(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 November 2021 (11.11.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/224599 A1

(51) International Patent Classification:
A01K 67/027 (2006.01) *C12N 15/90* (2006.01)
C07K 14/705 (2006.01) *C12Q 1/68* (2018.01)
C12N 15/877 (2010.01)

(21) International Application Number:
PCT/GB2021/051060

(22) International Filing Date:
04 May 2021 (04.05.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/020,128 05 May 2020 (05.05.2020) US
63/021,370 07 May 2020 (07.05.2020) US

(71) Applicant: **GENUS PLC** [GB/GB]; 1525 River Road, DeForest, Wisconsin 53532 (US).

(72) Inventors: **CIGAN, Andrew Mark**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **LIGHTNER, Jonathan Edward**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **CULBERTSON, Matthew Scott**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **CHRISTIANSON, William Thomas**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **BEATON, Benjamin**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **BURGER, Brian**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **BARNES, Dylan**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US). **CAMPBELL, Matthew**; c/o Genus PLC, 1525 River Road, DeForest, Wisconsin 53532 (US).

(74) Agent: **J A KEMP LLP**; 80 Turnmill Street, London EC1M 5QU (GB).

(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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, 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).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2021/224599 A1

(54) **Title:** METHODS FOR IMPROVING THE HEALTH OF PORCINE SPECIES BY TARGETED INACTIVATION OF CD163

(57) **Abstract:** The present disclosure relates methods and compositions useful for prevention of porcine reproductive and respiratory syndrome virus (PRRSv) in animals, including animals of the species Sus scrofa. The present teachings relate to swine wherein at least one allele of a CD163 gene has been inactivated, and to specific methods and nucleic acid sequences used in gene editing to inactivate the CD163 gene. Swine wherein both alleles of the CD163 gene are inactivated are resistant to porcine reproductive and respiratory syndrome virus (PRRSv). Elite lines comprising homozygous CD163 edited genes retain their superior properties

METHODS FOR IMPROVING THE HEALTH OF PORCINE SPECIES BY TARGETED INACTIVATION OF CD163

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to US Provisional Application 63/020,128 filed on May 5, 2020 and to US Provisional Application 63/021,370 filed on May 7, 2020. 63/020,128 and 63/021,370 are hereby incorporated by reference, each in their entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII-formatted sequence listing with a file named RD-12-2020-US2-SEQLST, created on March 10, 2021, and having a size of 137,058 bytes is filed concurrently with the specification. The sequence listing contained in this ASCII-formatted document is part of the specification and is herein incorporated by reference in its entirety.

FIELD

[0003] The present disclosure relates to methods for improving the health of porcine species. In particular, the present disclosure relates to methods of protecting *Sus scrofa* animals and elite lines from infection by porcine reproductive and respiratory syndrome virus (PRRSv) through targeted polynucleotide edits of CD163 to prevent infection and generate resistant animals, herds, and cell lines.

BACKGROUND

[0004] Viral infections are a major source of morbidity and mortality in the livestock industry. In particular, Porcine Reproductive and Respiratory Syndrome (PRRS) is a panzootic infectious disease of pigs, causing major economic losses to the world-wide pig industry. PRRS manifests in pigs of all ages but primarily causes late-term abortions and stillbirths in sows and respiratory disease in piglets. The causative agent of the disease is the positive-strand RNA PRRS virus (PRRSv). PRRS is the most economically important disease of domestic swine in North America, Europe and Asia, costing producers in North America more than $600 million annually.

[0005] Currently, there are no effective treatment programs for acute PRRS. As a result, when incidence of PRRS is detected on a farm, depopulation, sufficient cleaning/disinfection, and proper disposal of the carcasses must be used to eliminate the virus. In more extreme cases, whole herd depopulation-repopulation has been documented as an effective method of eliminating the PRRSv from endemically infected herds; however, this method results in significant loss.

[0006] Vaccines for PRRSv do exist; however, these vaccines have been unable to control the disease largely due to the genetic diversity within the structural proteins of the virus. Consequently, prevention of infection is currently the best control measure. As a prophylactic measure, farms in a country or zone where PRRSv exists must use stringent control measures, involving an assessment of the health status of replacement gilts and boars, as well as 45-60 days of isolation and acclimatization for incoming stock.

[0007] In recent years, more attention has been given to the role CD163 may play in the occurrence of PRRS. Despite the significant heterogeneity in strains of PRRSv, strains of PRRSv all share a tropism for CD163-positive cells. Although CD163 is a virus receptor, the CD163 scavenger receptor is also involved in the adhesion of monocytes to endothelial cells. Functions and a detailed description of CD163 are provided in Onofre, Gabriela *et al.*, *ACTA MEDICA*, 2009, 52, 57-61.

[0008] CD163 is a 130 kDa type 1 membrane protein considered to be a fusion receptor for the PRRS virus; it is mapped to chromosome 5 in pigs. The basic transcript encodes for a protein of 1076 amino acids. There are five reported isoforms of CD163; three of the isoforms display different splicing forms of their cytoplasmic domains. Generally, however, the genomic molecule sequence of CD163 comprises 17 exons coding for a peptide signal sequence, nine scavenger receptor cysteine-rich (SRCR) domains, two proline serine threonine (PST) linker domains, a cytoplasmic domain, and a short cytoplasmic tail. CD163 has been described as the receptor for PRRSv. Domain 5 (SRCR5) of the protein is the interaction site for the virus. Exon 7 of CD163 encodes the SRCR domain 5 (SRCR5) that serves as an interaction site for the PRRSv *in vitro*. Burkard (Burkard, C., PLoS Pathog. 2017, 23, 13, e1006206.) demonstrated that removal of CD163 exon 7 confers PRRSv resistance to porcine macrophages. The guides used in that work (set forth as targeting sequences including the PAM in SEQ ID NOs: 272 and 273), however, may lack sufficient activity and specificity for gene editing as part of a commercial breeding program. Further work by Whitworth and colleagues included

creating a 123 bp deletion in Exon 7 using guides as set forth (including the PAM) in SEQ ID NO: 354 and SEQ ID NO: 211 (Whitworth, K.M., Biol. Reprod., 2014, 91, 1-13). Whitworth *et al.* (Whitworth, K.M., Nature Biotechnology, 2016, 34, 20-22) reported the preparation of PRRSv resistant pigs by knocking out the function of CD163.

[0009] Genome editing includes altering the genome by deleting, inserting, or substituting specific nucleic acid sequences. The alteration can be gene- or location-specific. Genome editing can use site-directed nucleases, such as Cas proteins and their cognate polynucleotides.

[0010] Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) constitute the CRISPR-Cas system.

[0011] Cas9 is an exemplary Type II CRISPR Cas protein. Cas9 is an endonuclease that can be programmed by the tracrRNA/crRNA to cleave, in a site-specific manner, a DNA target sequence using two distinct endonuclease domains (HNH and RuvC/RNase H-like domains) (see U.S. Patent Application Publication No. 2014-0068797, published 6 Mar. 2014; *see also* Jinek, M., *et al.*, *Science*, 337:816-821 (2012) and *Karvelis et al. Genome Biology (2015) 16:253.*

[0012] The foregoing CD163 edits, while demonstrating some indication of efficacy against PRRSv, cannot be made as precisely and effectively as the edits disclosed herein. There is a need to improve the health of a porcine herd by editing the CD163 gene using guides for improved editing activity and reduced unintended edits, while conferring resistance to PRRSv.

SUMMARY

[0013] The present specification provides for and includes edited CD163 genes that confer PRRSv resistance on pigs comprising the edited gene.

[0014] In some embodiments, the present teachings provide for and include a CD163 gene edited to confer PRRSv resistance in *Sus scrofa* wherein the edit excises exon 7 and the edited gene can comprise a repaired genomic sequence as set forth in any one of SEQ ID NOs: 426-458 and 520-555. In some configurations, the edited gene can comprise a repaired genomic sequence selected from the group consisting of SEQ ID NOs: 426-458. In various configurations, the repaired gene sequence can comprise SEQ ID NO: 426, SEQ ID NO: 427, SEQ ID NO: 428, SEQ ID NO: 429, SEQ ID NO: 430, SEQ ID NO: 431, SEQ ID NO: 432, SEQ ID NO: 433, SEQ ID NO: 434, SEQ ID NO:

435, SEQ ID NO: 436, SEQ ID NO: 437, SEQ ID NO: 438, SEQ ID NO: 439, SEQ ID NO: 440, SEQ ID NO: 441, SEQ ID NO: 442, SEQ ID NO: 443, SEQ ID NO: 444, SEQ ID NO: 445, SEQ ID NO: 446, SEQ ID NO: 447, SEQ ID NO: 448, SEQ ID NO: 449, SEQ ID NO: 450, SEQ ID NO: 451, SEQ ID NO: 452, SEQ ID NO: 453, SEQ ID NO: 454, SEQ ID NO: 455, SEQ ID NO: 456, SEQ ID NO: 457, SEQ ID NO: 458, SEQ ID NO: 520, SEQ ID NO: 521, SEQ ID NO: 522, SEQ ID NO: 523, SEQ ID NO: 524, SEQ ID NO: 525, SEQ ID NO: 526, SEQ ID NO: 527, SEQ ID NO: 528, SEQ ID NO: 529, SEQ ID NO: 530, SEQ ID NO: 531, SEQ ID NO: 532, SEQ ID NO: 533, SEQ ID NO: 534, SEQ ID NO: 535, SEQ ID NO: 536, SEQ ID NO: 537, SEQ ID NO: 538, SEQ ID NO: 539, SEQ ID NO: 540, SEQ ID NO: 541, SEQ ID NO: 542, SEQ ID NO: 543, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 546, SEQ ID NO: 547, SEQ ID NO: 548, SEQ ID NO: 549, SEQ ID NO: 550, SEQ ID NO: 551, SEQ ID NO: 552, SEQ ID NO: 553, SEQ ID NO: 554, or SEQ ID NO: 555. In various configurations, the edit can be created using a pair of gRNAs, wherein the pair of gRNAs can be SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 237 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 230 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 237 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 229 and 261, SEQ ID NOs: 230 and 261, SEQ ID NOs: 231 and 261, SEQ ID NOs: 237 and 261, SEQ ID NOs: 241 and 261, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 219 and 258, SEQ ID NOs: 221 and 258, SEQ ID NOs: 224 and 258, SEQ ID NOs: 227 and 258, SEQ ID NOs: 219 and 261, SEQ ID NOs: 221 and 261, SEQ ID NOs: 224 and 261, SEQ ID NOs: 227 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 250 and 258, SEQ ID NOs: 249 and 261, or SEQ ID NOs: 250 and 261. In various configurations, the edit can be created using a pair of gRNAs, wherein the pair of targeting regions can be SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 227 and 258, SEQ ID NOs: 221 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, or SEQ ID NOs: 249 and 261.

[0015] In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 229 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 230 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 231 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 237 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 241 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 229 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 230 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs wherein the pair of targeting regions can be SEQ ID NOs: 231 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 237 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 241 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 229 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 230 and 261. In various configurations, the edit can be a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 231 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 237 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 241 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 219 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 221 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 224 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 227 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 219 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 221 and 258. In

various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 224 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 227 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 219 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 221 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 224 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 227 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 249 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 250 and 256. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 249 and 258. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 250 and 258. In various configurations, the edit can be created a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 249 and 261. In various configurations, the edit can be created using a pair of gRNAs wherein the pair of gRNAs can be SEQ ID NOs: 250 and 261. In various configurations, the repaired genomic sequence can comprise SEQ ID NO: 453 and the edit can be created using sequences set forth in SEQ ID NOs: 249 and 256.

[0016] In various configurations, the instant disclosure provides for and includes a *Sus scrofa* cell that can comprise the CD163 gene of the present teachings. In some configurations, the present teachings further provide for and include a cell line that can comprise a plurality of the *Sus scrofa* cell. In some configurations, the cell line can be a fibroblast cell line. In various configurations, the cell, plurality of cells, or cell line can be derived from PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65. The present teachings further provide for an embryo, piglet, or mature adult that can comprise a plurality of the cell.

[0017] The present disclosure also provides for a CD163 gene edited to confer PRRSv resistance in *Sus scrofa* wherein the edit creates a stop codon resulting in a predicted exon 7 amino acid sequence that can be selected from the group consisting of 506-517. In various configurations, the amino acid sequence can be set forth in SEQ ID

NO: 506, SEQ ID NO: 507, SEQ ID NO: 508, SEQ ID NO: 509, SEQ ID NO: 510, SEQ ID NO: 511, SEQ ID NO: 512, SEQ ID NO: 513, SEQ ID NO: 514, SEQ ID NO: 515, SEQ ID NO: 516, or SEQ ID NO: 517. In various configurations, the predicted amino acid sequence of exon 7 can be set forth in SEQ ID NO: 513. In some configurations, the edit can be created using gRNAs selected from the group consisting of SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs.: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 395, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID NOs:354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs. 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, and SEQ ID NOs: 366 and 397. In various configurations, the edit can be created using gRNAs selected from the group consisting of SEQ ID NOs: 351 and 365, SEQ ID NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 354 and 390, SEQ ID NOs: 358 and 394, SEQ ID NOs: 362 and 390, and SEQ ID NOs: 366 and 394. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 351 and 365. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 351 and 387. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 348 and 390. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 348 and 388. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 348 and 395. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 352 and 365. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 352 and 387. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 352 and 399. In various configurations, the gRNAs used to create the edit can be

SEQ ID NOs: 353 and 365. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 353 and 387. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 353 and 399. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 354 and 390. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 354 and 388. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 354 and 395. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 361. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 362. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 368. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 384. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 394. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 358 and 399. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 359 and 390. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 359 and 388. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 359 and 395. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 360 and 368. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 360 and 384. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 360 and 389. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 360 and 394. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 360 and 397. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 361 and 365. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 361 and 387. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 362 and 390. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 362 and 388. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 362 and 395. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 364 and 365. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 364 and 387. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 364 and 399. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 365 and 368. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 365 and 384. In various configurations, the gRNAs used to create the edit can be

SEQ ID NOs: 365 and 389. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 365 and 394. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 365 and 397. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 366 and 368. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 366 and 384. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 366 and 389. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 366 and 394. In various configurations, the gRNAs used to create the edit can be SEQ ID NOs: 366 and 397.

[0018] In various configurations, the edited CD163 gene can have a nucleic acid sequence selected from the group consisting of SEQ ID NO: 459-504. In various configurations, the repaired gene can have a nucleic acid sequence set forth in SEQ ID NO: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, or 504. In various configurations, the repaired gene can have a nucleic acid sequence as set forth in SEQ ID NO: 489. In various configurations the predicted amino acid sequence of exon 7 can be as set forth in SEQ ID NO: 513. In various configurations, the predicted amino acid sequence of the gene can be SEQ ID NO: 513, the repaired gene can have a nucleic acid sequence set forth in SEQ ID NO: 489, and the edit can be created using sequences set forth in SEQ ID NOs: 362 and 390.

[0019] The present disclosure provides for and includes a *Sus scrofa* cell that can comprise the CD163 gene edited to comprise an exogenous stop codon as described *supra*. The present disclosure also provides for a cell line that can comprise a plurality of the cell comprising the CD163 gene edited to comprise an exogenous stop codon. In some configurations, the cell line can be a fibroblast cell line. In various configurations, the cell can be derived from PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65. The present disclosure also provides for and includes an embryo, piglet, or mature adult comprising a plurality of the cell that can comprise the CD163 gene edited to comprise an exogenous stop codon.

[0020] In various embodiments, the present teachings provide for and include a pair of gRNAs for editing a Sus scrofa CD163 gene having a sequence as set forth in SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ

ID NOs: 237 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 230 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 237 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 229 and 261, SEQ ID NOs: 230 and 261, SEQ ID NOs: 231 and 261, SEQ ID NOs: 237 and 261, SEQ ID NOs: 241 and 261, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 219 and 258, SEQ ID NOs: 221 and 258, SEQ ID NOs: 224 and 258, SEQ ID NOs: 227 and 258, SEQ ID NOs: 219 and 261, SEQ ID NOs: 221 and 261, SEQ ID NOs: 224 and 261, SEQ ID NOs: 227 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 250 and 258, SEQ ID NOs: 249 and 261, SEQ ID NOs: 250 and 261, SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 395, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID NOs: 354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs: 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, or SEQ ID NOs: 366 and 397. In some configurations, the pair of gRNAs can have a sequence as set forth in SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 227 and 258, SEQ ID NOs: 221 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 249 and 261, SEQ ID NOs: 351 and 365, SEQ ID NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 354 and 390, SEQ ID NOs: 358 and 394, SEQ ID

NOs: 362 and 390, or SEQ ID NOs: 366 and 394. In various configurations, the pair of gRNAs can have a sequence as set forth in SEQ ID NOs: 249 and 256. In various configurations, the pair of gRNAs can have a sequence as set forth in SEQ ID NOs: 362 and 390.

[0021] The present disclosure provides for and includes a CRISPR complex for editing a CD163 gene of *Sus scrofa* comprising a pair of gRNAs of the present teachings.

[0022] The present disclosure provides for and includes a method for editing a CD163 gene of *Sus scrofa* comprising using a CRISPR-CAS complex comprising a pair of gRNAs of the present teachings.

[0023] The present disclosure provides for and includes a method for preparing a PRSSV resistant *Sus scrofa* cell by using a CRISPR-CAS complex comprising the pair of gRNAs of the present teachings.

[0024] The present disclosure provides for and includes a method of producing PRRSv resistant Sus scrofa animals, comprising: a) editing a CD163 gene of a *Sus scrofa* cell or a plurality of *Sus scrofa* cells using a CRISPR complex comprising a pair of gRNAs of the present teachings; and b) producing an animal from the cell or plurality of cells.

[0025] The present disclosure provides for and includes the use of a cell line according to the present teachings in producing PRRSv resistant animals.

[0026] The present disclosure also provides for and includes an embryo, piglet, or mature adult comprising a plurality of the cell according to the present teachings.

[0027] In various embodiments the present disclosure provides for and includes a method of determining the presence or absence of an edited sequence having 90% or 95% identity with a sequence set forth in SEQ ID NO: 453 comprising performing real time PCR with a) differentially labeled probes of sequences set forth in SEQ ID NO: 564 and SEQ ID NO: 558 or 561; b) a primer pair set forth in SEQ ID NOs: 562 and 563; and c) a primer pair set forth in SEQ ID NOs: 556 and 557 or SEQ ID NOs: 559 and 560. In some configurations, the edited sequence can have 100% identity with the sequence set forth in SEQ ID NO: 453. In some configurations, the edited sequence can have 90% identity with the sequence set forth in SEQ ID NO: 453. In some configurations, the edited sequence can have 95% identity with the sequence set forth in SEQ ID NO: 453.

[0028] In various embodiments, the present teachings provide for and include a PCR primer selected from the group consisting of SEQ ID NO: 556, SEQ ID NO: 557,

SEQ ID NO: 559, SEQ ID NO: 560, SEQ ID NO: 562, and SEQ ID NO: 563. In some configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 556. In various configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 557. In various configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 559. In various configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 560. In various configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 562. In various configurations, the PCR primer can have a sequence as set forth in SEQ ID NO: 563.

[0029] In some embodiments, the present disclosure provides for and includes a real time PCR probe selected from the group consisting of SEQ ID NO: 558, SEQ ID NO: 561, and SEQ ID NO: 564. In some configurations, the probe has a sequence as set forth in SEQ ID NO: 558. In various configurations, the probe has a sequence as set forth in SEQ ID NO: 561. In various configurations, the probe has a sequence as set forth in SEQ ID NO: 564.

[0030] In various embodiments, the present teachings provide for and include the use of PCR primers set forth in a) SEQ ID NO: 556 and 557 and SEQ ID NO: 562 and 563 or b) SEQ ID NO: 559 and 560 and SEQ ID NO: 562 and 563 to determine the presence or absence of an edited genome sequence set forth in SEQ ID NO: 453.

[0031] In various embodiments, the present teachings provide for and include the use of PCR probes set forth in a) SEQ ID NOs: 558 and 564 or b) SEQ ID NOs: 561 and 564 to determine the presence or absence of an edited genome sequence set forth in SEQ ID NO: 453.

[0032] In various embodiments, the present teachings provide for and include a method of creating a PRRSv resistant pig comprising editing the pig's genome to comprise a genomic sequence as set forth in SEQ ID NO: 453. In some configurations, the editing the pig's genome can comprise administering gRNAs having sequences set forth in SEQ ID NOs: 249 and 256. In various configurations, the administering comprises injecting pre-formed RNP complexes comprising the gRNAs and a CAS protein into a zygote, embryo, or MII oocyte. In various configurations, the pig is a PIC™ line 2 (Pig Improvement Company, Ltd, Basingstoke, UK), PIC™ line 3, PIC™ line 15, PIC™ line 19, PIC™ line 27, PIC™ line 62, or PIC™ line 65 pig.

[0033] In various embodiments, the present disclosure provides for and includes, a *Sus scrofa* animal comprising an edited gene that confers PRRSv resistance in *Sus*

scrofa wherein the edit excises exon 7 and the edited gene comprises a repaired genomic sequence set forth in SEQ ID NO: 453. In some configurations, the edit can be made with guideRNAs (gRNAs) having sequences set forth in SEQ ID NOs: 249 and 256. In various configurations, the animal can be an edited animal of PIC™ line 2, PIC™ line 3, PIC™ line 15, PIC™ line 19, PIC™ line 27, PIC™ line 62, or PIC™ line 65. In various configurations, the present disclosure provides for and includes a cell prepared from the animal of the present teachings. In various configurations, the present disclosure provides for and includes a cell line prepared from the cell according to the present teachings. In some configurations, the cell line can be a fibroblast cell line.

[0034] In some embodiments, the present teachings provide for and include a CD163 gene edited to confer PRRSv resistance in *Sus scrofa* wherein the edit excises exon 7 and the edited gene comprises a repaired genomic sequence set forth in SEQ ID NO: 453. In some configurations, the edit is created using sequences set forth in SEQ ID NOs: 249 and 256. In various configurations, the present disclosure provides for a *Sus scrofa* cell comprising the CD163 gene according to the present teachings. In some configurations, the present disclosure provides for a cell line comprising a plurality of the cell according to the present teachings. In some configurations, the cell line can be a fibroblast cell line. In various configurations, the cell can be isolated from PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65.

[0035] In various embodiments, the present disclosure provides for and includes a pair of gRNAs for editing a *Sus scrofa* CD163 gene comprising the guide sequences set forth in SEQ ID NOs: 249 and 256.

[0036] In various embodiments, the present teachings provide for and include a method of creating a PRRSv resistant pig comprising editing the pig's genome to comprise a genomic sequence as set forth in SEQ ID NO: 453. In some configurations, editing the pig's genome can comprise administering gRNAs having sequences set forth in SEQ ID NOs: 249 and 256. In some configurations, the administering can comprise injecting pre-formed RNP complexes comprising the gRNAs and a CAS protein into a zygote, embryo, or MII oocyte. In various configurations, the pig can be a PIC™ line 2, PIC™ line 3, PIC™ line 15, PIC™ line 19, PIC™ line 27, PIC™ line 62, or PIC™ line 65 pig.

DETAILED DESCRIPTION

[0037] The aspects of the present teachings include, but are not limited to, particular methods of improving the health of a porcine species by targeted inactivation of CD163, which can vary and are understood by skilled artisans. It is further to be understood that all terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting in any manner or scope. For example, as used in this specification and the appended claims, the singular forms "a," "an," and "the" can include plural referents unless the content clearly indicates otherwise. Further, all units, prefixes, and symbols may be denoted in their SI accepted forms. Numeric ranges recited within the specification are inclusive of the numbers defining the range and include each integer within the defined range.

[0038] So that the present application may be more readily understood, certain terms are first defined. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which aspects of the present application pertain. Many methods and materials similar, modified, or equivalent to those described herein can be used in the practice of the aspects of the present application without undue experimentation, the preferred materials and methods are described herein. In describing and claiming the aspects of the present application, the following terminology will be used in accordance with the definitions set out below.

[0039] As used herein, "animal cell" means a cell, including, but not limited to, a somatic cell, culture cell, gamete cell, blood cell, zygote, and embryonic cell. These animal cells can be reproductive or non-reproductive cells. As used herein, cells may be isolated from an animal or embryo and maintained in tissue culture. Also included are mixed cultures that can comprise gene edited cells of the present specification and a non-gene edited support cell or feeder cell.

[0040] As used herein, the terms "gene edited," "genetically edited," and "genome-edited," refer to the use of homing technology with naturally occurring or artificially engineered endonucleases, often referred to as "homing endonucleases," or "targeting endonucleases." "Genome-editing" and "gene editing," refer to altering the genome by deleting, inserting, or substituting specific nucleic acid sequences. The altering can be gene or location specific, but need not be altering the sequence of a gene *per se*. Genome editing can use endonucleases such as the CRISPR system to cut a nucleic acid, thereby generating a site for the alteration. Other endonucleases are

available and are suitable for use; however, off-site cutting and specificity can be significant problems. In systems like CRISPR and others, the nuclease can be directed to the target site by complexing with a polynucleotide, herein called a "target sequence," to introduce a site specific DSB. Not to be limited by theory, the DSB can then be repaired by endogenous non-homologous end joining (NHEJ) machinery.

[0041] A number of endonucleases are known that are suitable for, and have been adapted to, gene editing. Gene editing methods are known in the art, including Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems (*e.g.*, the CRISPR/Cas9 system), Transcription Activator-Like Effector Nucleases (TALENs), and Gene editing nucleases including Zinc Finger Nucleases (ZFNs). Gene edited animals can be distinguished from transgenic animals by the incorporation of exogenous DNA sequences, particularly sequences having identity with sequences from foreign species in the latter. Here, the terms include, and provide for, small deletions and insertions that do not introduce more than 10 nucleotides. The terms also encompass progeny animals such as those created by sexual crosses or asexual propagation from the initial gene edited animal.

[0042] As used herein, the term "repair template" or "repair sequences" refers to a polynucleotide introduced into a cell undergoing DSB repair at nuclease targeted locations in the genome to guide the repair and provide accurate and selective editing. Repair templates can be used to selectively change or delete sequences at a locus having a DSB and generally comprise a 5' genome hybridizing region and a 3' genome hybridizing region. In an aspect, the 5' genome hybridizing and 3' genome hybridizing regions can share at least 80% homology. In an aspect, the 5' genome hybridizing and 3' genome hybridizing regions can share identity. Further descriptions of the repair templates of the present disclosure are provided below. For deletion templates, a contiguous region of the genome can be separated by the deletion of one or more nucleotides. Targeted editing of individual bases can be prepared using repair templates comprising a contiguous region of a genome and having one or more base changes, including addition of a stop codon. The percentage identity of a repair template to a contiguous region of the genome can be between 80% and 100% using an ungapped alignment. As provided herein, a core region of identity within a repair template is provided that may be flanked by a flanking homology region sharing between 80% and 100% homology to the targeted chromosomal region. Not to be limited by theory, the

core region identity can increase the fidelity of the repair process while extended regions of homology on either side increase the efficiency and allow for genotypic variation among cells targeted for editing.

[0043] As used herein, the term "wild type" or "WT" refers to a phenotype, genotype, or gene that predominates in a natural population of pigs or line of pigs. When used to compare phenotypes and genotypes of gene edited cells and animals of specific lines, the term "wild type" refers to non-gene edited cells and animals of the same line. In an aspect, the present disclosure provides for comparison of edited pigs to non-edited pigs of a similar breed having a similar genetic background. In an aspect, the present disclosure provides for comparison of edited pigs to non-edited pigs of the same breeding line.

[0044] As used herein, the term "knock-out" refers to the disruption of gene function by reduction or elimination of its expression. Knock-outs may be generated through the creation of double-strand breaks (DSBs) in the chromosome which can then be repaired using either non-homologous end joining (NHEJ) or homologous recombination of DNA repair templates or targeting vectors by homology-directed repair (HDR). Not to be limited by theory, HDR knock-outs may also be prepared by microhomology-mediated end joining providing a repair template to insert, delete, or edit genomic sequences. Knock-outs may also be generated through replacement vectors, or hit-and-run vectors, or random insertion of a gene trap vector resulting in complete, partial, or conditional loss of gene function.

[0045] References herein to a deletion in a nucleotide sequence spanning a range are inclusive of all nucleotides in the listed range. For example, a 5 base pair deletion from nucleotide "a" to nucleotide "e" means that each of nucleotides "a," "b," "c," "d," and "e" have been deleted (where "b," "c," and "d" are between "a" and "e").

[0046] As used herein, the term "edit" includes alterations in the nucleotide sequence of a polynucleotide, such as, for example, a gene, coding DNA sequence (CDS), or non-coding DNA sequence, compared to the wild-type sequence. The term "edits" may include insertions, deletions, splice-donor site edits, point-edits, and the like.

[0047] As used herein, the term "clustered regularly interspaced short palindromic repeats" or "CRISPR" refers to a gene editing system utilizing a CRISPR segment of genetic material, and the RNA segments and enzymes it produces, to identify and modify specific DNA sequences in the genome of other organisms. CRISPR systems

include Type I, Type II, and Type III CRISPR systems. As used herein, the term "CRISPR associated protein" or "Cas" refers to a protein family that can be strictly associated with CRISPR elements and always occurs near a repeat cluster of CRISPR segments. For example, Cas proteins can include, but are not limited to, Cas9 family member proteins, Cas6 family member proteins (*e.g.*, Csy4 and Cas6), and Cas5 family member proteins. Examples of Cas protein families and methods of identifying the same have been disclosed in Haft, Daniel H., *et al.*, *PLoS Comput. Bio.*, 2005, 1, e60.

[0048] While not being limited by any particular scientific theory, a CRISPR nuclease can form a complex with a guide RNA (gRNA), which hybridizes with a complementary target nucleic acid molecule, thereby guiding the CRISPR nuclease to the target nucleic acid molecule. The crRNA comprises a repeat sequence and a spacer sequence which can be complementary to a specific protospacer sequence in an invading pathogen. It is the spacer sequence that can be designed to be complementary to target sequences in a eukaryotic genome. CRISPR nucleases can associate with their respective crRNAs in their active forms. The present specification provides for, and includes a crRNA that can comprise an RNA spacer comprising the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. As used herein, each of SEQ ID NOs: 22 to 271 and 347 to 425 provides for, and includes, the corresponding RNA sequence substituting uridine for the thymidine and ribose for deoxyribose. Also, as used herein, the SEQ ID NOs include the PAM (see below) which is present in the genome sequence targeted. Skilled artisans will recognize that the gRNA targeting this sequence would not include the PAM, and therefore would recognize that a gRNA having a sequence set forth in these SEQ ID NOs would only include the first 20 nucleotides, thus excluding the PAM.

