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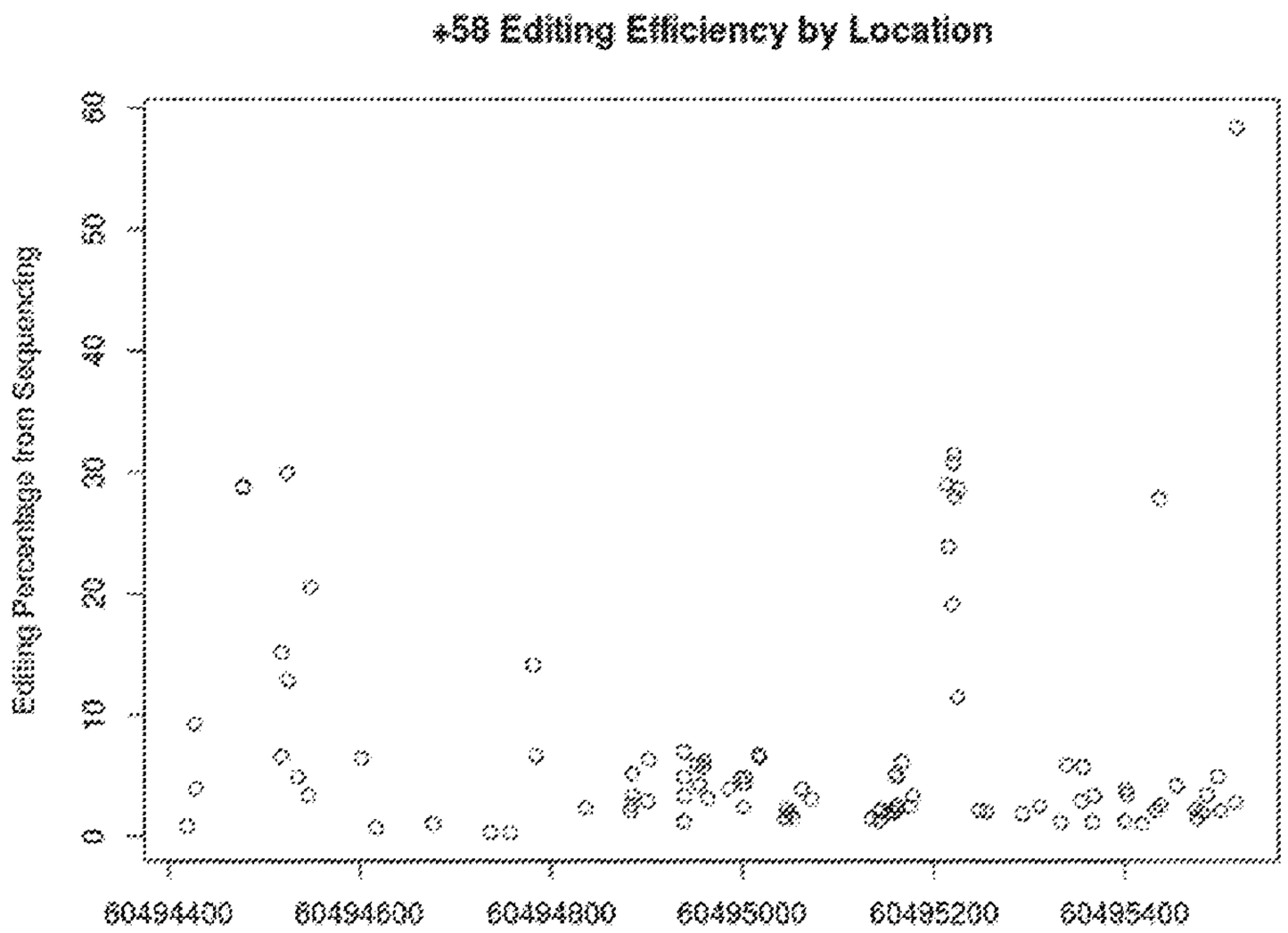
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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF HEMOGLOBINOPATHIES

Figure 1



(57) Abrégé/Abstract:
The present invention is directed to genome editing systems, reagents and methods for the treatment of hemoglobinopathies.

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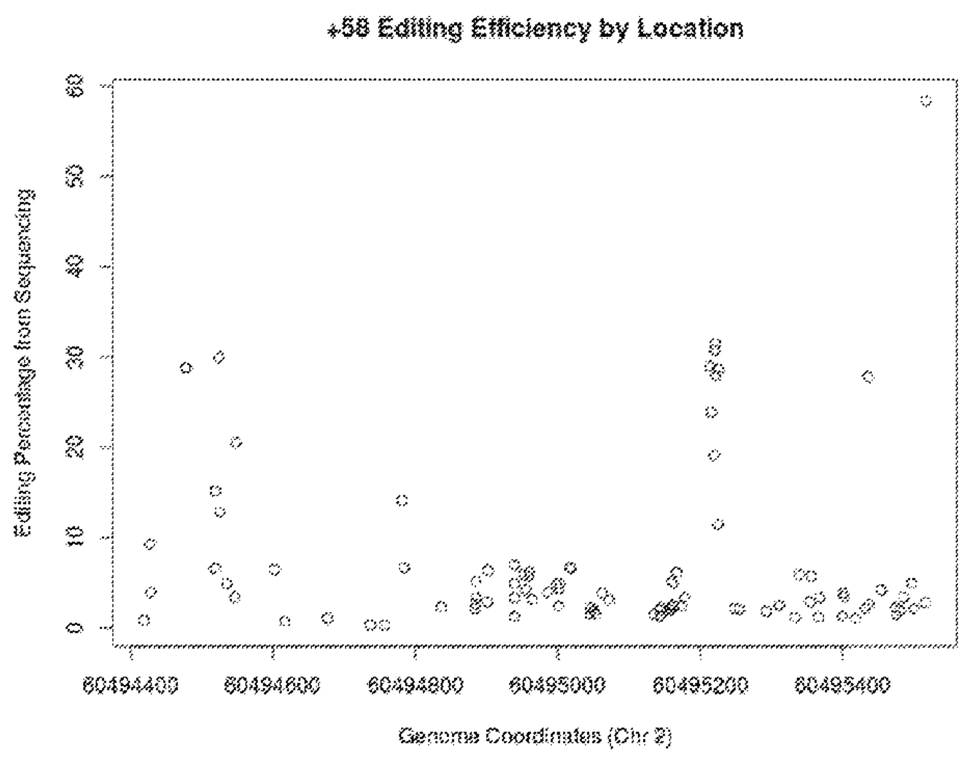
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(54) **Title**: COMPOSITIONS AND METHODS FOR THE TREATMENT OF HEMOGLOBINOPATHIES

Figure 1



(57) **Abstract**: The present invention is directed to genome editing systems, reagents and methods for the treatment of hemoglobino - pathies.

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COMPOSITIONS AND METHODS FOR THE TREATMENT OF HEMOGLOBINOPATHIES

RELATED APPLICATIONS

This application claims priority to U.S. Provisional patent application number 62/271,968, filed December 28, 2015, and U.S. Provisional patent application number 62/347,484, filed June 8, 2016. The entire contents of these applications are incorporated herein by reference.

BACKGROUND

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) evolved in bacteria as an adaptive immune system to defend against viral attack. Upon exposure to a virus, short segments of viral DNA are integrated into the CRISPR locus of the bacterial genome. RNA is transcribed from a portion of the CRISPR locus that includes the viral sequence. That RNA, which contains sequence complimentary to the viral genome, mediates targeting of a Cas9 protein to the sequence in the viral genome. The Cas9 protein cleaves and thereby silences the viral target.

Recently, the CRISPR/Cas system has been adapted for genome editing in eukaryotic cells. The introduction of site-specific single (SSBs) or double strand breaks (DSBs) allows for target sequence alteration through, for example, non-homologous end-joining (NHEJ) or homology-directed repair (HDR).

SUMMARY OF THE INVENTION

Without being bound by theory, the invention is based in part on the discovery that CRISPR systems, e.g., Cas9 CRISPR systems, e.g., as described herein, can be used to modify cells (e.g., hematopoietic stem and progenitor cells (HSPCs)) to increase fetal hemoglobin (HbF) expression and/or decrease expression of beta globin (e.g., a beta globin gene having a disease-causing mutation), and that such cells may be used to treat hemoglobinopathies, e.g., sickle cell disease and beta thalassemia.

Thus, in an aspect, the invention provides CRISPR systems (e.g., Cas CRISPR systems, e.g., Cas9 CRISPR systems, e.g., *S. pyogenes* Cas9 CRISPR systems) comprising one or more, e.g., one, gRNA molecule as described herein. Any of the gRNA molecules described herein may be used in such systems, and in the methods and cells described herein.

In an aspect, the invention provides a gRNA molecule that includes a tracr and crRNA, wherein the crRNA includes a targeting domain that is complementary with a target sequence of the BCL11A gene, a BCL11a enhancer, or a HFPH region.

In another aspect, the invention provides a gRNA molecule that includes a targeting domain that is complementary with a target sequence of the BCL11A gene, e.g., is complementary with a target

In an aspect, the invention provides a gRNA molecule that includes a tracr and crRNA, wherein the crRNA includes a targeting domain that is complementary with a target sequence of the BCL11A gene, a BCL11a enhancer, or a HFPH region.

In another aspect, the invention provides a gRNA molecule that includes a targeting domain that is complementary with a target sequence of the BCL11A gene, e.g., is complementary with a target sequence within a BCL11a coding region (e.g., within a BCL11a exon, e.g., within BCL11a exon 2).

In embodiments, the gRNA comprises a targeting domain that includes, e.g., consists of, any one of SEQ ID NO: 1 to SEQ ID NO: 85 or SEQ ID NO: 400 to SEQ ID NO: 1231.

In another aspect, the invention provides a gRNA molecule that includes a targeting domain that is complementary with a target sequence of a BCL11A enhancer.

In embodiments, the gRNA comprises a targeting domain that includes, e.g., consists of, any one of SEQ ID NO: 1232 to SEQ ID NO: 1499.

In embodiments, the gRNA to a target sequence of a BCL11a enhancer is to a target sequence within the +58 region of the BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 182 to SEQ ID NO: 277 or SEQ ID NO: 334 to SEQ ID NO: 341. In embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 341, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 247, SEQ ID NO: 245, SEQ ID NO: 249, SEQ ID NO: 244, SEQ ID NO: 199, SEQ ID NO: 251, SEQ ID NO: 250, SEQ ID NO: 334, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 336, or SEQ ID NO: 337. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 248. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 247. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 245. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 336. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 337. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 338. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 335. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 252. In an embodiment, the gRNA is a dgRNA that includes a crRNA that includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 248 – SEQ ID NO: 6607, and a tracr that includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 6660. In an embodiment, the gRNA is a dgRNA that includes a crRNA that includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 247 – SEQ ID NO: 6607, and a tracr that includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 6660. In an embodiment, the gRNA molecule is a sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 338 – SEQ ID NO: 6604 – UUUU. In an embodiment, the gRNA molecule is a sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 335 – SEQ ID NO: 6604 – UUUU. In an embodiment, the gRNA molecule is a sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 336 – SEQ ID NO: 6604 – UUUU. In an embodiment, the gRNA molecule is a

sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 245 – SEQ ID NO: 6604 – UUUU. In an embodiment, the gRNA molecule is a sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 337 – SEQ ID NO: 6604 – UUUU. In an embodiment, the gRNA molecule is a sgRNA molecule and includes, e.g., consists of, e.g., from 5' to 3', SEQ ID NO: 252 – SEQ ID NO: 6604 – UUUU.

In embodiments, the gRNA to a target sequence of a BCL11a enhancer is to a target sequence within the +62 region of the BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 278 to SEQ ID NO: 333. In embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 318, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 294, SEQ ID NO: 310, SEQ ID NO: 319, SEQ ID NO: 298, SEQ ID NO: 322, SEQ ID NO: 311, SEQ ID NO: 315, SEQ ID NO: 290, SEQ ID NO: 317, SEQ ID NO: 309, SEQ ID NO: 289, or SEQ ID NO: 281. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 318.

In embodiments, the gRNA to a target sequence of a BCL11a enhancer is to a target sequence within the +55 region of the BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1596 to SEQ ID NO: 1691. In embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1683, SEQ ID NO: 1638, SEQ ID NO: 1647, SEQ ID NO: 1609, SEQ ID NO: 1621, SEQ ID NO: 1617, SEQ ID NO: 1654, SEQ ID NO: 1631, SEQ ID NO: 1620, SEQ ID NO: 1637, SEQ ID NO: 1612, SEQ ID NO: 1656, SEQ ID NO: 1619, SEQ ID NO: 1675, SEQ ID NO: 1645, SEQ ID NO: 1598, SEQ ID NO: 1599, SEQ ID NO: 1663, SEQ ID NO: 1677, or SEQ ID NO: 1626.

In another aspect, the invention provides a gRNA molecule that includes a targeting domain that is complementary with a target sequence of a hereditary persistence of fetal hemoglobin (HPFH) region. In an embodiment, the HPFH region is the French HPFH region. In embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 86 to SEQ ID NO: 181 or SEQ ID NO: 1500 to SEQ ID NO: 1595. In embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 100, SEQ ID NO: 165, SEQ ID NO: 113, SEQ ID NO: 99, SEQ ID NO: 112, SEQ ID NO: 98, SEQ ID NO: 1580, SEQ ID NO: 106, SEQ ID NO: 1503, SEQ ID NO: 1589, SEQ ID NO: 160, SEQ ID NO: 1537, SEQ ID NO: 159, SEQ ID NO: 101, SEQ ID NO: 162, SEQ ID NO: 104, SEQ ID NO: 138, SEQ ID NO: 1536, SEQ ID NO: 1539, SEQ ID NO: 1585. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 100. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 165. In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 113.

In any of the aforementioned embodiments, the gRNA molecule may further have regions and/or properties described herein. In an aspect, the gRNA molecule (e.g., of any of the aforementioned aspects or embodiments) includes a targeting domain that includes, e.g., consists of, 17, 18, 19, 20,

21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences. In embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids disposed at the 3' end of the recited targeting domain sequence. In embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids disposed at the 5' end of the recited targeting domain sequence. In embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences do not include either the 5' or 3' nucleic acid of the recited targeting domain sequence. In any of the aforementioned aspects or embodiments, the targeting domain may consist of the recited targeting domain sequence.

In embodiments of the gRNA molecule, including in any of the aforementioned aspects and embodiments, the targeting domain includes 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids of any one of the recited targeting domain sequences. In other embodiments of the gRNA molecule, including in any of the aforementioned aspects and embodiments, the targeting domain consists of 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids of any one of the recited targeting domain sequences. In embodiments of the gRNA molecule, including in any of the aforementioned aspects and embodiments, the 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence) consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids disposed at the 3' end of the recited targeting domain sequence. In other embodiments of the gRNA molecule, including in any of the aforementioned aspects and embodiments, the 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids disposed at the 5' end of the recited targeting domain sequence. In other embodiments of the gRNA molecule, including in any of the aforementioned aspects and embodiments, the 17, 18, 19, 20, 21 (if present in the reference sequence), 22 (if present in the

reference sequence), 23 (if present in the reference sequence), or 24 (if present in the reference sequence), or 25 (if present in the reference sequence) consecutive nucleic acids of any one of the recited targeting domain sequences do not include either the 5' or 3' nucleic acid of the recited targeting domain sequence.

In an aspect, including in any of the aforementioned aspects or embodiments, the gRNA molecule includes a portion of the crRNA and a portion of the tracr that hybridize to form a flagpole, that includes SEQ ID NO: 6584 or 6585. In embodiments, the flagpole further includes a first flagpole extension, located 3' to the crRNA portion of the flagpole, wherein said first flagpole extension includes SEQ ID NO: 6586. In embodiments, the flagpole further includes a second flagpole extension located 3' to the crRNA portion of the flagpole and, if present, the first flagpole extension, wherein said second flagpole extension includes SEQ ID NO: 6587.

In an aspect, including in any of the aforementioned aspects or embodiments, the invention provides a gRNA molecule that includes a tracr that includes, e.g., consists of, SEQ ID NO: 6660 or SEQ ID NO: 6661. In embodiments, the crRNA portion of the flagpole includes SEQ ID NO: 6607 or SEQ ID NO: 6608.

In an aspect, including in any of the aforementioned aspects or embodiments, the invention provides a gRNA molecule that includes a tracr that includes SEQ ID NO: 6589 or 6590, and optionally, if a first flagpole extension is present, a first tracr extension, disposed 5' to SEQ ID NO: 6589 or 6590, said first tracr extension including SEQ ID NO: 6591.

In an aspect, including in any of the aforementioned aspects or embodiments, the invention provides a gRNA molecule wherein the targeting domain and the tracr are disposed on separate nucleic acid molecules (e.g., a dgRNA molecule).

In an aspect, including in any of the aforementioned aspects or embodiments, the invention provides a gRNA molecule wherein the targeting domain and the tracr are disposed on a single nucleic acid molecule (e.g., a sgRNA molecule), and wherein the tracr is disposed 3' to the targeting domain. In embodiments, the sgRNA molecule includes a loop, disposed 3' to the targeting domain and 5' to the tracr, e.g., a loop including, e.g., consisting of, SEQ ID NO: 6588.

In an aspect, including in any of the aforementioned aspects or embodiments, the invention provides a gRNA molecule including, from 5' to 3', [targeting domain]-:

(a) SEQ ID NO: 6601;

(b) SEQ ID NO: 6602;

(c) SEQ ID NO: 6603;

(d) SEQ ID NO: 6604; or

(e) any of (a) to (d), above, further including, at the 3' end, 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides, e.g., 4 uracil nucleotides. In embodiments, there are no intervening nucleotides between the targeting domain and the sequence of any of (a)-(e).

In another aspect, the invention provides CRISPR systems, e.g., Cas CRISPR systems, e.g., Cas9 CRISPR systems, e.g., *S. pyogenes* Cas9 CRISPR systems, the include one or more, e.g., one, gRNA molecule of any of the aforementioned aspects and embodiments. In an aspect, the invention provides a composition including a first gRNA molecule of any of the aforementioned aspects and embodiments, further including a Cas9 molecule. In embodiments, the Cas9 molecule is an active or inactive *s. pyogenes* Cas9. In embodiments, the first gRNA molecule and Cas9 molecule are present in a ribonuclear protein complex (RNP).

In another aspect, the invention provides compositions that include more than one gRNA, e.g., more than one gRNA molecule as described herein, e.g., more than one gRNA molecule of any of the aforementioned gRNA molecule aspects or embodiments. Thus, in a further aspect, the invention provides a composition of any of the aforementioned composition aspects and embodiments, further including a second gRNA molecule; a second gRNA molecule and a third gRNA molecule; or a second gRNA molecule, a third gRNA molecule, and a fourth gRNA molecule, wherein the second gRNA molecule, the third gRNA molecule (if present), and the fourth gRNA molecule (if present) are a gRNA molecule as described herein, e.g., a gRNA molecule of any of the aforementioned gRNA molecule aspects or embodiments. In an embodiment, each gRNA molecule of the composition is complementary to a different target sequence. In an embodiment, each gRNA molecule is complementary to target sequences within the same gene or region. In an aspect, the first gRNA molecule, the second gRNA molecule, the third gRNA molecule (if present), and the fourth gRNA molecule (if present) are complementary to target sequences not more than 20000 nucleotides, not more than 10000 nucleotides, not more than 6000, not more than 5000 nucleotides, not more than 4000, not more than 1000 nucleotides, not more than 500 nucleotides, not more than 400 nucleotides, not more than 300 nucleotides, not more than 200 nucleotides, not more than 100 nucleotides, not more than 90 nucleotides, not more than 80 nucleotides, not more than 70 nucleotides, not more than 60 nucleotides, not more than 50 nucleotides, not more than 40 nucleotides, not more than 30 nucleotides, not more than 20 nucleotides or not more than 10 nucleotides apart. In another aspect, each gRNA molecule of the composition is complementary to target sequence within a different gene or region.

Specific and preferred combinations of more than one gRNA molecule of the invention are described herein. In an aspect, the composition includes a first gRNA molecule and a second gRNA molecule,

wherein the first gRNA molecule and second gRNA molecule are complementary to different target sequences, and are:

(a) independently selected from the gRNA molecules described herein (e.g., above) to the BCL11a gene;

(b) independently selected from the gRNA molecules described herein (e.g., above) to the +58 BCL11a enhancer;

(c) independently selected from the gRNA molecules described herein (e.g., above) to the +62 BCL11a enhancer;

(d) independently selected from the gRNA molecules described herein (e.g., above) to the +55 BCL11a enhancer; or

(e) independently selected from the gRNA molecules described herein (e.g., above) to a HPFH region.

In an aspect, the composition includes a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are complementary to different target sequences, and:

(a) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the BCL11a gene, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +58 BCL11a enhancer;

(b) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the BCL11a gene, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +62 BCL11a enhancer;

(c) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the BCL11a gene, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +55 BCL11a enhancer;

(d) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the BCL11a gene, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to a HPFH region;

(e) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +58 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +62 BCL11a enhancer;

(f) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +58 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +55 BCL11a enhancer;

(g) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +58 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to a HPFH region;

(h) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +62 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +55 BCL11a enhancer;

(i) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +62 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to a HPFH region;

(j) the first gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to the +55 BCL11a enhancer, and the second gRNA molecule is selected from the gRNA molecules described herein (e.g., above) to a HPFH region;

In another aspect, the composition that includes a first gRNA molecule and a second gRNA molecule, includes:

(a) a first gRNA molecule that is selected from the gRNA molecules described herein (e.g., above) to a HPFH region, and a second gRNA molecule includes a targeting domain that is complementary to a target sequence of the beta globin gene; or

(b) a first gRNA molecule that is selected from the gRNA molecules described herein (e.g., above) to a BCL11a enhancer, e.g., a +58 BCL11a enhancer, a +55 BCL11a enhancer or a +62 BCL11a enhancer, and a second gRNA molecule includes a targeting domain that is complementary to a target sequence of the beta globin gene.

In any of the aforementioned composition aspects and embodiments, the composition may consist of, with respect to the gRNA components of the composition, a first gRNA molecule and a second gRNA molecule.

In any of the aforementioned composition aspects and embodiments, the compositions may be formulated in a medium suitable for electroporation.

In another aspect, the invention provides nucleic acids encoding the gRNA molecule(s) and/or Cas molecules of the CRISPR systems. Without being bound by theory, it is believed that delivering such

nucleic acids to cells will lead to the expression of the CRISPR system within the cell. In an aspect, the invention provides a nucleic acid sequence that encodes one or more gRNA molecules of any of the previous gRNA aspects and embodiments. In embodiments, the nucleic acid includes a promoter operably linked to the sequence that encodes the one or more gRNA molecules. In embodiments, the promoter is a promoter recognized by an RNA polymerase II or RNA polymerase III. In embodiments, the promoter is a U6 promoter or an HI promoter.

In further aspects, the nucleic acid further comprises sequence encoding a Cas9 molecule. In embodiments, the nucleic acid includes a promoter operably linked to the sequence that encodes a Cas9 molecule. In embodiments, the promoter is an EF-1 promoter, a CMV IE gene promoter, an EF-1α promoter, an ubiquitin C promoter, or a phosphoglycerate kinase (PGK) promoter.

In an aspect the invention provides a vector that includes the nucleic acid of any of the previous nucleic acid aspects and embodiments. In embodiments, the vector is selected from the group consisting of a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

In another aspect, the invention provides a composition including one or more gRNA molecules, e.g., as described herein, e.g., in any of the previous gRNA molecule aspects and embodiments, and nucleic acid encoding a Cas9 molecule.

In another aspect, the invention provides a composition including nucleic acid encoding one or more gRNA molecules (e.g., as described herein, e.g., of any of the previous gRNA molecule aspects and embodiments), and a Cas9 molecule.

In another aspect, the invention provides a composition, e.g., a composition of any of the aforementioned composition aspects and embodiments, further including a template nucleic acid. In another aspect, the invention provides a composition, e.g., a composition of any of the aforementioned composition aspects and embodiments, further including nucleic acid sequence encoding a template nucleic acid. In embodiments, the template nucleic acid includes a nucleotide that corresponds to a nucleotide of a target sequence of the gRNA molecule. In embodiments, the template nucleic acid includes nucleic acid encoding human beta globin, e.g., human beta globin including one or more of the mutations G16D, E22A and T87Q. In embodiments, the template nucleic acid includes nucleic acid encoding human gamma globin.

In another aspect, the invention provides cells that include (or at any time included) a gRNA molecule, CRISPR system, composition or nucleic acid (e.g., as described herein, e.g., as in any of the aforementioned aspects and embodiments). In such aspects, the invention provides cells that have been modified by such inclusion.

Thus, in an aspect, the invention provides a method of altering e.g., altering the structure, e.g., sequence, of a target sequence within nucleic acid of a cell, including contacting said cell with:

1) one or more gRNA molecules of any of the aforementioned gRNA molecule aspects and embodiments, and a Cas9 molecule (e.g., as described herein);

2) one or more gRNA molecules of any of the aforementioned gRNA molecule aspects and embodiments, and nucleic acid encoding a Cas9 molecule (e.g., as described herein);

3) nucleic acid encoding one or more gRNA molecules of any of the aforementioned gRNA molecule aspects and embodiments, and a Cas9 molecule (e.g., as described herein);

4) nucleic acid encoding one or more gRNA molecules of the aforementioned gRNA molecule aspects and embodiments, and nucleic acid encoding a Cas9 molecule (e.g., as described herein); or

5) any of 1) to 4), above, and a template nucleic acid (e.g., as described herein);

6) any of 1) to 4) above, and nucleic acid including sequence encoding a template nucleic acid (e.g., as described herein);

7) a composition as described herein (e.g., of any of the aforementioned composition aspects and embodiments); or

8) the vector of any of the aforementioned vector aspects and embodiments.

In embodiments, the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in a single composition. In other embodiments, the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in more than one composition. In embodiments, the more than one composition are delivered simultaneously or sequentially.

In embodiments, the cell is an animal cell. In embodiments, the cell the cell is a mammalian, primate, or human cell. In embodiments, the cell is a hempatopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs). In embodiments, the cell is a CD34+ cell. In embodiments, the cell is a CD34+/CD38-/CD90+/CD45RA- cell. In embodiments, the cell is a CD34+/CD90+/CD49f+ cell. In embodiments, the cell is a CD34+/CD38-/CD90+/CD45RA-/CD49f+ cell. In embodiments, the method includes a population of cells that has been enriched for HSPCs, e.g., for CD34+ cells.

In embodiments, the cell (e.g. population of cells) has been isolated from bone marrow. In embodiments, the cell (e.g. population of cells) has been isolated from mobilized peripheral blood. In embodiments, the cell (e.g. population of cells) has been isolated from umbilical cord blood.

In embodiments, the cell (e.g. population of cells) is autologous with respect to a patient to be administered said cell (e.g. population of cells). In embodiments, the cell (e.g. population of cells) is allogeneic with respect to a patient to be administered said cell (e.g. population of cells).

In embodiments, of the methods described herein, the altering results in an increase of fetal hemoglobin expression in the cell. In embodiments, of the methods described herein, the altering results in a reduction of fetal hemoglobin expression in the cell.

In another aspect, the invention provides a cell, altered by the method of any of the aforementioned method aspects and embodiments. In another aspect, the invention provides a cell, including a first gRNA molecule (e.g., as described herein), or a composition (e.g., as described herein), or a nucleic acid encoding the first gRNA molecule (e.g., as described herein), of any of the previous aspects and embodiments. In embodiments, the cell is an animal cell. In embodiments, the cell the cell is a mammalian, primate, or human cell. In embodiments, the cell is a hematopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs). In embodiments, the cell is a CD34+ cell. In embodiments, the cell is a CD34+/CD38-/CD90+/CD45RA- cell. In embodiments, the cell is a CD34+/CD90+/CD49f+ cell. In embodiments, the method includes a population of cells that has been enriched for HSPCs, e.g., for CD34+ cells.

In embodiments, the cell (e.g. population of cells) has been isolated from bone marrow. In embodiments, the cell (e.g. population of cells) has been isolated from mobilized peripheral blood. In embodiments, the cell (e.g. population of cells) has been isolated from umbilical cord blood.

In embodiments, the cell (e.g. population of cells) is autologous with respect to a patient to be administered said cell (e.g. population of cells). In embodiments, the cell (e.g. population of cells) is allogeneic with respect to a patient to be administered said cell (e.g. population of cells).

In embodiments, the cell includes, has included, or will include a second gRNA molecule, e.g., as described herein, e.g., of any of the previous gRNA molecule aspects and embodiments, or a nucleic acid encoding the second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule include nonidentical targeting domains.

In embodiments, expression of fetal hemoglobin is increased in the cell, e.g., relative to a cell of the same cell type that has not been modified to include a gRNA molecule (e.g., as described herein). In embodiments, expression of beta globin is decreased in the cell, e.g., relative to a cell of the same cell type that has not been modified to include a gRNA molecule (e.g., as described herein). In embodiments, expression of fetal hemoglobin is increased and expression of beta globin is decreased in the cell, e.g., relative to a cell of the same cell type that has not been modified to include a gRNA molecule (e.g., as described herein).

In an aspect, the cells of the invention (or methods comprising a cell) have been contacted with a stem cell expander, e.g., as described herein, e.g., compound 1, compound 2, compound 3 or compound 4. In an aspect, the cells of the invention (or methods comprising a cell) have been contacted with a stem cell expander, e.g., as described herein, e.g., compound 1, compound 2, compound 3, compound 4 or a combination thereof (e.g., compound 1 and compound 4). In an embodiment, the stem cell expander is compound 4. In an embodiment, the stem cell expander is a combination of compound 1 and compound 4. In embodiments, the contacting is ex vivo.

In another aspect, the invention provides methods of treatment, e.g., methods of treatment of hemoglobinopathies. In an aspect, the invention provides a method of treating a hemoglobinopathy, that includes administering to a patient a cell (e.g., a population of cells) of any of the previous cell or method aspects and embodiments.

In another aspect, the invention provides a method of increasing fetal hemoglobin expression in a mammal, including administering to a patient a cell of any of the previous cell or method aspects and embodiments. In an embodiment, the hemoglobinopathy is beta-thalassemia or sickle cell disease.

In another aspect, the invention provides a guide RNA molecule, e.g., as described herein, for use as a medicament, e.g., for use in the treatment of a disease. In embodiments, the disease is a hemoglobinopathy, e.g., beta-thalassemia or sickle cell disease.

In another aspect, the invention provides a gRNA molecule, e.g., as described herein, e.g., as described in any of the aforementioned gRNA molecule aspects and embodiments, wherein when a CRISPR system including the gRNA is introduced into a cell (e.g., a CD34+ cell, e.g., an HSC), at least about 15% of the indels produced include (a) a frameshift mutation; or (b) a large deletion, relative to unmodified target DNA, as measured by NGS. In embodiments, at least about 25% of the indels produced include (a) a frameshift mutation; or (b) a large deletion, relative to unmodified target DNA, as measured by NGS. In embodiments, at least about 40%, at least about 50%, at least about 60%, at least about 70% at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% of the indels produced include (a) a frameshift mutation; or (b) a large deletion, relative to unmodified target DNA, as measured by NGS.

In another aspect, the invention provides methods for the ex vivo expansion and modification of HSPCs (e.g., CD34+ cells). In an aspect, the invention provides a method of modifying cells (e.g., a population of cells) including:

(a) providing a population of cells;

(b) expanding said cells ex vivo in the presence of a stem cell expander; and

(c) introducing into said cells a first gRNA molecule (e.g., as described herein, e.g., of any of the aforementioned gRNA molecule aspects and embodiments), a nucleic acid molecule encoding the first gRNA molecule, or a composition (e.g., as described herein, e.g., of any of the aforementioned composition aspects and embodiments);

such that hemoglobin, e.g., fetal hemoglobin, expression is increased in said cells relative to the same cell type which have not been subjected to step (c).

In embodiments, the cells are introduced into a subject in need thereof, e.g., a subject that has a hemoglobinopathy, e.g., sickle cell disease or beta thalassemia.

In embodiments, the cells are or comprise CD34+ cells. In embodiments, the cells are or comprise HSPCs. In embodiments, the cells are isolated from bone marrow, mobilized peripheral blood or umbilical cord blood. In a preferred embodiment, the cells are isolated from bone marrow. In other embodiments, the cells are isolated from mobilized peripheral blood. In aspects, the moblized peripheral blood is isolated from a subject who has been administered a G-CSF. In aspects, the moblized peripheral blood is isolated from a subject who has been administered a moblization agent other than G-CSF, for example, Plerixafor® (AMD3100). In embodiments, the cells are an enriched population of cells.

In embodiments, the stem cell expander is compound 1, compound 2, compound 3, or compound 4, e.g., compound 4. In embodiments, the stem cell expander is compound 1, compound 2, compound 3, compound 4, or a combination thereof (e.g., a combination of compound 1 and compound 4). In embodiments, the cells are contacted with compound 4 at a concentration of from about 1 to about 200 micromolar (uM). In an embodiment, the concentration of compound 4 is about 75 micromolar (uM). In embodiments, the expanding said cells ex vivo in the presence of a stem cell expander occurs for a period of about 1-10 days, e.g., about 1-5 days, e.g., about 2-5 days, e.g., about 4 days.

In embodiments, the cells are autologous to a patient intended to be administered said cells. In embodiments, the cells are allogeneic to a patient intended to be administered said cells.

In embodiments, the expanding of step (b) is further in the presence of thrombopoietin (Tpo), Flt3 ligand (Flt-3L), human stem cell factor (SCF) and human interleukin-6 (IL-6). In embodiments, the thrombopoietin (Tpo), Flt3 ligand (Flt-3L), human stem cell factor (SCF) and human interleukin-6 (IL-6) are each at a concentration of about 50 ng/mL. In embodiments, the thrombopoietin (Tpo), Flt3 ligand (Flt-3L), human stem cell factor (SCF) and human interleukin-6 (IL-6) are each at a concentration of 50 ng/mL.

Additional aspects and embodiments are described below.

In an aspect, the invention provides a gRNA molecule that includes a tracr and crRNA, wherein the crRNA includes a targeting domain that is complementary with a target sequence of a BCL11A gene (e.g., a human BCL11a gene), a BCL11a enhancer (e.g., a human BCL11a enhancer), or a HFPH region (e.g., a human HPFH region).

In embodiments, the target sequence is of the BCL11A gene, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1 to SEQ ID NO: 85 or SEQ ID NO: 400 to SEQ ID NO: 1231. These embodiments are referred to herein as gRNA molecule embodiment 2.

In other embodiments, the target sequence is of a BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1232 to SEQ ID NO: 1499. These embodiments are referred to herein as gRNA molecule embodiment 3. In preferred embodiments, the target sequence is of a BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 182 to SEQ ID NO: 277 or SEQ ID NO: 334 to SEQ ID NO: 341. These embodiments are referred to herein as gRNA molecule embodiment 4. In more preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 341, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 247, SEQ ID NO: 245, SEQ ID NO: 249, SEQ ID NO: 244, SEQ ID NO: 199, SEQ ID NO: 251, SEQ ID NO: 250, SEQ ID NO: 334, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 336, or SEQ ID NO: 337. These embodiments are referred to herein as gRNA molecule embodiment 5. In still more preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 335, SEQ ID NO: 336, SEQ ID NO: 337, or SEQ ID NO: 338 (referred to herein as gRNA molecule embodiment 6), for example, includes, e.g., consists of, any one of SEQ ID NO: 248 or SEQ ID NO: 338 (referred to herein as gRNA molecule embodiment 7). In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 248 (referred to herein as gRNA molecule embodiment 8). In embodiments, the targeting domain includes, e.g., consists of, SEQ ID NO: 338 (referred to herein as gRNA molecule embodiment 9).

In other embodiments, the target sequence is of a BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 278 to SEQ ID NO: 333 (referred to herein as gRNA molecule embodiment 10). In preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 318, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 294, SEQ ID NO: 310, SEQ ID NO: 319, SEQ ID NO: 298, SEQ ID NO: 322, SEQ ID NO: 311, SEQ ID NO: 315, SEQ ID NO: 290, SEQ ID NO: 317, SEQ ID NO: 309, SEQ ID NO: 289, or SEQ ID NO: 281 (referred to herein as gRNA molecule embodiment 11). In one preferred embodiment, the targeting domain includes, e.g., consists of, SEQ ID NO: 318 (referred to herein as gRNA molecule embodiment 12).

In other embodiments, the target sequence is of a BCL11a enhancer, and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1596 to SEQ ID NO: 1691 (referred to herein as gRNA molecule embodiment 13). In preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 1683, SEQ ID NO: 1638, SEQ ID NO: 1647, SEQ ID NO: 1609, SEQ ID NO: 1621, SEQ ID NO: 1617, SEQ ID NO: 1654, SEQ ID NO: 1631, SEQ ID NO: 1620, SEQ ID NO: 1637, SEQ ID NO: 1612, SEQ ID NO: 1656, SEQ ID NO: 1619, SEQ ID NO: 1675, SEQ ID NO: 1645, SEQ ID NO: 1598, SEQ ID NO: 1599, SEQ ID NO: 1663, SEQ ID NO: 1677, or SEQ ID NO: 1626 (referred to herein as gRNA molecule embodiment 14).

In other embodiments, the target sequence is of a HFPH region (e.g., a French HPFH region), and the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 86 to SEQ ID NO: 181, SEQ ID NO: 1500 to SEQ ID NO: 1595, or SEQ ID NO: 1692 to SEQ ID NO: 1761 (referred to herein as gRNA molecule embodiment 15). In preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 100, SEQ ID NO: 165, SEQ ID NO: 113, SEQ ID NO: 99, SEQ ID NO: 112, SEQ ID NO: 98, SEQ ID NO: 1580, SEQ ID NO: 106, SEQ ID NO: 1503, SEQ ID NO: 1589, SEQ ID NO: 160, SEQ ID NO: 1537, SEQ ID NO: 159, SEQ ID NO: 101, SEQ ID NO: 162, SEQ ID NO: 104, SEQ ID NO: 138, SEQ ID NO: 1536, SEQ ID NO: 1539, SEQ ID NO: 1585 (referred to herein as gRNA molecule embodiment 16). In still more preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 1505, SEQ ID NO: 1589, SEQ ID NO: 1700, or SEQ ID NO: 1750 (referred to herein as gRNA molecule embodiment 17). In other preferred embodiments, the targeting domain includes, e.g., consists of, any one of SEQ ID NO: 100, SEQ ID NO: 165, or SEQ ID NO: 113 (referred to herein as gRNA molecule embodiment 18).

In embodiments, the gRNA molecule includes a targeting domain that includes, e.g., consists of, 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids of any targeting domain sequence described herein, for example a targeting domain sequence of Table 1 or Table 2. In embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids of any one of targeting domain sequences described herein, for example a targeting domain sequence of Table 1 or Table 2, are the 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids disposed at the 3' end of the recited targeting domain sequence. In other embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids of any one of the targeting domain sequences described herein, for example a targeting domain sequence of Table 1 or Table 2, are the 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids disposed at the 5' end of the recited targeting domain sequence. In other embodiments, the 17, 18, 19, 20, 21, 22, 23, or 24, preferably 20, consecutive nucleic acids of any one of the targeting domain sequences described herein, for example a targeting domain sequence of Table 1 or Table 2, do not include either the 5' or 3' nucleic acid of the recited targeting domain sequence.

In embodiments, the gRNA molecule includes a targeting domain that includes, e.g., consists of, 17, 18, 19, or 20, preferably 20, consecutive nucleic acids of any targeting domain sequence described herein, for example a targeting domain sequence of Table 5, Table 6, Table 7, Table 8 or Table 9. In embodiments, the 17, 18, 19, or 20, preferably 20, consecutive nucleic acids of any one of targeting domain sequences described herein, for example a targeting domain sequence of Table 5, Table 6, Table 7, Table 8 or Table 9, are the 17, 18, 19, or 20, preferably 20, consecutive nucleic acids disposed at the 3' end of the recited targeting domain sequence. In other embodiments, the 17, 18, 19, or 20, preferably 20, consecutive nucleic acids of any one of the targeting domain sequences described herein, for example a targeting domain sequence of Table 5, Table 6, Table 7, Table 8 or Table 9, are the 17, 18, 19, or 20, preferably 20, consecutive nucleic acids disposed at the 5' end of the recited targeting domain sequence. In other embodiments, the 17, 18, 19, or 20, preferably 20, consecutive nucleic acids of any one of the targeting domain sequences described herein, for example a targeting domain sequence of Table 5, Table 6, Table 7, Table 8 or Table 9, do not include either the 5' or 3' nucleic acid of the recited targeting domain sequence.

The following aspects describe features of the gRNA molecule that may be combined with any of the aforementioned aspects and embodiments. In embodiments, the gRNA molecule, including the gRNA molecule of any of the aforementioned aspects and embodiments, include a portion of the crRNA and a portion of the tracr that hybridize to form a flagpole that includes SEQ ID NO: 6584 or 6585. In embodiments, the flagpole further includes a first flagpole extension, located 3' to the crRNA portion of the flagpole, wherein said first flagpole extension includes SEQ ID NO: 6586. In embodiments, the flagpole further includes (in addition to or in alternative to the first flagpole extension) a second flagpole extension located 3' to the crRNA portion of the flagpole and, if present, the first flagpole extension, wherein said second flagpole extension includes SEQ ID NO: 6587.

In embodiments, including in any of the aforementioned aspects and embodiments, the tracr includes SEQ ID NO: 6660 or SEQ ID NO: 6661. In embodiments, the tracr includes SEQ ID NO: 7812, optionally further including, at the 3' end, an additional 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides. In embodiments, the crRNA includes, from 5' to 3', [targeting domain]-: a) SEQ ID NO: 6584; b) SEQ ID NO: 6585; c) SEQ ID NO: 6605; d) SEQ ID NO: 6606; e) SEQ ID NO: 6607; f) SEQ ID NO: 6608; or g) SEQ ID NO: 7806.

In embodiments, including in any of the aforementioned aspects and embodiments, the tracr includes, from 5' to 3': a) SEQ ID NO: 6589; b) SEQ ID NO: 6590; c) SEQ ID NO: 6609; d) SEQ ID NO: 6610; e) SEQ ID NO: 6660; f) SEQ ID NO: 6661; g) SEQ ID NO: 7812; h) SEQ ID NO: 7807; i) SEQ ID NO: 7808; j) SEQ ID NO: 7809; k) any of a) to j), above, further including, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides; l) any of a) to k), above, further including, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides,

e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides; or m) any of a) to l), above, further including, at the 5' end (e.g., at the 5' terminus), at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides.

In embodiments, the targeting domain and the tracr are disposed on separate nucleic acids molecules. In embodiments of such gRNA molecules, the nucleic acid molecule including the targeting domain includes SEQ ID NO: 6607, optionally disposed immediately 3' to the targeting domain, and the nucleic acid molecule including the tracr includes, e.g., consists of, SEQ ID NO: 6660.

In embodiments, the crRNA portion of the flagpole includes SEQ ID NO: 6607 or SEQ ID NO: 6608.

In embodiments, the tracr includes SEQ ID NO: 6589 or 6590, and optionally, if a first flagpole extension is present, a first tracr extension, disposed 5' to SEQ ID NO: 6589 or 6590, said first tracr extension including SEQ ID NO: 6591.

In embodiments, including in embodiments of any of the aforementioned aspects and embodiments, the targeting domain and the tracr are disposed on separate nucleic acid molecules. In other embodiments, including in embodiments of any of the aforementioned aspects and embodiments, the targeting domain and the tracr are disposed on a single nucleic acid molecule, for example, the tracr is disposed 3' to the targeting domain.

In embodiments, when the targeting domain and the tracr are disposed on a single nucleic acid molecule, the gRNA molecule further includes a loop, disposed 3' to the targeting domain and 5' to the tracr. In embodiments, the loop includes, e.g., consists of, SEQ ID NO: 6588.

In embodiments, when the targeting domain and the tracr are disposed on a single nucleic acid molecule, the gRNA molecule includes, from 5' to 3', [targeting domain]-: (a) SEQ ID NO: 6601; (b) SEQ ID NO: 6602; (c) SEQ ID NO: 6603; (d) SEQ ID NO: 6604; (e) SEQ ID NO: 7811; or (f) any of (a) to (e), above, further including, at the 3' end, 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides. In embodiments, the gRNA molecule includes, e.g., consists of, said targeting domain and SEQ ID NO: 7811, optionally disposed immediately 3' to said targeting domain.

In embodiments, including in any of the aforementioned aspects and embodiments, each of the nucleic acid residues of the gRNA molecule is an unmodified A, U, G or C nucleic acid residue and unmodified phosphate bonds between each residue of the nucleic acid molecule(s). In other embodiments, including in any of the aforementioned aspects and embodiments, one, or optionally more than one, of the nucleic acid molecules that make up the gRNA molecule includes: a) one or more, e.g., three, phosphorothioate modifications at the 3' end of said nucleic acid molecule or molecules; b) one or more, e.g., three, phosphorothioate modifications at the 5' end of said nucleic acid molecule or molecules; c) one or more, e.g., three, 2'-O-methyl modifications at the 3' end of

said nucleic acid molecule or molecules; d) one or more, e.g., three, 2'-O-methyl modifications at the 5' end of said nucleic acid molecule or molecules; e) a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues of said nucleic acid molecule or molecules; f) a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 5' residues of said nucleic acid molecule or molecules; or f) any combination thereof.

In preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 342; (b) SEQ ID NO: 343; or (c) SEQ ID NO: 1762 (referred to in this summary of invention as gRNA molecule embodiment 41).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 344, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 344, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 345, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 345, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 42).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 347; (b) SEQ ID NO: 348; or (c) SEQ ID NO: 1763 (referred to in this summary of invention as gRNA molecule embodiment 43).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 349, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 349, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 350, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 350, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 44).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 351; (b) SEQ ID NO: 352; or (c) SEQ ID NO: 1764 (referred to in this summary of invention as gRNA molecule embodiment 45).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 353, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting

of, SEQ ID NO: 353, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 354, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 354, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 46).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 355; (b) SEQ ID NO: 356; or (c) SEQ ID NO: 1765 (referred to in this summary of invention as gRNA molecule embodiment 47).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 357, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 357, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 358, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 358, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 48).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 359; (b) SEQ ID NO: 360; or (c) SEQ ID NO: 1766 (referred to in this summary of invention as gRNA molecule embodiment 49).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 361, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 361, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 362, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 362, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 50).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 363; (b) SEQ ID NO: 364; or (c) SEQ ID NO: 1767 (referred to in this summary of invention as gRNA molecule embodiment 51).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 365, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting

of, SEQ ID NO: 365, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 366, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 366, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 52).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 367; (b) SEQ ID NO: 368; or (c) SEQ ID NO: 1768 (referred to in this summary of invention as gRNA molecule embodiment 53).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 369, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 369, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 370, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 370, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 54).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 371; (b) SEQ ID NO: 372; or (c) SEQ ID NO: 1769 (referred to in this summary of invention as gRNA molecule embodiment 55).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 373, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 373, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 374, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 374, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 56).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 375; (b) SEQ ID NO: 376; or (c) SEQ ID NO: 1770 (referred to in this summary of invention as gRNA molecule embodiment 57).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 377, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting

of, SEQ ID NO: 377, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 378, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 378, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 58).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 379; (b) SEQ ID NO: 380; or (c) SEQ ID NO: 1771 (referred to in this summary of invention as gRNA molecule embodiment 59).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 381, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 381, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 382, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 382, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 60).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 383; (b) SEQ ID NO: 384; or (c) SEQ ID NO: 1772 (referred to in this summary of invention as gRNA molecule embodiment 61).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 385, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 385, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 386, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 386, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 62).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 387; (b) SEQ ID NO: 388; or (c) SEQ ID NO: 1773 (referred to in this summary of invention as gRNA molecule embodiment 63).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 389, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting

of, SEQ ID NO: 389, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 390, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 390, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 64).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 391; (b) SEQ ID NO: 392; or (c) SEQ ID NO: 1774 (referred to in this summary of invention as gRNA molecule embodiment 65).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 393, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 393, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 394, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 394, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 66).

In other preferred embodiments, the invention provides a gRNA molecule (e.g., that is a sgRNA molecule) including, e.g., consisting of, the sequence: (a) SEQ ID NO: 395; (b) SEQ ID NO: 396; or (c) SEQ ID NO: 1775 (referred to in this summary of invention as gRNA molecule embodiment 67).

In other preferred embodiments, the invention provides gRNA molecule (e.g., that is a dual gRNA molecule) including, e.g., consisting of: (a) a crRNA including, e.g., consisting of, SEQ ID NO: 397, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; (b) a crRNA including, e.g., consisting of, SEQ ID NO: 397, and a tracr including, e.g., consisting of, SEQ ID NO: 346; (c) a crRNA including, e.g., consisting of, SEQ ID NO: 398, and a tracr including, e.g., consisting of, SEQ ID NO: 6660; or (d) a crRNA including, e.g., consisting of, SEQ ID NO: 398, and a tracr including, e.g., consisting of, SEQ ID NO: 346 (referred to in this summary of invention as gRNA molecule embodiment 68).

In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, an indel is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule. In embodiments, including in embodiments comprising a targeting domain complementary to a target sequence of a +58 Enhancer region, the indel does not include a nucleotide of a GATA-1 and/or TAL-1 binding site. In embodiments, the indel does not interfere with the binding of GATA-1 and/or TAL-1 to their binding

sites. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a population of cells, an indel is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule in at least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%, of the cells of the population. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a population of cells, an indel that does not include a nucleotide of a GATA-1 and/or TAL-1 binding site is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule in at least about 20%, e.g., at least about 30%, e.g., at least about 35%, e.g., at least about 40%, e.g., at least about 45%, e.g., at least about 50%, e.g., at least about 55%, e.g., at least about 60%, e.g., at least about 65%, e.g., at least about 70%, e.g., at least about 75%, e.g., at least about 80%, e.g., at least about 85%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 99%, of the cells of the population. In embodiments, including in any of the aforementioned aspects and embodiments, the indel is an indel listed in any of Figure 25, Table 15, Table 26, Table 27 or Table 37. In embodiments, in at least about 30%, e.g., least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%, of the cells of the population, the indel is an indel listed in any of Figure 25, Table 15, Table 26, Table 27 or Table 37. In embodiments, the three most frequently detected indels in said population of cells include the indels associated with any gRNA molecule listed in any of Figure 25, Table 15, Table 26, Table 27 or Table 37. In embodiments, the indel (or pattern of top indels) is as measured by next generation sequencing (NGS), e.g., as described in the art. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, no off-target indels are formed in said cell, e.g., as detectible by next generation sequencing and/or a nucleotide insertional assay, e.g., assayed in an HPCS cell, e.g., a CD34+ cell. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a population of cells, no off-target indel is detected in more than about 5%, e.g., more than about 1%, e.g., more than about 0.1%, e.g., more than about 0.01%, of the cells of the population of cells, e.g., as detectible by next generation sequencing and/or a nucleotide insertional assay, e.g., assayed in a population of HPSC cells, e.g., a population of CD34+ cells.

In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, expression of fetal hemoglobin is increased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny. In embodiments, expression of fetal hemoglobin is increased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny, by at least about 20%, e.g., at least about 30%, e.g., at least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%. In embodiments, said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny, produces at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, levels of fetal hemoglobin (e.g., gamma globin) mRNA are increased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, expression of BCL11a mRNA is decreased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny. In embodiments, including in any of the aforementioned aspects and embodiments, the invention provides a gRNA molecule, wherein when a CRISPR system (e.g., an RNP as described herein) including the gRNA molecule is introduced into a cell, levels of BCL11a mRNA are decreased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny.

In any of the aforementioned aspects and embodiments that mention a cell, the cell is (or population of cells includes) a mammalian, primate, or human cell, e.g., is a human cell or population of human cells. In embodiments, the cell is (or population of cells includes) an HSPC, for example, is CD34+, for example, is CD34+CD90+. In embodiments, the cell (or population of cells) is autologous with respect to a patient to be administered said cell. In other embodiments, the cell (or population of cells) is allogeneic with respect to a patient to be administered said cell.

In another aspect, the invention provides a composition including: 1) one or more gRNA molecules (including a first gRNA molecule) described herein, for example, one or more gRNA molecules of any of the previous aspects and embodiments, and a Cas9 molecule, for example, as described herein; 2) one or more gRNA molecules (including a first gRNA molecule) described herein, for example, one or more gRNA molecules of any of the previous aspects and embodiments, and nucleic acid encoding a Cas9 molecule (described herein); 3) nucleic acid encoding one or more gRNA molecules

(including a first gRNA molecule) described herein, for example, one or more gRNA molecules of any of the previous aspects and embodiments, and a Cas9 molecule (described herein); 4) nucleic acid encoding one or more gRNA molecules (including a first gRNA molecule) described herein, for example, one or more gRNA molecules of any of the previous aspects and embodiments, and nucleic acid encoding a Cas9 molecule (described herein); or 5) any of 1) to 4), above, and a template nucleic acid; or 6) any of 1) to 4) above, and nucleic acid including sequence encoding a template nucleic acid.

In preferred embodiments, the invention provides a composition including a first gRNA molecule (e.g., described herein, e.g., a first gRNA molecule of any of the previous gRNA molecule aspects and embodiments), further including a Cas9 molecule (described herein).

In embodiments, the Cas9 molecule is an active or inactive *s. pyogenes* Cas9. In embodiments, the Cas9 molecule includes SEQ ID NO: 6611. In embodiments, the Cas9 molecule includes, e.g., consists of: (a) SEQ ID NO: 7821; (b) SEQ ID NO: 7822; (c) SEQ ID NO: 7823; (d) SEQ ID NO: 7824; (e) SEQ ID NO: 7825; (f) SEQ ID NO: 7826; (g) SEQ ID NO: 7827; (h) SEQ ID NO: 7828; (i) SEQ ID NO: 7829; (j) SEQ ID NO: 7830; or (k) SEQ ID NO: 7831.

In preferred embodiments, the first gRNA molecule and Cas9 molecule are present in a ribonuclear protein complex (RNP).

In embodiments, the invention provides a composition, e.g., a composition of any of the previous aspects and embodiments, further including a second gRNA molecule; a second gRNA molecule and a third gRNA molecule; or a second gRNA molecule, optionally, a third gRNA molecule, and, optionally, a fourth gRNA molecule, wherein the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are a gRNA molecule of a gRNA molecule described herein, e.g., a gRNA molecule of any of the previous gRNA molecule aspects and embodiments, and wherein each gRNA molecule of the composition is complementary to a different target sequence.

In embodiments, two or more of the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequences within the same gene or region. In embodiments, the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequences not more than 20000 nucleotides, not more than 10000 nucleotides, not more than 6000, not more than 5000 nucleotides, not more than 4000, not more than 1000 nucleotides, not more than 500 nucleotides, not more than 400 nucleotides, not more than 300 nucleotides, not more than 200 nucleotides, not more than 100 nucleotides, not more than 90 nucleotides, not more than 80 nucleotides, not more than 70 nucleotides, not more than 60

nucleotides, not more than 50 nucleotides, not more than 40 nucleotides, not more than 30 nucleotides, not more than 20 nucleotides or not more than 10 nucleotides apart.

In other embodiments, two or more of the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequence within different genes or regions.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition including a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of gRNA molecule embodiment 4, and are complementary to different target sequences;

(b) independently selected from the gRNA molecules of gRNA molecule embodiment 5, and are complementary to different target sequences;

c) independently selected from the gRNA molecules of gRNA molecule embodiment 6, and are complementary to different target sequences; or

(d) independently selected from the gRNA molecules of gRNA molecule embodiment 7, and are complementary to different target sequences; or

(e) independently selected from the gRNA molecules of any of gRNA molecule embodiments 41-56, and are complementary to different target sequences.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition including a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of gRNA molecule embodiment 10, and are complementary to different target sequences; or

(b) independently selected from the gRNA molecules of gRNA molecule embodiment 11, and are complementary to different target sequences.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of gRNA molecule embodiment 13, and are complementary to different target sequences; or

(b) independently selected from the gRNA molecules of gRNA molecule embodiment 14, and are complementary to different target sequences.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of gRNA molecule embodiment 16, and are complementary to different target sequences;

(b) independently selected from the gRNA molecules of gRNA molecule embodiment 17, and are complementary to different target sequences;

(c) independently selected from the gRNA molecules of gRNA molecule embodiment 18, and are complementary to different target sequences; or

(d) independently selected from the gRNA molecules of any of gRNA molecule embodiments 57-68, and are complementary to different target sequences.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 4, (b) selected from the gRNA molecules of gRNA molecule embodiment 5, (c) selected from the gRNA molecules of gRNA molecule embodiment 6, (d) selected from the gRNA molecules of gRNA molecule embodiment 7, or (e) selected from the gRNA molecules of any of gRNA molecule embodiments 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 10, or (b) selected from the gRNA molecules of gRNA molecule embodiment 11.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 4, (b) selected from the gRNA molecules of gRNA molecule embodiment 5, (c) selected from the gRNA molecules of gRNA molecule embodiment 6, (d) selected from the gRNA molecules of gRNA molecule embodiment 7, or (e) selected from the gRNA molecules of any of gRNA molecule embodiments 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 13, (b) selected from the gRNA molecules of gRNA molecule embodiment 14.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 4, (b) selected from the gRNA molecules of gRNA molecule embodiment 5, (c) selected from the gRNA molecules of gRNA molecule embodiment 6, (d) selected from the gRNA molecules of gRNA molecule embodiment 7, or (e) selected from the gRNA molecules of any of gRNA molecule embodiments 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 16, (b) selected from the gRNA molecules of gRNA molecule embodiment 17, (c) selected from the gRNA molecules of gRNA molecule embodiment 18, or (d) selected from the gRNA molecules of any of gRNA molecule embodiments 57-68.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 10, or (b) selected from the gRNA molecules of gRNA molecule embodiment 11; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 13, (b) selected from the gRNA molecules of gRNA molecule embodiment 14.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 10, or (b) selected from the gRNA molecules of gRNA molecule embodiment 11; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 16, (b) selected from the gRNA molecules of gRNA molecule embodiment 17, (c) selected from the gRNA molecules of gRNA molecule embodiment 18, or (d) selected from the gRNA molecules of any of gRNA molecule embodiments 57-68.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 13, (b) selected from the gRNA molecules of gRNA molecule embodiment 14; and (2) the second gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 16, (b) selected from the gRNA molecules of gRNA molecule embodiment 17, (c) selected from the gRNA molecules of gRNA molecule embodiment 18, or (d) selected from the gRNA molecules of any of gRNA molecule embodiments 57-68.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, including a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 16, (b) selected from the gRNA molecules of gRNA molecule embodiment 17, (c) selected from the gRNA molecules of gRNA molecule embodiment 18, or (d) selected from the gRNA molecules of any of gRNA molecule embodiments 57-68; and (2) the second gRNA molecule includes a targeting domain that is complementary to a target sequence of the beta globin gene; or

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of gRNA molecule embodiment 4, (b) selected from the gRNA molecules of gRNA molecule embodiment 5, (c) selected from the gRNA molecules of gRNA molecule embodiment 6, (d) selected from the gRNA molecules of gRNA molecule embodiment 7, (e) selected from the gRNA molecules of any of gRNA molecule embodiments 41-56, (f) selected from the gRNA molecules of gRNA molecule embodiment 10, (g) selected from the gRNA molecules of gRNA molecule embodiment 11, (h) selected from the gRNA molecules of gRNA molecule embodiment 13, or (i) selected from the gRNA molecules of gRNA molecule embodiment 14; and (2) the second gRNA molecule includes a targeting domain that is complementary to a target sequence of the beta globin gene.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, wherein the first gRNA molecule and the second gRNA molecule are independently selected from the gRNA molecules of any of gRNA molecule embodiments 41-68.

In embodiments of the composition, with respect to the gRNA molecule components of the composition, the composition consists of a first gRNA molecule and a second gRNA molecule.

In embodiments of the composition, each of said gRNA molecules is in a ribonuclear protein complex (RNP) with a Cas9 molecule described herein.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the invention provides a composition, further including a template nucleic acid, wherein the template nucleic acid includes a nucleotide that corresponds to a nucleotide at or near the target sequence of the first gRNA molecule. In embodiments, the template nucleic acid includes nucleic acid encoding: (a) human beta globin, e.g., human beta globin including one or more of the mutations G16D, E22A and T87Q, or fragment thereof; or (b) human gamma globin, or fragment thereof.

In embodiments, including in any of the aforementioned composition aspects and embodiments, the composition is formulated in a medium suitable for electroporation, for example, suitable for electroporation into HSPC cells. In embodiments of the composition which include one or more gRNA molecules, each of said gRNA molecules is in a RNP with a Cas9 molecule described herein, and wherein each of said RNP is at a concentration of less than about 10uM, e.g., less than about 3uM, e.g., less than about 1uM, e.g., less than about 0.5uM, e.g., less than about 0.3uM, e.g., less than about 0.1uM.

In another aspect, the invention provides a nucleic acid sequence that encodes one or more gRNA molecules described herein, for example, a gRNA molecule of any of the previous aspects and embodiments. In embodiments, the nucleic acid includes a promoter operably linked to the sequence that encodes the one or more gRNA molecules, for example, a promoter recognized by an RNA polymerase II or RNA polymerase III, for example, a U6 promoter or an HI promoter. In embodiments, the nucleic acid further encodes a Cas9 molecule, for example, described herein, for example, a Cas9 molecule that includes (e.g., consists of) any of SEQ ID NO: 6611, SEQ ID NO: 7821, SEQ ID NO: 7822, SEQ ID NO: 7823, SEQ ID NO: 7824, SEQ ID NO: 7825, SEQ ID NO: 7826, SEQ ID NO: 7827, SEQ ID NO: 7828, SEQ ID NO: 7829, SEQ ID NO: 7830, or SEQ ID NO: 7831. In embodiments, the nucleic acid includes a promoter operably linked to the sequence that encodes a Cas9 molecule, for example, an EF-1 promoter, a CMV IE gene promoter, an EF-1α promoter, an ubiquitin C promoter, or a phosphoglycerate kinase (PGK) promoter.

In another aspect, the invention provides a vector including the nucleic acid of any of the previous nucleic acid aspects and embodiments. In embodiments, the vector is selected from the group consisting of a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

In another aspect, the invention provides a method of altering a cell (e.g., a population of cells), (e.g., altering the structure (e.g., sequence) of nucleic acid) at or near a target sequence within said cell, including contacting (e.g., introducing into) said cell (e.g., population of cells) with: 1) one or more

gRNA molecules of any of the previous gRNA molecule aspects and embodiments and a Cas9 molecule (e.g., described herein); 2) one or more gRNA molecules of any of the previous gRNA molecule aspects and embodiments and nucleic acid encoding a Cas9 molecule (e.g., described herein); 3) nucleic acid encoding one or more gRNA molecules of any of the previous gRNA molecule aspects and embodiments and a Cas9 molecule (e.g., described herein); 4) nucleic acid encoding one or more gRNA molecules of any of the previous gRNA molecule aspects and embodiments and nucleic acid encoding a Cas9 molecule (e.g., described herein); 5) any of 1) to 4), above, and a template nucleic acid; 6) any of 1) to 4) above, and nucleic acid including sequence encoding a template nucleic acid; 7) the composition of any of the previous composition aspects and embodiments; or 8) the vector of any of the previous vector aspects and embodiments. In embodiments, the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in a single composition. In other embodiments, the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in more than one composition. In embodiments that include more than one composition, the more than one composition are delivered simultaneously or sequentially. In embodiments, the cell is an animal cell, for example, a mammalian, primate, or human cell. In embodiments, the cell, is a hematopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs), for example, the cell is a CD34+ cell, for example, the cell is a CD34+CD90+ cell. In embodiments, the cell is disposed in a composition including a population of cells that has been enriched for CD34+ cells. In embodiments, the cell (e.g. population of cells) has been isolated from bone marrow, mobilized peripheral blood, or umbilical cord blood. In embodiments, the cell is autologous with respect to a patient to be administered said cell. In other embodiments, the cell is allogeneic with respect to a patient to be administered said cell. In embodiments, the method results in an indel at or near a genomic DNA sequence complementary to the targeting domain of the one or more gRNA molecules, for example, an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37, for example an indel associated with the targeting domain of the gRNA molecule as shown in Figure 25, Table 15, Table 26, Table 27 or Table 37. In embodiments, the indel is an insertion or deletion of less than about 40 nucleotides, e.g., less than 30 nucleotides, e.g., less than 20 nucleotides, e.g., less than 10 nucleotides, for example, is a single nucleotide deletion. In embodiments, the method results in a population of cells wherein at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90% (e.g., at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%) of the cells of the population have been altered, e.g., include an indel. In embodiments, the method (e.g., the method performed on a population of cells, e.g., described herein) results in a cell (e.g., population of cells) that is capable of differentiating into a differentiated cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell exhibits an increased level of fetal hemoglobin, e.g., relative to an unaltered cell (e.g., population of cells). In

embodiments, the method results in a population of cells that is capable of differentiating into a population of differentiated cells, e.g., a population of cells of an erythroid lineage (e.g., a population of red blood cells), and wherein said population of differentiated cells has an increased fraction of F cells (e.g., at least about 15%, at least about 20% , at least about 25%, at least about 30%, or at least about 40% higher) e.g., relative to a population of unaltered cells. In embodiments, the method results in a cell that is capable of differentiating into a differentiated cell, e.g., a cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell produces at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell. In embodiments, the method is performed ex vivo. In other embodiments, the method is performed in vivo.

In another aspect, the invention provides a cell, altered by the method of altering a cell of any of the previous method aspects and embodiments.

In another aspect, the invention provides a cell, obtainable the method of altering a cell of any of the previous method aspects and embodiments.

In another aspect, the invention provides a cell, including a first gRNA molecule, e.g., described herein, e.g., of any of the previous gRNA molecule aspects and embodiments, or a composition, e.g., described herein, e.g., of any of the previous composition aspects and embodiments, a nucleic acid, e.g., described herein, e.g., of any of the previous nucleic acid aspects and embodiments, or a vector , e.g., described herein, e.g., of any of the previous vector aspects and embodiments. In embodiments, the cell further includes a Cas9 molecule, e.g., described herein, for example, a Cas9 molecule that includes, e.g., consists of, any of SEQ ID NO: 6611, SEQ ID NO: 7821, SEQ ID NO: 7822, SEQ ID NO: 7823, SEQ ID NO: 7824, SEQ ID NO: 7825, SEQ ID NO: 7826, SEQ ID NO: 7827, SEQ ID NO: 7828, SEQ ID NO: 7829, SEQ ID NO: 7830, or SEQ ID NO: 7831. In embodiments, the cell includes, has included, or will include a second gRNA molecule, e.g., described herein, e.g., of any of the previous gRNA molecule aspects and embodiments, or a nucleic acid encoding a second gRNA molecule, e.g., described herein, e.g., of any of the previous gRNA molecule aspects and embodiments, wherein the first gRNA molecule and second gRNA molecule include nonidentical targeting domains. In embodiments, expression of fetal hemoglobin is increased in said cell or its progeny (e.g., its erythroid progeny, e.g., its red blood cell progeny) relative to a cell or its progeny of the same cell type that has not been modified to include a gRNA molecule. In embodiments, the cell is capable of differentiating into a differentiated cell, e.g., a cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell exhibits an increased level of fetal hemoglobin, e.g., relative to a cell of the same type that has not been modified to include a gRNA molecule. In embodiments, the differentiated cell (e.g., cell of an erythroid lineage, e.g., red blood cell) produces at

least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin, e.g., relative to a cell of the same type that has not been modified to include a gRNA molecule. In embodiments, the cell has been contacted, e.g., contacted ex vivo, with a stem cell expander, e.g., described herein, e.g., a stem cell expander that is compound 1, compound 2, compound 3, compound 4, or a combination thereof (e.g., compound 1 and compound 4), for example, a stem cell expander that is compound 4. In embodiments, including in any of the aforementioned aspects and embodiments, the cell includes an indel at or near a genomic DNA sequence complementary to the targeting domain of the gRNA molecule (as described herein, e.g., a gRNA molecule of any of the aforementioned aspects and embodiments) introduced therein, for example, an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37, for example an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37 that is associated with the gRNA molecule (as described herein, e.g., a gRNA molecule of any of the aforementioned aspects and embodiments) introduced therein. In embodiments, the indel is an insertion or deletion of less than about 40 nucleotides, e.g., less than 30 nucleotides, e.g., less than 20 nucleotides, e.g., less than 10 nucleotides, for example, the indel is a single nucleotide deletion. In embodiments, including in any of the aforementioned cell aspects and embodiments, the cell is an animal cell, for example, the cell is a mammalian, a primate, or a human cell. In embodiments, including in any of the aforementioned cell aspects and embodiments, the cell is a hematopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs), for example, the cell is a CD34+ cell, for example, the cell is a CD34+CD90+ cell. In embodiments, including in any of the aforementioned cell aspects and embodiments, the cell (e.g. population of cells) has been isolated from bone marrow, mobilized peripheral blood, or umbilical cord blood. In embodiments, including in any of the aforementioned cell aspects and embodiments, the cell is autologous with respect to a patient to be administered said cell. In embodiments, the cell is allogeneic with respect to a patient to be administered said cell.

In another aspect, the invention provides a population of cells including the cell of any of the previous cell aspects and embodiments. In embodiments, at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90% (e.g., at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%) of the cells of the population are a cell according to any of the previous cell aspects and embodiments. In embodiments, the population of cells is capable of differentiating into a population of differentiated cells, e.g., a population of cells of an erythroid lineage (e.g., a population of red blood cells), and wherein said population of differentiated cells has an increased fraction of F cells (e.g., at least about 15%, at least about 20% , at least about 25%, at least about 30%, or at least about 40% higher) e.g., relative to a population of unmodified cells of the same type. In embodiments, the F cells of the population of differentiated cells produce an average of at least about 6 picograms (e.g., at least about 7 picograms,

at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell. In embodiments, the population includes: 1) at least 1e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered; 2) at least 2e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered; 3) at least 3e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered; 4) at least 4e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered; or 5) from 2e6 to 10e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered. In embodiments, at least about 40%, e.g., at least about 50%, (e.g., at least about 60%, at least about 70%, at least about 80%, or at least about 90%) of the cells of the population are CD34+ cells. In embodiments, at least about 10%, e.g., at least about 15%, e.g., at least about 20%, e.g., at least about 30% of the cells of the population are CD34+CD90+ cells. In embodiments, the population of cells is derived from bone marrow, peripheral blood (e.g., mobilized peripheral blood), umbilical cord blood, or induced pluripotent stem cells (iPSCs). In a preferred embodiment, the population of cells is derived from bone marrow. In embodiments, the population of cells includes, e.g., consists of, mammalian cells, e.g., human cells. In embodiments, the population of cells is autologous relative to a patient to which it is to be administered. In other embodiments, the population of cells is allogeneic relative to a patient to which it is to be administered.

In another aspect, the invention provides a composition including a cell of any of the previous cell aspects and embodiments, or the population of cells of any of previous population of cell aspects and embodiments. In embodiments, the composition includes a pharmaceutically acceptable medium, e.g., a pharmaceutically acceptable medium suitable for cryopreservation.

In another aspect, the invention provides a method of treating a hemoglobinopathy, including administering to a patient a cell of any of the previous cell aspects and embodiments, a population of cells of any of previous population of cell aspects and embodiments, or a composition of any of the previous composition aspects and embodiments. In embodiments, the hemoglobinopathy is a thalassemia, for example, beta-thalassemia, or sickle cell disease.

In another aspect, the invention provides a method of increasing fetal hemoglobin expression in a mammal, including administering to a patient a cell of any of the previous cell aspects and embodiments, a population of cells of any of previous population of cell aspects and embodiments, or a composition of any of the previous composition aspects and embodiments.

In another aspect, the invention provides a method of preparing a cell (e.g., a population of cells) including: (a) providing a cell (e.g., a population of cells) (e.g., a HSPC (e.g., a population of HSPCs)); (b) culturing said cell (e.g., said population of cells) ex vivo in a cell culture medium including a stem cell expander; and (c) introducing into said cell a first gRNA molecule (e.g.,

described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments), a nucleic acid molecule encoding a first gRNA molecule (e.g., described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments), a composition (e.g., described herein, for example, a composition of any of the previous composition aspects and embodiments), a nucleic acid (e.g., described herein, for example, a nucleic acid of any of the previous nucleic acid aspects and embodiments), or a vector (e.g., described herein, for example, a vector of any of the previous vector aspects and embodiments). In embodiments of said method, after said introducing of step (c), said cell (e.g., population of cells) is capable of differentiating into a differentiated cell (e.g., population of differentiated cells), e.g., a cell of an erythroid lineage (e.g., population of cells of an erythroid lineage), e.g., a red blood cell (e.g., a population of red blood cells), and wherein said differentiated cell (e.g., population of differentiated cells) produces increased fetal hemoglobin, e.g., relative to the same cells which have not been subjected to step (c). In embodiments, including in any of the aforementioned method aspects and embodiments, the stem cell expander is compound 1, compound 2, compound 3, compound 4 or a combination thereof (e.g., compound 1 and compound 4), for example, is compound 4. In embodiments, including in any of the aforementioned method aspects and embodiments, the cell culture medium includes thrombopoietin (Tpo), Flt3 ligand (Flt-3L), and human stem cell factor (SCF). In embodiments, including in any of the aforementioned method aspects and embodiments, the cell culture medium further includes human interleukin-6 (IL-6). In embodiments, including in any of the aforementioned method aspects and embodiments, the cell culture medium includes thrombopoietin (Tpo), Flt3 ligand (Flt-3L), and human stem cell factor (SCF) each at a concentration ranging from about 10 ng/mL to about 1000 ng/mL, for example, each at a concentration of about 50 ng/mL, e.g., at a concentration of 50 ng/mL. In embodiments, including in any of the aforementioned method aspects and embodiments, the cell culture medium includes human interleukin-6 (IL-6) at a concentration ranging from about 10 ng/mL to about 1000 ng/mL, for example, at a concentration of about 50 ng/mL, e.g., at a concentration of 50 ng/mL. In embodiments, including in any of the aforementioned method aspects and embodiments, the cell culture medium includes a stem cell expander at a concentration ranging from about 1 nM to about 1 mM, for example, at a concentration ranging from about 1 uM to about 100 uM, for example, .at a concentration ranging from about 50 uM to about 75 uM, for example, at a concentration of about 50 uM, e.g., at a concentration of 50 uM, or at a concentration of about 75 uM, e.g., at a concentration of 75 uM. In embodiments, including in any of the aforementioned method aspects and embodiments, the culturing of step (b) includes a period of culturing before the introducing of step (c), for example, the period of culturing before the introducing of step (c) is at least 12 hours, e.g., is for a period of about 1 day to about 3 days, e.g., is for a period of about 1 day to about 2 days, e.g., is for a period of about 2 days. In embodiments, including in any of the aforementioned method aspects and embodiments, the culturing of step (b) includes a period of culturing after the introducing of step (c), for example, the period of culturing after the introducing of step (c) is at least 12 hours, e.g., is for

a period of about 1 day to about 10 days, e.g., is for a period of about 1 day to about 5 days, e.g., is for a period of about 2 days to about 4 days, e.g., is for a period of about 2 days or is for a period of about 3 days or is for a period of about 4 days. In embodiments, including in any of the aforementioned method aspects and embodiments, the population of cells is expanded at least 4-fold, e.g., at least 5-fold, e.g., at least 10-fold, e.g., relative to cells which are not cultured according to step (b). In embodiments, including in any of the aforementioned method aspects and embodiments, the introducing of step (c) includes an electroporation, for example, an electroporation that includes 1 to 5 pulses, e.g., 1 pulse, and wherein each pulse is at a pulse voltage ranging from 700 volts to 2000 volts and has a pulse duration ranging from 10 ms to 100 ms. In embodiments, including in any of the aforementioned method aspects and embodiments, the electroporation includes, for example, consists of, 1 pulse. In embodiments, including in any of the aforementioned method aspects and embodiments, the pulse voltage ranges from 1500 to 1900 volts, e.g., is 1700 volts. In embodiments, including in any of the aforementioned method aspects and embodiments, the pulse duration ranges from 10 ms to 40 ms, e.g., is 20 ms. In embodiments, including in any of the aforementioned method aspects and embodiments, the cell (e.g., population of cells) provided in step (a) is a human cell (e.g., a population of human cells). In embodiments, including in any of the aforementioned method aspects and embodiments, the cell (e.g., population of cells) provided in step (a) is isolated from bone marrow, peripheral blood (e.g., mobilized peripheral blood), umbilical cord blood, or induced pluripotent stem cells (iPSCs), preferably bone marrow. In embodiments, including in any of the aforementioned method aspects and embodiments, the cell (e.g., population of cells) provided in step (a) is isolated from bone marrow, e.g., is isolated from bone marrow of a patient suffering from a hemoglobinopathy. In embodiments, including in any of the aforementioned method aspects and embodiments, the population of cells provided in step (a) is enriched for HSPCs, e.g., CD34+ cells. In embodiments, including in any of the aforementioned method aspects and embodiments, subsequent to the introducing of step (c), the cell (e.g., population of cells) is cryopreserved. In embodiments, including in any of the aforementioned method aspects and embodiments, subsequent to the introducing of step (c), the cell (e.g., population of cells) includes an indel at or near a genomic DNA sequence complementary to the targeting domain of the first gRNA molecule, for example, an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37, for example an indel shown in Figure 25, Table 15, Table 26, Table 27 or Table 37 as associated with the first gRNA molecule. In embodiments, including in any of the aforementioned method aspects and embodiments, after the introducing of step (c), at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% of the cells of the population of cells include an indel at or near a genomic DNA sequence complementary to the targeting domain of the first gRNA molecule, for example, an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37, for example an indel shown in Figure 25, Table 15, Table 26, Table 27 or Table 37 as associated with the first gRNA molecule.

In another aspect, the invention provides a cell (e.g., population of cells), obtainable by the method of preparing a cell of any of the previous aspects and embodiments. In another aspect, the invention provides a method of treating a hemoglobinopathy (for example a thalassemia (e.g., beta-thalassemia) or sickle cell disease), including administering to a human patient a composition including said cell (e.g., population of cells). In another aspect, the invention provides a method of increasing fetal hemoglobin expression in a human patient, including administering to said human patient a composition including said cell (e.g., population of cells). In embodiments, the human patient is administered a composition including at least about 1e6 cells (e.g., CD34+ cells, e.g., cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments) per kg body weight of the human patient, e.g., at least about 1e6 CD34+ cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments per kg body weight of the human patient. In embodiments, the human patient is administered a composition including at least about 2e6 cells (e.g., CD34+ cells, e.g., cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments) per kg body weight of the human patient, e.g., at least about 2e6 CD34+ cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments per kg body weight of the human patient. In embodiments, the human patient is administered a composition including from about 2e6 to about 10e6cells (e.g., CD34+ cells, e.g., cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments) per kg body weight of the human patient, e.g., at least about 2e6 to about 10e6 CD34+ cells obtainable by a method of preparing a cell of any of the previous aspects and embodiments per kg body weight of the human patient.

In another aspect, the invention provides a gRNA molecule described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments, a composition described herein, for example, a composition of any of the previous composition aspects and embodiments, a nucleic acid described herein, for example, a nucleic acid of any of the previous nucleic acid aspects and embodiments, a vector described herein, for example, a vector of any of the previous vector aspects and embodiments, a cell described herein, for example, a cell of any of the previous cell aspects and embodiments, or a population of cells described herein, for example, a population of cells of any of the previous population of cell aspects and embodiments, for use as a medicament.

In another aspect, the invention provides a gRNA molecule described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments, a composition described herein, for example, a composition of any of the previous composition aspects and embodiments, a nucleic acid described herein, for example, a nucleic acid of any of the previous nucleic acid aspects and embodiments, a vector described herein, for example, a vector of any of the previous vector aspects and embodiments, a cell described herein, for example, a cell of any of the previous cell aspects and embodiments, or a population of cells described herein, for example, a population of cells

of any of the previous population of cell aspects and embodiments, for use in the manufacture of a medicament.

In another aspect, the invention provides a gRNA molecule described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments, a composition described herein, for example, a composition of any of the previous composition aspects and embodiments, a nucleic acid described herein, for example, a nucleic acid of any of the previous nucleic acid aspects and embodiments, a vector described herein, for example, a vector of any of the previous vector aspects and embodiments, a cell described herein, for example, a cell of any of the previous cell aspects and embodiments, or a population of cells described herein, for example, a population of cells of any of the previous population of cell aspects and embodiments, for use in the treatment of a disease.

In another aspect, the invention provides a gRNA molecule described herein, for example, a gRNA molecule of any of the previous gRNA molecule aspects and embodiments, a composition described herein, for example, a composition of any of the previous composition aspects and embodiments, a nucleic acid described herein, for example, a nucleic acid of any of the previous nucleic acid aspects and embodiments, a vector described herein, for example, a vector of any of the previous vector aspects and embodiments, a cell described herein, for example, a cell of any of the previous cell aspects and embodiments, or a population of cells described herein, for example, a population of cells of any of the previous population of cell aspects and embodiments, for use in the treatment of a disease, wherein the disease is a hemoglobinopathy, for example a thalassemia (e.g., beta-thalassemia) or sickle cell disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Cas9 editing of the Bcl11a +58 erythroid enhancer region. Fraction of editing detected by NGS in HEK-293 Cas9GFP 24h post-delivery of crRNA targeting the +58 enhancer region and trRNA by lipofection. Each dot indicates a different crRNA, the trRNA was held constant. Genomic coordinates indicate location on Chromosome 2, e.g., in reference to hg38. (n=1)

Figure 2: Cas9 editing of the Bcl11a +62 erythroid enhancer region. Fraction of editing detected by NGS in HEK-293 Cas9GFP 24h post-delivery of crRNA targeting the +62 enhancer region and trRNA by lipofection. Each dot indicates a different crRNA, the trRNA was held constant. Genomic coordinates indicate location on Chromosome 2, e.g., in reference to hg38. (n=1)

Figure 3. Gating strategy for selection of cells after introduction of the Cas9 editing system.

Figure 4. Agarose gel electrophoresis of gene fragments from Cas9 editing system-treated CD34+ cells (shown are both CD34+CD90+ and CD34+CD90- cell populations, together with reference

unsorted cells). The upper band represents uncleaved homoduplex DNA and the lower bands indicate cleavage products resulting from heteroduplex DNA. The far left lane is a DNA ladder. Band intensity was calculated by peak integration of unprocessed images by ImageJ software. % gene modification (indel) was calculated as follows: % gene modification = 100 x (1 – (1- fraction cleaved)$^{1/2}$), and is indicated below each corresponding lane of the gel.

Figure 5. Erythroid enhancer region showing the sites of genomic DNA targeted by sgRNA molecules comprising targeting domains complimentary to the underlined nucleotides. Figure discloses SEQ ID NO: 2841.

Figure 6A/6B. NGS results for indel formation by sgEH1 (CR00276) (A) or sgEH2 (CR00275) (B) in CD34+ HSC cells. Insertions are in uppercase. Deletions are indicated by a dashed line. Regions of microhomology near the cutting site are highlighted in bold underline. Figure 6A discloses SEQ ID NOS 2842-2854 and Figure 6B discloses SEQ ID NOS 2855-2867, all respectively, in order of appearance.

Figure 6C/6D. NGS results for indel formation by sgEH8 (CR00273) (C) or sgEH9 (CR00277) (D) in CD34+ HSC cells. Insertions are in uppercase. Deletions are indicated by a dashed line. Regions of microhomology near the cutting site are highlighted in bold underline. Figure 6C discloses SEQ ID NOS 2868-2880 and Figure 6D discloses SEQ ID NOS 2881-2893, all respectively, in order of appearance.

Figure 7. NGS results and indel pattern formation across multiple donors for g7, g8 and g2. The sequences of the top 3 indels from two biological replicate experiments using g7 and g8 were identical, and the sequence of the top 3 indels using g2 were identical to those from a previous experiment. Figure discloses SEQ ID NOS 2894-2911, respectively, in order of appearance.

Figure 8. NGS results and indel pattern formation using a modified gRNA scaffold (BC). The sequences of the top 3 indels from these experiments are identical to those formed when using the standard gRNA scaffold. Figure discloses SEQ ID NOS 2912-2921, respectively, in order of appearance.

Figure 9. Comparison of indel pattern across different delivery methods. AVG % refers to the % of NGS reads exhibiting the indel indicated (Average of 2 experiments). Figure discloses SEQ ID NOS 2922-2946, respectively, in order of appearance.

Figure 10. Indel formation by co-introduction of g7 gRNA and g8 gRNA with either a non-extended flagpole region ("reg") or with a first flagpole extension and first tracr extension ("BC"). PAM sequences for the two gRNAs are boxed. Figure discloses SEQ ID NOS 2947-2955, respectively, in order of appearance.

Figure 11: Top gRNA sequences directed to +58 enhancer region of BCL11a gene in CD34+ cells, as measured by NGS (n=3).

Figure 12: Top gRNA sequences directed to +62 enhancer region of BCL11a gene in CD34+ cells, as measured by NGS (n=3).

Figure 13: Predicted excision sizes when 2 gRNA molecules targeting the BCL11a +62 enhancer locus are introduced into HEK293_Cas9 cells, when either CR00187 (light grey bars) or CR00202 (dark grey bars) is held constant and co-inserted into the cells with a second gRNA molecule including the targeting domain of the gRNA indicated.

Figure 14: Excision of genomic DNA within the +62 enhancer of BCL11a by addition of gRNA molecules including the targeting domain of CR00187 and the second gRNA molecule indicated in the graph. Stars (*) indicate excision observed at a frequency greater than 30%; Carats (^) indicate excision observed at a lower frequency (<30%). Those predicted excision products resulting in a fragment less than 50 nt could not be distinguished.

Figure 15: Excision of genomic DNA within the +62 enhancer of BCL11a by addition of gRNA molecules including the targeting domain of CR00202 and the second gRNA molecule indicated in the graph. Stars (*) indicate excision observed at a frequency greater than 30%; Carats (^) indicate excision observed at lower frequency (<30%).

Figure 16: % indel formation by 192 gRNA molecules targeted to the French HPFH region (*Sankaran VG et al. A functional element necessary for fetal hemoglobin silencing. NEJM (2011) 365:807-814.*)

Figure 17. Experimental scheme of Cas9:gRNA ribonucleoprotein (Cas9-RNP) delivery to primary human CD34+ HSPC for genome editing, followed by genetic and phenotypic characterization.

Figure 18. Cell viability following mock electroporation or electroporation of Cas9-RNP complexes into CD34+ HSPC. The HSPC were expanded *in vitro* for 48 hours after electroporation of RNP complexes and the cell viability monitored and percent viability determined. The names of gRNAs used to make RNP complexes are shown on x- axis and the corresponding cell viabilities on y-axis. The CRxxxx identifier indicates the targeting domain of the gRNA molecule.

Figure 19. Mismatch detection using T7 endonuclease assay. Human HSC were electroporated to deliver RNP complexes to introduce indels into +58 DHS region of BCL11A erythroid enhancer by NHEJ. PCR amplicons spanning the target region was subjected to the T7E1 assay and the resulting fragments analyzed by 2% agarose gel electrophoresis. Regions of +58 erythroid enhancer BCL11A were disrupted by both guide RNA formats (dual guide RNA – black; single guide RNA –gray (bottom graph and g7BCL11a-BC(1) and g7BCL11A-BC(2) from top graph)). The intensities of

Figure 26A: Phenotyping of edited and unedited cultures in CD34+ cell expansion medium by cell surface marker staining. All gRNA molecules were tested in the dgRNA format. The Tracr used was SEQ ID NO: 7808, and the crRNA had the following format and sequence with 2'O-Methyl (m) and Phosphorothioate Bond (*) modifications indicated:
mN*mN*mN*rNrNrNrNrNrNrNrNrNrNrNrNrNrNrNrNrGrUrUrUrUrArGrArGrCrUrArU*mG*mC*mU (SEQ ID NO: 2003), where N's are the residues of the indicated targeting domain. Shown are representative dot plots showing the fluorescence of cells stained with each antibody panel or corresponding isotype controls as described in Materials and Methods. Cells shown were pre-gated on the viable cell population by forward and side scatter properties and DAPI (4',6-Diamidino-2-Phenylindole) discrimination. The percentage of each cell population named at the top of the plot was determined by the gate indicated by the bold lined box.

Figure 26B: Phenotyping of edited and unedited cultures in CD34+ cell expansion medium by cell surface marker staining. All gRNA molecules were tested in the dgRNA format. The Tracr used was SEQ ID NO: 7808, and the crRNA had the following format and sequence with 2'O-Methyl (m) and Phosphorothioate Bond (*) modifications indicated:
mN*mN*mN*rNrNrNrNrNrNrNrNrNrNrNrNrNrNrNrNrGrUrUrUrUrArGrArGrCrUrArU*mG*mC*mU (SEQ ID NO: 2004), where N's are the residues of the indicated targeting domain. Shown is the percentage of each named cell population in edited and unedited cultures. Targeting domain of the edited cultures is as indicated.

Figure 27. % F cells in erythrocytes differentiated from populations of CD34+ cells edited with RNPs comprising dgRNAs targeting two sites within the HPFH region. "g2" is the positive control (targeting domain to a coding region of BCL11a gene); "cntrl" is negative control (introduction of Cas9 only).

Figure 28A. % Editing as determined by NGS in CD34+ HSPCs at day 2 following electroporation of RNP comprising the indicated dgRNA (unmod or modified as indicated). Controls include dgRNA comprising the targeting domain of CR00317 unmodified (CR00317-m), or electroporation in buffer only (No Cas9 or gRNA; "Mock").

Figure 28B. % Editing as determined by NGS in CD34+ HSPCs at day 2 following electroporation of RNP comprising the indicated dgRNA (unmod or modified as indicated). Controls include electroporation in buffer only (No Cas9 or gRNA; "Mock").

Figure 29. % Editing as determined by NGS in CD34+ HSPCs at day 2 following electroporation of RNP comprising the indicated sgRNA (unmod or modified as indicated. In each case the number corresponds to the CRxxxxxx identifier of the targeting domain, for example, Unmod sg312 refers to unmodified sgRNA comprising the targeting domain of CR00312). Controls include electroporation

of Cas9 protein only ("Cas9"), electroporation with buffer only ("mock"), or with no electroporation ("WT").

Figure 30A. Normalized % Erythrocytes (% F cells in "Mock" subtracted) that are HbF+ after electroporation of CD34+ HSPCs with RNP comprising the indicated dgRNA (unmod or modified as indicated) and then induced to differentiate by culture in erythroid differentiation medium. Controls include electroporation of buffer only ("mock").

Figure 30B. Normalized % Erythrocytes (% F cells in "Mock" subtracted) that are HbF+ after electroporation of CD34+ HSPCs with RNP comprising the indicated dgRNA (unmod or modified as indicated) and then in induced to differentiate by culture in erythroid differentiation medium. Controls include electroporation of buffer only ("mock").

Figure 31. Normalized % Erythrocytes (% F cells in "Mock" subtracted) that are HbF+ after electroporation of CD34+ HSPCs with RNP comprising the indicated sgRNA (unmod or modified as indicated. In each case the number corresponds to the CRxxxxxx identifier of the targeting domain, for example, Unmod sg312 refers to unmodified sgRNA comprising the targeting domain of CR00312) and then in induced to differentiate by culture in erythroid differentiation medium. Controls include electroporation of Cas9 protein and tracr only ("Cas9 + TracRNA only"), electroporation with buffer only ("Cells only + Pulse"), or with no electroporation ("Cells only no pulse").

Figure 32. Fold total cell expansion in culture at day 14 after erythroid differentiation after electroporation of RNP comprising the indicated sgRNA (in each case, the number corresponds to the CRxxxxxx identifier of the targeting domain, for example, Unmod sg312 refers to unmodified sgRNA comprising the targeting domain of CR00312) into CD34+ cells. Controls include electroporation of Cas9 protein and tracr only ("Cas9 + TracRNA only"), electroporation with buffer only ("Cells only + Pulse"), or with no electroporation ("Cells only no pulse").

Figure 33. Assessment of potential off-target sites cleaved by Cas9 directed by dgRNA molecules targeting the BCL11a enhancer region. Triangles represent the on-target site while open circles represent a potential off-target site for each guide RNA tested.

