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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING THE EXPRESSION OF MULTIPLE GENES

(57) Abstract: The present disclosure relates to site-specific disrupting agents for modulating, e.g., decreasing, expression of a target plurality of genes in a cell. In some embodiments, the target plurality of genes comprises pro-inflammatory genes, e.g., CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and IL-8. In some embodiments, the method comprises using a first site-specific disrupting agent that targets a first anchor sequence and a second site-specific disrupting agents that disrupts a second anchor sequence.



COMPOSITIONS AND METHODS FOR INHIBITING THE EXPRESSION OF MULTIPLE GENES**CROSS-REFERENCE TO RELATED APPLICATIONS**

The present application claims the benefit of U.S. provisional applications 63/085,013 filed
5 September 29, 2020, and 63/216,487 filed June 29, 2021. The contents of the aforementioned
applications are hereby incorporated by reference in their entirety.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in
10 ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on
September 29, 2021, is named O2057-7021WO_SL.txt and is 666,818 bytes in size.

BACKGROUND

Mis-regulation of gene expression is the underlying cause of many diseases (e.g., in mammals,
15 e.g., humans). A number of diseases and conditions are associated with pluralities of related genes. There
is a need for novel tools, systems, and methods to alter, e.g., decrease, expression of pluralities of
associated genes.

SUMMARY

20 The disclosure provides, among other things, site-specific disrupting agents or systems
comprising site-specific disrupting agents that may be used to modulate, e.g., decrease, expression of a
plurality of target genes, e.g., a first gene and a second gene, that are within an anchor sequence-mediated
conjunction (ASMC) comprising a first anchor sequence and a second anchor sequence. In one aspect, a
site-specific disrupting agent comprises a targeting moiety that binds specifically to a first anchor
25 sequence or proximal to the first anchor sequence in an ASMC. In some embodiments, binding of the
site-specific disrupting agent occurs in an amount sufficient to modulate, e.g., decrease, expression of the
plurality of target genes, e.g., the first gene and second gene. In some embodiments, the site-specific
disrupting agent further comprises an effector moiety. Generally, modulation of expression of a target
plurality of genes by a site-specific disrupting agent involves the binding of the site-specific disrupting
30 agent to or proximal to the first anchor sequence. In some embodiments, binding of the site-specific
disrupting agent to the first anchor sequence may disrupt binding of a nucleating polypeptide, e.g., CTCF,
to the first anchor sequence, e.g., thereby disrupting formation and/or maintenance of the ASMC, e.g.,
and thereby modulating, e.g., decreasing, expression of the plurality of genes. In some embodiments,
binding of the site-specific disrupting agent to or proximal to the first anchor sequence may localize a

functionality of an effector moiety to the first anchor sequence and/or ASMC, e.g., thereby disrupting formation and/or maintenance of the ASMC, e.g., and thereby modulating, e.g., decreasing, expression of the plurality of genes. In some embodiments, binding of the site-specific disrupting agent to or proximal to the first anchor sequence may localize a functionality of an effector moiety to the first anchor sequence and/or ASMC, e.g., thereby modulating, e.g., decreasing, expression of the plurality of genes. Without wishing to be bound by theory, in some embodiments it is thought that targeting a plurality of genes that are within the same ASMC may more effectively modulate, e.g., decrease, expression of the plurality of genes and/or more effectively achieve a therapeutic effect relating to the functionality of the plurality of genes. For example, in some embodiments a targeted plurality of genes may all be pro-inflammatory genes; targeting the plurality of pro-inflammatory genes for modulation, e.g., reduction, in expression as taught herein may more effectively decrease inflammation than targeting individual genes. Targeting a plurality of genes comprised within the same genomic complex, e.g., ASMC, (e.g., by targeting the ASMC or an anchor sequence of the ASMC) may have an additive or synergistic effect (e.g., with regard to expression modulation or stability/duration of modulation) that is greater than the effect of targeting individual genes of the plurality.

In some aspects, the disclosure provides a method of decreasing expression of a first gene and a second gene in a cell, comprising: contacting the cell with a site-specific disrupting agent comprising a targeting moiety that binds specifically to a first anchor sequence or a site proximal to a first anchor sequence, in an amount sufficient to decrease expression of the first and second genes, the first and second genes being within an anchor sequence-mediated conjunction that comprises the first anchor sequence and a second anchor sequence. In some embodiments, the first gene and the second gene are proinflammatory genes.

In some aspects, the disclosure is directed to a site-specific disrupting agent, comprising: a DNA-binding, e.g., a targeting moiety that binds specifically to or proximal to a first anchor sequence within a cell. In some embodiments, the first anchor sequence is part of an anchor sequence-mediated conjunction that further comprises a second anchor sequence, a first gene, and a second gene. In some embodiments, the first gene and the second gene are proinflammatory genes.

In some aspects, the disclosure is directed to a site-specific disrupting agent, comprising: a targeting moiety that binds specifically to or proximal to a first anchor sequence within a cell, wherein the first anchor sequence is part of an anchor sequence-mediated conjunction that further comprises a

second anchor sequence, a first gene, and a second gene, wherein the first gene and the second gene are proinflammatory genes.

In another aspect, the disclosure provides a system comprising a first site-specific disrupting agent comprising a first targeting moiety and optionally a first effector moiety, wherein the first site-specific disrupting agent binds specifically to a first anchor sequence (or proximal to the first anchor sequence) of an anchor sequence mediated conjunction (ASMC), comprising a first gene and a second gene, and a second site-specific disrupting agent comprising a second targeting moiety and optionally a second effector moiety, wherein the second site-specific disrupting agent binds to a second anchor sequence (or proximal to the second anchor sequence) of the ASMC.

In another aspect, the disclosure is directed to a method of decreasing expression of a first gene and a second gene in a cell, comprising: contacting the cell with a site-specific disrupting agent that comprises a targeting moiety that binds specifically to a first anchor sequence or a site proximal to a first anchor sequence, in an amount sufficient to decrease expression of the first and second genes, the first and second genes being within an anchor sequence-mediated conjunction that comprises the first anchor sequence and a second anchor sequence, wherein the first gene and the second gene are proinflammatory genes; thereby decreasing expression of the first and second genes.

In another aspect, the disclosure is directed to a method of decreasing expression of a first gene and a second gene in a cell, comprising:

contacting the cell with a system comprising a first site-specific disrupting agent comprising a first targeting moiety and optionally a first effector moiety that binds specifically to a first anchor sequence or a site proximal to a first anchor sequence and a second site-specific disrupting agent comprising a second targeting moiety and optionally a second effector moiety wherein the second site-specific disrupting agent binds to a second anchor sequence (or proximal to the second anchor sequence) of the ASMC, in an amount sufficient to decrease expression of the first and second genes, the first and second genes being within the ASMC, wherein the first gene and the second gene are proinflammatory genes.

In another aspect, the disclosure is directed to a reaction mixture comprising a cell (e.g., a human cell, e.g., a primary human cell) and a site-specific disrupting agent or a system as described herein.

In another aspect, the disclosure is directed to a method of treating a subject having an inflammatory disorder, comprising administering to the subject a site-specific disrupting agent or a system as described herein in an amount sufficient to treat the inflammatory disorder.

5 In another aspect, the disclosure is directed to a method of treating inflammation, e.g., local inflammation, in a subject having an infection, e.g., viral infection, e.g., COVID-19, comprising, administering to the subject a site-specific disrupting agent or a system as described herein in an amount sufficient to treat the inflammation.

10 In another aspect, the disclosure is directed to a human cell having decreased expression of a first gene and a second gene, wherein the first gene and the second gene are proinflammatory genes, wherein the cell comprises a disrupted (e.g., fully disrupted) anchor sequence-mediated conjunction that comprises the first and second genes. In some embodiments, the human cell was previously contacted with a site-specific disrupting agent or system described herein. In some embodiments, the human cell no longer
15 comprises a site-specific disrupting agent or system described herein.

In another aspect, the disclosure is directed to a human cell comprising a mutation at genomic coordinates chr4:74595464-74595486, chr4:74595457-74595479, chr4:74595460-74595482, chr4:74595472-74595494, chr4:75000088-75000110, chr4:75000091-75000113, chr4:75000085-
20 75000107, chr4:75000157-75000179, chr4:75000156-75000178, chr4:74595215-74595237, chr4:74595370-74595392, chr4:74595560-74595582, chr4:74595642-74595664, chr4:74595787-74595809, chr4:74528428-74528450, chr4:74528567-74528589, chr4:74528609-74528631, chr4:74789132-74789154, chr4:74789250-74789272, chr4:74789312-74789334, chr4:74964853-74964875, chr4:74964906-74964928, chr4:74965538-74965560, chr4:74965737-74965759,
25 chr4:75000031-75000053, chr4:75000115-75000137, chr4:75000231-75000253, chr4:74975146-74975168, chr4:74975369-74975391, chr4:74976318-74976340, chr4:74570348-74570370, chr4:74570503-74570525, or chr4:74570526-74570548, or within 5, 10, 15, 20, 30, 40, or 50 nucleotides of said region.

30 Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein.

All publications, patent applications, patents, and other references (e.g., sequence database reference numbers) mentioned herein are incorporated by reference in their entirety. For example, all

GenBank, Unigene, and Entrez sequences referred to herein, e.g., in any Table herein, are incorporated by reference. Unless otherwise specified, the sequence accession numbers specified herein, including in any Table herein, refer to the database entries current as of September 29, 2020. When one gene or protein references a plurality of sequence accession numbers, all of the sequence variants are encompassed.

5

DEFINITIONS

Anchor Sequence: The term “anchor sequence” as used herein, refers to a nucleic acid sequence recognized by a nucleating agent that binds sufficiently to form an anchor sequence-mediated
 10 conjunction, e.g., a complex. In some embodiments, an anchor sequence comprises one or more CTCF binding motifs. In some embodiments, an anchor sequence is not located within a gene coding region. In some embodiments, an anchor sequence is located within an intergenic region. In some embodiments, an anchor sequence is not located within either of an enhancer or a promoter. In some embodiments, an anchor sequence is located at least 400 bp, at least 450 bp, at least 500 bp, at least 550 bp, at least 600 bp,
 15 at least 650 bp, at least 700 bp, at least 750 bp, at least 800 bp, at least 850 bp, at least 900 bp, at least 950 bp, or at least 1kb away from any transcription start site. In some embodiments, an anchor sequence is located within a region that is not associated with genomic imprinting, monoallelic expression, and/or monoallelic epigenetic marks. In some embodiments, the anchor sequence has one or more functions selected from binding an endogenous nucleating polypeptide (e.g., CTCF), interacting with a second
 20 anchor sequence to form an anchor sequence mediated conjunction, or insulating against an enhancer that is outside the anchor sequence mediated conjunction. In some embodiments of the present disclosure, technologies are provided that may specifically target a particular anchor sequence or anchor sequences, without targeting other anchor sequences (e.g., sequences that may contain a nucleating agent (e.g., CTCF) binding motif in a different context); such targeted anchor sequences may be referred to as the
 25 “target anchor sequence”. In some embodiments, sequence and/or activity of a target anchor sequence is modulated while sequence and/or activity of one or more other anchor sequences that may be present in the same system (e.g., in the same cell and/or in some embodiments on the same nucleic acid molecule – e.g., the same chromosome) as the targeted anchor sequence is not modulated. In some embodiments, the anchor sequence comprises or is a nucleating polypeptide binding motif. In some embodiments, the
 30 anchor sequence is adjacent to a nucleating polypeptide binding motif.

Anchor Sequence-Mediated Conjunction: The term “anchor sequence-mediated conjunction” as used herein, refers to a DNA structure, in some cases, a complex, that occurs and/or is maintained via physical interaction or binding of at least two anchor sequences in the DNA by one or more polypeptides, such as nucleating polypeptides, or one or more proteins and/or a nucleic acid entity (such as RNA or

DNA), that bind the anchor sequences to enable spatial proximity and functional linkage between the anchor sequences (see, e.g. Figure 1).

Associated with: Two events or entities are “associated” with one another, as that term is used herein, if presence, level, form and/or function of one is correlated with that of the other. For example, in some embodiments, a particular entity (e.g., polypeptide, genetic signature, metabolite, microbe, etc.) is considered to be associated with a particular disease, disorder, or condition, if its presence, level, form and/or function correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof. In some embodiments, a DNA sequence is “associated with” a target genomic or transcription complex when the nucleic acid is at least partially within the target genomic or transcription complex, and expression of a gene in the DNA sequence is affected by formation or disruption of the target genomic or transcription complex.

Site-specific disrupting agent: As used herein, the term “site-specific disrupting agent” refers to an agent or entity that specifically inhibits, dissociates, degrades, and/or modifies one or more components of a genomic complex, e.g., ASMC, thereby modulating, e.g., decreasing, expression of a target plurality of genes as described herein. In some embodiments, a site-specific disrupting agent interacts with one or more components of a genomic complex. In some embodiments, a site-specific disrupting agent binds (e.g., directly or, in some embodiments, indirectly) to one or more genomic complex components. In some embodiments, a site-specific disrupting agent binds to an anchor sequence, e.g., a first and/or second anchor sequence, that may be part of an ASMC comprising a target plurality of genes. In some embodiments, a site-specific disrupting agent binds to a site proximal to an anchor sequence, e.g., a first and/or second anchor sequence, that may be part of an ASMC comprising a target plurality of genes. In some embodiments, a site-specific disrupting agent modifies one or more genomic complex components. In some embodiments, a site-specific disrupting agent comprises an oligonucleotide. In some embodiments, a site-specific disrupting agent comprises a polypeptide. In some embodiments, a site-specific disrupting agent comprises an antibody (e.g., a monospecific or multi-specific antibody construct) or antibody fragment. In some embodiments, a site-specific disrupting agent is directed to a particular genomic location and/or to a genomic complex by a targeting moiety, as described herein. In some embodiments, a site-specific disrupting agent comprises a genomic complex

component or variant thereof. In some embodiments, a site-specific disrupting agent comprises a targeting moiety. In some embodiments, a site-specific disrupting agent comprises an effector moiety. In some embodiments, a site-specific disrupting agent comprises a plurality of effector moieties. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and one or more effector moieties. In some embodiments, the site-specific disrupting agent specifically binds a first site in the genome with higher affinity than a second site in the genome (e.g., relative to any other site in the genome). In some embodiments, the site-specific disrupting agent preferentially inhibits, dissociates, degrades, and/or modifies one or more components of a first genomic complex relative to a second genomic complex (e.g., relative to any other genomic complex).

Domain: As used herein, the term “domain” refers to a section or portion of an entity. In some embodiments, a “domain” is associated with a particular structural and/or functional feature of the entity so that, when the domain is physically separated from the rest of its parent entity, it substantially or entirely retains the particular structural and/or functional feature. Alternatively, or additionally, in some embodiments, a domain may be or include a portion of an entity that, when separated from that (parent) entity and linked with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features that characterized it in the parent entity. In some embodiments, a domain is or comprises a section or portion of a molecule (e.g., a small molecule, carbohydrate, lipid, nucleic acid, polypeptide, etc.). In some embodiments, a domain is or comprises a section of a polypeptide. In some such embodiments, a domain is characterized by a particular structural element (e.g., a particular amino acid sequence or sequence motif, alpha-helix character, beta-sheet character, coiled-coil character, random coil character, etc.), and/or by a particular functional feature (e.g., binding activity, enzymatic activity, folding activity, signaling activity, etc.).

Effector moiety: As used herein, the term “effector moiety” refers to a domain with one or more functionalities that modulate, e.g., decrease, expression of a target plurality of genes in a cell when appropriately localized in the nucleus of a cell. In some embodiments, an effector moiety comprises a polypeptide. In some embodiments, an effector moiety comprises a polypeptide and a nucleic acid. A functionality associated with an effector moiety may directly affect expression of a target plurality of genes, e.g., blocking recruitment of a transcription factor that would stimulate expression of the gene. A functionality associated with an effector moiety may indirectly affect expression of a target plurality of genes, e.g., introducing epigenetic modifications or recruiting other factors that introduce epigenetic modifications that induce a change in chromosomal topology that inhibits expression of a target plurality of genes.

Genomic complex: As used herein, the term “genomic complex” is a complex that brings together two genomic sequence elements that are spaced apart from one another on one or more

chromosomes, via interactions between and among a plurality of protein and/or other components (potentially including, the genomic sequence elements). In some embodiments, the genomic sequence elements are anchor sequences to which one or more protein components of the complex binds. In some embodiments, a genomic complex may comprise an anchor sequence-mediated conjunction. In some
5 embodiments, a genomic sequence element may be or comprise a CTCF binding motif, a promoter and/or an enhancer. In some embodiments, a genomic sequence element includes at least one or both of a promoter and/or regulatory site (e.g., an enhancer). In some embodiments, complex formation is nucleated at the genomic sequence element(s) and/or by binding of one or more of the protein component(s) to the genomic sequence element(s). As will be understood by those skilled in the art, in
10 some embodiments, co-localization (e.g., conjunction) of the genomic sites via formation of the complex alters DNA topology at or near the genomic sequence element(s), including, in some embodiments, between them. In some embodiments, a genomic complex comprises an anchor sequence-mediated conjunction, which comprises one or more loops. In some embodiments, a genomic complex as described herein is nucleated by a nucleating polypeptide such as, for example, CTCF and/or Cohesin. In some
15 embodiments, a genomic complex as described herein may include, for example, one or more of CTCF, Cohesin, non-coding RNA (e.g., eRNA), transcriptional machinery proteins (e.g., RNA polymerase, one or more transcription factors, for example selected from the group consisting of TFIIA, TFIIB, TFIID, TFIIIE, TFIIF, TFIIF, etc.), transcriptional regulators (e.g., Mediator, P300, enhancer-binding proteins, repressor-binding proteins, histone modifiers, etc.), etc. In some embodiments, a genomic complex as
20 described herein includes one or more polypeptide components and/or one or more nucleic acid components (e.g., one or more RNA components), which may, in some embodiments, be interacting with one another and/or with one or more genomic sequence elements (e.g., anchor sequences, promoter sequences, regulatory sequences (e.g., enhancer sequences)) so as to constrain a stretch of genomic DNA into a topological configuration (e.g., a loop) that it does not adopt when the complex is not formed.

25 **Nucleic acid:** As used herein, in its broadest sense, the term “nucleic acid” refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, “*nucleic acid*” refers to an individual nucleic acid residue (e.g., a nucleotide and/or nucleoside); in some
30 embodiments, “*nucleic acid*” refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a “*nucleic acid*” is or comprises RNA; in some embodiments, a “*nucleic acid*” is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a nucleic acid in

that it does not utilize a phosphodiester backbone. For example, in some embodiments, a nucleic acid is, comprises, or consists of one or more "*peptide nucleic acids*", which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. Alternatively, or additionally, in some embodiments, a nucleic acid has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxy guanosine, and deoxycytidine). In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3 -methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5 -propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, 0(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a nucleic acid comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, a nucleic acid is partly or wholly single stranded; in some embodiments, a nucleic acid is partly or wholly double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a nucleic acid has enzymatic activity.

Operably linked: As used herein, the phrase "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A transcription control element "*operably linked*" to a functional element, e.g., gene, is associated in such a way that expression and/or activity of the functional element, e.g., gene, is achieved under conditions compatible with the transcription control element. In some embodiments, "*operably linked*" transcription control elements are contiguous (e.g., covalently linked) with coding elements, e.g., genes, of interest; in some embodiments, operably linked transcription control elements act in *trans* to or otherwise at a

distance from the functional element, e.g., gene, of interest. In some embodiments, operably linked means two nucleic acid sequences are comprised on the same nucleic acid molecule. In a further embodiment, operably linked may further mean that the two nucleic acid sequences are proximal to one another on the same nucleic acid molecule, e.g., within 1000, 500, 100, 50, or 10 base pairs of each other or directly adjacent to each other.

Peptide, Polypeptide, Protein: As used herein, the terms “peptide,” “polypeptide,” and “protein” refer to a compound comprised of amino acid residues covalently linked by peptide bonds, or by means other than 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 or by means other than 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.

Proximal: As used herein, “proximal” refers to a closeness of two sites, e.g., nucleic acid sites, such that binding of a site-specific disrupting agent at the first site and/or modification of the first site by a site-specific disrupting agent will produce the same or substantially the same effect as binding and/or modification of the other site. For example, a DNA-targeting moiety may bind to a first site that is proximal to an anchor sequence (the second site), and the effector moiety associated with said DNA-targeting moiety may epigenetically modify the first site such that the binding of the anchor sequence to an endogenous nucleating polypeptide modified, substantially the same as if the second site (the anchor sequence) had been bound and/or modified. In some embodiments, sites that are proximal to one another are less than 5000, 4000, 3000, 2000, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 10, or 5 base pairs from one another.

Sequence targeting polypeptide: As used herein, the term “sequence targeting polypeptide” as used herein, refers to a protein, such as an enzyme, e.g., Cas9 or a TALEN, that recognizes or specifically binds to a target nucleic acid sequence. In some embodiments, the sequence targeting polypeptide is a catalytically inactive protein, such as dCas9, a TAL effector molecule, or a Zn Finger molecule, that lacks endonuclease activity.

Specific binding: As used herein, the term “specific binding” refers to an ability to discriminate between possible binding partners in the environment in which binding is to occur. In some embodiments, a binding agent that interacts with one particular target when other potential targets are present is said to “bind specifically” to the target with which it interacts. In some embodiments, specific binding is assessed by detecting or determining degree of association between the binding agent and its partner; in some embodiments, specific binding is assessed by detecting or determining degree of

dissociation of a binding agent-partner complex. In some embodiments, specific binding is assessed by detecting or determining ability of the binding agent to compete with an alternative interaction between its partner and another entity. In some embodiments, specific binding is assessed by performing such detections or determinations across a range of concentrations.

5 **Subject:** As used herein, the term “subject” or “test subject” refers to any organism to which a provided compound or composition is administered in accordance with the present disclosure *e.g.*, for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (*e.g.*, mammals such as mice, rats, rabbits, non-human primates, and humans; insects; worms; *etc.*) and plants. In some embodiments, a subject may be suffering from, and/or susceptible to a disease, disorder,
10 and/or condition.

Substantially: As used herein, the term “substantially” refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the art will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term “substantially” may
15 therefore be used in some embodiments herein to capture potential lack of completeness inherent in many biological and chemical phenomena.

Symptoms are reduced: As used herein, the phrase “symptoms are reduced” may be used when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (*e.g.*, intensity, severity, *etc.*) and/or frequency. In some embodiments, a delay in the onset of a particular
20 symptom is considered one form of reducing the frequency of that symptom.

Target: An agent or entity is considered to “target” another agent or entity, in accordance with the present disclosure, if it binds specifically to the targeted agent or entity under conditions in which they come into contact with one another. In some embodiments, for example, an antibody (or antigen-binding fragment thereof) targets its cognate epitope or antigen. In some embodiments, a nucleic acid having a
25 particular sequence targets a nucleic acid of substantially complementary sequence. In some embodiments, a targeting moiety that specifically binds an anchor sequence targets the anchor sequence, the ASMC comprising the anchor sequence, and/or the plurality of genes within the ASMC.

Target plurality of genes: As used herein, the term “target plurality of genes” means a group of more than one gene (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or more genes) that is targeted for modulation, *e.g.*, of
30 expression. In some embodiments, a target plurality of genes is part of a targeted genomic complex. In some embodiments, each gene of a target plurality of genes has at least part (*e.g.*, part or all) of its genomic sequence as part of a target genomic complex, *e.g.*, at least partly inside an ASMC, which genomic complex is targeted by a site-specific disrupting agent as described herein. In some embodiments, modulation comprises inhibition of expression of the target plurality of genes. In some

embodiments, a target plurality of genes is modulated by contacting the target plurality of genes or a transcription control element operably linked to one or more of the target plurality of genes with a site-specific disrupting agent described herein. In some embodiments, one or more of a target plurality of genes is aberrantly expressed (e.g., over-expressed) in a cell, e.g., a cell in a subject (e.g., patient). In some embodiments, the target plurality of genes has related functionalities. For example, the genes of a target plurality of genes may all have a pro-inflammatory effect when expressed; the genes of such a target plurality of genes may be referred to herein as pro-inflammatory genes or target pro-inflammatory genes. In some embodiments, a gene of a target plurality of genes encodes a protein. In some embodiments, a gene of a target plurality of genes encodes a functional RNA.

Targeting moiety: As used herein, the term “targeting moiety” means an agent or entity that specifically interacts (e.g., targets) with a component or set of components, e.g., a component or components that participate in a genomic complex as described herein (e.g., an anchor sequence-mediated conjunction). In some embodiments, a targeting moiety in accordance with the present disclosure targets one or more target component(s) of a genomic complex as described herein. In some embodiments, a targeting moiety targets a genomic complex component that comprises a genomic sequence element (e.g., an anchor sequence). In some embodiments, a targeting moiety targets a genomic complex component other than a genomic sequence element. In some embodiments, a targeting moiety targets a plurality or combination of genomic complex components, which plurality in some embodiments may include a genomic sequence element. In some aspects, contributions of the present disclosure include the insight that inhibition, dissociation, degradation, and/or modification of one or more genomic complexes, e.g., comprising a target anchor sequence proximal to a target gene (e.g., fusion gene, e.g., fusion oncogene) and/or breakpoint, as described herein, can be achieved by targeting genomic complex component(s), including genomic sequence element(s), with site-specific disrupting agents. In some aspects, effective inhibition, dissociation, degradation, and/or modification of one or more genomic complexes, as described herein, can be achieved by targeting complex component(s) comprising genomic sequence element(s). In some embodiments, the present disclosure contemplates that improved (e.g., with respect to, for example, degree of specificity for a particular genomic complex as compared with other genomic complexes that may form or be present in a given system, effectiveness of the inhibition, dissociation, degradation, or modification [e.g., in terms of impact on number of complexes detected in a population]) inhibition, dissociation, degradation, or modification may be achieved by targeting one or more complex components that is not a genomic sequence element and, optionally, may alternatively or additionally include targeting a genomic sequence element, wherein improved inhibition, dissociation, degradation, or modification is relative to that typically achieved through targeting genomic sequence element(s) alone. In some embodiments, a site-specific disrupting agent as described herein promotes inhibition,

dissociation, degradation, or modification of a target genomic complex. For example, by way of non-limiting example, in some embodiments, a site-specific disrupting agent as described herein inhibits, dissociates, degrades (e.g., a component of), and/or modifies (e.g., a component of) an anchor sequence-mediated conjunction by targeting at least one component of a given genomic complex (e.g., comprising the anchor sequence-mediated conjunction). In some embodiments, a site-specific disrupting agent as described herein inhibits, dissociates, degrades (e.g., a component of), and/or modifies (e.g., a component of) a particular genomic complex (i.e., a target genomic complex) and does not inhibit, dissociate, degrade (e.g., a component of), and/or modify (e.g., a component of) at least one other particular genomic complex (i.e., a non-target genomic complex) that, for example, may be present in other cells (e.g., in non-target cells) and/or that may be present at a different site in the same cell (i.e., within a target cell). A site-specific disrupting agent as described herein may comprise a targeting moiety. In some embodiments, a targeting moiety also acts as an effector moiety (e.g., disrupting moiety); in some such embodiments a provided site-specific disrupting agent may lack any effector moiety (e.g., disrupting, modifying, or other effector moiety) separate (or meaningfully distinct) from the targeting moiety.

Therapeutically effective amount: As used herein, the term “therapeutically effective amount” means an amount of a substance (e.g., a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, an effective amount of a substance may vary depending on such factors as desired biological endpoint(s), substance to be delivered, target cell(s) or tissue(s), *etc.* For example, in some embodiments, an effective amount of compound in a formulation to treat a disease, disorder, and/or condition is an amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

Transcriptional control sequence: As used herein, the term “transcriptional control sequence” refers to a nucleic acid sequence that increases or decreases transcription of a gene. An “enhancing sequence” increases the likelihood of gene transcription. A “silencing or repressor sequence” decreases the likelihood of gene transcription. Examples of transcriptional control sequences include promoters and enhancers. In some embodiments, an ASMC comprises a transcriptional control sequence. Such a transcriptional control sequence is referred to as an internal transcriptional control sequence (e.g., an enhancing sequence that is comprised within an ASMC is referred to as an internal enhancing sequence).

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description of the embodiments of the disclosure will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosure, there are shown in the drawings embodiments, which are presently exemplified. It should be understood, however, that the disclosure is not limited to the precise arrangement and instrumentalities of the embodiments shown in the drawings.

Figure 1 shows a diagram showing exemplary positioning of gRNA sequences in an anchor sequence. Figure discloses SEQ ID NOS 244-245, respectively, in order of appearance.

Figure 2 shows a diagram showing exemplary positioning of gRNA sequences in an anchor sequence and restriction site information. Figure discloses SEQ ID NOS 246-247, respectively, in order of appearance.

Figure 3 shows a graph of expression (mRNA) of various chemokines in TNF-treated cells with and without treatment with a site-specific disrupting agent comprising a CRISPR/Cas molecule and first exemplary gRNA.

Figure 4 shows a graph of expression (mRNA) of various chemokines in TNF-treated cells with and without treatment with a site-specific disrupting agent comprising a CRISPR/Cas molecule and a second exemplary gRNA.

Figure 5 shows a diagram depicting different types of genomic complex, e.g., ASMCs, e.g., loops, and models for how to alter expression of genes contained within.

Figure 6 shows a graph of cytokine expression measured by RNA levels of CXCL1, CXCL2, CXCL3, and IL-8 in THP-1 cells treated with a site-specific disrupting agent comprising a CRISPR/Cas molecule and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 7 shows a graph of cytokine secretion (CXCL1 and IL-8) of THP-1 cells treated with site-specific disrupting agent comprising a CRISPR/Cas molecule and different sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 8 shows a graph (top) of cytokine expression (CXCL3) measured by RNA level in THP-1 cells treated with a site-specific disrupting agent comprising a CRISPR/Cas molecule and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes, and a flow chart (bottom) showing how cells were processed in the experiment.

Figure 9A shows a graph (top) of cytokine expression (CXCL1) measured by RNA level in THP-1 cells 3 weeks after treatment with a site-specific disrupting agent comprising a CRISPR/Cas molecule and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-

encoding genes, and a flow chart (bottom) showing how cells were processed in the experiment. **Figure 9B** shows a graph of cytokine expression (CXCL3) measured by RNA level in THP-1 cells 3 weeks after treatment with a site-specific disrupting agent comprising a CRISPR/Cas molecule and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 10 shows a graph of cytokine expression (CXCL1) measured by RNA level in THP-1 cells after treatment with a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule and a transcriptional repressor (KRAB) and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 11 shows a graph of cytokine expression (CXCL1) measured by RNA level in THP-1 cells after treatment with a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule and a histone methyltransferase (EZH2) and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 12 shows a graph of cytokine expression (CXCL1) measured by RNA level in THP-1 cells after treatment with a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule and a DNA methyltransferase (MQ1) and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 13 shows a graph (top) of cytokine expression (CXCL1) measured by RNA level in THP-1 cells after treatment with different site-specific disrupting agents for 72 hours, 3 weeks, or 4 weeks, and a flow chart (bottom) showing how cells were processed in the experiment.

Figure 14 shows a graph (top) of cytokine expression (CXCL3) measured by RNA level in THP-1 cells after treatment with different site-specific disrupting agents and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes, and a flow chart (bottom) showing how cells were processed in the experiment.

Figure 15 shows a graph (top) of cytokine expression (CXCL1) measured by RNA level in THP-1 cells after treatment with different site-specific disrupting agents and sgRNAs targeted to the anchor sequences of a genomic complex (e.g., ASMC) comprising cytokine-encoding genes.

Figure 16 shows human CXCL IGD and gene cluster organization. **Figure 16A** shows a schematic Insulated Genomic Domain (IGD) illustrating the two loops within CXCL1-8 gene cluster. CXCL8, CXCL6, and CXCL 1 genes reside on the left loop of the IGD. CXCL2-5 and CXCL7 genes reside on the right loop of the IGD. Investigation of the IGD data from different cell lines suggested that middle CTCF is only present in cells that secrete CXCL (e.g., not in lymphocytes).

Figure 16B shows guides were designed to the four different CTCF targets: Left CTCF-2, Left CTCF, Middle CTCF, and Right CTCF.

Figure 17 shows CXCL1-8 genes were downregulated when dCas9-EZH2 guide 30183 targeted Middle CTCF motif located within the CXCL1-8 cluster in TNF-alpha treated Human A549 lung cancer epithelial cells. Cells stimulated with TNF alpha were treated as control.

Figure 18 shows CXCL1, 2, 3, 8 genes were downregulated when dCas9-EZH2 guide 30183 targeted Middle CTCF motif located within the CXCL1-8 cluster in TNF-alpha treated Human IMR-90 normal lung fibroblast cells. Cells stimulated with TNF alpha were treated as control.

Figure 19 shows that CXCL1, 2, 3, 8 genes were downregulated when Controller A targeted Left CTCF motif located within the CXCL1-8 cluster in TNF-alpha treated Human monocytes. Cells stimulated with TNF alpha were treated as control.

Figure 20 shows mouse CXCL IGD and gene cluster organization. **Figure 20A** shows a schematic Insulated Genomic Domain (IGD) illustrating the two loops within CXCL gene cluster. **Figure 20B** illustrates the two loops within the CXCL1-5, 7 and 15 gene cluster. CXCL4, CXCL5, and CXCL7 genes reside on the left loop of the IGD. CXCL1-3 and CXCL15 genes reside on the right loop of the IGD. IGD guides were designed to the four different CTCF targets: Left (L), Middle 1 (M1), Middle 2 (M2), and Right (R) CTCF.

Figure 21A shows IGD guides were designed to the four different CTCF targets: Middle 1 (M1), Middle 2 (M2), and Right (R) CTCF.

Figure 21B shows in vitro downregulation of mouse CXCL IGD in Hep 1.6 using dCas9-MQ1. dCas9-MQ1 was transfected using guides targeting the right, or one of the two middle CTCF motifs in the CXCL gene cluster, which showed no down regulation in any of the seven CXCL genes after TNF alpha stimulation (orange). When dCas9-MQ1 was transfected using combination guides targeting both middle CTCF and right, the entire gene cluster was down regulated (blue).

Figure 22A shows schematic experimental design to determine the effect of dCas9-MQ1 on decreasing leukocyte filtration in inflamed lungs. Each mouse was treated with either LNP alone or with dCas9-MQ1 at 3 mg/kg targeting the two middle and right CTCF at -2 hour time point. The mice were stimulated with 5 mg/kg LPS at zero hours followed by a second dose of LNP alone or a dCas9-MQ1 at 3 mg/kg targeting the two middle and right CTCF at the +8 hour time point. Dexamethasone was administered intraperitoneal at 10mg/kg dose at time 0, 24, and 48 hours. The animals were terminated at 72 hours and bronchiolar lavage fluid were collected from the lungs for flow staining.

Figure 22B shows systemic administration of an dCas9-MQ1 decreased leukocyte infiltration in the inflamed lungs. Total leukocyte count/mL in the bronchiolar lavage fluid obtained from dCas9-MQ1 treated mice showed significant differences compared to LPS + disease animals.

Figure 23A shows the composition of infiltrating cells found in the bronchiolar lavage fluid obtained from an inflamed lung of a mice. The leukocyte cell types that make up the majority of the infiltrating cells are neutrophils, followed by B cells, T cells, macrophages and other types of hematopoietic cells.

Figure 23B shows dCas9-MQ1 decreased the count of neutrophils infiltrating the lungs with significant difference compared to the +LPS disease group.

Figure 24 shows the decrease of leukocyte cells in the BALF was lung specific and not due to the decrease of white blood cells in the peripheral blood. This graph illustrated that the effect of decreasing leukocyte count in the BALF with the dCas9-MQ1 treatment was lung specific and was not because the mouse itself had a decrease in leukocyte population. The hematopoietic cell population in the peripheral blood was similar across all groups.

Figures 25A-G show CXCL1-5, CXCL7, and CXCL15 gene expression was decreased in the lung tissue. After treating the animals with LNP alone or with dCas9-MQ1, the lung tissues were processed to check for CXCL gene expression by qPCR methods. All CXCL genes show downregulation when treated with dCA9-MQ1. CXCL2 expression was most downregulated.

Figure 26 shows decreasing CXCL expression and cellular recruitment to the site of inflammation had a beneficial downstream effect of decreasing the presence of other cytokines. The chemokine protein levels secreted in the BALF showed decrease in CXCL 1 and 2 protein levels. Decreasing CXCL expression and cellular recruitment to the site of inflammation had beneficial downstream effects of decreasing the presence of GM-CSF (Fig 26C) and IL6 (Fig. 26D).

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

The present disclosure provides technologies for modulating, e.g., decreasing, expression of a target plurality of genes in a cell, e.g., in a subject or patient, through the use of site-specific disrupting agent or a system comprising two or more site-specific disrupting agents. In some embodiments, a site-specific disrupting agent comprises a targeting moiety. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety. Without wishing to be bound by theory, a number of diseases and conditions are associated with groups of genes with related functionalities that are associated with a common genomic complex, e.g., ASMC. Modulation, e.g., disruption, of a genomic complex, e.g., ASMC, comprising (wholly or in part) a target plurality of genes may be an improved approach to altering (e.g., decreasing) expression of the target plurality of genes (e.g., with respect to improved efficiency, efficacy, and/or stability of alteration) over modulation of individual target genes. Said improvements may translate to corresponding improvements in the treatment of diseases and conditions associated with the target plurality of genes. For example, a plurality of genes may be

associated with a pro-inflammatory effect and a site-specific disrupting agent can target a genomic complex, e.g., ASMC, comprising (wholly or in part) the plurality of genes to modulate, e.g., decrease, expression of the plurality of genes and thereby achieve an anti-inflammatory effect (e.g., a superior anti-inflammatory effect relative to individually targeting the genes of the plurality). Examples of site-specific
5 disrupting agents, targeting moieties, effector moieties, and target pluralities of genes are provided herein.

A site-specific disrupting agent may modulate, e.g., decrease, expression of a target plurality of genes by one or more modalities. In some embodiments, a site-specific disrupting agent binds to a target site, e.g., anchor sequence, and physically or sterically competes for binding with other genomic complex components, e.g., a nucleating polypeptide. Without wishing to be bound by theory, physical or steric
10 blockage of an anchor sequence, e.g., such that binding of a genomic complex component (e.g., a nucleating polypeptide) to the anchor sequence is inhibited (e.g., prevented), is one mechanism by which a site-specific disrupting agent may modulate, e.g., decrease, expression of a target plurality of genes. A site-specific disrupting agent may destabilize the interaction of a genomic complex component (e.g., nucleating polypeptide) with an anchor sequence, e.g., by altering (e.g., decreasing) the affinity and/or
15 avidity at which the genomic complex component binds the anchor sequence. Blocking or destabilizing binding of a genomic complex component (e.g., nucleating polypeptide) to an anchor sequence may be accomplished by one or more means, including: epigenetic modification of the anchor sequence or a sequence proximal thereto, genetic modification of the anchor sequence or a sequence proximal thereto, or binding of the site-specific disrupting agent to the anchor sequence or a sequence proximal thereto.
20 Inhibiting (e.g., preventing) binding of a genomic complex component (e.g., a nucleating polypeptide) to an anchor sequence may inhibit (e.g., disrupt or prevent formation of) a genomic complex, e.g., ASMC. Inhibition of a genomic complex, e.g., ASMC, comprising, wholly or partly, a target plurality of genes may modulate, e.g., decrease, expression of the genes of the target plurality of genes. In some
25 embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety, and a second effector moiety. In some embodiments, the first effector moiety has a sequence that is different from the sequence of the second effector moiety. In some embodiments, the first effector moiety has a sequence that is identical to the sequence of the second effector moiety.

The disclosure further provides in part, a system comprising two or more site-specific disrupting agents, each comprising a targeting moiety and optionally an effector moiety. In some embodiments, the
30 targeting moieties target two or more different sequences (e.g., each site-specific disrupting agent may target a different sequence). In some embodiments, the first site-specific disrupting agent binds to a transcription regulatory element (e.g., a promoter or transcription start site (TSS)) operably linked to a target plurality of genes, e.g., human CXCL1-8 and the second site-specific disrupting agent binds to an anchor sequence of an anchor sequence mediated conjunction (ASMC) comprising a target plurality of

genes, e.g., human CXCL1-8. In some embodiments, modulation of expression of a target plurality of genes, e.g., human CXCL 1-8 by a system involves the binding of the first site-specific disrupting agent and second site-specific disrupting agent to the first and second DNA sequences, respectively. Binding of the first and second DNA sequences localizes the functionalities of the first and second effector moieties to those sites. Without wishing to be bound by theory, in some embodiments employing the functionalities of both the first and second site-specific disrupting agent effector moieties stably represses expression of a target plurality of gene associated with or comprising the first and/or second DNA sequences, e.g., wherein the first and/or second DNA sequences are or comprise sequences of the target plurality of gene or one or more operably linked transcription control elements.

Site-specific Disrupting Agents

In some embodiments, a site-specific disrupting agent comprises a targeting moiety. In some embodiments, the targeting moiety specifically binds a DNA sequence, e.g., an anchor sequence, and thereby modulates, e.g., disrupts, a genomic complex (e.g., ASMC) comprising said DNA sequence. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety. In some embodiments, the targeting moiety specifically binds a DNA sequence, thereby localizing the effector moiety's functionality to the DNA sequence, thereby modulating, e.g., disrupting, a genomic complex (e.g., ASMC) comprising said DNA sequence. In some embodiments, a site-specific disrupting agent comprises one targeting moiety and one effector moiety. In some embodiments, a site-specific disrupting agent comprises one targeting moiety and more than one effector moiety, e.g., two, three, four, or five effector moieties, each of which may be the same or different from another of the more than one effector moieties. In some embodiments, a site-specific disrupting agent may comprise two effector moieties where the first effector moiety comprises a different functionality than the second effector moiety. For example, a site-specific disrupting agent may comprise two effector moieties, where the first effector moiety comprises DNA methyltransferase functionality (e.g., comprises G9A or EZH2 or a functional fragment or variant thereof) and the second effector moiety comprises a transcriptional repressor functionality (e.g., comprises KRAB or a functional fragment or variant thereof). In some embodiments, a site-specific disrupting agent comprises effector moieties whose functionalities are complementary to one another with regard to decreasing expression of a target plurality of gene, where the functionalities together inhibit expression and, optionally, do not inhibit or negligibly inhibit expression when present individually. In some embodiments, a site-specific disrupting agent comprises a plurality of effector moieties, wherein each effector moiety complements each other effector moiety, each effector moiety decreases expression of a target plurality of gene.

In some embodiments, a site-specific disrupting agent comprises a combination of effector moieties whose functionalities synergize with one another with regard to decreasing expression of a target plurality of gene. Without wishing to be bound by theory, in some embodiments, epigenetic modifications to a genomic locus are cumulative, in that multiple transcription activating epigenetic markers (e.g., multiple different types of epigenetic markers and/or more extensive marking of a given type) individually together inhibit expression more effectively than individual modifications alone (e.g., producing a greater decrease in expression and/or a longer-lasting decrease in expression). In some embodiments, a site-specific disrupting agent comprises a plurality of effector moieties, wherein each effector moiety synergizes with each other effector moiety, e.g., each effector moiety decreases expression of a target plurality of gene. In some embodiments, a site-specific disrupting agent (comprising a plurality of effector moieties which synergize with one another) is more effective at inhibiting expression of a target plurality of gene, than a site-specific disrupting agent comprising an individual effector moiety. In some embodiments, a site-specific disrupting agent comprising said plurality of effector moieties is at least 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.55x, 1.6x, 1.65x, 1.7x, 1.75x, 1.8x, 1.85x, 1.9x, 1.95x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x as effective at decreasing expression of a target plurality of gene, than a site-specific disrupting agent comprising an individual effector moiety.

In some embodiments, a site-specific disrupting agent comprises one or more targeting moieties e.g., a Cas domain, TAL effector domain, or Zn Finger domain. In an embodiment, when system comprises two or more targeting moieties of the same type, e.g., two or more Cas domains, the targeting moieties specifically bind two or more different sequences. For example, in a site-specific disrupting agent system comprising two or more Cas domains, the two or more Cas domains may be chosen or altered such that they only appreciably bind the gRNA corresponding to their target sequence (e.g., and do not appreciably bind the gRNA corresponding to the target of another Cas domain).

In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety that are covalently linked, e.g., by a peptide bond. In some embodiments, the targeting moiety and effector moiety are situated on the same polypeptide chain, e.g., connected by one or more peptide bonds and/or a linker. In some embodiments, a site-specific disrupting agent comprises a fusion molecule, e.g., comprising the targeting moiety and effector moiety linked by a peptide bond and/or a linker. In some embodiments, a site-specific disrupting agent comprises a targeting moiety that is disposed N-terminal of an effector moiety on the same polypeptide chain. In some embodiments, a site-specific disrupting agent comprises a targeting moiety that is disposed C-terminal of an effector moiety

on the same polypeptide chain. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety that are covalently linked by a non-peptide bond. In some embodiments, a targeting moiety is conjugated to an effector moiety by a non-peptide bond. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and a plurality of effector moieties, wherein the targeting moiety and the plurality of effector moieties are covalently linked, e.g., by peptide bonds (e.g., the targeting moiety and plurality of effector moieties are all connected by a series of covalent bonds, although each individual moiety may not share a covalent bond with every other moiety).

In other embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety that are not covalently linked, e.g., that are non-covalently associated with one another. In some embodiments, a site-specific disrupting agent comprises a targeting moiety that non-covalently binds to an effector moiety or vice versa. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and a plurality of effector moieties, wherein the targeting moiety and at least one effector moiety are not covalently linked, e.g., are non-covalently associated with one another, and wherein the targeting moiety and at least one other effector moiety are covalently linked, e.g., by a peptide bond.

In some embodiments, a site-specific disrupting agent comprises a first effector moiety comprising G9A and a second effector moiety comprising KRAB. In some embodiments, a site-specific disrupting agent comprises a first effector moiety comprising G9A and a second effector moiety comprising EZH2. In some embodiments, a site-specific disrupting agent comprises a first effector moiety comprising EZH2 and a second effector moiety comprising KRAB.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety, wherein the C-terminal end of the effector moiety, e.g., an effector moiety chosen from, EZH2, or G9A or a functional variant or fragment thereof and the N-terminal end of the targeting moiety are covalently linked. In some embodiments, a site-specific disrupting agent comprises a targeting moiety and an effector moiety wherein the N-terminal end of the effector moiety, e.g., an effector moiety chosen from HDAC8, MQ1, DNMT3a/3L, KRAB, or a functional variant or fragment thereof and the C-terminal end of the targeting moiety are covalently linked. In some embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety and a second effector moiety, wherein, the C-terminal end of the first effector moiety, e.g., a effector moiety chosen from EZH2, G9A, or a functional variant or fragment thereof, and the N-terminal end of the targeting moiety are covalently linked and the C-terminal end of the targeting moiety and the N-terminal end of the second effector moiety, e.g., a

effector moiety chosen from HDAC8, MQ1, DNMT3a/3L, KRAB or a functional variant or fragment thereof are covalently linked. The covalent linkage may be, e.g., via a linker sequence.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety and a second effector moiety, wherein the first effector moiety is EZH2, or a functional variant or fragment thereof, e.g., wherein the first effector moiety comprises an amino acid sequence of SEQ ID NO: 17 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the first effector moiety is N-terminal of the targeting moiety; and the second effector moiety is KRAB, or a functional variant or fragment thereof, e.g., wherein the second effector moiety comprises an amino acid sequence of SEQ ID NO: 13 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the second effector moiety is C-terminal of the targeting moiety.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety and a second effector moiety, wherein the first effector moiety is EZH2, or a functional variant or fragment thereof, e.g., wherein the first effector moiety comprises an amino acid sequence of SEQ ID NO: 17 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the first effector moiety is N-terminal of the targeting moiety; and the second effector moiety is HDAC8, or a functional variant or fragment thereof, e.g., wherein the second effector moiety comprises an amino acid sequence of SEQ ID NO: 19 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the second effector moiety is C-terminal of the targeting moiety.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety and a second effector moiety, wherein the first effector moiety is G9A, or a functional variant or fragment thereof, e.g., wherein the first effector moiety comprises an amino acid sequence of SEQ ID NO: 67 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the first effector moiety is N-terminal of the targeting moiety; and the second effector moiety is KRAB, or a functional variant or fragment thereof, e.g., wherein the second effector moiety comprises an amino acid sequence of SEQ ID NO: 13 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity

thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the second effector moiety is C-terminal of the targeting moiety.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety, a first effector moiety and a second effector moiety, wherein the first effector moiety is G9A, or a functional variant or fragment thereof, e.g., wherein the first effector moiety comprises an amino acid sequence of SEQ ID NO: 67 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the first effector moiety is N-terminal of the targeting moiety; and the second effector moiety is EZH2, or a functional variant or fragment thereof, e.g., wherein the second effector moiety comprises an amino acid sequence of SEQ ID NO: 17 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, and the second effector moiety is C-terminal of the targeting moiety.

In some embodiments, the first effector moiety comprises a histone methyltransferase activity and the second effector moiety comprises a different histone methyltransferase activity. In some embodiments, the first effector moiety comprises a histone methyltransferase activity and the second effector moiety comprises the same histone methyltransferase activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises a histone deacetylase activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises a DNA methyltransferase activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises a DNA demethylase activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises a transcription repressor activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises a different histone demethylase activity. In some embodiments, the first effector moiety comprises a histone demethylase activity and the second effector moiety comprises the same histone demethylase activity. In some embodiments, the first effector moiety comprises a histone deacetylase activity and the second effector moiety comprises a DNA methyltransferase activity. In some embodiments, the first effector moiety comprises a histone deacetylase activity and the second effector moiety comprises a DNA demethylase activity. In some embodiments, the first effector moiety comprises a histone deacetylase activity and the second effector moiety comprises a transcription repressor activity. In some embodiments, the first effector moiety comprises a histone deacetylase activity and the second effector moiety comprises a different histone deacetylase activity. In some embodiments, the first effector

moiety comprises a histone deacetylase activity and the second effector moiety comprises the same histone deacetylase activity. In some embodiments, the first effector moiety comprises a DNA methyltransferase activity and the second effector moiety comprises a DNA demethylase activity. In some
 5 effector moiety comprises a transcription repressor activity. In some embodiments, the first effector moiety comprises a DNA methyltransferase activity and the second effector moiety comprises a different DNA methyltransferase activity. In some embodiments, the first effector moiety comprises a DNA methyltransferase activity and the second effector moiety comprises the same DNA methyltransferase activity. In some embodiments, the first effector moiety comprises a DNA demethylase activity and the
 10 second effector moiety comprises a transcription repressor activity. In some embodiments, the first effector moiety comprises a DNA demethylase activity and the second effector moiety comprises a different DNA demethylase activity. In some embodiments, the first effector moiety comprises a DNA demethylase activity and the second effector moiety comprises the same DNA demethylase activity. In some embodiments, the first effector moiety comprises a transcription repressor activity and the second
 15 effector moiety comprises a different transcription repressor activity. In some embodiments, the first effector moiety comprises a transcription repressor activity and the second effector moiety comprises the same transcription repressor activity.

In some embodiments, the first effector moiety comprises, DNMT3a/3l, MQ1, KRAB, G9A,
 20 HDAC8, or EZH2 and the second effector moiety comprises DNMT3a/3l, MQ1, KRAB, G9A, HDAC8, or EZH2.

Linkers

A site-specific disrupting agent may comprise one or more linkers. A linker may connect a targeting moiety to an effector moiety, an effector moiety to another effector moiety, or a targeting moiety
 25 to another targeting moiety. A linker may be a chemical bond, e.g., one or more covalent bonds or non-covalent bonds. In some embodiments, a linker is covalent. In some embodiments, a linker is non-covalent. In some embodiments, a linker is a peptide linker. Such a linker may be between 2-30, 5-30, 10-30, 15-30, 20-30, 25-30, 2-25, 5-25, 10-25, 15-25, 20-25, 2-20, 5-20, 10-20, 15-20, 2-15, 5-15, 10-15, 2-10, 5-10, or 2-5 amino acids in length, or greater than or equal to 2, 5, 10, 15, 20, 25, or 30 amino acids
 30 in length (and optionally up to 50, 40, 30, 25, 20, 15, 10, or 5 amino acids in length). In some embodiments, a linker can be used to space a first moiety from a second moiety, e.g., a targeting moiety from an effector moiety. In some embodiments, for example, a linker can be positioned between a targeting moiety and an effector moiety, e.g., to provide molecular flexibility of secondary and tertiary structures. In some embodiments, a site-specific disrupting agent may comprise a first effector moiety

linked to the targeting moiety via a first linker and a second effector moiety linked to the targeting moiety via a second linker. In some embodiments, the first linker has a sequence that is identical to the sequence of the second linker. In some embodiments, the first linker has a sequence that is not identical to the sequence of the second linker. In some embodiments, the first effector moiety is N-terminal of the targeting moiety. In some embodiments, the C-terminal of the targeting moiety. In some embodiments, the C-terminal end of the first effector moiety is linked to the N-terminal end of the targeting moiety via the first linker and the N-terminal end of the second effector moiety is linked to the C-terminal end of the targeting moiety via the second linker.

A linker may comprise flexible, rigid, and/or cleavable linkers described herein. In some embodiments, a linker includes at least one glycine, alanine, and serine amino acids to provide for flexibility. In some embodiments, a linker is a hydrophobic linker, such as including a negatively charged sulfonate group, polyethylene glycol (PEG) group, or pyrophosphate diester group. In some embodiments, a linker is cleavable to selectively release a moiety (e.g., polypeptide) from a modulating agent, but sufficiently stable to prevent premature cleavage.

In some embodiments, one or more moieties of a site-specific disrupting agent described herein are linked with one or more linkers.

As will be known by one of skill in the art, commonly used flexible linkers have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker). Flexible linkers may be useful for joining domains/moieties that require a certain degree of movement or interaction and may include small, non-polar (e.g., Gly) or polar (e.g., Ser or Thr) amino acids. Incorporation of Ser or Thr can also maintain the stability of a linker in aqueous solutions by forming hydrogen bonds with water molecules, and therefore reduce unfavorable interactions between a linker and moieties/domains.

Rigid linkers are useful to keep a fixed distance between domains/moieties and to maintain their independent functions. Rigid linkers may also be useful when a spatial separation of domains is critical to preserve the stability or bioactivity of one or more components in the fusion. Rigid linkers may have an alpha helix-structure or Pro-rich sequence, $(XP)_n$, with X designating any amino acid, preferably Ala, Lys, or Glu.

Cleavable linkers may release free functional domains/moieties in vivo. In some embodiments, linkers may be cleaved under specific conditions, such as presence of reducing reagents or proteases. *In*

vivo cleavable linkers may utilize reversible nature of a disulfide bond. One example includes a thrombin-sensitive sequence (e.g., PRS) between the two Cys residues. *In vitro* thrombin treatment of CPRSC (SEQ ID NO: 243) results in the cleavage of a thrombin-sensitive sequence, while a reversible disulfide linkage remains intact. Such linkers are known and described, e.g., in Chen et al. 2013. Fusion Protein Linkers: Property, Design and Functionality. Adv Drug Deliv Rev. 65(10): 1357–1369. *In vivo* cleavage of linkers in fusions may also be carried out by proteases that are expressed *vivo* under certain conditions, in specific cells or tissues, or constrained within certain cellular compartments. Specificity of many proteases offers slower cleavage of the linker in constrained compartments.

Examples of molecules suitable for use in linkers described herein include a negatively charged sulfonate group; lipids, such as a poly (–CH₂–) hydrocarbon chains, such as polyethylene glycol (PEG) group, unsaturated variants thereof, hydroxylated variants thereof, amidated or otherwise N-containing variants thereof; noncarbon linkers; carbohydrate linkers; phosphodiester linkers, or other molecule capable of covalently linking two or more components of a site-specific disrupting agent. Non-covalent linkers are also included, such as hydrophobic lipid globules to which the polypeptide is linked, for example through a hydrophobic region of a polypeptide or a hydrophobic extension of a polypeptide, such as a series of residues rich in leucine, isoleucine, valine, or perhaps also alanine, phenylalanine, or even tyrosine, methionine, glycine, or other hydrophobic residue. Components of a site-specific disrupting agent may be linked using charge-based chemistry, such that a positively charged component of a site-specific disrupting agent is linked to a negative charge of another component.

Nucleic acids

In one aspect, the disclosure provides nucleic acid sequences encoding a site-specific disrupting agent, a system, a targeting moiety and/or an effector moiety as described herein. A skilled artisan is aware that the nucleic acid sequences of RNA are identical to the corresponding DNA sequences, except that typically thymine (T) is replaced by uracil (U). It will be understood that when a nucleotide sequence is represented by a DNA sequence (e.g., comprising, A, T, G, C), this disclosure also provides the corresponding RNA sequence (e.g., comprising, A, U, G, C) in which “U” replaces “T.” Conventional notation is used herein to describe polynucleotide sequences: the left-hand end of a single-stranded polynucleotide sequence is the 5'-end; the left-hand direction of a double-stranded polynucleotide sequence is referred to as the 5'-direction.

It will be appreciated by those skilled in the art that due to the degeneracy of the genetic code, a multitude of nucleotide sequences encoding a site-specific disrupting agent comprising DNA-targeting

moiety and/or an effector moiety as described herein may be produced, some of which have similarity, e.g., 90%, 95%, 96%, 97%, 98%, or 99% identity to the nucleic acid sequences disclosed herein. For instance, codons AGA, AGG, CGA, CGC, CGG, and CGU all encode the amino acid arginine. Thus, at every position in the nucleic acid molecules of the disclosure where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide.

In some embodiments a nucleic acid sequence encoding a site-specific disrupting agent comprising a targeting moiety and/or one or more effector moieties may be part or all of a codon-optimized coding region, optimized according to codon usage in mammals, e.g., humans. In some embodiments, a nucleic acid sequence encoding a targeting moiety and/or one or more effector moieties is codon optimized for increasing the protein expression and/or increasing the duration of protein expression. In some embodiments, a protein produced by the codon optimized nucleic acid sequence is at least 1%, at least 2%, at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 40%, or at least 50% higher compared to levels of the protein when encoded by a nucleic acid sequence that is not codon optimized.

In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a polypeptide comprising one or more (e.g., one) DNA-targeting moiety and one or more effector moiety, e.g., wherein the effector moiety is or comprises MQ1, e.g., bacterial MQ1, or a functional variant or fragment thereof. In some embodiments, MQ1 is *Spiroplasma monobiae* MQ1, e.g., MQ1 from strain ATCC 33825 and/or corresponding to Uniprot ID P15840. In some embodiments, MQ1 effector moiety is encoded by a nucleotide sequence of SEQ ID NO: 10. In some embodiments, a nucleotide sequence described herein comprises a sequence of SEQ ID NO: 10 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

MQ1

AGCAAGGTGGAGAACAAGACCAAGAAGCTGCGGGTGTTCGAGGCCTTCGCCGGCATCGGCG
 CCCAGCGGAAGGCCCTGGAGAAGGTGCGGAAGGACGAGTACGAGATCGTGGGCCTGGCCG
 AGTGGTACGTGCCCCGCCATCGTGATGTACCAGGCCATCCACAACAACCTCCACACCAAGCTG
 GAGTACAAGAGCGTGAGCCGGGAGGAGATGATCGACTACCTGGAGAACAAGACCCTGAGCT
 GGAACAGCAAGAACCCCGTGAGCAACGGCTACTGGAAGCGGAAGAAGGACGACGAGCTGA
 AGATCATCTACAACGCCATCAAGCTGAGCGAGAAGGAGGGCAACATCTTCGACATCCGGGA

CCTGTACAAGCGGACCCTGAAGAACATCGACCTGCTGACCTACAGCTTCCCCTGCCAGGACC
 TGAGCCAGCAGGGCATCCAGAAGGGCATGAAGCGGGGCAGCGGCACCCGGAGCGGCCTGCT
 GTGGGAGATCGAGCGGGCCCTGGACAGCACCGAGAAGAACGACCTGCCCAAGTACCTGCTG
 ATGGAGAACGTGGGCGCCCTGCTGCACAAGAAGAACGAGGAGGAGCTGAACCAGTGGAAG
 5 CAGAAGCTGGAGAGCCTGGGCTACCAGAACAGCATCGAGGTGCTGAACGCCGCCGACTTCG
 GCAGCAGCCAGGCCCGGCGGGTGTTCATGATCAGCACCTGAACGAGTTCGTGGAGCT
 GCCCAAGGGCGACAAGAAGCCCAAGAGCATCAAGAAGGTGCTGAACAAGATCGTGAGCGA
 GAAGGACATCCTGAACAACCTGCTGAAGTACAACCTGACCGAGTTCAAGAAAACCAAGAGC
 AACATCAACAAGGCCAGCCTGATCGGCTACAGCAAGTTCAACAGCGAGGGCTACGTGTACG
 10 ACCCGAGTTCACCGGCCCCACCCTGACCGCCAGCGGCGCCAACAGCCGGATCAAGATCAA
 GGACGGCAGCAACATCCGGAAGATGAACAGCGACGAGACCTTCCTGTACATCGGCTTCGAC
 AGCCAGGACGGCAAGCGGGTGAACGAGATCGAGTTCCTGACCGAGAACCAGAAGATCTTCG
 TGTGCGGCAACAGCATCAGCGTGGAGGTGCTGGAGGCCATCATCGACAAGATCGGCGGC
 (SEQ ID NO: 10)

In some embodiments, MQ1 comprises an amino acid sequence of SEQ ID NO: 11. In some
 embodiments, MQ1 comprises an amino acid sequence of SEQ ID NO: 12. In some embodiments, an
 effector domain described herein comprises SEQ ID NO: 11 or 12, or a sequence with at least 80, 85, 90,
 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7,
 20 6, 5, 4, 3, 2, or 1 positions of difference thereto.

MQ1

MSKVENKTKKLRVFEAFAGIGAQRKALEKVRKDEYEIVGLAEWYVPAIVMYQAIHNNF
 HTKLEYKSVSREEMIDYLENKTLNWSKNPVSNGYWKRKKDDELKIIYNAIKLSEKEGN
 25 IFDIRDLKRTLKNIDLLTYSFPCQDLSQQGIQKGMKRGSGTRSGLLWEIERALDSTEKN
 DLPKYLLMENVGALLHKKNEEELNQWKQKLESLGYQNSIEVLNAADFGSSQARRRVF
 MISTLNEFVELPKGDKPKSIKKVLNKIVSEKDILNNLLKYNLTEFKKTKSNINKASLIGY
 SKFNSEGYVYDPEFTGPTLTASGANSRIKIKDGSNIRKMNSDETFLYIGFDSQDGKRVNEI
 EFLTENQKIFVCGNSISVEVLEAIIIDKIGG (SEQ ID NO: 11)

SKVENKTKKLRVFEAFAGIGAQRKALEKVRKDEYEIVGLAEWYVPAIVMYQAIHNNFHTKLEY
 KSVSREEMIDYLENKTLNWSKNPVSNGYWKRKKDDELKIIYNAIKLSEKEGNIFDIRDLKRTL

KNIDLLTYSFPCQDLSQQGIQKGMKRGSGTRSGLLWEIERALDSTEKNDLPKYLLMENVGALLH
 KKNEEELNQWKQKLESLGYQNSIEVLNAADFGSSQARRRVFMISTLNEFVELPKGDKKPKSIKK
 VLNKIVSEKDILNLLKYNLTEFKKTKSNINKASLIGYSKFNSEGYVVDPEFTGPTLTASGANSRI
 KIKDGSNIRKMNSDETFLYIGFDSQDGKRVNEIEFLTENQKIFVCGNSISVEVLEAIIDKIGG (SEQ
 ID NO: 12)

In some embodiments, MQ1 for use in a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to wildtype MQ1 (e.g., SEQ ID NO: 11 or SEQ ID NO: 12). In some embodiments, an MQ1 variant comprises one or more amino acid substitutions, deletions, or insertions relative to wildtype MQ1. In some embodiments, an MQ1 variant comprises a K297P substitution. In some embodiments, an MQ1 variant comprises a N299C substitution. In some embodiments, an MQ1 variant comprises a E301Y substitution. In some embodiments, an MQ1 variant comprises a Q147L substitution (e.g., and has reduced DNA methyltransferase activity relative to wildtype MQ1). In some embodiments, an MQ1 variant comprises K297P, N299C, and E301Y substitutions (e.g., and has reduced DNA binding affinity relative to wildtype MQ1). In some embodiments, an MQ1 variant comprises Q147L, K297P, N299C, and E301Y substitutions (e.g., and has reduced DNA methyltransferase activity and DNA binding affinity relative to wildtype MQ1). In some embodiments, the site-specific disrupting agent comprises one or more linkers described herein, e.g., connecting a moiety/domain to another moiety/domain. In some embodiments, the site-specific disrupting agent comprises a targeting moiety that is or comprises a CRISPR/Cas molecule, e.g., comprising a CRISPR/Cas protein, e.g., a dCas9 protein. In some embodiments, the site-specific disrupting agent is a fusion protein comprising an effector moiety that is or comprises MQ1 and a DNA-targeting moiety that is or comprises a CRISPR/Cas molecule, e.g., comprising a CRISPR/Cas protein, e.g., a dCas9 protein; e.g., a dCas9m4. In some embodiments, the site-specific disrupting agent comprises an additional moiety described herein. In some embodiments, the site-specific disrupting agent decreases expression of a target gene or a plurality of target genes (e.g., a target gene or a plurality of target gene described herein). In some embodiments, the site-specific disrupting agent may be used in methods of modulating, e.g., decreasing, gene expression, methods of treating a condition, or methods of epigenetically modifying a target gene or transcription control element described herein. In some embodiments, a system comprises two or more site-specific disrupting agents.

In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moiety, wherein the effector moiety is or comprises Krueppel-

associated box (KRAB) domain of Zinc Finger protein 10 e.g., as according to NP_056209.2 or the protein encoded by NM_015394.5 or a functional variant or fragment thereof. In some embodiments, KRAB is a synthetic KRAB construct. In some embodiments, KRAB for use in a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to wildtype KRAB (e.g., e.g., as according to NP_056209.2 or the protein encoded by NM_015394.5). In some embodiments, a KRAB variant comprises one or more amino acid substitutions, deletions, or insertions relative to wildtype KRAB. In some embodiments, a KRAB variant comprises a L37P substitution. In some embodiments, KRAB comprises an amino acid sequence of SEQ ID NO: 13:

10 KRAB
DAKSLTAWSR TLVTFKDV FVDFTREEWKLLDTAQQILYRNVMLENYKNLVSLGYQLTKPDVIL
RLEKGEEPWLVEREIHQETHPDSETAFEIKSSV (SEQ ID NO: 13)

In some embodiments, the KRAB effector moiety is encoded by a nucleotide sequence of SEQ ID NO: 14. In some embodiments, a nucleotide sequence described herein comprises a sequence of SEQ ID NO: 14 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

KRAB
20 GACGCCAAGAGCCTGACCGCCTGGAGCCGGACCCTGGTGACCTTCAAGGACGTGTTCGTGG
ACTTCACCCGGGAGGAGTGGAAGCTGCTGGACACCGCCCAGCAGATCCTGTACCGGAACGT
GATGCTGGAGAACTACAAGAACCTGGTGAGCCTGGGCTACCAGCTGACCAAGCCCGACGTG
ATCCTGCGGCTGGAGAAGGGCGAGGAGCCCTGGCTGGTGAGCGGGAGATCCACCAGGAGA
CCCACCCCGACAGCGAGACCGCCTTCGAGATCAAGAGCAGCGTG (SEQ ID NO: 14)

25 In some embodiments, KRAB for use in a polypeptide or a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to the KRAB sequence of SEQ ID NO: 13. In some embodiments, an KRAB variant comprises one or more amino acid substitutions, deletions, or insertions relative to SEQ ID NO: 13.

30 In some embodiments, the polypeptide or the site-specific disrupting agent is a fusion protein comprising an effector moiety that is or comprises KRAB and a targeting moiety, e.g., a Cas protein. In some embodiments, the polypeptide or the site-specific disrupting agent comprises an additional moiety described herein. In some embodiments, the polypeptide or the site-specific disrupting

agent decreases expression of a target gene or a plurality of target genes. In some embodiments, the polypeptide or the site-specific disrupting agent may be used in methods of modulating, e.g., decreasing, gene expression, methods of treating a condition, or methods of epigenetically modifying a target gene or a plurality of target genes, e.g., a transcription control element described herein.