[0049] Some CRISPR nucleases, such as CasX and Cas9, can require another non-coding RNA component, referred to as a *trans*-activating crRNA (tracrRNA), to have functional activity. A crRNA comprising a spacer sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425 can be covalently linked to the 5' end of a tracrRNA into one nucleic acid molecule in what is herein referred to as a "single guide RNA" (sgRNA), as described in Jinek, *et al.*, *Science*, 337, 2012, 816-821. As used herein, the tracrRNA is also referred to as "guide RNA backbone" or "the backbone" sequence. As used herein, gRNA includes both a single guide RNA or separate molecules comprising the spacer sequence in the crRNA for use with a separate

tracrRNA. The gRNA can guide the active Cas complex to a target site complementary to the spacer sequence in the crRNA, where the Cas nuclease can cleave the target site. The crRNA can have a region of complementarity to a potential DNA target sequence and a second region that can form base-pair hydrogen bonds with the tracrRNA to form a secondary structure, typically to form at least one stem structure. The region of complementarity to the DNA target sequence can be the spacer or spacer sequence. The tracrRNA and a crRNA can interact through a number of base-pair hydrogen bonds to form secondary RNA structures. Complex formation between tracrRNA/crRNA and Cas9 protein can result in a conformational change of the Cas9 protein that facilitates binding to DNA, endonuclease activities of the Cas9 protein, and crRNA-guided site-specific DNA cleavage by the endonuclease Cas9 (Svitashev *et al.*, Nature Communications, 2016, 7, 113274). In practice, the 20 nucleotides and the guide RNA backbone can be DNA (that can be expressed from a promoter to be transcribed *in vivo* (in cells) or *in vitro* (via T7 polymerase) to form an RNA guide or the guide RNA backbone can be chemically synthesized dual (crRNA and trRNA) guide or single guide RNA.

[0050] For a Cas9 protein/tracrRNA/crRNA complex to cleave a double-stranded DNA target sequence, the DNA target sequence can be adjacent to a cognate protospacer adjacent motif (PAM). By designing a crRNA to have an appropriate spacer sequence, the complex can be targeted to cleave at a locus of interest, *e.g.*, a locus at which sequence modification is desired.

[0051] A variety of Type II CRISPR-Cas system crRNA and tracrRNA sequences, and associated predicted secondary structures are known in the art (*see, e.g.*, Ran, F. A., *et al.*, *Nature*, 2015, 520, 186-191; Fonfara *et al.*, *Nucleic Acids Research*, 2014, 42, 2577-2590). The Type II CRISPR-Cas system of Ran *et al.*, is provided herein in an aspect.

[0052] The spacer of Type II CRISPR-Cas systems can hybridize to a nucleic acid target sequence that is located 5' or 3' of a PAM, depending upon the Cas protein to be used. A PAM can vary depending upon the Cas polypeptide to be used. For example, if Cas9 from *S. pyogenes* is used, the PAM can be a sequence in the nucleic acid target sequence that comprises the sequence 5'-NRR-3', wherein R can be either A or G, N is any nucleotide, and N is immediately 3' of the nucleic acid target sequence targeted by the nucleic acid target binding sequence. Preferably, the PAM of *S. pyogenes* comprises

5'-NGG-3'. In another example, if Cas9 from *S. thermophilus CRISPR3 (Sther CR3)* is used, the PAM can be a sequence in the nucleic acid target sequence that comprises the sequence 5'-nGGnG-3'. (*See* Sapranauskas *et al.*, *Nucleic Acids Research*, 2011, 39, 9275-9282).

[0053] Other Cas proteins recognize other PAMs, and one of skill in the art can determine the PAM for any particular Cas protein. A growing number of CAS proteins and systems are known in the art that are suitable for use with the targeting sequences of the present specification. See Shah *et al.*, *RNA Biol.*, 2013, 10, 891-899. A representative sample of CAS systems and their PAM sequences is provided in Table 1 below.

Table 1: Exemplary CRISPR/Cas Systems

Species/Variant of Cas9	PAM Sequence	
Streptococcus pyogenes (SP); SpCas9	3' NGG	Wu et al., *Nat Biotechnol.*, 2014, 32, 670-676
SpCas9 D1135E variant	3' NGG (reduced NAG binding)	Nishimasu *et al.*, *Science*, 2018, 361, 1259-1262.
SpCas9 VRER variant	3' NGCG	Nishimasu *et al.* (2018)
SpCas9 EQR variant	3' NGAG	Nishimasu *et al.* (2018)
SpCas9 VQR variant	3' NGAN or NGNG	Nishimasu *et al.* (2018)
Staphylococcus aureus (SA); SaCas9	3' NNGRRT or NNGRR(N)	Kleinstiver et al., Nature, 2015, 523, 481-485
Acidaminococcus sp. (AsCpf1) and Lachnospiraceae bacterium (LbCpf1)	5' TTTV	Fagerlund, R., *et al.*, 2015, *Genome Biology*, 16, 251
AsCpf1 RR variant	5' TYCV	Nishimasu *et al.*, *Mol Cell.*, 2017, 67, 139-147
LbCpf1 RR variant	5' TYCV	Nishimasu *et al.* (2017)
AsCpf1 RVR variant	5' TATV	Nishimasu *et al.* (2017)
Neisseria meningitidis (NM)	3' NNNNGAT	Hou et al., 2013, Proc. Natl. Acad. Sci. USA, 110, 15644-156449

Treponema denticola (TD)	3' NAAAAC	Sun *et al.*, *Biotechnol J.*, 2018, 13, e1700588
Campylobacter jejuni (Cj)	NNNNRYAC	Kim, et al., Nat Commun., 2017, 8, 14500.
Streptococcus thermophilus CR1 (St)	NNAGAAW	Toth et al., 2016, Biol Direct., 11, 46
Streptococcus thermophilus CR3	nGGnG	Müller *et al.*, *Mol Ther.*, 2016, 24,636-644

[0054] Methods that rely on any CRISPR/CAS system can have a PAM sequence of NRR (*e.g.*, NGG and NAA) as provided below for the *S. pyogenes* sequences or can have a PAM sequence of NGGNG as provided for the *S. thermophilus* sequences. Table 3 and the sequence listing provide the targeting sequences and chromosomal locations. It will also be appreciated by those of skill in the art that known CAS systems having different PAM requirements can be engineered to recognize and utilize the target sequences disclosed herein. Examples of modifications are presented in Table 1.

[0055] The terms "CRISPR/CasN or "CRISPR/CasN system" refer to a programmable nuclease system for genetic editing that includes a CasN (*e.g.*, Cas2, Cas5, Cas6, Cas9, etc.) protein, or derivative thereof, and one or more non-coding RNAs that can provide the function of a CRISPR RNA (crRNA) and trans-activating RNA (tracrRNA) for the CasN. The crRNA and tracrRNA can be used individually or can be combined to produce a "guide RNA" (gRNA). The crRNA or gRNA can provide a sequence that is complementary to the genomic target.

[0056] The term "Cpf1" or "CAS12" refers to another programmable RNA-guided endonuclease of a class 2 CRISPR-Cas system, described and used for gene editing purposes (Zetsche *et al.*, *Cell*, 163:759-771, 2015). This system can use a non-specific endonuclease unit from the Cpf1 protein family, with a specificity of cleavage conferred by a single crRNA (lacking tracrRNA). Similar to Cas9, the Cpf1 coding sequence can be fused to UTR sequences described herein to improve its stability, and thus the efficiency of the resulting gene editing method.

[0057] As used herein, the terms "transcription activator-like effector nucleases" or "TALENS" refer to nucleases engineered to enable the targeted alteration of a given DNA sequence. TALENs can comprise a non-specific DNA-cleaving nuclease fused to a TALE DNA-binding domain that can be engineered to allow targeted gene editing. A

"TALE DNA-binding domain" or "TALE" can be a polypeptide comprising one or more TALE repeat domains/units. The repeat domains can be involved in binding of the TALE to its cognate target DNA sequence. A single "repeat unit" (also referred to as a "repeat") can be 33–35 amino acids in length and exhibits at least some sequence homology with other TALE repeat sequences within a naturally occurring TALE protein. A "designed" DNA binding protein can be a protein not occurring in nature whose design/composition results principally from rational criteria. TALENS are discussed and disclosed in Joung *et al.*, *Nat. Rev. Mol. Cell. Biol.*, 2013, 14, 49-55. A "selected TALE" can be a protein not found in nature whose production results primarily from an empirical process such as phage display, interaction trap, or hybrid selection. The present specification provides for, and includes, the use of TALENS to the pigs described herein.

[0058] "Resistance" or "disease resistance" refers to the extent to which an organism can defend itself from and/or withstand the attack of a pathogen and remain unaffected. An organism may demonstrate complete resistance, meaning it remains virtually unaffected by a pathogen. Alternatively, an organism may demonstrate partial resistance, wherein the extent to which the pathogen affects the organisms can be less than a comparable organism with no resistance. Resistance may stem from a particular characteristic of the organism, allowing the organism to avoid the outcome of organism-pathogen interactions. Resistance can be demonstrated by the extent to which an organism can avoid the disease symptoms associated with, or reduce the incidence/severity of clinical signs, or reduce the clinical symptoms associated with a pathogen.

[0059] The terms "increased resistance" and "reduced susceptibility" refer to a statistically significant reduction of the incidence and/or severity of clinical signs or clinical symptoms which are associated with infection by a given pathogen. For example, "increased resistance" or "reduced susceptibility" refer to a statistically significant reduction of the incidence and/or severity of clinical signs or clinical symptoms which are associated with infection by PRRSv in an animal comprising a deleted or inactivated chromosomal sequence in a CD163 gene protein as compared to a control animal having an unmodified chromosomal sequence. The term "statistically significant reduction of clinical symptoms" means, but is not limited to, the frequency in the incidence of at least one clinical symptom in the modified group of subjects and, in some aspects, clinical

symptoms may be statistically reduced by at least 80% lower than in the non-modified control group after the challenge with the infectious agent.

[0060] As used herein, the terms "reduction of the incidence and/or severity of clinical signs" or "reduction of clinical symptoms" mean reducing the number of infected subjects in a group, reducing or eliminating the number of subjects exhibiting clinical signs of infection, or reducing the severity of any clinical signs that are present in one or more subjects, in comparison to wild-type infection in an otherwise similar genetic background. For example, these terms encompass any clinical signs of infection, lung pathology, viremia, antibody production, reduction of pathogen load, pathogen shedding, reduction in pathogen transmission, or reduction of any clinical sign symptomatic of PRRS when compared to an otherwise similar background. Comparisons of clinical signs between non-edited CD163 pigs and CD163 edited pigs can be individually or between herds. In an aspect, an individual pig does not present clinical signs. In an aspect, a herd of CD163 edited pigs present reduced clinical signs. In an aspect, the size of the herd can be at least 100 animals. In an aspect, CD163 edited pigs of the present specification can have reduced clinical signs of reproductive syndrome. In an aspect, a herd of CD163 edited pigs can have a reduced number of premature farrowings, reduced numbers of stillborn or mummified piglets, reduced numbers of PRRSv-positive piglets, or reductions in delays to return to service of sow. In an aspect, CD163 edited pigs of the present specification can have reduced clinical signs in sows and gilts including, but not limited to, reduced anorexia, reduced fever, reduced lethargy, reduced pneumonia, reduced agalactica, and reduced subcutaneous and hind limb edema. In an aspect, gilts and sows that are CD163 edited pigs of the present specification can have reduced red/blue discoloration of the ears and vulva. In an aspect, CD163 edited sows can exhibit reduced delays to return to estrus after weaning. The present specification provides for, and includes, reduced deaths among a herd of CD163 edited gilts and sows. Also included and provided for by the present specification are reduced clinical signs in finishing pigs. In an aspect, a herd of CD163 edited finishing pigs can have reduced respiratory clinical signs selected from the group consisting of fever, sneezing, hyperpnoea, dyspnea, coughing, pneumonia, lethargy, periocular edema, and oculo-nasal discharge. Preferably these clinical signs can be reduced in one or more animals of the present teachings by at least 10% in comparison to subjects not having a modification in the CD163 gene and having a similar background and that become

infected. In an aspect, clinical signs can be reduced in subjects of the present teachings by at least 80%. In another aspect, clinical signs can be reduced in pigs of the present teachings by at least 85%. In a further aspect, clinical signs can be reduced in pigs of the present teachings by at least 90%. In yet another aspect, clinical signs can be reduced in pigs of the present teachings by at least 95%. In aspects according to the present disclosure, clinical signs can be reduced by 80% to 100% relative to non-edited CD163 pigs.

[0061] The term "breeding" as used herein refers to a process comprising the selection of superior male and superior female animals use for creation of the next generation of offspring. This process further comprises the union of male and female gametes so that fertilization occurs. Such a union may be brought about by mating (copulation) or by *in vitro* or *in vivo* artificial methods. Such artificial methods can include, but are not limited to, artificial insemination, surgical assisted artificial insemination, *in vitro* fertilization, intracytoplasmic sperm injection, zona drilling, *in vitro* culture of fertilized oocytes, ovary transfer, and ovary splitting. The term "breeding" as used herein also can include transferring of a fertilized oocyte into the reproductive tract of a female animal in order to allow for more offspring from a particular elite female.

[0062] As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length promoter sequence, or the complete promoter sequence. In an aspect, the reference sequence can be the Sscrofa11.1 reference genome (GenBank accession: GCA_000003025.6).

[0063] The terms "percent identity" or "percent identical" as used herein in reference to two or more nucleotide or protein sequences is calculated by (i) comparing two optimally aligned sequences (nucleotide or protein) over a window of comparison, (ii) determining the number of positions at which the identical nucleic acid base (for nucleotide sequences) or amino acid residue (for proteins) occurs in both sequences to yield the number of matched positions, (iii) dividing the number of matched positions by the total number of positions in the window of comparison, and then (iv) multiplying this quotient by 100% to yield the percent identity. If the "percent identity" is being calculated in relation to a reference sequence without a particular comparison window being specified, then the percent identity can be determined by dividing the number of

matched positions over the region of alignment by the total length of the reference sequence. Accordingly, for purposes of the present application, when two sequences (query and subject) are optimally aligned (with allowance for gaps in their alignment), the "percent identity" for the query sequence can be equal to the number of identical positions between the two sequences divided by the total number of positions in the query sequence over its length (or a comparison window), which is then multiplied by 100%. When percentage of sequence identity is used in reference to proteins, it is recognized that residue positions which are not identical often can differ by conservative amino acid substitutions, where amino acid residues can be substituted for other amino acid residues with similar chemical properties (*e.g.*, charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity can be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity."

[0064] For optimal alignment of sequences to calculate their percent identity, various pair-wise or multiple sequence alignment algorithms and programs are known in the art, such as ClustalW or Basic Local Alignment Search Tool (BLAST®, National Library of Medicine, Bethesda, MD), etc., that can be used to compare the sequence identity or similarity between two or more nucleotide or protein sequences. Although other alignment and comparison methods are known in the art, the alignment and percent identity between two sequences (including the percent identity ranges described above) can be as determined by the ClustalW algorithm, *see, e.g.*, Chenna, R., *et al.*, *Nucleic Acids Research*, 2003, 31, 3497-3500; Thompson, J.D., *et al.*, *Nucleic Acids Research*, 1994, 22, 4673-4680; Larkin MA *et al.*, *Bioinformatics*, 2007, 23, 2947-2948; and Altschul, S.F., *et al.*, *J. Mol. Biol.*, 1990, 215, 403-410.

[0065] Identity to a sequence used herein can be expressed in terms of a percent identity between two sequences as determined according to alignment of the two sequences. The present specification provides for repair template sequences that can have at least 80% identity to a contiguous region of a genome. In an aspect, the repair template can have a 5' region and a 3' region sharing at least 80% identity to a contiguous region of the genome and a core region having identity to the chromosome sequences flanking the intended gene edit site.

[0066] The present specification provides for, and includes, but is not limited to, target sequences that can be 100% identical to the target sequences selected from the group consisting of SEQ ID NOs: 22 to 271 and 347 to 425. These target sequences can comprise a PAM sequence such as those listed on Table 1. Accordingly, when incorporated into a guide RNA, in an aspect the spacer region can share 100% identity to a sequence selected from the group of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. Also included are spacer sequences that can have from 15 to 20 nucleotides of each of SEQ ID NOs: 22 to 271 or from 15 to 20 nucleotides of each of SEQ ID NOs: 347 to 425. (The remainder of the sequence can comprise the PAM sequence which can be present in the genome, but not the gRNA molecules.) Also included and provided for are guide RNA spacer sequences that can have one or more mismatches to the target sequence. In an aspect, the mismatch can be at the distal end of the sequence target sequence to ensure that identity at the endonuclease cleavage site is maintained. Typically, the mismatches can occur at the 5' end of the target sequence relative to the nuclease site at the 3' end. In an aspect, a target sequence, or the spacer sequences of a guide RNA prepared therefrom, may have a single mismatch. In another aspect, a target sequence, or the spacer sequences of a guide RNA prepared therefrom, may have two (2) mismatches. In another aspect, a sequence, or the spacer sequences of a guide RNA prepared therefrom, may have three (3) mismatches. In another aspect, a sequence, or the spacer sequences of a guide RNA prepared therefrom, that may have less than four (4) mismatches are included. In some aspects, the mismatch regions can be limited to terminal nucleotides distal to the PAM sequences and cleavage site.

[0067] In an aspect, the target sequences and the spacer sequence of the gRNA guide can have at least 90% identity to a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. In a further aspect, the RNA guide can have at least 90% identity and 100% identity at 15 nucleotides at the 3' end of the sequence. In an aspect, the target sequences and the RNA guide can have at least 95% identity. In a further aspect, the RNA guide can have at least 95% identity or 100% identity at 15 nucleotides at the 3' end of the sequence. In an aspect, the target sequences and the RNA guide can have at least 96% identity. In a further aspect, the RNA guide can have at least 96% identity or 100% identity at 15 nucleotides at the 3' end of the sequence. In an

aspect, the target sequences and the RNA guide can have at least 97% identity. In a further aspect, the RNA guide can have at least 97% identity or 100% identity at 15 nucleotides at the 3' end of the sequence. In an aspect, the target sequences and the RNA guide can have at least 98% identity. In a further aspect, the RNA guide can have at least 98% identity or 100% identity at 15 nucleotides at the 3' end of the sequence. In an aspect, the target sequences and the RNA guide can have at least 99% identity. In a further aspect, the RNA guide can have at least 99% identity or 100% identity at 15 nucleotides at the 3' end of the sequence. In an aspect, the target sequences and the RNA guide can have at least 100% identity. Also included and provided for by the present specification are spacer sequences that can comprise the first 15 nucleotides each of SEQ ID NOs: 22 to 271 or the first 15 nucleotides each of SEQ ID NOs: 347 to 425. In an aspect, the spacer sequences can comprise the first 16 nucleotides in each of SEQ ID NOs: 22 to 271 or the first 16 nucleotides in each of SEQ ID NOs: 347 to 425. In another aspect, the spacer sequences can comprise the first 17 nucleotides in each of SEQ ID NOs: 22 to 271 or the first 17 nucleotides in each of SEQ ID NOs: 347 to 425. In an aspect, the spacer sequences can comprise the first 18 nucleotides in each of SEQ ID NOs: 22 to 271 or the first 18 nucleotides in each of SEQ ID NOs: 347 to 425. In yet another aspect, the spacer sequences can comprise the first 19 nucleotides in each of SEQ ID NOs: 22 to 271 or the first 19 nucleotides in each of SEQ ID NOs: 347 to 425.

[0068] The preferred meaning of "fertilization" as used herein to refer to pig breeding encompasses any technique that produces a viable embryo. Fertilization can include both insemination of female pigs, and *in vitro* or *ex vivo* fertilization. There are three commonly available ways to inseminate a sow, namely traditional artificial insemination (AI), intrauterine insemination (IUI), and deep intrauterine insemination (DIUI). These techniques all rely on providing a dose of semen, typically fresh and unfrozen, for insemination. *In vitro* fertilization (IVF) can be the harvesting of unfertilized oocytes(s) and the subsequent fertilization of those oocytes with semen *in vitro* (*i.e.*, in the laboratory) instead of *in vivo* (*i.e.*, in the live animal; insemination discussed above) as in standard ET. The fertilized oocytes(s) or embryo(s) from the oocyte donor can then be transferred into another female (embryo recipient). Embryo transfer (ET) can be the harvesting of fertilized oocytes(s) or embryo(s) from one female (embryo donor) and transfer of those embryo(s) into another female (embryo recipient) whose reproductive status can be synchronized with that of the donor.

[0069] As described in Cameron *et. al.* (*Nat Methods*, 2017, 14, 600-606), the CRISPR-Cas9 system can be used for genome editing in both basic research and biotechnology. The application of CRISPR and related technologies to gene or genome editing can be accompanied by unwanted off-target cleavage activity and possible pathogenic and other negative phenotypic consequences. To minimize off-target cleavage, a variety of genome-wide experimental methods have been recently developed but some methods are potentially biased due to cellular context or inefficiencies in recovering relevant cleavage sites. On approach, the SITE-SEQ® assay (Caribou Biosciences, Inc., Berkeley CA) disclosed in Cameron *et. al.* enables one to comprehensively list Cas9 cleavage sites in a sample genome, then probe those sites for cellular off-target editing in follow-up experiments through (1) extraction and purification of high-molecular weight genomic DNA, (2) execution of Cas9 Ribonucleoprotein (RNP) cleavage *in vitro*, (3) fragmentation, adapter ligation, and affinity purification to enrich for Cas9 cleaved fragments, and (4) amplification and indexing of SITE-SEQ® libraries for ILLUMINA® (ILLUMINA®, San Diego, CA) sequencing. (*See* Cameron *et al.*, *Nat Methods*, 2017, 14, 600-606.) Various other methods of discovering and reducing the number of potential off-target edits are known in the genome editing arts. While such bioinformatics tools can be invaluable for identifying suitable target sequences, the error rate is generally higher than desired and the approach can be limited when the system lacks extensive sequence data. Further, the fidelity of DSB break repair can vary, thus confounding the ability to predict and prevent unwanted off target effects. Further testing of *in silico* selected target sequences can identify select sequences that have higher efficiencies and fewer off target cuts. Testing in cell culture systems combined with high-throughput sequencing methods can be used to identify poor performing target constructs.

[0070] For example, the type II CRISPR system, which is derived from *Streptococcus pyogenes* (*S. pyogenes*), can be reconstituted in mammalian cells using Cas9, a specificity-determining CRISPR RNA (crRNA) comprising a backbone sequence and a sequence selected from the group consisting of the first twenty nucleotides of each of SEQ ID NOs: 22 to 271 and the first twenty nucleotides of each of SEQ ID NOs: 347 to 425, and an auxiliary trans-activating RNA (tracrRNA). The term "off target effect" broadly refers to any impact distinct from and not intended as a result of the on-target treatment or procedure. Examples of off-target edits can include

double strand breaks at unintended locations that lead to DNA insertions or unintended nucleotides or repair templates, deletions, or rearrangements. For some systems, for example the *S. pyogenes* and *S. thermophilus* CRISPR system, the crRNA and tracrRNA duplexes can be fused to generate a single-guide RNA (sgRNA). The first 20 nucleotides of the sgRNA can be complementary to the target DNA sequence and can be the spacer region, and those 20 nucleotides can be followed at the 3' end by a protospacer adjacent motif (PAM) in the genome (but not the guide). In an aspect, the first 20 nucleotides of the sgRNA and PAM sequence can be a sequence selected from the group consisting of SEQ ID NOs: 22 to 271 and 347 to 425. Accordingly, as provided below at Table 2 and SEQ ID NOs: 22 to 271 and 347 to 425, specific targeting of the Cas nuclease from either *S. pyogenes* or *S. thermophilus* can be accomplished combining the crRNAs with a backbone sequence. Although the 20-nucleotide guide sequence plus PAM sequence (*e.g.*, 23 to 25 nucleotides) of the sgRNA can provide tightly controlled targeting and cleavage, it has been discovered that off-target cleavage activity can still occur on DNA sequences with between 3-5 base pair mismatches in the PAM-distal part of the sgRNA-guiding sequence. Further, different types of guide RNA structures actually affect the cleavage precision, increasing or decreasing cleave on off-target sites. Various techniques, as well as a further description of off-target cleavage, are reviewed in Zhang *et al.*, *Mol Ther Nucleic Acids*, 2015, 4, e264. The mechanisms and effects of off-target cleavage are still poorly understood, meaning it can be difficult to predict and to compensate for its effects. However, the consequences can be severe; off-target cleavage can ultimately lead to genomic instability and disrupt the functionality of otherwise normal genes.

[0071] The present specification provides for, and includes, pigs that can have inactivating edits in both alleles of the CD163 gene and that can be resistant to infection with PRRSv. The CD163 pigs disclosed herein further do not comprise new sequences or polypeptides, nor do they comprise non-native amino acids resulting from frameshifts or missense mutations.

[0072] The gene edits disclosed herein allow for the protection of CD163-positive fetuses (*e.g.*, fetuses that have one or two wild-type CD163 alleles). CD163-positive fetuses can be protected from PRRSv infection while *in utero* so long as the dam possesses inactivating edits in both alleles of her CD163 genes (PCT/US2018/027944). For example, dams having inactivating edits in both alleles of the CD163 gene can be

mated with males having two wild-type CD163 alleles, and the resulting heterozygous fetuses will be protected from PRRSv infection *in utero*.

[0073] In an aspect, pigs having inactivating edits in one allele of CD163 can be generated using the methods of the present specification. These pigs having heterozygous CD163 alleles (one edited, one wild-type) can be bred with other pigs also having heterozygous CD163 alleles or homozygous edited CD163 alleles, and offspring from this breeding having homozygous edited CD163 alleles can be selected for resistance to infection with PRRSv. The present disclosure also provides for, and includes, porcine animals and populations, and methods for creating or improving porcine animals and populations, in which the animals can be homozygous for one or more particular genetic markers or alleles. In various aspects, the present disclosure provides for, and includes, animals, and methods for creating or improving crossing porcine animals and populations, by generating animals that can be heterozygous for one or more particular genetic markers or alleles, and crossing said animals with other animals heterozygous for one or more of the particular genetic markers or alleles to produce animals that can be homozygous for the one or more particular genetic markers or alleles. In some aspects, multiple rounds of crossing and/or back-crossing may be required to obtain homozygosity for each of the particular genetic markers and alleles. In other aspects, a single cross may be sufficient to obtain homozygosity.

[0074] Various techniques known in the art can be used to inactivate genes to make knock-out animals and/or to introduce edited genes into animals to produce founder animals and to make animal lines, in which the knockout or nucleic acid construct can be integrated into the genome. Such techniques can include, without limitation, pronuclear microinjection (U.S. Pat. No. 4,873,191), retrovirus mediated gene transfer into germ lines (Van der Putten *et al.*, *Proc. Natl. Acad. Sci. USA*, 1985, 82, 6148-1652), gene targeting into embryonic stem cells (Thompson *et al.*, *Cell*, 1989, 56, 313-321), electroporation of embryos (Lo, *Mol. Cell. Biol.*, 1983, 3, 1803-1814), sperm-mediated gene transfer (Lavitrano *et al.*, *Proc. Natl. Acad. Sci. USA*, 2002, 99, 14230-14235; Lavitrano *et al.*, *Reprod. Fert. Develop.*, 2006, 18, 19-23), and *in vitro* transformation of somatic cells, such as cumulus or mammary cells, or adult, fetal, or embryonic stem cells, followed by nuclear transplantation (Wilmut *et al.*, *Nature*, 1997, 385, 810-813 and Wakayama *et al.*, *Nature*, 1998, 394, 369-374). Pronuclear microinjection, sperm mediated gene transfer, and somatic cell nuclear transfer can be

other useful techniques. An animal can be genomically edited in that all of its cells have the edit, including its germ line cells. When methods are used that produce an animal that is mosaic in its modification, the animals may be mosaic and can be further inbred and progeny that are genomically edited can be produced and selected using standard methods. Cloning, for instance, may be used to make a mosaic animal if its cells are edited at the blastocyst stage, or genomic modification can take place when a single-cell is edited. In an aspect, an inactivated knock-out edit can be homozygous.

[0075] In an embryo/zygote microinjection aspect, a nucleic acid construct, mRNA, protein, polynucleotides, or combinations thereof, can be introduced into a fertilized egg. In an aspect, one or two cell fertilized eggs can be used. One and two cell fertilized eggs can provide a visible nuclear structure containing the genetic material from the sperm head and the egg within the protoplasm. In an aspect, pronuclear staged fertilized eggs can be obtained *in vitro* or *in vivo* (*i.e.*, surgically recovered from the oviduct of donor animals). In another aspect, *in vitro* fertilized eggs can be produced, for example, from collected swine ovaries by follicles aspirated using methods known in the art. *See, e.g.*, Agung *et al.*, *J Reprod Dev.*, 2013, 59, 103–106 and Appeltant *et al.*, *J Reprod Dev.*, 2016, 62, 439-449. In an aspect, mature oocytes can be provided for use in the *in vitro* fertilization methods. In another aspect, mature oocytes can be injected with the CRISPR/Cas gene editing system of the present specification.

[0076] The present specification further provides for *in vitro* fertilization of mature oocytes. In an aspect, the oocytes can be matured *in vitro* as provided above. In another aspect, mature oocytes can be collected from gilts. *In vitro* fertilization is performed according to established methods. See Appeltant *et al.*, *J Reprod Dev.*, 2011, 57, 9-16, and Fowler *et al.*, *Reprod Biol.*, 2018, 18, 203-211.

[0077] Zygotes or embryos can be obtained for germline editing by artificial insemination and flushing. The collected zygotes or embryos can then be edited using the methods provided herein. In an aspect, linearized nucleic acid constructs, mRNA, proteins, polynucleotides or combinations thereof can be injected or otherwise introduced, for example, by electroporation, into one of the pronuclei or into the cytoplasm of a zygote or embryo post-fertilization, or into a gamete cell pre-fertilization. In an aspect, a pre-formed RNP complex comprising the Cas nuclease protein and a guide RNA comprising a backbone of SEQ ID NO: 19 and a targeting sequence selected from any one of SEQ ID NOs: 22 to 271 and 347 to 425 can be prepared and injected

into the embryo, zygote, or oocyte. In another aspect, a pre-formed RNP complex comprising the Cas nuclease and a guide RNA comprising a backbone of SEQ ID NO: 19 and a spacer sequence selected from an RNA sequence of a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 can be prepared and injected into the embryo, zygote, or oocyte, together with a repair template comprising SEQ ID NOs: 1 to 13 listed in Table 6. Also included, and provided for, by the present specification are the combinations of spacer sequences and repair templates that can have the sequences of SEQ ID NOs:1 to 13 as recited in Table 6. In an aspect, the injected zygotes or embryos can be transferred to a recipient female (*e.g.*, into the oviducts of a recipient female) and allowed to develop in the recipient female to produce the transgenic or gene edited animals. In an aspect, the methods further provide for *in vitro* or *in vivo* fertilized zygotes or embryos that can be centrifuged at 15,000 x g for 5 minutes to sediment lipids allowing visualization of the pronucleus. The zygotes or embryos can be injected with using an EPPENDORF® FEMTOJET® (EPPENDORF® AG, Germany) injector and can be cultured until blastocyst formation. Rates of embryo cleavage and blastocyst formation and quality can be recorded.