Figure 34. Editing efficiency at targeted B2M locus in CD34+ hematopoietic stem cells by different Cas9 variants, as evaluated by NGS and Flow cytometry. NLS = SV40 NLS; His6 or His8 refers to 6 or 8 histidine residues, respectively (SEQ ID NOS 2969 and 2670, respectively); TEV = tobacco etch virus cleavage site; Cas9 = wild type S. pyogenes Cas9 – mutations or variants are as indicated).

Figure 35. Data show fold increase in cell number after a total of 10 days of culturing in expansion medium (3 days of culturing prior to electroporation and 7 days of culturing following

electroporation). With all the guide RNAs tested, including both single and dual guide RNA formats, cell expansion ranged from 2-7 fold. CR00312 and CR001128, indicated by arrows, demonstrated a 3-6 fold increase after 10 days of cell expansion. Lables "Crxxxx" refer to dgRNA having the indicated targeting domain; "sgxxxx" refer to sgRNA having the targeting domain of the CRxxxxx identifier with the same number (e.g., sg312 has the targeting domain of CR00312); "Unmod" indicates no modification to theRNA(s); "O'MePS", when used in relation to a dgRNA refers to a crRNA which has three 3' and three 5' 2'-OMe modifications and phosphorothioate bonds, paired with an unmodified tracr; "O'MePS", when used in relation to a sgRNA refers to gRNA which has three 5'-terminal 2'-OMe modifications and phosphorothioate bonds, three 3'-terminal phosphorothioate bonds, and three 2'OMe modifications of the 4th-to-last, 3rd-to-last and 2nd-to-last 3' nucelotides.

Figure 36. Gating strategy to distinguish different HSPC subpopulations.

Figure 37. Frequency of each hematopoietic subset after 48 hours ex vivo culture in the indicated medium (STF modified with IL6, Compound 4, or both IL6 and Compound 4), but before electroporation of a CRISPR system. STF = StemSpan SFEM.

Figure 38. Frequency of each hematopoietic subset 7 days after electroporation introducing a CRISPR system targeting the +58 enhancer of BCL11a, cultured in the indicated medium (STF modified with IL6, Compound 4, or both IL6 and Compound 4). STF = StemSpan SFEM.

Figure 39A. Cell viability upon gene editing using different RNP concentrations, with RNP containing dgRNAs targeting the +58 region.

Figure 39B. Cell viability upon gene editing using different RNP concentrations, with RNP containing sgRNAs targeting the +58 region.

Figure 40A. Gene editing efficiency measured by NGS upon gene editing using different RNP concentrations, with RNP containing dgRNAs targeting the +58 region.

Figure 40B Gene editing efficiency measured by NGS upon gene editing using different RNP concentrations, with RNP containing sgRNAs targeting the +58 region.

Figure 41A. Percentage of HbF induction measured by flow cytometry upon gene editing using different RNP concentrations, with RNP containing dgRNAs targeting the +58 region.

Figure 41B Percentage of HbF induction measured by flow cytometry upon gene editing using different RNP concentrations, with RNP containing sgRNAs targeting the +58 region.

Figure 42A. Gene editing efficiencies of RNPs with different Cas9 proteins. Gene editing was performed using different Cas9 variants (listed on the X-axis) coupled with either the unmodified

version of sgRNA CR00312 and sgRNA CR001128, or a modified version of sgRNA CR00312 and sgRNA CR001128. Edited cells were subjected to NGS to determine % edit (Y-axis).

Figure 42B. Induction of HbF+ cells upon gene editing using different Cas9 proteins. Gene editing was performed using different Cas9 variants (listed on the X-axis) coupled with either the unmodified version of sgRNA CR00312 and sgRNA CR001128, or a modified version of sgRNA CR00312 and sgRNA CR001128. Edited cells were differentiated into erythroid lineage and HbF production assessed by flow cytometry using an anti-HbF antibody conjugated with fluorophore.

Figure 43. % Editing as determined by NGS in CD34+ HSPCs by electroporation of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA sequences as indicated in Table 36). Editing was determined at either 2 days (black bars) or 6 days (gray bars) post-electroporation. Less than 1.5% editing was detected at each site after control electroporation of Cas9 protein and Tracr only ("none", data not shown). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 44. Percent of viable cells that are CD71+ after electroporation into CD34+ HSPCs of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA sequences as indicated in Table 36) and culture in erythroid differentiation conditions for 7 days. After electroporation, cells were maintained by Protocol 1 (black bars) or Protocol 2 (gray bars), as described in Example 4.7. Controls include electroporation of Cas9 protein and Tracr only ("none"). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 45. Percent of erythroid cells that are HbF+ after electroporation into CD34+ HSPCs of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA sequences as indicated in Table 36) and culture in erythroid differentiation conditions for 7 days. After electroporation, cells were maintained by Protocol 1 (black bars) or Protocol 2 (gray bars), as described in Example 4.7. Controls include electroporation of Cas9 protein and Tracr only ("none"). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 46. Percent of cells that are HbF+ after electroporation into CD34+ HSPCs of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA sequences as indicated in Table 36) and cultured in erythroid differentiation conditions for 14 days. After electroporation, cells were maintained by Protocol 1 (black bars) or Protocol 2 (gray bars), as described in Example 4.7. Controls include electroporation of Cas9 protein and Tracr only ("none"). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 47. Percent of cells that are HbF+ after electroporation into CD34+ HSPCs of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA

sequences as indicated in Table 36) and cultured in erythroid differentiation conditions for 21 days. After electroporation, cells were maintained by Protocol 1 (black bars) or Protocol 2 (gray bars), as described in Example 4.7. Controls include electroporation of Cas9 protein and Tracr only ("none"). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 48. Fold total cell expansion in erythroid differentiation culture for 7 (black bars) or 21 days (black bars) after electroporation into CD34+ HSPCs of RNP comprising the indicated dgRNA or sgRNA (unmod or modified as indicated) (labels refer to the gRNA sequences as indicated in Table 36). After electroporation, cells were maintained by Protocol 2, as described in Example 4.7. Controls include electroporation of Cas9 protein and Tracr only ("none"). Mean + standard deviation of 2 electroporation replicates is shown.

Figure 49. Assessment of potential off-target sites cleaved by Cas9 directed by dgRNA molecules targeting an HPFH region. Triangles represent the on-target site while open circles represent a potential off-target site for each guide RNA tested.

DEFINITIONS

The terms "CRISPR system," "Cas system" or "CRISPR/Cas system" refer to a set of molecules comprising an RNA-guided nuclease or other effector molecule and a gRNA molecule that together are necessary and sufficient to direct and effect modification of nucleic acid at a target sequence by the RNA-guided nuclease or other effector molecule. In one embodiment, a CRISPR system comprises a gRNA and a Cas protein, e.g., a Cas9 protein. Such systems comprising a Cas9 or modified Cas9 molecule are referred to herein as "Cas9 systems" or "CRISPR/Cas9 systems." In one example, the gRNA molecule and Cas molecule may be complexed, to form a ribonuclear protein (RNP) complex.

The terms "guide RNA," "guide RNA molecule," "gRNA molecule" or "gRNA" are used interchangeably, and refer to a set of nucleic acid molecules that promote the specific directing of a RNA-guided nuclease or other effector molecule (typically in complex with the gRNA molecule) to a target sequence. In some embodiments, said directing is accomplished through hybridization of a portion of the gRNA to DNA (e.g., through the gRNA targeting domain), and by binding of a portion of the gRNA molecule to the RNA-guided nuclease or other effector molecule (e.g., through at least the gRNA tracr). In embodiments, a gRNA molecule consists of a single contiguous polynucleotide molecule, referred to herein as a "single guide RNA" or "sgRNA" and the like. In other embodiments, a gRNA molecule consists of a plurality, usually two, polynucleotide molecules, which are themselves capable of association, usually through hybridization, referred to herein as a "dual guide RNA" or "dgRNA," and the like. gRNA molecules are described in more detail below, but generally include a targeting domain and a tracr. In embodiments the targeting domain and tracr are

disposed on a single polynucleotide. In other embodiments, the targeting domain and tracr are disposed on separate polynucleotides.

The term "targeting domain" as the term is used in connection with a gRNA, is the portion of the gRNA molecule that recognizes, e.g., is complementary to, a target sequence, e.g., a target sequence within the nucleic acid of a cell, e.g., within a gene.

The term "crRNA" as the term is used in connection with a gRNA molecule, is a portion of the gRNA molecule that comprises a targeting domain and a region that interacts with a tracr to form a flagpole region.

The term "target sequence" refers to a sequence of nucleic acids complimentary, for example fully complementary, to a gRNA targeting domain. In embodiments, the target sequence is disposed on genomic DNA. In an embodiment the target sequence is adjacent to (either on the same strand or on the complementary strand of DNA) a protospacer adjacent motif (PAM) sequence recognized by a protein having nuclease or other effector activity, e.g., a PAM sequence recognized by Cas9. In embodiments, the target sequence is a target sequence within a gene or locus that affects expression of a globin gene, e.g., that affects expression of beta globin or fetal hemoglobin (HbF). In embodiments, the target sequence is a target sequence within the globin locus. In embodiments, the target sequence is a target sequence within the BCL11a gene. In embodiments, the target sequence is a target sequence within the BCL11a ehancer region. In embodiments, the target sequence is a target sequence within a HPFH region.

The term "flagpole" as used herein in connection with a gRNA molecule, refers to the portion of the gRNA where the crRNA and the tracr bind to, or hybridize to, one another.

The term "tracr" as used herein in connection with a gRNA molecule, refers to the portion of the gRNA that binds to a nuclease or other effector molecule. In embodiements, the tracr comprises nucleic acid sequence that binds specifically to Cas9. In embodiments, the tracr comprises nucleic acid sequence that forms part of the flagpole.

The terms "Cas9" or "Cas9 molecule" refer to an enzyme from bacterial Type II CRISPR/Cas system responsible for DNA cleavage. Cas9 also includes wild-type protein as well as functional and non-functinal mutants thereof. In embodiments, the Cas9 is a Cas9 of *S. pyogenes*.

The term "complementary" as used in connection with nucleic acid, refers to the pairing of bases, A with T or U, and G with C. The term complementary refers to nucleic acid molecules that are completely complementary, that is, form A to T or U pairs and G to C pairs across the entire reference sequence, as well as molecules that are at least 80%, 85%, 90%, 95%, 99% complementary.

"Template Nucleic Acid" as used in connection with homology-directed repair or homologous recombination, refers to nucleic acid to be inserted at the site of modification by the CRISPR system donor sequence for gene repair (insertion) at site of cutting.

An "indel," as the term is used herein, refers to a nucleic acid comprising one or more insertions of nucleotides, one or more deletions of nucleotides, or a combination of insertions and delections of nucleotides, relative to a reference nucleic acid, that results after being exposed to a composition comprising a gRNA molecule, for example a CRISPR system. Indels can be determined by sequencing nucleic acid after being exposed to a composition comprising a gRNA molecule, for example, by NGS. With respect to the site of an indel, an indel is said to be "at or near" a reference site (e.g., a site complementary to a targeting domain of a gRNA molecule) if it comprises at least one insertion or deletion within about 10, 9 , 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide(s) of the reference site, or is overlapping with part or all of said refrence site (e.g., comprises at least one insertion or deletion overlapping with, or within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucelotides of a site complementary to the targeting domain of a gRNA molecule, e.g., a gRNA molecule described herein).

An "indel pattern," as the term is used herein, refers to a set of indels that results after exposure to a composition comprising a gRNA molecule. In an embodiment, the indel pattern consists of the top three indels, by frequency of appearance. In an embodiment, the indel pattern consists of the top five indels, by frequency of appearance. In an embodiment, the indel pattern consists of the indels which are present at greater than about 5% frequency relative to all sequencing reads. In an embodiment, the indel pattern consists of the indels which are present at greater than about 10% frequency relative to to total number of indel sequencing reads (i.e., those reads that do not consist of the unmodified reference nucleic acid sequence). In an embodiment, the indel pattern includes of any 3 of the top five most frequently observed indels. The indel pattern may be determined, for example, by sequencing cells of a population of cells which were exposed to the gRNA molecule.

An "off-target indel," as the term I used herein, refers to an indel at or near a site other than the target sequence of the targeting domain of the gRNA molecule. Such sites may comprise, for example, 1, 2, 3, 4, 5 or more mismatch nucleotides relative to the sequence of the targeting domain of the gRNA. In exemplary embodiments, such sites are detected using targeted sequencing of in silico predicted off-target sites, or by an insertional method known in the art.

The term "a" and "an" refers to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "about" when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or in some instances ±10%, or in some instances

±5%, or in some instances ±1%, or in some instances ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA, which comprises a nucleotide sequences or a partial nucleotide sequence encoding a protein that elicits an immune response therefore encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that the present invention includes, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to encode polypeptides that elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be synthesized or can be derived from a biological sample, or might be macromolecule besides a polypeptide. Such a biological sample can include, but is not limited to a tissue sample, a cell or a fluid with other biological components.

The term "autologous" refers to any material derived from the same individual into whom it is later to be re-introduced.

The term "allogeneic" refers to any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically

The term "xenogeneic" refers to a graft derived from an animal of a different species.

"Derived from" as that term is used herein, indicates a relationship between a first and a second molecule. It generally refers to structural similarity between the first molecule and a second molecule and does not connotate or include a process or source limitation on a first molecule that is derived from a second molecule.

The term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene, cDNA, or RNA, encodes a protein if transcription and translation of

mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

Unless otherwise specified, a "nucleotide sequence encoding an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. The phrase nucleotide sequence that encodes a protein or a RNA may also include introns to the extent that the nucleotide sequence encoding the protein may in some version contain an intron(s).

The term "effective amount" or "therapeutically effective amount" are used interchangeably herein, and refer to an amount of a compound, formulation, material, or composition, as described herein effective to achieve a particular biological result.

The term "endogenous" refers to any material from or produced inside an organism, cell, tissue or system.

The term "exogenous" refers to any material introduced from or produced outside an organism, cell, tissue or system.

The term "expression" refers to the transcription and/or translation of a particular nucleotide sequence driven by a promoter.

The term "transfer vector" refers to a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, and viruses. Thus, the term "transfer vector" includes an autonomously replicating plasmid or a virus. The term should also be construed to further include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, a polylysine compound, liposome, and the like. Examples of viral transfer vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

The term "expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, including cosmids, plasmids (e.g., naked or contained in liposomes)

and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

The term "homologous" or "identity" refers to the subunit sequence identity between two polymeric molecules, e.g., between two nucleic acid molecules, such as, two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit; e.g., if a position in each of two DNA molecules is occupied by adenine, then they are homologous or identical at that position. The homology between two sequences is a direct function of the number of matching or homologous positions; e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two sequences are homologous, the two sequences are 50% homologous; if 90% of the positions (e.g., 9 of 10), are matched or homologous, the two sequences are 90% homologous.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

The term "operably linked" or "transcriptional control" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences can be contiguous with each other and, e.g., where necessary to join two protein coding regions, are in the same reading frame.

The term "parenteral" administration of an immunogenic composition includes, e.g., subcutaneous (s.c.), intravenous (i.v.), intramuscular (i.m.), or intrasternal injection, intratumoral, or infusion techniques.

The term "nucleic acid" or "polynucleotide" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence

explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); and Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

The terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. A polypeptide includes a natural peptide, a recombinant peptide, or a combination thereof.

The term "promoter" refers to a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

The term "promoter/regulatory sequence" refers to a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.

The term "constitutive" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.

The term "inducible" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.

The term "tissue-specific" promoter refers to a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.

As used herein in connection with a messenger RNA (mRNA), a 5' cap (also termed an RNA cap, an RNA 7-methylguanosine cap or an RNA m7G cap) is a modified guanine nucleotide that has been added to the "front" or 5' end of a eukaryotic messenger RNA shortly after the start of transcription. The 5' cap consists of a terminal group which is linked to the first transcribed nucleotide. Its presence is critical for recognition by the ribosome and protection from RNases. Cap addition is coupled to transcription, and occurs co-transcriptionally, such that each influences the other. Shortly after the start of transcription, the 5' end of the mRNA being synthesized is bound by a cap-synthesizing complex associated with RNA polymerase. This enzymatic complex catalyzes the chemical reactions that are required for mRNA capping. Synthesis proceeds as a multi-step biochemical reaction. The capping moiety can be modified to modulate functionality of mRNA such as its stability or efficiency of translation.

As used herein, "in vitro transcribed RNA" refers to RNA, preferably mRNA, that has been synthesized in vitro. Generally, the in vitro transcribed RNA is generated from an in vitro transcription vector. The in vitro transcription vector comprises a template that is used to generate the in vitro transcribed RNA.

As used herein, a "poly(A)" is a series of adenosines attached by polyadenylation to the mRNA. In the preferred embodiment of a construct for transient expression, the polyA is between 50 and 5000 (SEQ ID NO: 6596), preferably greater than 64, more preferably greater than 100, most preferably greater than 300 or 400. poly(A) sequences can be modified chemically or enzymatically to modulate mRNA functionality such as localization, stability or efficiency of translation.

As used herein, "polyadenylation" refers to the covalent linkage of a polyadenylyl moiety, or its modified variant, to a messenger RNA molecule. In eukaryotic organisms, most messenger RNA (mRNA) molecules are polyadenylated at the 3' end. The 3' poly(A) tail is a long sequence of adenine nucleotides (often several hundred) added to the pre-mRNA through the action of an enzyme, polyadenylate polymerase. In higher eukaryotes, the poly(A) tail is added onto transcripts that contain a specific sequence, the polyadenylation signal. The poly(A) tail and the protein bound to it aid in protecting mRNA from degradation by exonucleases. Polyadenylation is also important for transcription termination, export of the mRNA from the nucleus, and translation. Polyadenylation occurs in the nucleus immediately after transcription of DNA into RNA, but additionally can also occur later in the cytoplasm. After transcription has been terminated, the mRNA chain is cleaved through the action of an endonuclease complex associated with RNA polymerase. The cleavage site is usually characterized by the presence of the base sequence AAUAAA near the cleavage site. After the mRNA has been cleaved, adenosine residues are added to the free 3' end at the cleavage site.

As used herein, "transient" refers to expression of a non-integrated transgene for a period of hours, days or weeks, wherein the period of time of expression is less than the period of time for expression of the gene if integrated into the genome or contained within a stable plasmid replicon in the host cell.

As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a disorder, e.g., a hemoglobinopathy, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a disorder, e.g., a hemoglobinopathy, resulting from the administration of one or more therapies (e.g., one or more therapeutic agents such as a gRNA molecule, CRISPR system, or modified cell of the invention). In specific embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a hemoglobinopathy disorder, not discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" refer to the inhibition of the progression of a disorder, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of a symptom of a hemoglobinopathy, e.g., sickle cell disease or beta-thalassemia.

The term "signal transduction pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of a cell. The phrase "cell surface receptor" includes molecules and complexes of molecules capable of receiving a signal and transmitting signal across the membrane of a cell.

The term "subject" is intended to include living organisms in which an immune response can be elicited (e.g., mammals, human).

The term, a "substantially purified" cell refers to a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some aspects, the cells are cultured in vitro. In other aspects, the cells are not cultured in vitro.

The term "therapeutic" as used herein means a treatment. A therapeutic effect is obtained by reduction, suppression, remission, or eradication of a disease state.

The term "prophylaxis" as used herein means the prevention of or protective treatment for a disease or disease state.

The term "transfected" or "transformed" or "transduced" refers to a process by which exogenous nucleic acid and/or ptotein is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid and/or protein. The cell includes the primary subject cell and its progeny.

The term "specifically binds," refers to a molecule recognizing and binding with a binding partner (e.g., a protein or nucleic acid) present in a sample, but which molecule does not substantially recognize or bind other molecules in the sample.

The term "bioequivalent" refers to an amount of an agent other than the reference compound, required to produce an effect equivalent to the effect produced by the reference dose or reference amount of the reference compound.

"Refractory" as used herein refers to a disease, e.g., a hemoglobinopathy, that does not respond to a treatment. In embodiments, a refractory hemoglobinopathy can be resistant to a treatment before or at the beginning of the treatment. In other embodiments, the refractory hemoglobinopathy can become resistant during a treatment. A refractory hemoglobinopathy is also called a resistant hemoglobinopathy.

"Relapsed" as used herein refers to the return of a disease (e.g., hemoglobinopathy) or the signs and symptoms of a disease such as a hemoglobinopathy after a period of improvement, e.g., after prior treatment of a therapy, e.g., hemoglobinopathy therapy.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. As another example, a range such as 95-99% identity, includes something with 95%, 96%, 97%, 98% or 99% identity, and includes subranges such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the breadth of the range.

The term "BCL11a" refers to B-cell lymphoma/leukemia 11A, a RNA polymerase II core promoter proximal region sequence-sepecific DNA binding protein, and the gene encoding said protein, together with all introns and exons. This gene encodes a C2H2 type zinc-finger protein. BCL11A has been found to play a role in the suppression of fetal hemoglobin production. BCL11a is also known

as B-Cell CLL/Lymphoma 11A (Zinc Finger Protein), CTIP1, EVI9, Ecotropic Viral Integration Site 9 Protein Homolog, COUP-TF-Interacting Protein 1, Zinc Finger Protein 856, KIAA1809, BCL-11A, ZNF856, EVI-9, and B-Cell CLL/Lymphoma 11A. The term encompasses all isoforms and splice variants of BLC11a. The human gene encoding BCL11a is mapped to chromosomal location 2p16.1 (by Ensembl). The human and murine amino acid and nucleic acid sequences can be found in a public database, such as GenBank, UniProt and Swiss-Prot., and the genomic sequence of human BCL11a can be found in GenBank at NC_000002.12. The BCL11a gene refers to this genomic location, including all introns and exons. There are multiple known isotypes of BCL11a.

The sequence of mRNA encoding isoform 1 of human BCL11a can be found at NM_022893.