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In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moiety, wherein the effector moiety is or comprises DNMT3a/3L complex, or a functional variant or fragment thereof. In some embodiments, the DNMT3a/3L complex is a fusion construct. In some embodiments the DNMT3a/3L complex comprises DNMT3A, e.g., human DNMT3A, e.g., as according to NP_072046.2 or the protein encoded by NM_022552.4) or the protein encoded by NM_022552.4 or a functional variant or fragment thereof, e.g., aa 679-912 of human DNMT3A, e.g., as according to NP_072046.2 or the protein encoded by NM_022552.4). In some embodiments the DNMT3a/3L complex comprises human DNMT3L or a functional fragment or variant thereof (e.g., as according to NP_787063.1 or the protein encoded by NM_175867.3 or a functional variant or fragment thereof, e.g., aa 274-386 of human DNMT3L as according to NP_787063.1 or the protein encoded by NM_175867.3). In some embodiments, DNMT3a/3L comprises an amino acid sequence of SEQ ID NO:15. In some embodiments, an effector moiety described herein comprises SEQ ID NO: 15, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

20

DNMT3A/3L (h)

NHDQEFDPPKVYPPVPAEKRPVLSLFDGIATGLLVLKDLGIQVDRIYASEVCEDSITVGMVRH
 QGKIMYVGDVRSVTQKHIEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDA
 RPKEGDDRPFFWLFENVVAMGVSDKRDISRFLSNPVMIDAKEVSAHRARYFWGNLPGMNRP
 LASTVNDKLELQECLEHGRIAKFSKVRTITTRSNSIKQGKDQHFVFMNEKEDILWCTEMERVFG
 FPVHYTDVSNMSRLARQRLGRSWSVPVIRHLFAPLKEYFACVSSGNSNANSRGPSFSSGLVPLS
 LRGSHMNPLEMFETVPVWRRQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVVDVTDTVRKD
 VEEWGPFDLVYGATPPLGHTCDRPPSWYLFQFHRLLQYARPKGPSRPFFWMFVDNLVLNKEDL
 DVASRFLEMEPV TIPDVHGGSLQNAVRVWSNIPAIRSRHWALVSEEELSLLAQNKQSSKLAALKW
 PTKLVKNCFLPLREYFKYFSTELTSSL (SEQ ID NO: 15)

30

In some embodiments, DNMT3a/3L is encoded by a nucleotide sequence of SEQ ID NO: 16. In some embodiments, a nucleic acid described herein comprises a sequence of SEQ ID NO: 16 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

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DNMT3A/3L (h)

AACCACGACCAGGAGTTCGACCCCCCAAGGTGTACCCCCCGTGCCCGCCGAGAAGCGGA
AGCCCATCCGGGTGCTGAGCCTGTTGACGGCATCGCCACCGGCCTGCTGGTGCTGAAGGAC
CTGGGCATCCAGGTGGACCGGTACATCGCCAGCGAGGTGTGCGAGGACAGCATCACCGTGG
10 GCATGGTGCGGCACCAGGGCAAGATCATGTACGTGGGCGACGTGCGGAGCGTGACCCAGAA
GCACATCCAGGAGTGGGGCCCCCTTCGACCTGGTGATCGGCGGCAGCCCCTGCAACGACCTG
AGCATCGTGAACCCCGCCCGGAAGGGCCTGTACGAGGGCACCGGCCGGCTGTTCTTCGAGTT
CTACCGGCTGCTGCACGACGCCCCGGCCCAAGGAGGGCGACGACCGGCCCTTCTTCTGGCTGT
TCGAGAACGTGGTGGCCATGGGCGTGAGCGACAAGCGGGACATCAGCCGGTTCCTGGAGAG
15 CAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCCGCCACCGGGCCCCGGTACTTCTGG
GGCAACCTGCCCGGCATGAACCGGCCCTGGCCAGCACCGTGAACGACAAGCTGGAGCTGC
AGGAGTGCCTGGAGCACGGCCGGATCGCCAAGTTCAGCAAGGTGCGGACCATCACACCCG
GAGCAACAGCATCAAGCAGGGCAAGGACCAGCACTTCCCCGTGTTTCATGAACGAGAAGGAG
GACATCCTGTGGTGCACCGAGATGGAGCGGGTGTTTCGGCTTCCCCGTGCACTACACCGACGT
20 GAGCAACATGAGCCGGCTGGCCCCGGCAGCGGCTGCTGGGCCGGAGCTGGAGCGTGCCCGTG
ATCCGGCACCTGTTTCGCCCCCTGAAGGAGTACTTCGCCTGCGTGAGCAGCGGCAACAGCAA
CGCCAACAGCCGGGGCCCCAGCTTCAGCAGCGGCCTGGTGCCCTGAGCCTGCGGGGCAGC
CACATGAATCCTCTGGAGATGTTTCGAGACAGTGCCCGTGTGGAGAAGGCAACCCGTGAGGG
TGCTGAGCCTCTTCGAGGACATTAAGAAGGAGCTGACCTCTCTGGGCTTTCTGGAATCCGGC
25 AGCGACCCCGGCCAGCTGAAACACGTGGTGGACGTGACCGACACAGTGAGGAAGGACGTGG
AAGAGTGGGGCCCCCTTTGACCTCGTGTATGGAGCCACACCTCCTCTCGGCCACACATGCGAT
AGGCCTCCCAGCTGGTATCTCTTCCAGTTCACAGACTGCTCCAGTACGCCAGACCTAAGCC
CGGCAGCCCCAGACCCTTCTTCTGGATGTTTCGTGGACAATCTGGTGCTGAACAAGGAGGATC
TGGATGTGGCCAGCAGATTTCTGGAGATGGAACCCGTGACAATCCCCGACGTGCATGGCGG
30 CTCTCTGCAGAACGCCGTGAGAGTGTGGTCCAACATCCCCGCCATTAGAAGCAGACACTGGG
CTCTGGTGAGCGAGGAGGAAGTGTCTCTGCTGGCCCAGAATAAGCAGTCCTCCAAGCTGGCC
GCCAAGTGGCCCAACAGCTGGTGAAGAAGTGTCTTCTGCCTCTGAGGGAGTATTTCAAGTA
TTTCAGCACCGAAGTACCAGCAGCCTG (SEQ ID NO: 16)

In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moiety, wherein the effector moiety is or comprises EZH2, e.g., as according to NP-004447.2 or NP_001190176.1 2 or the protein encoded by NM_004456.5 or NM_001203247.2 or a functional variant or fragment thereof. In some embodiments, MQ1 for use in a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to EZH2, e.g., as according to NP-004447.2 or NP_001190176.1 2 or the protein encoded by NM_004456.5 or NM_001203247.2. In some embodiments, an EZH2 variant comprises one or more amino acid substitutions, deletions, or insertions relative to wildtype EZH2. In some embodiments, EZH2 comprises an amino acid sequence of SEQ ID NO: 17:

EZH2

GQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRRADEVKSMFSSNRQKILERTEILNQEWKQRRRI
 QPVHILTSVSSLRGTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSPLQQNFMVEDETVLHNI
 PYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALGQYNDDDDDDGDDPEE
 REEKQKDLEDHRDDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEKYKELTEQQLPGALPPECT
 PNIDGPNAKSVQREQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKRKNTETALDNKPCGPQCYQ
 HLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNSSRPSTPTINVLESKDTDSREAGTETGGEN
 NDKEEEEKKDETSSSSEANSRCQTPMKPNIEPPENVEWSGAEASMFRVLIGTYIDNFCIAIRLI
 GTKTCRQVYEFVRVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHCRKIQLKKDGSSNHVYNYQP
 CDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQCNTKQCPCYLAVRECDPDLCLT
 CGAADHWDSKNVSCKNCSIQRGSKKHLALLAPSDVAGWGIFIKDPVQKNEFISEYCGEISQDEAD
 RRGKVYDKYMCNFLNNDNFVVDATRKGKIRFANHSVNPNCYAKVMMVNGDHRIGIFAKR
 AIQTGEELFFDYRYSQADALKYVGIEREMEIP (SEQ ID NO: 17)

In some embodiments, the EZH2 effector moiety is encoded by a nucleotide sequence of SEQ ID NO: 18. In some embodiments, a nucleotide sequence described herein comprises a sequence of SEQ ID NO: 18 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

EZH2

GGCCAGACCGGCAAGAAGAGCGAGAAGGGCCCCGTGTGCTGGCGGAAGCGGGTGAAGAGC
 GAGTACATGCGGCTGCGGCAGCTGAAGCGGTTCCGGCGGGCCGACGAGGTGAAGAGCATGT
 TCAGCAGCAACCGGCAGAAGATCCTGGAGCGGACCGAGATCCTGAACCAGGAGTGGAAGCA

GCGGCGAATCCAGCCCGTGCACATCCTGACCAGCGTGAGCAGCCTGCGGGGCACCCGGGAG
TGCAGCGTGACCAGCGACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAGACCCTGAACGC
CGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGGTGGAGG
ACGAGACCGTGCTGCACAACATCCCCTACATGGGCGACGAGGTGCTGGACCAGGACGGCAC
5 CTTTCATCGAGGAGCTGATCAAGAACTACGACGGCAAGGTGCACGGCGACCGGGAGTGCGGC
TTCATCAACGACGAGATCTTCGTGGAGCTGGTGAACGCCCTGGGCCAGTACAACGACGACG
ACGACGACGACGACGGCGACGACCCCGAGGAGCGGGAGGAGAAGCAGAAGGACCTGGAGG
ACCACCGGGACGACAAGGAGAGCCGGCCCCCCCCGGAAGTTCCCCAGCGACAAGATCTTCGA
GGCCATCAGCAGCATGTTCCCCGACAAGGGCACCGCCGAGGAGCTGAAGGAGAAGTACAAG
10 GAGCTGACCGAGCAGCAGCTGCCCCGGCGCCCTGCCCCCGAGTGACCCCCAACATCGACG
GCCCCAACGCCAAGAGCGTGACGCGGGAGCAGAGCCTGCACAGCTTCCACACCCTGTTCTG
CCGGCGGTGCTTCAAGTACGACTGCTTCCTGCACCCCTTCCACGCCACCCCCAACACCTACA
AGCGGAAGAACACCGAGACCGCCCTGGACAACAAGCCCTGCGGGCCCCAGTGCTACCAGCA
CCTGGAGGGGCGCCAAGGAGTTCGCCGCGCCCTGACCGCCGAGCGGATCAAGACCCCCCCC
15 AAGCGGCCCCGGCGGCCGGCGGGGCGGGCTGCCCAACAACAGCAGCCGGCCCAGCACCC
CCACCATCAACGTGCTGGAGAGCAAGGACACCGACAGCGACCGGGAGGCGCGCACCGAGA
CCGGCGGCGAGAAACAACGACAAGGAGGAGGAGGAGAAGAAGGACGAGACCAGCAGCAGC
AGCGAGGCCAACAGCCGGTGCCAGACCCCCATCAAGATGAAGCCCAACATCGAGCCCCCG
AGAACGTGGAGTGGAGCGGCGCCGAGGCCAGCATGTTCCGGGTGCTGATCGGCACCTACTA
20 CGACAACCTTCTGCGCCATCGCCCGGCTGATCGGCACCAAGACCTGCCGGCAGGTGTACGAGT
TCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCCCGCCGAGGACGTGGACACCCCCC
CCGGAAGAAGAAGCGGAAGCACCGGCTGTGGGCCGCCACTGCCGGAAGATCCAGCTGAAG
AAGGACGGCAGCAGCAACCACGTGTACAACCTACCAGCCCTGCGACCACCCCCGGCAGCCCT
GCGACAGCAGCTGCCCCCTGCGTGATCGCCCAGAACTTCTGCGAGAAGTTCTGCCAGTGCAGC
25 AGCGAGTGCCAGAACCGGTTCCCCGGCTGCCGGTGCAAGGCCAGTGCAACACCAAGCAGT
GCCCCTGCTACCTGGCCGTGCGGGAGTGCGACCCCCGACCTGTGCCTGACCTGCGGCGCCGCC
GACCACTGGGACAGCAAGAACGTGAGCTGCAAGAACTGCAGCATCCAGCGGGGCAGCAAG
AAGCACCTGCTGCTGGCCCCCAGCGACGTGGCCGGCTGGGGCATCTTCATCAAGGACCCCGT
GCAGAAGAACGAGTTCATCAGCGAGTACTGCGGCGAGATCATCAGCCAGGACGAGGCCGAC
30 CGGCGGGGCAAGGTGTACGACAAGTACATGTGCAGCTTCTGTTCAACCTGAACAACGACTT
CGTGGTGGACGCCACCCGGAAGGGCAACAAGATCCGGTTCGCCAACCACAGCGTGAACCCC
AACTGCTACGCCAAGGTGATGATGGTGAACGGCGACCACCGGATCGGCATCTTCGCCAAGC
GGGCCATCCAGACCGGCGAGGAGCTGTTCTTCGACTACCGGTACAGCCAGGCCGACGCCCT
GAAGTACGTGGGCATCGAGCGGGAGATGGAGATCCCC (SEQ ID NO: 18)

In some embodiments, EZH2 for use in a polypeptide or a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to the EZH2 sequence of SEQ ID NO: 17. In some embodiments, an EZH2 variant comprises one or more amino acid substitutions, deletions, or
 5 insertions relative to SEQ ID NO: 17.

In some embodiments, the polypeptide or the site-specific disrupting agent is a fusion protein comprising an effector moiety that is or comprises EZH2 and a targeting moiety. In some embodiments, the polypeptide or the site-specific disrupting agent comprises an additional moiety described herein. In
 10 some embodiments, the polypeptide or the site-specific disrupting agent decreases expression of a target gene or a plurality of target genes. In some embodiments, the polypeptide or the site-specific disrupting agent may be used in methods of modulating, e.g., decreasing, gene expression, methods of treating a condition, or methods of epigenetically modifying a target gene or a plurality of target genes, e.g., a transcription control element described herein.

In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moiety, wherein the effector moiety is or comprises HDAC8, e.g., as according to NP_001159890 or NP_060956.1 or the protein encoded by NM_001166418 or
 20 NM_018486.3 or a functional variant or fragment thereof. In some embodiments, HDAC8 comprises an amino acid sequence of SEQ ID NO: 19:

HDAC8

EEPEEPADSGQSLVPVYIYSPEYVSMCDLAKIPKRASMVHSLIEAYALHKQMRIVKPKVASMEE
 MATFHTDAYLQHLQKVSQEGDDHDPDSIEYGLGYDCPATEGIFDYAAAIGGATITAAQCLIDGM
 25 CKVAINWSGGWHHAKKDEASGFCYLNDVGLGKGRYYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNP
 KVMTVSLHKFSPGFFPGTGDVSDVGLGKGRYYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNP
 KAVVLQLGADTIAGDPMCSFNMTVPVGIGKCLKYILQWQLATLILGGGGYNLANTARCWTYLTG
 VILGKTLSSSEIPDHEFFTAYGPDYVLEITPSCRPDNRNEPHRIQQILNYIKGNLKHVV (SEQ ID NO:
 19)

In some embodiments, the HDAC8 effector moiety is encoded by a nucleotide sequence of SEQ ID NO: 66. In some embodiments, a nucleotide sequence described herein comprises a sequence of SEQ ID NO: 66 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

HDAC8

GAGGAGCCCCGAGGAGCCCCGCCGATAGCGGACAATCTCTGGTGCCCGTCTACATCTACAGCC
 CCGAATATGTGAGCATGTGTGATTCCCTCGCCAAGATCCCTAAGAGAGCCAGCATGGTGCAT
 5 TCTCTGATCGAGGCCTACGCTCTGCATAAGCAAATGAGGATCGTGAAGCCCAAGGTCGCCAG
 CATGGAAGAGATGGCCACCTTTACACCCGATGCCTACCTCCAACATCTCCAGAAGGTGTCCC
 AAGAGGGCGACGACGACCACCCCGACTCCATTGAGTACGGACTGGGCTATGATTGCCCCGC
 CACCGAGGGCATCTTTGACTATGCCGCCGCTATCGGCGGAGCTACCATCACAGCCGCCCAGT
 GTCTGATTGATGGCATGTGCAAGGTCGCCATCAACTGGTCCGGAGGCTGGCATCATGCCAAG
 10 AAGGATGAGGCCTCCGGCTTCTGTTATCTGAATGACGCCGTGCTGGGCATTCTGAGACTGAG
 GAGGAAATTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGAGTCGAA
 GATGCCTTCAGCTTCACCAGCAAGGTGATGACAGTCTCTCTGCACAAGTTCTCCCCCGGCTTC
 TTTCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGGCAAGGGAAGGTACTACAGCGTGA
 ACGTGCCCATTC AAGACGGCATCCAAGACGAGAAGTACTACCAGATCTGCGAGTCCGTGCTC
 15 AAGGAGGTCTACCAAGCCTTCAATCCTAAGGCTGTCGTGCTCCAAGTGGGAGCTGATACCAT
 TGCTGGCGATCCCATGTGCAGCTTCAATATGACACCCGTCGGAATCGGCAAGTGCCTCAAGT
 ACATCCTCCAGTGGCAGCTCGCCACCCTCATTCTCGGAGGAGGCGGATACAATCTGGCTAAT
 ACCGCCAGATGCTGGACCTATCTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAA
 TCCCCGACCACGAGTTTTTCACCGCTTACGGCCCCGACTACGTGCTGGAGATCACCCCCAGC
 20 TGCAGACCCGATAGAAACGAACCCCATAGAATCCAGCAAATTCTGAACTATATCAAGGGCA
 ACCTCAAGCACGTCTGTG (SEQ ID NO: 66)

In some embodiments, the HDAC8 for use in a polypeptide or a site-specific disrupting agent
 described herein is a variant, e.g., comprising one or more mutations, relative to the HDAC8 sequence of
 25 SEQ ID NO: 19. In some embodiments, an HDAC8 variant comprises one or more amino acid
 substitutions, deletions, or insertions relative to SEQ ID NO: 19.

In some embodiments, the polypeptide or the site-specific disrupting agent is a fusion protein
 comprising an effector moiety that is or comprises HDAC8 and a targeting moiety. In some
 30 embodiments, the polypeptide or the site-specific disrupting agent comprises an additional moiety
 described herein. In some embodiments, the polypeptide or the site-specific disrupting agent decreases
 expression of a target gene or a plurality of target genes. In some embodiments, the polypeptide or the
 site-specific disrupting agent may be used in methods of modulating, e.g., decreasing, gene expression,

methods of treating a condition, or methods of epigenetically modifying a target gene or a plurality of target genes, e.g., a transcription control element described herein.

In some embodiments, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moiety, wherein the effector moiety is or comprises G9A e.g., as according to NP_001350618.1 or the protein encoded by NM_001363689.1 or a functional variant or fragment thereof, e.g., aa967-1250 of comprises G9A e.g., as according to NP_001350618.1 or the protein encoded by NM_001363689.1. In some embodiments, G9A comprises an amino acid sequence of SEQ ID NO: 67:

G9A

GNRAIRTEKIIICRDVARGYENVPIPCVNGVDGEPEDYKYISENCETSTMNIDRNITHLQHCTCV
DDCSSSNCLCGQLSIRCWYDKDGRLLQEFNKIEPPLIFECNQACSCWRNCKNRVVQSGIKVRLQL
YRTAKMGWGVRLALQTIPQGTFFICEYVGELISDAEADVREDDSYLFDLDNKDGEVYCIDARYYG
NISRFINHLCDPNIIPVRVFMHLHQDLRFPIAFFSSRDIRTGEELGFDYGDRFWDIKSKYFTCQCGS
EKCKHSAEAIALEQSRLARLD (SEQ ID NO: 67)

In some embodiments, the G9A effector moiety is encoded by a nucleotide sequence of SEQ ID NO: 68. In some embodiments, a nucleotide sequence described herein comprises a sequence of SEQ ID NO: 68 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

G9A

GGAAATAGGGCTATCAGAACCGAGAAGATCATCTGTAGGGACGTGGCTAGAGGCTACGAGA
ACGTGCCCATTCTTTCGTGAATGGCGTGGATGGCGAACCTTGCCCCGAGGACTACAAATAC
ATCTCCGAGAACTGCGAAACCAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGC
ACTGCACATGTGTGGATGACTGCTCCTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGA
TGCTGGTACGACAAGGACGGCAGACTGCTGCAAGAGTTCAACAAGATCGAACCCCTCTCA
TCTTCGAGTGTAACCAAGCTTGCAGCTGCTGGAGAACTGCAAGAATAGAGTGGTCCAGAG
CGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCAAGATGGGATGGGGAGTGAGGGCT
CTGCAAACCATTCCTCAAGGCACCTTCATCTGCGAATACGTGGGCGAACTGATCTCCGACGC
CGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTTCGATCTGGACAATAAGGACGGCGAG

GTGTACTGCATCGACGCTAGATATTACGGCAACATCTCTAGATTCATCAACCACCTCTGCGA
 TCCCAACATCATTTCCCGTGAGGGTGTTTCATGCTGCACCAAGATCTGAGGTTCCCTAGAATCG
 CCTTCTTCAGCTCTAGAGACATCAGAACCGGCGAGGAGCTGGGCTTCGATTACGGCGATAGA
 TTCTGGGACATCAAGTCCAAGTACTTCACATGCCAGTGCGGCAGCGAGAAGTGTAAGCACA
 5 GCGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGAT (SEQ ID NO: 68)

In some embodiments, G9A for use in a polypeptide or a site-specific disrupting agent described herein is a variant, e.g., comprising one or more mutations, relative to the G9A sequence of SEQ ID NO: 67. In some embodiments, an G9A variant comprises one or more amino acid substitutions, deletions, or
 10 insertions relative to SEQ ID NO: 67.

In some embodiments, the polypeptide or the site-specific disrupting agent is a fusion protein comprising an effector moiety that is or comprises G9A and a targeting moiety. In some embodiments, the polypeptide or the site-specific disrupting agent comprises an additional moiety described herein. In
 15 some embodiments, the polypeptide or the site-specific disrupting agent decreases expression of a target gene or a plurality of target genes. In some embodiments, the polypeptide or the site-specific disrupting agent may be used in methods of modulating, e.g., decreasing, gene expression, methods of treating a condition, or methods of epigenetically modifying a target gene or a plurality of target genes, e.g., a transcription control element described herein.

20 *Systems*

Systems of the present disclosure may comprise two or more site-specific disrupting agents. In some embodiments, a site-specific disrupting agent system comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more, site-specific disrupting agents (and optionally no more than 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3,
 25 or 2). In some embodiments, system targets two or more different sequences (e.g., a 1st and 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th, 10th, 11th, 12th, and/or further DNA sequence, and optionally no more than a 20th, 19th, 18th, 17th, 16th, 15th, 14th, 13th, 12th, 11th, 10th, 9th, 8th, 6th, 5th, 4th, 3rd, or 2nd sequence). In some embodiments, system comprises a plurality of site-specific disrupting agents, wherein each member of the plurality of site-specific disrupting agents does not detectably bind, e.g., does not bind, to another
 30 member of the plurality of site-specific disrupting agents. In some embodiments, system comprises a first site-specific disrupting agent and a second site-specific disrupting agent, wherein the first site-specific disrupting agent does not detectably bind, e.g., does not bind, to the second site-specific disrupting agent.

In some embodiments, a system of the present disclosure comprises two or more site-specific disrupting agents, wherein the site-specific disrupting agents are present together in a composition, pharmaceutical composition, or mixture. In some embodiments, a system of the present disclosure comprises two or more site-specific disrupting agents, wherein one or more site-specific disrupting agents is not admixed with at least one other site-specific disrupting agent. In some embodiments, a system may comprise a first site-specific disrupting agent and a second site-specific disrupting agent, wherein the presence of the first site-specific disrupting agent in the nucleus of a cell does not overlap with the presence of the second site-specific disrupting agent in the nucleus of the same cell, wherein the system achieves a decrease in expression of a plurality of genes via the non-overlapping presences of the first and second site-specific disrupting agents. In some embodiments, the first site-specific disrupting agent and a second site-specific disrupting agent may act simultaneously or sequentially.

In some embodiments, the site-specific disrupting agents of a system each comprise a different targeting moiety (e.g., the first, second, third, or further site-specific disrupting agents each comprise different targeting moieties from one another). For example, a system may comprise a first site-specific disrupting agent and a second site-specific disrupting agent wherein the first site-specific disrupting agent comprises a first targeting moiety (e.g., a Cas9 domain, TAL effector domain, or Zn Finger domain), and the second site-specific disrupting agent comprises a second targeting moiety (e.g., a Cas9 domain, TAL effector domain, or Zn Finger domain) different from the first targeting moiety. In some embodiments, different can mean comprising distinct types of targeting moiety, e.g., the first targeting moiety comprises a Cas9 domain, and the second DNA-targeting moiety comprises a Zn finger domain. In other embodiments, different can mean comprising distinct variants of the same type of targeting moiety, e.g., the first targeting moiety comprises a first Cas9 domain (e.g., from a first species) and the second targeting moiety comprises a second Cas9 domain (e.g., from a second species).

In an embodiment, when a system comprises two or more targeting moieties of the same type, e.g., two or more Cas9 or Zn finger domains, the targeting moieties specifically bind two or more different sequences. For example, in a system comprising two or more Cas9 domains, the two or more Cas9 domains may be chosen or altered such that they only appreciably bind the gRNA corresponding to their target sequence (e.g., and do not appreciably bind the gRNA corresponding to the target of another Cas9 domain). In a further example, in a system comprising two or more effector moieties, the two or more effector moieties may be chosen or altered such that they only appreciably bind to their target sequence (e.g., and do not appreciably bind the target sequence of another effector moiety).

In some embodiments, a system comprises three or more site-specific disrupting agents and two or more site-specific disrupting agents comprise the same targeting moiety. For example, a system may comprise three site-specific disrupting agents, wherein the first and second site-specific disrupting agents both comprise a first targeting moiety and the third site-specific disrupting agent comprises a second different targeting moiety. For a further example, a system may comprise four site-specific disrupting agents, wherein the first and second site-specific disrupting agents both comprise a first targeting moiety and the third and fourth site-specific disrupting agents comprises a second different targeting moiety. For a further example, a system may comprise five site-specific disrupting agents, wherein the first and second site-specific disrupting agents both comprise a first targeting moiety, the third and fourth site-specific disrupting agents both comprise a second different targeting moiety, and the fifth site-specific disrupting agent comprises a third different targeting moiety. As described above, different can mean comprising different types of -targeting moieties or comprising distinct variants of the same type of targeting moiety.

In some embodiments, the site-specific disrupting agents of a system each bind to a different DNA sequence (e.g., the first, second, third, or further site-specific disrupting agents each bind DNA sequences that are different from one another). For example, a system may comprise a first site-specific disrupting agent and a second site-specific disrupting agent wherein the first site-specific disrupting agent binds to a first DNA sequence, and the second site-specific disrupting agent binds to a second DNA sequence. In some embodiments involving different DNA sequences, there is at least one position that is not identical between the DNA sequence bound by one site-specific disrupting agent and the DNA sequence bound by another site-specific disrupting agent, or there is at least one position present in the DNA sequence bound by one site-specific disrupting agent that is not present in the DNA sequence bound by another site-specific disrupting agent.

In some embodiments, the first DNA sequence may be situated on a first genomic DNA strand and the second DNA sequence may be situated on a second genomic DNA strand. In some embodiments, the first DNA sequence may be situated on the same genomic DNA strand as the second DNA sequence.

In some embodiments, a system comprises three or more site-specific disrupting agents and two or more site-specific disrupting agents bind the same DNA sequence. For example, a system may comprise three site-specific disrupting agents, wherein the first and site-specific disrupting agents both bind a first DNA sequence, and the third site-specific disrupting agent binds a second different DNA sequence. For a further example, a system may comprise four site-specific disrupting agents, wherein the

first and second site-specific disrupting agents both bind a first DNA sequence and the third and fourth site-specific disrupting agents both bind a second DNA sequence. For a further example, a system may comprise five site-specific disrupting agents, wherein the first and second site-specific disrupting agents both bind a first DNA sequence, the third and fourth site-specific disrupting agents both bind a second DNA sequence, and the fifth site-specific disrupting agent binds a third DNA sequence. As described above, different can mean that there is at least one position that is not identical between the DNA sequence bound by one site-specific disrupting agent and the DNA sequence bound by another site-specific disrupting agent, or that there is at least one position present in the DNA sequence bound by one site-specific disrupting agent that is not present in the DNA sequence bound by another site-specific disrupting agent.

In some embodiments, a system comprises two or more (e.g., two) site-specific disrupting agents and a plurality (e.g., two) of the site-specific disrupting agents comprise targeting moieties that bind to different DNA sequences. In such embodiments, a first targeting moiety may bind to a first DNA sequence and a second DNA-targeting moiety may bind to a second DNA sequence, wherein the first and the second DNA sequences are different and do not overlap. In some such embodiments, the first DNA sequence is separated from the second DNA sequence by at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 base pairs (and optionally, no more than 500, 400, 300, 200, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, or 50 base pairs). In some such embodiments, the first DNA sequence is separated from the second DNA sequence by no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 base pairs (and optionally, no base pairs, e.g., the first and second sequence are directly adjacent one another).

In some embodiments, the site-specific disrupting agents of a system each comprise a different effector moiety (e.g., the first, second, third, or further site-specific disrupting agents each comprise a different effector moiety from one another). For example, a system may comprise a first site-specific disrupting agent and a second site-specific disrupting agent wherein the first site-specific disrupting agent comprises a first effector moiety, and the second site-specific disrupting agent comprises a second effector moiety different from the first effector moiety. In some embodiments, the different effector moieties comprise distinct types of effector moiety. In other embodiments, the different effector moieties comprise distinct variants of the same type of effector moiety.

Targeting Moieties

Targeting moieties may specifically bind a DNA sequence, e.g., a DNA sequence associated with a target plurality of genes, e.g., an anchor sequence of an ASMC comprising the target plurality of genes. Any molecule or compound that specifically binds a DNA sequence may be used as a targeting moiety. In some embodiments, a targeting moiety comprises a nucleic acid, e.g., comprising a sequence that is complementary to an anchor sequence, e.g., an anchor sequence of an ASMC comprising the target plurality of genes. In some embodiments, the nucleic acid is an oligonucleotide that physically/sterically blocks binding a genomic complex component (e.g., a nucleating polypeptide, e.g., CTCF) to an anchor sequence. In some embodiments, the nucleic acid comprises a guide RNA (gRNA), e.g., compatible with a CRISPR/Cas molecule. In some embodiments, a targeting moiety comprises a CRISPR/Cas molecule, a TAL effector molecule, a Zn finger molecule, a tetR domain, a meganuclease, a peptide nucleic acid (PNA) or a nucleic acid molecule.

In some embodiments, a targeting moiety binds to its target sequence with a K_D of less than or equal to 500, 450, 400, 350, 300, 250, 200, 150, 100, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.005, 0.002, or 0.001 nM (and optionally, a K_D of at least 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01, 0.005, 0.002, or 0.001 nM). In some embodiments, a targeting moiety binds to its target sequence with a K_D of 0.001 nM to 500 nM, e.g., 0.1 nM to 5 nM, e.g., about 0.5 nM. In some embodiments, a targeting moiety binds to a non-target sequence with a K_D of at least 500, 600, 700, 800, 900, 1000, 2000, 5000, 10,000, or 100,000 nM (and optionally, does not appreciably bind to a non-target sequence). In some embodiments, a targeting moiety does not bind to a non-target sequence.

In some embodiments, a targeting moiety comprises a nucleic acid comprising a sequence selected from Table 7 or a sequence having at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

Table 7: Exemplary sequences

Name	Sequence	SEQ ID NO.
GD-28481	AGCCCCACCTTGTGGTCAGA	21
GD-28482	AGTGCTGCCTTCTGACCACA	22
GD-28483	GCTGCCTTCTGACCACAAGG	23

GD-28484	CCAGTATAAGCCCCACCTTG	24
GD-28485	CTGCCTGTCCCATAAGGAGG	25
GD-28486	GCACTGCCTGTCCCATAAGG	26
GD-28487	GGTCCTCCTCCTTATGGGAC	27
GD-28488	GCCTTGTTTTTCGGCTCTAGA	28
GD-28489	GCCATCTAGAGCCGAAAACA	29
GD-29251	CCAATGAAGATGAAACTGGG	30
GD-29252	AACGTGCTTGCCTAAGATTC	31
GD-29253	AGCCCTTAATCATATCTAGT	32
GD-29254	CAGAGCTTAAGACCTGTACT	33
GD-29255	GCCCACCTTGACCTTCACAA	34

In some embodiments, a targeting moiety comprises a nucleic acid comprising a sequence selected from Table 6 or a sequence having at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

5

Table 6: Exemplary guide sequence

Guide	Sequence	Strand	Genomic Coordinate
GD-30178	GTTACTGCGTAATTACCAGG (SEQ ID NO: 35)	+	GRCh37: chr4:74528428- 74528450
GD-30179	TATTACATCCTACCTATAAG (SEQ ID NO: 36)	+	GRCh37: chr4:74528567- 74528589
GD-30180	TGGGCTCTGGACTTAGATCG (SEQ ID NO: 37)	+	GRCh37: chr4:74528609- 74528631
GD-30181	TAAGTGGGCTATGTATACAC (SEQ ID NO: 38)	+	GRCh37: chr4:74789132- 74789154
GD-30182	TTTCTAAGTCTGTCACAAGG (SEQ ID NO: 39)	-	GRCh37: chr4:74789250- 74789272
GD-30183	AAAGTAATATGATCTAGGAA (SEQ ID NO: 40)	-	GRCh37: chr4:74789312- 74789334
GD-30184	GTTCGAGCGGCTGTGCGAGG (SEQ ID NO: 41)	+	GRCh37: chr4:74964853- 74964875

GD-30185	GCTCTGTGGCTCTCCGAGAA (SEQ ID NO: 42)	+	GRCh37: chr4:74964906- 74964928
GD-30186	GTGTGTGTGTTTCAACGTAG (SEQ ID NO: 43)	+	GRCh37: chr4:74965538- 74965560
GD-30187	GGAAGTCACTGGGAGCTGCG (SEQ ID NO: 44)	+	GRCh37: chr4:74965737- 74965759
GD-30200	GGCCACGGGTGTGTTCCAG (SEQ ID NO: 45)	-	GRCh37: chr4:75000031- 75000053
GD-30201	ATGGCCATTTGCAAAAGTCA (SEQ ID NO: 46)	+	GRCh37: chr4:75000115- 75000137
GD-30202	CCAAACTAGACAGATAAAGC (SEQ ID NO: 47)	+	GRCh37: chr4:75000231- 75000253
GD-30203	CCAGCATGACTCTAGCATGC (SEQ ID NO: 48)	-	GRCh37: chr4:74975146- 74975168
GD-30204	TGGCCAAGGTCTGATATGCA (SEQ ID NO: 49)	+	GRCh37: chr4:74975369- 74975391
GD-30205	TCATGAGTCCCAGAACATGT (SEQ ID NO: 50)	-	GRCh37: chr4:74976318- 74976340
GD-30206	GCGAAAGAAGTAGTAGCTAA (SEQ ID NO: 51)	-	GRCh37: chr4:74570348- 74570370
GD-30207	GACTAAGACTGGCAAATCTG (SEQ ID NO: 52)	-	GRCh37: chr4:74570503- 74570525
GD-30208	GACTAAGAGGAGCCGACATG (SEQ ID NO: 53)	+	GRCh37: chr4:74570526- 74570548
GD-30238	GAAAAACGGGTGTTGTGACG (SEQ ID NO: 54)	+	GRCh38: chr5:90661492- 90661514
GD-30239	TTTGTGAACTAAGGATTCTG (SEQ ID NO: 55)	-	GRCh38: chr5:90661646- 90661668
GD-30240	GTCCGTGTAGAGTTACCATG (SEQ ID NO: 56)	+	GRCh38: chr5:90661744- 90661766
GD-30241	GATGTATTCACAAGAGGACT	+	GRCh38: chr5:90785610-

	(SEQ ID NO: 57)		90785632
GD-30242	AATTACTACCTCATAGCTAG (SEQ ID NO: 58)	-	GRCm38: chr5:90909047- 90909069
GD-30592	GAAGGTAGAAATCCGCCACT (SEQ ID NO: 59)		GRCm38: chr5:90785724- 90785746
GD-30593	GAAACGCCGAGGTAATCAT (SEQ ID NO: 60)		GRCm38: chr5:90788137- 90788159
GD-30594	CAACTAAATTTCTAGCCCT (SEQ ID NO: 61)		GRCm38: chr5:90908926- 90908948
GD-28044	GACTCCAGTCTTTCTAGAAGA (SEQ ID NO: 62)		GRCm38: chr6:113076028- 113076047

In some embodiments, a targeting moiety comprises a nucleic acid comprising a sequence that is complementary to the sequence of an anchor sequence, e.g., of an ASMC comprising the target plurality
5 of genes, or having no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 positions of non-complementarity thereto.

In some embodiments, a targeting moiety comprises a nucleic acid comprising a sequence that at least partially overlapping with the region having genomic coordinates chr4:74595464-74595486, chr4:74595457-74595479, chr4:74595460-74595482, chr4:74595472-74595494, chr4:75000088-
10 75000110, chr4:75000091-75000113, chr4:75000085-75000107, chr4:75000157-75000179, chr4:75000156-75000178, chr4:74595215-74595237, chr4:74595370-74595392, chr4:74595560-74595582, chr4:74595642-74595664, chr4:74595787-74595809, chr4:74528428-74528450, chr4:74528567-74528589, chr4:74528609-74528631, chr4:74789132-74789154, chr4:74789250-74789272, chr4:74789312-74789334, chr4:74964853-74964875, chr4:74964906-74964928,
15 chr4:74965538-74965560, chr4:74965737-74965759, chr4:75000031-75000053, chr4:75000115-75000137, chr4:75000231-75000253, chr4:74975146-74975168, chr4:74975369-74975391, chr4:74976318-74976340, chr4:74570348-74570370, chr4:74570503-74570525, chr4:74570526-74570548, chr5:90785724-90785746, chr5:90788137-90788159, chr5:90908926-90908948, chr5:90661492-90661514, chr5:90661646-90661668, chr5:90661744-90661766, chr5:90785610-
20 90785632, chr5:90909047-90909069, or chr6:113076028-113076047 or a sequence that is within 5, 10, 15, 20, 30, 40, or 50 nucleotides of said region, or comprises a sequence having at least 70, 75, 80, 85, 90,

91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to a sequence at said genomic region, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

In some embodiments, a targeting moiety binds to a sequence at genomic positions
 5 chr4:74595464-74595486, chr4:74595457-74595479, chr4:74595460-74595482, chr4:74595472-
 74595494, chr4:75000088-75000110, chr4:75000091-75000113, chr4:75000085-75000107,
 chr4:75000157-75000179, chr4:75000156-75000178, chr4:74595215-74595237, chr4:74595370-
 74595392, chr4:74595560-74595582, chr4:74595642-74595664, chr4:74595787-74595809,
 chr4:74528428-74528450, chr4:74528567-74528589, chr4:74528609-74528631, chr4:74789132-
 10 74789154, chr4:74789250-74789272, chr4:74789312-74789334, chr4:74964853-74964875,
 chr4:74964906-74964928, chr4:74965538-74965560, chr4:74965737-74965759, chr4:75000031-
 75000053, chr4:75000115-75000137, chr4:75000231-75000253, chr4:74975146-74975168,
 chr4:74975369-74975391, chr4:74976318-74976340, chr4:74570348-74570370, chr4:74570503-
 74570525, chr4:74570526-74570548, chr5:90661492-90661514, chr5:90661646-90661668,
 15 chr5:90661744-90661766, chr5:90785610-90785632, chr5:90909047-90909069, chr5:90785724-
 90785746, chr5:90788137-90788159, chr5:90908926-90908948, or chr6:113076028-113076047.

In some embodiments, a targeting moiety binds to an anchor sequence or to a site proximal to an anchor sequence, e.g., an anchor sequence that is part of an ASMC comprising, wholly or in part, a target
 20 plurality of genes.

In some embodiments, a targeting moiety comprises a CRISPR/Cas molecule. In some
 embodiments, an effector moiety comprises a CRISPR/Cas molecule. A CRISPR/Cas molecule comprises
 a protein involved in the clustered regulatory interspaced short palindromic repeat (CRISPR) system, e.g.,
 25 a Cas protein, and optionally a guide RNA, e.g., single guide RNA (sgRNA).

CRISPR systems are adaptive defense systems originally discovered in bacteria and archaea.
 CRISPR systems use RNA-guided nucleases termed CRISPR-associated or “Cas” endonucleases (e. g.,
 Cas9 or Cpf1) to cleave foreign DNA. For example, in a typical CRISPR/Cas system, an endonuclease is
 30 directed to a target nucleotide sequence (e. g., a site in the genome that is to be sequence-edited) by
 sequence-specific, non-coding “guide RNAs” that target single- or double-stranded DNA sequences.
 Three classes (I-III) of CRISPR systems have been identified. The class II CRISPR systems use a single
 Cas endonuclease (rather than multiple Cas proteins). One class II CRISPR system includes a type II Cas
 endonuclease such as Cas9, a CRISPR RNA (“crRNA”), and a trans-activating crRNA (“tracrRNA”).

The crRNA contains a “guide RNA”, typically about 20-nucleotide RNA sequence that corresponds to a target DNA sequence. crRNA also contains a region that binds to the tracrRNA to form a partially double-stranded structure which is cleaved by RNase III, resulting in a crRNA/tracrRNA hybrid. A crRNA/tracrRNA hybrid then directs Cas9 endonuclease to recognize and cleave a target DNA sequence.

5 A target DNA sequence must generally be adjacent to a “protospacer adjacent motif” (“PAM”) that is specific for a given Cas endonuclease; however, PAM sequences appear throughout a given genome. CRISPR endonucleases identified from various prokaryotic species have unique PAM sequence requirements; examples of PAM sequences include 5’-NGG (*Streptococcus pyogenes*), 5’-NNAGAA (*Streptococcus thermophilus* CRISPR1), 5’-NGGNG (*Streptococcus thermophilus* CRISPR3), and 5’-NNNGATT (*Neisseria meningitidis*). Some endonucleases, e.g., Cas9 endonucleases, are associated with G-rich PAM sites, e. g., 5’-NGG (e.g., TGG, e.g., CGG, e.g., AGG), and perform blunt-end cleaving of the target DNA at a location 3 nucleotides upstream from (5’ from) the PAM site. Another class II CRISPR system includes the type V endonuclease Cpf1, which is smaller than Cas9; examples include AsCpf1 (from *Acidaminococcus* sp.) and LbCpf1 (from *Lachnospiraceae* sp.). Cpf1-associated CRISPR arrays are processed into mature crRNAs without the requirement of a tracrRNA; in other words, a Cpf1 system requires only Cpf1 nuclease and a crRNA to cleave a target DNA sequence. Cpf1 endonucleases, are associated with T-rich PAM sites, e. g., 5’-TTN. Cpf1 can also recognize a 5’-CTA PAM motif. Cpf1 cleaves a target DNA by introducing an offset or staggered double-strand break with a 4- or 5-nucleotide 5’ overhang, for example, cleaving a target DNA with a 5-nucleotide offset or staggered cut located 18 nucleotides downstream from (3’ from) from a PAM site on the coding strand and 23 nucleotides downstream from the PAM site on the complimentary strand; the 5-nucleotide overhang that results from such offset cleavage allows more precise genome editing by DNA insertion by homologous recombination than by insertion at blunt-end cleaved DNA. See, e.g., Zetsche et al. (2015) *Cell*, 163:759 – 771.

25 A variety of CRISPR associated (Cas) genes or proteins can be used in the technologies provided by the present disclosure and the choice of Cas protein will depend upon the particular conditions of the method. Specific examples of Cas proteins include class II systems including Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cpf1, C2C1, or C2C3. In some embodiments, a Cas protein, e.g., a Cas9 protein, may be from any of a variety of prokaryotic species. In some embodiments a particular Cas protein, e.g., a particular Cas9 protein, is selected to recognize a particular protospacer-adjacent motif (PAM) sequence. In some embodiments, a targeting moiety includes a sequence targeting polypeptide, such as a Cas protein, e.g., Cas9. In certain embodiments a Cas protein, e.g., a Cas9 protein, may be obtained from a bacteria or archaea or synthesized using known methods. In certain embodiments, a Cas

protein may be from a gram positive bacteria or a gram negative bacteria. In certain embodiments, a Cas protein may be from a Streptococcus (e.g., a *S. pyogenes*, or a *S. thermophilus*), a Francisella (e.g., an *F. novicida*), a Staphylococcus (e.g., an *S. aureus*), an Acidaminococcus (e.g., an *Acidaminococcus* sp. BV3L6), a Neisseria (e.g., an *N. meningitidis*), a Cryptococcus, a Corynebacterium, a Haemophilus, a Eubacterium, a Pasteurella, a Prevotella, a Veillonella, or a Marinobacter.

In some embodiments, a Cas protein requires a protospacer adjacent motif (PAM) to be present in or adjacent to a target DNA sequence for the Cas protein to bind and/or function. In some embodiments, the PAM is or comprises, from 5' to 3', NGG, YG, NNGRRT, NNNRRT, NGA, TYCV, TATV, NTTN, or NNNGATT, where N stands for any nucleotide, Y stands for C or T, R stands for A or G, and V stands for A or C or G. In some embodiments, a Cas protein is a protein listed in Table 1. In some embodiments, a Cas protein comprises one or more mutations altering its PAM. In some embodiments, a Cas protein comprises E1369R, E1449H, and R1556A mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises E782K, N968K, and R1015H mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises D1135V, R1335Q, and T1337R mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises S542R and K607R mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises S542R, K548V, and N552R mutations or analogous substitutions to the amino acids corresponding to said positions.

Table 1

Name	Enzyme	Species	# of AAs	PAM	Mutations to alter PAM recognition	Mutations to make catalytically dead
<i>FnCas9</i>	Cas9	<i>Francisella novicida</i>	1629	5'-NGG-3'	Wt	D11A/H969A/N995A
<i>FnCas9 RHA</i>	Cas9	<i>Francisella novicida</i>	1629	5'-YG-3'	E1369R/E1449H/R1556A	D11A/H969A/N995A
<i>SaCas9</i>	Cas9	<i>Staphylococcus aureus</i>	1053	5'-NNGRRT-3'	Wt	D10A/H557A
<i>SaCas9 KKH</i>	Cas9	<i>Staphylococcus aureus</i>	1053	5'-NNNRRT	E782K/N968K/R1015H	D10A/H557A

				-3'		
<i>SpCas9</i>	Cas9	<i>Streptococcus pyogenes</i>	1368	5'-NGG-3'	Wt	D10A/D839A/H840A/N863A
<i>SpCas9 VQR</i>	Cas9	<i>Streptococcus pyogenes</i>	1368	5'-NGA-3'	D1135V/R1335Q/T1337R	D10A/D839A/H840A/N863A
<i>AsCpf1 RR</i>	Cpf1	<i>Acidaminococcus sp. BV3L6</i>	1307	5'-TYCV-3'	S542R/K607R	E993A
<i>AsCpf1 RVR</i>	Cpf1	<i>Acidaminococcus sp. BV3L6</i>	1307	5'-TATV-3'	S542R/K548V/N552R	E993A
<i>FnCpf1</i>	Cpf1	<i>Francisella novicida</i>	1300	5'-NTTN-3'	Wt	D917A/E1006A/D1255A
<i>NmCas9</i>	Cas9	<i>Neisseria meningitidis</i>	1082	5'-NNNGATT-3'	Wt	D16A/D587A/H588A/N611A

In some embodiments, the Cas protein is catalytically active and cuts one or both strands of the target DNA site. In some embodiments, cutting the target DNA site is followed by formation of an alteration, e.g., an insertion or deletion, e.g., by the cellular repair machinery.

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In some embodiments, the Cas protein is modified to deactivate the nuclease, e.g., nuclease-deficient Cas9. Whereas wild-type Cas9 generates double-strand breaks (DSBs) at specific DNA sequences targeted by a gRNA, a number of CRISPR endonucleases having modified functionalities are available, for example: a “nickase” version of Cas9 generates only a single-strand break; a catalytically inactive Cas9 (“dCas9”) does not cut target DNA. In some embodiments, dCas9 binding to a DNA sequence may interfere with transcription at that site by steric hindrance. In some embodiments, dCas9 binding to an anchor sequence may interfere with (e.g., decrease or prevent) genomic complex (e.g., ASMC) formation and/or maintenance. In some embodiments, a targeting moiety comprises a catalytically inactive Cas9, e.g., dCas9, e.g., Cas9m4. Many catalytically inactive Cas9 proteins are known in the art. In some embodiments, dCas9 comprises mutations in each endonuclease domain of the Cas protein, e.g., D10A and H840A mutations.

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In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D11A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a H969A mutation or an

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analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a N995A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises D11A, H969A, and N995A mutations or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D10A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a H557A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises D10A and H557A mutations or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D839A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a H840A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a N863A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises D10A, D839A, H840A, and N863A mutations or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a E993A mutation or an analogous substitution to the amino acid corresponding to said position.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D917A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a E1006A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D1255A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises D917A, E1006A, and D1255A mutations or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D16A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D587A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a H588A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a N611A mutation or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises D16A, D587A, H588A, and N611A mutations or analogous substitutions to the amino acids corresponding to said positions.

In some aspects, a system described herein comprises, or a method described herein comprises the use of, a site-specific disrupting agent or a polypeptide comprising one or more (e.g., one) targeting moiety and one or more effector moieties (e.g., one or two effector moieties), wherein the one or more targeting moiety is or comprises a CRISPR/Cas molecule comprising a Cas protein, e.g., catalytically inactive Cas9 protein, e.g., sadCas9, dCas9, e.g., dCas9m4, or a functional variant or fragment thereof. In some embodiments, dCas9 comprises an amino acid sequence of SEQ ID NO: 5, 6, or 7:

Cas9

DKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
 TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
 YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTY
 NQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDL
 AEDAKLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIK
 RYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEE
 LLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPL
 ARGNSRFAWMTRKSEETITPWNFEEVVDKGASQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT
 VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGV
 EDRFNASLGTYHDLLKHKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK
 QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVS
 GQGDSLHEHIANLAGSPAIKKILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSR
 ERMKRIEIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIV
 PQSFLKDDSIDNKVLTRSDKNRGKSDNPSEEVVKKMKNYWRQLLNALITQRKFDNLTKAER
 GGLSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDF

QFYKVINNYHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKAT
 AKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTE
 VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKEL
 LGITIMERSSEKPNIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALP
 5 SKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAY
 NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYETRID
 LSQGGD (SEQ ID NO: 5)

dCas9 (Cas9m4)

10 DKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKR
 TARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEK
 YPTIYHLRKKLVDSTDKADRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLF
 EENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDA
 KLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDE
 15 HHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVK
 LNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYVVGPLARGN
 SRFAWMTRKSEETITPWNFEVVDKGASQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNE
 LTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRF
 NASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLFEDREMIEERLKYAHLFDDKVMKQLK
 20 RRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQG
 DSLHEHIANLAGSPAIIKQILQTVKVVDLVKVMGRHKPENIVIEARENQTTQKGQKNSRERM
 KRIEELGELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVAAIVPQSF
 LKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLS
 ELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYK
 25 VREINNYHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKATAKYF
 FYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQT
 GGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI
 TIMERSSEKPNIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
 VNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVL SAYNKH
 30 RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYETRIDLSQ
 LGGD (SEQ ID NO: 6)

Sa-dCas9

AKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRRHRIQ
RVKLLFDYNLLTDHSELGINPYEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTG
NELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYVKEAKQLLKVQKAYHQL
DQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYN
5 ALNDLNNLVITRDENEKLEYEKFQIENVFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEF
TNLKVYHDIKDITARKEIENAELLDQIAKILTIYQSSEDIQEELTNLNSLTQEEIEQISNLKGYTGT
HNLSLKAINLILDELWHTNDNQIAIFNRLKLVKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIK
VINAIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAKYLIEKIKLH
DMQEGKCLYSLEAIPLEDLLNPNFYEVDAIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYL
10 SSSDSKISYETFKKHILNLA KGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATRGLMN
LLRSYFRVNNLDVVKVSINGGFTSFLRRKWKFKKERNKGYKHAEDALIIANADFIKWKLD
KAKKVMENQMFEEKQAESMPEIETE QEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRKLINDT
LYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGD
KNPLYKYEEETGNYLTKYSKDNNGPVIKKIKYYGNKLNAHLDDITDDYPNSRNKVVKLSLKPYRF
15 DVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLLKISNQAEFIASFYKNDLIKINGEL
YRVIGVNNDLLNRIEVMIDITYREYLENMNDKRPPHIIKTIASKTQSIKKYSTDILGNLYEVKSK
KHPQIIKKG (SEQ ID NO: 7)

In some embodiments, the dCas9 is encoded by a nucleic acid sequence of SEQ ID NO: 8, or 9:

20 GACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCA
CCGACGAGTACAAGGTGCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAG
CATCAAGAAGAACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCCGAGGCCACC
CGGCTGAAGCGGACCGCCCGGCGGCTACACCCGGCGGAAGAACCGGATCTGCTACCTGC
AGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGA
25 GAGCTTCTGTTGGAGGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTG
GACGAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGCGGAAGAAGCTGGTGG
ACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCACATGATCAAGTTC
CGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGT
TCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGG
30 CGTGGACGCCAAGGCCATCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTG
ATCGCCAGCTGCCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCC
TGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCT
GAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCAGTAC
GCCGACCTGTTCTGCGCCGAAGAACCTGAGCGACGCCATCTGCTGAGCGACATCCTGCG

GGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGCGGTACGACGAG
CACCACCAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCAGAAAGTACA
AGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAG
CCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAG
5 CTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCA
GCATCCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTC
TACCCCTTCCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTA
CTACGTGGGCCCCCTGGCCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAATCCGAG
GAGACCATCACCCCCTGGAACCTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCT
10 TCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCA
CAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCG
AGGGCATGCGGAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGACCTGCT
GTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATC
GAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCA
15 CCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGA
GGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAG
GAGCGGCTGAAAACCTACGCCACCTGTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGC
GGCGGTACACCGGCTGGGGCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCA
GAGCGGCAAGACCATCCTGGACTTCCTGAAATCCGACGGCTTCGCCAACCGGAACCTTCATGC
20 AGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCAGGTGAGCGG
CCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAG
GGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGC
CCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGGGGCCAGAAGA
ACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCC
25 TGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCT
GCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTAC
GACGTGGCCGCCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGC
TGACCCGGAGCGACAAGGCCCGGGGCAAGAGCGACAACGTGCCAGCGAGGAGGTGGTGA
AGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTT
30 CGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATC
AAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCAGATCCTGGACAGCC
GGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCT
GAAATCCAAGCTGGTGAAGGACTTCGGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATC
AACAACTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCA

AGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCG
GAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTAC
AGCAACATCATGAACTTCTTCAAGACCGAGATCACCTGGCCAACGGCGAGATCCGGAAGC
GGCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTT
5 CGCCACCGTGC GGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAAACCGAGGTG
CAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCG
CCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTA
CAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAATCCGTGAA
GGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTC
10 CTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACA
GCCTGTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAA
GGGCAACGAGCTGGCCCTGCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACG
AGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACA
AGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGC
15 CGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGG
GAGCAGGCCGAGAACATCATCCACCTGTTCACCCTGACCAACCTGGGCGCCCCCGCCGCCTT
CAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGAC
GCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCT
GGGCGGCGAC (SEQ ID NO: 8)
20
GCCAAGCGGAACTACATCCTGGGCCTGGCCATCGGCATCACCAGCGTGGGCTACGGCATCAT
CGACTACGAGACCCGGGACGTGATCGACGCCGGCGTGCGGCTGTTCAAGGAGGCCAACGTG
GAGAACAACGAGGGCCGGCGGAGCAAGCGGGGCGCCCGGCGGCTGAAGCGGCGGCGGCGG
CACCGGATCCAGCGGGTGAAGAAGCTGCTGTTCGACTACAACCTGCTGACCGACCACAGCG
25 AGCTGAGCGGCATCAACCCCTACGAGGCCCGGGTGAAGGGCCTGAGCCAGAAGCTGAGCGA
GGAGGAGTTCAGCGCCGCCCTGCTGCACCTGGCCAAGCGGCGGGGCGTGACACAACGTGAAC
GAGGTGGAGGAGGACACCGGCAACGAGCTGAGCACCAAGGAGCAGATCAGCCGGAACAGC
AAGGCCCTGGAGGAGAAGTACGTGGCCGAGCTGCAGCTGGAGCGGCTGAAGAAGGACGGC
GAGGTGCGGGGCGAGCATCAACCGGTTCAAGACCAGCGACTACGTGAAGGAGGCCAAGCAGC
30 TGCTGAAGGTGCAGAAGGCCTACCACCAGCTGGACCAGAGCTTCATCGACACCTACATCGA
CCTGCTGGAGACCCGGCGGACCTACTACGAGGGCCCCGGCGAGGGCAGCCCCTTCGGCTGG
AAGGACATCAAGGAGTGGTACGAGATGCTGATGGGCCACTGCACCTACTTCCCCGAGGAGC
TGCGGAGCGTGAAGTACGCCTACAACGCCGACCTGTACAACGCCCTGAACGACCTGAACAA
CCTGGTGATCACCCGGGACGAGAACGAGAAGCTGGAGTACTACGAGAAGTTCCAGATCATC

GAGAACGTGTTCAAGCAGAAGAAGAAGCCCACCCTGAAGCAGATCGCCAAGGAGATCCTGG
TGAACGAGGAGGACATCAAGGGCTACCGGGTGACCAGCACCGGCAAGCCCGAGTTCACCAA
CCTGAAGGTGTACCACGACATCAAGGACATCACCGCCCCGGAAGGAGATCATCGAGAACGCC
GAGCTGCTGGACCAGATCGCCAAGATCCTGACCATCTACCAGAGCAGCGAGGACATCCAGG
5 AGGAGCTGACCAACCTGAACAGCGAGCTGACCCAGGAGGAGATCGAGCAGATCAGCAACCT
GAAGGGCTACACCGGCACCCACAACCTGAGCCTGAAGGCCATCAACCTGATCCTGGACGAG
CTGTGGCACACCAACGACAACCAGATCGCCATCTTCAACCGGCTGAAGCTGGTGCCCAAGA
AGGTGGACCTGAGCCAGCAGAAGGAGATCCCCACCACCCTGGTGGACGACTTCATCCTGAG
CCCCGTGGTGAAGCGGAGCTTCATCCAGAGCATCAAGGTGATCAACGCCATCATCAAGAAG
10 TACGGCCTGCCCCAACGACATCATCATCGAGCTGGCCCCGGGAGAAGAACAGCAAGGACGCCC
AGAAGATGATCAACGAGATGCAGAAGCGGAACCGGCAGACCAACGAGCGGATCGAGGAGA
TCATCCGGACCACCGGCAAGGAGAACGCCAAGTACCTGATCGAGAAGATCAAGCTGCACGA
CATGCAGGAGGGCAAGTGCCTGTACAGCCTGGAGGCCATCCCCCTGGAGGACCTGCTGAAC
AACCCTTCAACTACGAGGTGGACGCCATCATCCCCGGAGCGTGAGCTTCGACAACAGCTT
15 CAACAACAAGGTGCTGGTGAAGCAGGAGGAGAACAGCAAGAAGGGCAACCGGACCCCCCTT
CCAGTACCTGAGCAGCAGCGACAGCAAGATCAGCTACGAGACCTTCAAGAAGCACATCCTG
AACCTGGCCAAGGGCAAGGGCCGGATCAGCAAGACCAAGAAGGAGTACCTGCTGGAGGAG
CGGGACATCAACCGGTTTCAGCGTGCAGAAGGACTTCATCAACCGGAACCTGGTGGACACCC
GGTACGCCACCCGGGGCCTGATGAACCTGCTGCGGAGCTACTTCCGGGTGAACAACCTGGA
20 CGTGAAGGTGaatccATCAACGGCGGCTTCACCAGCTTCCTGCGGCGGAAGTGGAAGTTCAAG
AAGGAGCGGAACAAGGGCTACAAGCACACGCCGAGGACGCCCTGATCATCGCCAACGCCG
ACTTCATCTTCAAGGAGTGGAAGAAGCTGGACAAGGCCAAGAAGGTGATGGAGAACCAGAT
GTTTCGAGGAGAAGCAGGCCGAGAGCATGCCCCGAGATCGAGACCGAGCAGGAGTACAAGGA
GATCTTCATCACCCCCCACCAGATCAAGCACATCAAGGACTTCAAGGACTACAAGTACAGCC
25 ACCGGGTGGACAAGAAGCCCAACCGGAAGCTGATCAACGACACCCTGTACAGCACCCGGAA
GGACGACAAGGGCAACACCCTGATCGTGAACAACCTGAACGGCCTGTACGACAAGGACAAC
GACAAGCTGAAGAAGCTGATCAACAAGAGCCCCGAGAAGCTGCTGATGTACCACCACGACC
CCCAGACCTACCAGAAGCTGAAGCTGATCATGGAGCAGTACGGCGACGAGAAGAACCCCTT
GTACAAGTACTACGAGGAGACCGGCAACTACCTGACCAAGTACAGCAAGAAGGACAACGGC
30 CCCGTGATCAAGAAGATCAAGTACTACGGCAACAAGCTGAACGCCACCTGGACATCACCG
ACGACTACCCCAACAGCCGGAACAAGGTGGTGAAGCTGAGCCTGAAGCCCTACCGGTTCTGA
CGTGTACCTGGACAACGGCGTGTACAAGTTCGTGACCGTGAAGAACCTGGACGTGATCAAG
AAGGAGAACTACTACGAGGTGAACAGCAAGTGCTACGAGGAGGCCAAGAAGCTGAAGAAG
ATCAGCAACCAGGCCGAGTTCATCGCCAGCTTCTACAAGAACGACCTGATCAAGATCAACG

GCGAGCTGTACCGGGTGATCGGCGTGAACAACGACCTGCTGAACCGGATCGAGGTGAACAT
 GATCGACATCACCTACCGGGAGTACCTGGAGAACATGAACGACAAGCGGCCCCCCCCACATC
 ATCAAGACCATCGCCAGCAAGACCCAGAGCATCAAGAAGTACAGCACCGACATCCTGGGCA
 ACCTGTACGAGGTGaaatccAAGAAGCACCCCCAGATCATCAAGAAGGGC (SEQ ID NO: 9)

5

Guide RNA (gRNA)

In some embodiments, a targeting moiety may comprise a Cas molecule comprising or linked (e.g., covalently) to a gRNA. A gRNA is a short synthetic RNA composed of a “scaffold” sequence necessary for Cas-protein binding and a user-defined ~20 nucleotide targeting sequence for a genomic target. In practice, guide RNA sequences are generally designed to have a length of between 17 – 24 nucleotides (e.g., 19, 20, or 21 nucleotides) and be complementary to the targeted nucleic acid sequence. In some embodiments the gRNA comprises 3-6 flanking phosphorothioate (PS) linkages, e.g., 3 flanking PS linkages at each end. Custom gRNA generators and algorithms are available commercially for use in the design of effective guide RNAs. Gene editing has also been achieved using a chimeric “single guide RNA” (“sgRNA”), an engineered (synthetic) single RNA molecule that mimics a naturally occurring crRNA-tracrRNA complex and contains both a tracrRNA (for binding the nuclease) and at least one crRNA (to guide the nuclease to the sequence targeted for editing). Chemically modified sgRNAs have also been demonstrated to be effective for use with Cas proteins; see, for example, Hendel et al. (2015) Nature Biotechnol., 985 – 991.

20

In some embodiments, a gRNA comprises a nucleic acid sequence that is complementary to a DNA sequence associated with a target gene. In some embodiments, the DNA sequence is, comprises, or overlaps an expression control element that is operably linked to the target gene. In some embodiments, a gRNA comprises a nucleic acid sequence that is at least 90, 95, 99, or 100% complementary to a DNA sequence associated with a target gene. In some embodiments, a gRNA for use with a targeting moiety that comprises a Cas molecule is an sgRNA. In some embodiments, a gRNA binds to a nucleic acid sequence comprising a sequence selected from Table 4, Table 5, Table 6, Table 7 or a sequence having at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity thereto, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

30

In some embodiments, a gRNA for use with a CRISPR/Cas molecule specifically binds a target sequence associated with β -2-microglobulin expression. In some embodiments, a gRNA for use with a CRISPR/Cas molecule specifically binds a target sequence associated with one or more of CXCL1-8 gene expression. Such a gRNA may comprise a target-binding sequence selected from SEQ ID NOs: 20-62.

In some embodiments, a targeting moiety is or comprises a TAL effector molecule. A TAL effector molecule, e.g., a TAL effector molecule that specifically binds a DNA sequence, comprises a plurality of TAL effector domains or fragments thereof, and optionally one or more additional portions of naturally occurring TAL effectors (e.g., N- and/or C-terminal of the plurality of TAL effector domains). Many TAL effectors are known to those of skill in the art and are commercially available, e.g., from Thermo Fisher Scientific.

TALEs are natural effector proteins secreted by numerous species of bacterial pathogens including the plant pathogen *Xanthomonas* which modulates gene expression in host plants and facilitates bacterial colonization and survival. The specific binding of TAL effectors is based on a central repeat domain of tandemly arranged nearly identical repeats of typically 33 or 34 amino acids (the repeat-variable di-residues, RVD domain).

Members of the TAL effectors family differ mainly in the number and order of their repeats. The number of repeats ranges from 1.5 to 33.5 repeats and the C-terminal repeat is usually shorter in length (e.g., about 20 amino acids) and is generally referred to as a “half-repeat”. Each repeat of the TAL effector feature a one-repeat-to-one-base-pair correlation with different repeat types exhibiting different base-pair specificity (one repeat recognizes one base-pair on the target gene sequence). Generally, the smaller the number of repeats, the weaker the protein-DNA interactions. A number of 6.5 repeats has been shown to be sufficient to activate transcription of a reporter gene (Scholze et al., 2010).

Repeat to repeat variations occur predominantly at amino acid positions 12 and 13, which have therefore been termed “hypervariable” and which are responsible for the specificity of the interaction with the target DNA promoter sequence, as shown in Table 2 listing exemplary repeat variable di-residues (RVD) and their correspondence to nucleic acid base targets.

Table 2 – RVDs and Nucleic Acid Base Specificity

Target	Possible RVD Amino Acid Combinations												
A	NI	NN	CI	HI	KI								
G	NN	GN	SN	VN	LN	DN	QN	EN	HN	RH	NK	AN	FN
C	HD	RD	KD	ND	AD								
T	NG	HG	VG	IG	EG	MG	YG	AA	EP	VA	QG	KG	RG

Accordingly, it is possible to modify the repeats of a TAL effector to target specific DNA sequences. Further studies have shown that the RVD NK can target G. Target sites of TAL effectors also tend to include a T flanking the 5' base targeted by the first repeat, but the exact mechanism of this recognition is not known. More than 113 TAL effector sequences are known to date. Non-limiting
 5 examples of TAL effectors from *Xanthomonas* include, Hax2, Hax3, Hax4, AvrXa7, AvrXa10 and AvrBs3.

Accordingly, the TAL effector domain of the TAL effector molecule of the present disclosure may be derived from a TAL effector from any bacterial species (e.g., *Xanthomonas* species such as the African strain of *Xanthomonas oryzae* pv. *Oryzae* (Yu et al. 2011), *Xanthomonas*
 10 *campestris* pv. *raphani* strain 756C and *Xanthomonas oryzae* pv. *oryzicola* strain BLS256 (Bogdanove et al. 2011). As used herein, the TAL effector domain in accordance with the present disclosure comprises an RVD domain as well as flanking sequence(s) (sequences on the N-terminal and/or C-terminal side of the RVD domain) also from the naturally occurring TAL effector. It may comprise more or fewer repeats than the RVD of the naturally occurring TAL effector. The TAL effector molecule of the present
 15 disclosure is designed to target a given DNA sequence based on the above code and others known in the art. The number of TAL effector domains (e.g., repeats (monomers or modules)) and their specific sequence are selected based on the desired DNA target sequence. For example, TAL effector domains, e.g., repeats, may be removed or added in order to suit a specific target sequence. In an embodiment, the TAL effector molecule of the present disclosure comprises between 6.5 and 33.5 TAL effector domains,
 20 e.g., repeats. In an embodiment, TAL effector molecule of the present disclosure comprises between 8 and 33.5 TAL effector domains, e.g., repeats, e.g., between 10 and 25 TAL effector domains, e.g., repeats, e.g., between 10 and 14 TAL effector domains, e.g., repeats.

In some embodiments, the TAL effector molecule comprises TAL effector domains that
 25 correspond to a perfect match to the DNA target sequence. In some embodiments, a mismatch between a repeat and a target base-pair on the DNA target sequence is permitted as long as it allows for the function of the site-specific disrupting agent comprising the TAL effector molecule. In general, TALE binding is inversely correlated with the number of mismatches. In some embodiments, the TAL effector molecule of a site-specific disrupting agent of the present disclosure comprises no more than 7
 30 mismatches, 6 mismatches, 5 mismatches, 4 mismatches, 3 mismatches, 2 mismatches, or 1 mismatch, and optionally no mismatch, with the target DNA sequence. Without wishing to be bound by theory, in general the smaller the number of TAL effector domains in the TAL effector molecule, the smaller the number of mismatches will be tolerated and still allow for the function of the site-specific disrupting agent comprising the TAL effector molecule. The binding affinity is thought to depend on the sum of

matching repeat-DNA combinations. For example, TAL effector molecules having 25 TAL effector domains or more may be able to tolerate up to 7 mismatches.

In addition to the TAL effector domains, the TAL effector molecule of the present disclosure may
5 comprise additional sequences derived from a naturally occurring TAL effector. The length of the C-terminal and/or N-terminal sequence(s) included on each side of the TAL effector domain portion of the TAL effector molecule can vary and be selected by one skilled in the art, for example based on the studies of Zhang et al. (2011). Zhang et al., have characterized a number of C-terminal and N-terminal truncation mutants in Hax3 derived TAL-effector based proteins and have identified key elements, which contribute
10 to optimal binding to the target sequence and thus activation of transcription. Generally, it was found that transcriptional activity is inversely correlated with the length of N-terminus. Regarding the C-terminus, an important element for DNA binding residues within the first 68 amino acids of the Hax 3 sequence was identified. Accordingly, in some embodiments, the first 68 amino acids on the C-terminal side of the TAL effector domains of the naturally occurring TAL effector is included in the TAL effector molecule of a
15 site-specific disrupting agent of the present disclosure. Accordingly, in an embodiment, a TAL effector molecule of the present disclosure comprises 1) one or more TAL effector domains derived from a naturally occurring TAL effector; 2) at least 70, 80, 90, 100, 110, 120, 130, 140, 150, 170, 180, 190, 200, 220, 230, 240, 250, 260, 270, 280 or more amino acids from the naturally occurring TAL effector on the N-terminal side of the TAL effector domains; and/or 3) at least 68, 80, 90, 100, 110, 120, 130, 140, 150,
20 170, 180, 190, 200, 220, 230, 240, 250, 260 or more amino acids from the naturally occurring TAL effector on the C-terminal side of the TAL effector domains.