[0078] In an aspect, the CRISPR/Cas editing system and gRNA can be provided as a nucleic acid construct. In another aspect, the Cas nuclease may be provided as an mRNA together with a guide RNA comprising a backbone sequence and a targeting sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. In another aspect, the Cas nuclease may be provided as an mRNA together with a guide RNA comprising a backbone sequence and a targeting sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 together with a repair template listed in Table 6 and selected from the group consisting of SEQ ID NOs: 1 to 13. In a further aspect, the gene targeting complex can be provided by microinjection of a transcribable DNA encoding a Cas nuclease and a guide RNA comprising a backbone sequence and a targeting sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. In aspects according the present specification, the backbone sequence can be a sequence of SEQ ID NO: 19. Also included and provided for by the present specification are the combinations of targeting sequences and repair templates that can have one of the sequences recited in SEQ ID

NOs: 1 to 13. It would be understood by persons of skill in the art that various combinations of nuclease, gRNA, and optionally repair template sequences, can be introduced into oocytes, zygotes, blastula, and embryos to achieve the goals of the present methods. It will be further understood that other backbone sequences can be used in conjunction with the targeting sequences of SEQ ID NOs: 22 to 271 and SEQ ID NOs: 347 to 425 for use with other Cas nucleases. Any Cas nuclease having a PAM sequence of NGG or NGGNG can be suitable for the preparation of a backbone sequence for combination with the targeting sequences of the present specification. In various aspects, the desired edit can have a final genomic sequence of SEQ ID NOs: 426 to 505. In some configurations, the desired edit lacks exon 7 and can have a final nucleotide sequence as set forth in SEQ ID NOs: 426 to 458. In various configurations, the final genomic sequence can include an exogenous stop codon and can have a final genomic sequence as set forth in SEQ ID NOs: 459-504. In various configurations, these partial CD163 genes can have an exon 7 amino acid sequence as set forth in SEQ ID NOs: 506 to 517.

[0079] In an aspect, the injected zygotes or embryos can be transferred to a recipient female (*e.g.*, into the oviducts of a recipient female) and allowed to develop in the recipient female to produce the transgenic or gene edited animals. In an aspect, the methods further provide for *in vitro* or *in vivo* fertilized zygotes or embryos that can be centrifuged at 15,000 x g for 5 minutes to sediment lipids allowing visualization of the pronucleus. The zygotes or embryos can be injected using, for example, an EPPENDORF® FEMTOJET® injector and can be cultured until blastocyst formation. Rates of embryo cleavage and blastocyst formation and quality can be recorded.

[0080] The present specification provides for, and includes, a recipient sow having one or more embryos that can be injected with a CRISPR/Cas/gRNA combination according to the present disclosure. In an aspect, a pre-formed RNP complex can be provided comprising the Cas nuclease and a guide RNA comprising a backbone of SEQ ID NO: 19 and a spacer sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. In various aspects, two pre-formed RNP complexes can be provided comprising the Cas nuclease and a guide RNA comprising a backbone of SEQ ID NO: 19 and each comprising a different spacer sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. Also provided for, and included,

are recipient sows that can have one or more embryos injected with a pre-formed RNP complex that can comprise the Cas nuclease and a guide RNA comprising a targeting sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347-425. In an aspect, the pre-formed RNP complex can be prepared and injected into the embryo, zygote, or oocyte, together with a repair template listed in Table 6 (SEQ ID NOs: 1 to 13). In an aspect, a recipient sow can be a sow of a different line than the donor embryos.

[0081] Embryos or zygotes can be surgically transferred into uteri of synchronous recipients. Typically, 100-200 (or 150-200) embryos or zygotes can be deposited into the ampulla-isthmus junction of the oviduct using a catheter. After surgery, real-time ultrasound examination of pregnancy can be performed. In an aspect, the present specification can include a recipient sow having 1 to 100, but more typically 30 to 60 CRISPR/Cas/gRNA combination treated embryos wherein said embryos can comprise a gene edited CD163 gene comprising a sequence of SEQ ID NOs: 1 to 18 or 426 to 505. In an aspect, the transferred embryos can comprise a mosaic of edited cells. Also included are CD163 gene edited embryos that can be non-mosaic.

[0082] Methods of improving the health of a livestock animal or herd of livestock can comprise modifying a target sequence in the genome of an animal cell to form a truncated CD163 polypeptide. Also provided for, and included, are improved animals that can have truncated CD163 polypeptides. In some aspects, the predicted truncated polypeptides do not include non-native amino acids. Without being bound by theory, the truncated gene product can be rapidly digested *in vivo* by cellular proteases. In an aspect of the present specification, the truncated CD163 can result in the complete elimination of the protein. In a further aspect, the truncated CD163 can be non-detectable *in vivo*. In an aspect, the truncated CD163 can be non-detectable by immunofluorescence labelling and FACS analysis. In yet another aspect, the truncated CD163 can be non-detectable when using any of the expression analysis methods provided in the specification, including those provided in Example 5. In yet another aspect, the truncated CD163 can be non-detectable when using any of the expression analysis methods provided in the specification, including those provided in Example 8.

[0083] In another aspect, a gene edited CD163 pig can comprise a truncated CD163 gene with no more than 25 non-native amino acids. Not to be limited by theory, truncated CD163 proteins can be destabilized and targeted for degradation within the

cell. Accordingly, CD163 protein sequences and CD163 polypeptides comprising non-native amino acids can be non-detectable. In another aspect, the predicted truncated CD163 protein can comprise no more than 211 amino acids of the native protein. In an aspect, the truncated CD163 protein can be predicted to comprise no more than 144 amino acids of the native protein. In an aspect, the truncated CD163 protein can be predicted to comprise no more than 133 amino acids of the native protein. In yet another aspect, the truncated CD163 protein can be predicted to comprise no more than 129 amino acids of the native protein. In an aspect, the truncated CD163 protein can be predicted to comprise no more than 113 amino acids of the native protein. In yet another aspect, the truncated CD163 protein can be predicted to comprise no more than 108 amino acids of the native protein. In a further aspect, the truncated CD163 protein can be predicted to comprise no more than 93 amino acids of the native protein. In yet another aspect, the truncated CD163 protein can be predicted to comprise no more than 74 amino acids of the native protein. As provided herein, truncated CD163 proteins can comprise between 32 to 211 amino acids of native CD163 polypeptide sequences and no more than 25 non-native amino acids and can be undetectable in cells and cell extracts.

[0084] In other aspects, altered CD163 protein can have a truncation with a single amino acid substitution. In some aspects, the altered CD163 protein can have a predicted amino acid sequence of no more than 1,010 amino acids.

[0085] The present specification provides for, and includes, gene edited CD163 pigs that can have truncations of the CD163 protein (*e.g.*, amino acids 1 to 40 of CD163 reference sequence NP_999141). Not to be limited by theory, it is believed that truncations within the signal peptide (also known as the signal sequence) can result in a failure of the protein to translocate to the cellular membrane and the polypeptide is subsequently degraded within the cell. Accordingly, CD163 proteins having signal peptide truncations can result in CD163 null animals that are resistant to infection by PRRSv. In an aspect, the gene edited CD163 animals having proteins truncated in the signal sequence can comprise no more than 25 amino acids of non-native amino acid sequences. In an aspect, the gene edited pigs can comprise no more than the first 39 amino acids of the native CD163 protein. In an aspect, the gene edited pigs can comprise no more than the first 39 amino acids of the native CD163 protein and no more than 15 non-native amino acids. Also included, and provided for, by the present specification are gene edited pigs that can be predicted to comprise no more than the first 38 amino acids

of the native CD163 protein. In an aspect, the gene edited pigs can comprise no more than the first 38 amino acids of the native CD163 protein and no more than 15 non-native amino acids. Also included, and provided for, by the present specification are gene edited pigs that can be predicted to comprise no more than the first 36 amino acids of the native CD163 protein. In an aspect, the gene edited pigs can comprise no more than the first 36 amino acids of the native CD163 protein and no more than 15 non-native amino acids. Truncated proteins can be non-detectable using methods known to those of skill in the art.

[0086] In an aspect, the gene edited pigs can be predicted to comprise no more than the first 34 amino acids of the native CD163 protein and no more than 15 non-native amino acids. In an aspect, the gene edited pigs can be predicted to comprise no more than the first 32 amino acids of the native CD163 protein and no more than 15 non-native amino acids. Truncated proteins can be non-detectable using methods known to those of skill in the art.

[0087] Further, the present disclosure provides for and includes truncations of the CD163 protein that can include deletion of exon 7 (SEQ ID NOs: 426-458), sequences where an exogenous stop codon is introduced into exon 7 (SEQ ID NO: 459-504). These truncations can include CD163 proteins comprising no more than 1,010 amino acids.

[0088] Breeding techniques can be used to create animals that are homozygous for the inactivated gene from the initial homozygous or heterozygous founder or other heterozygous animals. Homozygous animals can be generated. Gene edited pigs described herein can be bred with other edited or wild-type pigs of interest to ultimately generate pigs that are homozygous or heterozygous for the edited gene. In an aspect, the homozygous animal can be an animal of any one of the lines generated by crosses with PIC™ elite porcine lines 2, 3, 15, 19, 27, 62, 65, and combinations thereof. In an aspect, the homozygous animal can be a hybrid animal prepared by a cross between animals of Line 2 and Line 3.

[0089] Gene editing has been used to address various diseases, including PRRS, in swine. While there have been several gene editing technologies developed over the past 15 years, generally these platforms can be designed to introduce a double-stranded break at a specific region of a genome. The introduced break can then be repaired by the cell's own machinery.

[0090] One repair pathway, non-homologous end-joining (NHEJ), is evolutionarily conserved throughout all kingdoms of life and is the predominant double-strand break repair pathway in mammalian cells. This repair process can result in the random insertion or deletion of nucleotides across the cut site. As a practical matter, this incomplete fidelity can be used to advantage by providing in trans a DNA repair template. In an aspect, a DNA repair template carrying an alternative allele of the targeted site (for example, a single base polymorphism or a single or multiple base insertion or deletion) can be co-delivered with the gene editing reagents, and through the use of the cell's homologous recombination machinery, the DNA repair template can direct the repair to generate a new allele. In aspects according to the present specification, DNA repair templates can be designed and selected based on the location of high efficiency of CRISPR/CAS cleavage (*e.g.*, high editing frequency from Table 3) and the ability to introduce deletions that can result in truncated proteins that comprise only wild type sequences. Repair templates can increase the efficiency and accuracy of gene editing. DNA repair templates suitable for the methods of the present application can include SEQ ID NOs: 1 to 13. In an aspect, the DNA repair templates can be paired with a guide sequence as described below in Table 6. Unlike transgenesis, genome editing according to the present specification does not result in the introduction of foreign DNA sequences into the genome.

[0091] However, as these exogenously added DNA repair templates have the possibility to randomly integrate in the genome, it is also advantageous to identify and use guide pairs that result in the deletion of DNA sequences such that the joined ends can result in the generation of an in-frame translational stop codon across the joined ends; when the cut sites of two guides are repaired by NHEJ in an end-to-end manner, this new DNA sequence, when transcribed into mRNA and translated into protein could terminate the production of the CD163 protein. Although function of this terminated CD163 protein can be lost, often, but not always, premature termination of protein synthesis can result in an unstable polypeptide that can be degraded and not detectable by standard methods. Guide pairs that cut exon 7 of the CD163 gene such that the joined ends form an exogenous stop codon can include SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 395, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID

NOs: 354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs: 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, and SEQ ID NOs: 366 and 397.

[0092] In one aspect, the present teachings can involve a simple, precise and reproducible single CD163 loss of function edit in elite pigs such that the edit occurs early in the CD163 gene. A single guide RNA-Cas protein combination and DNA repair template can be selected for directing a specific gene edit in elite pigs, based on several considerations and approaches: efficient cutting near the 5' end of the CD163 gene in tissue culture, bioinformatic review identifying guides with few mismatches in the pig genome, high specificity of on-target cutting as determined by biochemical prescreen, and the ability to target specific gene edits at an intended site in pig embryo-like cells. Examples of specific gene edits of CD163 are discussed in detail below. In an aspect, a specific gene edit according to the present specification can include a CD163 gene comprising a sequence selected from the group consisting of SEQ ID NOs: 1 to 18 and 426 to 505. In an aspect, cells comprising an edited CD163 gene can comprise at least one allele having a sequence selected from the group consisting of SEQ ID NOs:1 to 18 and 426 to 505. In an aspect, cells comprising two gene edited alleles of the CD163 gene can comprise, at both alleles, a sequence selected from the group consisting of SEQ ID NOs: 1 to 18 and 426 to 505. In an aspect, both gene edited CD163 genes can comprise the same sequence selected from the group consisting of SEQ ID NOs: 1 to 18 and 426-505 (*e.g.*, two alleles of SEQ ID NO: 1 or two alleles of SEQ ID NO: 2, etc.). In an aspect, the genome of a cell can comprise gene edited CD163 genes comprising one each of a sequence selected from the group consisting of SEQ ID NOs:1 to 18 and 426-505

(*e.g.*, an allele of SEQ ID NO: 1 with an allele of SEQ ID NO: 2, and all combinations thereof).

[0093] Also provided for, and included, are mixtures of cells that can comprise CD163 edited cells and non-gene edited cells. In an aspect, the mixture can be an embryo. In another aspect, the mixture can be a cell culture. In a further aspect, the mixture of cells does not comprise a reproductive cell. In an aspect, the present specification can provide for, and includes, a tissue culture of CD163 edited cells and methods to prepare such cultures. Cultures according to the present specification can include mixtures of CD163 edited cells (*e.g.*, cells having an allele of SEQ ID NO: 1 with cells having an allele of SEQ ID NO: 2, cells having an allele of SEQ ID NO: 1 with cells having an allele of SEQ ID NO: 3, and all combinations thereof). In an aspect, the tissue culture of non-reproductive cells can include cells comprising a single CD163 edit. In a further aspect, cells of a single CD163 edit in culture may comprise one or both edited alleles.

[0094] Specific examples of endonuclease and guide RNA backbone sequences that can be used in the products and methods of the present teachings are listed in Table 2.

Table 2: Endonuclease and Guide Backbone Sequences

Sequence Identity	**SEQ ID NO:**
Guide RNA Backbone (DNA sequence shown)	19
Streptococcus pyogenes endonuclease protein sequence	20
Streptococcus thermophilus CR3 endonuclease protein sequence	21

[0095] Elite porcine nucleus lines can be sequenced and aligned against a public reference for the CD163 gene. These data can be used for the development of gene editing reagents for the PRRS-resistance project. These sequences can be scanned for the presence of conserved RNA-guided CRISPR-Cas9 recognition sites which can consist of the 3 nucleotide or 5 nucleotide motif, nGG (AGG, CGG, TGG, GGG), nGGnG (AGGTG, CGGTG, TGGTG, GGGTG, AGGGG, CGGGG, TGGGG, GGGGG, AGGAG, CGGAG, TGGAG, GGGAG, AGGCG, CGGCG, TGGCG, GGGCG), respectively. This motif, called the PAM sequence, can be located adjacent to, and 3' of, a 20 nucleotide spacer sequence which can be used for base-pairing with the RNA.

Suitable sites can be identified, and crRNA guide or single guide sequences can be prepared, and are presented below in Table 3.

[0096] While not limited to any particular theory, when complexed, the guideRNA-Cas9 protein can recognize a DNA site for cleavage, which can then be repaired by cellular components by either non-homologous end joining (random repair) or by a DNA template repair pathway (homology directed repair, HDR).

[0097] Guide RNAs (gRNA) can be generated across exons 1 to exons 7 of CD163. Edits in the virus binding domain (Domain 5) of the CD163 protein can inhibit the ability of the virus to bind to the protein, thus preventing uptake of the virus into pig lung macrophages. When the edit in CD163 is homozygous in pigs, the animals can be resistant to PRRS infection. The present specification provides for, and includes, gRNA sequences including the crRNA sequences listed in Table 3 that can be selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. SEQ ID NOs: 22 to 271 and 347 to 425 are shown as DNA sequences. The corresponding RNA counterpart, and the presence of the PAM motif which is generally not included in gRNAs, would be apparent to a person of skill in the art. Also provided herein, are RNA sequences that can comprise sequences selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. SEQ ID NOs: 22 to 271 and 347 to 425 can be combined with the gRNA backbone sequence and expressed as RNA for use in the preparation of a gRNA and then mixed with the Cas nuclease to prepare an active ribonucleotide protein complex (sgRNP). The sgRNP complex can then be injected into a mature oocyte, a zygote, or early embryo, either alone or together with a repair template selected from the group consisting of SEQ ID NOs:1 to 13. After cleavage and template directed repair, the CD163 gene can be edited to incorporate the repair template sequences, and the edited genome of a CD163 gene edited cell can comprise a sequence selected from the group consisting of SEQ ID NOs: 1 to 13.

[0098] In one aspect, the present disclosure can include a simple, precise, and reproducible single CD163 loss of function edit in elite pigs such that the edit occurs early in the CD163 gene (exons 1-7). In an aspect, a pig can have a CD163 gene comprising a sequence selected from the group consisting of SEQ ID NOs: 1 to 18 and 426 to 505. Also included and provided for is a pig that can comprise SEQ ID NOs: 1 to 18 and 425 to 505 in a genomic region comprising the markers of Table 8. Also included

and provided for is a pig that can comprise SEQ ID NO: 2 in a genomic region comprising the markers of Table 8. Also included and provided for is a pig that can comprise SEQ ID NO: 426-458 in a genomic region comprising the markers of Table 8. Also included and provided for is a pig that can comprise SEQ ID NO: 459-504 in a genomic region comprising the markers of Table 8. Using porcine embryonic fibroblast cell lines derived from these elite pigs, 250 guide RNA and Cas9 protein combinations can be tested to identify gene editing pairs that efficiently cut and generate edits in the CD163 gene. Selected specific guides and endonuclease protein combinations found are listed in Table 3, along with the location of the target, their endonuclease activity (average editing frequency), and the position on CD163 of the edit. Greater average editing frequency combinations are preferred because they have more effective editing activity. Combinations having an average editing frequency greater than 1 are desired. In an aspect, combinations can have an average editing frequency greater than 5. In another aspect, combinations can have an average editing frequency greater than 10. In yet another aspect, combinations can have an average editing frequency greater than 15. The present disclosure provides for, and includes, combinations that can have an average editing frequency greater than 20. In an aspect, combinations can have an average editing frequency greater than 25. In yet another aspect, combinations can have an average editing frequency greater than 30.

[0099] As shown in Table 3, the average editing frequency varied greatly between target sequences. Moreover, sequences targeting overlapping sequences resulted in different editing frequencies depending on the Cas protein source. For example, SEQ ID NOs: 24 and 25 targeted overlapping locations and had a 10-fold difference in editing frequency. At this site, with these sequences, *S. pyogenes* provided a nearly a 20-fold higher editing frequency. In contrast, RNPs incorporating SEQ ID NOs: 33 and 34 resulted in indistinguishable average editing frequencies between the Cas proteins. While RNPs incorporating a Cas9 nuclease from *S. pyogenes* can provide superior average editing frequencies, that is not always the case. As shown for SEQ ID NOs: 60 and 61, RNPs incorporating the Cas9 protein from *S. thermophilus* resulted in superior editing frequencies (*e.g.*, 3.9 vs. 22.0). Accordingly, the present specification provides for efficient guide RNA sequences or efficient targeting sequences for targeting CD163 using the CRISPR/Cas system. In an aspect, RNPs can comprise a sequence selected

from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425.

[0100] All position designations herein refer to the Sscrofa11.1 reference genome (GenBank accession: GCA_000003025.6).

[0101] Target site sequences SEQ ID NOs: 22 to 271 and 347 to 425 can be editing target sequences within the porcine CD163 gene. In each target site sequence, the first 20 nucleotides can correspond to the guide RNA spacer, and the remaining 3 or 5 nucleotides can be the PAM sequences for *S. pyogenes* or *S. thermophilus*, respectively. The first 20 bases of SEQ ID NOs: 22 to 271 and 347 to 425 correspond to the DNA equivalent guide RNA sequences, with the appropriate ribonuclease bases substituted for deoxyribonuclease bases, although the DNA bases can be used instead of RNA bases as guides. Guide RNA sequences can be paired with *S. pyogenes* or *S. thermophilus* endonucleases to direct DNA breaks in the porcine genome in either coding or non-coding regions of the CD163 gene, which can result in DNA deletions and/or DNA nucleotide insertions. Depending on the locations of the target sites as disclosed in Table 3, pairs of guide/endonuclease combinations can be selected to delete regions of the CD163 gene between the pairs of guide/endonuclease combinations.

[0102] Target site sequences SEQ ID NOs: 212 to 271 and 347 to 425 are targeting sequences that can be used to design guides for deletion of exon 7 of the CD163 gene. Pairing of intron 6 targeting guides SEQ ID NOs: 212 to 253, and 262 to 271 with intron 7 targeting guides SEQ ID NOs: 254 to 261 using *S. pyogenes* can cause two double strand breaks on the CD163 gene, resulting in excision of exon 7. Pairing of targeting guides SEQ ID NOs: 249 and 256 also can result in excision of Exon 7. Pairing of some guides can result in the introduction of a stop codon in Exon 7, including SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs.: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 498, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID NOs: 354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID

NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs. 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, or SEQ ID NOs: 366 and 397.

[0103] For each targeting sequence, Table 3 lists its SEQ ID No., the species of CAS9 nuclease homing arm used, the location of the targeting sequence on the *Sus scrofa* genome including the PAM sequence, the editing efficiency as measured by the average fraction of edits for a particular guide in fetal fibroblast cell assays, and the exon on CD163 targeted.

Table 3: List of target sequences and editing activities in porcine fetal fibroblasts

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
22	*S. pyogenes*	chr5:63300192-63300214	12.9	Exon 1 / 15
23	*S. pyogenes*	chr5:63300222-63300244	1.4	Exon 1 / 15
24	*S. pyogenes*	chr5:63300236-63300258	5.8	Exon 1 / 15
25	*S. thermophilus*	chr5:63300236-63300260	0.3	Exon 1 / 15
26	*S. pyogenes*	chr5:63300250-63300272	8.6	Exon 1 / 15
27	*S. pyogenes*	chr5:63300251-63300273	0.1	Exon 1 / 15
28	*S. pyogenes*	chr5:63300275-63300297	25.7	Exon 1 / 15
29	*S. pyogenes*	chr5:63300288-63300310	24.9	Exon 1 / 15
30	*S. pyogenes*	chr5:63300293-63300315	12.8	Exon 1 / 15
31	*S. pyogenes*	chr5:63300305-63300327	0.4	Exon 1 / 15
32	*S. pyogenes*	chr5:63300308-63300330	3.4	Exon 1 / 15
33	*S. thermophilus*	chr5:63300308-63300332	3.7	Exon 1 / 15
34	*S. pyogenes*	chr5:63300327-63300349	5.3	Exon 1 / 15
35	*S. pyogenes*	chr5:63300336-63300358	3.5	Exon 1 / 15
36	*S. pyogenes*	chr5:63301950-63301972	14.4	Exon 2 / 15
37	*S. pyogenes*	chr5:63301951-63301973	16.3	Exon 2 / 15
38	*S. pyogenes*	chr5:63301962-63301984	13.6	Exon 2 / 15
39	*S. pyogenes*	chr5:63301981-63302003	3.6	Exon 2 / 15
40	*S. pyogenes*	chr5:63301995-63302017	21.8	Exon 2 / 15
41	*S. pyogenes*	chr5:63301997-63302019	8.2	Exon 2 / 15
42	*S. thermophilus*	chr5:63301997-63302021	10.1	Exon 2 / 15

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
43	*S. pyogenes*	chr5:63303121-63303143	16.4	Exon 3 / 15
44	*S. thermophilus*	chr5:63303121-63303145	16.1	Exon 3 / 15
45	*S. pyogenes*	chr5:63303129-63303151	31.4	Exon 3 / 15
46	*S. pyogenes*	chr5:63303136-63303158	22.8	Exon 3 / 15
47	*S. pyogenes*	chr5:63303137-63303159	42.9	Exon 3 / 15
48	*S. thermophilus*	chr5:63303137-63303161	10.7	Exon 3 / 15
49	*S. pyogenes*	chr5:63303140-63303162	36.7	Exon 3 / 15
50	*S. thermophilus*	chr5:63303140-63303164	32.6	Exon 3 / 15
51	*S. pyogenes*	chr5:63303158-63303180	21.1	Exon 3 / 15
52	*S. pyogenes*	chr5:63303166-63303188	23.6	Exon 3 / 15
53	*S. thermophilus*	chr5:63303166-63303190	22.1	Exon 3 / 15
54	*S. pyogenes*	chr5:63303169-63303191	40.0	Exon 3 / 15
55	*S. thermophilus*	chr5:63303169-63303193	19.5	Exon 3 / 15
56	*S. pyogenes*	chr5:63303181-63303203	9.9	Exon 3 / 15
57	*S. thermophilus*	chr5:63303181-63303205	10.4	Exon 3 / 15
58	*S. pyogenes*	chr5:63303184-63303206	20.6	Exon 3 / 15
59	*S. thermophilus*	chr5:63303184-63303208	2.8	Exon 3 / 15
60	*S. pyogenes*	chr5:63303189-63303211	3.9	Exon 3 / 15
61	*S. thermophilus*	chr5:63303189-63303213	22.0	Exon 3 / 15
62	*S. pyogenes*	chr5:63303190-63303212	18.1	Exon 3 / 15
63	*S. pyogenes*	chr5:63303191-63303213	23.5	Exon 3 / 15
64	*S. pyogenes*	chr5:63303209-63303231	23.6	Exon 3 / 15
65	*S. pyogenes*	chr5:63303213-63303235	4.1	Exon 3 / 15
66	*S. pyogenes*	chr5:63303214-63303236	16.2	Exon 3 / 15
67	*S. pyogenes*	chr5:63303220-63303242	14.6	Exon 3 / 15
68	*S. pyogenes*	chr5:63303226-63303248	15.4	Exon 3 / 15
69	*S. pyogenes*	chr5:63303243-63303265	21.6	Exon 3 / 15
70	*S. pyogenes*	chr5:63303250-63303272	1.5	Exon 3 / 15
71	*S. pyogenes*	chr5:63303251-63303273	30.3	Exon 3 / 15
72	*S. pyogenes*	chr5:63303278-63303300	20.1	Exon 3 / 15
73	*S. pyogenes*	chr5:63303278-63303300	1.5	Exon 3 / 15
74	*S. pyogenes*	chr5:63303282-63303304	23.9	Exon 3 / 15
75	*S. pyogenes*	chr5:63303283-63303305	20.8	Exon 3 / 15
76	*S. pyogenes*	chr5:63303294-63303316	33.8	Exon 3 / 15
77	*S. pyogenes*	chr5:63303299-63303321	8.2	Exon 3 / 15
78	*S. pyogenes*	chr5:63303305-63303327	6.1	Exon 3 / 15
79	*S. pyogenes*	chr5:63303315-63303337	25.2	Exon 3 / 15
80	*S. pyogenes*	chr5:63303319-63303341	28.7	Exon 3 / 15

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
81	*S. pyogenes*	chr5:63303338-63303360	4.5	Exon 3 / 15
82	*S. pyogenes*	chr5:63303339-63303361	2.8	Exon 3 / 15
83	*S. pyogenes*	chr5:63303357-63303379	18.3	Exon 3 / 15
84	*S. pyogenes*	chr5:63303358-63303380	26.6	Exon 3 / 15
85	*S. pyogenes*	chr5:63303374-63303396	16.1	Exon 3 / 15
86	*S. pyogenes*	chr5:63303378-63303400	35.4	Exon 3 / 15
87	*S. thermophilus*	chr5:63303378-63303402	40.0	Exon 3 / 15
88	*S. pyogenes*	chr5:63303379-63303401	24.4	Exon 3 / 15
89	*S. pyogenes*	chr5:63303380-63303402	25.6	Exon 3 / 15
90	*S. pyogenes*	chr5:63303406-63303428	28.1	Exon 3 / 15
91	*S. pyogenes*	chr5:63303413-63303435	13.7	Exon 3 / 15
92	*S. thermophilus*	chr5:63303413-63303437	19.0	Exon 3 / 15
93	*S. thermophilus*	chr5:63303419-63303443	15.2	Exon 3 / 15
94	*S. pyogenes*	chr5:63303421-63303443	16.5	Exon 3 / 15
95	*S. pyogenes*	chr5:63303428-63303450	35.8	Exon 3 / 15
96	*S. pyogenes*	chr5:63303441-63303463	3.4	Exon 3 / 15
97	*S. pyogenes*	chr5:63306087-63306109	19.8	Exon 4 / 15
98	*S. pyogenes*	chr5:63306091-63306113	4.5	Exon 4 / 15
99	*S. thermophilus*	chr5:63306091-63306115	3.1	Exon 4 / 15
100	*S. pyogenes*	chr5:63306098-63306120	29.9	Exon 4 / 15
101	*S. thermophilus*	chr5:63306098-63306122	31.5	Exon 4 / 15
102	*S. pyogenes*	chr5:63306101-63306123	29.2	Exon 4 / 15
103	*S. pyogenes*	chr5:63306108-63306130	6.9	Exon 4 / 15
104	*S. thermophilus*	chr5:63306108-63306132	14.7	Exon 4 / 15
105	*S. pyogenes*	chr5:63306116-63306138	21.6	Exon 4 / 15
106	*S. pyogenes*	chr5:63306127-63306149	26.4	Exon 4 / 15
107	*S. pyogenes*	chr5:63306140-63306162	6.9	Exon 4 / 15
108	*S. pyogenes*	chr5:63306144-63306166	35.2	Exon 4 / 15
109	*S. thermophilus*	chr5:63306144-63306168	3.3	Exon 4 / 15
110	*S. pyogenes*	chr5:63306147-63306169	8.8	Exon 4 / 15
111	*S. thermophilus*	chr5:63306147-63306171	3.2	Exon 4 / 15
112	*S. pyogenes*	chr5:63306148-63306170	5.2	Exon 4 / 15
113	*S. pyogenes*	chr5:63306149-63306171	28.0	Exon 4 / 15
114	*S. pyogenes*	chr5:63306193-63306215	9.3	Exon 4 / 15
115	*S. pyogenes*	chr5:63306236-63306258	14.2	Exon 4 / 15
116	*S. pyogenes*	chr5:63306251-63306273	26.3	Exon 4 / 15
117	*S. thermophilus*	chr5:63306251-63306275	4.4	Exon 4 / 15
118	*S. pyogenes*	chr5:63306257-63306279	1.3	Exon 4 / 15

