS
...	...	...	...	...	intron: FRAS1
140	G.A..TC.....	4	0.0	14	intergenic: ENSCAF000000004649-30498
↓			↓	↓	
# potential off-targets with 4 or less mismatches	PAM including non-canonical NGA and NAG	Off-target activity metrics			

FIG. 38B

Seq ID NO.	Human IL1A				Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
	Exon	UCSC genome assembly ID	Chromosome	Strand							
681	4	hg38	chr2	-	112,781,693	112,781,712	AUGGUGGUAGUAGCAACCAA	77	71	89	79
682	3	hg38	chr2	+	112,782,722	112,782,741	CAGAGACAGAUCAUAGG	75	69	90	78
683	5	hg38	chr2	+	112,779,511	112,779,530	UAUGUAUGCAGCAGCGUG	74	65	85	74
684	4	hg38	chr2	-	112,781,692	112,781,711	UGGUGGUAGUAGCAACCAAC	75	57	87	73
685	4	hg38	chr2	-	112,781,610	112,781,629	GGCCAUCGCCAAUGACUCAG	76	60	82	73
686	5	hg38	chr2	-	112,779,529	112,779,548	AGCCAAUGAUCAGUACCUCA	76	55	88	73
687	4	hg38	chr2	-	112,781,789	112,781,808	AGCUAUGGCCCCACUCCAUGA	76	60	80	72
688	4	hg38	chr2	-	112,781,709	112,781,728	UACCUCAAGGAGAGCAUGG	66	63	87	72
689	4	hg38	chr2	-	112,781,688	112,781,707	GGUAGUAGCAACCAACGGGA	78	59	77	71
690	4	hg38	chr2	+	112,781,800	112,781,819	GCCAUAGCUUACAUGAUAGA	76	43	92	70
691	3	hg38	chr2	+	112,782,719	112,782,738	AUUCAGAGACAGAUCAUCAA	68	51	92	70
692	5	hg38	chr2	+	112,779,633	112,779,652	ARGCUAAAAGGUGCUGACCU	66	57	86	70
693	4	hg38	chr2	+	112,781,674	112,781,693	CUUCUUCAGAACCUUCCCGU	74	55	77	69
694	5	hg38	chr2	+	112,779,621	112,779,640	ACAUGGCUACAGGAAGCUAAA	68	42	92	67
695	5	hg38	chr2	+	112,779,524	112,779,543	AGCCGUGAGGUACUGAUCAU	78	48	76	67

FIG. 39A

Seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
696	6	hg38	chr2	-	112,778,079	112,778,098	GGGUGCUUAUAAGUCAUCAA	78	55	69	67
697	4	hg38	chr2	-	112,781,712	112,781,731	GCUUACCUUCAAGGAGCA	69	50	80	66
698	4	hg38	chr2	+	112,781,704	112,781,723	UACCAUCCUUCUCCUUGA	69	48	82	66
699	4	hg38	chr2	+	112,781,629	112,781,648	CUCCAGGUCAUCAUCAGUGA	62	49	89	66
700	4	hg38	chr2	-	112,781,606	112,781,625	AUGGCCAAUGACUCAGAGGA	73	59	67	66
701	5	hg38	chr2	+	112,779,547	112,779,566	CUCGAUUAUACUUUGAUUG	77	30	92	66
702	4	hg38	chr2	+	112,781,713	112,781,732	GCUCUCCUUGAAGGUAAGCU	75	44	75	65
703	4	hg38	chr2	-	112,781,631	112,781,650	CAUCACUGAUGAUGACCUGG	67	52	75	65
704	5	hg38	chr2	+	112,779,548	112,779,567	UCGAUUAUACUUUGAUUGA	76	30	89	65
705	4	hg38	chr2	+	112,781,772	112,781,791	AUUGAUCCAUUGCAGCCUUCA	71	37	85	64
706	4	hg38	chr2	-	112,781,781	112,781,800	CCCACUCCAUAGAAGGCUGCA	62	43	85	63
707	5	hg38	chr2	+	112,779,559	112,779,578	UUUGAUUGAGGGCGUCAUUC	76	29	84	63
708	4	hg38	chr2	-	112,781,804	112,781,823	UCCUUCUAUCAUGUAAGCUA	75	44	67	62
709	4	hg38	chr2	+	112,781,634	112,781,653	GGUCAUACAUCAGUAGGAU	52	44	89	62
710	4	hg38	chr2	+	112,781,599	112,781,618	CUUACCUUCUUCUGAGUCAU	70	37	79	62