In some embodiments, a targeting moiety is or comprises a Zn finger molecule. A Zn finger molecule comprises a Zn finger protein, e.g., a naturally occurring Zn finger protein or engineered Zn
25 finger protein, or fragment thereof. Many Zn finger proteins are known to those of skill in the art and are commercially available, e.g., from Sigma-Aldrich.

In some embodiments, a Zn finger molecule comprises a non-naturally occurring Zn finger protein that is engineered to bind to a target DNA sequence of choice. See, for example, Beerli, et al.
30 (2002) Nature Biotechnol. 20:135-141; Pabo, et al. (2001) Ann. Rev. Biochem. 70:313-340; Isalan, et al. (2001) Nature Biotechnol. 19:656-660; Segal, et al. (2001) Curr. Opin. Biotechnol. 12:632-637; Choo, et al. (2000) Curr. Opin. Struct. Biol. 10:411-416; U.S. Pat. Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273; and U.S. Patent Publication Nos. 2005/0064474; 2007/0218528; 2005/0267061, all incorporated herein by

reference in their entireties.

An engineered Zn finger protein may have a novel binding specificity, compared to a naturally-occurring Zn finger protein. Engineering methods include, but are not limited to, rational design and various types of selection. Rational design includes, for example, using databases comprising triplet (or quadruplet) nucleotide sequences and individual Zn finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is associated with one or more amino acid sequences of zinc fingers which bind the particular triplet or quadruplet sequence. See, for example, U.S. Pat. Nos. 6,453,242 and 6,534,261, incorporated by reference herein in their entireties.

Exemplary selection methods, including phage display and two-hybrid systems, are disclosed in U.S. Pat. Nos. 5,789,538; 5,925,523; 6,007,988; 6,013,453; 6,410,248; 6,140,466; 6,200,759; and 6,242,568; as well as International Patent Publication Nos. WO 98/37186; WO 98/53057; WO 00/27878; and WO 01/88197 and GB 2,338,237. In addition, enhancement of binding specificity for zinc finger proteins has been described, for example, in International Patent Publication No. WO 02/077227.

In addition, as disclosed in these and other references, zinc finger domains and/or multi-fingered zinc finger proteins may be linked together using any suitable linker sequences, including for example, linkers of 5 or more amino acids in length. See, also, U.S. Pat. Nos. 6,479,626; 6,903,185; and 7,153,949 for exemplary linker sequences 6 or more amino acids in length. The proteins described herein may include any combination of suitable linkers between the individual zinc fingers of the protein. In addition, enhancement of binding specificity for zinc finger binding domains has been described, for example, in co-owned International Patent Publication No. WO 02/077227.

Zn finger proteins and methods for design and construction of fusion proteins (and polynucleotides encoding same) are known to those of skill in the art and described in detail in U.S. Pat. Nos. 6,140,081; 789,538; 6,453,242; 6,534,261; 5,925,523; 6,007,988; 6,013,453; and 6,200,759; International Patent Publication Nos. WO 95/19431; WO 96/06166; WO 98/53057; WO 98/54311; WO 00/27878; WO 01/60970; WO 01/88197; WO 02/099084; WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536; and WO 03/016496.

In addition, as disclosed in these and other references, Zn finger proteins and/or multi-fingered Zn finger proteins may be linked together, e.g., as a fusion protein, using any suitable linker sequences, including for example, linkers of 5 or more amino acids in length. See, also, U.S. Pat. Nos. 6,479,626;

6,903,185; and 7,153,949 for exemplary linker sequences 6 or more amino acids in length. The Zn finger molecules described herein may include any combination of suitable linkers between the individual zinc finger proteins and/or multi-fingered Zn finger proteins of the Zn finger molecule.

5 In certain embodiments, the targeting moiety comprises a Zn finger molecule comprising an engineered zinc finger protein that binds (in a sequence-specific manner) to a target DNA sequence. In some embodiments, the Zn finger molecule comprises one Zn finger protein or fragment thereof. In other
10 embodiments, the Zn finger molecule comprises a plurality of Zn finger proteins (or fragments thereof), e.g., 2, 3, 4, 5, 6 or more Zn finger proteins (and optionally no more than 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 Zn finger proteins). In some embodiments, the Zn finger molecule comprises at least three Zn finger proteins. In some embodiments, the Zn finger molecule comprises four, five or six fingers. In some
15 embodiments, the Zn finger molecule comprises 8, 9, 10, 11 or 12 fingers. In some embodiments, a Zn finger molecule comprising three Zn finger proteins recognizes a target DNA sequence comprising 9 or 10 nucleotides. In some embodiments, a Zn finger molecule comprising four Zn finger proteins
20 recognizes a target DNA sequence comprising 12 to 14 nucleotides. In some embodiments, a Zn finger molecule comprising six Zn finger proteins recognizes a target DNA sequence comprising 18 to 21 nucleotides.

In some embodiments, a Zn finger molecule comprises a two-handed Zn finger protein. Two
25 handed zinc finger proteins are those proteins in which two clusters of zinc finger proteins are separated by intervening amino acids so that the two zinc finger domains bind to two discontinuous target DNA sequences. An example of a two handed type of zinc finger binding protein is SIP1, where a cluster of four zinc finger proteins is located at the amino terminus of the protein and a cluster of three Zn finger proteins is located at the carboxyl terminus (see Remade, et al. (1999) EMBO Journal 18(18):5073-5084).
30 Each cluster of zinc fingers in these proteins is able to bind to a unique target sequence and the spacing between the two target sequences can comprise many nucleotides.

In some embodiments, a targeting moiety is or comprises a DNA-binding domain from a nuclease. For example, the recognition sequences of homing endonucleases and meganucleases such as I-
35 SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII are known. See also U.S. Pat. Nos. 5,420,032; 6,833,252; Belfort, et al. (1997) Nucleic Acids Res. 25:3379-3388; Dujon, et al. (1989) Gene 82:115-118; Perler, et al. (1994) Nucleic Acids Res. 22:1125-1127; Jasin (1996) Trends Genet. 12:224-228; Gimble, et al. (1996) J. Mol. Biol. 263:163-180; Argast, et al. (1998) J. Mol. Biol. 280:345-353 and the New England Biolabs catalogue. In addition, the

DNA-binding specificity of homing endonucleases and meganucleases can be engineered to bind non-natural target sites. See, for example, Chevalier, et al. (2002) *Molec. Cell* 10:895-905; Epinat, et al. (2003) *Nucleic Acids Res.* 31:2952-2962; Ashworth, et al. (2006) *Nature* 441:656-659; Paques, et al. (2007) *Current Gene Therapy* 7:49-66; U.S. Patent Publication No. 2007/0117128.

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In some embodiments, a targeting moiety comprises a nucleic acid. In some embodiments, a nucleic acid that may be included in a targeting moiety, may be or comprise DNA, RNA, and/or an artificial or synthetic nucleic acid or nucleic acid analog or mimic. For example, in some embodiments, a nucleic acid may be or include one or more of genomic DNA (gDNA), complementary DNA (cDNA), a peptide nucleic acid (PNA), a peptide-oligonucleotide conjugate, a locked nucleic acid (LNA), a bridged nucleic acid (BNA), a polyamide, a triplex-forming oligonucleotide, an antisense oligonucleotide, tRNA, mRNA, rRNA, miRNA, gRNA, siRNA or other RNAi molecule (e.g., that targets a non-coding RNA as described herein and/or that targets an expression product of a particular gene associated with a targeted genomic complex as described herein), etc. In some embodiments, a nucleic acid may include one or more residues that is not a naturally occurring DNA or RNA residue, may include one or more linkages that is/are not phosphodiester bonds (e.g., that may be, for example, phosphorothioate bonds, etc), and/or may include one or more modifications such as, for example, a 2' O modification such as 2'-OMeP. A variety of nucleic acid structures useful in preparing synthetic nucleic acids is known in the art (see, for example, WO2017/0628621 and WO2014/012081) those skilled in the art will appreciate that these may be utilized in accordance with the present disclosure.

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A nucleic acid suitable for use in a site-specific disrupting agent, e.g., in a targeting moiety, may include, but is not limited to, DNA, RNA, modified oligonucleotides (e.g., chemical modifications, such as modifications that alter backbone linkages, sugar molecules, and/or nucleic acid bases), and artificial nucleic acids. In some embodiments, a nucleic acid includes, but is not limited to, genomic DNA, cDNA, peptide nucleic acids (PNA) or peptide oligonucleotide conjugates, locked nucleic acids (LNA), bridged nucleic acids (BNA), polyamides, triplex forming oligonucleotides, modified DNA, antisense DNA oligonucleotides, tRNA, mRNA, rRNA, modified RNA, miRNA, gRNA, and siRNA or other RNA or DNA molecules.

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In some embodiments, a targeting moiety comprises a nucleic acid with a length from about 15-200, 20-200, 30-200, 40-200, 50-200, 60-200, 70-200, 80-200, 90-200, 100-200, 110-200, 120-200, 130-200, 140-200, 150-200, 160-200, 170-200, 180-200, 190-200, 215-190, 20-190, 30-190, 40-190, 50-190, 60-190, 70-190, 80-190, 90-190, 100-190, 110-190, 120-190, 130-190, 140-190, 150-190, 160-190, 170-190, 180-190, 15-180, 20-180, 30-180, 40-180, 50-180, 60-180, 70-180, 80-180, 90-180, 100-180, 110-

30

180, 120-180, 130-180, 140-180, 150-180, 160-180, 170-180, 15-170, 20-170, 30-170, 40-170, 50-170, 60-170, 70-170, 80-170, 90-170, 100-170, 110-170, 120-170, 130-170, 140-170, 150-170, 160-170, 15-160, 20-160, 30-160, 40-160, 50-160, 60-160, 70-160, 80-160, 90-160, 100-160, 110-160, 120-160, 130-160, 140-160, 150-160, 215-150, 20-150, 30-150, 40-150, 50-150, 60-150, 70-150, 80-150, 90-150, 100-150, 110-150, 120-150, 130-150, 140-150, 15-140, 20-140, 30-140, 40-140, 50-140, 60-140, 70-140, 80-140, 90-140, 100-140, 110-140, 120-140, 130-140, 15-130, 20-130, 30-130, 40-130, 50-130, 60-130, 70-130, 80-130, 90-130, 100-130, 110-130, 120-130, 215-120, 20-120, 30-120, 40-120, 50-120, 60-120, 70-120, 80-120, 90-120, 100-120, 110-120, 15-110, 20-110, 30-110, 40-110, 50-110, 60-110, 70-110, 80-110, 90-110, 100-110, 15-100, 20-100, 30-100, 40-100, 50-100, 60-100, 70-100, 80-100, 90-100, 15-90, 20-90, 30-90, 40-90, 50-90, 60-90, 70-90, 80-90, 15-80, 20-80, 30-80, 40-80, 50-80, 60-80, 70-80, 15-70, 20-70, 30-70, 40-70, 50-70, 60-70, 15-60, 20-60, 30-60, 40-60, 50-60, 15-50, 20-50, 30-50, 40-50, 15-40, 20-40, 30-40, 15-30, 20-30, or 15-20 nucleotides, or any range therebetween.

Effector Moieties

A site-specific disrupting agent of the present disclosure may comprise one or more effector moieties. An effector moiety has one or more functionalities that, when used as part of a site-specific disrupting agent described herein, modulate, e.g., decrease, expression of a target plurality of genes in a cell. In some embodiments, an effector moiety physically or sterically blocks an anchor sequence, e.g., such that binding of a genomic complex component (e.g., a nucleating polypeptide) to the anchor sequence is inhibited (e.g., prevented). In some embodiments, an effector moiety destabilizes the interaction of a genomic complex component (e.g., nucleating polypeptide) with an anchor sequence, e.g., by altering (e.g., decreasing) the affinity and/or avidity at which the genomic complex component binds the anchor sequence. For example, an effector moiety may recruit a factor that inhibits formation of or destabilizes a genomic complex, e.g., ASMC, or it may inhibit recruitment of a factor (e.g., a genomic complex component or transcription factor) necessary for formation or maintenance of a genomic complex (e.g., ASMC). In some embodiments, an effector moiety has epigenetic modification functionality in that it modulates the epigenetic landscape of the anchor sequence or a sequence proximal to the anchor sequence, e.g., by promoting (e.g., catalyzing) application or removal of one or more epigenetic modifications to the DNA or a histone associated thereto, to decrease expression of a target plurality of genes. In some embodiments, an effector moiety has genetic modification functionality, e.g., it introduces an alteration (e.g., an insertion, deletion, or substitution) to an anchor sequence or a sequence proximal thereto.

In some embodiments, an effector moiety comprises a CRISPR/Cas molecule, a TAL effector molecule, a Zn finger molecule, a tetR domain, or a meganuclease. In some embodiments, an effector moiety has genetic modification functionality, e.g., a CRISPR/Cas molecule, a TAL effector molecule, a Zn finger molecule with endonuclease activity capable of making a genetic alteration in a method
5 described herein.

In some embodiments, an effector moiety comprises a histone modifying functionality, e.g., a histone methyltransferase, histone demethylase, or histone deacetylase activity. In some embodiments, a histone methyltransferase functionality comprises H3K9 targeting methyltransferase activity. In some
10 embodiments, a histone methyltransferase functionality comprises H3K56 targeting methyltransferase activity. In some embodiments, a histone methyltransferase functionality comprises H3K27 targeting methyltransferase activity. In some embodiments, a histone methyltransferase or demethylase functionality transfers one, two, or three methyl groups. In some embodiments, a histone demethylase functionality comprises H3K4 targeting demethylase activity. In some embodiments, an effector moiety
15 comprises a protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, or a functional variant or fragment of any thereof, e.g., a SET domain of any thereof. In some embodiments, an effector moiety comprises a protein chosen from KDM1A (i.e., LSD1), KDM1B (i.e., LSD2), KDM2A, KDM2B, KDM5A, KDM5B, KDM5C, KDM5D, KDM4B, NO66, or a functional variant or fragment of any
20 thereof. In some embodiments, an effector moiety comprises a protein chosen from HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, or a functional variant or fragment of any thereof. In some embodiments, an effector moiety comprises a DNA modifying functionality, e.g., a DNA methyltransferase. In some embodiments, an effector moiety comprises a protein chosen from MQ1,
25 DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, or a functional variant or fragment of any thereof.

In some embodiments, an effector moiety comprises a transcription repressor. In some embodiments the transcription repressor blocks recruitment of a factor that stimulates or promotes
30 transcription, e.g., of the target gene. In some embodiments, the transcription repressor recruits a factor that inhibits transcription, e.g., of the target gene. In some embodiments, an effector moiety, e.g., transcription repressor, is or comprises a protein chosen from KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment of any thereof.

In some embodiments, an effector moiety comprises a protein having a functionality described herein. In some embodiments, an effector moiety comprises a protein selected from:

KRAB (e.g., as according to NP_056209.2 or the protein encoded by NM_015394.5);

a SET domain (e.g., the SET domain of:

5 SETDB1 (e.g., as according to NP_001353347.1 or the protein encoded by NM_001366418.1);

EZH2 (e.g., as according to NP_004447.2 or NP_001190176.1 or the protein encoded by NM_004456.5 or NM_001203247.2);

G9A (e.g., as according to NP_001350618.1 or the protein encoded by NM_001363689.1); or

SUV39H1 (e.g., as according to NP_003164.1 or the protein encoded by NM_003173.4));

10 histone demethylase LSD1 (e.g., as according to NP_055828.2 or the protein encoded by NM_015013.4);

FOG1 (e.g., the N-terminal residues of FOG1) (e.g., as according to NP_722520.2 or the protein encoded by NM_153813.3); or

KAP1 (e.g., as according to NP_005753.1 or the protein encoded by NM_005762.3);

a functional fragment or variant of any thereof, or

15 a polypeptide with a sequence that has at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any of the above-referenced sequences. In some embodiments, an effector moiety comprises a protein selected from:

DNMT3A (e.g., human DNMT3A) (e.g., as according to NP_072046.2

or the protein encoded by NM_022552.4);

20 DNMT3B (e.g., as according to NP_008823.1

or the protein encoded by NM_006892.4);

DNMT3L (e.g., as according to NP_787063.1

or the protein encoded by NM_175867.3);

DNMT3A/3L complex,

25 bacterial MQ1 (e.g., as according to CAA35058.1 obtained from strain ATCC 33825 or Uniprot ID P15840.3);

a functional fragment of any thereof, or

a polypeptide with a sequence that has at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any of the above-referenced sequences. In some embodiments, an effector moiety comprises a mature

30 bacterial MQ1 (e.g., as according to CAA35058.1 obtained from strain ATCC 33825 or Uniprot ID P15840.3

An exemplary effector moiety may include, but is not limited to: ubiquitin, bicyclic peptides as ubiquitin ligase inhibitors, transcription factors, DNA and protein modification enzymes such as

topoisomerases, topoisomerase inhibitors such as topotecan, DNA methyltransferases such as the DNMT family (e.g., DNMT3A, DNMT3B, DNMT3L), protein methyltransferases (e.g., viral lysine methyltransferase (vSET), protein-lysine N-methyltransferase (SMYD2), deaminases (e.g., APOBEC, UG1), histone methyltransferases such as enhancer of zeste homolog 2 (EZH2), PRMT1, histone-lysine-
5 N-methyltransferase (Setdb1), histone methyltransferase (SET2), euchromatic histone-lysine N-methyltransferase 2 (G9a), histone-lysine N-methyltransferase (SUV39H1), and G9a), histone deacetylase (e.g., HDAC1, HDAC2, HDAC3), enzymes with a role in DNA demethylation (e.g., the TET family enzymes catalyze oxidation of 5-methylcytosine to 5-hydroxymethylcytosine and higher oxidative derivatives), protein demethylases such as KDM1A and lysine-specific histone demethylase 1 (LSD1),
10 helicases such as DHX9, deacetylases (e.g., sirtuin 1, 2, 3, 4, 5, 6, or 7), kinases, phosphatases, DNA-intercalating agents such as ethidium bromide, SYBR green, and proflavine, efflux pump inhibitors such as peptidomimetics like phenylalanine arginyl β -naphthylamide or quinoline derivatives, nuclear receptor activators and inhibitors, proteasome inhibitors, competitive inhibitors for enzymes such as those involved in lysosomal storage diseases, protein synthesis inhibitors, nucleases (e.g., Cpf1, Cas9, zinc
15 finger nuclease), fusions of one or more thereof (e.g., dCas9-DNMT, dCas9-APOBEC, dCas9-UG1), and specific domains from proteins, such as a KRAB domain.

In some embodiments, a candidate domain may be determined to be suitable for use as an effector moiety by methods known to those of skill in the art. For example, a candidate effector moiety may be
20 tested by assaying whether, when the candidate effector moiety is present in the nucleus of a cell and appropriately localized (e.g., to a target gene or transcription control element operably linked to said target gene, e.g., via a Targeting moiety), the candidate effector moiety decreases expression of the target gene in the cell, e.g., decreases the level of RNA transcript encoded by the target gene (e.g., as measured by RNASeq or Northern blot) or decreases the level of protein encoded by the target gene (e.g., as
25 measured by ELISA).

In some embodiments, a site-specific disrupting agent comprises an effector moiety that does not bind (e.g., does not detectably bind) to another copy of the effector moiety, e.g., the effector moiety is monomeric and does not associate into multimers. In some embodiments, a site-specific disrupting agent
30 comprises an effector moiety that associates with a further copy of the effector moiety into a multimer, e.g., dimers, trimers, tetramers, or further. In some embodiments, a site-specific disrupting agent comprises a plurality of effector moieties, wherein each effector moiety does not detectably bind, e.g., does not bind, to another effector moiety. In some embodiments, an effector moiety for use in the compositions and methods described herein is functional in a monomeric, e.g., non-dimeric, state.

In some embodiments, an effector moiety comprises an epigenetic modifying moiety, e.g., that modulates the two-dimensional structure of chromatin (i.e., that modulate structure of chromatin in a way that would alter its two-dimensional representation).

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Epigenetic modifying moieties useful in methods and compositions of the present disclosure include agents that affect epigenetic markers, e.g., DNA methylation, histone methylation, histone acetylation, histone sumoylation, histone phosphorylation, and RNA-associated silencing. Exemplary epigenetic enzymes that can be targeted to a genomic sequence element as described herein include DNA methylases (e.g., DNMT3a, DNMT3b, DNMTL, MQ1), DNA demethylation (e.g., the TET family), histone methyltransferases, histone deacetylase (e.g., HDAC1, HDAC2, HDAC3), sirtuin 1, 2, 3, 4, 5, 6, or 7, lysine-specific histone demethylase 1 (LSD1), histone-lysine-N-methyltransferase (Setdb1), euchromatic histone-lysine N-methyltransferase 2 (G9a), histone-lysine N-methyltransferase (SUV39H1), enhancer of zeste homolog 2 (EZH2), viral lysine methyltransferase (vSET), histone methyltransferase (SET2), and protein-lysine N-methyltransferase (SMYD2). Examples of such epigenetic modifying agents are described, e.g., in de Groote et al. Nuc. Acids Res. (2012):1-18.

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In some embodiments, a site-specific disrupting agent, e.g., comprising an epigenetic modifying moiety, useful herein comprises or is a construct described in Kofler et al. Genome Medicine 7.59 (2015):1-3 incorporated herein by reference. For example, in some embodiments, a site-specific disrupting agent comprises or is a construct found in Table 1 of Kofler et al., e.g., histone deacetylase, histone methyltransferase, DNA demethylation, or H3K4 and/or H3K9 histone demethylase described in Table 1 (e.g., dCas9-p300, TALE-TET1, ZF-DNMT3A, or TALE-LSD1).

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Additional Moieties

A site-specific disrupting agent may further comprise one or more additional moieties (e.g., in addition to one or more targeting moieties and one or more effector moieties). In some embodiments, an additional moiety is selected from a tagging or monitoring moiety, a cleavable moiety (e.g., a cleavable moiety positioned between a targeting moiety and an effector moiety or at the N- or C-terminal end of a polypeptide), a small molecule, a membrane translocating polypeptide, or a pharmacological moiety.

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Exemplary Site-Specific Disrupting Agents

The following exemplary site-specific disrupting agents are presented for illustration purposes only and are not intended to be limiting.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g., comprising dCas9, e.g., an *S. aureus* dCas9), and an effector moiety comprising MQ1, e.g., bacterial MQ1. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 201 (e.g., a plasmid encoding the site-specific disrupting agent), and/or 202 (e.g., a nucleic acid (e.g., mRNA) encoding the site-specific disrupting agent), encoding the site-specific disrupting agent). In some embodiments, a nucleic acid described herein comprises a nucleic acid sequence of SEQ ID NO: 201 or 202 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto. In some embodiments, the targeting moiety is encoded by the nucleic acid sequence of SEQ ID NO: 9 and/or the effector moiety is encoded by the nucleic acid sequence of SEQ ID NO: 10.

Sa-dCas9-MQ1 (PL-27695) Plasmid DNA sequence:

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACA
GCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTG
GCGGGTGTGCGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCAT
ATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTTCGC
CATTTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAG
CTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGT
CACGACGTTGTAAAACGACGGCCAGTGAATTGACGCGTATTGGGATGGTACCTAATACGACT
CACTATAAGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCC
CCCAAGAAGAAGCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCCAAGCGGAAGTACA
TCCTGGGCCTGGCCATCGGCATCACCAGCGTGGGCTACGGCATCATCGACTACGAGACCCGG
GACGTGATCGACGCCGGCGTGCGGCTGTTCAAGGAGGCCAACGTGGAGAACAACGAGGGCC
GGCGGAGCAAGCGGGGCGCCCGGCGGCTGAAGCGGCGGCGGCGGCACCGGATCCAGCGGG
TGAAGAAGCTGCTGTTGACTACAACCTGCTGACCGACCACAGCGAGCTGAGCGGCATCAA
CCCCTACGAGGCCCGGGTGAAGGGCCTGAGCCAGAAGCTGAGCGAGGAGGAGTTCAGCGCC
GCCCTGCTGCACCTGGCCAAGCGGCGGGGCGTGACAAACGTGAACGAGGTGGAGGAGGACA
CCGGCAACGAGCTGAGCACCAAGGAGCAGATCAGCCGGAACAGCAAGGCCCTGGAGGAGA
AGTACGTGGCCGAGCTGCAGCTGGAGCGGCTGAAGAAGGACGGCGAGGTGCGGGGCGAGCA
TCAACCGGTTCAAGACCAGCGACTACGTGAAGGAGGCCAAGCAGCTGCTGAAGGTGCAGAA
GGCCTACCACCAGCTGGACCAGAGCTTCATCGACACCTACATCGACCTGCTGGAGACCCGGC

GGACCTACTACGAGGGCCCCGGCGAGGGCAGCCCCTTCGGCTGGAAGGACATCAAGGAGTG
GTACGAGATGCTGATGGGCCACTGCACCTACTTCCCCGAGGAGCTGCGGAGCGTGAAGTAC
GCCTACAACGCCGACCTGTACAACGCCCTGAACGACCTGAACAACCTGGTGATCACCCGGG
ACGAGAACGAGAAGCTGGAGTACTACGAGAAGTTCCAGATCATCGAGAACGTGTTCAAGCA
5 GAAGAAGAAGCCCACCCTGAAGCAGATCGCCAAGGAGATCCTGGTGAACGAGGAGGACAT
CAAGGGCTACCGGGTGACCAGCACCGGCAAGCCCGAGTTCACCAACCTGAAGGTGTACCAC
GACATCAAGGACATCACCGCCCGGAAGGAGATCATCGAGAACGCCGAGCTGCTGGACCAGA
TCGCCAAGATCCTGACCATCTACCAGAGCAGCGAGGACATCCAGGAGGAGCTGACCAACCT
GAACAGCGAGCTGACCCAGGAGGAGATCGAGCAGATCAGCAACCTGAAGGGCTACACCGG
10 CACCCACAACCTGAGCCTGAAGGCCATCAACCTGATCCTGGACGAGCTGTGGCACACCAAC
GACAACCAGATCGCCATCTTCAACCGGCTGAAGCTGGTGCCCAAGAAGGTGGACCTGAGCC
AGCAGAAGGAGATCCCCACCACCCTGGTGGACGACTTCATCCTGAGCCCCGTGGTGAAGCG
GAGCTTCATCCAGAGCATCAAGGTGATCAACGCCATCATCAAGAAGTACGGCCTGCCCAAC
GACATCATCATCGAGCTGGCCCGGGAGAAGAAGCAAGGACGCCCAGAAGATGATCAACG
15 AGATGCAGAAGCGGAACCGGCAGACCAACGAGCGGATCGAGGAGATCATCCGGACCACCG
GCAAGGAGAACGCCAAGTACCTGATCGAGAAGATCAAGCTGCACGACATGCAGGAGGGCA
AGTGCCTGTACAGCCTGGAGGCCATCCCCCTGGAGGACCTGCTGAACAACCCCTTCAACTAC
GAGGTGGACGCCATCATCCCCCGGAGCGTGAGCTTCGACAACAGCTTCAACAACAAGGTGC
TGGTGAAGCAGGAGGAGAACAGCAAGAAGGGCAACCGGACCCCCCTTCCAGTACCTGAGCAG
20 CAGCGACAGCAAGATCAGCTACGAGACCTTCAAGAAGCACATCCTGAACCTGGCCAAGGGC
AAGGGCCGGATCAGCAAGACCAAGAAGGAGTACCTGCTGGAGGAGCGGGACATCAACCGG
TTCAGCGTGCAGAAGGACTTCATCAACCGGAACCTGGTGGACACCCGGTACGCCACCCGGG
GCCTGATGAACCTGCTGCGGAGCTACTTCCGGGTGAACAACCTGGACGTGAAGGTGaatccAT
CAACGGCGGGCTTCACCAGCTTCCTGCGGCGGAAGTGGAAGTTCAAGAAGGAGCGGAACAAG
25 GGCTACAAGCACCACGCCGAGGACGCCCTGATCATCGCCAACGCCGACTTCATCTTCAAGGA
GTGGAAGAAGCTGGACAAGGCCAAGAAGGTGATGGAGAACCAGATGTTTCGAGGAGAAGCA
GGCCGAGAGCATGCCCAGATCGAGACCGAGCAGGAGTACAAGGAGATCTTCATCACCCCC
CACCAGATCAAGCACATCAAGGACTTCAAGGACTACAAGTACAGCCACCGGGTGGACAAGA
AGCCCAACCGGAAGCTGATCAACGACACCCTGTACAGCACCCGGAAGGACGACAAGGGCAA
30 CACCCTGATCGTGAACAACCTGAACGGCCTGTACGACAAGGACAACGACAAGCTGAAGAAG
CTGATCAACAAGAGCCCCGAGAAGCTGCTGATGTACCACCACGACCCCCAGACCTACCAGA
AGCTGAAGCTGATCATGGAGCAGTACGGCGACGAGAAGAACCCCTGTACAAGTACTACGA
GGAGACCGGCAACTACCTGACCAAGTACAGCAAGAAGGACAACGGCCCCGTGATCAAGAA
GATCAAGTACTACGGCAACAAGCTGAACGCCACCTGGACATCACCGACGACTACCCCAAC

AGCCGGAACAAGGTGGTGAAGCTGAGCCTGAAGCCCTACCGGTTTCGACGTGTACCTGGACA
ACGGCGTGTACAAGTTCGTGACCGTGAAGAACCTGGACGTGATCAAGAAGGAGAACTACTA
CGAGGTGAACAGCAAGTGCTACGAGGAGGCCAAGAAGCTGAAGAAGATCAGCAACCAGGC
CGAGTTCATCGCCAGCTTCTACAAGAACGACCTGATCAAGATCAACGGCGAGCTGTACCGG
5 GTGATCGGCGTGAACAACGACCTGCTGAACCGGATCGAGGTGAACATGATCGACATCACCT
ACCGGGAGTACCTGGAGAACATGAACGACAAGCGGCCCCCCCCACATCATCAAGACCATCGC
CAGCAAGACCCAGAGCATCAAGAAGTACAGCACCGACATCCTGGGCAACCTGTACGAGGTG
aatccAAGAAGCACCCCCAGATCATCAAGAAGGGCAAGCGGCCCGCCGCCACCAAGAAGGCC
GGCCAGGCCAAGAAGAAGAAGGCCCGGGACAGCAAGGTGGAGAACAAGACCAAGAAGCTG
10 CGGGTGTTTCGAGGCCTTCGCCGGCATCGGCGCCAGCGGAAGGCCCTGGAGAAGGTGCGGA
AGGACGAGTACGAGATCGTGGGCCTGGCCGAGTGGTACGTGCCCCGCCATCGTGATGTACCA
GGCCATCCACAACAACCTTCCACACCAAGCTGGAGTACAAGAGCGTGAGCCGGGAGGAGATG
ATCGACTACCTGGAGAACAAAGACCCTGAGCTGGAACAGCAAGAACCCCGTGAGCAACGGCT
ACTGGAAGCGGAAGAAGGACGACGAGCTGAAGATCATCTACAACGCCATCAAGCTGAGCGA
15 GAAGGAGGGCAACATCTTCGACATCCGGGACCTGTACAAGCGGACCCTGAAGAACATCGAC
CTGCTGACCTACAGCTTCCCCTGCCAGGACCTGAGCCAGCAGGGCATCCAGAAGGGCATGA
AGCGGGGCAGCGGCACCCGGAGCGGCCTGCTGTGGGAGATCGAGCGGGCCCTGGACAGCAC
CGAGAAGAACGACCTGCCCAAGTACCTGCTGATGGAGAACGTGGGCGCCCTGCTGCACAAG
AAGAACGAGGAGGAGCTGAACCAGTGGAAGCAGAAGCTGGAGAGCCTGGGCTACCAGAAC
20 AGCATCGAGGTGCTGAACGCCGCGACTTCGGCAGCAGCCAGGCCCGGCGGCGGGTGTTC
TGATCAGCACCCCTGAACGAGTTCGTGGAGCTGCCCAAGGGCGACAAGAAGCCCAAGAGCAT
CAAGAAGGTGCTGAACAAGATCGTGAGCGAGAAGGACATCCTGAACAACCTGCTGAAGTAC
AACCTGACCGAGTTCAAGAAaACCAAGAGCAACATCAACAAGGCCAGCCTGATCGGCTACA
GCAAGTTCAACAGCGAGGGCTACGTGTACGACCCCGAGTTCACCGGCCCCACCCTGACCGCC
25 AGCGGCGCCAACAGCCGGATCAAGATCAAGGACGGCAGCAACATCCGGAAGATGAACAGC
GACGAGACCTTCCTGTACATCGGCTTCGACAGCCAGGACGGCAAGCGGGTGAACGAGATCG
AGTTCCTGACCGAGAACCAGAAGATCTTCGTGTGCGGCAACAGCATCAGCGTGGAGGTGCT
GGAGGCCATCATCGACAAGATCGGCGGCCCCAGCAGCGGCGGCAAGCGGCCCGCCGCCACC
AAGAAGGCCGCGCCAGGCCAAGAAGAAGAAGGGCAGCTACCCCTACGACGTGCCCCGACTAC
30 GCCTGAGcgggcgcttaattaagctgccttctcggggcttgcccttctggccatgcccttcttcttcccttgccctgtaccttcttggtttgaataaa
gcctgagtaggaagtctagaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
aaattgtcttctcagcctgcagATCCCAATGGCGCGCCGAGCTTGGCTCGAGCATGGTCATAGCTGTTTC
CTGTGTGAAATTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTGT
AAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCG

TTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGA
GGCGGTTTGC GTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTTCGTT
CGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAG
GGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
5 AGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA
CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTG
GAAGCTCCCTCGTGGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTC
TCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGG
TCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA
10 TCCGTAACACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC
CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG
TGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGT
TACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTG
GTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTG
15 ATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTAAAGGGATTTTGGTCAT
GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTAAATCAA
TCTAAAGTATATATGAGTAACTTGGTCTGACAGTTAGAAAACTCATCGAGCATCAAATGA
AACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAAT
GAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGA
20 TTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAA
GTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCT
TTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAA
ACCGTTATTCATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAGGAC
AATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATT
25 TTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTCCAGGGATCGCAGTGGT
GAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAAT
TCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCA
TGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATAACAATCGATAGATTGTGCGACCTGA
TTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAA
30 TCGCGGCCTAGAGCAAGACGTTTCCCGTTGAATATGGCTCATACTCTTCCTTTTTCAATATTA
TTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAA
ATAAACAATAAGGGGTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAAC
CATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC (SEQ ID
NO: 201)

Sa-dCas9-MQ1 (MR-28126) Expressed mRNA sequence:

AGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCCAAGA
 AGAAGCGGAAGGTGGGCATCCACGGCGTGCCCCGCCGCCAAGCGGAACCTACATCCTGGG
 5 CCTGGCCATCGGCATCACCAGCGTGGGCTACGGCATCATCGACTACGAGACCCGGGACGTG
 ATCGACGCCGGCGTGCGGCTGTTCAAGGAGGCCAACGTGGAGAACAACGAGGGCCGGCGGA
 GCAAGCGGGGCGCCCCGGCGGCTGAAGCGGGCGGCGGCGGCACCGGATCCAGCGGGTGAAGA
 AGCTGCTGTTCTGACTACAACCTGCTGACCGACCACAGCGAGCTGAGCGGCATCAACCCCTAC
 GAGGCCCGGGTGAAGGGCCTGAGCCAGAAGCTGAGCGAGGAGGAGTTTCAGCGCCGCCCTGC
 10 TGCACCTGGCCAAGCGGCGGGGCGTGCACAACGTGAACGAGGTGGAGGAGGACACCGGCA
 ACGAGCTGAGCACCAAGGAGCAGATCAGCCGGAACAGCAAGGCCCTGGAGGAGAAGTACG
 TGGCCGAGCTGCAGCTGGAGCGGCTGAAGAAGGACGGCGAGGTGCGGGGCAGCATCAACC
 GGTTCAAGACCAGCGACTACGTGAAGGAGGCCAAGCAGCTGCTGAAGGTGCAGAAGGCCTA
 CCACCAGCTGGACCAGAGCTTCATCGACACCTACATCGACCTGCTGGAGACCCGGCGGACCT
 15 ACTACGAGGGCCCCGGCGAGGGCAGCCCCCTTCGGCTGGAAGGACATCAAGGAGTGGTACGA
 GATGCTGATGGGCCACTGCACCTACTTCCCCGAGGAGCTGCGGAGCGTGAAGTACGCCTACA
 ACGCCGACCTGTACAACGCCCTGAACGACCTGAACAACCTGGTGATCACCCGGGACGAGAA
 CGAGAAGCTGGAGTACTACGAGAAGTTCCAGATCATCGAGAACGTGTTCAAGCAGAAGAAG
 AAGCCCACCCTGAAGCAGATCGCCAAGGAGATCCTGGTGAACGAGGAGGACATCAAGGGCT
 20 ACCGGGTGACCAGCACCGGCAAGCCCCGAGTTCACCAACCTGAAGGTGTACCACGACATCAA
 GGACATCACCGCCCCGGAAGGAGATCATCGAGAACGCCGAGCTGCTGGACCAGATCGCCAAG
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 AGCTGACCCAGGAGGAGATCGAGCAGATCAGCAACCTGAAGGGCTACACCGGCACCCACAA
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 25 ATCGCCATCTTCAACCGGTGAAGCTGGTGCCCAAGAAGGTGGACCTGAGCCAGCAGAAGG
 AGATCCCCACCACCCTGGTGGACGACTTCATCCTGAGCCCCGTGGTGAAGCGGAGCTTCATC
 CAGAGCATCAAGGTGATCAACGCCATCATCAAGAAGTACGGCCTGCCCAACGACATCATCA
 TCGAGCTGGCCCCGGGAGAAGAACAGCAAGGACGCCAGAAGATGATCAACGAGATGCAGA
 AGCGGAACCGGCAGACCAACGAGCGGATCGAGGAGATCATCCGGACCACCGGCAAGGAGA
 30 ACGCCAAGTACCTGATCGAGAAGATCAAGCTGCACGACATGCAGGAGGGCAAGTGCCTGTA
 CAGCCTGGAGGCCATCCCCCTGGAGGACCTGCTGAACAACCCCTTCAACTACGAGGTGGAC
 GCCATCATCCCCCGGAGCGTGAGCTTCGACAACAGCTTCAACAACAAGGTGCTGGTGAAGC
 AGGAGGAGAACAGCAAGAAGGGCAACCGGACCCCCCTTCCAGTACCTGAGCAGCAGCGACA
 GCAAGATCAGCTACGAGACCTTCAAGAAGCACATCCTGAACCTGGCCAAGGGCAAGGGCCG

GATCAGCAAGACCAAGAAGGAGTACCTGCTGGAGGAGCGGGACATCAACCGGTTTCAGCGTG
CAGAAGGACTTCATCAACCGGAACCTGGTGGACACCCGGTACGCCACCCGGGGCCTGATGA
ACCTGCTGCGGAGCTACTTCCGGGTGAACAACCTGGACGTGAAGGTGaaatccATCAACGGCGG
CTTCACCAGCTTCTGCGGCGGAAGTGGAAGTTCAAGAAGGAGCGGAACAAGGGCTACAAG
5 CACCACGCCGAGGACGCCCTGATCATCGCCAACGCCGACTTCATCTTCAAGGAGTGGAAGA
AGCTGGACAAGGCCAAGAAGGTGATGGAGAACCAGATGTTTCGAGGAGAAGCAGGCCGAGA
GCATGCCCCGAGATCGAGACCGAGCAGGAGTACAAGGAGATCTTCATCACCCCCACCAGAT
CAAGCACATCAAGGACTTCAAGGACTACAAGTACAGCCACCGGGTGGACAAGAAGCCCAAC
CGGAAGCTGATCAACGACACCCTGTACAGCACCCGGAAGGACGACAAGGGCAACACCCTGA
10 TCGTGAACAACCTGAACGGCCTGTACGACAAGGACAACGACAAGCTGAAGAAGCTGATCAA
CAAGAGCCCCGAGAAGCTGCTGATGTACCACCACGACCCCCAGACCTACCAGAAGCTGAAG
CTGATCATGGAGCAGTACGGCGACGAGAAGAACCCCTGTACAAGTACTACGAGGAGACCG
GCAACTACCTGACCAAGTACAGCAAGAAGGACAACGGCCCCGTGATCAAGAAGATCAAGTA
CTACGGCAACAAGCTGAACGCCACCTGGACATCACCGACGACTACCCCAACAGCCGGAAC
15 AAGGTGGTGAAGCTGAGCCTGAAGCCCTACCGGTTTCGACGTGTACCTGGACAACGGCGTGT
ACAAGTTCGTGACCGTGAAGAACCTGGACGTGATCAAGAAGGAGAACTACTACGAGGTGAA
CAGCAAGTGCTACGAGGAGGCCAAGAAGCTGAAGAAGATCAGCAACCAGGCCGAGTTCATC
GCCAGCTTCTACAAGAACGACCTGATCAAGATCAACGGCGAGCTGTACCGGGTGATCGGCG
TGAACAACGACCTGCTGAACCGGATCGAGGTGAACATGATCGACATCACCTACCGGGAGTA
20 CCTGGAGAACATGAACGACAAGCGGCCCCCCCCACATCATCAAGACCATCGCCAGCAAGACC
CAGAGCATCAAGAAGTACAGCACCGACATCCTGGGCAACCTGTACGAGGTGaaatccAAGAAG
CACCCCCAGATCATCAAGAAGGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCGGCCAGGCCA
AGAAGAAGAAGGCCCGGGACAGCAAGGTGGAGAACAAGACCAAGAAGCTGCGGGTGTTTCG
AGGCCTTCGCCGGCATCGGCGCCCAGCGGAAGGCCCTGGAGAAGGTGCGGAAGGACGAGTA
25 CGAGATCGTGGGCTGGCCGAGTGGTACGTGCCCGCCATCGTGATGTACCAGGCCATCCACA
ACAACCTCCACACCAAGCTGGAGTACAAGAGCGTGAGCCGGGAGGAGATGATCGACTACCT
GGAGAACAAGACCCTGAGCTGGAACAGCAAGAACCCCGTGAGCAACGGCTACTGGAAGCG
GAAGAAGGACGACGAGCTGAAGATCATCTACAACGCCATCAAGCTGAGCGAGAAGGAGGG
CAACATCTTCGACATCCGGGACCTGTACAAGCGGACCCTGAAGAACATCGACCTGCTGACCT
30 ACAGCTTCCCCTGCCAGGACCTGAGCCAGCAGGGCATCCAGAAGGGCATGAAGCGGGGCAG
CGGCACCCGGAGCGGCCTGCTGTGGGAGATCGAGCGGGCCCTGGACAGCACCGAGAAGAAC
GACCTGCCCAAGTACCTGCTGATGGAGAACGTGGGCGCCCTGCTGCACAAGAAGAACGAGG
AGGAGCTGAACCAGTGGAAGCAGAAGCTGGAGAGCCTGGGCTACCAGAACAGCATCGAGG
TGCTGAACGCCGCCGACTTCGGCAGCAGCCAGGCCCGGCGGGGTGTTTCATGATCAGCAC

CCTGAACGAGTTCGTGGAGCTGCCCCAAGGGCGACAAGAAGCCCAAGAGCATCAAGAAGGTG
 CTGAACAAGATCGTGAGCGAGAAGGACATCCTGAACAACCTGCTGAAGTACAACCTGACCG
 AGTTCAAGAAaACCAAGAGCAACATCAACAAGGCCAGCCTGATCGGCTACAGCAAGTTCAA
 CAGCGAGGGCTACGTGTACGACCCCGAGTTCACCGGCCCCACCCTGACCGCCAGCGGCGCC
 5 AACAGCCGGATCAAGATCAAGGACGGCAGCAACATCCGGAAGATGAACAGCGACGAGACC
 TTCCTGTACATCGGCTTCGACAGCCAGGACGGCAAGCGGGTGAACGAGATCGAGTTCCTGAC
 CGAGAACCAGAAGATCTTCGTGTGCGGCAACAGCATCAGCGTGGAGGTGCTGGAGGCCATC
 ATCGACAAGATCGGCGGCCCCAGCAGCGGCGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCG
 GCCAGGCCAAGAAGAAGAAGGGCAGCTACCCCTACGACGTGCCCGACTACGCCTGAGcgggcg
 10 ctttaattaagctgccttctcggggcttgccttctggccatgcccttcttctcccttgcacctgtaccttggctttgaataaagcctgagtaggaagtcta
 gaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa (SEQ ID NO:
 202)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
 15 comprising dCas9, e.g., an *S. Pyogenes* dCas9 or a functional variant or mutant thereof; e.g., Cas9m4),
 and an effector moiety comprising MQ1, e.g., bacterial MQ1. In some embodiments, the site-specific
 disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 207 (e.g., a nucleic acid (e.g.,
 mRNA) encoding the site-specific disrupting agent). In some embodiments, a nucleic acid described
 herein comprises a nucleic acid sequence of SEQ ID NO: 207 or a sequence with at least 80, 85, 90, 95,
 20 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6,
 5, 4, 3, 2, or 1 positions of difference thereto. In some embodiments, the targeting moiety is encoded by
 the nucleic acid sequence of SEQ ID NO: 8 and/or the effector moiety is encoded by the nucleic acid
 sequence of SEQ ID NO: 10.

25 dCas9-MQ1 mRNA sequence (MR28125)

AAGGAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUAUAAGAGCCACCAUGGCCCCCAA
 GAAGAAGCGGAAGGUGGGCAUCCACGGCGUGCCCGCCGCCGACAAGAAGUACAGCAUCGG
 CCUGGCCAUUGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCC
 CAGCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACAGCAUCAAGAAGAACCUGAU
 30 CGGCGCCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGC
 CCGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUUCUUCAGCAA
 CGAGAUGGCCAAGGUGGACGACAGCUUCUUCACCGGCUGGAGGAGAGCUUCCUGGUGGA
 GGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUA
 CCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAA

GGCCGACCUGCGGCUGAUCUACCUGGCCCUGGCCCACAUGAUC AAGUUCGGGGGCCACUU
CCUGAUCGAGGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUCCAGCU
GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGC
CAAGGCCAUCCUGAGCGCCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUGAUCGCCCA
5 GCUGCCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCUGAGCCUGGGCCU
GACCCCCAACUUCAAGAGCAACUUCGACCUGGCGGAGGACGCCAAGCUGCAGCUGAGCAA
GGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCGGCGACCAGUACGCCGA
CCUGUUCUGGCGGCCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCUGCGGGU
GAACACCGAGAUCACCAAGGCCCCCCUGAGCGCCAGCAUGAUC AAGCGGUACGACGAGCA
10 CCACCAGGACCUGACCCUGCUGAAGGCCUGGUGCGGCAGCAGCUGCCCCGAGAAGUACAA
GGAGAUCUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCAG
CCAGGAGGAGUUCUACAAGUUCAUCAAGCCAUCCUGGAGAAGAUGGACGGCACCGAGGA
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15 CUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCCGAGAAGAUCCUGACCUUCCGGAU
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AUCCGAGGAGACCAUCACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGC
CCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCU
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20 GUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGAGCGGCGAGCAGAAGAAGGCCAU
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25 GACCGGGAGAUGAUCGAGGAGCGGCUGAAAACCUACGCCACCUGUUCGACGACAAGGUG
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AACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCUGAAAUCCGACGGC
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30 GCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUG
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CAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCA
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UGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGGCG
5 GCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGA
UCACCAAGCACGUGGCCCAGAUCCUGGACAGCCGGAUGAACACCAAGUACGACGAGAACG
ACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGCGACUUC
GGAAGGACUUCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACG
CCUACCUGAACGCCGUGGUGGGCACCGCCUGAUCAGAAGUACCCCAAGCUGGAGAGCG
10 AGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGAGCGAG
CAGGAGAUCCGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCGAACUUCUUC
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15 AAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCGGAAGAAGGACUGG
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GCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUGCUGGGCAU
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20 GGAGAACGGCCGGAAGCGGAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGCAACGAGCU
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GGACGAGAUCAUCGAGCAGAUACGCGAGUUCAGCAAGCGGGUGAUCUGGCCGACGCCAA
CCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAGGC
25 CGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUA
CUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUGGACGCCAC
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CGGCGACAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGCCCG
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30 UCGGCGCCCAGCGGAAGGCCUGGAGAAGGUGCGGAAGGACGAGUACGAGAUCGUGGGCC
UGGCCGAGUGGUACGUGCCCGCCAUCGUGAUGUACCAGGCCAUCCACAACAACUCCACA
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GACGAGCUGAAGAUCUACAACGCCAUCAAGCUGAGCGAGAAGGAGGGCAACAUCUUC

GACAUCCGGGACCUGUACAAGCGGACCCUGAAGAACAUCGACCUGCUGACCUACAGCUUC
 CCCUGCCAGGACCUGAGCCAGCAGGGCAUCCAGAAGGGGCAUGAAGCGGGGCAGCGGCACC
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 5 CUGAACCAGUGGAAGCAGAAGCUGGAGAGCCUGGGCUACCAGAACAGCAUCGAGGUGCU
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 GAACGAGUUCGUGGAGCUGCCCAAGGGCGACAAGAAGCCCAAGAGCAUCAAGAAGGUGC
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 AGUUCAAGAAAACCAAGAGCAACAUCAACAAGGCCAGCCUGAUCGGCUACAGCAAGUUA
 10 ACAGCGAGGGCUACGUGUACGACCCCGAGUUCACCGGCCCCACCCUGACCGCCAGCGGCG
 CCAACAGCCGGAUCAAGAUAAGGACGGCAGCAACAUCGGAAGAUGAACAGCGACGAGA
 CCUUCUGUACAUCGGCUUCGACAGCCAGGACGGCAAGCGGGUGAACGAGAUCGAGUUC
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 GCCAUAUCGACAAGAUCGGCGGGCCCCAGCAGCGGGCGGCAAGCGGCCCGCCGCCACCAAG
 15 AAGGCCGGCCAGGCCAAGAAGAAGAAGGGCAGCUACCCCUACGACGUGCCCGACUACGCC
 UGAGCGGCCGCUUAAUUAAGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU
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 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 207)

20

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 ID NOs: 203, 208, 73 or 74. In some embodiments, a site-specific disrupting agent described herein
 comprises an amino acid sequence of SEQ ID NO: 203, 208, 73 or 74, or a sequence with at least 80, 85,
 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9,
 25 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

Sa-dCas9-MQ1 Protein sequence:

MAPKKRKRKVGIIHGVPAAPAAKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRR
 SKRGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELGINPYEARVKGLSQKLSEEFSAALLHLA
 30 KRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYV
 KEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEKSPFGWKDIKEWYEMLMGHCTYF
 PEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYKFQIIENVFKQKKKPTLKQIAKEILV
 NEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTIYQSSEDIQEELTNLNS
 ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPPKKVDLSQQKEIPTTL

VDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIELAREKNSKDAQKMINEMQKRN RQTNERIEEII
 RTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNPNFNYEVD AII PRSVSFDNSFNKVLV
 KQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNLA KGKGRISKTKKEYLLEERDINRFSVQKDFI
 NRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHH AED
 5 ALIIANADFIFKEWK KLDKAKKVMENQMFE EKQAESMPEIETE QEYKEIFITPHQIKHIKDFKDYK
 YSHRVDK KPNRKLINDTL YSTRKDDKGNTLIVNNLNGLYDKDNDK LKKLINKSPEKLLMYHHD
 PQTYQKLKLIMEQY GDEKNPLYKY YEETGNYLTKY SKKDN GPVIKKIKYYGNKLNAHL DITDD
 YPNSRNKVVKLSLKP YRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLKKISNQ
 AEFIASFYKNDLIKINGEL YRVIGVNNDLLNRIEVNMIDITYREYLENMNDKRPPHIKTIASKTQSI
 10 KKYSTDILGNLYEVKSKKHPQIIKKGRPAATKKAGQAKKKKARDSKVENKTKKLRVFEAFAGI
 GAQRKALEKVRKDEYEIVGLAEWYVPAIVMYQAIHNNFHTKLEYKSVSREEMIDYLENKTL SW
 NSKNPVSNGYWKRKKDDELKIIYNAIKLSEKEGNIFDIRDL YKRTLKNIDLLTYSFPCQDLSQQGI
 QKGMKRGSGTRSGLLWEIERALDSTEKNDLPKYLLMENVGALLHKKNEEELNQWKQKLES LG
 YQNSIEVLNAADFGSSQARRRVFMISTLNEFVELPKGD KPKSIKKVLNKIVSEKDILNNLLKYNL
 15 TEFKKT KSNINKASLIGYSKFNSEGYVYDPEFTGPTLTASGANSRIKIKDGSNIRKMNSDETFLYIG
 FDSQDGKRVNEIEFLTENQKIFVCGNSISVEVLEAII DKIGGPSSGGKRPAATKKAGQAKKKKGSY
 PYDVDPDYA (SEQ ID NO: 203)

dCas9-MQ1 Protein sequence (corresponding to MR-28125):

20 MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL EESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLR LIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLI ALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLS DAILLSDILRVNT
 25 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFY PFLKDNREKIEK
 ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVT VKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT
 30 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL KSDGFANRNFMQLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPA IKKGILQTVKVVDELVKVMGRH KPENIVIEMAREN
 QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK

SKLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 5 ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIQISEFSKRVLAD
 ANLDKVLSAYNKHDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKARDSKVENKTKKLRVFEAFAGIGAQRKALE
 KVRKDEYEIVGLAEWYVPAIVMYQAIHNNFHTKLEYKSVSREEMIDYLENKTLSWNSKNPVS
 GYWKRRKKDELKIIYNAIKLSEKEGNIFDIRDLYKRTLKNIDLLTYSFPCQDLSQQGIQKGMKRG
 10 SGTRSGLLWEIERALDSTEKNDLPKYLLMENVGALLHKKNEEELNQWKQKLESLGYQNSIEVLN
 AADFGSSQARRRVFMISTLNEFVELPKGDKPKPSIKKVLNKKIVSEKDILNNLLKYNLTEFKKTKS
 NINKASLIGYSKFNSEGYVYDPEFTGPTLTASGANSRIKIKDGSNIRKMNSDETFLYIGFDSQDGK
 RVNEIEFLTENQKIFVCGNSISVEVLEAIIKIGGPSSGGKRPAATKKAGQAKKKKGSYPYDVPDY
 A (SEQ ID NO: 208)

15

Sa-dCas9-MQ1 without HA tag

MAPKKKRKVGIHGVPAAAKRNILGLAIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRR
 SKRGARRLKRHHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKLSSEEFSAALLHLA
 KRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLERLKKDGEVRGSINRFKTSYV
 20 KEAKQLLKVQKAYHQLDQSFIDTYIDLLETRRTYYEGPGEKSPFGWKDIKEWYEMLMGHCTYF
 PEELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYEYEFQIENVFQKQKKPTLKQIAKEILV
 NEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIENAEELDQIAKILTIYQSSEDIQEELTNLNS
 ELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTL
 VDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIELAREKNSKDAQKMINEMQKRNRTNERIEEII
 25 RTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNPNFYEVDAIIPRSVSFDNSFNKVLV
 KQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKEYLLEERDINRFSVQKDFI
 NRNLVDTRYATRGLMNNLSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHAED
 ALIIANADFIFKEWKLDKAKKVMENQMFEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYK
 YSHRVDKKPNRKLINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLLKLINKSPEKLLMYHHD
 30 PQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLN AHLDTDD
 YPNSRNKVVKLSLKPYPYFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLLKISNQ
 AEFASFYKNDLIKINGELYRVIGVNNDLLNRIEVMIDITYREYLENMNDKRPPHIIKTIASKTQSI
 KKYSTDILGNLYEVKSKKHPQIIKKGRPAATKKAGQAKKKKARDSKVENKTKKLRVFEAFAGI
 GAQRKALEKVRKDEYEIVGLAEWYVPAIVMYQAIHNNFHTKLEYKSVSREEMIDYLENKTLSW

NSKNPVSNNGYWKRKKDDELKIIYNAIKLSEKEGNIFDIRDLYKRTLKNIDLLTYSFPCQDLSQQGI
 QKGMKRGSGLLWEIERALDSTEKNDLPKYLLMENVGALLHKKNEEELNQWKQKLES LG
 YQNSIEVLNAADFGSSQARRRVFMISTLNEFVELPKGDKKPKSIKKVLNKIVSEKDILNNLLKYNL
 TEFKKTCSNINKASLIGYSKFNSEGYVYDPEFTGPTLTASGANSRIKIKDGSNIRKMNSDETFLYIG
 5 FDSQDGKRVNEIEFLTENQKIFVCGNSISVEVLEAIIKIGGPSSGGKRPAATKKAGQAKKKKGS
 (SEQ ID NO: 73)

dCas9-MQ1 without HA tag

MAPKKRKRKVGIIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLI
 10 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRILIYLAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIAS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADFLAAKNLS DAILSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 15 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
 ILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGSASAQSFIERMTNFDKNLPNEKV
 LPKHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS DGFANRNFQMQLIHDDSL
 20 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 QTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 25 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSEFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 30 SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKARDSKVENKTKKLRVFEAFAGIGAQRKALE
 KVRKDEYEIVGLAEWYVPAIVMYQAIHNNFHTKLEYKSVSREEMIDYLENKTLSWNSKNPVSN
 GYWKRKKDDELKIIYNAIKLSEKEGNIFDIRDLYKRTLKNIDLLTYSFPCQDLSQQGIQKGMKRGS
 GLLWEIERALDSTEKNDLPKYLLMENVGALLHKKNEEELNQWKQKLES LGYQNSIEVLN
 AADFGSSQARRRVFMISTLNEFVELPKGDKKPKSIKKVLNKIVSEKDILNNLLKYNL TEFKKTCS

NINKASLIGYSKFNSEGYVYDPEFTGPTLTASGANSRIKIKDGSNIRKMNSDETFLYIGFDSQDGK
RVNEIEFLTENQKIFVCGNSISVEVLEAIIKIGGPSSGGKRPAATKKAGQAKKKKGS (SEQ ID
NO: 74)

5 In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
comprising dCas9, e.g., an *S. pyogenes* dCas9), and an effector moiety comprising KRAB, e.g., a KRAB
domain. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence
of SEQ ID NOs: 204 (e.g., a plasmid encoding the site-specific disrupting agent) and/or 205 (e.g., a
nucleic acid (e.g., mRNA) encoding the site-specific disrupting agent). In some embodiments, a nucleic
10 acid described herein comprises a nucleic acid sequence of SEQ ID NO: 204 or 205 or a sequence with at
least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13,
12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto. In some embodiments, the targeting
moiety is encoded by the nucleic acid sequence of SEQ ID NO. 8 and the effector moiety is encoded by
the nucleic acid sequence of SEQ ID NO. 14.

15

Sp-dCas9-KRAB (PL-27687) Plasmid DNA sequence:

TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACA
GCTTGTCTGTAAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTG
GCGGGTGTCGGGGCTGGCTTAACCTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCAT
20 ATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTTCG
CATTACAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAG
CTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGT
CACGACGTTGTAAAACGACGGCCAGTGAATTGACGCGTATTGGGATGGTACCTAATACGACT
CACTATAAGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCC
25 CCCAAGAAGAAGCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCCGACAAGAAGTACAGC
ATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCGGTGATACCGACGAGTACAAGG
TGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCT
GATCGGCGCCCTGCTGTTTCGACAGCGGCGAGACCGCCGAGGCCACCCGGCTGAAGCGGACC
GCCC GGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCA
30 ACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGGTGG
GGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGCCTAC
CACGAGAAGTACCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGG
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ATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGTGC

AGACCTACAACCAGCTGTTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGACGCCAAGGC
 CATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCC
 GCGAGAAGAAGAACGGCCTGTTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCA
 ACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTA
 5 CGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCTTGG
 CCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGAT
 CACCAAGGCCCCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGACCTG
 ACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCCGAGAAGTACAAGGAGATCTTCTTCG
 ACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCGCCAGCCAGGAGGAGTTCTA
 10 CAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTG
 AACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCACCAGA
 TCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCTGAAG
 GACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCCCCCT
 GGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGaaatccGAGGAGACCATCACCCCTG
 15 GAACTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGGATGACC
 AACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGT
 ACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCC
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 AAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCGACAGCG
 20 TGGAGATCAGCGGCGTGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCT
 GAAGATCATCAAGGACAAGGACTTCTTGACAACGAGGAGAACGAGGACATCCTGGAGGA
 CATCGTGCTGACCCTGACCCTGTTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGAaAACCT
 ACGCCCACCTGTTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACACCGGCTG
 GGGCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATC
 25 CTGGACTTCCTGaaatccGACGGCTTCGCCAACCGGAACCTTCATGCAGCTGATCCACGACGACA
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 30 AGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAGCACCCCGTGG
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 CCCGGGGCAAGAGCGACAACGTGCCAGCGAGGAGGTGGTGAAGAAGATGAAGAACTACT

GGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACCTGACCAAGGC
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ACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCTG^{Gaaatcc}AAGCTGGTGAGCG
5 ACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAACCTACCACCACGCCCAC
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AGACCGAGATCACCTGGCCAACGGCGAGATCCGGAAGCGGCCCTGATCGAGACCAACGG
10 CGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTG
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AGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCC
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15 GAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGG
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20 GCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTG
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CGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCA
CCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACAAGCGGCCCGCCGC
25 CACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGCCAGCGACGCCAAGAGCCTGACCGC
CTGGAGCCGGACCCTGGTGACCTTCAAGGACGTGTTTCGTGGACTTCACCCGGGAGGAGTGG
AAGCTGCTGGACACCGCCCAGCAGATCCTGTACCGGAACGTGATGCTGGAGAACTACAAGA
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30 GCCTTCGAGATCAAGAGCAGCGTGAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAGGCCG
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^{cttaattaagctgccttctg}^{cgccg}^{gcttgccttctg}^{ccatgcccttctctcccttg}^{cacctgtacctcttg}^{gtcttgaataaagcctgagtaggaagtcta}
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^{tcag}ATCCCAATGGCGCGCCGAGCTTGGCTCGAGCATGGTCATAGCTGTTTCCTGTGTGAAAT

TGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCTTTCCAGTCGG
GAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCG
TATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTCGGCTGCGGCG
5 AGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCA
GGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTG
CTGGCGTTTTTCCATAGGCTCCGCCCTTGACGAGCATCACAAAATCGACGCTCAAGTCA
GAGGTGGCGAAACCCGACAGGACTATAAAGATAACCAGGCGTTTCCCCCTGGAAGCTCCCTC
GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGA
10 AGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCC
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TCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACA
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CGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAA
15 AAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGT
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GGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAA
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20 ATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAA
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CCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATC
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25 ATTCGTGATTGCGCCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAA
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30 AAACAACCTCTGGCGCATCGGGCTTCCCATACAATCGATAGATTGTGCGACCTGATTGCCCGA
CATTATCGCGAGCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGC
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AATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTA
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Sp-dCas9-KRAB (MR-28122) Expressed mRNA sequence:

5 AGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCCAAGA
AGAAGCGGAAGGTGGGCATCCACGGCGTGCCCCGCCGCGACAAGAAGTACAGCATCGGCCT
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AAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCG
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10 GCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTCAGCAACGAGATG
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GTACCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGACAAGGCCGACCTG
CGGCTGATCTACCTGGCCCTGGCCCACATGATCAAGTTCCGGGGGCCACTTCCTGATCGAGGG
15 CGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTTCATCCAGCTGGTGCAGACCTAC
AACCAGCTGTTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGA
GCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCTGCCCGGCGAGAA
GAAGAACGGCCTGTTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGA
GCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACACCTACGACGACGA
20 CCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTCTGGCCGCCAAGA
ACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCGAGATCACCAAGGC
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25 CAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAGCTGAACCGGGAG
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GCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTGAAGGACAACCG
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30 GGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGAC
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TGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCGGAAGCCCGCCTTCCT
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GCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGACCTGCTGAAGATCAT
CAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTGGAGGACATCGTGCTG
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5 AGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAAGACCATCCTGGACTTCC
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CAAGGAGGACATCCAGAAGGCCAGGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACAT
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GACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCGAGAACATCGTGATCGAGATGGCCC
10 GGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAGCAGCCGGGAGCGGATGAAGCGGATCG
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15 AGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATGAAGAATACTGGCGGCAGC
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ATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAACACCAAGTACGACGAGAACG
ACAAGCTGATCCGGGAGGTGAAGGTGATCACCTGaatccAAGCTGGTGAGCGACTTCCGGA
20 AGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAATACTACCACCACGCCCACGACGCCTA
CCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTC
GTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGA
TCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACATCATGAATTTCTTCAAGACCGAG
ATCACCTGGCCAACGGCGAGATCCGGAAGCGGCCCTGATCGAGACCAACGGCGAGACCG
25 GCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCC
CCAGGTGAACATCGTGAAGAAaACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATC
CTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGT
ACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAA
GGGCAAGAGCAAGAAGCTGaatccGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAG
30 CAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAG
GACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTTCGAGCTGGAGAACGGCCGGAAGCGGA
TGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCAGCAAGTACGT
GAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAG
CAGAAGCAGCTGTTTCGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCA

GCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTA
 CAACAAGCACCGGGACAAGCCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTACC
 CTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCG
 GTACACCAGCACCAAGGAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTG
 5 TACGAGACCCGGATCGACCTGAGCCAGCTGGGCGGCGACAAGCGGCCCGCCGCCACCAAGA
 AGGCCGGCCAGGCCAAGAAGAAGAAGGCCAGCGACGCCAAGAGCCTGACCGCCTGGAGCC
 GGACCCTGGTGACCTTCAAGGACGTGTTCTGTGGACTTCACCCGGGAGGAGTGGAAGCTGCTG
 GACACCGCCCAGCAGATCCTGTACCGGAACGTGATGCTGGAGAACTACAAGAACCTGGTGA
 GCCTGGGCTACCAGCTGACCAAGCCCGACGTGATCCTGCGGCTGGAGAAGGGCGAGGAGCC
 10 CTGGCTGGTGGAGCGGGAGATCCACCAGGAGACCCACCCGACAGCGAGACCGCCTTCGAG
 ATCAAGAGCAGCGTGAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCC
 AAGAAGAAGAAGGGCAGCTACCCCTACGACGTGCCCCGACTACGCCTGAGcgggccgcttaattaagctgc
 cttctcggggcttgcttcttgccatgcccttctctcccttgacactgtacctcttggtcttgaataaagcctgagtaggaagtctagaaaaaaaaa
 aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa (SEQ ID NO: 205)

15 In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ ID
 NOs: 206 or 75. In some embodiments, a nucleic acid described herein comprises a nucleic acid sequence
 of SEQ ID NO: 206 or 75 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or
 having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of
 20 difference thereto.

Sp-dCas9-KRAB Protein sequence:

MAPKKRKRKVGIIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLVESFLVEEDKKH
 25 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
 30 ILTFRIPYYVGPLARGNSRFWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN

QTTQKGQKNSRERMKRIEEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 5 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 10 SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKASDAKSLTAW SRTLVTFKDVFVDFTREEWK
 LLDTAQQILYRNVML ENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIQETHPDSETAFEIK
 SSVSGGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 206)

Sp-dCas9-KRAB Protein sequence without HA tag:

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLI
 15 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFHRLEESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 20 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
 ILTRIPYYVGPLARGNSRF AWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLEYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKS DGFANRNF MQLIHDDSL
 25 TFKEDIQKAQVSGQGDSLHEHIANLAGSPA IKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 QTTQKGQKNSRERMKRIEEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 30 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ

SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKASDAKSLTAWSTRLVTFKDVFDFTREEWK
 LLDTAQQILYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIK
 SSVSGGKRPAATKKAGQAKKKKGS (SEQ ID NO: 75)

5 In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
 comprising dCas9, e.g., an *S. aureus* dCas9) and an effector moiety comprising EZH2. In some
 embodiments, a site-specific disrupting agent comprises a targeting moiety comprising dCas9, e.g., an *S.*
pyogenes dCas9, e.g., mutant *S. pyogenes* Cas9, e.g., Cas9m4 and an effector moiety comprising EZH2.
 In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ
 10 ID NO: 209 (e.g., mRNA encoding the site-specific disrupting agent). In some embodiments, a nucleic
 acid described herein comprises a nucleic acid sequence of SEQ ID NO: 209 or a sequence with at least
 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11,
 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto. In some embodiments, the targeting moiety is
 encoded by the nucleic acid sequence of SEQ ID NO: 8 and/or the effector moiety is encoded by the
 15 nucleic acid sequence of SEQ ID NO: 18.