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
119	*S. pyogenes*	chr5:63306263-63306285	7.8	Exon 4 / 15
120	*S. pyogenes*	chr5:63306273-63306295	35.0	Exon 4 / 15
121	*S. pyogenes*	chr5:63306287-63306309	13.4	Exon 4 / 15
122	*S. pyogenes*	chr5:63306296-63306318	2.0	Exon 4 / 15
123	*S. pyogenes*	chr5:63306315-63306337	31.2	Exon 4 / 15
124	*S. pyogenes*	chr5:63306332-63306354	10.1	Exon 4 / 15
125	*S. pyogenes*	chr5:63306336-63306358	31.1	Exon 4 / 15
126	*S. thermophilus*	chr5:63306336-63306360	52.8	Exon 4 / 15
127	*S. pyogenes*	chr5:63306337-63306359	30.3	Exon 4 / 15
128	*S. pyogenes*	chr5:63306338-63306360	43.2	Exon 4 / 15
129	*S. pyogenes*	chr5:63306364-63306386	2.5	Exon 4 / 15
130	*S. pyogenes*	chr5:63306371-63306393	0.2	Exon 4 / 15
131	*S. thermophilus*	chr5:63306371-63306395	0.3	Exon 4 / 15
132	*S. pyogenes*	chr5:63309028-63309050	4.5	Exon 5 / 15
133	*S. pyogenes*	chr5:63309034-63309056	5.1	Exon 5 / 15
134	*S. pyogenes*	chr5:63309035-63309057	41.8	Exon 5 / 15
135	*S. thermophilus*	chr5:63309035-63309059	21.9	Exon 5 / 15
136	*S. pyogenes*	chr5:63309053-63309075	15.1	Exon 5 / 15
137	*S. pyogenes*	chr5:63309061-63309083	0.2	Exon 5 / 15
138	*S. pyogenes*	chr5:63309077-63309099	15.7	Exon 5 / 15
139	*S. thermophilus*	chr5:63309077-63309101	11.2	Exon 5 / 15
140	*S. pyogenes*	chr5:63309084-63309106	10.1	Exon 5 / 15
141	*S. thermophilus*	chr5:63309084-63309108	23.8	Exon 5 / 15
142	*S. pyogenes*	chr5:63309085-63309107	11.3	Exon 5 / 15
143	*S. pyogenes*	chr5:63309086-63309108	23.4	Exon 5 / 15
144	*S. pyogenes*	chr5:63309094-63309116	13.8	Exon 5 / 15
145	*S. pyogenes*	chr5:63309104-63309126	7.8	Exon 5 / 15
146	*S. pyogenes*	chr5:63309108-63309130	1.4	Exon 5 / 15
147	*S. pyogenes*	chr5:63309109-63309131	7.0	Exon 5 / 15
148	*S. pyogenes*	chr5:63309130-63309152	24.8	Exon 5 / 15
149	*S. pyogenes*	chr5:63309144-63309166	20.8	Exon 5 / 15
150	*S. pyogenes*	chr5:63309145-63309167	0.1	Exon 5 / 15
151	*S. pyogenes*	chr5:63309146-63309168	37.4	Exon 5 / 15
152	*S. pyogenes*	chr5:63309173-63309195	2.5	Exon 5 / 15
153	*S. pyogenes*	chr5:63309173-63309195	2.3	Exon 5 / 15
154	*S. pyogenes*	chr5:63309189-63309211	11.5	Exon 5 / 15
155	*S. pyogenes*	chr5:63309193-63309215	2.7	Exon 5 / 15
156	*S. pyogenes*	chr5:63309194-63309216	10.4	Exon 5 / 15

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
157	*S. pyogenes*	chr5:63309200-63309222	3.8	Exon 5 / 15
158	*S. thermophilus*	chr5:63309205-63309229	19.0	Exon 5 / 15
159	*S. pyogenes*	chr5:63309207-63309229	21.0	Exon 5 / 15
160	*S. pyogenes*	chr5:63309210-63309232	19.0	Exon 5 / 15
161	*S. pyogenes*	chr5:63309233-63309255	8.3	Exon 5 / 15
162	*S. pyogenes*	chr5:63309250-63309272	13.6	Exon 5 / 15
163	*S. pyogenes*	chr5:63309252-63309274	23.0	Exon 5 / 15
164	*S. pyogenes*	chr5:63309273-63309295	2.3	Exon 5 / 15
165	*S. thermophilus*	chr5:63309273-63309297	34.4	Exon 5 / 15
166	*S. pyogenes*	chr5:63309274-63309296	15.1	Exon 5 / 15
167	*S. pyogenes*	chr5:63309275-63309297	19.4	Exon 5 / 15
168	*S. thermophilus*	chr5:63309284-63309308	14.4	Exon 5 / 15
169	*S. pyogenes*	chr5:63309286-63309308	7.0	Exon 5 / 15
170	*S. pyogenes*	chr5:63309308-63309330	0.5	Exon 5 / 15
171	*S. thermophilus*	chr5:63309308-63309332	2.4	Exon 5 / 15
172	*S. pyogenes*	chr5:63309323-63309345	16.0	Exon 5 / 15
173	*S. pyogenes*	chr5:63309841-63309863	0.1	Exon 6 / 15
174	*S. pyogenes*	chr5:63309857-63309879	8.4	Exon 6 / 15
175	*S. thermophilus*	chr5:63309857-63309881	8.0	Exon 6 / 15
176	*S. pyogenes*	chr5:63309860-63309882	40.3	Exon 6 / 15
177	*S. thermophilus*	chr5:63309860-63309884	32.3	Exon 6 / 15
178	*S. pyogenes*	chr5:63309863-63309885	37.2	Exon 6 / 15
179	*S. pyogenes*	chr5:63309886-63309908	4.7	Exon 6 / 15
180	*S. thermophilus*	chr5:63309886-63309910	3.5	Exon 6 / 15
181	*S. pyogenes*	chr5:63309889-63309911	38.1	Exon 6 / 15
182	*S. pyogenes*	chr5:63309889-63309911	34.1	Exon 6 / 15
183	*S. thermophilus*	chr5:63309889-63309913	17.5	Exon 6 / 15
184	*S. pyogenes*	chr5:63309892-63309914	4.9	Exon 6 / 15
185	*S. pyogenes*	chr5:63309907-63309929	13.5	Exon 6 / 15
186	*S. pyogenes*	chr5:63309911-63309933	5.5	Exon 6 / 15
187	*S. pyogenes*	chr5:63309933-63309955	0.7	Exon 6 / 15
188	*S. thermophilus*	chr5:63309933-63309957	10.0	Exon 6 / 15
189	*S. pyogenes*	chr5:63309934-63309956	6.4	Exon 6 / 15
190	*S. pyogenes*	chr5:63309935-63309957	18.7	Exon 6 / 15
191	*S. pyogenes*	chr5:63309955-63309977	12.0	Exon 6 / 15
192	*S. pyogenes*	chr5:63309963-63309985	5.6	Exon 6 / 15
193	*S. pyogenes*	chr5:63309970-63309992	0.5	Exon 6 / 15
194	*S. pyogenes*	chr5:63309971-63309993	12.5	Exon 6 / 15

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
195	*S. pyogenes*	chr5:63309977-63309999	5.3	Exon 6 / 15
196	*S. pyogenes*	chr5:63310021-63310043	2.2	Exon 6 / 15
197	*S. pyogenes*	chr5:63310035-63310057	10.3	Exon 6 / 15
198	*S. pyogenes*	chr5:63310038-63310060	0.2	Exon 6 / 15
199	*S. pyogenes*	chr5:63310058-63310080	8.2	Exon 6 / 15
200	*S. pyogenes*	chr5:63310077-63310099	6.2	Exon 6 / 15
201	*S. pyogenes*	chr5:63310078-63310100	8.6	Exon 6 / 15
202	*S. pyogenes*	chr5:63310092-63310114	2.7	Exon 6 / 15
203	*S. pyogenes*	chr5:63310098-63310120	11.1	Exon 6 / 15
204	*S. thermophilus*	chr5:63310098-63310122	8.0	Exon 6 / 15
205	*S. pyogenes*	chr5:63310099-63310121	12.2	Exon 6 / 15
206	*S. pyogenes*	chr5:63310100-63310122	21.1	Exon 6 / 15
207	*S. thermophilus*	chr5:63310100-63310124	0.1	Exon 6 / 15
208	*S. pyogenes*	chr5:63310103-63310125	3.3	Exon 6 / 15
209	*S. pyogenes*	chr5:63310152-63310174	4.8	Exon 6 / 15
210	*S. pyogenes*	chr5:63323061-63323083	32.8	Exon 7 / 15
211	*S. pyogenes*	chr5:63323147-63323169	17.0	Exon 7 / 15
212	*S. pyogenes*	chr5:63322548-63322570	13.6	Intron 6
213	*S. pyogenes*	chr5:63322549-63322571	1.7	Intron 6
214	*S. pyogenes*	chr5:63322566-63322588	15.6	Intron 6
215	*S. pyogenes*	chr5:63322594-63322616	0.0	Intron 6
216	*S. pyogenes*	chr5:63322597-63322619	0.0	Intron 6
217	*S. pyogenes*	chr5:63322646-63322668	21.1	Intron 6
218	*S. pyogenes*	chr5:63322647-63322669	12.6	Intron 6
219	*S. pyogenes*	chr5:63322681-63322703	44.3	Intron 6
220	*S. pyogenes*	chr5:63322683-63322705	3.9	Intron 6
221	*S. pyogenes*	chr5:63322693-63322715	33.9	Intron 6
222	*S. pyogenes*	chr5:63322694-63322716	26.1	Intron 6
223	*S. pyogenes*	chr5:63322714-63322736	5.7	Intron 6
224	*S. pyogenes*	chr5:63322731-63322753	42.8	Intron 6
225	*S. pyogenes*	chr5:63322756-63322778	5.8	Intron 6
226	*S. pyogenes*	chr5:63322757-63322779	21.2	Intron 6
227	*S. pyogenes*	chr5:63322770-63322792	36.1	Intron 6
228	*S. pyogenes*	chr5:63322799-63322821	29.9	Intron 6
229	*S. pyogenes*	chr5:63322800-63322822	43.2	Intron 6
230	*S. pyogenes*	chr5:63322809-63322831	33.3	Intron 6
231	*S. pyogenes*	chr5:63322810-63322832	46.8	Intron 6
232	*S. pyogenes*	chr5:63322834-63322856	7.8	Intron 6

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
233	*S. pyogenes*	chr5:63322835-63322857	18.3	Intron 6
234	*S. pyogenes*	chr5:63322839-63322861	18.8	Intron 6
235	*S. pyogenes*	chr5:63322839-63322861	13.2	Intron 6
236	*S. pyogenes*	chr5:63322840-63322862	2.8	Intron 6
237	*S. pyogenes*	chr5:63322845-63322867	55.3	Intron 6
238	*S. pyogenes*	chr5:63322848-63322870	27.2	Intron 6
239	*S. pyogenes*	chr5:63322852-63322874	24.4	Intron 6
240	*S. pyogenes*	chr5:63322859-63322881	22.2	Intron 6
241	*S. pyogenes*	chr5:63322875-63322897	25.3	Intron 6
242	*S. pyogenes*	chr5:63322887-63322909	4.2	Intron 6
243	*S. pyogenes*	chr5:63322888-63322910	3.5	Intron 6
244	*S. pyogenes*	chr5:63322891-63322913	44.3	Intron 6
245	*S. pyogenes*	chr5:63322900-63322922	58.2	Intron 6
246	*S. pyogenes*	chr5:63322906-63322928	3.7	Intron 6
247	*S. pyogenes*	chr5:63322926-63322948	38.1	Intron 6
248	*S. pyogenes*	chr5:63322927-63322949	0.5	Intron 6
249	*S. pyogenes*	chr5:63322947-63322969	31.7	Intron 6
250	*S. pyogenes*	chr5:63322957-63322979	50.0	Intron 6
251	*S. pyogenes*	chr5:63322957-63322979	11.0	Intron 6
252	*S. pyogenes*	chr5:63322991-63323013	4.5	Intron 6
253	*S. pyogenes*	chr5:63322992-63323014	0.5	Intron 6
254	*S. pyogenes*	chr5:63323338-63323360	19.8	Intron 7
255	*S. pyogenes*	chr5:63323339-63323361	8.1	Intron 7
256	*S. pyogenes*	chr5:63323361-63323383	21.1	Intron 7
257	*S. pyogenes*	chr5:63323362-63323384	30.4	Intron 7
258	*S. pyogenes*	chr5:63323362-63323384	5.6	Intron 7
259	*S. pyogenes*	chr5:63323363-63323385	2.8	Intron 7
260	*S. pyogenes*	chr5:63323367-63323389	23.2	Intron 7
261	*S. pyogenes*	chr5:63323368-63323390	26.2	Intron 7
262	*S. thermophilus*	chr5:63322644-63322668	Activity not tested	Intron 6
263	*S. thermophilus*	chr5:63322647-63322671	Activity not tested	Intron 6
264	*S. thermophilus*	chr5:63322678-63322702	Activity not tested	Intron 6
265	*S. thermophilus*	chr5:63322681-63322705	Activity not tested	Intron 6
266	*S. thermophilus*	chr5:63322755-63322779	Activity not tested	Intron 6

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
267	*S. thermophilus*	chr5:63322807-63322831	Activity not tested	Intron 6
268	*S. thermophilus*	chr5:63322845-63322869	Activity not tested	Intron 6
269	*S. thermophilus*	chr5:63322850-63322874	Activity not tested	Intron 6
270	*S. thermophilus*	chr5:63322955-63322979	Activity not tested	Intron 6
271	*S. thermophilus*	chr5:63322989-63323013	Activity not tested	Intron 6
347	*S. pyogenes*	chr5:63323002-63323024	2.1	Exon 7
348	*S. pyogenes*	chr5:63323011-63323033	31.9	Exon 7
349	*S. pyogenes*	chr5:63323015-63323037	7.7	Exon 7
350	*S. pyogenes*	chr5:63323017-63323039	56.5	Exon 7
351	*S. pyogenes*	chr5:63323018-63323040	74.3	Exon 7
352	*S. pyogenes*	chr5:63323019-63323041	52.3	Exon 7
353	*S. pyogenes*	chr5:63323022-63323044	44.0	Exon 7
354	*S. pyogenes*	chr5:63323023-63323045	61.6	Exon 7
355	*S. pyogenes*	chr5:63323024-63323046	49.4	Exon 7
356	*S. pyogenes*	chr5:63323028-63323050	3.1	Exon 7
357	*S. pyogenes*	chr5:63323029-63323051	0.8	Exon 7
358	*S. pyogenes*	chr5:63323040-63323062	42.4	Exon 7
359	*S. pyogenes*	chr5:63323052-63323074	66.7	Exon 7
360	*S. pyogenes*	chr5:63323053-63323075	13.4	Exon 7
361	*S. pyogenes*	chr5:63323061-63323083	77.9	Exon 7
362	*S. pyogenes*	chr5:63323071-63323093	60.8	Exon 7
363	*S. pyogenes*	chr5:63323072-63323094	70.6	Exon 7
364	*S. pyogenes*	chr5:63323073-63323095	75.2	Exon 7
365	*S. pyogenes*	chr5:63323098-63323120	64.4	Exon 7
366	*S. pyogenes*	chr5:63323102-63323124	55.0	Exon 7
367	*S. pyogenes*	chr5:63323105-63323127	51.2	Exon 7
368	*S. pyogenes*	chr5:63323108-63323130	58.3	Exon 7
369	*S. pyogenes*	chr5:63323125-63323147	14.5	Exon 7
370	*S. pyogenes*	chr5:63323126-63323148	22.6	Exon 7
371	*S. pyogenes*	chr5:63323131-63323153	49.9	Exon 7
372	*S. pyogenes*	chr5:63323139-63323161	68.9	Exon 7
373	*S. pyogenes*	chr5:63323147-63323169	57.4	Exon 7
374	*S. pyogenes*	chr5:63323159-63323181	12.9	Exon 7
375	*S. pyogenes*	chr5:63323160-63323182	19.7	Exon 7

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
376	*S. pyogenes*	chr5:63323161-63323183	33.6	Exon 7
377	*S. pyogenes*	chr5:63323162-63323184	65.3	Exon 7
378	*S. pyogenes*	chr5:63323163-63323185	51.4	Exon 7
379	*S. pyogenes*	chr5:63323173-63323195	57.6	Exon 7
380	*S. pyogenes*	chr5:63323174-63323196	52.1	Exon 7
381	*S. pyogenes*	chr5:63323175-63323197	48.2	Exon 7
382	*S. pyogenes*	chr5:63323177-63323199	33.7	Exon 7
383	*S. pyogenes*	chr5:63323181-63323203	18.3	Exon 7
384	*S. pyogenes*	chr5:63323187-63323209	57.0	Exon 7
385	*S. pyogenes*	chr5:63323197-63323219	33.3	Exon 7
386	*S. pyogenes*	chr5:63323198-63323220	58.3	Exon 7
387	*S. pyogenes*	chr5:63323219-63323241	20.7	Exon 7
388	*S. pyogenes*	chr5:63323220-63323242	42.2	Exon 7
389	*S. pyogenes*	chr5:63323221-63323243	43.4	Exon 7
390	*S. pyogenes*	chr5:63323231-63323253	21.8	Exon 7
391	*S. pyogenes*	chr5:63323251-63323273	46.8	Exon 7
392	*S. pyogenes*	chr5:63323252-63323274	42.6	Exon 7
393	*S. pyogenes*	chr5:63323255-63323277	22.8	Exon 7
394	*S. pyogenes*	chr5:63323267-63323289	85.4	Exon 7
395	*S. pyogenes*	chr5:63323268-63323290	41.1	Exon 7
396	*S. pyogenes*	chr5:63323268-63323290	53.6	Exon 7
397	*S. pyogenes*	chr5:63323269-63323291	32.1	Exon 7
398	*S. pyogenes*	chr5:63323277-63323299	16.6	Exon 7
399	*S. pyogenes*	chr5:63323278-63323300	39.0	Exon 7
400	*S. pyogenes*	chr5:63323279-63323301	11.7	Exon 7
401	*S. pyogenes*	chr5:63323282-63323304	48.9	Exon 7
402	*S. pyogenes*	chr5:63323283-63323305	n/a	Exon 7
403	*S. pyogenes*	chr5:63323287-63323309	11.6	Exon 7
404	*S. pyogenes*	chr5:63323288-63323310	69.3	Exon 7
405	*S. pyogenes*	chr5:63323295-63323317	46.0	Exon 7
406	*S. pyogenes*	chr5:63323300-63323322	54.2	Exon 7
407	*S. thermophilus*	chr5:63323019-63323043	25.5	Exon 7
408	*S. thermophilus*	chr5:63323022-63323046	24.7	Exon 7
409	*S. thermophilus*	chr5:63323061-63323085	37.8	Exon 7
410	*S. thermophilus*	chr5:63323071-63323095	30.4	Exon 7
411	*S. thermophilus*	chr5:63323096-63323120	33.4	Exon 7
412	*S. thermophilus*	chr5:63323102-63323126	19.8	Exon 7
413	*S. thermophilus*	chr5:63323105-63323129	0.6	Exon 7

SEQ ID NO:	Cas9 Nuclease	Location of target	Average edited fraction	Position on CD163
414	*S. thermophilus*	chr5:63323159-63323183	14.8	Exon 7
415	*S. thermophilus*	chr5:63323160-63323184	7.8	Exon 7
416	*S. thermophilus*	chr5:63323161-63323185	3.6	Exon 7
417	*S. thermophilus*	chr5:63323163-63323187	7.9	Exon 7
418	*S. thermophilus*	chr5:63323175-63323199	33.8	Exon 7
419	*S. thermophilus*	chr5:63323175-63323199	11.5	Exon 7
420	*S. thermophilus*	chr5:63323219-63323243	8.1	Exon 7
421	*S. thermophilus*	chr5:63323253-63323277	32.6	Exon 7
422	*S. thermophilus*	chr5:63323275-63323299	0.2	Exon 7
423	*S. thermophilus*	chr5:63323277-63323301	12.3	Exon 7
424	*S. thermophilus*	chr5:63323280-63323304	26.0	Exon 7
425	*S. thermophilus*	chr5:63323295-63323319	30.5	Exon 7

[0104] Efficient guideRNA-Cas9 pairs which cut sites early in the CD163 gene (exons 1-4) were selected and further screened for potential off-target binding within the pig genome.

[0105] In some instances, the use of guide RNA and endonuclease has been observed to result in cleavage of DNA at unintended locations in the genome. Because the repair process for dsDNA breaks can be random, off-target cleavage events can result in undesirable changes to coding or regulatory regions in the genome. Therefore, in instances where a number of single or paired guides can perform an intended edit with similar editing frequencies, it is advantageous to consider off-target cleavage in choosing a guide or guide pair for editing experiments.

[0106] A number of computational and biochemical approaches for elucidating off-targets have been developed. Computational approaches can include, but are not limited to, Cas-OFFinder (Bae, S., *et al.*, Bioinformatics, 2014, 30, 1473-1475.), CRISPR-offinder (Zhao, C., *et al.*, Int. J. Biol. Sci., 2017, 13, 1470-1478), and CRISPR-OFF (Alkan, F., *et al.*, Genome Biol., 2018, 19, 177). Other computational approaches are readily available to those skilled in the art. Biochemical approaches can include, but are not limited to, GUIDE-Seq (Tsai, S.Q., *et al.*, Nat Biotechnol. 2015, 33, 187-197), SITE-SEQ® (Cameron, P., *et al.*, Nat Methods 2017, 14, 600-606), and CIRCLE-seq (Tsai, S.Q., *et al.*, Nat. Methods, 2017, 14, 607-614). Other biochemical approaches are readily available to those skilled in the art. While computational methods are relatively

fast and inexpensive compared to biochemical ones, biochemical approaches have been shown to be superior in identifying validated off-target edits.

[0107] A subset of guideRNAs that have demonstrated a high frequency of intended edits in porcine fetal fibroblasts, as described above, were assayed for specificity using SITE-SEQ®. Using naked gDNA and RNP editing reagents in vitro, SITE-SEQ® can provide a list of potential cleavage sites. Off-target cleavage in a cellular environment can be more complicated than that simulated biochemically *in vitro*. Factors such as effective RNP concentration and target availability due to chromatin state, among other factors, can contribute to the number of off-target edits realized in porcine cells or in edited pigs. Fortunately, biochemical methods such as SITE-SEQ® can provide researchers a list of sites to interrogate. These sites can be interrogated by methods that include, but are not limited to, TOPO cloning and sequencing of TOPO clones, ILLUMINA® amplicon sequencing, Nanopore sequencing, other NGS sequencing methods, and sequence capture (Gnirke, A., *et al.*, Nat. Biotechnol., 2009, 27, 182-189).

[0108] Screening was performed for biochemically-identified off-target sites in edited porcine fibroblasts and subsequently in injected embryos. Guides with validated off-target edits were de-prioritized for use in generating edited pigs. Animals generated using guides with known off-targets can be interrogated for the presence of off-target edits using the strategies outlined above. Animals that do contain off-target edits can either be removed from the breeding program or the off-target edits can be removed via breeding. This screening can include bioinformatic methods, such as BLAST® searching, to identify sequences in the *Sus scrofa* genome that contain 1-5 mismatches with the guideRNA, that could therefore allow for off-target binding. The number of potential off-target binding sites in the genome when allowing these mismatches in the computer algorithm is detailed below in Table 4.

Table 4: Mismatch Detection

		SEQ ID		**MISMATCHES ≤ 5**	
SITE	LOCATION- Spy coordinates	**SEQ ID Spy**	**SEQ ID Stherm**	Spy	Stherm C3
1	chr5:63300236-63300258	19	20	920	142
2	chr5:63300308-63300330	32	33	625	51
3	chr5:63301997-63302019	41	42	506	37

4	chr5:63303121-63303143	43	44	1625	201
5	chr5:63303137-63303159	47	48	474	32
6	chr5:63303140-63303162	49	50	548	49
7	chr5:63303166-63303188	52	53	692	92
8	chr5:63303169-63303191	54	55	782	94
9	chr5:63303181-63303203	56	57	1346	200
10	chr5:63303184-63303206	58	59	1873	382
11	chr5:63303189-63303211	60	61	1642	240
12	chr5:63303378-63303400	86	87	588	53
13	chr5:63303413-63303435	91	92	545	50
14	chr5:63306108-63306130	103	104	480	49
15	chr5:63306144-63306166	108	109	344	21
16	chr5:63306147-63306169	110	111	451	44
17	chr5:63306251-63306273	116	117	1343	65
18	chr5:63306336-63306358	125	126	718	66
19	chr5:63306371-63306393	130	131	1310	131
20	chr5:63309035-63309057	134	135	690	83
21	chr5:63309077-63309099	138	139	949	95
22	chr5:63309084-63309106	140	141	1234	99
23	chr5:63309273-63309295	164	165	418	47
24	chr5:63309308-63309330	170	171	747	95
25	chr5:63309857-63309879	174	175	795	47
26	chr5:63309860-63309882	176	177	736	94
27	chr5:63309886-63309908	179	180	1829	124
28	chr5:63309889-63309911	182	183	1575	94
29	chr5:63309933-63309955	187	188	871	95
30	chr5:63310098-63310120	203	204	533	73
31	chr5:63310100-63310122	206	207	537	53

[0109] One of the factors that contributes to cutting at these off-target sites can be whether the gene editing components are delivered as a DNA vector or as an RNA-protein complex; sites that may have as few as 1 or 2 base mismatches can be faithfully discriminated from the intended target site when the Cas9 and guide RNA can be delivered as a single guide ribonucleotide protein complex (sgRNP) rather than delivered on a DNA vector.

[0110] A second consideration to maximize specificity of a Cas9 guide-RNA reagent can be to prescreen guide RNA-protein pairs using *in vitro* biochemical methods. Several laboratories have published methods to screen for off-target edit sites. These biochemical approaches can identify potential off-target Cas9 cleavage sites in purified

genomic DNA. Using these assays, genomic DNA can be digested with a range of sgRNP concentrations, from limiting to saturating, thus permitting the recovery of both high- and low-cleavage-sensitivity off-target sites. *See, e.g.,* Cameron *et al.*, "SITE-SEQ®: A Genome-wide Method to Measure Cas9 Cleavage," Protocol Exchange (2017).

[0111] The off-target sites identified by off-target screening can be used to guide careful and comprehensive examination of possible off-target sites in cells, measuring both editing frequency and functional cellular consequence. Several selected guide RNA-Cas protein pairs can be screened, and the screening can demonstrate: 1) efficient cutting nearer to the 5' end of the CD163 gene, and 2) few mismatch sequences as determined by bioinformatic methods.

[0112] Using this guide selection criteria, several guide RNA-Cas protein pairs can be selected for cutting activity in porcine parthenotes. Porcine oocytes can be doubled using an electric current, injected with guide RNA-Cas protein, allowed to develop for 7 days, then harvested and DNA sequenced across the intended target site. Several guide RNA-Cas protein pairs can be identified to confer edits at a high frequency in parthenotes and are selected for further development.

[0113] The present specification provides for, and includes, methods for identifying and selecting optimal target sites for CRISPR/Cas mediated cleavage and gene editing. In an aspect, the method can comprise identifying a target region of a genome for editing; identifying all 20 nucleotide sequences in the target region; performing a bioinformatic screen to identify and remove sequences that match non-target sites in the pig genome that contain 1 to 4 mismatches and have a suitable PAM sequence based on the Cas protein; preparing CRISPR/Cas RNP complexes comprising a guide RNA backbone and target sequences, introducing the CRISPR/Cas RNP complexes into a porcine cell in culture, and determining the average editing frequency of the guide RNA/Cas combination. In an aspect, determining the average editing frequency of the guide RNA/Cas combination can comprise amplifying, by PCR, a region surrounding the target site, performing amplicon deep sequencing, and comparing it to untreated cells. In an aspect, the method provides for selecting preferred target sequences that can have an average editing frequency of at least 15. Also provided for are methods for identifying and selecting optimal target sites for CRISPR/Cas mediated cleavage and gene editing wherein the guide RNA and Cas proteins can be provided as part of an expression vector or vectors. Suitable cells are known to persons of skill in the

art and can include, but are not limited to, primary fetal fibroblasts of an intended porcine breeding line.

[0114] Multiple repair templates can be designed to generate in-frame stop codons. The repair templates listed, when used with appropriate guide RNAs in an endonuclease system, can generate an in-frame termination codon (TAA, TGA, TAG) after repair. Different lengths of repair templates can be used, having sequence identity on either side of the edit site. In an aspect, a repair template can share at least 15 nucleotides on either side of the CRISPR/Cas endonuclease cleavage site (*e.g.*, 15~15 nucleotides). In other aspects, repair templates can share more than 100 nucleotides on either side of the edit site (e.g., >100~>100). Repair templates can be single, double, or staggered strands of complementary DNA using overhangs on the ends. In addition, these templates are not limited to DNA but also could be RNA or modified nucleotides (inosine, for example) or a mixture of these bases and have 5' and or 3' ends protected from degradation. Because there are exonucleases that cleave nucleotides from the ends, protecting the ends with modified bases can prevent exonuclease digestion in the cell of both DNA and RNA. Not to be limited by theory, increasing the length of the region of identity on both sides of the edit site can increase the efficiency and specificity of the repair process (*e.g.*, homology arms). Included and provided for by the present specification are repair templates that can have 100% identity over at least 25 nucleotides on both sides of the edit site. This core identity region ensures that the desired edit (for example, deletions for SEQ ID NOs: 1 to 13) is accurate and efficient. In an aspect, the core identity region can be at least 40 nucleotides flanking the edit site. In yet another aspect, the core identity region can be at least 50 nucleotides on both sides of the edit site. In an aspect, the flanking core region can comprise 60 nucleotides of 100% identity. Also included in an aspect are repair templates that can have 70 identical nucleotides to the chromosomal region flanking the edit site. In an aspect, the core identity region can comprise 75 nucleotides of identity to the chromosomal region flanking the edit site. In an aspect, the core identity region can comprise 25 to 40 flanking nucleotides. In a further aspect, the core identity region can comprise 40 to 75 flanking nucleotides.