FIG. 39B

SEQ ID NO:	Human IL1B	UCSC genome assembly ID	Chromosome	Strand	start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
711	4	hg38	chr2	+	112,833,509	112,833,528	GGUGGUGGAGAUUCGUAGC	80	53	82	72
712	4	hg38	chr2	+	112,833,462	112,833,481	CAUGGCCACAACAACUGACG	76	57	82	72
713	5	hg38	chr2	-	112,832,797	112,832,816	CUUCGACACAUGGGGAUAACG	80	53	80	71
714	5	hg38	chr2	+	112,832,754	112,832,773	GUGCAGUUCAGUGAUCGUAC	79	54	82	71
715	2	hg38	chr2	-	112,836,196	112,836,215	UGAGCUGGCCAGUGAAAUGA	71	52	89	71
716	5	hg38	chr2	-	112,832,750	112,832,769	GAUCACUGAACUGCAGCGCUC	78	52	82	71
717	5	hg38	chr2	-	112,832,806	112,832,825	ACCUAUCUUCUUGGACACAU	73	48	88	70
718	4	hg38	chr2	-	112,833,538	112,833,557	GACCUCUGCCCUUGGAUGG	59	63	88	70
719	4	hg38	chr2	+	112,833,527	112,833,546	GCUGGAUGCCGCCAUCCAGA	70	55	78	68
720	4	hg38	chr2	-	112,833,499	112,833,518	UCCGACCACCACUACAGCAA	78	59	66	68
721	4	hg38	chr2	+	112,833,491	112,833,510	UGAAGCCCUUGCUGUAGUGG	73	58	72	68
722	6	hg38	chr2	-	112,831,398	112,831,417	UUCUCCAUGUCCUUUGUACA	71	45	89	68
723	6	hg38	chr2	+	112,831,310	112,831,329	CUUAUCAUCUUUCAACACGC	79	39	85	68
724	4	hg38	chr2	+	112,833,526	112,833,545	AGCUGGAUGCCGCCAUCCAG	69	53	80	67
725	4	hg38	chr2	-	112,833,453	112,833,472	UUUGGCCAUGGACAAGCUG	67	62	72	67

FIG. 40A

SEQ ID NO:	Human IL1B	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
726	4	hg38	chr2	+	112,833,402	112,833,421	GCUCAGGUCAUUCUCCUGGA	60	59	83	67
727	5	hg38	chr2	+	112,832,668	112,832,687	GUCCAUUCCUGUCCUUGG	65	65	70	67
728	4	hg38	chr2	-	112,833,500	112,833,519	CUCGACCAACACUACAGCA	74	47	77	66
729	4	hg38	chr2	-	112,833,488	112,833,507	CUACAGCAAGGGCUUCAGGC	63	47	89	66
730	5	hg38	chr2	-	112,832,807	112,832,826	AACCUAUCUUCUCCAGACACA	77	35	85	66
731	5	hg38	chr2	-	112,832,749	112,832,768	AUCACUGAACUGCAGGUCC	76	43	79	66
732	4	hg38	chr2	+	112,833,549	112,833,568	GCAGAGGUCCAGGUCCUGGA	51	61	83	65
733	5	hg38	chr2	+	112,832,802	112,832,821	UCCCAUGUGUCGAAGAAGAU	75	45	74	65
734	5	hg38	chr2	-	112,832,718	112,832,737	CAAAAAGCUUGGUGAUGUC	69	33	93	65
735	2	hg38	chr2	+	112,836,195	112,836,214	AUCAUUUACUGGCCAGCUC	79	35	79	64
736	2	hg38	chr2	+	112,836,185	112,836,204	GUAAUAAGCCAUCAUUUCAC	73	36	83	64
737	4	hg38	chr2	+	112,833,444	112,833,463	CAUCUUCUCCAGCUUGUCCA	63	49	81	64
738	5	hg38	chr2	+	112,832,728	112,832,747	AGCUUUUUUGUGUGAGUCC	69	39	83	64
739	6	hg38	chr2	+	112,831,350	112,831,369	UCCUUGAGGGCCCAAGGCCAC	59	54	79	64
740	6	hg38	chr2	-	112,831,294	112,831,313	UAAGCCCAUUCUACAGCUGG	62	60	69	64