EZH2-dCas9 mRNA (MR28938)

AGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAUAGAGCCACCAUGGCCCCCAAG
 20 AAGAAGCGGAAGGUGGGCGGCAGCGGCGGCAGCGGCCAGACCGGCAAGAAGAGCGAGAA
 GGGCCCCGUGUGCUGGCGGAAGCGGGUGAAGAGCGAGUACAUGCGGCUGCGGCAGCUGA
 AGCGGUUCCGGCGGGCCGACGAGGUGAAGAGCAUGUUCAGCAGCAACCGGCAGAAGAUGC
 UGGAGCGGACCGAGAUCCUGAACCAGGAGUGGAAGCAGCGGCGAAUCCAGCCCUGGCACA
 UCCUGACCAGCGUGAGCAGCCUGCGGGGCACCCGGGAGUGCAGCGUGACCAGCGACCUGG
 25 ACUUCCCCACCCAGGUGAUCCCCCUAAAGACCCUGAACGCCGUGGGCCAGCGUGCCCAUCA
 UGUACAGCUGGAGCCCCCUGCAGCAGAACUUAUGGUGGAGGACGAGACCGUGCUGCACA
 ACAUCCCCUACAUGGGCGACGAGGUGCUGGACCAGGACGGCACCUUCAUCGAGGAGCUGA
 UCAAGAACUACGACGGCAAGGUGCACGGCGACCGGGAGUGCGGCUUCAUAACGACGAGA
 UCUUCGUGGAGCUGGUGAACGCCUGGGCCAGUACAACGACGACGACGACGACGACGACG
 30 GCGACGACCCCGAGGAGCGGGAGGAGAAGCAGAAGGACCUGGAGGACCACCGGGACGACA
 AGGAGAGCCGGCCCCCCCCGGAAGUCCCCAGCGACAAGAUCUUCGAGGCCAUCAGCAGCA
 UGUUCCCCGACAAGGGCACCGCCGAGGAGCUGAAGGAGAAGUACAAGGAGCUGACCGAGC
 AGCAGCUGCCCGGCGCCUGCCCCCGAGUGCACCCCAACAUCGACGGCCCCAACGCCAA
 GAGCGUGCAGCGGGAGCAGAGCCUGCACAGCUUCCACACCCUGUUCUGCCGGCGGUGCUU

CAAGUACGACUGCUUCCUGCACCCCUUCCACGCCACCCCAACACCUACAAGCGGAAGAA
CACCGAGACCGCCCUGGACAACAAGCCCUGCGGCCCCCAGUGCUACCAGCACCUGGAGGG
CGCCAAGGAGUUCGCCGCCGCCUGACCGCCGAGCGGAUCAAGACCCCCCAAGCGGCCC
GGCGGCCGGCGGGGGCCGGCUGCCCAACAACAGCAGCCGGCCCAGCACCCCCACCAUCA
5 ACGUGCUGGAGAGCAAGGACACCGACAGCGACCGGGAGGCCGGCACCGAGACCGGCGGCG
AGAACAACGACAAGGAGGAGGAGGAGAAGAAGGACGAGACCAGCAGCAGCAGCAGGACC
AACAGCCGGUGCCAGACCCCCAUCAAGAUGAAGCCCAACAUCGAGCCCCCGAGAACGUG
GAGUGGAGCGGCGCCGAGGCCAGCAUGUUCGGGUGCUGAUCGGCACCUACUACGACAAC
UUCUGCGCCAUCGCCCGGCUGAUCGGCACCAAGACCUGCCGGCAGGUGUACGAGUUCGG
10 GUGAAGGAGAGCAGCAUCAUCGCCCCGCCCCCGCCGAGGACGUGGACACCCCCCCCCG
AAGAAGAAGCGGAAGCACCGGCUGUGGGCCGCCACUGCCGGAAGAUCAGCUGAAGAAG
GACGGCAGCAGCAACCACGUGUACAACUACCAGCCCUGCGACCACCCCCGGCAGCCCUGC
GACAGCAGCUGCCCCUGCGUGAUCGCCCAGAACUUCUGCGAGAAGUUCUGCCAGUGCAGC
AGCGAGUGCCAGAACCGGUUCCCCGGCUGCCGGUGCAAGGCCAGUGCAACACCAAGCAG
15 UGCCCCUGCUACCUGGCCGUGCGGGAGUGCGACCCCGACCUGUGCCUGACCUGCGGGCGCC
GCCGACCACUGGGACAGCAAGAACGUGAGCUGCAAGAACUGCAGCAUCCAGCGGGGCAGC
AAGAAGCACCUUGCUGCUGGGCCCCAGCGACGUGGCCGGCUGGGGCAUCUUCAUCAAGGAC
CCCGUGCAGAAGAACGAGUUCAUCAGCGAGUACUGCGGCGAGAUAUCAGCCAGGACGAG
GCCGACCGGCGGGGCAAGGUGUACGACAAGUACAUGUGCAGCUUCCUGUUCAACCUGAAC
20 AACGACUUCGUGGUGGACGCCACCCGGAAGGGCAACAAGAUCCGGUUCGCCAACCACAGC
GUGAACCCCAACUGCUACGCCAAGGUGAUGAUGGUGAACGGCGACCACCGGAUCGGCAUC
UUCGCCAAGCGGGCCAUCAGACCGGCGAGGAGCUGUUCUUCGACUACCGGUACAGCCAG
GCCGACGCCUGAAGUACGUGGGCAUCGAGCGGGAGAUUGGAGAUCCCAGCACCGGCGGC
AGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCCGACCCGACAAGAAGUAC
25 AGCAUCGGCCUGGCCAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUAC
AAGGUGCCCAGCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACAGCAUCAAGAAG
AACCUGAUCGGCGCCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAG
CGGACCGCCCGGCGGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUC
UUCAGCAACGAGAUGGCCAAGGUGGACGACAGCUUCUUCACCGGCUGGAGGAGAGCUUC
30 CUGGUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAG
GUGGCCUACCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGC
ACCGACAAGGCCGACCUGCGGCUGAUCUACCUGGCCUGGCCACAUGAUAAGUUCGG
GGCCACUUCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUC
AUCCAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGC

GUGGACGCCAAGGCCAUCCUGAGCGCCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUG
AUCGCCCAGCUGCCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCCUGAGC
CUGGGCCUGACCCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAG
CUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGGCCCAGAUCGGCGACCAG
5 UACGCCGACCUGUUCUGGGCCGCCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUC
CUGCGGGUGAACACCGAGAUACCAAGGCCCCCCUGAGCGCCAGCAUGAUCAAGCGGUAC
GACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUUGGUGCGGCAGCAGCUGCCCGAG
AAGUACAAGGAGAUCUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGC
GGCGCCAGCCAGGAGGAGUUCUACAAGUUAUCAAGCCCAUCCUGGAGAAGAUGGACGGC
10 ACCGAGGAGCUGCUGGUGAAGCUGAACC GGGAGGACCUGCUGCGGAAGCAGCGGACCUUC
GACAACGGCAGCAUCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGGCGG
CAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCUGACC
UUCGGGAUCCCCUACUACGUGGGCCCCUGGCCCGGGGCAACAGCCGGUUCGCCUGGAUG
ACCCGGAAAUCCGAGGAGACCAUACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGC
15 GCCAGCGCCCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAG
AAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACC
AAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGAGCGGCGAGCAGAAG
AAGGCCAUUCGUGGACCUGCUGUUAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAG
GAGGACUACUUAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGA
20 CCGGUUAACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGA
CUUCUGGACAACGAGGAGAACGAGGACAUCUGGAGGACAUCGUGCUGACCCUGACCCU
GUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAAACCUACGCCACCUGUUCGACGA
CAAGGUGAUGAAGCAGCUGAAGCGGCGGCGGUACACCGGCUGGGGCGGCUGAGCCGGAA
GCUGAUCAACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCUGAAAUC
25 CGACGGCUUCGCCAACCGGAACUUAUGCAGCUGAUCCACGACGACAGCCUGACCUUCAA
GGAGGACAUC CAGAAGGCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAU CGC
CAACCUGGCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGA
CGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCG
GGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGA
30 GGAGGGCAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCA
GCUGCAGAACGAGAAGCUGUACCUUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGA
CCAGGAGCUGGACAUCAACCGGCUGAGCGACUACGACGUGGCCGCCAUUCGUGCCCCAGAG
CUUCUGAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGGCCCGGGG
CAAGAGCGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGC

AGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGC
 GGGGCGGCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCC
 GGCAGAUACCAAGCACGUGGCCCAGAUCCUGGACAGCCGGAUGAACACCAAGUACGACG
 AGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGC
 5 GACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUAACAACUACCACCACGCC
 CACGACGCCUACCUGAACGCCGUGGUGGGCACCGCCCUGAUAAGAAGUACCCCAAGCUG
 GAGAGCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAA
 GAGCGAGCAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUAUGAA
 CUUCUUAAGACCGAGAUCACCCUGGCCAACGGCGAGAUCGGAAGCGGCCCCUGAUCGA
 10 GACCAACGGCGAGACCGGCGAGAUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCG
 GAAGGUGCUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAAACCGAGGUGCAGACCGGCG
 GCUUCAGCAAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCGGAAGA
 AGGACUGGGACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUGGCCUACAGCGUGC
 UGGUGGUGGCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUG
 15 CUGGGCAUCACCAUCAUGGAGCGGAGCAGCUUCGAGAAGAACCCCAUCGACUUCCUGGAG
 GCCAAGGGCUACAAGGAGGUGAAGAAGGACCUGAUAUCAAGCUGCCCAAGUACAGCCUG
 UUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGC
 AACGAGCUGGCCUGCCCAGCAAGUACGUGAACUUCUGUACCUGGCCAGCCACUACGAG
 AAGCUGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAG
 20 CACUACCUGGACGAGAUAUCGAGCAGAUACAGCGAGUUCAGCAAGCGGGUGAUCCUGGCC
 GACGCCAACCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGG
 GAGCAGGCCGAGAACAUAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCC
 UUCAAGUACUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUG
 GACGCCACCCUGAUCCACCAGAGCAUACCGGCCUGUACGAGACCCGGAUCGACCUGAGC
 25 CAGCUGGGCGGCGACAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCC
 AAGAAGAAGAAGGGCAGCUACCCCUACGACGUGCCCGACUACGCCUGAGCGGCCGCUUAA
 UUAAGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUUGCACCU
 GUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 30 AAAAA (SEQ ID NO: 209)

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ ID NOs: 210 or 76. In some embodiments, a nucleic acid described herein comprises a nucleic acid sequence of SEQ ID NO: 210 or 76 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity

thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

EZH2-dCas9 Protein Sequence (corresponding to MR-28938)

5 MAPKKKRKVGGSGSGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQK
ILERTEILNQEWKQRRIQPVHILTSVSSLRGTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSP
LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIEELIKNYDGKVHGDRECGFINDEIFVELVNALG
QYNDDDDDDDDGDDPEEREKQKDLEDHRDDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEK
YKELTEQQLPGALPPECTPNIDGPNAKSVQREQSLHSFHTLFCRRCFKYDCFLHPPHATPNTYKR
10 KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVL
ESKDTSDREAGTETGGENNDKEEEEEKDETSSSSEANSRCQTPIKMKNIEPPENVEWSGAEAS
MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVRKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
RKIQLKKDGSSNHVYNYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQC
TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCNCSIQRGSKKHLLLAPSDVAGWGIFIKDP
15 VQKNEFISEYCGEISQDEADRRGKVYDKYMCNFLNNDNFVVDATRKGNKIRFANHSVNPNC
YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGSGSGSGG
SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLVESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
20 TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNF
DLAEDAQLQSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYYVG
PLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLEY
25 FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVM
KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQV
SGQGDSLHEHIANLAGSPAICKGILQTVKVVDDELVKVMGRHKPENIVIEEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAAI
30 VPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA
TAKYFFYSNIMNFFKTEITLANGEIRKPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKT
EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKKGKSKKLKSVKE

LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
YKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSTGLYETRI
DLSQLGGDSGGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 210)

5

EZH2-dCas9 without HA tag

MAPKKKRKVGGSGGSGQTGKKSEKGPVCWRKRKVKSEYMRLRQLKRFRRADEVKSMFSSNRQK
ILERTEILNQEWKQRRIQPVHILTSVSSLRGTTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSP
LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALG
10 QYNDDDDDDDDGDDPEEREKQKDLLEDHRDDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEK
YKELTEQQLPGALPPECTPNIDGPNASVQREQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKR
KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNSSSRPSTPTINVL
ESKDTDSREAGTETGGENNDKEEEEKKDETSSSSEANSRCQTPIKMKPNIEPPENVEWSGAEAS
MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
15 RKIQLKKDGSSNHVYNYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQC
TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLAPSDVAGWGIFIKDP
VQKNEFISEYCGEISQDEADRRGKVYDKYMCNFLNNDVVDATRKGNKIRFANHSVNPNC
YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGGSGGSGG
SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
20 EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF
DLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
25 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYYVG
PLARGNSRFAWMTRKSEETITPWNFEEVVDKGGASQSFIERMTNFDKNLPNEKVLPKHSLLYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYAHLFDDKVM
KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDIQKAQV
30 SGQGDSLHEHIANLAGSPAIIKGILQTVKVVDLVKVMGRHKPENIVIEARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVA
VPQSFLKDDSIDNKVLTRSDKARGKSDNPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLESELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA

TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKT
 EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKE
 LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
 PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
 5 YNKHARDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
 SITGLYETRI
 DLSQLGGDSGGKRPAATKKAGQAKKKKGS (SEQ ID NO: 76)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
 comprising dCas9, e.g., an *S. aureus* dCas9), and an effector moiety comprising DNMT3 (e.g.,
 10 DNMT3a/3L). In some embodiments, a site-specific disrupting agent comprises a targeting moiety
 comprising dCas9, e.g., an *S. pyogenes* dCas9, e.g., mutant *S. pyogenes* Cas9, e.g., Cas9m4 and an
 effector moiety comprising DNMT3 (e.g., DNMT3a/3L). In some embodiments, the site-specific
 disrupting agent is encoded by the nucleic acid sequence of SEQ ID NO: 211 (e.g., mRNA encoding the
 site-specific disrupting agent). In some embodiments, a nucleic acid described herein comprises a nucleic
 15 acid sequence of SEQ ID NO: 211 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto,
 or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of
 difference thereto.

dCas9-DNMT3a/3L mRNA (MR-29414)

20 AGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAUUAAGAGCCACCAUGGCCCAAG
 AAGAAGCGGAAGGUGGGCAUCCACGCGUGCCCGCCGCGACAAGAAGUACAGCAUCGGC
 CUGGCCAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCC
 AGCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACAGCAUCAAGAAGAACCUGAUC
 GCGGCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCC
 25 CGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUUUCAGCAAC
 GAGAUGGCCAAGGUGGACGACAGCUUCUCCACCGGCUGGAGGAGAGCUUCCUGGUGGA
 GGAGGACAAGAAGCAGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUA
 CCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAA
 GGCCGACCUGCGGCUGAUCUACCUGGCCUGGCCACAUGAUCAGUUCGGGGCCACUU
 30 CCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUAUCCAGCU
 GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGC
 CAAGGCCAUCCUGAGCGCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUGAUCGCCCA
 GCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCUGAGCCUGGGCCU
 GACCCCAACUUAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGAGCAA

GGACACCUACGACGACGACCUGGACAACCUGCUGGGCCAGAU CGGGCGACCAGUACGCCGA
CCUGUUCUGGGCCGCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCUGCGGGU
GAACACCGAGAUACCAAGGCCCCCUGAGCGCCAGCAUGAUCAAGCGGUACGACGAGCA
CCACCAGGACCUGACCCUGCUGAAGGCCCUGGUGCGGCAGCAGCUGCCCCGAGAAGUACAA
5 GGAGAUCUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCCAG
CCAGGAGGAGUUCUACAAGUUCAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGA
GCUGCUGGUGAAGCUGAACC GGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGG
CAGCAUCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGGCGGCAGGAGGA
CUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAU CGAGAAGAUCUGACCUUCCGGAU
10 CCCCUCUACGUGGGCCCCCUGGCCCGGGCAACAGCCGGUUCGCCUGGAUGACCCGGAA
AUCCGAGGAGACCAUACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGC
CCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCU
GCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAA
GUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGAGCGGCGAGCAGAAGAAGGCCAU
15 CGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACU
ACUUCAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACCGGUUC
AACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCUG
GACAACGAGGAGAACGAGGACAUCUGGAGGACAUCGUGCUGACCCUGACCCUGUUCGAG
GACCGGGAGAUGAU CGAGGAGCGGCUGAAAACCUACGCCACCUGUUCGACGACAAGGUG
20 AUGAAGCAGCUGAAGCGGCGGCGGUACACCGGCUGGGGCGGCUGAGCCGGAAGCUGAUC
AACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCUGAAAUCGACGGC
UUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACCUUCAAGGAGGAC
AUCCAGAAGGCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAU CGCCAACCUG
GCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUG
25 GUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCGGGAGAA
CCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGAGGAGGG
CAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCA
GAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGA
GCUGGACAUCAACCGGCUGAGCGACUACGACGUGGCCGCCAU CGUGCCCCAGAGCUUCCU
30 GAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGGCCCGGGCAAGAG
CGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUGC
UGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGGCG
GCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGA
UCACCAAGCACGUGGCCCAGAUCUGGACAGCCGGAUGAACACCAAGUACGACGAGAACG

ACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGCGACUUC
GGAAGGACUUC CAGUUCUACAAGGUGCGGGAGAUAACAACUACCACCACGCCACGACG
CCUACCUGAACGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUGGAGAGCG
AGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGAGCGAG
5 CAGGAGAUCGGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC
AAGACCGAGAUCACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAAC
GGCGAGACCGGCGAGAUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUG
CUGAGCAUGCCCCAGGUGAACAUUCGUGAAGAAAACCGAGGUGCAGACCGGCGGCUUCAGC
AAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCCGGAAGAAGGACUGG
10 GACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUGGCCUACAGCGUGCUGGUGGUG
GCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUGCUGGGCAU
CACCAUCAUGGAGCGGAGCAGCUUCGAGAAGAACCCCAUCGACUUCUGGAGGCCAAGGG
CUACAAGGAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACAGCCUGUUCGAGCU
GGAGAACGGCCGGAAGCGGAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGCAACGAGCU
15 GGCCCUGCCCAGCAAGUACGUGAACUUCUGUACCUGGCCAGCCACUACGAGAAGCUGAA
GGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCU
GGACGAGAUCAUCGAGCAGAUACGCGAGUUCAGCAAGCGGGUGAUCUGGCCGACGCCAA
CCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAGGC
CGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUA
20 CUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUGGACGCCAC
CCUGAUCCACCAGAGCAUCACCGGCCUGUACGAGACCCGGAUCGACCUGAGCCAGCUGGG
CGGCGACAGCGCCGGCGGGCGGCAGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGGCGG
CCCCAAGAAGAAGCGGAAGGUGGCCGCCGCCGAGCAACCACGACCAGGAGUUCGACCC
CCCCAAGGUGUACCCCCCGUGCCCGCCGAGAAGCGGAAGCCCAUCCGGGUGCUGAGCCU
25 GUUCGACGGCAUCGCCACCGGCCUGCUGGUGCUGAAGGACCUGGGCAUCCAGGUGGACCG
GUACAUCGCCAGCGAGGUGUGCGAGGACAGCAUCACCGUGGGCAUGGUGCGGCACCAGGG
CAAGAUAUGUACGUGGGCGACGUGCGGAGCGUGACCCAGAAGCACAUCCAGGAGUGGG
GCCCCUUCGACCUGGUGAUCGGCGGCAGCCCCUGCAACGACCUGAGCAUCGUGAACCCCG
CCCGGAAGGGCCUGUACGAGGGCACCGGCCGGCUGUUCUUCGAGUUCUACCGGCUGCUGC
30 ACGACGCCCGGCCAAGGAGGGCGACGACCGGCCCUUCUUCUGGCUGUUCGAGAACGUGG
UGGCCAUGGGCGUGAGCGACAAGCGGGACAUCAGCCGGUUCUGGAGAGCAACCCCGUGA
UGAUCGACGCCAAGGAGGUGAGCGCCGCCACCGGGCCCGGUACUUCUGGGGCAACCUGC
CCGGCAUGAACCGGCCCCUGGCCAGCACCGUGAACGACAAGCUGGAGCUGCAGGAGUGCC
UGGAGCACGGCCGGAUCGCCAAGUUCAGCAAGGUGCGGACCAUACACCACCCGGAGCAACA

GCAUCAAGCAGGGCAAGGACCAGCACUCCCCGUGUUCAUGAACGAGAAGGAGGACAUCC
 UGUGGUGCACCGAGAUGGAGCGGGUGUUCGGCUCCCCGUGCACUACACCGACGUGAGCA
 ACAUGAGCCGGCUGGGCCCGGCAGCGGCUGCUGGGCCGGAGCUGGAGCGUGCCCGUGAUCC
 GGCACCUGUUCGCCCCCUGAAGGAGUACUUCGCCUGCGUGAGCAGCGGCAACAGCAACG
 5 CCAACAGCCGGGGCCCCAGCUUCAGCAGCGGCCUGGUGCCCCUGAGCCUGCGGGGCAGCC
 ACAUGAAUCCUCUGGAGAUGUUCGAGACAGUGCCCGUGUGGAGAAGGCAACCCGUGAGG
 GUGCUGAGCCUCUUCGAGGACAUAAGAAGGAGCUGACCUCUCUGGGCUUUCUGGAAUCC
 GGCAGCGACCCCGGCCAGCUGAAACACGUGGUGGACGUGACCGACACAGUGAGGAAGGAC
 GUGGAAGAGUGGGGGCCCCUUGACCUCGUGUAUGGAGCCACACCUCUCUGGCCACACA
 10 UGCGAUAGGCCUCCCAGCUGGUAUCUCUCCAGUCCACAGACUGCUCCAGUACGCCAGA
 CCUAAGCCCGGCAGCCCCAGACCCUUCUUCUGGAUGUUCGUGGACAAUCUGGUGCUGAAC
 AAGGAGGAUCUGGAUGUGGCCAGCAGAUUUCUGGAGAUGGAACCCGUGACAAUCCCCGA
 CGUGCAUGGGCGGCUCUCUGCAGAACGCCGUGAGAGUGUGGUCCAACAUCCCCGCCAUUAG
 AAGCAGACACUGGGCUCUGGUGAGCGAGGAGGAACUGUCUCUGCUGGCCCAGAAUAAGC
 15 AGUCCUCCAAGCUGGCCGCCAAGUGGCCACCAAGCUGGUGAAGAACUGCUUUCUGCCUC
 UGAGGGAGUAUUUCAAGUAUUUCAGCACCGAACUGACCAGCAGCCUGAGCGGGCGGCAAGC
 GGCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAAGGGCAGCUACCCCUACG
 ACGUGCCCGACUACGCCUGAGCGGCCGCUUAAUUAAGCUGCCUUCUGCGGGGCUUGCCUU
 CUGGCCAUGCCCUUCUUCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUG
 20 AGUAGGAAGUCUAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 211)

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 ID NOs: 211 or 77. In some embodiments, a construct described herein comprises an amino acid
 25 sequence of SEQ ID NO: 211 or 77 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity
 thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 positions of difference thereto.

dCas9-DNMT3a/3L Protein Sequence (corresponding to MR-29414)
 30 MAPKKRKRKVGIIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLLESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT

EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEK
 ILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 5 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 10 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 15 ELQKGNELALPSKYVNFLYLASHYEKLKGPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 SITGLYETRIDLSQLGGDSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSNHDQEFDPKVPYPPVP
 AEKRKPIRVLSLFDGIATGLLVKDLGIQVDRIASEVCEDSITVGMVRHQGKIMYVG D VRSVTQ
 KHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFEN
 20 VVAMGVSDKRDISRFLESNPVMIDAKEVSAHRARYFWGNLPGMNRPLASTVNDKLELQECLE
 HGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLAR
 QRLLGRSWSVPVIRHLFAPLKEYFACVSSGNSNANSRGPSFSSGLVPLSLRGSHMNPLEMFETVP
 VWRRQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVV DVTDTVRKDVEEWGPFDLVYGATP
 LGHTCDRPPSWYLFQFHRLQYARPKGPSRPFFWMFVDNLVLNKEDLDVASRFLEMEPV TIPD
 25 VHGGSLQNAV RVWSNIPAIRSRHWALVSEEELSLAQNKQSSKLA AKWPTKLVKNCFLPLREYF
 KYFSTELTSSLSGGKRPAATKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 212)

dCas-DNMT3a/3L (h) without HA tag

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 30 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFHRL EESFLVEEDKKH
 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDL DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQSKNGYAGYIDGGASQEEFYKF

IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
 ILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLEYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 5 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 QTTQKGQKNSRERMKRIEEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 10 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 15 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 SITGLYETRIDLSQLGGDSAGGGGSGGGGSGGGGSGPKKKRKVAAAGSNHDQEFDPKVPVPPV
 AEKRKPIRVLSLFDGIATGLLVKDLGIQVDRIASEVCEDSITVGMVRHQGKIMYVGDVRSVTQ
 KHIQEWGPFDLVIGGSPCNDLSIVNPARKGLYEGTGRLFFEFYRLLHDARPKEGDDRPFFWLFEN
 VVAMGVSDKRDISRFLSNPVMIDAKEVSAHRARYFWGNLPGMNRPLASTVNDKLELQECLE
 20 HGRIAKFSKVRTITTRSNSIKQGKDQHFPVFMNEKEDILWCTEMERVFGFPVHYTDVSNMSRLAR
 QRLLGRSWSVPVIRHLFAPLKEYFACVSSGNSNANSRGPSSFSSGLVPLSLRGSHMNPLEMFTVP
 VWRRQPVRVLSLFEDIKKELTSLGFLESGSDPGQLKHVVDTDTVRKDV EEWGPFDLVYGATP
 LGHTCDRPPSWYLFQFHRLLQYARPKGPSRPFFWFMFVDNLVLNKEDLDVASRFLMEPV TIPD
 VHGGSLQNAV RVWSNIPAIRSRHWALVSEEELSLAQNKQSSKLA AKWPTKL VKNCF LPLREYF
 25 KYFSTELTSSLSGGKRPAATKKAGQAKKKKGS (SEQ ID NO: 77)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
 comprising dCas9, e.g., an *S. aureus* dCas9), and an effector moiety comprising HDAC8, e.g., a HDAC8
 domain. In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising
 30 dCas9, e.g., an *S. pyogenes* dCas9, e.g., mutant *S. pyogenes* Cas9, e.g., Cas9m4, and an effector moiety
 comprising HDAC8, e.g., a HDAC8 domain. In some embodiments, the site-specific disrupting agent is
 encoded by the nucleic acid sequence of SEQ ID NO: 213 (e.g., mRNA encoding the site-specific
 disrupting agent).

In some embodiments, a nucleic acid described herein comprises a nucleic acid sequence of SEQ ID NO: 213 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

5 dCas9-HDAC8 mRNA (MR-29439)

AGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAAAGAGCCACCAUGGCCCCCAAG
AAGAAGCGGAAGGUGGGCAUCCACGGCGUGCCCCGCCGCCGACAAGAAGUACAGCAUCGGC
CUGGCCAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCC
AGCAAGAAGUUAAGGUGCUGGGCAACACCGACCGGCACAGCAUCAAGAAGAACCUGAUC
10 GCGCGCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCC
CGGCGGCGGUACACCCGGCGGAAGAACCUGAUCUGCUACCUGCAGGAGAUUUUAGCAAC
GAGAUGGCCAAGGUGGACGACAGCUUCUCCACCGGCUGGAGGAGAGCUUCCUGGUGGA
GGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUA
CCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAA
15 GGCCGACCUGCGGCUGAUCUACCUGGCCUGGCCACAUGAUCAGUUCGGGGCCACUU
CCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUAUCCAGCU
GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGC
CAAGGCCAUCCUGAGCGCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUGAUCGCCCA
GCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCUGAGCCUGGGCCU
20 GACCCCAACUUAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGAGCAA
GGACACCUACGACGACGACCUGGACAACCUGCUGGCCAGAUCCGGCGACCAGUACGCCGA
CCUGUUCUGGCCGCCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCUGCGGGU
GAACACCGAGAUCACCAAGGCCCCCGAGCGCCAGCAUGAUCAGCGGUACGACGAGCA
CCACCAGGACCUGACCCUGCUGAAGGCCUGGUGCGGCAGCAGCUGCCCGAGAAGUACAA
25 GGAGAUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCAG
CCAGGAGGAGUUCUACAAGUUAUCAAGCCCAUCCUGGAGAAGAUGGACGGCACCGAGGA
GCUGCUGGUGAAGCUGAACCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGG
CAGCAUCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGGCGGAGGAGGA
CUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCUGACCUUCCGGAU
30 CCCCUCUACGUGGGCCCCCGGGCCGAGCCGGUUCGCCUGGAUGACCCGGAA
AUCCGAGGAGACCAUCACCCCGGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGC
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ACUUCAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACCGGUUC
AACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCCUG
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5 GACCGGGAGAUGAUCGAGGAGCGGCUGAAAACCUACGCCCACCUGUUCGACGACAAGGUG
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AACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCCUGAAAUCCGACGGC
UUCGCCAACCGGAACUUCAUGCAGCUGAUCCACGACGACAGCCUGACCUUCAAGGAGGAC
AUCCAGAAGGCCAGGUGAGCGGCCAGGGCGACAGCCUGCAGGAGCACAUCGCCAACCUG
10 GCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUG
GUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCGGGAGAA
CCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGAGGAGGG
CAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCA
GAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGA
15 GCUGGACAUCAACCGGCUGAGCGACUACGACGUGGCCGCCAUCGUGCCCCAGAGCUUCCU
GAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGGCCCGGGCAAGAG
CGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUGC
UGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGGCG
GCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGA
20 UCACCAAGCACGUGGCCCAGAUCUGGACAGCCGGAUGAACACCAAGUACGACGAGAACG
ACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGCGACUCC
GGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCCACGACG
CCUACCUGAACGCCGUGGUGGGCACCGCCUGAUCAGAAGUACCCCAAGCUGGAGAGCG
AGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGAGCGAG
25 CAGGAGAUCCGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAACUUCUUC
AAGACCGAGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAAC
GGCGAGACCGGCGAGAUUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUG
CUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAAACCGAGGUGCAGACCGGCGGCUUCAGC
AAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCGGAAGAAGGACUGG
30 GACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUGGCCUACAGCGUGCUGGUGGUG
GCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUGCUGGGCAU
CACCAUCAUGGAGCGGAGCAGCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGG
CUACAAGGAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACAGCCUGUUCGAGCU
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GGCCCUGCCCAGCAAGUACGUGAACUUCCUGUACCUGGCCAGCCACUACGAGAAGCUGAA
GGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCU
GGACGAGAUCAUCGAGCAGAUACGCGAGUUCAGCAAGCGGGUGAUCCUGGCCGACGCCAA
CCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAGGC
5 CGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUAAGUA
CUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUGGACGCCAC
CCUGAUCCACCAGAGCAUCACCGGCCUGUACGAGACCCGGAUCGACCUGAGCCAGCUGGG
CGGCGACAGCGGCGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCGCGCCAGGCCAAGAAGAA
GAAGUCGGGCGGGGGUGGCUCAGAGGAGCCCGAGGAGCCCGCCGAUAGCGGACAAUCUCU
10 GGUGCCCGUCUACAUCUACAGCCCCGAAUAUGUGAGCAUGUGUGAUUCCCUCGCCAAGAU
CCCUAAGAGAGCCAGCAUGGUGCAUUCUCUGAUUCGAGGCCUACGCUCUGCAUAAGCAAAU
GAGGAUCGUGAAGCCCAAGGUCGCCAGCAUGGAAGAGAUGGCCACCUUUCACACCGAUGC
CUACCUCCAACAUCUCCAGAAGGUGUCCCAAGAGGGCGACGACGACCACCCCGACUCCAU
UGAGUACGGACUGGGCUAUGAUUGCCCCGCCACCGAGGGCAUCUUUGACUAUGCCGCCGC
15 UAUCGGCGGAGCUACCAUCACAGCCGCCAGUGUCUGAUUGAUGGCAUGUGCAAGGUCGC
CAUCAACUGGUCCGGAGGCUGGCAUCAUGCCAAGAAGGAUGAGGCCUCCGGCUUCUGUUA
UCUGAAUGACGCCGUGCUGGGCAUUCUGAGACUGAGGAGGAAAUUCGAGAGGAUUCUGU
ACGUGGAUCUGGAUCUGCAUCACGGAGAUGGAGUCGAAGAUGCCUUCAGCUUCACCAGCA
AGGUGAUGACAGUCUCUCUGCACAAGUUCUCCCCCGGCUUCUUUCCCGGAACCGGCGACG
20 UGUCCGACGUGGGACUGGGCAAGGGAAGGUACUACAGCGUGAACGUGCCCAUUCAAGAC
GGCAUCCAAGACGAGAAGUACUACCAGAUCUGCGAGUCCGUGCUCUAAAGGAGGUCUACCAA
GCCUCAAUCCUAAGGCUGUCGUGCUCUCAAACUGGGAGCUGAUACCAUUGCUGGCGAUCCC
AUGUGCAGCUUCAUAUGACACCCGUCGGAUUCGGCAAGUGCCUCAAGUACAUCUCCAG
UGGCAGCUCGCCACCCUCAUUCUCGGAGGAGGCGGAUACAAUCUGGCUAAUACCGCCAGA
25 UGCUGGACCUAUCUGACCGGCGUGAUUCUGGGCAAAACACUGAGCAGCGAAAUCCCCGAC
CACGAGUUUUUCACCGCUUACGGCCCCGACUACGUGCUGGAGAUACCCCCAGCUGCAGA
CCCGAUAGAAACGAACCCCAUAGAAUCCAGCAAAUUCUGAACUAUAUCAAGGGCAACCUC
AAGCACGUCGUGGGAGGUGGCGGAUCGGGAAAGCGGCCCGCCGCCACCAAGAAGGCCGGU
CAGGCCAAGAAGAAGAAGGGCAGCUACCCCUACGACGUGCCCGACUACGCCUGAGCGGCC
30 GCUUAAUUAAGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCUCCCUU
GCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAA (SEQ ID NO: 213)

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ ID NOs: 214 or 78. In some embodiments, a site-specific disrupting agent described herein comprises an amino acid sequence of SEQ ID NO: 214 or 78 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

dCas9-HDAC8 Protein Sequence (corresponding to MR 29439)

MAPKKKRKVGIIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEFSNEMAKVDDSFHRLSEESFLVEEDKKH
 10 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRILIYLAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
 15 ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 20 QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVA AIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 25 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSEEPPEPADSGQSLVPVYIYSPE
 30 YVSMCDSLAKIPKRASMVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHLQKVSQEG
 DDDHPDSIEYGLGYDCPATEGIFDYAAAIGGATITAAQCLIDGMCKVAINWSGGWHHAKKDEAS
 GFCYLND AVLGLRLRRKFERILYVDLDLHHGDGVEDAFSFTSKVMTVSLHKFSPGFFPGTGDVS
 DVGLGKGRYYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNPKAVVLQLGADTIAGDPMCSFN
 MTPVGIGKCLKYILQWQLATLILGGGGYNLANTARCWYLTGVILGKTLSSSEIPDHEFFTAYGPD

YVLEITPSCRPD RNEPHRIQQILNYIKGNLKHVVGGGGSGKRPAATKKAGQAKKKKGSYPYDVP
DYA (SEQ ID NO: 214)

dCas9-HDAC8 without HA tag

5 MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEFSNEMAKVDDSSFFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALS
LGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
10 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNREKIEK
ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
KIECFDSVEISGVEDRFNASLGT YHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
15 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF MQLIHDDSL
TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVA AIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
20 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
25 ANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSEEP EEPADSGQSLVPVYIYSPE
YVSMCDSLAKIPKRAS MVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHLQKVSQEG
DDDHPDSIEYGLGYDCPATEGIFDYAAAIGGATITAAQCLIDGMCKVAINWSGGWHHAKKDEAS
GFCYLND AVLGILRLRRKFERILYVDLDLHHGDGVEDAFSFTSKVMTVSLHKFSPGFFPGTGDVS
30 DVGLGKGRYYSVNVPIQDGIQDEKYYQICESVLKEVYQAFNP KAVVLQLGADTIAGDPMCSFN
MTPVGIGKCLKYILQWQLATLILGGGGYNLANTARCW TYLTGVILGKTLSS EIPDHEFFTAYGPD
YVLEITPSCRPD RNEPHRIQQILNYIKGNLKHVVGGGGSGKRPAATKKAGQAKKKKGS (SEQ ID
NO: 78)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g., comprising dCas9, e.g., an *S. aureus* dCas9), a first effector moiety comprising EZH2; e.g., an EZH2 domain, and a second effector moiety comprising HDAC8, e.g., an HDAC8 domain. In some

5 embodiments, a site-specific disrupting agent comprises a targeting moiety comprising dCas9, e.g., an *S. pyogenes* dCas9, e.g., a mutant *S. pyogenes* Cas9, e.g., Cas9m4; a first effector moiety comprising EZH2, e.g., an EZH2 domain; and a second effector moiety comprising HDAC8, e.g., an HDAC8 domain. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NO: 215 (e.g., mRNA encoding the site-specific disrupting agent). In some embodiments, a nucleic acid

10 described herein comprises a nucleic acid sequence of SEQ ID NO: 215 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

15 EZH2-dCas9-HDAC8 mRNA (MR-29447)

AGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGCCCCCAAG
 AAGAAGCGGAAGGUGGGCGGCAGCGGCGGCAGCGGCCAGACCGGCAAGAAGAGCGAGAA
 GGGCCCCGUGUGCUGGCGGAAGCGGGUGAAGAGCGAGUACAUGCGGCUGCGGCAGCUGA
 AGCGGUUCCGGCGGGCCGACGAGGUGAAGAGCAUGUUCAGCAGCAACCGGCAGAAGAUAUC
 20 UGGAGCGGACCGAGAUCCUGAACCAGGAGUGGAAGCAGCGGCGAAUCCAGCCCGUGCACA
 UCCUGACCAGCGUGAGCAGCCUGCGGGGCACCCGGGAGUGCAGCGUGACCAGCGACCUGG
 ACUUCCCCACCCAGGUGAUCCCCCUAAAGACCCUGAACGCCGUGGCCAGCGUGCCCAUCA
 UGUACAGCUGGAGCCCCCUGCAGCAGAACUUAUGGUGGAGGACGAGACCGUGCUGCACA
 ACAUCCCCUACAUGGGCGACGAGGUGCUGGACCAGGACGGCACCUUCAUCGAGGAGCUGA
 25 UCAAGAACUACGACGGCAAGGUGCACGGCGACCGGGAGUGCGGCUUCAUACGACGAGA
 UCUUCGUGGAGCUGGUGAACGCCCUUGGGCCAGUACAACGACGACGACGACGACGACGACG
 GCGACGACCCCGAGGAGCGGGAGGAGAAGCAGAAGGACCUGGAGGACCACCGGGACGACA
 AGGAGAGCCGGCCCCCCCCGGAAGUUCCCCAGCGACAAGAUCUUCGAGGCCAUCAGCAGCA
 UGUUCCCCGACAAGGGCACCGCCGAGGAGCUGAAGGAGAAGUACAAGGAGCUGACCGAGC
 30 AGCAGCUGCCCGGCGCCUGCCCCCGAGUGCACCCCCAACAUCGACGGCCCCAACGCCAA
 GAGCGUGCAGCGGGAGCAGAGCCUGCACAGCUUCCACACCCUGUUCUGCCGGCGGUGCUU
 CAAGUACGACUGCUUCCUGCACCCCCUCCACGCCACCCCCAACACCUACAAGCGGAAGAA
 CACCGAGACCGCCUGGACAACAAGCCCUGCGGCCCCCAGUGCUACCAGCACCUGGAGGG
 CGCCAAGGAGUUCGCCGCCGCCUGACCGCCGAGCGGAUCAAGACCCCCCCCCAAGCGGCCC

GGCGGCCGGCGGGCGGGGCGGCUGCCCAACAACAGCAGCCGGCCCAGCACCCCCACCAUCA
ACGUGCUGGAGAGCAAGGACACCGACAGCGACCGGGAGGCCGGCACCGAGACCGGGCGGCG
AGAACAACGACAAGGAGGAGGAGGAGAAGAAGGACGAGACCAGCAGCAGCAGCGAGGCC
AACAGCCGGUGCCAGACCCCCAUCAAGAUGAAGCCCAACAUCGAGCCCCCGAGAACGUG
5 GAGUGGAGCGGCGCCGAGGCCAGCAUGUUCGGGUGCUGAUCGGCACCUACUACGACAAC
UUCUGCGCCAUCGCCCCGGCUGAUCGGCACCAAGACCUGCCGGCAGGUGUACGAGUUCGG
GUGAAGGAGAGCAGCAUCAUCGCCCCCGCCCCGCGGAGGACGUGGACACCCCCCCCCGG
AAGAAGAAGCGGAAGCACCGGCUGUGGGCCGCCACUGCCGGAAGAUCAGCUGAAGAAG
GACGGCAGCAGCAACCACGUGUACAACUACCAGCCCUGCGACCACCCCCGGCAGCCCUGC
10 GACAGCAGCUGCCCCUGCGUGAUCGCCCAGAACUUCUGCGAGAAGUUCUGCCAGUGCAGC
AGCGAGUGCCAGAACCGGUUCCCCGGCUGCCGGUGCAAGGCCAGUGCAACACCAAGCAG
UGCCCCUGCUACCUGGCCGUGCGGGAGUGCGACCCCGACCUGUGCCUGACCUGCGGGCGCC
GCCGACCACUGGGACAGCAAGAACGUGAGCUGCAAGAACUGCAGCAUCCAGCGGGGCAGC
AAGAAGCACCUUGCUGCUGGCCCCCAGCGACGUGGCGCGCUGGGGCAUCUUCAUCAAGGAC
15 CCGUGCAGAAGAACGAGUUCAUCAGCGAGUACUGCGGCGAGAUAUCAGCCAGGACGAG
GCCGACCGGGCGGGGCAAGGUGUACGACAAGUACAUGUGCAGCUUCCUGUUCAACCUGAAC
AACGACUUCGUGGUGGACGCCACCCGGAAGGGCAACAAGAUCCGGUUCGCCAACCACAGC
GUGAACCCCAACUGCUACGCCAAGGUGAUGAUGGUGAACGGCGACCACCGGAUCGGCAUC
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20 GCCGACGCCUGAAGUACGUGGGCAUCGAGCGGGAGAUGGAGAUGCCAGCACCGGCGGC
AGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCCGACCCGACAAGAAGUAC
AGCAUCGGCCUGGCCAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUAC
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25 CGGACCGCCCGGCGGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUC
UUCAGCAACGAGAUGGCCAAGGUGGACGACAGCUUCUCCACCGGCUGGAGGAGAGCUUC
CUGGUGGAGGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAG
GUGGCCUACCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGC
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30 GGCCACUUCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUC
AUCCAGCUGGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGC
GUGGACGCCAAGGCCAUCCUGAGCGCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUG
AUCGCCAGCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCUGAGC
CUGGGCCUGACCCCCAACUUAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAG

CUGAGCAAGGACACCUACGACGACGACCUGGACAACCUGCUGGCCAGAUCCGGCGACCAG
UACGCCGACCUGUUCUUGGCCGCCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUC
CUGCGGGUGAACACCGAGAUACCAAGGCCCCCUGAGCGCCAGCAUGAUCAAGCGGUAC
GACGAGCACCACCAGGACCUGACCCUGCUGAAGGCCCUGGUGCGGCAGCAGCUGCCCGAG
5 AAGUACAAGGAGAUUCUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGC
GGCGCCAGCCAGGAGGAGUUCUACAAGUUCAUAAGCCCAUCCUGGAGAAGAUGGACGGC
ACCGAGGAGCUGCUGGUGAAGCUGAACCAGGAGGACCUGCUGCGGAAGCAGCGGACCUUC
GACAACGGCAGCAUCCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGG
CAGGAGGACUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCUGACC
10 UUCGGAUCCCCUACUACGUGGGCCCCCUGGCCCGGGGCAACAGCCGGUUCGCCUGGAUG
ACCCGGAAUCCGAGGAGACCAUACCCCCUGGAACUUCGAGGAGGUGGUGGACAAGGGC
GCCAGCGCCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAG
AAGGUGCUGCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACC
AAGGUGAAGUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGAGCGGCGAGCAGAAG
15 AAGGCCAUUCGUGGACCUGCUGUUAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAG
GAGGACUACUUAAGAAGAUUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGA
CCGGUUAACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGA
CUUCCUGGACAACGAGGAGAACGAGGACAUCUCCUGGAGGACAUCGUGCUGACCCUGACCCU
GUUCGAGGACCGGGAGAUGAUCGAGGAGCGGCUGAAAACCUACGCCACCUGUUCGACGA
20 CAAGGUGAUGAAGCAGCUGAAGCGGCGGCGGUACACCGGCUGGGGCGGCUGAGCCGGAA
GCUGAUCAACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCUGAAAUC
CGACGGCUUCGCCAACCGGAACUUAUGCAGCUGAUCCACGACGACAGCCUGACCUUCAA
GGAGGACAUCAGAGAAGGCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAUCCG
CAACCUGGCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGA
25 CGAGCUGGUGAAGGUGAUGGGCCGGCACAAGCCCGAGAACAUCGUGAUCGAGAUGGCCCG
GGAGAACCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGA
GGAGGGCAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCA
GCUGCAGAACGAGAAGCUGUACCUUACUACCUGCAGAACGGCCGGGACAUGUACGUGGA
CCAGGAGCUGGACAUCAACCGGCUGAGCGACUACGACGUGGCCGCCAUUCGUGCCCCAGAG
30 CUUCCUGAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGGCCCGGG
CAAGAGCGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGC
AGCUGCUGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGC
GGGGCGGCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCC
GGCAGAUACCAAGCACGUGGCCCAGAUCCUGGACAGCCGGAUGAACACCAAGUACGACG

AGAACGACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGC
GACUUCCGGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUAACAACUACCACCACGCC
CACGACGCCUACCUGAACGCCGUGGUGGGCACCGCCCUGAUCAAGAAGUACCCCAAGCUG
GAGAGCGAGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAA
5 GAGCGAGCAGGAGAUCCGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUCAUGAA
CUUCUUAAGACCGGAGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGA
GACCAACGGCGAGACCGGGGAGAUUGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCG
GAAGGUGCUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAAACCGAGGUGCAGACCGGCG
GCUUCAGCAAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCGGAAGA
10 AGGACUGGGACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUGGCCUACAGCGUGC
UGGUGGUGGCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUG
CUGGGCAUCACCAUCAUGGAGCGGAGCAGCUUCGAGAAGAACCCCAUCGACUUCUGGAG
GCCAAGGGCUACAAGGAGGUGAAGAAGGACCUGAUCAUCAAGCUGCCCAAGUACAGCCUG
UUCGAGCUGGAGAACGGCCGGAAGCGGAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGC
15 AACGAGCUGGGCCUGCCCAGCAAGUACGUGAACUUCUGUACCUGGCCAGCCACUACGAG
AAGCUGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAG
CACUACCUGGACGAGAUCAUCGAGCAGAUCAAGCGAGUUCAGCAAGCGGGUGAUCCUGGCC
GACGCCAACCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGG
GAGCAGGCCGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCC
20 UUCAAGUACUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUG
GACGCCACCCUGAUCCACCAGAGCAUACCGGCCUGUACGAGACCCGGAUCGACCUGAGC
CAGCUGGGCGGCGACAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAGGCCGGCCAGGCC
AAGAAGAAGAAGUCGGGCGGGGGUGGCUCAGAGGAGCCCGAGGAGCCCGCCGAUAGCGG
ACAAUCUCUGGUGCCCGUCUACAUCUACAGCCCCGAAUAUGUGAGCAUGUGUGAUUCCCU
25 CGCCAAGAUCCCUAAGAGAGCCAGCAUGGUGCAUUCUCUGAUCGAGGCCUACGCUCUGCA
UAAGCAAAUGAGGAUCGUGAAGCCCAAGGUCGCCAGCAUGGAAGAGAUGGCCACCUUUC
ACACCGAUGCCUACCUCCAACAUCUCCAGAAGGUGUCCCAAGAGGGCGACGACGACCACC
CCGACUCCAUGAGUACGGACUGGGCUAUGAUUGCCCCGCCACCGAGGGCAUCUUUGACU
AUGCCGCCGCUAUCGGCGGAGCUACCAUCACAGCCGCCAGUGUCUGAUUGAUGGCAUGU
30 GCAAGGUCGCCAUCAACUGGUCCGGAGGCUGGCAUCAUGCCAAGAAGGAUGAGGCCUCCG
GCUUCUGUUUUCUGAAUGACGCCGUGCUGGGCAUUCUGAGACUGAGGAGGAAAUUCGAG
AGGAUUCUGUACGUGGAUCUGGAUCUGCAUCACGGAGAUGGAGUCGAAGAUGCCUUCAG
CUUACACAGCAAGGUGAUGACAGUCUCUCUGCACAAGUUCUCCCCGGCUUCUUUCCCGG
AACCGGCGACGUGUCCGACGUGGGACUGGGCAAGGGAAGGUACUACAGCGUGAACGUGCC

CAUUCAAGACGGCAUCCAAGACGAGAAGUACUACCAGAUCUGCGAGUCCGUGCUCAAGGA
 GGUCUACCAAGCCUUCUAAUCCUAAAGGCUGUCGUGCUCCAACUGGGAGCUGAUACCAUUGC
 UGGCGAUCCCAUGUGCAGCUUCAUAUGACACCCGUCGGAAUCGGCAAGUGCCUCAAGUA
 CAUCCUCCAGUGGCAGCUCGCCACCCUCAUUCUCGGAGGAGGCGGAUACAAUCUGGCUAA
 5 UACCGCCAGAUGCUGGACCUAUCUGACCGGCGUGAUUCUGGGCAAAACACUGAGCAGCGA
 AAUCCCCGACCACGAGUUUUUACCGGCUUACGGCCCCGACUACGUGCUGGAGAUCACCCC
 CAGCUGCAGACCCGAUAGAAACGAACCCCAUAGAAUCCAGCAAAUUCUGAACUAUAUCAA
 GGGCAACCUCAAGCACGUCGUGGGAGGUGGCGGAUCGGGAAAGCGGCCCCGCCGCCACCAA
 GAAGGCCGGUCAGGCCAAGAAGAAGAAGGGGCAGCUACCCCUACGACGUGCCCGACUACGC
 10 CUGAGCGGCCGCUUAAUUAAGCUGCCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCU
 UCUCUCCCUUGCACCUGUACCUCUUGGUCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 215)

15 In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 ID NOs: 216 or 79. In some embodiments, a site-specific disrupting agent described herein comprises an
 amino acid sequence of SEQ ID NO: 216 or 79 or a sequence with at least 80, 85, 90, 95, 99, or 100%
 identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 positions of difference thereto.

20 EZH2-dCas9-HDAC8 Protein Sequence (corresponding to MR-29447)
 MAPKKKRKVGGSGSGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQK
 ILERTEILNQEWKQRRIQPVHILTSVSSLRGTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSP
 LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALG
 25 QYNDDDDDDDDGDDPEEREKQKDLEDHRDDKESRPPRKFPSDKIFEAISSMFPDKGTAEELKEK
 YKELTEQQLPGALPPECTPNIDGPNKSVQREQLHSFHTLFCRRCFKYDCFLHPFHATPNTYKR
 KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVL
 ESKDTS DREAGTETGGENNDKEEEEEKKDETSSSSEANSRCQTPIKMKNIEPPENVEWSGAEAS
 MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
 30 RKIQLKKDGSNNHVYNYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQCN
 TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLAPSDVAGWGIFIKDP
 VQKNEFISEYCGEISQDEADRRGKVYDKYMCSFLFNLNDFVVDATRKGNKIRFANHSVNPNC
 YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGGSGGSGG
 SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA

EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFFHRLEESFLVEEDKKHERHPIFGNIVDE
 VAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
 TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNF
 DLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
 5 IKRYDEHHQDLTLLKALVRQQLEPKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTRIPYYVG
 PLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSHLLYEY
 FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
 VEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVM
 10 KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQV
 SGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEEMARENQTTQKGQKNS
 RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAAI
 VPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQRKFDNLTKAE
 RGGLELKDAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
 15 FQFYKVVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA
 TAKYFFYSNIMNFFKTEITLANGEIRKPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKT
 EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEVGKSKKLKSVKE
 LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELAL
 PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVLSA
 20 YNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSLITGLYETRI
 DLSQLGGDSGGKRPAAATKKAGQAKKKKSGGGGSEEPPEPADSGQSLVPVYIYSPEYVSMCDLSA
 KIPKRASMVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHLQKVSQEGDDDDHPDSIEY
 GLGYDCPATEGIFDYAAAIGGATITAAQCLIDGMCKVAINWSGGWHHAKKDEASGFCYLNDAV
 LGILRLRRKFERILYVDLDLHHGDGVEDAFSFTSKVMTVSLHKFSPGFFPGTGDVSDVGLGKGRY
 25 YSVNVPIQDGIQDEKYYQICESVLKEVYQAFNPKAVVLQLGADTIAGDPMCSFNMTVPVGIGKCL
 KYILQWQLATLILGGGGYNLANTARCWTYLTGVILGKTLSSSEIPDHEFFTAYGPDYVLEITPSCR
 DRNEPHRIQQILNYIKGNLKHVVGGGGSGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID
 NO: 216)

30 EZH2-dCas9-HDAC8 Protein Sequence (corresponding to MR-29447)
 MAPKKKRVGGSGSGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQK
 ILERTEILNQEWKQRRIQPVHILTSVSSLRGTRECSVTSDDLDFPTQVIPLKTLNAVASVPIMYSWSP
 LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALG
 QYNDDDDDDDDGDDPEEREKQKDLEDHRDDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEK

YKELTEQQPLPGALPPECTPNIDGPNKSVQREQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKR
KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVL
ESKDTDSREAGTETGGENNDKEEEEKKDETSSSSEANSRCQTPIKMKNIEPPENVEWSGAEAS
MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
5 RKIQLKKDGSSNHVYNYQPCDHPRQPCDSSPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQC
TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLLAPSDVAGWGIFIKDP
VQKNEFISEYCGEISQDEADRRGKVYDKYMCSFLFNLNDFVVDATRKGKNKIRFANHSVNPNC
YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGGSGGSGG
SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
10 EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNIVDE
VAYHEKYPTIYHLRKKLVDSTDKADRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
TYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNF
DLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSASM
IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
15 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVG
PLARGNSRFAMTRKSEETITPWNFEVVDKKGASQSFIERMTNFDKNLPNEKVLPKHSLLEYEY
FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVM
KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQV
20 SGQGDSLHEHIANLAGSPAIKKGILQTVKVVDDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVA
VPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKMKNYWRQLLNAKLITQRKFDNLTKAE
RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
FQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA
25 TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVKKT
EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKE
LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLEFENGRKRMLASAGELQKGNELAL
PSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL
YNAKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSTGLYETRI
30 DLSQLGGDSGGKRPAAATKKAGQAKKKKSGGGGSEEPPEPADSGQSLVPVYIYSPEYVSMCD
SLAKIPKRASMVHSLIEAYALHKQMRIVKPKVASMEEMATFHTDAYLQHLQKVSQEGDDHDPDSIEY
GLGYDCPATEGIFDYAAAIGGATITAAQCLIDGMCKVAINWSGGWHHAKKDEASGFCYLND
AVLGILRLRRKFERILYVDLDLHHGDGVEDAFSFTSKVMTVSLHKFSPGFFPGTGDVSDVGLGKGRY
YSVNVPIQDGIQDEKYYQICESVLKEYYQAFNPKAVVLQLGADTIAGDPMCSFNMTVPVGIGKCL

KYILQWQLATLILGGGGYNLANTARCWTYLTGVILGKTLSSSEIPDHEFFTAYGPDYVLEITPSCR
DRNEPHRIQILNYIKGNLKHVVGGGGSGKRPAATKKAGQAKKKKGS (SEQ ID NO: 79)

5 In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g., comprising dCas9, e.g., an *S. aureus* dCas9), a first effector moiety comprising G9A; e.g., a G9A domain, and a second effector moiety comprising EZH2, e.g., an EZH2 domain. In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising dCas9, e.g., an *S. pyogenes* dCas9, e.g., a mutant *S. pyogenes* Cas9, e.g., Cas9m4; a first effector moiety comprising G9A, e.g., a G9A domain;
10 and a second effector moiety comprising EZH2, e.g., a EZH2 domain. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NO: 69 (e.g., mRNA encoding the site-specific disrupting agent). In some embodiments, a nucleic acid described herein comprises a nucleic acid sequence of SEQ ID NO: 69 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3,
15 2, or 1 positions of difference thereto.

G9A-dCas9-EZH2 (MR-29441) mRNA sequence

AGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCCAAGA
AGAAGCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCCTCGGGCGGGGGTGGCTCAGGAAG
20 CGCCGCTATCGCCGAAGTGCTGCTGAATGCCAGATGCGATCTGCATGCCGTGAACTACCACG
GCGACACCCCTCTGCATATCGCCGCTAGAGAGAGCTACCATGACTGTGTGCTGCTGTTTCTG
AGCAGAGGCGCCAATCCCGAGCTCAGAAACAAAGAGGGCGACACCGCTTGGGATCTGACAC
CCGAGAGATCCGACGTGTGGTTCGCTCTGCAACTGAATAGAAAAGTGAAGTGGGCGTCGG
CAATAGGGCCATTAGAACCGAGAAGATCATCTGTAGGGACGTGGCTAGGGGCTACGAGAAC
25 GTGCCCATCCCTTGTGTGAACGGAGTGGATGGAGAGCCTTGCCCCGAGGATTACAAATACAT
CAGCGAGAACTGCGAAACCTCCACCATGAATATCGATAGAAACATTACACACCTCCAGCAC
TGTACATGCGTGGACGATTGCAGCAGCAGCAACTGTCTGTGCGGCCAACTGAGCATCAGATG
CTGGTACGACAAGGATGGCAGACTGCTGCAAGAGTTCAACAAGATCGAACCCCTCTGATCT
TCGAGTGTAACCAAGCTTGCAGCTGTTGGAGGAACTGCAAGAATAGGGTCGTGCAGTCCGG
30 AATCAAGGTGAGACTGCAGCTGTATAGAACAGCTAAGATGGGATGGGGAGTCAGAGCTCTG
CAGACCATCCCCAAGGCACATTCATCTGTGAGTACGTCGGCGAACTCATCAGCGACGCTGA
GGCCGATGTGAGGGAGGACGACAGCTATCTCTTCGACCTCGACAACAAGGACGGCGAGGTG
TACTGCATCGACGCTAGATATTACGGCAACATCAGCAGATTCATCAACCACCTCTGCGACCC
CAATATCATCCCCGTGAGAGTGTTTCATGCTCCATCAAGATCTGAGATTCCCTAGGATCGCCTT

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GGGACATCAAGAGCAAGTACTTCACATGCCAATGCGGCAGCGAGAAATGCAAGCATAGCGC
CGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCTAGGCTGGACCCTCACCCCGAGCTGCTGC
CCGAAGTGGGATCTCTGCCTCCCGTGAATACCGGAGGTGGCGGATCGGGAGACAAGAAGTA
5 CAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCCGTGATCACCGACGAGTAC
AAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGA
ACCTGATCGGCGCCCTGCTGTTTCGACAGCGGCGAGACCGCCGAGGCCACCCGGCTGAAGCG
GACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC
AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGG
10 TGGAGGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGGACGAGGTGGC
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AAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCACATGATCAAGTTCCGGGGCCACTT
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GTGCAGACCTACAACCAGCTGTTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCCA
15 AGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCCAGCT
GCCCGGCGAGAAGAAGAACGGCCTGTTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACC
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20 AGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGA
CCTGACCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGAAGTACAAGGAGATCTTCT
TCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGGAGTT
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25 AGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTG
AAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCC
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CCCCTGGAACCTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGG
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30 ACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCG
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CGGCTGGGGCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAA
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5 ACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCAGGTGAGCGGCCAGGGCGA
CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTG
CAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCCGGCACAAGCCCCGAGAAC
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10 CACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACG
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CGCCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGG
AGCGACAAGGCCCGGGGCAAGAGCGACAACGTGCCAGCGAGGAGGTGGTGAAGAAGATG
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15 TGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCA
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ACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCTGAAATCCA
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CCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTAC
20 CCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA
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25 CGGCTTCAGCAAGGAGAGCATCCTGCCAAGCGGAACAGCGACAAGCTGATCGCCCGGAAG
AAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGC
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TGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGC
CAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCAAGTACAGCCTGTTC
30 GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAAC
GAGCTGGCCCTGCCAGCAAGTACGTGAACTTCCTGTACCTGGCCAGCCACTACGAGAAGCT
GAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTTCGTGGAGCAGCACAAGCACTA
CCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCC
AACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGG

CCGAGAACATCATCCACCTGTTACCCCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTAC
TTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCC
TGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGG
CGACAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAGGCCGCGCCAGGCCAAGAAGAAGAA
5 GTCGGGCGGGGGTGGCTCAGGACAGACCGGCAAAAAGTCCGAAAAGGGCCCCGTGTGCTGG
AGGAAGAGGGTCAAGAGCGAGTACATGAGGCTGAGACAGCTCAAGAGATTTAGGAGAGCC
GATGAGGTGAAGTCCATGTTCTCCAGCAACAGACAAAAGATTCTGGAGAGGACCGAGATCC
TCAACCAAGAGTGGAAGCAGAGAAGAATCCAGCCCGTGCACATTCTGACCTCCGTGAGCTC
TCTGAGGGGCACAAGAGAATGCTCCGTCACCAGCGATCTGGACTTCCCCACACAAGTGATCC
10 CCCTCAAGACACTGAACGCTGTGGCCAGCGTGCCCATCATGTATAGCTGGTCCCCTCTGCAA
CAGAACTTCATGGTGGAGGACGAGACAGTGCTGCACAATATCCCCTACATGGGAGATGAGG
TGCTGGACCAAGACGGCACCTTTATTGAGGAGCTGATTAAAACTACGATGGCAAGGTGCA
CGGCGATAGGGAGTGTGGCTTCATCAACGACGAGATCTTCGTGAGCTGGTGAATGCTCTGG
GCCAGTATAATGACGATGATGACGACGATGACGGCGACGACCCCGAAGAGAGAGAGGAGA
15 AGCAAAAGGATCTGGAGGACCATAGGGACGACAAAGAGTCTAGACCTCCTAGAAAGTTCCC
CTCCGACAAGATCTTCGAAGCCATCTCCTCCATGTTCCCCGACAAGGGCACAGCCGAGGAAC
TGAAGGAGAAGTATAAGGAACTCACAGAGCAACAGCTGCCCCGAGCTCTGCCTCCCGAGTG
CACCCCTAACATCGACGGCCCCAACGCCAAGAGCGTGACAGGGGAGCAATCCCTCCACAGC
TTCCATACCCTCTTCTGCAGAAGATGCTTTAAATACGATTGCTTTCTCCATCCTTTCCACGCC
20 ACACCCAACACCTACAAGAGGAAGAACACCGAAACCGCTCTGGACAATAAACCTTGCGGAC
CCCAGTGCTACCAGCATCTGGAAGGAGCCAAGGAATTTGCCGCTGCTCTGACAGCCGAGAG
AATTA AAAACCCCTCCCAAAAGACCCGGCGGCAGAAGGAGGGGCAGACTGCCTAATAACAGC
AGCAGACCCAGCACCCCTACCATTAACGTGCTGGAATCCAAGGACACCGACAGCGATAGAG
AGGCCGGCACAGAAACCGGCGGAGAGAAACAACGACAAGGAGGAGGAGGAGAAGAAAGAC
25 GAGACATCCTCCAGCAGCGAGGCTAATAGCAGATGCCAGACCCCTATCAAGATGAAACCTA
ATATCGAGCCCCCGAGAATGTGGAGTGGAGCGGCGCTGAGGCCTCCATGTTTAGAGTGCTG
ATCGGAACCTACTACGACAACTTCTGCGCTATCGCTAGACTGATTGGCACCAAGACATGCAG
ACAAGTGTACGAGTTCAGAGTCAAGGAGAGCTCCATTATCGCCCCCGCCCCGCGGAAGAT
GTGGACACCCCCCCCAGAAAGAAGAAAAGGAAGCATAGACTGTGGGCCGCCCCTGTAGAA
30 AGATCCAGCTCAAAAAGGACGGCAGCAGCAACCACGTGTACA ACTATCAGCCTTGTGACCA
CCCCAGACAACCTTGTGATTCCAGCTGCCCTTGCGTGATCGCCAGAACTTCTGCGAGAAGT
TCTGTCA GTGCAGCAGCGAGTGCCAAAATAGATTTCCCGGATGTAGGTGCAAAGCCCA GTGC
AATACCAAGCAGTGCCCTTGCTATCTGGCCGTGAGAGAGTGCGATCCCGATCTGTGTCTGAC
ATGTGGAGCTGCCGACCATTGGGACAGCAAGAATGTGAGCTGCAAGAACTGCAGCATCCAA

AGGGGAAGCAAAAAACATCTGCTGCTCGCCCCTTCCGATGTGGCCGGATGGGGAATCTTTAT
 CAAGGACCCCGTCCAGAAAAACGAGTTCATTTCCGAGTATTGCGGCGAGATCATCAGCCAA
 GACGAAGCTGATAGAAGAGGCAAAGTGTATGACAAATACATGTGCTCCTTCCTCTTCAACCT
 CAATAATGATTTTCGTGGTGGACGCCACAAGGAAGGGCAACAAGATTAGATTCGCCAACCCAC
 5 AGCGTCAATCCTAACTGCTATGCCAAGGTCATGATGGTCAACGGCGACCACAGAATTGGCAT
 CTTCGCTAAGAGGGCCATCCAGACCGGCGAGGAACTGTTCTTCGACTATAGATACTCCCAAG
 CCGACGCTCTGAAGTACGTGGGCATCGAGAGAGAGATGGAAATCCCCGGAGGTGGCGGATC
 GGGAAAGCGGCCCCGCCGCCACCAAGAAGGCCGGTCAGGCCAAGAAGAAGAAGGGCAGCTA
 CCCCTACGACGTGCCCCGACTACGCCTGAGCGGCCGCTTAATTAAGCTGCCTTCTGCGGGGCT
 10 TGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCC
 TGAGTAGGAAGTCTAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO. 69)

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 15 ID NOs: 70 or 80. In some embodiments, a site-specific disrupting agent described herein comprises an
 amino acid sequence of SEQ ID NO: 70 or 80 or a sequence with at least 80, 85, 90, 95, 99, or 100%
 identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 positions of difference thereto.

20 G9A-dCas9-EZH2 protein

MAPKKKRKVGIIHGVPAASGGGGSGSAAIAEVLLNARCDLHAVNYHGDTPHIAARESYPHDCVL
 LFLSRGANPELRNKEGDTAWDLTPERSDVWFALQLNRKLRLGVGNRAIRTEKIIICRDVARGYEN
 VPIPCVNGVDGEPCPEDYKYISENCETSTMNIDRNITHLQHCTCVDDCSSNCLCGQLSIRCWYD
 KDGRLLQEFNKIEPPLIFECNQACSCWRNCKNRVVQSGIKVRLQLYRTAKMGWGVRLQTIPTQG
 25 TFICEYVGELISDAEADVREDDSYLFDLDNKDGEVYCIDARYYGNISRFINHLCDPNIPVRVFML
 HQDLRFPRIAFFSSRDIRTGEELGFDYGDRFWDIKSKYFTCQCGSEKCKHSAEIALEQSRRLARLD
 PHPELLPELGSPPVNTGGGGSGDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSI
 KKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEE
 DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEGDL
 30 NPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRRLNLIQPLPGEKKNGLFG
 NLIASLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSD
 ILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQ
 EEFYKFIFKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKD
 NREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASQAQSFIERMTNFDK

NLPNEKVLPHKSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
 KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
 IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF MQ
 LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIV
 5 IEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
 DQELDINRLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKMKNYWRQLL
 NAKLITQRKFDNLTKAERGGSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
 VKVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVY
 DVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFAT
 10 VRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
 AKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
 MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIIEQISEFS
 KRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL
 DATLIHQ SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSGQTGKKSEKGPVC
 15 WRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQKILERTEILNQEWKQRRIQPVHILTSVSSLRG
 TRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSPLQQNFMVEDETVLHNIPYMGDEVLDQDG
 TFIEELIKNYDGKVHGDRECGFINDEIFVELVNALGQYNDDDDDDGDDPEEREKQKDLEDHR
 DDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEKYKELTEQQLPGALPPECTPNIDGPNAKSVQ
 REQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKRKNTETALDNKPCGPQCYQHLEGAKEFAAAA
 20 LTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVLESKDTSDREAGTETGGENNDKEEEEKKDE
 TSSSSEANSRCQTPIKMKPNIEPPENVEWSGAEASMFRLIGTYYDNFCAIARLIGTKTCRQVYEF
 RVKESSIIAPAPAEDVDTPPRKKRKHRLWAAHCRKIQLKKDGSSNHVYNYQPCDHPRQPCDSS
 CPCVIAQNFCFKCQCSSECQNRFPGRCKAQCNTKQCPCYLAVRECDPDLCLTCGAADHWDS
 KNVSCKNCSIQRGSKKHL LAPS DVAGWGIFIKDPVQKNEFISEYCGEISQDEADRRGKVYDKY
 25 MCSFLFNLNDFVVDATRKGNKIRFANHSVNPNCYAKVMMVNGDHRIGIFAKRAIQTGEELFFD
 YRYSQADALKYVGIEREMEIPGGGGSGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO.
 70)

G9A-dCas9-EZH2 protein without HA tag

30 MAPKKRKRKVGIIHGVPAAASGGGGSGSAAIAEVLLNARCDLHAVNYHGDTPHIAARES YHDCVL
 LFLSRGANPELRNKEGDTAWDLTPERSDVWFALQLNRKLRLGVGNRAIRTEKIIICRDVARGYEN
 VPIPCVNGVDGEPCPEDYKYISENCETSTMNIDRNITHLQHCTCVDDCSSNCLCGQLSIRCWYD
 KDGRLLQEFNKIEPPLIFECNQACSCWRNCKNRVVQSGIKVRLQLYRTAKMGWGVRLQTIPQG
 TFICEYVGELISDAEADVREDDSYLFDLDNKDGEVYCIDARYYGNISRFINHLCDPNIPVRVFML

HQDLRFPRIAFFSSRDIRTGEELGFDYGDRFWDIKSKYFTCQCGSEKCKHSAEAIALEQSRLARLD
PHELLPELGLSPPVNTGGGSGDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSI
KKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEE
DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLAHAMIKFRGHFLIEGDL
5 NPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFG
NLIALSLGLTPNFKSNFDLAEDAKLQLSKDQYADLFLAAKNLSDAILLSD
ILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQ
EEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKD
NREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDK
10 NLPNEKVLPHKSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQ
LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIV
IEMARENQTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
15 DQELDINRLSDYDVA AIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLL
NAKLITQRKFDNLTKAERGGSELKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVY
DVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFAT
VRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV
20 AKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKR
MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
KRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL
DATLIHQ SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKSGGGSGQTGKKSEKGPVC
WRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQKILERTEILNQEWKQRRIQPVHILTSVSSLRG
25 TRECSVTSDLDFTQVIPLKTLNAVASVPIMYSWSPLQQNFMVEDETVLHNIPYMGDEVLDQDG
TFIEELIKNYDGKVHGDRECGFINDEIFVELVNALGQYNDDDDDDGDDPEEREKQKDLEDHR
DDKESRPPRFPSDKIFEAISSMFPDKGTAEELKEKYKELTEQQLPGALPPECTPNIDGPNKSVQ
REQSLHSFHTLFCRRCFKYDCFLHPPHATPNTYKRKNTETALDNKPCGPQCYQHLEGAKEFAAA
LTAERIKTPPKRPGGRRRGRLPNSSRPSTPTINVLESKDTSDREAGTETGGENNDKEEEEKKDE
30 TSSSEANSRCQTPIKMKPNIEPPENVEWSGAEASMFRLIGTYYDNFCAIARLIGTKTCRQVYEF
RVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHCRKIQLKKDGSSNHVYNYQPCDHPRQPCDSS
CPCVIAQNFCFKCQCSSECQNRFPGCRCKAQCN TKQCPCYLAVRECDPDLCLTCGAADHWDS
KNVSCKNCSIQRGSKKHL LAPS DVAGWGIFIKDPVQKNEFISEYCGEISQDEADRRGKVYDKY

MCSFLFNLNNDFFVVDATRKGNKIRFANHSVNPNCYAKVMMVNGDHRIGIFAKRAIQTGEELFFD
YRYSQADALKYVGIEREMEIPGGGGSGKRPAATKKAGQAKKKKGS (SEQ ID NO. 80)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
5 comprising dCas9, e.g., an *S. aureus* dCas9), a first effector moiety comprising G9A; e.g., a G9A domain,
and a second effector moiety comprising KRAB, e.g., a KRAB domain. In some embodiments, a site-
specific disrupting agent comprises a targeting moiety comprising dCas9, e.g., an *S. pyogenes* dCas9, e.g.,
a mutant *S. pyogenes* Cas9, e.g., Cas9m4; a first effector moiety comprising G9A, e.g., a G9A domain;
and a second effector moiety comprising KRAB, e.g., a KRAB domain. In some embodiments, the site-
10 specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NO: 71 (e.g., mRNA
encoding the site-specific disrupting agent). In some embodiments, a nucleic acid described herein
comprises a nucleic acid sequence of SEQ ID NO: 71 or a sequence with at least 80, 85, 90, 95, 99, or
100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3,
2, or 1 positions of difference thereto.