[0115] In some aspects, the core identity region can be further flanked by additional regions of homology to the target site (the "flanking homology regions"). As provided herein, in an aspect, the flanking homology regions can comprise 100% identity to the target site. As examples, SEQ ID NOs: 1 to 13 shared 100% homology to the

CD163 region on either side of the targeted edit site as found in lines 2, 3, 15, 19, 27, 62, or 65 (*e.g.*, 100% homology in the core region and the flanking homology regions). Also included are repair templates that can comprise SEQ ID NOs: 1 to 13, wherein the core identity region can comprise 100% identity to 25 nucleotides of the genome on either side of the desired edit (*e.g.*, bases 50 to 100) and can be flanked by at least 80% identity to the genome on either side of the core region (*e.g.*, nucleotides 1 to 49 and 101 to 150 of SEQ ID NOs: 1 to 18). In another aspect, the core region can have 100% homology and the flanking homology regions can share 85% homology. In a further aspect, the core region can have 100% homology and the flanking homology regions can share 90% homology. In yet another aspect, the core region can have 100% homology and the flanking homology regions can share 95% homology. Also included in an aspect are repair templates that can have a core region that has 100% homology and the flanking homology regions share 97% homology. In some aspects, the flanking homology regions can share 99% homology. Not to be limited by theory, it is thought that increasing the length of the flanking homology regions can introduce the desired CD163 edits into related animals without further modifying the genome. That is, specific repair sequences incorporating polymorphisms or changes in the CD163 genome of other pigs are specifically included and provided for.

[0116] The present specification includes, and provides for, flanking homology regions that can have greater than 50 nucleotides on each side of the edit site. In an aspect, the flanking homology regions can have greater than 75 nucleotides on each side of the edit site. In an aspect, the flanking homology regions can have greater than 100 nucleotides on each side of the edit site. Also included are flanking homology regions that can have greater than 200 nucleotides on each side of the edit site. In aspects, the flanking homology regions can have between 30 and 1000 nucleotides on each side of the edit site.

[0117] The present specification includes, and provides for, additional modifications at the 5' and 3' ends of repair template. Repair templates are not limited to DNA but also could be RNA or modified nucleotides (inosine, for example) or a mixture of these bases and can have 5' and or 3' ends protected from degradation. Because there are exonucleases that cleave nucleotides from the ends, protecting the ends with modified bases can prevent exonuclease digestion in the cell of both DNA and RNA.

Accordingly, as provided herein, the 5' and 3' ends of a repair template comprising a flanking homology region can be modified to prevent degradation.

[0118] The present specification provides for, and includes, ribonucleoprotein (RNP) complexes that can comprise a guide polynucleotide and a Cas protein. In an aspect, the Cas protein can be a *S. thermophilus* Cas9. In another aspect, the Cas protein can be Cas9 from *S. pyogenes*. Other suitable Cas proteins are known in the art and exemplary Cas systems are described above in Table 1. The selection of suitable Cas proteins depends on the required PAM sequence combination, and methods of identifying Cas proteins and modifying Pam sequences are known. Similarly, Cas systems differ in their requirements for guide RNA backbone sequences (*e.g.*, tracrRNA sequences). In an aspect, the RNP complex can comprise a Cas protein and a guide nucleotide having at least 98% sequence identity to an RNA sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. In another aspect, the RNP complex can comprise a guide RNA comprising a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. In an aspect, the RNP complex can comprise a guide polynucleotide having 99% identity to a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. In an aspect, the guide polynucleotide of the RNP complex can comprise a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and 347 to 425. As provided herein, the RNP complex can comprise the sequences of any one of SEQ ID NOs: 22 to 271 and 347 to 425 combined with an RNA backbone as part of an sgRNA. In an aspect, the RNP complex can be pre-formed prior to injection into a target cell or can be injected or introduced separately.

[0119] Also provided for, and included, in the present specification are isolated guide RNAs that can comprise a spacer selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 and the first 20 nucleotides of each of SEQ ID NOs: 347 to 425. In an aspect, the Cas protein can be a protein comprising SEQ ID NOs: 20 or 21.

[0120] The present specification also includes, and provides for, DNA vectors that can encode guide RNAs for the preparation of CRISPR/Cas RNPs. In general, a vector encoding a guide RNA can comprise a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 or SEQ ID NOs:

347 to 425 and a guide RNA backbone arranged in cis and as part of a single transcription unit. Upon expression in a suitable cell, the DNA vector can produce an sgRNA of the present specification. Suitable expression vector backbones, including promoters, selectable markers and replication origins, are well known to persons of skill in the art. In practice, the 20 nucleotides and the guide RNA backbone can be DNA (when expressed from a promoter to be transcribed in vivo (in cells) or in vitro (via T7 polymerase) to form an RNA guide or the backbone can be chemically synthesized dual (crRNA and trRNA) guide or single guide RNA.

[0121] Specific guideRNAs can be paired to excise parts of the CD163 gene by using paired guideRNAs to delete sections of the gene located between those guides. Exemplary pairs of guideRNAs (listed as DNA sequences) are provided in Table 5. Guide pairs without an exon 7 amino acid sequence listed remove the entire exon. The complete amino acid sequence for these deletions is set forth in SEQ ID NO: 553. All guides in Table 5 can create the desired sequence by excising the DNA in between the two guides and NHEJ repair between the cut sites without the use of a DNA repair template. The guide pairs that result in the amino acid sequences set forth in SEQ ID NOs. 506-517 can introduce an exogenous stop codon across the cut ends of the DNA introduced by the guide.

Table 5 Exemplary Guide RNA Pairs

SEQ ID NO: (5')	Cut site (5')	SEQ ID NO: (3')	Cut site (3')	Deletion size (bp)	Repaired Sequence SEQ ID	Exon 7AA SEQ ID
229	63322816	256	63323377	561	426	N/A
230	63322814	256	63323377	562	427	N/A
231	63322826	256	63323377	551	428	N/A
237	63322861	256	63323377	516	429	N/A
241	63322891	256	63323377	486	430	N/A
229	63322816	258	63323378	562	431	N/A
230	63322814	258	63323378	563	432	N/A
231	63322826	258	63323378	552	433	N/A
237	63322861	258	63323378	517	434	N/A
241	63322891	258	63323378	487	435	N/A
229	63322816	261	63323373	558	436	N/A
230	63322814	261	63323373	559	437	N/A
231	63322826	261	63323373	548	438	N/A
237	63322861	261	63323373	513	439	N/A

241	63322891	261	63323373	483	440	N/A
219	63322697	256	63323377	680	441	N/A
221	63322709	256	63323377	668	442	N/A
224	63322747	256	63323377	630	443	N/A
227	63322786	256	63323377	591	444	N/A
219	63322697	258	63323378	681	445	N/A
221	63322709	258	63323378	669	446	N/A
224	63322747	258	63323378	631	447	N/A
227	63322786	258	63323378	592	448	N/A
219	63322697	261	63323373	677	449	N/A
221	63322709	261	63323373	665	450	N/A
224	63322747	261	63323373	627	451	N/A
227	63322786	261	63323373	588	452	N/A
249	63322963	256	63323377	414	453	N/A
250	63322973	256	63323377	404	454	N/A
249	63322963	258	63323378	415	455	N/A
250	63322973	258	63323378	405	456	N/A
249	63322963	261	63323373	411	457	N/A
250	63322973	261	63323373	401	458	N/A
351	63323023	365	63323103	80	459	506
351	63323023	387	63323235	212	460	506
348	63323027	390	63323236	209	461	507
348	63323027	388	63323236	209	462	507
348	63323027	395	63323284	257	463	507
352	63323035	365	63323103	68	464	508
352	63323035	387	63323235	200	465	508
352	63323035	399	63323283	248	466	508
353	63323038	365	63323103	65	467	509
353	63323038	387	63323235	197	468	509
353	63323038	399	63323283	245	469	509
354	63323039	390	63323236	197	470	509
354	63323039	388	63323236	197	471	509
354	63323039	395	63323284	245	472	509
358	63323056	361	63323077	21	473	510
358	63323056	362	63323087	31	474	510
358	63323056	368	63323124	68	475	510
358	63323056	384	63323203	147	476	510
358	63323056	394	63323272	216	477	510
358	63323056	399	63323283	227	478	511
359	63323057	390	63323236	179	479	511
359	63323057	388	63323236	179	480	511

359	63323057	395	63323284	227	481	511
360	63323058	368	63323124	66	482	511
360	63323058	384	63323203	145	483	511
360	63323058	389	63323237	179	484	511
360	63323058	394	63323272	214	485	511
360	63323058	397	63323285	227	486	511
361	63323077	365	63323103	26	487	512
361	63323077	387	63323235	158	488	512
362	63323087	390	63323236	149	489	513
362	63323087	388	63323236	149	490	512
362	63323087	395	63323284	197	491	513
364	63323089	365	63323103	14	492	514
364	63323089	387	63323235	146	493	514
364	63323089	399	63323283	194	494	514
365	63323103	368	63323124	21	495	515
365	63323103	384	63323203	100	496	516
365	63323103	389	63323237	134	497	516
365	63323103	394	63323272	169	498	516
365	63323103	397	63323285	182	499	516
366	63323118	368	63323124	6	500	517
366	63323118	384	63323203	85	501	517
366	63323118	389	63323237	119	502	517
366	63323118	394	63323272	154	503	517
366	63323118	397	63323285	167	504	517
354	63323039	211	63323163	123	505	518

[0122] In aspects, the guide RNA-Cas protein pairs can further comprise a DNA repair template. Exemplary guides and repair templates creating stop codons are listed in Table 6. These and other guides can be paired with other repair templates to generate in-frame stop codons or perturb, interfere, or eliminate splicing of exons, as examples of disrupting CD163 mRNA translation or processing. Other examples can include, but are not limited to, the elimination of the start ATG codon, or programming repair outcomes when using paired guide RNAs where a repair template could promote a single repair outcome over random non-homologous end joining when paired nucleases remove an exon (*e.g.*, deletion of exon 7 corresponding to domain 5) in CD163. Without being limited by theory, analysis reveals consistent reduction of bioinformatic mismatches with inclusion of the extra nG in the PAM (nGGnG) as well as reduced off-target cutting *in vitro* and *in vivo*. Reduction of mismatches can include those from an alignment

perspective (whether the DNA sequence has mismatches with the RNA guide sequence). *See* Table 4. A mismatch may not be an off-target edit (cutting or presence of indel, *in vitro* or *in vivo*, respectively), but an off-target edit is likely due to a mismatch. In the most preferred aspects, the methods disclosed herein produce no off-target edits in pigs, and the pigs disclosed herein have no off-target edits in their genomes.

Table 6: Guide RNAs and repair templates for editing Exon 2 of CD163

Table 6: Guide RNAs and repair templates for editing CD163

Region Chr5 Location of guideRNAs binding site	63301998 - 02017	63301997- 02016	63303213- 03232	63303283 - 03302	63303315- 03334	63303315- 03334	63303338- 03357	63303379- 03398	63306148 - 06167	63306263- 06282	63306364- 06383	63323147- 23166
Repair temp-late SEQ ID NO	1	2	3	4	5	6	7	8	9	10	11	12	13
Guide RNA spacer (DNA) SEQ ID NO	274	275	276	277	278	279	280	281	282	283	284	285	286
Guide RNA spacer (RNA) SEQ ID NO	287	288	289	290	291	292	293	294	295	296	297	298	299
WT Region SEQ ID NO.	300	301	302	303	304	305	306	307	308	309	310	311	312
Trans-lation of WT region	313	314	315	316	317	318	319	320	321	322	323	324	325
Translation of Deletion	326	327	328	329	330	331	332	333	334	335	336	337	338
Base deletion coordinates	chr5: 6330 1999-6330 2005	chr5: 6330 1999-6330 2005	chr5: 6330 3234-6330 3235	chr5: 6330 3304-6330 3307	chr5: 6330 3336-6330 3336	chr5: 6330 3337-6330 3337	chr5: 6330 3351-6330 3356	chr5: 6330 3399-6330 3401	chr5: 6330 3400-6330 3402	chr5:6 33061 56-63306 158	chr5: 6330 6282-6330 6284	chr5: 6330 6373-6330 6377	chr5: 6332 3159-6332 3162
Deleted bases (#)	CTT GGT C (7)	CTT GGT C (7)	GG (2)	GGC T (4)	G (1)	G (1)	CTT GTC (6)	GGG (3)	GGG (3)	TTC (3)	CTC (3)	CGA TC (5)	CGG C (4)

[0123] The stochastic natural cellular repair process of non-homologous end joining (NHEJ) repair sometimes involves inserting or deleting single or multiple nucleotides at the double-strand break. As a consequence of this repair process, if a double-strand break occurs in the coding region of a gene, a shift in the translational reading frame of the encoded mRNA can result until an in-frame stop codon, terminating protein translation. Although the translation of this naturally, accidentally mutated gene can produce a shortened protein relative to the protein product of an unmodified version, the amino acid sequence of this newly encoded polypeptide would be unique, possibly reducing or even improving the fitness of cells within the target organism. In an aspect, to preclude the creation of frame shifting mutations and the translation of this set of undesirable polypeptides, edited pigs can be screened for formation of an in-frame translational stop codon at or near the endonuclease cut site that is a consequence of a separate, naturally occurring NHEJ repair.

[0124] The excision of genomic DNA sequence using two intronic guideRNAs in conjunction with an endonuclease can be accomplished by NHEJ repairs that include, but are not limited to, the end-to-end joining of nuclease cut sites. Because introns are non-coding, NHEJ repair outcomes that include indels around the nuclease cut sites can accomplish the excision of the intended region of DNA which can include intron and/or exon sequences. Because some NHEJ repair outcomes occur more frequently than others, it is advantageous to consider repair outcome frequency when choosing a guideRNA pair for use in gene editing experiments. SEQ ID NO: 520-555 illustrate repair outcomes of exon 7 excisions observed in blastocysts from guide pairs as set forth in Table 7. The designed repair outcome for these guide pairs is listed in Table 5.

Table 7 Observed Repair Outcomes in Blastocysts

SEQ ID NO: (5')	SEQ ID NO: (3')	Repair outcome SEQ ID
249	261	520
249	261	521
249	261	522
249	261	523
249	261	524
249	261	525
249	261	526
249	261	527
249	256	528

249	256	529
249	256	530
249	256	531
249	256	532
249	256	533
249	256	534
249	256	535
241	258	536
241	258	537
241	258	538
241	258	539
241	258	540
241	258	541
241	258	542
241	258	543
241	258	544
221	261	545
221	261	546
221	261	547
221	261	548
221	261	549
229	256	550
229	256	551
229	256	552
229	256	553
229	256	554
229	256	555

[0125] In another aspect, during gene editing, in-frame stop codons that do not result in the addition of new amino acids can be created by including a DNA repair template together with the guide RNA-Cas protein pair. In an aspect, a DNA repair template can be a dsDNA. In another aspect, the DNA repair template can be a ssDNA. Co-introduction of a double- or single-stranded DNA repair template can be used to either delete or insert DNA nucleotides to form an in-frame translational stop codon (TAA, TGA, TAG) at or near the double-strand break site initiated by the endonuclease. DNA repair templates can further comprise polynucleotide modification templates containing several nucleotide changes in comparison to the native sequence, which can directly edit the target DNA sequence and can be co-transfected with the endonuclease editing reagents to generate edited CD163 genes with in-frame stop codons. In an aspect,

the encoded proteins from these gene edited CD163 genes, when transcribed into mRNA and then translated into protein, can only synthesize a shortened and non-functional form of the CD163 polypeptide. Not to be limited by theory, DNA repair templates having longer regions of sequence homology can be more efficient. In certain embodiments, DNA repair templates can contain regions of sequence identity (homology arms) within the DNA repair template which can flank the sequence change and can range from fewer than 50 nucleotides to greater than 1000 nucleotides.

[0126] Several aspects of Cas9-protein/guide RNA combinations together with DNA repair templates are shown in Table 6. As provided in Table 6, several Cas9-protein/guide RNA combinations can be paired with DNA repair templates having, but not being limited to, 75 bases of sequence homology on each side of the targeted deletion (50 core homology bases and flanking homology regions of 50 on either side). In an aspect, the protein-guide RNA complexes and template combinations can be transfected into cells. Without being limited by theory, repair of the double-strand break using the DNA repair template can direct the formation of an in-frame translational stop codon as the result of deletion of single or multiple nucleotides at or adjacent to the break site. Animals and cells obtained from this method demonstrate that endonuclease-directed double-strand breaks at the porcine CD163 gene can be repaired using a co-introduced DNA repair template. As provided herein, repair templates having a sequence of SEQ ID NOs: 1 to 13 can introduce an in-frame translational stop codon in Exon 2, thereby producing a shortened and non-functional CD163 protein.

[0127] Use of a DNA repair template according to the present specification is not limited to abolishing the function of CD163. The present specification further includes, and provides for, a repair template that can direct the removal or addition of nucleotides to the gene. In an aspect, the stability or half-life modulation of the encoded CD163 mRNA can be modulated by editing according to the present methods. In yet another aspect, DNA sequences which encode amino acids of the mature protein responsible for binding PRRS virus can be removed or replaced. In an aspect, CD163 expression and or activity can be reduced by at least 90% but not abolished. In an aspect of the present teachings, using the spacer sequences described with other repair templates would be contemplated by those skilled in the art to allow for the formation of an in frame stop codon by the removal of bases and the introduction of bases or a combination thereof.

[0128] Included, and provided for, by the present specification are pigs derived from elite porcine lines comprising edited CD163 genes. In aspects, the elite porcine lines can be PIC™ Line 15, PIC™ Line 17, PIC™ Line 27, PIC™ Line 65, PIC™ Line 14, PIC™ Line 62, PIC337, PIC800, PIC280, PIC327, PIC408, PIC™ 399, PIC410, PIC415, PIC359, PIC380, PIC837, PIC260, PIC265, PIC210, PIC™ Line 2, PIC™ Line 3, PIC™ Line 4, PIC™ Line 5, PIC™ Line 18, PIC™ Line 19, PIC™ Line 92, PIC95, PIC™ CAMBOROUGH® (Pig Improvement Company, Limited, Basingstoke, UK), PIC1070, PIC™ CAMBOROUGH® 40, PIC™ CAMBOROUGH® 22, PIC1050, PIC™ CAMBOROUGH® 29, PIC™ CAMBOROUGH® 48, or PIC™ CAMBOROUGH® x54. In various aspects, the elite porcine lines can be PIC™ elite porcine lines 2, 3, 15, 19, 27, 62, or 65. In another aspect, the pigs can comprise edited CD163 genes derived from elite porcine lines. In aspects, the pigs can comprise an edited CD163 in a CD163 genomic region having the genotypes shown in Table 8 to Table 14. Table 8 to Table 14 present each position on chromosome 5 in the vicinity of the CD163 gene in which a single nucleotide polymorphism exists.

[0129] Each table presents alleles that are homozygous in that line and provides a distinguishing genetic signature of the line, as well as its unique genome edited region. All the genetic signatures are based on the Sscrofa11.1 reference genome (GenBank accession: GCA_000003025.6). Accordingly, the transmission of the edited CD163 line can be followed, and animals comprising an edited CD163 gene obtained from the line can be identified. Pigs of other lines are heterozygous or opposite genotype at multiple alleles when compared to the lines of Table 8 to Table 14. In this way, the combination of genetic signatures in each line can be used to distinguish between pigs belonging to a particular line and pigs not belonging to a particular line. In an aspect, the genetic signatures provide for methods to breed and track the CD163 edited genomes from generation to generation. In an aspect, progeny generations that comprise the CD163 edited genomes having a genetic signature according to Table 8 to Table 14 can be prepared.

Table 8: Line 2 Genetic Signatures

Position	***Genotype***	***Position***	***Genotype***	***Position***	***Genotype***	***Position***	***Genotype***
60306428	C/C	60354925	C/C	63553656	G/G	65411831	C/C
60306527	C/C	60355415	G/G	63558087	G/G	65411848	G/G
60308030	C/C	60355420	T/T	63640722	G/G	65411854	T/T

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60320580	C/C	60355448	G/G	63792071	T/T	65411966	C/C
60322529	A/A	60355529	A/A	64009226	C/C	65412729	G/G
60324162	T/T	60355530	A/A	64459105	T/T	65413215	G/G
60327987	C/C	60355774	C/C	64460101	A/A	65413759	C/C
60328009	T/T	60356277	C/C	64527707	A/A	65417199	A/A
60335421	T/T	60356351	C/C	64577968	C/C	65417256	G/G
60338654	G/G	60356575	C/C	64943306	T/T	65417261	A/A
60344946	A/A	60356578	T/T	65242696	T/T	65417273	C/C
60345163	C/C	60356861	G/G	65242725	A/A	65417287	G/G
60345689	G/G	60356885	T/T	65242729	A/A	65417716	G/G
60345715	G/G	60356898	T/T	65242736	G/G	65417797	C/C
60345722	A/A	60356914	C/C	65249484	T/T	65417800	C/C
60345749	G/G	60357001	T/T	65260283	T/T	65419169	G/G
60345775	A/A	60357014	T/T	65269510	C/C	65419410	G/G
60345825	A/A	60358409	C/C	65273886	T/T	65420017	T/T
60346409	G/G	60358449	G/G	65276133	C/C	65420184	G/G
60346607	A/A	60358469	T/T	65277053	T/T	65420415	A/A
60346641	G/G	60358475	T/T	65277156	C/C	65420591	G/G
60346691	T/T	60358551	A/A	65277320	G/G	65420680	C/C
60346734	A/A	60358568	G/G	65280282	A/A	65420681	A/A
60347297	G/G	60358641	T/T	65282683	C/C	65420693	G/G
60350295	C/C	60358704	T/T	65283434	C/C	65420947	G/G
60350343	G/G	60358774	T/T	65283970	C/C	65420949	T/T
60350448	C/C	60368913	C/C	65284829	C/C	65421133	G/G
60350470	T/T	60368916	A/A	65285100	C/C	65421195	G/G
60350475	C/C	60368921	T/T	65285902	G/G	65421255	G/G
60350564	A/A	60368940	G/G	65285914	T/T	65421365	G/G
60350571	A/A	60368963	C/C	65286400	C/C	65421421	C/C
60350572	A/A	60416097	G/G	65294497	T/T	65422005	G/G
60350911	C/C	60543479	C/C	65383805	C/C	65422008	G/G
60351055	G/G	60614797	G/G	65405986	C/C	65422057	T/T
60351604	C/C	60614964	G/G	65410118	C/C	65422141	C/C
60351855	T/T	60920926	G/G	65410147	G/G	65422701	C/C
60351857	C/C	61479206	C/C	65410198	C/C	65422725	C/C
60351972	G/G	61479353	T/T	65410435	C/C	65425730	G/G
60352165	C/C	61653091	C/C	65410440	T/T	65601310	C/C
60352923	C/C	61783688	A/A	65410441	C/C	65602328	C/C
60353408	A/A	62091204	A/A	65410443	C/C	65711602	C/C
60353562	T/T	62416522	A/A	65410447	C/C	65761990	C/C
60353576	C/C	62476273	C/C	65411265	C/C	66293766	G/G
60353659	T/T	62687883	C/C	65411431	G/G	66296268	A/A
60353721	G/G	63044336	A/A	65411524	C/C		
60354428	G/G	63546615	C/C	65411698	G/G		

Table 9: Line 3 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60414864	T/T	64555715	G/G	64774093	G/G	66035868	A/A
60415813	C/C	64561148	G/G	64775512	G/G	66042409	C/C
60415854	G/G	64561209	G/G	64775565	C/C	66042542	C/C
60572371	A/A	64561659	G/G	64780089	C/C	66042551	C/C
60663464	C/C	64561828	T/T	64780152	C/C	66042557	A/A
60663499	A/A	64561873	G/G	64780196	C/C	66042563	G/G
61016527	G/G	64561935	C/C	64783169	G/G	66155827	A/A
61442674	T/T	64562021	C/C	64783774	C/C	66155859	A/A
61870484	C/C	64562771	C/C	64783896	G/G	66155863	T/T
61989476	G/G	64563462	G/G	64784000	T/T	66156160	A/A
62510031	G/G	64682243	C/C	64784139	C/C	66156183	G/G
62602514	T/T	64682929	G/G	64784235	T/T	66156533	G/G
63044336	A/A	64685072	T/T	64784410	A/A	66167582	C/C
63345577	G/G	64692673	G/G	64784653	A/A	66167809	C/C
63452493	A/A	64692905	T/T	64784725	C/C	66167823	T/T
63669675	G/G	64693058	T/T	64784844	A/A	66167877	A/A
63754659	T/T	64705228	G/G	64784875	T/T	66167888	C/C
63810627	C/C	64720067	A/A	64785001	A/A	66167905	A/A
63810632	G/G	64722426	T/T	64785078	C/C	66168135	C/C
63810707	T/T	64722539	C/C	64813032	G/G	66168261	C/C
63810733	G/G	64726936	G/G	64819974	C/C	66168311	G/G
63810819	C/C	64729340	C/C	64893099	G/G	66168615	G/G
63810857	C/C	64730596	G/G	64899922	A/A	66168688	G/G
63810861	G/G	64736752	G/G	64934758	C/C	66168742	G/G
64010555	T/T	64761650	G/G	65348500	G/G	66168880	G/G
64010599	A/A	64767369	G/G	65546268	A/A	66168947	T/T
64452338	T/T	64768411	G/G	65546327	G/G	66168952	A/A
64455982	C/C	64769978	T/T	65841270	T/T	66168993	A/A
64457532	T/T	64769989	C/C	65858531	G/G		
64458163	T/T	64770307	G/G	65979134	G/G		
64458711	G/G	64773053	A/A	65985520	C/C		

Table 10: Line 15 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60391943	G/G	61515967	T/T	63367266	G/G	64537982	T/T
60392234	T/T	61759785	C/C	63424311	T/T	64893205	A/A
60394009	A/A	61759821	C/C	63535058	T/T	64916422	G/G
60414796	T/T	61865586	A/A	63653754	G/G	65039734	C/C
60601475	A/A	61867498	C/C	63655538	T/T	65175496	C/C
60630898	A/A	62192420	G/G	63669242	A/A	65177506	G/G
60630910	A/A	62195570	A/A	63669944	C/C	65294016	C/C
60786421	G/G	62196101	C/C	63669946	G/G	65571397	A/A
60787517	G/G	62442547	G/G	63900332	A/A	65573608	T/T
60792231	G/G	62509196	T/T	64138082	C/C	65576707	C/C
60795724	C/C	62675268	G/G	64171653	C/C	65984917	G/G
60915104	C/C	62843969	G/G	64471845	G/G	65984984	T/T
61016227	A/A	62852334	A/A	64472363	A/A	65985058	T/T
61086145	T/T	63025151	A/A	64472504	T/T	65985392	C/C
61275101	C/C	63025152	C/C	64472616	T/T	66042472	C/C
61396087	A/A	63168616	A/A	64523343	A/A	66099282	A/A
61434042	G/G	63266682	T/T	64536599	G/G	66119075	T/T

Table 11: Line 19 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60320100	A/A	61390748	C/C	63174233	T/T	65031621	C/C
60320144	G/G	61465215	T/T	63174257	A/A	65031630	C/C
60320580	C/C	61567329	C/C	63393845	G/G	65031684	T/T
60507836	C/C	61567357	A/A	63393849	T/T	65222383	T/T
60512716	G/G	61567365	A/A	63393851	T/T	65222385	A/A
60564418	A/A	61567410	C/C	63860532	A/A	65242693	C/C
60666662	T/T	61567494	C/C	63861247	A/A	65497229	G/G
60683738	T/T	61567594	G/G	63862368	T/T	65499233	C/C
60782197	T/T	61975156	G/G	64138082	C/C	65502398	G/G
60782278	T/T	61975367	G/G	64171653	C/C	65562917	C/C
60782642	A/A	61975382	A/A	64181448	G/G	65566225	T/T
60782683	G/G	61975388	C/C	64402245	T/T	65567066	T/T
60782756	G/G	62363513	T/T	64455172	G/G	65894449	C/C
60782997	G/G	62367647	G/G	64514012	C/C	65904537	T/T
60783031	C/C	62378299	G/G	64514013	A/A	65959573	A/A
60783198	G/G	62610431	T/T	64522169	G/G	65998062	C/C
61131519	G/G	62952267	T/T	64522173	T/T	66158553	A/A
61131736	C/C	62965473	T/T	64522177	T/T	66158950	C/C
61189029	C/C	63108711	G/G	64522178	C/C	66159103	A/A
61359517	A/A	63109224	T/T	64794635	G/G	66159212	G/G

Table 12: Line 27 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60347934	A/A	62159353	T/T	62918655	T/T	64582272	A/A
60356984	C/C	62313847	C/C	63042154	C/C	64600735	G/G
60692943	C/C	62315464	A/A	63043261	G/G	65056396	A/A
60693260	G/G	62570257	C/C	63046692	G/G	65056671	T/T
60693769	G/G	62570793	A/A	63433705	G/G	65332987	A/A
60938885	T/T	62571313	T/T	63490990	A/A	65335837	C/C
60960728	A/A	62917536	G/G	63626018	A/A	65390844	A/A
61436306	G/G	62917597	T/T	63626423	T/T	65404042	A/A
61464114	C/C	62917618	C/C	63626826	C/C	65404045	A/A
61468375	G/G	62917629	T/T	63630149	A/A	65404063	C/C
61736415	A/A	62917780	G/G	63631098	C/C	65705261	G/G
61736429	G/G	62917866	T/T	63977777	A/A	65740018	T/T
61757961	G/G	62917889	T/T	63977821	G/G	65740030	A/A
61820110	A/A	62918448	G/G	64460204	C/C	66235840	G/G
61822513	T/T	62918456	A/A	64582243	C/C		
61858837	G/G	62918465	A/A	64582246	G/G		

Table 13: Line 62 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60336383	A/A	61760230	C/C	62483897	T/T	64484177	T/T
60343172	A/A	61927492	T/T	62847072	G/G	64689271	A/A
60345254	G/G	61927506	A/A	62874766	G/G	64758235	C/C
60447765	G/G	62006220	T/T	62897778	A/A	64790188	C/C
60452143	C/C	62115641	T/T	63100290	C/C	64895383	C/C
60452167	T/T	62169134	T/T	63219344	G/G	64991645	A/A
60462769	T/T	62368918	C/C	63361759	G/G	65045805	T/T
60653700	T/T	62471593	C/C	63388357	C/C	65146186	C/C
60654939	A/A	62474001	A/A	63442314	G/G	65368620	C/C
60654943	A/A	62474006	C/C	63590333	T/T	65432698	C/C
61085770	A/A	62474365	A/A	63670724	G/G	65542716	A/A
61108831	G/G	62480042	A/A	63714834	A/A	65637507	G/G
61117357	A/A	62480261	G/G	63792005	T/T	65771614	C/C
61234908	C/C	62480699	A/A	63985848	A/A	65771615	G/G
61331270	A/A	62480892	A/A	63985923	G/G	65772162	T/T
61388824	G/G	62481902	G/G	64131088	C/C	66033248	T/T
61755575	C/C	62482914	T/T	64471400	C/C	66033476	C/C
61759095	C/C	62483893	C/C	64483848	G/G	66033484	C/C

Table 14: Line 65 Genetic Signatures

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60322300	C/C	60783031	C/C	63383925	A/A	64759492	T/T

Position	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*	*Position*	*Genotype*
60411747	A/A	60783198	G/G	63392495	T/T	64795760	T/T
60412758	G/G	60791719	T/T	63549670	C/C	64797970	A/A
60412775	A/A	60900261	G/G	63593491	G/G	64798690	G/G
60412826	C/C	60911551	A/A	63609938	A/A	64860395	G/G
60413294	G/G	60912669	A/A	63641998	C/C	64860396	C/C
60459719	A/A	61223574	A/A	63678793	G/G	64862131	C/C
60462179	C/C	61486607	C/C	63784770	G/G	65371202	T/T
60492928	A/A	61756324	G/G	63810897	C/C	65371472	G/G
60492938	T/T	61832527	C/C	63810903	C/C	65371901	G/G
60666662	T/T	61895566	C/C	63810920	G/G	65371970	C/C
60722708	C/C	61895833	A/A	63810922	T/T	65372072	A/A
60770183	G/G	62354225	C/C	63810928	G/G	65409493	A/A
60770197	C/C	62364072	G/G	63872757	C/C	65437347	G/G
60777431	C/C	62383525	G/G	63944636	G/G	65573362	T/T
60781592	T/T	62399452	T/T	63944765	C/C	65601655	G/G
60782197	T/T	62404734	A/A	64138639	C/C	65747069	T/T
60782278	T/T	62432982	C/C	64194054	G/G	65843843	A/A
60782642	A/A	62841388	G/G	64242768	T/T	65849424	T/T
60782683	G/G	62931496	T/T	64270989	G/G	66248756	A/A
60782756	G/G	63156406	C/C	64753488	G/G		
60782997	G/G	63156417	T/T	64758888	A/A		
60783006	G/G	63266198	A/A	64758900	C/C		

[0130] Elite PIC™ lines 2, 3, 15, 19, 27, 62 and 65 are lines selected for superior commercial phenotypes. In an aspect, the CD163 gene edited cells and animals can be free of deleterious alleles that are present in wild populations and in many commercial herds. In aspects, CD163 gene edited cells and animals can be free of one of more of the deleterious alleles selected from the group consisting of epetheliogenesis imperfecta, melanotic skin tumors, dermatosis vegetans, abnormal mamae, shortened vertebral column, kinky tail, rudimentary tail, Hairlessness, Hairlessness (2), Woolly hair, Hydrocephalus, Tassels, Legless, Three-legged, Syndactyly, Polydactyly, Pulawska factor, Heterochromia iridis, Congenital tremor A III, Congenital tremor A IV, Congenital ataxia, Hind leg paralysis, Bentleg, Thickleg, Malignant hyperthermia, Hemophilia (von Willebrand's disease), Leukemia, Hemolytic disease, edema, Acute respiratory distress (“barker”), Rickets, 25 Renal hypoplasia, Renal cysts, Uterus aplasia, Porcine Stress Syndrome (PSS), halothane (HAL), Dipped Shoulder (Humpy Back, Kinky Back, Kyphosis), Hyperostosis, Mammary Hypoplasia, and Undeveloped Udder. As provided herein, the improved methods of preparing CD163 gene edited animals can avoid introducing new mutations at the deleterious loci or generating new amorphic, hypomorphic, hypermorphic,

neomorphic, antimorphic mutations at non-target sites. These latter changes can be particularly undesirable in elite lines as they can interfere with genes involved in desirable traits that can be controlled by multiple loci in a continuous, quantitative way in a population. Many such Quantitative Trait Loci (QTL) are known and can be typically characterized by a bell-shaped curve the trait value can be plotted against the number of observed animals. Such polygenic inheritance of traits can be common among traits recognized as commercially important such as, but not limited to, backfat, average daily feed intake, lifetime daily gain, and loin depth.