The peptide sequence of isoform 1 of human BCL11a is:

```
          10         20         30         40         50
MSRRKQGKPQ HLSKREFSPE PLEAILTDDE PDHGPLGAPE GDHDLLTCGQ
          60         70         80         90        100
CQMNFPLGDI LIFIEHKRKQ CNGSLCLEKA VDKPPSPSPI EMKKASNPVE
         110        120        130        140        150
VGIQVTPEDD DCLSTSSRGI CPKQEHIADK LLHWRGLSSP RSAHGALIPT
         160        170        180        190        200
PGMSAEYAPQ GICKDEPSSY TCTTCKQPFT SAWFLLQHAQ NTHGLRIYLE
         210        220        230        240        250
SEHGSPLTPR VGIPSGLGAE CPSQPPLHGI HIADNNPFNL LRIPGSVSRE
         260        270        280        290        300
ASGLAEGRFP PTPPLFSPPP RHHLDPHRIE RLGAEEMALA THHPSAFDRV
         310        320        330        340        350
LRLNPMAMEP PAMDFSRRLR ELAGNTSSPP LSPGRPSPMQ RLLQPFQPGS
         360        370        380        390        400
KPPFLATPPL PPLQSAPPPS QPPVKSKSCE FCGKTFKFQS NLVVHRRSHT
         410        420        430        440        450
GEKPYKCNLC DHACTQASKL KRHMKTHMHK SSPMTVKSDD GLSTASSPEP
         460        470        480        490        500
GTSDLVGSAS SALKSVVAKF KSENDPNLIP ENGDEEEEED DEEEEEEEEE
         510        520        530        540        550
EEEELTESER VDYGFGLSLE AARHHENSSR GAVVGVGDES RALPDVMQGM
         560        570        580        590        600
VLSSMQHFSE AFHQVLGEKH KRGHLAEAEG HRDTCDEDSV AGESDRIDDG
         610        620        630        640        650
TVNGRGCSPG ESASGGLSKK LLLGSPSSLS PFSKRIKLEK EFDLPPAAMP
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660 670 680 690 700

NTENVYSQWL AGYAASRQLK DPFLSFGDSR QSPFASSSEH SSENGSLRFS

710 720 730 740 750

TPPGELDGGI SGRSGTGSGG STPHISGPGP GRPSSKEGRR SDTCEYCGKV

760 770 780 790 800

FKNCSNLTVH RRSHTGERPY KCELCNYACA QSSKLTRHMK THGQVGKDVY

810 820 830

KCEICKMPFS VYSTLEKHMK KWHSDRVLNN DIKTE

SEQ ID NO: 2005 (Identifier Q9H165-1; and NM_022893.3; and accession ADL14508.1).

The sequences of other BCL11a protein isoforms are provided at:

Isoform 2: Q9H165-2

Isoform 3: Q9H165-3

Isoform 4: Q9H165-4

Isoform 5: Q9H165-5

Isoform 6: Q9H165-6

As used herein, a human BCL11a protein also encompasses proteins that have over its full length at least about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity with BCL11a isolorm 1-6, wherein such proteins still have at least one of the functions of BCL11a.

The term “globin locus” as used herein refers to the region of human chromosome 11 comprising genes for embryonic (ε), fetal (G(γ) and A(γ)), adult globin genes (γ and β), locus control regions and DNase I hypersensitivity sites.

The term “complementary” as used in connection with nucleic acid, refers to the pairing of bases, A with T or U, and G with C. The term complementary refers to nucleic acid molecules that are completely complementary, that is, form A to T or U pairs and G to C pairs across the entire reference sequence, as well as molecules that are at least 80%, 85%, 90%, 95%, 99% complementary.

The term “HPFH” refers to hereditary persistence of fetal hemoglobin, and is characterized in increased fetal hemoglobin in adult red blood cells. The term “HPFH region” refers to a genomic site which, when modified (e.g., mutated or deleted), causes increased HbF production in adult red blood cells, and includes HPFH sites identified in the literature (see e.g., the Online Mendelian Inheritance in Man: http://www.omim.org/entry/141749). In an exemplary embodiment, the HPFH region is a region within or encompassing the beta globin gene cluster on chromosome 11p15. In an exemplary

embodiment, the HPFH region is within or emcompasses at least part of the delta globin gene. In an exemplary embodiment, the HPFH region is a region of the promoter of HBG1. In an exemplary embodiment, the HPFH region is a region of the promoter of HBG1. In an exemplary embodiment, the HPFH region is a region described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the French breakpoint deletional HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Algerian HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Sri Lankan HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the HPFH-3 as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the HPFH-2 as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an embodiment, the HPFH-1 region is the HPFH-3 as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Sri Lankan $(\delta\beta)^0$-thalassemia HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Sicilian $(\delta\beta)^0$-thalassemia HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Macedonian $(\delta\beta)^0$-thalassemia HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the Kurdish β^0-thalassemia HPFH as described in Sankaran VG et al. NEJM (2011) 365:807-814. In an exemplary embodiment, the HPFH region is the region located at Chr11:5213874-5214400 (hg18). In an exemplary embodiment, the HPFH region is the region located at Chr11:5215943-5215046 (hg18). In an exemplary embodiment, the HPFH region is the region located at Chr11:5234390-5238486 (hg38).

"BCL11a enhancer" as the term is used herein, refers to nucleic acid sequence which affects, e.g., enhances, expression or function of BCL11a. See e.g., Bauer et al., Science, vol. 342, 2013, pp. 253-257. The BCL11a enhancer may be, for example, operative only in certain cell types, for example, cells of the erythroid lineage. One example of a BCL11a enhancer is the nucleic acid sequence between exon 2 and exon 3 of the BCL11a gene gene (e.g., the nucleic acid at or corresponding to positions +55: Chr2:60497676- 60498941; +58: Chr2:60494251- 60495546; +62: Chr2:60490409-60491734 as recorded in hg38). In an embodiment, the BCL11a Enhancer is the +62 region of the nucleic acid sequence between exon 2 and exon 3 of the BCL11a gene. In an embodiment, the BCL11a Enhancer is the +58 region of the nucleic acid sequence between exon 2 and exon 3 of the BCL11a gene. In an embodiment, the BCL11a Enhancer is the +55 region of the nucleic acid sequence between exon 2 and exon 3 of the BCL11a gene.

The terms "hematopoietic stem and progenitor cell" or "HSPC" are used interchangeably, and refer to a population of cells comprising both hematopoietic stem cells ("HSCs") and hematopoietic progenitor cells ("HPCs"). Such cells are characterized, for example, as CD34+. In exemplary

embodiments, HSPCs are isolated from bone marrow. In other exemplary embodiments, HSPCs are isolated from peripheral blood. In other exemplary embodiments, HSPCs are isolated from umbilical cord blood.

"Stem cell expander" as used herein refers to a compound which causes cells, e.g., HSPCs, HSCs and/or HPCs to proliferate, e.g., increase in number, at a faster rate relative to the same cell types absent said agent. In one exemplary aspect, the stem cell expander is an inhibitor of the aryl hydrocarbon receptor pathway. Additional examples of stem cell expanders are provided below. In embodiments, the proliferation, e.g., increase in number, is accomplished *ex vivo*.

"Engraftment" or "engraft" refers to the incorporation of a cell or tissue, e.g., a population of HSPCs, into the body of a recipient, e.g., a mammal or human subject. In one example, engraftment includes the growth, expansion and/or differention of the engrafted cells in the recipient. In an example, engraftment of HSPCs includes the differentiation and growth of said HSPCs into erythroid cells within the body of the recipient.

The term "Hematopoietic progenitor cells" (HPCs) as used herein refers to primitive hematopoietic cells that have a limited capacity for self-renewal and the potential for multilineage differentiation (e.g., myeloid, lymphoid), mono-lineage differentiation (e.g., myeloid or lymphoid) or cell-type restricted differentiation (e.g., erythroid progenitor) depending on placement within the hematopoietic hierarchy (Doulatov et al., Cell Stem Cell 2012).

"Hematopoietic stem cells" (HSCs) as used herein refer to immature blood cells having the capacity to self-renew and to differentiate into more mature blood cells comprising granulocytes (e.g., promyelocytes, neutrophils, eosinophils, basophils), erythrocytes (e.g., reticulocytes, erythrocytes), thrombocytes (e.g., megakaryoblasts, platelet producing megakaryocytes, platelets), and monocytes (e.g., monocytes, macrophages). HSCs are interchangeably described as stem cells throughout the specification. It is known in the art that such cells may or may not include CD34+ cells. CD34+ cells are immature cells that express the CD34 cell surface marker. CD34+ cells are believed to include a subpopulation of cells with the stem cell properties defined above. It is well known in the art that HSCs are multipotent cells that can give rise to primitive progenitor cells (e.g., multipotent progenitor cells) and/or progenitor cells committed to specific hematopoietic lineages (e.g., lymphoid progenitor cells). The stem cells committed to specific hematopoietic lineages may be of T cell lineage, B cell lineage, dendritic cell lineage, Langerhans cell lineage and/or lymphoid tissue-specific macrophage cell lineage. In addition, HSCs also refer to long term HSC (LT-HSC) and short term HSC (ST-HSC). ST-HSCs are more active and more proliferative than LT-HSCs. However, LT-HSC have unlimited self renewal (i.e., they survive throughout adulthood), whereas ST-HSC have limited self renewal (i.e., they survive for only a limited period of time). Any of these HSCs can be used in any of the methods described herein. Optionally, ST-HSCs are useful because they are highly proliferative and

thus, quickly increase the number of HSCs and their progeny. Hematopoietic stem cells are optionally obtained from blood products. A blood product includes a product obtained from the body or an organ of the body containing cells of hematopoietic origin. Such sources include un-fractionated bone marrow, umbilical cord, peripheral blood (e.g., mobilized peripheral blood, e.g., moblized with a mobilization agent such as G-CSF or Plerixafor® (AMD3100)), liver, thymus, lymph and spleen. All of the aforementioned crude or un-fractionated blood products can be enriched for cells having hematopoietic stem cell characteristics in ways known to those of skill in the art. In an embodiment, HSCs are characterized as CD34+/CD38-/CD90+/CD45RA-. In embodiments, the HSC s are characterized as CD34+/CD90+/CD49f+ cells.

"Expansion" or "Expand" in the context of cells refers to an increase in the number of a characteristic cell type, or cell types, from an initial cell population of cells, which may or may not be identical. The initial cells used for expansion may not be the same as the cells generated from expansion.

"Cell population" refers to eukaryotic mammalian, preferably human, cells isolated from biological sources, for example, blood product or tissues and derived from more than one cell.

"Enriched" when used in the context of cell population refers to a cell population selected based on the presence of one or more markers, for example, CD34+.

The term "CD34+ cells" refers to cells that express at their surface CD34 marker. CD34+ cells can be detected and counted using for example flow cytometry and fluorescently labeled anti-CD34 antibodies.

"Enriched in CD34+ cells" means that a cell population has been selected based on the presence of CD34 marker. Accordingly, the percentage of CD34+ cells in the cell population after selection method is higher than the percentage of CD34+ cells in the initial cell population before selecting step based on CD34 markers. For example, CD34+ cells may represent at least 50%, 60%, 70%, 80% or at least 90% of the cells in a cell population enriched in CD34+ cells.

The terms "F cell" and "F-cell" refer to cells, ususally erythrocytes (e.g., red blood cells) which contain and/or produce (e.g., express) fetal hemoglobin. For example, an F-cell is a cell that contains or produces detectible levels of fetal hemoglobin. For example, an F-cell is a cell that contains or produces at least 5 picograms of fetal hemoglobin. In another example, an F-cell is a cell that contains or produces at least 6 picograms of fetal hemoglobin. In another example, an F-cell is a cell that contains or produces at least 7 picograms of fetal hemoglobin. In another example, an F-cell is a cell that contains or produces at least 8 picograms of fetal hemoglobin. In another example, an F-cell is a cell that contains or produces at least 9 picograms of fetal hemoglobin. In another example, an F-cell is a cell that contains or produces at least 10 picograms of fetal hemoglobin. Levels of fetal

hemoglobin may be measured using an assay described herein or by other method known in the art, for example, flow cytometry using an anti-fetal hemoglobin detection reagent, high performance liquid chromatography, mass spectrometry, or enzyme-linked immunoabsorbent assay.

Unless otherwise stated, all genome or chromosome coordinates are are according to hg38.

DETAILED DESCRIPTION

The gRNA molecules, compositions and methods described herein relate to genome editing in eukaryotic cells using the CRISPR/Cas9 system. In particular, the gRNA molecules, compositions and methods described herein relate to regulation of globin levels and are useful, for example, in regulating expression and production of globin genes and protein. The gRNA molecules, compositions and methods can be useful in the treatment of hemoglobinopathies.

I . gRNA Molecules

A gRNA molecule may have a number of domains, as described more fully below, however, a gRNA molecule typically comprises at least a crRNA domain (comprising a targeting domain) and a tracr. The gRNA molecules of the invention, used as a component of a CRISPR system, are useful for modifying (e.g., modifying the sequence) DNA at or near a target site. Such modifications include deletions and or insertions that result in, for example, reduced or eliminated expression of a functional product of the gene comprising the target site. These uses, and additional uses, are described more fully below.

In an embodiment, a unimolecular, or sgRNA comprises, preferably from 5' to 3' : a crRNA (which contains a targeting domain complementary to a target sequence and a region that forms part of a flagpole (i.e., a crRNA flagpole region)); a loop; and a tracr (which contains a domain complementary to the crRNA flagpole region, and a domain which additionally binds a nuclease or other effector molecule, e.g., a Cas molecule, e.g., aCas9 molecule), and may take the following format (from 5’ to 3’):

[targeting domain] – [crRNA flagpole region] – [optional first flagpole extension] – [loop] – [optional first tracr extension] – [tracr flagpole region] – [tracr nuclease binding domain].

In embodiments, the tracr nuclease binding domain binds to a Cas protein, e.g., a Cas9 protein.

In an embodiment, a bimolecular, or dgRNA comprises two polynucleotides; the first, preferably from 5' to 3': a crRNA (which contains a targeting domain complementary to a target sequence and a region that forms part of a flagpole; and the second, preferrably from 5’ to 3’: a tracr (which contains a domain complementary to the crRNA flagpole region, and a domain which additionally binds a nuclease or other effector molecule, e.g., a Cas molecule, e.g., Cas9 molecule), and may take the following format (from 5’ to 3’):

Polynucleotide 1 (crRNA): [targeting domain] – [crRNA flagpole region] – [optional first flagpole extension] – [optional second flagpole extension]

Polynucleotide 2 (tracr): [optional first tracr extension] – [tracr flagpole region] - [tracr nuclease binding domain]

In embodiments, the tracr nuclease binding domain binds to a Cas protein, e.g., a Cas9 protein.

In some aspects, the targeting domain comprises or consists of a targeting domain sequence described herein, e.g., a targeting domain described in Table 1, 2, 3, 4, 5, or 6, or a targeting domain comprising or consisting of 17, 18, 19, 20, 21, 22, 23, 24, or 25 (preferably 20) consecutive nucleotides of a targeting domain sequence described in Table 1, 2, 3, 4, 5, or 6.

In some aspects, the flagpole, e.g., the crRNA flagpole region, comprises, from 5' to 3': GUUUUAGAGCUA (SEQ ID NO: 6584).

In some aspects, the flagpole, e.g., the crRNA flagpole region, comprises, from 5' to 3': GUUUAAGAGCUA (SEQ ID NO: 6585).

In some aspects the loop comprises, from 5' to 3': GAAA (SEQ ID NO: 6588).

In some aspects the tracr comprises, from 5' to 3':
UAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG
UGC (SEQ ID NO: 6589) and is preferably used in a gRNA molecule comprising SEQ ID NO 6584.

In some aspects the tracr comprises, from 5' to 3':
UAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG
UGC (SEQ ID NO: 6590) and is preferably used in a gRNA molecule comprising SEQ ID NO 6585.

In some aspects, the gRNA may also comprise, at the 3' end, additional U nucleic acids. For example the gRNA may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 U nucleic acids at the 3' end (SEQ ID NO: 2006). In an embodiment, the gRNA comprises an additional 4 U nucleic acids at the 3' end. In the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) may comprise, at the 3' end, additional U nucleic acids. For example, the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 U nucleic acids at the 3' end (SEQ ID NO: 2006). In an embodiment, in the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) comprises an additional 4 U nucleic acids at the 3' end. In an embodiment of a dgRNA, only the polynucleotide comprising the tracr comprises the additional U nucleic acid(s), e.g., 4 U nucleic acids. In an emebodiment of a dgRNA, only the polynucleotide

comprising the targeting domain comprises the additional U nucleic acid(s). In an embodiment of a dgRNA, both the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr comprise the additional U nucleic acids, e.g., 4 U nucleic acids.

In some aspects, the gRNA may also comprise, at the 3' end, additional A nucleic acids. For example the gRNA may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 A nucleic acids at the 3' end (SEQ ID NO: 2007). In an embodiment, the gRNA comprises an additional 4 A nucleic acids at the 3' end. In the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) may comprise, at the 3' end, additional A nucleic acids. For example, the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 A nucleic acids at the 3' end (SEQ ID NO: 2007). In an embodiment, in the case of dgRNA, one or more of the polynucleotides of the dgRNA (e.g., the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr) comprises an additional 4 A nucleic acids at the 3' end. In an embodiment of a dgRNA, only the polynucleotide comprising the tracr comprises the additional A nucleic acid(s), e.g., 4 A nucleic acids. In an emebodiment of a dgRNA, only the polynucleotide comprising the targeting domain comprises the additional A nucleic acid(s). In an embodiment of a dgRNA, both the polynucleotide comprising the targeting domain and the polynucleotide comprising the tracr comprise the additional U nucleic acids, e.g., 4 A nucleic acids.

In embodiments, one or more of the polynucleotides of the gRNA molecule may comprise a cap at the 5' end.

In an embodiment, a unimolecular, or sgRNA comprises, preferably from 5' to 3': a crRNA (which contains a targeting domain complementary to a target sequence; a crRNA flagpole region; first flagpole extension; a loop; a first tracr extension (which contains a domain complementary to at least a portion of the first flagpole extension); and a tracr (which contains a domain complementary to the crRNA flagpole region, and a domain which additionally binds a Cas9 molecule). In some aspects, the targeting domain comprises a targeting domain sequence described herein, e.g., a targeting domain described in Table 1, 2, 3, 4, 5, or 6, or a targeting domain comprising or consisting of 17, 18, 19, 20, 21, 22, 23, 24, or 25 (preferably 20) consecutive nucleotides of a targeting domain sequence described in Table 1, 2, 3, 4, 5, or 6, for example the 3' 17, 18, 19, 20, 21, 22, 23, 24 or 25 (preferably 20) consecutive nucleotides of a targeting domain sequence described in Table 1, 2, 3, 4, 5, or 6.

In aspects comprising a first flagpole extension and/or a first tracr extension, the flagpole, loop and tracr sequences may be as described above. In general any first flagpole extension and first tracr extension may be employed, provided that they are complementary. In embodiments, the first

flagpole extension and first tracr extension consist of 3, 4, 5, 6, 7, 8, 9, 10 or more complementary nucleotides.

In some aspects, the first flagpole extension comprises, from 5' to 3': UGCUG (SEQ ID NO: 6586). In some aspects, the first flagpole extension consists of SEQ ID NO: 6586.

In some aspects, the first tracr extension comprises, from 5' to 3': CAGCA (SEQ ID NO: 6591). In some aspects, the first tracr extension consists of SEQ ID NO: 6591.

In an embodiment, a dgRNA comprises two nucleic acid molecules. In some aspects, the dgRNA comprises a first nucleic acid which contains, preferably from 5' to 3' : a targeting domain complementary to a target sequence; a crRNA flagpole region; optionally a first flagpole extension; and, optionally, a second flagpole extension; and a second nucleic acid (which may be referred to herein as a tracr), and comprises at least a domain which binds a Cas molecule, e.g., a Cas9 molecule) comprising preferably from 5' to 3': optionally a first tracr extension; and a tracr (which contains a domain complementary to the crRNA flagpole region, and a domain which additionally binds a Cas, e.g., Cas9, molecule). The second nucleic acid may additionally comprise, at the 3' end (e.g., 3' to the tracr) additional U nucleic acids. For example the tracr may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 U nucleic acids at the 3' end (SEQ ID NO: 2006) (e.g., 3' to the tracr). The second nucleic acid may additionally or alternately comprise, at the 3' end (e.g., 3' to the tracr) additional A nucleic acids. For example the tracr may comprise an additional 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 A nucleic acids at the 3' end (SEQ ID NO: 2007) (e.g., 3' to the tracr). In some aspects, the targeting domain comprises a targeting domain sequence described herein, e.g., a targeting domain described in Table 1, 2, 3, 4, 5, or 6, or a targeting domain comprising or consisting of 17, 18, 19, 20, 21, 22, 23, 24, or 25 (preferably 20) consecutive nucleotides of a targeting domain sequence described in Table 1, 2, 3, 4, 5, or 6.

In aspects involving a dgRNA, the crRNA flagpole region, optional first flagpole extension, optional first tracr extension and tracr sequences may be as described above.

In some aspects, the optional second flagpole extension comprises, from 5' to 3': UUUUG (SEQ ID NO: 6587).

In embodiments, the 3' 1, 2, 3, 4, or 5 nucleotides, the 5' 1, 2, 3, 4, or 5 nucleotides, or both the 3' and 5' 1, 2, 3, 4, or 5 nucleotides of the gRNA molecule (and in the case of a dgRNA molecule, the polynucleotide comprising the targeting domain and/or the polynucleotide comprising the tracr) are modified nucleic acids, as described more fully in section XIII, below.

The domains are discussed briefly below:

1) The Targeting Domain:

Guidance on the selection of targeting domains can be found, e.g., in Fu Y el al. NAT BIOTECHNOL 2014 (doi: 10.1038/nbt.2808) and Sternberg SH el al. NATURE 2014 (doi: 10.1038/naturel3011).

The targeting domain comprises a nucleotide sequence that is complementary, e.g., at least 80, 85, 90, 95, or 99% complementary, e.g., fully complementary, to the target sequence on the target nucleic acid. The targeting domain is part of an RNA molecule and will therefore comprise the base uracil (U), while any DNA encoding the gRNA molecule will comprise the base thymine (T). While not wishing to be bound by theory, it is believed that the complementarity of the targeting domain with the target sequence contributes to specificity of the interaction of the gRNA molecule/Cas9 molecule complex with a target nucleic acid. It is understood that in a targeting domain and target sequence pair, the uracil bases in the targeting domain will pair with the adenine bases in the target sequence.

In an embodiment, the targeting domain is 5 to 50, e.g., 10 to 40, e.g., 10 to 30, e.g., 15 to 30, e.g., 15 to 25 nucleotides in length. In an embodiment, the targeting domain is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 nucleotides in length. In an embodiment, the targeting domain is 16 nucleotides in length. In an embodiment, the targeting domain is 17 nucleotides in length. In an embodiment, the targeting domain is 18 nucleotides in length. In an embodiment, the targeting domain is 19 nucleotides in length. In an embodiment, the targeting domain is 20 nucleotides in length. In an embodiment, the targeting domain is 21 nucleotides in length. In an embodiment, the targeting domain is 22 nucleotides in length. In an embodiment, the targeting domain is 23 nucleotides in length. In an embodiment, the targeting domain is 24 nucleotides in length. In an embodiment, the targeting domain is 25 nucleotides in length. In embodiments, the aforementioned 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides comprise the 5'- 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides from a targeting domain described in Table 1, 2, 3, 4, 5, or 6. In embodiments, the aforementioned 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides comprise the 3'- 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides from a targeting domain described in Table 1, 2, 3, 4, 5, or 6.

Without being bound by theory, it is believed that the 8, 9, 10, 11 or 12 nucleic acids of the targeting domain disposed at the 3' end of the targeting domain is important for targeting the target sequence, and may thus be referred to as the "core" region of the targeting domain. In an embodiment, the core domain is fully complementary with the target sequence.

The strand of the target nucleic acid with which the targeting domain is complementary is referred to herein as the target sequence. In some aspects, the target sequence is disposed on a chromosome, e.g., is a target within a gene. In some aspects the target sequence is disposed within an exon of a gene. In some aspects the target sequence is disposed within an intron of a gene. In some aspects, the target sequence comprises, or is proximal (e.g., within 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, or 1000 nucleic acids) to a binding site of a regulatory element, e.g., a promoter or transcription factor binding

site, of a gene of interest. Some or all of the nucleotides of the domain can have a modification, e.g., modification found in Section XIII herein.

2) crRNA Flagpole Region:

The flagpole contains portions from both the crRNA and the tracr. The crRNA flagpole region is complementary with a portion of the tracr, and in an embodiment, has sufficient complementarity to a portion of the tracr to form a duplexed region under at least some physiological conditions, for example, normal physiological conditions. In an embodiment, the crRNA flagpole region is 5 to 30 nucleotides in length. In an embodiment, the crRNA flagpole region is 5 to 25 nucleotides in length. The crRNA flagpole region can share homology with, or be derived from, a naturally occurring portion of the repeat sequence from a bacterial CRISPR array. In an embodiment, it has at least 50% homology with a crRNA flagpole region disclosed herein, e.g., an S. pyogenes, or S. thermophilus, crRNA flagpole region.

In an embodiment, the flagpole, e.g., the crRNA flagpole region, comprises SEQ ID NO: 6584. In an embodiment, the flagpole, e.g., the crRNA flagpole region, comprises sequence having at least 50%, 60%, 70%, 80%, 85%, 90%, 95% or 99% homology with SEQ ID NO: 6584. In an embodiment, the flagpole, e.g., the crRNA flagpole region, comprises at least 5, 6, 7, 8, 9, 10, or 11 nucleotides of SEQ ID NO: 6584. In an embodiment, the flagpole, e.g., the crRNA flagpole region, comprises SEQ ID NO: 6585. In an embodiment, the flagpole comprises sequence having at least 50%, 60%, 70%, 80%, 85%, 90%, 95% or 99% homology with SEQ ID NO: 6585. In an embodiment, the flagpole, e.g., the crRNA flagpole region, comprises at least 5, 6, 7, 8, 9, 10, or 11 nucleotides of SEQ ID NO: 6585.

Some or all of the nucleotides of the domain can have a modification, e.g., modification described in Section XIII herein.

3) First Flagpole Extension

When a tracr comprising a first tracr extension is used, the crRNA may comprise a first flagpole extension. In general any first flagpole extension and first tracr extension may be employed, provided that they are complementary. In embodiments, the first flagpole extension and first tracr extension consist of 3, 4, 5, 6, 7, 8, 9, 10 or more complementary nucleotides.

The first flagpole extension may comprise nucleotides that are complementary, e.g., 80%, 85%, 90%, 95% or 99%, e.g., fully complementary, with nucleotides of the first tracr extension. In some aspects, the first flagpole extension nucleotides that hybridize with complementary nucleotides of the first tracr extension are contiguous. In some aspects, the first flagpole extension nucleotides that hybridize with complementary nucleotides of the first tracr extension are discontinuous, e.g., comprises two or more regions of hybridization separated by nucleotides that do not base pair with nucleotides of the first tracr extension. In some aspects, the first flagpole extension comprises at least 2, 3, 4, 5, 6, 7, 8,

9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides. In some aspects, the first flagpole extension comprises, from 5' to 3': UGCUG (SEQ ID NO: 6586). In some aspects, the first flagpole extension consists of SEQ ID NO: 6586. In some aspects the first flagpole extension comprises nucleic acid that is at least 80%, 85%, 90%, 95% or 99% homology to SEQ ID NO: 6586.

Some or all of the nucleotides of the first tracr extension can have a modification, e.g., modification found in Section XIII herein.

3) The Loop

A loop serves to link the crRNA flagpole region (or optionally the first flagpole extension, when present) with the tracr (or optionally the first tracr extension, when present) of a sgRNA. The loop can link the crRNA flagpole region and tracr covalently or non-covalently. In an embodiment, the linkage is covalent. In an embodiment, the loop covalently couples the crRNA flagpole region and tracr. In an embodiment, the loop covalently couples the first flagpole extension and the first tracr extension. In an embodiment, the loop is, or comprises, a covalent bond interposed between the crRNA flagpole region and the domain of the tracr which hybridizes to the crRNA flagpole region. Typically, the loop comprises one or more, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.

In dgRNA molecules the two molecules can be associated by virtue of the hybridization between at least a portion of the crRNA (e.g., the crRNA flagpole region) and at least a portion of the tracr (e.g., the domain of the tracr which is complementary to the crRNA flagpole region).

A wide variety of loops are suitable for use in sgRNAs. Loops can consist of a covalent bond, or be as short as one or a few nucleotides, e.g., 1 , 2, 3, 4, or 5 nucleotides in length. In an embodiment, a loop is 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 or more nucleotides in length. In an embodiment, a loop is 2 to 50, 2 to 40, 2 to 30, 2 to 20, 2 to 10, or 2 to 5 nucleotides in length. In an embodiment, a loop shares homology with, or is derived from, a naturally occurring sequence. In an embodiment, the loop has at least 50% homology with a loop disclosed herein. In an embodiment, the loop comprises SEQ ID NO: 6588.

Some or all of the nucleotides of the domain can have a modification, e.g., modification described in Section XIII herein.

4) The Second Flagpole Extension

In an embodiment, a dgRNA can comprise additional sequence, 3' to the crRNA flagpole region or, when present, the first flagpole extension, referred to herein as the second flagpole extension. In an embodiment, the second flagpole extension is, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, or 2-4 nucleotides in length. In an embodiment, the second flagpole extension is 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more nucleotides in length. In an embodiment, the second flagpole extension comprises SEQ ID NO: 6587.

5) The Tracr:

The tracr is the nucleic acid sequence required for nuclease, e.g., Cas9, binding. Without being bound by theory, it is believed that each Cas9 species is associated with a particular tracr sequence. Tracr sequences are utilized in both sgRNA and in dgRNA systems. In an embodiment, the tracr comprises sequence from, or derived from, an *S. pyogenes* tracr. In some aspects, the tracr has a portion that hybridizes to the flagpole portion of the crRNA, e.g., has sufficient complementarity to the crRNA flagpole region to form a duplexed region under at least some physiological conditions (sometimes referred to herein as the tracr flagpole region or a tracr domain complementary to the crRNA flagpole region). In embodiments, the domain of the tracr that hybridizes with the crRNA flagpole region comprises at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides that hybridize with complementary nucleotides of the crRNA flagpole region. In some aspects, the tracr nucleotides that hybridize with complementary nucleotides of the crRNA flagpole region are contiguous. In some aspects, the tracr nucleotides that hybridize with complementary nucleotides of the crRNA flagpole region are discontinuous, e.g., comprises two or more regions of hybridization separated by nucleotides that do not base pair with nucleotides of the crRNA flagpole region. In some aspects, the portion of the tracr that hybridizes to the crRNA flagpole region comprises, from 5' to 3': UAGCAAGUUAAAA (SEQ ID NO: 6597). In some aspects, the portion of the tracr that hybridizes to the crRNA flagpole regioncomprises, from 5' to 3': UAGCAAGUUUAAA (SEQ ID NO: 6598). In embodiments, the sequence that hybridizes with the crRNA flagpole region is disposed on the tracr 5'- to the sequence of the tracr that additionally binds a nuclease, e.g., a Cas molecule, e.g., a Cas9 molecule.

The tracr further comprises a domain that additionally binds to a nuclease, e.g., a Cas molecule, e.g., a Cas9 molecule. Without being bound by theory, it is believed that Cas9 from different species bind to different tracr sequences. In some aspects, the tracr comprises sequence that binds to a *S. pyogenes* Cas9 molecule. In some aspects, the tracr comprises sequence that binds to a Cas9 molecule disclosed herein. In some aspects, the domain that additionally binds a Cas9 molecule comprises, from 5' to 3': UAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 6599). In some aspects the domain that additionally binds a Cas9 molecule comprises, from 5' to 3': UAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 6600).

In some embodiments, the tracr comprises SEQ ID NO: 6589. In some embodiments, the tracr comprises SEQ ID NO: 6590.

Some or all of the nucleotides of the tracr can have a modification, e.g., modification found in Section XIII herein. In embodiments, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA),

e.g., any of the gRNA or gRNA components described above, comprises an inverted abasic residue at the 5' end, the 3' end or both the 5' and 3' end of the gRNA. In embodiments, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises one or more phosphorothioate bonds between residues at the 5' end of the polynucleotide, for example, a phosphrothioate bond between the first two 5' residues, between each of the first three 5' residues, between each of the first four 5' residues, or between each of the first five 5' residues. In embodiments, the gRNA or gRNA component may alternatviely or additionally comprise one or more phosphorothioate bonds between residues at the 3' end of the polynucleotide, for example, a phosphrothioate bond between the first two 3' residues, between each of the first three 3' residues, between each of the first four 3' residues, or between each of the first five 5' residues. In an embodiment, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises a phosphorothioate bond between each of the first four 5' residues (e.g., comprises, e.g., consists of, three phosphorothioate bonds at the 5' end(s)), and a phosphorothioate bond between each of the first four 3' residues (e.g., comprises, e.g., consists of, three phosphorothioate bonds at the 3' end(s)). In an embodiment, any of the phosphorothioate modificaitons described above are combined with an inverted abasic residue at the 5' end, the 3' end, or both the 5' and 3' ends of the polynucleotide. In such embodiments, the inverted abasic nucleotide may be linked to the 5' and/or 3' nucelotide by a phosphate bond or a phosphorothioate bond. In embodiments, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises one or more nucleotides that include a 2' O-methyl modification. In embodiments, each of the first 1, 2, 3, or more of the 5' residues comprise a 2' O-methyl modification. In embodiments, each of the first 1, 2, 3, or more of the 3' residues comprise a 2' O-methyl modification. In embodiments, the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues comprise a 2' O-methyl modification. In embodiments, each of the first 1, 2, 3 or more of the 5' residues comprise a 2' O-methyl modification, and each of the first 1, 2, 3 or more of the 3' residues comprise a 2' O-methyl modification. In an embodiment, each of the first 3 of the 5' residues comprise a 2' O-methyl modification, and each of the first 3 of the 3' residues comprise a 2' O-methyl modification. In embodiments, each of the first 3 of the 5' residues comprise a 2' O-methyl modification, and the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues comprise a 2' O-methyl modification. In embodiments, any of the 2' O-methyl modfications, e.g., as described above, may be combined with one or more phosphorothioate modifications, e.g., as described above, and/or one or more inverted abasic modifications, e.g., as described above. In an embodiment, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises, e.g., consists of, a phosphorothioate bond between each of the first four 5' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a phosphorothioate bond between each of the first four 3' residues (e.g., comprises, e.g., consists of three phosphorothioate

bonds at the 5' end of the polynucleotide(s)), a 2' O-methyl modification at each of the first three 5' residues, and a 2' O-methyl modification at each of the first three 3' residues. In an embodiment, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises, e.g., consists of, a phosphorothioate bond between each of the first four 5' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a phosphorothioate bond between each of the first four 3' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a 2' O-methyl modification at each of the first three 5' residues, and a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues.

In an embodiment, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises, e.g., consists of, a phosphorothioate bond between each of the first four 5' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a phosphorothioate bond between each of the first four 3' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a 2' O-methyl modification at each of the first three 5' residues, a 2' O-methyl modification at each of the first three 3' residues, and an additional inverted abasic residue at each of the 5' and 3' ends.

In an embodiment, the gRNA (e.g., the sgRNA or the tracr and/or crRNA of a dgRNA), e.g., any of the gRNA or gRNA components described above, comprises, e.g., consists of, a phosphorothioate bond between each of the first four 5' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a phosphorothioate bond between each of the first four 3' residues (e.g., comprises, e.g., consists of three phosphorothioate bonds at the 5' end of the polynucleotide(s)), a 2' O-methyl modification at each of the first three 5' residues, and a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues, and an additional inverted abasic residue at each of the 5' and 3' ends.

In an embodiment, the gRNA is a dgRNA and comprises, e.g., consists of:

crRNA:

mN*mN*mN*NNNNNNNNNNNNNNNNNNGUUUUAGAGCUAU*mG*mC*mU (SEQ ID NO: 2008), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus); and

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660) (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a dgRNA and comprises, e.g., consists of:

crRNA:

mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAGCUAU*mG*mC*mU (SEQ ID NO: 2009), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus); and

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a dgRNA and comprises, e.g., consists of:

crRNA:

mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 2010), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus); and

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660) (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a dgRNA and comprises, e.g., consists of:

crRNA:

mN*mN*mN*NNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 2011), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus); and

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346), where m indicates a base with 2'O-Methyl modification, and * indicates a phosphorothioate bond (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a dgRNA and comprises, e.g., consists of:

crRNA:

NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 2012), where N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus); and

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346), where m indicates a base with 2'O-Methyl modification, and * indicates a phosphorothioate bond (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a sgRNA and comprises, e.g., consists of:

NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 2013), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a sgRNA and comprises, e.g., consists of:

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 2014), where m indicates a base with 2'O-Methyl modification, * indicates a phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

In an embodiment, the gRNA is a sgRNA and comprises, e.g., consists of:

mN*mN*mN*NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 2015), where m indicates a base with 2'O-Methyl modification, * indicates a

phosphorothioate bond, and N's indicate the residues of the targeting domain, e.g., as described herein, (optionally with an inverted abasic residue at the 5' and/or 3' terminus).

6) First Tracr Extension

Where the gRNA comprises a first flagpole extension, the tracr may comprise a first tracr extension. The first tracr extension may comprise nucleotides that are complementary, e.g., 80%, 85%, 90%, 95% or 99%, e.g., fully complementary, with nucleotides of the first flagpole extension. In some aspects, the first tracr extension nucleotides that hybridize with complementary nucleotides of the first flagpole extension are contiguous. In some aspects, the first tracr extension nucleotides that hybridize with complementary nucleotides of the first flagpole extension are discontinuous, e.g., comprises two or more regions of hybridization separated by nucleotides that do not base pair with nucleotides of the first flagpole extension. In some aspects, the first tracr extension comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides. In some aspects, the first tracr extension comprises SEQ ID NO: 6591. In some aspects the first tracr extension comprises nucleic acid that is at least 80%, 85%, 90%, 95% or 99% homology to SEQ ID NO: 6591.

Some or all of the nucleotides of the first tracr extension can have a modification, e.g., modification found in Section XIII herein.

In some embodiments, the sgRNA may comprise, from 5' to 3', disposed 3' to the targeting domain:

a)

GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAA AGUGGCACCGAGUCGGUGC (SEQ ID NO: 6601);

b)

GUUUAAGAGCUAGAAAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAA AGUGGCACCGAGUCGGUGC (SEQ ID NO: 6602);

c)

GUUUUAGAGCUAUGCUGGAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 6603);

d)

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGC (SEQ ID NO: 6604);

e) any of a) to d), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides;

f) any of a) to d), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides; or

g) any of a) to f), above, further comprising, at the 5' end (e.g., at the 5' terminus, e.g., 5' to the targeting domain), at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides. In embodiments, any of a) to g) above is disposed directly 3' to the targeting domain.

In an embodiment, a sgRNA of the invention comprises, e.g., consists of, from 5' to 3': [targeting domain]-
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAA
AGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 7811).

In an embodiment, a sgRNA of the invention comprises, e.g., consists of, from 5' to 3': [targeting domain]-
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUC
AACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 7807).

In some embodiments, the dgRNA may comprise:

A crRNA comprising, from 5' to 3', preferrably disposed directly 3' to the targeting domain:

a) GUUUUAGAGCUA (SEQ ID NO: 6584);

b) GUUUAAGAGCUA (SEQ ID NO: 6585);

c) GUUUUAGAGCUAUGCUG (SEQ ID NO: 6605);

d) GUUUAAGAGCUAUGCUG (SEQ ID NO: 6606);

e) GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 6607);

f) GUUUAAGAGCUAUGCUGUUUUG (SEQ ID NO: 6608); or

g) GUUUUAGAGCUAUGCU (SEQ ID NO: 7806):

and a tracr comprising, from 5' to 3':

a)
UAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG
UGC (SEQ ID NO: 6589);

b)
UAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGG
UGC (SEQ ID NO: 6590);

c)

CAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGA GUCGGUGC (SEQ ID NO: 6609);

d)

CAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGA GUCGGUGC (SEQ ID NO: 6610);

e)

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660);

f)

AACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6661);

g)

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGC (SEQ ID NO: 7812)

h)

GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 7807);

i)

AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG UCGGUGCUUU (SEQ ID NO: 7808);

j)

GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAGUCGGUGCUUU (SEQ ID NO: 7809);

k) any of a) to j), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides;

l) any of a) to j), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides; or

m) any of a) to l), above, further comprising, at the 5' end (e.g., at the 5' terminus), at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides.

In an embodiment, the sequence of k), above comprises the 3' sequence UUUUUU, e.g., if a U6 promoter is used for transcription. In an embodiment, the sequence of k), above, comprises the 3'

sequence UUUU, e.g., if an HI promoter is used for transcription. In an embodiment, sequence of k), above, comprises variable numbers of 3' U's depending, e.g., on the termination signal of the pol-III promoter used. In an embodiment, the sequence of k), above, comprises variable 3' sequence derived from the DNA template if a T7 promoter is used. In an embodiment, the sequence of k), above, comprises variable 3' sequence derived from the DNA template, e.g., if in vitro transcription is used to generate the RNA molecule. In an embodiment, the sequence of k), above, comprises variable 3' sequence derived from the DNA template, e.g, if a pol-II promoter is used to drive transcription.

In an embodiment, the crRNA comprises, e.g., consists of, a targeting domain and, disposed 3' to the targeting domain (e.g., disposed directly 3' to the targeting domain), a sequence comprising, e.g., consisting of, SEQ ID NO: 6607, and the tracr comprises, e.g., consists of AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In an embodiment, the crRNA comprises, e.g., consists of, a targeting domain and, disposed 3' to the targeting domain (e.g., disposed directly 3' to the targeting domain), a sequence comprising, e.g., consisting of, SEQ ID NO: 6608, and the tracr comprises, e.g., consists of, AACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6661).

In an embodiment, the crRNA comprises, e.g., consists of, a targeting domain and, disposed 3' to the targeting domain (e.g., disposed directly 3' to the targeting domain), a sequence comprising, e.g., consisting of, GUUUUAGAGCUAUGCU (SEQ ID NO: 7806), and the tracr comprises, e.g., consists of,
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUC AACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 7807).

In an embodiment, the crRNA comprises, e.g., consists of, a targeting domain and, disposed 3' to the targeting domain (e.g., disposed directly 3' to the targeting domain), a sequence comprising, e.g., consisting of, GUUUUAGAGCUAUGCU (SEQ ID NO: 7806), and the tracr comprises, e.g., consists of,
AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG UCGGUGCUUU (SEQ ID NO: 7808).

In an embodiment, the crRNA comprises, e.g., consists of, a targeting domain and, disposed 3' to the targeting domain (e.g., disposed directly 3' to the targeting domain), a sequence comprising, e.g., consisting of, GUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 6607), and the tracr comprises, e.g., consists of,
GUUGGAACCAUUCAAAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UGAAAAAGUGGCACCGAGUCGGUGCUUU (SEQ ID NO: 7809).

II. Targeting Domains Useful for Altering Expression of Globin Genes

Provided in the tables below are targeting domains for gRNA molecules for use in the various aspect of the present invention, for example, in altering expression of globin genes, for example, a fetal hemoglobin gene or a hemoglobin beta gene.

Table 1: gRNA targeting domains directed to BLC11a exon regions

Id.	Target	Target Region	Strand	Targeting Site (hg38)	gRNA Targeting Domain	SEQ ID NO:
53335_5_1	BCL11a	Exon 5	+	chr2:60451170-60451195	CAGUCAUUAUUUAUUAUGAAUAAGC	400
53335_5_2	BCL11a	Exon 5	+	chr2:60451196-60451221	GGAAAUAAUUCACAUGCCAAUUAUU	401
53335_5_3	BCL11a	Exon 5	+	chr2:60451217-60451242	UAUUUGGCUAUCUUUUCACUAAGCU	402
53335_5_4	BCL11a	Exon 5	+	chr2:60451233-60451258	CACUAAGCUAGGUAAUCUAGCCAGA	403
53335_5_5	BCL11a	Exon 5	+	chr2:60451236-60451261	UAAGCUAGGUAAUCUAGCCAGAAGG	404
53335_5_6	BCL11a	Exon 5	+	chr2:60451241-60451266	UAGGUAAUCUAGCCAGAAGGUGGAU	405
53335_5_7	BCL11a	Exon 5	+	chr2:60451245-60451270	UAAUCUAGCCAGAAGGUGGAUAGGU	406
53335_5_8	BCL11a	Exon 5	+	chr2:60451261-60451286	UGGAUAGGUAGGAUUUUCCCCACUU	407
53335_5_9	BCL11a	Exon 5	+	chr2:60451268-60451293	GUAGGAUUUUCCCCACUUAGGUUCA	408
53335_5_10	BCL11a	Exon 5	+	chr2:60451295-60451320	GCCUGUAUGUGAAUCUACAGCACAC	409
53335_5_11	BCL11a	Exon 5	+	chr2:60451359-60451384	UGCUAUGUUGUUUCUAAUUCUCUUC	410
53335_5_12	BCL11a	Exon 5	+	chr2:60451414-60451439	UGAGAAAAGUUCUGUGCAAAUUAAC	411
53335_5_13	BCL11a	Exon 5	+	chr2:60451415-60451440	GAGAAAAGUUCUGUGCAAAUUAACU	412
53335_5_14	BCL11	Exon 5	+	chr2:60451462-	CCUGUUUGUAGAUGUAACU	413

	a			60451487	UAUUUG	
53335_5_15	BCL11a	Exon 5	+	chr2:60451477-60451502	AACUUAUUUGUGGCACUAAUUUCUA	414
53335_5_16	BCL11a	Exon 5	+	chr2:60451625-60451650	GUCUGCAUUAAGAUAUAUUUCCUGA	415
53335_5_17	BCL11a	Exon 5	+	chr2:60451630-60451655	CAUUAAGAUAUAUUUCCUGAAGGUU	416
53335_5_18	BCL11a	Exon 5	+	chr2:60451631-60451656	AUUAAGAUAUAUUUCCUGAAGGUUU	417
53335_5_19	BCL11a	Exon 5	+	chr2:60451687-60451712	ACUACUGACAUUUAUCACCUUCUUU	418
53335_5_20	BCL11a	Exon 5	+	chr2:60451754-60451779	UUAAAAGACAAAUUCAAAUCCUGCA	419
53335_5_21	BCL11a	Exon 5	+	chr2:60451786-60451811	CACCAAAAGCAUAUAUUUGAAAAAC	420
53335_5_22	BCL11a	Exon 5	+	chr2:60451792-60451817	AAGCAUAUAUUUGAAAAACAGGAUU	421
53335_5_23	BCL11a	Exon 5	+	chr2:60451819-60451844	GCGUGCCAUAUUAUGCAUUAUUUAA	422
53335_5_24	BCL11a	Exon 5	+	chr2:60451847-60451872	UAUGCAAAUUAUAAGUCAGACAGUU	423
53335_5_25	BCL11a	Exon 5	+	chr2:60451971-60451996	UUGAAAAUUUAUGCCAUCUGAUAAG	424
53335_5_26	BCL11a	Exon 5	+	chr2:60452037-60452062	AAAUCUGUUCCUCCUACCCACCCGA	425
53335_5_27	BCL11a	Exon 5	+	chr2:60452038-60452063	AAUCUGUUCCUCCUACCCACCCGAU	426
53335_5_28	BCL11a	Exon 5	+	chr2:60452048-60452073	UCCUACCCACCCGAUGGGUGUCUGU	427
53335_5_29	BCL11a	Exon 5	+	chr2:60452055-60452080	CACCCGAUGGGUGUCUGUAGGAAAC	428
53335_5_30	BCL11a	Exon 5	+	chr2:60452203-60452228	AGACAACAUAGAAUGUAUAGAAACA	429
53335_5_31	BCL11a	Exon 5	+	chr2:60452204-60452229	GACAACAUAGAAUGUAUAGAAACAA	430
53335_5_32	BCL11	Exon 5	+	chr2:60452205-	ACAACAUAGAAUGUAUAGA	431

	a			60452230	AACAAG	
53335_5_33	BCL11a	Exon 5	+	chr2:60452209-60452234	CAUAGAAUGUAUAGAAACAAGGGGU	432
53335_5_34	BCL11a	Exon 5	+	chr2:60452210-60452235	AUAGAAUGUAUAGAAACAAGGGGUU	433
53335_5_35	BCL11a	Exon 5	+	chr2:60452211-60452236	UAGAAUGUAUAGAAACAAGGGGUUG	434
53335_5_36	BCL11a	Exon 5	+	chr2:60452237-60452262	GGACUCAUGCGCAUUUCCACAAUAC	435
53335_5_37	BCL11a	Exon 5	+	chr2:60452245-60452270	GCGCAUUUCCACAAUACAGGUAAUU	436
53335_5_38	BCL11a	Exon 5	+	chr2:60452249-60452274	AUUUCCACAAUACAGGUAAUUAGGU	437
53335_5_39	BCL11a	Exon 5	+	chr2:60452253-60452278	CCACAAUACAGGUAAUUAGGUUGGC	438
53335_5_40	BCL11a	Exon 5	+	chr2:60452263-60452288	GGUAAUUAGGUUGGCUGGUUUCAGA	439
53335_5_41	BCL11a	Exon 5	+	chr2:60452264-60452289	GUAAUUAGGUUGGCUGGUUUCAGAA	440
53335_5_42	BCL11a	Exon 5	+	chr2:60452269-60452294	UAGGUUGGCUGGUUUCAGAAGGGCC	441
53335_5_43	BCL11a	Exon 5	+	chr2:60452270-60452295	AGGUUGGCUGGUUUCAGAAGGGCCA	442
53335_5_44	BCL11a	Exon 5	+	chr2:60452291-60452316	GCCAGGGCAUCACUCAUGACAGCGA	443
53335_5_45	BCL11a	Exon 5	+	chr2:60452298-60452323	CAUCACUCAUGACAGCGAUGGUCCA	444
53335_5_46	BCL11a	Exon 5	+	chr2:60452299-60452324	AUCACUCAUGACAGCGAUGGUCCAC	445
53335_5_47	BCL11a	Exon 5	+	chr2:60452311-60452336	AGCGAUGGUCCACGGGCCCUCUCUA	446
53335_5_48	BCL11a	Exon 5	+	chr2:60452312-60452337	GCGAUGGUCCACGGGCCCUCUCUAU	447
53335_5_49	BCL11a	Exon 5	+	chr2:60452335-60452360	AUGGGACUGAUUCACUGUUCCAAUG	448
53335_5_50	BCL11	Exon 5	+	chr2:60452336-	UGGGACUGAUUCACUGUUC	449

	a			60452361	CAAUGU	
53335_5_51	BCL11a	Exon 5	+	chr2:60452383-60452408	UUUUUAUUAAAAAAUAUAAAUAAAA	450
53335_5_52	BCL11a	Exon 5	+	chr2:60452392-60452417	AAAAAUAUAAAUAAAAUGGCGCUGC	451
53335_5_53	BCL11a	Exon 5	+	chr2:60452398-60452423	AUAAAUAAAAUGGCGCUGCAGGCCU	452
53335_5_54	BCL11a	Exon 5	+	chr2:60452402-60452427	AUAAAAUGGCGCUGCAGGCCUAGGC	453
53335_5_55	BCL11a	Exon 5	+	chr2:60452406-60452431	AAUGGCGCUGCAGGCCUAGGCUGGA	454
53335_5_56	BCL11a	Exon 5	+	chr2:60452416-60452441	CAGGCCUAGGCUGGAAGGACUCUGC	455
53335_5_57	BCL11a	Exon 5	+	chr2:60452436-60452461	UCUGCAGGACUCUGUCUUCGCACAA	456
53335_5_58	BCL11a	Exon 5	+	chr2:60452444-60452469	ACUCUGUCUUCGCACAACGGCUUCU	457
53335_5_59	BCL11a	Exon 5	+	chr2:60452447-60452472	CUGUCUUCGCACAACGGCUUCUUGG	458
53335_5_60	BCL11a	Exon 5	+	chr2:60452478-60452503	CUGUCAGAAAACAUCACAAACUAGC	459
53335_5_61	BCL11a	Exon 5	+	chr2:60452505-60452530	GAUGACAGACCACGCUGACGUCGAC	460
53335_5_62	BCL11a	Exon 5	+	chr2:60452506-60452531	AUGACAGACCACGCUGACGUCGACU	461
53335_5_63	BCL11a	Exon 5	+	chr2:60452509-60452534	ACAGACCACGCUGACGUCGACUGGG	462
53335_5_64	BCL11a	Exon 5	+	chr2:60452531-60452556	GGGCGGCACGCGUCCACCCCACCCC	463
53335_5_65	BCL11a	Exon 5	+	chr2:60452532-60452557	GGCGGCACGCGUCCACCCCACCCCU	464
53335_5_66	BCL11a	Exon 5	+	chr2:60452533-60452558	GCGGCACGCGUCCACCCCACCCCUG	465
53335_5_67	BCL11a	Exon 5	+	chr2:60452534-60452559	CGGCACGCGUCCACCCCACCCCUGG	466
53335_5_68	BCL11	Exon 5	+	chr2:60452559-	GGGCUUCAAAUUUUCUCAG	467

	a			60452584	AACUUA	
53335_5_69	BCL11a	Exon 5	+	chr2:60452560-60452585	GGCUUCAAAUUUUCUCAGAACUUAA	468
53335_5_70	BCL11a	Exon 5	+	chr2:60452607-60452632	AAACUGCCACACAUCUUGAGCUCUC	469
53335_5_71	BCL11a	Exon 5	+	chr2:60452608-60452633	AACUGCCACACAUCUUGAGCUCUCU	470
53335_5_72	BCL11a	Exon 5	+	chr2:60452623-60452648	UGAGCUCUCUGGGUACUACGCCGAA	471
53335_5_73	BCL11a	Exon 5	+	chr2:60452624-60452649	GAGCUCUCUGGGUACUACGCCGAAU	472
53335_5_74	BCL11a	Exon 5	+	chr2:60452625-60452650	AGCUCUCUGGGUACUACGCCGAAUG	473
53335_5_75	BCL11a	Exon 5	+	chr2:60452626-60452651	GCUCUCUGGGUACUACGCCGAAUGG	474
53335_5_76	BCL11a	Exon 5	+	chr2:60452649-60452674	GGGGGUGUGUGAAGAACCUAGAAAG	475
53335_5_77	BCL11a	Exon 5	+	chr2:60452653-60452678	GUGUGUGAAGAACCUAGAAAGAGGU	476
53335_5_78	BCL11a	Exon 5	+	chr2:60452662-60452687	GAACCUAGAAAGAGGUUGGAGACAG	477
53335_5_79	BCL11a	Exon 5	-	chr2:60451215-60451240	CUUAGUGAAAAGAUAGCCAAAUAAU	478
53335_5_80	BCL11a	Exon 5	-	chr2:60451256-60451281	GGGAAAAUCCUACCUAUCCACCUUC	479
53335_5_81	BCL11a	Exon 5	-	chr2:60451281-60451306	CACAUACAGGCCGUGAACCUAAGUG	480
53335_5_82	BCL11a	Exon 5	-	chr2:60451282-60451307	UCACAUACAGGCCGUGAACCUAAGU	481
53335_5_83	BCL11a	Exon 5	-	chr2:60451283-60451308	UUCACAUACAGGCCGUGAACCUAAG	482
53335_5_84	BCL11a	Exon 5	-	chr2:60451299-60451324	ACCUGUGUGCUGUAGAUUCACAUAC	483
53335_5_85	BCL11a	Exon 5	-	chr2:60451350-60451375	UAGAAACAACAUAGCAAAUUAAAAU	484
53335_5_86	BCL11	Exon 5	-	chr2:60451394-	UCUCAGUUUGGUAUUUUUU	485

	a			60451419	ACUGCU	
53335_5_87	BCL11a	Exon 5	-	chr2:60451411-60451436	AAUUUGCACAGAACUUUUCUCAGUU	486
53335_5_88	BCL11a	Exon 5	-	chr2:60451446-60451471	ACAAACAGGUGGUAAAAAUUCUUUC	487
53335_5_89	BCL11a	Exon 5	-	chr2:60451462-60451487	CAAAUAAGUUACAUCUACAAACAGG	488
53335_5_90	BCL11a	Exon 5	-	chr2:60451465-60451490	CCACAAAUAAGUUACAUCUACAAAC	489
53335_5_91	BCL11a	Exon 5	-	chr2:60451552-60451577	GAUUUUAGAGGGGGGAAAUUAUAGG	490
53335_5_92	BCL11a	Exon 5	-	chr2:60451555-60451580	GCAGAUUUUAGAGGGGGGAAAUUAU	491
53335_5_93	BCL11a	Exon 5	-	chr2:60451565-60451590	GAAAUUUGGGGCAGAUUUUAGAGGG	492
53335_5_94	BCL11a	Exon 5	-	chr2:60451566-60451591	GGAAAUUUGGGGCAGAUUUUAGAGG	493
53335_5_95	BCL11a	Exon 5	-	chr2:60451567-60451592	AGGAAAUUUGGGGCAGAUUUUAGAG	494
53335_5_96	BCL11a	Exon 5	-	chr2:60451568-60451593	CAGGAAAUUUGGGGCAGAUUUUAGA	495
53335_5_97	BCL11a	Exon 5	-	chr2:60451569-60451594	UCAGGAAAUUUGGGGCAGAUUUUAG	496
53335_5_98	BCL11a	Exon 5	-	chr2:60451582-60451607	UUUAGUACUUACAUCAGGAAAUUUG	497
53335_5_99	BCL11a	Exon 5	-	chr2:60451583-60451608	CUUUAGUACUUACAUCAGGAAAUUU	498
53335_5_100	BCL11a	Exon 5	-	chr2:60451584-60451609	UCUUUAGUACUUACAUCAGGAAAUU	499
53335_5_101	BCL11a	Exon 5	-	chr2:60451592-60451617	AUAAAACUUCUUUAGUACUUACAUC	500
53335_5_102	BCL11a	Exon 5	-	chr2:60451648-60451673	UCUAAUUUUAGGUUCCCAAACCUUC	501
53335_5_103	BCL11a	Exon 5	-	chr2:60451664-60451689	GUUUCUUAUUAAAUAUUCUAAUUUU	502
53335_5_10	BCL11	Exon 5	-	chr2:60451707-	CUGGGCGAGCGGUAAAUCC	503

4	a			60451732	UAAAGA	
53335_5_105	BCL11a	Exon 5	-	chr2:60451723-60451748	UUAGGAAAGAACAUCACUGGGCGAG	504
53335_5_106	BCL11a	Exon 5	-	chr2:60451730-60451755	AUUUAGCUUAGGAAAGAACAUCACU	505
53335_5_107	BCL11a	Exon 5	-	chr2:60451731-60451756	AAUUUAGCUUAGGAAAGAACAUCAC	506
53335_5_108	BCL11a	Exon 5	-	chr2:60451746-60451771	UUGAAUUUGUCUUUUAAUUUAGCUU	507
53335_5_109	BCL11a	Exon 5	-	chr2:60451776-60451801	UAUAUGCUUUUGGUGGCUACCAUGC	508
53335_5_110	BCL11a	Exon 5	-	chr2:60451788-60451813	CUGUUUUUCAAAUAUAUGCUUUUGG	509
53335_5_111	BCL11a	Exon 5	-	chr2:60451791-60451816	AUCCUGUUUUUCAAAUAUAUGCUUU	510
53335_5_112	BCL11a	Exon 5	-	chr2:60451827-60451852	GCAUACCAUUAAAUAAUGCAUAAUA	511
53335_5_113	BCL11a	Exon 5	-	chr2:60451882-60451907	AUUGCUGUUUAUUAAUGCUGAAGUG	512
53335_5_114	BCL11a	Exon 5	-	chr2:60451949-60451974	CAAGGAGAAUCAAAAUGCAAAACUU	513
53335_5_115	BCL11a	Exon 5	-	chr2:60451950-60451975	UCAAGGAGAAUCAAAAUGCAAAACU	514
53335_5_116	BCL11a	Exon 5	-	chr2:60451972-60451997	GCUUAUCAGAUGGCAUAAAUUUUUCA	515
53335_5_117	BCL11a	Exon 5	-	chr2:60451987-60452012	GGGAACUAGGUUACCGCUUAUCAGA	516
53335_5_118	BCL11a	Exon 5	-	chr2:60452005-60452030	GGGCAGGAGAUGUAGGAGGGGAACU	517
53335_5_119	BCL11a	Exon 5	-	chr2:60452012-60452037	GAGAAAUGGGCAGGAGAUGUAGGAG	518
53335_5_120	BCL11a	Exon 5	-	chr2:60452013-60452038	UGAGAAAUGGGCAGGAGAUGUAGGA	519
53335_5_121	BCL11a	Exon 5	-	chr2:60452014-60452039	UUGAGAAAUGGGCAGGAGAUGUAGG	520
53335_5_12	BCL11	Exon 5	-	chr2:60452017-	GAUUUGAGAAAUGGGCAGG	521

2	a			60452042	AGAUGU	
53335_5_123	BCL11a	Exon 5	-	chr2:60452026-60452051	GGAGGAACAGAUUUGAGAAAUGGGC	522
53335_5_124	BCL11a	Exon 5	-	chr2:60452030-60452055	GGUAGGAGGAACAGAUUUGAGAAAU	523
53335_5_125	BCL11a	Exon 5	-	chr2:60452031-60452056	GGGUAGGAGGAACAGAUUUGAGAAA	524
53335_5_126	BCL11a	Exon 5	-	chr2:60452049-60452074	UACAGACACCCAUCGGGUGGGUAGG	525
53335_5_127	BCL11a	Exon 5	-	chr2:60452052-60452077	UCCUACAGACACCCAUCGGGUGGGU	526
53335_5_128	BCL11a	Exon 5	-	chr2:60452056-60452081	UGUUUCCUACAGACACCCAUCGGGU	527
53335_5_129	BCL11a	Exon 5	-	chr2:60452057-60452082	CUGUUUCCUACAGACACCCAUCGGG	528
53335_5_130	BCL11a	Exon 5	-	chr2:60452060-60452085	UACCUGUUUCCUACAGACACCCAUC	529
53335_5_131	BCL11a	Exon 5	-	chr2:60452061-60452086	GUACCUGUUUCCUACAGACACCCAU	530
53335_5_132	BCL11a	Exon 5	-	chr2:60452111-60452136	AAUUUUUAAUGCUUAUAAGACAAUG	531
53335_5_133	BCL11a	Exon 5	-	chr2:60452155-60452180	ACACAAUAAAUGUUGGAGCUUUAGG	532
53335_5_134	BCL11a	Exon 5	-	chr2:60452158-60452183	UUGACACAAUAAAUGUUGGAGCUUU	533
53335_5_135	BCL11a	Exon 5	-	chr2:60452167-60452192	UGCUUAACAUUGACACAAUAAAUGU	534
53335_5_136	BCL11a	Exon 5	-	chr2:60452256-60452281	CCAGCCAACCUAAUUACCUGUAUUG	535
53335_5_137	BCL11a	Exon 5	-	chr2:60452295-60452320	ACCAUCGCUGUCAUGAGUGAUGCCC	536
53335_5_138	BCL11a	Exon 5	-	chr2:60452323-60452348	GAAUCAGUCCCAUAGAGAGGGCCCG	537
53335_5_139	BCL11a	Exon 5	-	chr2:60452330-60452355	GAACAGUGAAUCAGUCCCAUAGAGA	538
53335_5_14	BCL11	Exon 5	-	chr2:60452331-	GGAACAGUGAAUCAGUCCC	539

0	a			60452356	AUAGAG	
53335_5_141	BCL11a	Exon 5	-	chr2:60452357-60452382	UAAAAACAAAAAAACAGACCCACAU	540
53335_5_142	BCL11a	Exon 5	-	chr2:60452423-60452448	GAGUCCUGCAGAGUCCUUCCAGCCU	541
53335_5_143	BCL11a	Exon 5	-	chr2:60452517-60452542	GCGUGCCGCCCAGUCGACGUCAGCG	542
53335_5_144	BCL11a	Exon 5	-	chr2:60452547-60452572	AAAUUUGAAGCCCCCAGGGGUGGGG	543
53335_5_145	BCL11a	Exon 5	-	chr2:60452550-60452575	AGAAAAUUUGAAGCCCCCAGGGGUG	544
53335_5_146	BCL11a	Exon 5	-	chr2:60452551-60452576	GAGAAAAUUUGAAGCCCCCAGGGGU	545
53335_5_147	BCL11a	Exon 5	-	chr2:60452552-60452577	UGAGAAAAUUUGAAGCCCCCAGGGG	546
53335_5_148	BCL11a	Exon 5	-	chr2:60452555-60452580	UUCUGAGAAAAUUUGAAGCCCCCAG	547
53335_5_149	BCL11a	Exon 5	-	chr2:60452556-60452581	GUUCUGAGAAAAUUUGAAGCCCCCA	548
53335_5_150	BCL11a	Exon 5	-	chr2:60452557-60452582	AGUUCUGAGAAAAUUUGAAGCCCCC	549
53335_5_151	BCL11a	Exon 5	-	chr2:60452602-60452627	CUCAAGAUGUGUGGCAGUUUUCGGA	550
53335_5_152	BCL11a	Exon 5	-	chr2:60452606-60452631	AGAGCUCAAGAUGUGUGGCAGUUUU	551
53335_5_153	BCL11a	Exon 5	-	chr2:60452616-60452641	UAGUACCCAGAGAGCUCAAGAUGUG	552
53335_5_154	BCL11a	Exon 5	-	chr2:60452646-60452671	UCUAGGUUCUUCACACACCCCCAUU	553
53335_4_1	BCL11a	Exon 4	+	chr2:60457199-60457224	UUAAUUUUUUUUAUUUUUUUCAAUAA	554
53335_4_2	BCL11a	Exon 4	+	chr2:60457200-60457225	UAAUUUUUUUUAUUUUUUCAAUAAA	555
53335_4_3	BCL11a	Exon 4	+	chr2:60457209-60457234	UUAUUUUUUCAAUAAAGGGACAAAA	556
53335_4_4	BCL11	Exon 4	+	chr2:60457210-	UAUUUUUUCAAUAAAGGGA	557

	a			60457235	CAAAAU	
53335_4_5	BCL11a	Exon 4	+	chr2:60457222-60457247	AAAGGGACAAAAUGGGUGUAUGAAC	558
53335_4_6	BCL11a	Exon 4	+	chr2:60457259-60457284	ACAACUGCCAAAAAAACACAGACAG	559
53335_4_7	BCL11a	Exon 4	+	chr2:60457312-60457337	CCAGAAACAAAUACAAUAAAAAGCC	560
53335_4_8	BCL11a	Exon 4	+	chr2:60457328-60457353	UAAAAAGCCAGGUUGUAAUGACCUU	561
53335_4_9	BCL11a	Exon 4	+	chr2:60457401-60457426	CAAAUUAAGUGCCUCUGUUUUGAAC	562
53335_4_10	BCL11a	Exon 4	+	chr2:60457402-60457427	AAAUUAAGUGCCUCUGUUUUGAACA	563
53335_4_11	BCL11a	Exon 4	+	chr2:60457480-60457505	AGUUACGACAAACAGCUUUCAUUAC	564
53335_4_12	BCL11a	Exon 4	+	chr2:60457491-60457516	ACAGCUUUCAUUACAGGAAUAGAAA	565
53335_4_13	BCL11a	Exon 4	+	chr2:60457545-60457570	AUUUACAAAAAAGUAUUGACUAAAG	566
53335_4_14	BCL11a	Exon 4	+	chr2:60457546-60457571	UUUACAAAAAAGUAUUGACUAAAGC	567
53335_4_15	BCL11a	Exon 4	+	chr2:60457616-60457641	UAAAUAUAAAGCACCAUUUAGUUUU	568
53335_4_16	BCL11a	Exon 4	+	chr2:60457642-60457667	GGCAAUGAAAAAAACUGCAAAACAU	569
53335_4_17	BCL11a	Exon 4	+	chr2:60457695-60457720	UCUUUCUUUCUUUUACUGCAUAUGA	570
53335_4_18	BCL11a	Exon 4	+	chr2:60457706-60457731	UUUACUGCAUAUGAAGGUAAGAUGC	571
53335_4_19	BCL11a	Exon 4	+	chr2:60457714-60457739	AUAUGAAGGUAAGAUGCUGGAAUGU	572
53335_4_20	BCL11a	Exon 4	+	chr2:60457715-60457740	UAUGAAGGUAAGAUGCUGGAAUGUA	573
53335_4_21	BCL11a	Exon 4	+	chr2:60457725-60457750	AGAUGCUGGAAUGUAGGGUGAUAGA	574
53335_4_22	BCL11	Exon 4	+	chr2:60457730-	CUGGAAUGUAGGGUGAUAG	575

	a			60457755	AAGGAA	
53335_4_23	BCL11a	Exon 4	+	chr2:60457731-60457756	UGGAAUGUAGGGUGAUAGAAGGAAA	576
53335_4_24	BCL11a	Exon 4	+	chr2:60457792-60457817	UUAGCUUGCAGUACUGCAUACAGUA	577
53335_4_25	BCL11a	Exon 4	+	chr2:60457799-60457824	GCAGUACUGCAUACAGUAUGGCAGC	578
53335_4_26	BCL11a	Exon 4	+	chr2:60457806-60457831	UGCAUACAGUAUGGCAGCAGGAAAA	579
53335_4_27	BCL11a	Exon 4	+	chr2:60457817-60457842	UGGCAGCAGGAAAAAGGAACAAAAA	580
53335_4_28	BCL11a	Exon 4	+	chr2:60457863-60457888	ACAGCCAUCCAUGUGACAUUCUAGC	581
53335_4_29	BCL11a	Exon 4	+	chr2:60457887-60457912	CAGGCUCCCCCAAACCGCCAUUAUA	582
53335_4_30	BCL11a	Exon 4	+	chr2:60457996-60458021	CCUGCCAAAUUAAAAAAAUAUACUG	583
53335_4_31	BCL11a	Exon 4	+	chr2:60458031-60458056	UCUUUUUUUUUUCCACUACCAAAAA	584
53335_4_32	BCL11a	Exon 4	+	chr2:60458178-60458203	AAAGACCAUAAAUGUAUUUUAGCAU	585
53335_4_33	BCL11a	Exon 4	+	chr2:60458274-60458299	UUUUUUUUUUUUACAACCUGAAGAG	586
53335_4_34	BCL11a	Exon 4	+	chr2:60458287-60458312	CAACCUGAAGAGCGGUGUGUAUCCA	587
53335_4_35	BCL11a	Exon 4	+	chr2:60458317-60458342	UAGAAUUUCCACUACCAUUUUUAAA	588
53335_4_36	BCL11a	Exon 4	+	chr2:60458350-60458375	AAGUCUUGUAACACCACCAAGACAA	589
53335_4_37	BCL11a	Exon 4	+	chr2:60458564-60458589	UAAGUAAGCUCAAUAGUCAAGUAAA	590
53335_4_38	BCL11a	Exon 4	+	chr2:60458568-60458593	UAAGCUCAAUAGUCAAGUAAAUGGC	591
53335_4_39	BCL11a	Exon 4	+	chr2:60458677-60458702	UUCCCUUAAGUAUAGACCUGUAAAC	592
53335_4_40	BCL11	Exon 4	+	chr2:60458678-	UCCCUUAAGUAUAGACCUG	593

	a			60458703	UAAACU	
53335_4_41	BCL11a	Exon 4	+	chr2:60458717-60458742	GUGCACUUAAUUGUCCUAUCUGAGC	594
53335_4_42	BCL11a	Exon 4	+	chr2:60458775-60458800	AUUAGAGAAAAGAUACAGAUAUCAC	595
53335_4_43	BCL11a	Exon 4	+	chr2:60458806-60458831	AGUCAAGUGCUAUUUGAACACCAAC	596
53335_4_44	BCL11a	Exon 4	+	chr2:60458807-60458832	GUCAAGUGCUAUUUGAACACCAACU	597
53335_4_45	BCL11a	Exon 4	+	chr2:60458808-60458833	UCAAGUGCUAUUUGAACACCAACUG	598
53335_4_46	BCL11a	Exon 4	+	chr2:60458904-60458929	GUUAACACAAAUAGCACACAGUGUA	599
53335_4_47	BCL11a	Exon 4	+	chr2:60458930-60458955	GGAAAAGAAAUGAAGUACAACUUUU	600
53335_4_48	BCL11a	Exon 4	+	chr2:60458931-60458956	GAAAAGAAAUGAAGUACAACUUUUA	601
53335_4_49	BCL11a	Exon 4	+	chr2:60459074-60459099	CUUAUAUACCUGUUCUAGUUUUAAA	602
53335_4_50	BCL11a	Exon 4	+	chr2:60459165-60459190	UAUUGUCAGCCUCUUCCUUUCAAUA	603
53335_4_51	BCL11a	Exon 4	+	chr2:60459174-60459199	CCUCUUCCUUUCAAUAUGGUAUACA	604
53335_4_52	BCL11a	Exon 4	+	chr2:60459223-60459248	UGUCCACUUGACAACCAAGUAGAUC	605
53335_4_53	BCL11a	Exon 4	+	chr2:60459238-60459263	CAAGUAGAUCUGGAUCUAUUUCUUU	606
53335_4_54	BCL11a	Exon 4	+	chr2:60459322-60459347	UUACUAGUGUAUUUAAUUGCGUUCC	607
53335_4_55	BCL11a	Exon 4	+	chr2:60459323-60459348	UACUAGUGUAUUUAAUUGCGUUCCA	608
53335_4_56	BCL11a	Exon 4	+	chr2:60459378-60459403	UCAUUGUUUAAAAAAAAUAAAACUU	609
53335_4_57	BCL11a	Exon 4	+	chr2:60459379-60459404	CAUUGUUUAAAAAAAAUAAAACUUU	610
53335_4_58	BCL11	Exon 4	+	chr2:60459413-	AGCCCAUUUCUUUUAAGCU	611

	a			60459438	CUCACC	
53335_4_59	BCL11a	Exon 4	+	chr2:60459435-60459460	ACCAGGAGCAAAGUAGCUUUUAUAC	612
53335_4_60	BCL11a	Exon 4	+	chr2:60459470-60459495	AGUUUUGUUUAUAAAAUUAAACUAA	613
53335_4_61	BCL11a	Exon 4	+	chr2:60459490-60459515	ACUAAAGGAAAAAUGAUGAUUAACU	614
53335_4_62	BCL11a	Exon 4	+	chr2:60459499-60459524	AAAAUGAUGAUUAACUAGGACAUAA	615
53335_4_63	BCL11a	Exon 4	+	chr2:60459500-60459525	AAAUGAUGAUUAACUAGGACAUAAU	616
53335_4_64	BCL11a	Exon 4	+	chr2:60459513-60459538	CUAGGACAUAAUGGGUCAUCUUUUU	617
53335_4_65	BCL11a	Exon 4	+	chr2:60459564-60459589	AUAUAGAAUUAUAUGCUAGUUCCUA	618
53335_4_66	BCL11a	Exon 4	+	chr2:60459632-60459657	UAACAAGUAGAAAGAACCAUCGAUG	619
53335_4_67	BCL11a	Exon 4	+	chr2:60459649-60459674	CAUCGAUGUGGUUUUAAUAGAUCCA	620
53335_4_68	BCL11a	Exon 4	+	chr2:60459718-60459743	GUUUUUCUGUUAAUUUGUCAAUUCA	621
53335_4_69	BCL11a	Exon 4	+	chr2:60459818-60459843	UCAGUGCUAUCUAUUCUGUCUAUAG	622
53335_4_70	BCL11a	Exon 4	+	chr2:60459819-60459844	CAGUGCUAUCUAUUCUGUCUAUAGA	623
53335_4_71	BCL11a	Exon 4	+	chr2:60459900-60459925	UAUGUAUUACAGAAUGUAUGCAGCA	624
53335_4_72	BCL11a	Exon 4	+	chr2:60459937-60459962	CUCUCUCUCUCUUUUUCUCUCAGAA	625
53335_4_73	BCL11a	Exon 4	+	chr2:60459943-60459968	CUCUCUUUUUCUCUCAGAACGGAAC	626
53335_4_74	BCL11a	Exon 4	+	chr2:60459977-60460002	CAACAUGUUUGCUCAGCAACGAAUU	627
53335_4_75	BCL11a	Exon 4	+	chr2:60459978-60460003	AACAUGUUUGCUCAGCAACGAAUUA	628
53335_4_76	BCL11	Exon 4	+	chr2:60460068-	ACAUGUAAAUUAUUGCACA	629

	a			60460093	AGAGAA	
53335_4_77	BCL11a	Exon 4	+	chr2:60460126-60460151	GCCCCAUACAGAUCAUGCAUUCAAA	630
53335_4_78	BCL11a	Exon 4	+	chr2:60460140-60460165	AUGCAUUCAAACGGUGAGAACAUAA	631
53335_4_79	BCL11a	Exon 4	+	chr2:60460193-60460218	AAAGAAAAAGAAAAAGAAAAAAAC	632
53335_4_80	BCL11a	Exon 4	+	chr2:60460201-60460226	AGAAAAAGAAAAAAAACAGGUGUGC	633
53335_4_81	BCL11a	Exon 4	+	chr2:60460269-60460294	UGUUUGUUUGUUUGUUUAAAUCACA	634
53335_4_82	BCL11a	Exon 4	+	chr2:60460270-60460295	GUUUGUUUGUUUGUUUAAAUCACAU	635
53335_4_83	BCL11a	Exon 4	+	chr2:60460291-60460316	ACAUGGGACUAGAAAAAAAUCCUAC	636
53335_4_84	BCL11a	Exon 4	+	chr2:60460292-60460317	CAUGGGACUAGAAAAAAAUCCUACA	637
53335_4_85	BCL11a	Exon 4	+	chr2:60460297-60460322	GACUAGAAAAAAAUCCUACAGGGAG	638
53335_4_86	BCL11a	Exon 4	+	chr2:60460298-60460323	ACUAGAAAAAAAUCCUACAGGGAGU	639
53335_4_87	BCL11a	Exon 4	+	chr2:60460299-60460324	CUAGAAAAAAAUCCUACAGGGAGUG	640
53335_4_88	BCL11a	Exon 4	+	chr2:60460303-60460328	AAAAAAAUCCUACAGGGAGUGGGGC	641
53335_4_89	BCL11a	Exon 4	+	chr2:60460306-60460331	AAAAUCCUACAGGGAGUGGGGCUGG	642
53335_4_90	BCL11a	Exon 4	+	chr2:60460307-60460332	AAAUCCUACAGGGAGUGGGGCUGGA	643
53335_4_91	BCL11a	Exon 4	+	chr2:60460313-60460338	UACAGGGAGUGGGGCUGGAGGGCGA	644
53335_4_92	BCL11a	Exon 4	+	chr2:60460314-60460339	ACAGGGAGUGGGGCUGGAGGGCGAU	645
53335_4_93	BCL11a	Exon 4	+	chr2:60460315-60460340	CAGGGAGUGGGGCUGGAGGGCGAUG	646
53335_4_94	BCL11	Exon 4	+	chr2:60460319-	GAGUGGGGCUGGAGGGCGA	647

	a			60460344	UGGGGA	
53335_4_95	BCL11a	Exon 4	+	chr2:60460320-60460345	AGUGGGGCUGGAGGGCGAUGGGGAA	648
53335_4_96	BCL11a	Exon 4	+	chr2:60460321-60460346	GUGGGGCUGGAGGGCGAUGGGGAAG	649
53335_4_97	BCL11a	Exon 4	+	chr2:60460326-60460351	GCUGGAGGGCGAUGGGGAAGGGGAG	650
53335_4_98	BCL11a	Exon 4	+	chr2:60460335-60460360	CGAUGGGGAAGGGGAGUGGUGAAAA	651
53335_4_99	BCL11a	Exon 4	+	chr2:60460336-60460361	GAUGGGGAAGGGGAGUGGUGAAAAA	652
53335_4_100	BCL11a	Exon 4	+	chr2:60460337-60460362	AUGGGGAAGGGGAGUGGUGAAAAAG	653
53335_4_101	BCL11a	Exon 4	+	chr2:60460338-60460363	UGGGGAAGGGGAGUGGUGAAAAGG	654
53335_4_102	BCL11a	Exon 4	+	chr2:60460345-60460370	GGGGAGUGGUGAAAAAGGGGUGUC	655
53335_4_103	BCL11a	Exon 4	+	chr2:60460348-60460373	GAGUGGUGAAAAAGGGGGUGUCAGG	656
53335_4_104	BCL11a	Exon 4	+	chr2:60460349-60460374	AGUGGUGAAAAAGGGGGUGUCAGGU	657
53335_4_105	BCL11a	Exon 4	+	chr2:60460356-60460381	AAAAAGGGGGUGUCAGGUGGGAGUG	658
53335_4_106	BCL11a	Exon 4	+	chr2:60460357-60460382	AAAAGGGGGUGUCAGGUGGGAGUGA	659
53335_4_107	BCL11a	Exon 4	+	chr2:60460360-60460385	AGGGGGUGUCAGGUGGGAGUGAGGG	660
53335_4_108	BCL11a	Exon 4	+	chr2:60460361-60460386	GGGGGUGUCAGGUGGGAGUGAGGGA	661
53335_4_109	BCL11a	Exon 4	+	chr2:60460362-60460387	GGGGUGUCAGGUGGGAGUGAGGGAG	662
53335_4_110	BCL11a	Exon 4	+	chr2:60460442-60460467	GUGCCAUUUUUUCAUGUGUUUCUCC	663
53335_4_111	BCL11a	Exon 4	+	chr2:60460443-60460468	UGCCAUUUUUUCAUGUGUUUCUCCA	664
53335_4_11	BCL11	Exon 4	+	chr2:60460462-	UCUCCAGGGUACUGUACAC	665

2	a			60460487	GCUAAA	
53335_4_113	BCL11a	Exon 4	+	chr2:60460505-60460530	ACAUUUGUAAACGUCCUUCCCCACC	666
53335_4_114	BCL11a	Exon 4	+	chr2:60460527-60460552	ACCUGGCCAUGCGUUUUCAUGUGCC	667
53335_4_115	BCL11a	Exon 4	+	chr2:60460544-60460569	CAUGUGCCUGGUGAGCUUGCUACUC	668
53335_4_116	BCL11a	Exon 4	+	chr2:60460545-60460570	AUGUGCCUGGUGAGCUUGCUACUCU	669
53335_4_117	BCL11a	Exon 4	+	chr2:60460551-60460576	CUGGUGAGCUUGCUACUCUGGGCAC	670
53335_4_118	BCL11a	Exon 4	+	chr2:60460579-60460604	CAUAGUUGCACAGCUCGCAUUUAUA	671
53335_4_119	BCL11a	Exon 4	+	chr2:60460595-60460620	GCAUUUAUAAGGCCUUUCGCCCGUG	672
53335_4_120	BCL11a	Exon 4	+	chr2:60460607-60460632	CCUUUCGCCCGUGUGGCUUCUCCUG	673
53335_4_121	BCL11a	Exon 4	+	chr2:60460686-60460711	UCGCUGCGUCUGCCCUCUUUUGAGC	674
53335_4_122	BCL11a	Exon 4	+	chr2:60460687-60460712	CGCUGCGUCUGCCCUCUUUUGAGCU	675
53335_4_123	BCL11a	Exon 4	+	chr2:60460696-60460721	UGCCCUCUUUUGAGCUGGGCCUGCC	676
53335_4_124	BCL11a	Exon 4	+	chr2:60460697-60460722	GCCCUCUUUUGAGCUGGGCCUGCCC	677
53335_4_125	BCL11a	Exon 4	+	chr2:60460702-60460727	CUUUUGAGCUGGGCCUGCCCGGGCC	678
53335_4_126	BCL11a	Exon 4	+	chr2:60460715-60460740	CCUGCCCGGGCCCGGACCACUAAUA	679
53335_4_127	BCL11a	Exon 4	+	chr2:60460716-60460741	CUGCCCGGGCCCGGACCACUAAUAU	680
53335_4_128	BCL11a	Exon 4	+	chr2:60460717-60460742	UGCCCGGGCCCGGACCACUAAUAUG	681
53335_4_129	BCL11a	Exon 4	+	chr2:60460776-60460801	CCCGAGAUCCCUCCGUCCAGCUCCC	682
53335_4_13	BCL11	Exon 4	+	chr2:60460777-	CCGAGAUCCCUCCGUCCAG	683

0	a			60460802	CUCCCC	
53335_4_131	BCL11a	Exon 4	+	chr2:60460780-60460805	AGAUCCCUCCGUCCAGCUCCCCGGG	684
53335_4_132	BCL11a	Exon 4	+	chr2:60460785-60460810	CCUCCGUCCAGCUCCCCGGGCGGUG	685
53335_4_133	BCL11a	Exon 4	+	chr2:60460812-60460837	GAGAAGCGCAAACUCCCGUUCUCCG	686
53335_4_134	BCL11a	Exon 4	+	chr2:60460827-60460852	CCGUUCUCCGAGGAGUGCUCCGACG	687
53335_4_135	BCL11a	Exon 4	+	chr2:60460830-60460855	UUCUCCGAGGAGUGCUCCGACGAGG	688
53335_4_136	BCL11a	Exon 4	+	chr2:60460837-60460862	AGGAGUGCUCCGACGAGGAGGCAAA	689
53335_4_137	BCL11a	Exon 4	+	chr2:60460848-60460873	GACGAGGAGGCAAAAGGCGAUUGUC	690
53335_4_138	BCL11a	Exon 4	+	chr2:60460865-60460890	CGAUUGUCUGGAGUCUCCGAAGCUA	691
53335_4_139	BCL11a	Exon 4	+	chr2:60460869-60460894	UGUCUGGAGUCUCCGAAGCUAAGGA	692
53335_4_140	BCL11a	Exon 4	+	chr2:60460870-60460895	GUCUGGAGUCUCCGAAGCUAAGGAA	693
53335_4_141	BCL11a	Exon 4	+	chr2:60460887-60460912	CUAAGGAAGGGAUCUUUGAGCUGCC	694
53335_4_142	BCL11a	Exon 4	+	chr2:60460890-60460915	AGGAAGGGAUCUUUGAGCUGCCUGG	695
53335_4_143	BCL11a	Exon 4	+	chr2:60460902-60460927	UUGAGCUGCCUGGAGGCCGCGUAGC	696
53335_4_144	BCL11a	Exon 4	+	chr2:60460935-60460960	CACUGCGAGUACACGUUCUCCGUGU	697
53335_4_145	BCL11a	Exon 4	+	chr2:60460936-60460961	ACUGCGAGUACACGUUCUCCGUGUU	698
53335_4_146	BCL11a	Exon 4	+	chr2:60460944-60460969	UACACGUUCUCCGUGUUGGGCAUCG	699
53335_4_147	BCL11a	Exon 4	+	chr2:60460948-60460973	CGUUCUCCGUGUUGGGCAUCGCGGC	700
53335_4_14	BCL11	Exon 4	+	chr2:60460949-	GUUCUCCGUGUUGGGCAUC	701

8	a			60460974	GCGGCC	
53335_4_149	BCL11a	Exon 4	+	chr2:60460950-60460975	UUCUCCGUGUUGGGCAUCGCGGCCG	702
53335_4_150	BCL11a	Exon 4	+	chr2:60460951-60460976	UCUCCGUGUUGGGCAUCGGGCCGG	703
53335_4_151	BCL11a	Exon 4	+	chr2:60460955-60460980	CGUGUUGGGCAUCGCGGCCGGGGGC	704
53335_4_152	BCL11a	Exon 4	+	chr2:60460992-60461017	UUCUCGAGCUUGAUGCGCUUAGAGA	705
53335_4_153	BCL11a	Exon 4	+	chr2:60460993-60461018	UCUCGAGCUUGAUGCGCUUAGAGAA	706
53335_4_154	BCL11a	Exon 4	+	chr2:60460994-60461019	CUCGAGCUUGAUGCGCUUAGAGAAG	707
53335_4_155	BCL11a	Exon 4	+	chr2:60461007-60461032	CGCUUAGAGAAGGGGCUCAGCGAGC	708
53335_4_156	BCL11a	Exon 4	+	chr2:60461008-60461033	GCUUAGAGAAGGGGCUCAGCGAGCU	709
53335_4_157	BCL11a	Exon 4	+	chr2:60461009-60461034	CUUAGAGAAGGGGCUCAGCGAGCUG	710
53335_4_158	BCL11a	Exon 4	+	chr2:60461031-60461056	CUGGGGCUGCCCAGCAGCAGCUUUU	711
53335_4_159	BCL11a	Exon 4	+	chr2:60461036-60461061	GCUGCCCAGCAGCAGCUUUUUGGAC	712
53335_4_160	BCL11a	Exon 4	+	chr2:60461046-60461071	AGCAGCUUUUUGGACAGGCCCCCCG	713
53335_4_161	BCL11a	Exon 4	+	chr2:60461059-60461084	ACAGGCCCCCCGAGGCCGACUCGCC	714
53335_4_162	BCL11a	Exon 4	+	chr2:60461060-60461085	CAGGCCCCCCGAGGCCGACUCGCCC	715
53335_4_163	BCL11a	Exon 4	+	chr2:60461061-60461086	AGGCCCCCCGAGGCCGACUCGCCCG	716
53335_4_164	BCL11a	Exon 4	+	chr2:60461072-60461097	GGCCGACUCGCCCGGGGAGCAGCCG	717
53335_4_165	BCL11a	Exon 4	+	chr2:60461099-60461124	GCCAUUAACAGUGCCAUCGUCUAUG	718
53335_4_16	BCL11	Exon 4	+	chr2:60461112-	CCAUCGUCUAUGCGGUCCG	719

6	a			60461137	ACUCGC	
53335_4_167	BCL11a	Exon 4	+	chr2:60461144-60461169	CGAGUCUUCGUCGCAAGUGUCCCUG	720
53335_4_168	BCL11a	Exon 4	+	chr2:60461151-60461176	UCGUCGCAAGUGUCCCUGUGGCCCU	721
53335_4_169	BCL11a	Exon 4	+	chr2:60461157-60461182	CAAGUGUCCCUGUGGCCCUCGGCCU	722
53335_4_170	BCL11a	Exon 4	+	chr2:60461162-60461187	GUCCCUGUGGCCCUCGGCCUCGGCC	723
53335_4_171	BCL11a	Exon 4	+	chr2:60461165-60461190	CCUGUGGCCCUCGGCCUCGGCCAGG	724
53335_4_172	BCL11a	Exon 4	+	chr2:60461189-60461214	GUGGCCGCGCUUAUGCUUCUCGCCC	725
53335_4_173	BCL11a	Exon 4	+	chr2:60461195-60461220	GCGCUUAUGCUUCUCGCCCAGGACC	726
53335_4_174	BCL11a	Exon 4	+	chr2:60461198-60461223	CUUAUGCUUCUCGCCCAGGACCUGG	727
53335_4_175	BCL11a	Exon 4	+	chr2:60461202-60461227	UGCUUCUCGCCCAGGACCUGGUGGA	728
53335_4_176	BCL11a	Exon 4	+	chr2:60461223-60461248	UGGAAGGCCUCGCUGAAGUGCUGCA	729
53335_4_177	BCL11a	Exon 4	+	chr2:60461253-60461278	CUGAGCACCAUGCCCUGCAUGACGU	730
53335_4_178	BCL11a	Exon 4	+	chr2:60461254-60461279	UGAGCACCAUGCCCUGCAUGACGUC	731
53335_4_179	BCL11a	Exon 4	+	chr2:60461258-60461283	CACCAUGCCCUGCAUGACGUCGGGC	732
53335_4_180	BCL11a	Exon 4	+	chr2:60461259-60461284	ACCAUGCCCUGCAUGACGUCGGGCA	733
53335_4_181	BCL11a	Exon 4	+	chr2:60461264-60461289	GCCCUGCAUGACGUCGGGCAGGGCG	734
53335_4_182	BCL11a	Exon 4	+	chr2:60461312-60461337	GACCGCGCCCCGCGAGCUGUUCUCG	735
53335_4_183	BCL11a	Exon 4	+	chr2:60461315-60461340	CGCGCCCCGCGAGCUGUUCUCGUGG	736
53335_4_18	BCL11	Exon 4	+	chr2:60461330-	GUUCUCGUGGUGGCGCGCC	737

4	a			60461355	GCCUCC	
53335_4_185	BCL11a	Exon 4	+	chr2:60461446-60461471	CCUCUUCCUCCUCGUCCCCGUUCUC	738
53335_4_186	BCL11a	Exon 4	+	chr2:60461447-60461472	CUCUUCCUCCUCGUCCCCGUUCUCC	739
53335_4_187	BCL11a	Exon 4	+	chr2:60461453-60461478	CUCCUCGUCCCCGUUCUCCGGGAUC	740
53335_4_188	BCL11a	Exon 4	+	chr2:60461457-60461482	UCGUCCCCGUUCUCCGGGAUCAGGU	741
53335_4_189	BCL11a	Exon 4	+	chr2:60461458-60461483	CGUCCCCGUUCUCCGGGAUCAGGUU	742
53335_4_190	BCL11a	Exon 4	+	chr2:60461459-60461484	GUCCCCGUUCUCCGGGAUCAGGUUG	743
53335_4_191	BCL11a	Exon 4	+	chr2:60461481-60461506	UUGGGGUCGUUCUCGCUCUUGAACU	744
53335_4_192	BCL11a	Exon 4	+	chr2:60461490-60461515	UUCUCGCUCUUGAACUUGGCCACCA	745
53335_4_193	BCL11a	Exon 4	+	chr2:60461508-60461533	GCCACCACGGACUUGAGCGCGCUGC	746
53335_4_194	BCL11a	Exon 4	+	chr2:60461529-60461554	CUGCUGGCGCUGCCCACCAAGUCGC	747
53335_4_195	BCL11a	Exon 4	+	chr2:60461535-60461560	GCGCUGCCCACCAAGUCGCUGGUGC	748
53335_4_196	BCL11a	Exon 4	+	chr2:60461536-60461561	CGCUGCCCACCAAGUCGCUGGUGCC	749
53335_4_197	BCL11a	Exon 4	+	chr2:60461542-60461567	CCACCAAGUCGCUGGUGCCGGGUUC	750
53335_4_198	BCL11a	Exon 4	+	chr2:60461543-60461568	CACCAAGUCGCUGGUGCCGGGUUCC	751
53335_4_199	BCL11a	Exon 4	+	chr2:60461544-60461569	ACCAAGUCGCUGGUGCCGGGUUCCG	752
53335_4_200	BCL11a	Exon 4	+	chr2:60461550-60461575	UCGCUGGUGCCGGGUUCCGGGGAGC	753
53335_4_201	BCL11a	Exon 4	+	chr2:60461553-60461578	CUGGUGCCGGGUUCCGGGGAGCUGG	754
53335_4_20	BCL11	Exon 4	+	chr2:60461556-	GUGCCGGGUUCCGGGGAGC	755

2	a			60461581	UGGCGG	
53335_4_203	BCL11a	Exon 4	+	chr2:60461571-60461596	GAGCUGGCGGUGGAGAGACCGUCGU	756
53335_4_204	BCL11a	Exon 4	+	chr2:60461586-60461611	AGACCGUCGUCGGACUUGACCGUCA	757
53335_4_205	BCL11a	Exon 4	+	chr2:60461587-60461612	GACCGUCGUCGGACUUGACCGUCAU	758
53335_4_206	BCL11a	Exon 4	+	chr2:60461588-60461613	ACCGUCGUCGGACUUGACCGUCAUG	759
53335_4_207	BCL11a	Exon 4	+	chr2:60461589-60461614	CCGUCGUCGGACUUGACCGUCAUGG	760
53335_4_208	BCL11a	Exon 4	+	chr2:60461618-60461643	CGAUUUGUGCAUGUGCGUCUUCAUG	761
53335_4_209	BCL11a	Exon 4	+	chr2:60461634-60461659	GUCUUCAUGUGGCGCUUCAGCUUGC	762
53335_4_210	BCL11a	Exon 4	+	chr2:60461639-60461664	CAUGUGGCGCUUCAGCUUGCUGGCC	763
53335_4_211	BCL11a	Exon 4	+	chr2:60461640-60461665	AUGUGGCGCUUCAGCUUGCUGGCCU	764
53335_4_212	BCL11a	Exon 4	+	chr2:60461651-60461676	CAGCUUGCUGGCCUGGGUGCACGCG	765
53335_4_213	BCL11a	Exon 4	+	chr2:60461660-60461685	GGCCUGGGUGCACGCGUGGUCGCAC	766
53335_4_214	BCL11a	Exon 4	+	chr2:60461673-60461698	GCGUGGUCGCACAGGUUGCACUUGU	767
53335_4_215	BCL11a	Exon 4	+	chr2:60461674-60461699	CGUGGUCGCACAGGUUGCACUUGUA	768
53335_4_216	BCL11a	Exon 4	+	chr2:60461690-60461715	GCACUUGUAGGGCUUCUCGCCCGUG	769
53335_4_217	BCL11a	Exon 4	+	chr2:60461699-60461724	GGGCUUCUCGCCCGUGUGGCUGCGC	770
53335_4_218	BCL11a	Exon 4	+	chr2:60461711-60461736	CGUGUGGCUGCGCCGGUGCACCACC	771
53335_4_219	BCL11a	Exon 4	+	chr2:60461757-60461782	GUCUUGCCGCAGAACUCGCAUGACU	772
53335_4_22	BCL11	Exon 4	+	chr2:60461767-	AGAACUCGCAUGACUUGGA	773

0	a			60461792	CUUGAC	
53335_4_221	BCL11a	Exon 4	+	chr2:60461768-60461793	GAACUCGCAUGACUUGGACUUGACC	774
53335_4_222	BCL11a	Exon 4	+	chr2:60461769-60461794	AACUCGCAUGACUUGGACUUGACCG	775
53335_4_223	BCL11a	Exon 4	+	chr2:60461770-60461795	ACUCGCAUGACUUGGACUUGACCGG	776
53335_4_224	BCL11a	Exon 4	+	chr2:60461774-60461799	GCAUGACUUGGACUUGACCGGGGGC	777
53335_4_225	BCL11a	Exon 4	+	chr2:60461775-60461800	CAUGACUUGGACUUGACCGGGGCU	778
53335_4_226	BCL11a	Exon 4	+	chr2:60461778-60461803	GACUUGGACUUGACCGGGGGCUGGG	779
53335_4_227	BCL11a	Exon 4	+	chr2:60461779-60461804	ACUUGGACUUGACCGGGGGCUGGGA	780
53335_4_228	BCL11a	Exon 4	+	chr2:60461782-60461807	UGGACUUGACCGGGGGCUGGGAGGG	781
53335_4_229	BCL11a	Exon 4	+	chr2:60461785-60461810	ACUUGACCGGGGGCUGGGAGGGAGG	782
53335_4_230	BCL11a	Exon 4	+	chr2:60461786-60461811	CUUGACCGGGGGCUGGGAGGGAGGA	783
53335_4_231	BCL11a	Exon 4	+	chr2:60461787-60461812	UUGACCGGGGGCUGGGAGGGAGGAG	784
53335_4_232	BCL11a	Exon 4	+	chr2:60461790-60461815	ACCGGGGGCUGGGAGGGAGGAGGGG	785
53335_4_233	BCL11a	Exon 4	+	chr2:60461800-60461825	GGGAGGGAGGAGGGGCGGAUUGCAG	786
53335_4_234	BCL11a	Exon 4	+	chr2:60461803-60461828	AGGGAGGAGGGGCGGAUUGCAGAGG	787
53335_4_235	BCL11a	Exon 4	+	chr2:60461804-60461829	GGGAGGAGGGGCGGAUUGCAGAGGA	788
53335_4_236	BCL11a	Exon 4	+	chr2:60461807-60461832	AGGAGGGGCGGAUUGCAGAGGAGGG	789
53335_4_237	BCL11a	Exon 4	+	chr2:60461808-60461833	GGAGGGGCGGAUUGCAGAGGAGGGA	790
53335_4_23	BCL11	Exon 4	+	chr2:60461809-	GAGGGGCGGAUUGCAGAGG	791

8	a			60461834	AGGGAG	
53335_4_239	BCL11a	Exon 4	+	chr2:60461810-60461835	AGGGGCGGAUUGCAGAGGAGGGAGG	792
53335_4_240	BCL11a	Exon 4	+	chr2:60461811-60461836	GGGGCGGAUUGCAGAGGAGGGAGGG	793
53335_4_241	BCL11a	Exon 4	+	chr2:60461812-60461837	GGGCGGAUUGCAGAGGAGGGAGGGG	794
53335_4_242	BCL11a	Exon 4	+	chr2:60461822-60461847	CAGAGGAGGGAGGGGGGGCGUCGCC	795
53335_4_243	BCL11a	Exon 4	+	chr2:60461826-60461851	GGAGGGAGGGGGGGCGUCGCCAGGA	796
53335_4_244	BCL11a	Exon 4	+	chr2:60461827-60461852	GAGGGAGGGGGGGCGUCGCCAGGAA	797
53335_4_245	BCL11a	Exon 4	+	chr2:60461830-60461855	GGAGGGGGGGCGUCGCCAGGAAGGG	798
53335_4_246	BCL11a	Exon 4	+	chr2:60461842-60461867	UCGCCAGGAAGGGCGGCUUGCUACC	799
53335_4_247	BCL11a	Exon 4	+	chr2:60461846-60461871	CAGGAAGGGCGGCUUGCUACCUGGC	800
53335_4_248	BCL11a	Exon 4	+	chr2:60461851-60461876	AGGGCGGCUUGCUACCUGGCUGGAA	801
53335_4_249	BCL11a	Exon 4	+	chr2:60461872-60461897	GGAAUGGUUGCAGUAACCUUUGCAU	802
53335_4_250	BCL11a	Exon 4	+	chr2:60461873-60461898	GAAUGGUUGCAGUAACCUUUGCAUA	803
53335_4_251	BCL11a	Exon 4	+	chr2:60461877-60461902	GGUUGCAGUAACCUUUGCAUAGGGC	804
53335_4_252	BCL11a	Exon 4	+	chr2:60461878-60461903	GUUGCAGUAACCUUUGCAUAGGGCU	805
53335_4_253	BCL11a	Exon 4	+	chr2:60461882-60461907	CAGUAACCUUUGCAUAGGGCUGGGC	806
53335_4_254	BCL11a	Exon 4	+	chr2:60461887-60461912	ACCUUUGCAUAGGGCUGGGCCGGCC	807
53335_4_255	BCL11a	Exon 4	+	chr2:60461888-60461913	CCUUUGCAUAGGGCUGGGCCGGCCU	808
53335_4_25	BCL11	Exon 4	+	chr2:60461889-	CUUUGCAUAGGGCUGGGCC	809

6	a			60461914	GGCCUG	
53335_4_257	BCL11a	Exon 4	+	chr2:60461896-60461921	UAGGGCUGGGCCGGCCUGGGGACAG	810
53335_4_258	BCL11a	Exon 4	+	chr2:60461899-60461924	GGCUGGGCCGGCCUGGGGACAGCGG	811
53335_4_259	BCL11a	Exon 4	+	chr2:60461900-60461925	GCUGGGCCGGCCUGGGGACAGCGGU	812
53335_4_260	BCL11a	Exon 4	+	chr2:60461949-60461974	UCUCUAAGUCUCCUAGAGAAAUCCA	813
53335_4_261	BCL11a	Exon 4	+	chr2:60461952-60461977	CUAAGUCUCCUAGAGAAAUCCAUGG	814
53335_4_262	BCL11a	Exon 4	+	chr2:60461953-60461978	UAAGUCUCCUAGAGAAAUCCAUGGC	815
53335_4_263	BCL11a	Exon 4	+	chr2:60461956-60461981	GUCUCCUAGAGAAAUCCAUGGCGGG	816
53335_4_264	BCL11a	Exon 4	+	chr2:60461971-60461996	CCAUGGCGGGAGGCUCCAUAGCCAU	817
53335_4_265	BCL11a	Exon 4	+	chr2:60461997-60462022	GGAUUCAACCGCAGCACCCUGUCAA	818
53335_4_266	BCL11a	Exon 4	+	chr2:60462004-60462029	ACCGCAGCACCCUGUCAAAGGCACU	819
53335_4_267	BCL11a	Exon 4	+	chr2:60462005-60462030	CCGCAGCACCCUGUCAAAGGCACUC	820
53335_4_268	BCL11a	Exon 4	+	chr2:60462011-60462036	CACCCUGUCAAAGGCACUCGGGUGA	821
53335_4_269	BCL11a	Exon 4	+	chr2:60462012-60462037	ACCCUGUCAAAGGCACUCGGGUGAU	822
53335_4_270	BCL11a	Exon 4	+	chr2:60462015-60462040	CUGUCAAAGGCACUCGGGUGAUGGG	823
53335_4_271	BCL11a	Exon 4	+	chr2:60462020-60462045	AAAGGCACUCGGGUGAUGGGUGGCC	824
53335_4_272	BCL11a	Exon 4	+	chr2:60462021-60462046	AAGGCACUCGGGUGAUGGGUGGCCA	825
53335_4_273	BCL11a	Exon 4	+	chr2:60462041-60462066	GGCCAGGGCCAUCUCUUCCGCCCCC	826
53335_4_27	BCL11	Exon 4	+	chr2:60462053-	CUCUUCCGCCCCCAGGCGC	827

4	a			60462078	UCUAUG	
53335_4_275	BCL11a	Exon 4	+	chr2:60462056-60462081	UUCCGCCCCCAGGCGCUCUAUGCGG	828
53335_4_276	BCL11a	Exon 4	+	chr2:60462057-60462082	UCCGCCCCCAGGCGCUCUAUGCGGU	829
53335_4_277	BCL11a	Exon 4	+	chr2:60462058-60462083	CCGCCCCCAGGCGCUCUAUGCGGUG	830
53335_4_278	BCL11a	Exon 4	+	chr2:60462059-60462084	CGCCCCCAGGCGCUCUAUGCGGUGG	831
53335_4_279	BCL11a	Exon 4	+	chr2:60462076-60462101	UGCGGUGGGGGUCCAAGUGAUGUCU	832
53335_4_280	BCL11a	Exon 4	+	chr2:60462079-60462104	GGUGGGGGUCCAAGUGAUGUCUCGG	833
53335_4_281	BCL11a	Exon 4	+	chr2:60462082-60462107	GGGGGUCCAAGUGAUGUCUCGGUGG	834
53335_4_282	BCL11a	Exon 4	+	chr2:60462092-60462117	GUGAUGUCUCGGUGGUGGACUAAAC	835
53335_4_283	BCL11a	Exon 4	+	chr2:60462093-60462118	UGAUGUCUCGGUGGUGGACUAAACA	836
53335_4_284	BCL11a	Exon 4	+	chr2:60462094-60462119	GAUGUCUCGGUGGUGGACUAAACAG	837
53335_4_285	BCL11a	Exon 4	+	chr2:60462095-60462120	AUGUCUCGGUGGUGGACUAAACAGG	838
53335_4_286	BCL11a	Exon 4	+	chr2:60462096-60462121	UGUCUCGGUGGUGGACUAAACAGGG	839
53335_4_287	BCL11a	Exon 4	+	chr2:60462097-60462122	GUCUCGGUGGUGGACUAAACAGGGG	840
53335_4_288	BCL11a	Exon 4	+	chr2:60462102-60462127	GGUGGUGGACUAAACAGGGGGGGAG	841
53335_4_289	BCL11a	Exon 4	+	chr2:60462103-60462128	GUGGUGGACUAAACAGGGGGGGAGU	842
53335_4_290	BCL11a	Exon 4	+	chr2:60462106-60462131	GUGGACUAAACAGGGGGGGAGUGGG	843
53335_4_291	BCL11a	Exon 4	+	chr2:60462125-60462150	AGUGGGUGGAAAGCGCCCUUCUGCC	844
53335_4_29	BCL11	Exon 4	+	chr2:60462129-	GGUGGAAAGCGCCCUUCUG	845

2	a			60462154	CCAGGC	
53335_4_293	BCL11a	Exon 4	+	chr2:60462154-60462179	CGGAAGCCUCUCUCGAUACUGAUCC	846
53335_4_294	BCL11a	Exon 4	+	chr2:60462167-60462192	CGAUACUGAUCCUGGUAUUCUUAGC	847
53335_4_295	BCL11a	Exon 4	+	chr2:60462174-60462199	GAUCCUGGUAUUCUUAGCAGGUUAA	848
53335_4_296	BCL11a	Exon 4	+	chr2:60462175-60462200	AUCCUGGUAUUCUUAGCAGGUUAAA	849
53335_4_297	BCL11a	Exon 4	+	chr2:60462176-60462201	UCCUGGUAUUCUUAGCAGGUUAAAG	850
53335_4_298	BCL11a	Exon 4	+	chr2:60462203-60462228	GUUAUUGUCUGCAAUAUGAAUCCCA	851
53335_4_299	BCL11a	Exon 4	+	chr2:60462208-60462233	UGUCUGCAAUAUGAAUCCCAUGGAG	852
53335_4_300	BCL11a	Exon 4	+	chr2:60462211-60462236	CUGCAAUAUGAAUCCCAUGGAGAGG	853
53335_4_301	BCL11a	Exon 4	+	chr2:60462215-60462240	AAUAUGAAUCCCAUGGAGAGGUGGC	854
53335_4_302	BCL11a	Exon 4	+	chr2:60462216-60462241	AUAUGAAUCCCAUGGAGAGGUGGCU	855
53335_4_303	BCL11a	Exon 4	+	chr2:60462220-60462245	GAAUCCCAUGGAGAGGUGGCUGGGA	856
53335_4_304	BCL11a	Exon 4	+	chr2:60462244-60462269	AAGGACAUUCUGCACCUAGUCCUGA	857
53335_4_305	BCL11a	Exon 4	+	chr2:60462245-60462270	AGGACAUUCUGCACCUAGUCCUGAA	858
53335_4_306	BCL11a	Exon 4	+	chr2:60462259-60462284	CUAGUCCUGAAGGGAUACCAACCCG	859
53335_4_307	BCL11a	Exon 4	+	chr2:60462260-60462285	UAGUCCUGAAGGGAUACCAACCCGC	860
53335_4_308	BCL11a	Exon 4	+	chr2:60462261-60462286	AGUCCUGAAGGGAUACCAACCCGCG	861
53335_4_309	BCL11a	Exon 4	+	chr2:60462266-60462291	UGAAGGGAUACCAACCCGCGGGGUC	862
53335_4_31	BCL11	Exon 4	+	chr2:60462267-	GAAGGGAUACCAACCCGCG	863

0	a			60462292	GGGUCA	
53335_4_311	BCL11a	Exon 4	+	chr2:60462268-60462293	AAGGGAUACCAACCCGCGGGGUCAG	864
53335_4_312	BCL11a	Exon 4	+	chr2:60462345-60462370	GCGUGUUGCAAGAGAAACCAUGCAC	865
53335_4_313	BCL11a	Exon 4	+	chr2:60462352-60462377	GCAAGAGAAACCAUGCACUGGUGAA	866
53335_4_314	BCL11a	Exon 4	+	chr2:60462384-60462409	UUGCAAGUUGUACAUGUGUAGCUGC	867
53335_4_315	BCL11a	Exon 4	+	chr2:60462385-60462410	UGCAAGUUGUACAUGUGUAGCUGCU	868
53335_4_316	BCL11a	Exon 4	-	chr2:60457269-60457294	GGAAAAACCACUGUCUGUGUUUUUU	869
53335_4_317	BCL11a	Exon 4	-	chr2:60457295-60457320	GUUUCUGGUCUUUGUUAAGUUCUAU	870
53335_4_318	BCL11a	Exon 4	-	chr2:60457315-60457340	CCUGGCUUUUUAUUGUAUUUGUUUC	871
53335_4_319	BCL11a	Exon 4	-	chr2:60457338-60457363	AAGAUGACCAAAGGUCAUUACAACC	872
53335_4_320	BCL11a	Exon 4	-	chr2:60457352-60457377	AAAAAAAAAAAUAAAAGAUGACCAA	873
53335_4_321	BCL11a	Exon 4	-	chr2:60457415-60457440	UGCUUAUGUGCCCUGUUCAAAACAG	874
53335_4_322	BCL11a	Exon 4	-	chr2:60457459-60457484	AACUUGAAAUUUUAUCUUUUACUAU	875
53335_4_323	BCL11a	Exon 4	-	chr2:60457460-60457485	UAACUUGAAAUUUUAUCUUUUACUA	876
53335_4_324	BCL11a	Exon 4	-	chr2:60457522-60457547	AUGGCAAUGCAGAAUAUUUUGUUAU	877
53335_4_325	BCL11a	Exon 4	-	chr2:60457546-60457571	GCUUUAGUCAAUACUUUUUUGUAAA	878
53335_4_326	BCL11a	Exon 4	-	chr2:60457613-60457638	ACUAAAUGGUGCUUUAUAUUUAGAU	879
53335_4_327	BCL11a	Exon 4	-	chr2:60457632-60457657	GUUUUUUUCAUUGCCAAAAACUAAA	880
53335_4_32	BCL11	Exon 4	-	chr2:60457786-	AUGCAGUACUGCAAGCUAA	881

8	a			60457811	UAACGU	
53335_4_329	BCL11a	Exon 4	-	chr2:60457858-60457883	AAUGUCACAUGGAUGGCUGUCAUAG	882
53335_4_330	BCL11a	Exon 4	-	chr2:60457859-60457884	GAAUGUCACAUGGAUGGCUGUCAUA	883
53335_4_331	BCL11a	Exon 4	-	chr2:60457860-60457885	AGAAUGUCACAUGGAUGGCUGUCAU	884
53335_4_332	BCL11a	Exon 4	-	chr2:60457870-60457895	GGAGCCUGCUAGAAUGUCACAUGGA	885
53335_4_333	BCL11a	Exon 4	-	chr2:60457874-60457899	UGGGGGAGCCUGCUAGAAUGUCACA	886
53335_4_334	BCL11a	Exon 4	-	chr2:60457896-60457921	GAGAAGCCAUAUAAUGGCGGUUUGG	887
53335_4_335	BCL11a	Exon 4	-	chr2:60457897-60457922	UGAGAAGCCAUAUAAUGGCGGUUUG	888
53335_4_336	BCL11a	Exon 4	-	chr2:60457898-60457923	AUGAGAAGCCAUAUAAUGGCGGUUU	889
53335_4_337	BCL11a	Exon 4	-	chr2:60457899-60457924	GAUGAGAAGCCAUAUAAUGGCGGUU	890
53335_4_338	BCL11a	Exon 4	-	chr2:60457904-60457929	UUACAGAUGAGAAGCCAUAUAAUGG	891
53335_4_339	BCL11a	Exon 4	-	chr2:60457907-60457932	ACAUUACAGAUGAGAAGCCAUAUAA	892
53335_4_340	BCL11a	Exon 4	-	chr2:60457999-60458024	CCACAGUAUAUUUUUUUAAUUUGGC	893
53335_4_341	BCL11a	Exon 4	-	chr2:60458003-60458028	GCUGCCACAGUAUAUUUUUUUAAUU	894
53335_4_342	BCL11a	Exon 4	-	chr2:60458030-60458055	UUUUGGUAGUGGAAAAAAAAAGAC	895
53335_4_343	BCL11a	Exon 4	-	chr2:60458046-60458071	GGUAUCAAUGUACCUUUUUUGGUAG	896
53335_4_344	BCL11a	Exon 4	-	chr2:60458052-60458077	UUAAAAGGUAUCAAUGUACCUUUUU	897
53335_4_345	BCL11a	Exon 4	-	chr2:60458072-60458097	UUUAACUGUUGCUUGUUCUCUUAAA	898
53335_4_34	BCL11	Exon 4	-	chr2:60458130-	UUGAAUUAAAUGUUCAUCU	899

6	a			60458155	AGUGUU	
53335_4_347	BCL11a	Exon 4	-	chr2:60458161-60458186	UGGUCUUUUUUCUGUAUUUCUAGAA	900
53335_4_348	BCL11a	Exon 4	-	chr2:60458186-60458211	UGAUUCCUAUGCUAAAAUACAUUUA	901
53335_4_349	BCL11a	Exon 4	-	chr2:60458231-60458256	UUAAGAUUAUAUAGUACUUAAAUAU	902
53335_4_350	BCL11a	Exon 4	-	chr2:60458260-60458285	AAAAAAAAAAACAUACAUUGGGGAA	903
53335_4_351	BCL11a	Exon 4	-	chr2:60458265-60458290	UUGUAAAAAAAAAAAAACAUACAUUG	904
53335_4_352	BCL11a	Exon 4	-	chr2:60458266-60458291	GUUGUAAAAAAAAAAAAACAUACAUU	905
53335_4_353	BCL11a	Exon 4	-	chr2:60458267-60458292	GGUUGUAAAAAAAAAAAAACAUACAU	906
53335_4_354	BCL11a	Exon 4	-	chr2:60458293-60458318	AUGCCUUGGAUACACACCGCUCUUC	907
53335_4_355	BCL11a	Exon 4	-	chr2:60458312-60458337	AAAUGGUAGUGGAAAUUCUAUGCCU	908
53335_4_356	BCL11a	Exon 4	-	chr2:60458328-60458353	CUUGUUAUCCAUUUAAAAAUGGUAG	909
53335_4_357	BCL11a	Exon 4	-	chr2:60458334-60458359	ACAAGACUUGUUAUCCAUUUAAAAA	910
53335_4_358	BCL11a	Exon 4	-	chr2:60458366-60458391	GCAUUUUAGGGUUCCAUUGUCUUGG	911
53335_4_359	BCL11a	Exon 4	-	chr2:60458369-60458394	ACUGCAUUUUAGGGUUCCAUUGUCU	912
53335_4_360	BCL11a	Exon 4	-	chr2:60458383-60458408	AUGUUUAGGGGGGAACUGCAUUUUA	913
53335_4_361	BCL11a	Exon 4	-	chr2:60458384-60458409	UAUGUUUAGGGGGGAACUGCAUUUU	914
53335_4_362	BCL11a	Exon 4	-	chr2:60458398-60458423	AAAAAACACUUCAUUAUGUUUAGGG	915
53335_4_363	BCL11a	Exon 4	-	chr2:60458399-60458424	UAAAAAACACUUCAUUAUGUUUAGG	916
53335_4_36	BCL11	Exon 4	-	chr2:60458400-	UUAAAAAACACUUCAUUAU	917

4	a			60458425	GUUUAG	
53335_4_365	BCL11a	Exon 4	-	chr2:60458401-60458426	UUUAAAAAACACUUCAUUAUGUUUA	918
53335_4_366	BCL11a	Exon 4	-	chr2:60458402-60458427	UUUUAAAAAACACUUCAUUAUGUUU	919
53335_4_367	BCL11a	Exon 4	-	chr2:60458478-60458503	UGAUUGCUUUCGCUUCUACAGUGCA	920
53335_4_368	BCL11a	Exon 4	-	chr2:60458535-60458560	GACGCAACAUUGCAAGCGCUGUGAA	921
53335_4_369	BCL11a	Exon 4	-	chr2:60458562-60458587	UACUUGACUAUUGAGCUUACUUACU	922
53335_4_370	BCL11a	Exon 4	-	chr2:60458634-60458659	AAAAAAAUUGAACACAAUCUCAUUG	923
53335_4_371	BCL11a	Exon 4	-	chr2:60458682-60458707	UCCCAGUUUACAGGUCUAUACUUAA	924
53335_4_372	BCL11a	Exon 4	-	chr2:60458683-60458708	UUCCCAGUUUACAGGUCUAUACUUA	925
53335_4_373	BCL11a	Exon 4	-	chr2:60458696-60458721	GCACUGUACAAUUUUCCCAGUUUAC	926
53335_4_374	BCL11a	Exon 4	-	chr2:60458734-60458759	GAGUAUAAAAUAAACCUGCUCAGAU	927
53335_4_375	BCL11a	Exon 4	-	chr2:60458764-60458789	AUCUUUUCUCUAAUCAGAGAUACAG	928
53335_4_376	BCL11a	Exon 4	-	chr2:60458829-60458854	AAUAAAAGCUAGCAUCUGCCCCAGU	929
53335_4_377	BCL11a	Exon 4	-	chr2:60458897-60458922	UGUGCUAUUUGUGUUAACAUGGAAG	930
53335_4_378	BCL11a	Exon 4	-	chr2:60458903-60458928	ACACUGUGUGCUAUUUGUGUUAACA	931
53335_4_379	BCL11a	Exon 4	-	chr2:60459085-60459110	ACUAUUUGCCAUUUAAAACUAGAAC	932
53335_4_380	BCL11a	Exon 4	-	chr2:60459114-60459139	AAUAAUAUGAUUUAUUAGCACAACG	933
53335_4_381	BCL11a	Exon 4	-	chr2:60459152-60459177	AGGCUGACAAUAAGGUUUGACAGAG	934
53335_4_38	BCL11	Exon 4	-	chr2:60459153-	GAGGCUGACAAUAAGGUUU	935

2	a			60459178	GACAGA	
53335_4_383	BCL11a	Exon 4	-	chr2:60459154-60459179	AGAGGCUGACAAUAAGGUUUGACAG	936
53335_4_384	BCL11a	Exon 4	-	chr2:60459165-60459190	UAUUGAAAGGAAGAGGCUGACAAUA	937
53335_4_385	BCL11a	Exon 4	-	chr2:60459177-60459202	CCUUGUAUACCAUAUUGAAAGGAAG	938
53335_4_386	BCL11a	Exon 4	-	chr2:60459183-60459208	UUAAGACCUUGUAUACCAUAUUGAA	939
53335_4_387	BCL11a	Exon 4	-	chr2:60459229-60459254	GAUCCAGAUCUACUUGGUUGUCAAG	940
53335_4_388	BCL11a	Exon 4	-	chr2:60459240-60459265	CAAAAGAAAUAGAUCCAGAUCUACU	941
53335_4_389	BCL11a	Exon 4	-	chr2:60459271-60459296	UUUAAUAAUGUCUUUUUAAAAAUAC	942
53335_4_390	BCL11a	Exon 4	-	chr2:60459323-60459348	UGGAACGCAAUUAAAUACACUAGUA	943
53335_4_391	BCL11a	Exon 4	-	chr2:60459348-60459373	UUGAAUGUGUAAUGUGCAAAAGCCC	944
53335_4_392	BCL11a	Exon 4	-	chr2:60459418-60459443	CUCCUGGUGAGAGCUUAAAAGAAAU	945
53335_4_393	BCL11a	Exon 4	-	chr2:60459419-60459444	GCUCCUGGUGAGAGCUUAAAAGAAA	946
53335_4_394	BCL11a	Exon 4	-	chr2:60459439-60459464	ACCAGUAUAAAAGCUACUUUGCUCC	947
53335_4_395	BCL11a	Exon 4	-	chr2:60459547-60459572	UUCUAUAUUGUAUUUCUCACAACAA	948
53335_4_396	BCL11a	Exon 4	-	chr2:60459588-60459613	AGCAUUGGGUGAGGUAAUAAACCUU	949
53335_4_397	BCL11a	Exon 4	-	chr2:60459602-60459627	UUGUAGCUUAAUUCAGCAUUGGGUG	950
53335_4_398	BCL11a	Exon 4	-	chr2:60459607-60459632	UAAACUUGUAGCUUAAUUCAGCAUU	951
53335_4_399	BCL11a	Exon 4	-	chr2:60459608-60459633	AUAAACUUGUAGCUUAAUUCAGCAU	952
53335_4_40	BCL11	Exon 4	-	chr2:60459651-	CUUGGAUCUAUUAAAACCA	953

0	a			60459676	CAUCGA	
53335_4_401	BCL11a	Exon 4	-	chr2:60459674-60459699	AUUUGGUUUUAAAAUAUGAGUGCCU	954
53335_4_402	BCL11a	Exon 4	-	chr2:60459696-60459721	AACAGAACAAGUUUAUUCUAUCAUU	955
53335_4_403	BCL11a	Exon 4	-	chr2:60459749-60459774	UGUAUCAAUUGGAAAGGAAGAAAAA	956
53335_4_404	BCL11a	Exon 4	-	chr2:60459760-60459785	AAAGGGUUAAAUGUAUCAAUUGGAA	957
53335_4_405	BCL11a	Exon 4	-	chr2:60459765-60459790	UCUCUAAAGGGUUAAAUGUAUCAAU	958
53335_4_406	BCL11a	Exon 4	-	chr2:60459782-60459807	UAUGAGCUAAAUGUCUGUCUCUAAA	959
53335_4_407	BCL11a	Exon 4	-	chr2:60459783-60459808	CUAUGAGCUAAAUGUCUGUCUCUAA	960
53335_4_408	BCL11a	Exon 4	-	chr2:60459855-60459880	UAACGUGAGGAGGAAAAACAGUCUU	961
53335_4_409	BCL11a	Exon 4	-	chr2:60459870-60459895	UACAGUUUUAUUUUAUAACGUGAGG	962
53335_4_410	BCL11a	Exon 4	-	chr2:60459873-60459898	AUGUACAGUUUUAUUUUAUAACGUG	963
53335_4_411	BCL11a	Exon 4	-	chr2:60460023-60460048	CUUAGACAGCAUGUAUGGUAUGUUA	964
53335_4_412	BCL11a	Exon 4	-	chr2:60460033-60460058	UUUUUUUUAAACUUAGACAGCAUGUA	965
53335_4_413	BCL11a	Exon 4	-	chr2:60460130-60460155	ACCGUUUGAAUGCAUGAUCUGUAUG	966
53335_4_414	BCL11a	Exon 4	-	chr2:60460131-60460156	CACCGUUUGAAUGCAUGAUCUGUAU	967
53335_4_415	BCL11a	Exon 4	-	chr2:60460132-60460157	UCACCGUUUGAAUGCAUGAUCUGUA	968
53335_4_416	BCL11a	Exon 4	-	chr2:60460314-60460339	AUCGCCCUCCAGCCCCACUCCCUGU	969
53335_4_417	BCL11a	Exon 4	-	chr2:60460403-60460428	GAAUAAUGAUAUAAAAACUGAAUAG	970
53335_4_41	BCL11	Exon 4	-	chr2:60460448-	UACCCUGGAGAAACACAUG	971

8	a			60460473	AAAAAA	
53335_4_419	BCL11a	Exon 4	-	chr2:60460468-60460493	AUGCCUUUUAGCGUGUACAGUACCC	972
53335_4_420	BCL11a	Exon 4	-	chr2:60460522-60460547	AUGAAAACGCAUGGCCAGGUGGGGA	973
53335_4_421	BCL11a	Exon 4	-	chr2:60460526-60460551	GCACAUGAAAACGCAUGGCCAGGUG	974
53335_4_422	BCL11a	Exon 4	-	chr2:60460527-60460552	GGCACAUGAAAACGCAUGGCCAGGU	975
53335_4_423	BCL11a	Exon 4	-	chr2:60460528-60460553	AGGCACAUGAAAACGCAUGGCCAGG	976
53335_4_424	BCL11a	Exon 4	-	chr2:60460531-60460556	ACCAGGCACAUGAAAACGCAUGGCC	977
53335_4_425	BCL11a	Exon 4	-	chr2:60460536-60460561	AGCUCACCAGGCACAUGAAAACGCA	978
53335_4_426	BCL11a	Exon 4	-	chr2:60460553-60460578	CUGUGCCCAGAGUAGCAAGCUCACC	979
53335_4_427	BCL11a	Exon 4	-	chr2:60460610-60460635	CCACAGGAGAAGCCACACGGGCGAA	980
53335_4_428	BCL11a	Exon 4	-	chr2:60460617-60460642	UCACUGUCCACAGGAGAAGCCACAC	981
53335_4_429	BCL11a	Exon 4	-	chr2:60460618-60460643	CUCACUGUCCACAGGAGAAGCCACA	982
53335_4_430	BCL11a	Exon 4	-	chr2:60460631-60460656	GAACUGUAGCAAUCUCACUGUCCAC	983
53335_4_431	BCL11a	Exon 4	-	chr2:60460670-60460695	ACGCAGCGACACUUGUGAGUACUGU	984
53335_4_432	BCL11a	Exon 4	-	chr2:60460671-60460696	GACGCAGCGACACUUGUGAGUACUG	985
53335_4_433	BCL11a	Exon 4	-	chr2:60460701-60460726	GCCCGGGCAGGCCCAGCUCAAAAGA	986
53335_4_434	BCL11a	Exon 4	-	chr2:60460702-60460727	GGCCCGGGCAGGCCCAGCUCAAAAG	987
53335_4_435	BCL11a	Exon 4	-	chr2:60460718-60460743	CCAUAUUAGUGGUCCGGGCCCGGGC	988
53335_4_43	BCL11	Exon 4	-	chr2:60460722-	CGCCCCAUAUUAGUGGUCC	989

6	a			60460747	GGGCCC	
53335_4_437	BCL11a	Exon 4	-	chr2:60460723-60460748	ACGCCCCAUAUUAGUGGUCCGGGCC	990
53335_4_438	BCL11a	Exon 4	-	chr2:60460728-60460753	GGAGCACGCCCCAUAUUAGUGGUCC	991
53335_4_439	BCL11a	Exon 4	-	chr2:60460729-60460754	GGGAGCACGCCCCAUAUUAGUGGUC	992
53335_4_440	BCL11a	Exon 4	-	chr2:60460734-60460759	GUGGAGGGAGCACGCCCCAUAUUAG	993
53335_4_441	BCL11a	Exon 4	-	chr2:60460754-60460779	GGGGCGCAGCGGCACGGGAAGUGGA	994
53335_4_442	BCL11a	Exon 4	-	chr2:60460755-60460780	CGGGGCGCAGCGGCACGGGAAGUGG	995
53335_4_443	BCL11a	Exon 4	-	chr2:60460758-60460783	UCUCGGGGCGCAGCGGCACGGGAAG	996
53335_4_444	BCL11a	Exon 4	-	chr2:60460764-60460789	GAGGGAUCUCGGGGCGCAGCGGCAC	997
53335_4_445	BCL11a	Exon 4	-	chr2:60460765-60460790	GGAGGGAUCUCGGGGCGCAGCGGCA	998
53335_4_446	BCL11a	Exon 4	-	chr2:60460770-60460795	UGGACGGAGGGAUCUCGGGGCGCAG	999
53335_4_447	BCL11a	Exon 4	-	chr2:60460778-60460803	CGGGGAGCUGGACGGAGGGAUCUCG	1000
53335_4_448	BCL11a	Exon 4	-	chr2:60460779-60460804	CCGGGGAGCUGGACGGAGGGAUCUC	1001
53335_4_449	BCL11a	Exon 4	-	chr2:60460780-60460805	CCCGGGGAGCUGGACGGAGGGAUCU	1002
53335_4_450	BCL11a	Exon 4	-	chr2:60460787-60460812	CACACCGCCCGGGGAGCUGGACGGA	1003
53335_4_451	BCL11a	Exon 4	-	chr2:60460788-60460813	CCACACCGCCCGGGGAGCUGGACGG	1004
53335_4_452	BCL11a	Exon 4	-	chr2:60460791-60460816	UCUCCACACCGCCCGGGGAGCUGGA	1005
53335_4_453	BCL11a	Exon 4	-	chr2:60460795-60460820	CGCUUCUCCACACCGCCCGGGAGC	1006
53335_4_45	BCL11	Exon 4	-	chr2:60460801-	AGUUUGCGCUUCUCCACAC	1007

4	a			60460826	CGCCCG	
53335_4_455	BCL11a	Exon 4	-	chr2:60460802-60460827	GAGUUUGCGCUUCUCCACACCGCCC	1008
53335_4_456	BCL11a	Exon 4	-	chr2:60460803-60460828	GGAGUUUGCGCUUCUCCACACCGCC	1009
53335_4_457	BCL11a	Exon 4	-	chr2:60460829-60460854	CUCGUCGGAGCACUCCUCGGAGAAC	1010
53335_4_458	BCL11a	Exon 4	-	chr2:60460830-60460855	CCUCGUCGGAGCACUCCUCGGAGAA	1011
53335_4_459	BCL11a	Exon 4	-	chr2:60460837-60460862	UUUGCCUCCUCGUCGGAGCACUCCU	1012
53335_4_460	BCL11a	Exon 4	-	chr2:60460849-60460874	AGACAAUCGCCUUUUGCCUCCUCGU	1013
53335_4_461	BCL11a	Exon 4	-	chr2:60460884-60460909	AGCUCAAAGAUCCCUUCCUUAGCUU	1014
53335_4_462	BCL11a	Exon 4	-	chr2:60460913-60460938	GUGGCUCGCCGGCUACGCGGCCUCC	1015
53335_4_463	BCL11a	Exon 4	-	chr2:60460921-60460946	UACUCGCAGUGGCUCGCCGGCUACG	1016
53335_4_464	BCL11a	Exon 4	-	chr2:60460929-60460954	AGAACGUGUACUCGCAGUGGCUCGC	1017
53335_4_465	BCL11a	Exon 4	-	chr2:60460937-60460962	CAACACGGAGAACGUGUACUCGCAG	1018
53335_4_466	BCL11a	Exon 4	-	chr2:60460957-60460982	CUGCCCCCGGCCGCGAUGCCCAACA	1019
53335_4_467	BCL11a	Exon 4	-	chr2:60460975-60461000	CUCGAGAAGGAGUUCGACCUGCCCC	1020
53335_4_468	BCL11a	Exon 4	-	chr2:60460993-60461018	UUCUCUAAGCGCAUCAAGCUCGAGA	1021
53335_4_469	BCL11a	Exon 4	-	chr2:60461043-60461068	GGGGCCUGUCCAAAAAGCUGCUGCU	1022
53335_4_470	BCL11a	Exon 4	-	chr2:60461044-60461069	GGGGGCCUGUCCAAAAAGCUGCUGC	1023
53335_4_471	BCL11a	Exon 4	-	chr2:60461067-60461092	GCUCCCCGGGCGAGUCGGCCUCGGG	1024
53335_4_47	BCL11	Exon 4	-	chr2:60461068-	UGCUCCCCGGGCGAGUCGG	1025

2	a			60461093	CCUCGG	
53335_4_473	BCL11a	Exon 4	-	chr2:60461069-60461094	CUGCUCCCCGGGCGAGUCGGCCUCG	1026
53335_4_474	BCL11a	Exon 4	-	chr2:60461070-60461095	GCUGCUCCCCGGGCGAGUCGGCCUC	1027
53335_4_475	BCL11a	Exon 4	-	chr2:60461071-60461096	GGCUGCUCCCCGGGCGAGUCGGCCU	1028
53335_4_476	BCL11a	Exon 4	-	chr2:60461077-60461102	GGCCGCGGCUGCUCCCCGGGCGAGU	1029
53335_4_477	BCL11a	Exon 4	-	chr2:60461085-60461110	CUGUUAAUGGCCGCGGCUGCUCCCC	1030
53335_4_478	BCL11a	Exon 4	-	chr2:60461086-60461111	ACUGUUAAUGGCCGCGGCUGCUCCC	1031
53335_4_479	BCL11a	Exon 4	-	chr2:60461097-60461122	UAGACGAUGGCACUGUUAAUGGCCG	1032
53335_4_480	BCL11a	Exon 4	-	chr2:60461103-60461128	ACCGCAUAGACGAUGGCACUGUUAA	1033
53335_4_481	BCL11a	Exon 4	-	chr2:60461115-60461140	CCGGCGAGUCGGACCGCAUAGACGA	1034
53335_4_482	BCL11a	Exon 4	-	chr2:60461131-60461156	GACGAAGACUCGGUGGCCGGCGAGU	1035
53335_4_483	BCL11a	Exon 4	-	chr2:60461139-60461164	ACACUUGCGACGAAGACUCGGUGGC	1036
53335_4_484	BCL11a	Exon 4	-	chr2:60461143-60461168	AGGGACACUUGCGACGAAGACUCGG	1037
53335_4_485	BCL11a	Exon 4	-	chr2:60461146-60461171	CACAGGGACACUUGCGACGAAGACU	1038
53335_4_486	BCL11a	Exon 4	-	chr2:60461167-60461192	CACCUGGCCGAGGCCGAGGGCCACA	1039
53335_4_487	BCL11a	Exon 4	-	chr2:60461168-60461193	CCACCUGGCCGAGGCCGAGGGCCAC	1040
53335_4_488	BCL11a	Exon 4	-	chr2:60461175-60461200	AGCGCGGCCACCUGGCCGAGGCCGA	1041
53335_4_489	BCL11a	Exon 4	-	chr2:60461176-60461201	AAGCGCGGCCACCUGGCCGAGGCCG	1042
53335_4_49	BCL11	Exon 4	-	chr2:60461182-	AAGCAUAAGCGCGGCCACC	1043

0	a			60461207	UGGCCG	
53335_4_491	BCL11a	Exon 4	-	chr2:60461188-60461213	GGCGAGAAGCAUAAGCGCGGCCACC	1044
53335_4_492	BCL11a	Exon 4	-	chr2:60461196-60461221	AGGUCCUGGGCGAGAAGCAUAAGCG	1045
53335_4_493	BCL11a	Exon 4	-	chr2:60461214-60461239	UCAGCGAGGCCUUCCACCAGGUCCU	1046
53335_4_494	BCL11a	Exon 4	-	chr2:60461215-60461240	UUCAGCGAGGCCUUCCACCAGGUCC	1047
53335_4_495	BCL11a	Exon 4	-	chr2:60461221-60461246	CAGCACUUCAGCGAGGCCUUCCACC	1048
53335_4_496	BCL11a	Exon 4	-	chr2:60461233-60461258	CUCAGCUCCAUGCAGCACUUCAGCG	1049
53335_4_497	BCL11a	Exon 4	-	chr2:60461263-60461288	GCCCUGCCCGACGUCAUGCAGGGCA	1050
53335_4_498	BCL11a	Exon 4	-	chr2:60461268-60461293	GCCGCGCCCUGCCCGACGUCAUGCA	1051
53335_4_499	BCL11a	Exon 4	-	chr2:60461269-60461294	AGCCGCGCCCUGCCCGACGUCAUGC	1052
53335_4_500	BCL11a	Exon 4	-	chr2:60461304-60461329	GCUCGCGGGGCGCGGUCGUGGGCGU	1053
53335_4_501	BCL11a	Exon 4	-	chr2:60461305-60461330	AGCUCGCGGGGCGCGGUCGUGGGCG	1054
53335_4_502	BCL11a	Exon 4	-	chr2:60461310-60461335	AGAACAGCUCGCGGGGCGCGGUCGU	1055
53335_4_503	BCL11a	Exon 4	-	chr2:60461311-60461336	GAGAACAGCUCGCGGGGCGCGGUCG	1056
53335_4_504	BCL11a	Exon 4	-	chr2:60461317-60461342	CACCACGAGAACAGCUCGCGGGGCG	1057
53335_4_505	BCL11a	Exon 4	-	chr2:60461322-60461347	CGCGCCACCACGAGAACAGCUCGCG	1058
53335_4_506	BCL11a	Exon 4	-	chr2:60461323-60461348	GCGCGCCACCACGAGAACAGCUCGC	1059
53335_4_507	BCL11a	Exon 4	-	chr2:60461324-60461349	GGCGCGCCACCACGAGAACAGCUCG	1060
53335_4_50	BCL11	Exon 4	-	chr2:60461350-	UACGGCUUCGGGCUGAGCC	1061

8	a			60461375	UGGAGG	
53335_4_509	BCL11a	Exon 4	-	chr2:60461353-60461378	GACUACGGCUUCGGGCUGAGCCUGG	1062
53335_4_510	BCL11a	Exon 4	-	chr2:60461356-60461381	CUGGACUACGGCUUCGGGCUGAGCC	1063
53335_4_511	BCL11a	Exon 4	-	chr2:60461366-60461391	CUCGCUCUCCCUGGACUACGGCUUC	1064
53335_4_512	BCL11a	Exon 4	-	chr2:60461367-60461392	UCUCGCUCUCCCUGGACUACGGCUU	1065
53335_4_513	BCL11a	Exon 4	-	chr2:60461373-60461398	ACUGCCUCUCGCUCUCCCUGGACUA	1066
53335_4_514	BCL11a	Exon 4	-	chr2:60461380-60461405	CUCCUCGACUGCCUCUCGCUCUCCC	1067
53335_4_515	BCL11a	Exon 4	-	chr2:60461512-60461537	GCCAGCAGCGCGCUCAAGUCCGUGG	1068
53335_4_516	BCL11a	Exon 4	-	chr2:60461515-60461540	AGCGCCAGCAGCGCGCUCAAGUCCG	1069
53335_4_517	BCL11a	Exon 4	-	chr2:60461544-60461569	CGGAACCCGGCACCAGCGACUUGGU	1070
53335_4_518	BCL11a	Exon 4	-	chr2:60461545-60461570	CCGGAACCCGGCACCAGCGACUUGG	1071
53335_4_519	BCL11a	Exon 4	-	chr2:60461548-60461573	UCCCCGGAACCCGGCACCAGCGACU	1072
53335_4_520	BCL11a	Exon 4	-	chr2:60461562-60461587	UCUCCACCGCCAGCUCCCCGGAACC	1073
53335_4_521	BCL11a	Exon 4	-	chr2:60461569-60461594	GACGGUCUCUCCACCGCCAGCUCCC	1074
53335_4_522	BCL11a	Exon 4	-	chr2:60461592-60461617	CCCCCAUGACGGUCAAGUCCGACGA	1075
53335_4_523	BCL11a	Exon 4	-	chr2:60461608-60461633	CACAUGCACAAAUCGUCCCCCAUGA	1076
53335_4_524	BCL11a	Exon 4	-	chr2:60461665-60461690	AACCUGUGCGACCACGCGUGCACCC	1077
53335_4_525	BCL11a	Exon 4	-	chr2:60461712-60461737	UGGUGGUGCACCGGCGCAGCCACAC	1078
53335_4_52	BCL11	Exon 4	-	chr2:60461713-	CUGGUGGUGCACCGGCGCA	1079

6	a			60461738	GCCACA	
53335_4_527	BCL11a	Exon 4	-	chr2:60461726-60461751	AUUUCAGAGCAACCUGGUGGUGCAC	1080
53335_4_528	BCL11a	Exon 4	-	chr2:60461734-60461759	ACGUUCAAAUUUCAGAGCAACCUGG	1081
53335_4_529	BCL11a	Exon 4	-	chr2:60461737-60461762	AAGACGUUCAAAUUUCAGAGCAACC	1082
53335_4_530	BCL11a	Exon 4	-	chr2:60461766-60461791	UCAAGUCCAAGUCAUGCGAGUUCUG	1083
53335_4_531	BCL11a	Exon 4	-	chr2:60461794-60461819	UCCGCCCCUCCUCCCUCCCAGCCCC	1084
53335_4_532	BCL11a	Exon 4	-	chr2:60461848-60461873	CAGCCAGGUAGCAAGCCGCCCUUCC	1085
53335_4_533	BCL11a	Exon 4	-	chr2:60461868-60461893	AAAGGUUACUGCAACCAUUCCAGCC	1086
53335_4_534	BCL11a	Exon 4	-	chr2:60461891-60461916	CCCAGGCCGGCCCAGCCCUAUGCAA	1087
53335_4_535	BCL11a	Exon 4	-	chr2:60461909-60461934	GUCUAGCCCACCGCUGUCCCCAGGC	1088
53335_4_536	BCL11a	Exon 4	-	chr2:60461913-60461938	ACACGUCUAGCCCACCGCUGUCCCC	1089
53335_4_537	BCL11a	Exon 4	-	chr2:60461942-60461967	CUCUAGGAGACUUAGAGAGCUGGCA	1090
53335_4_538	BCL11a	Exon 4	-	chr2:60461943-60461968	UCUCUAGGAGACUUAGAGAGCUGGC	1091
53335_4_539	BCL11a	Exon 4	-	chr2:60461947-60461972	GAUUUCUCUAGGAGACUUAGAGAGC	1092
53335_4_540	BCL11a	Exon 4	-	chr2:60461963-60461988	GGAGCCUCCCGCCAUGGAUUUCUCU	1093
53335_4_541	BCL11a	Exon 4	-	chr2:60461974-60461999	CCAAUGGCUAUGGAGCCUCCCGCCA	1094
53335_4_542	BCL11a	Exon 4	-	chr2:60461989-60462014	GUGCUGCGGUUGAAUCCAAUGGCUA	1095
53335_4_543	BCL11a	Exon 4	-	chr2:60461995-60462020	GACAGGGUGCUGCGGUUGAAUCCAA	1096
53335_4_54	BCL11	Exon 4	-	chr2:60462008-	CCCGAGUGCCUUUGACAGG	1097

4	a			60462033	GUGCUG	
53335_4_545	BCL11a	Exon 4	-	chr2:60462016-60462041	ACCCAUCACCCGAGUGCCUUUGACA	1098
53335_4_546	BCL11a	Exon 4	-	chr2:60462017-60462042	CACCCAUCACCCGAGUGCCUUUGAC	1099
53335_4_547	BCL11a	Exon 4	-	chr2:60462046-60462071	CGCCUGGGGGCGGAAGAGAUGGCCC	1100
53335_4_548	BCL11a	Exon 4	-	chr2:60462052-60462077	AUAGAGCGCCUGGGGGCGGAAGAGA	1101
53335_4_549	BCL11a	Exon 4	-	chr2:60462061-60462086	CCCCACCGCAUAGAGCGCCUGGGGG	1102
53335_4_550	BCL11a	Exon 4	-	chr2:60462064-60462089	GACCCCCACCGCAUAGAGCGCCUGG	1103
53335_4_551	BCL11a	Exon 4	-	chr2:60462065-60462090	GGACCCCCACCGCAUAGAGCGCCUG	1104
53335_4_552	BCL11a	Exon 4	-	chr2:60462066-60462091	UGGACCCCCACCGCAUAGAGCGCCU	1105
53335_4_553	BCL11a	Exon 4	-	chr2:60462067-60462092	UUGGACCCCCACCGCAUAGAGCGCC	1106
53335_4_554	BCL11a	Exon 4	-	chr2:60462091-60462116	UUUAGUCCACCACCGAGACAUCACU	1107
53335_4_555	BCL11a	Exon 4	-	chr2:60462143-60462168	GAGAGAGGCUUCCGGCCUGGCAGAA	1108
53335_4_556	BCL11a	Exon 4	-	chr2:60462144-60462169	CGAGAGAGGCUUCCGGCCUGGCAGA	1109
53335_4_557	BCL11a	Exon 4	-	chr2:60462151-60462176	UCAGUAUCGAGAGAGGCUUCCGGCC	1110
53335_4_558	BCL11a	Exon 4	-	chr2:60462156-60462181	CAGGAUCAGUAUCGAGAGAGGCUUC	1111
53335_4_559	BCL11a	Exon 4	-	chr2:60462163-60462188	AGAAUACCAGGAUCAGUAUCGAGAG	1112
53335_4_560	BCL11a	Exon 4	-	chr2:60462180-60462205	ACCCCUUUAACCUGCUAAGAAUACC	1113
53335_4_561	BCL11a	Exon 4	-	chr2:60462227-60462252	AUGUCCUUCCCAGCCACCUCUCCAU	1114
53335_4_56	BCL11	Exon 4	-	chr2:60462228-	AAUGUCCUUCCCAGCCACC	1115

2	a			60462253	UCUCCA	
53335_4_563	BCL11a	Exon 4	-	chr2:60462261-60462286	CGCGGGUUGGUAUCCCUUCAGGACU	1116
53335_4_564	BCL11a	Exon 4	-	chr2:60462267-60462292	UGACCCCGCGGGUUGGUAUCCCUUC	1117
53335_4_565	BCL11a	Exon 4	-	chr2:60462279-60462304	ACGGAAGUCCCCUGACCCCGCGGGU	1118
53335_4_566	BCL11a	Exon 4	-	chr2:60462283-60462308	GAACACGGAAGUCCCCUGACCCCGC	1119
53335_4_567	BCL11a	Exon 4	-	chr2:60462284-60462309	CGAACACGGAAGUCCCCUGACCCCG	1120
53335_4_568	BCL11a	Exon 4	-	chr2:60462303-60462328	UAAGAAUCUACUUAGAAAGCGAACA	1121
53335_4_569	BCL11a	Exon 4	-	chr2:60462333-60462358	UCUUGCAACACGCACAGAACACUCA	1122
53335_4_570	BCL11a	Exon 4	-	chr2:60462365-60462390	UUGCAAACAGCCAUUCACCAGUGCA	1123
53335_3_1	BCL11a	Exon 3	+	chr2:60468716-60468741	UAAGGCUCAACUUACAAAUACCCUG	1124
53335_3_2	BCL11a	Exon 3	+	chr2:60468717-60468742	AAGGCUCAACUUACAAAUACCCUGC	1125
53335_3_3	BCL11a	Exon 3	+	chr2:60468718-60468743	AGGCUCAACUUACAAAUACCCUGCG	1126
53335_3_4	BCL11a	Exon 3	+	chr2:60468740-60468765	GCGGGGCAUAUUCUGCACUCAUCCC	1127
53335_3_5	BCL11a	Exon 3	+	chr2:60468745-60468770	GCAUAUUCUGCACUCAUCCCAGGCG	1128
53335_3_6	BCL11a	Exon 3	+	chr2:60468746-60468771	CAUAUUCUGCACUCAUCCCAGGCGU	1129
53335_3_7	BCL11a	Exon 3	+	chr2:60468747-60468772	AUAUUCUGCACUCAUCCCAGGCGUG	1130
53335_3_8	BCL11a	Exon 3	+	chr2:60468773-60468798	GGAUUAGAGCUCCAUGUGCAGAACG	1131
53335_3_9	BCL11a	Exon 3	+	chr2:60468774-60468799	GAUUAGAGCUCCAUGUGCAGAACGA	1132
53335_3_10	BCL11	Exon 3	+	chr2:60468775-	AUUAGAGCUCCAUGUGCAG	1133

	a			60468800	AACGAG	
53335_3_11	BCL11a	Exon 3	+	chr2:60468778-60468803	AGAGCUCCAUGUGCAGAACGAGGGG	1134
53335_3_12	BCL11a	Exon 3	+	chr2:60468783-60468808	UCCAUGUGCAGAACGAGGGGAGGAG	1135
53335_3_13	BCL11a	Exon 3	-	chr2:60468739-60468764	GGAUGAGUGCAGAAUAUGCCCCGCA	1136
53335_3_14	BCL11a	Exon 3	-	chr2:60468740-60468765	GGGAUGAGUGCAGAAUAUGCCCCGC	1137
53335_3_15	BCL11a	Exon 3	-	chr2:60468765-60468790	ACAUGGAGCUCUAAUCCCCACGCCU	1138
53335_3_16	BCL11a	Exon 3	-	chr2:60468766-60468791	CACAUGGAGCUCUAAUCCCCACGCC	1139
53335_3_17	BCL11a	Exon 3	-	chr2:60468787-60468812	GCCUCUCCUCCCCUCGUUCUGCACA	1140
53335_3_18	BCL11a	Exon 3	-	chr2:60468814-60468839	UCACAGAUAAACUUCUGCACUGGAG	1141
53335_3_19	BCL11a	Exon 3	-	chr2:60468815-60468840	UUCACAGAUAAACUUCUGCACUGGA	1142
53335_3_20	BCL11a	Exon 3	-	chr2:60468816-60468841	UUUCACAGAUAAACUUCUGCACUGG	1143
53335_3_21	BCL11a	Exon 3	-	chr2:60468819-60468844	UUCUUUCACAGAUAAACUUCUGCAC	1144
53335_2_1	BCL11a	Exon 2	+	chr2:60545963-60545988	CAUUUACCUGCUAUGUGUUCCUGUU	1145
53335_2_2	BCL11a	Exon 2	+	chr2:60545964-60545989	AUUUACCUGCUAUGUGUUCCUGUUU	1146
53335_2_3	BCL11a	Exon 2	+	chr2:60545965-60545990	UUUACCUGCUAUGUGUUCCUGUUUG	1147
53335_2_4	BCL11a	Exon 2	+	chr2:60546009-60546034	ACGUUGAUAAACAAUCGUCAUCCUC	1148
53335_2_5	BCL11a	Exon 2	+	chr2:60546019-60546044	ACAAUCGUCAUCCUCUGGCGUGACC	1149
53335_2_6	BCL11a	Exon 2	+	chr2:60546035-60546060	GGCGUGACCUGGAUGCCAACCUCCA	1150
53335_2_7	BCL11	Exon 2	+	chr2:60546036-	GCGUGACCUGGAUGCCAAC	1151

	a			60546061	CUCCAC	
53335_2_8	BCL11a	Exon 2	+	chr2:60546041-60546066	ACCUGGAUGCCAACCUCCACGGGAU	1152
53335_2_9	BCL11a	Exon 2	+	chr2:60546063-60546088	GAUUGGAUGCUUUUUUCAUCUCGAU	1153
53335_2_10	BCL11a	Exon 2	+	chr2:60546069-60546094	AUGCUUUUUUCAUCUCGAUUGGUGA	1154
53335_2_11	BCL11a	Exon 2	+	chr2:60546070-60546095	UGCUUUUUUCAUCUCGAUUGGUGAA	1155
53335_2_12	BCL11a	Exon 2	+	chr2:60546071-60546096	GCUUUUUUCAUCUCGAUUGGUGAAG	1156
53335_2_13	BCL11a	Exon 2	+	chr2:60546075-60546100	UUUUCAUCUCGAUUGGUGAAGGGGA	1157
53335_2_14	BCL11a	Exon 2	+	chr2:60546078-60546103	UCAUCUCGAUUGGUGAAGGGGAAGG	1158
53335_2_15	BCL11a	Exon 2	+	chr2:60546106-60546131	CUUAUCCACAGCUUUUUCUAAGCAG	1159
53335_2_16	BCL11a	Exon 2	+	chr2:60546162-60546187	CGAUAAAAAUAAGAAUGUCCCCCAA	1160
53335_2_17	BCL11a	Exon 2	+	chr2:60546163-60546188	GAUAAAAAUAAGAAUGUCCCCCAAU	1161
53335_2_18	BCL11a	Exon 2	+	chr2:60546175-60546200	AAUGUCCCCCAAUGGGAAGUUCAUC	1162
53335_2_19	BCL11a	Exon 2	+	chr2:60546188-60546213	GGGAAGUUCAUCUGGCACUGCCCAC	1163