15

G9A-dCas9-KRAB (MR-29942) mRNA sequence

AGGAAATAAGAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCCAAGA
AGAAGCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCCTCGGGCGGGGGTGGCTCAGGAAG
CGCCGCTATCGCCGAAGTGCTGCTGAATGCCAGATGCGATCTGCATGCCGTGAACTACCACG
20 GCGACACCCCTCTGCATATCGCCGCTAGAGAGAGCTACCATGACTGTGTGCTGCTGTTTCTG
AGCAGAGGGCGCAATCCCGAGCTCAGAAACAAAGAGGGCGACACCGCTTGGGATCTGACAC
CCGAGAGATCCGACGTGTGGTTCGCTCTGCAACTGAATAGAAAAGTGGAGACTGGGCGTCGG
CAATAGGGCCATTAGAACCGAGAAGATCATCTGTAGGGACGTGGCTAGGGGCTACGAGAAC
GTGCCCATCCCTTGTGTGAACGGAGTGGATGGAGAGCCTTGCCCCGAGGATTACAAATACAT
25 CAGCGAGAACTGCGAAACCTCCACCATGAATATCGATAGAAACATTACACACCTCCAGCAC
TGTACATGCGTGACGATTGCAGCAGCAGCAACTGTCTGTGCGGCAACTGAGCATCAGATG
CTGGTACGACAAGGATGGCAGACTGCTGCAAGAGTTCAACAAGATCGAACCCCTCTGATCT
TCGAGTGTAACCAAGCTTGCAGCTGTTGGAGGAACTGCAAGAATAGGGTCGTGCAGTCCGG
AATCAAGGTGAGACTGCAGCTGTATAGAACAGCTAAGATGGGATGGGGAGTCAGAGCTCTG
30 CAGACCATCCCCAAGGCACATTCATCTGTGAGTACGTCGGCGAACTCATCAGCGACGCTGA
GGCCGATGTGAGGGAGGACGACAGCTATCTCTTCGACCTCGACAACAAGGACGGCGAGGTG
TACTGCATCGACGCTAGATATTACGGCAACATCAGCAGATTCATCAACCACCTCTGCGACCC
CAATATCATCCCCGTGAGAGTGTTTCATGCTCCATCAAGATCTGAGATTCCCTAGGATCGCCTT
CTTCAGCTCTAGAGACATTAGAACCGGCGAGGAGCTGGGATTCGACTACGGCGACAGGTTCT

GGGACATCAAGAGCAAGTACTTCACATGCCAATGCGGCAGCGAGAAATGCAAGCATAGCGC
CGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCTAGGCTGGACCCTCACCCCGAGCTGCTGC
CCGAACCTGGGATCTCTGCCTCCCGTGAATACCGGAGGTGGCGGATCGGGAGACAAGAAGTA
CAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGCGGTGATCACCGACGAGTAC
5 AAGGTGCCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGACCGGCACAGCATCAAGAAGA
ACCTGATCGGCGCCCTGCTGTTCGACAGCGGCGAGACCGCCGAGGCCACCCGGCTGAAGCG
GACCGCCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTGCTACCTGCAGGAGATCTTC
AGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGAGAGCTTCCTGG
TGGAGGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAACATCGTGACGAGGTGGC
10 CTACCACGAGAAGTACCCACCATCTACCACCTGCGGAAGAAGCTGGTGGACAGCACCGAC
AAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCACATGATCAAGTTCCGGGGCCACTT
CCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGACAAGCTGTTCATCCAGCTG
GTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCAGCGGCGTGGACGCCA
AGGCCATCCTGAGCGCCCCGGCTGAGCAAGAGCCGGCGGCTGGAGAACCTGATCGCCAGCT
15 GCCCGGCGAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCCCTGAGCCTGGGCCTGACC
CCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCTGCAGCTGAGCAAGGACA
CCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGACCAGTACGCCGACCTGTTC
CTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACATCCTGCGGGTGAACACCG
AGATCACCAAGGCCCCCCTGAGCGCCAGCATGATCAAGCGGTACGACGAGCACCACCAGGA
20 CCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCCGAGAAGTACAAGGAGATCTTCT
TCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGGCCAGCCAGGAGGAGTT
CTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACCGAGGAGCTGCTGGTGAAG
CTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACAACGGCAGCATCCCCACC
AGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGAGGACTTCTACCCCTTCCTG
25 AAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGGATCCCCTACTACGTGGGCC
CCCTGGCCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAAATCCGAGGAGACCATCAC
CCCCTGGAACCTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCCCAGAGCTTCATCGAGCGG
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ACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTACGTGACCGAGGGCATGCG
30 GAAGCCCGCCTTCCTGAGCGGCGAGCAGAAGAAGGCCATCGTGACCTGCTGTTCAAGACC
AACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCAAGAAGATCGAGTGCTTCG
ACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAGCCTGGGCACCTACCACGA
CCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTG
GAGGACATCGTGCTGACCCTGACCCTGTTCGAGGACCGGGAGATGATCGAGGAGCGGCTGA

AAACCTACGCCCACCTGTTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGGCGGTACAC
CGGCTGGGGCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGAGCGGCAA
GACCATCCTGGACTTCCTGAAATCCGACGGCTTCGCCAACCGGAACCTTCATGCAGCTGATCC
ACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAGGTGAGCGGCCAGGGCGA
5 CAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCCATCAAGAAGGGCATCCTG
CAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCGGCACAAGCCCCGAGAAC
ATCGTGATCGAGATGGCCCCGGGAGAACCAGACCACCCAGAAGGGCCAGAAGAAGAGCCGG
GAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCAGCCAGATCCTGAAGGAG
CACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACG
10 GCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGC
CGCCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACAACAAGGTGCTGACCCGG
AGCGACAAGGCCCGGGGCAAGAGCGACAACGTGCCCAGCGAGGAGGTGGTGAAGAAGATG
AAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCAGCGGAAGTTCGACAACC
TGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCCGGCTTCATCAAGCGGCA
15 GCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCCAGATCCTGGACAGCCGGATGAAC
ACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGGTGATCACCTGAAATCCA
AGCTGGTGGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTGCGGGAGATCAACAATA
CCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCGCCCTGATCAAGAAGTAC
CCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAGATGA
20 TCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTACTTCTTCTACAGCAACAT
CATGAACTTCTTCAAGACCGAGATCACCTGGCCAACGGCGAGATCCGGAAGCGGCCCTG
ATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGGCCGGGACTTCGCCACCG
TGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAAACCGAGGTGCAGACCGG
CGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACAAGCTGATCGCCCCGGAAG
25 AAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCCTACAGCGTGC
TGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAAATCCGTGAAGGAGCTGC
TGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCCATCGACTTCCTGGAGGC
CAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTC
GAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGAGCTGCAGAAGGGCAAC
30 GAGCTGGCCCTGCCAGCAAGTACGTGAACCTTCCTGTACCTGGCCAGCCACTACGAGAAGCT
GAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCGTGGAGCAGCACAAGCACTA
CCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGTGATCCTGGCCGACGCC
AACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAGCCCATCCGGGAGCAGG
CCGAGAACATCATCCACCTGTTACCTGACCAACCTGGGCGCCCCCGCCGCCTTCAAGTAC

TTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAGGTGCTGGACGCCACCC
 TGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCTGAGCCAGCTGGGCGG
 CGACAGCGGCGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAAGAA
 GTCGGGCGGGGGTGGCTCAGACGCTAAGTCTCTGACCGCTTGGAGCAGAACACTGGTCACCT
 5 TCAAGGACGTGTTTCGTCGACTTCACAAGAGAGGAGTGGAAACTGCTGGACACCGCCCAGCA
 GATCCTCTATAGAAACGTCATGCTGGAGAACTACAAGAATCTGGTGTCTCTGGGCTACCAGC
 TGACCAAGCCCCGACGTGATTCTGAGGCTGGAGAAGGGCGAGGAGCCTTGGCTGGTGGAGAG
 AGAGATCCACCAAGAAACCCACCCCGACAGCGAAACCGCCTTCGAGATCAAGAGCAGCGTG
 GGAGGTGGCGGATCGGGAAAGCGGCCCCGCCGCCACCAAGAAGGCCGGTCAGGCCAAGAAG
 10 AAGAAGGGCAGCTACCCCTACGACGTGCCCCGACTACGCCTGAGCGGCCGCTTAATTAAGCT
 GCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGG
 TCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID
 NO. 71)

15

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 ID NOs: 72 or 81. In some embodiments, a site-specific disrupting agent described herein comprises an
 amino acid sequence of SEQ ID NO: 72 or 81 or a sequence with at least 80, 85, 90, 95, 99, or 100%
 identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 20 positions of difference thereto.

G9A-dCas9-KRAB protein

MAPKKRKRKVGIHGVPAASGGGSGSAAIAEVLLNARCDLHAVNYHGDTPHIAARESYPHDCVL
 LFLSRGANPELRNKEGDTAWDLTPERSDVWFALQLNRKLRLGVGNRAIRTEKIIICRDVARGYEN
 25 VPIPCVNGVDGEPCPEDYKYISENCETSTMNIDRNITHLQHCTCVDDCSSNCLCGQLSIRCWYD
 KDGRLQEFNKIEPPLIFECNQACSCWRNCKNRVVQSGIKVRLQLYRTAKMGWGVRLQITIPQG
 TFICEYVGELISDAEADVREDDSYLFDLDNKDGEVYCIDARYYGNISRFINHLCDPNIIPVRVFML
 HQDLRFPIAFFSSRDIRTGEELGFDYGDRFWDIKSKYFTCQCGSEKCKHSAEAIALEQSRRLARLD
 PHPELLPELGSPPVNTGGGSGDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSI
 30 KKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEE
 DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDL
 NPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFG
 NLIALSLGLTPNFKSNFDLAEDAKLQLSKDQYADLFLAAKNLSDAILSD
 ILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQ

EEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKD
 NREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
 NLPNEKVLPHKSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
 KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
 5 IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQ
 LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIV
 IEMARENQTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
 DQELDINRLSDYDVA AIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKMKNYWRQLL
 NAKLITQRKFDNLTKAERGGSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
 10 VKVITLKSCLVSDFRKDFQFYKVINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVY
 DVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFAT
 VRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGGDSPTVAYSVLVV
 AKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
 MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS
 15 KRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL
 DATLIHQ SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSDAKSLTAW SRTLVT
 FKDV FVDFTREEWKLLDTAQQILYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREI
 HQETHPDSETAFEIKSSVGGGGSGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO. 72)

20 G9A-dCas9-KRAB protein without HA tag
 MAPKKKRKVGIHGVPAASGGGGSGSAAIAEVLLNARCDLHAVNYHGDTPLHIAARES YHDCVL
 LFLSRGANPELRNKEGDTAWDLTPERSDVWFALQLNRKLRLGVGNRAIRTEKIIICRDVARGYEN
 VPIPCVNGVDGEPCPEDYKYISENCETSTMNIDRNITHLQHCTCVDDCSSNCLCGQLSIRCWYD
 KDGRLLQEFNKIEPPLIFECNQACSCWRNCKNRVVQSGIKVRLQLYRTAKMGWGVRALQTIPQG
 25 TFICEYVGELISDAEADVREDDSYLFDLDNKDGEVYCIDARYYGNISRFINHLCDPNII PVRVFML
 HQDLRFPRIAFFSSRDIRTGEELGFDYGDRFWDIKSKYFTCCQCGSEKCKHSAEAIALEQSRLARLD
 PHPELLPELGS LPPVNTGGGGSGDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSI
 KKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFHRLEESFLVEE
 DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAHMIKFRGHFLIEGDL
 30 NPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG
 NLIASLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSD
 ILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQ
 EEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKD
 NREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK

NLPNEKVLPHKSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL
 KEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM
 IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQ
 LIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKILQTVKVVDELVKVMGRHKPENIV
 5 IEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
 DQELDINRLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLL
 NAKLITQRKFDNLTKAERGGSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
 VKVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVV
 DVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFAT
 10 VRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVV
 AKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKR
 MLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIIEQISEFS
 KRVLADANLDKVL SAYNKH RD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL
 DATLIHQ SITGLYETRIDLSQLGGDSGGKRPAAATKKAGQAKKKKSGGGGSDAKSLTAW SRTLVT
 15 FKDV FVDFTREEWKLLDTAQQILYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREI
 HQETHPDSETAFEIKSSVGGGGSGKRPAAATKKAGQAKKKKGS (SEQ ID NO. 81)

In some embodiments, a site-specific disrupting agent comprises a targeting moiety (e.g.,
 comprising dCas9, e.g., an *S. aureus* dCas9), a first effector moiety comprising EZH2; e.g., an EZH2
 20 domain, and a second effector moiety comprising KRAB, e.g., a KRAB domain. In some embodiments, a
 site-specific disrupting agent comprises a targeting moiety comprising dCas9, e.g., an *S. pyogenes* dCas9,
 e.g., a mutant *S. pyogenes* Cas9, e.g., Cas9m4; a first effector moiety comprising EZH2, e.g., an EZH2
 domain; and a second effector moiety comprising KRAB, e.g., a KRAB domain. In some embodiments,
 the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NO: 85 (e.g., mRNA
 25 encoding the site-specific disrupting agent). In some embodiments, a nucleic acid described herein
 comprises a nucleic acid sequence of SEQ ID NO: 85 or a sequence with at least 80, 85, 90, 95, 99, or
 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3,
 2, or 1 positions of difference thereto.

30 EZH2-dCas9-KRAB (MR-29948) mRNA sequence

AGGAAATAAGAGAGAAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCCAAGA
 AGAAGCGGAAGGTGGGCGGCAGCGGCGGCAGCGGCCAGACCGGCAAGAAGAGCGAGAAGG
 GCCCCGTGTGCTGGCGGAAGCGGGTGAAGAGCGAGTACATGCGGCTGCGGCAGCTGAAGCG
 GTTCCGGCGGGCCGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGCAGAAGATCCTGGAG

CGGACCGAGATCCTGAACCAGGAGTGGAAGCAGCGGCGAATCCAGCCCGTGACATCCTGA
CCAGCGTGAGCAGCCTGCGGGGCACCCGGGAGTGACGCGTGACCAGCGACCTGGACTTCCC
CACCCAGGTGATCCCCCTAAAGACCCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCT
GGAGCCCCCTGCAGCAGAACTTCATGGTGGAGGACGAGACCGTGCTGCACAACATCCCCTA
5 CATGGGCGACGAGGTGCTGGACCAGGACGGCACCTTCATCGAGGAGCTGATCAAGAACTAC
GACGGCAAGGTGCACGGCGACCGGGAGTGCGGCTTCATCAACGACGAGATCTTCGTGGAGC
TGGTGAACGCCCTGGGCCAGTACAACGACGACGACGACGACGACGACGACGGCGACGACCCCGA
GGAGCGGGAGGAGAAGCAGAAGGACCTGGAGGACCACCGGGACGACAAGGAGAGCCGGCC
CCCCCGGAAGTTCCCCAGCGACAAGATCTTCGAGGCCATCAGCAGCATGTTCCCCGACAAGG
10 GCACCGCCGAGGAGCTGAAGGAGAAGTACAAGGAGCTGACCGAGCAGCAGCTGCCCCGGCG
CCCTGCCCCCGAGTGACCCCCAACATCGACGGCCCCAACGCCAAGAGCGTGACGCGGGA
GCAGAGCCTGCACAGCTTCCACACCCTGTTCTGCCGGCGGTGCTTCAAGTACGACTGCTTCC
TGCACCCCTTCCACGCCACCCCCAACACCTACAAGCGGAAGAACACCGAGACCGCCCTGGA
CAACAAGCCCTGCGGCCCCCAGTGCTACCAGCACCTGGAGGGCGCCAAGGAGTTCGCCGCC
15 GCCCTGACCGCCGAGCGGATCAAGACCCCCCCCCAAGCGGCCCGGCGGCGGCGGGGCC
GGCTGCCCAACAACAGCAGCCGGGCCAGCACCCCCACCATCAACGTGCTGGAGAGCAAGGA
CACCGACAGCGACCGGGAGGCCGGCACCGAGACCGGCGGCGAGAACAACGACAAGGAGGA
GGAGGAGAAGAAGGACGAGACCAGCAGCAGCAGCAGGCCAACAGCCGGTGCCAGACCCC
CATCAAGATGAAGCCCAACATCGAGCCCCCGAGAACGTGGAGTGGAGCGGCGCCGAGGCC
20 AGCATGTTCCGGGTGCTGATCGGCACCTACTACGACAACCTTCTGCGCCATCGCCCGGCTGAT
CGGCACCAAGACCTGCCGGCAGGTGTACGAGTTCCGGGTGAAGGAGAGCAGCATCATCGCC
CCCGCCCCCGCCGAGGACGTGGACACCCCCCCCCGGAAGAAGAAGCGGAAGCACCGGCTGT
GGGCGGCCCACTGCCGGAAGATCCAGCTGAAGAAGGACGGCAGCAGCAACCACGTGTACAA
CTACCAGCCCTGCGACCACCCCCGGCAGCCCTGCGACAGCAGCTGCCCCTGCGTGATCGCCC
25 AGAACTTCTGCGAGAAGTTCTGCCAGTGCAGCAGCGAGTGCCAGAACCGGTTCCCCGGCTGC
CGGTGCAAGGCCAGTGCAACACCAAGCAGTGCCCCCTGCTACCTGGCCGTGCGGGAGTGCG
ACCCCGACCTGTGCCTGACCTGCGGCGCCGCCGACCACTGGGACAGCAAGAACGTGAGCTG
CAAGAACTGCAGCATCCAGCGGGGCAGCAAGAAGCACCTGCTGCTGGCCCCAGCGACGTG
GCCGGCTGGGGCATCTTCATCAAGGACCCCGTGCGAGAAGAACGAGTTCATCAGCGAGTACT
30 GCGGCGAGATCATCAGCCAGGACGAGGCCGACCGGCGGGGCAAGGTGTACGACAAGTACAT
GTGCAGCTTCCTGTTCAACCTGAACAACGACTTCGTGGTGGACGCCACCCGGAAGGGCAACA
AGATCCGGTTCGCCAACCACAGCGTGAAACCCCAACTGCTACGCCAAGGTGATGATGGTGAA
CGGCGACCAACCGGATCGGCATCTTCGCCAAGCGGGCCATCCAGACCGGCGAGGAGCTGTTT
TTCGACTACCGGTACAGCCAGGCCGACGCCCTGAAGTACGTGGGCATCGAGCGGGAGATGG

AGATCCCCAGCACCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGGCGGCAGCGG
GCCGACCCGACAAGAAGTACAGCATCGGCCTGGCCATCGGCACCAACAGCGTGGGCTGGGC
CGTGATCACCGACGAGTACAAGGTGCCAGCAAGAAGTTCAAGGTGCTGGGCAACACCGAC
CGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGCTGTTTCGACAGCGGCGAGACCGCCG
5 AGGCCACCCGGCTGAAGCGGACCGCCCGGCGGCGGTACACCCGGCGGAAGAACCGGATCTG
CTACCTGCAGGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGC
TGGAGGAGAGCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGGCACCCCATCTTCGGCAA
CATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCACCATCTACCACCTGCGGAAGAAG
CTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTACCTGGCCCTGGCCCACATGAT
10 CAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGACGTGGAC
AAGCTGTTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGAGAACCCCATCAACGC
CAGCGGCGTGGACGCCAAGGCCATCCTGAGCGCCCGGCTGAGCAAGAGCCGGCGGCTGGAG
AACCTGATCGCCCAGCTGCCCCGGCGAGAAGAAGAACGGCCTGTTTCGGCAACCTGATCGCCC
TGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAGGACGCCAAGCT
15 GCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCGGCGAC
CAGTACGCCGACCTGTTCTTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGAGCGACAT
CCTGCGGGTGAACACCGAGATCACCAAGGCCCCCTGAGCGCCAGCATGATCAAGCGGTAC
GACGAGCACCAACAGGACCTGACCCTGCTGAAGGCCCTGGTGCGGCAGCAGCTGCCCGAGA
AGTACAAGGAGATCTTCTTCGACCAGAGCAAGAACGGCTACGCCGGCTACATCGACGGCGG
20 CGCCAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCCATCCTGGAGAAGATGGACGGCACC
GAGGAGCTGCTGGTGAAGCTGAACCGGGAGGACCTGCTGCGGAAGCAGCGGACCTTCGACA
ACGGCAGCATCCCCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGGCAGGA
GGACTTCTACCCCTTCTGAAGGACAACCGGGAGAAGATCGAGAAGATCCTGACCTTCCGG
ATCCCCTACTACGTGGGCCCCCTGGCCCGGGGCAACAGCCGGTTCGCCTGGATGACCCGGAA
25 ATCCGAGGAGACCATCACCCCTGGAACCTTCGAGGAGGTGGTGGACAAGGGCGCCAGCGCC
CAGAGCTTCATCGAGCGGATGACCAACTTCGACAAGAACCTGCCCAACGAGAAGGTGCTGC
CCAAGCACAGCCTGCTGTACGAGTACTTCACCGTGTACAACGAGCTGACCAAGGTGAAGTA
CGTGACCGAGGGCATGCGGAAGCCCGCCTTCTGAGCGGCGAGCAGAAGAAGGCCATCGTG
GACCTGCTGTTCAAGACCAACCGGAAGGTGACCGTGAAGCAGCTGAAGGAGGACTACTTCA
30 AGAAGATCGAGTGCTTCGACAGCGTGGAGATCAGCGGCGTGGAGGACCGGTTCAACGCCAG
CCTGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAG
GAGAACGAGGACATCCTGGAGGACATCGTGCTGACCCTGACCCTGTTTCGAGGACCGGGAGA
TGATCGAGGAGCGGCTGAAAACCTACGCCACCTGTTTCGACGACAAGGTGATGAAGCAGCT
GAAGCGGCGGCGGTACACCGGCTGGGGCCGGCTGAGCCGGAAGCTGATCAACGGCATCCGG

GACAAGCAGAGCGGCAAGACCATCCTGGACTTCCTGAAATCCGACGGCTTCGCCAACCGGA
ACTTCATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCA
GGTGAGCGGCCAGGGCGACAGCCTGCACGAGCACATCGCCAACCTGGCCGGCAGCCCCGCC
ATCAAGAAGGGCATCCTGCAGACCGTGAAGGTGGTGGACGAGCTGGTGAAGGTGATGGGCC
5 GGCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACCCAGAAGG
GCCAGAAGAACAGCCGGGAGCGGATGAAGCGGATCGAGGAGGGCATCAAGGAGCTGGGCA
GCCAGATCCTGAAGGAGCACCCCGTGGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCT
GTACTACCTGCAGAACGGCCGGGACATGTACGTGGACCAGGAGCTGGACATCAACCGGCTG
AGCGACTACGACGTGGCCGCCATCGTGCCCCAGAGCTTCCTGAAGGACGACAGCATCGACA
10 ACAAGGTGCTGACCCGGAGCGACAAGGCCCGGGGCAAGAGCGACAACGTGCCCAGCGAGG
AGGTGGTGAAGAAGATGAAGAACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATCACCCA
GCGGAAGTTCGACAACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCC
GGCTTCATCAAGCGGCAGCTGGTGGAGACCCGGCAGATCACCAAGCACGTGGCCAGATCC
TGGACAGCCGGATGAACACCAAGTACGACGAGAACGACAAGCTGATCCGGGAGGTGAAGG
15 TGATCACCTGAAATCCAAGCTGGTGAGCGACTTCCGGAAGGACTTCCAGTTCTACAAGGTG
CGGGAGATCAACAACCTACCACCACGCCCACGACGCCTACCTGAACGCCGTGGTGGGCACCG
CCCTGATCAAGAAGTACCCCAAGCTGGAGAGCGAGTTCGTGTACGGCGACTACAAGGTGTA
CGACGTGCGGAAGATGATCGCCAAGAGCGAGCAGGAGATCGGCAAGGCCACCGCCAAGTA
CTTCTTCTACAGCAACATCATGAACTTCTTCAAGACCGAGATCACCTGGCCAACGGCGAGA
20 TCCGGAAGCGGCCCCCTGATCGAGACCAACGGCGAGACCGGCGAGATCGTGTGGGACAAGGG
CCGGGACTTCGCCACCGTGCGGAAGGTGCTGAGCATGCCCCAGGTGAACATCGTGAAGAAA
ACCGAGGTGCAGACCGGCGGCTTCAGCAAGGAGAGCATCCTGCCCAAGCGGAACAGCGACA
AGCTGATCGCCCGGAAGAAGGACTGGGACCCCAAGAAGTACGGCGGCTTCGACAGCCCCAC
CGTGGCCTACAGCGTGCTGGTGGTGGCCAAGGTGGAGAAGGGCAAGAGCAAGAAGCTGAA
25 ATCCGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGGAGCAGCTTCGAGAAGAACCCC
ATCGACTTCCTGGAGGCCAAGGGCTACAAGGAGGTGAAGAAGGACCTGATCATCAAGCTGC
CCAAGTACAGCCTGTTTCGAGCTGGAGAACGGCCGGAAGCGGATGCTGGCCAGCGCCGGCGA
GCTGCAGAAGGGCAACGAGCTGGCCCTGCCAGCAAGTACGTGAACTTCCTGTACCTGGCC
AGCCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTTCTGTGG
30 AGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCAGCAAGCGGGT
GATCCTGGCCGACGCCAACCTGGACAAGGTGCTGAGCGCCTACAACAAGCACCGGGACAAG
CCCATCCGGGAGCAGGCCGAGAACATCATCCACCTGTTACCCCTGACCAACCTGGGCGCCCC
CGCCGCCTTCAAGTACTTCGACACCACCATCGACCGGAAGCGGTACACCAGCACCAAGGAG
GTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACCCGGATCGACCT

GAGCCAGCTGGGCGGCGACAGCGGCGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCGGCCA
 GGCCAAGAAGAAGAAGTTCGGGCGGGGGTGGCTCAGACGCTAAGTCTCTGACCGCTTGAGC
 AGAACACTGGTCACCTTCAAGGACGTGTTTCGTCGACTTCACAAGAGAGGAGTGGAACTGC
 TGGACACCGCCCAGCAGATCCTCTATAGAAACGTCATGCTGGAGAACTACAAGAATCTGGT
 5 GTCTCTGGGCTACCAGCTGACCAAGCCCGACGTGATTCTGAGGCTGGAGAAGGGCGAGGAG
 CCTTGGCTGGTGGAGAGAGAGATCCACCAAGAAACCCACCCCGACAGCGAAACCGCCTTCG
 AGATCAAGAGCAGCGTGGGAGGTGGCGGATCGGGAAAGCGGCCCCGCCGCCACCAAGAAGG
 CCGGTCAGGCCAAGAAGAAGAAGGGCAGCTACCCCTACGACGTGCCCCGACTACGCCTGAGC
 GGCCGCTTAATTAAGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTT
 10 GCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAA (SEQ ID NO. 85)

In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 15 ID NOs: 86 or 82. In some embodiments, a site-specific disrupting agent described herein comprises an
 amino acid sequence of SEQ ID NO: 86 or 82 or a sequence with at least 80, 85, 90, 95, 99, or 100%
 identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 positions of difference thereto.

20 EZH2-dCas9-KRAB protein

MAPKKKRKVGGSGGSGQTGKKSEKGPVCWRKRVKSEYMRLRQLKRFRRADEVKSMFSSNRQK
 ILERTEILNQEWKQRRIQPVHILTSVSSLRGTTRECSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSP
 LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALG
 QYNDDDDDDGDDPEEREKQKDLEDHRDDKESRPPRFPSDKIFEAISSMFPDKGTAEELKEK
 25 YKELTEQQLPGALPPECTPNIDGPNASVQREQSLHSFHTLFCRRCFKYDCFLHPFHATPNTYKR
 KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVL
 ESKDTSDDREAGTETGGENNDKEEEEKKDETSSSSEANSRCQTPIKMKNIEPPENVEWSGAEAS
 MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
 RKIQLKKDGSSNHVYNYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQC
 30 TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCISIQRGSKKHLALLAPSDVAGWGIFIKDP
 VQKNEFISEYCGEISQDEADRRGKVYDKYMCNFLNNDVVDATRKGNKIRFANHSVNPNC
 YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGGSGGSGG
 SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
 EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNIVDE

VAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
 TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNF
 DLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASM
 IKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
 5 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVG
 PLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY
 FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
 VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVM
 KQLKRRRYTGWGRLSRKLINGIRDKQSGKTIIDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQV
 10 SGQGDSLHEHIANLAGSPAIIKQILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNS
 RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAI
 VPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAE
 RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
 FQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA
 15 TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNVIVKKT
 EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKE
 LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELAL
 PSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANLDKVLSA
 YNKHARDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSTGLYETRI
 20 DLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSDAKSLTAWSRTLVTFKDVFDVDFTREEWKL
 LDTAQQILYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKS
 SVGGGGSGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO. 86)

EZH2 -dCas9-KRAB protein without HA tag

25 MAPKKKRVVGGSGGSGQTGKKSEKGPVCWRKRKSEYMRLRQLKRFRRADEVKSMFSSNRQK
 ILERTEILNQEWKQRRIQPVHILTSVSSLRGTRCSVTSDLDFPTQVIPLKTLNAVASVPIMYSWSP
 LQQNFMVEDETVLHNIPYMGDEVLDQDGTFFIELIKNYDGKVHGDRECGFINDEIFVELVNALG
 QYNDDDDDDDDGDDPEEREKQKQDLEDHRDDKESRPPRKFPSPDKIFEAISSMFPDKGTAEELKEK
 YKELTEQQLPGALPPECTPNIDGPNKSVQREQLHSFHTLFCRRCFKYDCFLHPFHATPNTYKR
 30 KNTETALDNKPCGPQCYQHLEGAKEFAAALTAERIKTPPKRPGGRRRGRLPNNSSRPSTPTINVL
 ESKDTSDDREAGTETGGENNDKEEEEEKDETSSSSEANSRCQTPIKMKPNIEPPENVEWSGAEAS
 MFRVLIGTYYDNFCAIARLIGTKTCRQVYEFVRKESSIIAPAPAEDVDTPPRKKKRKHRLWAAHC
 RKIQLKKGSSNHVYNYQPCDHPRQPCDSSCPCVIAQNFCEKFCQCSSECQNRFPGCRCKAQC
 TKQCPCYLAVRECDPDLCLTCGAADHWDSKNVSCKNCSIQRGSKKHLLAPSDVAGWGIFIKDP

VQKNEFISEYCGEISQDEADRRGKVYDKYMCSFLFNLNDFVVDATRKGNKIRFANHSVNPNC
 YAKVMMVNGDHRIGIFAKRAIQTGEELFFDYRYSQADALKYVGIEREMEIPSTGGSGGSGGSGG
 SGGSGRPDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
 EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGNIVDE
 5 VAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQ
 TYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF
 DLAEDAKLQLSKDTYDDDLNLLAQIGDQYADFLAAKNLSDAILLSDILRVNTEITKAPLSASM
 IKRYDEHHQDLTLLKALVRQQLEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
 EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPLKDNREKIEKILTRIPYYVG
 10 PLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLEYEY
 FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISG
 VEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVM
 KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQV
 SGQGDSLHEHIANLAGSPAICKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS
 15 RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAAI
 VPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKMKKNYWRQLLNAKLITQRKFDNLTKAE
 RGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKD
 FQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMIKSEQEIGKA
 TAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIVKKT
 20 EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKE
 LLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELAL
 PSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSA
 YNKHDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSTGLYETRI
 DLSQLGGDSGGKRPAATKKAGQAKKKKSGGGGSDAKSLTAWSRTLVTFKDVFDFTREEWKL
 25 LDTAQQILYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAFEIKS
 SVGGGGSGKRPAATKKAGQAKKKKGS (SEQ ID NO. 82)

In some embodiments, a site-specific disrupting agent comprises a CRISPR/Cas molecule
 comprising Cas9. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid
 30 sequence of SEQ ID NO: 217 (e.g., mRNA encoding the site-specific disrupting agent). In some
 embodiments, a nucleic acid described herein comprises a nucleic acid sequence of SEQ ID NO: 217 or a
 sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17,
 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

Cas9 mRNA (MR-28127)

AGGAAAUAAGAGAGAAAAGAAGAGUAAGAAGAAAUAUAAGAGCCACCAUGGCCCCCAAG
AAGAAGCGGAAGGUGGGCAUCCACGGCGUGCCCGCCGCCGACAAGAAGUACAGCAUCGGC
CUGGACAUCGGCACCAACAGCGUGGGCUGGGCCGUGAUCACCGACGAGUACAAGGUGCCC
5 AGCAAGAAGUUCAAGGUGCUGGGCAACACCGACCGGCACAGCAUCAAGAAGAACCUGAUC
GGCGCCCUGCUGUUCGACAGCGGCGAGACCGCCGAGGCCACCCGGCUGAAGCGGACCGCC
CGGCGGCGGUACACCCGGCGGAAGAACCGGAUCUGCUACCUGCAGGAGAUCUUCAGCAAC
GAGAUGGCCAAGGUGGACGACAGCUUCUCCACCGGCUGGAGGAGAGCUUCCUGGUGGA
GGAGGACAAGAAGCACGAGCGGCACCCCAUCUUCGGCAACAUCGUGGACGAGGUGGCCUA
10 CCACGAGAAGUACCCACCAUCUACCACCUGCGGAAGAAGCUGGUGGACAGCACCGACAA
GGCCGACCUGCGGCUGAUCUACCUGGCCUGGCCACAUGAUCAGUUCGGGGGCCACUU
CCUGAUCGAGGGCGACCUGAACCCCGACAACAGCGACGUGGACAAGCUGUUCAUCCAGCU
GGUGCAGACCUACAACCAGCUGUUCGAGGAGAACCCCAUCAACGCCAGCGGCGUGGACGC
CAAGGCCAUCCUGAGCGCCCGGCUGAGCAAGAGCCGGCGGCUGGAGAACCUGAUCGCCCA
15 GCUGCCCGGCGAGAAGAAGAACGGCCUGUUCGGCAACCUGAUCGCCUGAGCCUGGGCCU
GACCCCAACUUCAAGAGCAACUUCGACCUGGCCGAGGACGCCAAGCUGCAGCUGAGCAA
GGACACCUACGACGACGACCUGGACAACCUGCUGGCCCAGAUCCGGCGACCAGUACGCCGA
CCUGUUCUGGCCGCCAAGAACCUGAGCGACGCCAUCCUGCUGAGCGACAUCCUGCGGGU
GAACACCGAGAUACCAAGGCCCCCCUGAGCGCCAGCAUGAUCAGCGGUACGACGAGCA
20 CCACCAGGACCUGACCCUGCUGAAGGCCUGGUGCGGCAGCAGCUGCCCGAGAAGUACAA
GGAGAUCUUCUUCGACCAGAGCAAGAACGGCUACGCCGGCUACAUCGACGGCGGCGCCAG
CCAGGAGGAGUUCUACAAGUUCAUCAAGCCAUCCUGGAGAAGAUGGACGGCACCGAGGA
GCUGCUGGUGAAGCUGAACCGGGAGGACCUGCUGCGGAAGCAGCGGACCUUCGACAACGG
CAGCAUCCCCACCAGAUCCACCUGGGCGAGCUGCACGCCAUCCUGCGGCGGCAGGAGGA
25 CUUCUACCCCUUCCUGAAGGACAACCGGGAGAAGAUCGAGAAGAUCUGACCUUCCGGAU
CCCCUACUACGUGGGGCCCCUGGCCCGGGGCAACAGCCGGUUCGCCUGGAUGACCCGGAA
AUCCGAGGAGACCAUCACCCCUUGGAACUUCGAGGAGGUGGUGGACAAGGGCGCCAGCGC
CCAGAGCUUCAUCGAGCGGAUGACCAACUUCGACAAGAACCUGCCCAACGAGAAGGUGCU
GCCCAAGCACAGCCUGCUGUACGAGUACUUCACCGUGUACAACGAGCUGACCAAGGUGAA
30 GUACGUGACCGAGGGCAUGCGGAAGCCCGCCUUCUGAGCGGCGAGCAGAAGAAGGCCAU
CGUGGACCUGCUGUUCAAGACCAACCGGAAGGUGACCGUGAAGCAGCUGAAGGAGGACU
ACUUCAAGAAGAUCGAGUGCUUCGACAGCGUGGAGAUCAGCGGCGUGGAGGACCGGUUC
AACGCCAGCCUGGGCACCUACCACGACCUGCUGAAGAUCAUCAAGGACAAGGACUUCUG
GACAACGAGGAGAACGAGGACAUCUGGAGGACAUCGUGCUGACCCUGACCCUGUUCGAG

GACCGGGAGAUGAUCGAGGAGCGGCUGAAAACCUACGCCCACCUGUUCGACGACAAGGUG
AUGAAGCAGCUGAAGCGGCGGGCGGUACACCGGCUGGGGCGGCUGAGCCGGAAGCUGAUC
AACGGCAUCCGGGACAAGCAGAGCGGCAAGACCAUCCUGGACUUCCUGAAAUCCGACGGC
UUCGCCAACCGGAACUUAUGCAGCUGAUCCACGACGACAGCCUGACCUUCAAGGAGGAC
5 AUCCAGAAGGCCCAGGUGAGCGGCCAGGGCGACAGCCUGCACGAGCACAUCGCCAACCU
GCCGGCAGCCCCGCCAUCAAGAAGGGCAUCCUGCAGACCGUGAAGGUGGUGGACGAGCUG
GUGAAGGUGAUGGGCCGGCACAAGCCCCGAGAACAUCGUGAUCGAGAUGGCCCCGGGAGAA
CCAGACCACCCAGAAGGGCCAGAAGAACAGCCGGGAGCGGAUGAAGCGGAUCGAGGAGGG
CAUCAAGGAGCUGGGCAGCCAGAUCUGAAGGAGCACCCCGUGGAGAACACCCAGCUGCA
10 GAACGAGAAGCUGUACCUGUACUACCUGCAGAACGGCCGGGACAUGUACGUGGACCAGGA
GCUGGACAUAACCGGCUGAGCGACUACGACGUGGACCACAUCGUGCCCCAGAGCUUCCU
GAAGGACGACAGCAUCGACAACAAGGUGCUGACCCGGAGCGACAAGAACCGGGGCAAGAG
CGACAACGUGCCCAGCGAGGAGGUGGUGAAGAAGAUGAAGAACUACUGGCGGCAGCUGC
UGAACGCCAAGCUGAUCACCCAGCGGAAGUUCGACAACCUGACCAAGGCCGAGCGGGGCG
15 GCCUGAGCGAGCUGGACAAGGCCGGCUUCAUCAAGCGGCAGCUGGUGGAGACCCGGCAGA
UCACCAAGCACGUGGCCCAGAUCUGGACAGCCGGAUGAACACCAAGUACGACGAGAACG
ACAAGCUGAUCCGGGAGGUGAAGGUGAUCACCCUGAAAUCCAAGCUGGUGAGCGACUUC
GGAAGGACUUCCAGUUCUACAAGGUGCGGGAGAUCAACAACUACCACCACGCCACGACG
CCUACCUGAACGCCGUGGUGGGCACCGCCCUGAUAAGAAGUACCCCAAGCUGGAGAGCG
20 AGUUCGUGUACGGCGACUACAAGGUGUACGACGUGCGGAAGAUGAUCGCCAAGAGCGAG
CAGGAGAUCCGGCAAGGCCACCGCCAAGUACUUCUUCUACAGCAACAUAUGAACUUCUUC
AAGACCGAGAUACCCUGGCCAACGGCGAGAUCCGGAAGCGGCCCCUGAUCGAGACCAAC
GGCGAGACCGGCGAGAUUCGUGUGGGACAAGGGCCGGGACUUCGCCACCGUGCGGAAGGUG
CUGAGCAUGCCCCAGGUGAACAUCGUGAAGAAAACCGAGGUGCAGACCGGCGGCUUCAGC
25 AAGGAGAGCAUCCUGCCCAAGCGGAACAGCGACAAGCUGAUCGCCCCGGAAGAAGGACUGG
GACCCCAAGAAGUACGGCGGCUUCGACAGCCCCACCGUGGCCUACAGCGUGCUGGUGGUG
GCCAAGGUGGAGAAGGGCAAGAGCAAGAAGCUGAAAUCCGUGAAGGAGCUGCUGGGCAU
CACCAUCAUGGAGCGGAGCAGCUUCGAGAAGAACCCCAUCGACUUCCUGGAGGCCAAGGG
CUACAAGGAGGUGAAGAAGGACCUGAUAUCAAGCUGCCCAAGUACAGCCUGUUCGAGCU
30 GGAGAACGGCCGGAAGCGGAUGCUGGCCAGCGCCGGCGAGCUGCAGAAGGGCAACGAGCU
GGCCUGCCCAGCAAGUACGUGAACUUCUGUACCUGGCCAGCCACUACGAGAAGCUGAA
GGGCAGCCCCGAGGACAACGAGCAGAAGCAGCUGUUCGUGGAGCAGCACAAGCACUACCU
GGACGAGAUAUCGAGCAGAUCAGCGAGUUCAGCAAGCGGGUGAUCUGGCCGACGCCAA
CCUGGACAAGGUGCUGAGCGCCUACAACAAGCACCGGGACAAGCCCAUCCGGGAGCAGGC

CGAGAACAUCAUCCACCUGUUCACCCUGACCAACCUGGGCGCCCCCGCCGCCUUCAAGUA
 CUUCGACACCACCAUCGACCGGAAGCGGUACACCAGCACCAAGGAGGUGCUGGACGCCAC
 CCUGAUCCACCAGAGCAUCACCGGCCUGUACGAGACCCGGAUCGACCUGAGCCAGCUGGG
 CGGCGACAGCGGCGGCAAGCGGCCCCGCCGCCACCAAGAAGGCCGGCCAGGCCAAGAAGAA
 5 GAAGGGCAGCUACCCCUACGACGUGCCCGACUACGCCUGAGCGGCCGCUUAAUUAAGCUG
 CCUUCUGCGGGGCUUGCCUUCUGGCCAUGCCCUUCUUCUCCCUUGCACCUGUACCUCU
 UGGUCUUUGAAUAAAGCCUGAGUAGGAAGUCUAGAAAAAAAAAAAAAAAAAAAAAAAAAAAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ
 ID NO: 217)

10 In some embodiments, a site-specific disrupting agent comprises the amino acid sequence of SEQ
 ID NOs: 218 or 84. In some embodiments, a site-specific disrupting agent described herein comprises an
 amino acid sequence of SEQ ID NO: 218 or 84 or a sequence with at least 80, 85, 90, 95, 99, or 100%
 identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1
 15 positions of difference thereto.

Cas9 Protein Sequence (corresponding to MR-28127)

MAPKKRKRKVGIIHGVPAADKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKH
 20 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEK
 25 ILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLEYEFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 30 QTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS

MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 5 SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 218)

Cas9 Protein Sequence (corresponding to MR-28127) without HA tag

MAPKKRKRKVGIIHGVPAAADKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
 GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFHRLSEESFLVEEDKKH
 10 ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHMIKFRGHFLIEGDLNPDNS
 DVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
 EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQSKNGYAGYIDGGASQEEFYKF
 IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEK
 15 ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKV
 LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
 KIECFDSVEISGVEDRFNASLGTYHDLLKIIKD KDFLDNEENEDILEDIVLTTLTFEDREMIEERLKT
 YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL
 TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
 20 QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
 RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKKNYWRQLLNAKLITQ
 RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
 SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMI
 AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
 25 MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPPKKYGGFDSPTVAYSVLVVAKVEKG
 KSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
 ELQKGNELALPSKYVNFLYLASHYEKLKGGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLAD
 ANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ
 SITGLYETRIDLSQLGGDSGGKRPAATKKAGQAKKKKGS (SEQ ID NO: 84)

30

In some embodiments, a site-specific disrupting agent comprises a nuclear localization sequence (NLS). In some embodiments, the site-specific disrupting agent comprises an NLS, e.g., an SV40 NLS at the N-terminus. In some embodiments, the site-specific disrupting agent comprises an NLS, e.g., an SV40 NLS at the C-terminus. In some embodiments, the site-specific disrupting agent comprises

an NLS, e.g., a nucleoplasmin NLS at the C-terminus. In some embodiments, the site-specific disrupting agent comprises a first NLS at the N-terminus and a second NLS at the C-terminus. In some embodiments the first and the second NLS have the same sequence. In some embodiments, the first and the second NLS have different sequences. In some embodiments, the site-specific disrupting agent comprises a first NLS at the N-terminus, a second NLS, and a third NLS at the C-terminus. In some embodiments, at least two NLSs have the same sequence. In some embodiments, the first and the second NLS have the same sequence and the third NLS has a different sequence than the first and the second NLS. In some embodiments, the site-specific disrupting agent comprises an SV40 NLS, e.g., the site-specific disrupting agent comprises a sequence according to PKKKRK (SEQ ID NO: 63). In some embodiments, the site-specific disrupting agent comprises a nucleoplasmin NLS, e.g., the site-specific disrupting agent comprises a sequence of KRPAATKKAGQAKKK (SEQ ID NO: 64). In some embodiments, the site-specific disrupting agent comprises a C-terminal sequence comprising one or more of, e.g., any one or both of: a nucleoplasmin nuclear localization sequence and an HA-tag. In some embodiments, the site-specific disrupting agent comprises an epitope tag, e.g., an HA tag: YPYDVDPDYA (SEQ ID NO: 65). In some embodiments, the site-specific disrupting agent may comprise two copies of the epitope tag.

While an epitope tag is useful in many research contexts, it is sometimes desirable to omit an epitope tag in a therapeutic context. Accordingly, in some embodiments, the site-specific disrupting agent lacks an epitope tag. In some embodiments, a site-specific disrupting agent described herein comprises a sequence provided herein (or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto), but lacking the HA tag of SEQ ID NO: 65. In some embodiments, a nucleic acid described herein comprises a sequence provided herein (or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto), but lacking a region encoding the HA tag of SEQ ID NO: 65. In some embodiments, the site-specific disrupting agent does not comprise an NLS. In some embodiments, the site-specific disrupting agent does not comprise an epitope tag. In some embodiments the site-specific disrupting agent does not comprise an HA tag. In some embodiments, the site-specific disrupting agent does not comprise an HA tag sequence according to SEQ ID NO: 65.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising a Zn finger molecule and an effector moiety comprising EZH2 or a functional fragment or variant thereof. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 219, 220, 222, 223, 233, or 234, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity

thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising a Zn finger molecule and an effector moiety comprising DNMT3 (e.g., DNMT3a or DNMT3L) or a functional fragment or variant thereof. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 221, 231, or 236-239, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising a Zn finger molecule and an effector moiety comprising G9A or a functional fragment or variant thereof. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 224, 225, or 227-230, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

In some embodiments, a site-specific disrupting agent comprises a targeting moiety comprising a Zn finger molecule and an effector moiety comprising HDAC8 or a functional fragment or variant thereof. In some embodiments, the site-specific disrupting agent is encoded by the nucleic acid sequence of SEQ ID NOs: 226, 232, 235, or 240-242, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

Table 3. Additional Exemplary Site-Specific Disrupting Agent Encoding Sequences

Ref. number and (SEQ ID NO)	Agent Description	Sequence
MR-29950 (SEQ ID NO: 219)	EZH2-CXCL1 ZF6 T7	AGGAAATAAGAGAGAAAGAAGAGTAAGAAGAAATATAAGAGCCACCATGGCCCCAAGAAGAA GCGAAGGTGGCGGCAGCGGCGGCAGCGGCCAGACCGGCAAGAAAGAGCGAAGAGGGCCCCGTG TGCTGGCGAAGCGGGTGAAGAGCGAGTACATGCGGCTGCGGCAGCTGAAGCGGTTCGCGCGGGC CGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGCAGAAAGATCCTGGAGCGGACCGAGATCCTGA ACGAGGTGGAAGCAGCGCGGAATCCAGCCCCGTGCACATCCTGACCAAGCGTGAGCAGCCTGCCG GGCACCCGGGAGTGACGCTGACCAAGCGACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAGAC CCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGGT GGAGGACGAGACCGTGTGCAACAATCCCTACATGGCGACGAGGTGCTGGACCAAGACGGCA CCTTCATCGAGAGCTGATCAAGAACTACGACGGCAAGGTGCACGGCGACCGGAGTGCGGGCTTC ATCAACGACGAGATCTTCGTGGAGCTGGTGAACGCCCTGGGCCAGTACAACGACGACGACGACGA CGACGCGGCACGACCCCGAGGAGCGGGAGGAGAGAGAGAGGACCTGGAGGACCAACCGGGAC GACAAGGAGAGCCCGGCCCGGAAAGTTCGCCAGCGACAAGATCTTCGAGGCCATCAGCAGCAT GTTCCCCGACAAGGGCACCGCCGAGGAGCTGAAGGAGAAAGTACAAGGAGCTGACCGAGCAGCAG CTGCCCCGGCCCTGCCCCCGGAGTGCAACCCCAACATCGACGGCCCCAACGCCAAGAGCGGTGCA GCGGGAGCAGAGCCTGCACAGCTTCCACACCCCTGTTCTGCCGGCGGTGCTTCAAGTACGACTGCTT CCTGCACCCCTTCCACGCCACCCCAACACCTACAAGCGGAAGAACACCGAGACCGCCCTGGACA ACAAGCCCTGCGGCCCCCAAGTGCTACCAAGCACCTGGAGGGGCCCAAGGAGTTTCGCCCGCCCTG

		ACCGCCGAGCGGATCAAGACCCCCCAAGCGGCCCGCGCGCGCGCGCGCGCTGCCCAA CAACAGCAGCCGGCCAGCACCCCCACCATCAACGTGCTGGAGAGCAAGGACACCGACAGCGACC GGGAGCCCGCACCGAGACCGGCGGAGAAACGACAAGGAGGAGGAGAGAAAGGACG AGACCAGCAGCAGCAGGAGCCAAACAGCCGCTGCCAGACCCCCCATCAAGATGAAGCCCAACATC GAGCCCCCGAGAACGTGGAGTGGAGCGGCGCGGAGCCAGCATGTTCCGGGTGCTGATCGGGCAC CTACTACGACAACCTTCTGCGCCATCGCCCGGCTGATCGGCACCAAGACCTGCCGGCAGGTGTACGA GTTCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCCGCGGAGGACGTGGACACCCCCCCCC GGAAGAAGAGCGGAAGCACCGGCTGTGGGCCGCCCACTGCCGGAAGATCCAGCTGAAGAAGGA CGCAGCAGCAACCAACGTGTACAACCTACCAAGCCCTGCGACCAACCCCCGCGAGCCCTGCCGACAGCA GCTGCCCCCTGCGTGATCGCCCCAGAACTTCTGCGAGAAAGTTCTGCCAGTGCAGCAGCGAGTGCCAGA ACCGGTTCCCCGGCTGCCGGTGCAAGGCCCAAGTGCAACACCAAGCAAGTGCCCCCTGCTACCTGGCCG TGCGGGAGTGCGACCCCGACCTGTGCTGACCTGCGGCCCGCGACCACTGGGACAGCAAGAAGAAC GTGAGCTGCAAGAACTGCAGCATCCAGCGGGGCAAGCAAGAACCTGCTGCTGCGCCCCCAGCGA CGTGCGCCGGCTGGGGCATCTTCATCAAGGACCCCGTGCAAGAAACGAGTTTCATCAGCGAGTACT GCGGCGAGATCATCAGCCAGGACGAGGCCGACCGCGGGGCAAGGTGTACGACAAGTACATGTGC AGCTTCCTGTTCAACCTGAACAACGACTTCTGTGTGGACGCCACCCGGAAGGGCAACAAGATCCG GTTCCGCAACCAACAGCGTGAACCCCAACTGCTACGCCAAGGTGATGATGGTGAACGGCGACCCACC GGATCGGCATCTTCGCCAAGCGGGCCAATCCAGACCGGCGAGGAGCTGTTCTTCGACTACCGGTACA GCCAGGCCGACGCCCTGAAGTACGTGGCATCGAGCGGAGATGGAGATCCCCGCGCAGCAGCGGA TCCCTGGAGCCCCGGGAAAAGCCTTACAAGTGTCCCGAGTGCGGAAAGAGCTTCAGCAGAGCCGA TAATCTGACCGGACCAAAAGGACCCACACCGGAGAGAAAGCCTTATAAGTGTCCCGAATGCCGGCA AAAGCTTTTCTAGAAAGCGATCATCTGACCAACCAACAGAGGACACACCCGGAGAAAACCTTAC AAATGCCCCCGAGTGGGCAAAAGCTTCTCCAGAGCAGCAATCTGTTGAGACACCAAGGACCCCA CACCGGCAAAACCCTATAAATGCCCCGAATGTGGCAAGAGCTTTAGCACATCCGGCGAGCTGG
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		TGAGGCATCAAGAACACATACCGGCGAGAGCCCTACAAGTGCCCCGAGTGTGGAAGCTTC AGCACCCACCTCGATCTGATCAGACACACAGAGGACCCATACCGGAGAGAAACCTACAAAATGTCC CGAGTGCAGAAAGTCCTTAGCCAGCTGGCCCATCTGAGAGCTCATAAAGGACACACACCGGCG AGAAGCCTTACAAGTGTCCCGAGTGGGAAATCCTTCTCCAACTGGCCCATCTGAGGGCCCCACC AGAGAAACCCACACCGGCAAAAAGACCTCCGCTAGCGGCAGCGGCGGCGGCAAGCG GCCCCGCCACCAAGAAAGGCCGCCAGGCCAAGAAAGAAAGGCGAGCTACCCCTACGACGTGC CCGACTACGCCCTGAGCGGCCGCTTAATTAAAGCTGCCCTTCTGCGGGCTTGCCCTTCTGGCCATGCCCT TCTTCTCCTTGTCACTGTACCTCTTGTGCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAA
MR- 29949 (SEQ ID NO: 220)	EZH2- CXCL1 ZF1 T7	AGGAAATAAGAGAGAAAGAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAGAA GCGAAAGGTGGCGGCAGCGGCGGCAGCGGCCAGACCGCAAGAAAGAGCGAAGAGGCCCCCGTG TGCTGGCGAAAGCGGTGAAGAGCGAGTACATGCGGCTGCGGCACTGAAAGCGGTTCCGCGGGC CGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGCAGAAATCCTGGAGCGGACCGAGATCCTGA ACCAGGAGTGGAAGCAGCGCGGAATCCAGCCCCGTGCACATCCTGACCAAGCGTGAGCAGCCTGCCG GGCACCCGGGAGTGCAGCGTGACCAAGCGACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAAGAC CCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGGT GGAGGACGAGACCGTGTGCACAAACATCCCCCTACATGGGCGACGAGGTGCTGGACCAGGACGGCA CCTTCATCGAGGAGCTGATCAAGAACTACGACGGCAAGGTGACGCGGACCCGGAGTGGGGCTTC ATCAACGACGAGATCTTCGTGGAGCTGGTGAACGCCCTGGGCCAGTACAACGACGACGACGACGA CGACGACGGCGACGACCCCGAGGAGCGGGAGGAGAGCAGAAAGACCTGGAGGACCAACCGGGGAC GACAAGGAGAGCGGGCCCCCGGAAAGTTCCCCAGCGACAAAGATCTTCGAGGCCATCAGCAGCAT GTTCCCCGACAAGGGCACCGCGGAGGAGCTGAAGGAGAAAGTACAAGGAGCTGACCGGAGCAGCAG CTGCCCCGGCCCTGCCCCCGGAGTGCAACCCCAACATCGACGGCCCCAACGCCAAGAGCGGTGCA

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		AAAGACCAGCGCTAGCGGCAGCGGGCGGGCAGCGGGGGCAACACGACGAGGAGTTTCGACCCCC CCAAAGGTGTACCCCGCGTGCCTCCCGCCGAGAAAGCGGAAGCCCATCCGGGTGCTGAGCCTGTTCGACG GCATCGCCACCGGCGCTGCTGTGTCTGAAGGACCTGGGCATCCAGGTGACCGGTACATCGCCAGC GAGGTGTGCGAGGACAGCATCACCGTGGGCATGCTGCGGCACCAAGGGCAAGATCATGTACGTGGG CGACGTGCGGAGCGTGACCCAGAAAGCACATCCAGGAGTGGGGCCCCCTTCGACCTGGTGATCGGGCG GCAGCCCCCTGCAACGACCTGAGCATCGTGAAACCCCGCCCGGAAGGGCCTGTACGAGGGCAACCGGC CGGCTGTTCTTCGAGTTCTACCGGCTGCTGCACGACGCGCCCGGCCAAGGAGGGCGACGACCGGCC TTCTTCTGGCTGTTTCGAGAACGTGGTGGCCATGGGCGTGAGCGACAAGCGGGACATCAGCCGGTTC CTGAGAGCAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCCGCCACCGGGCCCGTACTT CTGGGGCAACCTGCCCCGGCATGAACCGGCCCTGGCCAGCACCGTGAACGACAAGCTGGAGCTGC AGGAGTGCCTGGAGCACGGCCGGATCGCCAAAGTTCAAGCAAGGTGCGGACCATCACCAACCCGGAGC AACAGCATCAAGCAGGGCAAGGACACGACACTTCCCGGTTCATGAACGAGAAAGGAGGACATCCT GTGTGCACCGAGATGGAGCGGGTGTTCGGCTTCCCGGTGCACACTACACCGACGTGAGCAACAATGA GCCGGCTGGCCCGCAGCGGTGCTGGGCCGGAGCTGGAGCGTGCCCGTGATCCGGCACCTGTTCCG CCCCCTGAAGGAGTACTTCGCCCTGCGTGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCC AGCTTCAGCAGCGGCCCTGGTGCCCTGAGCCTGCGGGGGCAGCCACATGAATCCTCTCTGGAGATGTTT GAGACAGTGCCCGTGTGGAGAAAGGCAACCCGTGAGGTGCTGAGCCTCTTCGAGGACATTAAAGAA GGAGCTGACCTCTCTGGGCTTTCGTGAAATCCGGCAGCGACCCCGGCCAGCTGAAACACGTTGGTGGA CGTGACCGACACAGTGAGGAAGGACGTGAAAGAGTGGGGCCCCCTTTGACCTCGTGTATGGAGCCA CACCTCCTCTCGGCCACACATGCGATAGGCCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCT CCAGTACGCCAGACCTAAGCCCCGGCAGCCCCAGACCCCTTCTCTGGATGTTCTGGACAAATCTGGT GCTGAACAAGGAGGATCTGGATGTGGCCAGCAGATTTCCTGGAGATGGAAACCCGTGACAAATCCCCCG ACGTGCATGGCGGCTCTCTGCAGAACGCCGTGAGAGTGTGGTCCAAACATCCCCGCCATTAGAAGCA GACACTGGGCTCTGGTGAGCGAGGAGGAACTGTCTCTGCTGGCCCAAGATAAGCAGTCTCTCAAG
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		CTGGCCGCCAAGTGGCCCAACCAAGCTGGTGAAGAACTGCTTTCTGCCTCTGAGGGAGTATTTCAAG TATTTACAGCACCGAAGCTGACAGCAGCCTGAGCGGGCGCAAGCGGCCCGCCGCCACCAAGAAAGGC CGGCCAGGCCAAGAAGAAGAGGCGAGCTACCCCTACGACGTGCCCGACTACGCTGAGCGGCCG CTTAATTAAAGCTGCCCTTCTGCGGGCTTGCCTTCTGCCCATGCCCTTCTTCTCTCCCTTGCACTGTGA CCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAAAATAAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA
MR- 29908 (SEQ ID NO: 222)	EZH2- CXCL1 ZF5 T7	AGGAAATAAGAGAGAAAGAAGAGTAAGAAGAAATAATAAGAGCCACCATGGCCCCCAAGAAGAA GCGGAAGGTGGGCGCAGCGGCGGCAGCGGCCAGACCGGCAAGAAAGAGCGAAGAGGGCCCCGTG TGCTGGCGGAAGCGGGTGAAGAGCGAGTACATGCGGCTGCGGCAGCTGAAGCGGTTCCGGCGGGC CGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGCAGAAAGATCCTGGAGCGGACCGAGATCCTGA ACCAGGAGTGGAAGCAGCGGGCAATCCAGCCCCGTGCACATCCTGACCAGCGTGAGCAGCCTGCGG GGCACCCGGGAGTGACGCGTGACCAAGCAGCACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAAGAC CCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGGT GGAGGACGAGACCGGTGTGCACAAACATCCCCCTACATGGGCGACGAGGTGCTGGACCAGGACGGCA CCTTCATCGAGGAGCTGATCAAGAATAACGACCGGCAAGGTGCACCGGCGACCGGGAGTGCGGGCTTC ATCAACGACGAGATCTTCGTGGAGCTGGTGAACGCCCTGGGCCAGTACAACGACGACGACGACGA CGACGACGGCGACGACCCCGAGGAGCGGGGAGGAGAGCAAGGACCTGGAGGACCAACCGGGGAC GACAAGGAGAGCGCGGCCCCCGGAAGTTCCCCAGCGACAAGATCTTCGAGGCCATCAGCAGCAT GTTCCCCGACAAAGGCGACCGCGGAGGAGCTGAAGGAGAAAGTACAAGGAGCTGACCGAGCAGCAG CTGCCCCGGCCCTGCCCCCGGAGTGCAACCCCAACATCGACGGCCCCCAACGCCAAGAGCGGTGCA GCGGGAGCAGAGCCTGCACAGCTTCCACACCTGTTCTGCCGGCGGTGCTTCAAGTACGACTGCTT CCTGCACCCCTTCCACGCCACCCCCAACACCTACAAGCGGAAGAACACCGAGACCGCCCTGGACA ACAAGCCCTGCGGCCCCCAAGTGTACCAAGCACCTGAGGGGCGCAAGGAGTTGCGCGCCGCCCTG ACCGCCGAGCGGATCAAGACCCCCCAAGCGGCCCGCGGCGCGGGCGGGCTGCCCAA

		CAACAGCAGCCGGCCAGCACCCCCACCATCAACGTGCTGGAGAGCAAGGACACCGACAGCGACC GGGAGCCGGCACCGAGACCGGGCGGAGAAACGACAAAGGAGGAGGAGAGAAAGGACG AGACCAGCAGCAGCAGGAGGCCAACAGCCGGTGCCAGACCCCATCAAGATGAAGCCCAACATC GAGCCCCCGAGAACGTGGAGTGGAGCGGCGGAGGCCAGCATGTTCCGGGTGCTGATCGGCAC CTACTACGACAACTTCTGCGCCATCGCCCGGCTGATCGGCCAACAGACCTGCCGGCAGGTGTACGA GTTCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCGGAGGACGTGGACACCCCCCCCC GGAGAGAGCGGGAAGCACCGGCTGTGGGCGGCCCACTGCCGGAAGATCCAGCTGAAGAAAGGA CGGACAGCAACACGTGTACAACCTACAGCCCTGCGACCAACCCCGGAGCCCTGCGACAGCA GCTGCCCCCTGCGTGATCGCCCGAGAACTTCTGCGAGAAAGTTCTGCCAGTGCAGCAGCGAGTGCCAGA ACCGGTTCCCCGGCTGCCGGTGCAAGGCCCAAGTGCAACACCAAGCAGTGCCCCCTGCTACCTGGCCG TGCGGGAGTGCGACCCCGACCTGTGCTGACCTGCGGGCGCCGACCACTGGGACAGCAAGAAC GTGAGCTGCAAGAACTGCAGCATCCAGCGGGGCGAGCAAGAACCTGTCTGTGCTGGCCCCCAGCGA CGTGCCCGGCTGGGCGATCTTCATCAAGGACCCCGTGCGAAGAAACGAGTTTCATCAGCGAGTACT GCGGCGAGATCATCAGCCAGGACGAGGCGGACCGGGGGCAAGGTGTACGACAAGTACATGTGC AGCTTCCTGTTCACCTGAACAACGACTTCGTGTTGGACGCCACCCGGAAGGGCAACAAGATCCG GTTGCCAACCCACAGCGTGAACCCCAACTGCTACGCCAAAGGTGATGATGGTGAAACGGCGACCCACC GGATCGGCATCTTCGCCAAGCGGGCCAATCCAGACCGGGCGAGGAGCTGTTCTTCGACTACCGGTACA GCCAGGCCGACGCCCTGAAGTACGTGGGCAATCGAGCGGAGATGGAGATCCCCGGCAGCAGCGGA TCCCTGGAGCCCGGCGAAAGCCTTACAAGTGCCCGGAGTGTGGCAATCCTTTAGCACCCCGGA AATCTGACCGTCCACCAGAGAAACACATACCGGGCGAGAAACCCTACAAGTGTCCCGAGTGGCGCAA ATCCTTCAGCCAGCTGGCCCATCTGAGAGCCCCATCAAAGGACCCCATACCGGGCGAGAAACCTTACAA GTGTCCCGGAATGTGGAAAGTCTTTAGCAGCCCCCGCCGATCTGACAAAGACATCAAGAAACCCACAC CGGCGAGAAAGCCCTATAATGTCCCGAGTGTGGAAAGTCTTCAGCCAGAGCGGCAATCTGACCG AGCATCAAGAAACCCATACCGGCGAAAGCCCTATAAGTGCCCCGAATGCGGAAAGAGCTTCTCC
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		CCTGCACCCCTTCCACGCCACCCCCAACACCTACAAGCGGAAGAACACCGAGACCGCCCTGGACA ACAAGCCCTGCGGCCCCCAAGTGTACCAAGCACCTGGAGGGGCCAAGGAGTTGCGCCGCCGCCCTG ACCGCCGAGCGGATCAAGACCCCCCAAGCGCCCCGCGGCCGCGCGGGCGGCTGCCCAA CAACAGCAGCCGGCCAGCACCCCCACCATCAACGTGTGGAGAGCAAGGACACCGACAGCGACC GGGAGCCGGCACCGAGACCGGGCGGAGAACACGACAAAGGAGGAGGAGAAAGAGACG AGACCAGCAGCAGCAGCGAGGCCAACAGCCGGTGCCAGACCCCCCATCAAGATGAAGCCCAACATC GAGCCCCCGAGAACGTGGAGTGGAGCGGCGCGAGGCCAGCATGTTCCGGGTGCTGATCGGCAC CTACTACGACAACCTTCTGCGCCATCGCCGGCTGATCGGCACCAAGACCTGCCGGCAGGTGTACGA GTTCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCCCGCGGAGACGTGGACACCCCCCCCC GGAAGAAAGCGGAAGCACCCGGCTGTGGGCCGCCCACTGCCGGAAGATCCAGCTGAAGAAAGGA CGGCAGCAGCAACCAGTGTACAACACTACAGCCCTGCGACCAACCCCGGACGCCCTGCCGACAGCA GCTGCCCTGCGTGATCGCCAGAACCTTCTGCGAGAAAGTTCTGCCAGTGCAGCAGCGAGTGCCAGA ACCGGTTCCCCGGCTGCCGTGCAAGGCCCAAGTGCACCAAGCAAGTGCCTGTCTACCTGGCCG TGCGGAGTGCACCCCGACCTGTGCTGACCTGCGGCCGCCGACCACTGGGACAGCAAGAAC GTGAGCTGCAAGAACTGCAGCATCCAGCGGGGCGAGCAAGAAAGCACCTGTCTGTGCTGCCCGCAGCGA CGTGGCCGGCTGGGGCATCTTCATCAAGGACCCCGTGCAGAAAGAACGAGTTTCATCAGCGAGTACT GCGGCGAGATCATCAGCCAGGACGAGGCCGACCGGCGGGGCAAGGTGTACGACAAGTACATGTGC AGCTTCCTGTTCAACCTGAACAACGACTTCTGTGTGGACGCCACCCGGAAGGGCAACAAGATCCG GTTGCCAACCAACAGCGTGAACCCCAACTGTCTACGCCAAGGTGATGATGTTGAACGGCGACCAAC GGATCGGCATCTTCGCCAAGCGGGCCATCCAGACCGGGGAGGAGCTGTTCTTCGACTACCGGTACA GCCAGGCCGACGCCCTGAAGTACGTGGGCATCGAGCGGGGAGATGGAGATCCCCGGCAGCAGCGGA TCCCTGGAGCCCCGGCGAAAACCCCTATAAGTGCCCCGAGTGCGGCAAGAGCTTTAGCGATCCCCGG CCATCTGTGAGGCATCAGAGGACCCACACCGGGCAAGAGCCTTACAAAATGCCCCGAGTGTGGAA AAAGCTTCAGCAGAGCGATCATCTGTACCAACCCATCAGAGGACACATACCGGCGAGAAAGCCTTAT
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		AAATGCCCGGAATGTGGAAAGAGCTTCTCCAGAAAGCGACCACTCTGACCAACCACCAAGAGGACCCCA TACCGGAGAAAACCTTACAAAATGCCCGGAGTGTGGAAAGTCCCTTCAGCTCCCCCGCCGATCTGAC AAGACATCAGAGAACCCACACCGGCGAAAAACCTTATAAATGTCCCGAGTGTGGCAAAAAGCTTCT CCGACAAAGAGGATCTGACAAAGACACCAAGAGACCCACACCGGCGAGAAACCTTATAAATGTCC GAATGCGGAAAAAGCTTTAGCAGAAACGACGCTCTGACCCGAACACCAAGAGAACACATACCCGGAGA GAAACCTTATAAATGTCCCGAGTGGGAAAAATCCTTCAGCACCAACCGGGGCTCTGACAGAGCATCA GAGGACACACACCGGCAAAAAGACCTCCGCTAGCGGCAAGCGGGCGGCAAGCGGCGGCAAGCGG CCCGCCGCCACCAAGAAAGCGCGGCCAGGCCAAGAAAGAAAGAGGCAAGCTACCCCTACGACGTGCC CGACTACGCCTGAGCGGGCCGCTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTT CTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAGTCTAGAAAAAAA AAAAAAA AAAAAAA AAAAAAA
MR- 29906 (SEQ ID NO: 224)	G9A-CXCL1 ZF4 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAGAAATATAAGAGCCACCATGCCCCCAAGAAAGAA GCGGAAAGGTGGGCAATCCACGGCGTGCCCGCCGCTCGGGCGGGGTGGCTCAGGAAATAGGGCTA TCAGAAACCGAGAAAGATCATCTGTAGGGACGTGGCTAGAGGCTACGAGAACGTGCCCATTCCTTGC GTGAATGGCGTGGATGGCGAACCTTGCCCCGAGGACTACAAATACATCTCCGAGAACTGCGGAAAC CAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGTACGACAAGGACGCGAGACTGCT GCAAGAGTTCAACAAGATCGAACCCCTCTCATCTTCGAGTGTAAACCAAGCTTGCAGCTGTGGAG AAACTGCAAGAAATAGAGTGGTCCAGAGCGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCA AGATGGGATGGGGAGTGAGGGCTCTGCAAACCATTTCCCAAGGCACCTTCATCTGCGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTTCGATCTGGA CAATAAGGACGGCGAGGTGTACTGCAATCGACGCTAGATATTACGGCAACATCTCTAGATTTCATCAA CCACCTCTGCGATCCCAACATCATTTCCCGTGAGGGTGTTCATGCTGACCAAGATCTGAGGTTCCCT