[0131] Similarly, traits associated with productivity can be multigenic and controlled by multiple QTLs. These traits were typically measured by visual inspection but methods now can include ultrasound to measure backfat thickness (bfp), loin depth (ldp) and intramuscular fat (uip). As provided in Table 15, elite lines can have desirable phenotypic traits including high backfat and loin depth while having high lifetime daily gain. Similarly, the sows of the elite lines can be fecund and have large litters, few still-born, and sufficient teats to ween and nurse the piglets.

Table 15: Desirable Phenotypic Traits

LINE	TRT	average	standard deviation
2	Backfat, mm	7.8	1.81
3	Backfat, mm	9.44	2.77
15	Backfat, mm	8.16	2.03
65	Backfat, mm	7.45	1.88
2	Average Daily Feed Intake, kg	1.99	0.24
3	Average Daily Feed Intake, kg	2.13	0.25
15	Average Daily Feed Intake, kg	2.22	0.24
65	Average Daily Feed Intake, kg	2.2	0.27
2	Lifetime Daily Gain, grams/day	683.83	73.53
3	Lifetime Daily Gain, grams/day	704.23	80.15
15	Lifetime Daily Gain, grams/day	755.61	69.32
65	Lifetime Daily Gain, grams/day	800.6	84.48
2	Loin depth, mm	65.31	7.09
3	Loin depth, mm	62.61	7.14

15	Loin depth, mm	67.17	7.15
65	Loin depth, mm	81.2	8
2	Total Born per litter	13.71	3.19
3	Total Born per litter	15.06	3.41
15	Total Born per litter	10.39	2.71
65	Total Born per litter	10.31	2.52
2	Still born per litter	1.19	1.43
3	Still born per litter	1.25	1.50
15	Still born per litter	1.22	1.37
65	Still born per litter	0.77	1.16
2	Teat number	15.43	1.27
3	Teat number	15.44	1.29

[0132] The present specification provides for, and includes, gene edited pigs of selected elite lines that can be homozygous for CD163 knockout edits ($CD163^{-/-}$). In an aspect, the line can be selected from the group consisting of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, Line 65, and progeny thereof comprising the edited CD163 genes described herein. Gene edited lines 2, 3, 15, 19, 27, 62, and 65 can comprise CD163 genomic regions as provided at Table 8 to Table 14, and can be readily distinguished from each other, from unimproved lines, and from other elite lines. In aspects according to the present specification, the heterozygous and homozygous pigs of the present specification can be free of off-site mutations. The present specification provides for, and includes, pigs and cells that can have edited CD163 genes comprising SEQ ID NOs: 1 to 18 and 426 to 505 and that can share a genetic signature comprising at least 90% of the genotypic markers of Table 8 to Table 14. The present specification provides for, and includes, pigs and cells that can have edited CD163 genes comprising SEQ ID NO: 2 and sharing a genetic signature comprising at least 90% of the genotypic markers of Table 8 to Table 14. In an aspect, the genetic signature of a CD163 edited pig or cell can share a genetic signature comprising at least 95% of the genotypic markers of Table 8 to Table 14. Also included are CD163 edited pigs or cells that can share a genetic signature comprising at least 97% of the genotypic markers of Table 8 to Table 14. In an aspect, the CD163 edited pigs or cells can share a genetic signature comprising

at least 98% of the genotypic markers of Table 8 to Table 14. Another aspect provides for CD163 edited pigs or cells that can share a genetic signature comprising at least 99% of the genotypic markers of Table 8 to Table 14.

[0133] As provided herein, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, can retain desirable commercial traits and can be free of deleterious off-target mutations and can comprise an edited CD163 gene comprising any of SEQ ID NOs: 1 to 18 and 426-505. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, can comprise at least 90% of the loin depth of the non-edited pig line and can comprise an edited CD163 gene comprising any of SEQ ID NOs: 1 to 18 and 426-505. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, can comprise at least 90% of the lifetime daily gain of the non-edited pig line. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, can comprise at least 90% of the average daily feed intake of the non-edited pig line. The present specification provides for, and includes, pigs and cells that can have edited CD163 genes comprising SEQ ID NOs: 1 to 18 and 426 to 505 and can share a genetic signature comprising at least 90% of the genotypic markers of Table 8 to Table 14.

[0134] As provided herein, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, can retain desirable reproductive traits. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, comprising a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can comprise at least 90% of the total born per litter of the non-edited pig line. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, comprising a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can comprise no more than 110% of the number of still born piglets compared to the non-edited pig line. In aspects of the present disclosure, the gene-edited CD163$^{-/-}$ animals and cells of Lines 2, 3, 15, 19, 27, 62, and 65, comprising a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can comprise at least 90% of the average number of teats compared to the non-edited pig line.

[0135] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 2 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 2 animals. In an aspect, gene edited

CD163$^{-/-}$ animals and cells of Line 2 can have backfat that is at least 97% of the unedited Line 2 animal.

[0136] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 3 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 3 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 3 can have backfat that is at least 95% of the unedited Line 3 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 3 can have backfat that is at least 97% of the unedited Line 2 animal.

[0137] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 15 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 15 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 15 can have backfat that is at least 95% of the unedited Line 15 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 15 can have backfat that is at least 97% of the unedited Line 2 animal.

[0138] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 19 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 19 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 19 can have backfat that is at least 95% of the unedited Line 19 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 19 have can backfat that is at least 97% of the unedited Line 2 animal.

[0139] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 27 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs: 1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 27 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 27 can have backfat that is at least 95% of the unedited Line 27 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 27 can have backfat that is at least 97% of the unedited Line 2 animal.

[0140] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 62 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 62 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 62 can have backfat that is at least 95% of the unedited Line 62 animal. In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 62 having a gene-edited CD163$^{-/-}$ of SEQ ID NO: 2 can have backfat that is at least 95% of the amount found in unedited Line 62 animals. In an aspect, gene edited CD163$^{-/-}$

animals and cells of Line 62 can have backfat that is at least 97% of the unedited Line 2 animal.

[0141] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 65 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have backfat that is at least 90% of the amount found in unedited Line 65 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 65 can have backfat that is at least 95% of the unedited Line 65 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 65 can have backfat that is at least 97% of the unedited Line 2 animal.

[0142] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 2 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 2 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 2 can have a number of total born per litter that is at least 95% of the unedited Line 2 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 2 can have a number of total born per litter that is at least 97% of the unedited Line 2 animal.

[0143] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 3 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs: 1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 3 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 3 can have a number of total born per litter that is at least 95% of the unedited Line 3 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 3 can have a number of total born per litter that is at least 97% of the unedited Line 3 animal.

[0144] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 15 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs: 1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 15 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 15 can have a number of total born per litter that is at least 95% of the unedited Line 15 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 15 can have a number of total born per litter that is at least 97% of the unedited Line 15 animal.

[0145] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 19 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 19 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 19 can have a number of total

born per litter that is at least 95% of the unedited Line 19 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 19 can have a number of total born per litter that is at least 97% of the unedited Line 19 animal.

[0146] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 27 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 27 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 27 can have a number of total born per litter that is at least 95% of the unedited Line 27 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 27 can have a number of total born per litter that is at least 97% of the unedited Line 27 animal.

[0147] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 62 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 62 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 62 can have a number of total born per litter that is at least 95% of the unedited Line 62 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 62 can have a number of total born per litter that is at least 97% of the unedited Line 62 animal.

[0148] In an aspect, the gene edited CD163$^{-/-}$ animals and cells of Line 65 having a gene-edited CD163$^{-/-}$ of SEQ ID NOs:1 to 18 or 426 to 505 can have a number of total born per litter that is at least 90% of the amount found in unedited Line 65 animals. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 65 can have a number of total born per litter that is at least 95% of the unedited Line 65 animal. In an aspect, gene edited CD163$^{-/-}$ animals and cells of Line 65 can have a number of total born per litter that is at least 97% of the unedited Line 65 animal.

[0149] The present specification provides for and includes CD163 edited animals comprising the germplasm of PIC™ Line 15, PIC™ Line 17, PIC™ Line 27, PIC™ Line 65, PIC™ Line 14, PIC™ Line 62, PIC337, PIC800, PIC280, PIC327, PIC408, PIC™ 399, PIC410, PIC415, PIC359, PIC380, PIC837, PIC260, PIC265, PIC210, PIC™ Line 2, PIC™ Line 3, PIC™ Line 4, PIC™ Line 5, PIC™ Line 18, PIC™ Line 19, PIC™ Line 92, PIC95, PIC™ CAMBOROUGH®, PIC1070, PIC™ CAMBOROUGH® 40, PIC™ CAMBOROUGH® 22, PIC1050, PIC™ CAMBOROUGH® 29, PIC™ CAMBOROUGH® 48, or PIC™ CAMBOROUGH® x54. As used herein, the term germplasm includes an intact genome present in cells or nuclei and comprising

chromosomes. The term germplasm may include any gamete, germ cell, or any somatic cell from which an animal can be cloned. The edited germplasm can comprise an edit having an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The edited germplasm can comprise an edit having an edited genomic sequence of any one of SEQ ID NOs: 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, or 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 15. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 17. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 27. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 65. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 14. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 62. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC337. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC800. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC280. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC327. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC408. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ 399. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC410. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC415. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC359. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC380. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC837. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC260. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC265. The

edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC210. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 2. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 3. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 4. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 5. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 18. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 19. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 92. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC95. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH®. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC1070. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH® 40. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH® 22. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC1050. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH® 29. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH® 48. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ CAMBOROUGH® x54.

[0150] The present specification provides for and includes CD163 edited animals comprising the germplasm of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65. The edited germplasm can comprise an edit having an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The edited germplasm can comprise an edit having an edited genomic sequence of any one of SEQ ID NOs: 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501,

502, 503, 504, or 505. The edited germplasm can comprise an edit having an edited genomic sequence as set forth in SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 2 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 3 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 15 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 19 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to 80%, 85%, 90%, 95% similar or identical to PIC™ Line 27 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 62 with a CD163 edited sequence comprising SEQ ID NO: 453. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 65 with a CD163 edited sequence comprising SEQ ID NO: 453.

[0151] The edited germplasm can comprise an edit having an edited genomic sequence as set forth in SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 2 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 3 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 15 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 19 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to 80%, 85%, 90%, 95% similar or identical to PIC™ Line 27 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 62 with a CD163 edited sequence comprising SEQ ID NO: 489. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 65 with a CD163 edited sequence comprising SEQ ID NO: 489.

The edited germplasm can have a predicted exon 7 amino acid sequence from any one of SEQ ID NOs: 506-517. The edited germplasm can have a predicted exon 7 amino acid sequence as set forth in SEQ ID NO: 513.

[0152] The edited germplasm can comprise an edit having an edited genomic sequence as set forth in SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 2 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 3 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 15 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 19 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to 80%, 85%, 90%, 95% similar or identical to PIC™ Line 27 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 62 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can comprise a genome that is 80%, 85%, 90%, 95% similar or identical to PIC™ Line 65 with a CD163 edited sequence comprising SEQ ID NO: 505. The edited germplasm can have a predicted exon 7 amino acid sequence as set forth in SEQ ID NO: 518.

[0153] The present specification also provides for and includes cells of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65. In some embodiments, a cell of PIC™ Line 2 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 2 can comprise an editing genomic sequence as set forth in SEQ ID NO: 453. In some embodiments, a cell of PIC™ Line 3 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 3 can comprise an editing genomic sequence as set forth in SEQ ID NO: 453. In some embodiments, a cell of PIC™ Line 15 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 15 can comprise an edited genomic sequence as set forth in SEQ Id NO: 453. In some embodiments, a cell of PIC™ Line 19 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 19 can comprise an editing genomic sequence as set forth in

SEQ Id NO: 453. In some embodiments, a cell of PIC™ Line 27 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 27 can comprise an editing genomic sequence as set forth in SEQ ID NO: 453. In some embodiments, a cell of PIC™ Line 62 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 62 can comprise an editing genomic sequence as set forth in SEQ Id NO: 453. In some embodiments, a cell of PIC™ Line 65 can comprise an edited genomic sequence of any one of SEQ ID NOs: 426 to 505. The cell of PIC™ Line 65 can comprise an editing genomic sequence as set forth in SEQ Id NO: 453. The edited germplasm can have a predicted exon 7 amino acid sequence as set forth in SEQ ID NO: 513.

[0154] The present specification provides for, and includes, hybrid animals that can comprise the CD163 gene edits characterized by SEQ ID NOs: 1 to 18 and 426 to 505. In some configurations, gene edit can comprise SEQ ID NO: 453. In an aspect, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® (PIC™ UK Limited, Basingstoke, UK) line. CAMBOROUGH® pigs are hybrids that can be prepared by a cross between Line 2 and Line 3. In an aspect, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® line having an edited CD163 gene of SEQ ID NO: 2. In an aspect, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® line having an edited CD163 gene of SEQ ID NO: 426-458. In various aspects, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® line having an edited CD163 gene of SEQ ID NO: 453. In an aspect, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® line having an edited CD163 gene of SEQ ID NO: 459-504. In various aspects, the hybrid animal can be a $CD163^{-/-}$ hybrid animal of the CAMBOROUGH® line having an edited CD163 gene sequence of SEQ ID NO: 489. CAMBOROUGH® hybrid pigs are pigs that have large litters with uniform and vigorous piglets. CAMBOROUGH® hybrid pigs have a long productive life and have a low mortality. CAMBOROUGH® $CD163^{-/-}$ hybrid pigs retain these desirable commercial traits. In an aspect, CAMBOROUGH® $CD163^{-/-}$ hybrid pigs have litter sizes that are indistinguishable from non-gene edited CAMBOROUGH® hybrid pigs. In aspects according to the present specification, the heterozygous and homozygous pigs of the present specification are free of off-site mutations.

[0155] The present specification further provides, and includes, methods for preparing CD163 gene edited hybrid animals. In an aspect, a first parent can comprise a

CD163 gene edited boar, gilt, or sow of one of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65 for crossing to a second parent. In an aspect, the genome of a gene edited first parent can comprise a sequence selected from the group consisting of SEQ ID NOs: 1 to 18 or 426 to 505. In some aspects, the genome can comprise SEQ ID NO: 453. In various aspects, the genome can comprise SEQ ID NO: 489. In various aspects, the genome can comprise SEQ ID NO: 505. In an aspect, the method can comprise a second parent selected from the group consisting of a CD163 gene edited boar, gilt, or sow of one of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65. Also provided for, and included, by the present specification are methods of preparing CD163 edited animals that can comprise crossing a progeny of any one of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65.

[0156] In an aspect, the present specification provides for heterozygous pigs from lines 2, 3, 15, and 65 (Table 8, Table 9, Table 10, and Table 14, respectively) that can have at least one copy of the CD163 gene successfully edited in a knock-out edit. These edited pigs can exhibit a healthy phenotype with no noticeable deleterious effects from the edit. In another aspect, the specification provides for pigs from lines 19, 27, and 62 (Table 11, Table 12, and Table 13, respectively) that can be edited using the methods disclosed herein to generate edited pigs with healthy phenotypes. The heterozygous pigs can be crossed with non-edited animals of the corresponding line to produce F1 heterozygous pigs. In an embodiment, heterozygous pigs of lines 2, 3, 15, and 65 can be crossed to a second heterozygous pig of lines 2, 3, 15, and 65, and homozygous CD163 edited pigs can be produced in Mendelian proportions. Notably, the gene edits can be unique and identifiable using SEQ ID NOs: 1 to 18 and 426 to 505 and share genetic signatures comprising at least 90% of the genotypic markers of Table 8 to Table 14, thereby enabling the detection and breeding of the CD163 gene edited genomes in any progeny generation. In an aspect, the genetic signatures can share 95% or more of the genotypic markers of Table 8 to Table 14. In another aspect, the genetic signatures share 97% or more of the genotypic markers of Table 8 to Table 14. Also included are genetic signatures that can share 98% or more of the genotypic markers of Table 8 to Table 14. Importantly, the linkage of the CD163 edits to the genomic regions identifiable using the genotypic markers of Table 8 to Table 14 can enable the preparation of any progeny animal having the desired edits and the tracking of the edited regions in any number of

progeny generations. In aspects according to the present specification, the heterozygous and homozygous pigs of the present specification can be free of off-site mutations.

[0157] The present specification provides for, and includes, an embryo or zygote that can be obtained from an elite line of pigs. In an aspect, the embryo or zygote can be obtained from an elite porcine line selected from the group consisting of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, or Line 65. In an aspect, the embryo or zygote can be a frozen embryo or zygote. In another aspect, the embryo or zygote can be a frozen blastocyst. As provided herein, the embryos can be prepared from *in vitro* matured oocytes collected from estrus synchronized gilts. The surrounding cumulus cells can be removed from the *in vitro* matured oocytes and incubated with washed boar spermatozoa and incubated. After incubation, presumptive zygotes can be microinjected with an RNP mixture comprising the CRISPR-Cas endonuclease and guide RNA combinations comprising the first 20 nucleotides of SEQ ID NOs: 22 to 271 or 347 to 425 listed in Table 3. Injected embryos can be transferred to a surrogate female at the 1 to 4 cell stage. In an aspect, the RNP mixture can further include repair templates listed in Table 6 (SEQ ID NOs: 1 to 13). In an aspect, injected zygotes can be surgically implanted into the oviducts of estrus synchronized, un-mated surrogate females by a mid-line laparotomy under general anesthesia (each surrogate receives 40-60 injected embryos).

[0158] The present specification provides for, and includes, gene edited pigs of selected elite lines that can be $CD163^{-/-}$. In an aspect, the line can be PIC™ Line 15, PIC™ Line 17, PIC™ Line 27, PIC™ Line 65, PIC™ Line 14, PIC™ Line 62, PIC337, PIC800, PIC280, PIC327, PIC408, PIC™ 399, PIC410, PIC415, PIC359, PIC380, PIC837, PIC260, PIC265, PIC210, PIC™ Line 2, PIC™ Line 3, PIC™ Line 4, PIC™ Line 5, PIC™ Line 18, PIC™ Line 19, PIC™ Line 92, PIC95, PIC™ CAMBOROUGH®, PIC1070, PIC™ CAMBOROUGH® 40, PIC™ CAMBOROUGH® 22, PIC1050, PIC™ CAMBOROUGH® 29, PIC™ CAMBOROUGH® 48, or PIC™ CAMBOROUGH® x54. In an aspect, the line can be selected from the group consisting of PIC™ Line 2, Line 3, Line 15, Line 19, Line 27, Line 62, Line 65, and progeny thereof comprising the edited CD163 genes described herein. Gene edited lines 2, 3, 15, 19, 27, 62, and 65 comprise CD163 genomic regions as provided above at Table 8 to Table 14, and can be readily distinguished from each other, from unimproved lines, and from other elite lines. Similarly, progeny of lines 2, 3, 15, 19, 27, 62, and 65 comprising

the CD163 genomic regions as provided in Table 8 to Table 14 can be identified. Accordingly, the present specification provides for progeny pigs that can have a $CD163^{-/-}$ genomic region.

[0159] The present specification provides for and includes hybrid porcine lines comprising an edited CD163 gene of the present teachings. In some aspects, the hybrid porcine line can be produced by crossing an edited PIC™ line with at least one other edited PIC™ line. In some aspects, the porcine line can be produced by serial crosses to introduce germplasm from three or more porcine lines. In an aspect, the line can be PIC™ Line 15, PIC™ Line 17, PIC™ Line 27, PIC™ Line 65, PIC™ Line 14, PIC™ Line 62, PIC337, PIC800, PIC280, PIC327, PIC408, PIC™ 399, PIC410, PIC415, PIC359, PIC380, PIC837, PIC260, PIC265, PIC210, PIC™ Line 2, PIC™ Line 3, PIC™ Line 4, PIC™ Line 5, PIC™ Line 18, PIC™ Line 19, PIC™ Line 92, PIC95, PIC™ CAMBOROUGH®, PIC1070, PIC™ CAMBOROUGH® 40, PIC™ CAMBOROUGH® 22, PIC1050, PIC™ CAMBOROUGH® 29, PIC™ CAMBOROUGH® 48, or PIC™ CAMBOROUGH® x54.

[0160] In various aspects, PIC™ Line 65 is sold under the trade name PIC337. In various aspects, PIC™ line 62 is sold under the tradename PIC408. In various aspects, hybrid pigs made by crossing PIC™ lines 15 and 17 are sold under the tradenames PIC800 or PIC280. In various aspects, PIC™ Line 27 is sold under the tradename PIC327. In various aspects, hybrids created from crossing PIC™ Line 65 and PIC™ Line 62 is sold under the tradenames PIC399, PIC410 or PIC415. In various aspects, hybrids created from crossing PIC™ Line 65 and Pic Line 27 are sold under the tradename PIC359. In various aspects, hybrids prepared from crossing PIC™ Line 800 pigs (which is a hybrid of PIC™ Line 15 and PIC™ Line 17) to PIC™ Line 65 pigs are sold under the tradenames PIC380 or PIC837. In various aspects, PIC™ Line 14 is sold under the trade name PIC260. In various aspects, hybrids created from crossing PIC™ Line 14 and PIC™ Line 65 are sold under the tradename PIC265. In various aspects, hybrids created by crossing PIC™ Line 2 and PIC™ Line 3 are sold under the tradenames PIC210, PIC™ CAMBOROUGH®, and PIC1050. In various aspects, hybrids of PIC™ Line 3 and PIC™ Line 92 are sold under the tradename PIC95. In various configurations, hybrids made from crossing PIC™ Line 19 and PIC™ Line 3 are sold under the tradename PIC1070. In various aspects, hybrids created by crossing PIC™ Line 18 and PIC™ Line 3 are sold under the tradename PIC™ CAMBOROUGH® 40. In

various aspects, hybrids created from crossing PIC™ Line 19 and PIC1050 (which is itself a hybrid of PIC™ lines 2 and 3) are sold under the tradename PIC™ CAMBOROUGH® 22. In various aspects, hybrids created from crossing PIC™ Line 2 and PIC1070 (which is itself a hybrid of PIC™ lines 19 and 3) are sold under the tradename PIC™ CAMBOROUGH® 29. In various aspects, hybrids created from crossing PIC™ Line 18 and PIC1050 (which is itself a hybrid of PIC™ lines 2 and 3) are sold under the tradename PIC™ CAMBOROUGH® 48. In various aspects, hybrids created from crossing PIC™ Line 4 and PIC™ Line 5 are sold under the tradename PIC™ CAMBOROUGH® x54. The present teachings provide for and include pigs of any of the forgoing lines or hybrids comprising a CD163 edit of the present teachings.

[0161] In various aspects, the present teachings provide for an include pigs comprising an edited CD163 gene comprising an edited sequence set forth in SEQ ID NOs: 453, 489, or 505 wherein the pig can be a pig of PIC™ Line 15, PIC™ Line 17, PIC™ Line 27, PIC™ Line 65, PIC™ Line 14, PIC™ Line 62, PIC™ Line 2, PIC™ Line 3, PIC™ Line 4, PIC™ Line 5, PIC™ Line 18, PIC™ Line 19, PIC™ Line 92, or a hybrid of two or more of these lines. In various aspects, the hybrid pig can be a cross of PIC™ Line 15 and PIC™ Line 17, PIC™ Line 65 and PIC™ Line 62, PIC™ Line 65 and PIC™ Line 27, a serial hybrid of PIC™ Line 15 and PIC™ Line 17, wherein the hybrid offspring is then crossed to PIC™ Line 65, PIC™ Line 14 and PIC™ Line 65, PIC™ Line 2 and PIC™ Line 3, PIC™ Line 3 and PIC™ Line 92, PIC™ Line 19 and PIC™ Line 3, PIC™ Line 18 and PIC™ Line 3, a serial hybrid between a hybrid pig of PIC™ Line 2 and PIC™ Line 3 and a pig of PIC™ Line 19, a serial hybrid between a hybrid pig of PIC™ Line 19 and PIC™ Line 3 and a pig of PIC™ Line 2, a serial hybrid between a hybrid of PIC™ Line 2 and PIC™ Line 3 crossed to a pig of PIC™ Line 18, or a hybrid of PIC™ Line 4 and PIC™ Line 5.

[0162] In various aspects, the hybrid line comprising an edited CD163 gene can be produced by crossing two or more of lines 2, 3, 15, 19, 27, 62, or 65. In some aspects, the hybrid line comprising an edited CD163 gene can be a CAMBOROUGH® line. CAMBOROUGH® pigs are hybrids that can be prepared by a cross between Line 2 and Line 3. In various aspects, the hybrid line can comprise a PIC™ 837 hybrid line comprising an edited CD163 gene. PIC™ 837 pigs are hybrids that can be prepared by crossing PIC™ Line 800 pigs to PIC™ Line 65 pigs.

[0163] The present specification provides for, and includes, hybrid CD163$^{-/-}$ progeny lines comprising one CD163$^{-/-}$ genomic region that can be obtained from lines 2, 3, 15, 19, 27, 62, or 65, and a second CD163$^{-/-}$ genomic region that can be obtained from a different line selected from lines 2, 3, 15, 19, 27, 62, or 65. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise a CD163$^{-/-}$ allele in a genomic region according to Table 8 and a CD163$^{-/-}$ allele in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise a CD163$^{-/-}$ allele in a genomic region according to Table 8 and a CD163$^{-/-}$ allele in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise a CD163$^{-/-}$ allele in a genomic region according to Table 9 and a CD163$^{-/-}$ allele in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise a CD163$^{-/-}$ allele in a genomic region according to Table 9 and a CD163$^{-/-}$ allele in a genomic region according to Table 14.

[0164] The present specification provides for, and includes, hybrid animals that can comprise the CD163 gene edits characterized by SEQ ID NOs: 1 to 18 and 426 to 505. In an aspect, the hybrid animals comprising the CD163 gene edits can be characterized by SEQ ID NO: 2. In an aspect, the hybrid animals comprising the CD163 gene edits can be characterized by SEQ ID NO: 426-458. In an aspect, the hybrid animals comprising the CD163 gene edits can be characterized by SEQ ID NO: 453. In an aspect, the hybrid animals comprising the CD163 gene edits can be characterized by SEQ ID NO: 459-504. In an aspect, the hybrid animals comprising the CD163 gene edits can be characterized by SEQ ID NO: 489. In an aspect, the hybrid animal can be a CD163$^{-/-}$ hybrid animals of the CAMBOROUGH® line. CAMBOROUGH® pigs are hybrids that can be prepared by a cross between Line 2 and Line 3.

[0165] In various aspects, the hybrid pig can comprise SEQ ID NO: 2 in a genomic region according to Table 8 and SEQ ID NO: 2 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 453 in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 2 in a genomic region according to Table 8 and SEQ ID NO: 2 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453

in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 2 in a genomic region according to Table 9 and SEQ ID NO: 2 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453 in a genomic region according to Table 9 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 9 and SEQ ID NO: 489 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 2 in a genomic region according to Table 8 and SEQ ID NO: 2 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 453 in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 10.In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 2 in a genomic region according to Table 8 and SEQ ID NO: 2 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453 in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 2 in a genomic region according to Table 9 and SEQ ID NO: 2 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453 in a genomic region according to Table 9 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 9 and SEQ ID NO: 489 in a genomic region according to Table 10.In aspects according to the present specification, the heterozygous and homozygous pigs of the present specification can be free of off-site mutations.