53335_2_20	BCL11a	Exon 2	+	chr2:60546193-60546218	GUUCAUCUGGCACUGCCCACAGGUG	1164
53335_2_21	BCL11a	Exon 2	+	chr2:60546196-60546221	CAUCUGGCACUGCCCACAGGUGAGG	1165
53335_2_22	BCL11a	Exon 2	+	chr2:60546213-60546238	AGGUGAGGAGGUCAUGAUCCCCUUC	1166
53335_2_23	BCL11a	Exon 2	+	chr2:60546225-60546250	CAUGAUCCCCUUCUGGAGCUCCCAA	1167
53335_2_24	BCL11a	Exon 2	+	chr2:60546226-60546251	AUGAUCCCCUUCUGGAGCUCCCAAC	1168
53335_2_25	BCL11	Exon 2	+	chr2:60546232-	CCCUUCUGGAGCUCCCAAC	1169

	a			60546257	GGGCCG	
53335_2_26	BCL11a	Exon 2	+	chr2:60546237-60546262	CUGGAGCUCCCAACGGGCCGUGGUC	1170
53335_2_27	BCL11a	Exon 2	+	chr2:60546257-60546282	UGGUCUGGUUCAUCAUCUGUAAGAA	1171
53335_2_28	BCL11a	Exon 2	+	chr2:60546267-60546292	CAUCAUCUGUAAGAAUGGCUUCAAG	1172
53335_2_29	BCL11a	Exon 2	+	chr2:60546272-60546297	UCUGUAAGAAUGGCUUCAAGAGGCU	1173
53335_2_30	BCL11a	Exon 2	+	chr2:60546278-60546303	AGAAUGGCUUCAAGAGGCUCGGCUG	1174
53335_2_31	BCL11a	Exon 2	+	chr2:60546282-60546307	UGGCUUCAAGAGGCUCGGCUGUGGU	1175
53335_2_32	BCL11a	Exon 2	-	chr2:60545956-60545981	ACACAUAGCAGGUAAAUGAGAAGCA	1176
53335_2_33	BCL11a	Exon 2	-	chr2:60545972-60545997	UUUGCCCCAAACAGGAACACAUAGC	1177
53335_2_34	BCL11a	Exon 2	-	chr2:60545985-60546010	UCAUCUAGAGGAAUUUGCCCCAAAC	1178
53335_2_35	BCL11a	Exon 2	-	chr2:60546002-60546027	ACGAUUGUUUAUCAACGUCAUCUAG	1179
53335_2_36	BCL11a	Exon 2	-	chr2:60546033-60546058	GAGGUUGGCAUCCAGGUCACGCCAG	1180
53335_2_37	BCL11a	Exon 2	-	chr2:60546045-60546070	UCCAAUCCCGUGGAGGUUGGCAUCC	1181
53335_2_38	BCL11a	Exon 2	-	chr2:60546053-60546078	AAAAAGCAUCCAAUCCCGUGGAGGU	1182
53335_2_39	BCL11a	Exon 2	-	chr2:60546057-60546082	AUGAAAAAAGCAUCCAAUCCCGUGG	1183
53335_2_40	BCL11a	Exon 2	-	chr2:60546060-60546085	GAGAUGAAAAAAGCAUCCAAUCCCG	1184
53335_2_41	BCL11a	Exon 2	-	chr2:60546114-60546139	GGCAGCCUCUGCUUAGAAAAAGCUG	1185
53335_2_42	BCL11a	Exon 2	-	chr2:60546140-60546165	UCGAGCACAAACGGAAACAAUGCAA	1186
53335_2_43	BCL11	Exon 2	-	chr2:60546154-	CAUUCUUAUUUUUAUCGAG	1187

	a			60546179	CACAAA	
53335_2_44	BCL11a	Exon 2	-	chr2:60546183-60546208	CAGUGCCAGAUGAACUUCCCAUUGG	1188
53335_2_45	BCL11a	Exon 2	-	chr2:60546184-60546209	GCAGUGCCAGAUGAACUUCCCAUUG	1189
53335_2_46	BCL11a	Exon 2	-	chr2:60546185-60546210	GGCAGUGCCAGAUGAACUUCCCAUU	1190
53335_2_47	BCL11a	Exon 2	-	chr2:60546186-60546211	GGGCAGUGCCAGAUGAACUUCCCAU	1191
53335_2_48	BCL11a	Exon 2	-	chr2:60546211-60546236	AGGGGAUCAUGACCUCCUCACCUGU	1192
53335_2_49	BCL11a	Exon 2	-	chr2:60546212-60546237	AAGGGGAUCAUGACCUCCUCACCUG	1193
53335_2_50	BCL11a	Exon 2	-	chr2:60546234-60546259	CACGGCCCGUUGGGAGCUCCAGAAG	1194
53335_2_51	BCL11a	Exon 2	-	chr2:60546235-60546260	CCACGGCCCGUUGGGAGCUCCAGAA	1195
53335_2_52	BCL11a	Exon 2	-	chr2:60546236-60546261	ACCACGGCCCGUUGGGAGCUCCAGA	1196
53335_2_53	BCL11a	Exon 2	-	chr2:60546248-60546273	AUGAUGAACCAGACCACGGCCCGUU	1197
53335_2_54	BCL11a	Exon 2	-	chr2:60546249-60546274	GAUGAUGAACCAGACCACGGCCCGU	1198
53335_2_55	BCL11a	Exon 2	-	chr2:60546257-60546282	UUCUUACAGAUGAUGAACCAGACCA	1199
53335_1_1	BCL11a	Exon 1	+	chr2:60553216-60553241	CGAGAAUUCCCGUUUGCUUAAGUGC	1200
53335_1_2	BCL11a	Exon 1	+	chr2:60553217-60553242	GAGAAUUCCCGUUUGCUUAAGUGCU	1201
53335_1_3	BCL11a	Exon 1	+	chr2:60553218-60553243	AGAAUUCCCGUUUGCUUAAGUGCUG	1202
53335_1_4	BCL11a	Exon 1	+	chr2:60553234-60553259	UAAGUGCUGGGGUUUGCCUUGCUUG	1203
53335_1_5	BCL11a	Exon 1	+	chr2:60553244-60553269	GGUUUGCCUUGCUUGCGGCGAGACA	1204
53335_1_6	BCL11	Exon 1	+	chr2:60553247-	UUGCCUUGCUUGCGGCGAG	1205

	a			60553272	ACAUGG	
53335_1_7	BCL11a	Exon 1	+	chr2:60553248-60553273	UGCCUUGCUUGCGGCGAGACAUGGU	1206
53335_1_8	BCL11a	Exon 1	+	chr2:60553254-60553279	GCUUGCGGCGAGACAUGGUGGGCUG	1207
53335_1_9	BCL11a	Exon 1	+	chr2:60553255-60553280	CUUGCGGCGAGACAUGGUGGGCUGC	1208
53335_1_10	BCL11a	Exon 1	+	chr2:60553256-60553281	UUGCGGCGAGACAUGGUGGGCUGCG	1209
53335_1_11	BCL11a	Exon 1	+	chr2:60553291-60553316	CGGCGGCGGCGGCGGCGGCGGCGGG	1210
53335_1_12	BCL11a	Exon 1	+	chr2:60553298-60553323	GGCGGCGGCGGCGGCGGGCGGACGA	1211
53335_1_13	BCL11a	Exon 1	+	chr2:60553303-60553328	CGGCGGCGGCGGGCGGACGACGGCU	1212
53335_1_14	BCL11a	Exon 1	+	chr2:60553313-60553338	GGGCGGACGACGGCUCGGUUCACAU	1213
53335_1_15	BCL11a	Exon 1	+	chr2:60553314-60553339	GGCGGACGACGGCUCGGUUCACAUC	1214
53335_1_16	BCL11a	Exon 1	+	chr2:60553322-60553347	ACGGCUCGGUUCACAUCGGGAGAGC	1215
53335_1_17	BCL11a	Exon 1	+	chr2:60553323-60553348	CGGCUCGGUUCACAUCGGGAGAGCC	1216
53335_1_18	BCL11a	Exon 1	+	chr2:60553335-60553360	CAUCGGGAGAGCCGGGUUAGAAAGA	1217
53335_1_19	BCL11a	Exon 1	+	chr2:60553406-60553431	AAAUAAAUUAGAAAUAAUACAAAGA	1218
53335_1_20	BCL11a	Exon 1	+	chr2:60553412-60553437	AUUAGAAAUAAUACAAAGAUGGCGC	1219
53335_1_21	BCL11a	Exon 1	+	chr2:60553413-60553438	UUAGAAAUAAUACAAAGAUGGCGCA	1220
53335_1_22	BCL11a	Exon 1	+	chr2:60553427-60553452	AAGAUGGCGCAGGGAAGAUGAAUUG	1221
53335_1_23	BCL11a	Exon 1	+	chr2:60553428-60553453	AGAUGGCGCAGGGAAGAUGAAUUGU	1222
53335_1_24	BCL11	Exon 1	+	chr2:60553441-	AAGAUGAAUUGUGGGAGAG	1223

	a			60553466	CCGUCA	
53335_1_25	BCL11a	Exon 1	-	chr2:60553227-60553252	GGCAAACCCCAGCACUUAAGCAAAC	1224
53335_1_26	BCL11a	Exon 1	-	chr2:60553228-60553253	AGGCAAACCCCAGCACUUAAGCAAA	1225
53335_1_27	BCL11a	Exon 1	-	chr2:60553253-60553278	AGCCCACCAUGUCUCGCCGCAAGCA	1226
53335_1_28	BCL11a	Exon 1	-	chr2:60553349-60553374	CUCUGGAGUCUCCUUCUUUCUAACC	1227
53335_1_29	BCL11a	Exon 1	-	chr2:60553371-60553396	AAGGCACUGAUGAAGAUAUUUUCUC	1228
53335_1_30	BCL11a	Exon 1	-	chr2:60553395-60553420	UUUCUAAUUUAUUUUGGAUGUCAAA	1229
53335_1_31	BCL11a	Exon 1	-	chr2:60553406-60553431	UCUUUGUAUUAUUUCUAAUUUAUUU	1230
53335_1_32	BCL11a	Exon 1	-	chr2:60553463-60553488	AAAAAAGCUUAAAAAAAAGCCAUGA	1231

Table 2: gRNA targeting domains targeting BCL11a Intron 2 (e.g., to a BCL11a Enhancer)

Id.	**Target**	**Target Region**	**Strand**	**Targeting Site (hg38)**	**gRNA Targeting Domain**	**SEQ ID NO:**
53335_I2_1	BCL11a	Intron 2	+	chr2:60494277-60494302	GGGAGUUUGGCUUCUCAUCUGUGCA	1232
53335_I2_2	BCL11a	Intron 2	+	chr2:60494289-60494314	UCUCAUCUGUGCAUGGCCUCUAAAC	1233
53335_I2_3	BCL11a	Intron 2	+	chr2:60494290-60494315	CUCAUCUGUGCAUGGCCUCUAAACU	1234
53335_I2_4	BCL11a	Intron 2	+	chr2:60494302-60494327	UGGCCUCUAAACUGGGCAGUGACCA	1235
53335_I2_5	BCL11a	Intron 2	+	chr2:60494307-60494332	UCUAAACUGGGCAGUGACCAUGGCC	1236
53335_I2_6	BCL11a	Intron 2	+	chr2:60494324-60494349	CCAUGGCCUGGUCACCUCCCCACUC	1237
53335_I2_7	BCL11a	Intron 2	+	chr2:60494330-	CCUGGUCACCUCCCCACU	1238

				60494355	CUGGACC	
53335_I2_8	BCL11a	Intron 2	+	chr2:60494331-60494356	CUGGUCACCUCCCCACUCUGGACCU	1239
53335_I2_9	BCL11a	Intron 2	+	chr2:60494351-60494376	GACCUGGGUUGCCCCUCUGUAAACA	1240
53335_I2_10	BCL11a	Intron 2	+	chr2:60494354-60494379	CUGGGUUGCCCCUCUGUAAACAAGG	1241
53335_I2_11	BCL11a	Intron 2	+	chr2:60494418-60494443	AAAUCUUAUGCAAUUUUUGCCAAGA	1242
53335_I2_12	BCL11a	Intron 2	+	chr2:60494419-60494444	AAUCUUAUGCAAUUUUUGCCAAGAU	1243
53335_I2_13	BCL11a	Intron 2	+	chr2:60494426-60494451	UGCAAUUUUUGCCAAGAUGGGAGUA	1244
53335_I2_14	BCL11a	Intron 2	+	chr2:60494427-60494452	GCAAUUUUUGCCAAGAUGGGAGUAU	1245
53335_I2_15	BCL11a	Intron 2	+	chr2:60494428-60494453	CAAUUUUUGCCAAGAUGGGAGUAUG	1246
53335_I2_16	BCL11a	Intron 2	+	chr2:60494440-60494465	AGAUGGGAGUAUGGGGAGAAGAG	1247
53335_I2_17	BCL11a	Intron 2	+	chr2:60494446-60494471	GAGUAUGGGGAGAGAAGAGUGGAAA	1248
53335_I2_18	BCL11a	Intron 2	+	chr2:60494477-60494502	AGAGCUCAGUGAGAUGAGAUCAA	1249
53335_I2_19	BCL11a	Intron 2	+	chr2:60494478-60494503	GAGCUCAGUGAGAUGAGAUCAAA	1250
53335_I2_20	BCL11a	Intron 2	+	chr2:60494479-60494504	AGCUCAGUGAGAUGAGAUCAAAG	1251
53335_I2_21	BCL11a	Intron 2	+	chr2:60494518-60494543	UCAUUCCAUCUCCCUAAUCUCCAAU	1252
53335_I2_22	BCL11a	Intron 2	+	chr2:60494533-60494558	AAUCUCCAAUUGGCAAAGCCAGACU	1253
53335_I2_23	BCL11a	Intron 2	+	chr2:60494534-60494559	AUCUCCAAUUGGCAAAGCCAGACUU	1254
53335_I2_24	BCL11a	Intron 2	+	chr2:60494535-60494560	UCUCCAAUUGGCAAAGCCAGACUUG	1255
53335_I2_25	BCL11a	Intron 2	+	chr2:60494548-	AAGCCAGACUUGGGGCA	1256

				60494573	AUACAGAC	
53335_I2_26	BCL11a	Intron 2	+	chr2:60494617-60494642	UAUCACCAAAUGUUCUUUCUUCAGC	1257
53335_I2_27	BCL11a	Intron 2	+	chr2:60494631-60494656	CUUUCUUCAGCUGGAAUUUAAAAUA	1258
53335_I2_28	BCL11a	Intron 2	+	chr2:60494649-60494674	UAAAAUAUGGACUCAUCCGUAAAAU	1259
53335_I2_29	BCL11a	Intron 2	+	chr2:60494675-60494700	GGAAUAAUAAUAGUAUAUGCUUCAU	1260
53335_I2_30	BCL11a	Intron 2	+	chr2:60494676-60494701	GAAUAAUAAUAGUAUAUGCUUCAUA	1261
53335_I2_31	BCL11a	Intron 2	+	chr2:60494766-60494791	GCUUGUGAACUAAAAUGCUGCCUCC	1262
53335_I2_32	BCL11a	Intron 2	+	chr2:60494806-60494831	ACACCUCAGCAGAAACAAAGUUAUC	1263
53335_I2_33	BCL11a	Intron 2	+	chr2:60494830-60494855	CAGGCCCUUUCCCCAAUUCCUAGUU	1264
53335_I2_34	BCL11a	Intron 2	+	chr2:60494831-60494856	AGGCCCUUUCCCCAAUUCCUAGUUU	1265
53335_I2_35	BCL11a	Intron 2	+	chr2:60494844-60494869	AAUUCCUAGUUUGGGUCAGAAGAAA	1266
53335_I2_36	BCL11a	Intron 2	+	chr2:60494845-60494870	AUUCCUAGUUUGGGUCAGAAGAAAA	1267
53335_I2_37	BCL11a	Intron 2	+	chr2:60494851-60494876	AGUUUGGGUCAGAAGAAAAGGGAAA	1268
53335_I2_38	BCL11a	Intron 2	+	chr2:60494852-60494877	GUUUGGGUCAGAAGAAAAGGGAAAA	1269
53335_I2_39	BCL11a	Intron 2	+	chr2:60494857-60494882	GGUCAGAAGAAAAGGGAAAGGGAG	1270
53335_I2_40	BCL11a	Intron 2	+	chr2:60494864-60494889	AGAAAAGGGAAAAGGGAGAGGAAAA	1271
53335_I2_41	BCL11a	Intron 2	+	chr2:60494883-60494908	GGAAAAAGGAAAAGAAUAUGACGUC	1272
53335_I2_42	BCL11a	Intron 2	+	chr2:60494884-60494909	GAAAAAGGAAAAGAAUAUGACGUCA	1273
53335_I2_43	BCL11a	Intron 2	+	chr2:60494885-	AAAAAGGAAAAGAAUAU	1274

				60494910	GACGUCAG	
53335_I2_44	BCL11a	Intron 2	+	chr2:60494886-60494911	AAAAGGAAAAGAAUAUGACGUCAGG	1275
53335_I2_45	BCL11a	Intron 2	+	chr2:60494889-60494914	AGGAAAAGAAUAUGACGUCAGGGGG	1276
53335_I2_46	BCL11a	Intron 2	+	chr2:60494901-60494926	UGACGUCAGGGGAGGCAAGUCAGU	1277
53335_I2_47	BCL11a	Intron 2	+	chr2:60494902-60494927	GACGUCAGGGGAGGCAAGUCAGUU	1278
53335_I2_48	BCL11a	Intron 2	+	chr2:60494928-60494953	GGAACACAGAUCCUAACACAGUAGC	1279
53335_I2_49	BCL11a	Intron 2	+	chr2:60494939-60494964	CCUAACACAGUAGCUGGUACCUGAU	1280
53335_I2_50	BCL11a	Intron 2	+	chr2:60494955-60494980	GUACCUGAUAGGUGCCUAUAUGUGA	1281
53335_I2_51	BCL11a	Intron 2	+	chr2:60494959-60494984	CUGAUAGGUGCCUAUAUGUGAUGGA	1282
53335_I2_52	BCL11a	Intron 2	+	chr2:60494960-60494985	UGAUAGGUGCCUAUAUGUGAUGGAU	1283
53335_I2_53	BCL11a	Intron 2	+	chr2:60494963-60494988	UAGGUGCCUAUAUGUGAUGGAUGGG	1284
53335_I2_54	BCL11a	Intron 2	+	chr2:60494986-60495011	GGUGGACAGCCCGACAGAUGAAAAA	1285
53335_I2_55	BCL11a	Intron 2	+	chr2:60494998-60495023	GACAGAUGAAAAAUGGACAAUUAUG	1286
53335_I2_56	BCL11a	Intron 2	+	chr2:60495001-60495026	AGAUGAAAAAUGGACAAUUAUGAGG	1287
53335_I2_57	BCL11a	Intron 2	+	chr2:60495002-60495027	GAUGAAAAAUGGACAAUUAUGAGGA	1288
53335_I2_58	BCL11a	Intron 2	+	chr2:60495003-60495028	AUGAAAAAUGGACAAUUAUGAGGAG	1289
53335_I2_59	BCL11a	Intron 2	+	chr2:60495017-60495042	AUUAUGAGGAGGGGAGAGUGCAGAC	1290
53335_I2_60	BCL11a	Intron 2	+	chr2:60495018-60495043	UUAUGAGGAGGGGAGAGUGCAGACA	1291
53335_I2_61	BCL11a	Intron 2	+	chr2:60495019-	UAUGAGGAGGGGAGAGU	1292

				60495044	GCAGACAG	
53335_I2_62	BCL11a	Intron 2	+	chr2:60495045-60495070	GGAAGCUUCACCUCCUUUACAAUUU	1293
53335_I2_63	BCL11a	Intron 2	+	chr2:60495046-60495071	GAAGCUUCACCUCCUUUACAAUUUU	1294
53335_I2_64	BCL11a	Intron 2	+	chr2:60495057-60495082	UCCUUUACAAUUUUGGGAGUCCACA	1295
53335_I2_65	BCL11a	Intron 2	+	chr2:60495062-60495087	UACAAUUUUGGGAGUCCACACGGCA	1296
53335_I2_66	BCL11a	Intron 2	+	chr2:60495135-60495160	CAUCACCAAGAGAGCCUUCCGAAAG	1297
53335_I2_67	BCL11a	Intron 2	+	chr2:60495144-60495169	GAGAGCCUUCCGAAAGAGGCCCCCC	1298
53335_I2_68	BCL11a	Intron 2	+	chr2:60495145-60495170	AGAGCCUUCCGAAAGAGGCCCCCCU	1299
53335_I2_69	BCL11a	Intron 2	+	chr2:60495152-60495177	UCCGAAAGAGGCCCCCCUGGGCAAA	1300
53335_I2_70	BCL11a	Intron 2	+	chr2:60495162-60495187	GCCCCCCUGGGCAAACGGCCACCGA	1301
53335_I2_71	BCL11a	Intron 2	+	chr2:60495167-60495192	CCUGGGCAAACGGCCACCGAUGGAG	1302
53335_I2_72	BCL11a	Intron 2	+	chr2:60495215-60495240	CCACCCACGCCCCCACCCUAAUCAG	1303
53335_I2_73	BCL11a	Intron 2	+	chr2:60495230-60495255	CCCUAAUCAGAGGCCAAACCCUUCC	1304
53335_I2_74	BCL11a	Intron 2	+	chr2:60495293-60495318	ACUAGCUUCAAAGUUGUAUUGACCC	1305
53335_I2_75	BCL11a	Intron 2	+	chr2:60495333-60495358	AAGAGUAGAUGCCAUAUCUCUUUUC	1306
53335_I2_76	BCL11a	Intron 2	+	chr2:60495354-60495379	UUUCUGGCCUAUGUUAUUACCUGUA	1307
53335_I2_77	BCL11a	Intron 2	+	chr2:60495366-60495391	GUUAUUACCUGUAUGGACUUUGCAC	1308
53335_I2_78	BCL11a	Intron 2	+	chr2:60495400-60495425	CUAUCUGCUCUUACUUAUGCACACC	1309
53335_I2_79	BCL11a	Intron 2	+	chr2:60495401-	UAUCUGCUCUUACUUAU	1310

				60495426	GCACACCU	
53335_I2_80	BCL11a	Intron 2	+	chr2:60495402-60495427	AUCUGCUCUUACUUAUGCACACCUG	1311
53335_I2_81	BCL11a	Intron 2	+	chr2:60495486-60495511	CCUGUCUAGCUGCCUUCCUUAUCAC	1312
53335_I2_82	BCL11a	Intron 2	+	chr2:60495500-60495525	UUCCUUAUCACAGGAAUAGCACCCA	1313
53335_I2_83	BCL11a	Intron 2	+	chr2:60495543-60495568	GAGUAGAACCCCCUAUAAACUAGUC	1314
53335_I2_84	BCL11a	Intron 2	+	chr2:60495554-60495579	CCUAUAAACUAGUCUGGUUUGCCCA	1315
53335_I2_85	BCL11a	Intron 2	+	chr2:60495555-60495580	CUAUAAACUAGUCUGGUUUGCCCAU	1316
53335_I2_86	BCL11a	Intron 2	+	chr2:60495556-60495581	UAUAAACUAGUCUGGUUUGCCCAUG	1317
53335_I2_87	BCL11a	Intron 2	+	chr2:60495566-60495591	UCUGGUUUGCCCAUGGGGCACAGUC	1318
53335_I2_88	BCL11a	Intron 2	+	chr2:60495578-60495603	AUGGGGCACAGUCAGGCUGUUUUCC	1319
53335_I2_89	BCL11a	Intron 2	+	chr2:60495579-60495604	UGGGGCACAGUCAGGCUGUUUUCCA	1320
53335_I2_90	BCL11a	Intron 2	+	chr2:60495582-60495607	GGCACAGUCAGGCUGUUUUCCAGGG	1321
53335_I2_91	BCL11a	Intron 2	+	chr2:60495583-60495608	GCACAGUCAGGCUGUUUUCCAGGGU	1322
53335_I2_92	BCL11a	Intron 2	+	chr2:60495584-60495609	CACAGUCAGGCUGUUUUCCAGGGUG	1323
53335_I2_93	BCL11a	Intron 2	+	chr2:60495661-60495686	ACACACGUAUGUGUUGUGAUCCCUG	1324
53335_I2_94	BCL11a	Intron 2	+	chr2:60495674-60495699	UUGUGAUCCCUGUGGUUUGAGAGUU	1325
53335_I2_95	BCL11a	Intron 2	+	chr2:60495706-60495731	UCCCUAAAAGUCAAAAUAUUCUCAA	1326
53335_I2_96	BCL11a	Intron 2	+	chr2:60495707-60495732	CCCUAAAAGUCAAAAUAUUCUCAAU	1327
53335_I2_97	BCL11a	Intron 2	+	chr2:60495735-	CCCUCAAUCAGCACAUA	1328

				60495760	CACACAAA	
53335_I2_98	BCL11a	Intron 2	+	chr2:60495742-60495767	UCAGCACAUACACACAAAAGGUACC	1329
53335_I2_99	BCL11a	Intron 2	+	chr2:60495775-60495800	UGUAAUUCUUUUCCUGCUCAAAGAC	1330
53335_I2_100	BCL11a	Intron 2	+	chr2:60495820-60495845	CCCCAACCAAAAACCCUUGCCACCA	1331
53335_I2_101	BCL11a	Intron 2	+	chr2:60495821-60495846	CCCAACCAAAAACCCUUGCCACCAU	1332
53335_I2_102	BCL11a	Intron 2	+	chr2:60495828-60495853	AAAAACCCUUGCCACCAUGGGAGCC	1333
53335_I2_103	BCL11a	Intron 2	+	chr2:60495829-60495854	AAAACCCUUGCCACCAUGGGAGCCU	1334
53335_I2_104	BCL11a	Intron 2	+	chr2:60495830-60495855	AAACCCUUGCCACCAUGGGAGCCUG	1335
53335_I2_105	BCL11a	Intron 2	+	chr2:60495839-60495864	CCACCAUGGGAGCCUGGGGCAGAGA	1336
53335_I2_106	BCL11a	Intron 2	+	chr2:60495867-60495892	CACAGUGAAGUCAAACUGUAAUUCC	1337
53335_I2_107	BCL11a	Intron 2	+	chr2:60495877-60495902	UCAAACUGUAAUUCCAGGCUCUAAA	1338
53335_I2_108	BCL11a	Intron 2	+	chr2:60495913-60495938	UUUUUCUGAGAGUCUCUAAAUUACA	1339
53335_I2_109	BCL11a	Intron 2	+	chr2:60495914-60495939	UUUUCUGAGAGUCUCUAAAUUACAA	1340
53335_I2_110	BCL11a	Intron 2	+	chr2:60495972-60495997	ACACCUAAGAAACAUACUGCAGCUC	1341
53335_I2_111	BCL11a	Intron 2	+	chr2:60496001-60496026	AAAGAGAACAAACAAACCAAAGAGA	1342
53335_I2_112	BCL11a	Intron 2	+	chr2:60496002-60496027	AAGAGAACAAACAAACCAAAGAGAA	1343
53335_I2_113	BCL11a	Intron 2	+	chr2:60496011-60496036	AACAAACCAAAGAGAAGGGAUCCAG	1344
53335_I2_114	BCL11a	Intron 2	+	chr2:60496048-60496073	UAUGUGAAAAGUCAAUUGAUAAUGA	1345
53335_I2_115	BCL11a	Intron 2	+	chr2:60496055-	AAAGUCAAUUGAUAAUG	1346

				60496080	AAGGCUUU	
53335_I2_116	BCL11a	Intron 2	+	chr2:60496063-60496088	UUGAUAAUGAAGGCUUUAGGAUAAC	1347
53335_I2_117	BCL11a	Intron 2	+	chr2:60496066-60496091	AUAAUGAAGGCUUUAGGAUAACCGG	1348
53335_I2_118	BCL11a	Intron 2	+	chr2:60496067-60496092	UAAUGAAGGCUUUAGGAUAACCGGA	1349
53335_I2_119	BCL11a	Intron 2	+	chr2:60496068-60496093	AAUGAAGGCUUUAGGAUAACCGGAG	1350
53335_I2_120	BCL11a	Intron 2	+	chr2:60496098-60496123	AUGAUUGAAAGCAAUGCACCUGUGC	1351
53335_I2_121	BCL11a	Intron 2	+	chr2:60496104-60496129	GAAAGCAAUGCACCUGUGCAGGAAA	1352
53335_I2_122	BCL11a	Intron 2	+	chr2:60496111-60496136	AUGCACCUGUGCAGGAAAUGGAUUA	1353
53335_I2_123	BCL11a	Intron 2	+	chr2:60496118-60496143	UGUGCAGGAAAUGGAUUACGGAAAC	1354
53335_I2_124	BCL11a	Intron 2	+	chr2:60496119-60496144	GUGCAGGAAAUGGAUUACGGAAACA	1355
53335_I2_125	BCL11a	Intron 2	+	chr2:60496153-60496178	UCAUGAAAUCCCAGAAAACCAGAAC	1356
53335_I2_126	BCL11a	Intron 2	+	chr2:60496154-60496179	CAUGAAAUCCCAGAAAACCAGAACC	1357
53335_I2_127	BCL11a	Intron 2	+	chr2:60496164-60496189	CAGAAAACCAGAACCGGGAAAGUUC	1358
53335_I2_128	BCL11a	Intron 2	+	chr2:60496171-60496196	CCAGAACCGGGAAAGUUCUGGAAGU	1359
53335_I2_129	BCL11a	Intron 2	+	chr2:60496198-60496223	GAAAAACAAAUCAUGACUUAAGCAA	1360
53335_I2_130	BCL11a	Intron 2	+	chr2:60496238-60496263	CGUUUACAGAAUGCCUUGUCCCACG	1361
53335_I2_131	BCL11a	Intron 2	-	chr2:60494308-60494333	AGGCCAUGGUCACUGCCCAGUUUAG	1362
53335_I2_132	BCL11a	Intron 2	-	chr2:60494327-60494352	CCAGAGUGGGGAGGUGACCAGGCCA	1363
53335_I2_133	BCL11a	Intron 2	-	chr2:60494333-	CCAGGUCCAGAGUGGGG	1364

				60494358	AGGUGACC	
53335_I2_134	BCL11a	Intron 2	-	chr2:60494341-60494366	GGGGCAACCCAGGUCCAGAGUGGGG	1365
53335_I2_135	BCL11a	Intron 2	-	chr2:60494344-60494369	AGAGGGGCAACCCAGGUCCAGAGUG	1366
53335_I2_136	BCL11a	Intron 2	-	chr2:60494345-60494370	CAGAGGGGCAACCCAGGUCCAGAGU	1367
53335_I2_137	BCL11a	Intron 2	-	chr2:60494346-60494371	ACAGAGGGGCAACCCAGGUCCAGAG	1368
53335_I2_138	BCL11a	Intron 2	-	chr2:60494356-60494381	CUCCUUGUUUACAGAGGGGCAACCC	1369
53335_I2_139	BCL11a	Intron 2	-	chr2:60494365-60494390	UAUUACAACCUCCUUGUUUACAGAG	1370
53335_I2_140	BCL11a	Intron 2	-	chr2:60494366-60494391	UUAUUACAACCUCCUUGUUUACAGA	1371
53335_I2_141	BCL11a	Intron 2	-	chr2:60494367-60494392	UUUAUUACAACCUCCUUGUUUACAG	1372
53335_I2_142	BCL11a	Intron 2	-	chr2:60494401-60494426	UAAGAUUUAUAAGACAUUAGGGUAU	1373
53335_I2_143	BCL11a	Intron 2	-	chr2:60494407-60494432	AUUGCAUAAGAUUUAUAAGACAUUA	1374
53335_I2_144	BCL11a	Intron 2	-	chr2:60494408-60494433	AAUUGCAUAAGAUUUAUAAGACAUU	1375
53335_I2_145	BCL11a	Intron 2	-	chr2:60494440-60494465	CUCUUCUCUCCCCAUACUCCCAUCU	1376
53335_I2_146	BCL11a	Intron 2	-	chr2:60494477-60494502	UUGAUAUCUCAUCUCACUGAGCUCU	1377
53335_I2_147	BCL11a	Intron 2	-	chr2:60494478-60494503	UUUGAUAUCUCAUCUCACUGAGCUC	1378
53335_I2_148	BCL11a	Intron 2	-	chr2:60494526-60494551	CUUUGCCAAUUGGAGAUUAGGGAGA	1379
53335_I2_149	BCL11a	Intron 2	-	chr2:60494532-60494557	GUCUGGCUUUGCCAAUUGGAGAUUA	1380
53335_I2_150	BCL11a	Intron 2	-	chr2:60494533-60494558	AGUCUGGCUUUGCCAAUUGGAGAUU	1381
53335_I2_151	BCL11a	Intron 2	-	chr2:60494541-	UUGCCCCAAGUCUGGCU	1382

				60494566	UUGCCAAU	
53335_I2_152	BCL11a	Intron 2	-	chr2:60494554-60494579	GAACCAGUCUGUAUUGCCCCAAGUC	1383
53335_I2_153	BCL11a	Intron 2	-	chr2:60494599-60494624	GGUGAUAAAUCAUUAGGAAUGAGCU	1384
53335_I2_154	BCL11a	Intron 2	-	chr2:60494610-60494635	AAAGAACAUUUGGUGAUAAAUCAUU	1385
53335_I2_155	BCL11a	Intron 2	-	chr2:60494625-60494650	AAAUUCCAGCUGAAGAAAGAACAUU	1386
53335_I2_156	BCL11a	Intron 2	-	chr2:60494668-60494693	AUAUACUAUUAUUAUUCCUAUUUUA	1387
53335_I2_157	BCL11a	Intron 2	-	chr2:60494745-60494770	AAGCUCGGAGCACUUACUCUGCUCU	1388
53335_I2_158	BCL11a	Intron 2	-	chr2:60494765-60494790	GAGGCAGCAUUUUAGUUCACAAGCU	1389
53335_I2_159	BCL11a	Intron 2	-	chr2:60494789-60494814	UGAGGUGUAACUAAUAAAUACCAGG	1390
53335_I2_160	BCL11a	Intron 2	-	chr2:60494792-60494817	UGCUGAGGUGUAACUAAUAAAUACC	1391
53335_I2_161	BCL11a	Intron 2	-	chr2:60494812-60494837	GGGCCUGAUAACUUUGUUUCUGCUG	1392
53335_I2_162	BCL11a	Intron 2	-	chr2:60494837-60494862	UGACCCAAACUAGGAAUUGGGGAAA	1393
53335_I2_163	BCL11a	Intron 2	-	chr2:60494838-60494863	CUGACCCAAACUAGGAAUUGGGGAA	1394
53335_I2_164	BCL11a	Intron 2	-	chr2:60494843-60494868	UUCUUCUGACCCAAACUAGGAAUUG	1395
53335_I2_165	BCL11a	Intron 2	-	chr2:60494844-60494869	UUUCUUCUGACCCAAACUAGGAAUU	1396
53335_I2_166	BCL11a	Intron 2	-	chr2:60494845-60494870	UUUUCUUCUGACCCAAACUAGGAAU	1397
53335_I2_167	BCL11a	Intron 2	-	chr2:60494851-60494876	UUUCCCUUUUCUUCUGACCCAAACU	1398
53335_I2_168	BCL11a	Intron 2	-	chr2:60494942-60494967	CCUAUCAGGUACCAGCUACUGUGUU	1399
53335_I2_169	BCL11a	Intron 2	-	chr2:60494961-	CAUCCAUCACAUAUAGG	1400

				60494986	CACCUAUC	
53335_I2_170	BCL11a	Intron 2	-	chr2:60494972-60494997	GGCUGUCCACCCAUCCAUCACAUAU	1401
53335_I2_171	BCL11a	Intron 2	-	chr2:60494998-60495023	CAUAAUUGUCCAUUUUUCAUCUGUC	1402
53335_I2_172	BCL11a	Intron 2	-	chr2:60494999-60495024	UCAUAAUUGUCCAUUUUUCAUCUGU	1403
53335_I2_173	BCL11a	Intron 2	-	chr2:60495058-60495083	GUGUGGACUCCCAAAAUUGUAAAGG	1404
53335_I2_174	BCL11a	Intron 2	-	chr2:60495061-60495086	GCCGUGUGGACUCCCAAAAUUGUAA	1405
53335_I2_175	BCL11a	Intron 2	-	chr2:60495080-60495105	GAAAUAAUUUGUAUGCCAUGCCGUG	1406
53335_I2_176	BCL11a	Intron 2	-	chr2:60495111-60495136	GGAGAAUUGGAUUUUAUUUCUCAAU	1407
53335_I2_177	BCL11a	Intron 2	-	chr2:60495112-60495137	UGGAGAAUUGGAUUUUAUUUCUCAA	1408
53335_I2_178	BCL11a	Intron 2	-	chr2:60495129-60495154	GAAGGCUCUCUUGGUGAUGGAGAAU	1409
53335_I2_179	BCL11a	Intron 2	-	chr2:60495137-60495162	CUCUUUCGGAAGGCUCUCUUGGUGA	1410
53335_I2_180	BCL11a	Intron 2	-	chr2:60495143-60495168	GGGGGCCUCUUUCGGAAGGCUCUCU	1411
53335_I2_181	BCL11a	Intron 2	-	chr2:60495152-60495177	UUUGCCCAGGGGGGCCUCUUUCGGA	1412
53335_I2_182	BCL11a	Intron 2	-	chr2:60495156-60495181	GCCGUUUGCCCAGGGGGGCCUCUUU	1413
53335_I2_183	BCL11a	Intron 2	-	chr2:60495166-60495191	UCCAUCGGUGGCCGUUUGCCCAGGG	1414
53335_I2_184	BCL11a	Intron 2	-	chr2:60495167-60495192	CUCCAUCGGUGGCCGUUUGCCCAGG	1415
53335_I2_185	BCL11a	Intron 2	-	chr2:60495168-60495193	UCUCCAUCGGUGGCCGUUUGCCCAG	1416
53335_I2_186	BCL11a	Intron 2	-	chr2:60495169-60495194	CUCUCCAUCGGUGGCCGUUUGCCCA	1417
53335_I2_187	BCL11a	Intron 2	-	chr2:60495170-	CCUCUCCAUCGGUGGCC	1418

				60495195	GUUUGCCC	
53335_I2_188	BCL11a	Intron 2	-	chr2:60495183-60495208	GAGGACUGGCAGACCUCUCCAUCGG	1419
53335_I2_189	BCL11a	Intron 2	-	chr2:60495186-60495211	GAAGAGGACUGGCAGACCUCUCCAU	1420
53335_I2_190	BCL11a	Intron 2	-	chr2:60495202-60495227	GGGCGUGGGUGGGGUAGAAGAGGAC	1421
53335_I2_191	BCL11a	Intron 2	-	chr2:60495207-60495232	GGUGGGGGCGUGGGUGGGGUAGAAG	1422
53335_I2_192	BCL11a	Intron 2	-	chr2:60495216-60495241	UCUGAUUAGGGUGGGGGCGUGGGUG	1423
53335_I2_193	BCL11a	Intron 2	-	chr2:60495217-60495242	CUCUGAUUAGGGUGGGGGCGUGGGU	1424
53335_I2_194	BCL11a	Intron 2	-	chr2:60495218-60495243	CCUCUGAUUAGGGUGGGGGCGUGGG	1425
53335_I2_195	BCL11a	Intron 2	-	chr2:60495221-60495246	UGGCCUCUGAUUAGGGUGGGGGCGU	1426
53335_I2_196	BCL11a	Intron 2	-	chr2:60495222-60495247	UUGGCCUCUGAUUAGGGUGGGGGCG	1427
53335_I2_197	BCL11a	Intron 2	-	chr2:60495227-60495252	AGGGUUUGGCCUCUGAUUAGGGUGG	1428
53335_I2_198	BCL11a	Intron 2	-	chr2:60495228-60495253	AAGGGUUUGGCCUCUGAUUAGGGUG	1429
53335_I2_199	BCL11a	Intron 2	-	chr2:60495229-60495254	GAAGGGUUUGGCCUCUGAUUAGGGU	1430
53335_I2_200	BCL11a	Intron 2	-	chr2:60495230-60495255	GGAAGGGUUUGGCCUCUGAUUAGGG	1431
53335_I2_201	BCL11a	Intron 2	-	chr2:60495233-60495258	CCAGGAAGGGUUUGGCCUCUGAUUA	1432
53335_I2_202	BCL11a	Intron 2	-	chr2:60495234-60495259	UCCAGGAAGGGUUUGGCCUCUGAUU	1433
53335_I2_203	BCL11a	Intron 2	-	chr2:60495246-60495271	UUUAUCACAGGCUCCAGGAAGGGUU	1434
53335_I2_204	BCL11a	Intron 2	-	chr2:60495251-60495276	UUGCUUUUAUCACAGGCUCCAGGAA	1435
53335_I2_205	BCL11a	Intron 2	-	chr2:60495252-	GUUGCUUUUAUCACAGG	1436

				60495277	CUCCAGGA	
53335_I2_206	BCL11a	Intron 2	-	chr2:60495256-60495281	AACAGUUGCUUUUAUCACAGGCUCC	1437
53335_I2_207	BCL11a	Intron 2	-	chr2:60495263-60495288	GCAAGCUAACAGUUGCUUUUAUCAC	1438
53335_I2_208	BCL11a	Intron 2	-	chr2:60495318-60495343	AUCUACUCUUAGACAUAACACACCA	1439
53335_I2_209	BCL11a	Intron 2	-	chr2:60495319-60495344	CAUCUACUCUUAGACAUAACACACC	1440
53335_I2_210	BCL11a	Intron 2	-	chr2:60495347-60495372	AAUAACAUAGGCCAGAAAAGAGAUA	1441
53335_I2_211	BCL11a	Intron 2	-	chr2:60495364-60495389	GCAAAGUCCAUACAGGUAAUAACAU	1442
53335_I2_212	BCL11a	Intron 2	-	chr2:60495376-60495401	GCUGAUUCCAGUGCAAAGUCCAUAC	1443
53335_I2_213	BCL11a	Intron 2	-	chr2:60495426-60495451	CGAUACAGGGCUGGCUCUAUGCCCC	1444
53335_I2_214	BCL11a	Intron 2	-	chr2:60495440-60495465	AGAUGGCUGAAAAGCGAUACAGGGC	1445
53335_I2_215	BCL11a	Intron 2	-	chr2:60495444-60495469	AGUGAGAUGGCUGAAAAGCGAUACA	1446
53335_I2_216	BCL11a	Intron 2	-	chr2:60495445-60495470	UAGUGAGAUGGCUGAAAAGCGAUAC	1447
53335_I2_217	BCL11a	Intron 2	-	chr2:60495462-60495487	GACUUGGGAGUUAUCUGUAGUGAGA	1448
53335_I2_218	BCL11a	Intron 2	-	chr2:60495482-60495507	UAAGGAAGGCAGCUAGACAGGACUU	1449
53335_I2_219	BCL11a	Intron 2	-	chr2:60495483-60495508	AUAAGGAAGGCAGCUAGACAGGACU	1450
53335_I2_220	BCL11a	Intron 2	-	chr2:60495489-60495514	CCUGUGAUAAGGAAGGCAGCUAGAC	1451
53335_I2_221	BCL11a	Intron 2	-	chr2:60495501-60495526	UUGGGUGCUAUUCCUGUGAUAAGGA	1452
53335_I2_222	BCL11a	Intron 2	-	chr2:60495505-60495530	GACCUUGGGUGCUAUUCCUGUGAUA	1453
53335_I2_223	BCL11a	Intron 2	-	chr2:60495524-	CUACUCUGAGGUACUGA	1454

				60495549	UGGACCUU	
53335_I2_224	BCL11a	Intron 2	-	chr2:60495525-60495550	UCUACUCUGAGGUACUGAUGGACCU	1455
53335_I2_225	BCL11a	Intron 2	-	chr2:60495532-60495557	AGGGGGUUCUACUCUGAGGUACUGA	1456
53335_I2_226	BCL11a	Intron 2	-	chr2:60495541-60495566	CUAGUUUAUAGGGGGUUCUACUCUG	1457
53335_I2_227	BCL11a	Intron 2	-	chr2:60495554-60495579	UGGGCAAACCAGACUAGUUUAUAGG	1458
53335_I2_228	BCL11a	Intron 2	-	chr2:60495555-60495580	AUGGGCAAACCAGACUAGUUUAUAG	1459
53335_I2_229	BCL11a	Intron 2	-	chr2:60495556-60495581	CAUGGGCAAACCAGACUAGUUUAUA	1460
53335_I2_230	BCL11a	Intron 2	-	chr2:60495557-60495582	CCAUGGGCAAACCAGACUAGUUUAU	1461
53335_I2_231	BCL11a	Intron 2	-	chr2:60495578-60495603	GGAAAACAGCCUGACUGUGCCCCAU	1462
53335_I2_232	BCL11a	Intron 2	-	chr2:60495579-60495604	UGGAAAACAGCCUGACUGUGCCCCA	1463
53335_I2_233	BCL11a	Intron 2	-	chr2:60495604-60495629	GGCAGAGAAUGUCUGCACCCCACCC	1464
53335_I2_234	BCL11a	Intron 2	-	chr2:60495630-60495655	UGACGUUAUAUGUAAGCAUCACAAC	1465
53335_I2_235	BCL11a	Intron 2	-	chr2:60495684-60495709	GGAAGCUCCAAACUCUCAAACCACA	1466
53335_I2_236	BCL11a	Intron 2	-	chr2:60495685-60495710	GGGAAGCUCCAAACUCUCAAACCAC	1467
53335_I2_237	BCL11a	Intron 2	-	chr2:60495710-60495735	CCCAUUGAGAAUAUUUUGACUUUUA	1468
53335_I2_238	BCL11a	Intron 2	-	chr2:60495711-60495736	GCCCAUUGAGAAUAUUUUGACUUUU	1469
53335_I2_239	BCL11a	Intron 2	-	chr2:60495738-60495763	CCUUUUGUGUGUAUGUGCUGAUUGA	1470
53335_I2_240	BCL11a	Intron 2	-	chr2:60495739-60495764	ACCUUUUGUGUGUAUGUGCUGAUUG	1471
53335_I2_241	BCL11a	Intron 2	-	chr2:60495768-	AGCAGGAAAAGAAUUAC	1472

				60495793	AGUUUUCC	
53335_I2_242	BCL11a	Intron 2	-	chr2:60495790-60495815	GGUAUUGAAUUGCCUGUCUUUGAGC	1473
53335_I2_243	BCL11a	Intron 2	-	chr2:60495816-60495841	GGCAAGGGUUUUUGGUUGGGGGAAG	1474
53335_I2_244	BCL11a	Intron 2	-	chr2:60495817-60495842	UGGCAAGGGUUUUUGGUUGGGGGAA	1475
53335_I2_245	BCL11a	Intron 2	-	chr2:60495818-60495843	GUGGCAAGGGUUUUUGGUUGGGGGA	1476
53335_I2_246	BCL11a	Intron 2	-	chr2:60495822-60495847	CAUGGUGGCAAGGGUUUUUGGUUGG	1477
53335_I2_247	BCL11a	Intron 2	-	chr2:60495823-60495848	CCAUGGUGGCAAGGGUUUUUGGUUG	1478
53335_I2_248	BCL11a	Intron 2	-	chr2:60495824-60495849	CCCAUGGUGGCAAGGGUUUUUGGUU	1479
53335_I2_249	BCL11a	Intron 2	-	chr2:60495825-60495850	UCCCAUGGUGGCAAGGGUUUUUGGU	1480
53335_I2_250	BCL11a	Intron 2	-	chr2:60495829-60495854	AGGCUCCCAUGGUGGCAAGGGUUUU	1481
53335_I2_251	BCL11a	Intron 2	-	chr2:60495836-60495861	CUGCCCCAGGCUCCCAUGGUGGCAA	1482
53335_I2_252	BCL11a	Intron 2	-	chr2:60495837-60495862	UCUGCCCCAGGCUCCCAUGGUGGCA	1483
53335_I2_253	BCL11a	Intron 2	-	chr2:60495842-60495867	CCUUCUCUGCCCCAGGCUCCCAUGG	1484
53335_I2_254	BCL11a	Intron 2	-	chr2:60495845-60495870	GUGCCUUCUCUGCCCCAGGCUCCCA	1485
53335_I2_255	BCL11a	Intron 2	-	chr2:60495854-60495879	GACUUCACUGUGCCUUCUCUGCCCC	1486
53335_I2_256	BCL11a	Intron 2	-	chr2:60495893-60495918	AAAAAUGACAGCACCAUUUAGAGCC	1487
53335_I2_257	BCL11a	Intron 2	-	chr2:60495978-60496003	UUUCCAGAGCUGCAGUAUGUUUCUU	1488
53335_I2_258	BCL11a	Intron 2	-	chr2:60496020-60496045	GGGUGACCUCUGGAUCCCUUCUCUU	1489
53335_I2_259	BCL11a	Intron 2	-	chr2:60496035-	ACUUUUCACAUAUGAGG	1490

				60496060	GUGACCUC	
53335_I2_260	BCL11a	Intron 2	-	chr2:60496045-60496070	UUAUCAAUUGACUUUUCACAUAUGA	1491
53335_I2_261	BCL11a	Intron 2	-	chr2:60496046-60496071	AUUAUCAAUUGACUUUUCACAUAUG	1492
53335_I2_262	BCL11a	Intron 2	-	chr2:60496090-60496115	GCAUUGCUUUCAAUCAUCUCCCCUC	1493
53335_I2_263	BCL11a	Intron 2	-	chr2:60496119-60496144	UGUUUCCGUAAUCCAUUUCCUGCAC	1494
53335_I2_264	BCL11a	Intron 2	-	chr2:60496165-60496190	AGAACUUUCCCGGUUCUGGUUUUCU	1495
53335_I2_265	BCL11a	Intron 2	-	chr2:60496166-60496191	CAGAACUUUCCCGGUUCUGGUUUUC	1496
53335_I2_266	BCL11a	Intron 2	-	chr2:60496174-60496199	CCGACUUCCAGAACUUUCCCGGUUC	1497
53335_I2_267	BCL11a	Intron 2	-	chr2:60496180-60496205	GUUUUUCCGACUUCCAGAACUUUCC	1498
53335_I2_268	BCL11a	Intron 2	-	chr2:60496233-60496258	GACAAGGCAUUCUGUAAACGUGUAU	1499

In embodiments, the gRNA targeting domain consists of the 20 3' nt of any one of the sequences in the table above.

Exemplary preferred gRNA tareting domains useful in the compositions and methods of the invention are described in the tables below.

Table 5. Preferred Guide RNA Targeting Domains Directed to Coding Regions of the BCL11a Gene

Id.	**Target**	**gRNA Targeting Domain**	**Targeting Site (Chr2)**	**SEQ ID NO:**
CR000044	BCL11a	ACAGGCCGUGAACCUAAGUG	60678414-60678436	1
CR000056	BCL11a	UAGAGGGGGGAAAUUAUAGG	60678685-60678707	2
CR000068	BCL11a	CGAGCGGUAAAUCCUAAAGA	60678840-60678862	3
CR000079	BCL11a	AGACAAAUUCAAAUCCUGCA	60678895-60678917	4
CR000091	BCL11a	GCUUUUGGUGGCUACCAUGC	60678909-60678931	5
CR000102	BCL11a	AAUUUAUGCCAUCUGAUAAG	60679112-60679134	6
CR000033	BCL11a	UGUUCCUCCUACCCACCCGA	60679178-60679200	7

CR000045	BCL11a	AUAAAUGUUGGAGCUUUAGG	60679288-60679310	8
CR000069	BCL11a	CAACCUAAUUACCUGUAUUG	60679389-60679411	9
CR000080	BCL11a	CGCUGUCAUGAGUGAUGCCC	60679428-60679450	10
CR000092	BCL11a	GGCAUCACUCAUGACAGCGA	60679432-60679454	11
CR000103	BCL11a	AGUCCCAUAGAGAGGGCCCG	60679456-60679478	12
CR000115	BCL11a	ACUGAUUCACUGUUCCAAUG	60679476-60679498	13
CR000034	BCL11a	AGGACUCUGUCUUCGCACAA	60679577-60679599	14
CR000058	BCL11a	CCACGCUGACGUCGACUGGG	60679650-60679672	15
CR000070	BCL11a	GUUCUUCACACACCCCCAUU	60679779-60679801	16
CR000081	BCL11a	GCGCUUCUCCACACCGCCCG	60687934-60687956	17
CR000093	BCL11a	GUCUGGAGUCUCCGAAGCUA	60688006-60688028	18
CR000116	BCL11a	AAAGAUCCCUUCCUUAGCUU	60688017-60688039	19
CR000035	BCL11a	UCGCCGGCUACGCGGCCUCC	60688046-60688068	20
CR000047	BCL11a	CGGAGAACGUGUACUCGCAG	60688070-60688092	21
CR000071	BCL11a	GAGCUUGAUGCGCUUAGAGA	60688133-60688155	22
CR000082	BCL11a	CCCCCCGAGGCCGACUCGCC	60688200-60688222	23
CR000094	BCL11a	CACCAUGCCCUGCAUGACGU	60688394-60688416	24
CR000105	BCL11a	GAGAGCGAGAGGGUGGACUA	60688506-60688528	25
CR000117	BCL11a	CACGGACUUGAGCGCGCUGC	60688649-60688671	26
CR000036	BCL11a	GCCCACCAAGUCGCUGGUGC	60688676-60688698	27
CR000048	BCL11a	CCCACCAAGUCGCUGGUGCC	60688677-60688699	28
CR000060	BCL11a	GGCGGUGGAGAGACCGUCGU	60688712-60688734	29
CR000072	BCL11a	GCCGCAGAACUCGCAUGACU	60688898-60688920	30
CR000083	BCL11a	CCGCCCCCAGGCGCUCUAUG	60689194-60689216	31
CR000095	BCL11a	UGGGGGUCCAAGUGAUGUCU	60689217-60689239	32
CR000106	BCL11a	CUCAACUUACAAAUACCCUG	60695857-60695879	33
CR000118	BCL11a	AGUGCAGAAUAUGCCCCGCA	60695872-60695894	34
CR000037	BCL11a	GCAUAUUCUGCACUCAUCCC	60695881-60695903	35
CR000049	BCL11a	GAGCUCUAAUCCCCACGCCU	60695898-60695920	36
CR000061	BCL11a	AGAGCUCCAUGUGCAGAACG	60695914-60695936	37
CR000084	BCL11a	GAUAAACUUCUGCACUGGAG	60695947-60695969	38
CR000096	BCL11a	UAGCUGUAGUGCUUGAUUUU	60695981-60696003	39
CR000107	BCL11a	UAUCCAAAUUCAUACAAUAG	60716451-60716473	40
CR000119	BCL11a	AUGCCCUGAGUAUCCCAACA	60717068-60717090	41
CR000038	BCL11a	UAGUAACAGACCCAUGUGCU	60717232-60717254	42

CR000050	BCL11a	AUACUUCAAGGCCUCAAUGA	60717661-60717683	43
CR000062	BCL11a	GACUAGGUAGACCUUCAUUG	60717672-60717694	44
CR000073	BCL11a	CUGAGCUAGUGGGGGCUCUU	60720773-60720795	45
CR000085	BCL11a	CUGAGCACAUUCUUACGCCU	60721291-60721313	46
CR000097	BCL11a	UUUAUAAGACAUUAGGGUAt	60721534-60721556	47
CR000108	BCL11a	UGAUAGGUGCCUAUAUGUGA	60722096-60722118	48
CR000120	BCL11a	UCCACCCAUCCAUCACAUAU	60722105-60722127	49
CR000039	BCL11a	CUUCAAAGUUGUAUUGACCC	60722434-60722456	50
CR000051	BCL11a	AAACCAGACUAGUUUAUAGG	60722687-60722709	51
CR000063	BCL11a	UUUGAGACAGUUACCCCUUC	60723706-60723728	52
CR000074	BCL11a	AACCUGAGGGCUAGUUUCUA	60724541-60724563	53
CR000086	BCL11a	GCCUGGACCCACCGCUUCAU	60725058-60725080	54
CR000109	BCL11a	AAACCAGUGAGGUCAUCUAU	60725857-60725879	55
CR000121	BCL11a	ACCUGCUAUGUGUUCCUGUU	60773104-60773126	56
CR000040	BCL11a	UAGAGGAAUUUGCCCCAAAC	60773118-60773140	57
CR000052	BCL11a	GAUAAACAAUCGUCAUCCUC	60773150-60773172	58
CR000064	BCL11a	UGGCAUCCAGGUCACGCCAG	60773166-60773188	59
CR000075	BCL11a	GACCUGGAUGCCAACCUCCA	60773176-60773198	60
CR000087	BCL11a	AAAAGCAUCCAAUCCCGUGG	60773190-60773212	61
CR000110	BCL11a	GAUGCUUUUUUCAUCUCGAU	60773204-60773226	62
CR000122	BCL11a	CCACAGCUUUUUCUAAGCAG	60773247-60773269	63
CR000041	BCL11a	CCCCCAAUGGGAAGUUCAUC	60773316-60773338	64
CR000053	BCL11a	AUCAUGACCUCCUCACCUGU	60773344-60773366	65
CR000065	BCL11a	AGGAGGUCAUGAUCCCCUUC	60773354-60773376	66
CR000088	BCL11a	CCCCUUCUGGAGCUCCCAAC	60773367-60773389	67
CR000099	BCL11a	GCUCCCAACGGGCCGUGGUC	60773378-60773400	68
CR000111	BCL11a	ACAGAUGAUGAACCAGACCA	60773390-60773412	69
CR000123	BCL11a	UCUGUAAGAAUGGCUUCAAG	60773408-60773430	70
CR000042	BCL11a	CAAUUAUUAGAGUGCCAGAG	60773459-60773481	71
CR000054	BCL11a	GCCUUGCUUGCGGCGAGACA	60780385-60780407	72
CR000066	BCL11a	ACCAUGUCUCGCCGCAAGCA	60780386-60780408	73
CR000077	BCL11a	GAGUCUCCUUCUUUCUAACC	60780482-60780504	74
CR000089	BCL11a	GAAUUGUGGGAGAGCCGUCA	60780582-60780604	75
CR000100	BCL11a	AAAAUAGAGCGAGAGUGCAC	60781166-60781188	76
CR000112	BCL11a	GCCCCUGGCGUCCACACGCG	60781306-60781328	77

CR000124	BCL11a	CUCCUCGUUCUUUAUUCCUC	60781716-60781738	78
CR000043	BCL11a	GACUGCCCGCGCUUUGUCCU	60781815-60781837	79
CR000055	BCL11a	UUCCCAGGGACUGGGACUCC	60781838-60781860	80
CR000078	BCL11a	UGGCUCCUGGGUGUGCGCCU	60782123-60782145	81
CR000090	BCL11a	UGAAGCCCGCUUUUUGACAG	60782254-60782276	82
CR000101	BCL11a	AGGGUGGAGACGGGUCGCCA	60782526-60782548	83
CR000113	BCL11a	GCUCAGGGUCUCGCGGGCCA	60782543-60782565	84
CR000059	BCL11a	UGAGUCCGAGCAGAAGAAGA	73160981-73161003	85

Table 6: Preferred Guide RNA Targeting Domains directed to the French HPFH (French HPFH; Sankaran VG et al. A functional element necessary for fetal hemoglobin silencing. NEJM (2011) 365:807-814.)

Id.	**Target Name**	**Strand**	**gRNA Targeting Domain**	**Genomic Target Location**	**SEQ ID NO:**
CR001016	HPFH	-	UCUUAAACCAACCUGCUCAC	chr11:5234538-5234558	86
CR001017	HPFH	+	CAGGUUGGUUUAAGAUAAGC	chr11:5234543-5234563	87
CR001018	HPFH	+	AGGUUGGUUUAAGAUAAGCA	chr11:5234544-5234564	88
CR001019	HPFH	-	UUAAGGGAAUAGUGGAAUGA	chr11:5234600-5234620	89
CR001020	HPFH	-	AGGGCAAGUUAAGGGAAUAG	chr11:5234608-5234628	90
CR001021	HPFH	+	CCCUUAACUUGCCCUGAGAU	chr11:5234613-5234633	91
CR001022	HPFH	-	CCAAUCUCAGGGCAAGUUAA	chr11:5234616-5234636	92
CR001023	HPFH	-	GCCAAUCUCAGGGCAAGUUA	chr11:5234617-5234637	93
CR001024	HPFH	-	UGACAGAACAGCCAAUCUCA	chr11:5234627-5234647	94
CR001025	HPFH	-	AUGACAGAACAGCCAAUCUC	chr11:5234628-5234648	95
CR001026	HPFH	-	GAGAUAUGUAGAGGAGAACA	chr11:5234670-5234690	96
CR001027	HPFH	-	GGAGAUAUGUAGAGGAGAAC	chr11:5234671-5234691	97
CR001028	HPFH	-	UGCGGUGGGGAGAUAUGUAG	chr11:5234679-5234699	98
CR001029	HPFH	-	CUGCUGAAAGAGAUGCGGUG	chr11:5234692-5234712	99
CR001030	HPFH	-	ACUGCUGAAAGAGAUGCGGU	chr11:5234693-5234713	100
CR001031	HPFH	-	AACUGCUGAAAGAGAUGCGG	chr11:5234694-5234714	101
CR001032	HPFH	-	AACAACUGCUGAAAGAGAUG	chr11:5234697-5234717	102

CR001033	HPFH	-	UCUGCAAAAAUGAAACUAGG	chr11:5234731-5234751	103
CR001034	HPFH	-	ACUUCUGCAAAAAUGAAACU	chr11:5234734-5234754	104
CR001035	HPFH	+	CAUUUUUGCAGAAGUGUUUU	chr11:5234739-5234759	105
CR001036	HPFH	+	AGUGUUUUAGGCUAAUAUAG	chr11:5234751-5234771	106
CR001037	HPFH	-	UUGGAGACAAAAAUCUCUAG	chr11:5234883-5234903	107
CR001038	HPFH	+	UCUAGAGAUUUUUGUCUCCA	chr11:5234882-5234902	108
CR001039	HPFH	+	CUAGAGAUUUUUGUCUCCAA	chr11:5234883-5234903	109
CR001040	HPFH	+	GUCUCCAAGGGAAUUUUGAG	chr11:5234895-5234915	110
CR001041	HPFH	+	CCAAGGGAAUUUUGAGAGGU	chr11:5234899-5234919	111
CR001042	HPFH	-	CCAACCUCUCAAAAUUCCCU	chr11:5234902-5234922	112
CR001043	HPFH	+	GGAAUUUUGAGAGGUUGGAA	chr11:5234904-5234924	113
CR001044	HPFH	+	UGCUUGCUUCCUCCUUCUUU	chr11:5234953-5234973	114
CR001045	HPFH	-	AAGAAUUUACCAAAAGAAGG	chr11:5234965-5234985	115
CR001046	HPFH	-	AGGAAGAAUUUACCAAAAGA	chr11:5234968-5234988	116
CR001047	HPFH	-	AAAAAUUAGAGUUUUAUUAU	chr11:5234988-5235008	117
CR001048	HPFH	-	UUUUUUAAAUAUUCUUUUAA	Chr11:5235023-5235045	118
CR001049	HPFH	+	UAUUUACCAGUUAUUGAAAU	chr11:5235062-5235082	119
CR001050	HPFH	+	CCAGUUAUUGAAAUAGGUUC	chr11:5235068-5235088	120
CR001051	HPFH	-	CCAGAACCUAUUUCAAUAAC	chr11:5235071-5235091	121
CR001052	HPFH	+	UUCUGGAAACAUGAAUUUUA	chr11:5235085-5235105	122
CR001053	HPFH	+	AUUUUGAAUGUUUAAAAUUA	chr11:5235151-5235171	123
CR001054	HPFH	-	AAAUUUAAUCUGGCUGAAUA	chr11:5235216-5235236	124
CR001055	HPFH	-	GAACUUCGUUAAAUUUAAUC	chr11:5235226-5235246	125
CR001056	HPFH	+	AUUAAAUUUAACGAAGUUCC	chr11:5235227-5235247	126
CR001057	HPFH	+	UUAAAUUUAACGAAGUUCCU	chr11:5235228-5235248	127
CR001058	HPFH	-	UUCUGUACUAGCAUAUUCCC	chr11:5235248-5235268	128
CR001059	HPFH	+	UGUGUUCUUAAAAAAAAAUG	Chr11:5235275-5235297	129
CR001060	HPFH	+	AAAAAUGUGGAAUUAGACCC	chr11:5235293-5235313	130
CR001061	HPFH	-	CUACUGGGAUCUUCAUUCCU	chr11:5235313-5235333	131
CR001062	HPFH	-	ACUACUGGGAUCUUCAUUCC	chr11:5235314-5235334	132

CR001063	HPFH	-	GAAAAGAGUGAAAAACUACU	chr11:5235328-5235348	133
CR001064	HPFH	-	AGAAAAGAGUGAAAAACUAC	chr11:5235329-5235349	134
CR001065	HPFH	+	GAAUUCAAAUAAUGCCACAA	chr11:5235349-5235369	135
CR001066	HPFH	-	UGUGUAUUUGUCUGCCAUUG	chr11:5235366-5235386	136
CR001067	HPFH	+	CACCCAUGAGCAUAUCCAAA	chr11:5235384-5235404	137
CR001068	HPFH	-	UUCCUUUUGGAUAUGCUCAU	chr11:5235389-5235409	138
CR001069	HPFH	-	CUUCCUUUUGGAUAUGCUCA	chr11:5235390-5235410	139
CR001070	HPFH	+	CAUGAGCAUAUCCAAAAGGA	chr11:5235388-5235408	140
CR001071	HPFH	+	UAUCCAAAAGGAAGGAUUGA	chr11:5235396-5235416	141
CR001072	HPFH	-	UUUCCUUCAAUCCUUCCUUU	chr11:5235402-5235422	142
CR001073	HPFH	+	AAGGAAGGAUUGAAGGAAAG	chr11:5235403-5235423	143
CR001074	HPFH	+	GAAGGAUUGAAGGAAAGAGG	chr11:5235406-5235426	144
CR001075	HPFH	+	GAGGAGGAAGAAAUGGAGAA	chr11:5235422-5235442	145
CR001076	HPFH	+	AGGAAGAAAUGGAGAAAGGA	chr11:5235426-5235446	146
CR001077	HPFH	+	GAAGGAAGAGGGGAAGAGAG	chr11:5235448-5235468	147
CR001078	HPFH	+	GAAGAGGGGAAGAGAGAGGA	chr11:5235452-5235472	148
CR001079	HPFH	+	AGGGGAAGAGAGAGGAUGGA	chr11:5235456-5235476	149
CR001080	HPFH	+	GGGGAAGAGAGAGGAUGGAA	chr11:5235457-5235477	150
CR001081	HPFH	+	AAGAGAGAGGAUGGAAGGGA	chr11:5235461-5235481	151
CR001082	HPFH	+	AGAGAGGAUGGAAGGGAUGG	chr11:5235464-5235484	152
CR001083	HPFH	+	GGAAGGGAUGGAGGAGAAGA	chr11:5235473-5235493	153
CR001084	HPFH	+	GAAGAAGGAAAAAUAAAUAA	Chr11:5235483-5235505	154
CR001085	HPFH	+	AGGAAAAAUAAAUAAUGGAG	Chr11:5235488-5235510	155
CR001086	HPFH	+	AAAUAAAUAAUGGAGAGGAG	chr11:5235498-5235518	156
CR001087	HPFH	+	UGGAGAGGAGAGGAGAAAAA	chr11:5235508-5235528	157
CR001088	HPFH	+	AGAGGAGAGGAGAAAAAAGG	chr11:5235511-5235531	158
CR001089	HPFH	+	GAGGAGAGGAGAAAAAAGGA	chr11:5235512-5235532	159
CR001090	HPFH	+	AGGAGAGGAGAAAAAAGGAG	chr11:5235513-5235533	160
CR001091	HPFH	+	AGGAGAAAAAAGGAGGGGAG	chr11:5235518-5235538	161
CR001092	HPFH	+	GAGAGGAGAGGAGAAGGGAU	chr11:5235535-5235555	162

CR001093	HPFH	+	AGAGGAGAGGAGAAGGGAUA	chr11:5235536-5235556	163
CR001094	HPFH	+	GAAGAGAAAGAGAAAGGGAA	Chr11:5235553-5235575	164
CR001095	HPFH	+	AAGAGAGGAAAGAAGAGAAG	chr11:5235581-5235601	165
CR001096	HPFH	+	GAGAGAAAAGAAACGAAGAG	Chr11:5235598-5235620	166
CR001097	HPFH	+	AGAGAAAAGAAACGAAGAGA	Chr11:5235599-5235621	167
CR001098	HPFH	+	GAGAAAAGAAACGAAGAGAG	Chr11:5235600-5235622	168
CR001099	HPFH	+	AAAGAAACGAAGAGAGGGGA	chr11:5235609-5235629	169
CR001100	HPFH	+	AAGAAACGAAGAGAGGGGAA	chr11:5235610-5235630	170
CR001101	HPFH	+	GGAAGGGAAGGAAAAAAAAG	chr11:5235626-5235646	171
CR001102	HPFH	+	AAGACUGACAGUUCAAAUUU	chr11:5235672-5235692	172
CR001103	HPFH	+	ACUGACAGUUCAAAUUUUGG	chr11:5235675-5235695	173
CR001104	HPFH	+	UUCAAAUUUUGGUGGUGAUA	chr11:5235683-5235703	174
CR001105	HPFH	+	AAUAGAAACUCAAACUCUGU	chr11:5235709-5235729	175
CR001106	HPFH	+	GUACAAUAGUAUAACCCCUU	chr11:5235739-5235759	176
CR001107	HPFH	-	CUAUUAAAGGUUUUCCAAAG	chr11:5235756-5235776	177
CR001108	HPFH	-	ACUAUUAAAGGUUUUCCAAA	chr11:5235757-5235777	178
CR001109	HPFH	-	UACUAUUAAAGGUUUUCCAA	chr11:5235758-5235778	179
CR001110	HPFH	-	GCAUUUGUGGAUACUAUUAA	chr11:5235769-5235789	180
CR001111	HPFH	+	UUAAUAGUAUCCACAAAUGC	chr11:5235769-5235789	181
CR001132	HPFH	-	UAUCAAGCAUCCAGCAUUUG	chr11:5235782-5235802	1500
CR001133	HPFH	-	UAUCUAAAAAUGUAAUUGCU	chr11:5235814-5235834	1501
CR001134	HPFH	-	AGCAUUUCUAUACAUGUCUU	chr11:5235862-5235882	1502
CR001135	HPFH	+	UAAUCAUAAAAACCUCAAAC	chr11:5235893-5235913	1503
CR001136	HPFH	-	UUUAAGUGGCUACCGGUUUG	chr11:5235908-5235928	1504
CR001137	HPFH	-	GUAAGCAUUUAAGUGGCUAC	chr11:5235915-5235935	1505
CR001138	HPFH	-	ACUGUUGGUAAGCAUUUAAG	chr11:5235922-5235942	1506
CR001139	HPFH	-	UAAUUUAUCAAUUCUACUGU	chr11:5235937-5235957	1507
CR001140	HPFH	+	ACAGUAGAAUUGAUAAAUUA	chr11:5235937-5235957	1508
CR001141	HPFH	+	CAAAUGCAUUUUACAGCAUU	chr11:5236027-5236047	1509
CR001142	HPFH	+	GGUUGAUUAAAAGUAACCAG	chr11:5236048-5236068	1510

CR001143	HPFH	-	AUAUAGUUUGAACUCACCUC	Chr11:5236059-5236081	1511
CR001144	HPFH	+	UUUAUUUGUAUAUAGAAAGA	chr11:5236090-5236110	1512
CR001145	HPFH	+	UGCCUGAGAUUCUGAUCACA	chr11:5236119-5236139	1513
CR001146	HPFH	+	GCCUGAGAUUCUGAUCACAA	chr11:5236120-5236140	1514
CR001147	HPFH	+	CCUGAGAUUCUGAUCACAAG	chr11:5236121-5236141	1515
CR001148	HPFH	-	CCCCUUGUGAUCAGAAUCUC	chr11:5236124-5236144	1516
CR001149	HPFH	+	AAGGGGAAAUGUUAUAAAAU	chr11:5236138-5236158	1517
CR001150	HPFH	+	AGGGGAAAUGUUAUAAAAUA	chr11:5236139-5236159	1518
CR001151	HPFH	+	UGUUAUAAAAUAGGGUAGAG	chr11:5236147-5236167	1519
CR001152	HPFH	-	CAAAGUUUAAAGGUCAUUCA	chr11:5236175-5236195	1520
CR001153	HPFH	-	UAACUUGUAACAAAGUUUAA	chr11:5236185-5236205	1521
CR001154	HPFH	+	CAAGUUAUUUUUCUGUAACC	chr11:5236198-5236218	1522
CR001155	HPFH	-	AAUAUCUUUCGUUGGCUUCC	chr11:5236219-5236239	1523
CR001156	HPFH	-	AAUUAUUCAAUAUCUUUCGU	chr11:5236227-5236247	1524
CR001157	HPFH	+	GAUAUUGAAUAAUUCAAGAA	chr11:5236233-5236253	1525
CR001158	HPFH	+	AUUGAAUAAUUCAAGAAAGG	chr11:5236236-5236256	1526
CR001159	HPFH	+	GAAUAAUUCAAGAAAGGUGG	chr11:5236239-5236259	1527
CR001160	HPFH	+	AUUCAAGAAAGGUGGUGGCA	chr11:5236244-5236264	1528
CR001161	HPFH	+	UAUUUUAGAAGUAGAGAAAA	chr11:5236313-5236333	1529
CR001162	HPFH	+	AUUUUAGAAGUAGAGAAAAU	chr11:5236314-5236334	1530
CR001163	HPFH	+	GAAAAUGGGAGACAAAUAGC	chr11:5236328-5236348	1531
CR001164	HPFH	+	AAAAUGGGAGACAAAUAGCU	chr11:5236329-5236349	1532
CR001165	HPFH	+	AGCUGGGCUUCUGUUGCAGU	chr11:5236345-5236365	1533
CR001166	HPFH	+	GCUGGGCUUCUGUUGCAGUA	chr11:5236346-5236366	1534
CR001167	HPFH	+	GCCAUUUCUAUUAUCAGACU	chr11:5236383-5236403	1535
CR001168	HPFH	-	UCCAAGUCUGAUAAUAGAAA	chr11:5236387-5236407	1536
CR001169	HPFH	+	UUAUCAGACUUGGACCAUGA	chr11:5236393-5236413	1537
CR001170	HPFH	-	CACGACUGACAUCACCGUCA	chr11:5236410-5236430	1538
CR001171	HPFH	+	UCAGUCGUGAACACAAGAAU	chr11:5236421-5236441	1539
CR001172	HPFH	+	CAGUCGUGAACACAAGAAUA	chr11:5236422-5236442	1540

CR001173	HPFH	+	GGCCACAUUUGUGAGUUUAG	chr11:5236443-5236463	1541
CR001174	HPFH	-	UACCACUAAACUCACAAAUG	chr11:5236448-5236468	1542
CR001175	HPFH	+	UAAAAUCAGAAAUACAGUCU	chr11:5236471-5236491	1543
CR001176	HPFH	+	AAAAGAUGUACUUAGAUAUG	chr11:5236528-5236548	1544
CR001177	HPFH	+	UGUACUUAGAUAUGUGGAUC	chr11:5236534-5236554	1545
CR001178	HPFH	+	AGCUCAGAAAGAAUACAACC	chr11:5236557-5236577	1546
CR001179	HPFH	+	ACCAGGUCAAGAAUACAGAA	chr11:5236574-5236594	1547
CR001180	HPFH	-	UCCAUUCUGUAUUCUUGACC	chr11:5236578-5236598	1548
CR001181	HPFH	-	CUGUCAUUUUUAACAGGUAG	chr11:5236646-5236666	1549
CR001182	HPFH	-	CAUCAUCUGUCAUUUUUAAC	chr11:5236652-5236672	1550
CR001183	HPFH	-	AAACACAUUCUAAGAUUUUA	chr11:5236691-5236711	1551
CR001184	HPFH	+	AAUCUUAGAAUGUGUUUGUG	chr11:5236694-5236714	1552
CR001185	HPFH	+	AUCUUAGAAUGUGUUUGUGA	chr11:5236695-5236715	1553
CR001186	HPFH	+	UUAGAAUGUGUUUGUGAGGG	chr11:5236698-5236718	1554
CR001187	HPFH	-	CAAUUUUCUUAUAUAUGAAU	chr11:5236734-5236754	1555
CR001188	HPFH	+	UUGAUUCUAAAAAAAAAUGUU	Chr11:5236746-5236768	1556
CR001189	HPFH	+	AAAUGUUAGGUAAAUUCUUA	chr11:5236764-5236784	1557
CR001190	HPFH	+	GGUAAAUUCUUAAGGCCAUG	chr11:5236772-5236792	1558
CR001191	HPFH	-	AGAUCAAAUAACAGUCCUCA	chr11:5236790-5236810	1559
CR001192	HPFH	+	GUCUGUUAAUUCCAAAGACU	chr11:5236812-5236832	1560
CR001193	HPFH	-	AAAGUGAAAAGCCAAGUCUU	chr11:5236826-5236846	1561
CR001194	HPFH	+	CCUGAAAUGAUUUUACACAU	chr11:5236858-5236878	1562
CR001195	HPFH	-	CCAAUGUGUAAAAUCAUUUC	chr11:5236861-5236881	1563
CR001196	HPFH	+	CUGAAAUGAUUUUACACAUU	chr11:5236859-5236879	1564
CR001197	HPFH	+	AUUUUACACAUUGGGAGAUC	chr11:5236867-5236887	1565
CR001198	HPFH	+	GGUUACAUGUUUAUUCUAUA	chr11:5236888-5236908	1566
CR001199	HPFH	+	UCUAUAUGGAUUGCAUUGAG	chr11:5236902-5236922	1567
CR001200	HPFH	+	AGGAUUUGUAUAACAGAAUA	chr11:5236922-5236942	1568
CR001201	HPFH	+	UUUUCUUUUCUCUUCUGAGA	Chr11:5236945-5236967	1569
CR001202	HPFH	-	GCACUCUAGCUUGGGCAAUA	chr11:5236984-5237004	1570

CR001203	HPFH	-	UGCACUCUAGCUUGGGCAAU	chr11:5236985-5237005	1571
CR001204	HPFH	-	UGCACCAUUGCACUCUAGCU	Chr11:5236985-5237007	1572
CR001205	HPFH	-	GCUAUUCAGGUGGCUGAGGC	chr11:5237061-5237081	1573
CR001206	HPFH	+	ACCUGAAUAGCUGGGACUGC	Chr11:5237065-5237087	1574
CR001207	HPFH	+	GCAGGCAUGCACCACACGCC	Chr11:5237083-5237105	1575
CR001208	HPFH	-	UACAAAAUCAGCCGGGCGUG	chr11:5237102-5237122	1576
CR001209	HPFH	-	GGCUUGUAAACCCAGCACUU	chr11:5237208-5237228	1577
CR001210	HPFH	-	CUGGCUGGAUGCGGUGGCUC	chr11:5237229-5237249	1578
CR001211	HPFH	+	CUGAGCCACCGCAUCCAGCC	chr11:5237227-5237247	1579
CR001212	HPFH	-	CUUAUCCUGGCUGGAUGCGG	chr11:5237235-5237255	1580
CR001213	HPFH	+	CACCGCAUCCAGCCAGGAUA	chr11:5237233-5237253	1581
CR001214	HPFH	-	GACCUUAUCCUGGCUGGAUG	chr11:5237238-5237258	1582
CR001215	HPFH	-	CUUUUAGACCUUAUCCUGGC	chr11:5237244-5237264	1583
CR001216	HPFH	+	GCCAGGAUAAGGUCUAAAAG	chr11:5237244-5237264	1584
CR001217	HPFH	-	UCCACUUUUAGACCUUAUCC	chr11:5237248-5237268	1585
CR001218	HPFH	+	AAUAGCAUCUACUCUUGUUC	chr11:5237271-5237291	1586
CR001219	HPFH	+	CUCUUGUUCAGGAAACAAUG	chr11:5237282-5237302	1587
CR001220	HPFH	+	GGAAACAAUGAGGACCUGAC	chr11:5237292-5237312	1588
CR001221	HPFH	+	GAAACAAUGAGGACCUGACU	chr11:5237293-5237313	1589
CR001222	HPFH	+	ACCUGACUGGGCAGUAAGAG	chr11:5237305-5237325	1590
CR001223	HPFH	-	ACCACUCUUACUGCCCAGUC	chr11:5237309-5237329	1591
CR001224	HPFH	+	AAGAGUGGUGAUUAAUAGAU	chr11:5237320-5237340	1592
CR001225	HPFH	+	AGAGUGGUGAUUAAUAGAUA	chr11:5237321-5237341	1593
CR001226	HPFH	+	AGAAUCGAACUGUUGAUUAG	chr11:5237356-5237376	1594
CR001227	HPFH	+	UCGAACUGUUGAUUAGAGGU	chr11:5237360-5237380	1595
CR003027	HPFH	+	CGAACUGUUGAUUAGAGGUA	chr11:5237361-5237381	1692
CR003028	HPFH	+	AUGAUUUUAAUCUGUGACCU	chr11:5237386-5237406	1693
CR003029	HPFH	+	UAAUCUGUGACCUUGGUGAA	chr11:5237393-5237413	1694
CR003030	HPFH	+	AAUCUGUGACCUUGGUGAAU	chr11:5237394-5237414	1695
CR003031	HPFH	-	AGCUACUUGCCCAUUCACCA	chr11:5237406-5237426	1696

CR003032	HPFH	+	UAGCUAUCUAAUGACUAAAA	chr11:5237421-5237441	1697
CR003033	HPFH	+	AUGACUAAAAUGGAAAACAC	chr11:5237431-5237451	1698
CR003034	HPFH	+	AAAUACCCAUGCUGAGUCUG	chr11:5237482-5237502	1699
CR003035	HPFH	-	AGGCACCUCAGACUCAGCAU	chr11:5237490-5237510	1700
CR003036	HPFH	-	UAGGCACCUCAGACUCAGCA	chr11:5237491-5237511	1701
CR003037	HPFH	+	GCUGAGUCUGAGGUGCCUAU	chr11:5237492-5237512	1702
CR003038	HPFH	-	UAUUUAUAUAGAUGUCCUAU	chr11:5237510-5237530	1703
CR003039	HPFH	-	CAUAUAUCAAACAAUGUACU	chr11:5237535-5237555	1704
CR003040	HPFH	+	CCAGUACAUUGUUUGAUAUA	chr11:5237533-5237553	1705
CR003041	HPFH	-	CCAUAUAUCAAACAAUGUAC	chr11:5237536-5237556	1706
CR003042	HPFH	+	CAGUACAUUGUUUGAUAUAU	chr11:5237534-5237554	1707
CR003043	HPFH	+	CAUUGUUUGAUAUAUGGGUU	chr11:5237539-5237559	1708
CR003044	HPFH	+	GAUAUAUGGGUUUGGCACUG	chr11:5237547-5237567	1709
CR003045	HPFH	+	UAUGGGUUUGGCACUGAGGU	chr11:5237551-5237571	1710
CR003046	HPFH	+	GGGUUUGGCACUGAGGUUGG	chr11:5237554-5237574	1711
CR003047	HPFH	+	GCACUGAGGUUGGAGGUCAG	chr11:5237561-5237581	1712
CR003048	HPFH	+	CAGAGGUUAGAAAUCAGAGU	chr11:5237578-5237598	1713
CR003049	HPFH	+	AGAGGUUAGAAAUCAGAGUU	chr11:5237579-5237599	1714
CR003050	HPFH	+	UAGAAAUCAGAGUUGGGAAU	chr11:5237585-5237605	1715
CR003051	HPFH	+	AGAAAUCAGAGUUGGGAAUU	chr11:5237586-5237606	1716
CR003052	HPFH	+	GUUGGGAAUUGGGAUUAUAC	chr11:5237596-5237616	1717
CR003053	HPFH	-	CUUUGUAUUCAUCACACUCU	chr11:5237654-5237674	1718
CR003054	HPFH	+	AUGAAUACAAAGUUAAAUGA	chr11:5237662-5237682	1719
CR003055	HPFH	-	UAAAUGUUGGUGUUCAUUAA	chr11:5237689-5237709	1720
CR003056	HPFH	-	UGAGAUUUCACAUUAAAUGU	chr11:5237702-5237722	1721
CR003057	HPFH	+	ACAUUUAAUGUGAAAUCUCA	chr11:5237702-5237722	1722
CR003058	HPFH	-	UAAAAUCAUCGGGGAUUUUG	chr11:5237749-5237769	1723
CR003059	HPFH	-	CUAAAAUCAUCGGGGAUUUU	chr11:5237750-5237770	1724
CR003060	HPFH	-	UCUAAAAUCAUCGGGGAUUU	chr11:5237751-5237771	1725
CR003061	HPFH	-	ACUGAGUUCUAAAAUCAUCG	chr11:5237758-5237778	1726

CR003062	HPFH	-	UACUGAGUUCUAAAAUCAUC	chr11:5237759-5237779	1727
CR003063	HPFH	-	AUACUGAGUUCUAAAAUCAU	chr11:5237760-5237780	1728
CR003064	HPFH	+	UAAUUAGUGUAAUGCCAAUG	chr11:5237786-5237806	1729
CR003065	HPFH	+	AAUUAGUGUAAUGCCAAUGU	chr11:5237787-5237807	1730
CR003066	HPFH	+	AAUGCCAAUGUGGGUUAGAA	chr11:5237796-5237816	1731
CR003067	HPFH	-	ACUUCCAUUCUAACCCACAU	chr11:5237803-5237823	1732
CR003068	HPFH	+	AAUGGAAGUCAACUUGCUGU	chr11:5237814-5237834	1733
CR003069	HPFH	+	CUUGCUGUUGGUUUCAGAGC	chr11:5237826-5237846	1734
CR003070	HPFH	+	CUGUUGGUUUCAGAGCAGGU	chr11:5237830-5237850	1735
CR003071	HPFH	+	UUCAGAGCAGGUAGGAGAUA	chr11:5237838-5237858	1736
CR003072	HPFH	+	AGUGAAAAGCUGAAACAAAA	chr11:5237877-5237897	1737
CR003073	HPFH	+	AAGCUGAAACAAAAAGGAAA	chr11:5237883-5237903	1738
CR003074	HPFH	+	UGAAACAAAAAGGAAAAGGU	chr11:5237887-5237907	1739
CR003075	HPFH	+	GAAACAAAAAGGAAAAGGUA	chr11:5237888-5237908	1740
CR003076	HPFH	+	GGAAAAGGUAGGGUGAAAGA	chr11:5237898-5237918	1741
CR003077	HPFH	+	GAAAAGGUAGGGUGAAAGAU	chr11:5237899-5237919	1742
CR003078	HPFH	+	AAAGAUGGGAAAUGUAUGUA	chr11:5237913-5237933	1743
CR003079	HPFH	+	GAUGGGAAAUGUAUGUAAGG	chr11:5237916-5237936	1744
CR003080	HPFH	+	UGUAAGGAGGAUGAGCCACA	chr11:5237929-5237949	1745
CR003081	HPFH	+	GGAGGAUGAGCCACAUGGUA	chr11:5237934-5237954	1746
CR003082	HPFH	+	GAGGAUGAGCCACAUGGUAU	chr11:5237935-5237955	1747
CR003083	HPFH	+	GAUGAGCCACAUGGUAUGGG	chr11:5237938-5237958	1748
CR003084	HPFH	-	AGUAUACCUCCCAUACCAUG	chr11:5237947-5237967	1749
CR003085	HPFH	+	AUGGUAUGGGAGGUAUACUA	chr11:5237948-5237968	1750
CR003086	HPFH	+	GGAGGUAUACUAAGGACUCU	chr11:5237956-5237976	1751
CR003087	HPFH	+	GAGGUAUACUAAGGACUCUA	chr11:5237957-5237977	1752
CR003088	HPFH	+	ACUCUAGGGUCAGAGAAAUA	chr11:5237971-5237991	1753
CR003089	HPFH	+	CUCUAGGGUCAGAGAAAUAU	chr11:5237972-5237992	1754
CR003090	HPFH	-	AAGAAUGUGAAUUUUGUAGA	chr11:5238004-5238024	1755
CR003091	HPFH	+	UUCUACAAAAUUCACAUUCU	chr11:5238003-5238023	1756

CR003092	HPFH	+	ACAAAAUUCACAUUCUUGGC	chr11:5238007-5238027	1757
CR003093	HPFH	+	CAAAAUUCACAUUCUUGGCU	chr11:5238008-5238028	1758
CR003094	HPFH	+	UUCACAUUCUUGGCUGGGUG	chr11:5238013-5238033	1759
CR003095	HPFH	+	AGGGUGGAUCACCUGAUGUU	chr11:5238071-5238093	1760
CR003096	HPFH	-	GAUCUCGAACUCCUAACAUC	chr11:5238090-5238110	1761

In some aspects of the invention, it is preferred that the gRNA molecule to an HPFH region comprise, e.g., consist of, a targeting domain of a gRNA capable of producing at least about a 20% increase in F cells relative to control, e.g., in an assay described in Example 4. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 113. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 99. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 112. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 98. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1580. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 106. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1589. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1503. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 160. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1537. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 159. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 101. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 162. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 104. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 138. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1536. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1539. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 1585.

In some aspects of the invention, e.g., as indicated herein, it may be beneficial to include gRNA molecules targeting more than one, e.g., two, target sites (e.g., a first gRNA molecule and a second gRNA molecule). In some embodiments, the two target sites are both located in an HPFH region. In such aspects, any combination of more than one, e.g., two, gRNA molecules (e.g., a first gRNA

molecule and a second gRNA molecule) comprising targeting domains listed in Table 6 may be used. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 100 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 165 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 113 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 99 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 112 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 98 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and

the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1580 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 106 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 104, respectively. In an aspect,

the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1503 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID

NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1589 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 160

and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1537 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g.,

consisting of, SEQ ID NO: 159 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 159 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains

comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 101 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains

comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 162 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains

comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 104 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include

targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 138 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the

second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1539, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1536 and SEQ ID NO: 1585, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1539 and SEQ ID NO: 1585, respectively. In

an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 165, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 113, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 99, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 112, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 98, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1580, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 106, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1503, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1589, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 160, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1537, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 159, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 101, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 162, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 104, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 138, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1536, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1585 and SEQ ID NO: 1539, respectively.

Table 7: Preferred Guide RNA Targeting Domains directed to the +58 Enhancer Region of the BCL11a Gene (i.e., to a BCL11a Enhancer)

Id.	Exon/ Feature	Strand	Targeting domain	Locations	SEQ ID NO:
CR00242	58	+	UAUGCAAUUUUUGCCAAGAU	Chr2:60494419-60494441	182
CR00243	58	+	UUUUGCCAAGAUGGGAGUAU	Chr2:60494427-60494449	183
CR00244	58	+	UUUGCCAAGAUGGGAGUAUG	Chr2:60494428-60494450	184
CR00245	58	+	UCAGUGAGAUGAGAUAUCAA	Chr2:60494477-60494499	185
CR00246	58	+	CAGUGAGAUGAGAUAUCAAA	Chr2:60494478-60494500	186
CR00247	58	+	CCAUCUCCCUAAUCUCCAAU	Chr2:60494518-60494540	187
CR00248	58	-	CCAAUUGGAGAUUAGGGAGA	Chr2:60494518-60494540	188
CR00249	58	-	GCUUUGCCAAUUGGAGAUUA	Chr2:60494524-60494546	189
CR00250	58	-	GGCUUUGCCAAUUGGAGAUU	Chr2:60494525-60494547	190
CR00251	58	+	AAUUGGCAAAGCCAGACUUG	Chr2:60494535-60494557	191
CR00252	58	-	AGUCUGUAUUGCCCCAAGUC	Chr2:60494546-60494568	192
CR00253	58	+	AGACUUGGGGCAAUACAGAC	Chr2:60494548-60494570	193
CR00255	58	-	ACAUUUGGUGAUAAAUCAUU	Chr2:60494602-60494624	194
CR00256	58	+	CCAAAUGUUCUUUCUUCAGC	Chr2:60494617-60494639	195
CR00257	58	+	AUAAUAGUAUAUGCUUCAUA	Chr2:60494676-60494698	196
CR00258	58	-	CGGAGCACUUACUCUGCUCU	Chr2:60494737-60494759	197
CR00259	58	-	AGCAUUUUAGUUCACAAGCU	Chr2:60494757-60494779	198
CR00260	58	-	UGUAACUAAUAAAUACCAGG	Chr2:60494781-60494803	199
CR00261	58	-	AGGUGUAACUAAUAAAUACC	Chr2:60494784-60494806	200
CR00264	58	-	UCUGACCCAAACUAGGAAUU	Chr2:60494836-60494858	201
CR00266	58	+	AAGGAAAAGAAUAUGACGUC	Chr2:60494883-60494905	202
CR00267	58	+	AGGAAAAGAAUAUGACGUCA	Chr2:60494884-60494906	203
CR00268	58	+	GGAAAAGAAUAUGACGUCAG	Chr2:60494885-60494907	204
CR00269	58	+	GAAAAGAAUAUGACGUCAGG	Chr2:60494886-60494908	205
CR00270	58	+	UCAGGGGGAGGCAAGUCAGU	Chr2:60494901-60494923	206
CR00271	58	+	CAGGGGGAGGCAAGUCAGUU	Chr2:60494902-60494924	207
CR00272	58		AUCACAUAUAGGCACCUAUC	Chr2:60494939-60494961	208
CR00273	58		CAGGUACCAGCUACUGUGUU	Chr2:60494939-60494961	209
CR00274	58		ACUAUCCACGGAUAUACACU	Chr2:60494939-60494961	210
CR00275	58	+	CACAGUAGCUGGUACCUGAU	Chr2:60494939-60494961	211
CR00276	58	-	AUCACAUAUAGGCACCUAUC	Chr2:60494953-60494975	212
CR00277	58	+	UGAUAGGUGCCUAUAUGUGA	Chr2:60494955-60494977	213
CR00278	58	+	AGGUGCCUAUAUGUGAUGGA	Chr2:60494959-60494981	214

CR00279	58	+	GGUGCCUAUAUGUGAUGGAU	Chr2:60494960-60494982	215
CR00280	58	-	UCCACCCAUCCAUCACAUAU	Chr2:60494964-60494986	216
CR00281	58	+	ACAGCCCGACAGAUGAAAAA	Chr2:60494986-60495008	217
CR00282	58	+	AUGAAAAAUGGACAAUUAUG	Chr2:60494998-60495020	218
CR00283	58	+	AAAAAUGGACAAUUAUGAGG	Chr2:60495001-60495023	219
CR00284	58	+	AAAAUGGACAAUUAUGAGGA	Chr2:60495002-60495024	220
CR00285	58	+	AAAUGGACAAUUAUGAGGAG	Chr2:60495003-60495025	221
CR00286	58	+	GAGGAGGGGAGAGUGCAGAC	Chr2:60495017-60495039	222
CR00287	58	+	AGGAGGGGAGAGUGCAGACA	Chr2:60495018-60495040	223
CR00288	58	+	CUUCACCUCCUUUACAAUUU	Chr2:60495045-60495067	224
CR00289	58	+	UUCACCUCCUUUACAAUUUU	Chr2:60495046-60495068	225
CR00290	58	-	GACUCCCAAAAUUGUAAAGG	Chr2:60495050-60495072	226
CR00291	58	-	GUGGACUCCCAAAAUUGUAA	Chr2:60495053-60495075	227
CR00292	58	+	UUUUGGGAGUCCACACGGCA	Chr2:60495062-60495084	228
CR00293	58	-	AAUUUGUAUGCCAUGCCGUG	Chr2:60495072-60495094	229
CR00294	58	+	CCAAGAGAGCCUUCCGAAAG	Chr2:60495135-60495157	230
CR00295	58	-	CCAGGGGGGCCUCUUUCGGA	Chr2:60495144-60495166	231
CR00296	58	+	CCUUCCGAAAGAGGCCCCCC	Chr2:60495144-60495166	232
CR00297	58	+	CUUCCGAAAGAGGCCCCCCU	Chr2:60495145-60495167	233
CR00298	58	+	AAGAGGCCCCCCUGGGCAAA	Chr2:60495152-60495174	234
CR00299	58	-	CGGUGGCCGUUUGCCCAGGG	Chr2:60495158-60495180	235
CR00300	58	-	UCGGUGGCCGUUUGCCCAGG	Chr2:60495159-60495181	236
CR00301	58	-	AUCGGUGGCCGUUUGCCCAG	Chr2:60495160-60495182	237
CR00302	58	-	CAUCGGUGGCCGUUUGCCCA	Chr2:60495161-60495183	238
CR00303	58	+	CCUGGGCAAACGGCCACCGA	Chr2:60495162-60495184	239
CR00304	58	-	CCAUCGGUGGCCGUUUGCCC	Chr2:60495162-60495184	240
CR00305	58	+	GCAAACGGCCACCGAUGGAG	Chr2:60495167-60495189	241
CR00306	58	-	CUGGCAGACCUCUCCAUCGG	Chr2:60495175-60495197	242
CR00307	58	-	GGACUGGCAGACCUCUCCAU	Chr2:60495178-60495200	243
CR00308	58	-	UCUGAUUAGGGUGGGGGCGU	Chr2:60495213-60495235	244
CR00309	58	+	CACGCCCCCACCCUAAUCAG	Chr2:60495215-60495237	245
CR00310	58	-	UUGGCCUCUGAUUAGGGUGG	Chr2:60495219-60495241	246
CR00311	58	-	UUUGGCCUCUGAUUAGGGUG	Chr2:60495220-60495242	247
CR00312	58	-	GUUUGGCCUCUGAUUAGGGU	Chr2:60495221-60495243	248
CR00313	58	-	GGUUUGGCCUCUGAUUAGGG	Chr2:60495222-60495244	249

CR00314	58	-	AAGGGUUUGGCCUCUGAUUA	Chr2:60495225-60495247	250
CR00315	58	-	GAAGGGUUUGGCCUCUGAUU	Chr2:60495226-60495248	251
CR00316	58	-	UUGCUUUUAUCACAGGCUCC	Chr2:60495248-60495270	252
CR00317	58	-	CUAACAGUUGCUUUUAUCAC	Chr2:60495255-60495277	253
CR00318	58	+	CUUCAAAGUUGUAUUGACCC	Chr2:60495293-60495315	254
CR00319	58	-	ACUCUUAGACAUAACACACC	Chr2:60495311-60495333	255
CR00320	58	+	UAGAUGCCAUAUCUCUUUUC	Chr2:60495333-60495355	256
CR00321	58	-	CAUAGGCCAGAAAAGAGAUA	Chr2:60495339-60495361	257
CR00322	58	+	GGCCUAUGUUAUUACCUGUA	Chr2:60495354-60495376	258
CR00323	58	-	GUCCAUACAGGUAAUAACAU	Chr2:60495356-60495378	259
CR00324	58	+	UACCUGUAUGGACUUUGCAC	Chr2:60495366-60495388	260
CR00325	58	-	UUCCAGUGCAAAGUCCAUAC	Chr2:60495368-60495390	261
CR00326	58	+	UGCUCUUACUUAUGCACACC	Chr2:60495400-60495422	262
CR00327	58	+	GCUCUUACUUAUGCACACCU	Chr2:60495401-60495423	263
CR00328	58	+	CUCUUACUUAUGCACACCUG	Chr2:60495402-60495424	264
CR00329	58	-	CAGGGCUGGCUCUAUGCCCC	Chr2:60495418-60495440	265
CR00330	58	-	GCUGAAAAGCGAUACAGGGC	Chr2:60495432-60495454	266
CR00331	58	-	GAUGGCUGAAAAGCGAUACA	Chr2:60495436-60495458	267
CR00332	58	-	AGAUGGCUGAAAAGCGAUAC	Chr2:60495437-60495459	268
CR00333	58	-	GGGAGUUAUCUGUAGUGAGA	Chr2:60495454-60495476	269
CR00334	58	-	AAGGCAGCUAGACAGGACUU	Chr2:60495474-60495496	270
CR00335	58	-	GAAGGCAGCUAGACAGGACU	Chr2:60495475-60495497	271
CR00336	58	-	GAUAAGGAAGGCAGCUAGAC	Chr2:60495481-60495503	272
CR00337	58	+	CUAGCUGCCUUCCUUAUCAC	Chr2:60495486-60495508	273
CR00338	58	-	UGGGUGCUAUUCCUGUGAUA	Chr2:60495497-60495519	274
CR00339	58	+	UAUCACAGGAAUAGCACCCA	Chr2:60495500-60495522	275
CR00340	58	-	CUGAGGUACUGAUGGACCUU	Chr2:60495516-60495538	276
CR00341	58	-	UCUGAGGUACUGAUGGACCU	Chr2:60495517-60495539	277
CR001124	58	-	UUAGGGUGGGGGCGUGGGUG	Chr2:60495214-60495236	334
CR001125	58	-	UUUUAUCACAGGCUCCAGGA	Chr2:60495215-60495237	335
CR001126	58	-	UUUAUCACAGGCUCCAGGAA	Chr2:60495216-60495238	336
CR001127	58	-	CACAGGCUCCAGGAAGGGUU	Chr2:60495220-60495242	337
CR001128	58	+	AUCAGAGGCCAAACCCUUCC	Chr2:60495236-60495258	338
CR001129	58	-	CUCUGAUUAGGGUGGGGGCG	Chr2:60495244-60495266	339
CR001130	58	-	GAUUAGGGUGGGGGCGUGGG	Chr2:60495249-60495271	340

CR001131	58	-	AUUAGGGUGGGGGCGUGGGU	Chr2:60495250-60495272	341

In some aspects of the invention, it is preferred that the gRNA molecule to the +58 Enhancer region of BCL11a comprise a targeting domain of a gRNA listed in Figure 11. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 341. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 246. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 248. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 247. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 245. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 249. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 244. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 199. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 251. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 250. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 334. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 185. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 186. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 336. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 337.

In some aspects of the invention, e.g., as indicated herein, it may be beneficial to include gRNA moleucles targeting more than one, e.g., two, target sites (e.g., a first gRNA molecule and a second gRNA molecule). In some embodiments, the two target sites are both located in the +58 BCL11a enhancer region. In such aspects, any combination of more than one, e.g., two, gRNA molecules (e.g., a first gRNA molecule and a second gRNA molecule) comprising targeting domains listed in Table 7 may be used. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the

second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 341 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 246 and SEQ ID NO: 337, respectively. In an aspect,

the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 186, respectively. In an

aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 248 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 334,

respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 247 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID

NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 245 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249

and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 249 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of,

SEQ ID NO: 244 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 244 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g.,

consisting of, SEQ ID NO: 199 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 199 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains

comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 251 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting

domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 250 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include

targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 334 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 185 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the

second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 186 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 336 and SEQ ID NO: 337, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 251, respectively. In an aspect, the

first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 336, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 246, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 248, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 247, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 245, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 249, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 244, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 199, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 251, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 250, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 334, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 185, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 186, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 337 and SEQ ID NO: 336, respectively.

In embodiments, the aspects of the invention relate to or incorporate a gRNA molecule that is complementary to a target sequence within the +58 enhancer region of the BCL11a gene which is

disposed 3' to the GATA-1 binding site, e.g., 3' to the GATA1 binding site and TAL-1 binding site. In embodiments, the CRISPR system comprising a gRNA molecule described herein induces one or more indels at or near the target site. In embodiments, the indel does not comprise a nucleotide of a GATA-1 binding site and/or does not comprise a nucleotide of a TAL-1 binding site. In embodiments, the CRISPR system induces one or more frameshift indels at or near the target site, e.g., at an overall frameshift indel rate of at least about 10%, at least about 20%, at least about 30%, at least about 40%, or at least about 50%, e.g., as measured by NGS. In embodiments, the overall indel rate is at least about 70%, at least about 80%, at least about 90%, or at least about 95%, e.g., as measured by NGS.

Table 8: Preferred Guide RNA Targeting Domains directed to the +62 Enhancer Region of the BCL11a Gene (i.e., to a BCL11a Enhancer)

Id.	**Exon/ Feature**	**Strand**	**Targeting domain**	**Locations**	**SEQ ID NO:**
CR00171	62	-	AGCUCUGGAAUGAUGGCUUA	Chr2:60490354-60490376	278
CR00172	62	-	AUUGUGGAGCUCUGGAAUGA	Chr2:60490361-60490383	279
CR00173	62	-	CUGGAAUAGAAAAUUGGAGU	Chr2:60490384-60490406	280
CR00174	62	-	UGGGUACGGGGAACUAAGAC	Chr2:60490403-60490425	281
CR00175	62	+	UUAGUUCCCCGUACCCAUCA	Chr2:60490409-60490431	282
CR00176	62	-	UAUUUUCCUUGAUGGGUACG	Chr2:60490415-60490437	283
CR00177	62	-	AUAUUUUCCUUGAUGGGUAC	Chr2:60490416-60490438	284
CR00178	62	-	AAUAUUUUCCUUGAUGGGUA	Chr2:60490417-60490439	285
CR00179	62	-	GGAAGGAAAUGAGAACGGAA	Chr2:60490456-60490478	286
CR00180	62	-	AGGAAGGAAAUGAGAACGGA	Chr2:60490457-60490479	287
CR00181	62	+	AUACUUCAAGGCCUCAAUGA	Chr2:60490520-60490542	288
CR00182	62	-	GACUAGGUAGACCUUCAUUG	Chr2:60490531-60490553	289
CR00183	62	-	UUCUUCUUGCUAAGGUGACU	Chr2:60490547-60490569	290
CR00184	62	-	GAGAUUGAUUCUUCUUGCUA	Chr2:60490555-60490577	291
CR00185	62	-	GUGUUCUAUGAGGUUGGAGA	Chr2:60490580-60490602	292
CR00186	62	-	GGAUGAGUGUUCUAUGAGGU	Chr2:60490586-60490608	293
CR00187	62	-	CAUGGGAUGAGUGUUCUAUG	Chr2:60490590-60490612	294
CR00188	62	-	UGAGUCAGGGAGUGGUGCAU	Chr2:60490607-60490629	295
CR00189	62	-	AUGAGUCAGGGAGUGGUGCA	Chr2:60490608-60490630	296
CR00190	62	+	CCACUCCCUGACUCAUAUCU	Chr2:60490615-60490637	297
CR00191	62	-	UAAGGCCUAGAUAUGAGUCA	Chr2:60490620-60490642	298
CR00192	62	-	GUAAGGCCUAGAUAUGAGUC	Chr2:60490621-60490643	299

CR00193	62	+	AUAUCUAGGCCUUACAUUGC	Chr2:60490629-60490651	300
CR00194	62	-	AAAUUAAUUAGAGGCAUAGA	Chr2:60490656-60490678	301
CR00195	62	-	GAAAUUAAUUAGAGGCAUAG	Chr2:60490657-60490679	302
CR00196	62	-	GAACACAUGAAAUUAAUUAG	Chr2:60490665-60490687	303
CR00197	62	+	CCAAUGAGUUUCUUCAAUAC	Chr2:60490688-60490710	304
CR00198	62	-	CAAAUAUAAUAGAAGCAAGU	Chr2:60490721-60490743	305
CR00199	62	-	AAAUAACUUCCCUUUUAGGA	Chr2:60490821-60490843	306
CR00200	62	-	GGAAAAAUAACUUCCCUUUU	Chr2:60490825-60490847	307
CR00201	62	-	UUUUGAACAGAAAUGAUAUU	Chr2:60490846-60490868	308
CR00202	62	-	AGUUCAAGUAGAUAUCAGAA	Chr2:60490905-60490927	309
CR00203	62	-	AAGUUCAAGUAGAUAUCAGA	Chr2:60490906-60490928	310
CR00204	62	-	GGGUGGCUGUUUAAAGAGGG	Chr2:60490994-60491016	311
CR00205	62	-	GUGGGGUGGCUGUUUAAAGA	Chr2:60490997-60491019	312
CR00206	62	-	UGUGGGGUGGCUGUUUAAAG	Chr2:60490998-60491020	313
CR00207	62	+	UGCCAACCAGACUGUGCGCC	Chr2:60491051-60491073	314
CR00208	62	-	AACCUGGCGCACAGUCUGGU	Chr2:60491053-60491075	315
CR00209	62	+	AACCAGACUGUGCGCCAGGU	Chr2:60491055-60491077	316
CR00210	62	-	UACCAACCUGGCGCACAGUC	Chr2:60491057-60491079	317
CR00211	62	-	UCUGUCAGACUUUACCAACC	Chr2:60491069-60491091	318
CR00212	62	+	AUAUGUGAAGCCCAACUACG	Chr2:60491118-60491140	319
CR00213	62	-	AGUUGCACAACCACGUAGUU	Chr2:60491128-60491150	320
CR00214	62	-	GAGUUGCACAACCACGUAGU	Chr2:60491129-60491151	321
CR00215	62	+	CUAUAGCUGACUUUCAACCA	Chr2:60491151-60491173	322
CR00216	62	-	AACUUCUUUGCAGAUGACCA	Chr2:60491168-60491190	323
CR00217	62	-	UUGCAUUGAGGAUGCGCAGG	Chr2:60491199-60491221	324
CR00218	62	-	UUUUUGCAUUGAGGAUGCGC	Chr2:60491202-60491224	325
CR00221	62	+	GACCUCAUUUUGAUGCCAGA	Chr2:60491281-60491303	326
CR00222	62	-	UGCCCUCUGGCAUCAAAAUG	Chr2:60491283-60491305	327
CR00223	62	+	AUGCCAGAGGGCAGCAAACA	Chr2:60491293-60491315	328
CR00224	62	-	CUGUACUUAAUAGCUGAAGG	Chr2:60491326-60491348	329
CR00225	62	-	ACUGUACUUAAUAGCUGAAG	Chr2:60491327-60491349	330
CR00227	62	+	CAGCUAUUAAGUACAGUAAA	Chr2:60491333-60491355	331
CR00228	62	-	GAGCAUUUCAAAUGAUAGUU	Chr2:60491360-60491382	332
CR00229	62	+	CAUUUGAAAUGCUCCCGGCC	Chr2:60491369-60491391	333

In some aspects of the invention, it is preferred that the gRNA molecule to the +62 Enhancer region of BCL11a comprise a targeting domain of a gRNA listed in Figure 12. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 318. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 312. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 313. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 294. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 310. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 319. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 298. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 322. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 311. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 315. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 290. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 317. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 309. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 289. In an aspect, the gRNA molecule includes a targeting domain comprising, e.g., consisting of, SEQ ID NO: 281.

In some aspects of the invention, e.g., as indicated herein, it may be beneficial to include gRNA moleucles targeting more than one, e.g., two, target sites (e.g., a first gRNA molecule and a second gRNA molecule). In some embodiments, the two target sites are both located in the +62 BCL11a enhancer region. In such aspects, any combination of more than one, e.g., two, gRNA molecules (e.g., a first gRNA molecule and a second gRNA molecule) comprising targeting domains listed in Table 8 may be used. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 318 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the

second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 312 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 313 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 294 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 310 and SEQ ID NO: 281, respectively. In an aspect, the

first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 319 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 311, respectively. In an

aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 298 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 322 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 312,

respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 311 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID

NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 315 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 290 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317

and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 317 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of,

SEQ ID NO: 309 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 289, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 309 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 289 and SEQ ID NO: 281, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 312, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 313, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains

comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 294, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 310, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 319, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 298, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 322, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 311, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 315, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 290, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 317, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 309, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 281 and SEQ ID NO: 289, respectively.

Table 9. Preferred Guide RNA Targeting Domains directed to the +55 Enhancer Region of the BCL11a Gene (i.e., to a BCL11a Enhancer)

ID	**Species**	**Strand**	**Location**	**Targeting Domain Sequence**	**SEQ ID NO:**
CR002142	h	+	Chr2:60498031 -60498053	CCUGGCAGACCCUCAAGAGC	1596
CR002143	h	-	Chr2:60498031 -60498053	CCUGGCAGACCCUCAAGAGC	1597
CR002144	h	+	Chr2:60498032 -60498054	CUGGCAGACCCUCAAGAGCA	1598
CR002145	h	+	Chr2:60498033 -60498055	UGGCAGACCCUCAAGAGCAG	1599
CR002146	h	-	Chr2:60498040 -60498062	CCCUCAAGAGCAGGGGUCUU	1600
CR002147	h	-	Chr2:60498041 -60498063	CCUCAAGAGCAGGGGUCUUC	1601
CR001262	h	+	Chr1:55039155 -55039177	UAAGGCCAGUGGAAAGAAUU	1602
CR002148	h	+	Chr2:60498045 -60498067	AAGAGCAGGGGUCUUCUCUU	1603
CR002149	h	+	Chr2:60498046 -60498068	AGAGCAGGGGUCUUCUCUUU	1604

CR002150	h	+	Chr2:60498047 -60498069	GAGCAGGGGUCUUCUCUUUG	1605
CR002151	h	+	Chr2:60498048 -60498070	AGCAGGGGUCUUCUCUUUGG	1606
CR002152	h	+	Chr2:60498051 -60498073	AGGGGUCUUCUCUUUGGGGG	1607
CR002153	h	+	Chr2:60498068 -60498090	GGGAGGACAUCACUCUUAGC	1608
CR002154	h	+	Chr2:60498069 -60498091	GGAGGACAUCACUCUUAGCA	1609
CR001261	h	-	Chr1:55039269 -55039291	GCCAGACUCCAAGUUCUGCC	1610
CR002155	h	+	Chr2:60498073 -60498095	GACAUCACUCUUAGCAGGGC	1611
CR002156	h	+	Chr2:60498074 -60498096	ACAUCACUCUUAGCAGGGCU	1612
CR002157	h	+	Chr2:60498075 -60498097	CAUCACUCUUAGCAGGGCUG	1613
CR002158	h	+	Chr2:60498091 -60498113	GCUGGGGUGAGUCAAAAGUC	1614
CR002159	h	+	Chr2:60498092 -60498114	CUGGGGUGAGUCAAAAGUCU	1615
CR002160	h	+	Chr2:60498099 -60498121	GAGUCAAAAGUCUGGGAGAA	1616
CR002161	h	+	Chr2:60498102 -60498124	UCAAAAGUCUGGGAGAAUGG	1617
CR002162	h	+	Chr2:60498107 -60498129	AGUCUGGGAGAAUGGAGGUG	1618
CR002163	h	+	Chr2:60498110 -60498132	CUGGGAGAAUGGAGGUGUGG	1619
CR002164	h	+	Chr2:60498111 -60498133	UGGGAGAAUGGAGGUGUGGA	1620
CR002165	h	+	Chr2:60498112 -60498134	GGGAGAAUGGAGGUGUGGAG	1621
CR002166	h	+	Chr2:60498120 -60498142	GGAGGUGUGGAGGGGAUAAC	1622
CR001263	h	+	Chr1:55039180 -55039202	GGCAGCGAGGAGUCCACAGU	1623
CR002167	h	+	Chr2:60498121 -60498143	GAGGUGUGGAGGGGAUAACU	1624
CR002168	h	+	Chr2:60498137 -60498159	AACUGGGUCAGACCCCAAGC	1625
CR002169	h	+	Chr2:60498141 -60498163	GGGUCAGACCCCAAGCAGGA	1626
CR002170	h	+	Chr2:60498142 -60498164	GGUCAGACCCCAAGCAGGAA	1627
CR002171	h	-	Chr2:60498149 -60498171	CCCCAAGCAGGAAGGGCCUC	1628
CR002172	h	-	Chr2:60498150 -60498172	CCCAAGCAGGAAGGGCCUCU	1629
CR002173	h	-	Chr2:60498151 -60498173	CCAAGCAGGAAGGGCCUCUA	1630
CR002174	h	+	Chr2:60498157 -60498179	AGGAAGGGCCUCUAUGUAGA	1631

CR002175	h	+	Chr2:60498158 -60498180	GGAAGGGCCUCUAUGUAGAC	1632
CR002176	h	+	Chr2:60498165 -60498187	CCUCUAUGUAGACGGGUGUG	1633
CR002177	h	-	Chr2:60498165 -60498187	CCUCUAUGUAGACGGGUGUG	1634
CR002178	h	+	Chr2:60498175 -60498197	GACGGGUGUGUGGCUCCUUA	1635
CR002179	h	-	Chr2:60498190 -60498212	CCUUAAGGUGACCCAGCAGC	1636
CR002180	h	+	Chr2:60498192 -60498214	UUAAGGUGACCCAGCAGCCC	1637
CR002181	h	+	Chr2:60498193 -60498215	UAAGGUGACCCAGCAGCCCU	1638
CR002182	h	-	Chr2:60498201 -60498223	CCCAGCAGCCCUGGGCACAG	1639
CR002183	h	-	Chr2:60498202 -60498224	CCAGCAGCCCUGGGCACAGA	1640
CR001261	h	-	Chr1:55039269 -55039291	GCCAGACUCCAAGUUCUGCC	1641
CR002184	h	+	Chr2:60498204 -60498226	AGCAGCCCUGGGCACAGAAG	1642
CR002185	h	-	Chr2:60498209 -60498231	CCCUGGGCACAGAAGUGGUG	1643
CR002186	h	-	Chr2:60498210 -60498232	CCUGGGCACAGAAGUGGUGC	1644
CR002187	h	+	Chr2:60498211 -60498233	CUGGGCACAGAAGUGGUGCG	1645
CR002188	h	+	Chr2:60498240 -60498262	UGCCAACAGUGAUAACCAGC	1646
CR002189	h	+	Chr2:60498241 -60498263	GCCAACAGUGAUAACCAGCA	1647
CR001262	h	+	Chr1:55039155 -55039177	UAAGGCCAGUGGAAAGAAUU	1648
CR002190	h	-	Chr2:60498242 -60498264	CCAACAGUGAUAACCAGCAG	1649
CR002191	h	+	Chr2:60498255 -60498277	CCAGCAGGGCCUGUCAGAAG	1650
CR002192	h	-	Chr2:60498255 -60498277	CCAGCAGGGCCUGUCAGAAG	1651
CR002193	h	+	Chr2:60498261 -60498283	GGGCCUGUCAGAAGAGGCCC	1652
CR002194	h	-	Chr2:60498264 -60498286	CCUGUCAGAAGAGGCCCUGG	1653
CR002195	h	+	Chr2:60498271 -60498293	GAAGAGGCCCUGGACACUGA	1654
CR002196	h	+	Chr2:60498275 -60498297	AGGCCCUGGACACUGAAGGC	1655
CR002197	h	+	Chr2:60498276 -60498298	GGCCCUGGACACUGAAGGCU	1656
CR001264	h	-	Chr1:55039149 -55039171	UCUUUCCACUGGCCUUAACC	1657
CR002198	h	-	Chr2:60498278 -60498300	CCCUGGACACUGAAGGCUGG	1658

CR002199	h	-	Chr2:60498279 -60498301	CCUGGACACUGAAGGCUGGG	1659
CR002200	h	+	Chr2:60498287 -60498309	CUGAAGGCUGGGCACAGCCU	1660
CR002201	h	+	Chr2:60498288 -60498310	UGAAGGCUGGGCACAGCCUU	1661
CR002202	h	+	Chr2:60498289 -60498311	GAAGGCUGGGCACAGCCUUG	1662
CR002203	h	+	Chr2:60498301 -60498323	CAGCCUUGGGGACCGCUCAC	1663
CR002204	h	-	Chr2:60498304 -60498326	CCUUGGGGACCGCUCACAGG	1664
CR002205	h	-	Chr2:60498313 -60498335	CCGCUCACAGGACAUGCAGC	1665
CR002206	h	-	Chr2:60498341 -60498363	CCGACAACUCCCUACCGCGA	1666
CR002207	h	-	Chr2:60498350 -60498372	CCCUACCGCGACCCCUAUCA	1667
CR002208	h	-	Chr2:60498351 -60498373	CCUACCGCGACCCCUAUCAG	1668
CR002209	h	-	Chr2:60498355 -60498377	CCGCGACCCCUAUCAGUGCC	1669
CR002210	h	-	Chr2:60498361 -60498383	CCCCUAUCAGUGCCGACCAA	1670
CR002211	h	-	Chr2:60498362 -60498384	CCCUAUCAGUGCCGACCAAG	1671
CR002212	h	-	Chr2:60498363 -60498385	CCUAUCAGUGCCGACCAAGC	1672
CR002213	h	-	Chr2:60498373 -60498395	CCGACCAAGCACACAAGAUG	1673
CR002214	h	-	Chr2:60498377 -60498399	CCAAGCACACAAGAUGCACA	1674
CR002215	h	+	Chr2:60498380 -60498402	AGCACACAAGAUGCACACCC	1675
CR002216	h	+	Chr2:60498384 -60498406	CACAAGAUGCACACCCAGGC	1676
CR002217	h	+	Chr2:60498385 -60498407	ACAAGAUGCACACCCAGGCU	1677
CR001264	h	-	Chr1:55039149 -55039171	UCUUUCCACUGGCCUUAACC	1678
CR002218	h	+	Chr2:60498389 -60498411	GAUGCACACCCAGGCUGGGC	1679
CR002219	h	+	Chr2:60498396 -60498418	ACCCAGGCUGGGCUGGACAG	1680
CR002220	h	+	Chr2:60498397 -60498419	CCCAGGCUGGGCUGGACAGA	1681
CR002221	h	-	Chr2:60498397 -60498419	CCCAGGCUGGGCUGGACAGA	1682
CR002222	h	+	Chr2:60498398 -60498420	CCAGGCUGGGCUGGACAGAG	1683
CR002223	h	-	Chr2:60498398 -60498420	CCAGGCUGGGCUGGACAGAG	1684
CR002224	h	+	Chr2:60498415 -60498437	GAGGGGUCCCACAAGAUCAC	1685

CR002225	h	+	Chr2:60498416-60498438	AGGGGUCCCACAAGAUCACA	1686
CR002226	h	-	Chr2:60498422-60498444	CCCACAAGAUCACAGGGUGU	1687
CR001263	h	+	Chr1:55039180-55039202	GGCAGCGAGGAGUCCACAGU	1688
CR002227	h	-	Chr2:60498423-60498445	CCACAAGAUCACAGGGUGUG	1689
CR002228	h	+	Chr2:60498431-60498453	UCACAGGGUGUGCCCUGAGA	1690
CR002229	h	+	Chr2:60498434-60498456	CAGGGUGUGCCCUGAGAAGG	1691

In some aspects of the invention, it is preferred that the gRNA molecule to the +55 Enhancer region of BCL11a comprise a targeting domain comprising, e.g., consisting of a targeting domain sequence selected from SEQ ID NO: 1683, SEQ ID NO: 1638, SEQ ID NO: 1647, SEQ ID NO: 1609, SEQ ID NO: 1621, SEQ ID NO: 1617, SEQ ID NO: 1654, SEQ ID NO: 1631, SEQ ID NO: 1620, SEQ ID NO: 1637, SEQ ID NO: 1612, SEQ ID NO: 1656, SEQ ID NO: 1619, SEQ ID NO: 1675, SEQ ID NO: 1645, SEQ ID NO: 1598, SEQ ID NO: 1599, SEQ ID NO: 1663, SEQ ID NO: 1677, and SEQ ID NO: 1626.

In some aspects of the invention, e.g., as indicated herein, it may be beneficial to include gRNA moleucles targeting more than one, e.g., two, target sites (e.g., a first gRNA molecule and a second gRNA molecule). In some embodiments, the two target sites are both located in the +55 BCL11a enhancer region. In such aspects, any combination of more than one, e.g., two, gRNA molecules (e.g., a first gRNA molecule and a second gRNA molecule) comprising, e.g., consisting of, targeting domains listed in Table 9 may be used, that target different target sites of the +55 BCL11a enhancer region. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID

NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1683 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g.,

consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1638 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1647 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1619, respectively. In

an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1609 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of,

SEQ ID NO: 1621 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1621 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule

include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1617 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1654 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1631 and SEQ ID

NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1620 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting

domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1637 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and

the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1612 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1617,

respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1656 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of,

SEQ ID NO: 1619 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1619 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA

molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1675 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1612, respectively. In

an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1645 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO:

1598 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1598 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1599 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include

targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1677, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1663 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA

molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1677 and SEQ ID NO: 1626, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1638, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1647, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1609, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID

NO: 1621, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1617, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1654, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1631, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1620, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1637, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1612, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1656, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1619, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1675, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1645, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1598, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1599, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1663, respectively. In an aspect, the first gRNA molecule and the second gRNA molecule include targeting domains comprising, e.g., consisting of, SEQ ID NO: 1626 and SEQ ID NO: 1677, respectively.

III. Methods for Designing gRNAs

Methods for designing gRNAs are described herein, including methods for selecting, designing and validating target sequences. Exemplary targeting domains are also provided herein. Targeting Domains discussed herein can be incorporated into the gRNAs described herein.

Methods for selection and validation of target sequences as well as off-target analyses are described, e.g., in. Mali el al. , 2013 SCIENCE 339(6121): 823-826; Hsu et al , 2013 NAT BIOTECHNOL, 31 (9): 827-32; Fu et al , 2014 NAT BIOTECHNOL, doi: 10.1038/nbt.2808. PubMed PM ID: 24463574; Heigwer et al, 2014 NAT METHODS ll (2): 122-3. doi: 10.1038/nmeth.2812. PubMed PMID:

24481216; Bae el al , 2014 BIOINFORMATICS PubMed PMID: 24463181 ; Xiao A el al , 2014 BIOINFORMATICS PubMed PMID: 24389662.

For example, a software tool can be used to optimize the choice of gRNA within a user's target sequence, e.g., to minimize total off-target activity across the genome. Off target activity may be other than cleavage. For each possible gRNA choice e.g., using S. pyogenes Cas9, the tool can identify all off-target sequences (e.g., preceding either NAG or NGG PAMs) across the genome that contain up to certain number (e.g., 1 , 2, 3, 4, 5, 6, 7, 8, 9, or 10) of mismatched base-pairs. The cleavage efficiency at each off-target sequence can be predicted, e.g., using an experimentally-derived weighting scheme. Each possible gRNA is then ranked according to its total predicted off-target cleavage; the top-ranked gRNAs represent those that are likely to have the greatest on-target and the least off-target cleavage. Other functions, e.g., automated reagent design for CRISPR construction, primer design for the on-target Surveyor assay, and primer design for high-throughput detection and quantification of off-target cleavage via next-gen sequencing, can also be included in the tool. Candidate gRNA molecules can be evaluated by art-known methods or as described herein.

Although software algorithms may be used to generate an initial list of potential gRNA molecules, cutting efficiency and specificity will not necessarily reflect the predicted values, and gRNA molecules typically require screening in specific cell lines, e.g., primary human cell lines, e.g., human HSPCs, e.g., human CD34+ cells, to determine, for example, cutting efficiency, indel formation, cutting specificity and change in desired phenotype. These properties may be assayed by the methods described herein.

In aspects of the invention, a gRNA comprising the targeting domain of CR00312 (SEQ ID NO: 248, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR00312 #1:

GUUUGGCCUCUGAUUAGGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 342)

sgRNA CR00312 #2:

mG*mU*mU*UGGCCUCUGAUUAGGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 343)

sgRNA CR00312 #3:

mG*mU*mU*UGGCCUCUGAUUAGGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1762))

dgRNA CR00312 #1:

crRNA: GUUUGGCCUCUGAUUAGGGUGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 344)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR00312 #2:

crRNA:
mG*mU*mU*UGGCCUCUGAUUAGGGUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 345)

tracr:
mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR00312 #3:

crRNA:
mG*mU*mU*UGGCCUCUGAUUAGGGUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 345)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001128 (SEQ ID NO: 338, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001128 #1:

AUCAGAGGCCAAACCCUUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 347)

sgRNA CR001128 #2:

mA*mU*mC*AGAGGCCAAACCCUUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 348)

sgRNA CR001128 #3:

mA*mU*mC*AGAGGCCAAACCCUUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1763)

dgRNA CR001128 #1:

crRNA: AUCAGAGGCCAAACCCUUCCGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 349)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001128 #2:

crRNA: mA*mU*mC*AGAGGCCAAACCCUUCCGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 350)

tracr: mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001128 #3:

crRNA: mA*mU*mC*AGAGGCCAAACCCUUCCGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 350)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001126 (SEQ ID NO: 336, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001126 #1:

UUUAUCACAGGCUCCAGGAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 351)

sgRNA CR001126 #2:

mU*mU*mU*AUCACAGGCUCCAGGAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 352)

sgRNA CR001126 #3:

mU*mU*mU*AUCACAGGCUCCAGGAAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1764)

dgRNA CR001126 #1:

crRNA: UUUAUCACAGGCUCCAGGAAGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 353)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001126 #2:

crRNA: mU*mU*mU*AUCACAGGCUCCAGGAAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 354)

tracr:
mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001126 #3:

crRNA: mU*mU*mU*AUCACAGGCUCCAGGAAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 354)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR00311 (SEQ ID NO: 247, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR00311 #1:

UUUGGCCUCUGAUUAGGGUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 355)

sgRNA CR00311 #2:

mU*mU*mU*GGCCUCUGAUUAGGGUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 356)

sgRNA CR00311 #3:

mU*mU*mU*GGCCUCUGAUUAGGGUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1765)

dgRNA CR00311 #1:

crRNA: UUUGGCCUCUGAUUAGGGUGGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 357)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR00311 #2:

crRNA:

mU*mU*mU*GGCCUCUGAUUAGGGUGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 358)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR00311 #3:

crRNA:

mU*mU*mU*GGCCUCUGAUUAGGGUGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 358)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR00309 (SEQ ID NO: 245, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR00309 #1:

CACGCCCCCACCCUAAUCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 359)

sgRNA CR00309 #2:

mC*mA*mC*GCCCCCACCCUAAUCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 360)

sgRNA CR00309 #3:

mC*mA*mC*GCCCCCACCCUAAUCAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1766)

dgRNA CR00309 #1:

crRNA: CACGCCCCCACCCUAAUCAGGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 361)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR00309 #2:

crRNA: mC*mA*mC*GCCCCCACCCUAAUCAGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 362)

tracr:
mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR00309 #3:

crRNA: mC*mA*mC*GCCCCCACCCUAAUCAGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 362)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001127 (SEQ ID NO: 337, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001127 #1:

CACAGGCUCCAGGAAGGGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 363)

sgRNA CR001127 #2:

mC*mA*mC*AGGCUCCAGGAAGGGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 364)

sgRNA CR001127 #3:

mC*mA*mC*AGGCUCCAGGAAGGGUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1767)

dgRNA CR001127 #1:

crRNA: CACAGGCUCCAGGAAGGGUUGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 365)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001127 #2:

crRNA: mC*mA*mC*AGGCUCCAGGAAGGGUUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 366)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001127 #3:

crRNA: mC*mA*mC*AGGCUCCAGGAAGGGUUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 366)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR00316 (SEQ ID NO: 252, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR00316 #1:

UUGCUUUUAUCACAGGCUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 367)

sgRNA CR00316 #2:

mU*mU*mG*CUUUUAUCACAGGCUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 368)

sgRNA CR00316 #3:

mU*mU*mG*CUUUUAUCACAGGCUCCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1768)

dgRNA CR00316 #1:

crRNA: UUGCUUUUAUCACAGGCUCCGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 369)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR00316 #2:

crRNA: mU*mU*mG*CUUUUAUCACAGGCUCCGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 370)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR00316 #3:

crRNA: mU*mU*mG*CUUUUAUCACAGGCUCCGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 370)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001125 (SEQ ID NO: 335, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001125 #1:

UUUUAUCACAGGCUCCAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 371)

sgRNA CR001125 #2:

mU*mU*mU*UAUCACAGGCUCCAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 372)

sgRNA CR001125 #3:

mU*mU*mU*UAUCACAGGCUCCAGGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1769)

dgRNA CR001125 #1:

crRNA: UUUUAUCACAGGCUCCAGGAGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 373)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001125 #2:

crRNA: mU*mU*mU*UAUCACAGGCUCCAGGAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 374)

tracr: mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001125 #3:

crRNA: mU*mU*mU*UAUCACAGGCUCCAGGAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 374)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001030 (SEQ ID NO: 100, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001030 #1:

ACUGCUGAAAGAGAUGCGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 375)

sgRNA CR001030 #2:

mA*mC*mU*GCUGAAAGAGAUGCGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 376)

sgRNA CR001030 #3:

mA*mC*mU*GCUGAAAGAGAUGCGGUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1770)

dgRNA CR001030 #1:

crRNA: ACUGCUGAAAGAGAUGCGGUGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 377)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001030 #2:

crRNA:

mA*mC*mU*GCUGAAAGAGAUGCGGUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 378)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001030 #3:

crRNA:

mA*mC*mU*GCUGAAAGAGAUGCGGUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 378)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001028 (SEQ ID NO: 98, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention , including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001028 #1:

UGCGGUGGGGAGAUAUGUAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 379)

sgRNA CR001028 #2:

mU*mG*mC*GGUGGGGAGAUAUGUAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 380)

sgRNA CR001028 #3:

mU*mG*mC*GGUGGGGAGAUAUGUAGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1771)

dgRNA CR001028 #1:

crRNA: UGCGGUGGGGAGAUAUGUAGGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 381)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001028 #2:

crRNA: mU*mG*mC*GGUGGGGAGAUAUGUAGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 382)

tracr: mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001028 #3:

crRNA: mU*mG*mC*GGUGGGGAGAUAUGUAGGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 382)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001221 (SEQ ID NO: 1589, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001221 #1:

GAAACAAUGAGGACCUGACUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 383)

sgRNA CR001221 #2:

mG*mA*mA*ACAAUGAGGACCUGACUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 384)

sgRNA CR001221 #3:

mG*mA*mA*ACAAUGAGGACCUGACUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1772)

dgRNA CR001221 #1:

crRNA: GAAACAAUGAGGACCUGACUGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 385)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001221 #2:

crRNA: mG*mA*mA*ACAAUGAGGACCUGACUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 386)

tracr: mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001221 #3:

crRNA: mG*mA*mA*ACAAUGAGGACCUGACUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 386)

tracr: AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR001137 (SEQ ID NO: 1505, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR001137 #1:

GUAAGCAUUUAAGUGGCUACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 387)

sgRNA CR001137 #2:

mG*mU*mA*AGCAUUUAAGUGGCUACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 388)

sgRNA CR001137 #3:

mG*mU*mA*AGCAUUUAAGUGGCUACGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1773)

dgRNA CR001137 #1:

crRNA: GUAAGCAUUUAAGUGGCUACGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 389)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR001137 #2:

crRNA:

mG*mU*mA*AGCAUUUAAGUGGCUACGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 390)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR001137 #3:

crRNA:

mG*mU*mA*AGCAUUUAAGUGGCUACGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 390)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR003035 (SEQ ID NO: 1505, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR003035 #1:

AGGCACCUCAGACUCAGCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 391)

sgRNA CR003035 #2:

mA*mG*mG*CACCUCAGACUCAGCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU (SEQ ID NO: 392)

sgRNA CR003035 #3:

mA*mG*mG*CACCUCAGACUCAGCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U (SEQ ID NO: 1774)

dgRNA CR003035 #1:

crRNA: AGGCACCUCAGACUCAGCAUGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 393)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR003035 #2:

crRNA: mA*mG*mG*CACCUCAGACUCAGCAUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 394)

tracr:

mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR003035 #3:

crRNA: mA*mG*mG*CACCUCAGACUCAGCAUGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 394)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC
GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In aspects of the invention, a gRNA comprising the targeting domain of CR003085 (SEQ ID NO: 1750, underlined below), e.g., one of the gRNA molecules described below, is useful in the CRISPR systems, methods, cells and other aspects and embodiments of the invention, including in aspects involving more than one gRNA molecule, e.g., described herein:

sgRNA CR003085 #1:

AUGGUAUGGGAGGUAUACUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUA
GUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU (SEQ ID NO: 395)

sgRNA CR003085 #2:

mA*mU*mG*GUAUGGGAGGUAUACUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA
AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCU*mU*mU*mU
(SEQ ID NO: 396)

sgRNA CR003085 #3:

mA*mU*mG*GUAUGGGAGGUAUACUAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA
AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCmU*mU*mU*U
(SEQ ID NO: 1775)

dgRNA CR003085 #1:

crRNA: AUGGUAUGGGAGGUAUACUAGUUUUAGAGCUAUGCUGUUUUG (SEQ ID NO: 397)

tracr:
AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC
GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660)

dgRNA CR003085 #2:

crRNA:
mA*mU*mG*GUAUGGGAGGUAUACUAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 398)

tracr:
mA*mA*mC*AGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUG
GCACCGAGUCGGUGCUUUU*mU*mU*mU (SEQ ID NO: 346)

dgRNA CR003085 #3:

crRNA:

mA*mU*mG*GUAUGGGAGGUAUACUAGUUUUAGAGCUAUGCUGUU*mU*mU*mG (SEQ ID NO: 398)

tracr:

AACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACC GAGUCGGUGCUUUUUUU (SEQ ID NO: 6660).

In each of the gRNA molecules described above, a "*" denotes a phosphorothioate bond between the adjacent nucleotides, and "mN" (where N = A, G, C or U) denotes a 2'-OMe modified nucleotide. In embodiments, any of the gRNA molecules described herein, e.g., described above, is complexed with a Cas9 molecule, e.g., as described herein, to form a ribonuclear protein complex (RNP). Such RNPs are particularly useful in the methods, cells, and other aspects and embodiments of the invention, e.g., described herein.

IV. Cas Molecules

Cas9 Molecules

In preferred embodiments, the Cas molecule is a Cas9 molecule. Cas9 molecules of a variety of species can be used in the methods and compositions described herein. While the S. pyogenes Cas9 molecule are the subject of much of the disclosure herein, Cas9 molecules of, derived from, or based on the Cas9 proteins of other species listed herein can be used as well. In other words, other Cas9 molecules, e.g., S. thermophilus, Staphylococcus aureus and/or Neisseria meningitidis Cas9 molecules, may be used in the systems, methods and compositions described herein. Additional Cas9 species include: Acidovorax avenae, Actinobacillus pleuropneumoniae, Actinobacillus succinogenes, Actinobacillus suis, Actinomyces sp., cycliphilus denitrificans, Aminomonas paucivorans, Bacillus cereus, Bacillus smithii, Bacillus thuringiensis, Bacteroides sp., Blastopirellula marina, Bradyrhiz' obium sp., Brevibacillus latemsporus, Campylobacter coli, Campylobacter jejuni, Campylobacter lad, Candidatus Puniceispirillum, Clostridiu cellulolyticum, Clostridium perfringens, Corynebacterium accolens, Corynebacterium diphtheria, Corynebacterium matruchotii, Dinoroseobacter sliibae, Eubacterium dolichum, gamma proteobacterium, Gluconacetobacler diazotrophicus, Haemophilus parainfluenzae, Haemophilus sputorum, Helicobacter canadensis, Helicobacter cinaedi, Helicobacter mustelae, Ilyobacler polytropus, Kingella kingae, Lactobacillus crispatus, Listeria ivanovii, Listeria monocytogenes, Listeriaceae bacterium, Methylocystis sp., Methylosinus trichosporium, Mobiluncus mulieris, Neisseria bacilliformis, Neisseria cinerea, Neisseria flavescens, Neisseria lactamica. Neisseria sp., Neisseria wadsworthii, Nitrosomonas sp., Parvibaculum lavamentivorans, Pasteurella multocida, Phascolarctobacterium succinatutens, Ralstonia syzygii, Rhodopseudomonas palustris,

Rhodovulum sp., Simonsiella muelleri, Sphingomonas sp., Sporolactobacillus vineae, Staphylococcus lugdunensis, Streptococcus sp., Subdoligranulum sp., Tislrella mobilis, Treponema sp., or Verminephrobacter eiseniae.

A Cas9 molecule, as that term is used herein, refers to a molecule that can interact with a gRNA molecule (e.g., sequence of a domain of a tracr) and, in concert with the gRNA molecule, localize (e.g., target or home) to a site which comprises a target sequence and PAM sequence.

In an embodiment, the Cas9 molecule is capable of cleaving a target nucleic acid molecule, which may be referred to herein as an active Cas9 molecule. In an embodiment, an active Cas9 molecule, comprises one or more of the following activities: a nickase activity, i.e., .the ability to cleave a single strand, e.g., the non-complementary strand or the complementary strand, of a nucleic acid molecule; a double stranded nuclease activity, i.e., the ability to cleave both strands of a double stranded nucleic acid and create a double stranded break, which in an embodiment is the presence of two nickase activities; an endonuclease activity; an exonuclease activity; and a helicase activity, i.e., the ability to unwind the helical structure of a double stranded nucleic acid.

In an embodiment, an enzymatically active Cas9 molecule cleaves both DNA strands and results in a double stranded break. In an embodiment, a Cas9 molecule cleaves only one strand, e.g., the strand to which the gRNA hybridizes to, or the strand complementary to the strand the gRNA hybridizes with.

In an embodiment, an active Cas9 molecule comprises cleavage activity associated with an HNH-like domain. In an embodiment, an active Cas9 molecule comprises cleavage activity associated with an N-terminal RuvC-like domain. In an embodiment, an active Cas9 molecule comprises cleavage activity associated with an HNH-like domain and cleavage activity associated with an N-terminal RuvC-like domain. In an embodiment, an active Cas9 molecule comprises an active, or cleavage competent, HNH-like domain and an inactive, or cleavage incompetent, N-terminal RuvC-like domain. In an embodiment, an active Cas9 molecule comprises an inactive, or cleavage incompetent, HNH-like domain and an active, or cleavage competent, N-terminal RuvC-like domain.

In an embodiment, the ability of an active Cas9 molecule to interact with and cleave a target nucleic acid is PAM sequence dependent. A PAM sequence is a sequence in the target nucleic acid. In an embodiment, cleavage of the target nucleic acid occurs upstream from the PAM sequence. Active Cas9 molecules from different bacterial species can recognize different sequence motifs (e.g., PAM sequences). In an embodiment, an active Cas9 molecule of S. pyogenes recognizes the sequence motif NGG and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Mali el ai, SCIENCE 2013; 339(6121): 823- 826. In an embodiment, an active Cas9 molecule of S. thermophilus recognizes the sequence motif NGGNG and NNAG AAW (W = A or T) and directs cleavage of a core target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from these sequences. See, e.g., Horvath et al., SCIENCE 2010; 327(5962): 167- 170,

and Deveau et al , J BACTERIOL 2008; 190(4): 1390- 1400. In an embodiment, an active Cas9 molecule of S. mulans recognizes the sequence motif NGG or NAAR (R - A or G) and directs cleavage of a core target nucleic acid sequence 1 to 10, e.g., 3 to 5 base pairs, upstream from this sequence. See, e.g., Deveau et al. , J BACTERIOL 2008; 190(4): 1 390- 1400.

In an embodiment, an active Cas9 molecule of S. aureus recognizes the sequence motif NNGRR (R = A or G) and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Ran F. et al., NATURE, vol. 520, 2015, pp. 186-191. In an embodiment, an active Cas9 molecule of N. meningitidis recognizes the sequence motif NNNNGATT and directs cleavage of a target nucleic acid sequence 1 to 10, e.g., 3 to 5, base pairs upstream from that sequence. See, e.g., Hou et al., PNAS EARLY EDITION 2013, 1 -6. The ability of a Cas9 molecule to recognize a PAM sequence can be determined, e.g., using a transformation assay described in Jinek et al , SCIENCE 2012, 337:816.

Some Cas9 molecules have the ability to interact with a gRNA molecule, and in conjunction with the gRNA molecule home (e.g., targeted or localized) to a core target domain, but are incapable of cleaving the target nucleic acid, or incapable of cleaving at efficient rates. Cas9 molecules having no, or no substantial, cleavage activity may be referred to herein as an inactive Cas9 (an enzymatically inactive Cas9), a dead Cas9, or a dCas9 molecule. For example, an inactive Cas9 molecule can lack cleavage activity or have substantially less, e.g., less than 20, 10, 5, 1 or 0.1 % of the cleavage activity of a reference Cas9 molecule, as measured by an assay described herein.

Exemplary naturally occurring Cas9 molecules are described in Chylinski et al , RNA Biology 2013; 10:5, 727-737. Such Cas9 molecules include Cas9 molecules of a cluster 1 bacterial family, cluster 2 bacterial family, cluster 3 bacterial family, cluster 4 bacterial family, cluster 5 bacterial family, cluster 6 bacterial family, a cluster 7 bacterial family, a cluster 8 bacterial family, a cluster 9 bacterial family, a cluster 10 bacterial family, a cluster 1 1 bacterial family, a cluster 12 bacterial family, a cluster 13 bacterial family, a cluster 14 bacterial family, a cluster 1 bacterial family, a cluster 16 bacterial family, a cluster 17 bacterial family, a cluster 1 8 bacterial family, a cluster 19 bacterial family, a cluster 20 bacterial family, a cluster 21 bacterial family, a cluster 22 bacterial family, a cluster 23 bacterial family, a cluster 24 bacterial family, a cluster 25 bacterial family, a cluster 26 bacterial family, a cluster 27 bacterial family, a cluster 28 bacterial family, a cluster 29 bacterial family, a cluster 30 bacterial family, a cluster 31 bacterial family, a cluster 32 bacterial family, a cluster 33 bacterial family, a cluster 34 bacterial family, a cluster 35 bacterial family, a cluster 36 bacterial family, a cluster 37 bacterial family, a cluster 38 bacterial family, a cluster 39 bacterial family, a cluster 40 bacterial family, a cluster 41 bacterial family, a cluster 42 bacterial family, a cluster 43 bacterial family, a cluster 44 bacterial family, a cluster 45 bacterial family, a cluster 46 bacterial family, a cluster 47 bacterial family, a cluster 48 bacterial family,. a cluster 49 bacterial family, a

cluster 50 bacterial family, a cluster 5 1 bacterial family, a cluster 52 bacterial family, a cluster 53 bacterial family, a cluster 54 bacterial family, a cluster 55 bacterial family, a cluster 56 bacterial family, a cluster 57 bacterial family, a cluster 58 bacterial family, a cluster 59 bacterial family, a cluster 60 bacterial family, a cluster 61 bacterial family, a cluster 62 bacterial family, a cluster 63 bacterial family, a cluster 64 bacterial family, a cluster 65 bacterial family, a cluster 66 bacterial family, a cluster 67 bacterial family, a cluster 68 bacterial family, a cluster 69 bacterial family, a cluster 70 bacterial family, a cluster 71 bacterial family, a cluster 72 bacterial family, a cluster 73 bacterial family, a cluster 74 bacterial family, a cluster 75 bacterial family, a cluster 76 bacterial family, a cluster 77 bacterial family, or a cluster 78 bacterial family.

Exemplary naturally occurring Cas9 molecules include a Cas9 molecule of a cluster 1 bacterial family. Examples include a Cas9 molecule of: S. pyogenes (e.g., strain SF370, MGAS 10270, MGAS 10750, MGAS2096, MGAS315, MGAS5005, MGAS6180, MGAS9429, NZ131 and SSI- 1), S. thermophilus (e.g., strain LMD-9), S. pseudoporcinus (e.g., strain SPIN 20026), S. mutans (e.g., strain UA 159, NN2025), S. macacae (e.g., strain NCTC1 1558), S. gallolylicus (e.g., strain UCN34, ATCC BAA-2069), S. equines (e.g., strain ATCC 9812, MGCS 124), S. dysdalactiae (e.g., strain GGS 124), S. bovis (e.g., strain ATCC 700338), S. cmginosus (e.g.; strain F021 1), S. agalactia* (e.g., strain NEM316, A909), Listeria monocytogenes (e.g., strain F6854), Listeria innocua (L. innocua, e.g., strain Clip l 1262), EtUerococcus italicus (e.g., strain DSM 15952), or Enterococcus faecium (e.g., strain 1,231 ,408). Additional exemplary Cas9 molecules are a Cas9 molecule of Neisseria meningitidis (Hou et al. PNAS Early Edition 2013, 1 -6) and a S. aureus Cas9 molecule.

In an embodiment, a Cas9 molecule, e.g., an active Cas9 molecule or inactive Cas9 molecule, comprises an amino acid sequence: having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology with; differs at no more than 1%, 2%, 5%, 10%, 15%, 20%, 30%, or 40% of the amino acid residues when compared with; differs by at least 1, 2, 5, 10 or 20 amino acids but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or is identical to; any Cas9 molecule sequence described herein or a naturally occurring Cas9 molecule sequence, e.g., a Cas9 molecule from a species listed herein or described in Chylinski et al. , RNA Biology 2013, 10:5, 726-T,1 Hou et al. PNAS Early Edition 2013, 1-6.

In an embodiment, a Cas9 molecule comprises an amino acid sequence having 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% homology with; differs at no more than 1%, 2%, 5%, 10%, 15%, 20%, 30%, or 40% of the amino acid residues when compared with; differs by at least 1, 2, 5, 10 or 20 amino acids but by no more than 100, 80, 70, 60, 50, 40 or 30 amino acids from; or is identical to; *S. pyogenes* Cas9:

```
Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                  10                  15
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Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20 25 30
Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35 40 45
Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60
Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65 70 75 80
Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85 90 95
Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100 105 110
His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115 120 125
His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130 135 140
Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145 150 155 160
Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165 170 175
Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180 185 190
Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195 200 205
Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210 215 220
Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225 230 235 240
Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245 250 255
Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260 265 270
Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275 280 285
Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290 295 300
Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser

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305                 310                 315                 320
Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335
Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350
Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365
Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380
Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400
Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415
Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430
Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445
Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460
Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480
Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495
Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510
Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525
Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540
Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560
Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575
Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590
Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605
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Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
610 615 620
Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625 630 635 640
His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
645 650 655
Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
660 665 670
Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
675 680 685
Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
690 695 700
Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705 710 715 720
His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
725 730 735
Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
740 745 750
Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
755 760 765
Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
770 775 780
Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785 790 795 800
Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
805 810 815
Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
820 825 830
Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845
Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860
Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865 870 875 880
Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
885 890 895
Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp

900 905 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr

915 920 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp

930 935 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser

945 950 955 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg

965 970 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val

980 985 990

Val Gly Thr Ala Leu Ile Lys Lys Tyr Pro Lys Leu Glu Ser Glu Phe

995 1000 1005

Val Tyr Gly Asp Tyr Lys Val Tyr Asp Val Arg Lys Met Ile Ala Lys

1010 1015 1020

Ser Glu Gln Glu Ile Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser

1025 1030 1035 1040

Asn Ile Met Asn Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu

1045 1050 1055

Ile Arg Lys Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile

1060 1065 1070

Val Trp Asp Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser

1075 1080 1085

Met Pro Gln Val Asn Ile Val Lys Lys Thr Glu Val Gln Thr Gly Gly

1090 1095 1100

Phe Ser Lys Glu Ser Ile Leu Pro Lys Arg Asn Ser Asp Lys Leu Ile

1105 1110 1115 1120

Ala Arg Lys Lys Asp Trp Asp Pro Lys Lys Tyr Gly Gly Phe Asp Ser

1125 1130 1135

Pro Thr Val Ala Tyr Ser Val Leu Val Val Ala Lys Val Glu Lys Gly

1140 1145 1150

Lys Ser Lys Lys Leu Lys Ser Val Lys Glu Leu Leu Gly Ile Thr Ile

1155 1160 1165

Met Glu Arg Ser Ser Phe Glu Lys Asn Pro Ile Asp Phe Leu Glu Ala

1170 1175 1180

Lys Gly Tyr Lys Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys

1185 1190 1195 1200

Tyr Ser Leu Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser
1205 1210 1215
Ala Gly Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr
1220 1225 1230
Val Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser
1235 1240 1245
Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys His
1250 1255 1260
Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys Arg Val
1265 1270 1275 1280
Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys
1285 1290 1295
His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu
1300 1305 1310
Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp
1315 1320 1325
Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp
1330 1335 1340
Ala Thr Leu Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg Ile
1345 1350 1355 1360
Asp Leu Ser Gln Leu Gly Gly Asp
1365

(SEQ ID NO: 6611)

In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes one or more mutations to positively charged amino acids (e.g., lysine, arginine or histidine) that introduce an uncharged or nonpolar amino acid, e.g., alanine, at said position. In embodiments, the mutation is to one or more positively charged amino acids in the nt-groove of Cas9. In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes a mutatation at position 855 of SEQ ID NO: 6611, for example a mutation to an uncharged amino acid, e.g., alanine, at position 855 of SEQ ID NO: 6611. In embodiments, the Cas9 molecule has a mutation only at position 855 of SEQ ID NO: 6611, relative to SEQ ID NO: 6611, e.g., to an uncharged amino acid, e.g., alanine. In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes a mutatation at position 810, a mutation at position 1003, and/or a mutation at position 1060 of SEQ ID NO: 6611, for example a mutation to alanine at position 810, position 1003, and/or position 1060 of SEQ ID NO: 6611. In embodiments, the Cas9 molecule has a mutation only at position 810, position 1003, and position 1060 of SEQ ID NO: 6611, relative to SEQ ID NO: 6611, e.g., where each mutation is to an uncharged amino acid, for example, alanine. In embodiments, the

Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes a mutatation at position 848, a mutation at position 1003, and/or a mutation at position 1060 of SEQ ID NO: 6611, for example a mutation to alanine at position 848, position 1003, and/or position 1060 of SEQ ID NO: 6611. In embodiments, the Cas9 molecule has a mutation only at position 848, position 1003, and position 1060 of SEQ ID NO: 6611, relative to SEQ ID NO: 6611, e.g., where each mutation is to an uncharged amino acid, for example, alanine. In embodiments, the Cas9 molecule is a Cas9 molecule as described in Slaymaker et al., *Science Express*, available online December 1, 2015 at Science DOI: 10.1126/science.aad5227.

In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes one or more mutations. In embodiments, the Cas9 variant comprises a mutation at position 80 of SEQ ID NO: 6611, e.g., includes a leucine at position 80 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a C80L mutation). In embodiments, the Cas9 variant comprises a mutation at position 574 of SEQ ID NO: 6611, e.g., includes a glutamic acid at position 574 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a C574E mutation). In embodiments, the Cas9 variant comprises a mutation at position 80 and a mutation at position 574 of SEQ ID NO: 6611, e.g., includes a leucine at position 80 of SEQ ID NO: 6611, and a glutamic acid at position 574 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a C80L mutation and a C574E mutation). Without being bound by theory, it is believed that such mutations improve the solution properties of the Cas9 molecule.

In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes one or more mutations. In embodiments, the Cas9 variant comprises a mutation at position 147 of SEQ ID NO: 6611, e.g., includes a tyrosine at position 147 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a D147Y mutation). In embodiments, the Cas9 variant comprises a mutation at position 411 of SEQ ID NO: 6611, e.g., includes a threonine at position 411 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a P411T mutation). In embodiments, the Cas9 variant comprises a mutation at position 147 and a mutation at position 411 of SEQ ID NO: 6611, e.g., includes a tyrosine at position 147 of SEQ ID NO: 6611, and a threonine at position 411 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a D147Y mutation and a P411T mutation). Without being bound by theory, it is believed that such mutations improve the targeting efficiency of the Cas9 molecule, e.g., in yeast.

In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes one or more mutations. In embodiments, the Cas9 variant comprises a mutation at position 1135 of SEQ ID NO: 6611, e.g., includes a glutamic acid at position 1135 of SEQ ID NO: 6611 (i.e., comprises, e.g., consists of, SEQ ID NO: 6611 with a D1135E mutation). Without being bound by

theory, it is believed that such mutations improve the selectivity of the Cas9 molecule for the NGG PAM sequence versus the NAG PAM sequence.

In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes one or more mutations that introduce an uncharged or nonpolar amino acid, e.g., alanine, at certain positions. In embodiments, the Cas9 molecule is a *S. pyogenes* Cas9 variant of SEQ ID NO: 6611 that includes a mutatation at position 497, a mutation at position 661, a mutation at position 695 and/or a mutation at position 926 of SEQ ID NO: 6611, for example a mutation to alanine at position 497, position 661, position 695 and/or position 926 of SEQ ID NO: 6611. In embodiments, the Cas9 molecule has a mutation only at position 497, position 661, position 695, and position 926 of SEQ ID NO: 6611, relative to SEQ ID NO: 6611, e.g., where each mutation is to an uncharged amino acid, for example, alanine. Without being bound by theory, it is believed that such mutations reduce the cutting by the Cas9 molecule at off-target sites

It will be understood that the mutations described herein to the Cas9 molecule may be combined, and may be combined with any of the fusions or other modifications described herein, and the Cas9 molecule tested in the assays described herein.

Various types of Cas molecules can be used to practice the inventions disclosed herein. In some embodiments, Cas molecules of Type II Cas systems are used. In other embodiments, Cas molecules of other Cas systems are used. For example, Type I or Type III Cas molecules may be used. Exemplary Cas molecules (and Cas systems) are described, e.g., in Haft et ai, PLoS COMPUTATIONAL BIOLOGY 2005, 1(6): e60 and Makarova et al , NATURE REVIEW MICROBIOLOGY 201 1 , 9:467-477, the contents of both references are incorporated herein by reference in their entirety.

In an embodiment, the Cas9 molecule comprises one or more of the following activities: a nickase activity; a double stranded cleavage activity (e.g., an endonuclease and/or exonuclease activity); a helicase activity; or the ability, together with a gRNA molecule, to localize to a target nucleic acid.

Altered Cas9 Molecules

Naturally occurring Cas9 molecules possess a number of properties, including: nickase activity, nuclease activity (e.g., endonuclease and/or exonuclease activity); helicase activity; the ability to associate functionally with a gRNA molecule; and the ability to target (or localize to) a site on a nucleic acid (e.g., PAM recognition and specificity). In an embodiment, a Cas9 molecules can include all or a subset of these properties. In typical embodiments, Cas9 molecules have the ability to interact with a gRNA molecule and, in concert with the gRNA molecule, localize to a site in a nucleic acid. Other activities, e.g., PAM specificity, cleavage activity, or helicase activity can vary more widely in Cas9 molecules.

Cas9 molecules with desired properties can be made in a number of ways, e.g., by alteration of a parental, e.g., naturally occurring Cas9 molecules to provide an altered Cas9 molecule having a desired property. For example, one or more mutations or differences relative to a parental Cas9 molecule can be introduced. Such mutations and differences comprise: substitutions (e.g., conservative substitutions or substitutions of non-essential amino acids); insertions; or deletions. In an embodiment, a Cas9 molecule can comprises one or more mutations or differences, e.g., at least 1 , 2, 3, 4, 5, 10, 15, 20, 30, 40 or 50 mutations but less than 200, 100, or 80 mutations relative to a reference Cas9 molecule.

In an embodiment, a mutation or mutations do not have a substantial effect on a Cas9 activity, e.g. a Cas9 activity described herein. In an embodiment, a mutation or mutations have a substantial effect on a Cas9 activity, e.g. a Cas9 activity described herein. In an embodiment, exemplary activities comprise one or more of PAM specificity, cleavage activity, and helicase activity. A mutation(s) can be present, e.g., in: one or more RuvC-like domain, e.g., an N- terminal RuvC-like domain; an HNH-like domain; a region outside the RuvC-like domains and the HNH-like domain. In some embodiments, a mutation(s) is present in an N-terminal RuvC- like domain. In some embodiments, a mutation(s) is present in an HNH-like domain. In some embodiments, mutations are present in both an N-terminal RuvC-like domain and an HNH-like domain.

Whether or not a particular sequence, e.g., a substitution, may affect one or more activity, such as targeting activity, cleavage activity, etc, can be evaluated or predicted, e.g., by evaluating whether the mutation is conservative or by the method described in Section ΠΙ. In an embodiment, a "non-essential" amino acid residue, as used in the context of a Cas9 molecule, is a residue that can be altered from the wild-type sequence of a Cas9 molecule, e.g., a naturally occurring Cas9 molecule, e.g., an active Cas9 molecule, without abolishing or more preferably, without substantially altering a Cas9 activity (e.g., cleavage activity), whereas changing an "essential" amino acid residue results in a substantial loss of activity (e.g., cleavage activity).

Cas9 Molecules with altered PAM recognition or no PAM recognition

Naturally occurring Cas9 molecules can recognize specific PAM sequences, for example the PAM recognition sequences described above for S. pyogenes, S. thermophilus, S. mutans, S. aureus and N. meningitidis.

In an embodiment, a Cas9 molecule has the same PAM specificities as a naturally occurring Cas9 molecule. In other embodiments, a Cas9 molecule has a PAM specificity not associated with a naturally occurring Cas9 molecule, or a PAM specificity not associated with the naturally occurring Cas9 molecule to which it has the closest sequence homology. For example, a naturally occurring Cas9 molecule can be altered, e.g., to alter PAM recognition, e.g., to alter the PAM sequence that the Cas9 molecule recognizes to decrease off target sites and/or improve specificity; or eliminate a PAM

recognition requirement. In an embodiment, a Cas9 molecule can be altered, e.g., to increase length of PAM recognition sequence and/or improve Cas9 specificity to high level of identity to decrease off target sites and increase specificity. In an embodiment, the length of the PAM recognition sequence is at least 4, 5, 6, 7, 8, 9, 10 or 15 amino acids in length. Cas9 molecules that recognize different PAM sequences and/or have reduced off- target activity can be generated using directed evolution. Exemplary methods and systems that can be used for directed evolution of Cas9 molecules are described, e.g., in Esvelt el al , Nature 2011, 472(7344): 499-503. Candidate Cas9 molecules can be evaluated, e.g., by methods described herein.

Non-Cleaving and Modified-Cleavage Cas9 Molecules

In an embodiment, a Cas9 molecule comprises a cleavage property that differs from naturally occurring Cas9 molecules, e.g., that differs from the naturally occurring Cas9 molecule having the closest homology. For example, a Cas9 molecule can differ from naturally occurring Cas9 molecules, e.g., a Cas9 molecule of S. pyogenes, as follows: its ability to modulate, e.g., decreased or increased, cleavage of a double stranded break (endonuclease and/or exonuclease activity), e.g., as compared to a naturally occurring Cas9 molecule (e.g., a Cas9 molecule of S. pyogenes); its ability to modulate, e.g., decreased or increased, cleavage of a single strand of a nucleic acid, e.g., a non-complimentary strand of a nucleic acid molecule or a complementary strand of a nucleic acid molecule (nickase activity), e.g., as compared to a naturally occurring Cas9 molecule (e.g., a Cas9 molecule of S. pyogenes); or the ability to cleave a nucleic acid molecule, e.g., a double stranded or single stranded nucleic acid molecule, can be eliminated.

Modified Cleavage active Cas9 Molecules

In an embodiment, an active Cas9 molecule comprises one or more of the following activities: cleavage activity associated with an N-terminal RuvC-like domain; cleavage activity associated with an HNH-like domain; cleavage activity associated with an HNH domain and cleavage activity associated with an N-terminal RuvC-like domain.

In an embodiment, the Cas9 molecule is a Cas9 nickase, e.g., cleaves only a single strand of DNA. In an embodiment, the Cas9 nickase includes a mutation at position 10 and/or a mutation at position 840 of SEQ ID NO: 6611, e.g., comprises a D10A and/or H840A mutation to SEQ ID NO: 6611.

Non-Cleaving inactive Cas9 Molecules

In an embodiment, the altered Cas9 molecule is an inactive Cas9 molecule which does not cleave a nucleic acid molecule (either double stranded or single stranded nucleic acid molecules) or cleaves a nucleic acid molecule with significantly less efficiency, e.g., less than 20, 10, 5, 1 or 0.1 % of the cleavage activity of a reference Cas9 molecule, e.g., as measured by an assay described herein. The reference Cas9 molecule can by a naturally occurring unmodified Cas9 molecule, e.g., a naturally

occurring Cas9 molecule such as a Cas9 molecule of S. pyogenes, S. thermophilus, S. aureus or N. meningitidis. In an embodiment, the reference Cas9 molecule is the naturally occurring Cas9 molecule having the closest sequence identity or homology. In an embodiment, the inactive Cas9 molecule lacks substantial cleavage activity associated with an N- terminal RuvC-like domain and cleavage activity associated with an HNH-like domain.

In an embodiment, the Cas9 molecule is dCas9. Tsai et al. (2014), Nat. Biotech. 32:569-577.

A catalytically inactive Cas9 molecule may be fused with a transcription repressor. An inactive Cas9 fusion protein complexes with a gRNA and localizes to a DNA sequence specified by gRNA's targeting domain, but, unlike an active Cas9, it will not cleave the target DNA. Fusion of an effector domain, such as a transcriptional repression domain, to an inactive Cas9 enables recruitment of the effector to any DNA site specified by the gRNA. Site specific targeting of a Cas9 fusion protein to a promoter region of a gene can block or affect polymerase binding to the promoter region, for example, a Cas9 fusion with a transcription factor (e.g., a transcription activator) and/or a transcriptional enhancer binding to the nucleic acid to increase or inhibit transcription activation. Alternatively, site specific targeting of a a Cas9- fusion to a transcription repressor to a promoter region of a gene can be used to decrease transcription activation.

Transcription repressors or transcription repressor domains that may be fused to an inactive Cas9 molecule can include ruppel associated box (KRAB or SKD), the Mad mSIN3 interaction domain (SID) or the ERF repressor domain (ERD).

In another embodiment, an inactive Cas9 molecule may be fused with a protein that modifies chromatin. For example, an inactive Cas9 molecule may be fused to heterochromatin protein 1 (HPl), a histone lysine methyltransferase (e.g., SUV39H 1 , SUV39H2, G9A, ESET/SETDB 1 , Pr-SET7/8, SUV4-20H 1,RIZ1), a histone lysine demethylates (e.g., LSD1/BHC1 10, SpLsdl/Sw, l/Safl 10, Su(var)3-3, JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1 , JMJD2D, Rph 1 , JARID 1 A/RBP2, JARI DIB/PLU-I, JAR1D 1C/SMCX, JARID1 D/SMCY, Lid, Jhn2, Jmj2), a histone lysine deacetylases (e.g., HDAC1, HDAC2, HDAC3, HDAC8, Rpd3, Hos 1 , Cir6, HDAC4, HDAC5, HDAC7, HDAC9, Hdal , Cir3, SIRT 1 , SIRT2, Sir2, Hst 1 , Hst2, Hst3, Hst4, HDAC 1 1) and a DNA methylases (DNMT1,DNMT2a/DMNT3b, MET1). An inactive Cas9-chomatin modifying molecule fusion protein can be used to alter chromatin status to reduce expression a target gene.

The heterologous sequence (e.g., the transcription repressor domain) may be fused to the N- or C-terminus of the inactive Cas9 protein. In an alternative embodiment, the heterologous sequence (e.g., the transcription repressor domain) may be fused to an internal portion (i.e., a portion other than the N-terminus or C-terminus) of the inactive Cas9 protein.

The ability of a Cas9 molecule/gRNA molecule complex to bind to and cleave a target nucleic acid can be evaluated, e.g., by the methods described herein in Section III. The activity of a Cas9 molecule, e.g., either an active Cas9 or a inactive Cas9, alone or in a complex with a gRNA molecule may also be evaluated by methods well-known in the art, including, gene expression assays and chromatin-based assays, e.g., chromatin immunoprecipitation (ChiP) and chromatin in vivo assay (CiA).

Other Cas9 Molecule Fusions

In embodiments, the Cas9 molecule, e.g, a Cas9 of *S. pyogenes*, may additionally comprise one or more amino acid sequences that confer additional activity.

In some aspects, the Cas9 molecule may comprise one or more nuclear localization sequences (NLSs), such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the Cas9 molecule comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the carboxy-terminus, or a combination of these (e.g. one or more NLS at the amino-terminus and one or more NLS at the carboxy terminus). When more than one NLS is present, each may be selected independently of the others, such that a single NLS may be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. In some embodiments, an NLS is considered near the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-terminus. Typically, an NLS consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface, but other types of NLS are known. Non-limiting examples of NLSs include an NLS sequence comprising or derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO: 6612); the NLS from nucleoplasmin (e.g. the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK (SEQ ID NO: 6613); the c-myc NLS having the amino acid sequence PAAKRVKLD (SEQ ID NO: 6614) or RQRRNELKRSP (SEQ ID NO: 6615); the hRNPA1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 6616); the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 6617) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO: 6618) and PPKKARED (SEQ ID NO: 6619) of the myoma T protein; the sequence PQPKKKPL (SEQ ID NO: 6620) of human p53; the sequence SALIKKKKKMAP (SEQ ID NO: 6621) of mouse c-ab1 IV; the sequences DRLRR (SEQ ID NO: 6622) and PKQKKRK (SEQ ID NO: 6623) of the influenza virus NS1; the sequence RKLKKKIKKL (SEQ ID NO: 6624) of the Hepatitis virus delta antigen; the sequence REKKKFLKRR (SEQ ID NO: 6625) of the mouse Mx1 protein; the sequence KRKGDEVDGVDEVAKKKSKK (SEQ ID NO: 6626) of the human poly(ADP-ribose) polymerase;

and the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: 6627) of the steroid hormone receptors (human) glucocorticoid. Other suitable NLS sequences are known in the art (e.g., Sorokin, Biochemistry (Moscow) (2007) 72:13, 1439-1457; Lange J Biol Chem. (2007) 282:8, 5101-5).

In an embodiment, the Cas9 molecule, e.g., *S. pyogenes* Cas9 molecule, comprises a NLS sequence of SV40, e.g., disposed N terminal to the Cas9 molecule. In an embodiment, the Cas9 molecule, e.g., *S. pyogenes* Cas9 molecule, comprises a NLS sequence of SV40 disposed N-terminal to the Cas9 molecule and a NLS sequence of SV40 disposed C terminal to the Cas9 molecule. In an embodiment, the Cas9 molecule, e.g., *S. pyogenes* Cas9 molecule, comprises a NLS sequence of SV40 disposed N-terminal to the Cas9 molecule and a NLS sequence of nucleoplasmin disposed C-terminal to the Cas9 molecule. In any of the aforementioned embodiments, the molecule may additionally comprise a tag, e.g., a His tag, e.g., a His(6) tag or His(8) tag (SEQ ID NOS 2969 and 2970, respectively), e.g., at the N terminus or the C terminus.

In some aspects, the Cas9 molecule may comprise one or more amino acid sequences that allow the Cas9 molecule to be specifically recognized, for example a tag. In one embodiment, the tag is a Histidine tag, e.g., a histidine tag comprising at least 3, 4, 5, 6, 7, 8, 9, 10 or more histidine amino acids (SEQ ID NO: 2971). In embodiments, the histidine tag is a His6 tag (six histidines) (SEQ ID NO: 2969). In other embodiments, the histidine tag is a His8 tag (eight histidines) (SEQ ID NO: 2970). In embodiments, the histidine tag may be separated from one or more other portions of the Cas9 molecule by a linker. In embodiments, the linker is GGS. An example of such a fusion is the Cas9 molecule iProt106520.

In some aspects, the Cas9 molecule may comprise one or more amino acid sequences that are recognized by a protease (e.g., comprise a protease cleavage site). In embodiments, the cleavage site is the tobacco etch virus (TEV) cleavage site, e.g., comprises the sequence ENLYFQG (SEQ ID NO: 7810). In some aspects the protease cleavage site, e.g., the TEV cleavage site is disposed between a tag, e.g., a His tag, e.g., a His6 or His8 tag (SEQ ID NOS 2969 and 2970, respectively), and the remainder of the Cas9 molecule. Without being bound by theory it is believed that such introduction will allow for the use of the tag for, e.g., purification of the Cas9 molecule, and then subsequent cleavage so the tag does not interfere with the Cas9 molecule function.

In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal NLS, and a C-terminal NLS (e.g., comprises, from N- to C-terminal NLS-Cas9-NLS), e.g., wherein each NLS is an SV40 NLS (PKKKRKV (SEQ ID NO: 6612)). In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal NLS, a C-terminal NLS, and a C-terminal His6 tag (SEQ ID NO: 2969) (e.g., comprises, from N- to C-terminal NLS-Cas9-NLS-His tag), e.g., wherein each NLS is an SV40 NLS (PKKKRKV (SEQ ID NO: 6612)). In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal

His tag (e.g., His6 tag (SEQ ID NO: 2969)), an N-terminal NLS, and a C-terminal NLS (e.g., comprises, from N- to C-terminal His tag-NLS-Cas9-NLS), e.g., wherein each NLS is an SV40 NLS (PKKKRKV (SEQ ID NO: 6612)). In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal NLS and a C-terminal His tag (e.g., His6 tag (SEQ ID NO: 2969)) (e.g., comprises from N- to C- terminal His tag-Cas9-NLS), e.g., wherein the NLS is an SV40 NLS (PKKKRKV (SEQ ID NO: 6612)). In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal His tag and a C-terminal His tag (e.g., His6 tag (SEQ ID NO: 2969)) (e.g., comprises from N- to C- terminal NLS-Cas9-His tag), e.g., wherein the NLS is an SV40 NLS (PKKKRKV (SEQ ID NO: 6612)). In embodiments, the Cas9 molecule (e.g., a Cas9 molecule as described herein) comprises an N-terminal His tag (e.g., His8 tag (SEQ ID NO: 2970)), an N-terminal cleavage domain (e.g., a tobacco etch virus (TEV) cleavage domain (e.g., comprises the sequence ENLYFQG (SEQ ID NO: 7810))), an N-terminal NLS (e.g., an SV40 NLS; SEQ ID NO: 6612), and a C-terminal NLS (e.g., an SV40 NLS; SEQ ID NO: 6612) (e.g., comprises from N- to C-terminal His tag-TEV-NLS-Cas9-NLS). In any of the aforementioned embodiments the Cas9 has the sequence of SEQ ID NO: 6611. Alternatively, in any of the aforementioned embodiments, the Cas9 has a sequence of a Cas9 variant of SEQ ID NO: 6611, e.g., as described herein. In any of the aforementioned embodiments, the Cas9 molecule comprises a linker between the His tag and another portion of the molecule, e.g., a GGS linker. Amino acid sequences of exemplary Cas9 molecules described above are provided below. "iProt" identifiers match those in Figure 60.

iProt105026 (also referred to as iProt106154, iProt106331, iProt106545, and PID426303, depending on the preparation of the protein) (SEQ ID NO: 7821):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL
FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK
HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE
KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLKDDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
KLESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKRPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI

REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL SQLGGDSRAD PKKKRKVHHH HHH

iProt106518 (SEQ ID NO: 7822):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL FDSGETAEAT RLKRTARRRY TRRKNRILYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI EEFDSVEISG VEDRFNASLG TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR LSDYDVDHIV PQSFLKDDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP KLESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKRPL IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL SQLGGDSRAD PKKKRKVHHH HHH

iProt106519 (SEQ ID NO: 7823):

MGSSHHHHHH HHENLYFQGS MDKKYSIGLD IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL HEHIANLAGS PAIKKGILQT VKVVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVDH IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS KLVSDFRKDF QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGDGG GSPKKKRKV

iProt106520 (SEQ ID NO: 7824):

MAHHHHHHGG SPKKKRKVDK KYSIGLDIGT NSVGWAVITD EYKVPSKKFK
VLGNTDRHSI KKNLIGALLF DSGETAEATR LKRTARRRYT RRKNRICYLQ EIFSNEMAKV
DDSFFHRLEE SFLVEEDKKH ERHPIFGNIV DEVAYHEKYP TIYHLRKKLV DSTDKADLRL
IYLALAHMIK FRGHFLIEGD LNPDNSDVDK LFIQLVQTYN QLFEENPINA SGVDAKAILS
ARLSKSRRLE NLIAQLPGEK KNGLFGNLIA LSLGLTPNFK SNFDLAEDAK LQLSKDTYDD
DLDNLLAQIG DQYADLFLAA KNLSDAILLS DILRVNTEIT KAPLSASMIK RYDEHHQDLT
LLKALVRQQL PEKYKEIFFD QSKNGYAGYI DGGASQEEFY KFIKPILEKM DGTEELLVKL
NREDLLRKQR TFDNGSIPHQ IHLGELHAIL RRQEDFYPFL KDNREKIEKI LTFRIPYYVG
PLARGNSRFA WMTRKSEETI TPWNFEEVVD KGASAQSFIE RMTNFDKNLP NEKVLPKHSL
LYEYFTVYNE LTKVKYVTEG MRKPAFLSGE QKKAIVDLLF KTNRKVTVKQ LKEDYFKKIE
CFDSVEISGV EDRFNASLGT YHDLLKIIKD KDFLDNEENE DILEDIVLTL TLFEDREMIE
ERLKTYAHLF DDKVMKQLKR RRYTGWGRLS RKLINGIRDK QSGKTILDFL KSDGFANRNF
MQLIHDDSLT FKEDIQKAQV SGQGDSLHEH IANLAGSPAI KKGILQTVKV VDELVKVMGR
HKPENIVIEM ARENQTTQKG QKNSRERMKR IEEGIKELGS QILKEHPVEN TQLQNEKLYL
YYLQNGRDMY VDQELDINRL SDYDVDHIVP QSFLKDDSID NKVLTRSDKN RGKSDNVPSE
EVVKKMKNYW RQLLNAKLIT QRKFDNLTKA ERGGLSELDK AGFIKRQLVE
TRQITKHVAQ ILDSRMNTKY DENDKLIREV KVITLKSKLV SDFRKDFQFY KVREINNYHH
AHDAYLNAVV GTALIKKYPK LESEFVYGDY KVYDVRKMIA KSEQEIGKAT
AKYFFYSNIM NFFKTEITLA NGEIRKRPLI ETNGETGEIV WDKGRDFATV RKVLSMPQVN
IVKKTEVQTG GFSKESILPK RNSDKLIARK KDWDPKKYGG FDSPTVAYSV LVVAKVEKGK
SKKLKSVKEL LGITIMERSS FEKNPIDFLE AKGYKEVKKD LIIKLPKYSL FELENGRKRM
LASAGELQKG NELALPSKYV NFLYLASHYE KLKGSPEDNE QKQLFVEQHK HYLDEIIEQI
SEFSKRVILA DANLDKVLSA YNKHRDKPIR EQAENIIHLF TLTNLGAPAA FKYFDTTIDR
KRYTSTKEVL DATLIHQSIT GLYETRIDLS QLGGDSRADP KKKRKV

iProt106521 (SEQ ID NO: 7825):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL
FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK
HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE
KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLKDDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
KLESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKRPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI
REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL
SQLGGDSRAD HHHHHH

iProt106522 (SEQ ID NO: 7826):

MAHHHHHHGG SDKKYSIGLD IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL HEHIANLAGS PAIKKGILQT VKVVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVDH IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS KLVSDFRKDF QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGDSR ADPKKKRKV

iProt106658 (SEQ ID NO: 7827):

MGSSHHHHHH HHENLYFQGS MDKKYSIGLD IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL HEHIANLAGS PAIKKGILQT VKVVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVDH IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS KLVSDFRKDF QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGDGG GSPKKKRKV

iProt106745 (SEQ ID NO: 7828):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK

HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE
KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLKDDSI DNAVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
KLESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKRPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI
REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL
SQLGGDSRAD PKKKRKVHHH HHH

iProt106746 (SEQ ID NO: 7829):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL
FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK
HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE
KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEALY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLKDDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
ALESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKAPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI
REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL
SQLGGDSRAD PKKKRKVHHH HHH

iProt106747 (SEQ ID NO: 7830):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL
FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK
HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE

KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTNFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGRL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMQLIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLADDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRQITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
ALESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKAPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI
REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL
SQLGGDSRAD PKKKRKVHHH HHH

iProt106884 (SEQ ID NO: 7831):

MAPKKKRKVD KKYSIGLDIG TNSVGWAVIT DEYKVPSKKF KVLGNTDRHS IKKNLIGALL
FDSGETAEAT RLKRTARRRY TRRKNRICYL QEIFSNEMAK VDDSFFHRLE ESFLVEEDKK
HERHPIFGNI VDEVAYHEKY PTIYHLRKKL VDSTDKADLR LIYLALAHMI KFRGHFLIEG
DLNPDNSDVD KLFIQLVQTY NQLFEENPIN ASGVDAKAIL SARLSKSRRL ENLIAQLPGE
KKNGLFGNLI ALSLGLTPNF KSNFDLAEDA KLQLSKDTYD DDLDNLLAQI GDQYADLFLA
AKNLSDAILL SDILRVNTEI TKAPLSASMI KRYDEHHQDL TLLKALVRQQ LPEKYKEIFF
DQSKNGYAGY IDGGASQEEF YKFIKPILEK MDGTEELLVK LNREDLLRKQ RTFDNGSIPH
QIHLGELHAI LRRQEDFYPF LKDNREKIEK ILTFRIPYYV GPLARGNSRF AWMTRKSEET
ITPWNFEEVV DKGASAQSFI ERMTAFDKNL PNEKVLPKHS LLYEYFTVYN ELTKVKYVTE
GMRKPAFLSG EQKKAIVDLL FKTNRKVTVK QLKEDYFKKI ECFDSVEISG VEDRFNASLG
TYHDLLKIIK DKDFLDNEEN EDILEDIVLT LTLFEDREMI EERLKTYAHL FDDKVMKQLK
RRRYTGWGAL SRKLINGIRD KQSGKTILDF LKSDGFANRN FMALIHDDSL TFKEDIQKAQ
VSGQGDSLHE HIANLAGSPA IKKGILQTVK VVDELVKVMG RHKPENIVIE MARENQTTQK
GQKNSRERMK RIEEGIKELG SQILKEHPVE NTQLQNEKLY LYYLQNGRDM YVDQELDINR
LSDYDVDHIV PQSFLKDDSI DNKVLTRSDK NRGKSDNVPS EEVVKKMKNY
WRQLLNAKLI TQRKFDNLTK AERGGLSELD KAGFIKRQLV ETRAITKHVA QILDSRMNTK
YDENDKLIRE VKVITLKSKL VSDFRKDFQF YKVREINNYH HAHDAYLNAV VGTALIKKYP
KLESEFVYGD YKVYDVRKMI AKSEQEIGKA TAKYFFYSNI MNFFKTEITL ANGEIRKRPL
IETNGETGEI VWDKGRDFAT VRKVLSMPQV NIVKKTEVQT GGFSKESILP KRNSDKLIAR
KKDWDPKKYG GFDSPTVAYS VLVVAKVEKG KSKKLKSVKE LLGITIMERS SFEKNPIDFL
EAKGYKEVKK DLIIKLPKYS LFELENGRKR MLASAGELQK GNELALPSKY VNFLYLASHY
EKLKGSPEDN EQKQLFVEQH KHYLDEIIEQ ISEFSKRVIL ADANLDKVLS AYNKHRDKPI
REQAENIIHL FTLTNLGAPA AFKYFDTTID RKRYTSTKEV LDATLIHQSI TGLYETRIDL
SQLGGDSRAD PKKKRKVHHH HHH

Nucleic Acids Encoding Cas9 Molecules

Nucleic acids encoding the Cas9 molecules, e.g., an active Cas9 molecule or an inactive Cas9 molecule are provided herein.

Exemplary nucleic acids encoding Cas9 molecules are described in Cong et al , SCIENCE 2013, 399(6121):819-823; Wang et al , CELL 2013, 153(4):910-918; Mali et al. , SCIENCE 2013, 399(6121):823-826; Jinek et al, SCIENCE 2012, 337(6096):816-821.

In an embodiment, a nucleic acid encoding a Cas9 molecule can be a synthetic nucleic acid sequence. For example, the synthetic nucleic acid molecule can be chemically modified, e.g., as described in Section XIII. In an embodiment, the Cas9 mRNA has one or more of, e.g., all of the following properties: it is capped, polyadenylated, substituted with 5-methylcytidine and/or pseudouridine.

In addition or alternatively, the synthetic nucleic acid sequence can be codon optimized, e.g., at least one non-common codon or less-common codon has been replaced by a common codon. For example, the synthetic nucleic acid can direct the synthesis of an optimized messenger mRNA, e.g., optimized for expression in a mammalian expression system, e.g., described herein.

Provided below is an exemplary codon optimized nucleic acid sequence encoding a Cas9 molecule of S. pyogenes.

atggataaaa agtacagcat cgggctggac atcggtacaa actcagtggg gtgggccgtg 60

attacggacg agtacaaggt accctccaaa aaatttaaag tgctgggtaa cacggacaga 120

cactctataa agaaaaatct tattggagcc ttgctgttcg actcaggcga gacagccgaa 180

gccacaaggt tgaagcggac cgccaggagg cggtatacca ggagaaagaa ccgcatatgc 240

tacctgcaag aaatcttcag taacgagatg gcaaaggttg acgatagctt tttccatcgc 300

ctggaagaat cctttcttgt tgaggaagac aagaagcacg aacggcaccc catctttggc 360

aatattgtcg acgaagtggc atatcacgaa aagtacccga ctatctacca cctcaggaag 420

aagctggtgg actctaccga taaggcggac ctcagactta tttatttggc actcgcccac 480

atgattaaat ttagaggaca tttcttgatc gagggcgacc tgaacccgga caacagtgac 540

gtcgataagc tgttcatcca acttgtgcag acctacaatc aactgttcga agaaaaccct 600

ataaatgctt caggagtcga cgctaaagca atcctgtccg cgcgcctctc aaaatctaga 660

agacttgaga atctgattgc tcagttgccc ggggaaaaga aaaatggatt gtttggcaac 720

ctgatcgccc tcagtctcgg actgacccca aatttcaaaa gtaacttcga cctggccgaa 780

gacgctaagc tccagctgtc caaggacaca tacgatgacg acctcgacaa tctgctggcc 840

cagattgggg atcagtacgc cgatctcttt ttggcagcaa agaacctgtc cgacgccatc 900

ctgttgagcg atatcttgag agtgaacacc gaaattacta aagcacccct tagcgcatct 960

atgatcaagc ggtacgacga gcatcatcag gatctgaccc tgctgaaggc tcttgtgagg 1020

caacagctcc ccgaaaaata caaggaaatc ttctttgacc agagcaaaaa cggctacgct 1080

ggctatatag atggtggggc agtcaggag gaattctata aattcatcaa gcccattctc 1140

gagaaaatgg acggcacaga ggagttgctg gtcaaactta acagggagga cctgctgcgg 1200

aagcagcgga cctttgacaa cgggtctatc ccccaccaga ttcatctggg cgaactgcac 1260

gcaatcctga ggaggcagga ggatttttat cctttcctta aagataaccg cgagaaaata 1320

gaaaagattc ttacattcag gatcccgtac tacgtgggac ctctcgcccg gggcaattca 1380

cggtttgcct ggatgacaag gaagtcagag gagactatta caccttggaa cttcgaagaa 1440

gtggtggaca agggtgcatc tgcccagtct ttcatcgagc ggatgacaaa ttttgacaag 1500

aacctcccta atgagaaggt gctgcccaaa cattctctgc tctacgagta ctttaccgtc 1560

tacaatgaac tgactaaagt caagtacgtc accgagggaa tgaggaagcc ggcattcctt 1620

agtggagaac agaagaaggc gattgtagac ctgttgttca agaccaacag gaaggtgact 1680

gtgaagcaac ttaaagaaga ctactttaag aagatcgaat gttttgacag tgtggaaatt 1740

tcaggggttg aagaccgctt caatgcgtca ttggggactt accatgatct tctcaagatc 1800

ataaaggaca aagacttcct ggacaacgaa gaaaatgagg atattctcga agacatcgtc 1860

ctcaccctga ccctgttcga agacagggaa atgatagaag agcgcttgaa aacctatgcc 1920

cacctcttcg acgataaagt tatgaagcag ctgaagcgca ggagatacac aggatgggga 1980

agattgtcaa ggaagctgat caatggaatt agggataaac agagtggcaa gaccatactg 2040

gatttcctca aatctgatgg cttcgccaat aggaacttca tgcaactgat tcacgatgac 2100

tctcttacct tcaaggagga cattcaaaag gctcaggtga gcgggcaggg agactccctt 2160

catgaacaca tcgcgaattt ggcaggttcc cccgctatta aaaagggcat ccttcaaact 2220

gtcaaggtgg tggatgaatt ggtcaaggta atgggcagac ataagccaga aaatattgtg 2280

atcgagatgg cccgcgaaaa ccagaccaca cagaagggcc agaaaaatag tagagagcgg 2340

atgaagagga tcgaggaggg catcaaagag ctgggatctc agattctcaa agaacacccc 2400

gtagaaaaca cacagctgca gaacgaaaaa ttgtacttgt actatctgca gaacggcaga 2460

gacatgtacg tcgaccaaga cttgatatt aatagactgt ccgactatga cgtagaccat 2520

atcgtgcccc agtccttcct gaaggacgac tccattgata acaaagtctt gacaagaagc 2580

gacaagaaca ggggtaaaag tgataatgtg cctagcgagg aggtggtgaa aaaaatgaag 2640

aactactggc gacagctgct taatgcaaag ctcattacac aacggaagtt cgataatctg 2700

acgaaagcag agagaggtgg cttgtctgag ttggacaagg cagggtttat taagcggcag 2760

ctggtggaaa ctaggcagat cacaaagcac gtggcgcaga ttttggacag ccggatgaac 2820

acaaaatacg acgaaaatga taaactgata cgagaggtca aagttatcac gctgaaaagc 2880

aagctggtgt ccgatttttcg gaaagacttc cagttctaca aagttcgcga gattaataac 2940

taccatcatg ctcacgatgc gtacctgaac gctgttgtcg ggaccgcctt gataaagaag 3000

tacccaaagc tggaatccga gttcgtatac ggggattaca aagtgtacga tgtgaggaaa 3060

atgatagcca agtccgagca ggagattgga aaggccacag ctaagtactt cttttattct 3120

aacatcatga attttttaa gacggaaatt accctggcca acggagagat cagaaagcgg 3180

ccccttatag agacaaatgg tgaaacaggt gaaatcgtct gggataaggg cagggatttc 3240

gctactgtga ggaaggtgct gagtatgcca caggtaaata tcgtgaaaaa aaccgaagta 3300

cagaccggag gattttccaa ggaaagcatt ttgcctaaaa gaaactcaga caagctcatc 3360

gcccgcaaga aagattggga ccctaagaaa tacgggggat ttgactcacc caccgtagcc 3420

tattctgtgc tggtggtagc taaggtggaa aaaggaaagt ctaagaagct gaagtccgtg 3480

aaggaactct tgggaatcac tatcatggaa agatcatcct ttgaaaagaa ccctatcgat 3540

ttcctggagg ctaagggtta caaggaggtc aagaaagacc tcatcattaa actgccaaaa 3600

tactctctct tcgagctgga aaatggcagg aagagaatgt tggccagcgc cggagagctg 3660

caaaagggaa acgagcttgc tctgccctcc aaatatgtta attttctcta tctcgcttcc 3720

cactatgaaa agctgaaagg gtctcccgaa gataacgagc agaagcagct gttcgtcgaa 3780

cagcacaagc actatctgga tgaaataatc gaacaaataa gcgagttcag caaaagggtt 3840

atcctggcgg atgctaattt ggacaaagta ctgtctgctt ataacaagca ccgggataag 3900

cctattaggg aacaagccga gaatataatt cacctcttta cactcacgaa tctcggagcc 3960

cccgccgcct tcaaatactt tgatacgact atcgaccgga aacggtatac cagtaccaaa 4020

gaggtcctcg atgccaccct catccaccag tcaattactg gcctgtacga aacacggatc 4080

gacctctctc aactgggcgg cgactag 4107

(SEQ ID NO: 6628)

If the above Cas9 sequences are fused with a peptide or polypeptide at the C-terminus (e.g., an inactive Cas9 fused with a transcription repressor at the C-terminus), it is understood that the stop codon will be removed.

VI. Functional Analysis of Candidate Molecules

Candidate Cas9 molecules, candidate gRNA molecules, candidate Cas9 molecule/gRNA molecule complexes, can be evaluated by art-known methods or as described herein. For example, exemplary methods for evaluating the endonuclease activity of Cas9 molecule are described, e.g., in Jinek el al., SCIENCE 2012; 337(6096):8 16-821.

VII. Template Nucleic Acids (For Introduction of Nucleic Acids)

The term "template nucleic acid" or “donor template” as used herein refers to a nucleic acid to be inserted at or near a target sequence that has been modified, e.g., cleaved, by a CRISPR system of the present invention. In an embodiment, nucleic acid sequence at or near the target sequence is modified to have some or all of the sequence of the template nucleic acid, typically at or near cleavage site(s). In an embodiment, the template nucleic acid is single stranded. In an alternate embodiment, the template nucleic acid is double stranded. In an embodiment, the template nucleic acid is DNA, e.g., double stranded DNA. In an alternate embodiment, the template nucleic acid is single stranded DNA.

In embodiments, the template nucleic acid comprises sequence encoding a globin protein, e.g., a beta globin, e.g., comprises a beta globin gene. In an embodiment, the beta globin encoded by the nucleic acid comprises one or more mutations, e.g., anti-sickling mutations. In an embodiment, the beta globin encoded by the nucleic acid comprises the mutation T87Q. In an embodiment, the beta globin encoded by the nucleic acid comprises the mutation G16D. In an embodiment, the beta globin encoded by the nucleic acid comprises the mutation E22A. In an embodiment, the beta globin gene comprises the mutations G16D, E22A and T87Q. In embodiments, the template nucleic acid further comprises one or more regulatory elements, e.g., a promoter (e.g., a human beta globin promoter), a 3’ enhancer, and/or at least a portion of a globin locus control regoin (e.g., one or more DNAseI hypersensitivity sites (e.g., HS2, HS3 and/or HS4 of the human globin locus)).

In other embodiments, the template nucleic acid comprises sequence encoding a gamma globin, e.g., comprises a gamma globin gene. In embodiments, the template nucleic acid comprises sequence encoding more than one copy of a gamma globin protein, e.g., comprises two or more, e.g., two, gamma globin gene sequences. In embodiments, the template nucleic acid further comprises one or more regulatory elements, e.g., a promotor and/or enhancer.

In an embodiment, the template nucleic acid alters the structure of the target position by participating in a homology directed repair event. In an embodiment, the template nucleic acid alters the sequence of the target position. In an embodiment, the template nucleic acid results in the incorporation of a modified, or non-naturally occurring base into the target nucleic acid.