		AGAAATCGCCTTCTCAGCTCTAGAGACATCAGAAACCGGCGAGGAGCTGGGCTTCGATTACGGCGGAT AGATTCTGGGACATCAAGTCCAAAGTACTTACATGCCAGTGCAGCGCAGCAGAGAGTGTAAAGCACAG CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGATGCGAGCAGCGGATCCCTGG AGCCCGGAGAAAAAGCCTTACAAATGCCCGAGTGGGCAAGTCTTTCAGCCAGCTGGCTCATCTGA GAGCTCATCAAAAGGACCCACACCGGCGAGAAAGCCCTATAAGTGGCCCCGAGTGCCGGAATAATCCTTC TCCCAGAGCAGCAATCTCGTCAGACACAGAGGACCCACACCGGCGGAGAAACCTTACAAAGTGTCC CGAATGTGGAAGTCTTCTCCCAAAAGAGCTCTCTGATCGCCCATCAGAGAACACATACCGGCGGA AAACCCCTACAAAGTGCCCCGAGTGTGGCAAAAGCTTTTCCACCCACCGGCAATCTGACCGTGCATCA AAGAAACCCACACCGGCGAAAAACCCCTACAAATGCCCGAGTGTGGCAATCTTCTCCGACCCCG GCCATCTGGTGAGGCCACAGAGGACACACACCGGCGAGAAACCTTTATAATGTCCCCGAATGCGGC AAGTCCTTTAGCACCGGCTCTCTGGTGAGACATCAGAGGACACATACCGGCGAAAGCCTTAC AAGTGTCGCCGAGTGTGGCAAAAGCTTCAGCCAGAACAGCACACTGACAGAGCATCAGAGAACCCCA TACCGGCAAAAGACCGCTAGCGGCAAGCGGCGGCGCAGCGGCGGCAAGCGGCCCGCGGCC ACCAAGAAGCGCGCCAGGCCAAAGAAAGAAAGAGGAGCTACCCCTACGACGTGCCCGACTACGC CTGAGCGGCCGCTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTCTCTCC CTTGCACTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA AAA
MR- 29904 (SEQ ID NO: 225)	G9A-CXCL1 ZF5 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAGAAATATAAGAGCCACCATTGCCCCCAAGAAAGAA GCGGAAAGGTGGGCATCCACGGCGTGCCCGCCGCTCGGGCGGGGTGGCTCAGGAAATAGGGGCTA TCAGAAACCGAGAAAGATCATCTGTAGGGACGTGGCTAGAGGCTACGAGAACGTGCCCATTCCTTGC GTGAATGGCGTGGATGGCGAACCTTGCCCCGAGGACTACAAATACATCTCCGAGAACTGCGGAAAC CAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGTACGACAAGGACGCGCAGACTGCT

		GCAAGAGTTCAACAAAGATCGAACCCCTCTCATCTTCGAGTGTAACCAAGCTTGACGCTGCTGGAG AAACTGCAAGAAATAGAGTGGTCCAGAGCGGCAATCAAGGTGAGACTGCAACTGTACAGAACCGCCA AGATGGGATGGGAGTGAGGGCTCTGCAACCAATTCCTCCAAAGGCACCTTCATCTCGGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTCGATCTGGA CAATAAGGACGGCGAGGTGTACTGCATCGACGCTAGATATTACGGCAACATCTCTAGATTTCATCAA CCACCTCTGCGATCCCAACATCATTCCTCGTGAGGGTGTTCATGTGTGACCAAGATCTGAGGTTCCCT AGAAATCGCCTTCTTCAGCTCTAGAGACATCAGAACCGGCGAGGAGCTGGGCTTCGATTACGGCGAT AGATTCTGGACATCAAGTCCAAGTACTTCACATGCCAGTGCAGCGCAGAGAAAGTGTAAAGCACAG CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGATGGCAGCAGCGGATCCCCTGG AGCCCGGCGAAAAAGCCTTACAAAGTGCCCCGAGTGTGGCAAAATCCTTTAGCACCCACCGGAAATCTG ACCGTCCACCAGAGAACACATACCGGGCGAGAAACCCCTACAAAGTGTCCCGAGTGTGCGGCAAAATCCTT CAGCCAGCTGGCCCATCTGAGAGCCCATCAAAAGGACCCATACCGGGCGAGAAACCTTACAAAGTGTC CCGAATGTGGAAGTCTTTAGCAGCCCCCGCGGATCTGACAAGACATCAAAAGAACCCACACCGGC GAGAAGCCCTATAAATGTCCCGAGTGTGGAAGTCTTCAGCCAGAGCGGCAATCTGACCGAGCA TCAAAGAAACCCATACCGGGCGAAAAAGCCCTATAAGTGCCCCGAATGCGGAAAAAGCTTCTCCACAA GCGGCGAGCTGGTGAGACACCAAGGACACATACCGGGCGAAAAAGCCCTTATAATGCCCCGAGTGC GGCAAGAGCTTCTCTAGAAAGGACAACTCTGAAGAACCCACCAAGAACACACACACCGGCGAGAAAGCC CTACAAATGCCCGGAGTGCGGCAAGAGCTTTAGCCAGTCCAGCAACCTCGTGAGACATCAGAGGA CACATACCGGAAAAAGACCAAGCGCTAGCGGCAGCGGCGGCGCAGCGGCGGCAAGCGGCCCGC CGCCACCAAGAAAGGCCGCCAGGCCAAAGAAAGAAAGAGGCGAGCTACCCCTACGACGTGCCCCGACT ACGCTGAGCGGCCGCTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCT CTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAA AAAAA AAAAA AAAAA
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MR- 29890 (SEQ ID NO: 226)	CXCL1 ZF4- HDAC8 SP6	AGGAAAATAAGAGAGAAAGAAGAGTAAGAAAGAAATATAAGAGCCACCATTGGCCCCCAAGAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCTCCCGCCGGCAGCAGCGGATCCCTGGAGCCCGGAGAAA AGCCTTACAAATGCCCGAGTGGCAAGTCCTTCAGCCAGCTGGCTCATCTGAGAGCTCATCAAA GGACCCACACCGGGAGAAAGCCCTATAAGTGCCCCGAGTGGGAAAATCCTTCTCCAGAGCAGC AATCTCGTCAGACACCAGAGGACCCACACCGGGGAGAAACCTTACAAGTGTCCCGAATGTGGAAA GTCTTCTCCCAAAGAGCTCTGTATCGCCCATCAGAGAACACATACCGGGGAAAACCCCTACAA GTGCCCCGAGTGTGGCAAAGCTTTTCCACCAACCGGCAATCTGACCGTGCATCAAGAAACCCACAC CGCGAAAACCTTACAAATGCCCGGAGTGTGGCAAATCCTTCTCCGACCCCGGCCATCTGGTGAG GCACAGAGGACACACACCGGGGAGAAACCTTATAATGTCCCCGAATGCGGCAAGTCCTTTAGCA CCAGCGGCTCTGTGTGAGACATCAGAGGACACATACCGGGGAAAAGCCTTACAAGTGTCCCGAG TGTGGCAAAGCTTACGCCAGAACAGCACACTGACAGAGCATCAGAGAACCCATACCGGGCAAAA GACCAGCGTAGCGGACGCGGGCGGCAGCGGGCGGAGGAGCCCGAGAGCCCGCGGATAGC GGACAAATCTGTGTGCCGTCTACATCTACAGCCCCGAATATGTAGCATGTGTGATTCCTTCGCC AAGATCCCTAAGAGAGCCAGCATGTGTGCTTCTGTATCGAGGCCTACGCTCTGCATAAGCAAATG AGGATCGTGAAGCCCCAAGGTGCGCCAGCATGGAAGAGATGGCCACCTTTCACACCGATGCCTACCTC CAACATCTCCAGAAAGGTGTCCCAAAGAGGGCGACGACGACCCACCCCGACTCCATTGAGTACGGACT GGGCTATGATTGCCCGCCACCGAGGGCATCTTTGACTATGCCCGCGCTATCGGCGGAGCTACCAT CACAGCCGCCAGTGTCTGATTGATGGCATGTGCAAGGTGCGCAATCAACTGGTCCGGAGGCTGGCA TCATGCCAAGAAGGATGAGGCCTCCGGCTTCTGTTATCTGAATGACGCCGTGCTGGGCATTCTGAG ACTGAGGAGGAAATTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGAGTCG AAGATGCCCTTCAGCTTCACCCAGCAAGGTGATGACAGTCTCTCTGCACAAAGTTCTCCCCCGGCTTCTT TCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGGCAAGGGAAAGGTACTACAGCGTGAACGTGC CCATTCAAGACGGCATCCAAGACGAGAAAGTACTACCAGATCTGCGAGTCCGTGCTCAAGGAGGTC TACCAAGCCTTCAATCCTAAGGCTGTGCTGCTCCAACTGGGAGCTGATACCATTTGCTGGCGATCCC
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		ATGTGCAGCTTCAATATGACACCCGTCGGAAATCGGCAAGTGCCCTCAAGTACATCTCCAGTGGCAG CTCGCCACCCTCATTTCTCGGAGGAGCGGATACAAATCTGGCTAATACCGCCAGATGCTGGACCTAT CTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCACGAGTTTTTACCCGCT TACGGCCCCGACTACGTGCTGGAGATCACCCCCAGCTGCAGACCCGATAGAAACGAAACCCCATAG AATCCAGCAAAATTTCTGAACATATATCAAGGGCAACCTCAAGCACGCTCGTGGGAGGTGGCGGATCGG GAAAGCGGCCCGCCACCAAGAAAGGCCGGTCAGGCCAAGAAAGAAAGGGCAGCTACCCCTA CGACGTGCCCGACTACGCCCTGAGCGGCGCTTAATTAAAGCTGCCCTTCTGCGGGGCTTGCCCTTCTGG CCATGCCCTTCTCTCCCTTGCACTGTACCTCTTGCTTTGAATAAAGCCTGAGTAGGAAGTC TAGAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA AAAAAATAAAAAAATAAAAAA
MR- 29886 (SEQ ID NO: 227)	G9A-CXCL1 ZF6 SP6	AGGAAATAAGAGAGAAAGAAAGAGTAAGAAAGAAATATAAGAGCCACCCTGGCCCCAAGAAGAA GCGGAAGGTGGGCAATCCACGGCGTGCCCCCGCCCTCGGGCGGGGTGGCTCAGGAATAAGGGCTA TCAGAACCGAGAAGATCATCTGTAGGAGCGTGGCTAGAGGCTACGAGAACGTGCCCATTTCTTGC GTGAATGGCGTGGATGGGAAACCTTGCCCCGAGGACTACAAATACATCTCCGAGAACCTGCGAAAC CAGCACAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGGTACGACAAGGACGGCAGACTGCT GCAAGAGTTCAACAAGATCGAAACCCCTCTCATCTTCTGAGTGTAAACCAAGCTTGCAAGCTGCTGGAG AAACTGCAAGAAATAGAGTGGTCCAGAGCGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCA AGATGGGATGGGAGTGAGGGCTCTGCAAAACCATTTCCCCAAGGCACCTTCATCTGCGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTCGATCTGGA CAATAAGGACGGCGAGGTGTACTGCAATCGACGCTAGATATTACGGCAACATCTCTAGATTTCATCAA CCACCTCTGCGATCCCAACATCATTTCCCGTGAGGGTGTTCATGTGTGACCAAGATCTGAGGTTCCCT AGATCGCCTTCTTCAGCTCTAGAGACATCAGAACCGCGGAGGAGCTGGGCTTCGATTACGGCGAT AGATTCTGGACATCAAGTCCAAGTACTTCACATGCCAGTGCGGCAGCGAAGTGTAAAGCACAG

		CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGATGGCAGCAGCGGATCCCTGG AGCCCGGCGAAAAGCCTTACAAAGTGTCCTCCGAGTGCGGAAAGAGCTTCAGCAGAGCCGATAATCTG ACCGAGCACCAAGGACCCACACCGGAGAGAGCCTTATAAGTGTCCCGAATGCGGCAAAAGCTT TTCTAGAAAGCGATCATCTGACCAACCACAGAGGACACACACCGGAGAAAAACCTTACAAATGCC CCGAGTGGCGGCAAAAGCTTCTCCAGAGCAGCAATCTGGTGAGACACCAAGAGACCCACACCCGGC GAAAAACCCCTATAAATGCCCCGAAATGTGGCAAGAGCTTTAGCACATCCGGCGAGCTGGTGAGGGCA TCAAGAACACATACCGGCGGAGAGCCCTACAAAGTGCCCCGAGTGTGGAATAAGCTTCAGCACCC ACCTCGATCTGATCAGACACAGAGGACCCATACCGGAGAGAAAAACCTACAAATGTCCCGAGTGC GGAAAGTCCTTTAGCCAGCTGGCCCATCTGAGAGCTCATCAAAAGGACACACACCGGGCGAGAAAGCC TTACAAAGTGTCCTCCGAGTGCGGAAAATCCTTCTCCCAACTGGCCCCATCTGAGGGCCCCACCCAGAGAAC CCACACCGGCAAAAAGACCTCCGCTAGCGGCGAGCGGGCGGCAAGCGGGCGGCAAGCGGCCCGCCG CCACCAAGAAAGCGCGCCAGGCCAAAGAAAGAAAGGGCAGCTACCCCTACGACGTGCCCGGACTAC GCCTGAGCGGCGCTTAATTAAAGCTGCCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCT CCCTTGCACTGTACCTCTTGCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA AAAAA
MR- 29873 (SEQ ID NO: 228)	G9A-CXCL1 ZF3 SP6	AGGAAATAAGAGAGAAAAGAAAGTAAGAAAGAAATAAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGCATCCACGGCGTGCCCGCGCCTCGGGCGGGGTGGCTCAGGAATAAGGGCTA TCAGAAACCGAGAAAGATCATCTGTAGGGACGTGGCTAGAGGCTACGAGAACGTGCCCATTCCTTGC GTGAATGGCGTGGATGGGAAACCTTGCCCCCGAGGACTACAAATACATCTCCGAGAACTGCGAAAC CAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGTAACGACAAGGACGCGCAGACTGCT GCAAGAGTTCAACAAGATCGAACCCCTCTCTCATCTTCGAGTGTAACCAAGCTTGCACTGCTGGAG AAACTGCAAGAATAGAGTGGTCCAGAGCGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCA

		AGATGGGATGGGGAGTGAGGGCTCTGCAACCAATTCCTCCCAAGGCACCTTCATCTGCGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGACGTGAGAGAGGACGACAGCTATCTCTCGATCTGGA CAATAAGGACGGCAGGTGTACTGCAATCGACGTAGATATTACGGCAACATCTCTAGATTATCAAA CCACCTCTGCGATCCCAACATCATTCCTCGTGAGGGTGTTCATGCTGCACCAAGATCTGAGGTTCCCT AGAAATCGCCTTCTTCAGCTCTAGAGACATCAGAACCCGGCAGGAGCTGGGCTTCGATTACGGCGGAT AGATTCTGGGACATCAAGTCCAAGTACTTCACATGCCAGTGGCAGCGAGAGAAAGTGTAAAGCACAG CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGATGGCAGCAGCGGATCCCTGG AGCCCGCGCAAAACCTTATAAATGCCCGAGTGTGGCAAGAGCTTTTCCGACCCCGACACCTCG TGAGGCATCAGAGAACACATACCGGCGAGAAACCTTACAAAGTCCCCCGAATGCGGCAAAATCCTTC TCTAGAAAGGACAACTGTGAAAAACCATCAAAAGAACCCATACCGGCGAGAAAGCCCTATAAATGTCC CGAGTGTGGAAAGAGCTTCAGCCACAAAGAACGCTCTGCAGAACCATCAGAGGACCCATACCGGGCG AAAAGCCTTATAAGTGGCCCGAGTGGGAAATTCCTTTTCTAGAGGACGAGCTGAAATGTGCACC AAAGGACACATACCGGAGAGAAACCTTACAAATGCCCGAGTGTGGCAAGTCTTCAGCACCTCC GGCAATCTGGTGAGGCACCAAAAGGACACACACCGGCGAAACCTTACAAAGTGTCCCGAGTGGCG AAAAAGCTTTTCCAGAACAGCACACTGACCCGAACACCAAAAGGACCCACACCGGAGAGAAAACTT ATAATGTCCCGAGTGTGGAAAGTCTTTAGCCAGTCCGGCAATCTGACAGAGCATCAAAAGAAACCC ACACCGGCAAAAGACCTCCGCTAGCGGCAGCGGCGGCGGCAAGCGGCCCGCCGCC ACCAAGAGGCCGCCAGGCCAAGAAAGAAAGAGGCGAGCTACCCCTACGACGTGCCCGACTACGC CTGAGCGGCCGCTTAATTAAAGCTGCCTTCTGCGGGCTTGCCTTCTTGCCATGCCCTTCTCTCTCC CTTGCACTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAGTCTAGAAAAAAGAAAAA AAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAA AAA
MR- 29872	G9A-CXCL1 ZF2 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATAAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCCGCCCTCGGGCGGGGTGCTCAGGAAATAGGGCTA

(SEQ ID NO: 229)	TCAGAACCGAGAAAGATCATCTGTAGGGACGTGGCTAGAGGCTACGAGAACGTGCCCATTCCTTGC GTGAATGGCGTGGATGGCGAACCTTGGCCCCGAGGACTACAAATACATCTCCGAGAACTGCGAAAC CAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGTACGACAAGGACGGCAGACTGCT GCAAGAGTTCAACAAGATCGAACCCCCCTCTCATCTTCGAGTGTAAACCAAGCTTGCAGCTGCTGGAG AAACTGCAAGAAATAGAGTGGTCCAGAGCGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCA AGATGGGATGGGGAGTGAAGGCTCTGTCAACCAATTCGCCAAGGCAACCTTTCATCTGCGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTCGATCTGGA CAATAAGGACGGCGAGGTGTA CTGCAATCGACGCTAGATATTACGGCAACATCTCTAGATTCAACAA CCACCTCTGCGATCCCAACATCATTTCCCGTGAGGGTGTTCATGCTGCACCAAGATCTGAGGTTCCCT AGAAATCGCCTTCTTCAGCTCTAGAGACATCAGAACCGGGCAGGAGCTGGGCTTCGATTACGGCGGAT AGATTCTGGGACATCAAGTCCAAAGTACTTCACATGCCAGTGCAGCGCAGGAGAAAGTGTAAAGCACAG CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGATGCGAGCAGCGGATCCCTGG AGCCCCGGCGAAAAACCCCTATAAGTGCCCCGAGTGCGGCAAGAGCTTTAGCGATCCCCGGCCATCTG GTGAGGCATCAGAGGACCCACACCGGGCGAAAAAGCCTTACAAATGCCCCGAGTGTGGAAAAAAGCTT CAGCAGAAAGCGATCATCTGACCCACCCATCAGAGGACACACATACCGGGCGAGAAAGCCTTATAAATGCC CCGAATGTGGAAAGAGCTTCTCCAGAAAGCGACCATCTTGACCAACCAACAGAGGACCCATACCGGA GAAAAACCTTACAAATGCCCCGAGTGTGGAAAGTCTTTCAGCTCCCCCGCGATCTGACAAGACAT CAGAGAACCCACACCGGCGAAAAACCTTATAAATGTCCCGAGTGTGGCAAAAGCTTCTCCGACAA GAAGGATCTGACAAAGACACCAAGGACCCACACCGGGCGAGAAACCTTATAAATGTCCCCGAATGCG GAAAAAGCTTTAGCAGAAACGACGCTCTGACCGAACACCCAGAGAACACATACCGGAGAGAAAAACCC TATAAATGTCCCGAGTGGGAAAAATCCTTCAGCACCAACCGGCGCTCTGACAGAGCATCAGAGGAC ACACACCGGCAAAAGACCTCCGCTAGCGGCGAGCGGGCGGCGAGCGGGCGCAAGCGGCCCGCC GCCACCAAGAAAGGCCGCGCAGGCCAAGAAAGAAAGGCGAGCTACCCCTACGACGTGCCCGACTA
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		CGCCTGAGCGGCGCTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTC TCCCTTGCACTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAA AAAAA AAAAA AAAAA
MR- 29871 (SEQ ID NO: 230)	G9A-CXCL1 ZF1 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCAATCCACGGCGTGCCCGCGCTCGGGCGGGGTGGCTCAGGAATAGGGCTA TCAGAACCGAGAAAGATCATCTGTAGGACGTGGCTAGAGGCTACGAGAACGTGCCCCATTCCTTGC GTGAATGGCGTGGATGGGAACCTTGCCCCGAGGACTACAAATACATCTCCGAGAACTGCGAAAC CAGCACAAATGAACATCGACAGAAACATCACCCACCTCCAGCACTGCACATGTGTGGATGACTGCTC CTCCAGCAACTGTCTGTGCGGCCAGCTCTCCATCAGATGCTGGTACGACAAGGACGGCAGACTGCT GCAAGAGTTCAACAAAGATCGAACCCCTCTCATCTTCGAGTGTAACCAAGCTTGACAGCTGCTGGAG AAACTGCAAGAAATAGAGTGGTCCAGAGCGGCATCAAGGTGAGACTGCAACTGTACAGAACCGCCA AGATGGGATGGGAGTGAGGGCTCTGCAAAACCATTCGCCAAGGCACCTTCATCTGCGAATACGTG GGCGAACTGATCTCCGACGCCGAAGCTGACGTGAGAGAGGACGACAGCTATCTCTCGATCTGGA CAATAAGGACGGCGAGGTGTACTGCATCGACGCTAGATATTACGGCAACATCTCTAGATTTCATCAA CCACCTCTGCGATCCCAACATCATCTCCCGTGAGGGTGTTCATGTGTGCAACCAAGATCTGAGGTTCCCT AGAAATCGCCTTCTTCAGCTCTAGAGACATCAGAACCGCGGAGGAGCTGGGCTTCGATTACGGCGAT AGATTCTGGGACATCAAGTCCAAAGTACTTCACATGCCAGTGCAGCGAGAAAGTGTAAAGCACAG CGCTGAGGCCATTGCTCTGGAGCAGTCTAGACTGGCCAGACTGGATGGCAGCAGCGGATCCCTGG AGCCCGGCGAAAAAGCCTTACAAATGTCCCGAATGCGGAAAGAGCTTCAGCAGAGCCGACAACTG ACCGAACATCAGAGAAACCATACCGGAGAAAAACCTTACAAATGTCCCGAGTGCGGCAAAAGCTT CTCCCAAGCCGGACATCTGGCCAGCCACCAAGGACACATACCGGGCGAGAAACCCCTACAAAGTGCC CCGAGTGGCGCAAGTCTCTCTAGATCCGATGAGCTGGTTCAGACATCAGAGAACCCATACCGGCG AGAAAGCCTTATAAGTGGCCCCGAATGTGGCAAGTCTTCAGCCAGAGAGCTCATCTGGAGAGGCATC

		AAAGAACACACACCGGAGAGAAACCTTACAAAGTGTCCCGAGTGTGGAAAGAGCTTCTCCAGAAAGG GACGAGCTGAACGTCCACCAAGAAACCCATACCGGGGAAAGCCCTATAAATGCCCCGAGTGTGG AAAATCCTTTTCTAGATCCGACCATCTGACAAACCACAGAGGACCCATACCGGAGAGAAAGCCCTA CAAATGCCCCGAGTGTGGAAAAAGCTTCTCTAGAAACGATGCTCTGACAGAGCACCAAGGACCC ACACCGGCAAAAAGACCGCTAGCGGCAGCGGGCGGCAAGCGGGCCCCGCCG CACCAAGAAAGCGCGCCAGGCCAAGAAAGGAGAGTACCCCTACGACGTGCCCGACTACG CCTGAGCGGCGCTTAATTAAGCTGCCCTTCTGCGGGCTTGCCTTCTTGCCCATGCCCTTCTTCTCTC CCTTGACCTGTACCTCTTGGTCTTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA AAAA
MR- 29870 (SEQ ID NO: 231)	CXCL1 ZF2- DNMT3a-3L SP6	AGGAAATAAGAGAGAAAGAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCTCCCGCGCGGAGCAGCGGATCCCTGGAGCCCCGGGAAA AACCCTATAAGTGCCTCGAGTGGGCAAGAGCTTTAGCGATCCCGGCCATCTGGTGAGGCATCAGA GGACCCACACCGGGGAAAGCCTTACAAATGCCCGGAGTGTGGAAAAAGCTTCAGCAGAAAGCGAT CATCTGACCAACCCATCAGAGGACACATACCGGGCGAGAAAGCCTTATAAATGCCCCGAAATGTGGAAA GAGCTTCTCCAGAAAGCGACCATCTGACCAACCAACCAGAGGACCCATACCGGAGAAAAAACCTTACA AATGCCCGGAGTGTGGAAAGTCTTTCAGCTCCCGCGCGGATCTGACAAAGACATCAGAGAAACCCACA CCGGCGAAAAACCTTATAAATGTCCCGAGTGTGGCAAAAGCTTCTCCGACAAGAGGATCTGACA AGACACCAAAAGGACCCACACCGGGGAGAAACCTTATAAATGTCCCGAATGCGGAAAAAGCTTTAG CAGAAACGACGCTCTGACCGAAACACAGAGAAACACATACCGGGAGAGAAACCCCTATAAATGTCCCCG AGTGCGGAAAAATCCTTCAGCACCAACCGGGCGCTCTGACAGAGCATCAGAGGACACACACCGGCAAA AAGACCTCCGCTAGCGGCAAGCGGGCGGCGAGCGGGGCAACCAACGACAGGAGTTCCGACCCCCC CAAGGTGTACCCCCCGTGCCTCCCGCGAGAAAGCGGAAGCCCATCCCGGTGCTGAGCCTGTTCCGACG GCATCGCACCGGCTGCTGCTGTGCTGAAGGACCTGGGCATCCAGGTGACCGGTACATCGCCAGC

		GAGGTGTGCGAGGACAGCATCACCGTGGGCATGTCGGGCACCAAGGGCAAGATCATGTACGTGGG CGACGTGCGGAGCGTGACCCAGAGACACATCCAGGAGTGGGGCCCTTCGACCTGGTGATCGGCG GCAGCCCTGTCAACGACCTGAGCATCGTGAACCCCGCCCGGAAGGCTGTACGAGGGCACCGGC CGGCTGTTCTTCGAGTTCTACCGGCTGCTGCACGACGCCCCGGCCCAAGGAGGGCACGACCGGCC TTCTTCTGGCTGTTTCGAGAACGTGGTGGCCATGGGCGTGAGCGACAAAGCGGGACATCAGCCCGTTT CTGGAGAGCAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCCGCCACCGGGCCCGGTACTT CTGGGGCAACCTGCCCCGCATGAACCGGCCCTGGCCAGCACCGTGAACGACAAAGCTGGAGCTGC AGGAGTGCCTGAGCACGGCCGATCGCCAAAGTTCAGCAAGGTGCGGACCATCACCCGGAGC AACAGCATCAAGCAGGGCAAGGACCAGCACTTCCCCGTGTTTCATGAACGAGAAAGGAGACATCCT GTGTGCACCCGAGATGGAGCGGGTGTTCGGCTTCCCCGTGCACACTACACCGACGTGAGCAACATGA GCCGGCTGGCCCGCAGCGGCTGCTGGGCCGAGCTGGAGCGTGGCCGTGATCCGGCACCTGTTCTG CCCCCTGAAGGAGTACTTCGCCCTGCGTGAGCAGCGGCAACAGCAACGCCGGGGCCCC AGCTTCAGCAGCGGCTGTGCCCCCTGAGCCTGCGGGGAGCCACATGAATCCTCTCTGGAGATGTTT GAGACAGTGCCCCGTGTGGAGAAAGGCAACCCGTGAGGTGCTGAGCCTCTTCGAGGACATTAAAGAA GGAGCTGACCTCTCTGGGCTTTCTGGAATCCGGCAGCGACCCCGGCCAGCTGAAACACACGTGGTGA CGTGACCCGACACAGTGAGGAAGGACGTGGAAAGAGTGGGGCCCCCTTTGACCTCGTGTATGGAGCCA CACCTCCTCTCGGCCACACATGCGATAGGCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCT CCAGTACGCCAGACCTAAGCCCCGGCAGCCCCAGACCCCTTCTTCTGGATGTTCTGGACAAATCTGGT GCTGAACAAAGGAGGATCTGGATGTGGCAGCAGATTCTTGAGATGGAACCCGTGACAATCCCCG ACGTGCATGGCGGCTCTCTGCAGAACGCCGTGAGAGTGTGGTCCAAACATCCCCGCCATTAGAAAGCA GACACTGGGCTCTGGTGAGCGAGGAGGAACCTGTCTCTGTGGCCCCAGAAATAGCAGTCCTCCAAG CTGGCCGCCAAGTGGCCCCACCAAGCTGGTGAAGAACTGCTTCTGCTCTGAGGGAGTATTTCAAG TATTTTCAGCACCCGAACCTGACCAGCAGCTGAGCGGGCGGCAAGCGGCCCGCCACCAAGAAAGGC CGGCCAGGCCAAGAAGAAGAGGCAGCTACCCCTACGACGTGCCCCGACTACGCTGAGCGGCCG
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		CTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACTGTGTA CCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAAGAAAAAAGAAAAAAGAAAAA AAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAA
MR- 29867 (SEQ ID NO: 232)	CXCL1 ZF6- HDAC8 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAAGGTGGGCATCCACGGCGTGCCCGCCCGCGGCAGCAGCGGATCCCTGGAGCCCCGGCGAAA AGCCTTACAAGTGTCCCGAGTGCGGAAAGAGCTTCAGCAGAGCCGATAATCTGACCGAGCACCCAA AGGACCCACACCGGAGAGAAAGCCTTATAAGTGTCCCGAATGCCGCAAAAGCTTTTCTAGAAAGCGA TCATCTGACCAACCACAGAGGACACACACCGGAGAAAAACCTTACAAATGCCCGAGTGCGGCA AAAGCTTCTCCAGAGCAGCAATCTGGTGAGACACCAAGGACCCACACCGGCGAAAAACCCCTAT AAATGCCCCGAATGTGGCAAGAGCTTTAGCACATCCGGCGAGCTGGTGAGGCATCAAAAGAAACACA TACCGGCGAGAAAGCCCTACAAAGTGCCCGAGTGTTGGAAAAAGCTTCAGCACCCACCTCGATCTGAT CAGACACAGAGGACCCATACCGGAGAGAAAAACCTTACAAATGTCCCGAGTGCGGAAAAAGTCTCTTA GCCAGCTGGCCCATCTGAGAGCTCATCAAAGGACACACACCGGCGAGAAAGCCTTACAAGTGTCCC GAGTGGGAAAAATCCTTCTCCCAACTGGCCCATCTGAGGGCCACACAGAGAAACCCACACCGGCAA AAAGACCTCCGCTAGCGGCAGCGGGCGGCGAGCGGGCGAGGAGCCCGAGGAGCCCGCCGAT AGCGGACAAATCTCTGGTGCCCGTCTACATCTACAGCCCCGAATATGTGAGCATGTGTGATTCCCTC GCCAAGATCCCTAAGAGAGCCAGCATGGTGCAATCTCTGATCGAGGCTACGCTCTGCATAAGCAA ATGAGGATCGTGAAAGCCCAAGGTCCGACAGCATGGAAGAGATGGCCACCTTTACACCCGATGCCTA CCTCCAACATCTCCAGAAGGTGTCCCAAGAGGGGACGACGACCAACCCCGACTCCATTGAGTACG GACTGGGCTATGATTGCCCCGCCACCGAGGGCATCTTTGACTATGCCCGCCGCTATCGGGCGGAGCTA CCATCACAGCCGCCAGTGTCTGATTGATGGCATGTGCAAGGTGCGCATCAACTGGTCCGGAGGCT GGCATCATGCCAAGAAAGGATGAGGCCCTCCGGCTTCTGTATCTGAATGACGCCGTGCTGGGCATTTC TGAGACTGAGGAGGAAATTTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCAGCGAGATGGA GTCGAAAGATGCCTTCAGCTTCACCAGCAAGGTGATGACAGTCTCTCTGCACAAAGTTCTCCCCCGGC

		TTCTTTCCCGGAACCGGCGACGTGTCCGACGTGGGCAAGGGAAGGTACTACAGCGTGAA CGTGCCCAATTCAAGACGGGCAATCCAAGACGAGAAAGTACTACAGATCTGCGAGTCCGTGCTCAAGG AGGTCTACCAAGCCTTCAATCCTAAGGCTGTGCTGCTCCAACTGGAGCTGATACCAATTGCTGGCG ATCCCATGTGCAGCTTCAATATGACACCCGTCGGAATCGGCAAGTGCCCTCAAGTACATCCTCCAGT GGCAGCTCGCCACCCCTCATTTCTCGGAGGAGGCGGATACAATCTGGCTAATACCGCCAGATGCTGGA CCTATCTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCAACGAGTTTTC CCGCTTACGGCCCCGACTACGTGCTGGAGATCACCCCCAGCTGCAGACCCGATAGAAACGAAACCCC ATAGAAATCCAGCAAAATTTGAACTATATCAAGGGCAACCTCAAGCACGTGCTGGAGGTGGCGGA TCGGGAAAGCGGCCCGCCGCCACCAAGAAAGGCCGTCAGGCCAAGAAAGAAAGGGCAGCTACC CCTACGACGTGCCCCACTACGCCCTGAGCGGCCGCTTAATTAAAGTGCCTTCTGCGGGGCTTGCCCTTC TGGCCATGCCCTTCTTCTCCTTGTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAA GTCTAGAAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA AAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
MR- 29850 (SEQ ID NO: 233)	EZH2- CXCL1 ZF4 T7	AGGAAATAAGAGAGAAAAAGAGTAAGAAGAAATATAAGAGCCACCATGCCCCCAAGAAAGAA GCGGAAAGGTGGCGGCAGCGGCGGCAGCGGCCAGACCCGGCAAGAAAGCGAGAAAGGGCCCCGTG TGCTGGCGGAAGCGGGTGAAGAGCGAGTACATGCGGCTGCGGCAGCTGAAGCGGTTCGCGGCGGGC CGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGCAGAAAGATCCTGGAGCGGACCGAGATCCTGA ACCAAGAGTGAAGCAGCGCGGAATCCAGCCCGTGACATCCTGACCAAGCGTGAGCAGCCTGCGG GGCACCCGGGAGTGCAGCGTGACCAAGCAGACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAAGAC CCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGGT GGAGGACGAGACCGGTGCTGCACAACATCCCCCTACATGGGCGACGAGGTGCTGGACCAGGACGGCA CCTTCATCGAGGAGCTGATCAAGAACTACGACGGCAAGGTGCACGGCGACCGGGAGTGCGGGCTTC ATCAACGACGAGATCTTCGTGGAGCTGTTGAACGCCCTGGGCCAGTACAACGACGACGACGACGA CGACGACGGCAGACCCCGAGGAGCGGGAGGAGAAAGCAGAAAGACCTGGAGGACCAACCGGGAC

		GACAAGAGAGCCGGCCCCCGGAAGTTCCCCAGCGACAAGATCTTCGAGGCCATCAGCAGCAT GTTCCCCGACAAGGGCACCGCCGAGGAGCTGAAGGAGAAAGTACAAGGAGCTGACCGAGCAGCAG CTGCCCGGCCCTGCCCCCGGAGTGACCCCCAACATCGACGGCCCCAACGCCAAGAGCGTGCA GCGGAGCAGAGCCTGCACAGCTTCCACACCCCTGTTCTGCCGGCGTGCTTCAAGTACGACTGCTT CCTGCACCCCTTCCACGCCACCCCCAACACCTACAAGCGGAAGAACACCCGAGACCCGCTGGACA ACAAGCCCTGCGGCCCCAGTGCTACCAAGCACCTGGAGGGGCCAAGGAGTTGCGCGCCGCCCTG ACCGCCGAGCGGATCAAGACCCCCCAAGCGGCCCGCGGCCGCGCGGGCTGCCCAA CAACAGCAGCGGCCAGCACCCCCACCATCAACGTGTGGAGAGCAAGGACACCGACAGCGACC GGGAGGCCGCACCGAGACCGGGCGGAGAAACGACAAGGAGGAGGAGAGAAAGAGACG AGACCAGCAGCAGCAGCGAGGCCAACAGCCGGTGCCAGACCCCCCATCAAGATGAAGCCCCAACATC GAGCCCCCGAGAACGTGGAGTGGAGCGGCGCGGAGCCAGCATGTTCCGGGTGCTGATCGGGCAC CTACTACGACAACCTTCTGCGCCATCGCCCCGCTGATCGGCACCAAGACCTGCCGGCAGGTGTACGA GTTCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCGCGGAGACGTGGACACCCCCCCCC GGAAGAAGAGCGGAAGCACCGGCTGTGGGCCGCCACTGCCGGAAGATCCAGCTGAAGAAGGA CGGCAGCAGCAACCAGTGTACAACCTACCAGCCCTGCGACCAACCCCCCGCAGCCCTGCCGACAGCA GCTGCCCTGCGTGATCGCCCCAGAACTTCTGCGAGAAAGTTCTGCCAGTGCAGCAGCGAGTGCCAGA ACCGGTTCCCCGGCTGCCGGTGCAAGGCCCAAGTGCAACACCAAGCAAGTGCCCCCTGCTACCTGGCCG TGCGGAGTGCACCCCGACCTGTGCTGACCTGCGGCCCGCCGACCACTGGGACAGCAAGAAC GTGAGCTGCAAGAACTGCAGCATCCAGCGGGCAGCAAGAAGACCTGCTGTGCCCCCAGCGA CGTGGCCGGCTGGGGCATCTTCATCAAGGACCCCGTGCAAGAACGAGTTTCATCAGCGAGTACT GCGGCGAGATCATCAGCCAGGACGAGGCCACCGGGGGGCAAGGTGTACGACAAGTACATGTGC AGCTTCCTGTTCAACCTGAACAACGACTTCGTGTGTGACGCCACCCGGAAGGGCAACAAGATCCG GTTCCGCAACCAAGCGGTGAACCCCAACTGCTACGCCAAGGTGATGATGTTGAACGGCGACCAACC GGATCGGCATCTTCGCCAAGCGGGCCATCCAGACCGGGGAGGAGCTGTTCTTCGACTACCGGTACA
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		GCCAGGCCGACGCCCTGAAGTACGTGGGCATCGAGCGGAGATGGAGATCCCCGGCAGCAGCGGA TCCCTGGAGCCCCGGAGAAAGCCTTACAAAATGCCCCGAGTGGCGCAAGTCCTTCAGCCAGCTGGCT CATCTGAGAGCTCATCAAAGGACCCACACCGGGGAGAAAGCCCTATAAGTGCCCCGAGTGCGGAAA ATCCTTCTCCAGAGCAGCAATCTGTCAGACACACAGAGAGACCCACACCGGCGAGAAACCTTACA AGTGTCGCCGAAATGTGGAAAGTCCCTTCTCCCAAAGAGCTCTCTGATCGCCCATCAGAGAAACACATA CCGGCGAAAAACCCCTACAAGTGCCCCGAGTGTGGCAAAGCTTTTCCACCCACCGGCAATCTGACCG TGCAATCAAAGAACCCACACCGGGGAAAAACCCCTACAAATGCCCCGAGTGTGGCAAAATCCTTCTCCG ACCCGGCCATCTGTTGAGCACAGAGGACACACACCGGGGAGAAACCTTATAAATGTCCCGAA TGCGGCAAGTCCTTTAGCACAGCGGCTCTCTGTGTGAGACATCAGAGGACACATACCGGGCGAAAA GCCTTACAAGTGTCGCCGAGTGTGGCAAAGCTTCAGCCAGAAACAGCACACTGACAGAGCATCAGA GAACCCATACCGGCAAAAAGACCAGCGCTAGCGGCAAGCGGCGGCGGCGGCAAGCGGGCC CGCCGCCACCAAGAAAGGCCGGCCAGGCCAAGAAAGAAAGAGGCGAGCTACCCCTACGACGTGCCCG ACTACGCCCTGAGCGGCCGCTTAATTAAAGCTGCCTTCTGCGGGCTTGCCCTTCTGCGCATGCCCTTCT TCTCTCCCTTGCACTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAGTCTAGAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAA
MR- 29849 (SEQ ID NO: 234)	EZH2- CXCL1 ZF3 T7	AGGAAATAAGAGAGAAAGAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGCGGCAGCGGGCGGCAGCGGCCAGACCGGCAAGAAAGAGCGAGAAAGGGCCCCGTG TGCTGGCGGAAGCGGGTGAAGAGCGAGTACATGCGGCTGCGGCAGCTGAAGCGGTTCCGGCGGGC CGACGAGGTGAAGAGCATGTTTCAGCAGCAACCGGGCAGAAAGATCCTGGAGCGGACCGAGATCCTGA ACCAGGAGTGGAAGCAGCGGGCAATCCAGCCCCGTGCACATCCTGACCGCGTGAGCAGCCTGCCG GGCACCCGGGAGTGACGCTGACCAAGCGACCTGGACTTCCCCACCCAGGTGATCCCCCTAAAAGAC CCTGAACGCCGTGGCCAGCGTGCCCATCATGTACAGCTGGAGCCCCCTGCAGCAGAACTTCATGCT GGAGGACGAGACCGTGTGTGCACAAACATCCCCCTACATGGGCGACGAGGTGCTGGACCGACGCGCA

		CCTTCATCGAGGAGCTGATCAAGAACTACGACGGCAAGGTGCACGGCGACCGGGAGTGCGGGCTTC ATCAACGACGAGATCTTCGTGGAGCTGGTGAACGCCCTGGGCCAGTACAACGACGACGACGACGA CGACGAGGCGACGACCCCGAGGAGCGGGAGGAGAAAGCAGAAAGACCTGGAGGACCAACCGGGAC GACAAGGAGAGCCGGCCCCCCCCGGAAGTTCCCCAGCGACAAAGATCTTCGAGGCCATCAGCAGCAT GTTCCCCGACAAAGGGCACCGCCGAGGAGCTGAAGGAGAAAGTACAAGGAGCTGACCGGACGACGAG CTGCCCGGCCCTGCCCCCCGAGTGCAACCCCCAACATCGACGGCCCCAACGCCAAGAGCGGTGCA GCGGGAGCAGAGCCTGCACAGCTTCCACACCCCTGTTCTGCGGGGGTGTCTCAAGTACGACTGCTT CCTGCACCCCTTCCACGCCACCCCCAACACCTACAAGCGGAAGAACACCGAGACCGCCCTGGACA ACAAGCCCTGCGGGCCCCAGTGCTACCAAGCACCTGGAGGGGCCAAAGGAGTTGCGCGCCGCCCTG ACCGCCGAGCGGATCAAGACCCCCCCCCAAGCGGCCCGGGCGGGCGGGCGGGCTGCCCAA CAACAGCAGCCGGCCCCAGCACCCCCACCATCAACGTGTGGAGAGCAAGGACACCGGACAGCGACC GGGAGCGCGCACCGAGACCGGGCGGCGAGAACAAACGACAAGGAGGAGGAGAGAAAGAGGACG AGACCAGCAGCAGCAGCGAGGCCAACAGCCGGTGCCAGACCCCCCATCAAGATGAAGCCCAACATC GAGCCCCCGAGAACGTGGAGTGGAGCGGCGCGGAGCCAGCATGTTCCGGGTGCTGATCGGCAC CTACTACGACAACTTCTGCGCCATCGCCCCGGCTGATCGGCACCAAGACCTGCCGGCAGGTGTACGA GTTCCGGGTGAAGGAGAGCAGCATCATCGCCCCCGCCCCCGCGAGGACGTGGACACCCCCCCCC GGAAAGAAAGCGGAAGCACCGGCTGTGGGGCGGCCACTGCCGGAAGATCCAGCTGAAGAAAGGA CGGCAGCAGCAACCACGTGTACAACCTACCAAGCCCTGCGACCAACCCCGGCGAGCCCTGCGACAGCA GCTGCCCTGCGTGATCGCCAGAACTTCTGCGAGAAAGTTCTGCCAGTGCAAGCAGAGTGCCAGA ACCGGTTCCCCGGCTGCCGGTGCAAGGCCCAAGTGCAACACCAAGCAGTGCCCCCTGCTACCTGGCCG TGCGGGAGTGCGACCCCGACCTGTGCTGACCTGCGGGCGCCGACCACTGGGACAGCAAGAAAC GTGAGCTGCAAGAACTGCAGCATCCAGCGGGGCGAGCAAGAAAGCACTGTCTGTGCTGGCCCCCAGCGA CGTGCGCGGCTGGGGCATCTTCATCAAGGACCCCGTGCAGAAAGAAAGAGTTTCATCAGCGAGTACT GCGGCGAGATCATCAGCCAGGACGAGGCCGACCGGGGGCAAGGTGTACGACAAGTACATGTGC
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		AGCTTCCTGTTCAACCTGAACAACGACTTCGTGTGGACGCCACCCGGAAGGGCAACAAGATCCG GTTCCGCAACCAACAGCGGTGAACCCCAACTGCTACGCCAAGGTGATGATGTTGTAACGGCGACCAACC GGATCGGCATCTTCGCCAAGCGGGCCATCCAGACCGGGGAGGAGCTGTTCTTCGACTACCGGTACA GCCAGGCCGACGCCCTGAAGTACGTGGGCATCGAGCGGGAGATGGAGATCCCCCGGCAGCAGCGGA TCCCTGGAGCCCCGGGAAAAACCCTATAAATGCCCCGAGTGTGGCAAAGAGCTTTTCCGACCCCCGGA CATCTCGTGAGGCATCAGAGAACACATACCGGGGAGAAACCCTACAAGTGCCCCGGAATGCGGCAA ATCCTTCTCTAGAAAGGACAATCTGAAAAAACCATCAAAAGAACCCATACCGGGCGAGAAAGCCCTATA AATGTCCCAGTGTGAAAGAGCTTCAGCCACAAGAACGCTCTGCAAGAACCATCAGAGGACCCCAT ACCGGGGAAAAAGCCTTATAAGTGCCCCGAGTGGGAAAAATCCTTTTCTAGAAAGGACGAGCTGAA TGTGCACCAAAAGGACACATACCGGAGAGAAACCCTACAAATGCCCCGAGTGGCGCAAGTCCTTCA GCACCTCCGGCAATCTGGTGAGGCACCAAAAGGACACACACCGGGGAAAAAACCTTACAAAGTGTCCTC GAGTGGGAAAAAAGCTTTTCCCAAGAACAGCACACTGACCGAACACCAAGGACCCACACCGGGAGA GAAACCTTATAAATGTCCCGAGTGTGAAAAAGTCTTTAGCCAGTCCGGCAATCTGACAGAGCATCA AAGAACCCACACCGGCAAAAAGACCTCCGCTAGCGGCAGCGGGCGGCAAGCGGCAAGCGG CCCGCCGCCACCAAGAAAGGCCGGCCAGGCCAAGAAAGAAAGGGCAGCTACCCCTACGACGTGCC CGACTACGCCTGAGCGGGCCGCTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCCATGCCCTT CTTCTCTCCCTTGCACTGTACCTCTTGTGCTTTGAAATAAAGCCTGAGTAGGAAGTCTAGAAAAAAA AAAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAA AAAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAAATAAAAA
MR- 29848 (SEQ ID NO: 235)	CXCL1 ZF3- HDAC8 SP6	AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAAGGTGGGCATCCACGGCGTGCCCGCCCGCGGCAGCAGCGGATCCCTGGAGCCCCGGCGAAA AACCCTATAAATGCCCCGAGTGTGGCAAAGAGCTTTTCCGACCCCGGACACCTCGTGAGGCATCAGA GAACACATACCGGGGAGAAACCCTACAAGTGCCCCGGAATGCGGCAAAATCCTTCTCTAGAAAAAGGAC AATCTGAAAAACCATCAAGAACCCATACCGGGGAGAGGCCCTATAAATGTCCCGAGTGTGGA

		GAGCTTCAGCCACAAGAACGCTCTGCGAAGAACCATCAGAGGACCCATACCGGGGAAAGCCTTATA AGTGCCCCGAGTGCGGAAATCCTTTTCTAGAAGGACGAGCTGAATGTGCACCAAAAGGACACAT ACCGGAGAGAAACCTTACAAATGCCCGAGTGC GGCAAGTCCTTACGACCTCCGGCAATCTGGT GAGGCACCAAGGACACACACCGGCGAAACCTTACAAAGTGTCCCGAGTGC GGAAAAAGCTTTT CCCAGAACAGCACACTGACCGAACACCAAGGACCCACACCGGAGAGAAACCTTATAAATGTCCC GAGTGTGGAAAGTCTTTAGCCAGTCCGGCAATCTGACAGAGCATCAAGAAACCCACACCGGCAA AAAGACCTCCGCTAGCGGCAGCGGCGGCGCAGCGGCGGAGAGCCGAGGAGCCCGCCGAT AGCGGACAATCTGTGTGCCGTCTACATCTACAGCCCCGAATATGTGAGCATGTGTGATTCCCTC GCCAAGATCCCTAAGAGAGCCAGCATGGTGCATTCTCTGATCGAGGCCTACGCTCTGCATAAGCAA ATGAGGATCGTGAAAGCCCCAAGGTCGCCAGCATGGAAAGAGATGGCCACCTTTACACCCGATGCCTA CCTCCAACATCTCCAGAAGGTGTCCCAAGAGGGCGACGACGCCACCCGACTCCATTGAGTACG GACTGGGCTATGATTGCCCGCCACCGAGGGCATCTTTGACTATGCCGCCGCTATCGGCGGAGCTA CCATCACAGCCGCCAGTGTCTGATTGATTGGCATGTGCAAGGTGCGCATCAACTGTGCCGAGGCT GGCATCATGCCAAGAAGGATGAGGCCTCCGGCTTCTGTATCTGAATGACGCCGTGCTGGGCATTC TGAGACTGAGGAGGAAATTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGA GTCGAAGATGCCCTTCAGCTTCACCCAGCAAGGTGATGACAGTCTCTCTGCACAAAGTTCTCCCCCGGC TTCTTTCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGGCAAGGGAAGGTACTACAGCGTGAA CGTGCCCAATTCAAGACGGCATCCAAAGACGAGAAAGTACTACAGATCTGCGAGTCCGTGCTCAAGG AGGTCTACCAAGCCTTCAATCCTAAGGCTGTGTGCTCCAACTGGGAGCTGATACCAATTGTGGCG ATCCCATGTGCAGCTTCAATATGACACCCGTCGGAAATCGGCAAGTGCCCTCAAGTACATCCTCCAGT GGCAGCTCGCCACCCCTCATTTCTCGGAGGAGGCGGATACAAATCTGGCTAATACCGCCAGATGCTGGA CCTATCTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCAACGAGTTTTTCA CCGCTTACGGGCCCGACTACGTGTGGAGATCACCCCGAGCTGCAGACCCGATAGAAACGAAACCCC ATAGAAATCCAGCAAATTTCTGAACTATATCAAGGGCAACCTCAAGCACGTGCTGGGAGGTGGCGGA
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		TCGGGAAAGCGGCCCGCCGCCAACCAAGAGAGCCGGTCAGGCCAAGAAAGAGAGGCGAGCTACC CCTACGACGTGCCCGACTACGCCCTGAGCGGCCGCTTAATTAAAGTGCCTTCTGCGGGGCTTGCCCTTC TGCCATGCCCTTCTTCTCCTTGCACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAA GTCTAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAA AAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAAAGAAAAA
MR- 29847 (SEQ ID NO: 236)	CXCL1 ZF6- DNMT3a-3L SP6	AGGAAATAAGAGAGAAAGAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCCGCCCGCGGAGCAGCGGATCCCTGGAGCCCGCGGAAA AGCCTTACAAGTGTCCCGAGTGGGAAAGAGCTTCAGCAGAGCCGATAATCTGACCGAGCACCAA AGGACCCACACCCGGAGAGAAAGCCTTATAAGTGTCCCGAATGCGGCAAAAGCTTTTCTAGAAAGCGA TCATCTGACCAACCCACAGAGGACACACACCGGAGAAAAACCTTACAAAATGCCCGGAGTGCGGCA AAAGCTTCTCCACAGAGCAGCAATCTGGTGAGACACCAAAGGACCCACACCGGCGAAAAACCCCTAT AAATGCCCCGAATGTGGCAAGAGCTTTAGCACATCCGGGAGCTGGTGAGGCATCAAAAGAACACA TACCGGCGAGAAAGCCCTACAAAGTGCCCGAGTGTGGAAAAAGCTTCAGCACCCACCTCGATCTGAT CAGACACCAGAGAGCCCATACCGGAGAGAAAAACCCCTACAAATGTCCCGAGTGCAGAAAGTCCTTA GCCAGCTGGCCCATCTGAGAGCTCATCAAAAGGACACACACCGGCGAGAAAGCCTTACAAGTGTCCC GAGTGCGGAAATCTCTTCTCCCAACTGGCCCATCTGAGGGCCCCACCCAGAGAACCCACACCCGGCAA AAAGACCTCCGCTAGCGGCAGCGGGCGGCGCAGCGGCGGCAACACAGACAGGAGTTCTGACCCCC CCAAGGTGTACCCCCCGTGCCCGCCGAGAAAGCGGAAGCCCCATCCGGGTGCTGAGCCTGTTCGACG GCATCGCACCCGGCCTGTGTGTCTGAAGGACCTGGGCATCCAGGTGGACCGGTACATCGCCAGC GAGGTGTGCGAGGACAGCATCACCGTGGGCATGGTGCGGCACCCAGGGCAAGATCATGTACGTGGG CGACGTGCGGAGCGTGACCCAGAAAGCACATCCAGGAGTGGGGCCCCCTTCGACCTGGTGATCGGGC GCAGCCCCGTGCAACGACCTGAGCATCGTGAACCCCGCCCCGGAAGGGCCTGTACGAGGGCAACCGGC CGGCTGTTCTTCGAGTTCTACCGGCTGTGTGACGACGCCCCGGCCCCAAGGAGGGCGACGACCGGCC TTCTTCTGGCTGTCGAGAACGTGGTGGCCATGGGCGTGAGCGCAAGCGGGACATCAGCCGGTTC

		CTGGAGAGCAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCGCCCAACCGGGCCCGGTACTT CTGGGGCAACCTGCCCGGCATGAACCGGCCCTGGCCAGCACCGTGAACGACAAGCTGGAGCTGC AGGAGTGCCTGGAGCACGGCCGGATCGCCAAGTTACGAAGGTGCGGACCATCACACCCGGAGC AACAGCATCAAGCAGGGCAAGGACCAGCACTTCCCCGTGTTTCATGAACGAGAAAGGAGGACATCCT GTGGTGACCCGAGATGGAGCGGGGTGTTCCGGCTTCCCCGTGCACACTACACCCGACGTGAGCAACATGA GCCGGCTGGCCCGCAGCGGCTGCTGGGCCGGAGCTGGAGCGTGCCCGTGATCCCGGCACCTGTTCCG CCCCCTGAAGGAGTACTTCGCCCTGCGTGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCC AGCTTCAGCAGCGGCTGTGTGCCCTGAGCCTGCGGGGAGCCACATGAATCCTCTCTGGAGATGTTT GAGACAGTGCCCGTGTGGAGAAAGGCAACCCGTGAGGGTGTCTGAGCCTCTTCGAGGACATTAAAGAA GGAGCTGACCTCTCTCTGGGCTTTCTGGAATCCGGCAGCGACCCCGGCCAGCTGAAACACACGTGGTGGA CGTGACCGACACAGTGAGGAAAGGACGTGGAAAGAGTGGGGCCCCCTTTGACCTCGTGTATGGAGCCA CACCTCCTCTCGGCCACACATGCGATAGGCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCT CCAGTACGCCAGACCTAAGCCCGGCAGCCCCAGACCCCTTCTTCTGGATGTTCTGGACAATCTGGT GCTGAACAAGGAGGATCTGGATGTGGCCAGCAGATTCTGGAGATGGAACCCGTGACAATCCCCG ACGTGCATGGCGGCTCTCTGCAGAACGCCGTGAGAGTGTGGTCCAAACATCCCCGCCATTAGAAAGCA GACACTGGGCTCTGGTGAGCGAGGAGGAACTGTCTCTGCTGGCCCCAGAAATAGCAGTCCTCCAAG CTGGCCGCCAAGTGGCCCAACCAAGCTGGTGAAGAACTGCTTCTGCTCTGAGGGAGTATTTCAAG TATTTACGACCCGAACCTGACCAAGCAGCCTGAGCGGCGGCAAGCGGCCCGCCACCAAGAAAGGC CGGCCAGGCCAAGAAGAAGAGGCGAGCTACCCCTACGACGTGCCCGACTACGCTGAGCGGCCG CTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTA CCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAAGTCTAGAAAAAATAAAAAAATAAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA
MR- 29846	CXCL1 ZF4- DNMT3a-3L	AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATAATAAGAGCCACCATTGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCGCAGCAGCGGATCCCTGGAGCCCGGAGAAA

(SEQ ID NO: 237)	SP6	AGCCTTACAAAATGCCCCGAGTGCGGCAAGTCCTTCAGCCAGCTGGCTCATCTGAGAGCTCATCAA GGACCCACACCGGCGAGAAGCCCTATAAGTGCCCCGAGTGCGGAAAATCCTTCTCCCAAGACGAGC AATCTCGTCAGACACAGAGGACCCACACCGGCGAGAAACCTTACAAGTGTCCCGAATGTGAAA GTCTTCTCCCAAAGAGCTCTGTATCGCCCATCAGAGAACACATACCGGCGAAAAACCTTACAA GTGCCCCGAGTGTGGCAAAAGCTTTTCCACCCACCGGCAATCTGACCGTGCATCAAAAGAAACCCACAC CGGCGAAAAACCTTACAAATGCCCCGAGTGTGGCAAAATCCTTCTCCGACCCCGGCCATCTGGTGAG GCACGAGGACACACACCGGCGAGAAACCTTATAAATGTCCCGAATGCGGCAAGTCCTTTTAGCA CCAGCGGCTCTCTGGTGAGACATCAGAGGACACATACCGGCGAAAAGCCTTACAAGTGTCCCGAG TGTGGCAAAAGCTTACGCCAGAACAGCACACTGACAGAGCATCAGAGAACCCATACCGGCAAAAA GACCAGCGTAGCGGCAGCGGCGGCGGCAAGCGGCGGCAACCCACGACGAGGAGTTCGACCCCCC AGGTGTACCCCCCGTGCCCCGCGAGAAAGCGGAAGCCCATCCGGGTGTGAGCCTGTTCTCGACGGC ATCGCCACCGGCGCTGTGTGTGTAAGGACCTGGGCATCCAGGTGACCGGTACATCGCCAGCGGA GGTGTGCGAGGACATCACCGTGGGCATGGTGCGGCACCAAGGCAAGATCATGTACGTGGGCG ACGTGCGGAGCGTAGCCAGAACAGACATCCAGGAGTGGGCCCCCTTCGACCTGTTGATCGGCGGC AGCCCCTGCAACGACCTGAGCATCGTGAACCCCGCCCGGAAAGGGCCTGTACGAGGGCACCGGGCCG GCTGTTCTTCGAGTTCTACCGGCTGCTGCACGACGCCCCGGCCCAAGGAGGGCGACGACCGGCCCTT CTTCTGGCTGTTTCGAGAACGTGGTGGCCATGGGCGTGAGCGGACAAAGCGGGACATCAGCCGGTTCCT GGAGAGCAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCCGCCACCGGGCCCGGTACTTCT GGGCAACCTGCCCCGCGATGAACCGGCCCCCTGGCCAGCACCGTGAACGACAAGCTGGAGCTGCAG GAGTGCCCTGGAGCACGGCCGGATCGCCAAAGTTCAGCAAGGTGCGGACCATCACCAACCGGAGCAA CAGCATCAAGCAGGGCAAGGACCAAGCACTTCCCCGTGTTTCATGAACGAGAAGGAGGACATCCTGT GGTGACCGGAGATGGAGCGGGTGTTCGGCTTCCCCCGTGCACTACACCGACGTGAGCAACATGAGC CGGCTGGCCCCGCGAGCGGCTGTGGGCGGAGCTGGAGCGGTGCCCGTGTATCCGGCACCTGTTCCGCC CCCCTGAAGGAGTACTTCGCTGCGTGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCCAG
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		CTTCAGCAGCGGCCTGTGTGCCCTGAGCCTGGGGGCGAGCCACATGAATCCTCTGGAGATGTTCTGA GACAGTGCCCGTGTGAGAGAAAGGCAACCCGTGAGGGTGTCTGAGCCTCTTCGAGGACATTAAGAAAG AGCTGACCTCTCTGGGCTTTCTGGAAATCCGGCAGCGACCCCGGCCAGCTGAACACACGTGTGTGGACG TGACCGACACAGTGAGGAAGGACGTGGAAGAGTGGGGCCCTTTGACCTCGTGTATGGAGCCACA CCTCCTCTCGGCCACACATGCGATAGGCCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCTCC AGTACGCCAGACCTAAGCCCGGCGAGCCCGAGACCCCTTCTTCTGGATGTTCGTGGACAATCTGGTGC TGAAACAAGGAGGATCTGGATGTGGCCAGCAGATTTCTGGAGATGGAACCCGTGACAATCCCCGAC GTGCATGGCGGCTCTTCAGAAACGCCGTGAGAGTGTGTCTCAACATCCCCGCCATTAGAAGCAGA CACTGGGCTCTGTGAGCGGAGGAACTGTCTCTGTCTGGCCAGAAATAAGCAGTCCTCCAAAGCTG GCCGCCAAGTGGCCCAACCAAGCTGGTGAAGAACTGCTTTCTGCCTCTGAGGGAGTATTTCAAGTAT TTCAGCACCGAACTGACCAGCAGCCTGAGCGGGGCAAGCGGCCCGCCACCAAGAAAGGCCGG CCAGGCCAAGAAAGAAAGGCGAGCTACCCCTACGACGTGCCCGACTACGCCGTGAGCGGCCGCTT AATTAAAGCTGCCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGACCTGTACCT CTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAAAATAAAAAAATAAAAAA AAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAAATAAAAAA
MR- 29845 (SEQ ID NO: 238)	CXCL1 ZF3- DNMT3a-3L SP6	AGGAAATAAGAGAGAAAGAAAGTAAGAAAGAAATAAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCTCCCGCGCGGCGAGCAGCGGATCCCTGGAGCCCGCGGAA AACCTATAAATGCCCCGAGTGTGGCAAGAGCTTTTCCGACCCCGGACACCTCGTGAGGCATCAGA GAACACATACCGGCGAGAAACCCCTACAAAGTGCCCCCGAATGCGGCAAAATCCTTCTCTAGAAAAAGGAC AATCTGAAAAAACCATCAAAAGAAACCCATACCGGGGAGAAAGCCCTATAAATGTCCCGAGTGTGGAAA GAGCTTCAGCCACAAGAACGCTCTGCGAGAAACCATCAGAGGACCCATACCGGGCGAAAGCCTTATA AGTGCCCCGAGTGGGAAATCCTTTTCTAGAAAGGACGAGCTGAAATGTGCACCAAAAGGACACAT ACCGGAGAGAAACCCCTACAAATGCCCGGAGTGGGCAAGTCCCTTCAGCACCTCCGGCAATCTGGT GAGGCACCAAGGACACACACCGGCGAAACCTTACAAAGTGTCCCGAGTGGGAAAAAGCTTTT