FIG. 40B

Seq ID No.	Canine IL1A			Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
	Exon	UCSC genome assembly ID	Chromosome								
741	5	canFam3	chr17	+	36,975,147	36,975,166	GUCGAACUUAUACUUUGAUUG	94	49	92	78
742	6	canFam3	chr17	-	36,973,236	36,973,255	UUGCCAGUGAAGUUUGACAU	80	64	87	77
743	5	canFam3	chr17	-	36,975,140	36,975,159	AGUAUAGUUCGACAAACAGG	94	60	75	76
744	4	canFam3	chr17	-	36,977,120	36,977,139	UGGUAGUGGUGGCAGCCAAU	83	55	88	75
745	4	canFam3	chr17	+	36,977,194	36,977,213	ACAUGCAGUCCUCAUGAAGU	85	54	83	74
746	5	canFam3	chr17	+	36,975,233	36,975,252	AAGUUGUAUGCUACUGAUCU	84	49	89	74
747	4	canFam3	chr17	-	36,977,206	36,977,225	GAGCUGUGACCCACUUCUUG	87	55	77	73
748	5	canFam3	chr17	-	36,975,143	36,975,162	CAAAGUAUAGUUCGACAAAC	94	37	85	72
749	4	canFam3	chr17	-	36,977,121	36,977,140	GUGGUAGUGGUGGCAGCCAA	68	61	87	72
750	2	canFam3	chr17	-	36,978,664	36,978,683	CCUGAAGAACUGUUACAGGU	78	56	78	71
751	4	canFam3	chr17	+	36,977,216	36,977,235	GUCACAGCUCAUAUCAUAGA	82	42	88	70
752	5	canFam3	chr17	+	36,975,108	36,975,127	UCUGUAAUGCAGCAGUCAUG	69	50	87	69
753	4	canFam3	chr17	+	36,977,141	36,977,160	AUUUCCUUGAAGGUUAGCU	79	44	84	69
754	4	canFam3	chr17	+	36,977,193	36,977,212	GACAUGCAGUCCUCAUGAAG	82	49	75	69
755	4	canFam3	chr17	+	36,977,051	36,977,070	AAUGUCUCCAGGUCAUCAU	82	54	70	69

FIG. 41A

SEQ ID NO:	Canine IL1A			Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
	Exon	UCSC genome assembly ID	Chromosome								
756	4	canFam3	chr17	+	36,977,162	36,977,181	GGAUGUCUUUGAGAUUUCAG	69	56	81	69
757	6	canFam3	chr17	+	36,973,122	36,973,141	CCACUGACCUUUGAGCAAUAC	91	33	83	69
758	4	canFam3	chr17	-	36,977,062	36,977,081	AUUCACACCCGAUGAGACC	91	45	69	68
759	6	canFam3	chr17	+	36,973,230	36,973,249	GCUCCCAUGUCAAAACUUCAC	83	35	86	68
760	6	canFam3	chr17	-	36,973,237	36,973,256	UUUGCCAGUGAAGUUUGACA	79	43	80	68
761	5	canFam3	chr17	-	36,975,105	36,975,124	GACUGCUGCAUUACAGAAUU	86	30	85	67
762	4	canFam3	chr17	-	36,977,149	36,977,168	GACAUCCAGCUUACCUUCA	83	29	85	66
763	4	canFam3	chr17	-	36,977,140	36,977,159	GUUACCUUCAAGGAAAUG	69	55	75	66
764	5	canFam3	chr17	+	36,975,232	36,975,251	GAAUUGUAUGCUACUGAUC	91	28	81	66
765	5	canFam3	chr17	+	36,975,169	36,975,188	GUUGUCAUUCAGGAUUAUU	77	38	84	66
766	4	canFam3	chr17	+	36,977,102	36,977,121	CUUCUUUAGAAUCUCCCAU	70	43	82	65
767	2	canFam3	chr17	-	36,978,668	36,978,687	AAGACCUUGAAGAACUGUUC	82	36	74	64
768	2	canFam3	chr17	+	36,978,686	36,978,705	UUCAAAGAGGUCAGGAACUU	74	35	84	64
769	6	canFam3	chr17	+	36,973,182	36,973,201	UUUGAUUUUUCUAGAGUCAC	75	38	78	64
770	2	canFam3	chr17	+	36,978,673	36,978,692	AGUUCUUCAGGUCUUCAAAG	72	51	66	63