Mutations in a gene or pathway described herein may be corrected using one of the approaches discussed herein. In an embodiment, a mutation in a gene or pathway described herein is corrected by homology directed repair (HDR) using a template nucleic acid. In an embodiment, a mutation in a gene or pathway described herein is corrected by homologous recombination (HR) using a template nucleic acid. In an embodiment, a mutation in a gene or pathway described herein is corrected by Non-Homologous End Joining (NHEJ) repair using a template nucleic acid. In other embodiments, nucleic acid encoding molecules of interest may be inserted at or near a site modified by a CRISPR system of the present invention. In embodiments, the template nucleic acid comprises regulatory elements, e.g., one or more promotors and/or enhancers, operably linked to the nucleic acid sequence encoding a molecule of interest, e.g., as described herein.

HDR or HR Repair and Template Nucleic Acids

As described herein, nuclease-induced homology directed repair (HDR) or homologous recombination (HR) can be used to alter a target sequence and correct (e.g., repair or edit) a mutation in the genome. While not wishing to be bound by theory, it is believed that alteration of the target sequence occurs by repair based on a donor template or template nucleic acid. For example, the donor template or the template nucleic acid provides for alteration of the target sequence. It is contemplated that a plasmid donor or linear double stranded template can be used as a template for homologous recombination. It is further contemplated that a single stranded donor template can be used as a template for alteration of the target sequence by alternate methods of homology directed repair (e.g., single strand annealing) between the target sequence and the donor template. Donor template-effected alteration of a target sequence may depend on cleavage by a Cas9 molecule. Cleavage by Cas9 can comprise a double strand break, one single strand break, or two single strand breaks.

In an embodiment, a mutation can be corrected by either a single double-strand break or two single strand breaks. In an embodiment, a mutation can be corrected by providing a template and a CRISPR/Cas9 system that creates (1) one double strand break, (2) two single strand breaks, (3) two double stranded breaks with a break occurring on each side of the target sequence, (4) one double stranded break and two single strand breaks with the double strand break and two single strand breaks occurring on each side of the target sequence, (5) four single stranded breaks with a pair of single stranded breaks occurring on each side of the target sequence, or (6) one single strand break.

Double strand break mediated correction

In an embodiment, double strand cleavage is effected by a Cas9 molecule having cleavage activity associated with an HNH-like domain and cleavage activity associated with a RuvC-like domain, e.g., an N-terminal RuvC-like domain, e.g., a wild type Cas9. Such embodiments require only a single gRNA.

Single strand break mediated correction

In other embodiments, two single strand breaks, or nicks, are effected by a Cas9 molecule having nickase activity, e.g., cleavage activity associated with an HNH-like domain or cleavage activity associated with an N-terminal RuvC-like domain. Such embodiments require two gRNAs, one for placement of each single strand break. In an embodiment, the Cas9 molecule having nickase activity cleaves the strand to which the gRNA hybridizes, but not the strand that is complementary to the strand to which the gRNA hybridizes. In an embodiment, the Cas9 molecule having nickase activity does not cleave the strand to which the gRNA hybridizes, but rather cleaves the strand that is complementary to the strand to which the gRNA hybridizes.

In an embodiment, the nickase has HNH activity, e.g., a Cas9 molecule having the RuvC activity inactivated, e.g., a Cas9 molecule having a mutation at D10, e.g., the D10A mutation. D10A inactivates RuvC; therefore, the Cas9 nickase has (only) HN H activity and will cut on the strand to which the gRNA hybridizes (e.g., the complementary strand, which does not have the NGG PAM on it). In other embodiments, a Cas9 molecule having an H840, e.g., an H840A, mutation can be used as a nickase. H840A inactivates HNH; therefore, the Cas9 nickase has (only) RuvC activity and cuts on the non-complementary strand (e.g., the strand that has the NGG PAM and whose sequence is identical to the gRNA).

In an embodiment, in which a nickase and two gRNAs are used to position two single strand nicks, one nick is on the + strand and one nick is on the - strand of the target nucleic acid. The PAMs are outwardly facing. The gRNAs can be selected such that the gRNAs are separated by, from about 0-50, 0- 100, or 0-200 nucleotides. In an embodiment, there is no overlap between the target sequence that is complementary to the targeting domains of the two gRNAs. In an embodiment, the gRNAs do not overlap and are separated by as much as 50, 100, or 200 nucleotides. In an embodiment, the use of two gRNAs can increase specificity, e.g., by decreasing off-target binding (Ran el cil., CELL 2013).

In an embodiment, a single nick can be used to induce HDR. It is contemplated herein that a single nick can be used to increase the ratio of HDR, HR or NHEJ at a given cleavage site.

Placement of the double strand break or a single strand break relative to target position

The double strand break or single strand break in one of the strands should be sufficiently close to target position such that correction occurs. In an embodiment, the distance is not more than 50, 100, 200, 300, 350 or 400 nucleotides. While not wishing to be bound by theory, it is believed that the

break should be sufficiently close to target position such that the break is within the region that is subject to exonuclease-mediated removal during end resection. If the distance between the target position and a break is too great, the mutation may not be included in the end resection and, therefore, may not be corrected, as donor sequence may only be used to correct sequence within the end resection region.

In an embodiment, in which a gRNA (unimolecular (or chimeric) or modular gRNA) and Cas9 nuclease induce a double strand break for the purpose of inducing HDR- or HR-mediated correction, the cleavage site is between 0-200 bp (e.g., 0 to 175, 0 to 150, 0 to 125, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 25 to 200, 25 to 175, 25 to 150, 25 to 125, 25 to 100, 25 to 75, 25 to 50, 50 to 200, 50 to 175, 50 to 150, 50 to 125, 50 to 100, 50 to 75, 75 to 200, 75 to 175, 75 to 150, 75 to 1 25, 75 to 100 bp) away from the target position. In an embodiment, the cleavage site is between 0- 100 bp (e.g., 0 to 75, 0 to 50, 0 to 25, 25 to 100, 25 to 75, 25 to 50, 50 to 100, 50 to 75 or 75 to 100 bp) away from the target position.

In an embodiment, in which two gRNAs (independently, unimolecular (or chimeric) or modular gRNA) complexing with Cas9 nickases induce two single strand breaks for the purpose of inducing HDR-mediated correction, the closer nick is between 0-200 bp (e.g., 0 to 175, 0 to 150, 0 to 125, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 25 to 200, 25 to 175, 25 to 150, 25 to 125, 25 to 100, 25 to 75, 25 to 50, 50 to 200, 50 to 175, 50 to 150, 50 to 125, 50 to 100, 50 to 75, 75 to 200, 75 to 175, 75 to 150, 75 to 125, 75 to 100 bp) away from the target position and the two nicks will ideally be within 25-55 bp of each other (e.g., 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 30 to 55, 30 to 50, 30 to 45, 30 to 40, 30 to 35, 35 to 55, 35 to 50, 35 to 45, 35 to 40, 40 to 55, 40 to 50, 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20, 10 or 5 bp away from each other). In an embodiment, the cleavage site is between 0- 100 bp (e.g., 0 to 75, 0 to 50, 0 to 25, 25 to 100, 25 to 75, 25 to 50, 50 to 100, 50 to 75 or 75 to 100 bp) away from the target position.

In one embodiment, two gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double-strand break on both sides of a target position. In an alternate embodiment, three gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double strand break (i.e., one gRNA complexes with a Cas9 nuclease) and two single strand breaks or paired single stranded breaks (i.e., two gRNAs complex with Cas9 nickases) on either side of the target position (e.g., the first gRNA is used to target upstream (i.e., 5') of the target positionand the second gRNA is used to target downstream (i.e., 3') of the target position). In another embodiment, four gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to generate two pairs of single stranded breaks (i.e., two pairs of two gRNAs complex with Cas9 nickases) on either side of the target position (e.g., the first gRNA is used to target upstream (i.e., 5') of the target position and the second gRNA is used to target downstream

(i.e., 3') of the target position). The double strand break(s) or the closer of the two single strand nicks in a pair will ideally be within 0-500 bp of the target position (e.g., no more than 450, 400, 350, 300, 250, 200, 150, l00, 50 or 25 bp from the target position). When nickases are used, the two nicks in a pair are within 25-55 bp of each other (e.g., between 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 50 to 55, 45 to 55, 40 to 55, 35 to 55, 30 to 55, 30 to 50, 35. to 50, 40 to 50 , 45 to 50, 35 to 45, or 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20 or 10 bp).

In one embodiment, two gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double-strand break on both sides of a target position. In an alternate embodiment, three gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double strand break (i.e., one gRNA complexes with a Cas9 nuclease) and two single strand breaks or paired single stranded breaks (i.e., two gRNAs complex with Cas9 nickases) on two target sequences (e.g., the first gRNA is used to target an upstream (i.e., 5') target sequence and the second gRNA is used to target a downstream (i.e., 3') target sequence of an insertion site. In another embodiment, four gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to generate two pairs of single stranded breaks (i.e., two pairs of two gRNAs complex with Cas9 nickases) on either side of an insertion site (e.g., the first gRNA is used to target an upstream (i.e., 5') target sequence described herein, and the second gRNA is used to target a downstream (i.e., 3') target sequence described herein). The double strand break(s) or the closer of the two single strand nicks in a pair will ideally be within 0-500 bp of the target position (e.g., no more than 450, 400, 350, 300, 250, 200, 150, 100, 50 or 25 bp from the target position). When nickases are used, the two nicks in a pair are within 25-55 bp of each other (e.g., between 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 50 to 55, 45 to 55, 40 to 55, 35 to 55, 30 to 55, 30 to 50, 35 to 50, 40 to 50 , 45 to 50, 35 to 45, or 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20 or 10 bp).

Length of the homology arms

The homology arm should extend at least as far as the region in which end resection may occur, e.g., in order to allow the resected single stranded overhang to find a complementary region within the donor template. The overall length could be limited by parameters such as plasmid size or viral packaging limits. In an embodiment, a homology arm does not extend into repeated elements, e.g., ALU repeats, LINE repeats. A template may have two homology arms of the same or different lengths.

Exemplary homology arm lengths include at least 25, 50, 100, 250, 500, 750 or 1000 nucleotides.

Target position, as used herein, refers to a site on a target nucleic acid (e.g., the chromosome) that is modified by a Cas9 molecule-dependent process. For example, the target position can be a modified

Cas9 molecule cleavage of the target nucleic acid and template nucleic acid directed modification, e.g., correction, of the target position. In an embodiment, a target position can be a site between two nucleotides, e.g., adjacent nucleotides, on the target nucleic acid into which one or more nucleotides is added. The target position may comprise one or more nucleotides that are altered, e.g., corrected, by a template nucleic acid. In an embodiment, the target position is within a target sequence (e.g., the sequence to which the gRN A binds). In an embodiment, a target position is upstream or downstream of a target sequence (e.g., the sequence to which the gRNA binds).

Typically, the template sequence undergoes a breakage mediated or catalyzed recombination with the target sequence. In an embodiment, the template nucleic acid includes sequence that corresponds to a site on the target sequence that is cleaved by a Cas9 mediated cleavage event. In an embodiment, the template nucleic acid includes sequence that corresponds to both, a first site on the target sequence that is cleaved in a first Cas9 mediated event, and a second site on the target sequence that is cleaved in a second Cas9 mediated event.

In an embodiment, the template nucleic acid can include sequence which results in an alteration in the coding sequence of a translated sequence, e.g., one which results in the substitution of one amino acid for another in a protein product, e.g., transforming a mutant allele into a wild type allele, transforming a wild type allele into a mutant allele, and/or introducing a stop codon, insertion of an amino acid residue, deletion of an amino acid residue, or a nonsense mutation.

In other embodiments, the template nucleic acid can include sequence which results in an alteration in a non-coding sequence, e.g., an alteration in an exon or in a 5' or 3' non-translated or non-transcribed region. Such alterations include an alteration in a control element, e.g., a promoter, enhancer, and an alteration in a cis-acting or trans-acting control element.

The template nucleic acid can include sequence which, when integrated, results in:

decreasing the activity of a positive control element;

increasing the activity of a positive control element;

decreasing the activity of a negative control element;

increasing the activityof a negative control element;

decreasing the expression of a gene;

increasing the expression of a gene;

increasing resistance to a disorder or disease;

increasing resistance to viral entry;

correcting a mutation or altering an unwanted amino acid residue;

conferring, increasing, abolishing or decreasing a biological property of a gene product, e.g., increasing the enzymatic activity of an enzyme, or increasing the ability of a gene product to interact with another molecule.

The template nucleic acid can include sequence which results in:

a change in sequence of 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12 or more nucleotides of the target sequence.

In an embodiment, the template nucleic acid is 20+/- 10, 30+/- 10, 40+/- 10, 50+/- 10, 60+/- 10, 70+/- 10, 80+/- 10, 90+/- 10, 100+/- 10, 1 10+/- 10, 120+/- 10, 130+/- 10, 140+/- 10, 150+/- 10, 160+/- 10, 170+/- 10, 1 80+/- 10, 190+/- 10, 200+/- 10, 210+/-10, 220+/- 10, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-2000, 2000-3000 or more than 3000 nucleotides in length.

A template nucleic acid comprises the following components:

[5' homology arm]-[insertion sequence]-[3' homology arm].

The homology arms provide for recombination into the chromosome, which can replace the undesired element, e.g., a mutation or signature, with the replacement sequence. In an embodiment, the homology arms flank the most distal cleavage sites.

In an embodiment, the 3' end of the 5' homology arm is the position next to the 5' end of the replacement sequence. In an embodiment, the 5' homology arm can extend at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, or 2000 nucleotides 5' from the 5' end of the replacement sequence.

In an embodiment, the 5' end of the 3' homology arm is the position next to the 3' end of the replacement sequence. In an embodiment, the 3' homology arm can extend at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, or 2000 nucleotides 3' from the 3' end of the replacement sequence.

It is contemplated herein that one or both homology arms may be shortened to avoid including certain sequence repeat elements, e.g., Alu repeats, LINE elements. For example, a 5' homology arm may be shortened to avoid a sequence repeat element. In other embodiments, a 3' homology arm may be shortened to avoid a sequence repeat element. In some embodiments, both the 5' and the 3' homology arms may be shortened to avoid including certain sequence repeat elements.

It is contemplated herein that template nucleic acids for correcting a mutation may designed for use as a single-stranded oligonucleotide (ssODN). When using a ssODN, 5' and 3' homology arms may range up to about 200 base pairs (bp) in length, e.g., at least 25, 50, 75, 100, 125, 150, 175, or 200 bp

in length. Longer homology arms are also contemplated for ssODNs as improvements in oligonucleotide synthesis continue to be made.

NHEJ Approaches for Gene Targeting

As described herein, nuclease-induced non-homologous end-joining (NHEJ) can be used to target gene-specific knockouts. Nuclease-induced NHEJ can also be used to remove (e.g., delete) sequence in a gene of interest.

While not wishing to be bound by theory, it is believed that, in an embodiment, the genomic alterations associated with the methods described herein rely on nuclease-induced NHEJ and the error-prone nature of the NHEJ repair pathway. NHEJ repairs a double-strand break in the DNA by joining together the two ends; however, generally, the original sequence is restored only if two compatible ends, exactly as they were formed by the double-strand break, are perfectly ligated. The DNA ends of the double-strand break are frequently the subject of enzymatic processing, resulting in the addition or removal of nucleotides, at one or both strands, prior to rejoining of the ends. This results in the presence of insertion and/or deletion (indel) mutations in the DNA sequence at the site of the NHEJ repair. Two-thirds of these mutations may alter the reading frame and, therefore, produce a non-functional protein. Additionally, mutations that maintain the reading frame, but which insert or delete a significant amount of sequence, can destroy functionality of the protein. This is locus dependent as mutations in critical functional domains are likely less tolerable than mutations in non-critical regions of the protein.

The indel mutations generated by NHEJ are unpredictable in nature; however, at a given break site certain indel sequences are favored and are over represented in the population. The lengths of deletions can vary widely; most commonly in the 1 -50 bp range, but they can easily reach greater than 100-200 bp. Insertions tend to be shorter and often include short duplications of the sequence immediately surrounding the break site. However, it is possible to obtain large insertions, and in these cases, the inserted sequence has often been traced to other regions of the genome or to plasmid DNA present in the cells.

Because NHEJ is a mutagenic process, it can also be used to delete small sequence motifs as long as the generation of a specific final sequence is not required. If a double-strand break is targeted near to a short target sequence, the deletion mutations caused by the NHEJ repair often span, and therefore remove, the unwanted nucleotides. For the deletion of larger DNA segments, introducing two double-strand breaks, one on each side of the sequence, can result in NHEJ between the ends with removal of the entire intervening sequence. Both of these approaches can be used to delete specific DNA sequences; however, the error-prone nature of NHEJ may still produce indel mutations at the site of repair.

Both double strand cleaving Cas9 molecules and single strand, or nickase, Cas9 molecules can be used in the methods and compositions described herein to generate NHEJ- mediated indels. NHEJ-mediated indels targeted to the gene, e.g., a coding region, e.g., an early coding region of a gene of interest can be used to knockout (i.e., eliminate expression of) a gene of interest. For example, early coding region of a gene of interest includes sequence immediately following a transcription start site, within a first exon of the coding sequence, or within 500 bp of the transcription start site (e.g., less than 500, 450, 400, 350, 300, 250, 200, 150, 100 or 50 bp).

Placement of double strand or single strand breaks relative to the target position

In an embodiment, in which a gRNA and Cas9 nuclease generate a double strand break for the purpose of inducing NHEJ-mediated indels, a gRNA, e.g., a unimolecular (or chimeric) or modular gRNA molecule, is configured to position one double-strand break in close proximity to a nucleotide of the target position. In an embodiment, the cleavage site is between 0-500 bp away from the target position (e.g., less than 500, 400, 300, 200, 100, 50, 40, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 bp from the target position).

In an embodiment, in which two gRNAs complexing with Cas9 nickases induce two single strand breaks for the puipose of inducing NHEJ-mediated indels, two gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position two single-strand breaks to provide for NHEJ repair a nucleotide of the target position. In an embodiment, the gRNAs are configured to position cuts at the same position, or within a few nucleotides of one another, on different strands, essentially mimicking a double strand break. In an embodiment, the closer nick is between 0-30 bp away from the target position (e.g., less than 30, 25, 20, 1 , 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 bp from the target position), and the two nicks are within 25-55 bp of each other (e.g., between 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 50 to 55, 45 to 55, 40 to 55, 35 to 55, 30 to 55, 30 to 50, 35 to 50, 40 to 50 , 45 to 50, 35 to 45, or 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20 or 10 bp). In an embodiment, the gRNAs are configured to place a single strand break on either side of a nucleotide of the target position.

Both double strand cleaving Cas9 molecules and single strand, or nickase, Cas9 molecules can be used in the methods and compositions described herein to generate breaks both sides of a target position. Double strand or paired single strand breaks may be generated on both sides of a target position to remove the nucleic acid sequence between the two cuts (e.g., the region between the two breaks is deleted). In one embodiment, two gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to position a double-strand break on both sides of a target position (e.g., the first gRNA is used to target upstream (i.e., 5') of the mutation in a gene or pathway described herein, and the second gRNA is used to target downstream (i.e., 3') of the mutation in a gene or pathway described herein). In an alternate embodiment, three gRNAs, e.g., independently,

unimolecular (or chimeric) or modular gRNA, are configured to position a double strand break (i.e., one gRNA complexes with a Cas9 nuclease) and two single strand breaks or paired single stranded breaks (i.e., two gRNAs complex with Cas9 nickases) on either side of a target position (e.g., the fu st gRNA is used to target upstream (i.e., 5') of the mutation in a gene or pathway described herein, and the second gRNA is used to target downstream (i.e., 3') of the mutation in a gene or pathway described herein). In another embodiment, four gRNAs, e.g., independently, unimolecular (or chimeric) or modular gRNA, are configured to generate two pairs of single stranded breaks (i.e., two pairs of two gRNAs complex with Cas9 nickases) on either side of the target position (e.g., the first gRNA is used to target upstream (i.e., 5') of the mutation in a gene or pathway described herein, and the second gRNA is used to target downstream (i.e., 3') of the mutation in a gene or pathway described herein). The double strand break(s) or the closer of the two single strand nicks in a pair will ideally be within 0-500 bp of the target position (e.g., no more than 450, 400, 350, 300, 250, 200, 150, 100, 50 or 25 bp from the target position). When nickases are used, the two nicks in a pair are within 25-55 bp of each other (e.g., between 25 to 50, 25 to 45, 25 to 40, 25 to 35, 25 to 30, 50 to 55, 45 to 55, 40 to 55, 35 to 55, 30 to 55, 30 to 50, 35 to 50, 40 to 50 , 45 to 50, 35 to 45, or 40 to 45 bp) and no more than 100 bp away from each other (e.g., no more than 90, 80, 70, 60, 50, 40, 30, 20 or 10 bp).

In other embodiments, the insertion of template nucleic acid may be mediated by microhomology end joining (MMEJ). See, e.g., Saksuma et al., "MMEJ-assisted gene knock-in using TALENs and CRISPR-Cas9 with the PITCh systems." *Nature Protocols* **11**, 118–133 (2016) doi:10.1038/nprot.2015.140 Published online 17 December 2015, the contents of which are incorporated by reference in their entirety.

VIII. Systems Comprising More Than One gRNA Molecule

While not intending to be bound by theory, it has been surprisingly shown herein that the targeting of two target sequences (e.g., by two gRNA molecule/Cas9 molecule complexes which each induce a single- or double-strand break at or near their respective target sequences) located in close proximity on a continuous nucleic acid induces excision (e.g., deletion) of the nucleic acid sequence (or at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% of the nucleic acid sequence) located between the two target sequences. In some aspects, the present disclosure provides for the use of two or more gRNA molecules that comprise targeting domains targeting target sequences in close proximity on a continuous nucleic acid, e.g., a chromosome, e.g., a gene or gene locus, including its introns, exons and regulatory elements. The use may be, for example, by introduction of the two or more gRNA molecules, together with one or more Cas9 molecules (or nucleic acid encoding the two or more gRNA molecules and/or the one or more Cas9 molecules) into a cell.

In some aspects, the target sequences of the two or more gRNA molecules are located at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000,

4000, 5000, 6000, 7000, 8000, 9000, 10,000, 11,000, 12,000, 13,000, 14,000, or 15,000 nucleotides apart on a continuous nucleic acid, but not more than 25,000 nucleotides apart on a continuous nucleic acid. In an embodiment, the target sequences are located about 4000 nucleotides apart. In an embodiment, the target sequences are located about 6000 nucleotides apart.

In some aspects, the plurality of gRNA molecules each target sequences within the same gene or gene locus. In another aspect, the plurality of gRNA molecules each target sequences within 2 or more different genes.

In some aspects, the invention provides compositions and cells comprising a plurality, for example, 2 or more, for example, 2, gRNA molecules of the invention, wherein the plurality of gRNA molecules target sequences less than 15,000, less than 14,000, less than 13,000, less than 12,000, less than 11,000, less than 10,000, less than 9,000, less than 8,000, less than 7,000, less than 6,000, less than 5,000, less than 4,000, less than 3,000, less than 2,000, less than 1,000, less than 900, less than 800, less than 700, less than 600, less than 500, less than 400, less than 300, less than 200, less than 100, less than 90, less than 80, less than 70, less than 60, less than 50, less than 40, or less than 30 nucleotides apart. In an embodiment, the target sequences are on the same strand of duplex nulceic acid. In an embodiment, the target sequences are on different strands of duplex nucleic acid.

In one embodiment, the invention provides a method for excising (e.g., deleting) nucleic acid disposed between two gRNA binding sites disposed less than 25,000, less than 20,000, less than 15,000, less than 14,000, less than 13,000, less than 12,000, less than 11,000, less than 10,000, less than 9,000, less than 8,000, less than 7,000, less than 6,000, less than 5,000, less than 4,000, less than 3,000, less than 2,000, less than 1,000, less than 900, less than 800, less than 700, less than 600, less than 500, less than 400, less than 300, less than 200, less than 100, less than 90, less than 80, less than 70, less than 60, less than 50, less than 40, or less than 30 nucleotides apart on the same or different strands of duplex nucleic acid. In an embodiment, the method provides for deletion of more than 50%, more than 60%, more than 70%, more than 80%, more than 85%, more than 86%, more than 87%, more than 88%, more than 89%, more than 90%, more than 91%, more than 92%, more than 93%, more than 94%, more than 95%, more than 96%, more than 97%, more than 98%, more than 99%, or 100% of the nucleotides disposed between the PAM sites associated with each gRNA binding site. In embodiments, the deletion further comprises of one or more nucleotides within one or more of the PAM sites associated with each gRNA binding site. In embodiments, the deletion also comprises one or more nucleotides outside of the region between the PAM sites associated with each gRNA binding site.

In one aspect, the two or more gRNA molecules comprise targeting domains targeting target sequences flanking a gene regulatory element, e.g., a promotor binding site, an enhancer region, or a

repressor region, such that excision of the intervening sequence (or a portion of the intervening sequence) causes up- or down-regulation of a gene of interest.

In an embodiment, the two or more gRNA molecules include targeting domains comprising, e.g., consisting of, a targeting domain sequence of Table 1 or Table 5. In aspects, the two or more gRNA molecules comprise targeting domains that are complementary with sequences in the same gene. In aspects, the two or more gRNA molecules comprise targeting domains that are complementary with sequences of different genes. In an embodiment, the two or more gRNA molecules include targeting domains comprising, e.g., consisting of, a targeting domain sequence of Table 6. In an embodiment, the two or more gRNA molecules include targeting domains comprising, e.g., consisting of, a targeting domain sequence of Table 2, Table 7, Table 8 and/or Table 9. In aspects, the two or more gRNA molecules comprise targeting domains that are complementary with sequences in the same gene. In aspects, the two or more gRNA molecules comprise targeting domains that are complementary with sequences of different genes. In an embodiment, the two or more gRNA molecules are selected from the gRNA molecules of Table 7, Table 8 and/or Table 9. In an embodiment, the first and second gRNA molecules comprise targeting domains comprising, e.g., consisting of, targeting domain sequences selected from Tables 1-9, and are selected from different tables, e.g., and comprise targeting domains that are complementary with sequences of different genes.

In one aspect, the two or more gRNA molecules comprise targeting domains targeting target sequences flanking a gene regulatory element, e.g., a promotor binding site, an enhancer region, or a repressor region, such that excision of the intervening sequence (or a portion of the intervening sequence) causes up- or down-regulation of a gene of interest. By way of example, the two or more gRNA molecules comprise targeting domains targeting target sequences flanking a GATA1 binding site (or portion thereof) or TAL1 binding site (or portion thereof) of the erythroid enhancer region of the BCL11a gene (e.g., within the +55, +58 or +62 region). In other embodiments, the gRNA molecule or molecules do not result in a disruption of the GATA1 binding site or TAL1 binding site within the BCL11a Enhancer.

In an embodiment, the two or more gRNA molecules comprise targeting domains that comprise, e.g., consist of, targeting domains selected from Table 1. In an embodiment, the two or more gRNA molecules comprise targeting domains that comprise, e.g., consist of, targeting domains selected from Table 2. In an embodiment, the two or more gRNA molecules comprise targeting domains that comprise, e.g., consist of, targeting domains selected from Table 3. In an embodiment, the two or more gRNA molecules comprise targeting domains that comprise, e.g., consist of, targeting domains selected from Table 4. In an embodiment, the two or more gRNA molecules comprise targeting domains that comprise, e.g., consist of, targeting domains selected from Table 5. In an embodiment,

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Claims:

1. A gRNA molecule comprising a tracr and crRNA, wherein the crRNA comprises a targeting domain that is complementary with a target sequence of a BCL11A gene (e.g., a human BCL11a gene), a BCL11a enhancer (e.g., a human BCL11a enhancer), or a HFPH region (e.g., a human HPFH region).

2. A gRNA molecule of claim 1, wherein the target sequence is of the BCL11A gene, and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 1 to SEQ ID NO: 85 or SEQ ID NO: 400 to SEQ ID NO: 1231.

3. A gRNA molecule of claim 1, wherein the target sequence is of a BCL11a enhancer, and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 1232 to SEQ ID NO: 1499.

4. A gRNA molecule of claim 1, wherein the target sequence is of a BCL11a enhancer, and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 182 to SEQ ID NO: 277 or SEQ ID NO: 334 to SEQ ID NO: 341.

5. A gRNA molecule of claim 4, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 341, SEQ ID NO: 246, SEQ ID NO: 248, SEQ ID NO: 247, SEQ ID NO: 245, SEQ ID NO: 249, SEQ ID NO: 244, SEQ ID NO: 199, SEQ ID NO: 251, SEQ ID NO: 250, SEQ ID NO: 334, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 336, or SEQ ID NO: 337.

6. A gRNA molecule of claim 4, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 247, SEQ ID NO: 248, SEQ ID NO: 335, SEQ ID NO: 336, SEQ ID NO: 337, or SEQ ID NO: 338.

7. A gRNA molecule of claim 4, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 248 or SEQ ID NO: 338.

8. A gRNA molecule of claim 4, wherein the targeting domain comprises, e.g., consists of, SEQ ID NO: 248.

9. A gRNA molecule of claim 4, wherein the targeting domain comprises, e.g., consists of, SEQ ID NO: 338.

10. A gRNA molecule of claim 1, wherein the target sequence is of a BCL11a enhancer, and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 278 to SEQ ID NO: 333.

11. A gRNA molecule of claim 10, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 318, SEQ ID NO: 312, SEQ ID NO: 313, SEQ ID NO: 294, SEQ ID NO: 310, SEQ ID NO: 319, SEQ ID NO: 298, SEQ ID NO: 322, SEQ ID NO: 311, SEQ ID NO: 315, SEQ ID NO: 290, SEQ ID NO: 317, SEQ ID NO: 309, SEQ ID NO: 289, or SEQ ID NO: 281.

12. A gRNA molecule of claim 10, wherein the targeting domain comprises, e.g., consists of, SEQ ID NO: 318.

13. A gRNA molecule of claim 1, wherein the target sequence is of a BCL11a enhancer, and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 1596 to SEQ ID NO: 1691.

14. A gRNA molecule of claim 13, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 1683, SEQ ID NO: 1638, SEQ ID NO: 1647, SEQ ID NO: 1609, SEQ ID NO: 1621, SEQ ID NO: 1617, SEQ ID NO: 1654, SEQ ID NO: 1631, SEQ ID NO: 1620, SEQ ID NO: 1637, SEQ ID NO: 1612, SEQ ID NO: 1656, SEQ ID NO: 1619, SEQ ID NO: 1675, SEQ ID NO: 1645, SEQ ID NO: 1598, SEQ ID NO: 1599, SEQ ID NO: 1663, SEQ ID NO: 1677, or SEQ ID NO: 1626.

15. A gRNA molecule of claim 1, wherein the target sequence is of a HFPH region (e.g., a French HPFH region), and the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 86 to SEQ ID NO: 181, SEQ ID NO: 1500 to SEQ ID NO: 1595, or SEQ ID NO: 1692 to SEQ ID NO: 1761.

16. A gRNA molecule of claim 15, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 100, SEQ ID NO: 165, SEQ ID NO: 113, SEQ ID NO: 99, SEQ ID NO: 112, SEQ ID NO: 98, SEQ ID NO: 1580, SEQ ID NO: 106, SEQ ID NO: 1503, SEQ ID NO: 1589, SEQ ID NO: 160, SEQ ID NO: 1537, SEQ ID NO: 159, SEQ ID NO: 101, SEQ ID NO: 162, SEQ ID NO: 104, SEQ ID NO: 138, SEQ ID NO: 1536, SEQ ID NO: 1539, SEQ ID NO: 1585.

17. A gRNA molecule of claim 15, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 1505, SEQ ID NO: 1589, SEQ ID NO: 1700, or SEQ ID NO: 1750.

18. A gRNA molecule of claim 15, wherein the targeting domain comprises, e.g., consists of, any one of SEQ ID NO: 100, SEQ ID NO: 165, or SEQ ID NO: 113.

19. The gRNA molecule of any of claims 2-18, wherein the targeting domain comprises, e.g., consists of, 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences.

20. The gRNA molecule of claim 16, wherein the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids disposed at the 3' end of the recited targeting domain sequence.

21. The gRNA molecule of claim 16, wherein the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences are the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids disposed at the 5' end of the recited targeting domain sequence.

22. The gRNA molecule of claim 16, wherein the 17, 18, 19, 20, 21, 22, 23, or 24 consecutive nucleic acids of any one of the recited targeting domain sequences do not comprise either the 5' or 3' nucleic acid of the recited targeting domain sequence.

23. The gRNA molecule of any of claims 2-22, wherein the targeting domain consists of the recited targeting domain sequence.

24. The gRNA molecule of any of the previous claims, wherein a portion of the crRNA and a portion of the tracr hybridize to form a flagpole comprising SEQ ID NO: 6584 or 6585.

25. The gRNA molecule of claim 24, wherein the flagpole further comprises a first flagpole extension, located 3' to the crRNA portion of the flagpole, wherein said first flagpole extension comprises SEQ ID NO: 6586.

26. The gRNA molecule of claim 24 or 25, wherein the flagpole further comprises a second flagpole extension located 3' to the crRNA portion of the flagpole and, if present, the first flagpole extension, wherein said second flagpole extension comprises SEQ ID NO: 6587.

27. The gRNA molecule of any of claims 1-26, wherein the tracr comprises SEQ ID NO: 6660 or SEQ ID NO: 6661.

28. The gRNA molecule of any of claims 1-26, wherein the tracr comprises SEQ ID NO: 7812, optionally further comprising, at the 3' end, an additional 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides.

29. The gRNA molecule of any of claims 1-28, wherein the crRNA comprises, from 5' to 3', [targeting domain]-:

a) SEQ ID NO: 6584;

b) SEQ ID NO: 6585;

c) SEQ ID NO: 6605;

d) SEQ ID NO: 6606;

e) SEQ ID NO: 6607;

f) SEQ ID NO: 6608; or

g) SEQ ID NO: 7806.

30. The gRNA molecule of any of claims 1-23 or 29, wherein the tracr comprises, from 5' to 3':

a) SEQ ID NO: 6589;

b) SEQ ID NO: 6590;

c) SEQ ID NO: 6609;

d) SEQ ID NO: 6610;

e) SEQ ID NO: 6660;

f) SEQ ID NO: 6661;

g) SEQ ID NO: 7812;

h) SEQ ID NO: 7807;

i) (SEQ ID NO: 7808;

j) SEQ ID NO: 7809;

k) any of a) to j), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 uracil (U) nucleotides;

l) any of a) to k), above, further comprising, at the 3' end, at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides; or

m) any of a) to l), above, further comprising, at the 5' end (e.g., at the 5' terminus), at least 1, 2, 3, 4, 5, 6 or 7 adenine (A) nucleotides, e.g., 1, 2, 3, 4, 5, 6, or 7 adenine (A) nucleotides.

31. The gRNA molecule of any of claims 1-23, wherein the targeting domain and the tracr are disposed on separate nucleic acid molecules, and wherein the nucleic acid molecule comprising the targeting domain comprises SEQ ID NO: 6607, optionally disposed immediately 3' to the targeting domain, and the nucleic acid molecule comprising the tracr comprises, e.g., consists of, SEQ ID NO: 6660.

32. The gRNA molecule of any of claims 27-28, wherein the crRNA portion of the flagpole comprises SEQ ID NO: 6607 or SEQ ID NO: 6608.

33. The gRNA molecule of any of claims 1-26, wherein the tracr comprises SEQ ID NO: 6589 or 6590, and optionally, if a first flagpole extension is present, a first tracr extension, disposed 5' to SEQ ID NO: 6589 or 6590, said first tracr extension comprising SEQ ID NO: 6591.

34. The gRNA molecule of any of claims 1-33, wherein the targeting domain and the tracr are disposed on separate nucleic acid molecules.

35. The gRNA molecule of any of claims 1-33, wherein the targeting domain and the tracr are disposed on a single nucleic acid molecule, and wherein the tracr is disposed 3' to the targeting domain.

36. The gRNA molecule of claim 35, further comprising a loop, disposed 3' to the targeting domain and 5' to the tracr.

37. The gRNA molecule of claim 36, wherein the loop comprises SEQ ID NO: 6588.

38. The gRNA molecule of any of claims 1-23, comprising, from 5' to 3', [targeting domain]-:

(a) SEQ ID NO: 6601;

(b) SEQ ID NO: 6602;

(c) SEQ ID NO: 6603;

(d) SEQ ID NO: 6604;

(e) SEQ ID NO: 7811; or

(f) any of (a) to (e), above, further comprising, at the 3' end, 1, 2, 3, 4, 5, 6 or 7 uracil (U) nucleotides.

39. The gRNA molecule of any of claims 1-40, wherein the targeting domain and the tracr are disposed on a single nucleic acid molecule, and wherein said nucleic acid molecule comprises, e.g., consists of, said targeting domain and SEQ ID NO: 7811, optionally disposed immediately 3' to said targeting domain.

40. The gRNA molecule of any of claims 1-39 wherein one, or optionally more than one, of the nucleic acid molecules comprising the gRNA molecule comprises:

a) one or more, e.g., three, phosphorothioate modifications at the 3' end of said nucleic acid molecule or molecules;

b) one or more, e.g., three, phosphorothioate modifications at the 5' end of said nucleic acid molecule or molecules;

c) one or more, e.g., three, 2'-O-methyl modifications at the 3' end of said nucleic acid molecule or molecules;

d) one or more, e.g., three, 2'-O-methyl modifications at the 5' end of said nucleic acid molecule or molecules;

e) a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 3' residues of said nucleic acid molecule or molecules;

f) a 2' O-methyl modification at each of the 4th-to-terminal, 3rd-to-terminal, and 2nd-to-terminal 5' residues of said nucleic acid molecule or molecules; or

f) any combination thereof.

41. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 342;

(b) SEQ ID NO: 343; or

(c) SEQ ID NO: 1762.

42. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 344, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 344, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 345, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 345, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

43. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 347;

(b) SEQ ID NO: 348; or

(c) SEQ ID NO: 1763.

44. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 349, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 349, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 350, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 350, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

45. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 351;

(b) SEQ ID NO: 352; or

(c) SEQ ID NO: 1764.

46. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 353, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 353, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 354, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 354, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

47. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 355;

(b) SEQ ID NO: 356; or

(c) SEQ ID NO: 1765.

48. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 357, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 357, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 358, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 358, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

49. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 359;

(b) SEQ ID NO: 360; or

(c) SEQ ID NO: 1766.

50. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 361, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 361, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 362, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 362, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

51. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 363;

(b) SEQ ID NO: 364; or

(c) SEQ ID NO: 1767.

52. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 365, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 365, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 366, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 366, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

53. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 367;

(b) SEQ ID NO: 368; or

(c) SEQ ID NO: 1768.

54. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 369, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 369, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 370, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 370, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

55. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 371;

(b) SEQ ID NO: 372; or

(c) SEQ ID NO: 1769.

56. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 373, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 373, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 374, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 374, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

57. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 375;

(b) SEQ ID NO: 376; or

(c) SEQ ID NO: 1770.

58. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 377, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 377, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 378, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 378, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

59. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 379;

(b) SEQ ID NO: 380; or

(c) SEQ ID NO: 1771.

60. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 381, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 381, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 382, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 382, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

61. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 383;

(b) SEQ ID NO: 384; or

(c) SEQ ID NO: 1772.

62. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 385, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 385, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 386, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 386, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

63. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 387;

(b) SEQ ID NO: 388; or

(c) SEQ ID NO: 1773.

64. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 389, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 389, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 390, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 390, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

65. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 391;

(b) SEQ ID NO: 392; or

(c) SEQ ID NO: 1774.

66. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 393, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 393, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 394, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 394, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

67. A gRNA molecule of claim 1, comprising, e.g., consisting of, the sequence:

(a) SEQ ID NO: 395;

(b) SEQ ID NO: 396; or

(c) SEQ ID NO: 1775.

68. A gRNA molecule of claim 1, comprising, e.g., consisting of:

(a) a crRNA comprising, e.g., consisting of, SEQ ID NO: 397, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660;

(b) a crRNA comprising, e.g., consisting of, SEQ ID NO: 397, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346;

(c) a crRNA comprising, e.g., consisting of, SEQ ID NO: 398, and a tracr comprising, e.g., consisting of, SEQ ID NO: 6660; or

(d) a crRNA comprising, e.g., consisting of, SEQ ID NO: 398, and a tracr comprising, e.g., consisting of, SEQ ID NO: 346.

69. A gRNA molecule of any of claims 1-68, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a cell, an indel is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule.

70. The gRNA molecule of claim 69, wherein the indel does not comprise a nucleotide of a GATA-1 or TAL-1 binding site.

71. A gRNA molecule of any of claims 1-70, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a population of cells, an indel is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule in at least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%, of the cells of the population.

72. A gRNA molecule of any of claims 1-68, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a population of cells, an indel that does not comprise a nucleotide of a GATA-1 or TAL-1 binding site is formed at or near the target sequence complementary to the targeting domain of the gRNA molecule in at least about 20%, e.g., at least about 30%, e.g., at least about 35%, e.g., at least about 40%, e.g., at least about 45%, e.g., at least about 50%, e.g., at least about 55%, e.g., at least about 60%, e.g., at least about 65%, e.g., at least about 70%, e.g., at least about 75%, e.g., at least about 80%, e.g., at least about 85%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 99%, of the cells of the population.

73. The gRNA molecule of any of claims 71-72, wherein in at least about 30%, e.g., least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%, of the cells of the population, the indel is an indel listed in any of Figure 25, Table 15, Table 26, Table 27 or Table 37.

74. The gRNA molecule of any of claims 71-73, wherein the three most frequently detected indels in said population of cells comprise the indels associated with any gRNA molecule listed in any of Figure 25, Table 15, Table 26, Table 27 or Table 37.

75. The gRNA molecule of any of claims 69-74, wherein the indel is as measured by next generation sequencing (NGS).

76. A gRNA molecule of any of claims 1-75, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a cell, expression of fetal hemoglobin is increased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny.

77. A gRNA molecule of claim 76, wherein expression of fetal hemoglobin is increased in said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny, by at least about 20%, e.g., at least about 30%, e.g., at least about 40%, e.g., at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90%, e.g., at least about 95%, e.g., at least about 96%, e.g., at least about 97%, e.g., at least about 98%, e.g., at least about 99%.

78, A gRNA molecule of any of claims 76-77, wherein said cell or its progeny, e.g., its erythroid progeny, e.g., its red blood cell progeny, produces at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell.

79. The gRNA molecule of any of claims 1-78, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a cell, no off-target indels are formed in said cell, e.g., as detectible by next generation sequencing and/or a nucleotide insertional assay.

80. The gRNA molecule of any of claims 1-78, wherein when a CRISPR system (e.g., an RNP as described herein) comprising the gRNA molecule is introduced into a population of cells, no off-target indel is detected in more than about 5%, e.g., more than about 1%, e.g., more than about 0.1%, e.g., more than about 0.01%, of the cells of the population of cells, e.g., as detectible by next generation sequencing and/or a nucleotide insertional assay.

81. The gRNA molecule of any of claims 69-80, wherein the cell is (or population of cells comprises) a mammalian, primate, or human cell, e.g., is a human cell.

82. The gRNA molecule of claim 81, wherein the cell is (or population of cells comprises) an HSPC.

83. The gRNA molecule of claim 82, wherein the HSPC is CD34+.

84. The gRNA molecule of claim 83, wherein the HSPC is CD34+CD90+.

85. The gRNA molecule of any of claims 69-84, wherein the cell is autologous with respect to a patient to be administered said cell.

86. The gRNA molecule of any of claims 69-84, wherein the cell is allogeneic with respect to a patient to be administered said cell.

87. A composition comprising:

1) one or more gRNA molecules (including a first gRNA molecule) of any of claims 1-86 and a Cas9 molecule;

2) one or more gRNA molecules (including a first gRNA molecule) of any of claims 1-86 and nucleic acid encoding a Cas9 molecule;

3) nucleic acid encoding one or more gRNA molecules (including a first gRNA molecule) of any of claims 1-86 and a Cas9 molecule;

4) nucleic acid encoding one or more gRNA molecules (including a first gRNA molecule) of any of claims 1-86 and nucleic acid encoding a Cas9 molecule; or

5) any of 1) to 4), above, and a template nucleic acid; or

6) any of 1) to 4) above, and nucleic acid comprising sequence encoding a template nucleic acid.

88. A composition comprising a first gRNA molecule of any of claims 1-86, further comprising a Cas9 molecule.

89. The composition of claim 87 or 88, wherein the Cas9 molecule is an active or inactive *s. pyogenes* Cas9.

90. The composition of claim 87-89, wherein the Cas9 molecule comprises SEQ ID NO: 6611.

91. The composition of claim 87-89, wherein the Cas9 molecule comprises, e.g., consists of:

(a) SEQ ID NO: 7821;

(b) SEQ ID NO: 7822;

(c) SEQ ID NO: 7823;

(d) SEQ ID NO: 7824;

(e) SEQ ID NO: 7825;

(f) SEQ ID NO: 7826;

(g) SEQ ID NO: 7827;

(h) SEQ ID NO: 7828;

(i) SEQ ID NO: 7829;

(j) SEQ ID NO: 7830; or

(k) SEQ ID NO: 7831.

92. The composition of any of claims 88-91, wherein the first gRNA molecule and Cas9 molecule are present in a ribonuclear protein complex (RNP).

93. The composition of any of claims 87-92, further comprising a second gRNA molecule; a second gRNA molecule and a third gRNA molecule; or a second gRNA molecule, optionally, a third gRNA molecule, and, optionally, a fourth gRNA molecule, wherein the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are a gRNA molecule of any of claims 1-68, and wherein each gRNA molecule of the composition is complementary to a different target sequence.

94. The composition of claim 93, wherein two or more of the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequences within the same gene or region.

95. The composition of claim 93 or 94, wherein the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequences not more than 20000 nucleotides, not more than 10000 nucleotides, not more than 6000, not more than 5000 nucleotides, not more than 4000, not more than 1000 nucleotides, not more than 500 nucleotides, not more than 400 nucleotides, not more than 300 nucleotides, not more than 200 nucleotides, not more than 100 nucleotides, not more than 90 nucleotides, not more than 80 nucleotides, not more than 70 nucleotides, not more than 60 nucleotides, not more than 50 nucleotides, not more than 40 nucleotides, not more than 30 nucleotides, not more than 20 nucleotides or not more than 10 nucleotides apart.

96. The composition of claim 93, wherein two or more of the first gRNA molecule, the second gRNA molecule, the optional third gRNA molecule, and the optional fourth gRNA molecule are complementary to target sequence within different genes or regions.

97. The composition of any of claims 94-95, comprising a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of claim 4, and are complementary to different target sequences;

(b) independently selected from the gRNA molecules of claim 5, and are complementary to different target sequences;

c) independently selected from the gRNA molecules of claim 6, and are complementary to different target sequences; or

(d) independently selected from the gRNA molecules of claim 7, and are complementary to different target sequences; or

(e) independently selected from the gRNA molecules of any of claims 41-56, and are complementary to different target sequences.

98. The composition of any of claims 94-95, comprising a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of claim 10, and are complementary to different target sequences; or

(b) independently selected from the gRNA molecules of claim 11, and are complementary to different target sequences.

99. The composition of any of claims 94-95, comprising a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of claim 13, and are complementary to different target sequences; or

(b) independently selected from the gRNA molecules of claim 14, and are complementary to different target sequences.

100. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein the first gRNA molecule and second gRNA molecule are:

(a) independently selected from the gRNA molecules of claim 16, and are complementary to different target sequences;

(b) independently selected from the gRNA molecules of claim 17, and are complementary to different target sequences;

(c) independently selected from the gRNA molecules of claim 18, and are complementary to different target sequences; or

(d) independently selected from the gRNA molecules of any of claims 57-68, and are complementary to different target sequences.

101. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 4, (b) selected from the gRNA molecules of claim 5, (c) selected from the gRNA molecules of claim 6, (d) selected from the gRNA molecules of claim 7, or (e) selected from the gRNA molecules of any of claims 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 10, or (b) selected from the gRNA molecules of claim 11.

102. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 4, (b) selected from the gRNA molecules of claim 5, (c) selected from the gRNA molecules of claim 6, (d) selected from the gRNA molecules of claim 7, or (e) selected from the gRNA molecules of any of claims 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 13, (b) selected from the gRNA molecules of claim 14.

103. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 4, (b) selected from the gRNA molecules of claim 5, (c) selected from the gRNA molecules of claim 6, (d) selected from the gRNA molecules of claim 7, or (e) selected from the gRNA molecules of any of claims 41-56; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 16, (b) selected from the gRNA molecules of claim 17, (c) selected from the gRNA molecules of claim 18, or (d) selected from the gRNA molecules of any of claims 57-68.

104. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 10, or (b) selected from the gRNA molecules of claim 11; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 13, (b) selected from the gRNA molecules of claim 14.

105. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 10, or (b) selected from the gRNA molecules of claim 11; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 16, (b) selected from the gRNA molecules of claim 17, (c) selected from the gRNA molecules of claim 18, or (d) selected from the gRNA molecules of any of claims 57-68.

106. The composition of any of claims 94-96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 13, (b) selected from the gRNA molecules of claim 14; and

(2) the second gRNA molecule is: (a) selected from the gRNA molecules of claim 16, (b) selected from the gRNA molecules of claim 17, (c) selected from the gRNA molecules of claim 18, or (d) selected from the gRNA molecules of any of claims 57-68.

107. The composition of claim 96, comprising a first gRNA molecule and a second gRNA molecule, wherein:

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 16, (b) selected from the gRNA molecules of claim 17, (c) selected from the gRNA molecules of claim 18, or (d) selected from the gRNA molecules of any of claims 57-68; and (2) the second gRNA molecule comprises a targeting domain that is complementary to a target sequence of the beta globin gene; or

(1) the first gRNA molecule is: (a) selected from the gRNA molecules of claim 4, (b) selected from the gRNA molecules of claim 5, (c) selected from the gRNA molecules of claim 6, (d) selected from the gRNA molecules of claim 7, (e) selected from the gRNA molecules of any of claims 41-56, (f) selected from the gRNA molecules of claim 10, (g) selected from the gRNA molecules of claim 11, (h) selected from the gRNA molecules of claim 13, or (i) selected from the gRNA molecules of claim 14; and (2) the

second gRNA molecule comprises a targeting domain that is complementary to a target sequence of the beta globin gene.

108. The composition of claim 94-96, wherein the first gRNA molecule and the second gRNA molecule are independently selected from the gRNA molecules of any of claims 41-68.

109. The composition of any of claims 87-108, wherein with respect to the gRNA molecule components of the composition, the composition consists of a first gRNA molecule and a second gRNA molecule.

110. The composition of any one of claims 87-109, wherein each of said gRNA molecules is in a ribonuclear protein complex (RNP) with a Cas9 molecule described herein, e.g., a Cas9 molecule of any of claims 90 or 91.

111. The composition of any of claims 87-110, comprising a template nucleic acid, wherein the template nucleic acid comprises a nucleotide that corresponds to a nucleotide at or near the target sequence of the first gRNA molecule.

112. The composition of any of claims 111, wherein the template nucleic acid comprises nucleic acid encoding:

(a) human beta globin, e.g., human beta globin comprising one or more of the mutations G16D, E22A and T87Q, or fragment thereof; or

(b) human gamma globin, or fragment thereof.

113. The composition of any of claims 87-112, formulated in a medium suitable for electroporation.

114. The composition of any of claims 87-113, wherein each of said gRNA molecules is in a RNP with a Cas9 molecule described herein, and wherein each of said RNP is at a concentration of less than about 10uM, e.g., less than about 3uM, e.g., less than about 1uM, e.g., less than about 0.5uM, e.g., less than about 0.3uM, e.g., less than about 0.1uM.

115. A nucleic acid sequence that encodes one or more gRNA molecules of any of claims 1-68.

116. The nucleic acid sequence of claim 115, wherein the nucleic acid comprises a promoter operably linked to the sequence that encodes the one or more gRNA molecules.

117. The nucleic acid sequence of claim 116, wherein the promoter is a promoter recognized by an RNA polymerase II or RNA polymerase III.

118. The nucleic acid sequence of claim 117, wherein the promoter is a U6 promoter or an HI promoter.

119. The nucleic acid sequence of any of claims 115-118, wherein the nucleic acid further encodes a Cas9 molecule.

120. The nucleic acid sequence of claim 119, wherien the Cas9 molecule comprises any of SEQ ID NO: 6611, SEQ ID NO: 7821, SEQ ID NO: 7822, SEQ ID NO: 7823, SEQ ID NO: 7824, SEQ ID NO: 7825, SEQ ID NO: 7826, SEQ ID NO: 7827, SEQ ID NO: 7828, SEQ ID NO: 7829, SEQ ID NO: 7830, or SEQ ID NO: 7831.

121. The nucleic acid sequence of any of claims 119-120, wherein said nucleic acid comprises a promoter operably linked to the sequence that encodes a Cas9 molecule.

122. The nucleic acid sequence of claim 121, wherein the promoter is an EF-1 promoter, a CMV IE gene promoter, an EF-1α promoter, an ubiquitin C promoter, or a phosphoglycerate kinase (PGK) promoter.

123. A vector comprising the nucleic acid of any of claims 115-122.

124. The vector of claim 123, wherein in the vector is selected from the group consisting of a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

125. A method of altering a cell (e.g., a population of cells), (e.g., altering the structure (e.g., sequence) of nucleic acid) at or near a target sequence within said cell, comprising contacting (e.g., introducing into) said cell (e.g., population of cells) with:

1) one or more gRNA molecules of any of claims 1-68 and a Cas9 molecule;

2) one or more gRNA molecules of any of claims 1-68 and nucleic acid encoding a Cas9 molecule;

3) nucleic acid encoding one or more gRNA molecules of any of claims 1-68 and a Cas9 molecule;

4) nucleic acid encoding one or more gRNA molecules of any of claims 1-68 and nucleic acid encoding a Cas9 molecule;

5) any of 1) to 4), above, and a template nucleic acid;

6) any of 1) to 4) above, and nucleic acid comprising sequence encoding a template nucleic acid;

7) the composition of any of claims 87-114; or

8) the vector of any of claims 123-124.

126. The method of claim 125, wherein the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in a single composition.

127. The method of claim 125, wherein the gRNA molecule or nucleic acid encoding the gRNA molecule, and the Cas9 molecule or nucleic acid encoding the Cas9 molecule, are formulated in more than one composition.

128. The method of claim 127, wherein the more than one composition are delivered simultaneously or sequentially.

129. The method of any of claims 125-128, wherein the cell is an animal cell.

130. The method of any of claims 125-128, wherein the cell is a mammalian, primate, or human cell.

131. The method of claim 130, wherein the cell is a hematopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs).

132. The method of any of claims 125-131, wherein the cell is a CD34+ cell.

133. The method of any of claims 125-132, wherein the cell is a CD34+CD90+ cell.

134. The method of any of claims 125-133, wherein the cell is disposed in a composition comprising a population of cells that has been enriched for CD34+ cells.

135. The method of any of claims 125-134, wherein the cell (e.g. population of cells) has been isolated from bone marrow, mobilized peripheral blood, or umbilical cord blood.

136. The method of any of claims 125-135, wherein the cell is autologous or allogeneic with respect to a patient to be administered said cell.

137. The method of any of claims 125-136, wherein the altering results in an indel at or near a genomic DNA sequence complementary to the targeting domain of the one or more gRNA molecules.

138. The method of claim 137, wherein the indel is an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37.

139. The method of claim 137-138, wherein the indel is an insertion or deletion of less than about 40 nucleotides, e.g., less than 30 nucleotides, e.g., less than 20 nucleotides, e.g., less than 10 nucleotides.

140. The method of claim 139, wherein the indel is a single nucleotide deletion.

141. The method of any of claims 137-140, wherein the method results in a population of cells wherein at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90% (e.g., at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%) of the cells of the population have been altered, e.g., comprise an indel.

142. The method of any of claims 125-141, wherein the altering results in a cell (e.g., population of cells) that is capable of differentiating into a differentiated cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell exhibits an increased level of fetal hemoglobin, e.g., relative to an unaltered cell (e.g., population of cells).

143. The method of any of claims 125-142, wherein the altering results in a population of cells that is capable of differentiating into a population of differentiated cells, e.g., a population of cells of an erythroid lineage (e.g., a population of red blood cells), and wherein said population of differentiated cells has an increased fraction of F cells (e.g., at least about 15%, at least about 20% , at least about 25%, at least about 30%, or at least about 40% higher) e.g., relative to a population of unaltered cells.

144. The method of any of claims 125-142, wherein the altering results in a cell that is capable of differentiating into a differentiated cell, e.g., a cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell produces at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell.

145. A cell, altered by the method of any of claims 125-144.

146. A cell, obtainable by the method of any of claims 125-144.

147. A cell, comprising a first gRNA molecule of any of claims 1-68, or a composition of any of claims 87-114, a nucleic acid of any of claims 115-122, or a vector of any of claims 123-124.

148. The cell of claim 147, comprising a Cas9 molecule.

149. The cell of claim 148, wherein the Cas9 molecule comprises any of SEQ ID NO: 6611, SEQ ID NO: 7821, SEQ ID NO: 7822, SEQ ID NO: 7823, SEQ ID NO: 7824, SEQ ID NO: 7825, SEQ ID NO: 7826, SEQ ID NO: 7827, SEQ ID NO: 7828, SEQ ID NO: 7829, SEQ ID NO: 7830, or SEQ ID NO: 7831.

150. The cell of any of claims 145-149, wherein the cell comprises, has comprised, or will comprise a second gRNA molecule of any of claims 1-68, or a nucleic acid encoding a second gRNA molecule of any of claims 1-68, wherein the first gRNA molecule and second gRNA molecule comprise nonidentical targeting domains.

151. The cell of any of claims 145-150, wherein expression of fetal hemoglobin is increased in said cell or its progeny (e.g., its erythroid progeny, e.g., its red blood cell progeny) relative to a cell or its progeny of the same cell type that has not been modified to comprise a gRNA molecule.

152. The cell of any of claims 145-150, wherein the cell is capable of differentiating into a differentiated cell, e.g., a cell of an erythroid lineage (e.g., a red blood cell), and wherein said differentiated cell exhibits an increased level of fetal hemoglobin, e.g., relative to a cell of the same type that has not been modified to comprise a gRNA molecule.

153. The cell of claim 152, wherein the differentiated cell (e.g., cell of an erythroid lineage, e.g., red blood cell) produces at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin, e.g., relative to a cell of the same type that has not been modified to comprise a gRNA molecule.

154. The cell of any of claims 145-153, that has been contacted with a stem cell expander.

155. The cell of claim 154, wherein the stem cell expander is compound 1, compound 2, compound 3, compound 4, or a combination thereof (e.g., compound 1 and compound 4).

156. The cell of claim 155, wherein the stem cell expander is compound 4.

157. A cell, e.g., a cell of any of claims 145-156, comprising an indel at or near a genomic DNA sequence complementary to the targeting domain of a gRNA molecule of any of claims 1-68.

158. The cell of claim 157, wherein the indel is an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37.

159. The cell of any of claims 157-158, wherein the indel is an insertion or deletion of less than about 40 nucleotides, e.g., less than 30 nucleotides, e.g., less than 20 nucleotides, e.g., less than 10 nucleotides..

160. The cell of any of claims 157-159, wherein the indel is a single nucleotide deletion.

161. The cell of any of claims 145-160, wherein the cell is an animal cell.

162. The cell of claim 161, wherein the cell is a mammalian, a primate, or a human cell.

163. The cell of any of claims 145-162, wherein the cell is a hematopoietic stem or progenitor cell (HSPC) (e.g., a population of HSPCs).

164. The cell of any of claims 145-163, wherein the cell is a CD34+ cell.

165. The cell of claim 164, wherein the cell is a CD34+CD90+ cell.

166. The cell of any of claims 145-165, wherein the cell (e.g. population of cells) has been isolated from bone marrow, mobilized peripheral blood, or umbilical cord blood.

167. The cell of any of claims 145-166, wherein the cell is autologous with respect to a patient to be administered said cell.

168. The cell of any of claims 145-166, wherein the cell is allogeneic with respect to a patient to be administered said cell.

169. A population of cells comprising the cell of any of claims 145-168.

170. The population of cells of claim 169, wherein at least about 50%, e.g., at least about 60%, e.g., at least about 70%, e.g., at least about 80%, e.g., at least about 90% (e.g., at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99%) of the cells of the population are a cell according to any one of claims 145-168.

171. The population of cells of any of claims 169-170, wherein the population of cells is capable of differentiating into a population of differentiated cells, e.g., a population of cells of an erythroid lineage (e.g., a population of red blood cells), and wherein said population of differentiated cells has an increased fraction of F cells (e.g., at least about 15%, at least about 20% , at least about 25%, at least about 30%, or at least about 40% higher) e.g., relative to a population of unmodified cells of the same type.

172. The population of cells of claim 171, wherein the F cells of the population of differentiated cells produce an average of at least about 6 picograms (e.g., at least about 7 picograms, at least about 8 picograms, at least about 9 picograms, at least about 10 picograms, or from about 8 to about 9 picograms, or from about 9 to about 10 picograms) fetal hemoglobin per cell.

173. The population of cells of any of claims 169-172, comprising:

1) at least 1e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered;

2) at least 2e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered;

3) at least 3e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered;

4) at least 4e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered; or

5) from 2e6 to 10e6 CD34+ cells/kg body weight of the patient to whom the cells are to be administered.

174. The population of cells of any of claims 169-173, wherein at least about 40%, e.g., at least about 50%, (e.g., at least about 60%, at least about 70%, at least about 80%, or at least about 90%) of the cells of the population are CD34+ cells.

175. The population of cells of claim 174, wherein at least about 10%, e.g., at least about 15%, e.g., at least about 20%, e.g., at least about 30% of the cells of the population are CD34+CD90+ cells.

176. The population of cells of any of claims 169-175, wherein the population of cells is derived from bone marrow.

177. The population of cells of any of claims 169-176, wherein the population of cells comprises, e.g., consists of, mammalian cells, e.g., human cells.

178. The population of cells of any of claims 169-177, wherein the population of cells is autologous relative to a patient to which it is to be administered.

179. The population of cells of any of claims 169-177, wherein the population of cells is allogeneic relative to a patient to which it is to be administered.

180. A composition comprising a cell of any of claims 145-168, or the population of cells of any of claims 169-179.

181. The composition of claim 180, comprising a pharmaceutically acceptable medium, e.g., a pharmaceutically acceptable medium suitable for cryopreservation.

182. A method of treating a hemoglobinopathy, comprising administering to a patient a cell of any of claims 145-168, a population of cells of any of claims 169-179, or a composition of any of claims 180-181.

183. A method of increasing fetal hemoglobin expression in a mammal, comprising administering to a patient a cell of any of claims 145-168, a population of cells of any of claims 169-179, or a composition of any of claims 180-181.

184. The method of claim 182, wherein the hemoglobinopathy is beta-thalassemia or sickle cell disease.

185. A method of preparing a cell (e.g., a population of cells) comprising:

(a) providing a cell (e.g., a population of cells) (e.g., a HSPC (e.g., a population of HSPCs));

(b) culturing said cell (e.g., said population of cells) ex vivo in a cell culture medium comprising a stem cell expander; and

(c) introducing into said cell a first gRNA molecule of any of claims 1-68, a nucleic acid molecule encoding a first gRNA molecule of any of claims 1-68, a composition of any of claims 87-114, a nucleic acid of any of claims 115-122, or a vector of any of claims 123-124.

186. The method of claim 185, wherein after said introducing of step (c), said cell (e.g., population of cells) is capable of differentiating into a differentiated cell (e.g., population of differentiated cells), e.g., a cell of an erythroid lineage (e.g., population of cells of an erythroid lineage), e.g., a red blood cell (e.g., a population of red blood cells), and wherein said differentiated cell (e.g., population of differentiated cells) produces increased fetal hemoglobin, e.g., relative to the same cells which have not been subjected to step (c).

187. The method of any of claims 185-186, wherein the stem cell expander is compound 1, compound 2, compound 3, compound 4 or a combination thereof (e.g., compound 1 and compound 4).

188. The method of claim 187, wherein the stem cell expander is compound 4.

189. The method of any of claims 185-188, wherein the cell culture medium comprises thrombopoietin (Tpo), Flt3 ligand (Flt-3L), and human stem cell factor (SCF).

190. The method of claim 189, wherein the cell culture medium further comprises human interleukin-6 (IL-6).

191. The method of claim 189-190, wherein the cell culture medium comprises thrombopoietin (Tpo), Flt3 ligand (Flt-3L), and human stem cell factor (SCF) each at a concentration ranging from about 10 ng/mL to about 1000 ng/mL.

192. The method of claim 191, wherein the cell culture medium comprises thrombopoietin (Tpo), Flt3 ligand (Flt-3L), and human stem cell factor (SCF) each at a concentration of about 50 ng/mL, e.g, at a concentration of 50 ng/mL.

193. The method of any of claims 190-192, wherein the cell culture medium comprises human interleukin-6 (IL-6) at a concentration ranging from about 10 ng/mL to about 1000 ng/mL.

194. The method of claim 193, wherein the cell culture medium comprises human interleukin-6 (IL-6) at a concentration of about 50 ng/mL, e.g, at a concentration of 50 ng/mL.

195. The method of any of claims 185-194, wherein the cell culture medium comprises a stem cell expander at a concentration ranging from about 1 nM to about 1 mM.

196. The method of claim 195, wherein the cell culture medium comprises a stem cell expander at a concentration ranging from about 1 uM to about 100 uM.

197. The method of claim 196, wherein the cell culture medium comprises a stem cell expander at a concentration ranging from about 50 uM to about 75 uM.

198. The method of claim 197, wherein the cell culture medium comprises a stem cell expander at a concentration of about 50 uM, e.g., at a concentration of 50 uM.

199. The method of claim 197, wherein the cell culture medium comprises a stem cell expander at a concentration of about 75 uM, e.g., at a concentration of 75 uM.

200. The method of any of claims 185-199, wherein the culturing of steb (b) comprises a period of culturing before the introducing of step (c).

201. The method of claim 200, wherein the period of culturing before the introducing of step (c) is at least 12 hours, e.g., is for a period of about 1 day to about 3 days, e.g., is for a period of about 1 day to about 2 days, e.g., is for a period of about 2 days.

202. The method of any of claims 185-201, wherein the culturing of step (b) comprises a period of culturing after the introducing of step (c).

203. The method of claim 202, wherein the period of culturing after the introducing of step (c) is at least 12 hours, e.g., is for a period of about 1 day to about 10 days, e.g., is for a period of about 1 day to about 5 days, e.g., is for a period of about 2 days to about 4 days, e.g., is for a period of about 2 days or is for a period of about 3 days or is for a period of about 4 days.

204. The method of any of claims 185-203, wherein the population of cells is expanded at least 4-fold, e.g., at least 5-fold, e.g, at least 10-fold, e.g., relative to cells which are not cultured according to step (b).

205. The method of any of claims 185-204, wherein the introducing of step (c) comprises an electroporation.

206. The method of claim 205, wherein the electroporation comprises 1 to 5 pulses, e.g., 1 pulse, and wherein each pulse is at a pulse voltage ranging from 700 volts to 2000 volts and has a pulse duration ranging from 10 ms to 100 ms.

207. The method of claim 206, wherein the electroporation comprises 1 pulse.

208. The method of any of claims 206-207, wherein the pulse voltage ranges from 1500 to 1900 volts, e.g., is 1700 volts.

209. The method of any of claims 206-208, wherein the pulse duration ranges from 10 ms to 40 ms, e.g., is 20 ms.

210. The method of any of claims 185-209, wherein the cell (e.g., population of cells) provided in step (a) is a human cell (e.g., a population of human cells).

211. The method of claim 210, wherein the cell (e.g., population of cells) provided in step (a) is isolated from bone marrow, peripheral blood (e.g., mobilized peripheral blood) or umbilical cord blood.

212. The method of claim 211, wherein the cell (e.g., population of cells) provided in step (a) is isolated from bone marrow, e.g., is isolated from bone marrow of a patient suffering from a hemoglobinopathy.

213. The method of any of claims 185-212, wherein the population of cells provided in step (a) is enriched for CD34+ cells.

214. The method of any of claims 185-213, wherein subsequent to the introducing of step (c), the cell (e.g., population of cells) is cryopreserved.

215. The method of any of claims 185-214, wherein subsequent to the introducing of step (c), the cell (e.g., population of cells) comprises an indel at or near a genomic DNA sequence complementary to the targeting domain of the first gRNA molecule.

216. The method of any of claims 132, wherein the indel is an indel shown on Figure 25, Table 15, Table 26, Table 27 or Table 37.

217. The method of any of claims 185-216, wherein after the introducing of step (c), at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% of the cells of the population of

cells comprise an indel at or near a genomic DNA sequence complementary to the targeting domain of the first gRNA molecule.

218. The method of claim 217, wherein the indel in each of said cells of the population of cells is an indel shown in Figure 25, Table 15, Table 26, Table 27 or Table 37.

219. A cell (e.g., population of cells), obtainable by the method of any of claims 185-218.

220. A method of treating a hemoglobinopathy, comprising administering to a human patient a composition comprising a cell (e.g., a population of cells) of claim 219.

221. A method of increasing fetal hemoglobin expression in a human patient, comprising administering to said human patient a composition comprising a cell (e.g., a population of cells) of claim 219.

222. The method of claim 220, wherein the hemoglobinopathy is beta-thalassemia or sickle cell disease.

223. The method of any of claims 220-222, wherein the human patient is administered a composition comprising at least about 1e6 cells of claim 219 per kg body weight of the human patient, e.g., at least about 1e6 CD34+ cells of claim 219 per kg body weight of the human patient.

224. The method claim 223, wherein the human patient is administered a composition comprising at least about 2e6 cells of claim 219 per kg body weight of the human patient, e.g., at least about 2e6 CD34+ cells of claim 219 per kg body weight of the human patient.

225. The method claim 223, wherein the human patient is administered a composition comprising from about 2e6 to about 10e6 cells of claim 219 per kg body weight of the human patient, e.g., at least about 2e6 to about 10e6 CD34+ cells of claim 219 per kg body weight of the human patient.

226. A gRNA molecule of any of claims 1-86, a composition of any of claims 87-114 or 180-181, a nucleic acid of any of claims 115-122, a vector of any of claims 123-124, a cell of any of claims 145-168 or 219, or a population of cells of any of claims 169-179, for use as a medicament.

227. A gRNA molecule of any of claims 1-86, a composition of any of claims 87-114 or 180-181, a nucleic acid of any of claims 115-122, a vector of any of claims 123-124, a cell of any of claims 145-168 or 219, or a population of cells of any of claims 169-179, for use in the manufacture of a medicament.

228. A gRNA molecule of any of claims 1-86, a composition of any of claims 87-114 or 180-181, a nucleic acid of any of claims 115-122, a vector of any of claims 123-124, a cell of any of claims 145-168 or 219, or a population of cells of any of claims 169-179, for use in the treatment of a disease.

229. A gRNA molecule of any of claims 1-86, a composition of any of claims 87-114 or 180-181, a nucleic acid of any of claims 115-122, a vector of any of claims 123-124, a cell of any of claims 145-168 or 219, or a population of cells of any of claims 169-179, for use in the treatment of a disease, wherein the disease is a hemoglobinopathy.

230. A gRNA molecule of any of claims 1-86, a composition of any of claims 87-114 or 180-181, a nucleic acid of any of claims 115-122, a vector of any of claims 123-124, a cell of any of claims 145-168 or 219, or a population of cells of any of claims 169-179, for use in the treatment of a disease, wherein the hemoglobinopathy is beta-thalassemia or sickle cell disease.

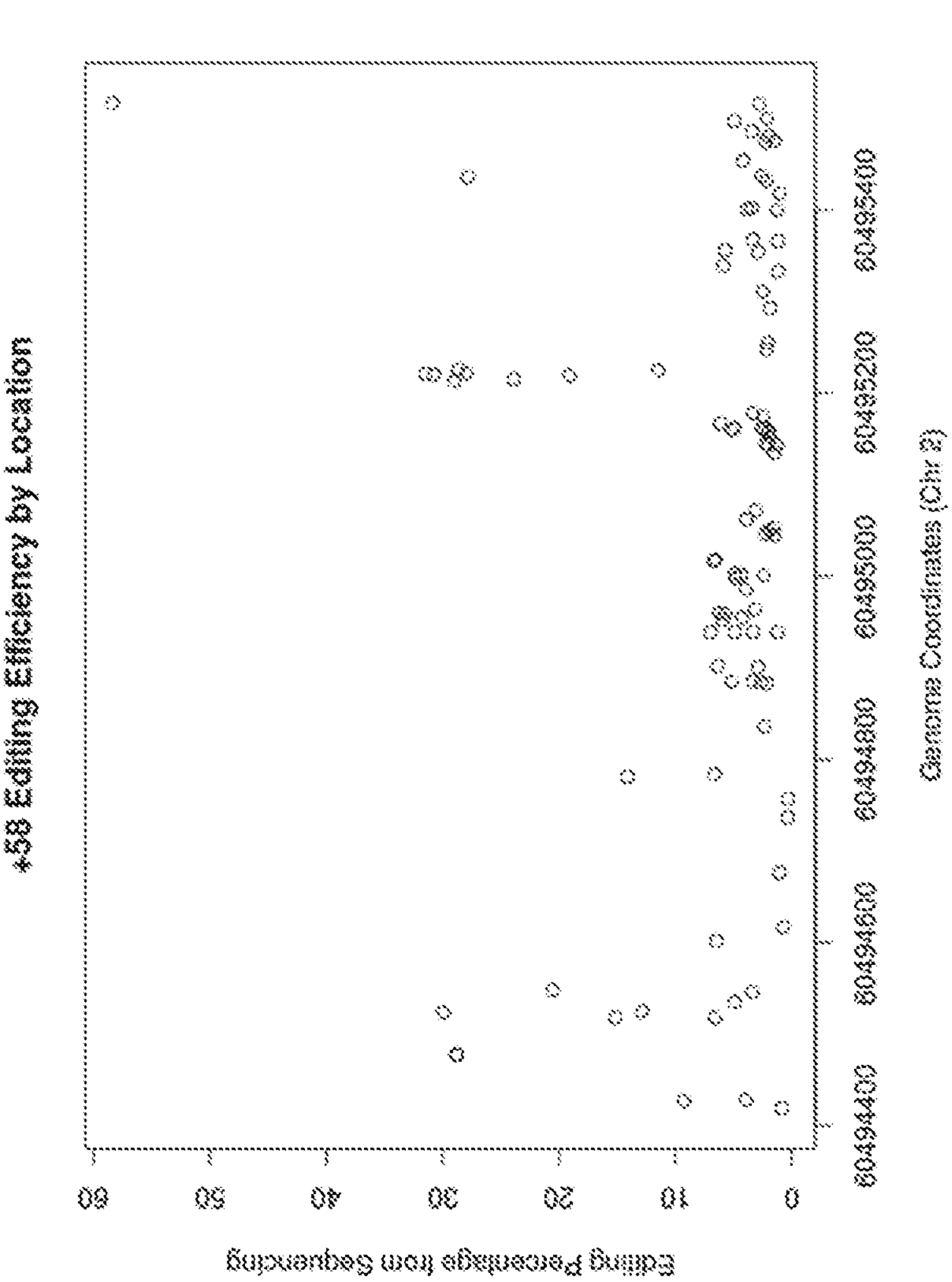

+58 Editing Efficiency by Location
Editing Percentage from Sequencing
0
10
20
30
40
50
60
Genome Coordinates (Chr 2)
60494400
60494600
60494800
60495000
60495200
60495400

Figure 1

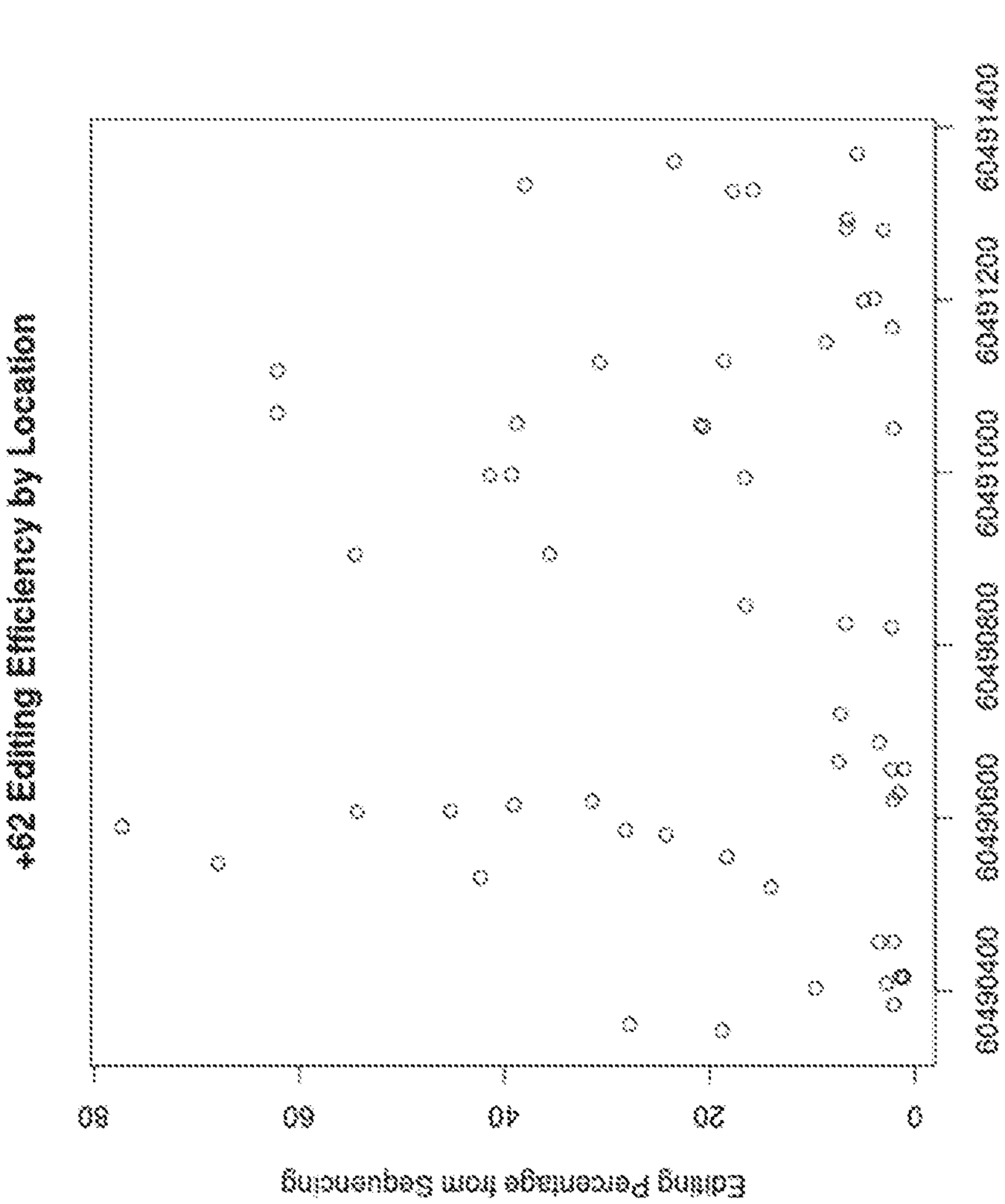

+62 Editing Efficiency by Location
Editing Percentage from Sequencing
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40
60
80
Genome Coordinates
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60491000
60491200
60491400
60490400
60490600

Figure 2

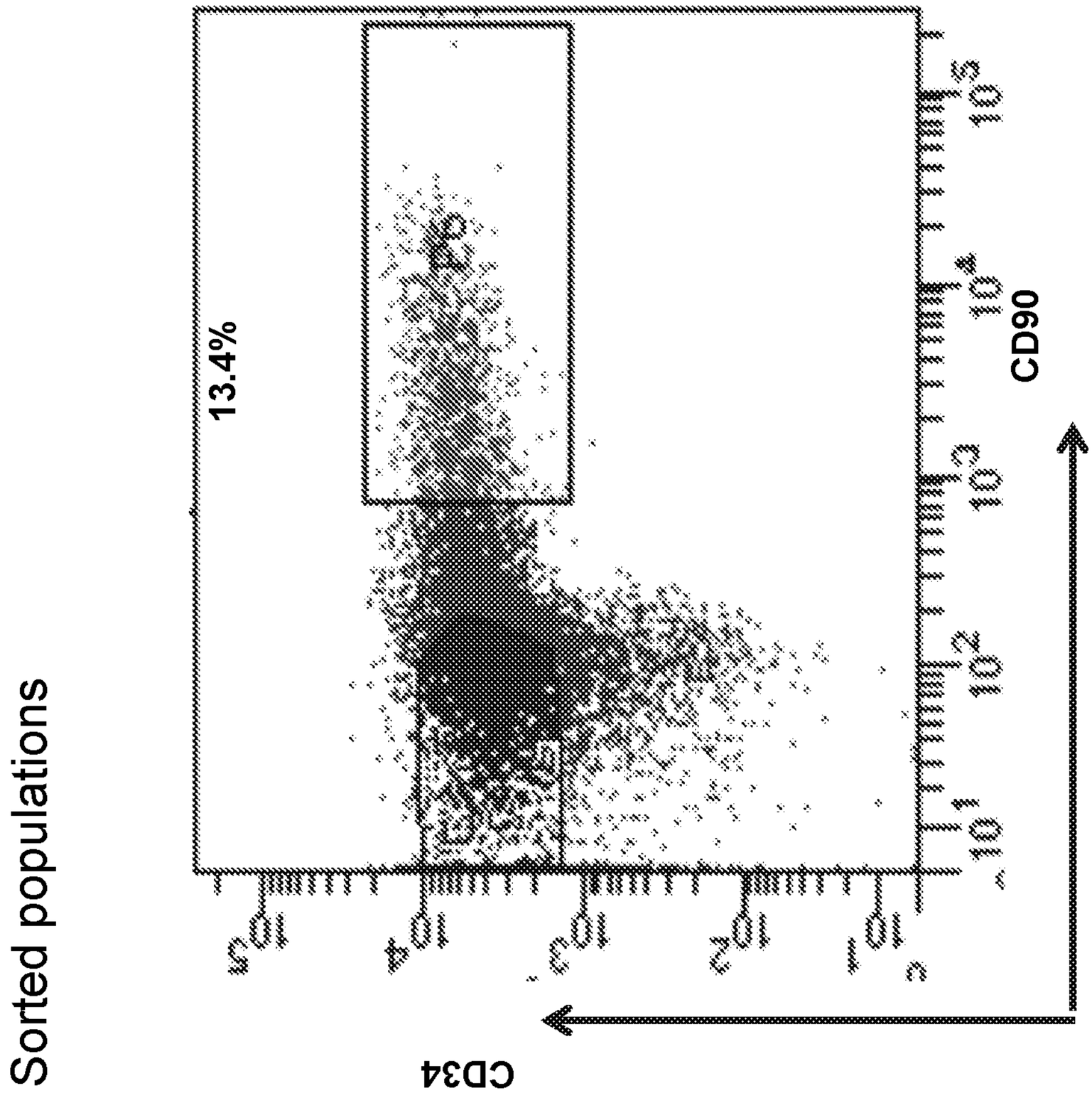


Figure 3

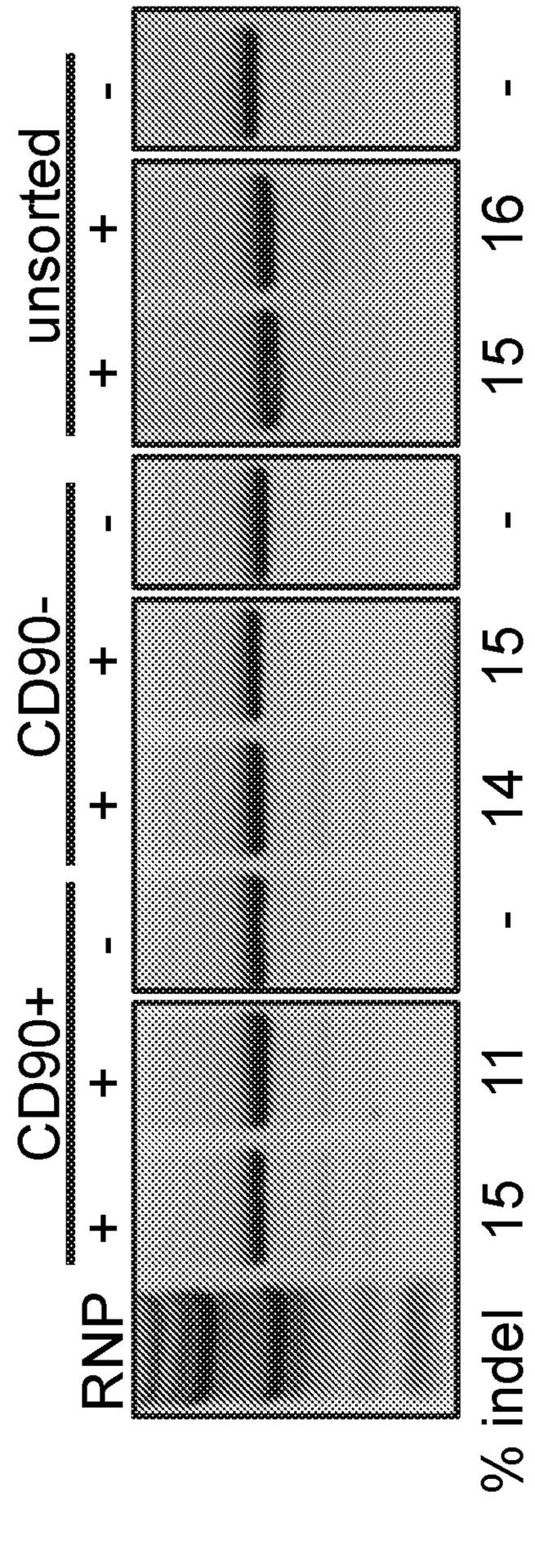


Figure 4

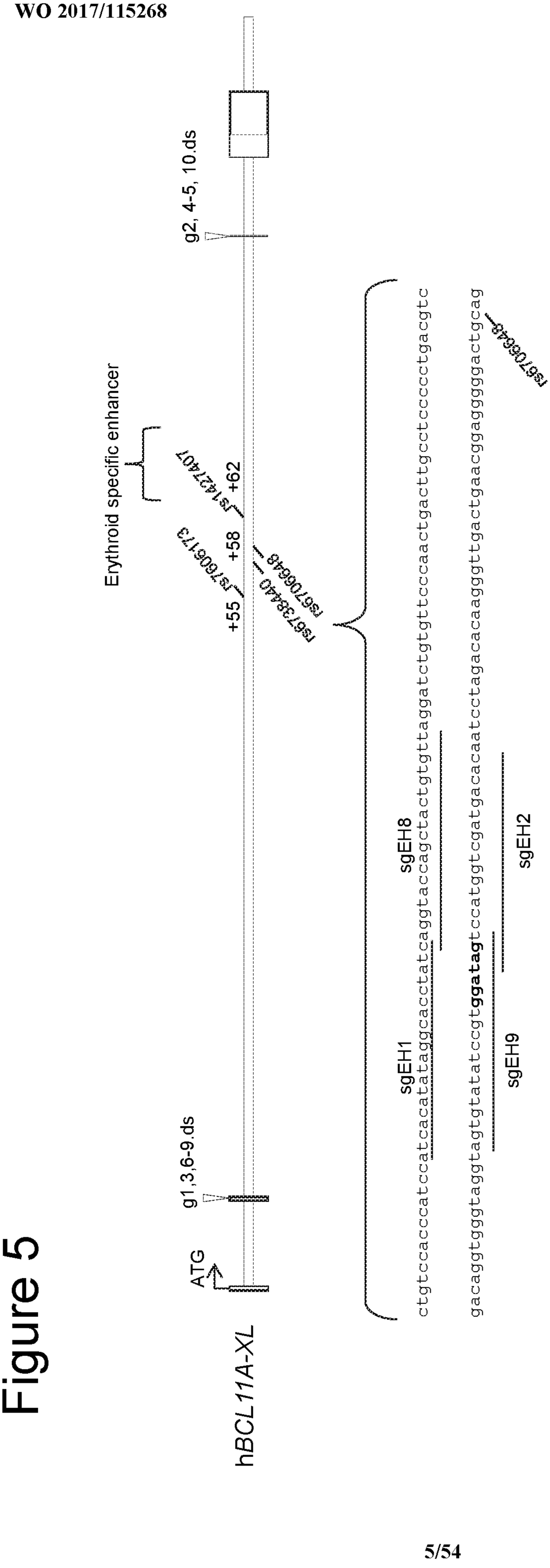

g2, 4-5, 10.ds
Erythroid specific enhancer
+62
+58
+55
rs142740
rs76061
rs67066
rs73844
rs67066
g1,3,6-9.ds
ATG
hBCL11A-XL
sgEH1
sgEH8
sgEH9
sgEH2
rs67066
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gacaggtgggtaggtagtgtatatccgtggatagtccatggtcgatgacacaatcctagacacaaagggttgactgaacggaggggactgcagtcctagacacaaagggttgactgaacggaggggactgcag

Figure 5

Figure 6A/B

A

sgEH1	Total indel %	Average %
exp1.1	67	17
exp1.2	82	10
exp2.1	95	4
exp2.2	92	20
		14
		7
		26
		16
		7
		22
		20
		6

B

sgEH2	Total indel %	Average %
exp1.1	83	23
exp1.2	84	8
exp2.1	92	7
exp2.2	81	24
		8
		7
		24
		8
		7
		12
		11
		9

The same microhomology was used for both sgRNAs.