		CCCAGAACAGCACACTGACCGAACACCAAGGACCCACACCGGAGAGAAACCTTATAAATGTCCC GAGTGTGAAAGTCTTTAGCCAGTCCGGCAATCTGACAGAGCATCAAGAACCACACCGGCAA AAAGACCTCCGCTAGCGGACGCGGCGGCAAGCGGCAACACAGACCAGGAGTTCGACCCCC CCAAGGTGTACCCCCCGTGCCTCCGCGGAGAAAGCGGAAAGCCCATCCGGGTGCTGAGCCTGTTGACG GCATCGCCACCCGGCCTGCTGGTGTGAAGGACCTGGGCATCCAGGTGGACCGGTACATCGCCAGC GAGGTGTGCGAGGACAGCATCACCGTGGGCATGGTGGGCACCAAGGGCAAGATCATGTACGTGGG CGACGTGCGGAGCGTGACCCAGAGACATCCAGGAGTGGGGCCCTTCGACCTGGTGATCGGGG GCAGCCCCGTGCAACGACCTGAGCATCGTGAACCCCGCCCGGAAGGGCCTGTACGAGGGCAACCGG CGGCTGTTCTTCGAGTTCTACCGGCTGCTGCACGACGCCCCGGCCCAAGGAGGGGACGACCGGCCCC TTCTTCTGGCTGTTGAGAACGTGGTGGCCATGGGCGTGAGCGACAAAGCGGGACATCAGCCGGTTC CTGGAGAGCAAACCCCGTGATGATCGACGCCAAGGAGGTGAGCGCCGCCACCGGGCCCCGGTACTT CTGGGGCAACCTGCCCCGGCATGAACCGGCCCTGGCCAGCACCGGTGAACGACAAAGCTGGAGCTGC AGGAGTGCCCTGAGCACGGCCGGATCGCCAAGTTCAGCAAGGTGCGGACCATCACCCCGGAGC AACAGCATCAAGCAGGGCAAGGACCACTTCCCCGTGTTTCATGAACGAGAGGAGGACATCCT GTGTGCACCCGAGATGGAGCGGGTGTTCGGCTTCCCCGTGCACACTACACCGACGTGAGCAACATGA GCCGGCTGGCCCCGGCAGCGGCTGCTGGGCCGGAGCTGGAGCGTGCCCCGTGATCCGGCACCTGTTCG CCCCCCTGAAGGAGTACTTCGCCTGCGTGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCC AGCTTCAGCAGCGGCTGCTGCCCTGAGCCTGCGGGGAGCCACATGAATCCTCTGGAGATGTTTC GAGACAGTGCCCCGTGTGAGAGGCAACCCGTGAGGTGCTGAGCCTCTTCGAGGACATTAAAGAA GGAGCTGACCTCTCTGGGCTTTCTGGAATCCGGCAGCGACCCCCGGCCAGCTGAAACACAGTGGTGA CGTGACCCGACACAGTGAGGAAAGGACGTGGAAAGAGTGGGGCCCCCTTTGACCTCGTGTATGGAGCCA CACCTCCTCTCGGCCACACATGCGATAGGCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCT CCAGTACGCCAGACCTAAGCCCCGGCAGCCCCAGACCCCTTCTTCTGGATGTTCTGTGGACAATCTGCT GCTGAACAAGGAGGATCTGGATGTGGCCAGCAGATTTCTGGAGATGGAACCCCGTGACAATCCCCG
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		ACGTGCATGGGGCTCTCTGCAGAACGCCGTGAGAGTGTGTCTCCAACATCCCCGCCATTAGAAGCA GACACTGGGCTCTGGTGAGCGAGGAGGAACACTGTCTCTGTCTGTGCCCCAGAAATAAGCAGTCCTCCAAG CTGGCCGCCAAGTGGCCCCACCAGCTGGTGAAAGAACTGCTTCTGCTCTGAGGGAGTATTTCAAG TATTTAGCACCCGAACCTGACCAGCAGCCTGAGCGGCGGCAAGCGGCCCGCCGCCACCAAGAAAGGC CGGCCAGGCCAAGAAAGAAAGGGCAGCTACCCCTACGACGTGCCCGACTACGCTGAGCGGCGCG CTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCTTCTGGCCATGCCCTTCTTCTCTCCCTTGCACTGTA CCTCTTGCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAATAAAAAAATAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AGGAAATAAGAGAGAAAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAAGAA GCGGAAGGTGGGCATCCACGGCGTGCCCGCCGCGCAGCAGCGGATCCCTGGAGCCCCGGCGAAA AGCCTTACAAATGTCCCGAATGCGGAAAGAGCTTCAGCAGAGCCGACAAATCTGACCGAACATCAG AGAAACCATACCGGAGAAAAACCTTACAAATGTCCCGAGTGGCGCAAAGCTTCTCCCAAAGCCGG ACATCTGGCCAGCCACCAGGACACATACCGGCGAGAAACCTACAAGTGCCCCGAGTGGCGCA AGTCTTCTCTAGATCCGATGAGCTGGTCAGACATCAGAGAACCCATACCGGCGAGAAAGCCTTATA AGTGCCCCGAATGTGGCAAGTCTTCAGCCAGAGAGCTCATCTGGAGAGGCATCAAAAGAACACAC ACGGGAGAGAAACCTTACAAAGTGTCCTCCGAGTGTGGAAAGAGCTTCTCCAGAAAGGACGAGCTGAA CGTCCACCAAGAAACCCATACCGGCGAAAGCCCTATAAATGCCCGAGTGTGGAAAAATCCTTTTC TAGATCCGACCATCTGACAAACCCACCCAGAGGACCCATACCGGAGAGAAAGCCCTACAAATGCCCGG AGTGTGAAAAAGCTTCTTAGAAAAAGTGTCTGTACAGAGCACCAAAAGGACCCACACCGGCAAA AAGACCAGCGTAGCGGCAGCGGCGGCGGAGCGGCGGCAACCCAGCACAGGAGTTCGACCCCCC CAAGGTGTACCCCCCGTGCCCGCCGAGAAAGCGGAAGCCCCATCCGGGTGCTGAGCCTGTTCCGACG GCATCGCCACCGGCCCTGCTGGTGTGAAGGACCTGGGCATCCAGGTGGACCGGTACATCGCCAGC GAGGTGTGCGAGGACAGCATCACCGTGGGCAATGGTGGGCAACAGGGCAAGATCATGTACGTGGG CGACGTGCGGAGCGTGACCCAGAAAGCACATCCAGGAGTGGGGCCCCCTTCGACCTGGTGATCGGGG
MR-29787 (SEQ ID NO: 239)	CXCL1 ZF1-DNMT3a-3L SP6	

		GCAGCCCTGCAACGACCTGAGCATCGTGAAACCCCGCCCGGAAGGGCCTGTACGAGGGCAACCGGC CGGCTGTTCTTCGAGTTCTACCGGCTGTCTGACGACGCGCCGCGCCCAAGAGGGCGACGACCGGCC TTCTTCTGGCTGTTGAGAACGTGGTGGCCATGGCGTGAGCGACAAGCGGGACATCAGCCGGTTC CTGAGAGCAACCCCGTGTATGATCGACGCCAAGAGGTGAGCGCCGCCACCGGGCCCGGTACTT CTGGGGCAACCTGCCCCGGCATGAACCGGCCCTGGCCAGCACCCGTGAACGACAAGCTGGAGCTGC AGGAGTGCCTGGAGCACGGCCGGATCGCCAAAGTTCAAGCAAGGTGCGGACCATCACCAACCCGGAGC AACAGCATCAAGCAGGGCAAGGACACAGCACTTCCCGGTGTTATGAACGAGAAAGGAGGACATCCT GTGTGCAACCGAGATGGAGCGGGTGTTCGGCTTCCCGTGCACTACACCGACGTGAGCAACATGA GCCGGCTGGCCCGGCAGCGGCTGCTGGGCCGGAGCTGGAGCGTGCCCGTGATCCGGCACCTGTTCCG CCCCCTGAAGGAGTACTTCGCCCTGCGTGAGCAGCGGCAACAGCAACGCCAACAGCCGGGGCCCC AGCTTCAGCAGCGGCCCTGGTGCCCTGAGCCTGCGGGGCGAGCCACATGAATCCTCTGTGGAGATGTT GAGACAGTGCCCGTGTGGAGAAAGGCAACCCGTGAGGTGCTGAGCCTCTTCGAGGGACATTAAAGAA GGAGCTGACCTCTCTGGGCTTCTGTGAATCCGGCAGCGACCCCGGCCAGCTGAAACACGTGGTGGA CGTGACCGACACAGTGAGGAAGGACGTGAAAGAGTGGGGCCCCCTTGACCTCGTGTATGGAGCCA CACCTCCTCTCGGCCACACATGCGATAGGCCCTCCAGCTGGTATCTCTTCCAGTTCCACAGACTGCT CCAGTACGCCAGACCTAAAGCCCGGCAGCCCCAGACCCCTTCTTCTGGATGTTCTGTGGACAACTCTGGT GCTGAACAAGGAGGATCTGGATGTGGCCAGCAGATTTCTGGAGATGGAAACCCGTGACAAATCCCCCG ACGTGCAATGGCGGCTCTCTGCAGAACGCCGTGAGAGTGTGGTCCAACATCCCCGCCATTAGAAGCA GACACTGGGCTCTGTGAGCGAGGAGGAACTGTCTCTGTGCCCCAGAATAAGCAGTCCTCCAAG CTGGCCGCCAAGTGGCCCCAACCAAGCTGGTGAAGAACTGCTTCTGCTCTGAGGGAGTATTTCAAG TATTTCAACACCCGAACCTGACCAGCAGCCTGAGCGGGGGCAAGCGGCCCGCCGCCAACCAAGAGGC CGGCCAGGCCAAGAAAGAAAGAGGACAGTACCCCTACGACGTGCCCGGACTACGCCCTGAGCGGGCCG CTTAATTAAAGCTGCCTTCTGCGGGGCTTGCCCTTCTGGCCAATGCCCTTCTCTCTCTGACACCTGTA CCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGTCTAGAAAAAAGAAAAAAGAAAAAAGAAAAA
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MR- 29783 (SEQ ID NO: 240)	CXCL1 ZF5- HDAC8 SP6	AAAAA AGGAAATAAGAGAGAGAAAGAGTAAGAAAGAAATATAAGAGCCACCATGGCCCCCAAGAAGAA GCGGAAAGGTGGGCATCCACGGCGTGCCCGCGCGGAGCAGCGGATCCCTGGAGCCCCGGGAAA AGCCTTACAAAGTGGCCCGAGTGTGGCAATTCCTTTAGCACACCACCGGAAATCTGACCGTCCACCAGA GAACACATACCGGGGAGAAAACCCCTACAAAGTGTCCCGAGTGCGGCAAAATCCCTTCAGCCAGCTGGCC CATCTGAGAGCCCATCAAAAGGACCCATACCGGGGAGAAACCTTACAAGTGTCCCGAAATGTGGA GTCCCTTAGCAGCCCCCGCGATCTGACAAAGACATCAAAAGAACCCACACCGGCGAGAAAGCCCTATA AATGTCCCGAGTGTGAAAGTCCCTTCAGCCAGAGCGGCAATCTGACCGAGCATCAAAAGAACCCCAT ACCGGGGAAAAGCCCTATAAGTGCCCCGAATGCGGAAAAAGCTTCTCCACAAAGCGGCGAGCTGGT GAGACACCAAAGGACACATACCGGGCGAAAAGCCTTATAAATGCCCGGAGTGCGGGCAAGAGCTTCT CTAGAAAGGACAAATCTGAAGAACCAACCAAGAACACACACCGGCGAGAAAGCCCTACAAATGCCCC GAGTGGCGCAAGAGCTTTAGCCAGTGCCAGCAACCTCGTGAGACATCAGAGGACACATACCGGAAA AAAGACAGCGTAGCGGCAGCGGCGGCGGCAAGCGGCGGAGGAGGCCGAGAGCCCCGCCGAT AGCGGACAAATCTCTGTGTCCTACATCTACAGCCCCGAATATGTGAGCATGTGTGATTCCCTC GCCAAGATCCCTAAGAGAGCCAGCATGGTGCATTCTCTGATCGAGGCCCTACGCTCTGCATAAGCAA ATGAGGATCGTGAAAGCCCCAAGGTCGCCAGCATGGAAAGAGATGGCCACCTTTTCACACCGATGCCTA CCTCCAACATCTCCAGAAAGGTGTCCCAAGAGGGCGACGACGACCCCGACTCCATTGAGTACG GACTGGGCTATGATTGCCCGCCACCGAGGGCATCTTTGACTATGCCGCCGCTATCGGCGGAGCTA CCATCACAGCCGCCAGTGTCTGATTGATGGCATGTGCAAGGTGCGCATCAACTGGTCCGGAGGCT GGCATCATGCCAAGAAGGATGAGGCCCTCCGGCTTCTGTTATCTGAATGACGCCGCTGCTGGGCATTC TGAGACTGAGGAGGAAATTCTGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGA GTCGAAGATGCCCTTCAGCTTCACCGCAAGGTGATGACAGTCTCTCTGCACAAAGTTCTCCCCCGGC TTCTTTCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGCAAGGGAAGGTACTACAGCGTGAA CGTGCCCATTCAAAGACGGCATCCAAGACGAGAAAGTACTACAGATCTGCGAGTCCGTGCTCAAGG
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		AGGTCTACCAAGCCTTCAAATCCTAAGGCTGTGCTGCTCCAACCTGGGAGCTGATACCAATTGCTGGCG ATCCCATGTGCAGCTTCAATAATGACACCCGTCGGAAATCGGCAAGTGCCCTCAAGTACATCCTCCAGT GGCAGCTCGCCACCCCTCATTTCTCGGAGGAGCGGATACAATCTGGCTAATACCGCCAGATGCTGGA CCTATCTGACCGCGGTGATTTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCAACGAGTTTTC CCGTTACGGCCCCGACTACGTGCTGGAGATCACCCCCAGCTGCAGACCCCGATAGAAACGAAACCCC ATAGAAATCCAGCAAATTTCTGAACATATATCAAGGGCAACCTCAAAGCACGTGCTGGGAGGTGGCGGA TCGGGAAAGCGGCCCGCCGCCACCAAGAAAGGCCGGTCAGGCCAAGAAAGAAAGGGCAGCTACC CCTACGACGTGCCCGACTACGCTGAGCGCGCTTAATTAAAGCTGCCTTCTGCGGGCTTGCCCTC TGGCCATGCCCTTCTTCTCTCCCTTGCACTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAA GTCTAGAAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA AAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
MR- 29782 (SEQ ID NO: 241)	CXCL1 ZF2- HDAC8 SP6	AGGAAATAAGAGAGAAAGAAAGAGTAAGAAAGAAATAATAAGAGCCACCATGGCCCCCAAGAAGAA GCGAAAGGTGGGCAATCCACGGCGTGCCTCCCGCGGAGCAGCAGCGGATCCCTGGAGCCCCGCGAAA AACCTATAAGTGCCCGAGTGCGGCAAGAGCTTTAGCGATCCCGGCCATCTGGTGAGGCATCAGA GGACCCACACCGGGGAAAGCCTTACAAATGCCCGGAGTGTGGAAAAAGCTTCAGCAGAAAGCGAT CATCTGACCAACCATCAGAGGACACATACCGGGCGAGAAAGCCTTATAAATGCCCGGAAATGTGGAAA GAGCTTCTCAGAAAGCGACCATCTGACCAACCAACAGAGGACCCATACCGGAGAAAAACCTTACA AATGCCCGAGTGTGGAAAGTCTTTCAGCTCCCGCCGATCTGACAAAGACATCAGAGAACCCACA CCGGCGAAAAACCTTATAAATGTCCCGAGTGTGGCAAAAGCTTCTCCGACAAAGAGATCTGACA AGACACCAAAAGGACCCACACCGGGGAGAAACCTTATAAATGTCCCGAATGCGGAAAAAGCTTTAG CAGAAACGACGCTCTGACCGAAACACAGAGAACACATACCGGAGAGAAACCTTATAAATGTCCCG AGTGCGGAAAAATCCTTTCAGCACCAACCGCGCTCTGACAGAGCATCAGAGGACACACACCGGCAAA AAGACCTCCGCTAGCGGCAAGCGGGCGGCGAGCGGGGAGGAGCCCGAGGAGCCCGCCGATA GCGGACAATCTCTGTGCGCGTCTACATCTACAGCCCCGAATATGTGAGCATGTGTGATTCCCTCG

		CCAAGATCCCTAAGAGAGCCAGCATGTTGTCATTCTCTGATCGAGGCCCTACGCTCTGCATAAGCAAA TGAGGATCGTGAAGCCCAAGGTCGCCAGCATGGAAGAGATGGCCACCTTTACACCCGATGCCTAC CTCCAACATCTCCAGAAAGGTGTCCCAAGAGGGCGACGACGACCACCCGACTCCATTGAGTACGG ACTGGGCTATGATTGCCCGCCACCGAGGGCATCTTTGACTATGCCGCCGCTATCGGCGGAGCTAC CATCACAGCCGCCAGTGTCTGATTGATGGCATGTGCAAGTCCGCATCAACTGGTCCGGAGGGCTG GCATCATGCCAAGAAAGGATGAGGCCCTCCGGCTTCTGTTATCTGAATGACGCCGTGCTGGGCATTCT GAGACTGAGGAGGAAATTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGAG TCGAAGATGCCTTCAGCTTCACCAGCAAGGTGATGACAGTCTCTCTGCACAAAGTTCTCCCCGGCTT CTTTCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGGCAAGGGAAGGTACTACAGCGTGAACG TGCCCATTCAAAGACGGCATCCAAGACGAGAAAGTACTACCAGATCTGCGAGTCCGTGCTCAAGGAG GTCTACCAAGCCTTCAATCCTAAGGTGTGCTGCTCCAAGTGGGAGCTGATACCATTTGCTGGCGAT CCCATGTGCAGCTTCAATATGACACCCGTCGGAAATCGGCAAGTGCCTCAAGTACATCCTCCAGTGG CAGCTCGCCACCTCATTTCTCGGAGGAGGCGGATACAATCTGGCTAATACCGCCAGATGCTGGACC TATCTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCACGAGTTTTTCACC GCTTACGGCCCCGACTACGTGCTGGAGATCACCCCCAGCTGCAGACCCGATAGAAACGAACCCCAT AGAATCCAGCAAAATTCTGAATAATCAAGGGCAACCTCAAGCACGTGCGTGGGAGGTGGCGGATC GGGAAAGCGGCCCGCCGCCACCAAGAAAGGCCGGTCAAGGCCAAGAAAGAAAGGGCAGCTACCCC TACGACGTGCCCGACTACGCTGAGCGGCCGCTTAATTAAGCTGCCTTCTGCGGGGCTTGCCTTCTG GCCATGCCCTTCTTCTCTCCCTTGACCTGTACCTCTTGGTCTTTGAATAAAGCCTGAGTAGGAAGT CTAGAAAAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAA
MR- 29780 (SEQ ID	CXCL1 ZF1- HDAC8 SP6	AGGAAATAAGAGAGAAAGAGAGTAAGAAAGAAATAAAGAGCCACCATGGCCCCCAAGAAGAA GCGGAAGGTGGCATCCACGGCGTGCCCGCCCGCGCAGCAGCGGATCCCTGGAGCCCGCGGAAA AGCCTTACAAATGTCCGAATGCGGAAGAGCTTCAGCAGAGCCGACAATCTGACCGAACATCAG

NO: 242)	AGAACCCATACCGGAGAAAAACCTTACAAATGTCCCGAGTGGGCAAAAGCTTCTCCCAAGCCGG ACATCTGGCCAGCCACCAAGGACACATACCGGCGAGAAACCCTACAAAGTGCCCCCGAGTGCGGCA AGTCCTTCTAGATCCGATGAGCTGGTCAACATCAGAGAACCCATACCGGCGAGAAAGCCTTATA AGTGCCCCGAAATGTGGCAAGTCTTCAGCCAGAGAGCTCATCTGGAGAGGCATCAAAAGAACACAC ACCGGAGAGAAAAACCTTACAAAGTGTCCCGAGTGTGGAAAGAGCTTCTCCAGAAAGGACGAGCTGAA CGTCCACCAAGAAACCCATACCGGCGGAAAAAGCCCTATAAATGCCCGGAGTGTGGAAATCCTTTTC TAGATCCGACCATCTGACAAACCCACAGAGAGACCCATACCGGAGAGAAAGCCCTACAAATGCCCCCG AGTGTGGAATAAGCTTCTAGAAACGATGCTCTGACAGAGACCAAGAGACCCACACCGGCAAA AAGACGAGCGCTAGCGGCAGCGGGCGGCGAGCGGCGGAGAGCCCGAGGAGCCCGCGCGATA GCGGACAAATCTCTGGTGCCCGTCTACATCTACAGCCCCCGAATATGTGAGCATGTGTGATTCCCTCG CCAAAGATCCCTAAGAGAGCCAGCATGGTGCAATCTCTGATCGAGGCCCTACGCTCTGCATAAGCAAA TGAGGATCGTGAAGCCCAAGGTCGCCAGCATGGAAGAGATGGCCACCTTTACACCCGATGCCCTAC CTCCAAACATCTCCAGAAAGGTGTCCCAAGAGGGCGACGACGACCCCGACTCCATTGAGTACGG ACTGGGCTATGATTGCCCCCGCACCGAGGGCATCTTTGACTATGCCCGCGCTATCGGCGGAGCTAC CATCACAGCCGCCAGTGTCTGATTGATGGCATGTGCAAGGTGCGCCATCAACTGGTCCGGAGGCTG GCATCATGCCAAGAAAGGATGAGGGCTTCCGGCTTCTGTTATCTGAATGACGCCGTGCTGGGCATTCT GAGACTGAGGAGGAAATTTCGAGAGGATTCTGTACGTGGATCTGGATCTGCATCACGGAGATGGAG TCGAAGATGCCCTTCAGCTTCAACCAGCAAGGTGATGACAGTCTCTGTGCAAAAGTTCTCCCCCGGCTT CTTTCCCGGAACCGGCGACGTGTCCGACGTGGGACTGGGCAAGGGAAGGTACTACAGCGTGAACG TGCCCATTCAGACGGGCATCCAAAGACGAGAAAGTACTACCAGATCTGCGAGTCCGTGCTCAAGGAG GTCTACCAAGCCCTTCAATCCTAAGGCTGTCTGCTCCAACTGGGAGCTGATACCATTTGCTGGCGAT CCCATGTGCAGCTTCAATATGACACCCGTCGGAATCGGCAAGTGCCTCAAGTACATCCTCCAGTGG CAGCTCGCCACCCCTCATTTCTCGGAGGAGGCGGATACAAATCTGGCTAATACCGCCAGATGCTGGACC TATCTGACCGGCGTGATTCTGGGCAAAACACTGAGCAGCGAAATCCCCGACCGAGTTTTCACC
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		GCTTACGGCCCCGACTACGTGCTGGAGATCACCCCCCAGCTGCAGACCCGATAGAAACGAACCCCAT AGATCCAGCAAAATTCTGA ACTATATCAAGGGCAACCTCAAGCACGTCGTGGGAGGTGGCGGATC GGGAAAGCGGCCCGCCACCAAGAAAGCCGGTCAAGGCCAAGAAAGAAAGGGCAGCTACCCC TACGACGTGCCCGACTACGCCTGAGCGGCCCGCTTAATTAAAGCTGCCCTTCTGCGGGGCTTGCCTTCTG GCCATGCCCTTCTTCTCTCCCTTGCACCTGTACCTCTTGGTCTTTTGAAATAAAGCCTGAGTAGGAAGT CTAGAAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA AAAAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAA
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In some embodiments, a nucleic acid for use in a method or composition described herein (e.g., a nucleic acid encoding a site-specific disrupting agent) comprises a nucleic acid sequence of any one of SEQ ID NOs: 69, 71, 85, 201, 202, 204, 205, 207, 209, 211, 213, 215, 217, or 219-242, a complementary or reverse complementary sequence of any thereof, or comprises a sequence with at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any thereof. In some embodiments, a site-specific disrupting agent for use in a method or composition described herein comprises an amino acid sequence of any one of SEQ ID NOs: 70, 72-82, 84, 86, 203, 206, 208, 210, 212, 214, 216, or 218, or comprises a sequence with at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any thereof. In some embodiments, a site-specific disrupting agent for use in a method or composition described herein comprises an amino acid sequence encoded by any one of SEQ ID NOs: 69, 71, 85, 201, 202, 204, 205, 207, 209, 211, 213, 215, 217, or 219-242, or an amino acid sequence with at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any thereof.

Functional Characteristics

A site-specific disrupting agent or a system of the present disclosure can be used to modulate, e.g., decrease, expression of a target plurality of genes in a cell. In some embodiments, modulating expression comprises decreasing the level of RNA, e.g., mRNA, encoded by each of the target plurality of genes. In some embodiments, modulating expression comprises decreasing the level of protein encoded by each of the target plurality of genes. In some embodiments, modulating expression comprises both decreasing the level of mRNA and protein encoded by each of the target plurality of genes. In some embodiments, the expression of a gene of the target plurality of genes in a cell contacted by or comprising the site-specific disrupting agent is at least 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.55x, 1.6x, 1.65x, 1.7x, 1.75x, 1.8x, 1.85x, 1.9x, 1.95x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x lower than the level of expression of the gene in a cell not contacted by or comprising the site-specific disrupting agent or system. In some embodiments, the expression of each gene of the target plurality of genes in a cell contacted by or comprising the site-specific disrupting agent or system is at least 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.55x, 1.6x, 1.65x, 1.7x, 1.75x, 1.8x, 1.85x, 1.9x, 1.95x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 30x, 40x, 50x, 60x, 70x, 80x, 90x, or 100x lower than the level of expression of the gene in a cell not contacted by or comprising the site-specific disrupting agent or system. Expression of a gene may be assayed by methods known to those of skill in the art, including RT-PCR, ELISA, Western blot, and the methods of Examples 2 or 4-19.

A site-specific disrupting agent or a system of the present disclosure can be used to decrease binding of a nucleating polypeptide, e.g., CTCF, to an anchor sequence. In some embodiments, contacting a cell or administering a site-specific disrupting agent or a system results in a decrease in binding of a nucleating polypeptide, e.g., CTCF, to an anchor sequence (e.g., an anchor sequence of an ASMC comprising a target plurality of genes). In some embodiments, contacting a cell or administering a site-specific disrupting agent or a system results in a complete loss of binding or a decrease of at least 50, 60, 70, 80, 90, 95, or 99%, e.g., as measured by ChIP and/or quantitative PCR, relative to the binding of the nucleating polypeptide (e.g., CTCF) to the anchor sequence prior to treatment with the site-specific disrupting agent or a system or in the absence of the site-specific disrupting agent or a system.

A site-specific disrupting agent or a system of the present disclosure can be used to disrupt a genomic complex (e.g., ASMC) comprising a target plurality of cells. In some embodiments, contacting a cell or administering a site-specific disrupting agent or a system results in a decrease in the level of a genomic complex (e.g., ASMC) comprising the target plurality of genes relative to the level of the complex prior to treatment with the site-specific disrupting agent or a system or in the absence of the site-specific disrupting agent or a system. In some embodiments, contacting a cell or administering a site-specific disrupting agent or a system results in a complete loss of the genomic complex, e.g., ASMC, or a decrease of at least 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95, or 99%, e.g., as measured by ChIA-PET, ELISA (e.g., to assess gene expression changes), CUT&RUN, ATAC-SEQ, ChIP and/or quantitative PCR, relative to the level of the complex prior to treatment with the site-specific disrupting agent or a system or in the absence of the site-specific disrupting agent or a system.

A site-specific disrupting agent or a system of the present disclosure can be used to modulate, e.g., decrease, expression of a target plurality of genes in a cell for a time period. In some embodiments, the expression of a gene of the target plurality of genes in a cell contacted by or comprising the site-specific disrupting agent or a system is appreciably decreased for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, 7, 10, or 14 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely). In some embodiments, the expression of each gene of the target plurality of genes in a cell contacted by or comprising the site-specific disrupting agent or a system is appreciably decreased for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, 7, 10, or 14 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely). Optionally, the expression of a gene of the target plurality of genes in a cell contacted by or comprising the site-specific

disrupting agent or a system is appreciably decreased for no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 years. Optionally, the expression of each gene of the target plurality of genes in a cell contacted by or comprising the site-specific disrupting agent or a system is appreciably decreased for no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 years.

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A site-specific disrupting agent or a system may comprise a plurality of effector moieties, where each effector moiety has a different functionality from each other effector moiety. For example, a site-specific disrupting agent or a system may comprise a first effector moiety comprising histone deacetylase functionality and a second effector moiety comprising DNA methyltransferase functionality. In some
10 embodiments, a site-specific disrupting agent comprises a combination of effector moieties whose functionalities are complementary to one another with regard to modulating, e.g., decreasing, expression of a target plurality of genes, e.g., where the functionalities together decrease expression and, optionally, do not decrease or negligibly decrease expression when present individually.

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In some embodiments, a site-specific disrupting agent or a system comprises a combination of effector moieties whose functionalities synergize with one another with regard to modulating, e.g., decreasing, expression of a target plurality of genes. Without wishing to be bound by theory, it is thought that epigenetic modifications to a genomic locus are cumulative, in that multiple repressive epigenetic markers (e.g., multiple different types of epigenetic markers and/or more extensive marking of a given
20 type) together reduce expression more effectively than individual modifications alone (e.g., producing a greater decrease in expression and/or a longer-lasting decrease in expression). In some embodiments, a site-specific disrupting agent or a system comprises a plurality of effector moieties that synergize with each other, e.g., each effector moiety decreases expression of a target gene. In some embodiments, a site-specific disrupting agent comprising a plurality of different effector moieties which synergize with one
25 another is more effective at modulating, e.g., decreasing, expression of a target plurality of genes than a site-specific disrupting agent comprising only one of the plurality of different effector moieties or a non-synergistic combination of effector moieties. In some embodiments, such a site-specific disrupting agent is at least 1.05x (i.e., 1.05 times), 1.1x, 1.15x, 1.2x, 1.25x, 1.3x, 1.35x, 1.4x, 1.45x, 1.5x, 1.55x, 1.6x, 1.65x, 1.7x, 1.75x, 1.8x, 1.85x, 1.9x, 1.95x, 2x, 3x, 4x, 5x, 6x, 7x, 8x, 9x, 10x, 20x, 30x, 40x, 50x, 60x,
30 70x, 80x, 90x, or 100x as effective at modulating, e.g., decreasing, expression of a target plurality of genes than a site-specific disrupting agent or a system comprising only one of the plurality of different effector moieties or a non-synergistic combination of effector moieties.

Target Sites

A site-specific disrupting agent or a system disclosed herein is useful for modulating, e.g., decreasing, expression of a target plurality of genes in cell, e.g., in a subject or patient. A target plurality of genes may include any gene known to those of skill in the art. A target plurality of genes comprises at least two genes. In some embodiments, a targeted plurality of genes comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 genes (and optionally, no more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 30 genes), e.g., a first gene and a second gene, and optionally a third gene, a fourth gene, a fifth gene, a sixth gene, a seventh gene, an eighth gene, a ninth gene, a tenth gene, an eleventh gene, a twelfth gene, a thirteenth gene, a fourteenth gene, a fifteenth gene, a sixteenth gene, a seventeenth gene, an eighteenth gene, a nineteenth gene, and/or a twentieth gene. In some embodiments, a targeted plurality of genes comprises 2-20, 2-18, 2-16, 2-14, 2-12, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-18, 3-16, 3-14, 3-12, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-18, 4-16, 4-14, 4-12, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-18, 5-16, 5-14, 5-12, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-18, 6-16, 6-14, 6-12, 6-10, 6-9, 6-8, 6-7, 7-20, 7-18, 7-16, 7-14, 7-12, 7-10, 7-9, 7-8, 8-20, 8-18, 8-16, 8-14, 8-12, 8-10, 8-9, 9-20, 9-18, 9-16, 9-14, 9-12, 9-10, 10-20, 10-18, 10-16, 10-14, 10-12, 12-20, 12-18, 12-16, 12-14, 14-20, 14-18, 14-16, 16-20, 16-18, or 18-20 genes.

In some embodiments, two or more (e.g., all) genes of a target plurality of genes are associated with a disease or condition in a subject, e.g., a mammal, e.g., a human, bovine, horse, sheep, chicken, rat, mouse, cat, or dog. In some embodiments, the disease or condition is an inflammatory disease, e.g., an immune mediated inflammatory disease. In some embodiments, the disease or condition is one or more of rheumatoid arthritis, inflammatory arthritis, gout, asthma, neutrophilic asthma, neutrophilic dermatosis, paw edema, acute respiratory disease syndrome (ARDS), COVID-19, psoriasis, inflammatory bowel disease, infection (e.g., by a pathogen, e.g., a bacteria, a virus, or a fungus), external injury (e.g., scrapes or foreign objects), effects of radiation or chemical injury, osteoarthritis, osteoarthritic joint pain, joint pain, inflammatory pain, acute pain, chronic pain, cystitis, bronchitis, dermatitis, cardiovascular disease, neurodegenerative disease, liver disease, lung disease, kidney disease, pain, swelling, stiffness, tenderness, redness, warmth, or elevated biomarkers related to disease states (e.g., cytokines, immune receptors, or inflammatory markers). In some embodiments, the inflammatory disorder is associated with an infection, e.g., by a virus, e.g., Sars-Cov-2 virus. In some embodiments, the inflammatory disorder is an autoimmune disorder. In some embodiments, the inflammatory disorder is associated with hypoxia. In some embodiments, the inflammatory disorder is associated with ARDS, hypoxia, and/or sepsis. In some embodiments, the infection is a super infection, e.g., caused by more than one pathogen, e.g., a first virus or a bacterium, or a fungus, and a second virus, or a bacterium, or a fungus. In some embodiments, the

inflammatory disorder may change lung cell composition, e.g., decreased AT2 cells and/or increased dendritic cell, macrophages, neutrophils, NK cells, fibroblasts, leukocytes, lymphatic endothelial cells and/or vascular endothelial cells. In some embodiments, the disorder is associated with one or more comorbidities, e.g., respiratory infections, obesity, gastroesophageal reflux disease, skin lesions, and/or obstructive sleep apnea.

In some embodiments, two or more (e.g., all) genes of a target plurality of genes are aberrantly expressed, e.g., over-expressed, in a cell, e.g., in a subject, e.g., a human subject.

In some embodiments, two or more (e.g., all) genes of a target plurality of genes have related functionalities. Without wishing to be bound by theory, it is thought that genes with related functionalities are frequently positioned in close proximity to one another in the genome and are also frequently found within (wholly or in part) common genomic complexes, e.g., ASMCs. Modulating, e.g., decreasing, expression of a target plurality of genes where two or more (e.g., all) of the genes of the plurality have related functionalities may be accomplished efficiently and effectively by targeting a genomic complex, e.g., ASMC, comprising said interrelated genes.

In some embodiments, one, two, three, or more (e.g., all) genes of a target plurality of genes are cytokines, e.g., chemokines, an interleukin, a transcription factor (e.g., an interferon regulatory transcription factor), intercellular adhesion molecule (ICAM), or an interferon receptor. In some embodiments, two or more (e.g., all) genes of a target plurality of genes are cytokines, an interleukin, a transcription factor (e.g., an interferon regulatory transcription factor), intercellular adhesion molecule (ICAM), or an interferon receptor. In some embodiments, the target plurality of genes are mammalian gene, e.g., mouse genes, human genes.

In some embodiments, two or more (e.g., all) genes of a target plurality of genes have pro-inflammatory functionality. In some embodiments, two or more (e.g., all) genes of a target plurality of genes may act as a chemoattractant for immune cells, e.g., neutrophils. For example, genes having pro-inflammatory functionality (also referred to herein as pro-inflammatory genes) include CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, IL8, CXCL15, CCL2, CCL7, CCL9, IL1A, IL1B, CSF2, IRF1, ICAM1, ICAM4, ICAM5, IFNAR2, IL10RB, or IFNGR2. In some embodiments, a target plurality of genes comprises two or more of CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, IL8, CXCL15, CCL2, CCL7, CCL9, IL1A, IL1B, CSF2, IRF1, ICAM1, ICAM4, ICAM5, IFNAR2, IL10RB, or IFNGR2. In some embodiments, the plurality of genes comprises one or more genes more human

CXCL family. In some embodiments, a target plurality of genes comprises CXCL1 (e.g., nucleic acid sequence encoding an RNA according to NM_002089 or a nucleic acid encoding a polypeptide according to P09341, or a mutant thereof), CXCL2 (e.g., nucleic acid sequence encoding an RNA according to NM_001511 or a nucleic acid encoding a polypeptide according to P19875, or a mutant thereof), CXCL3 (e.g., nucleic acid sequence encoding an RNA according to NM_002090 or a nucleic acid encoding a polypeptide according to P19876, or a mutant thereof), CXCL4 (e.g., nucleic acid sequence encoding an RNA according to NM_002619 or NM_001363352, or a nucleic acid encoding a polypeptide according to P02776, or a mutant thereof), CXCL5 (e.g., nucleic acid sequence encoding an RNA according to NM_002994 or a nucleic acid encoding a polypeptide according to P42830, or a mutant thereof), CXCL6 (e.g., nucleic acid sequence encoding an RNA according to NM_002993 or a nucleic acid encoding a polypeptide according to P80162, or a mutant thereof), CXCL7 (e.g., nucleic acid sequence encoding an RNA according to NM_002704 or a nucleic acid encoding a polypeptide according to P02775, or a mutant thereof), and IL8 (e.g., nucleic acid sequence encoding an RNA according to NM_000584 or NM_001354840, or a nucleic acid encoding a polypeptide according to P10145, or a mutant thereof). In some embodiments, the plurality of genes comprises one or more genes more mouse CXCL family. In some embodiments, a target plurality of genes comprises CXCL1(e.g., nucleic acid sequence encoding an RNA according to NM_008176.3 or a nucleic acid encoding a polypeptide according to P12850, or a mutant thereof), CXCL2 (e.g., nucleic acid sequence encoding an RNA according to NM_009140.2 or a nucleic acid encoding a polypeptide according to P10889, or a mutant thereof), CXCL3 (e.g., nucleic acid sequence encoding an RNA according to NM_203320.3 or a nucleic acid encoding a polypeptide according to Q6W5C0, or a mutant thereof), CXCL4 (e.g., nucleic acid sequence encoding an RNA according to NM_019932 or a nucleic acid encoding a polypeptide according to Q9Z126, or a mutant thereof), CXCL5 (e.g., nucleic acid sequence encoding an RNA according to NM_009141.3 or a nucleic acid encoding a polypeptide according to P50228, or a mutant thereof), CXCL7 (e.g., nucleic acid sequence encoding an RNA according to NM_023785.3 or a nucleic acid encoding a polypeptide according to Q9EQI5, or a mutant thereof), and CXCL15 (e.g., nucleic acid sequence encoding an RNA according to NM_011339 or a nucleic acid encoding a polypeptide according to Q9WVL7, or a mutant thereof). In some embodiments, a target plurality of genes comprises CCL2, CCL7, CCL9, IL1A, and IL1B. In some embodiments, a target plurality of genes comprises CSF2, IRF1, ICAM1, ICAM4, and ICAM5. In some embodiments, a target plurality of genes comprises IFNAR2, IL10RB, and IFNGR2.

In some embodiments, inhibition expression of two or more (e.g., all) genes of a target plurality of genes may modulate expression of other genes encoding a protein, e.g., cytokines, e.g., decreasing

CXCL expression and cellular recruitment of CXCL to the site of inflammation, reduces presence of GM-CSF, and/or IL-6 in the site of inflammation.

In some embodiments, a target plurality of genes is part of a genomic complex, e.g., ASMC. As used herein, referring to a target plurality of genes being part of a genomic complex, e.g., ASMC, means that each of the genes of the plurality are at least partly comprised within the genomic complex, e.g., ASMC. Referring to a target plurality of genes as part of a genomic complex, e.g., ASMC, is used interchangeably with reference to a genomic complex, e.g., ASMC, comprising a target plurality of genes. For example, a target plurality of genes may consist of two genes positioned adjacent one another in the genome wherein a first anchor sequence is disposed within the first of the genes and a second anchor sequence is disposed outside of the second of the genes distal to the first gene. An ASMC formed by association of said first and second anchor sequence would wholly comprise the second of the genes and partly comprise the first of the genes; a plurality of genes consisting of these two genes would be part of this ASMC. In some embodiments, each gene of a target plurality of genes is wholly within a genomic complex, e.g., ASMC, (e.g., no portion of the transcript encoding gene sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, each gene of a target plurality of genes is partly within a genomic complex, e.g., ASMC, (e.g., some portion of the transcript encoding gene sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, at least one gene of a target plurality of genes is wholly within a genomic complex, e.g., ASMC, (e.g., no portion of the transcript encoding gene sequence is outside of the genomic complex, e.g., ASMC), and at least one gene of a target plurality of genes is partly within a genomic complex, e.g., ASMC, (e.g., some portion of the transcript encoding gene sequence is outside of the genomic complex, e.g., ASMC).

A gene of a target plurality of genes may include coding sequences, e.g., exons, and/or non-coding sequences, e.g., introns, 3'UTR, or 5'UTR. In some embodiments, a gene of a target plurality of genes is operably linked to a transcription control element. In some embodiments, a transcription control element of a gene of a target plurality of genes is also part of the genomic complex, e.g., ASMC, that the gene is part of. Referring to a transcription control element operably linked to a gene as part of a genomic complex, e.g., ASMC, can be understood in the same sense as described above in reference to the target plurality of genes. In some embodiments, each transcription control element operably linked to a gene of a target plurality of genes is wholly within a genomic complex, e.g., ASMC, (e.g., no portion of the transcription control element sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, each transcription control element of a target plurality of genes is partly within a genomic complex, e.g., ASMC, (e.g., some portion of the transcription control element sequence is outside of the

genomic complex, e.g., ASMC). In some embodiments, each transcription control element of a target plurality of genes is completely outside of the genomic complex, e.g., ASMC, (e.g., each transcription control element sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, at least one transcription control element operably linked to a gene of a target plurality of genes is wholly within a genomic complex, e.g., ASMC (e.g., no portion of the transcription control element sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, at least one transcription control element operably linked to another gene of the target plurality of genes is partly within the genomic complex, e.g., ASMC, (e.g., some portion of the transcript encoding gene sequence is outside of the genomic complex, e.g., ASMC). In some embodiments, at least one transcription control element operably linked to a gene of a target plurality of genes is completely outside of the genomic complex, e.g., ASMC.

In some embodiments, a site-specific disrupting agent or a system targets a target plurality of genes by binding to an anchor sequence, e.g., an anchor sequence that is part of a genomic complex (e.g., ASMC) comprising the target plurality of genes. In some embodiments, a targeting moiety binds to the anchor sequence. In some embodiments, binding of a genomic complex component, e.g., nucleating polypeptide, to an anchor sequence nucleates complex formation, e.g., anchor sequence-mediated conjunction formation. Each anchor sequence-mediated conjunction comprises one or more anchor sequences, e.g., a plurality of anchor sequences. In some embodiments, an anchor sequence-mediated conjunction can be disrupted to alter, e.g., inhibit, expression of a target plurality of genes. Such disruptions may modulate gene expression by, e.g., changing topological structure of DNA, e.g., by modulating the ability of a gene of the plurality to interact with a transcription control element (e.g., enhancing and silencing/repressive sequences).

A targeting moiety suitable for use in a site-specific disrupting agent or a system may bind, e.g., specifically bind, to a site that is proximal to an anchor sequence, e.g., an anchor sequence that is part of a genomic complex (e.g., ASMC) comprising the target plurality of genes. As used herein, proximal refers to a closeness of two sites, e.g., nucleic acid sites, such that binding of a site-specific disrupting agent or a system at the first site and/or modification of the first site by the site-specific disrupting agent will produce the same or substantially the same effect as binding and/or modification of the other site. For example, a targeting moiety may bind to a first site that is proximal to an anchor sequence that is part of a genomic complex (e.g., ASMC) comprising the target plurality of genes (the second site), and an effector moiety associated with said targeting moiety may epigenetically modify the first site such that genomic complex (e.g., ASMC) comprising the anchor sequence is modified, substantially the same as if the second site had been bound and/or modified. In some embodiments, a site proximal to a target gene (e.g.,

an exon, intron, or splice site within the target gene), proximal to a transcription control element operably linked to the target gene, or proximal to an anchor sequence is within 5000, 4000, 3000, 2000, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 25, 20, 15, 10, or 5 base pairs from the target gene (e.g., an exon, intron, or splice site within the target gene), transcription control element, or anchor sequence (and optionally at least 5, 10, 20, 25, 50, 100, 200, or 300 base pairs from the target gene (e.g., an exon, intron, or splice site within the target gene), transcription control element, or anchor sequence). In some embodiments, a site proximal to an anchor sequence is a site that is less than 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 20, 10, or 5 base pairs from the anchor sequence (and optionally at least 5, 10, 20, 25, 50, 100, 200, or 300 base pairs). In some embodiments, a site proximal to an anchor sequence is a site that is less than 800, 700, 600, 500, 400, or 300 base pairs from the anchor sequence (and optionally at least 5, 10, 20, 25, 50, 100, 200, or 300 base pairs).

A targeting moiety suitable for use in a site-specific disrupting agent or a system described herein may bind, e.g., specifically bind, to a site comprising at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides or base pairs (and optionally no more 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, or 10 nucleotides or base pairs). In some embodiments, a targeting moiety binds to a site comprising 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides or base pairs.

Genomic Complexes

Genomic complexes relevant to the present disclosure include stable structures that comprise a plurality of polypeptide and/or nucleic acid (particularly ribonucleic acid) components and that co-localize two or more genomic sequence elements (e.g., anchor sequences, promoter and/or enhancer elements). In some embodiments, relevant genomic complexes comprise anchor-sequence-mediated conjunctions (e.g., genomic loops). In some embodiments, genomic sequence elements that are (i.e., in three-dimensional space) in genomic complexes include transcriptional promoter and/or regulatory (e.g., enhancer or repressor) sequences. Alternatively, or additionally, in some embodiments, genomic sequence elements that are in genomic complexes include binding sites for one or more of CTCF, YY1, etc. In some embodiments, a genomic complex comprises a target plurality of genes. In some embodiments, two or more (e.g., all) genes of a target plurality of genes are located in a single loop of an ASMC. In some embodiments, two or more (e.g., all) genes of a target plurality of genes are located in different loops of an ASMC.

In some embodiments, a genomic complex whose incidence is decreased in accordance with the present disclosure comprises, or consists of, one or more components chosen from: a genomic sequence element (e.g., an anchor sequence, e.g., a CTCF binding motif, a YY1 binding motif, etc., that may, in some embodiments, be recognized by a nucleating component), one or more polypeptide components (e.g., one or more nucleating polypeptides, one or more transcriptional machinery proteins, and/or one or more transcriptional regulatory proteins), and/or one or more non-genomic nucleic acid components (e.g., non-coding RNA and/or an mRNA, for example, transcribed from a gene associated with the genomic complex).

In some embodiments, a genomic complex component is part of a genomic complex, wherein the genomic complex brings together two genomic sequence elements that are spaced apart from one another on a chromosome, e.g., via an interaction between and among a plurality of protein and/or other components.

In some embodiments, a genomic sequence element is an anchor sequences to which one or more protein components of the complex binds; thus, in some embodiments, a genomic complex comprises an anchor-sequence-mediated conjunction. In some embodiments, a genomic sequence element comprises a CTCF binding motif, a promoter and/or an enhancer. In some embodiments, a genomic sequence element includes at least one or both of a promoter and/or regulatory site (e.g., an enhancer). In some embodiments, complex formation is nucleated at the genomic sequence element(s) and/or by binding of one or more of the protein component(s) to the genomic sequence element(s).

Genomic sequence elements involved in genomic complexes as described herein, may be non-contiguous with one another. In some embodiments with noncontiguous genomic sequence elements (e.g., anchor sequences, promoters, and/or transcriptional regulatory sequences), a first genomic sequence element (e.g., anchor sequence, promoter, or transcriptional regulatory sequence) may be separated from a second genomic sequence element (e.g., anchor sequence, promoter, or transcriptional regulatory sequence) by about 500bp to about 500Mb, about 750bp to about 200Mb, about 1kb to about 100Mb, about 25kb to about 50Mb, about 50kb to about 1Mb, about 100kb to about 750kb, about 150kb to about 500kb, or about 175kb to about 500kb. In some embodiments, a first genomic sequence element (e.g., anchor sequence, promoter, or transcriptional regulatory sequence) is separated from a second genomic sequence element (e.g., anchor sequence, promoter, or transcriptional regulatory sequence) by about 500bp, 600bp, 700bp, 800bp, 900bp, 1kb, 5kb, 10kb, 15kb, 20kb, 25kb, 30kb, 35kb, 40kb, 45kb, 50kb,

55kb, 60kb, 65kb, 70kb, 75kb, 80kb, 85kb, 90kb, 95kb, 100kb, 125kb, 150kb, 175kb, 200kb, 225kb, 250kb, 275kb, 300kb, 350kb, 400kb, 500kb, 600kb, 700kb, 800kb, 900kb, 1Mb, 2Mb, 3Mb, 4Mb, 5Mb, 6Mb, 7Mb, 8Mb, 9Mb, 10Mb, 15Mb, 20Mb, 25Mb, 50Mb, 75Mb, 100Mb, 200Mb, 300Mb, 400Mb, 500Mb, or any size therebetween.

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Anchor Sequence-Mediated Conjunction

In some embodiments, a genomic complex relevant to the present disclosure is or comprises an anchor sequence-mediated conjunction (ASMC). In some embodiments, an anchor-sequence-mediated conjunction is formed when nucleating polypeptide(s) bind to anchor sequences in the genome and interactions between and among these proteins and, optionally, one or more other components, forms a conjunction in which the anchor sequences are physically co-localized. In many embodiments described herein, one or more genes is associated with an anchor-sequence-mediated conjunction; in such embodiments, the anchor sequence-mediated conjunction typically includes one or more anchor sequences, one or more genes, and one or more transcriptional control sequences, such as an enhancing or silencing sequence. In some embodiments, a transcriptional control sequence is within, partially within, or outside an anchor sequence-mediated conjunction. In some embodiments, the ASMC comprises an internal enhancing sequence, e.g., an enhancer. In some embodiments, an ASMC comprises a target plurality of genes.

20 In some embodiments, a genomic complex as described herein (e.g., an anchor sequence-mediated conjunction) is or comprises a genomic loop, such as an intra-chromosomal loop. In certain embodiments, genomic complex as described herein (e.g., an anchor sequence-mediated conjunction) comprises a plurality of genomic loops. One or more genomic loops may include a first anchor sequence, a nucleic acid sequence, a transcriptional control sequence, and a second anchor sequence. In some
25 embodiments, at least one genomic loop includes, in order, a first anchor sequence, a transcriptional control sequence, and a second anchor sequence; or a first anchor sequence, a nucleic acid sequence, and a second anchor sequence. In yet some embodiments, either one or both of nucleic acid sequences and transcriptional control sequence is located within a genomic loop. In yet some embodiments, either one or both of nucleic acid sequences and transcriptional control sequence is located outside a genomic loop. In
30 some embodiments, one or more genomic loops comprise a transcriptional control sequence. In some embodiments, genomic complex (e.g., an anchor sequence-mediated conjunction) includes a TATA box, a CAAT box, a GC box, or a CAP site.

In some embodiments, an anchor sequence-mediated conjunction comprises a plurality of genomic loops; in some such embodiments, an anchor sequence-mediated conjunction comprises at least one of an anchor sequence, a nucleic acid sequence, and a transcriptional control sequence in one or more genomic loops.

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Types of Loops

In some embodiments, a genomic loop comprises one or more, e.g., 2, 3, 4, 5, or more, genes, e.g., a target plurality of genes. In some embodiments, two or more, e.g., 2, 3, 4, 5, or more, genes of the target plurality of genes are transcribed in the same direction. In some embodiments, all genes of the target plurality of genes are transcribed in the same direction.

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In some embodiments, the present disclosure provides methods of modulating (e.g., decreasing) expression of a target plurality of genes in a loop comprising inhibiting, dissociating, degrading, and/or modifying a genomic complex that achieves co-localization of genomic sequences that are outside of, not part of, or comprised within (i) a gene whose expression is modulated (e.g., of a target plurality of genes); and/or (ii) one or more associated transcriptional control sequences that influence transcription of a gene whose expression is modulated.

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In some embodiments, the present disclosure provides methods of modulating (e.g., decreasing) transcription of a target plurality of genes comprising inhibiting formation of and/or destabilizing a complex that achieves co-localization of genomic sequences that are non-contiguous with (i) a gene whose expression is modulated (e.g., of a target plurality of genes); and/or (ii) associated transcriptional control sequences that influence transcription of a gene whose expression is modulated.

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In some embodiments, an anchor sequence-mediated conjunction is associated with one or more, e.g., 2, 3, 4, 5, or more, transcriptional control sequences. In some embodiments, a gene of a target plurality of genes (e.g., one, two, or more, e.g., all of the target plurality of genes) is non-contiguous with one or more transcriptional control sequences. In some embodiments where a gene is non-contiguous with its transcriptional control sequence(s), a gene may be separated from one or more transcriptional control sequences by about 100bp to about 500Mb, about 500bp to about 200Mb, about 1kb to about 100Mb, about 25kb to about 50Mb, about 50kb to about 1Mb, about 100kb to about 750kb, about 150kb to about 500kb, or about 175kb to about 500kb. In some embodiments, a gene is separated from a transcriptional control sequence by about 100bp, 300bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1kb, 5kb, 10kb, 15kb, 20kb, 25kb, 30kb, 35kb, 40kb, 45kb, 50kb, 55kb, 60kb, 65kb, 70kb, 75kb, 80kb, 85kb, 90kb, 95kb, 100kb, 125kb, 150kb, 175kb, 200kb, 225kb, 250kb, 275kb, 300kb, 350kb, 400kb, 500kb, 600kb, 700kb,

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800kb, 900kb, 1Mb, 2Mb, 3Mb, 4Mb, 5Mb, 6Mb, 7Mb, 8Mb, 9Mb, 10Mb, 15Mb, 20Mb, 25Mb, 50Mb, 75Mb, 100Mb, 200Mb, 300Mb, 400Mb, 500Mb, or any size therebetween.

Anchor Sequences

In general, an anchor sequence is a genomic sequence element to which a genomic complex component, e.g., nucleating polypeptide, binds specifically. In some embodiments, binding of a genomic complex component to an anchor sequence nucleates complex formation.

Each anchor sequence-mediated conjunction comprises one or more anchor sequences, e.g., a plurality. In some embodiments, anchor sequences can be manipulated or altered to form and/or stabilize naturally occurring loops, to form one or more new loops (e.g., to form exogenous loops or to form non-naturally occurring loops with exogenous or altered anchor sequences), or to inhibit formation of or destabilize naturally occurring or exogenous loops. Such alterations may modulate gene expression by, e.g., changing topological structure of DNA, e.g., by thereby modulating ability of a target gene to interact with gene regulation and control factors (e.g., enhancing and silencing/repressor sequences). In some embodiments, chromatin structure is modified by substituting, adding or deleting one or more nucleotides within an anchor sequence-mediated conjunction. In some embodiments, chromatin structure is modified by substituting, adding, or deleting one or more nucleotides within an anchor sequence of an anchor sequence-mediated conjunction.

In some embodiments, an anchor sequence comprises a common nucleotide sequence, e.g., a CTCF-binding motif:

N(T/C/G)N(G/A/T)CC(A/T/G)(C/G)(C/T/A)AG(G/A)(G/T)GG(C/A/T)(G/A)(C/G)(C/T/A)(G/A/C)
(SEQ ID NO:1), where N is any nucleotide.

A CTCF-binding motif may also be in an opposite orientation, e.g.,
(G/A/C)(C/T/A)(C/G)(G/A)(C/A/T)GG(G/T)(G/A)GA(C/T/A)(C/G)(A/T/G)CC(G/A/T)N(T/C/G)N
(SEQ ID NO:2). In some embodiments, an anchor sequence comprises SEQ ID NO:1 or SEQ ID NO:2 or a sequence at least 75%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to either SEQ ID NO:1 or SEQ ID NO:2.

In some embodiments, an anchor sequence comprises a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif. In some embodiments, an anchor sequence comprises a nucleating polypeptide binding motif, e.g., a YY1-binding motif: CCGCCATNTT (SEQ ID NO: 3), where N is any nucleotide. A YY1-binding motif may also be in an

opposite orientation, e.g., AANATGGCGG (SEQ ID NO: 4), where N is any nucleotide. In some embodiments, an anchor sequence comprises SEQ ID NO:3 or SEQ ID NO:4 or a sequence at least 75%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to either SEQ ID NO:3 or SEQ ID NO:4.

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In some embodiments, an anchor sequence-mediated conjunction comprises at least a first anchor sequence and a second anchor sequence. For example, in some embodiments, a first anchor sequence and a second anchor sequence may each comprise a common nucleotide sequence, e.g., each comprises a CTCF binding motif. In some embodiments, a first anchor sequence and a second anchor sequence may each comprise a USF1 binding motif. In some embodiments, a first anchor sequence and a second anchor sequence may each comprise a YY1 binding motif. In some embodiments, a first anchor sequence and a second anchor sequence may each comprise a TAF3 binding motif. In some embodiments, a first anchor sequence and a second anchor sequence may each comprise a ZNF143 binding motif.

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In some embodiments, a first anchor sequence and second anchor sequence comprise different sequences, e.g., a first anchor sequence comprises a CTCF binding motif, and a second anchor sequence comprises an anchor sequence other than a CTCF binding motif. In some embodiments, a first anchor sequence comprises a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif, and a second anchor sequence comprises a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif, wherein the first and second anchor sequences do not both comprise a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif. In some embodiments, each anchor sequence comprises a common nucleotide sequence (e.g., a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif) and one or more flanking nucleotides on one or both sides of a common nucleotide sequence.

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Two anchor sequences (e.g., each comprising a CTCF binding motif, a USF1 binding motif, a YY1 binding motif, a TAF3 binding motif, or a ZNF143 binding motif) that can form a conjunction may be present in a genome in any orientation, e.g., in the same orientation (tandem) either 5'-3' (left tandem, or 3'-5' (right tandem), or convergent orientation, where one anchor sequence is oriented 5'-3' and the other is oriented 3'-5'.

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Two CTCF-binding motifs (e.g., contiguous or non-contiguous CTCF binding motifs) that can form a conjunction may be present in a genome in any orientation, e.g., in the same orientation (tandem)

either 5'-3' (left tandem, e.g., the two CTCF-binding motifs that comprise SEQ ID NO:1) or 3'-5' (right tandem, e.g., the two CTCF-binding motifs comprise SEQ ID NO:2), or convergent orientation, where one CTCF-binding motif comprises SEQ ID NO:1 and another other comprises SEQ ID NO:2.

CTCFBDB 2.0: Database For CTCF binding motifs And Genome Organization (on the world wide web

at insulatordb.uthsc.edu/) can be used to identify CTCF binding motifs associated with a target gene.

In some embodiments, an anchor sequence comprises a CTCF binding motif associated with a target plurality of genes, wherein the target plurality of genes is associated with a disease, disorder and/or condition. In some embodiments, an anchor sequence comprises a CTCF binding motif associated with a target plurality of genes, wherein the genes of the target plurality of genes have related functionalities. In some embodiments, an anchor sequence comprises a CTCF binding motif associated with a target plurality of genes, wherein the target plurality of genes (e.g., two or more, e.g., all, of the plurality) are aberrantly expressed in a cell of a subject.

In some embodiments, chromatin structure may be modified by substituting, adding, or deleting one or more nucleotides within at least one anchor sequence, e.g., a nucleating polypeptide binding motif. One or more nucleotides may be specifically targeted, e.g., a targeted alteration, for substitution, addition or deletion within an anchor sequence, e.g., a nucleating polypeptide binding motif.

In some embodiments, an anchor sequence-mediated conjunction may be altered by changing an orientation of at least one common nucleotide sequence, e.g., a nucleating polypeptide binding motif. In

some embodiments, an anchor sequence comprises a nucleating polypeptide binding motif, e.g., CTCF binding motif, and a targeting moiety introduces an alteration in at least one nucleating polypeptide binding motif, e.g., altering binding affinity for a nucleating polypeptide.

In some embodiments, an anchor sequence-mediated conjunction may be altered by introducing an exogenous anchor sequence. In some embodiments, addition of a non-naturally occurring or exogenous anchor sequence to destabilize or inhibit formation of a naturally occurring anchor sequence-mediated conjunction, e.g., by inducing a non-naturally occurring loop to form, alters (e.g., decreases) transcription of a nucleic acid sequence.

Other Compositions

Nucleic acids and Vectors

The present disclosure is further directed, in part, to nucleic acids encoding a site-specific disrupting agent or a system described herein. In some embodiments, a site-specific disrupting agent may be provided via a composition comprising a nucleic acid encoding a site-specific disrupting agent, e.g., a targeting moiety and/or effector moiety of the site-specific disrupting agent, wherein the nucleic acid is

associated with sufficient other sequences to achieve expression of the site-specific disrupting agent in a system of interest (e.g., in a particular cell, tissue, organism, etc). In some embodiments, system may be provided via a composition comprising a first nucleic acid encoding a first site-specific disrupting agent, e.g., a first targeting moiety and/or a first effector moiety of the first site-specific disrupting agent, and a
5 second nucleic acid encoding a second site-specific disrupting agent, e.g., a second targeting moiety and/or a second effector moiety of the second site-specific disrupting agent wherein the first and/ or the second nucleic acid is associated with sufficient other sequences to achieve expression of the site-specific disrupting agents in a system of interest (e.g., in a particular cell, tissue, organism, etc).

10 In some particular embodiments, the present disclosure provides compositions of nucleic acids that encode a site-specific disrupting agent or polypeptide or nucleic acid portion thereof (e.g., a targeting moiety and/or effector moiety comprising a polypeptide and/or nucleic acid). In some particular embodiments, the present disclosure provides compositions of nucleic acids that encode a first site-specific disrupting agent and a second site-specific disrupting agent, or polypeptides or nucleic acid
15 portions thereof (e.g., a targeting moiety and/or effector moiety comprising a polypeptide and/or nucleic acid). In some such embodiments, provided nucleic acids may include DNA, RNA, or any other nucleic acid moiety or entity as described herein, and may be prepared by any technology described herein or otherwise available in the art (e.g., synthesis, cloning, amplification, in vitro or in vivo transcription, etc). In some embodiments, provided nucleic acids that encode one or more site-specific disrupting agents, or
20 polypeptides or nucleic acid portions thereof may be operationally associated with one or more replication, integration, and/or expression signals appropriate and/or sufficient to achieve integration, replication, and/or expression of the provided nucleic acid in a system of interest (e.g., in a particular cell, tissue, organism, etc.).

25 In some embodiments, a composition for delivering a site-specific disrupting agent or a system described herein comprises a vector, e.g., a viral vector, comprising one or more nucleic acids encoding a site-specific disrupting agent or polypeptide or nucleic acid portion thereof. In some embodiments, a first vector comprises a first nucleic acid encoding a first site-specific disrupting agent, and second vector comprises a second nucleic acid encoding a second site-specific disrupting agent. In some embodiments a
30 single vector comprises a first nucleic acid encoding a first site-specific disrupting agent and a second nucleic acid encoding a second site-specific disrupting agent.

In some embodiments, a composition for delivering a site-specific disrupting agent or a system described herein is or comprises RNA, e.g., mRNA, comprising one or more nucleic acids encoding one or more components of a site-specific disrupting agent or polypeptide or nucleic acid portion thereof.

5 Nucleic acids as described herein or nucleic acids encoding a protein described herein, may be incorporated into a vector. Vectors, including those derived from retroviruses such as lentivirus, are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Examples of vectors include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. An expression vector may be
10 provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and described in a variety of virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno- associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable
15 markers.

Expression of natural or synthetic nucleic acids is typically achieved by operably linking a nucleic acid encoding the gene of interest to a promoter and incorporating the construct into an expression vector. Vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors
20 contain transcription and translation terminators, initiation sequences, and promoters useful for expression of the desired nucleic acid sequence.

Additional promoter elements, e.g., enhancing sequences, may regulate frequency of transcriptional initiation. Typically, these sequences are located in a region 30-110 bp upstream of a
25 transcription start site, although a number of promoters have recently been shown to contain functional elements downstream of transcription start sites as well. Spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In a thymidine kinase (tk) promoter, spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements
30 can function either cooperatively or independently to activate transcription.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. In some embodiments of

a suitable promoter is Elongation Growth Factor-1 α (EF-1 α). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, an actin promoter, a myosin promoter, a hemoglobin promoter, and a creatine kinase promoter. The present disclosure should not be interpreted to be limited to use of any particular promoter or category of promoters (e.g., constitutive promoters). For example, in some embodiments, inducible promoters are contemplated as part of the present disclosure. In some embodiments, use of an inducible promoter provides a molecular switch capable of turning on expression of a polynucleotide sequence to which it is operatively linked, when such expression is desired. In some embodiments, use of an inducible promoter provides a molecular switch capable of turning off expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

In some embodiments, an expression vector to be introduced can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In some aspects, a selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate transcriptional control sequences to enable expression in the host cells. Useful selectable markers may include, for example, antibiotic-resistance genes, such as neo, etc.

In some embodiments, reporter genes may be used for identifying potentially transfected cells and/or for evaluating the functionality of transcriptional control sequences. In general, a reporter gene is a gene that is not present in or expressed by a recipient source (of a reporter gene) and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity or visualizable fluorescence. Expression of a reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, a construct with a minimal 5' flanking region that shows highest level of expression of reporter

gene is identified as a promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for ability to modulate promoter-driven transcription.

Cells

5 The present disclosure is further directed, in part, to cells comprising a site-specific disrupting agent or a system described herein. Any cell, e.g., cell line, e.g., a cell line suitable for expression of a recombinant polypeptide, known to one of skill in the art is suitable to comprise a site-specific disrupting agent described herein. In some embodiments, a cell, e.g., cell line, may be used to express a site-specific disrupting agent, a system comprising one or more site-specific agents, or nucleic acid or polypeptide
10 portion thereof. In some embodiments, a cell, e.g., cell line, may be used to express or amplify a nucleic acid, e.g., a vector, encoding a site-specific disrupting agent. In some embodiments, a cell, e.g., cell line, may be used to express or amplify one or more nucleic acids, e.g., a vector, encoding a first site-specific disrupting agent and a vector encoding a second site specific disrupting agent. In some embodiments, a cell comprises a nucleic acid encoding a site-specific disrupting agent described herein. In some
15 embodiments, a cell comprises a first nucleic acid encoding a first site-specific disrupting agent and a second nucleic acid encoding a second site-specific disrupting agent described herein.

 In some embodiments, a cell comprises a nucleic acid encoding a site-specific disrupting agent or nucleic acid or polypeptide portion thereof, and the nucleic acid is integrated into the genomic DNA of
20 the cell. In some embodiments, a cell comprises a nucleic acid encoding a site-specific disrupting agent or nucleic acid or polypeptide portion thereof, and the nucleic acid is disposed on a vector. In some embodiments, a cell comprises a first nucleic acid encoding a first site-specific disrupting agent and a second nucleic acid encoding a second site-specific disrupting agent or nucleic acid or polypeptide portion thereof, and the first and the second nucleic acid are integrated into the genomic DNA of the cell.
25 In some embodiments, a cell comprises a first nucleic acid encoding a first site-specific disrupting agent and a second nucleic acid encoding a second site-specific disrupting agent or nucleic acid or polypeptide portion thereof, and the first and the second nucleic acid are disposed on a vector.

 Examples of cells that may comprise and/or express a site-specific disrupting agent or a system
30 herein include, but are not limited to, hepatocytes, stellate cells, Kupffer cells, neuronal cells, endothelial cells, alveolar cells, epithelial cells, myocytes, synovial layer, chondrocytes, immune cells, and lymphocytes.

The present disclosure is further directed, in part, to a cell made by a method or process described herein. In some embodiments, the disclosure provides a cell produced by, providing a site-specific disrupting agent described herein, providing the cell, and contacting the cell with the site-specific disrupting agent (or a nucleic acid encoding the site-specific disrupting agent, or a composition comprising said site-specific disrupting agent or nucleic acid). In some embodiments, the disclosure provides a cell produced by, providing system described herein, providing the cell, and contacting the cell with the system (or a first nucleic acid encoding the first site-specific disrupting agent and second nucleic acid encoding the second site-specific disrupting agent, or a composition comprising said system or nucleic acids). Without wishing to be bound by theory, a cell contacted with a site-specific disrupting agent or a system described herein may exhibit: a decrease in expression of a target plurality of genes; a modification of epigenetic markers associated with the target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes; a genetic modification of a gene of the target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes; and/or a decrease (e.g., the absence of) in the level of a genomic complex, e.g., ASMC, comprising a target plurality of genes, compared to a similar cell that has not been contacted by the site-specific disrupting agent. In some embodiments, a cell exhibiting said decrease in expression of a target plurality of genes, modification of epigenetic markers, and/or genetic modification does not comprise the site-specific disrupting agent. The decrease in expression of a target plurality of genes, modification of epigenetic markers, and/or genetic modification may persist, e.g., for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, 7, 10, or 14 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely) after contact with the site-specific disrupting agent. In some embodiments, a cell previously contacted by a site-specific disrupting agent retains the decrease in expression of a target plurality of genes, modification of epigenetic markers, and/or genetic modification after the site-specific disrupting agent is no longer present in the cell, e.g., for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, 7, 10, or 14 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely) after the site-specific disrupting agent is no longer present in the cell. In some embodiments, the cell is a mammalian, e.g., human, cell. In some embodiments, the cell is a somatic cell and/or a primary cell.

Kits

The present disclosure further directed, in part, to a kit comprising a site-specific disrupting agent, a system, nucleic acid encoding a site-specific disrupting agent, or a first nucleic acid encoding the first site-specific disrupting agent and a second nucleic acid encoding the second site-specific disrupting agent described herein. In some embodiments, a kit comprises a site-specific disrupting agent, a system, or nucleic acid encoding the same and instructions for the use of said site-specific disrupting agent or the system. In some embodiments, a kit comprises a nucleic acid encoding the site-specific disrupting agent or a component thereof (e.g., a polypeptide or nucleic acid portion of the site-specific disrupting agent) and instructions for the use of said nucleic acid and/or said site-specific disrupting agent. In some
10 embodiments, a kit comprises a cell comprising a nucleic acid encoding the site-specific disrupting agent or a component thereof (e.g., a polypeptide or nucleic acid portion of the site-specific disrupting agent) and instructions for the use of said cell, nucleic acid, and/or said site-specific disrupting agent. In some embodiments, a kit comprises or a first nucleic acid encoding the first site-specific disrupting agent and a second nucleic acid encoding the second site-specific disrupting agent or a component thereof (e.g., a
15 polypeptide or nucleic acid portion of the first and the second site-specific disrupting agent) and instructions for the use of said nucleic acids and/or said system. In some embodiments, a kit comprises a cell comprising a nucleic acid encoding or a first nucleic acid encoding the first site-specific disrupting agent and a second nucleic acid encoding the second site-specific disrupting agent or a component thereof (e.g., a polypeptide or nucleic acid portion of the first and the second site-specific disrupting agent) and
20 instructions for the use of said cell, nucleic acid, and/or said system.

In some embodiments, a kit comprises a unit dosage of a site-specific disrupting agent, or a unit dosage of a nucleic acid, e.g., a vector, encoding a site-specific disrupting agent described herein. In some
25 embodiments, a kit comprises a unit dosage of a system, or a unit dosage of a first and a second nucleic acid, e.g., a vector, encoding a first site-specific disrupting agent and a second site-specific disrupting agent described herein.

Methods of Making a Site-Specific Disrupting Agent

In some embodiments, a site-specific disrupting agent or a system comprises one or more proteins
30 and may thus be produced by methods of making proteins. As will be appreciated by one of skill, methods of making proteins or polypeptides (which may be included in modulating agents as described herein) are routine in the art. See, in general, Smales & James (Eds.), *Therapeutic Proteins: Methods and Protocols* (Methods in Molecular Biology), Humana Press (2005); and Crommelin, Sindelar & Meibohm (Eds.), *Pharmaceutical Biotechnology: Fundamentals and Applications*, Springer (2013).