FIG. 41B

Seq ID No:	Canine IL1B			Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
	Exon	ucsc genome assembly ID	Chromosome								
771	5	canFam3	chr17	-	37,021,899	37,021,918	UGAUGCAGCCCAUGCAAAUCGG	92	64	87	81
772	5	canFam3	chr17	+	37,021,888	37,021,907	CUUGCAGUCCACCGAUGCA	94	50	90	78
773	5	canFam3	chr17	-	37,021,818	37,021,837	UCGGGCUUCUCCACCUCAAUG	89	63	81	78
774	3	canFam3	chr17	-	37,023,344	37,023,363	AAAUGUGAAGGUGAGACCAU	78	66	80	75
775	6	canFam3	chr17	-	37,020,531	37,020,550	CCACAUGAGCUUUGUGCACG	90	51	85	75
776	4	canFam3	chr17	+	37,022,218	37,022,237	CCUCAUCUACCAGAGAAACUG	75	59	88	74
777	4	canFam3	chr17	+	37,022,177	37,022,196	ACUCUUGUUACAGAGCUGGU	78	57	84	73
778	5	canFam3	chr17	-	37,021,902	37,021,921	GAGUGAUGCAGCCCAUGCAAU	86	53	82	73
779	2	canFam3	chr17	-	37,023,937	37,023,956	UGGCUUACUCCAGGUCAGUG	78	63	79	73
780	6	canFam3	chr17	+	37,020,421	37,020,440	ACUCACCUUCUAGCUGUAGGG	87	48	82	72
781	3	canFam3	chr17	-	37,023,356	37,023,375	UGAUGGCCCCUGGAAAUUGA	70	55	90	71
782	5	canFam3	chr17	-	37,021,884	37,021,903	AUCGGUGGACUGCAAGUUAC	93	43	78	71
783	4	canFam3	chr17	-	37,022,220	37,022,239	CACAGUUCUCUGGUAGAUGA	81	56	77	71
784	2	canFam3	chr17	-	37,023,957	37,023,976	CGAACUCACAGUGAAAUAGA	77	46	91	71
785	6	canFam3	chr17	+	37,020,422	37,020,441	CUCACCUUCUAGCUGUAGGGU	81	50	82	71

FIG. 42A

Canine IL1B				Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
Seq ID No:	Exon	ucsc genome assembly ID	Chromosome								
786	5	canFam3	chr17	-	37,021,820	37,021,839	CUUCGGGCUUCUCCACCUCAA	87	42	86	71
787	4	canFam3	chr17	+	37,022,191	37,022,210	GCUGGUGGGAGACUUGCAAC	88	50	75	71
788	6	canFam3	chr17	-	37,020,533	37,020,552	UCCACAUAGAGCUUUGUGCA	76	46	90	71
789	6	canFam3	chr17	-	37,020,429	37,020,448	AAAGCCCACCCUACAGCUAG	89	52	68	70
790	5	canFam3	chr17	+	37,021,842	37,021,861	CAUAUGAGUUAGACAGCAC	90	50	69	70
791	6	canFam3	chr17	-	37,020,452	37,020,471	CUGUCCUGUGUGAUGAAGGA	69	54	87	70
792	3	canFam3	chr17	-	37,023,345	37,023,364	GAAUUGUGAAGGUGAGACCA	71	55	84	70
793	6	canFam3	chr17	-	37,020,504	37,020,523	AAGUAAUAAACAAGAUACUG	71	57	80	69
794	4	canFam3	chr17	-	37,022,146	37,022,165	CGUGUCAGUCAUUGUAGCUU	92	33	82	69
795	6	canFam3	chr17	+	37,020,445	37,020,464	CUUCCAUCCUUCACACAC	74	44	88	69
796	4	canFam3	chr17	+	37,022,230	37,022,249	GAGAACUGUGGUUCAGGUCU	78	51	80	69
797	2	canFam3	chr17	+	37,023,946	37,023,965	GGAGUAGCCAUCAUUCAC	83	40	84	69
798	2	canFam3	chr17	-	37,023,946	37,023,965	GUGAAAUGAUGGCUUACUCC	88	45	75	69
799	5	canFam3	chr17	-	37,021,819	37,021,838	UUCGGGCUUCCACCUCAAU	93	32	79	68
800	6	canFam3	chr17	+	37,020,540	37,020,559	AGCUCAUGUGGAACACCACU	88	39	76	68