Figure 6C/D

C

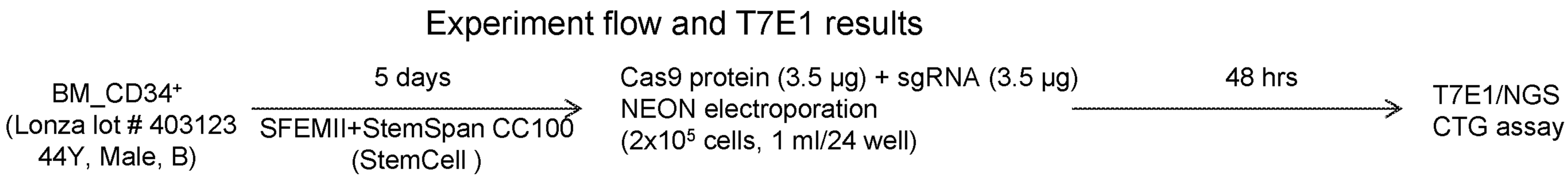
	Total indel %	ggcacctatcaggtaccagctactgt-gttaggatctgtgttcccaactgacttgcctccc	GATA1 deleted	Average %
		ggcacctatcaggtaccagctactgt**T**gttaggatctgtgttcccaactgacttgcctccc	No	8
sgEH8.exp1.1	30	ggcacctatcaggtaccagctact---gttaggatctgtgttcccaactgacttgcctccc	No	3
		ggcacctatcaggtaccagcta--------------ctgtgttcccaactgacttgcctccc	No	2
		ggcacctatcaggtaccagctactgt**T**gttaggatctgtgttcccaactgacttgcctccc	No	9
sgEH1.exp1.2	29	ggcacctatcaggtaccagctact---gttaggatctgtgttcccaactgacttgcctccc	No	4
		ggcacctatcaggtaccagcta--------------ctgtgttcccaactgacttgcctccc	No	1
		ggcacctatcaggtaccagctactgt**T**gttaggatctgtgttcccaactgacttgcctccc	No	9
sgEH1.exp2.1	32	ggcacctatcaggtaccagctact---gttaggatctgtgttcccaactgacttgcctccc	No	4
		ggcacctatcaggtaccagcta--------------ctgtgttcccaactgacttgcctccc	No	1
		ggcacctatcaggtaccagctactgt**T**gttaggatctgtgttcccaactgacttgcctccc	No	9
sgEH1.exp2.2	32	ggcacctatcaggtaccagctact---gttaggatctgtgttcccaactgacttgcctccc	No	4
		ggcacctatcaggtaccagcta--------------ctgtgttcccaactgacttgcctccc	No	1

D

	Total indel %	ttttcatctgtcgggctgtccacccatccatcacatataggcacctatcaggtaccagct	GATA1 deleted	Average %
		ttttcatctgtcgggctgtccacccatccatca--tataggcacctatcaggtaccagct	No	41
sgEH9.exp1.1	81	ttttcatctgtcgggctgtccacccatccat-----ataggcacctatcaggtaccagct	No	3
		ttttcatctgtcgggctgtccacccatccatca----------------ggtaccagct	Yes	3
		ttttcatctgtcgggctgtccacccatccatca--tataggcacctatcaggtaccagct	No	42
sgEH9.exp1.2	80	ttttcatctgtcgggctgtccacccatccat-----ataggcacctatcaggtaccagct	No	4
		ttttcatctgtcgggctgtccacccatccatca----------------ggtaccagct	Yes	2
		ttttcatctgtcgggctgtccacccatccatca--tataggcacctatcaggtaccagct	No	44
sgEH9.exp2.1	86	ttttcatctgtcgggctgtccacccatccatca----------------ggtaccagct	Yes	5
		ttttcatctgtcgggctgtccacccatccat-----ataggcacctatcaggtaccagct	No	4
		ttttcatctgtcgggctgtccacccatccatca--tataggcacctatcaggtaccagct	No	47
sgEH9.exp2.2	92	ttttcatctgtcgggctgtccacccatccat-----ataggcacctatcaggtaccagct	No	4
		ttttcatctgtcgggctgtccacccatccatca----------------ggtaccagct	Yes	3

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Figure 7



	Total indel %	Sequences of the top 3 most frequent indels	Average %
Ref		CCTGTGGGCAGTGCCAGATGAACTTCC-CATTGGGGGACATTCTTATTTTTATCGAGCACA	
g7_1_1		CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	23
g7_1_2	88	CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	14
g7_1_3		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	5
g7_2_1		CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	24
g7_2_2	92	CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	14
g7_2_3		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	5
Ref		TTTTATCGAGCACAAACGGAAA**CAA**TG**CAA**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	
g8_1_1		TTTTATCGAGCACAAACGGAAA-----**CAA**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	39
g8_1_2	92	TTTTATCGAGCACAAACGGAAACAATG-A**A**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	15
g8_1_3		TTTTATCGAGCACAAACGGAAA**CAA**T-**CAA**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	3
g8_2_1		TTTTATCGAGCACAAACGGAAA-----**CAA**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	35
g8_2_2	94	TTTTATCGAGCACAAACGGAAACAATG-A**A**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	19
g8_2_3		TTTTATCGAGCACAAACGGAAACAAT-**CAA**TGGCAGCCTCTGCTTAGAAAAAGCTGTGGA	3
Ref		ATGAGT**GCAG**AATATGCCCC**GCAG**GTATTTGTAAGTTGAGCCTTATTTCTTCTACGAAA	
g2_1_1	85	ATGAGT--------------**GCAG**GTATTTGTAAGTTGAGCCTTATTTCTTCTAC-AAA	15
g2_1_2		ATGAGTGCAGAATAT-----**GCAG**GTATTTGTAAGTTGAGCCTTATTTCTTCTAC-AAA	12
g2_1_3		ATGAGTGCAGAATATGCCC-**GCAG**GTATTTGTAAGTTGAGCCTTATTTCTTCTACGAAA	10

Figure 8

GGGCAGTGCCAGATGAACTTCCCATTGGGGGACATTCTTATTTTTATCGAGCACAAACGGAAACAATGCAATGGCAGC

g7 g8

Reg: 5′-GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT

BC: 5′-GTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTT

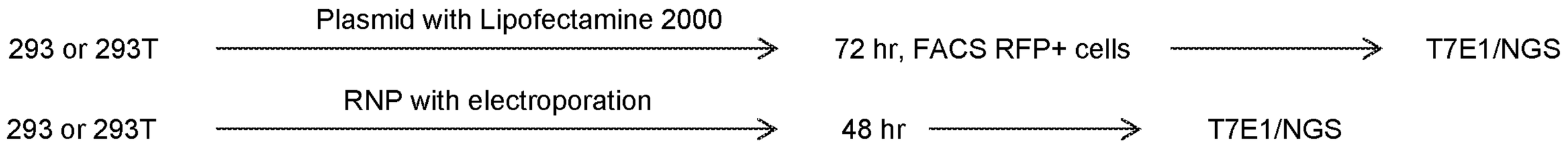
Experiment flow

BM_CD34+ (Lonza lot # 402754 Donor 27328: 33 years, Female, Race: C) → 5 days, SFEMII+StemSpan CC100 (StemCell) → Cas9 protein (3.75ug) + sgRNA (3.75 ug) NEON electroporation ($2x10^5$ cells, 1 ml/24 well) → 48 hrs → T7E1/NGS CTG

	Total Indel %	Sequences of the top 3 most frequent indels	Average %
g7Ref		AGTGCCAGATGAACTTCC-CATTGGGGGACATTCTTATTTTTA	
g7BC_1_1		AGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTA	19
g7BC_1_2	38	AGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTA	8
g7BC_1_3		AGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTA	6
g7BC_2_1		AGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTA	13
g7BC_2_2	33	AGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTA	5
g7BC_2_3		AGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTA	4

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Figure 9



cell line_number Vehicle/method	Total Indel %	CCTGTGGGCAGTGCCAGATGAACTTCC-CATTGGGGGACATTCTTATTTTTATCGAGCACA	AVG %
293_300K Plasmid/Lipo2000	71	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	13%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	6%
		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	5%
293_600K Plasmid/Lipo2000	56	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	14%
		CCTGTGGGCAGTGCCAGATGAACTTC---ATTGGGGGACATTCTTATTTTTATCGAGCACA	9%
		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	3%
293_1500K RNP/electroporation.1	96	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	22%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	9%
		CCTGTGGGCAGTGCCAGATGAA------CATTGGGGGACATTCTTATTTTTATCGAGCACA	5%
293_1500K RNP/electroporation.1	96	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	21%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	10%
		CCTGTGGGCAGTGCCAGATGAA------CATTGGGGGACATTCTTATTTTTATCGAGCACA	5%
293T_300K Plasmid/Lipo2000	73	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	15%
		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	5%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	6%
293T_600K Plasmid/Lipo2000	69	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	10%
		CCTGTGGGCAGTGCCAGATGAACTTCCCCATTGGGGGACATTCTTATTTTTATCGAGCACA	5%
		CCTGTGGGCAGTGCCAGATGAACTTCC---TTGGGGGACATTCTTATTTTTATCGAGCACA	3%
293T_1500K RNP/electroporation.1	72	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	10%
		CCTGTGGGCAGTGCCAGATGAA------CATTGGGGGACATTCTTATTTTTATCGAGCACA	7%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	4%
293T_1500K RNP/electroporation.1	75	CCTGTGGGCAGTGCCAGATGAACTTC--CATTGGGGGACATTCTTATTTTTATCGAGCACA	11%
		CCTGTGGGCAGTGCCAGATGAA------CATTGGGGGACATTCTTATTTTTATCGAGCACA	7%
		CCTGTGGGCAGTGCCAGATGAACTT---CATTGGGGGACATTCTTATTTTTATCGAGCACA	5%

Figure 10

Sequences of the top 2 most frequent indels	Average hit% (% of overall indel)
g7Ref GGGCAGTGCCAGATGAACTTCCCATTGGGGGACATTCTTATTTTTATCGAGCACAAACGGAAACAATGCAATGGCAGCCTCTGCTTAGAAAAAGC	
g7/8reg_1_1 GGGCAGTGCCAGATGAACTTCCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	64
g7/8reg_1_2 GGGCAGTGCCAGATGAACTTCCCATTGGGGGACATTCTTATTTTTATCGAGCACAAAC------AATGCAATGGCAGCCTCTGCTTAGAAAAAGC	7
g7/8reg_2_1 GGGCAGTGCCAGATGAACTTCCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	64
g7/8reg_2_2 GGGCAGTGCCAGATGAACTTCCCATTGGGGGACATTCTTATTTTTATCGAGCACAAAC------AATGCAATGGCAGCCTCTGCTTAGAAAAAGC	8
g7/8BC_1_1 GGGCAGTGCCAGATGAACTTCCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	39
g7/8BC_1_2 GGGCAGTGCCAGATGAACTTCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	4
g7/8BC_2_1 GGGCAGTGCCAGATGAACTTCCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	26
g7/8BC_2_1 GGGCAGTGCCAGATGAACTTCC----------AATGGCAGCCTCTGCTTAGAAAAAGC	9

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PCT/IB2016/058007

Figure 11

Targeting Domain ID	Species	Strand	Target Sequence Location	mean % indel formation in CD34+ cells (NGS)	SD
CR001131	h	-	Chr2:60495250-60495272	54%	5%
CR000310	h	-	Chr2:60495219-60495241	51%	9%
CR000312	h	-	Chr2:60495221-60495243	46%	4%
CR000311	h	-	Chr2:60495220-60495242	44%	2%
CR000309	h	+	Chr2:60495215-60495237	38%	7%
CR000313	h	-	Chr2:60495222-60495244	36%	7%
CR000308	h	-	Chr2:60495213-60495235	33%	9%
CR000260	h	-	Chr2:60494781-60494803	33%	2%
CR000315	h	-	Chr2:60495226-60495248	32%	6%
CR000314	h	-	Chr2:60495225-60495247	31%	5%
CR001124	h	-	Chr2:60495214-60495236	26%	2%
CR000245	h	+	Chr2:60494477-60494499	25%	1%
CR000246	h	+	Chr2:60494478-60494500	24%	0%
CR001126	h	-	Chr2:60495216-60495238	17%	6%
CR001127	h	-	Chr2:60495220-60495242	7%	2%

WO 2017/115268
PCT/IB2016/058007
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Figure 12

Targeting Domain ID	Species	Strand	Location	Mean % indel formation in CD34+ cells (NGS)	SD (%)
CR000211	h	-	Chr2:60491069-60491091	59%	5
CR000205	h	-	Chr2:60490997-60491019	54%	1
CR000206	h	-	Chr2:60490998-60491020	53%	6
CR000187	h	-	Chr2:60490590-60490612	46%	2
CR000203	h	-	Chr2:60490906-60490928	44%	0
CR000212	h	+	Chr2:60491118-60491140	40%	2
CR000191	h	-	Chr2:60490620-60490642	38%	8
CR000215	h	+	Chr2:60491151-60491173	37%	7
CR000204	h	-	Chr2:60490994-60491016	35%	2
CR000208	h	-	Chr2:60491053-60491075	32%	7
CR000183	h	-	Chr2:60490547-60490569	31%	7
CR000210	h	-	Chr2:60491057-60491079	29%	7
CR000202	h	-	Chr2:60490905-60490927	28%	6
CR000182	h	-	Chr2:60490531-60490553	28%	4
CR000174	h	-	Chr2:60490403-60490425	28%	7

Figure 13

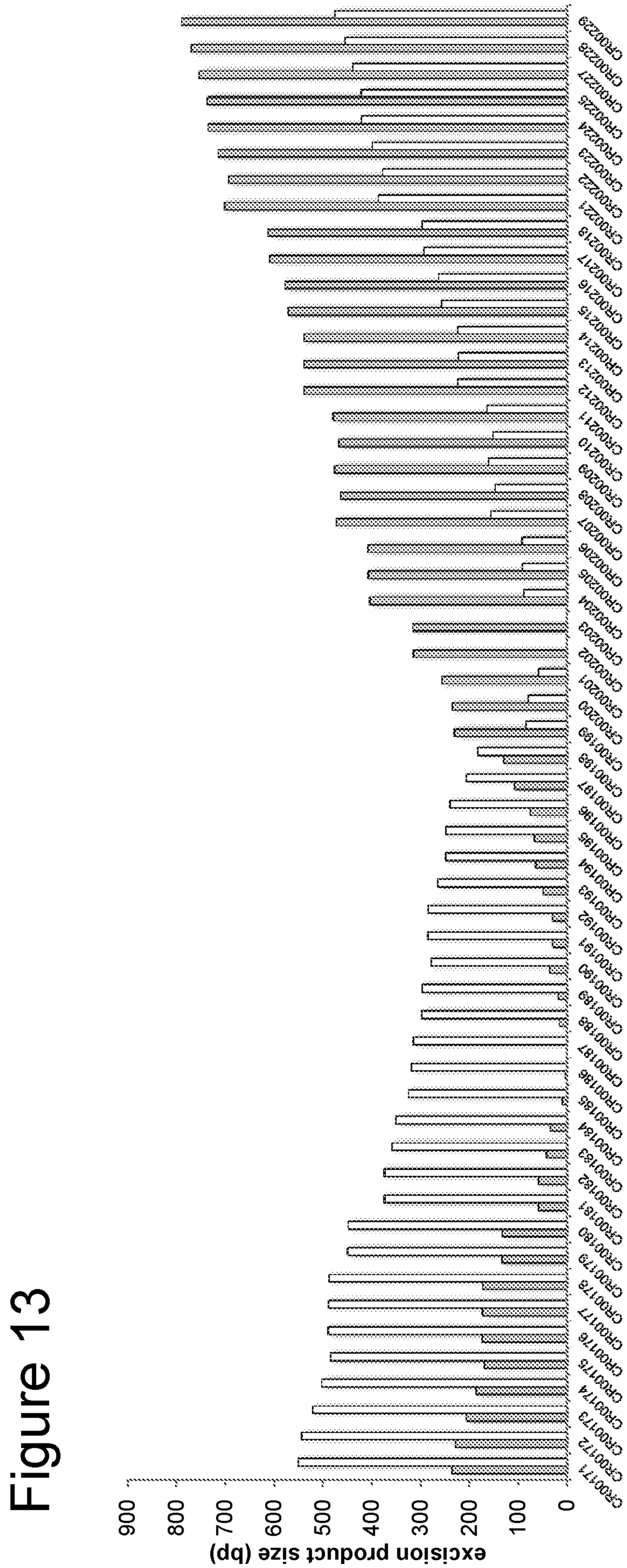

CR00229
CR00228
CR00227
CR00225
CR00224
CR00223
CR00222
CR00221
CR00219
CR00217
CR00216
CR00215
CR00214
CR00213
CR00212
CR00211
CR00210
CR00209
CR00208
CR00207
CR00206
CR00205
CR00204
CR00203
CR00202
CR00201
CR00200
CR00199
CR00198
CR00197
CR00196
CR00195
CR00194
CR00193
CR00192
CR00191
CR00190
CR00189
CR00188
CR00187
CR00186
CR00185
CR00184
CR00183
CR00182
CR00181
CR00180
CR00179
CR00178
CR00177
CR00176
CR00175
CR00174
CR00173
CR00172
CR00171
900
800
700
600
500
400
300
200
100
0
excision product size (bp)
CR00187
CR00202

Figure 14

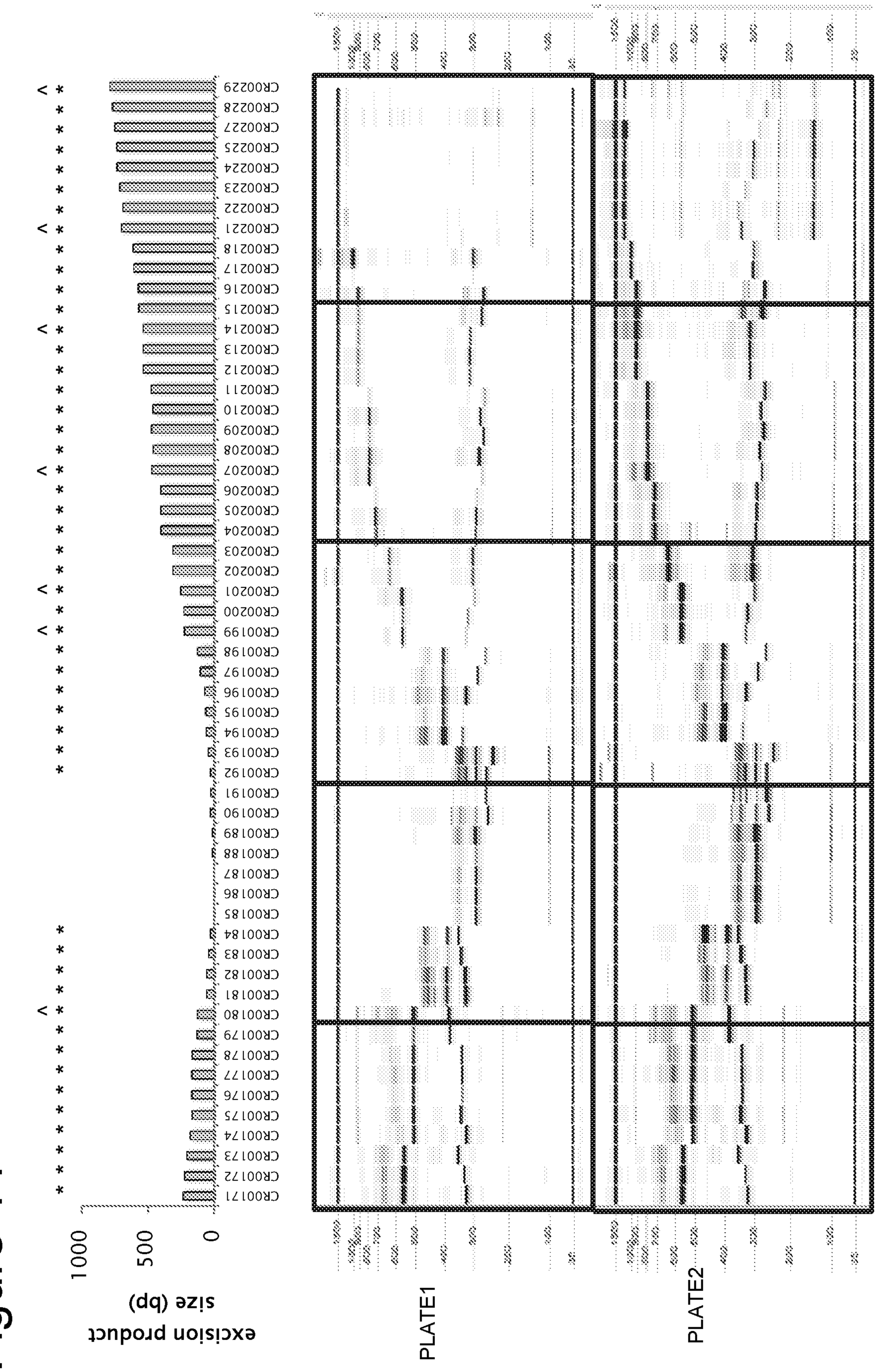
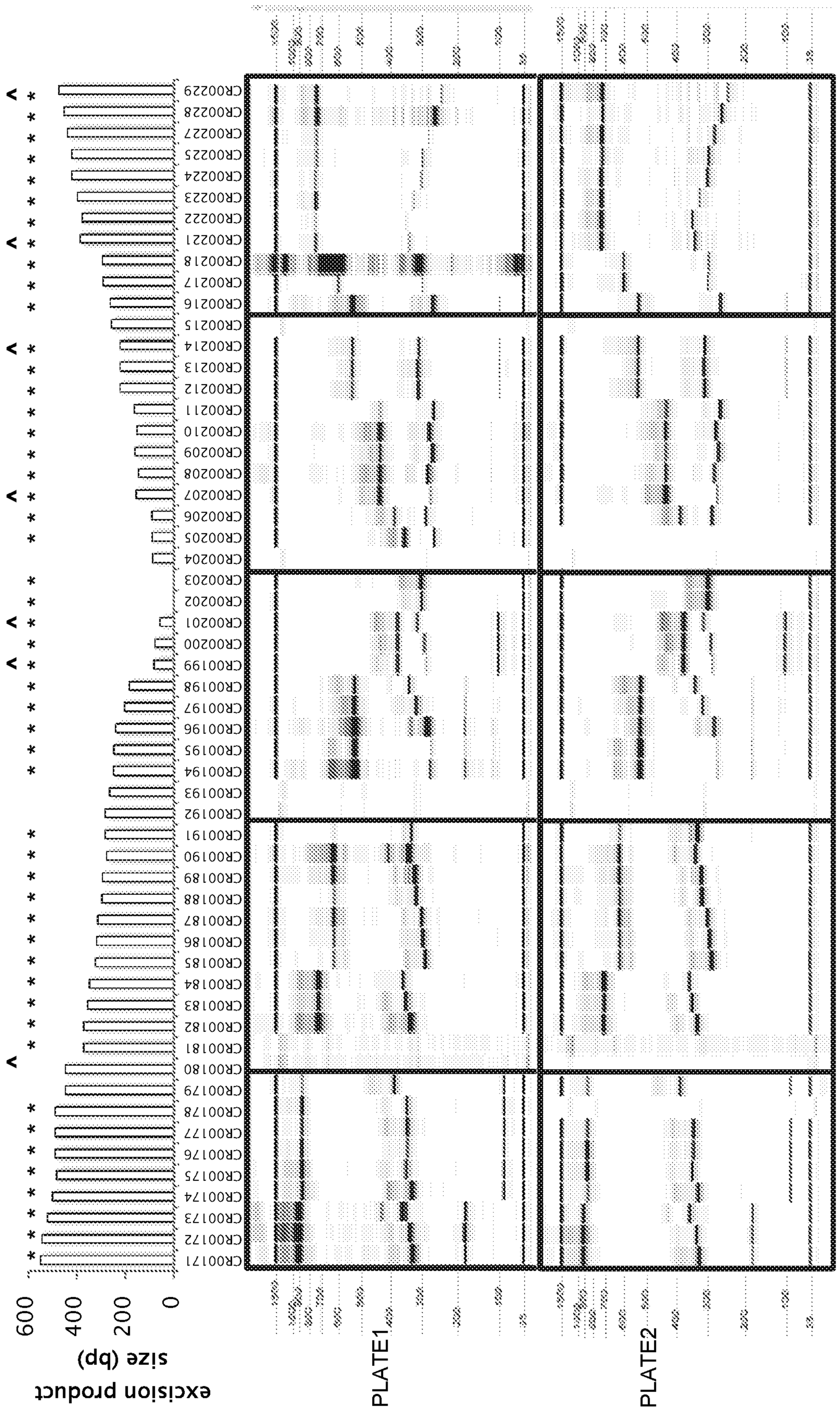

excision product
size (bp)
1000
500
0
CR00229
CR00228
CR00227
CR00225
CR00224
CR00223
CR00222
CR00221
CR00218
CR00217
CR00216
CR00215
CR00214
CR00213
CR00212
CR00211
CR00210
CR00209
CR00208
CR00207
CR00206
CR00205
CR00204
CR00203
CR00202
CR00201
CR00200
CR00199
CR00198
CR00197
CR00196
CR00195
CR00194
CR00193
CR00192
CR00191
CR00190
CR00189
CR00188
CR00187
CR00186
CR00185
CR00184
CR00183
CR00182
CR00181
CR00180
CR00179
CR00178
CR00177
CR00176
CR00175
CR00174
CR00173
CR00172
CR00171
PLATE1
PLATE2

Figure 15


excision product
size (bp)
600
400
200
0
CR00229
CR00228
CR00227
CR00225
CR00224
CR00223
CR00222
CR00221
CR00218
CR00217
CR00216
CR00215
CR00214
CR00213
CR00212
CR00211
CR00210
CR00209
CR00208
CR00207
CR00206
CR00205
CR00204
CR00203
CR00202
CR00201
CR00200
CR00199
CR00198
CR00197
CR00196
CR00195
CR00194
CR00193
CR00192
CR00191
CR00190
CR00189
CR00188
CR00187
CR00186
CR00185
CR00184
CR00183
CR00182
CR00181
CR00180
CR00179
CR00178
CR00177
CR00176
CR00175
CR00174
CR00173
CR00172
CR00171
PLATE1
PLATE2

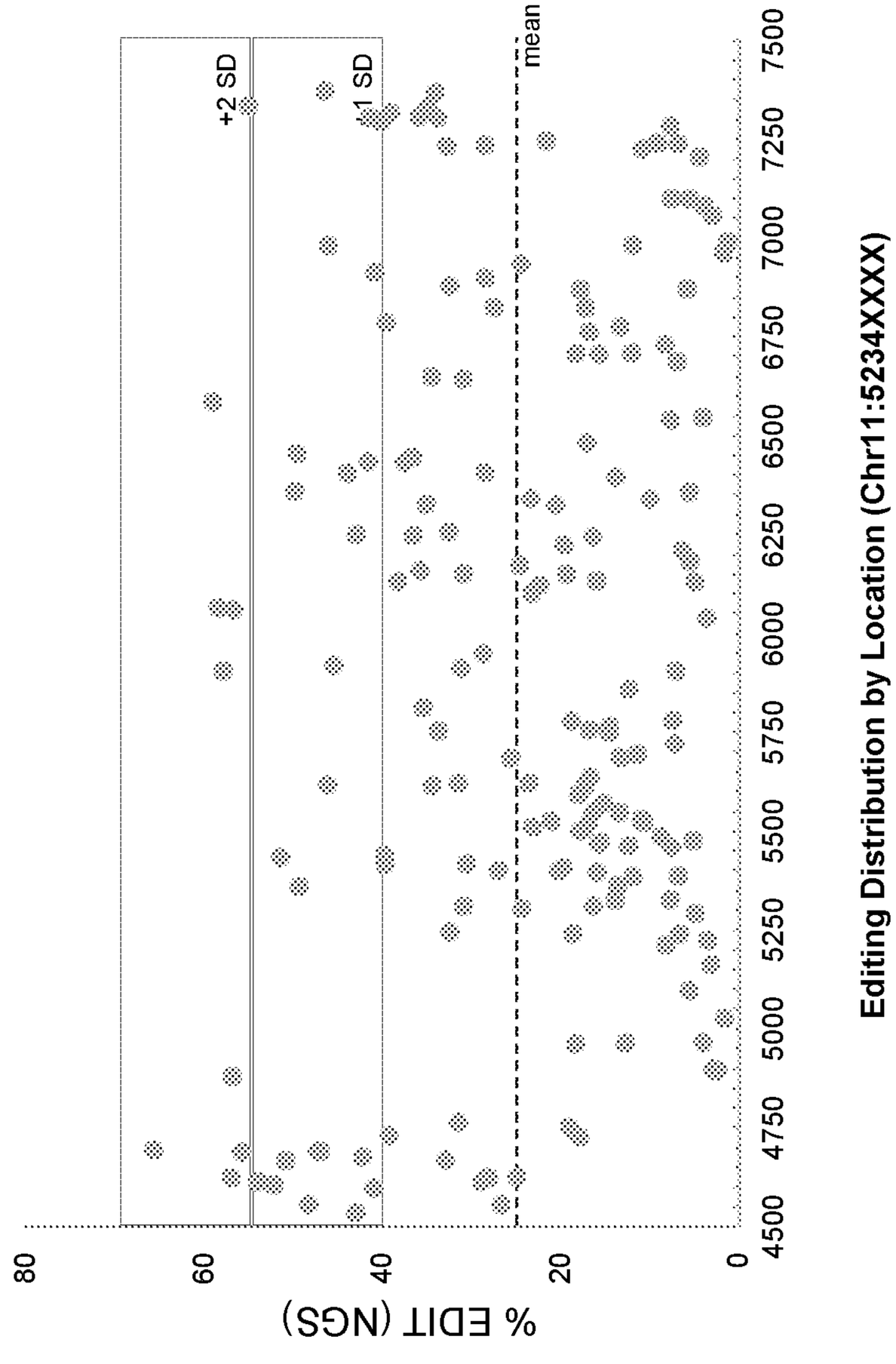

Editing Distribution by Location (Chr11:5234XXXX)
% EDIT (NGS)
0
20
40
60
80
4500
4750
5000
5250
5500
5750
6000
6250
6500
6750
7000
7250
7500
mean
+1 SD
+2 SD

Figure 16

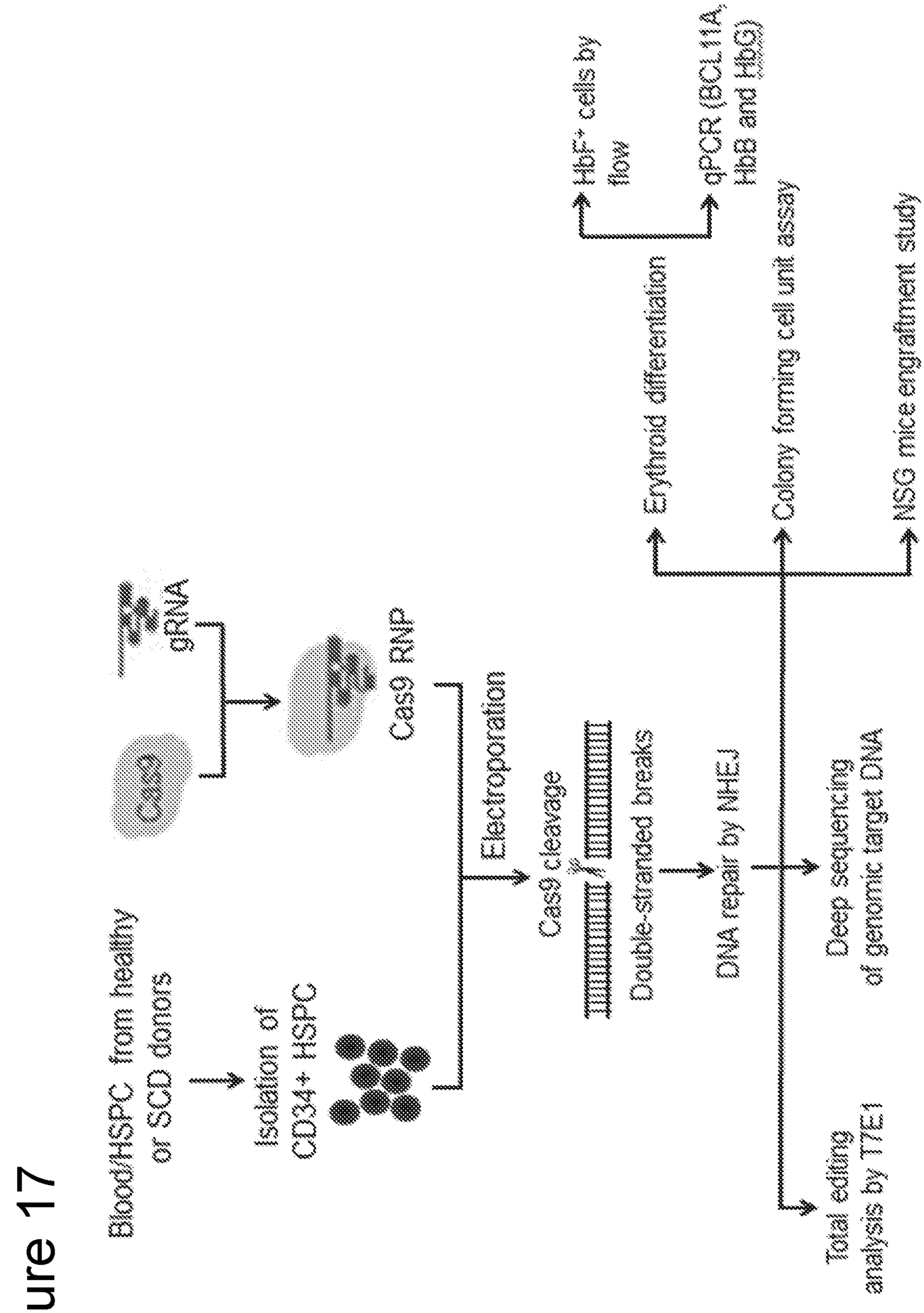
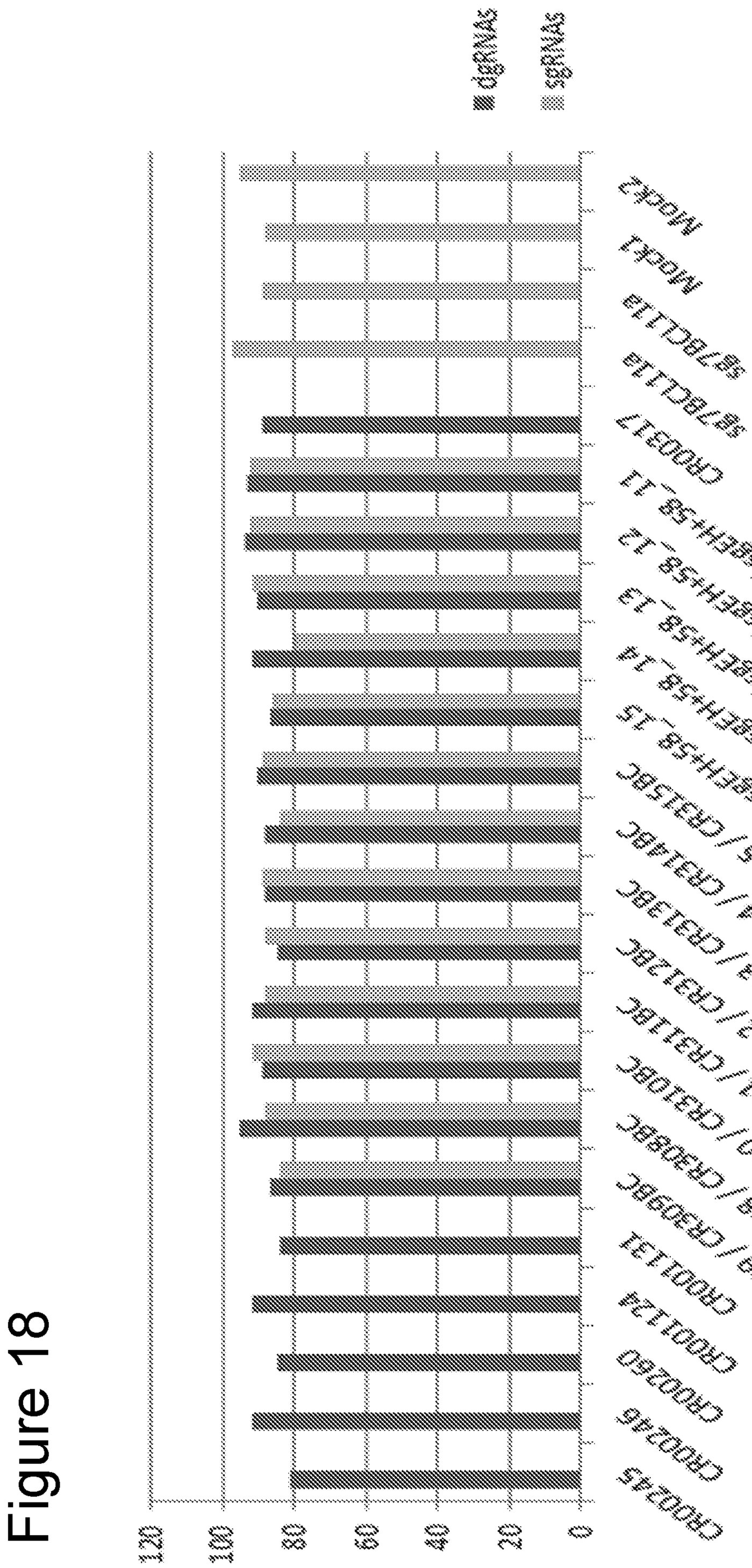
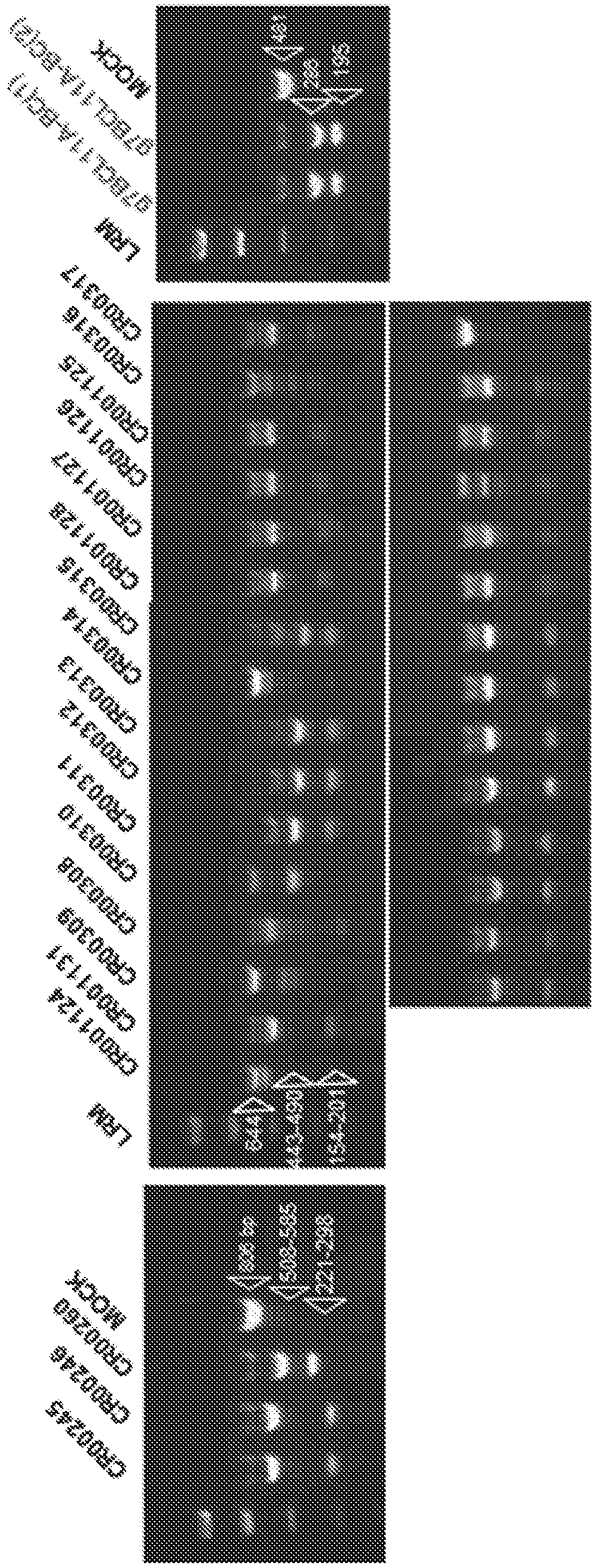

Abort/HSPC from healthy or SCD donors
Isolation of CD34+ HSPC
gRNA
Cas9
Cas9 RNP
Electroporation
Cas9 cleavage
Double-stranded breaks
DNA repair by NHEJ
Deep sequencing of genomic target DNA
Total editing analysis by TIDE
HbF+ cells by flow
qPCR (BCL11A, Hbb and Hbg)
Erythroid differentiation assay
Colony forming cell unit assay
NSG mice engraftment study

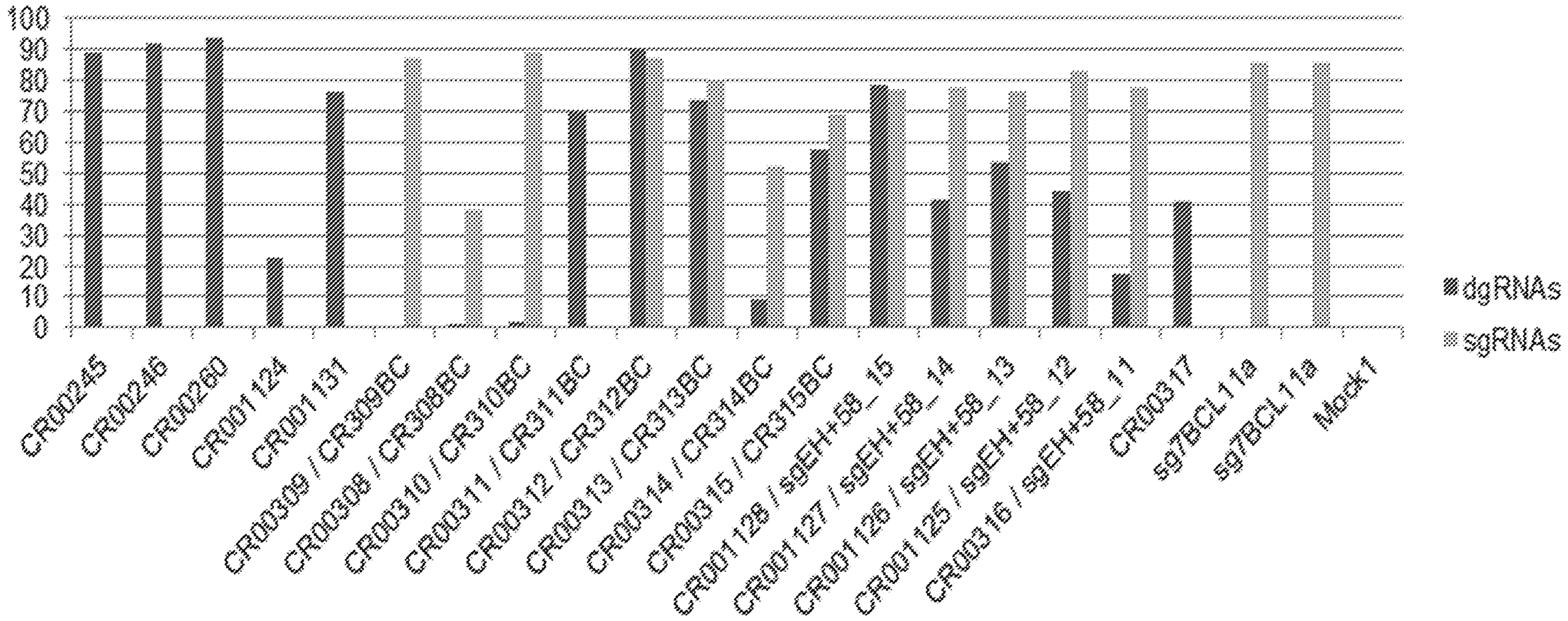
Figure 17


Sorbas
Desmas
Figure 18
120
100
80
60
40
20
0


MOCK
CR003126
CR003118
CR003117
CR003114
CR003113
CR003134
CR003133
CR003128
CR003137
CR003126
CR003125
CR003136
CR003134
CR003133
CR003130
CR003131
CR003128
CR003137
CR003139
CR003138

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Figure 20



NGS Indel (%)	CR00245	CR00246	CR00260	CR001124	CR001131	CR00309/ CR309BC	CR00308/ CR308BC	CR00310/ CR310BC	CR00311/ CR311BC	CR00312/ CR312BC	CR00313/ CR313BC
dgRNAs	88.65	91.38	93.68	22.74	76.33	0.83	1.55	1.87	69.43	89.83	73.84
sgRNAs						86.83	38.28	89.05		86.84	80.35

NGS Indel (%)	CR00314/ CR314BC	CR00315/ CR315BC	CR001128/ sgEH+58_15	CR001127/ sgEH+58_14	CR001126/ sgEH+58_13	CR001125/ sgEH+58_12	CR00316/ sgEH+58_11	CR00317	sg7BCL11a	sg7BCL11a	Mock1
dgRNAs	9.09	57.34	78.2	41.39	53.68	44.35	17.21	40.69			
sgRNAs	51.82	69.17	77.07	77.46	76.18	82.9	77.37		85.57	85.8	

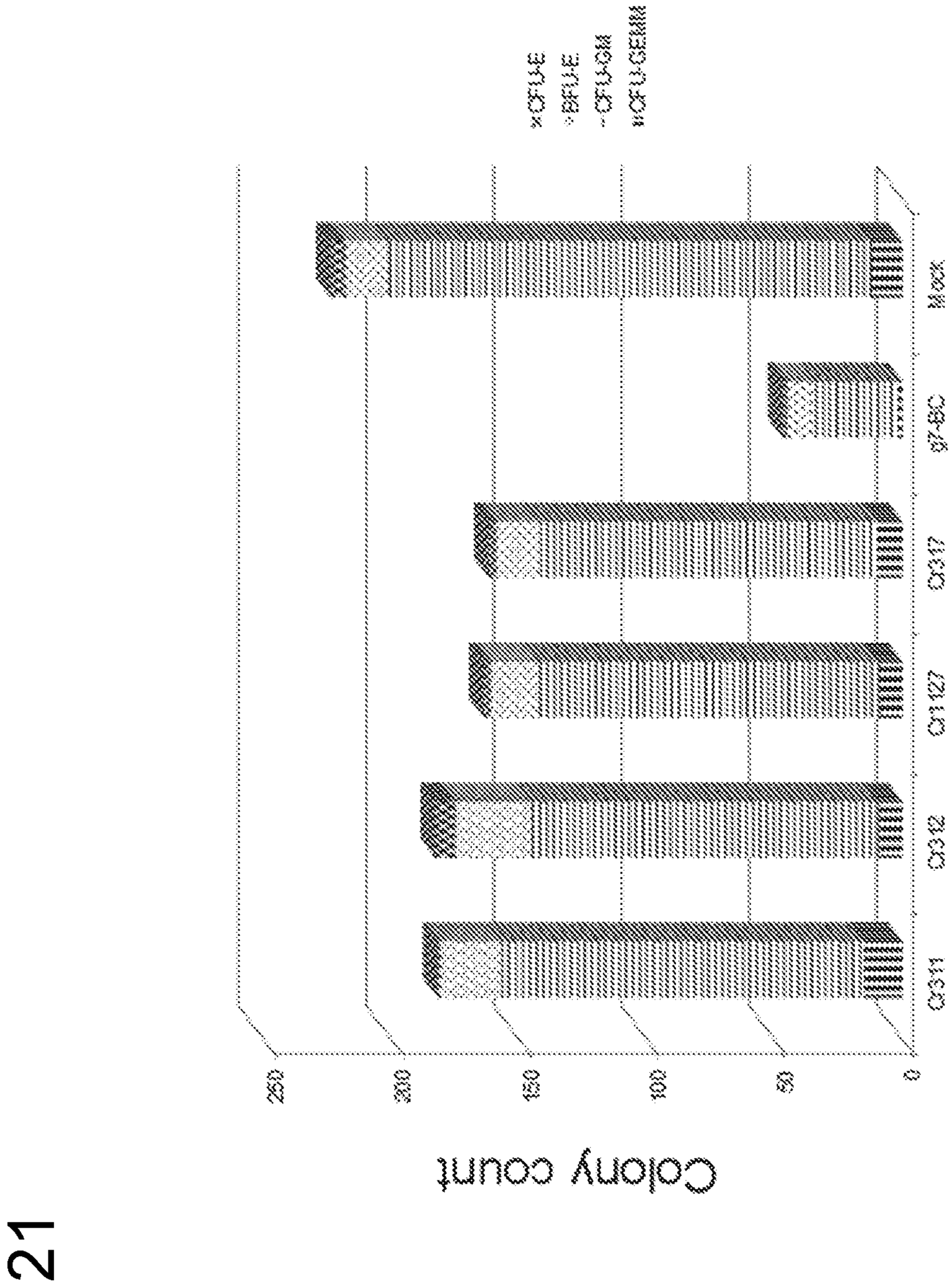

Cr311
Cr312
Cr127
Cr313
Cr314
Cr315
Cr316
Cr317
Mock
0
50
100
150
200
250
Colony count

Figure 21

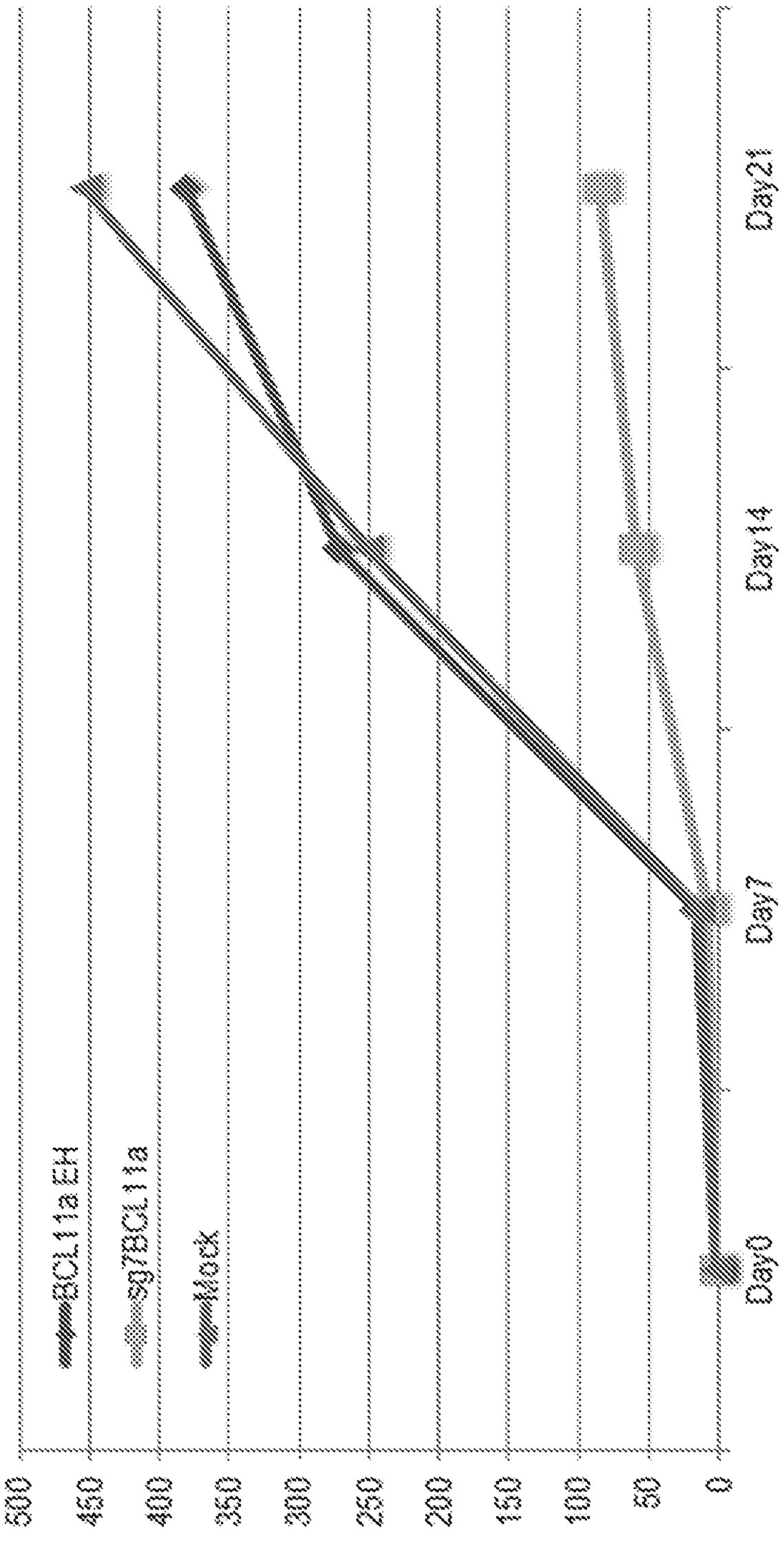

Day21
Day14
Day7
Day0
BCL11a EH
sg7BCL11a
Mock
Fold expansion
60
45
40
35
30
25
20
15
10
5
0

Figure 22

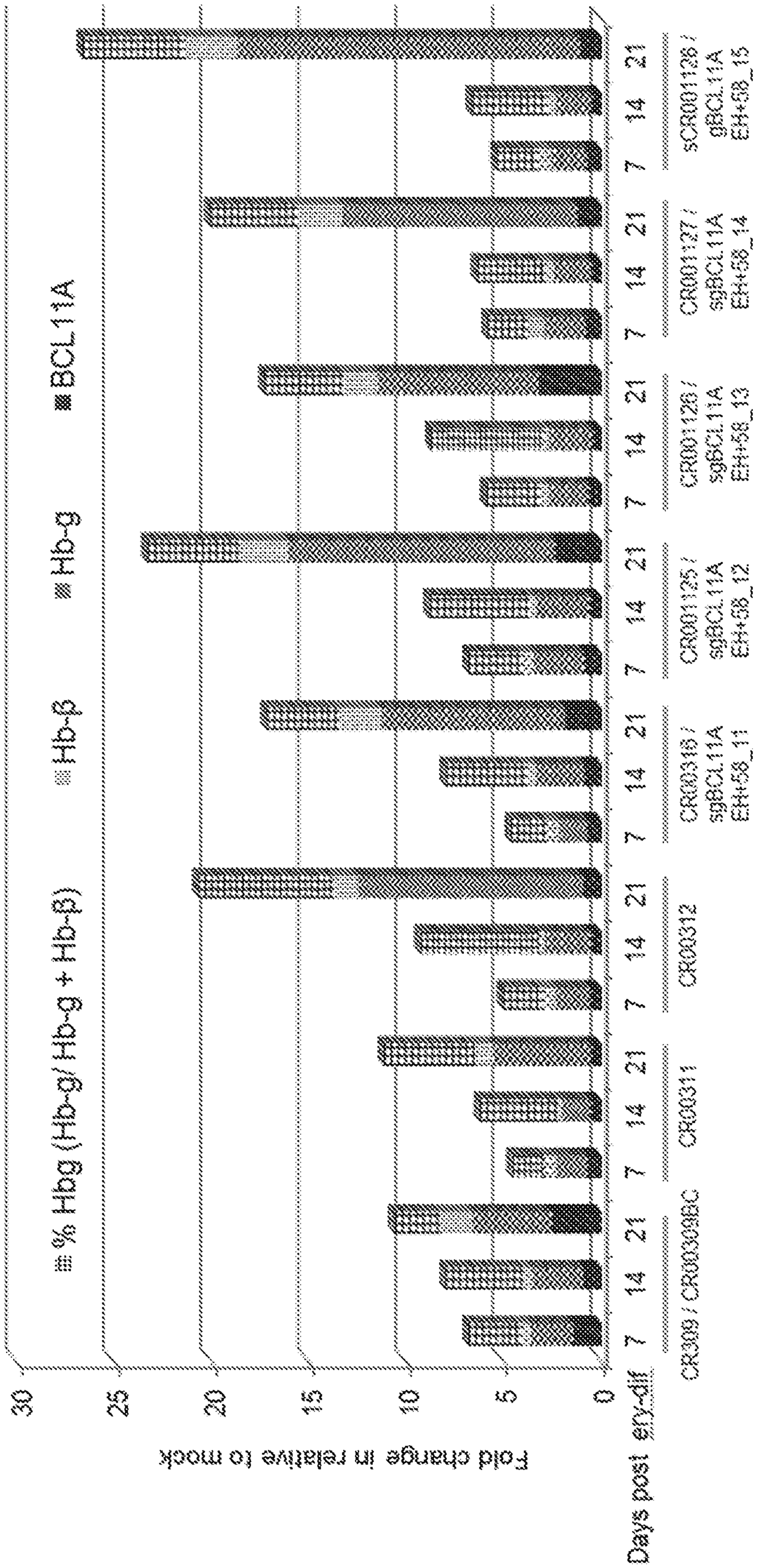

Hb-β
Hb-g
BCL11A
% Hbg (Hb-g/ Hb-g + Hb-β)
Fold change in relative to mock
30
25
20
15
10
5
0
Days post EPO-d7
7 14 21 7 14 21
CR309 / CR00309BC
CR00311
CR00312
7 14 21 7 14 21
CR00313 / sgBCL11A EH+58_11
CR00125 / sgBCL11A EH+58_12
CR00126 / sgBCL11A EH+58_13
7 14 21 7 14 21
CR00127 / sgBCL11A EH+58_14
CR00128 / gBCL11A EH+58_15

Figure 23

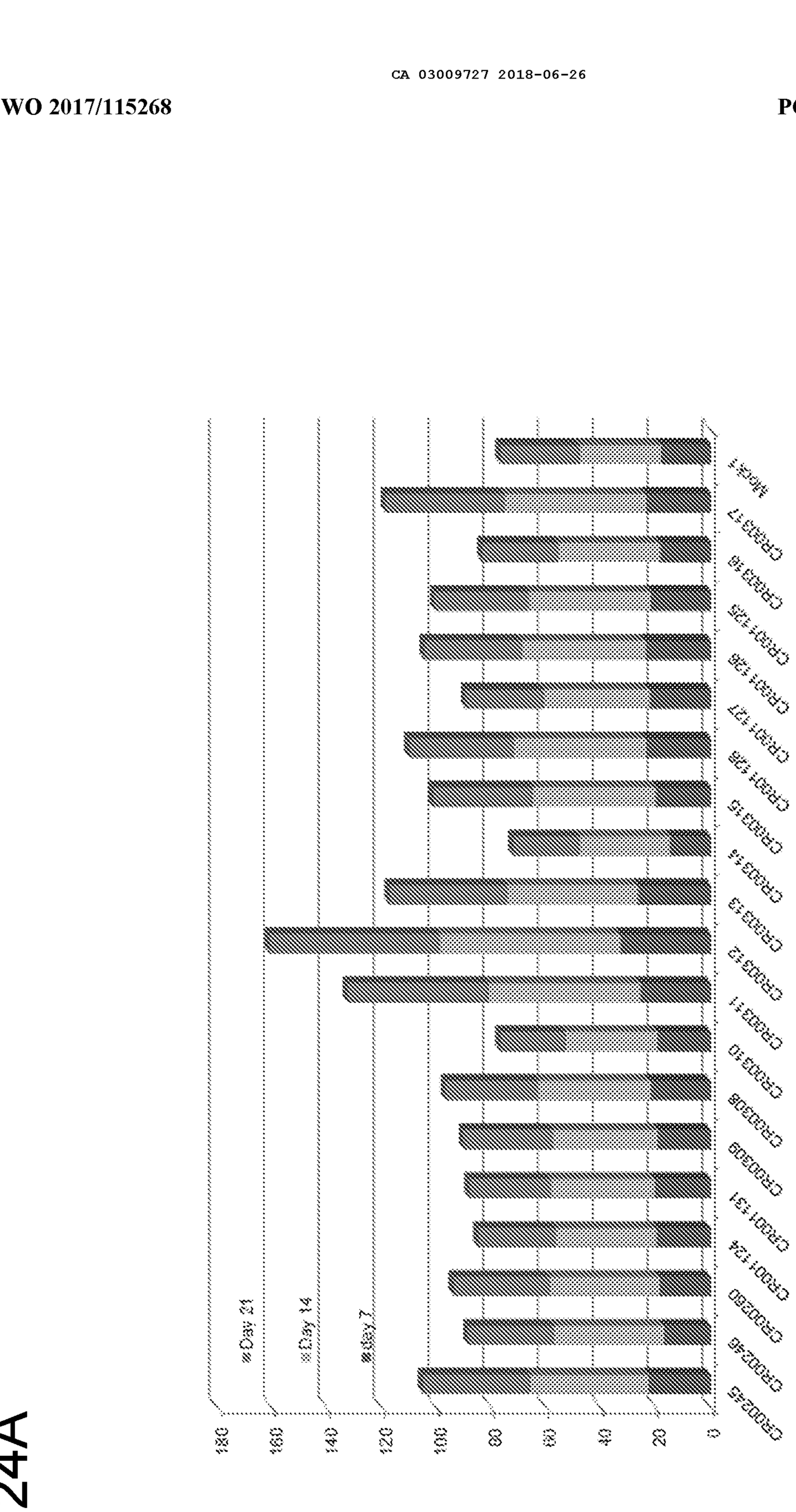

Day 21
Day 14
Day 7
0
20
40
60
80
100
120
140
160
180
CR00245
CR00246
CR00260
CR00124
CR00131
CR00309
CR00308
CR00310
CR00311
CR00312
CR00313
CR00316
CR00315
CR00128
CR00127
CR00128
CR00125
CR00317
Mock

Figure 24A

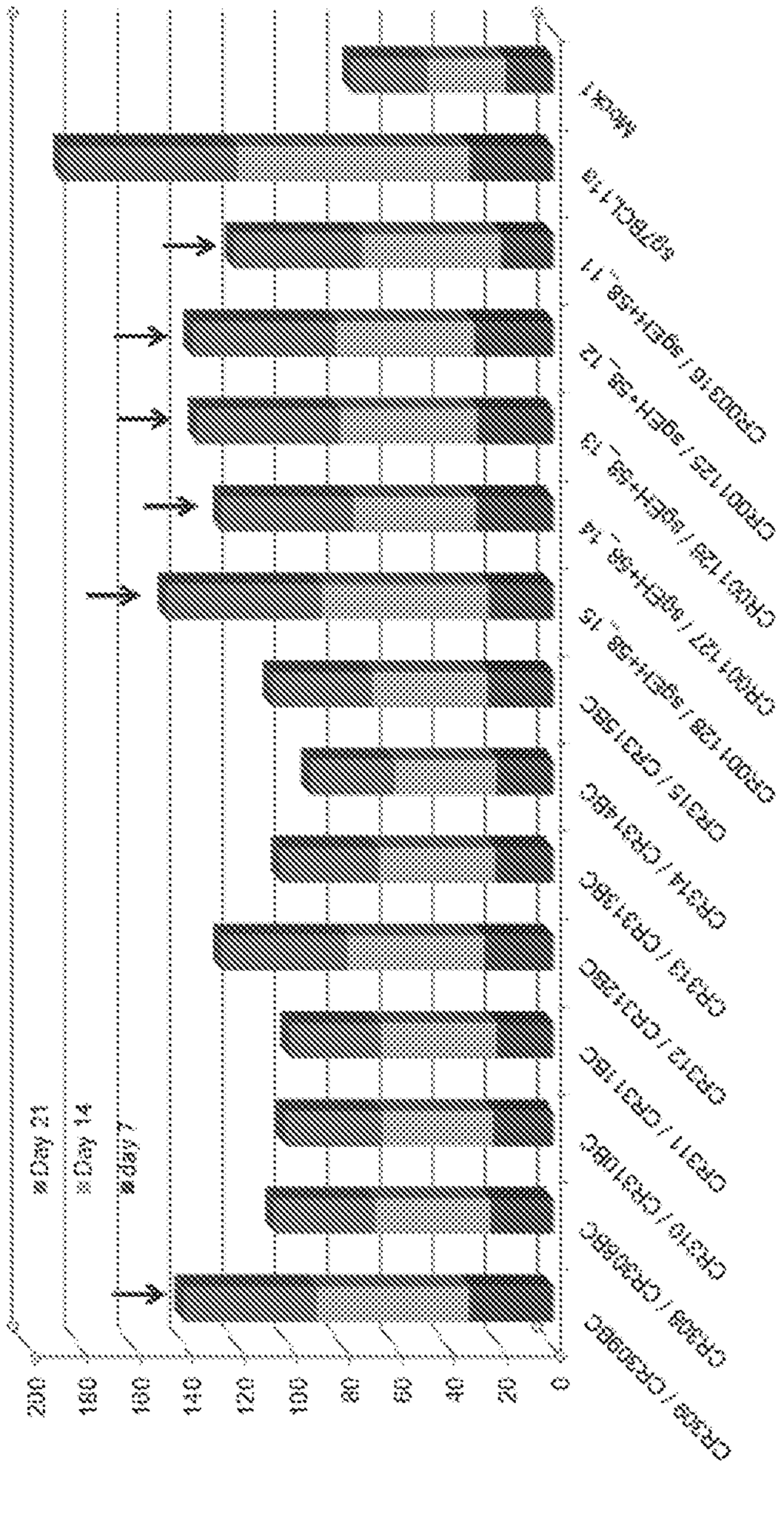

Day 21
Day 14
day 7
200
180
160
140
120
100
80
60
40
20
0
CR4038 / CR4039EC
CR4039 / CR4040EC
CR4019 / CR4030EC
CR4031 / CR4010EC
CR4012 / CR4013EC
CR4013 / CR4014EC
CR4014 / CR4015EC
CR4015 / CR4016EC
CR4004 / CR4028EC
CR4028 / CR4027EC
CR4027 / CR4026EC
CR4026 / CR4025EC
CR4025 / CR4024EC
CR4024 / CR4023EC
CR4023 / CR4022EC
CR4022 / CR4021EC
CR4021 / CR4020EC
CR4020 / CR4009EC
CR4009 / CR4008EC
CR4008 / CR4007EC
CR4007 / CR4006EC
CR4006 / CR4005EC
CR4005 / CR4004EC

Figure 24B

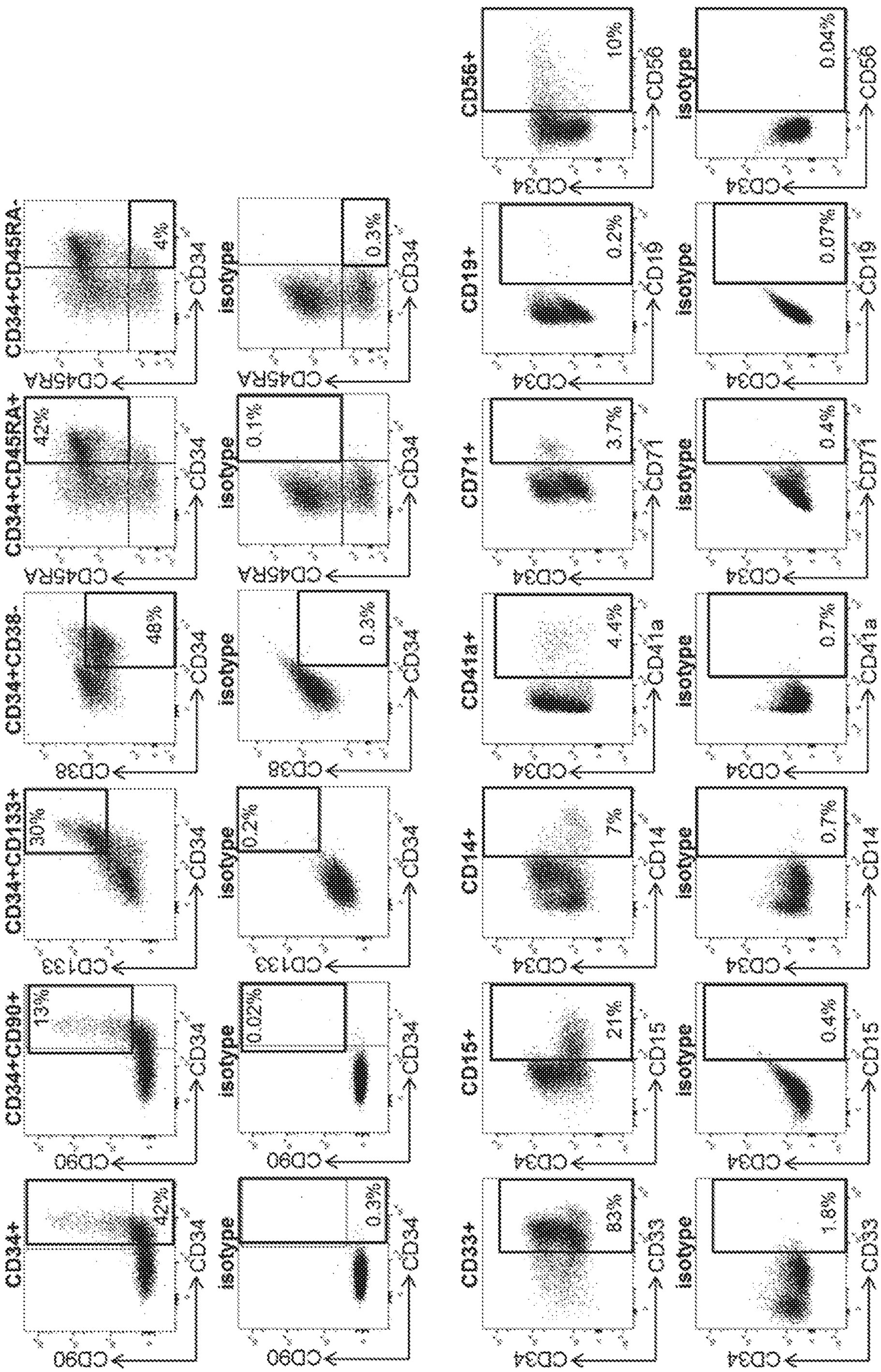
Figure 25

Tal-1 site GATA-1 site

crRNA	Indel (%)		WT	Rate
CR00311	86	GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGCCTCTGATTAGG-TGGGGGCGTGGGTGGGGTAGAAGAG	1 bp deletion	9%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGCCTCTG----------GGGGCGTGGGTGGGGTAGAAGAG	9 bp deletion	2%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGC----------GTGGGTGGGGTAGAAGAG	19 bp deletion	3%
CR00312	94	GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGC----------GTGGGTGGGGTAGAAGAG	19 bp deletion	6%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGCCTCTGATTAGG-TGGGGGCGTGGGTGGGGTAGAAGAG	1 bp deletion	5%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGG---------- CGTGGGTGGGGTAGAAGAG	24 bp deletion	2%
CR00313	90	GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGC----------GTGGGTGGGGTAGAAGAG	19 bp deletion	9%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGG----------TGGGGGCGTGGGTGGGGTAGAAGAG	13 bp deletion	3%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGG----------GGGCGTGGGTGGGGTAGAAGAG	16 bp deletion	3%
CR00315	73	GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGGC----------GTGGGTGGGGTAGAAGAG	19 bp deletion	5%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGG----------GCGTGGGTGGGGTAGAAGAG	18 bp deletion	3%
		GTCTAGTGCAAGCTAACAGTTGCTTTTATCACAGGCTCCAGGAAGGGTTTGG----------GTGGGGTAGAAGAG	24 bp deletion	1%

(antisense strand)

Figure 26A


CD34+CD45RA-
4%
CD34
CD45RA
isotype
0.3%
CD34
CD45RA
CD56+
10%
CD56
CD34
isotype
0.04%
CD56
CD34
CD34+CD45RA+
42%
CD34
CD45RA
isotype
0.1%
CD34
CD45RA
CD19+
0.2%
CD19
CD34
isotype
0.07%
CD19
CD34
CD34+CD38-
48%
CD34
CD38
isotype
0.3%
CD34
CD38
CD71+
3.7%
CD71
CD34
isotype
0.4%
CD71
CD34
CD41a+
4.4%
CD41a
CD34
isotype
0.7%
CD41a
CD34
CD34+CD133+
30%
CD34
CD133
isotype
0.2%
CD34
CD133
CD14+
7%
CD14
CD34
isotype
0.7%
CD14
CD34
CD34+CD90+
13%
CD34
CD90
isotype
0.02%
CD34
CD90
CD15+
21%
CD15
CD34
isotype
0.4%
CD15
CD34
CD34+
42%
CD34
CD90
isotype
0.3%
CD34
CD90
CD33+
83%
CD33
CD34
isotype
1.8%
CD33
CD34

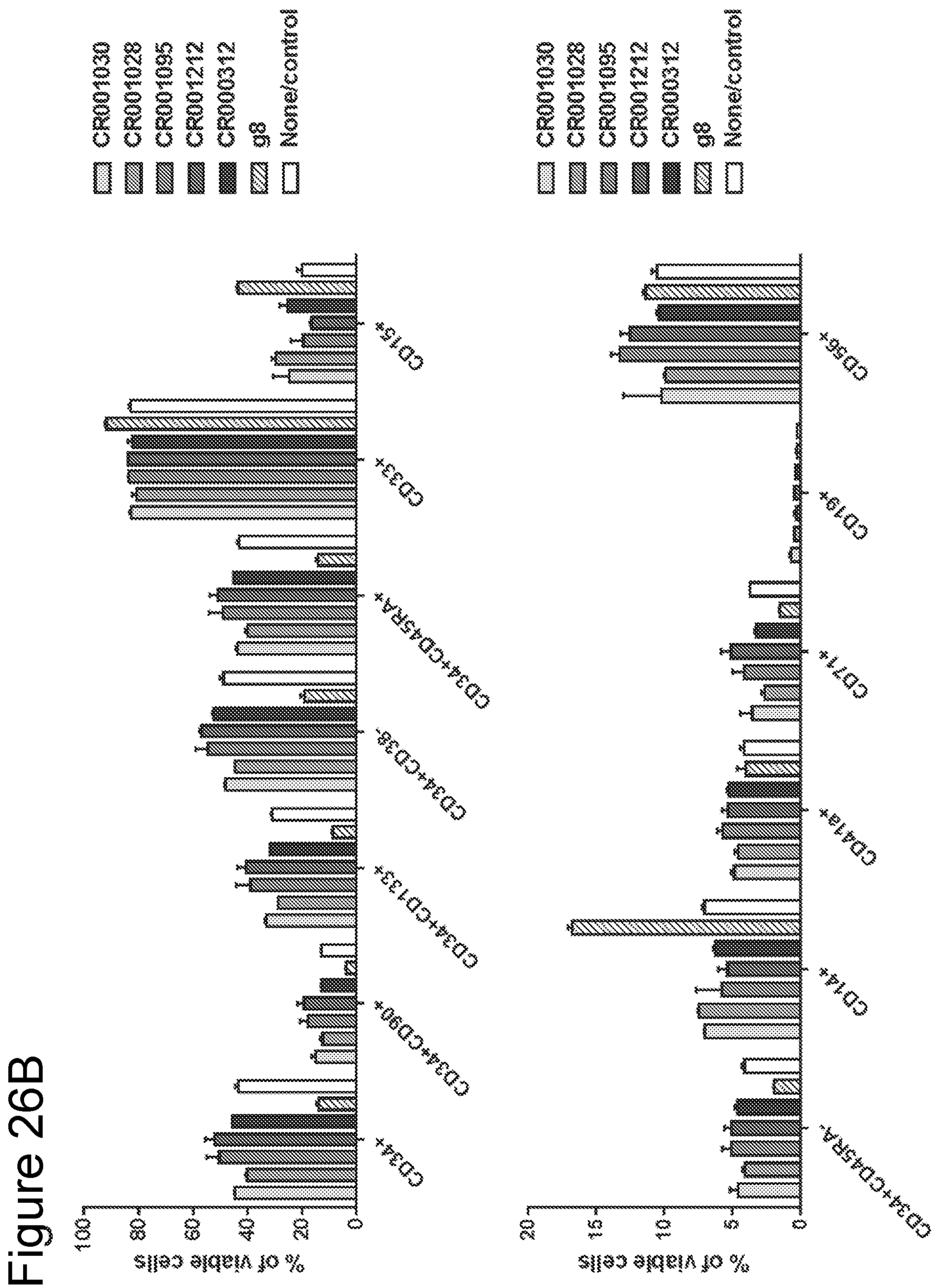
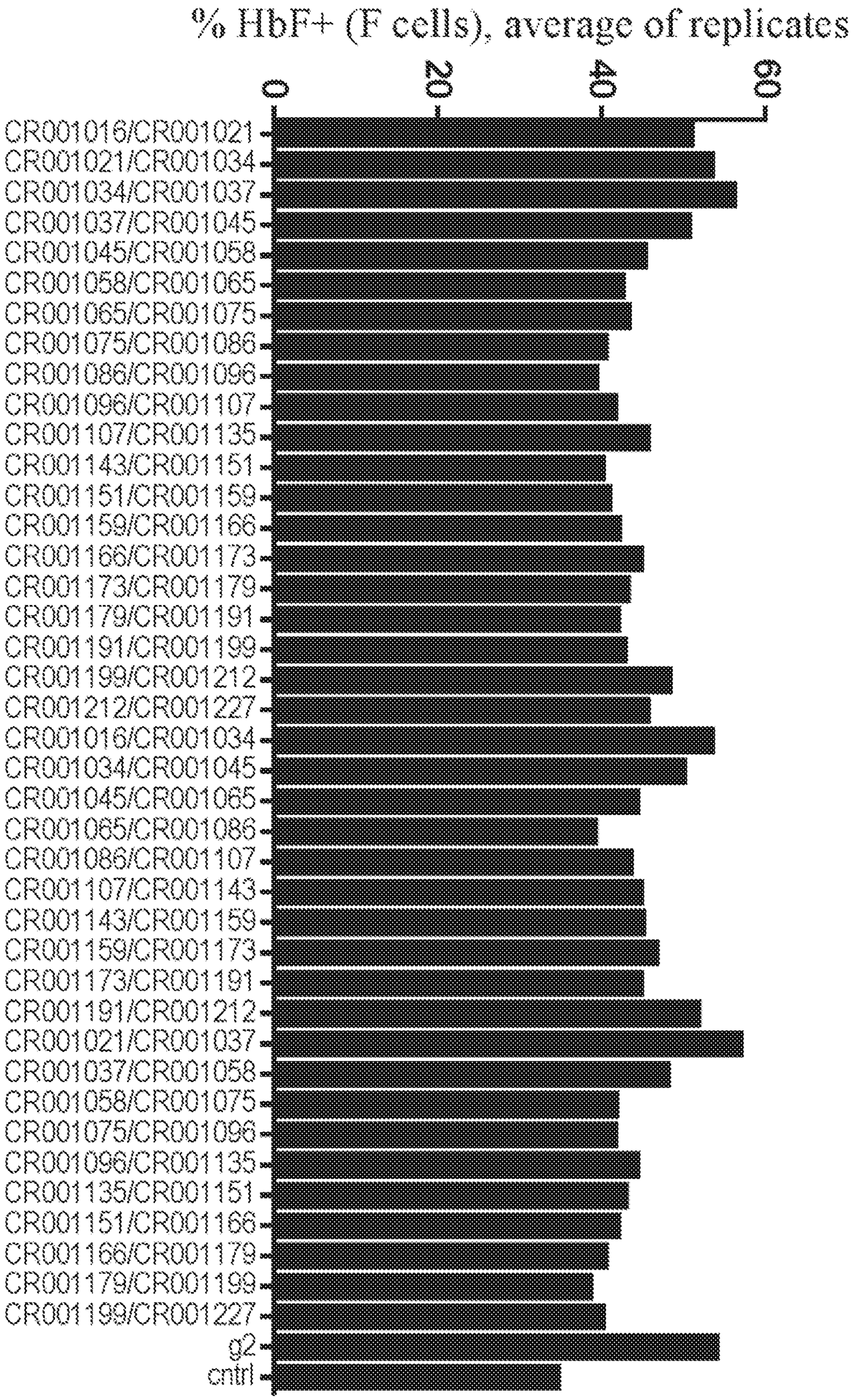

CR001030
CR001028
CR001035
CR001212
CR000312
g8
None/control
% of viable cells
CD56+
CD19+
CD71+
CD4+3a+
CD14+
CD34+CD45RA+
CD16+
CD33+
CD34+CD45RA+
CD34+CD38-
CD34+CD133+
CD34+CD90+
CD34+

Figure 26B

Figure 27



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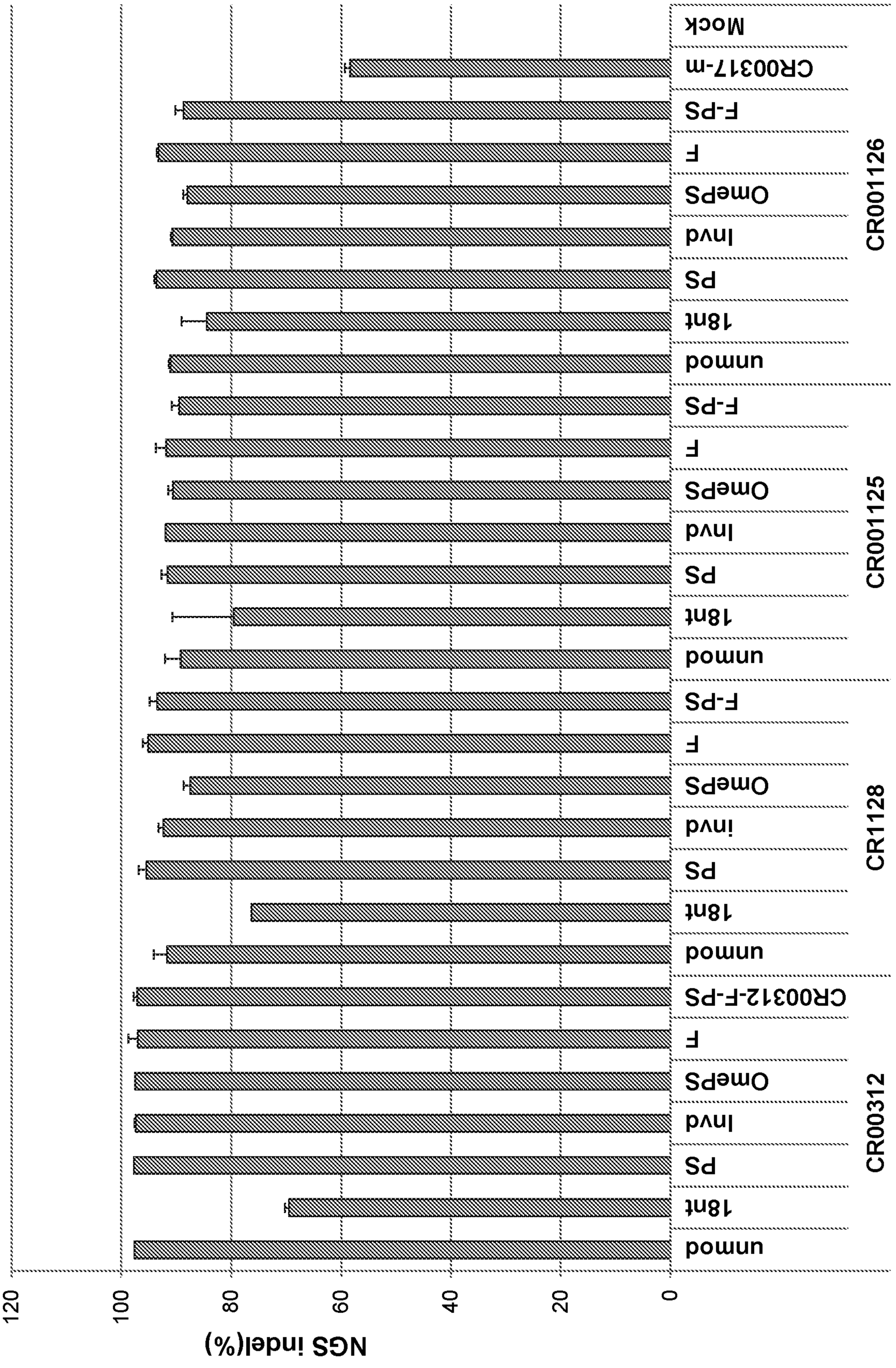

CR001126
Mock
CR00317-m
F-PS
F
OmePS
Invd
PS
18nt
unmod
CR001125
F-PS
unmod
18nt
PS
Invd
OmePS
F
CR00312-F-PS
CR1128
F-PS
unmod
18nt
PS
Invd
OmePS
F
CR00312
F-PS
unmod
18nt
PS
Invd
OmePS
F
CR00312-F-PS
unmod
18nt
PS
Invd
OmePS
F
F-PS
unmod
NGS indel(%)
120
100
80
60
40
20
0

Figure 28A

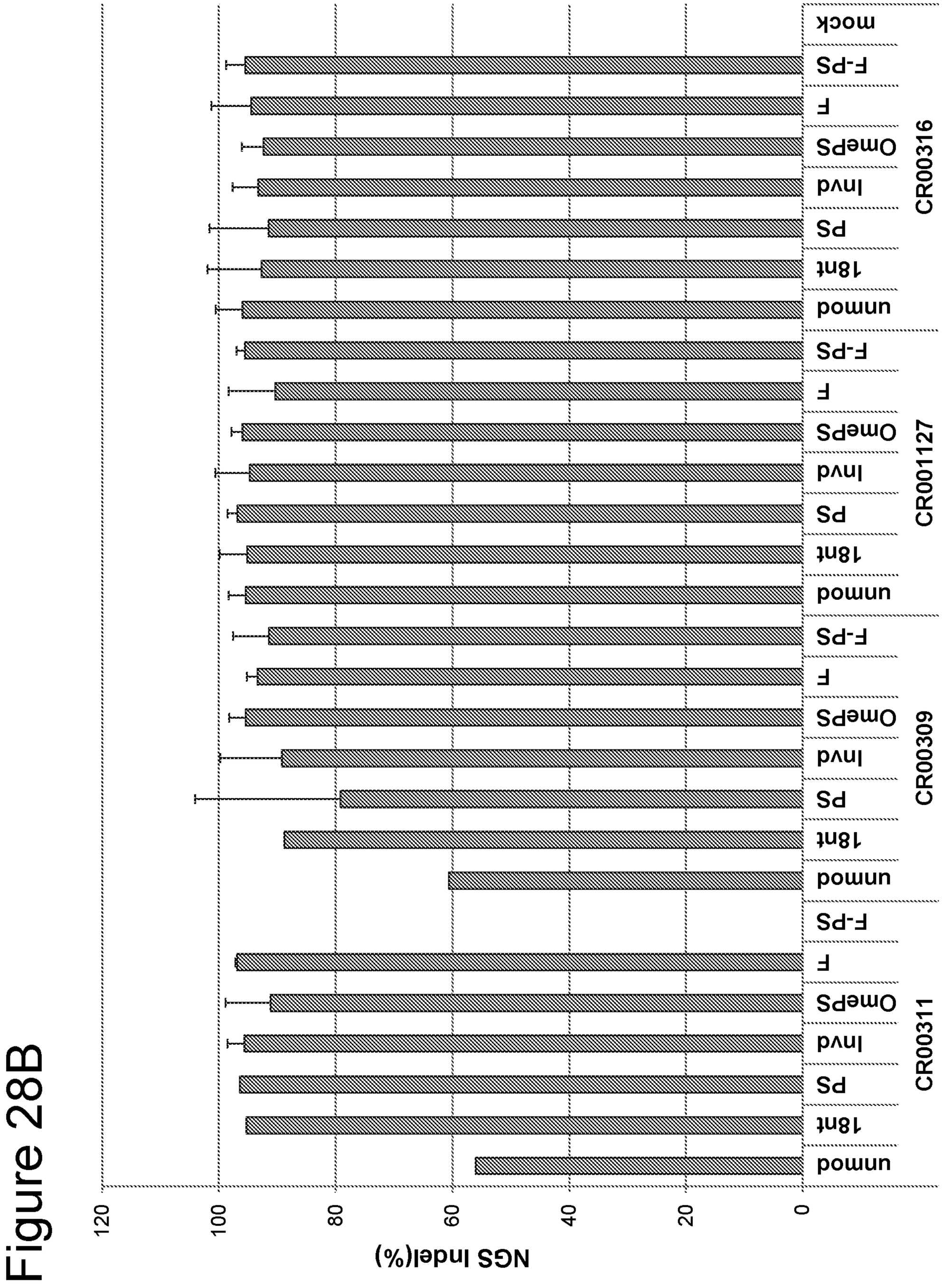

CR00316
mock
F-PS
F
OmePS
Invd
PS
18nt
unmod
CR001127
F-PS
unmod
18nt
PS
Invd
OmePS
F
F-PS
CR00309
unmod
18nt
PS
Invd
OmePS
F
F-PS
CR00311
unmod
18nt
PS
Invd
OmePS
F
F-PS
unmod
18nt
PS
Invd
OmePS
F
F-PS
mock
0
20
40
60
80
100
120
NGS Indel(%)

Figure 28B


NGS-Indel(%)
0
20
40
60
80
100
120
Mock
WT
PS sg316 Cas9
PS sg1127
PS sg309
PS sg31
PS sg1126
PS sg1125
PS sg1128
PS sg312
MOePS sg316
OMePS sg1127
OMePS sg309
OMePS sg31
OMePS sg1126-1
OMePS sg1125
OMePS sg1128
OMePS sg312
18 sg316
18 sg1127
18 sg309
18 sg31
18 sg1126
18 sg1125
18 sg1128
18 sg312
unmod sg316
unmod sg1127
unmod-sg309
unmod sg31
unmod sg1126
unmod sg1125
unmod sg1128
unmod sg312

Figure 29

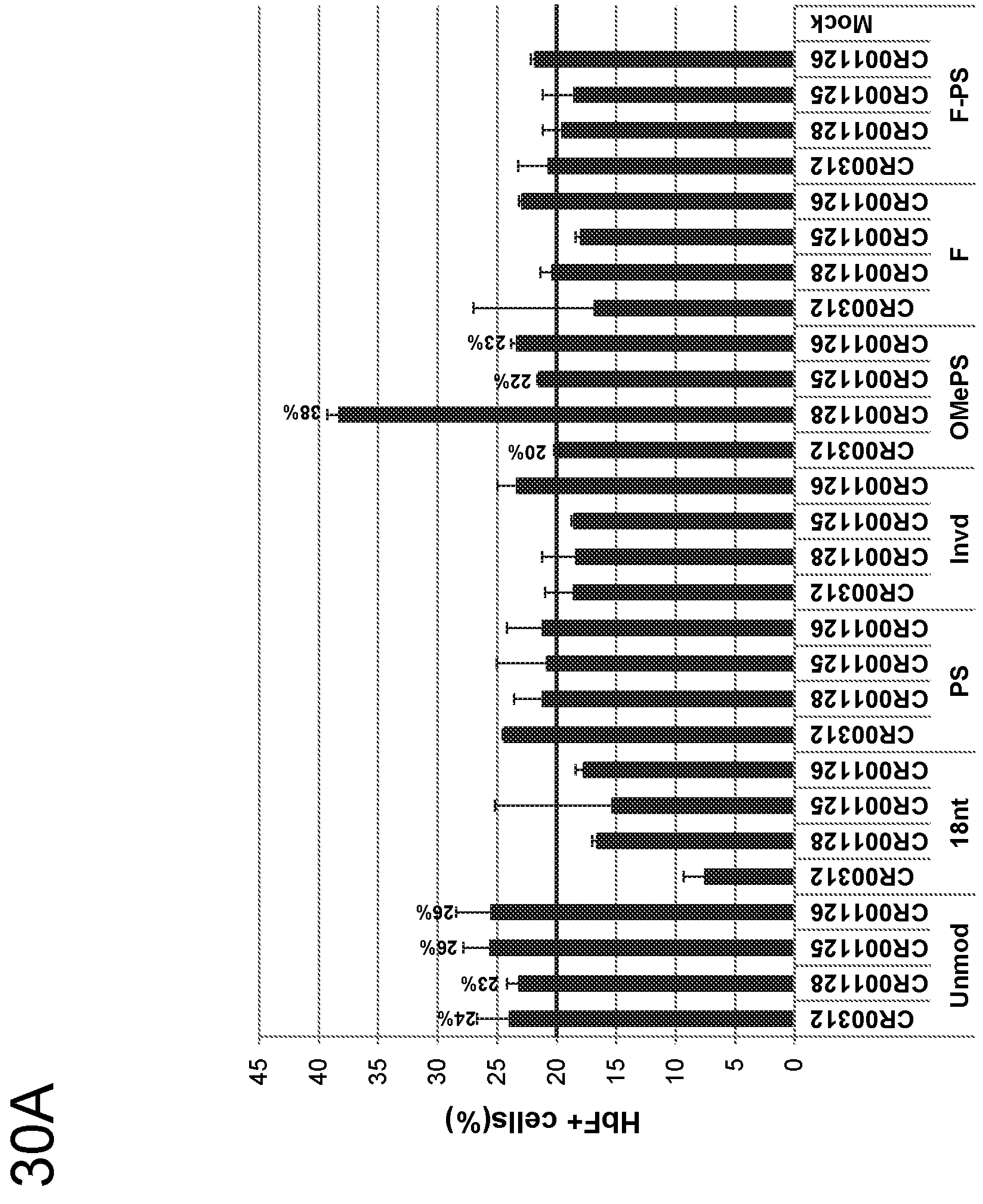
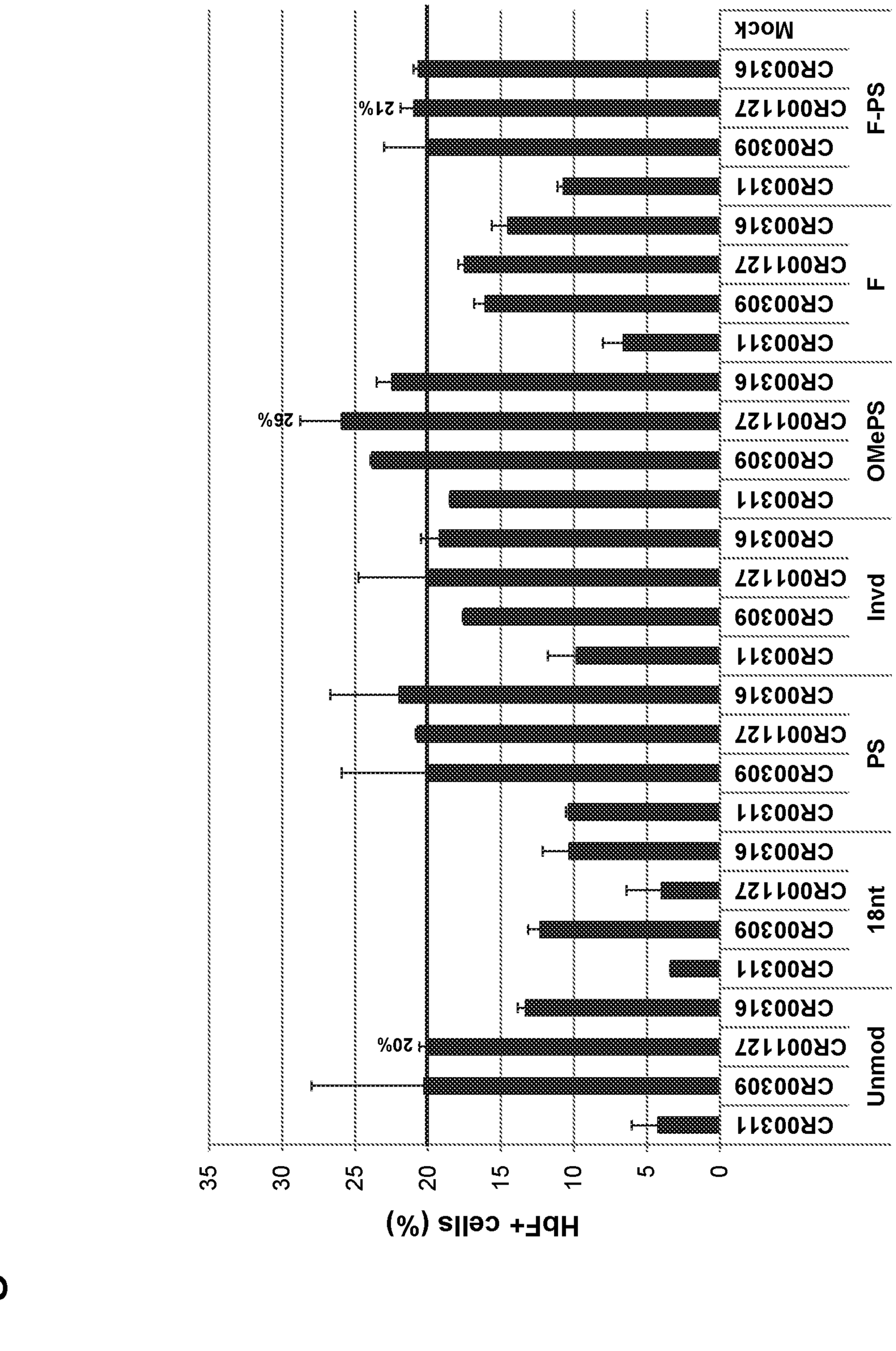