[0166] In an aspect, the hybrid animal can be a CD163$^{-/-}$ hybrid animals of the CAMBOROUGH® line. CAMBOROUGH® pigs are hybrids that can be prepared by a cross between Line 2 and Line 3. In an aspect, the hybrid pig can comprise SEQ ID NO:

426 to 505 in a genomic region according to Table 8 and SEQ ID NO: 426-505 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 453 in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, the hybrid pig can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 426 to 505 in a genomic region according to Table 8 and SEQ ID NO: 426 to 505 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453 in a genomic region according to Table 8 and SEQ ID NO: 489 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 8 and SEQ ID NO: 453 in a genomic region according to Table 14. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 426 to 505 in a genomic region according to Table 9 and SEQ ID NO: 426 to 505 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 453 in a genomic region according to Table 9 and SEQ ID NO: 453 in a genomic region according to Table 10. In an aspect, a hybrid CD163$^{-/-}$ progeny line can comprise SEQ ID NO: 489 in a genomic region according to Table 9 and SEQ ID NO: 489 in a genomic region according to Table 10.

[0167] In various aspects, an edited pig of PIC™ line 2, PIC™ line 3, PIC™ line 15, PIC™ line 19, PIC™ line 27, PIC™ line 62, or PIC™ line 65 can have a CD163 gene comprising a predicted amino acid sequence as set forth in SEQ ID NO: 518. In various aspects, the edited CD163 gene can have a 123 bp deletion as set forth in SEQ ID NO: 505. In various configurations, this deletion can have an Exon 7 Amino Acid sequence of AHRKPRLV----------
TVVSLLGGAHFGEGSGQIWAEEFQCEGHESHLSLCPVAPRPDGTCSHSRDVGVV CS (SEQ ID NO: 518). In various aspects, a pig having an edited CD163 gene comprising an amino acid sequence set forth in SEQ ID NO: 518 can be a hybrid offspring between two PIC™ lines. In various aspects, the pig can have an edited CD163 Exon 7 sequence comprising a nucleotide sequence set forth in SEQ ID NO: 505. In various aspects, the pig can be an offspring of a cross between PIC™ line 2 and PIC™ line 3. In aspects according to the present specification, the heterozygous and homozygous pigs of the present specification can be free of off-site mutations.

[0168] Importantly, the CD163 edited pigs and cells of the present specification retain their desirable commercial phenotypes. The edited pigs can exhibit a healthy phenotype with no noticeable acute deleterious effects from the edit. In an aspect, the pigs of lines 2, 3, 15, 19, 27, 62, and 65, can retain the commercially desirable phenotypes as provided in Table 15.

[0169] Methods for improving the health of existing herds of livestock can comprise modifying the CD163 gene locus using the methods described above. In an aspect, the method can comprise introducing into a pig cell an endonuclease or a polynucleotide encoding said endonuclease, and a guide polynucleotide comprising a sequence selected from the group consisting of the first 20 nucleotides of each of SEQ ID NOs: 22 to 271 or the first 20 nucleotides of each of 347 to 425, incubating the cell under conditions that permit the endonuclease to act upon the DNA at, or near, the target sequence and thereby induce recombination, homology-directed repair, or non-homologous end joining at or near the target site, identifying at least one cell having a modification at said target sequence, and producing an animal from an animal cell. In aspects of the present specification, the endonuclease can be an RNP complex of a guide RNA and a Cas protein from *S. thermophilus*. In another aspect, the endonuclease can be an RNP complex of a guide RNA and a Cas protein from *S. pyogenes*.

[0170] In an aspect, the method can further comprise providing to the cell a repair guide comprising a sequence selected from the group consisting of nucleotides 50 to 100 of SEQ ID NOs: 1 to 13. In another aspect, a repair guide can comprise a sequence selected from the group consisting of nucleotides 50 to 100 of SEQ ID NOs: 1 to 13 and can further comprise 85% homology to nucleotides 1 to 49 and 101 to 150 of SEQ ID NOs: 1 to 13. In an aspect, the repair guide can comprise a sequence selected from the group consisting of SEQ ID NOs: 1 to 13. As provided herein, the method for improving the health of existing herds provides for the maintenance of desirable commercial phenotypes as discussed above. In an aspect, the desirable commercial phenotypes can be at least 90% of the phenotypes observed in herds of non-edited pigs having a similar genetic background.

[0171] The CD163 gene edited locus can be introduced into the herd by conventional breeding or by methods incorporating artificial insemination. To prepare homozygous animals, crosses between parents having at least one gene edited CD163 locus comprising a sequence of SEQ ID NOs: 1 to 18 or 426 to 505 can be performed

and homozygous progeny (present at a 1:4 Mendelian ratio) can be selected. As provided herein, a combination of parents and one gene edited CD163 locus can be suitable for improving the herd. Further breeding of animals having the CD163 gene edited loci can improve the health of the herd until all the animals of the herd comprise a CD163 gene edited locus as described. Notably, the health of the herd can improve significantly well before the herd has been bred fully to a CD163 gene edited herd. Specifically, and as discussed above, pig fetuses in a PRRSv resistant gene edited CD163 sow are themselves protected from PRRSv. Further, one of ordinary skill in the art would know that, as the numbers of homozygous CD163 edited animals increase, suitable pig vectors for PRRSv transmission decrease (*e.g.*, herd immunity develops). Accordingly, the present methods can provide for improving a herd by introducing herd immunity.

[0172] One useful method of detecting the desired edit is to use real-time PCR. PCR primers flanking the region of interest and a probe that specifically anneals to the region of interest. The probe is labelled with both a fluorophore and a quencher. In the PCR reaction, the primers and probe hybridize in a sequence-dependent manner to the complementary DNA strand of the region of interest. Because the probe is intact, the fluorophore and quencher are in close proximity and the quencher absorbs fluorescence emitted by the fluorophore. The polymerase extends from the primers and begins DNA synthesis. When the polymerase reaches the probe, the exonuclease activity of the polymerase cleaves the hybridized probe. As a result of cleavage, the fluorophore is separated from the quencher and fluoresces. This fluorescence is detected by the real time instrument. These steps are repeated for each PCR cycle and allow detection of specific products.

[0173] In the instant application, three separate sets of primers and probes were designed. The first set of primers (SEQ ID NO: 556 and 557) flanked the unedited genomic sequence comprising SEQ ID NO: 249 and a probe (SEQ ID NO: 558) which binds to the unedited genomic DNA in between the primers. The second set of primers (SEQ ID NO: 559 and 560) flanked the unedited genomic sequence comprising SEQ ID NO: 256, and a probe (SEQ ID NO: 561) binds the unedited between the primers. The final set of primers (SEQ ID NO: 562 and 563) flanked the desired Exon 7 deletion edit created by excision of the sequence between the cut sites of SEQ ID NO: 249 and SEQ ID NO: 256. A probe (SEQ ID NO: 564) was designed to bind the desired edit in between these primers. A commercial real-time PCR kit was then used to probe various

animals for the desired edit. A variety of commercial real-time PCR kits exist including, such as, but without limitation, PRIMETIME® from IDT, TAQMAN® (Roche Molecular Systems, Inc, Pleasonton, CA) from Applied Biosystems, and various kits from Qiagen and Bio-Rad. Skilled persons will recognize that any such kit can be used with the primers and methods of the present teachings to achieve like results.

EXAMPLES

Example 1

[0174] This Example illustrates target site selection of a porcine CD163 gene knockout in pig cells.

[0175] A *Streptococcus* Cas9/gRNA RNA-directed DNA endonuclease was used to generate DNA sequence insertions, deletions, and combinations thereof, in the porcine CD163 gene (Sscrofa11.1, GenBank accession: GCA_000003025.6), whereby guide RNAs and protein combinations were delivered singly or as pairs, such that sequence changes in the CD163 gene reduce or abolish the function, stability, or expression of the CD163 messenger RNA or protein. The sequence of the CD163 gene from 120 nucleotides upstream (chr5:63300192) of the translational start site to 59 nucleotides downstream of CD163 exon 7 (chr5:63323390), was screened for the guide RNA binding sites having either an adjacent nGG or nGGnG PAM sequence required for cutting by the Cas9 proteins derived from *Streptococcus pyogenes* or *Streptococcus thermophilus CRISPR3 (S. thermophilus CR3),* respectively, the sequences of which are in the sequence listing filed herewith, as listed in Table 2. The DNA sequences for the target sites for editing, the locations of the target sites on the CD163 gene, and the editing activity (measured as described in Example 3) are provided in Table 3. Guide RNA molecules had the same sequence as the target, with corresponding RNA nucleotides, without the PAM sequence (nGG for *S. pyogenes* and nGGnG for *S. thermophilus*). Guide polynucleotide molecules could also consist of DNA bases or mixtures of DNA and RNA bases. The target site sequences are listed in the sequence listing filed herewith as SEQ ID NOs: 22 to 271 and 347 to 425. The target site sequences shown are conserved across pig germplasm and screened by DNA sequencing.

Example 2

[0176] This example illustrates nucleofection for the delivery of guide RNA/Cas9 endonuclease to porcine fetal fibroblasts.

[0177] To test the DNA cutting activity in living cells to produce an edited porcine CD163 allele, the CRISPR-Cas endonuclease and guide RNA targeting the sequences listed in Table 3 were nucleofected into porcine fetal fibroblast cells. Porcine fetal fibroblast (PFF) cells lines were prepared from 28-35 day-old fetuses and 3.2 µg of Cas9 protein (*S. pyogenes* or *S. thermophilus*) and 2.2 µg of *in vitro* transcribed single guide RNA were combined in water to a total volume of 2.23 µl, then were nucleofected into PFF cells using a Lonza electroporator. In preparation for nucleofection, PFF cells were harvested using TrypLE express (recombinant Trypsin), upon which the culture medium was removed from cells, washed 1 X with HBSS or DPBS, and incubated for 3–5 minutes at 38.5°C in the presence of TrypLE. Cells were then harvested with complete medium. Cells were pelleted via centrifugation (300 x g for 5 minutes at room temperature), supernatant was discarded, then the cells were resuspended in 10 mL PBS to obtain single cell suspension counting cells using trypan blue staining.

[0178] The appropriate amount of cells was pelleted by centrifugation (300 x g for 5 minutes at room temperature), the supernatant was discarded, and the cells were resuspended in nucleofection buffer P3 at a final concentration of $7.5x10^6$ cells/ml. 20 µl of the cell suspension were added to each well of a nucleofection plate containing the RNP mixture using a multichannel pipette, then mixed gently to resuspend the cells. The RNP/cell mixture was transferred in the nucleofection plate, nucleofected with program CM138 (supplied by the manufacturer). 80 µl of warm Embryonic Fibroblast Medium, EFM, (Dulbecco's Modified Eagle's Medium (DMEM) containing 2.77 mM glucose, 1.99 mM L-glutamine, and 0.5 mM sodium pyruvate, supplemented with 100 µM 2-Mercaptoethanol, 1X Eagle's minimum essential medium non-essential amino acids (MEM NEAA), 100 µg/mL Penicillin-Streptomycin, and 12% Fetal Bovine Serum were added to each well after nucleofection. The suspensions were mixed gently by pipetting, then 100 µl were transferred to a 12 well plate containing 900 µl of EFM pre-incubated at 38.5°C. The plate was then incubated at 38.5°C, 5% CO_2 for 48 hours. Forty-eight hours post nucleofection, genomic DNA was prepared from transfected and control PFF cells, 15 µl of QUICKEXTRACT™ DNA Extraction Solution (Lucigen, Madison, WI) were added to pelleted cells then lysed by incubating for 10 mins at 37° C, for 8 mins at 65° C, for 5 mins at 95° C, then lysate was held at 4° C until used for DNA sequencing.

Example 3

[0179] This example illustrates the editing frequency of guide RNA/Cas9 combinations directed against porcine CD163.

[0180] Nucleotide sequence changes were introduced into the porcine CD163 gene by delivering Cas9 protein complexed with guide RNAs to fetal fibroblast cells as described in Example 2.

[0181] To evaluate DNA double strand cleavage at a porcine CD163 genomic target site mediated by the guide RNA/Cas endonuclease system, a region of approximately 250 bp genomic DNA surrounding the target site was amplified by PCR and the PCR product was then examined by amplicon deep sequencing for the presence of edits. After transfection in triplicate, PFF genomic DNA was extracted as described in Example 2. The region surrounding the intended target site was PCR amplified with NEB Q5 Polymerase, adding sequences necessary for amplicon-specific barcodes and ILLUMINA® (ILLUMINA®, San Diego, CA) sequencing using tailed primers through two rounds of PCR. The resulting PCR amplifications were deep sequenced on an ILLUMINA® MISEQ® Personal Sequencer (ILLUMINA®, San Diego, CA). The resulting reads were examined for the presence of edits at the expected site of cleavage by comparison to control experiments where the Cas9 protein and guide RNA were omitted from the transfection or by comparison to the reference genome. To calculate the frequency of NHEJ edits for a target site, Cas9 protein, guide RNA combination, the total number of edited reads (amplicon sequences containing insertions or deletions when compared to the DNA sequences from control treatments or reference genome) were divided by total read number (wild-type plus edited reads) of an appropriate length containing a perfect match to the barcode and forward primer. Total read counts averaged approximately 7000 per sample and NHEJ activity is expressed as the average (n=3) edited fraction in Table 3. As shown in Table 3, from 0 to 58.2% of the reads contained edits and the average editing frequency across all combinations was approximately 16%. This example demonstrates that, in fetal fibroblast cells, the porcine CD163 gene nucleotide sequence was edited through the stimulation of double-strand breaks mediated by transfecting Cas9 protein from either *S. pyogenes* or *S. thermophilus* complexed with various guide RNAs.

Example 4

[0182] This example illustrates generation of an in-frame stop codon using DNA repair template.

[0183] DNA repair templates were used to introduce stop codons in the porcine CD163 gene when co-delivered with Cas9 protein and guide RNA. The endonuclease-guide complexes and repair templates disclosed in Table 6 were generated and used to edit CD163 genes in blastocysts. The base deletions were verified by the methods of Example 5.

Example 5

[0184] This example illustrates the molecular characterization of edited animal genomes.

[0185] A tissue sample was taken from an animal whose genome was been edited according to the examples herein. Tail, ear notch, or blood samples were suitable tissue types. The tissue sample was frozen at -20°C within 1 hour of sampling to preserve integrity of the DNA in the tissue sample.

[0186] DNA was extracted from tissue samples after proteinase K digestion in lysis buffer. Characterization was performed on two different sequence platforms, short sequence reads using the ILLUMINA® platform and long sequence reads on an Oxford NANOPORE™ platform (Oxford NANOPORE™ Technologies, Oxford, UK).

[0187] For short sequence reads, two-step PCR was used to amplify and sequence the region of interest. The first step was a locus-specific PCR which amplified the locus of interest from the DNA sample using a combined locus-specific primer with a vendor-specific primer. The second step attached the sequencing index and adaptor sequences to the amplicon from the first step so that sequencing could occur.

[0188] The locus-specific primers for the first step PCR were chosen so that they amplified a region <300bp such that ILLUMINA® paired-end sequencing reads could span the amplified fragment. Multiple amplicons were preferred to provide redundancy should deletions or naturally occurring point mutations prevent primers from correctly binding. Sequence data for the amplicon was generated using an ILLUMINA® sequencing platform (MISEQ®, ILLUMINA®, San Diego, CA). Sequence reads are analyzed to characterize the outcome of the editing process.

[0189] For long sequence reads, two-step PCR was used to amplify and sequence the region of interest. The first step is a locus-specific PCR which amplified the locus of

interest from the DNA sample using a combined locus-specific primer with a vendor-specific adapter. The second step PCR attached the sequencing index to the amplicon from the first-step PCR so that the DNA was ready for preparing a sequencing library. The step 2 PCR products underwent a set of chemical reactions from a vendor kit to polish the ends of the DNA and ligate on the adapter containing the motor protein to allow access to the pores for DNA strand-based sequencing.

[0190] The locus specific primers for the first step PCR range were designed to amplify different regions of the CD163 gene and amplified regions differed in length. Normalized DNA is then mixed with vendor supplied loading buffer and is loaded onto the NANOPORE™ flowcell.

[0191] Long sequence reads, while having lower per base accuracy than short reads, are very useful for observing the long range context of the sequence around the target site.

Example 6

[0192] This example illustrates methods of making pigs having edited CD163 genes conferring PRRSv resistance.

[0193] Porcine oocytes were isolated, fertilized, and then the resulting zygotes are edited to generate gene edited pigs.

[0194] CD163 RNP complexes were microinjected into the cytoplasm of *in vivo* or *in vitro* fertilized porcine one-cell zygotes. These zygotes were then incubated to generate edited multicellular embryos and transferred to surrogate gilts via standard methods to birth gene edited pigs. To prepare embryo donors and surrogates, pubertal gilts from PIC™ Line 2, Line 3, Line 15, and Line 65 were subjected to estrus synchronization by treatment with 0.22% altrenogest solution (20–36 mg/animal) for 14 days. Follicular growth was induced by the administration of PMSG 36 hours following the last dose of Matrix, and ovulation was induced by the administration of hCG 82 hours after PMSG administration. To generate *in vivo* fertilized zygotes, females in standing heat were then artificially inseminated (AI) with boar semen from the corresponding PIC™ line. *In vivo* derived zygotes were recovered surgically 12-24 hours after AI by retrograde flushing the oviduct with sterile TL-HEPES medium supplemented with 0.3% BSA (w/v). Fertilized zygotes were subjected to a single 2 - 50 picoliter (pl) cytoplasmic injection of Cas9 protein and guide RNA complex (25 - 50

ng/µl and 12.5 - 35 ng/µl) targeting CD163 and cultured in PZM5 medium (Yoshioka, K., *et al.*, Biol. Reprod., 2002, 60: 112-119; Suzuki, C., *et al.*, Reprod. Fertil. Dev., 2006 18, 789-795; Yoshioka, K., J. Reprod. Dev. 2008, 54, 208-213). Injected zygotes were surgically implanted into the oviducts of estrus synchronized, un-mated surrogate females by a mid-line laparotomy under general anesthesia (each surrogate received 20-60 injected embryos).

[0195] *In vitro* fertilized embryos for gene editing were derived from non-fertilized PIC™ oocytes. Immature oocytes from estrus synchronized PIC™ gilts were collected from medium size (3–6 mm) follicles. Oocytes with evenly dark cytoplasm and intact surrounding cumulus cells were then selected for maturation. Cumulus oocyte complexes were placed in a well containing 500 µl of maturation medium, TCM-199 (Invitrogen) with 3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 10 ng/ml EGF, 0.5 µg/ml luteinizing hormone (LH), 0.5 µg/ml FSH, 10 ng/ml gentamicin (Sigma), and 10% follicular fluid for 42–44 h at 38.5 °C and 5% CO_2, in humidified air. At the end of the maturation, the surrounding cumulus cells were removed from the oocytes by vortexing for 3 min in the presence of 0.1% hyaluronidase. Then, *in vitro* matured oocytes were placed in 100 µl droplets of IVF medium (modified Tris-buffered medium containing 113.1 mM NaCl, 3 mM KCl, 7.5 mM $CaCl_2$, 11 mM glucose, 20 mM Tris, 2 mM caffeine, 5 mM sodium pyruvate, and 2 mg/ml bovine serum albumin (BSA)) in groups of 25–30 oocytes and were fertilized according to established protocol (Abeydeera, *Biol. Reprod.,* 57:729–734, 1997) using fresh extended boar semen. One ml of extended semen was mixed with Dulbecco's Phosphate Buffered Saline (DPBS) containing 1 mg/ml BSA to a final volume of 10 ml and centrifuged at 1000 × g, 25° C for 4 minutes, and spermatozoa were washed in DPBS three times. After the final wash, spermatozoa were re-suspended in mTBM medium and added to oocytes at a final concentration of 1×10^5 spermatozoa/ml, and co-incubated for 4-5 h at 38.5° C and 5% CO_2. Presumptive zygotes were microinjected 5 hours post IVF and transferred to a surrogate female after 18-42 hours (1-4 cell stage). Each surrogate receives 20-60 injected embryos. Pregnancies were confirmed by lack of return to estrus (21 days) and ultrasound at 28 days post embryo transfer.

[0196] To establish the frequency of Cas9-guide RNA targeted gene editing in porcine embryos, uninjected control zygotes and injected surplus zygotes generated by *in vitro* fertilization were cultivated in PZM3 or PZM5 medium at 38.5° C for 5-7 days.

Blastocysts were harvested at day 7 post cultivation and the genomic DNA isolated for next generation sequencing.

Example 7

[0197] This example illustrates the generation and characterization of gene edited pigs.

[0198] Animals from PIC™ lines 2, 3, 15, and 65 were edited by the methods described in Example 6. Successful edits were confirmed using the methods of Example 5. Fibroblast cell lines were grown from collagenase treated ear notch samples extracted from the edited animals and deposited with the American Type Culture Collection (ATCC®). The ATCC® has an address of 10801 University Boulevard, Manassas, VA 20110-2209. A representative sample of CD163 edited PIC™ Line 2 was deposited with the ATCC on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125814. A representative sample of CD163 edited PIC™ Line 3 was deposited with the ATCC on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125815. A representative sample of CD163 edited PIC™ Line 15 was deposited with the ATCC on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125816. A representative sample of CD163 edited PIC™ Line 65 was deposited with the ATCC® on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125813. Each deposit was made according to the Budapest Treaty. Representative animals from each line were confirmed to have heterozygous edits as specified in Table 16.

Table 16: Verified Gene Edited Animals

Line number	CD163 Allele 1	CD163 Allele 2	ATCC Deposit No.
2	Wild type sequence	**Deleted nucleotides chr5:** 63301999-63302005	PTA-125814
3	Wild type sequence	**Deleted nucleotides chr5:** 63301999-63302005	PTA-125815
15	Wild type sequence	**Deleted nucleotides chr5:** 63301999-63302005	PTA-125816
65	Wild type sequence	**Deleted nucleotides chr5:** 63301999-63302005	PTA-125813

Each of the animals in Table 16 presented a healthy phenotype with deleted nucleotides 63301999-63302005 (in exon 2) from chromosome 5.

[0199] Additional cell lines were grown from collagenase treated ear notch samples from unedited animals from PIC™ lines 19, 27, and 62, were deposited with the ATCC. A representative sample of PIC™ Line 19 was deposited with the ATCC® on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125811. A representative sample of PIC™ Line 27 was deposited with the ATCC on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125907. A representative sample of PIC™ Line 62 was deposited with the ATCC on April 3, 2019 and assigned ATTC® Patent Deposit Number PTA-125812. Each deposit was made according to the Budapest Treaty. Using conventional cloning methods, animals are generated from the cell lines deposited as PTA-125811, PTA-125812, and PTA-125907 and edited using the methods of Example 6 in order to generate edited lines.

[0200] Single nucleotide polymorphisms (SNPs) in the vicinity of the CD163 gene were analyzed for each of the deposited PIC™ lines (2, 3, 15, 19, 27, 62, and 65) and SNP profiles of each of the lines that are capable of distinguishing each line are selected. The starting dataset for defining the line signatures was a collection of 330 whole genome sequenced animals from the 7 deposited PIC™ lines. A 6 Mb region of Chromosome 5 centered on the CD163 gene was extracted for the signatures, and variation within and between the lines was examined for each nucleotide in this region in order to identify a relatively small number of SNPs that together formed a signature for the line.

[0201] To be a candidate for inclusion in the signature of a given line, the following criteria were imposed on each chromosomal position: sequence coverage had to exist for 90% of animals in the line; the above had to be true for at least 5 of the 7 lines; and all animals with data in the target line must have had the same homozygous genotype. For each of the other lines, a genotype frequency for this genotype was calculated across all the sequenced animals for that line. A cutoff was imposed of at least 30% of the difference between the highest and lowest per-line genotype frequency of the other 6 lines (*i.e.*, there must be a spread of genotype frequencies within the 6 lines).

[0202] A combination of metrics for discriminating power and even distribution were used to select a subset of genotypes that could define each line. Table 8 to Table 14 provide the positions on chromosome 5 for the SNPs for which the homozygous allele

was fixed in each porcine line. These sets of homozygous alleles distinguish each porcine line from the other lines. The genotype listed indicates which allele is homozygous at each position, as indicated in Table 8 to Table 14.

Example 8

[0203] This example compares differing levels of PRRSv resistance of immune cells isolated from wild type and gene edited pigs.

[0204] CD163 surface expression analysis was conducted on Monocyte Derived Macrophages (MoMØs) recovered from edited and wild type animals, each with and without edits to the CD163 gene according to the methods described in the previous examples. The edits tested are presented in Table 17. Four edits comprise deletions of various sizes as shown, and the fifth comprises a 2 base pair insertion; all edits in Table 15 are in exon 2. All deletions or insertions result in a truncated CD163 polypeptide. CD163 expression was assessed by immunofluorescence labelling and FACS analysis using clone 2A10/11, a mouse anti-pig CD163 monoclonal antibody. CD163 edited cells, MoMØs, lacked a functional epitope on the surface of the cell as evident from cell surface expression analysis.

Table 17: CD163 gene edits tested for protein expression

Region Chr5 Location of guideRNAs binding site	63301997-02016	63301997-02016	63301997-02016	63301997-02016	63301997-02016
SEQ ID NO	14	15	16	17	18
Guide RNA spacer (DNA) SEQ ID NO	42	42	42	42	42
Guide RNA spacer (RNA) SEQ ID NO	288	288	288	288	288
Translation of Deletion	339	340	341	342	343

Base deletion or insertion coordinates	chr5:63302012-63302013	chr5:63302009-63302041	chr5:63302012-63302013	chr5:63302005-63302020	chr5:63302011-63302020
Deleted or inserted bases (#)	TC (2 bp deletion)	SEQ ID NO:344; (33 bp deletion)	TC (2 bp insertion)	SEQ ID NO:345; (16 bp deletion)	SEQ ID NO: 346; (10 bp deletion)

[0205] To test the two base pair deletion edit to CD163 in homozygous edited cells for PRRSv viral infection, MoMØs were infected with PRRSv type 1 and type 2. Twenty-four (24) hours post infectivity, in a microscope field of view, cells were counted to determine the number of cells which contain replicating PRRSv. CD163 homozygous edited MoMØ cells were found to be not permissive to both PRRSv types 1 and 2.

Example 9

[0206] This example demonstrates the use of two guides for removal of exon 7 from *S. scrofa* CD163 in porcine fibroblasts.

[0207] In order to remove CD163 exon 7 DNA sequences which encode SRCR5 of the mature CD163 polypeptide, DNA sequences located in the intronic regions 450 bp upstream and 59 bp downstream of CD163 exon 7 were examined for *Streptococcus pyogenes* (NGG) and *Streptococcus* thermophilus (NGGNG) Cas9 protein and guideRNA recognition sites. 48 sites were identified within the 450 bp of intron 6 and 10 sites were identified within a 59 bp region of intron 7. The ability of these 58 sites to bind to Cas9 and guideRNAs to direct gene editing was first tested using single guides in porcine fetal fibroblasts. A subset of these single guideRNA-Cas9 proteins—the guideRNAs which generated a high frequency of edits across the spacer recognition site--were further tested as guide pairs for their ability to remove CD163 exon 7. Guides were introduced to porcine fetal fibroblasts by nucleofection as described in Example 2: each guide was prepared as an RNP and then the two sets of complexes were combined in a total volume of 2.23 µl prior to transfection. Editing frequency for guide pairs was determined as described in Example 3. The frequencies of NHEJ-mediated repairs whereby the endonuclease cut sites are brought together resulting in the deletion of CD163 exon 7 are shown in Table 18.

Table 18. End-to-end repair frequencies using paired guideRNAs for CD163 exon 7 deletion in porcine fetal fibroblasts

SEQ ID NO: (5')	Cut site (5')	SEQ ID NO: (3')	Cut site (3')	Deletion size (bp)	Desired repair outcome (%)
229	63322816	256	63323377	561	23.3
230	63322814	256	63323377	562	24.7
231	63322826	256	63323377	551	15.3
237	63322861	256	63323377	516	20.7
241	63322891	256	63323377	486	20.0
229	63322816	258	63323378	562	5.3
230	63322814	258	63323378	563	7.0
231	63322826	258	63323378	552	4.3
237	63322861	258	63323378	517	15.7
241	63322891	258	63323378	487	5.7
229	63322816	261	63323373	558	15.0
230	63322814	261	63323373	559	18.0
231	63322826	261	63323373	548	0.0
237	63322861	261	63323373	513	12.0
241	63322891	261	63323373	483	14.0
219	63322697	256	63323377	680	28.0
221	63322709	256	63323377	668	33.7
224	63322747	256	63323377	630	27.0
227	63322786	256	63323377	591	22.7
219	63322697	258	63323378	681	7.7
221	63322709	258	63323378	669	13.0
224	63322747	258	63323378	631	7.3
227	63322786	258	63323378	592	6.3
219	63322697	261	63323373	677	14.5
221	63322709	261	63323373	665	20.7

224	63322747	261	63323373	627	14.7
227	63322786	261	63323373	588	11.3
249	63322963	256	63323377	414	38.0
250	63322973	256	63323377	404	36.7
249	63322963	258	63323378	415	20.3
250	63322973	258	63323378	405	14.7
249	63322963	261	63323373	411	17.7
250	63322973	261	63323373	401	11.7

[0208] The excision guides had a wide range of editing frequencies for the desired edit.

[0209] Example 10

[0210] This example illustrates the excision of Exon 7 in porcine blastocysts using dual guide RNAs.

[0211] A subset of guides screened in porcine fetal fibroblasts were additionally tested for their ability to remove CD163 exon 7 in porcine blastocysts. The subset of guides to be tested in porcine embryos was chosen based on a combination of their efficacy in generating exon 7 deletions in porcine fibroblasts and low number of off-target edits for each guide in the pair (see Detailed Description). Edited porcine embryos were generated as described above. Briefly, oocytes recovered from slaughterhouse ovaries were *in vitro* fertilized as described in Example 6. The sgRNP solution was injected into the cytoplasm of presumptive zygotes at 4-5 hours post-fertilization by using a single pulse from a FEMTOJET® 4i microinjector (Eppendorf; Hamburg, Germany) with settings at pi=200 hPa, ti=0.25 s, pc=15 hPa. Glass capillary pipettes (Sutter Instrument, Navato, CA, USA) with an outer diameter of 1.2 mm and an inner diameter of 0.94 mm were pulled to a very fine point of < 0.5 µm. Microinjection was performed in TL-Hepes (ABT360, LLC) supplemented with 3 mg/ml BSA (Proliant) on the heated stage of an inverted microscope equipped with Narishige (Narishige International USA, Amityville, NY) micromanipulators. Following injections, presumptive zygotes were cultured for 7 days in PZM5 (Cosmo Bio, Co LTD, Tokyo, Japan) in an incubator environment of 5% CO_2, 5% O_2, 90% N_2. Editing frequency of

blastocysts was determined as described in Example 3. The frequencies of end-to-end NHEJ repairs resulting in the deletion of CD163 exon 7 are shown in Table 19.