FIG. 42B

Equine IL1A														
Seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score			
801	5	equCab3	chr15	+	16,365,277	16,365,296	AAGUUGUAAUGUACUGAUCG	95	56	87	79			
802	5	equCab3	chr15	+	16,365,215	16,365,234	CAUCAUUCAGAGUACACUGG	84	61	84	76			
803	5	equCab3	chr15	+	16,365,212	16,365,231	GGGCAUCAUUCAGAGUACAC	83	57	89	76			
804	5	equCab3	chr15	+	16,365,275	16,365,294	GGAGUUGUAAUGUACUGAU	84	51	89	75			
805	6	equCab3	chr15	-	16,364,107	16,364,126	UUGCCAGUGAAAUUUGACAU	76	61	85	74			
806	4	equCab3	chr15	+	16,367,084	16,367,103	GUCAUAGCUUGCAUCAUAGA	88	40	89	72			
807	4	equCab3	chr15	-	16,367,074	16,367,093	AAGCUAUGACCCACUUCUG	77	59	79	72			
808	4	equCab3	chr15	-	16,366,996	16,367,015	GCUGAACUUCAGGAGAGCG	79	56	82	72			
809	4	equCab3	chr15	-	16,366,977	16,366,996	GUGGUGCUGGUGGCAGCCAA	65	66	85	72			
810	4	equCab3	chr15	+	16,366,958	16,366,977	CUUCUUCAGAGUCUUCUCCGU	80	55	82	72			
811	6	equCab3	chr15	+	16,363,989	16,364,008	AGGACAGACCCUUDAGCAGUA	88	46	81	72			
812	5	equCab3	chr15	-	16,365,184	16,365,203	AGUGUAAUUCGAGACACAUC	89	44	82	71			
813	6	equCab3	chr15	+	16,363,990	16,364,009	GGACAGACCCUUDAGCAGUAC	95	46	73	71			
814	4	equCab3	chr15	-	16,366,976	16,366,995	UGGUGCUGGUGGCAGCCAAC	76	53	83	70			
815	5	equCab3	chr15	+	16,365,191	16,365,210	CUCGAAUACACUUAUGAUG	89	31	90	70			

FIG. 43A

Equine IL1A				Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
Seq ID NO:	Exon	UCSC genome assembly ID										
816	2	equCab3	chr15	-	16,369,063	16,369,082	CCUGAAGAACUGUUAACAGGU	70	53	80	68	
817	4	equCab3	chr15	+	16,366,997	16,367,016	GCUCUCCUUGAAGUUCAGCU	79	49	77	68	
818	5	equCab3	chr15	+	16,365,276	16,365,295	GAAGUGGAAUGUACUGAUC	90	36	79	68	
819	4	equCab3	chr15	-	16,367,065	16,367,084	CCCACUCCUGAGGACUGCA	68	48	86	67	
820	4	equCab3	chr15	+	16,367,062	16,367,081	CCAUGCAGUCCUCAGGAAGU	66	55	79	67	
821	4	equCab3	chr15	+	16,367,009	16,367,028	GUUCAGCUUGGAGUUCUUAG	84	38	79	67	
822	4	equCab3	chr15	+	16,366,907	16,366,926	AAUGGCUUCCAGGUCAUCAU	85	49	67	67	
823	5	equCab3	chr15	+	16,365,192	16,365,211	UCGAAUUAACACUUUGAUUGA	84	32	86	67	
824	6	equCab3	chr15	+	16,364,101	16,364,120	GCACCCAUUGUCAAUUUUCAC	85	31	85	67	
825	4	equCab3	chr15	+	16,367,055	16,367,074	AAUGUAUCCCAUGCAGUCCUC	84	46	67	66	
826	4	equCab3	chr15	+	16,367,018	16,367,037	GGAGUCUUUAGAGGUUUCAG	73	59	65	66	
827	6	equCab3	chr15	-	16,364,087	16,364,106	GGGUGCUUAUAUCAUGAAG	84	48	67	66	
828	6	equCab3	chr15	-	16,364,000	16,364,019	AGAUGAACCCGUACUGCUAA	94	35	71	66	
829	2	equCab3	chr15	+	16,369,078	16,369,097	UCAGGUCUUCAAAGAGGUCA	68	49	73	63	
830	4	equCab3	chr15	-	16,367,005	16,367,024	GACAUCCAAAGCUGAACUCCA	79	33	78	63	