HbF+ cells(%)
0
5
10
15
20
25
30
35
40
45
Unmod
CR00312
CR001128
CR001125
CR001126
24%
23%
26%
26%
18nt
CR00312
CR001128
CR001125
CR001126
PS
CR00312
CR001128
CR001125
CR001126
F-PS
CR00312
CR001128
CR001125
CR001126
F
CR00312
CR001128
CR001125
CR001126
OMePS
CR00312
CR001128
CR001125
CR001126
38%
22%
23%
20%
Invd
CR00312
CR001128
CR001125
CR001126
PS
CR00312
CR001128
CR001125
CR001126
Mock

Figure 30A

Figure 30B


HbF+ cells (%)
35
30
25
20
15
10
5
0
20%
25%
21%
Unmod
18nt
PS
OMePS
F
F-PS
Invd
CR00311
CR00309
CR001127
CR00316
Mock

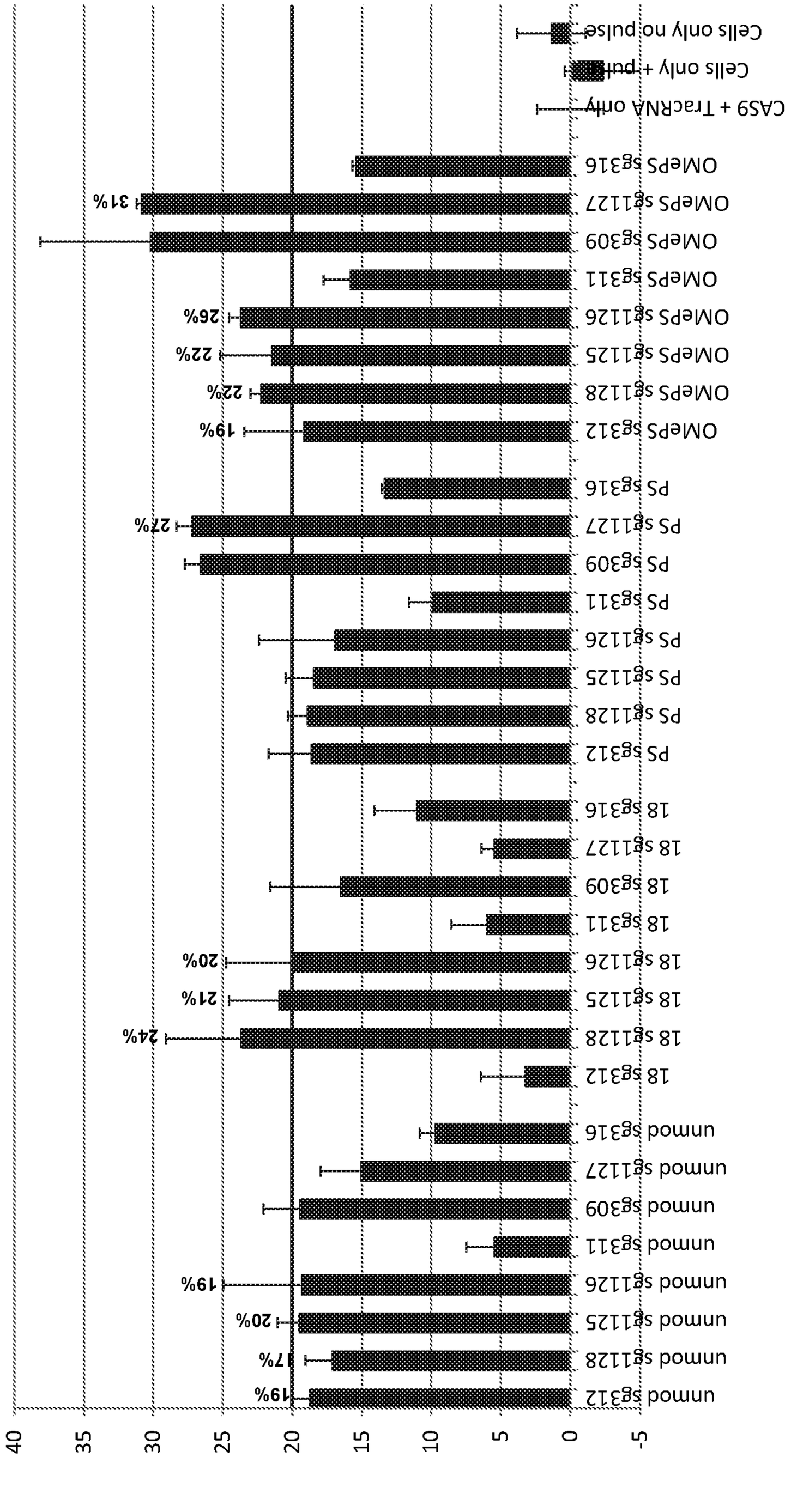

Cells only no pulse
Cells only + pulse
CAS9 + TracRNA only
OMePS sg316
OMePS sg1127
31%
OMePS sg309
OMePS sg311
OMePS sg1126
26%
OMePS sg1125
22%
OMePS sg1128
22%
OMePS sg312
19%
PS sg316
PS sg1127
27%
PS sg309
PS sg311
PS sg1126
PS sg1125
PS sg1128
PS sg312
18 sg316
18 sg1127
18 sg309
18 sg311
18 sg1126
20%
18 sg1125
21%
18 sg1128
24%
18 sg312
unmod sg316
unmod sg1127
unmod sg309
unmod sg311
unmod sg1126
19%
unmod sg1125
20%
unmod sg1128
17%
unmod sg312
19%
40
35
30
25
20
15
10
5
0
-5

Figure 31

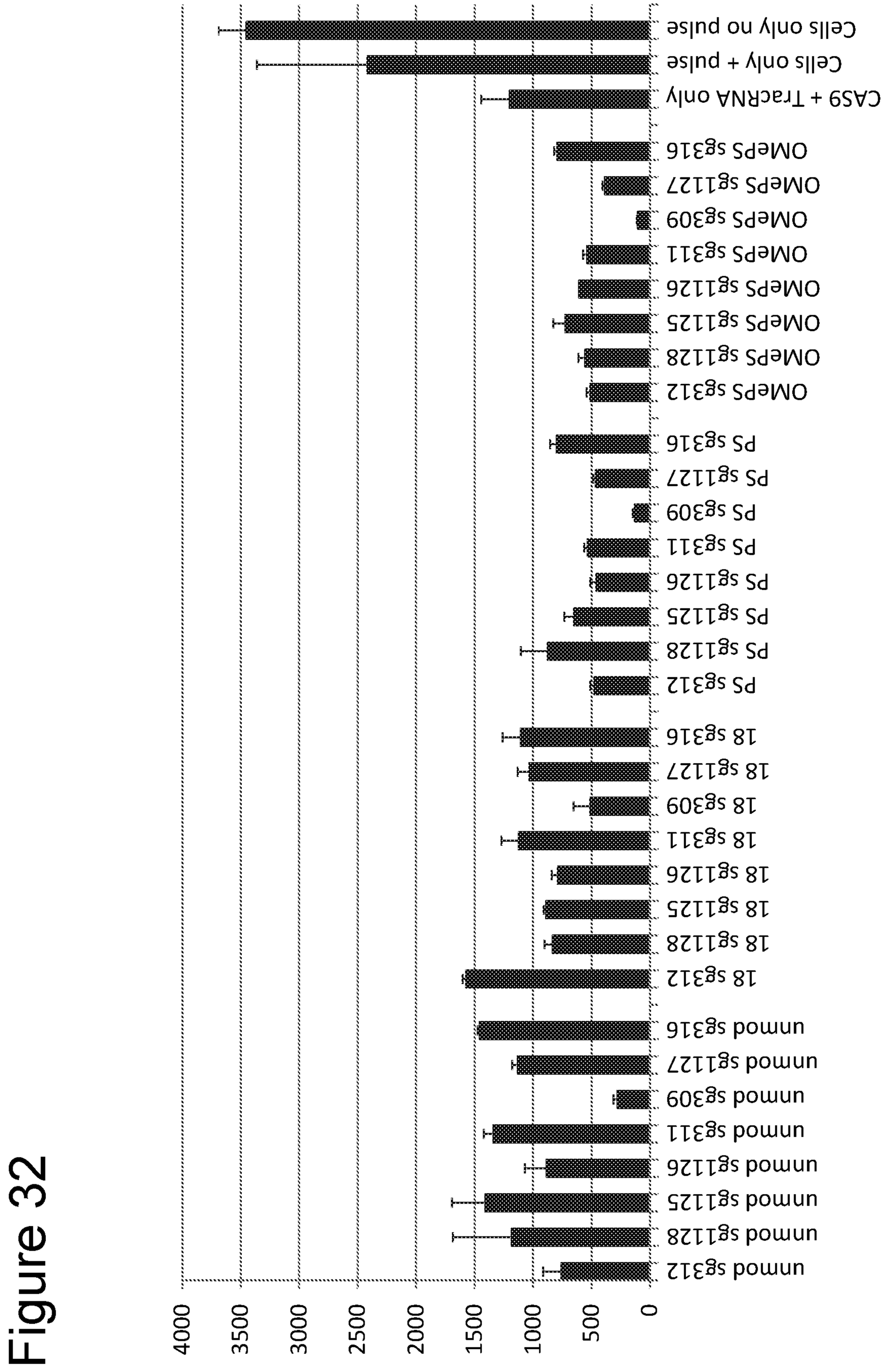

Cells only no pulse
Cells only + pulse
CAS9 + TracRNA only
OMePS sg316
OMePS sg1127
OMePS sg309
OMePS sg311
OMePS sg1126
OMePS sg1125
OMePS sg1128
OMePS sg312
PS sg316
PS sg1127
PS sg309
PS sg311
PS sg1126
PS sg1125
PS sg1128
PS sg312
18 sg316
18 sg1127
18 sg309
18 sg311
18 sg1126
18 sg1125
18 sg1128
18 sg312
unmod sg316
unmod sg1127
unmod sg309
unmod sg311
unmod sg1126
unmod sg1125
unmod sg1128
unmod sg312
4000
3500
3000
2500
2000
1500
1000
500
0

Figure 32

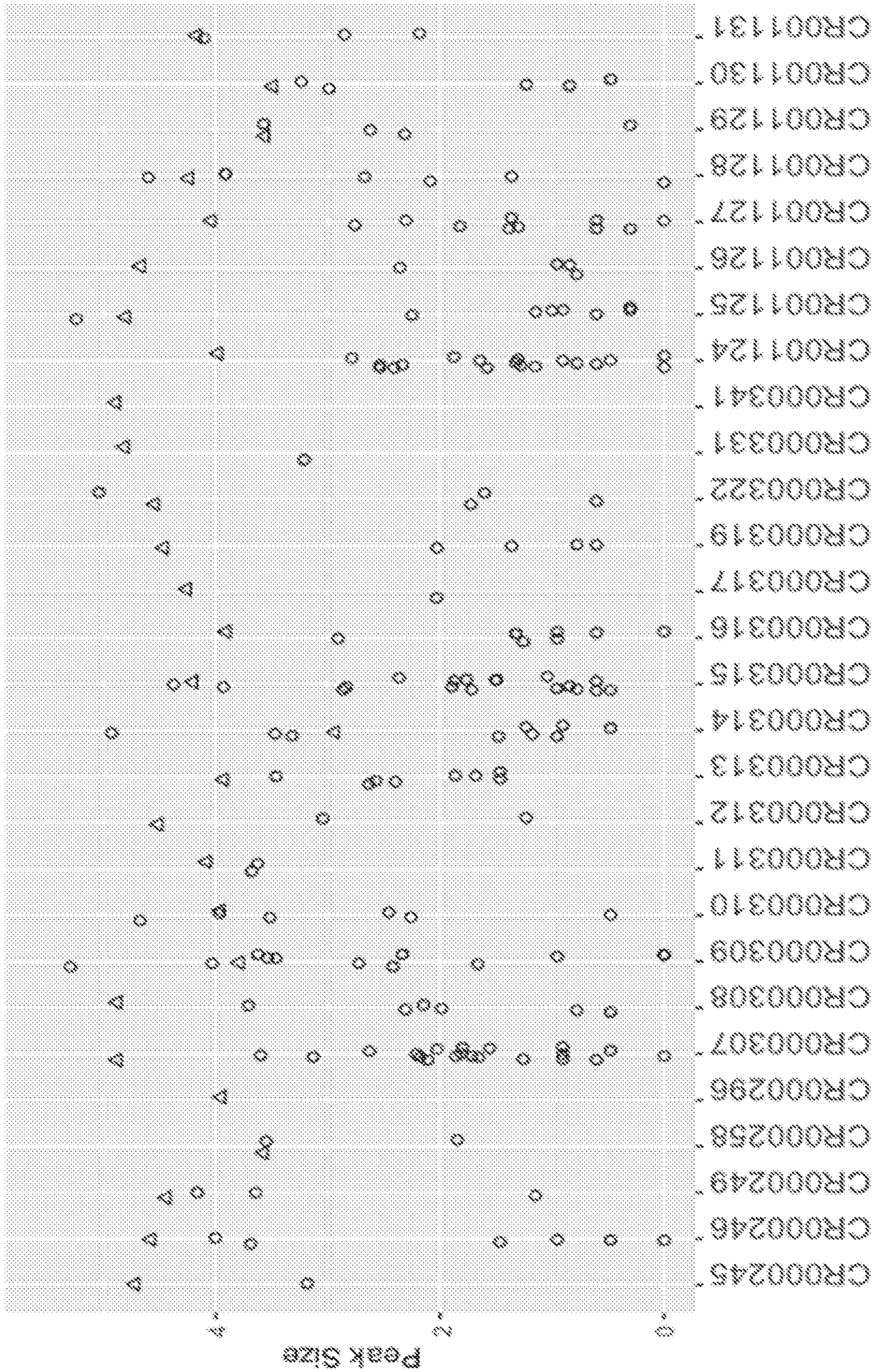
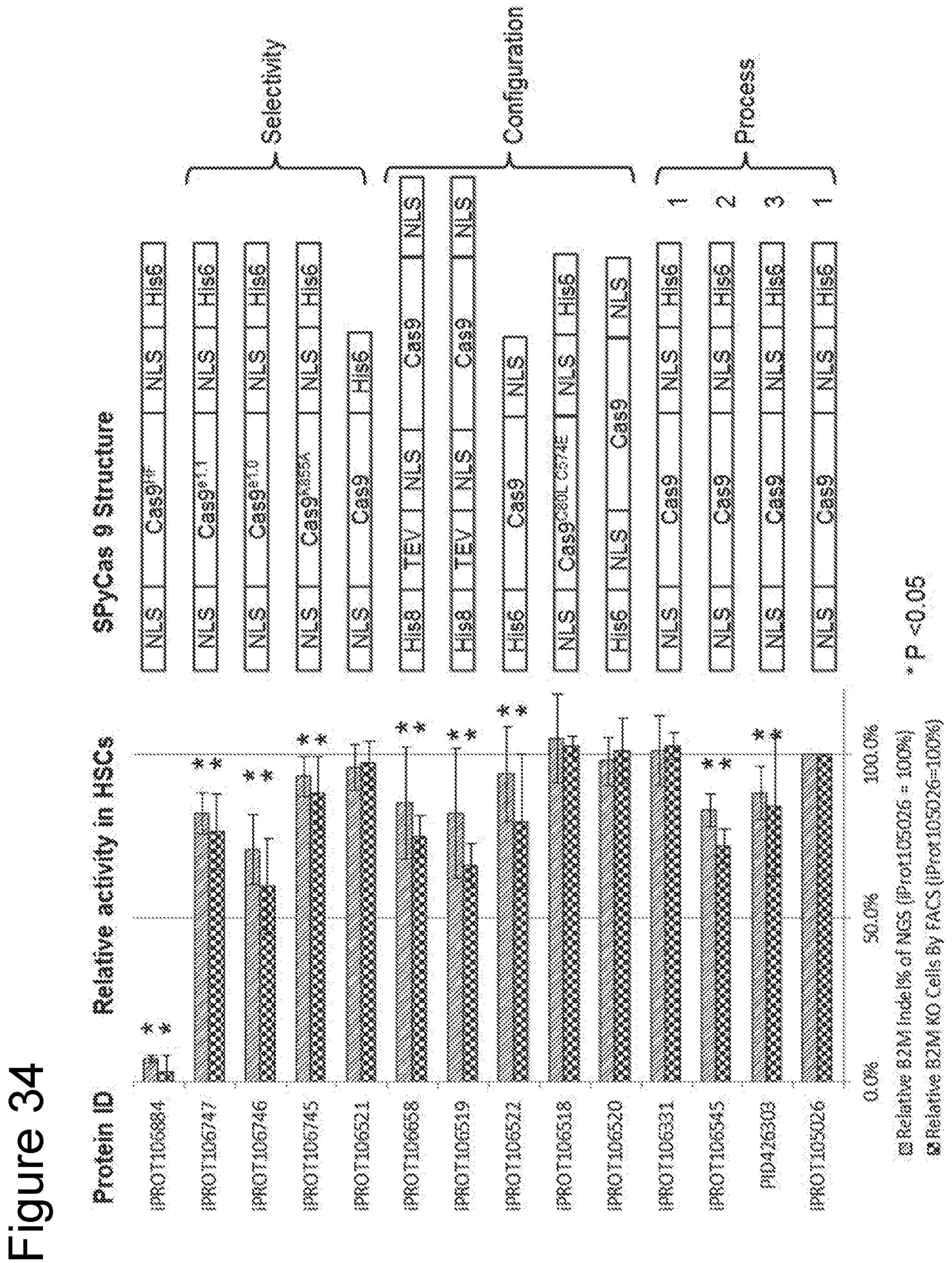

Guide Name
CR000244
CR000245
CR000246
CR000249
CR000258
CR000296
CR000307
CR000308
CR000309
CR000310
CR000311
CR000312
CR000313
CR000314
CR000316
CR000317
CR000319
CR000322
CR000331
CR000341
CR000124
CR000125
CR000126
CR000127
CR000128
CR000129
CR000130
CR000131
Peak Size
0
2
4

Figure 33

Figure 34


Selectivity
Configuration
Process
SpyCas 9 Structure
NLS
Cas9WT
NLS
His6
Cas9T1
Cas9R63A
Cas9R63A/R74E
TEV
Cas9LC674E
1
2
3
Relative activity in HSCs
* p <0.05
Protein ID
iPROT106824
iPROT106747
iPROT106746
iPROT106745
iPROT106521
iPROT106658
iPROT106519
iPROT106522
iPROT106518
iPROT106520
iPROT106331
iPROT106545
iPROT105026
0.0%
50.0%
100.0%
Relative B2M Indel% of NGS (iProt105026 = 100%)
Relative B2M KO Cells by FACS (iProt105026=100%)

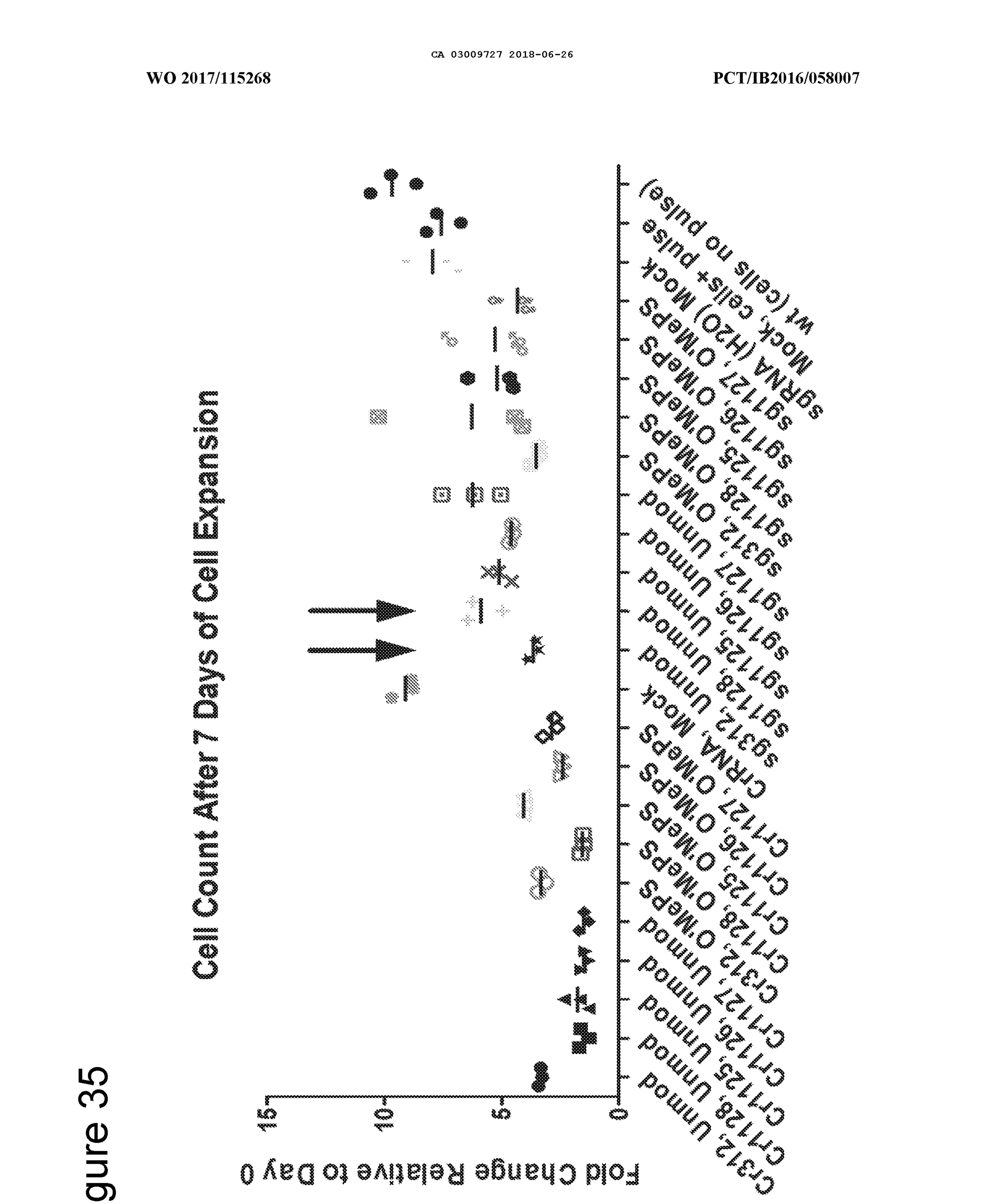
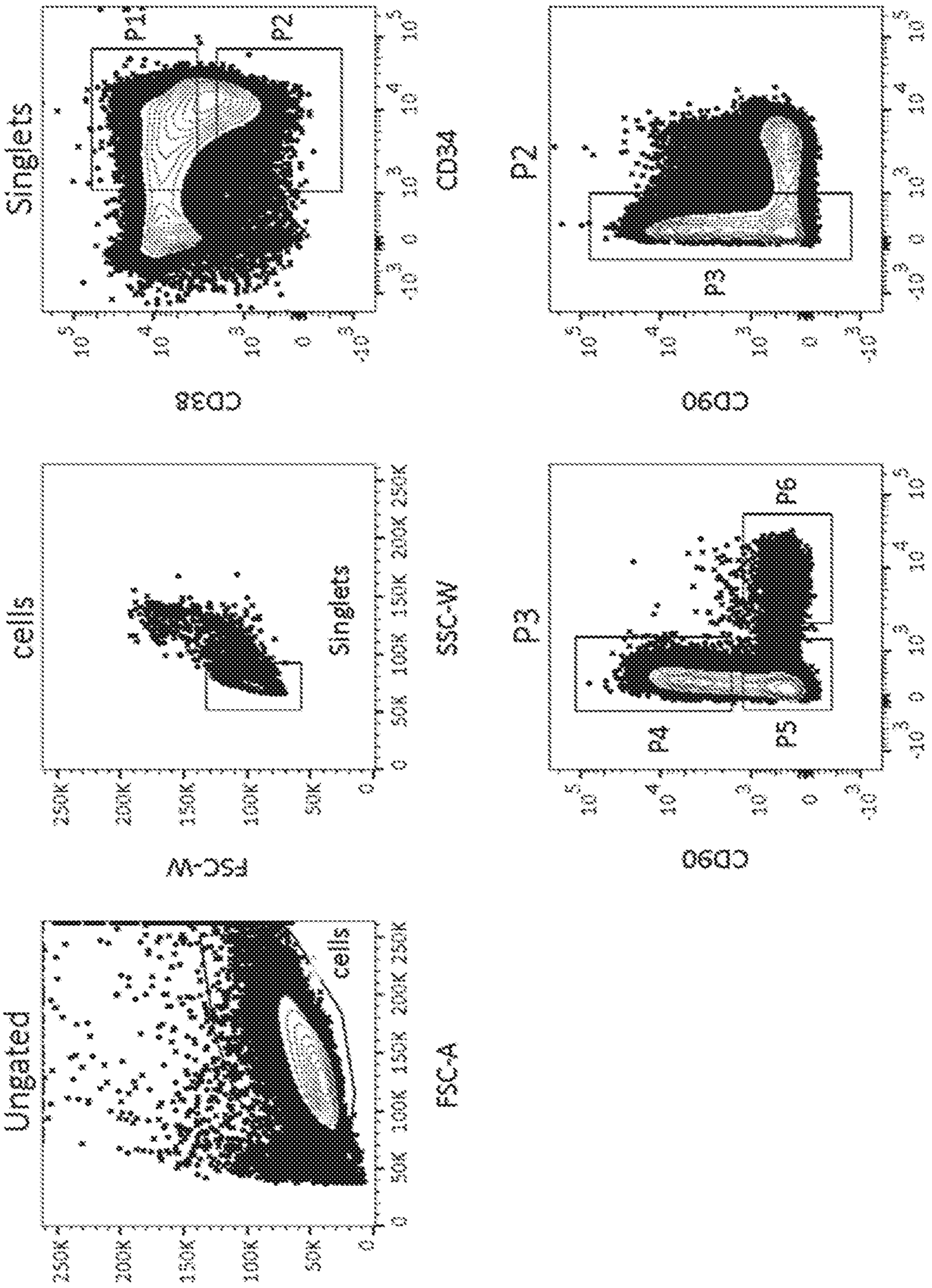

Cell Count After 7 Days of Cell Expansion
Fold change Relative to Day 0
0
5
10
15
Powder
UmmoD
C3132, C71128
C71425
C71428
C71427
C71429
C71426
C71431
C71432
C71433
C71434
C71435
C71436
C71437
C71438
C71439
C71440
C71441
C71442
C71443
C71444
C71445
C71446
C71447
C71448
C71449
C71450
C71451
C71452
C71453
C71454
C71455
C71456
C71457
C71458
C71459
C71460
C71461
C71462
C71463
C71464
C71465
C71466
C71467
C71468
C71469
C71470

Figure 3


Unstained
cells
SSC-A
FSC-A
cells
Singlets
SSC-W
FSC-W
P3
P4
P5
P6
CD49f
CD90
P2
P3
CD45RA
CD90
Singlets
CD34
CD38

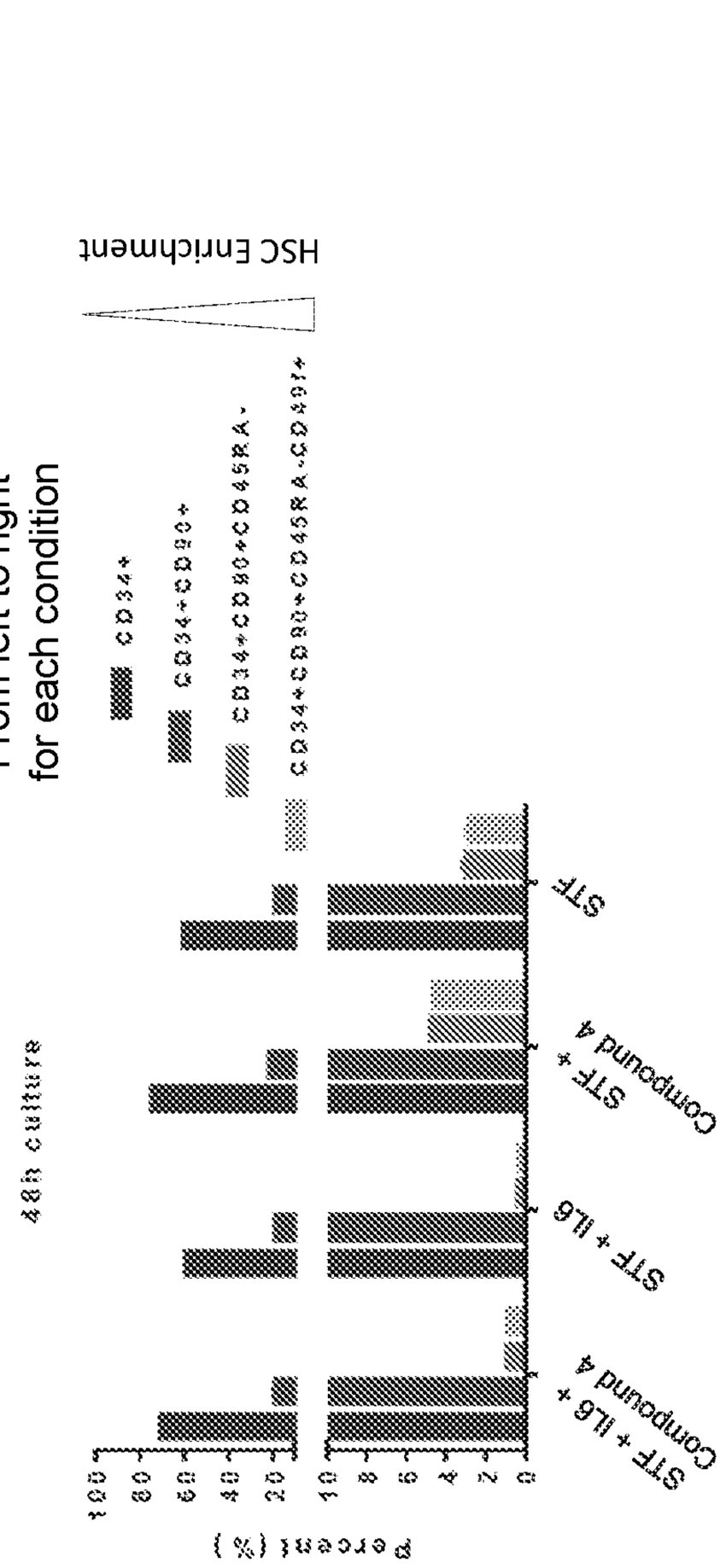

From left to right
for each condition
CD34+
CD34+CD90+
CD34+CD90+CD45RA-
CD34+CD90+CD45RA-CD49f+
HSC Enrichment
48h culture
Percent (%)
0
2
4
6
8
10
0
20
40
60
80
100
STF
STF + Compound 4
STF + IL6
STF + IL6 + Compound 4

Figure 37

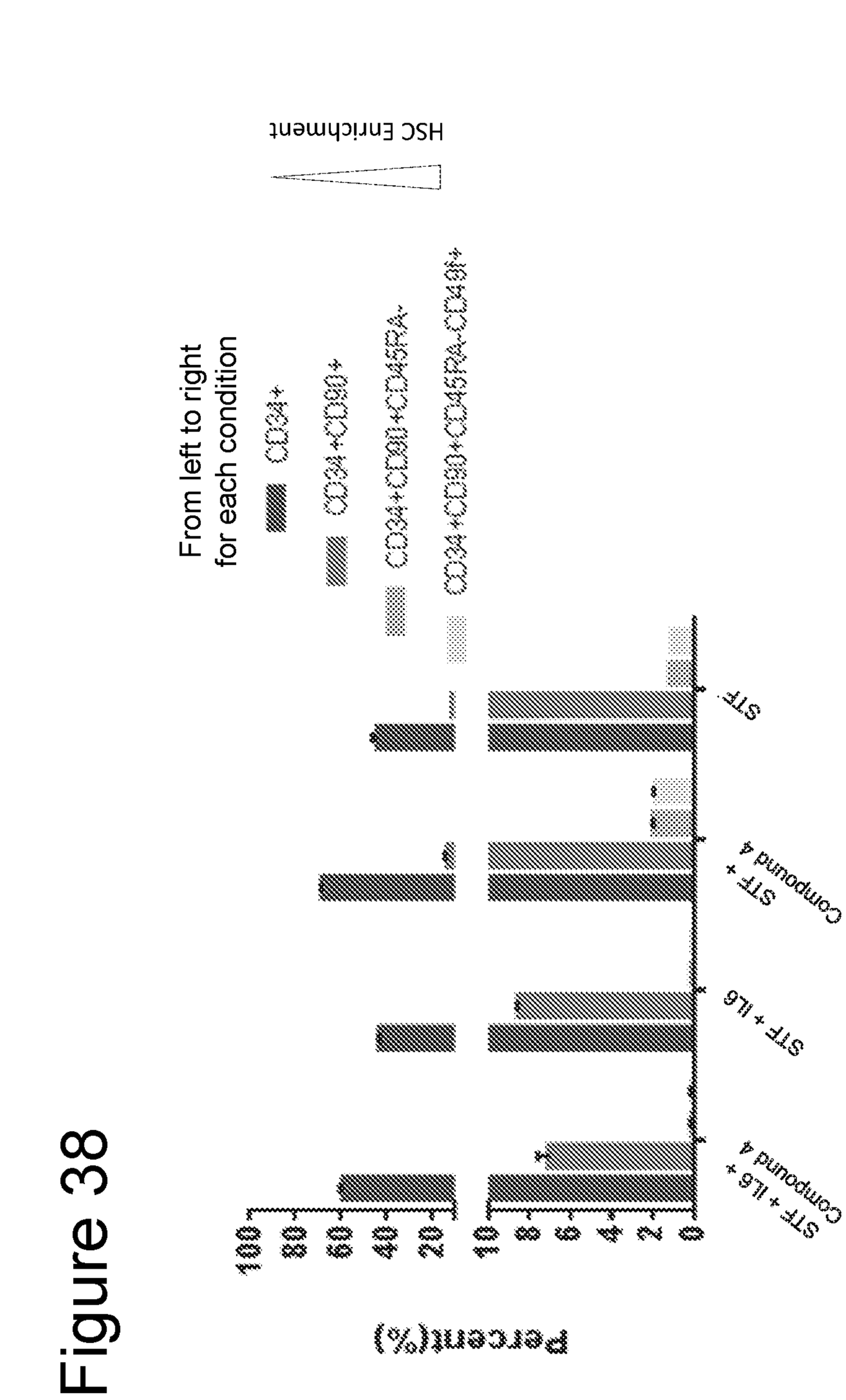

From left to right
for each condition
CD34+
CD34+CD90+
CD34+CD38-CD45RA-
CD34+CD90+CD45RA-CD49f+
HSC Enrichment
STF
STF + Compound 4
STF + IL6
STF + IL6 + Compound 4
100
80
60
40
20
10
8
6
4
2
0
Percent(%)

Figure 38

Figure 39B

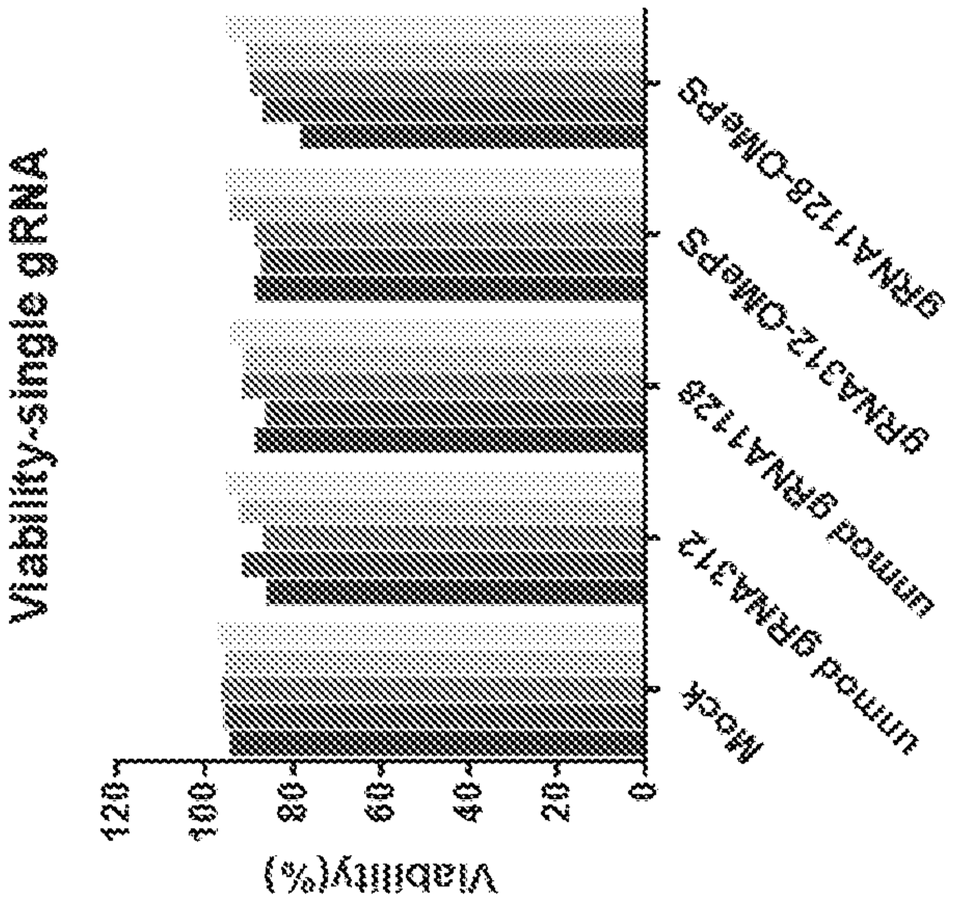


Figure 39A

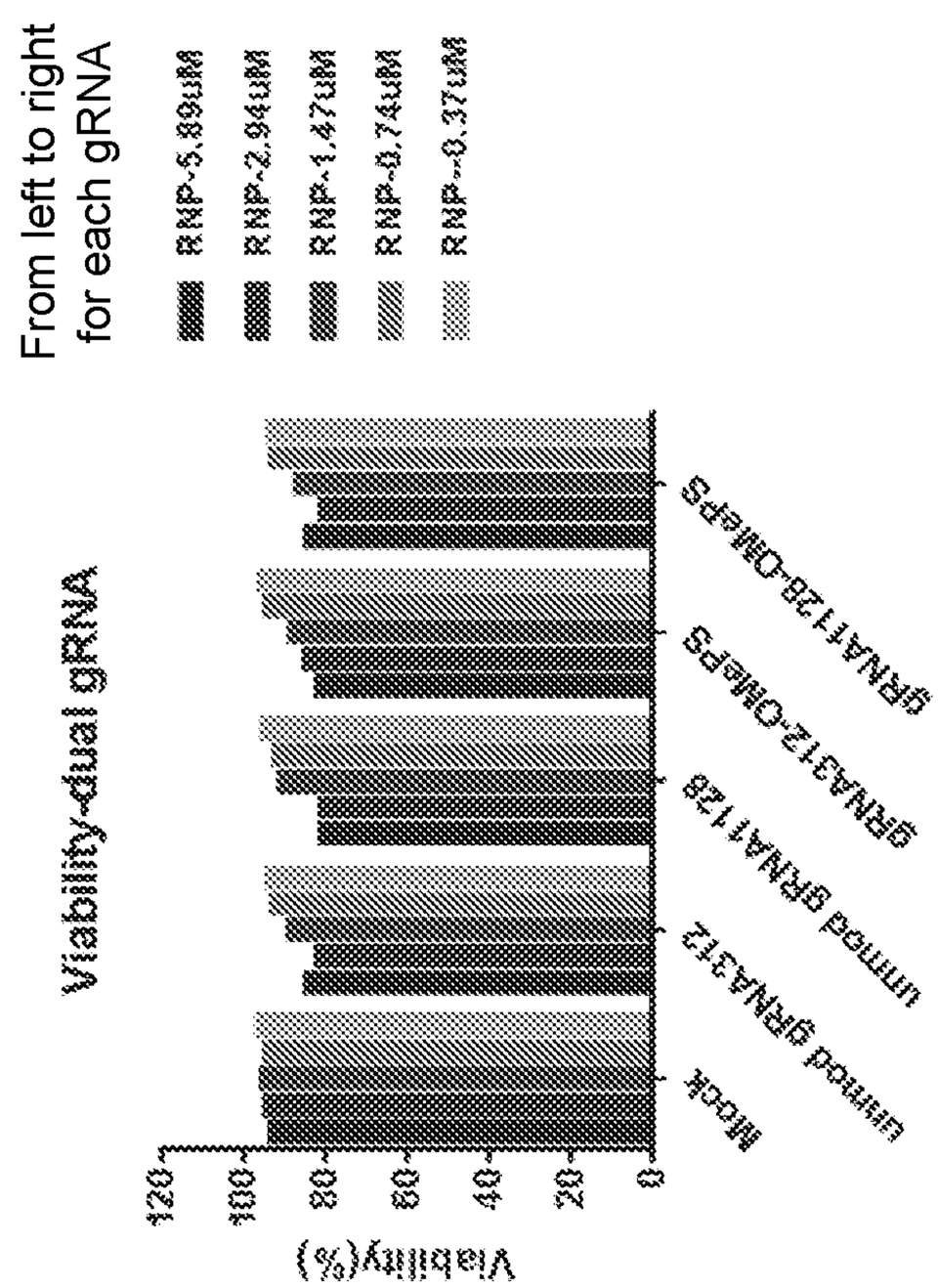


Figure 40B

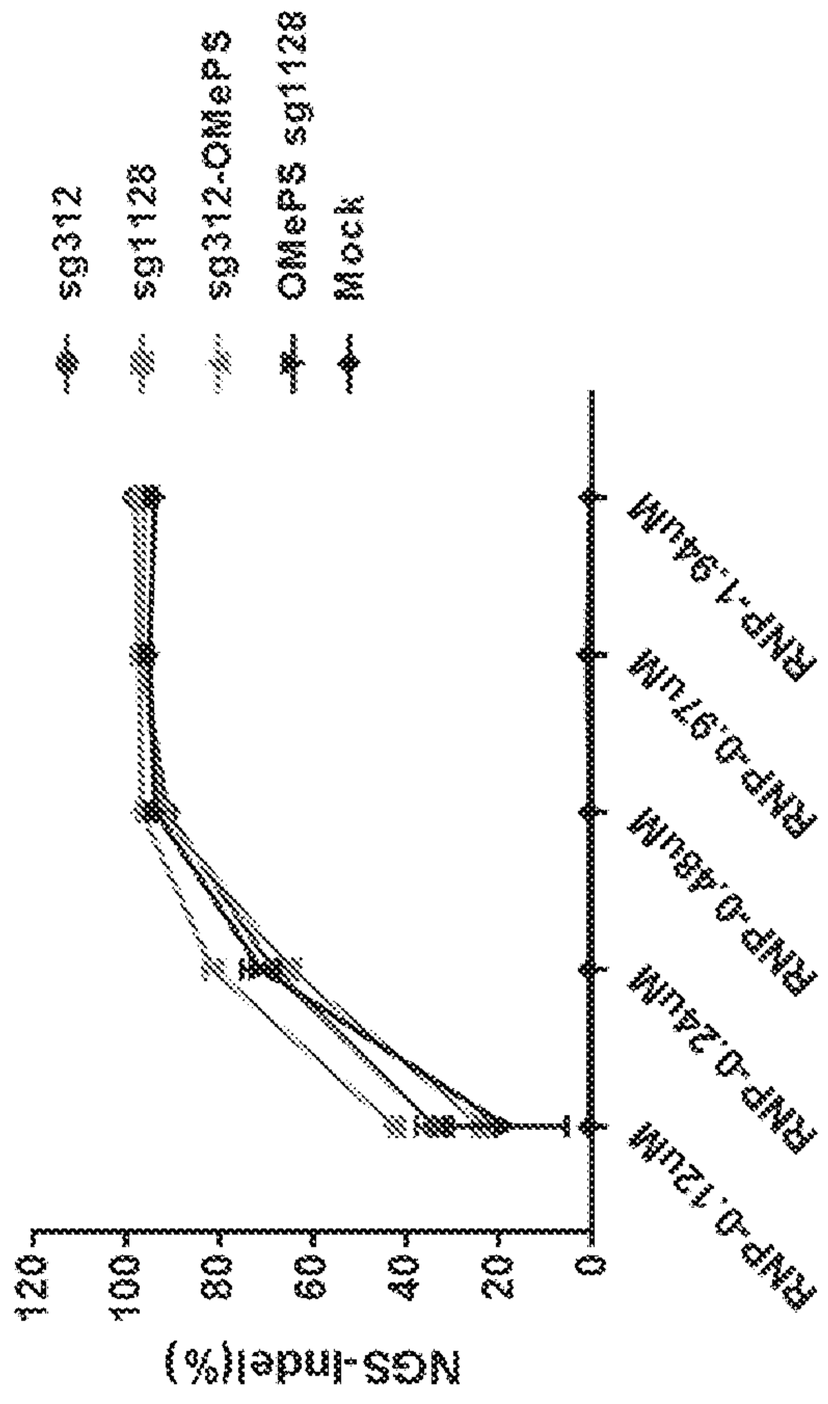


Figure 40A

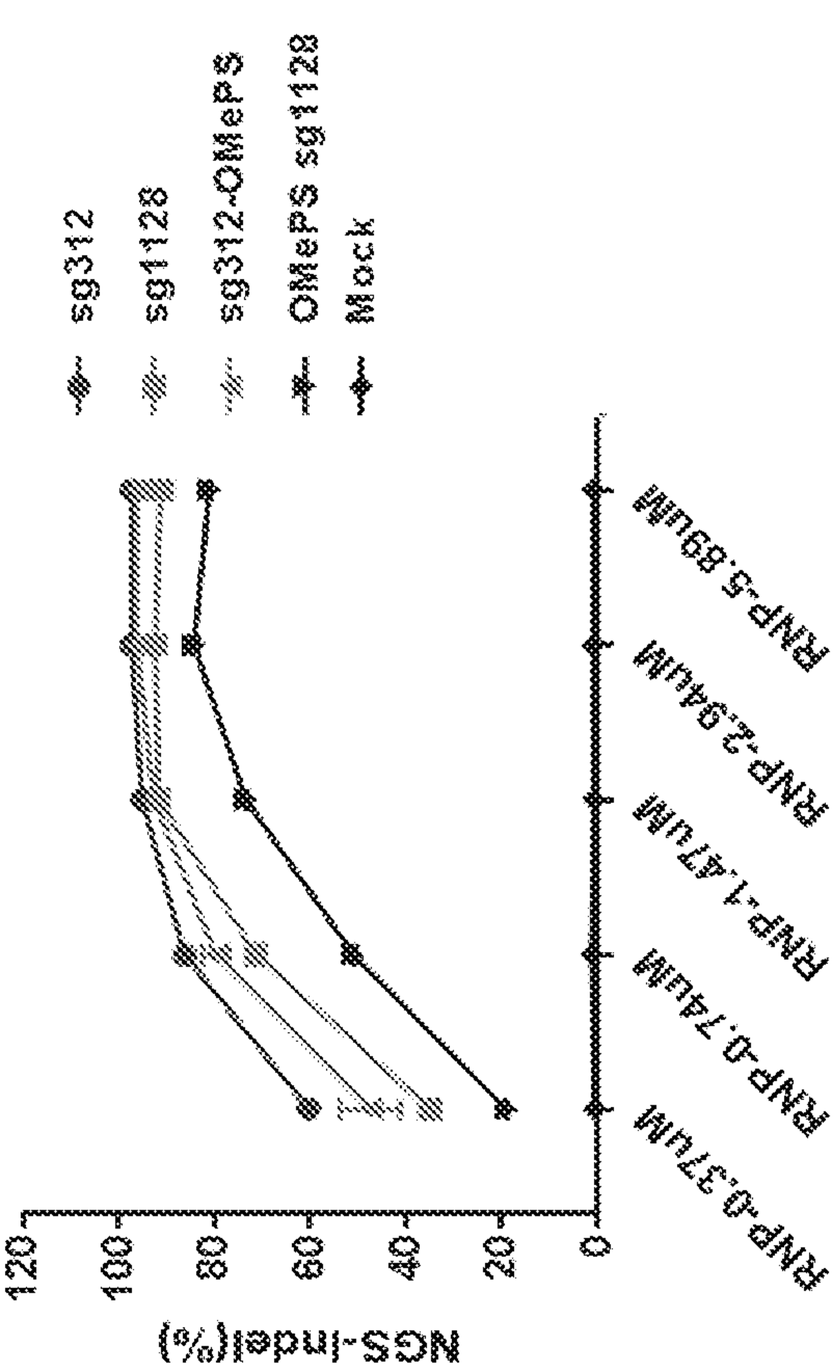


Figure 41A

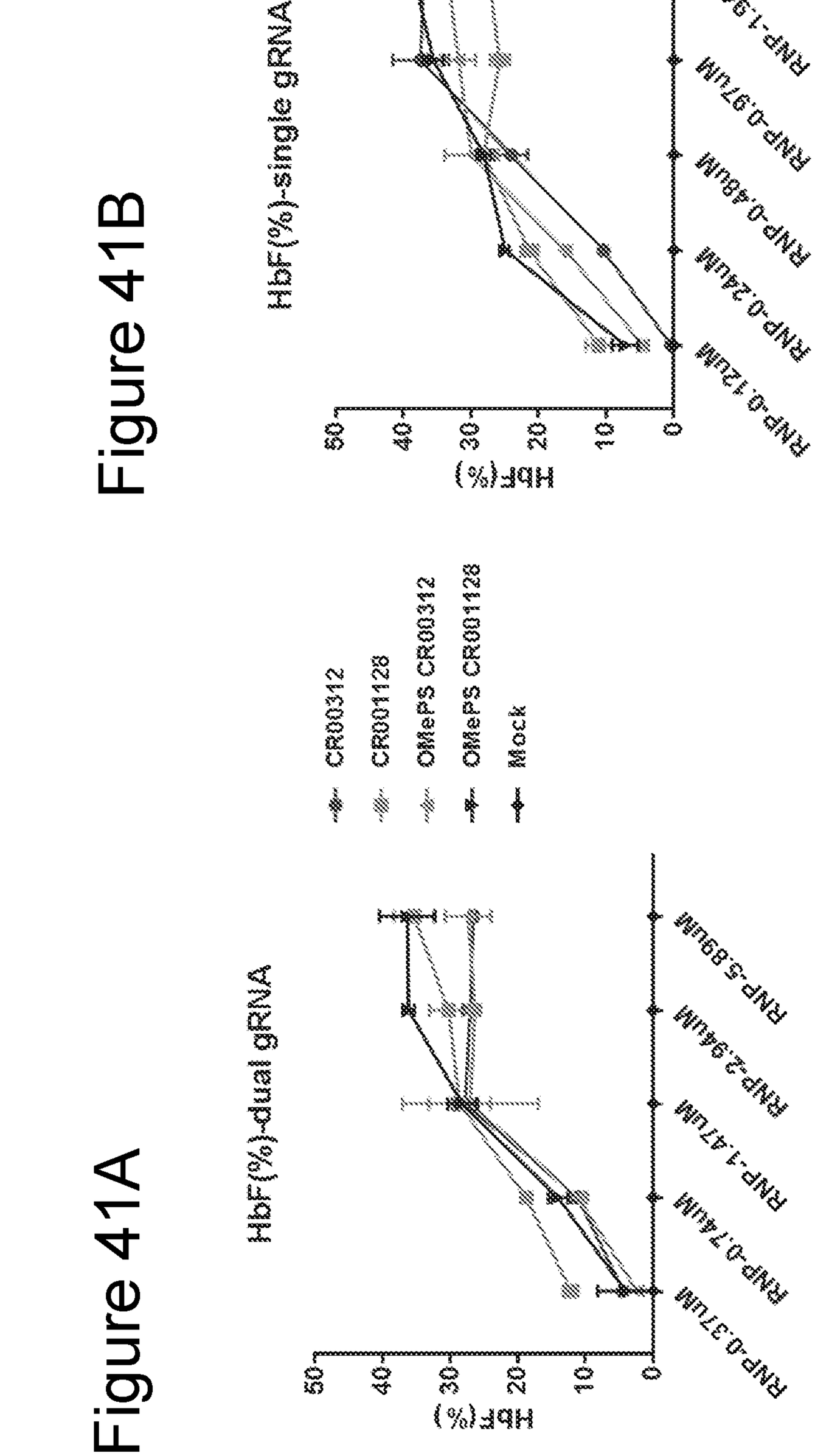


Figure 41B

Figure 42B

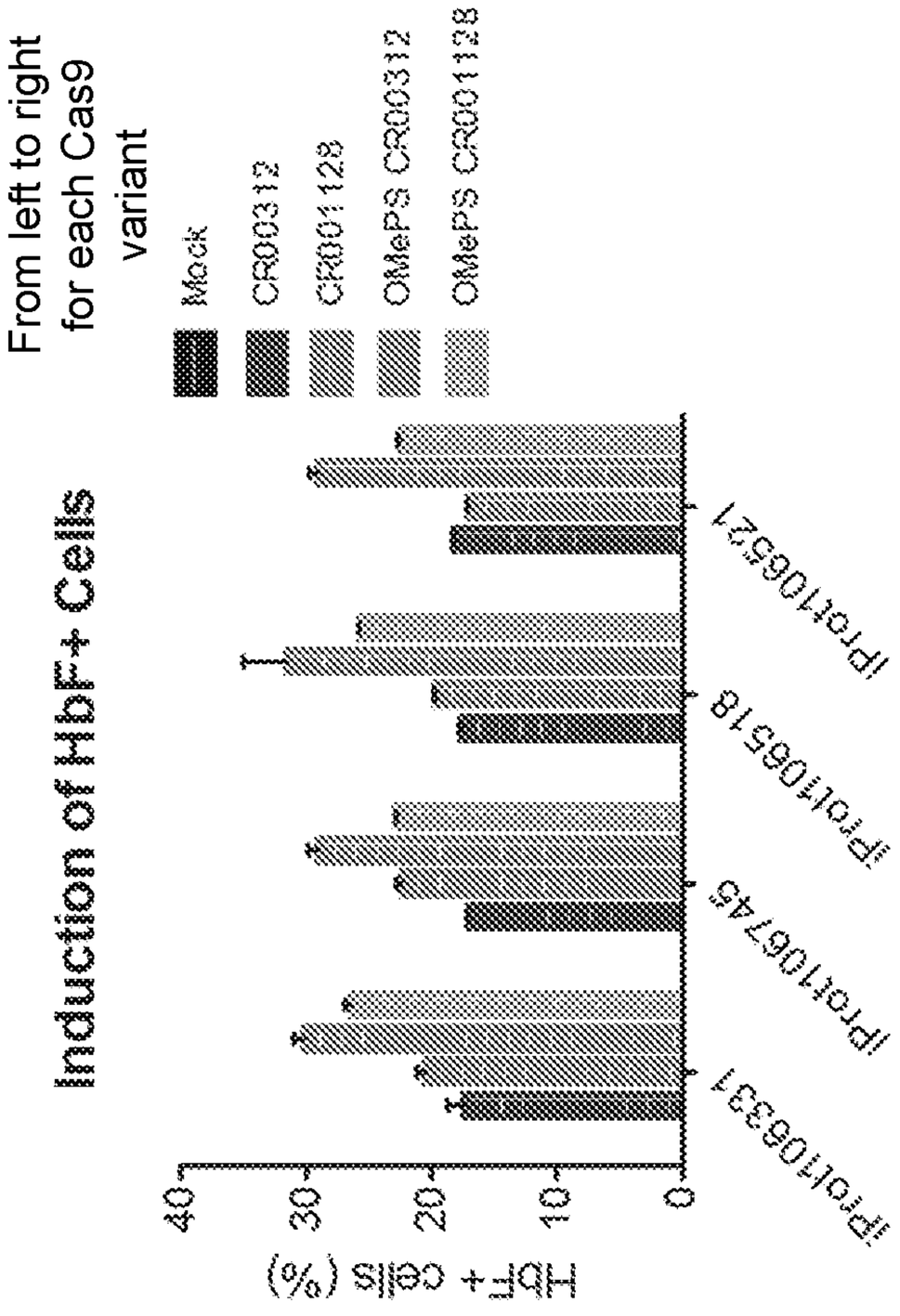


Figure 42A

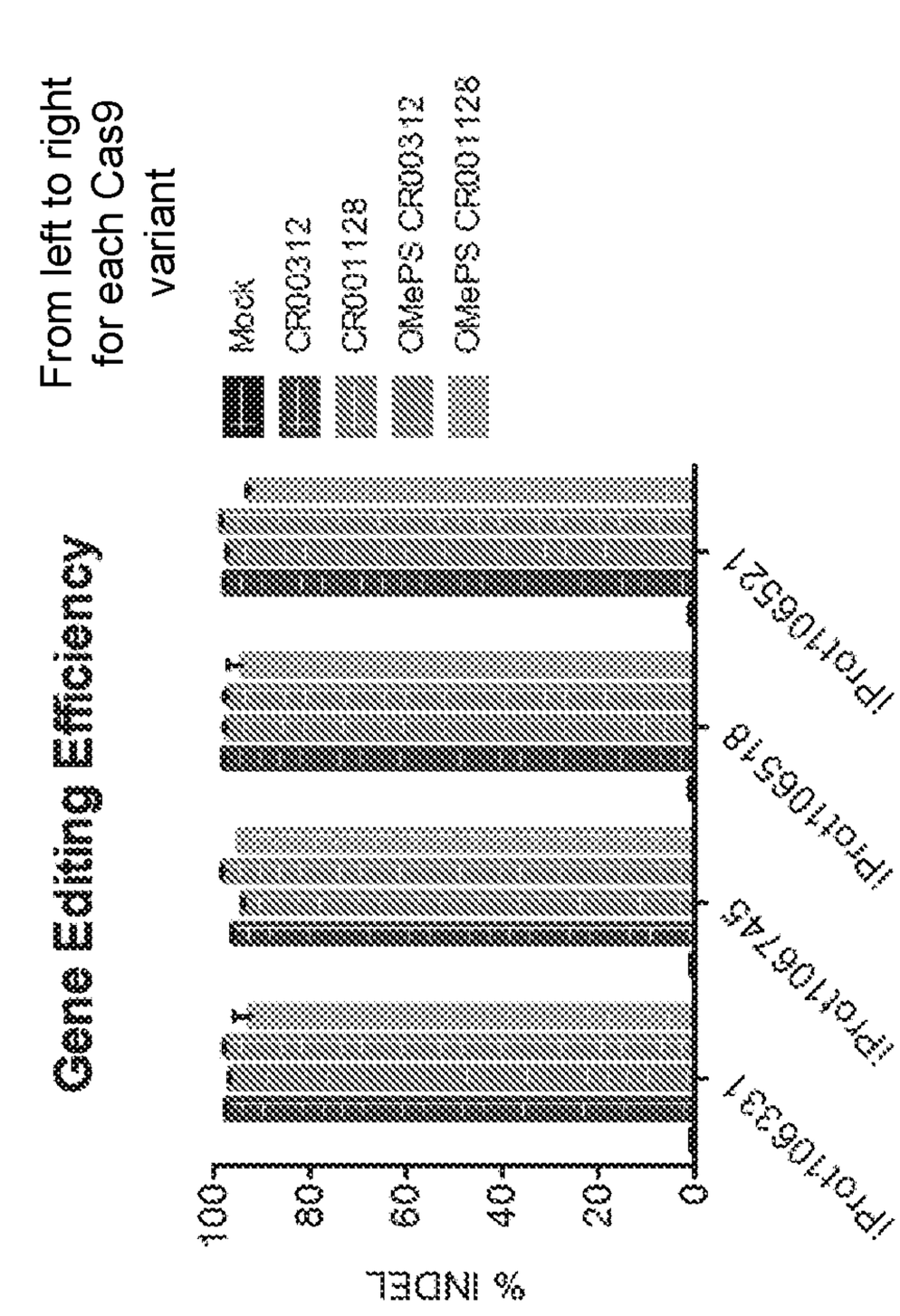


Figure 43

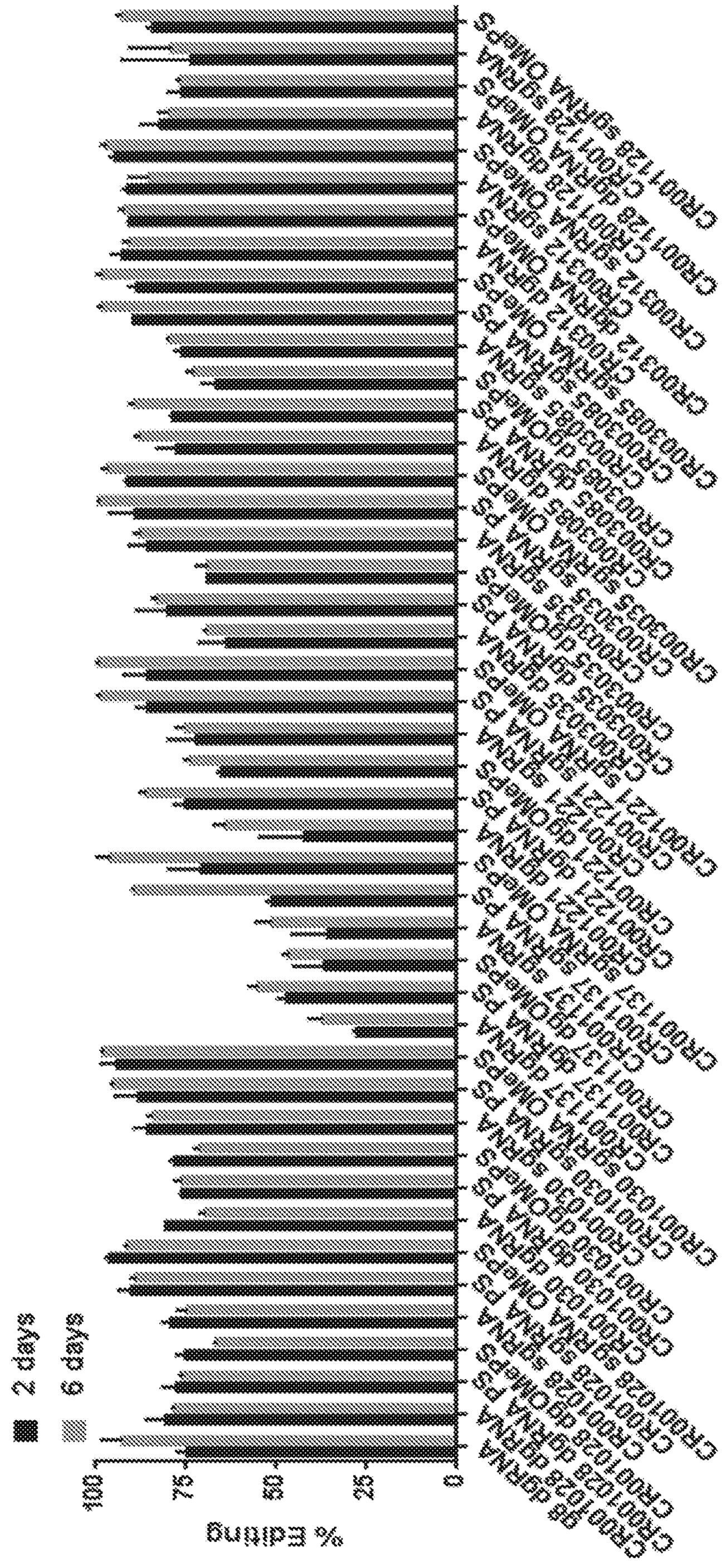


Figure 44

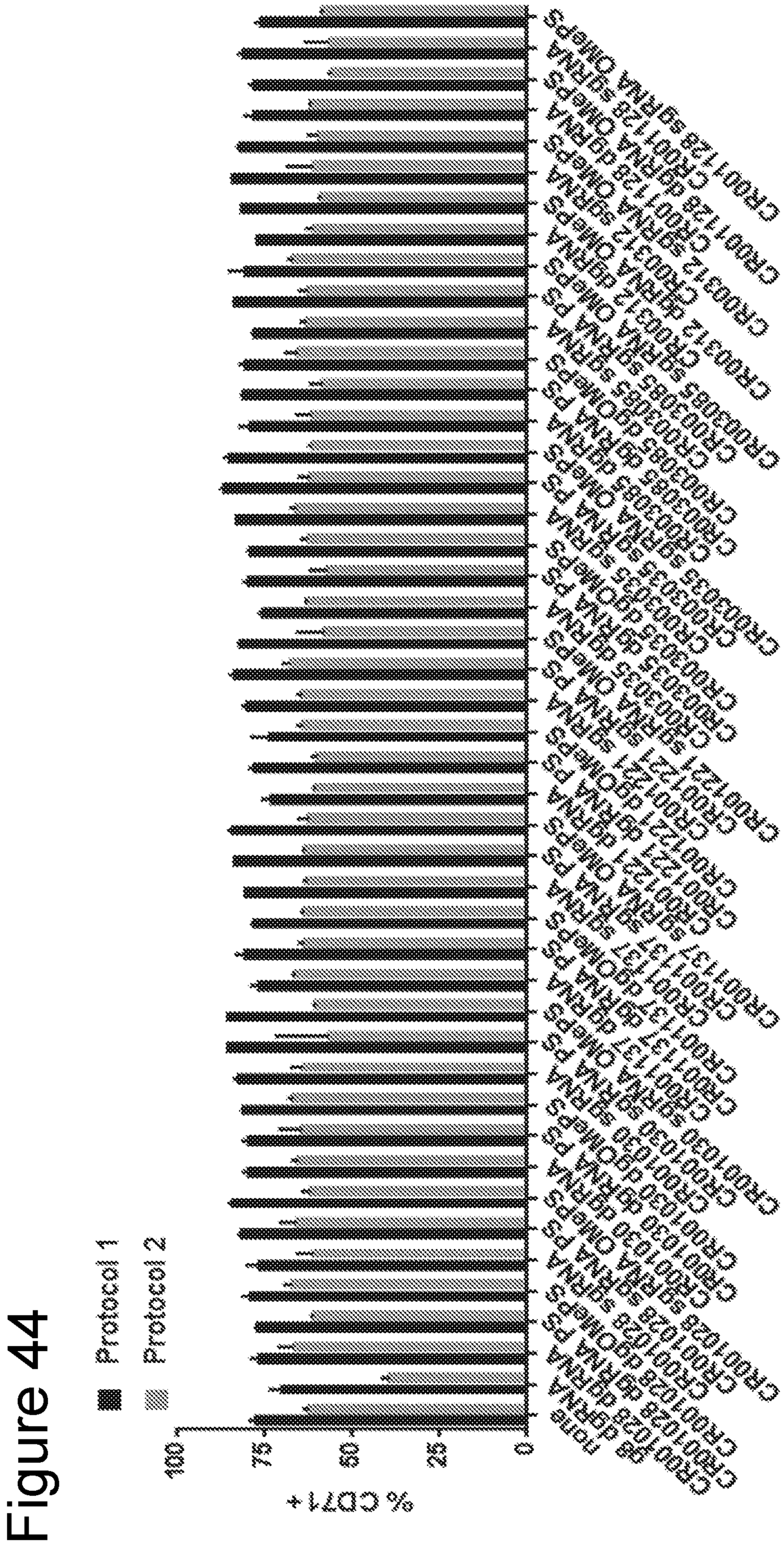


Figure 45

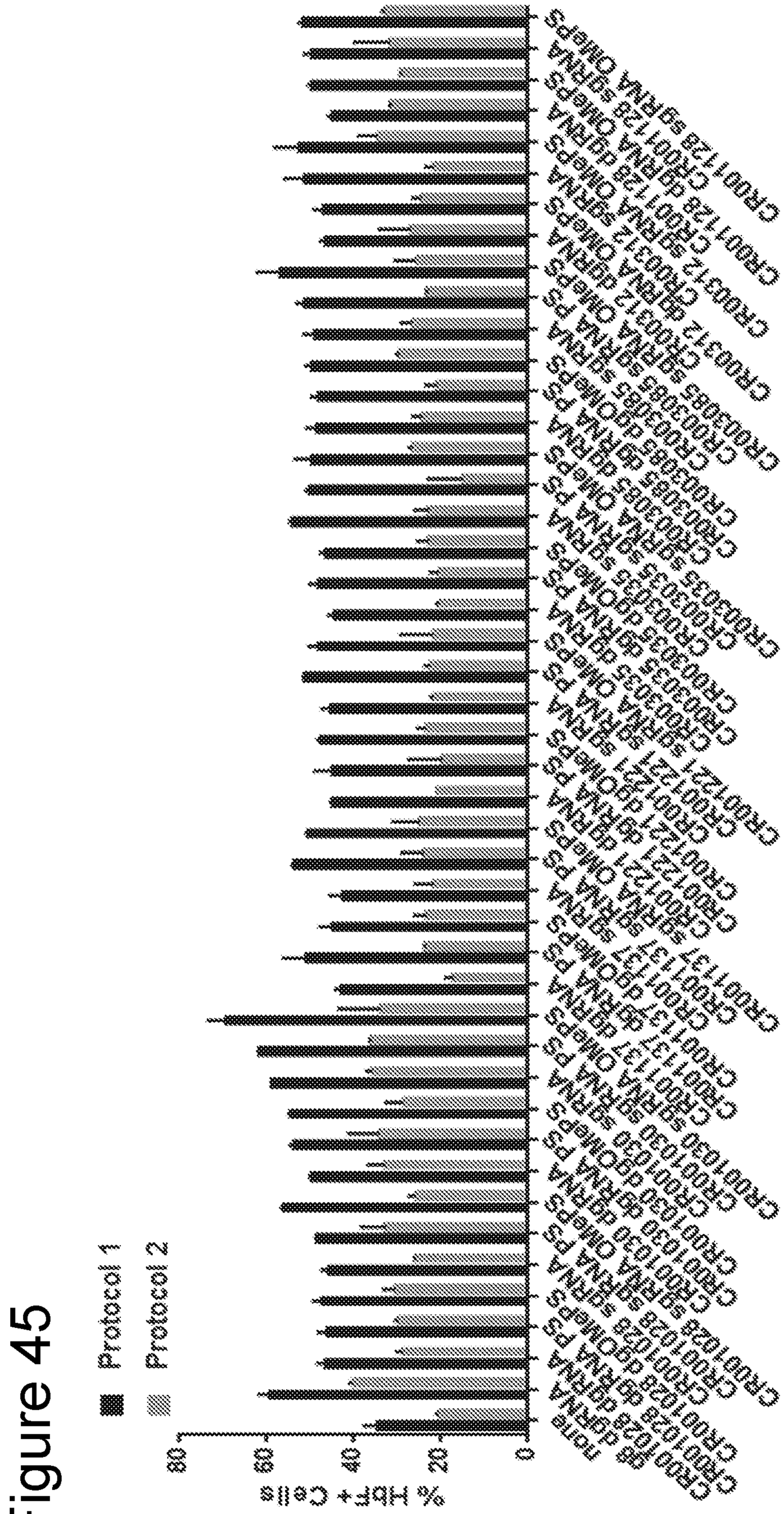
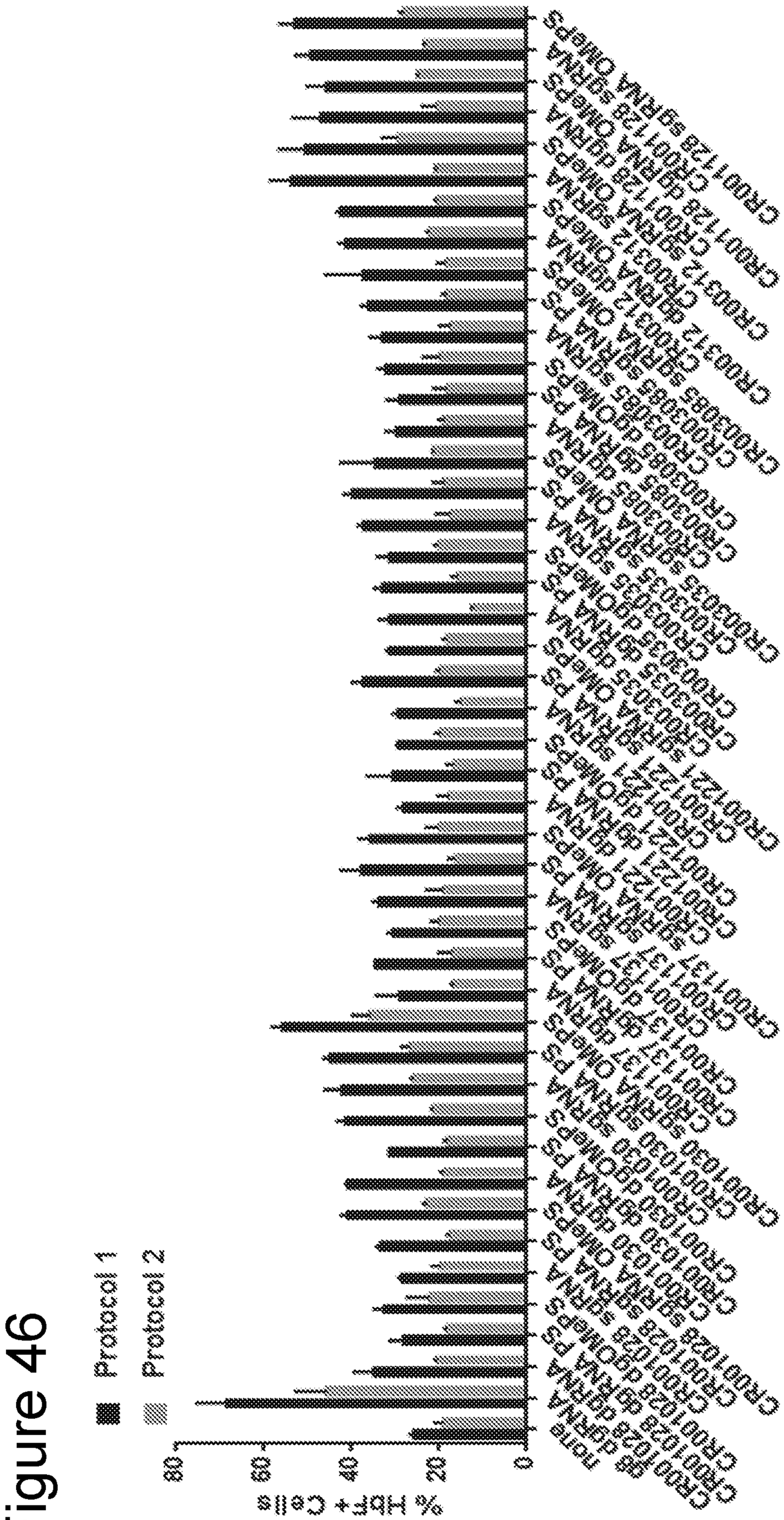


Figure 46



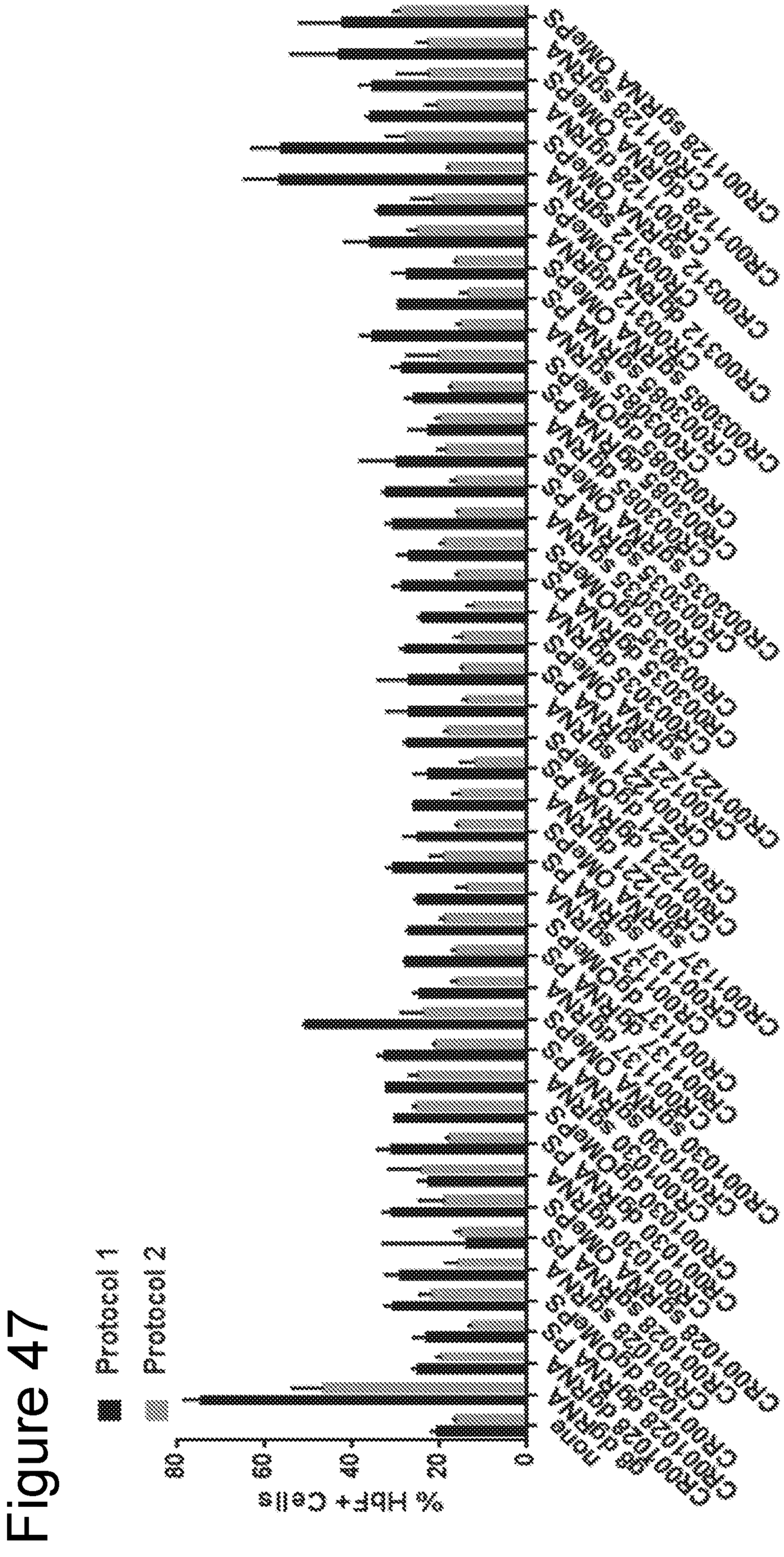
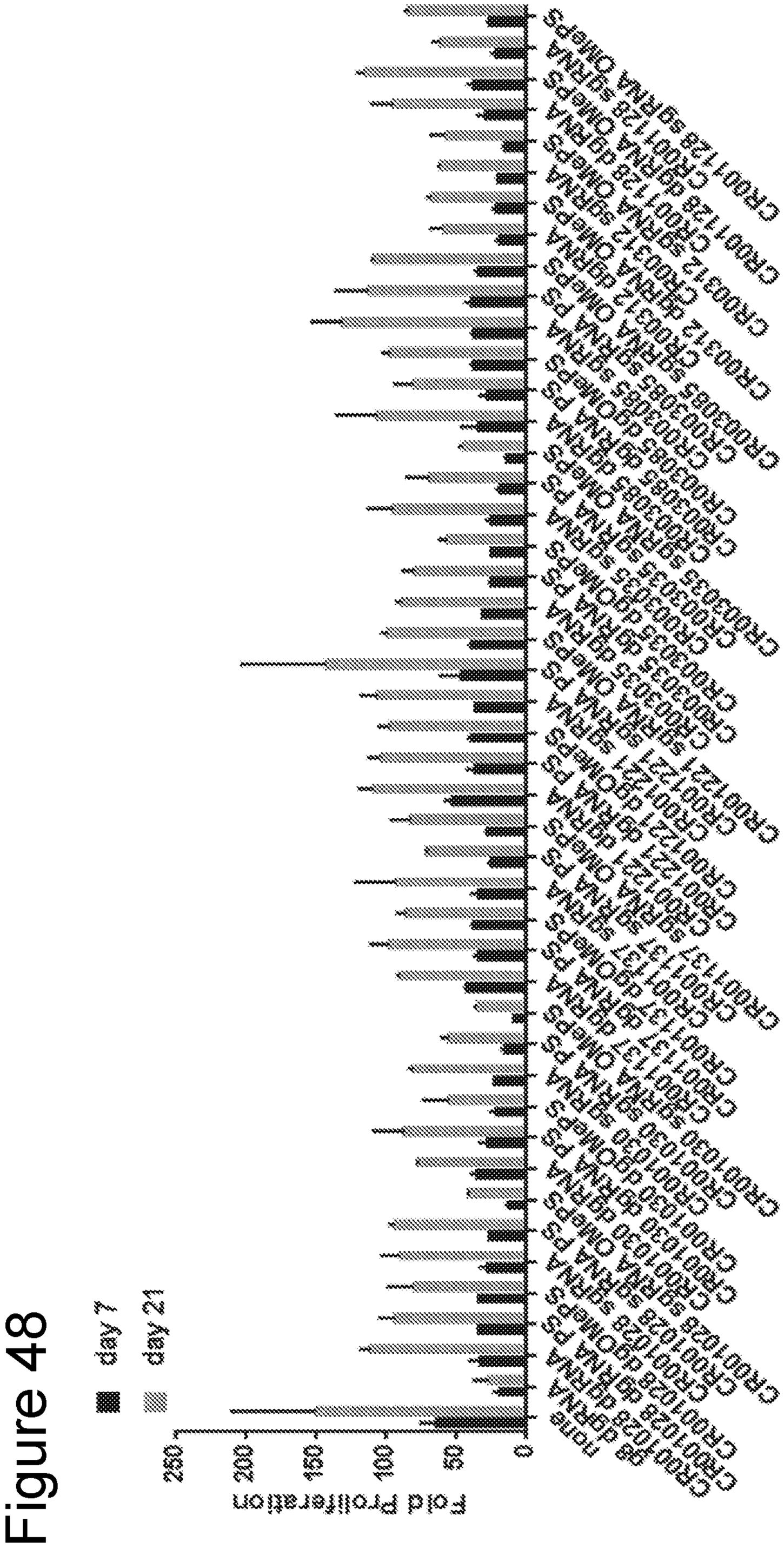


Figure 47

Figure 48


day 7
day 21
Fold Proliferation
250
200
150
100
50
0
CR001428 SQRMA OMeRS
CR001428 CR001428 SQRMA
CR001428 SQRMA OMeRS
CR001428 SQRMA OMeRS
CR001428 SQRMA OMeRS

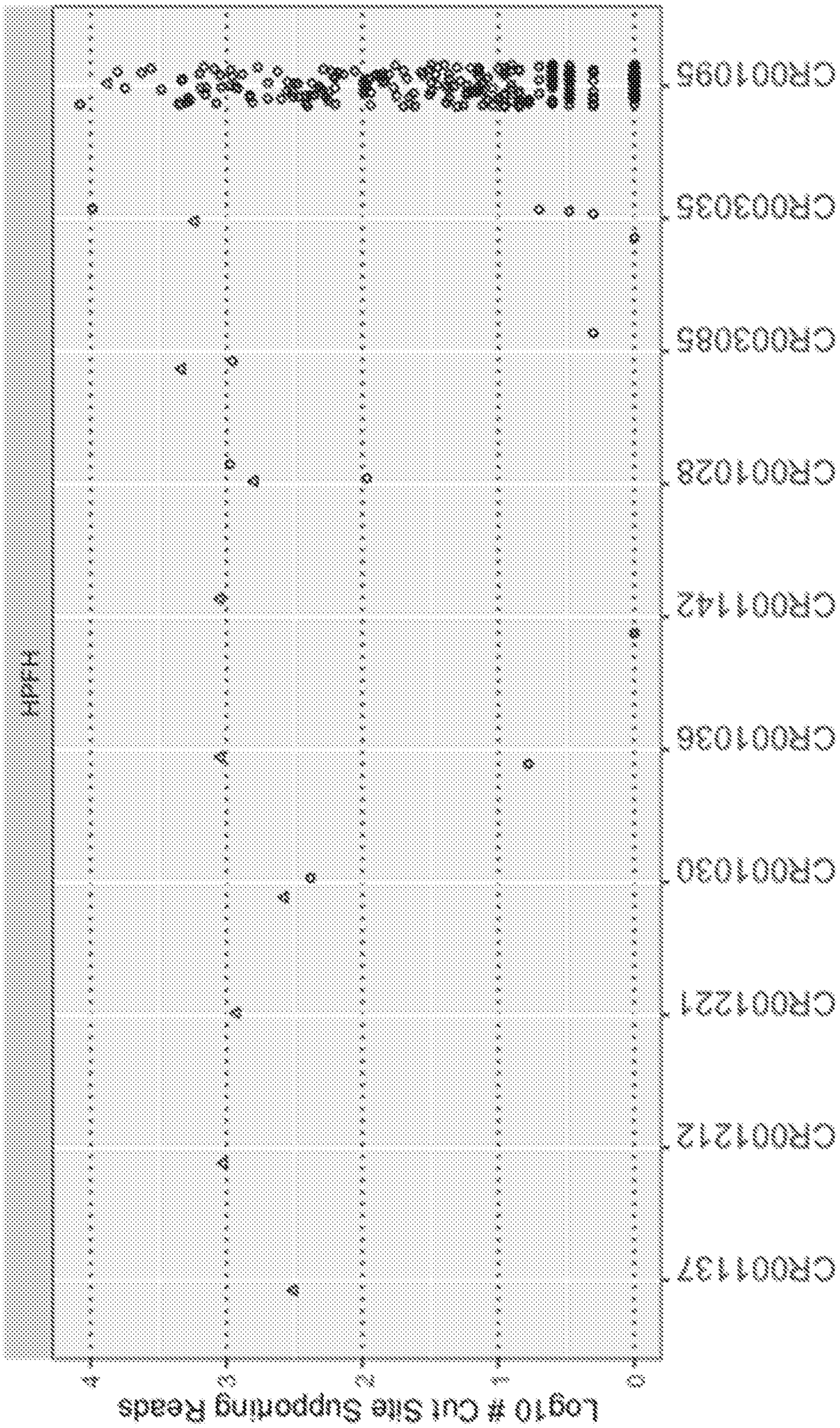

CR001092
CR003035
CR003085
CR001028
CR001042
CR001038
CR001030
CR001227
CR001212
CR001137
Guide Name
Hit%
0
25
50
75
100
log10 # Cut Site Supporting Reads

Figure 49

WO 2017/115268

INTERNATIONAL SEARCH REPORT

Intern PCT/IB2016/058007
PCT/IB2016/058007

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. ☐ forming part of the international application as filed:

      ☐ 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:

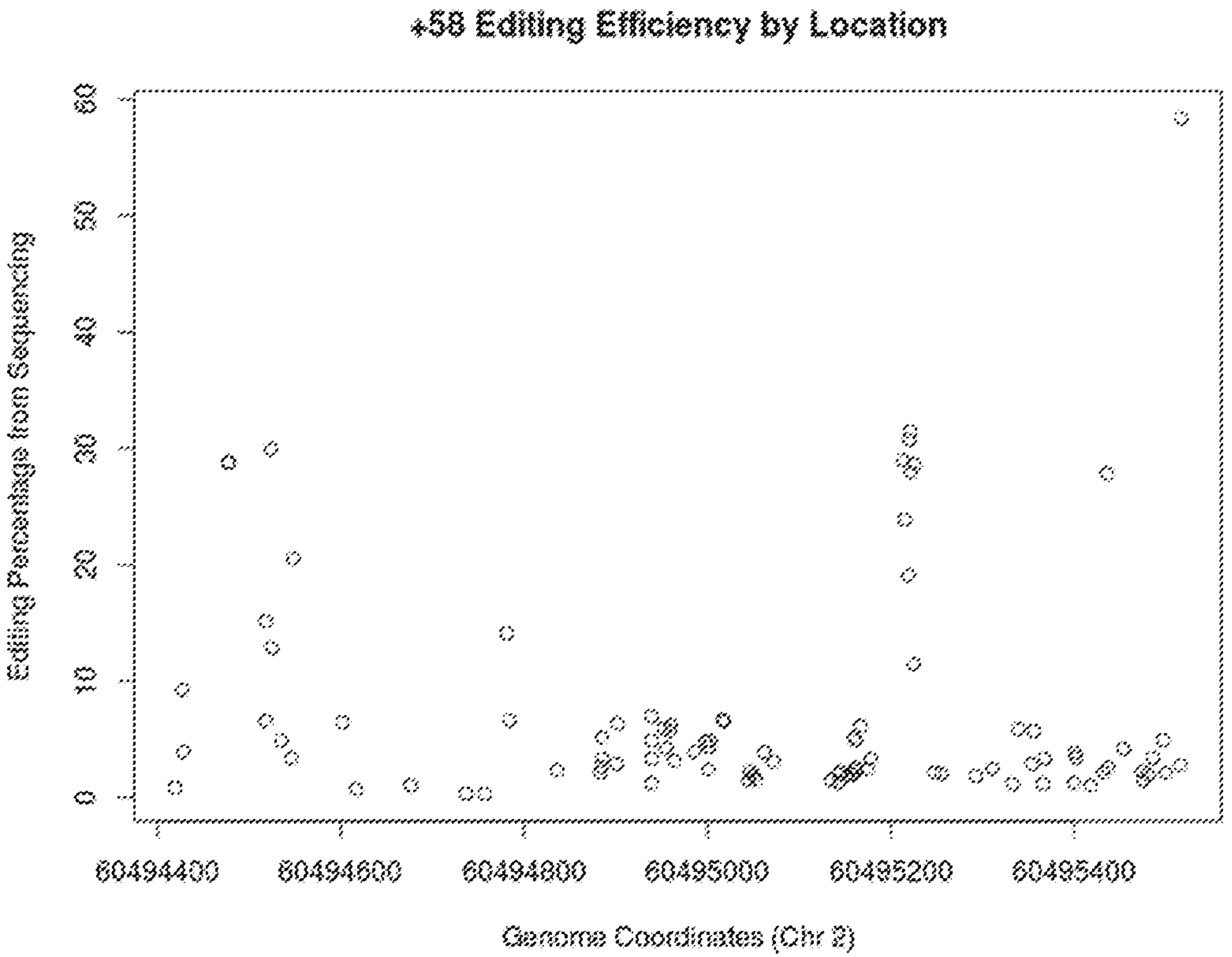
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ 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:

Figure 1


+58 Editing Efficiency by Location
Editing Percentage from Sequencing
0
10
20
30
40
50
60
60494400
60494600
60494800
60495000
60495200
60495400
Genome Coordinates (Chr 2)