Table 19 End-to-end repair frequencies using paired guideRNAs for in CD163 exon 7 deletion in porcine embryos

SEQ ID NO: (5')	Cut site (5')	SEQ ID NO: (3')	Cut site (3')	Deletion size (bp)	Desired repair outcome (%)
229	63322816	256	63323377	561	38.0
230	63322814	256	63323377	562	21.0
231	63322826	256	63323377	551	24.0
241	63322891	256	63323377	486	29.0
229	63322816	258	63323378	562	7.0
231	63322826	258	63323378	552	12.0
241	63322891	258	63323378	487	20.0
219	63322697	256	63323377	680	35.0
221	63322709	256	63323377	668	36.0
224	63322747	256	63323377	630	24.0
227	63322786	256	63323377	591	0.0
227	63322786	258	63323378	592	0.0
221	63322709	261	63323373	665	15.0
249	63322963	256	63323377	414	44.0
250	63322973	256	63323377	404	14.0
249	63322963	258	63323378	415	7.0
249	63322963	261	63323373	411	53.0

[0212] This example demonstrates that a number of guide pairs can be used to delete CD163 exon 7, but the efficiency can vary greatly between guide pairs and cell types.

Example 11

[0213] This example demonstrates the use of two guides to introduce a premature stop codon into exon 7 of *S. scrofa* CD163.

[0214] Guides within exon 7 of CD163 were screened by bioinformatic methods for their ability to generate an in-frame stop codon when the cuts generated by those guides are ligated together during NHEJ. Bioinformatic predictions were tested in porcine fetal fibroblasts as described in Example 2: guides were complexed separately before being combined in a total volume of 2.23 µl prior to transfection. Editing frequency was determined as described in Example 3. The frequencies of end-to-end NHEJ repairs resulting in the introduction of a premature stop codon in exon 7 of CD163 are shown in Table 20.

Table 20. End-to-end repair frequencies for introduction of a stop codon in CD163 exon 7 deletion in porcine fetal fibroblasts

SEQ ID NO: (5')	Cut site (5')	SEQ ID NO: (3')	Cut site (3')	Deletion size (bp)	Desired repair outcome (%)
351	63323023	365	63323103	80	50.5
351	63323023	387	63323235	212	1.0
348	63323027	390	63323236	209	53.5
348	63323027	388	63323236	209	50.0
348	63323027	395	63323284	257	31.0
352	63323035	365	63323103	68	29.0
352	63323035	387	63323235	200	12.5
352	63323035	399	63323283	248	21.0
353	63323038	365	63323103	65	21.0
353	63323038	387	63323235	197	6.0
353	63323038	399	63323283	245	11.0
354	63323039	390	63323236	197	55.5
354	63323039	388	63323236	197	18.0
354	63323039	395	63323284	245	17.0
358	63323056	361	63323077	21	0.0
358	63323056	362	63323087	31	4.0
358	63323056	368	63323124	68	37.0
358	63323056	384	63323203	147	34.0

358	63323056	394	63323272	216	56.0
358	63323056	399	63323283	227	34.0
359	63323057	390	63323236	179	13.0
359	63323057	388	63323236	179	3.0
359	63323057	395	63323284	227	4.0
360	63323058	368	63323124	66	7.5
360	63323058	384	63323203	145	6.0
360	63323058	389	63323237	179	0.0
360	63323058	394	63323272	214	16.5
360	63323058	397	63323285	227	0.0
361	63323077	365	63323103	26	42.0
361	63323077	387	63323235	158	0.0
362	63323087	390	63323236	149	45.0
362	63323087	388	63323236	149	17.5
362	63323087	395	63323284	197	22.5
364	63323089	365	63323103	14	0.0
364	63323089	387	63323235	146	14.5
364	63323089	399	63323283	194	35.5
365	63323103	368	63323124	21	0.0
365	63323103	384	63323203	100	17.0
365	63323103	389	63323237	134	0.0
365	63323103	394	63323272	169	32.5
365	63323103	397	63323285	182	1.0
366	63323118	368	63323124	6	0.0
366	63323118	384	63323203	85	29.0
366	63323118	389	63323237	119	14.0
366	63323118	394	63323272	154	48.5
366	63323118	397	63323285	167	10.0
354	63323039	211	63323163	123	29.0

[0215] This example illustrates that guide pairs designed to introduce a stop codon have a wide variety of editing efficiencies in porcine fibroblasts.

Example 12

[0216] This example illustrates the editing efficiency of guides introducing stop codons in porcine blastocysts.

[0217] A subset of guides screened in porcine fetal fibroblasts were additionally tested for their ability to introduce a premature stop codon in exon 7 of CD163 in porcine embryos. The subset of guides to be tested in porcine embryos was chosen based on a combination of their efficacy in introducing a premature stop codon in exon 7 of CD163 in porcine fibroblasts and the lack of observed off-targets for each guide in the pair as described *supra*. Editing frequency was determined as described in Example 3. The frequencies of end-to-end NHEJ repairs resulting in the introduction of a premature stop codon in exon 7 of CD163 are shown in Table 21.

Table 21 End-to-end repair frequencies for introduction of a stop codon in CD163 exon 7 deletion in porcine blastocysts

SEQ ID NO: (5')	Cut site (5')	SEQ ID NO: (3')	Cut site (3')	Deletion size (bp)	Desired repair outcome (%)
351	63323023	365	63323103	80	39.6
348	63323027	390	63323236	209	31
348	63323027	388	63323236	209	35.4
354	63323039	390	63323236	197	30.4
358	63323056	394	63323272	216	29.2
362	63323087	390	63323236	149	31
366	63323118	394	63323272	154	33
354	63323039	211	63323163	123	27.4

[0218] This example demonstrates that guide pairs that can be used to introduce a premature stop codon into exon 7 of CD163 have a wide variety of editing efficiencies.

Example 13

[0219] This example illustrates variable repair outcomes for NHEJ repair.

[0220] A subset of guideRNAs designed to delete CD163 exon 7 were tested for their ability to delete the exon 7 coding and flanking regions in porcine blastocysts, as described in Example 9. Editing frequency of blastocysts was determined as described in Example 3. In this example, a subset of the DNA sequences observed in porcine blastocysts *in vivo* as the result of NHEJ-mediated repair are shown for five guideRNA pairs in Table 7. It is known that simple nucleotide deletions also occur, as well as more

complex NHEJ-mediated repair DNA sequences which contain deletions, insertions, rearrangements, inversions, and any combination thereof. Without being limited by theory, these varied repair outcomes also occur as a result of endonuclease cutting of DNA using single and paired guides in porcine blastocysts. Table 7 also shows that the frequencies of the observed DNA sequence associated with each repair outcome vary between guideRNA pairs. In some, but not all, instances the frequency of DNA sequence associated with the end-to-end joining of cut sites of the paired guideRNA was the most highly represented repair outcome. For some guideRNA pairs there was a single predominant DNA repair outcome, while for other guideRNA pairs there were DNA repair outcomes that occured with equal frequency. Most of the DNA repair outcomes shown for these five guideRNA pairs resulted in the deletion of CD163 exon 7 DNA sequences corresponding to the SRCR 5 domain. Therefore, the decision for which guideRNA pair to use for generation of edited pigs depends largely on the tolerance for multiple alleles in a population.

[0221] This example demonstrates that the NHEJ-mediated repair outcomes that result when two intronic guideRNAs are used to delete genomic DNA vary between guideRNA pairs, not only in the manner in which DNA breaks are resolved but in the frequency of these resolutions. It is advantageous to screen guideRNA pairs in fibroblasts or embryos to observe DNA repair outcomes of single or dual guideRNAs for the generation of edited animals.

Example 14

[0222] This example illustrates a real time PCR assay for identifying the presence of the spacer sequence set forth in SEQ ID NO: 249 and/or the desired Exon 7 excision edit in cells.

[0223] Two sets of primers and two probes were designed for this assay. One set of primers were designed to flank the spacer sequence set forth in SEQ ID NO: 249 in the unedited genome. The sequences of these primers are set forth in SEQ ID NOs: 556 and 557. A probe of sequence SEQ ID NO: 558 and labeled with the HEX fluorescent moiety was designed to anneal to the unedited genome between the PCR primers. The other set of primers, having sequences set forth in SEQ ID NOs: 562 and 563, were designed to flank the desired edit sequence. A probe, having a sequence set forth in SEQ ID NO: 564 and labeled with the FAM fluorescent moiety, was designed to anneal to nucleotides spanning the joining region of the edit. Real time PCR was performed using both primer sets and with genomic DNA extracted from tail and/or ear samples of pigs

that had known allelic status (wild type, homozygous, or heterozygous). 5 µl of 2x PRIMETIME® Master Mix (Integrated DNA Technologies, Coralville, IA) was mixed with 0.5 µl of each primer (10 µM), 0.5 µl of each probe (2.5 µM), and 2 µl of genomic DNA. PCR was performed with 3 minutes initial denaturing at 95° C, then 35 cycles of: 95° C for 15 seconds, 64° C for 30 seconds, and 72° C for 30 seconds. A final elongation was performed at 72° C for 2 minutes, and then the reaction was held in the cycler at 4° C. Fluorescence was measured and charted and, as expected, the homozygotes were close to the y axis (representing the FAM moiety wavelength), the heterozygotes grouped near the center of the chart, and the wild type pigs grouped close to the X axis (representing the HEX moiety wavelength). The assay was therefore successful in detecting the edit based on the spacer sequence set forth in SEQ ID NO: 249.

Example 15

[0224] This example illustrates a real time PCR assay for identifying the presence of the spacer sequence set forth in SEQ ID NO: 256 and/or the desired Exon 7 excision edit in cells.

[0225] Two sets of primers and two probes were designed for this assay. One set of primers flanked the spacer sequence set forth in SEQ ID NO: 256. The sequence of these primers is set forth in SEQ ID NOs: 559 and 560. A probe, having a sequence set forth in SEQ ID NO: 561 and labeled with the HEX fluorescent moiety, was designed to anneal to the unedited version of the spacer sequence. The other set of primers and probe are designed to target the desired edit and are described in Example 14 (SEQ ID NOs: 562-564.) Real time PCR was performed as described in Example 14, but with the instant primers and probes. Fluorescence was charted and, as expected, the homozygotes were close to the y axis (representing the FAM moiety), the heterozygotes grouped near the center of the chart, and the wild type pigs grouped close to the X axis (representing the HEX moiety). The assay was therefore successful in detecting the edit based on the spacer sequence set forth in SEQ ID NO: 256.

Example 16

[0226] This example illustrates a comparison of CD163 CRISPR-CAS gRNA activity in cells between previously published guide pairs and guide pairs of the present teachings.

[0227] Each pair of guides was tested in porcine fibroblasts as described in Example 2. Each pair was further tested in porcine blastocysts as described in Example 10. The results are shown below in Table 22.

Table 22 Comparative Activity in Porcine Cells

guide pair SEQ ID NOs:	Source	% desired repair fibroblasts	% desired repair blastocysts
272 and 273	Burkard 2017	6	20
249 and 256	Present Disclosure	38	44
354 and 211	Whitworth 2014	17	n.d.
362 and 390	Present Disclosure	45	31

[0228] These data illustrate that the guides of the present disclosure provide at least a two-fold improvement in the percentage of cells that have the desired edit relative to the guides that were previously disclosed in the literature. Therefore, the guides of the present disclosure are more efficient than the guides previously disclosed in the literature.

Example 17

[0229] This example illustrates CD163 edited pigs challenged with PRRSv Type I.

[0230] Pigs from PIC™ Line 2 were edited with guides as set forth in SEQ ID NOs: 249 and 256 as described in Example 6. Edits were confirmed as described in Example 5. Edited pigs were then crossbred to create pigs that were homozygous for the edit. These homozygous edited pigs were inoculated with 3 ml of PRSSv Type I (SD13-15) having 10^4 to 10^5 virions (4-5 log $TCID_{50}$). 1.5 ml was administered intramuscularly with a 21 gauge needle. The remaining 1.5 ml was administered intranasally. Serum samples were obtained on Day 0 (prior to inoculation on that day), Day 3, Day 5, Day 10, Day 14, and Day 21. Realtime PCR to determine the presence of virus in the serum samples using TETRACORE® EZ-PRRSV MPX 4.0 Master Mix and Enzyme with ROX (TETRACORE®, Rockville, MD) according to manufacturer directions. The real time PCR EU adjusted counts are shown in Table 23. The counts have been inverted using standard methods known in the art to make the data more intuitive—higher numbers indicate more virus detected.

Table 23 Realtime PCR EU Adjusted Counts for Type I PRRSv Challenge

Animal	CD163	Day 0	Day 3	Day 5	Day 10	Day 14	Day 21

2	WT	0	19.8	22.6	20	19.6	18.3
4	WT	0	17	17.7	18.6	16.8	17.5
5	WT	0	18.9	21.5	17.3	14.2	13.6
6	WT	0	15.8	19.7	15.6	14.5	13.4
17	WT	0	17.1	17.8	18.5	18	15.8
18	WT	0	15.8	17.9	17	16.8	15
20	WT	0	17.2	20.3	18.8	17	12.8
3	Edit	0	0	0	0	0	0
7	Edit	0	0	0	0	0	0
14	Edit	0	0	0	0	0	0
19	Edit	0	0	0	0	0	0

[0231] No PRRSv was detected in the serum of the edited animals throughout the experiment.

[0232] The serum samples were also subjected to ELISA using the IDEXX PRRS X3 antibody test kit; the test was performed by an accredited Veterinary Diagnostic Laboratory. The results are shown as a Sample:Positive ratio. Ratios greater than or equal to 0.40 are considered positive. The ratios for each sample are shown in Table 24.

Table 24 ELISA S/P Results for Type I PRRSv Challenge

Animal	**CD163**	**Day 0**	**Day 3**	**Day 5**	**Day 10**	**Day 14**	**Day 21**
2	WT	0.0	0.0	0.0	0.5	0.6	1.4
4	WT	0.0	0.0	0.0	0.1	0.2	0.8
5	WT	0.0	0.0	0.0	0.9	1.3	1.8
6	WT	0.0	0.0	0.0	1.3	1.3	1.9
17	WT	0.0	0.0	0.0	0.2	0.2	1.1
18	WT	0.0	0.0	0.0	0.3	0.3	1.4
20	WT	0.0	0.0	0.0	0.3	0.4	1.2
3	Edit	0.0	0.0	0.0	0.0	0.0	0.0
7	Edit	0.0	0.0	0.0	0.0	0.0	0.0
14	Edit	0.0	0.0	0.0	0.0	0.0	0.0
19	Edit	0.0	0.0	0.0	0.0	0.0	0.0

[0233] The edited pigs do not have any positive ratios. In contrast, by day 21, all of the wild type pigs have positive ratios. This further illustrates that there is no PRRSv circulating in the edited pigs' serum.

[0234] This example illustrates that pigs edited with guides of SEQ ID NOs: 249 and 256 are resistant to PRRSv Type I virus infection.

Example 18

[0235] This example illustrates CD163 edited pigs challenged with PRRSv Type II.

[0236] Pigs from PIC™ Lines 2 and 3 were edited with guides as set forth in SEQ ID NOs: 249 and 256 as described in Example 6. Edits were confirmed as described in Example 5. Edited pigs were then crossbred to create pigs that were homozygous for the edit. These homozygous edited pigs were then inoculated with 3 ml of PRRSv Type II (NVSL 97-7895) having 10^4 to 10^5 virions (4-5 log $TCID_{50}$). 1.5 ml was administered intramuscularly with a 21 gauge needle. The remaining 1.5 ml was administered intranasally. Serum samples were obtained on Day 0 (prior to inoculation on that day), Day 3, Day 5, Day 10, Day 14, and Day 21. Realtime PCR to determine the presence of virus in the serum samples using TETRACORE® EZ-PRRSV MPX 4.0 Master Mix and Enzyme with ROX according to manufacturer directions. The NA adjusted counts for real time PCR are shown in Table 25. The numbers were inverted to make them more intuitive—the higher the count, the more virus is present.

Table 25 Realtime PCR NA Adjusted Counts for Type II PRRSv Challenge

Animal	CD163	Day 0	Day 3	Day 5	Day 10	Day 14	Day 21
21	WT	0.0	18.4	22.0	21.9	18.0	16.1
23	WT	0.0	16.4	22.0	21.8	20.0	19.7
25	WT	N/A	17.2	21.2	21.9	19.2	19.8
30	WT	0.0	16.2	17.8	21.4	21.1	17.6
33	WT	0.0	18.5	N/A	21.8	18.5	15.3
35	WT	N/A	17.0	22.9	21.8	20.5	20.9
36	WT	0.0	16.7	21.4	21.9	19.9	17.4
38	WT	0.0	16.0	18.1	20.2	20.9	9.4
24	Edit	N/A	1.9	0.0	0.0	0.0	0.0
37	Edit	0.0	0.0	0.0	0.0	0.0	0.0

[0237] The edited pigs have very little to no virus counts relative to the wild type pigs.

[0238] The serum samples were also subjected to ELISA using the IDEXX PRRS X3 antibody test kit; the test was performed by an accredited Veterinary Diagnostic Laboratory. The results are shown as a Sample:Positive ratio. Ratios greater than or equal to 0.40 are considered positive. The ratios for each sample are shown in Table 26.

Table 26 ELISA S/P Ratios for Type II PRRSv Challenge

Animal	Edit	Day 0	Day 3	Day 5	Day 10	Day 14	Day 21
21	WT	0.0	0.0	0.0	1.7	2.0	1.9

23	WT	0.0	0.0	0.0	1.2	1.7	1.9
25	WT	N/A	0.0	0.0	1.2	1.6	1.7
30	WT	0.0	0.0	0.0	0.4	0.6	0.6
33	WT	0.0	0.0	N/A	0.7	0.6	0.7
35	WT	N/A	0.0	0.0	1.3	1.3	1.4
36	WT	0.0	0.0	0.0	1.2	1.5	1.8
38	WT	0.0	0.0	0.0	0.5	0.9	1.0
24	Edit	N/A	0.2	0.1	0.2	0.1	0.1
37	Edit	0.0	0.0	0.0	0.0	0.0	0.0

[0239] The edited pigs do not have any positive ratios; in contrast, by day 10, all of the wild type pigs have positive ratios. Taken together, these data illustrate that there is no virus circulating in the edited pigs' blood.

[0240] This example illustrates that the pigs with a CD163 gene edited with SEQ ID NOs: 249 and 256 are resistant to PRRSv Type II infection.

[0241] The contents of each of the foregoing references and applications are incorporated herein by reference in their entireties. Having described the present disclosure in detail, it will be apparent that modifications and variations are possible without departing from the scope of the teachings defined in the appended claims.

WHAT IS CLAIMED IS:

1. A CD163 gene edited to confer PRRSv resistance in *Sus scrofa* wherein the edit excises exon 7 and the edited gene comprises a repaired genomic sequence selected from the group consisting of SEQ ID NOs: 426-458 and 520-555.

2. The edited CD163 gene according to claim 1, wherein the edit is created using a pair of guideRNA (gRNAs) for exon 7 excision sites selected from the group consisting of SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 237 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 230 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 237 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 229 and 261, SEQ ID NOs: 230 and 261, SEQ ID NOs: 231 and 261, SEQ ID NOs: 237 and 261, SEQ ID NOs: 241 and 261, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 219 and 258, SEQ ID NOs: 221 and 258, SEQ ID NOs: 224 and 258, SEQ ID NOs: 227 and 258, SEQ ID NOs: 219 and 261, SEQ ID NOs: 221 and 261, SEQ ID NOs: 224 and 261, SEQ ID NOs: 227 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 250 and 258, SEQ ID NOs: 249 and 261, and SEQ ID NOs: 250 and 261.

3. The edited CD163 gene according to claim 1, wherein the edit is created using a pair of gRNAs for exon 7 excision sites selected from the group consisting of SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 227 and 258, SEQ ID NOs: 221 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, and SEQ ID NOs: 249 and 261.

4. The edited CD163 gene according to claim 1, wherein the repaired genomic sequence is set forth in SEQ ID NO: 453.

5. The edited CD163 gene according to claim 1, wherein the edit is created using sequences set forth in SEQ ID NOs: 249 and 256.

6. The edited CD163 gene according to claim 1, wherein the repaired genomic sequence is set forth in SEQ ID NO: 453 and the edit is created using sequences set forth in SEQ ID NOs: 249 and 256.

7. A *Sus scrofa* cell comprising the CD163 gene of any one of claims 1-6.

8. A cell line comprising a plurality of the cell according to claim 7.

9. The cell line according to claim 8, wherein the cell line is a fibroblast cell line.

10. The cell according to claim 7, wherein the cell is derived from PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65.

11. A CD163 gene edited to confer PRRSv resistance in *Sus scrofa* wherein the edit creates a stop codon resulting in a predicted exon 7 amino acid sequence selected from the group consisting of SEQ ID NOs: 506-517.

12. The CD163 gene according to claim 11, wherein the edit is created using gRNAs selected from the group consisting of SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 395, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID NOs: 354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs: 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, and SEQ ID NOs: 366 and 397.

13. The CD163 gene according to claim 11, wherein the edit is created using gRNAs selected from the group consisting of SEQ ID NOs: 351 and 365, SEQ NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 354 and 390, SEQ ID NOs: 358 and 394, SEQ ID NOs: 362 and 390, and SEQ ID NOs: 366 and 394.

14. The CD163 gene according to claim 11, wherein the repaired gene comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 459-504.

15. The CD163 gene according to claim 11, wherein the predicted amino acid sequence of CD163 exon 7 is set forth in SEQ ID NO: 513.

16. The CD163 gene according to claim 11, wherein the repaired gene has a nucleic acid sequence set forth in SEQ ID NO: 489.

17. The CD163 gene according to claim 11, wherein the edit is created using sequences set forth in SEQ ID NOs: 362 and 390.

18. The CD163 gene according to claim 11, wherein the predicted amino acid sequence of the gene is set forth in SEQ ID NO: 513, the repaired gene has a nucleic acid sequence set forth in SEQ ID NO: 489, and the edit is created using sequences set forth in SEQ ID NOs: 362 and 390.

19. A *Sus scrofa* cell comprising the CD163 gene of any one of claims 11-18.

20. A cell line comprising a plurality of the cell according to claim 19.

21. The cell line according to claim 20, wherein the cell line is a fibroblast cell line.

22. The cell according to claim 19, wherein the cell is derived from PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65.

23. A pair of gRNAs for editing a *Sus scrofa* CD163 gene selected from the group consisting of SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 237 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 230 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 237 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 229 and 261, SEQ ID NOs: 230 and 261, SEQ ID NOs: 231 and 261, SEQ ID NOs: 237 and 261, SEQ ID NOs: 241 and 261, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 219 and 258, SEQ ID NOs: 221 and 258, SEQ ID NOs: 224 and 258, SEQ ID NOs: 227 and 258, SEQ ID NOs: 219 and 261, SEQ ID NOs: 221 and 261, SEQ ID NOs: 224 and 261, SEQ ID NOs: 227 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 250 and 258, SEQ ID NOs: 249 and 261, SEQ ID NOs: 250 and 261, SEQ ID NOs: 351 and 365, SEQ ID NOs: 351 and 387, SEQ NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 348 and 395, SEQ ID NOs: 352 and 365, SEQ ID NOs: 352 and 387, SEQ ID NOs: 352 and 399, SEQ ID NOs: 353 and 365, SEQ ID NOs: 353 and 387, SEQ ID NOs: 353 and 399, SEQ ID NOs: 354 and 390, SEQ ID NOs: 354 and 388, SEQ ID NOs: 354 and 395, SEQ ID NOs: 358 and 361, SEQ ID NOs: 358 and 362, SEQ ID NOs: 358 and 368, SEQ ID NOs: 358 and 384, SEQ ID NOs: 358 and 394, SEQ ID NOs: 358 and 399, SEQ ID NOs: 359 and 390, SEQ ID NOs: 359 and 388, SEQ ID NOs: 359 and 395, SEQ ID NOs: 360 and 368, SEQ ID NOs: 360 and 384, SEQ ID

NOs: 360 and 389, SEQ ID NOs: 360 and 394, SEQ ID NOs: 360 and 397, SEQ ID NOs: 361 and 365, SEQ ID NOs: 361 and 387, SEQ ID NOs: 362 and 390, SEQ ID NOs: 362 and 388, SEQ ID NOs: 362 and 395, SEQ ID NOs: 364 and 365, SEQ ID NOs: 364 and 387, SEQ ID NOs: 364 and 399, SEQ ID NOs: 365 and 368, SEQ ID NOs: 365 and 384, SEQ ID NOs: 365 and 389, SEQ ID NOs: 365 and 394, SEQ ID NOs: 365 and 397, SEQ ID NOs: 366 and 368, SEQ ID NOs: 366 and 384, SEQ ID NOs: 366 and 389, SEQ ID NOs: 366 and 394, and SEQ ID NOs: 366 and 397.

24. The pair of gRNAs according to claim 23, wherein the pair of gRNAs is selected from the group consisting of SEQ ID NOs: 229 and 256, SEQ ID NOs: 230 and 256, SEQ ID NOs: 231 and 256, SEQ ID NOs: 241 and 256, SEQ ID NOs: 229 and 258, SEQ ID NOs: 231 and 258, SEQ ID NOs: 241 and 258, SEQ ID NOs: 219 and 256, SEQ ID NOs: 221 and 256, SEQ ID NOs: 224 and 256, SEQ ID NOs: 227 and 256, SEQ ID NOs: 227 and 258, SEQ ID NOs: 221 and 261, SEQ ID NOs: 249 and 256, SEQ ID NOs: 250 and 256, SEQ ID NOs: 249 and 258, SEQ ID NOs: 249 and 261, SEQ ID NOs: 351 and 365, SEQ ID NOs: 348 and 390, SEQ ID NOs: 348 and 388, SEQ ID NOs: 354 and 390, SEQ ID NOs: 358 and 394, SEQ ID NOs: 362 and 390, and SEQ ID NOs: 366 and 394.

25. A CRISPR complex for editing a CD163 gene of *Sus scrofa* comprising the pair of gRNAs of any one of claims 23-24.

26. A method for editing a CD163 gene of *Sus scrofa* comprising using a CRISPR-CAS complex comprising the pair of gRNAs of any one of claims 23-24.

27. A method for preparing a PRSSv resistant *Sus scrofa* cell by using a CRISPR-CAS complex comprising the pair of gRNAs of any one of claims 23-24.

28. A method of producing PRRSv resistant *Sus scrofa* animals, comprising:

a) editing a CD163 gene of a *Sus scrofa* cell or a plurality of *Sus scrofa* cells using a CRISPR complex comprising the pair of gRNAs of any one of claims 23-24; and

b) producing an animal from the cell or plurality of cells.

29. Use of a cell line according to claim 8 in producing PRRSv resistant animals.

30. Use of a cell line according to claim 19 in producing PRRSv resistant animals.

31. An embryo, piglet, or mature adult comprising a plurality of the cell according to any one of claims 7-10 or 19-22.

32. A method of determining the presence or absence of an edited sequence having 90% identity with a sequence set forth in SEQ ID NO: 453 comprising performing real time

PCR with

a) differentially labeled probes of sequences set forth in SEQ ID NO: 564 and SEQ ID NO: 558 or 561

b) a primer pair set forth in SEQ ID NOs: 562 and 563; and

c) a primer pair set forth in SEQ ID NOs: 556 and 557 or SEQ ID NOs: 559 and 560.

33. A method according to claim 32, wherein the edited sequence has 100% identity with the sequence set forth in SEQ ID NO: 453.

34. A PCR primer selected from the group consisting of SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 559, SEQ ID NO: 560, SEQ ID NO: 562, and SEQ ID NO: 563.

35. A real time PCR probe selected from the group consisting of SEQ ID NO: 558, SEQ ID NO: 561, and SEQ ID NO: 564.

36. Use of PCR primers set forth in a) SEQ ID NO: 556 and 557 and SEQ ID NO: 562 and 563 or b) SEQ ID NO: 559 and 560 and SEQ ID NO: 562 and 563 to determine the presence or absence of an edited genome sequence set forth in SEQ ID NO: 453.

37. Use of PCR probes set forth in a) SEQ ID NOs: 558 and 564 or b) SEQ ID NOs: 561 and 564 to determine the presence or absence of an edited genome sequence set forth in SEQ ID NO: 453.

38. A method of creating a PRRSv resistant pig comprising editing the pig's genome to comprise a genomic sequence as set forth in SEQ ID NO: 453.

39. A method according to claim 38, wherein editing the pig's genome comprises administering gRNAs having sequences set forth in SEQ ID NOs: 249 and 256.

40. A method according to claim 39, wherein the administering comprises injecting pre-formed RNP complexes comprising the gRNAs and a CAS protein into a zygote, embryo, or MII oocyte.

41. A method according to claim 38, wherein the pig is a PIC line 2, PIC line 3, PIC line 15, PIC line 19, PIC line 27, PIC line 62, or PIC line 65 pig.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2021/051060

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/027 C07K14/705 C12N15/877 C12N15/90 C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01K C12Q C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/073237 A1 (UNIV COURT UNIV OF EDINBURGH [GB]) 26 April 2018 (2018-04-26)	1-3, 7-15, 19-32, 34,35
Y	figures 1A, 1C	11-15
A		4-6, 16-18, 33,36-41
	----- -/--	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2021	19/07/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Deleu, Laurent

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2021/051060

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHRISTINE BURKARD ET AL: "Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function", PLOS PATHOGENS, vol. 13, no. 2, 23 February 2017 (2017-02-23), page e1006206, XP055554369, DOI: 10.1371/journal.ppat.1006206 figures 1, 2	1-3, 7-15, 19-32, 34,35
Y	K. M. WHITWORTH ET AL: "Use of the CRISPR/Cas9 System to Produce Genetically Engineered Pigs from In Vitro-Derived Oocytes and Embryos", BIOLOGY OF REPRODUCTION, vol. 91, no. 3, 6 August 2014 (2014-08-06) , pages 78-78, XP055242497, US ISSN: 0006-3363, DOI: 10.1095/biolreprod.114.121723 table 6	11-15

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2021/051060

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2021/051060

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018073237 A1	26-04-2018	AU 2017344936 A1	04-04-2019
		CA 3037451 A1	26-04-2018
		CN 109862786 A	07-06-2019
		EP 3525581 A1	21-08-2019
		JP 2019533445 A	21-11-2019
		KR 20190067212 A	14-06-2019
		PH 12019500624 A1	19-08-2019
		RU 2019110035 A	05-10-2020
		US 2020045945 A1	13-02-2020
		WO 2018073237 A1	26-04-2018