FIG. 43B

Seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
831	4	equCab3	chr15	-	16,422,442	16,422,461	GCAUGUCGUGUCAUCAUUG	94	51	79	74
832	4	equCab3	chr15	-	16,422,510	16,422,529	CUCAGCUCCAUGGCGAUGG	67	67	78	71
833	4	equCab3	chr15	+	16,422,466	16,422,485	AAGUCUUGUUGUAAAGUUGG	72	51	84	69
834	5	equCab3	chr15	-	16,421,656	16,421,675	CUGCAGGCUUGUCCACCUCAA	62	61	84	69
835	5	equCab3	chr15	+	16,421,639	16,421,658	GGUUGUAUUCUCUCCAUUG	64	60	84	69
836	6	equCab3	chr15	+	16,420,228	16,420,247	CUCACCUCCAGCUGUAGGGU	76	49	82	69
837	5	equCab3	chr15	-	16,421,721	16,421,740	AUUCAGUGACUGCAGACUC	64	56	84	68
838	5	equCab3	chr15	+	16,421,699	16,421,718	GGGAUUUAUGGUAUAUGUCC	70	58	77	68
839	5	equCab3	chr15	-	16,421,689	16,421,708	CAUAAUCCUGGUGUGUC	66	43	94	68
840	6	equCab3	chr15	+	16,420,227	16,420,246	ACUCACCUCCAGCUGUAGGG	75	49	82	68
841	4	equCab3	chr15	+	16,422,481	16,422,500	GUUGGUGGAGAAUUGAAGC	77	45	79	67
842	4	equCab3	chr15	-	16,422,377	16,422,396	CCUCCAGGAUGAUGACUUG	58	49	93	67
843	5	equCab3	chr15	+	16,421,679	16,421,698	ACAUGCACCGGACAGCACCA	66	63	73	67
844	6	equCab3	chr15	-	16,420,339	16,420,358	UUCUGCAUGAGCUUUGGCA	57	49	94	67
845	6	equCab3	chr15	-	16,420,235	16,420,254	GAAGCCACCCUACAGCUGG	72	60	69	67

FIG. 44A

Seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
846	4	equCab3	chr15	+	16,422,500	16,422,519	CUGGAUGCCCCCAUCGCCCA	64	54	80	66
847	4	equCab3	chr15	+	16,422,467	16,422,486	AGUCUUGUUGAAAGUUGGU	75	44	81	66
848	5	equCab3	chr15	-	16,421,699	16,421,718	GGACAUAUACCAUAAAUCCC	69	46	84	66
849	6	equCab3	chr15	-	16,420,270	16,420,289	AAGAACCUUGUACCUUGUCUUG	63	48	86	66
850	6	equCab3	chr15	-	16,420,257	16,420,276	UGUCUUGUGGGAUGAAAGAU	71	50	77	66
851	4	equCab3	chr15	-	16,422,400	16,422,419	GAUACCCGUUCCUUGCUCAC	60	53	84	65
852	5	equCab3	chr15	-	16,421,635	16,421,654	GGAGAGAUACAACCAACA	57	55	82	65
853	6	equCab3	chr15	+	16,420,284	16,420,303	GUUCUUUUCCUUGAGGCCCA	63	44	86	65
854	6	equCab3	chr15	+	16,420,256	16,420,275	CAUCUUUCAUCCCAAGAC	70	37	90	65
855	2	equCab3	chr15	+	16,423,392	16,423,411	GCAGUAAGUCAUCAUGUCAC	60	51	81	64
856	4	equCab3	chr15	+	16,422,463	16,422,482	UGAAAGUCUUUGUUGUAAAGU	75	29	87	64
857	6	equCab3	chr15	-	16,420,269	16,420,288	AGAACCUGUACCUUGUCUUGU	58	46	87	64
858	6	equCab3	chr15	-	16,420,258	16,420,277	CUGUCUUUGUGGAUGAAAGA	70	42	80	64
859	6	equCab3	chr15	-	16,420,238	16,420,257	UGGGAAGCCCAACCCUACAGC	83	38	70	64
860	6	equCab3	chr15	+	16,420,224	16,420,243	UUCACUCACCCUCCAGCUGUA	58	49	84	64

FIG. 44B

SEQ ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
861	5	felCat9	chrA3	-	105,117,073	105,117,092	AAGUUGGGUGCUACUGAUCU	86	55	87	76
862	6	felCat9	chrA3	+	105,118,589	105,118,608	GCCAGUGAAAUUUGACAUGG	81	59	87	76
863	4	felCat9	chrA3	-	105,115,232	105,115,251	CUUCCAGGUCAUCAAGCAGUG	73	64	87	75
864	5	felCat9	chrA3	-	105,117,159	105,117,178	UUGGAAUUAACACUUUGACUG	85	50	89	75
865	5	felCat9	chrA3	-	105,117,198	105,117,217	UCUGUAAUGCAGCGGCGCG	90	61	75	75
866	4	felCat9	chrA3	+	105,115,148	105,115,167	GCUUACCCUCAAGAAGAGUG	80	61	82	74
867	4	felCat9	chrA3	+	105,115,168	105,115,187	UGGUGAUGGGUGGCAGCCAAU	79	55	86	73
868	4	felCat9	chrA3	-	105,115,060	105,115,079	GUCAUAGCUUGCAUCAUAGA	89	40	88	72
869	4	felCat9	chrA3	-	105,115,082	105,115,101	CUGUACAGUCCUUAUGAAGU	86	52	78	72
870	6	felCat9	chrA3	+	105,118,587	105,118,606	UUGCCAGUGAAAUUUGACAU	80	51	86	72
871	2	felCat9	chrA3	+	105,113,230	105,113,249	CCUGAAGAACUGUUACAGGU	75	56	79	70
872	4	felCat9	chrA3	+	105,115,070	105,115,089	AAGCUAUGACCCACUUCUAG	86	52	71	70
873	4	felCat9	chrA3	-	105,115,119	105,115,138	UUAGAGGUUUCAGAAAGUACU	74	57	79	70
874	4	felCat9	chrA3	-	105,115,146	105,115,165	CUCUUCUUGAGGGUUAAGCUG	81	57	73	70
875	4	felCat9	chrA3	-	105,115,147	105,115,166	ACUCUUCUUGAGGGUUAAGCU	87	42	81	70

FIG. 45A

SEQ ID NO:	Feline IL1A				Strand	Start coordinate	End coordinate	crRNA sequence	Off-target score	On-target score	Frameshift score	Overall score
	Exon	UCSC genome assembly ID	Chromosome									
876	4	felCat9	chrA3	+	105,115,167	105,115,186	GUGGUGAUGGGCGAGCCAA	64	61	86	70	
877	5	felCat9	chrA3	+	105,117,164	105,117,183	AAAGUGUAAUUCGAAAAGCA	78	52	80	70	
878	6	felCat9	chrA3	-	105,118,704	105,118,723	CGACUGACCUUUAGCAAUAC	93	34	80	69	
879	5	felCat9	chrA3	+	105,117,166	105,117,185	AGUGUAAUUCGAAAAGCAGG	79	56	68	68	
880	5	felCat9	chrA3	+	105,117,201	105,117,220	AGCCGUGCAUUACAGAAUC	88	34	83	68	
881	5	felCat9	chrA3	-	105,117,206	105,117,225	AUCCAGAUUCUGUAAUGCAG	73	57	75	68	
882	4	felCat9	chrA3	-	105,115,135	105,115,154	GGUAGCUGGGGUGUCUUAG	80	39	82	67	
883	5	felCat9	chrA3	-	105,117,074	105,117,093	GAAGUUGGGUGCUACUGAUC	89	32	82	67	
884	5	felCat9	chrA3	+	105,117,163	105,117,182	CAAAGUGUAAUUCGAAAAGC	87	36	79	67	
885	6	felCat9	chrA3	+	105,118,588	105,118,607	UGCCAGUGAAAUUUGACAUG	76	40	84	67	
886	6	felCat9	chrA3	-	105,118,593	105,118,612	GCCCCAUGUCAAAUUUCAC	88	31	84	67	
887	4	felCat9	chrA3	-	105,115,083	105,115,102	UCUGUACAGUCCUCAUGAAG	84	48	68	66	
888	4	felCat9	chrA3	-	105,115,156	105,115,175	CAUCACCACACUCUUCUUGA	77	39	82	66	
889	4	felCat9	chrA3	+	105,115,226	105,115,245	AUCCUCACUGCUGAUGACC	78	43	76	66	
890	5	felCat9	chrA3	-	105,117,088	105,117,107	UUUUCACUGCUAUAAGAAGUU	77	39	82	66	

FIG. 45B

seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	Strand	start coordinate	End coordinate	sgRNA target sequence	Off-target score	On-target score	Frameshift score	Overall score
891	4	felCat9	chrA3	+	105,052,988	105,053,007	GAGUCUUAGGCAUGCCGUGU	94	59	88	80
892	4	felCat9	chrA3	-	105,053,005	105,053,024	CAUGGCCACAAUGACCGACA	92	68	76	79
893	5	felCat9	chrA3	+	105,053,695	105,053,714	UGAUGCGGCCCAUACAACAC	96	57	78	77
894	5	felCat9	chrA3	+	105,053,656	105,053,675	ACCCAUCAUCUGUGACACGU	84	59	84	76
895	6	felCat9	chrA3	+	105,055,070	105,055,089	CCGAUGAGCUUUGGCACG	90	53	85	76
896	5	felCat9	chrA3	-	105,053,791	105,053,810	GGUACAUAUUCUGUCCAUUG	83	59	84	75
897	6	felCat9	chrA3	+	105,055,073	105,055,092	CAUGAGCUUUGUGCACGGGG	85	61	79	75
898	5	felCat9	chrA3	-	105,053,706	105,053,725	CGUGUAGUCCUGUGAUUGUA	92	42	87	74
899	6	felCat9	chrA3	+	105,055,068	105,055,087	UUCGCAUGAGCUUUGUGCA	86	44	92	74
900	4	felCat9	chrA3	+	105,052,997	105,053,016	GCAUGCCGUGUCGGUCAUUG	96	48	75	73
901	5	felCat9	chrA3	+	105,053,757	105,053,776	UGUCUGGCUCAUACGAGCUU	95	40	86	73
902	6	felCat9	chrA3	-	105,055,183	105,055,202	GGUACUCACCUCCAGCUGUA	83	53	84	73
903	2	felCat9	chrA3	-	105,050,631	105,050,650	CAUGGCCACUUCAGAAACCU	81	58	76	72
904	5	felCat9	chrA3	+	105,053,655	105,053,674	AACCCAUCAUCUGUGACACG	83	50	83	72
905	5	felCat9	chrA3	+	105,053,758	105,053,777	GUCUGGCUCAUACGAGCUUC	93	38	84	72

FIG. 46A

Feline IL1B												
Seq ID NO:	Exon	UCSC genome assembly ID	Chromosome	strand	start coordinate	End coordinate	sgRNA target sequence	Off-target score	On-target score	Frameshift score	Overall score	
906	5	felCat9	chrA3	+	105,053,774	105,053,793	CUUCGGGCUUCCACCUCAA	86	47	84	72	
907	6	felCat9	chrA3	+	105,055,069	105,055,088	UCCGCAUGAGCUUUGGCAC	87	49	79	72	
908	6	felCat9	chrA3	-	105,055,180	105,055,199	ACUCACCUCAGCUGUAGGG	79	53	83	72	
909	5	felCat9	chrA3	-	105,053,752	105,053,771	CGUAUGAGCCAGACAGCACC	88	48	78	71	
910	5	felCat9	chrA3	+	105,053,795	105,053,814	GGACAGAAUUGAACCACAA	79	55	80	71	
911	5	felCat9	chrA3	-	105,053,812	105,053,831	AACGUCCCCAGUUACCUUGU	87	40	86	71	
912	6	felCat9	chrA3	+	105,055,172	105,055,191	GAAACCCACCCUACAGCUGG	83	57	72	71	
913	6	felCat9	chrA3	-	105,055,179	105,055,198	CUCACCUCAGCUGUAGGGU	81	50	82	71	
914	2	felCat9	chrA3	-	105,050,673	105,050,692	GUAGUAAGCCAUCAUUUCAC	83	41	86	70	
915	3	felCat9	chrA3	-	105,051,268	105,051,287	GUCUCACCUUUAUCUUUUUCG	82	36	91	70	
916	4	felCat9	chrA3	-	105,052,931	105,052,950	CCUCAUUCUCCAGAAAACUG	68	56	85	70	
917	4	felCat9	chrA3	+	105,052,975	105,052,994	AGCCCGACAAACAAGAGUCUU	87	43	78	70	
918	5	felCat9	chrA3	+	105,053,663	105,053,682	AUCUGUGACACGUGGGAUGA	84	46	79	70	
919	6	felCat9	chrA3	+	105,055,150	105,055,169	UGUCCUGUGUGAUGAAAGAU	80	47	83	70	
920	4	felCat9	chrA3	-	105,052,972	105,052,991	ACUCUUGUUGUCCGGCCUGGU	77	46	85	69	

FIG. 46B