A protein or polypeptide of compositions of the present disclosure can be biochemically synthesized by employing standard solid phase techniques. Such methods include exclusive solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis. These methods can be used when a peptide is relatively short (e.g., 10 kDa) and/or when it cannot be produced by recombinant techniques (i.e., not encoded by a nucleic acid sequence) and therefore involves different chemistry.

Solid phase synthesis procedures are well known in the art and further described by John Morrow Stewart and Janis Dillaha Young, *Solid Phase Peptide Syntheses*, 2nd Ed., Pierce Chemical Company, 1984; and Coin, I., et al., *Nature Protocols*, 2:3247-3256, 2007.

For longer peptides, recombinant methods may be used. Methods of making a recombinant therapeutic polypeptide are routine in the art. See, in general, Smales & James (Eds.), *Therapeutic Proteins: Methods and Protocols* (Methods in Molecular Biology), Humana Press (2005); and Crommelin, Sindelar & Meibohm (Eds.), *Pharmaceutical Biotechnology: Fundamentals and Applications*, Springer (2013).

Exemplary methods for producing a therapeutic pharmaceutical protein or polypeptide involve expression in mammalian cells, although recombinant proteins can also be produced using insect cells, yeast, bacteria, or other cells under control of appropriate promoters. Mammalian expression vectors may comprise non-transcribed elements such as an origin of replication, a suitable promoter, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and termination sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, splice, and polyadenylation sites may be used to provide other genetic elements required for expression of a heterologous DNA sequence. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described in Green & Sambrook, *Molecular Cloning: A Laboratory Manual* (Fourth Edition), Cold Spring Harbor Laboratory Press (2012).

In cases where large amounts of the protein or polypeptide are desired, it can be generated using techniques such as described by Brian Bray, *Nature Reviews Drug Discovery*, 2:587-593, 2003; and Weissbach & Weissbach, 1988, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp 421-463.

Various mammalian cell culture systems can be employed to express and manufacture recombinant protein. Examples of mammalian expression systems include CHO cells, COS cells, HeLa

and BHK cell lines. Processes of host cell culture for production of protein therapeutics are described in Zhou and Kantardjieff (Eds.), *Mammalian Cell Cultures for Biologics Manufacturing* (Advances in Biochemical Engineering/Biotechnology), Springer (2014). Compositions described herein may include a vector, such as a viral vector, e.g., a lentiviral vector, encoding a recombinant protein. In some
5 embodiments, a vector, e.g., a viral vector, may comprise a nucleic acid encoding a recombinant protein. Purification of protein therapeutics is described in Franks, *Protein Biotechnology: Isolation, Characterization, and Stabilization*, Humana Press (2013); and in Cutler, *Protein Purification Protocols* (Methods in Molecular Biology), Humana Press (2010). Formulation of protein therapeutics is described in Meyer (Ed.), *Therapeutic Protein Drug Products: Practical Approaches to formulation in the*
10 Laboratory, Manufacturing, and the Clinic, Woodhead Publishing Series (2012). Proteins comprise one or more amino acids. Amino acids include any compound and/or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in
15 some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some
20 embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some
25 embodiments, such modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term “amino acid” may
30 be used to refer to a free amino acid; in some embodiments it may be used to refer to an amino acid residue of a polypeptide.

Pharmaceutical Compositions, Formulation, Delivery, and Administration

The present disclosure is further directed, in part, to pharmaceutical compositions comprising a site-specific disrupting agent described herein, and to pharmaceutical compositions comprising nucleic acids encoding a site-specific disrupting agent or a system described herein.

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As used herein, the term “pharmaceutical composition” refers to an active agent (e.g., a site-specific disrupting agent or a system, or nucleic acid encoding the same), formulated together with one or more pharmaceutically acceptable carriers (e.g., pharmaceutically acceptable carriers known to those of skill in the art). In some embodiments, active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, a pharmaceutical composition comprises a site-specific disrupting agent or a system of the present disclosure.

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In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; trans-dermally; or nasally, pulmonary, and/or to other mucosal surfaces, for example, as aerosols, aqueous solutions, or suspensions. In some embodiments, the composition may be lyophilized or spray dried. In some embodiments, the composition may be formulated for pulmonary administration and/or intravenous administration.

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As used herein, the term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

30

As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or

portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. In some embodiments, for example, materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; 5 cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and 10 aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

As used herein, the term “pharmaceutically acceptable salt”, refers to salts of such compounds 15 that are appropriate for use in pharmaceutical contexts, *i.e.*, salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, et al. describes pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977). In 20 some embodiments, pharmaceutically acceptable salts include, but are not limited to, nontoxic acid addition salts, which are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods used in the art such as ion exchange. In some embodiments, pharmaceutically acceptable salts include, but are 25 not limited to, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, 30 oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. In some embodiments, pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using

counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl having from 1 to 6 carbon atoms, sulfonate, and aryl sulfonate.

In various embodiments, the present disclosure provides pharmaceutical compositions described herein with a pharmaceutically acceptable excipient. Pharmaceutically acceptable excipient includes an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients may be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous.

Pharmaceutical preparations may be made following conventional techniques of pharmacy involving milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing, and filling for hard gelatin capsule forms. When a liquid carrier is used, a preparation can be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous solution or suspension. Such a liquid formulation may be administered directly per os.

In some embodiments, pharmaceutical compositions may be formulated for delivery to a cell and/or to a subject via any route of administration. Modes of administration to a subject may include injection, infusion, inhalation, intranasal, intraocular, topical delivery, inter-cannular delivery, or ingestion. Injection includes, without limitation, intravenous, intramuscular, intra-arterial, intrathecal, intraventricular, intracapsular, intra-orbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, bronchial, sub-capsular, subarachnoid, intraspinal, intra-cerebrospinal, and intra-sternal injection and infusion. In some embodiments, administration includes aerosol inhalation, e.g., with nebulization. In some embodiments, administration is systemic (e.g., oral, rectal, nasal, sublingual, buccal, or parenteral), enteral (e.g., system-wide effect, but delivered through the gastrointestinal tract), or local (e.g., local application on the skin, intravitreal injection). In some embodiments, one or more compositions is administered systemically. In some embodiments, administration is non-parenteral and a therapeutic is a parenteral therapeutic. In some embodiments, administration may be bronchial (e.g., by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, inter-dermal, transdermal, etc.), enteral, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (e. g. intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (e.g., by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may be a single dose. In some embodiments, administration may

involve dosing that is intermittent (e.g., a plurality of doses separated in time) and/or periodic (e.g., individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (e.g., perfusion) for at least a selected period of time. In some
5 treatment or over a period of time as a treatment regimen. In some embodiments, administrations may be given as needed, e.g., for as long as symptoms associated with the disease, disorder or condition persist. In some embodiments, repeated administrations may be indicated for the remainder of the subject's life. Treatment periods may vary and could be, e.g., one day, two days, three days, one week, two weeks, one month, two months, three months, six months, a year, or longer.

10 In some embodiments, administration is provided using a respiratory delivery device, e.g., nebulizer, e.g., metered-dose inhaler, e.g., dry powder inhaler. Some of the commercially available dry powder inhalers include Spinhaler (Fisons Pharmaceuticals, Rochester, NY) and Rotahaler (GSK, RTP, NC). In some embodiments, the nebulizer may include a jet nebulizer, an ultrasonic nebulizer, and/or a
15 vibrating mesh nebulizer.

Dosage

Pharmaceutical compositions according to the present disclosure may be delivered in a therapeutically effective amount. A precise therapeutically effective amount is an amount of a
20 composition that will yield the most effective results in terms of efficacy of treatment in a given subject. This amount will vary depending upon a variety of factors, including but not limited to characteristics of a therapeutic compound (including activity, pharmacokinetics, pharmacodynamics, and bioavailability), physiological condition of a subject (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage, and type of medication), nature of a pharmaceutically
25 acceptable carrier or carriers in a formulation, and/or route of administration.

In some aspects, the present disclosure provides methods of delivering a therapeutic comprising administering a composition as described herein to a subject, wherein a genomic complex modulating agent is a therapeutic and/or wherein delivery of a therapeutic causes changes in gene expression relative
30 to gene expression in absence of a therapeutic.

Methods as provided in various embodiments herein may be utilized in any some aspects delineated herein. In some embodiments, one or more compositions is/are targeted to specific cells, or one or more specific tissues.

For example, in some embodiments one or more compositions is/are targeted to epithelial, connective, muscular, and/or nervous tissue or cells. In some embodiments a composition is targeted to a cell or tissue of a particular organ system, e.g., respiratory system (pharynx, larynx, trachea, bronchi, lungs, diaphragm), cardiovascular system (heart, vasculature); digestive system (esophagus, stomach, liver, gallbladder, pancreas, intestines, colon, rectum and anus); endocrine system (hypothalamus, pituitary gland, pineal body or pineal gland, thyroid, parathyroids, adrenal glands); excretory system (kidneys, ureters, bladder); lymphatic system (lymph, lymph nodes, lymph vessels, tonsils, adenoids, thymus, spleen); integumentary system (skin, hair, nails); muscular system (e.g., skeletal muscle); nervous system (brain, spinal cord, nerves); reproductive system (ovaries, uterus, mammary glands, testes, vas deferens, seminal vesicles, prostate); skeletal system (bone, cartilage); and/or combinations thereof. In some embodiments, a composition is targeted to a cell, e.g., endothelial, alveolar, epithelial, hepatocytes, stellate cells, Kupffer cells, synovial layer, chondrocytes, fibroblast cells, ductal epithelial cells, epithelial enterocytes, goblet cells, basal cells, and/or immune cells. In some embodiments, a composition is targeted to a cell of an organ, e.g., nasal cells, lung cells, ileum cells, cardiac cells, optic cells, liver cells, bladder cells, pancreatic cells, kidney cells, neural cells, prostate cells, testis cells, In some embodiments, a composition of the present disclosure crosses a blood-brain-barrier, a placental membrane, or a blood-testis barrier. In some embodiments, a composition is targeted to a cell expressing an ACE-2 receptor.

In some embodiments, a pharmaceutical composition as provided herein is administered systemically.

In some embodiments, administration is non-parenteral and a therapeutic is a parenteral therapeutic.

In some embodiments, a pharmaceutical composition of the present disclosure has improved PK/PD, e.g., increased pharmacokinetics or pharmacodynamics, such as improved targeting, absorption, or transport (e.g., at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90% improved or more) as compared to a active agent alone. In some embodiments, a pharmaceutical composition has reduced undesirable effects, such as reduced diffusion to a nontarget location, off-target activity, or toxic metabolism, as compared to a therapeutic alone (e.g., at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90% or more reduced, as compared to an active agent alone). In some embodiments, a composition increases efficacy and/or decreases toxicity of a therapeutic (e.g., at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 75%, 80%, 90% or more) as compared to an active agent alone.

Pharmaceutical compositions described herein may be formulated for example including a carrier, such as a pharmaceutical carrier and/or a polymeric carrier, e.g., a liposome or vesicle, and delivered by known methods to a subject in need thereof (e.g., a human or non-human agricultural or domestic animal, e.g., cattle, dog, cat, horse, poultry). Such methods include transfection (e.g., lipid-mediated, cationic polymers, calcium phosphate); electroporation or other methods of membrane disruption (e.g., nucleofection) and viral delivery (e.g., lentivirus, retrovirus, adenovirus, AAV). Methods of delivery are also described, e.g., in Gori et al., *Delivery and Specificity of CRISPR/Cas9 Genome Editing Technologies for Human Gene Therapy*. Human Gene Therapy. July 2015, 26(7): 443-451. doi:10.1089/hum.2015.074; and Zuris et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. *Nat Biotechnol.* 2014 Oct 30;33(1):73–80.

Lipid Nanoparticles

Site-specific disrupting agents as described herein can be delivered using any biological delivery system/formulation including a particle, for example, a nanoparticle delivery system. Nanoparticles include particles with a dimension (e.g. diameter) between about 1 and about 1000 nanometers, between about 1 and about 500 nanometers in size, between about 1 and about 100 nm, between about 30 nm and about 200 nm, between about 50 nm and about 300 nm, between about 75 nm and about 200 nm, between about 100 nm and about 200 nm, and any range therebetween. A nanoparticle has a composite structure of nanoscale dimensions. In some embodiments, nanoparticles are typically spherical although different morphologies are possible depending on the nanoparticle composition. The portion of the nanoparticle contacting an environment external to the nanoparticle is generally identified as the surface of the nanoparticle. In some embodiments, nanoparticles have a greatest dimension ranging between 25 nm and 200 nm. Nanoparticles as described herein comprise delivery systems that may be provided in any form, including but not limited to solid, semi-solid, emulsion, or colloidal nanoparticles. A nanoparticle delivery system may include but not limited to lipid-based systems, liposomes, micelles, microvesicles, exosomes, or gene gun. In one embodiment, the nanoparticle is a lipid nanoparticle (LNP). In some embodiments, the LNP is a particle that comprises a plurality of lipid molecules physically associated with each other by intermolecular forces. In some embodiments, an LNP may comprise multiple components, e.g., 3-4 components. In one embodiment, the site-specific disrupting agent or a pharmaceutical composition comprising said site-specific disrupting agent (or a nucleic acid encoding the same, or pharmaceutical composition comprising a nucleic acid encoding said site specific disrupting agent) is encapsulated in an LNP. In one embodiment, the system or a pharmaceutical composition comprising said system (or a nucleic acid encoding the same, or pharmaceutical composition comprising nucleic acid encoding the said system) is encapsulated in an LNP. In some embodiments, the nucleic acid

encoding the first site-specific disrupting agent and the nucleic acid encoding the second site-specific disrupting agent are present in same LNP. In some embodiments, the nucleic acid encoding the first site-specific disrupting agent and the nucleic acid encoding the second site-specific disrupting agent are present in different LNPs. Preparation of LNPs and the modulating agent encapsulation may be used/and
5 or adapted from Rosin et al, Molecular Therapy, vol. 19, no. 12, pages 1286-2200, December 2011). In some embodiments, lipid nanoparticle compositions disclosed herein are useful for expression of protein encoded by mRNA. In some embodiments, nucleic acids, when present in the lipid nanoparticles, are resistant in aqueous solution to degradation with a nuclease.

10 In some embodiments, the LNP formulations may include a CCD lipid, a neutral lipid, and/or a helper lipid. In some embodiments, the LNP formulation comprises an ionizable lipid. In some embodiments, an ionizable lipid may be a cationic lipid, an ionizable cationic lipid, or an amine-containing lipid that can be readily protonated. In some embodiments, the lipid is a cationic lipid that can exist in a positively charged or neutral form depending on pH. In some embodiments, the cationic
15 lipid is a lipid capable of being positively charged, e.g., under physiological conditions. In some embodiments, the lipid particle comprises a cationic lipid in formulation with one or more of neutral lipids, ionizable amine-containing lipids, biodegradable alkyn lipids, steroids, phospholipids including polyunsaturated lipids, structural lipids (e.g., sterols), PEG, cholesterol and polymer conjugated lipids. In some embodiments, LNP formulation (e.g., MC3 and/or SSOP) includes cholesterol, PEG, and/or a
20 helper lipid. The LNPs may be, e.g., microspheres (including uni-lamellar and multi-lamellar vesicles, lamellar phase lipid bilayers that, in some embodiments, are substantially spherical. In some embodiments, the LNP can comprise an aqueous core, e.g., comprising a nucleic acid encoding a site-specific disrupting agent or a system as disclosed herein. In some embodiments of the present disclosure, the cargo for the LNP formulation includes at least one guide RNA. In some embodiments, the
25 cargo, e.g., a nucleic acid encoding a site-specific disrupting agent, or a system as disclosed herein, may be adsorbed to the surface of an LNP, e.g., an LNP comprising a cationic lipid. In some embodiments, the cargo, e.g., a nucleic acid encoding a site-specific disrupting agent, or a system as disclosed herein may be associated with the LNP. In some embodiments, the cargo, e.g., a nucleic acid encoding a site-specific disrupting agent, or a system as disclosed herein, may be encapsulated, e.g., fully encapsulated and/or
30 partially encapsulated in an LNP.

In some embodiments, an LNP comprising a cargo may be administered for systemic delivery, e.g., delivery of a therapeutically effective dose of cargo that can result in a broad exposure of an active agent within an organism. Systemic delivery of lipid nanoparticles can be for example, intravenous,

pulmonary, bronchial, intraarterial, subcutaneous, and intraperitoneal delivery. In some embodiments, systemic delivery of lipid nanoparticles is by intravenous delivery. In some embodiments, an LNP comprising a cargo may be administered for local delivery, e.g., delivery of an active agent directly to a target site within an organism. In some embodiments, an LNP may be locally delivered into a disease site, e.g., a tumor, other target site, e.g., a site of inflammation, or to a target organ, e.g., the liver, lung, stomach, colon, pancreas, uterus, breast, lymph nodes, and the like. In some embodiments, an LNP as disclosed herein may be locally delivered to a specific cell, e.g., hepatocytes, stellate cells, Kupffer cells, endothelial, alveolar, and/or epithelial cells. In some embodiments, an LNP as disclosed herein may be locally delivered to a specific tumor site, e.g., subcutaneous, orthotopic.

The LNPs may be formulated as a dispersed phase in an emulsion, micelles, or an internal phase in a suspension. In some embodiments, the LNPs are biodegradable. In some embodiments, the LNPs do not accumulate to cytotoxic levels or cause toxicity in vivo at a therapeutically effective dose. In some embodiments, the LNPs do not accumulate to cytotoxic levels or cause toxicity in vivo after repeat administrations at a therapeutically effective dose. In some embodiments, the LNPs do not cause an innate immune response that leads to a substantially adverse effect at a therapeutically effective dose.

In some embodiments, the LNP used, comprises the formula (6Z,9Z,28Z,31Z)-heptatriacont-6,9,28,31-tetraene-19-yl 4-(dimethylamino) butanoate or ssPalmO-phenyl-P4C2 (ssPalmO-Phe, SS-OP). In some embodiments, the LNP formulation comprises the formula, (6Z,9Z,28Z,31Z)-heptatriacont-6,9,28,31-tetraene-19-yl 4-(dimethylamino)butanoate (MC3), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), Cholesterol, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2k-DMG), e.g., MC3 LNP or ssPalmO-phenyl-P4C2 (ssPalmO-Phe, SS-OP), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), Cholesterol, 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2k-DMG), e.g., SSOP-LNP.

Liposomes are spherical vesicle structures composed of a uni- or multi-lamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer. Liposomes may be anionic, neutral or cationic. Liposomes are biocompatible, nontoxic, can deliver both hydrophilic and lipophilic drug molecules, protect their cargo from degradation by plasma enzymes, and transport their load across biological membranes and the blood brain barrier (BBB) (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review).

Vesicles can be made from several different types of lipids; however, phospholipids are most commonly used to generate liposomes as drug carriers. Vesicles may comprise without limitation DOTMA, DOTAP, DOTIM, DDAB, alone or together with cholesterol to yield DOTMA and cholesterol, DOTAP and cholesterol, DOTIM and cholesterol, and DDAB and cholesterol. Methods for preparation of multilamellar vesicle lipids are known in the art (see for example U.S. Pat. No. 6,693,086, the teachings of which relating to multilamellar vesicle lipid preparation are incorporated herein by reference). Although vesicle formation can be spontaneous when a lipid film is mixed with an aqueous solution, it can also be expedited by applying force in the form of shaking by using a homogenizer, sonicator, or an extrusion apparatus (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review). Extruded lipids can be prepared by extruding through filters of decreasing size, as described in Templeton et al., Nature Biotech, 15:647-652, 1997, the teachings of which relating to extruded lipid preparation are incorporated herein by reference.

Methods and compositions provided herein may comprise a pharmaceutical composition administered by a regimen sufficient to alleviate a symptom of a disease, disorder, and/or condition. In some aspects, the present disclosure provides methods of delivering a therapeutic by administering compositions as described herein.

Uses

The present disclosure is further directed to uses of the site-specific disrupting agents or systems disclosed herein. Among other things, in some embodiments such provided technologies may be used to achieve modulation, e.g., repression, of expression of a target plurality of genes and, for example, enable control of the activity, delivery, and penetrance of one or more products of a target plurality of genes, e.g., in a cell. In some embodiments, a cell is a mammalian, e.g., human, cell. In some embodiments, a cell is a somatic cell. In some embodiments, a cell is a primary cell. For example, in some embodiments, a cell is a mammalian somatic cell. In some embodiments, a mammalian somatic cell is a primary cell. In some embodiments, a mammalian somatic cell is a non-embryonic cell.

Modulating Gene Expression

The present disclosure is further directed, in part, to a method of modulating, e.g., decreasing, expression of a target plurality of genes, comprising providing a site-specific disrupting agent or a system described herein (or a nucleic acid encoding the same, or pharmaceutical composition comprising said site-specific disrupting agent or nucleic acid), and contacting the target plurality of genes, an anchor

sequence associated with the target plurality of genes, and/or a genomic complex (e.g., ASMC) comprising the target plurality of genes with the site-specific disrupting agent or a system. In some embodiments, modulating, e.g., decreasing, expression of a target plurality of genes comprises modulation of transcription of a gene of the target plurality of genes as compared with a reference value, e.g., transcription of the gene in the absence of the site-specific disrupting agent or a system. In some
 5 embodiments, the method of modulating, e.g., decreasing, expression of a target plurality of genes is used ex vivo, e.g., on a cell from a subject, e.g., a mammalian subject, e.g., a human subject. In some embodiments, the cell is a mammalian, e.g., human, cell. In some embodiments, the cell is a somatic cell. In some embodiments, the cell is a primary cell. In some embodiments, the method of modulating, e.g.,
 10 decreasing, expression of a target plurality of genes is used in vivo, e.g., on a mammalian subject, e.g., a human subject. In some embodiments, the method of modulating, e.g., decreasing, expression of a target plurality of genes is used in vitro, e.g., on a cell or cell line described herein.

Without wishing to be bound by theory, in some embodiments it is thought that a site-specific
 15 disrupting agent or a system may modulate the expression of a target plurality of genes by binding to an anchor sequence of a genomic complex, e.g., ASMC, comprising the target plurality of genes, and having one, two, or all of the following effects: physically or sterically blocking (e.g., competitively inhibiting) binding of a genomic complex component (e.g., nucleating polypeptide) to the anchor sequence; epigenetically modifying the target plurality of genes, a transcription control element operably linked to
 20 the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes (e.g., thereby decreasing and/or eliminating binding of a genomic complex component, e.g., nucleating polypeptide, to the anchor sequence); or genetically modifying the target plurality of genes, a transcription control
 25 element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes (e.g., thereby decreasing and/or eliminating binding of a genomic complex component, e.g., nucleating polypeptide, to the anchor sequence).

In some embodiments, a method described herein modulates, e.g., decreases, the expression of
 30 two or more genes of a target plurality of genes. In some embodiments, a method described herein modulates, e.g., decreases, the expression of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 (and optionally, no more than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 30) genes of a target plurality of genes. In some embodiments, a method described herein modulates, e.g., decreases, the expression of 2-20, 2-18, 2-16, 2-14, 2-12, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-

18, 3-16, 3-14, 3-12, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-18, 4-16, 4-14, 4-12, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-18, 5-16, 5-14, 5-12, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-18, 6-16, 6-14, 6-12, 6-10, 6-9, 6-8, 6-7, 7-20, 7-18, 7-16, 7-14, 7-12, 7-10, 7-9, 7-8, 8-20, 8-18, 8-16, 8-14, 8-12, 8-10, 8-9, 9-20, 9-18, 9-16, 9-14, 9-12, 9-10, 10-20, 10-18, 10-16, 10-14, 10-12, 12-20, 12-18, 12-16, 12-14, 14-20, 14-18, 14-16, 16-20, 16-18, or 18-20 genes of a target plurality of genes. In some embodiments, a method described herein modulates, e.g., decreases, the expression of each gene (e.g., all genes) of a target plurality of genes.

In some embodiments, a method described herein modulates, e.g., decreases, the expression of a gene of a target plurality of genes, wherein one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., all) of the genes is a cytokine, an interleukin, a transcription factor (e.g., an interferon regulatory transcription factor), intercellular adhesion molecule (ICAM), or an interferon receptor. In some embodiments, a method described herein modulates, e.g., decreases, the level of RNA, e.g., mRNA, produced from one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., all) genes of the target plurality of genes. In some embodiments, modulating expression comprises decreasing the level of protein produced from one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., all) genes of the target plurality of genes. In some embodiments, modulating expression comprises both decreasing the level of mRNA and protein produced from one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, e.g., all) genes of the target plurality of genes. In some embodiments, the decrease is a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to pre-treatment levels or levels in the absence of the site-specific disrupting agent.

In some embodiments, a method described herein modulates, e.g., decreases, the expression of one or more of (e.g., 1, 2, 3, or all of) human CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and IL8, e.g., upon stimulation of the cell with TNF-alpha, e.g., using an assay of any of Examples 2 or 4-11. In some embodiments, a method described herein modulates, e.g., decreases, the expression of one or more of (e.g., 1, 2, 3, or all of) mice CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL7, and CXCL15, e.g., upon stimulation of the cell with TNF-alpha, e.g., using an assay of Example 14.

In some embodiments, a method described herein decreases binding of a nucleating polypeptide, e.g., CTCF, to an anchor sequence. In some embodiments, contacting a cell or administering a site-specific disrupting agent results in a decrease in binding of a nucleating polypeptide, e.g., CTCF, to an anchor sequence (e.g., an anchor sequence of an ASMC comprising a target plurality of genes). In some embodiments, contacting a cell or administering a site-specific disrupting agent results in a complete loss of binding or a decrease of at least 50, 60, 70, 80, 90, 95, or 99%, e.g., as measured by ChIP and/or

quantitative PCR, relative to the binding of the nucleating polypeptide (e.g., CTCF) to the anchor sequence prior to treatment with the site-specific disrupting agent or the system or in the absence of the site-specific disrupting agent or the system.

5 The present disclosure is further directed, in part, to a method of treating a condition associated with over-expression of a target plurality of genes in a subject, comprising administering to the subject a site-specific disrupting agent, a system, a nucleic acid, a vector, a cell, or a pharmaceutical composition described herein. Conditions associated with over-expression of particular genes are known to those of skill in the art. Such conditions include, but are not limited to, metabolic disorders, neuromuscular
10 disorders, cancer (e.g., solid tumors), fibrosis, diabetes, urea disorders, immune disorders, inflammation, and arthritis. In some embodiments, the disorder is an auto-immune disorder. In some embodiments, the disorder is associated with or caused by an infection, e.g., a viral infection, e.g., SARS-Cov2 viral infection.

15 The present disclosure is further directed, in part, to a method of modulating, e.g., decreasing, expression of a target plurality of genes, in a cell in a subject, e.g., a human subject. In some embodiments, the subject has a disease or condition. In some embodiments, the disease is an inflammatory disease, e.g., an immune mediated inflammatory disease. In some embodiments, the disease or condition is one or more of rheumatoid arthritis, gout, asthma, neutrophilic asthma, neutrophilic
20 dermatosis, paw edema, acute respiratory disease syndrome (ARDS), COVID-19, psoriasis, inflammatory bowel disease, infection (e.g., by a pathogen, e.g., a bacteria, a viruses, or a fungus), external injury (e.g., scrapes or foreign objects), effects of radiation or chemical injury, osteoarthritis, osteoarthritic joint pain, joint pain, inflammatory pain, acute pain, chronic pain, cystitis, bronchitis, dermatitis, cardiovascular disease, neurodegenerative disease, liver disease, lung disease, kidney disease, pain, swelling, stiffness,
25 tenderness, redness, warmth, or elevated biomarkers related to disease states (e.g., cytokines, immune receptors, or inflammatory markers). In some embodiments, the inflammatory disorder is associated with an infection, e.g., by a virus, e.g., Sars-Cov-2 virus. In some embodiments, the inflammatory disorder is an autoimmune disorder.

30 Methods and compositions as provided herein may treat a condition associated with over-expression of a target plurality of genes by stably or transiently altering (e.g., decreasing) transcription of a target plurality of genes. In some embodiments, such a modulation persists for at least about 1 hr to about 30 days, or at least about 2 hrs, 6 hrs, 12 hrs, 18 hrs, 24 hrs, 2 days, 3, days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19

days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, or longer or any time therebetween. In some embodiments, such a modulation persists for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, or 7 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely). Optionally, such a modulation persists for no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 years.

In some embodiments, a method or composition provided herein may decrease expression of a gene of a target plurality of genes in a cell by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (and optionally up to 100%) relative to expression of the gene of the target plurality of genes in a cell not contacted by the composition or treated with the method. In some embodiments, a method or composition provided herein may decrease expression of each gene of a target plurality of genes in a cell by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (and optionally up to 100%) relative to expression of each gene of the target plurality of genes in a cell not contacted by the composition or treated with the method.

In some embodiments, a method provided herein may modulate, e.g., decrease, expression of a target plurality of genes by disrupting a genomic complex, e.g., an anchor sequence-mediated conjunction, comprising said target plurality of genes. In some embodiments, a method described herein disrupts a genomic complex (e.g., ASMC). In some embodiments, contacting a cell or administering a site-specific disrupting agent results in a decrease in the level of a genomic complex (e.g., ASMC) comprising the target plurality of genes relative to the level of the complex prior to treatment with the site-specific disrupting agent or the system or in the absence of the site-specific disrupting agent or the system. In some embodiments, contacting a cell or administering a site-specific disrupting agent results in a complete loss of the genomic complex, e.g., ASMC, or a decrease of at least 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 95, or 99%, e.g., as measured by ChIA-PET, ELISA (e.g., to assess gene expression changes), CUT&RUN, ATAC-SEQ, ChIP and/or quantitative PCR, relative to the level of the complex prior to treatment with the site-specific disrupting agent or the system or in the absence of the site-specific disrupting agent or the system.

In some embodiments, methods and compositions as provided herein may treat a condition associated with cascade of inflammation or cytokine storm by decreasing recruitment of cytokines in the site of inflammation. In some embodiments, the cascade of inflammation and/or cytokine storm is associated with an inflammatory disorder, e.g., a viral mediated inflammatory disorder, e.g., COVID-19 infection. In some embodiments, the inflammatory disorder is associated with an infection, e.g., by a

virus, e.g., Sars-Cov-2 virus. In some embodiments, the inflammatory disorder is an autoimmune disorder. In some embodiments, the inflammatory disorder is associated with hypoxia. In some embodiments, the inflammatory disorder is associated with ARDS, hypoxia, and/or sepsis. In some embodiments, the infection is a super infection, e.g., caused by more than one pathogen, e.g., a first virus
5 or a bacterium, or a fungus, and a second virus, or a second bacterium, or a second fungus.

Epigenetic Modification

The present disclosure is further directed, in part, to a method of epigenetically modifying: one or more (e.g., all) genes of a target plurality of genes; a transcription control element operably linked to the target plurality of genes; an anchor sequence proximal to the target plurality of genes or associated with
10 an anchor sequence-mediated conjunction comprising the target plurality of genes; or a site proximal to said anchor sequence, the method comprising providing a site-specific disrupting agent, a system, a nucleic acid encoding the site-specific disrupting agent, nucleic acids encoding the components of the system, or pharmaceutical composition comprising said site-specific disrupting agent, system or nucleic acid; and contacting the one or more (e.g., all) genes of the target plurality of genes, a transcription
15 control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence with the site-specific disrupting agent or the system, thereby epigenetically modifying the target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of
20 genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence.

In some embodiments, a method of epigenetically a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target
25 plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises increasing or decreasing DNA methylation of the target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said
30 anchor sequence. In some embodiments, a method of epigenetically modifying target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises increasing or decreasing histone methylation of a histone associated with the target plurality of genes, a transcription

control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence. In some embodiments, a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises decreasing histone acetylation of a histone associated with the target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence. In some embodiments, a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises increasing or decreasing histone sumoylation of a histone associated with the target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence. In some embodiments, a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises increasing or decreasing histone phosphorylation of a histone associated with the target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence.

In some embodiments, a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence may decrease the level of the epigenetic modification by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (and optionally up to 100%) relative to the level of the epigenetic modification at that site in a cell not contacted by the composition or treated with the method. In some embodiments, a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence

proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence may increase the level of the epigenetic modification by at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 300, 400, 500, 600, 700, 800, 900, or 1000% (and optionally up to 200, 300, 400, 500, 600, 700, 800, 900, 1000, or 2000%) relative to the level of the epigenetic modification at that site in a cell not contacted by the composition or treated with the method. In some embodiments epigenetic modification of a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence may modify the level of expression of the target plurality of genes, e.g., as described herein.

In some embodiments, an epigenetic modification produced by a method described herein persists for at least about 1 hour to about 30 days, or at least about 2 hours, 6 hours, 12 hours, 18 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, or longer or any time there between. In some embodiments, such a modulation persists for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or at least 1, 2, 3, 4, 5, 6, or 7 days, or at least 1, 2, 3, 4, or 5 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or at least 1, 2, 3, 4, or 5 years (e.g., indefinitely). Optionally, such a modulation persists for no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 years.

In some embodiments, a site-specific disrupting agent or a system for use in a method of epigenetically modifying a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence comprises an effector moiety that comprises an epigenetic modifying moiety. For example, an effector moiety may comprise an epigenetic modifying moiety with DNA methyltransferase activity, and an endogenous or naturally occurring target sequence (e.g., a gene of a target plurality of genes, a transcription control element operably linked to the target plurality of genes, an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, or a site proximal to said anchor sequence) may be altered to increase its methylation (e.g., decreasing interaction of a transcription factor with a portion of a gene of a target plurality of genes or transcription control element, decreasing binding of a nucleating protein to an anchor sequence, and/or disrupting or preventing an anchor sequence-mediated conjunction), or may be

altered to decrease its methylation (e.g., increasing interaction of a transcription factor with a portion of a gene of a target plurality of genes or transcription control element, increasing binding of a nucleating protein to an anchor sequence, and/or promoting or increasing strength of an anchor sequence-mediated conjunction).

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Genetic Modification

The present disclosure is further directed, in part, to a method of genetically modifying one or more (e.g., one, two, three, or all) genes of a target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes, the method comprising providing a site-specific disrupting agent or a system or nucleic acid encoding the same or pharmaceutical composition comprising said site-specific disrupting agent, system or nucleic acid; and contacting the one or more (e.g., one, two, three, or all) genes of the target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes with the site-specific disrupting agent, thereby genetically modifying the target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes.

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Genetic modification may comprise introducing one or more of an insertion, deletion, or substitution into a gene of a target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes. In some embodiments, an insertion comprises addition of at least 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, or 2000 nucleotides (and optionally no more than 3000, 2500, 2000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, or 200 nucleotides). In some embodiments, an insertion comprises addition of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, or 100 nucleotides (and optionally no more than 200, 150, 100, 90, 80, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide). In some embodiments, the insertion comprises addition of 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 nucleotides. In some embodiments, a deletion comprises removal of at least 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, or 2000 nucleotides (and optionally no more than 3000, 2500, 2000, 1500, 1000, 900, 800, 700, 600, 500, 400, 300, or 200 nucleotides). In some embodiments, a deletion comprises removal of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

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15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, or 100 nucleotides (and optionally no more than 200, 150, 100, 90, 80, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide). In some embodiments, the deletion comprises removal of 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 nucleotides. In some embodiments, a substitution comprises alteration of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, or 100 nucleotides (and optionally no more than 200, 150, 100, 90, 80, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 nucleotide). In some embodiments, the substitution comprises alteration of 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 nucleotides.

In some embodiments, a genetic modification comprises an insertion, deletion, or substitution to an anchor sequence, e.g., associated with an ASMC comprising the target plurality of genes. In some embodiments, the genetic modification alters (e.g., decreases or increases) the binding of a genomic complex component, e.g., a nucleating polypeptide, to the anchor sequence. In some embodiments, the genetic modification abrogates (e.g., via an insertion, deletion, or substitution), wholly or in part, an anchor sequence, thereby decreasing or abolishing the binding of a nucleating polypeptide to the anchor sequence, e.g., and decreasing the presence of or abolishing an ASMC comprising said anchor sequence. Without wishing to be bound by theory, the disclosure contemplates use of a site-specific disrupting agent with genetic modification functionality to introduce an insertion, deletion, or substitution into an anchor sequence to decrease or eliminate the anchor sequence's participation in a genomic complex, e.g., ASMC, that comprises a target plurality of genes. As described elsewhere herein, such an alteration is expected to disrupt the genomic complex, e.g., ASMC, and may decrease expression of the target plurality of genes.

In some embodiments, the genetic modification comprises insertion of a sequence comprising an anchor sequence. Without wishing to be bound by theory, the disclosure contemplates use of a site-specific disrupting agent or a system with genetic modification functionality to introduce an exogenous anchor sequence into a gene of a target plurality of genes, a transcription control element operably linked to the target plurality of genes, or an anchor sequence proximal to the target plurality of genes or associated with an anchor sequence-mediated conjunction comprising the target plurality of genes. It is thought that the presence of a new anchor sequence may disrupt the formation and/or maintenance of a genomic complex, e.g., ASMC, comprising the target plurality of genes, thereby modulating, e.g., decreasing, expression of the target plurality of genes.

The following examples are provided to further illustrate some embodiments of the present disclosure but are not intended to limit the scope of the disclosure; it will be understood by their

exemplary nature that other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

EXAMPLES

Example 1: Decreasing Expression of an Exemplary Plurality of Genes

This example describes, in part, experiments demonstrating decreasing expression of a target plurality of genes comprising CXCL1, CXCL2, CXCL3, and IL8 using a site-specific disrupting agent comprising a CRISPR/Cas molecule and guide RNAs comprising the given guide sequences.

Transfection of RNPs comprising a site-specific disrupting agent comprising mRNA encoding CRISPR/Cas molecule (Cas9) and sgRNA Cas9/guide RNP complex was carried out by electroporation into THP-1 cells. Cells were cultured in RPMI + 10% FBS. A parental line was also analyzed for comparison.

350k cells were plated in quadruplicate for each edited cell line and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to 2 wells for each cell line. The remaining 2 wells are untreated as a control.

The edited and parental cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol.

RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1, CXCL2, CXCL3 & IL8 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific). CXCL1-3 & IL7 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells.

The CTCF anchors at both boundaries of the Insulated Genomic Domain (IGD) were located using ChIP-seq data, and the CTCF anchor sequences were identified computationally using the known CTCF position weight matrix ([JASPAR](#)). CRISPR (Sp Cas9) guides were chosen to target the CTCF anchor sequence.

The guides sequences are listed in the table below.

Table 4.

Guide Sequence	Genomic Coordinates	Guide
GGGGCCACTAGGGACAGGAT (SEQ ID NO: 20) (non-target control)	chr19:55627120-55627139	GD-28662
AGCCCCACCTTGTGGTCAGA (SEQ ID NO: 21)	chr4:74595464-74595486	GD-28481
AGTGCTGCCTTCTGACCACA	chr4:74595457-74595479	GD-28482

(SEQ ID NO: 22)		
GCTGCCTTCTGACCACAAGG		GD-28483
(SEQ ID NO: 23)	chr4:74595460-74595482	
CCAGTATAAGCCCCACCTTG		GD-28484
(SEQ ID NO: 24)	chr4:74595472-74595494	
CTGCCTGTCCCATAAGGAGG		GD-28485
(SEQ ID NO: 25)	chr4:75000088-75000110	
GCACTGCCTGTCCCATAAGG		GD-28486
(SEQ ID NO: 26)	chr4:75000091-75000113	
GGTCCTCCTCCTTATGGGAC		GD-28487
(SEQ ID NO: 27)	chr4:75000085-75000107	
GCCTTGTTTTCGGCTCTAGA		GD-28488
(SEQ ID NO: 28)	chr4:75000157-75000179	
GCCATCTAGAGCCGAAAACA		GD-28489
(SEQ ID NO: 29)	chr4:75000156-75000178	

Example 2: Cytokine expression decrease in THP-1 cells at 72 hours

This example describes, in part, experiments demonstrating decreasing expression of a target plurality of genes comprising CXCL1, CXCL2, CXCL3, and IL8 using a site-specific disrupting agent comprising a CRISPR/Cas molecule and guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

sgRNA and mRNA encoding Cas9 RNPs were electroporated into THP-1 cells. sgRNA sequences (from Example 1) were chosen to target one of the CTCF sites of the ASMC comprising CXCL1, CXCL2, CXCL3, and IL8. The transfected cells were incubated with 10ng/ml TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the manufacturer's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1, CXCL2, CXCL3 & IL8 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1-3 & IL8 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results in Figure 6 show that a site-specific disrupting agent comprising a CRISPR/Cas molecule and an sgRNA can be used to decrease CXCL1, CXCL2, CXCL3, and IL8 expression in THP-1 cells, and that expression is decreased at 72 hours post-treatment. The results also show that LNP delivery of said site-specific disrupting agent can be used to deliver effective amounts of the agent to the target cells.

Similar results were seen when the experiment was repeated with sgRNAs targeting the other CTCF site of the ASMC comprising CXCL1, CXCL2, CXCL3, and IL8 (data not shown).

Example 3: Cytokine protein secretion of THP-1 cells decreased by site-specific modulating agent

This example describes, in part, experiments demonstrating decreasing secretion of CXCL1 and IL-8, two genes of a target plurality of genes, by treating cells using a site-specific disrupting agent comprising a CRISPR/Cas molecule and guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

THP-1 cells were electroporated with RNPs comprising sgRNAs and mRNA encoding a site-specific disrupting agent comprising an exemplary CRISPR/Cas molecule (Cas9) as in previous Examples. sgRNAs (from Example 1) were targeted to one of the CTCF sites of the ASMC comprising CXCL1 and IL-8. Cells were stimulated with 10ng/ml TNF alpha for 24hours. After that time, cell supernatants were collected and frozen at -80 degrees °C. Supernatants from cells contacted with 4 different sgRNAs, in addition to the mRNA encoding the CRISPR/Cas molecule, as well as an untransfected positive control were screened for CXCL1 and IL-8 protein levels on a cytokine panel by Myriad Genetics Inc. Figure 7 shows diminished levels of CXCL1 and IL8 were seen for each supernatant obtained from cells treated with sgRNA and CRISPR/Cas molecule RNPs, demonstrating a phenotypic response to ASMC disruption (e.g., by disrupting anchor sequence and nucleating polypeptide interactions, e.g., disrupting CTCF binding). This data is in agreement with the decreased mRNA expression seen by qPCR in Example 2.

Similar results were seen when the experiment was repeated with sgRNAs targeting the other CTCF site of the ASMC comprising CXCL1 and IL8 (data not shown).

Example 4: CXCL3 expression decrease as measured by qPCR

This example describes, in part, experiments demonstrating decreasing expression of CXCL3 by treating THP-1 cells using a site-specific disrupting agent comprising a CRISPR/Cas molecule and a variety of guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Cells were transfected with mRNA encoding a CRISPR/Cas molecule (Cas9) and sgRNA targeted to either of the CTCF sites of the ASMC comprising the target plurality of genes using LNPs. sgRNAs (from Example 1) used target the left or right CTCF site as indicated in Figure 9A. Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr® Spark™ from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental

control into 24 well plates (see Figure 8 flow chart). One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well. Untreated parental cells were plated with and without 10ng/ml TNF alpha.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL3 TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL3 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 8 graph) that a site-specific disrupting agent comprising a CRISPR/Cas molecule and several different sgRNAs can be used to decrease CXCL3 expression in THP-1 cells. The results also show that LNP delivery of said site-specific disrupting agent can be used to deliver effective amounts of the agent to the target cells. The results also demonstrate that targeting the anchor sequence on either side of the ASMC comprising the target plurality of genes can decrease expression of the target plurality of genes.

Similar results were seen when the experiment was repeated with sgRNAs targeting the other CTCF site of the ASMC comprising CXCL1, CXCL2, CXCL3, and IL8 (data not shown).

Example 5: CXCL1 and CXCL3 Expression is Decreased 3 Weeks Post-Transfection

This example describes, in part, experiments demonstrating a stable decrease in expression of CXCL1 and CXCL3 in THP-1 cells three weeks post-transfection with a site-specific disrupting agent comprising a CRISPR/Cas molecule and a variety of guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

Cells and LNP were prepared, and samples analyzed as in Example 4, except that transfected cells were incubated for 3 weeks before TNF alpha stimulation (see Figure 9A flow chart).

The results show (Figure 9A and 9B) that a site-specific disrupting agent comprising a CRISPR/Cas molecule and several different sgRNAs can be used to stably decrease CXCL1 and CXCL3 expression in THP-1 cells up to and including 3 weeks after treatment with LNPs comprising the agent(s). The results also demonstrate that targeting the anchor sequence on either side of the ASMC comprising the target plurality of genes can stably decrease expression of the target plurality of genes.

Example 6: Agents comprising KRAB effector moieties decrease CXCL1 expression

This example describes, in part, experiments demonstrating a decrease in expression of CXCL1 in THP-1 cells after transfection with a site-specific disrupting agent comprising a CRISPR/Cas molecule fused to a transcriptional repressor and a variety of guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Cells were transfected with mRNA encoding a CRISPR/Cas molecule fused to a transcriptional repressor, dCas9-KRAB, and sgRNA (from Example 1) targeted to either CTCF site of the ASMC comprising the target plurality of genes using LNPs. Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr[®] Spark[™] from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well. Untreated parental cells were plated with and without 10ng/ml TNF alpha. Transfection with mRNA encoding a CRISPR/Cas molecule (Cas9) and the sgRNAs (per the Examples 2, 4, and 5) was performed as a positive control.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript[®] RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1 specific TaqMan primer/probe sets with the TaqMan[®] Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 10) that a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule fused to a transcriptional repressor, KRAB, and targeted to CTCF sites by different sgRNAs can be used to decrease CXCL1 expression in THP-1 cells. The decrease in expression was seen 72 hours post-transfection.

Example 7: Agents comprising EZH2 effector moieties decrease CXCL1 expression

This example describes, in part, experiments demonstrating a decrease in expression of CXCL1 in THP-1 cells after transfection with a site-specific disrupting agent comprising a CRISPR/Cas molecule fused to a histone methyltransferase (EZH2) and a variety of guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Cells were transfected with mRNA encoding a catalytically inactive CRISPR/Cas molecule (dCas9) fused to a histone deacetylase, dCas9-EZH2, and

sgRNA (from Example 1) targeted to either CTCF site of the ASMC comprising the target plurality of genes using LNPs. Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr® Spark™ from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well. Untreated parental cells were plated with and without 10ng/ml TNF alpha.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 11) that a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule fused to a histone methyltransferase (EZH2) and targeted to CTCF sites by different sgRNAs can be used to decrease CXCL1 expression in THP-1 cells. The decrease in expression was seen 72 hours post-transfection.

Example 8: Agents comprising MQ1 effector moieties decrease CXCL1 expression

This example describes, in part, experiments demonstrating a decrease in expression of CXCL1 in THP-1 cells after transfection with a site-specific disrupting agent comprising a CRISPR/Cas molecule fused to a DNA methyltransferase (MQ1) and a variety of guide RNAs targeted to the anchor sequences of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Cells were transfected with mRNA encoding a catalytically inactive CRISPR/Cas molecule (dCas9) fused to MQ1, dCas9-MQ1, and sgRNA (from Example 1) targeted to either CTCF site of the ASMC comprising the target plurality of genes using LNPs. Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr® Spark™ from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well. Untreated parental cells were plated with and without 10ng/ml TNF alpha.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA

samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1 and CXCL3 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 and 3 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 12) that a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule fused to DNA methyltransferase (MQ1), and targeted to CTCF sites by different sgRNAs can be used to decrease CXCL1 expression in THP-1 cells. The decrease in expression was seen 72 hours post-transfection. Similar results were seen measuring CXCL3 expression (data not shown).

Example 9: Durable CXCL1 Decrease in Expression After Cas9 or dCas9-EZH2 Treatment

This example describes, in part, experiments demonstrating a stable decrease in expression of CXCL1 in THP-1 cells up to 4 weeks after transfection with a site-specific disrupting agent comprising either a CRISPR/Cas molecule or a catalytically inactive CRISPR/Cas molecule fused to a histone deacetylase (EZH2) and an sgRNA targeted to an anchor sequence of the ASMC comprising the target plurality of genes comprising CXCL1.

Using the ATx™ Scalable Transfection System (MaxCyte), THP-1 cells grown in RPMI + 10% FBS were electroporated with mRNA encoding either of the site-specific disrupting agents (Cas9 or dCas9-EZH2) and sgRNA (from Example 1) at 5 million cells per condition in processing assemblies. Samples of the transfected cells were harvested and incubated with TNF alpha for 24hrs. This was repeated each week carried out to 4 weeks. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen) following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1, CXCL2, CXCL3 & IL8 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show that (Figure 13) a site-specific disrupting agent comprising a CRISPR/Cas molecule, or a catalytically inactive CRISPR/Cas molecule fused to a histone methyltransferase (EZH2) and targeted to a CTCF site by an sgRNA can be used to decrease CXCL1 expression in THP-1 cells. The

decrease in expression is durable up to at least 4 weeks, and is also observed at 72 hours and 3 weeks post-transfection.

Example 10: CXCL3 Expression Decreases Upon Treatment with EZH2-dCas9-KRAB and sgRNA

This example describes, in part, experiments demonstrating a decrease in expression of CXCL3 in THP-1 cells after transfection with a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule fused to a histone methyltransferase (EZH2) and a transcriptional repressor (KRAB) and a variety of guide RNAs targeted to an anchor sequence of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Several different site-specific disrupting agents were tested: G9A-dCas9-EZH2 (G9A fused to dCas9 fused to EZH2), G9A-dCas9-KRAB, and EZH2-dCas9-KRAB. Cells were transfected with mRNA encoding the site-specific disrupting agent, and sgRNA targeted to a CTCF site of the ASMC comprising the target plurality of genes using LNPs. The sgRNA was chosen to target a genomic DNA site proximal to the left CTCF site but some distance removed from the left CTCF site (e.g., 80, 160, 235, or 300 nucleotides from the CTCF site). Exemplary guide sequences targeting genomic DNA sites proximal to the left CTCF site, but some distance removed from the left CTCF site are given in Table 5.

Table 5

Guide	Guide Sequence	Genomic Coordinates	SEQ ID No.
GD-29251	CCAATGAAGATGAACTGGG	chr4:74595215-74595237	30
GD-29252	AACGTGCTTGCCTAAGATTC	chr4:74595370-74595392	31
GD-29253	AGCCCTTAATCATATCTAGT	chr4:74595560-74595582	32
GD-29254	CAGAGCTTAAGACCTGTACT	chr4:74595642-74595664	33
GD-29255	GCCACCTTGACCTTCACAA	chr4:74595787-74595809	34

Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr® Spark™ from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well.

Untreated parental cells were plated with and without 10ng/ml TNF alpha.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and

analyzed by quantitative PCR using individual CXCL1 and CXCL3 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 and 3 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 14) that a site-specific disrupting agent comprising a catalytically inactive CRISPR/Cas molecule fused to a histone methyltransferase (EZH2) and a transcriptional repressor (KRAB), and targeted to a site proximal to a CTCF an sgRNA can be used to decrease CXCL3 expression in THP-1 cells. Similar results were seen measuring CXCL1 expression (data not shown).

Example 11: CXCL1 Expression Decreases Upon Treatment with Site-Specific Disrupting Agents and sgRNA

This example describes, in part, experiments demonstrating a decrease in expression of CXCL1 in THP-1 cells after transfection with various exemplary site-specific disrupting agents including: a catalytically inactive CRISPR/Cas molecule fused to a DNA methyltransferase (DNMT33a/3l); a catalytically inactive CRISPR/Cas molecule fused to a histone deacetylase (HDAC8); or a catalytically inactive CRISPR/Cas molecule fused to a histone deacetylase (HDAC8) and a histone methyltransferase (EZH2), and a variety of guide RNAs targeted to an anchor sequence of the ASMC comprising the target plurality of genes.

THP-1 cells were grown in RPMI + 10% FBS. Several different site-specific disrupting agents were tested: dCas9-DNMT3a/3l (DNMT3a/3l fused to dCas9), dCas9-HDAC8, and EZH2-dCas9-HDAC8. Cells were transfected with mRNA encoding the site-specific disrupting agent, and sgRNA (from Example 1) targeted to either CTCF site of the ASMC comprising the target plurality of genes using LNPs. Lipid nanoparticle (LNP) formulation using SSOP lipid mixture was carried out by using the NanoAssemblr® Spark™ from Precision NanoSystems Inc. For experimental conditions, 350k cells/well were plated 72hrs after LNP transfection for each transfected condition and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added to each well. Untreated parental cells were plated with and without 10ng/ml TNF alpha.

The transfected cells were incubated with TNF alpha for 24hrs. After that time, DNA and RNA were isolated using the AllPrep DNA/RNA Kit (Qiagen), following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. The results show (Figure 15) that a site-specific disrupting agent comprising dCas9-DNMT3a/3l, dCas9-HDAC8, or EZH2-dCas9-HDAC8 can be used to decrease CXCL1 expression in THP-1 cells and that these agents were effective at decreasing cytokine expression when targeted to CTCF sites by several different sgRNAs.

Example 12: CXCL Gene Cluster Expression Decreases Upon Treatment with dCas9-EZH2 and guide 30183 in Human A549 lung cancer epithelial cells and IMR-90 cells

This example demonstrates CXCL gene cluster expression decreases in Human A549 lung cancer epithelial cells and IMR-90 cells when treated with dCas9-EZH2 and guide 30183 (Controller 1). Human A549 cells (ATCC® CCL-185) & IMR-90 cells (ATCC®-CCL-186) were plated at 15,000 cells per well in a flat bottom cell culture treated plate in 100 μ l of media. A549 cells received F12/K ATCC®-30-2004 media and IMR-90 cells received EMEM ATCC®-30-2003 media. Both complete medias were made with 10% FBS (VWR cat# 97068-085). After 24 hours adhering to the plate, LNPs containing guide 30183 and EZH2-dCas9 controller were added to the media at a final concentration of 2 μ g/ml SSOP lipid mix. After 6 hours, media was replaced with 100 μ l of appropriate media and cells were incubated for 72 hours. After completion of 72-hour incubation, TNF alpha (Sigma Cat# 654205) was added to designated wells at 10ng/ml final concentration and incubated for 24 hours. After 24 hours, RNA was isolated using the NucleoSpin® 96 RNA Core Kit (Macherey-Nagel Inc, cat# 740466.4) following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific). Gene expression was quantified relative to the human ABL1 reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells. Data showed that expression of genes in CXCL gene cluster (specifically, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and IL-8) levels were down between 40-70% in Human A549 lung cancer epithelial cells when treated with dCas9-EZH2 (Fig. 17). The expression of genes in CXCL gene cluster, (specifically, CXCL1, CXCL2, CXCL3, and IL-8) levels were down about 50% in IMR-90 cells when the middle CTCF was target with dCas9-EZH2 and GD-30183 (Fig. 18).

Example 13: CXCL Gene Cluster Expression Decreases Upon Treatment with dCas9-EZH2 and guide GD-28481 in Human monocytes

This example demonstrates CXCL gene cluster expression decreases in Human monocyte cells when treated with dCas based effector (Controller A).

Transfection of the Cas9/guide RNP complex was carried out by electroporation into THP-1 cells (ATCC-TIB-202) by Synthego.

Upon receiving edited cell lines, vials were thawed, and cells were cultured in RPMI + 10% FBS (VWR cat# 97068-085) for one week to allow cells to recover from freezing and thawing. A parental unedited THP1 cell line was also analyzed for comparison.

350,000 cells were plated in quadruplicate for the edited cell line and the parental control into 24 well plates. One hour later 10ng/ml of TNF alpha (Sigma Cat# 654205) was added. Untreated control wells were also used to compare fold increase in chemokine expression.

The edited and parental cells were incubated with TNF alpha for 24hrs. Afterwards, DNA and RNA were isolated using the DNA/RNA All Prep Kit (Qiagen) following the Manufacture's protocol. RNA samples were reverse transcribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual CXCL1, CXCL2, CXCL3 & IL8 specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific).

CXCL1-3 & IL8 gene expression was quantified relative to the human GAPDH reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells.

Data showed, at 24 hours post-dosing gene expression of CXCL1, CXCL2, CXCL3, and IL-8, decreased 65%, 55%, 88%, and 52% in monocytes treated with controller A compared to the CXCL1, CXCL2, CXCL3, and IL-8 gene expression respectively in untreated monocytes (Fig. 19).

Example 14: Mouse CXCL Gene Cluster Expression Decreases Upon Treatment with dCas9-MQ1 and sgRNA Targeting three Anchor Sequences In Hep 1.6 Cells

This example demonstrates mouse CXCL gene cluster expression downregulates when treated with dCas9-MQ1 and sgRNA targeting three anchor sequences in Hep 1.6 cells.

Mouse cells HEPA 1.6 (ATCC® CRL-1830) were plated at 10k cells per well in a flat bottom cell culture treated plate in 100µl of media (DMEM Gibco Cat# 11995-065, 10% FBS VWR cat# 97068-085). After 24 hours adhering to the plate, the cultures were divided in four treatment groups and three control

groups. LNPs containing (i) guides GD-30594 and dCas9-MQ1 controller targeting Right CTCF, (ii) guide GD-30592 with dCas9-MQ1 effector targeting middle CTCF 1, (iii) guide GD-30593 with dCas9-MQ1 effector targeting middle CTCF and (iv) a combination of GD-30594, GD-30592 and GD-30593 with dCas9-MQ1 targeting both middle and right CTCF were added to the cell cultures under treatment group at a final concentration of 2µg/mL SSOP lipid mix. Untreated cells, cells treated with LNP, and cells treated with TNF and a LNP containing a transfection control guide were used as controls. After 6 hours, media was replaced with 100µl of DMEM and cells were incubated for 72 hours. After completion of 72hr incubation, TNF alpha (Sigma Cat# 654245) is added to designated wells at 10ng/ml final concentration and incubated for 24 hours. After 24hrs, RNA was isolated using the NucleoSpin® 96 RNA Core Kit (Macherey-Nagel, cat# 740466.4) following the Manufacture's protocol. RNA samples were retrotranscribed to cDNA using LunaScript® RT SuperMix Kit (New England Biolabs) and analyzed by quantitative PCR using individual specific TaqMan primer/probe sets with the TaqMan® Fast Advanced Master Mix (Thermo Fisher Scientific). Gene expression was quantified relative to the mouse HPRT reference gene using the $\Delta\Delta C_t$ method. Changes in gene expression were further quantified by measuring the fold increase in gene expression after TNF alpha treatment directly compared to the levels of gene expression in the untreated cells.

Data demonstrated, cells treated with dCas9-MQ1 transfected using guides targeting the right or one of the two middle CTCF motifs in the CXCL gene cluster, showed some down regulation in all of the seven CXCL genes after TNF alpha stimulation (Fig. 21B). However, the entire CXCL gene cluster was significantly more downregulated when cells were treated with dCas9-MQ1 transfected using a combination of guides targeting both middle and right CTCF (Fig. 21B).

Example 15: Systemic Administration of dCas9-MQ1 Demonstrates A Significant Decrease In Leukocyte Infiltration In The Inflamed Lungs

This example demonstrates that systemic administration of dCas9-MQ1 decrease leukocyte infiltration in vivo in mouse lungs.

Murine lipopolysaccharide (LPS) lung inflammation model was used to study acute inflammation in the lungs. LNP comprising DOTAP 1% PEG short ncRNA was used as control. Each mouse received 3mg/kg dose of LNP-DOTAP or dCas9-MQ1 at -2 hour time via intravenous administration point. The mice were stimulated with 5mg/kg of LPS via oral aspiration at 0 hours. A second dose of LNP-DOTAP or dCas9-MQ1 at 3 mg/kg was administered at +8 hour time point. Dexamethasone was administered intraperitoneally at 10mg/kg dose at times 0, 24, and 48 hours. The animals were terminated at 72 hours

and bronchiolar lavage fluid were collected from the lungs for flow staining. Reduction in neutrophil infiltration in BALF was used as a measure to understand the severity of inflammatory response.

The bronchiolar lavage fluid collected from the lungs of dCas9-MQ1 treated animals showed about 5.0×10^5 leukocyte count/mL. The sham group, i.e., healthy mice receiving no treatment did not have any significant presence of leukocyte in bronchiolar lavage fluid (BALF). The LPS treated mice, Dexamethasone treated mice, and LNP-DOTAP treated mice showed, about 8.0×10^5 leukocyte count/mL, about 7.2×10^5 leukocyte count/mL, and about 6.0×10^5 leukocyte count/mL in the bronchiolar lavage fluid respectively (Fig. 22B). 56% decrease in neutrophils infiltration in broncho-alveolar lavage fluid (BALF) was also observed in mice 72 hours after treatment with dCas9-MQ1 compared to the disease control.

Example 16: Systemic Administration of dCas9-MQ1 Demonstrates A Significant Decrease In Neutrophil Infiltration In BALF

This example analyzes BALF obtained in Example 15 to assess the cell population.

Flow cytometry analysis using the following staining panels below were used to assess the cell population in the BALF obtained in example 15 and the percentage of cells present in the BALF at the time of termination were documented (Fig. 23A). Neutrophil count in the BALF were also graphed using the antibody staining panel below.

alveolar macrophages: CD45⁺, Siglec F⁺, CD11b⁺, CD11c⁺

Neutrophils: CD45⁺, Siglec F⁻, CD11b⁺, CD11c⁻, Ly-6G⁺

T cells: CD45⁺, Siglec F⁻, CD11c⁻, CD3⁺

B cells: CD45⁺, Siglec F⁻, CD11c⁻, B220⁺ (Fig. 23B)

Analysis showed, the leukocyte cell types that make up the majority of the infiltrating cells are neutrophils, followed by B cells, T cells, macrophages and other types of hematopoietic cells (Fig. 23A). The controller decreased the number of neutrophils infiltrating the lungs with significant difference compared to the +LPS disease group (Fig. 23B).

Example 17: The Decrease Of Leukocyte Cells In The BALF Is Lung Specific

This example demonstrates that the reduction of Leukocyte cells in the BALF were lung specific suggesting the decrease was resulted from dCas9-MQ1 treatment.

Murine lipopolysaccharide (LPS) lung inflammation model was used to study acute inflammation in the lungs. LNP comprising DOTAP 1% PEG short ncRNA was used as control. Each mouse received

3mg/kg dose of LNP-DOTAP or dCas9-MQ1 at -2 hour time via intravenous administration point. The mice were stimulated with 5mg/kg of LPS via oral aspiration at 0 hours. A second dose of LNP-DOTAP or dCas9-MQ1 at 3 mg/kg was administered at +8 hour time point. Dexamethasone was administered intraperitoneally at 10mg/kg dose at times 0, 24, and 48 hours. The animals were terminated at 72 hours and bronchiolar lavage fluid were collected from the lungs for flow staining. Peripheral blood was collected at 72h termination. Flow analysis using CD45⁺ antibody staining was used to determine the leukocyte population in the peripheral blood in each group. The leukocyte count obtained for each group were plotted in a graph.

The graph illustrated that the effect of decreasing leukocyte count in the BALF with the controller treatment was lung specific suggesting the decrease in leukocyte count was due to dCas9-MQ1 treatment instead of the mouse itself had a decrease in leukocyte population which would have shown lower leukocyte count in peripheral blood as well. The hematopoietic cell population in the peripheral blood was found to be similar across all groups (Fig 24).

Example 18: Systemic Administration of dCas9-MQ1 Demonstrates CXCL Gene Expression Is Decreased In The Lung Tissue

This example demonstrates CXCL gene cluster expression downregulates in lung tissue upon systemic administration of dCas9-MQ1.

BALF was collected using the method described in example 15. Following BALF collection, half of the left lung lobe was snapped frozen to store for qPCR analysis. The lung tissue was homogenized, and RNA was extracted for qPCR analysis quantifying specifically for CXCL1-7 and CXCL15. Gene expression was quantified relative to the mouse GAPDH reference gene using the $\Delta\Delta C_t$ method.

Data showed that the CXCL gene cluster expression was downregulated to varying extent upon in the lung tissue samples obtained from mice treated with dCas9-MQ1 compared to CXCL gene cluster expression in lung tissue samples obtained from mice that were not treated with dCas9-MQ1 (Fig. 25).

Example 19: Decreasing CXCL Expression Has A Beneficial Downstream Effect Of Decreasing Cellular Recruitment and The Presence Of Other Cytokines To The Site Of Inflammation

Over-expression of the CXCL gene cluster produces chemokines that attract neutrophils. Chemokines that recruit inflammatory cells to the lung promote local inflammation, leading to severe pathogenesis. This example demonstrates downregulating CXCL expression has a beneficial downstream effect of reducing cellular recruitment leading to a reduction in the presence of other cytokines at the site of inflammation, suggesting downregulating CXCL expression is a promising method to reduce the severity of inflammation pathogenesis.

BALF was collected using the method described in Example 15. Following BALF collection, half of the left lung lobe was snapped frozen to store for qPCR analysis. The lung tissue was homogenized, and RNA was extracted for qPCR analysis quantifying specifically for the total count of CXCL1, CXCL2, GM-CSF, and IL-6 protein in the BALF using multiplexing Luminex® instrument.

5 Data demonstrated that the lung tissues obtained from mice treated with dCas9-MQ1 showed a lower expression of CXCL1, CXCL2, GM-CSF, and IL-6 compared to the CXCL1, CXCL2, GM-CSF, and IL-6 expression found in the lung tissues obtained from mice that were not treated with dCas9-MQ1.

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EQUIVALENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Some aspects, advantages, and
15 modifications are within the scope of the following claims.

CLAIMS

We claim:

1. A method of decreasing expression of a first gene and a second gene in a cell, comprising:
contacting the cell with a site-specific disrupting agent that comprises a targeting moiety that binds specifically to a first anchor sequence or a site proximal to a first anchor sequence, in an amount sufficient to decrease expression of the first and second genes,
the first and second genes being within an anchor sequence-mediated conjunction that comprises the first anchor sequence and a second anchor sequence,
wherein optionally the first gene and the second gene are proinflammatory genes;
thereby decreasing expression of the first and second genes.
2. A site-specific disrupting agent, comprising:
a DNA-binding, e.g., a targeting moiety that binds specifically to or proximal to a first anchor sequence within a cell,
wherein the first anchor sequence is part of an anchor sequence-mediated conjunction that further comprises a second anchor sequence, a first gene, and a second gene,
wherein optionally the first gene and the second gene are proinflammatory genes.
3. The site-specific disrupting agent of claim 2, wherein the first or second anchor sequence is located between IL-8 and RASSF6; between the IL-8 enhancer and RASSF6; between CXCL1 and CXCL4; between CXCL2 and EPGN; or between the E2 enhancer and EPGN.
4. The site-specific disrupting agent of claim 2 or 3, wherein the site-specific disrupting agent further comprises an effector moiety.
5. The site-specific disrupting agent of any of claims 2-4 wherein the targeting moiety comprises a TAL effector molecule, a CRISPR/Cas molecule (e.g., a catalytically inactive CRISPR/Cas protein), a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide.
6. The site-specific disrupting agent of any of claims 2-5, wherein the effector moiety comprises an effector described herein, e.g., MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, EZH2, HDAC8, KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6, HDAC7, HDAC8, HDAC9, HDAC10,

HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2 or DNMT3, or a functional variant or fragment of any thereof.

7. The site-specific disrupting agent of any of claims 2-6, wherein the effector moiety is linked to the targeting moiety via a linker.

8. The site-specific disrupting agent of any of claims 2-7, wherein the effector moiety is C-terminal of the targeting moiety.

9. The site-specific disrupting agent of any of claims 2-7, wherein the effector moiety is N-terminal of the targeting moiety.

10. The site-specific disrupting agent of any of claims 2-9, wherein the effector moiety is encoded by a nucleotide sequence chosen from any of SEQ ID NOs: 10, 14, 16, 18, 66, 68, or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

11. The site-specific disrupting agent of any of claims 2-10, wherein the effector moiety comprises an amino acid sequence according to any of SEQ ID NOs: 11, 12, 13, 15, 17, 19, 67 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

12. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is MQ1 or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 11 or 12 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, wherein optionally the effector moiety is C-terminal of the targeting moiety.

13. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is KRAB, or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 13 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, wherein optionally the effector moiety is C-terminal of the targeting moiety.

14. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is DNMT3a/3L, or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 15 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, wherein optionally the effector moiety is C-terminal of the targeting moiety.

15. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is EZH2, or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 17 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto.

16. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is HDAC8, or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 19 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, wherein optionally the effector moiety is C-terminal of the targeting moiety.

17. The site-specific disrupting agent of any of claims 2-11, wherein the effector moiety is G9A, or a functional variant or fragment thereof, e.g., wherein the effector moiety comprises an amino acid sequence of SEQ ID NO: 67 or a sequence with at least 80, 85, 90, 95, 99, or 100% identity thereto, or having no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 positions of difference thereto, wherein optionally the effector moiety is N-terminal of the targeting moiety.

18. The site-specific disrupting agent of any of claims 2-17, which further comprises a second effector moiety.

19. The site-specific disrupting agent of claim 18, wherein the targeting moiety is situated between the first effector moiety and the second effector moiety.

20. The site-specific disrupting agent of any of claims 2-19, wherein the effector moiety comprises a polymer e.g., an oligonucleotide; e.g., a gRNA.

21. The site-specific disrupting agent of claim 20, wherein the oligonucleotide has a sequence that comprises a complement of the anchor sequence or to a sequence proximal to the anchor sequence.

22. The site-specific disrupting agent of any of claims 2-21, wherein the targeting moiety further comprises a gRNA, e.g., a gRNA that binds a genomic locus comprising at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62, e.g., wherein the gRNA comprises a sequence that comprises at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62.

23. The site-specific disrupting agent of any of claims 2-22, wherein the targeting domain comprises a CRISPR/Cas molecule, e.g., a catalytically inactive CRISPR/ Cas protein, and a gRNA, e.g., a gRNA that binds a genomic locus comprising at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62, e.g., wherein the gRNA comprises a sequence that comprises at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62 and the effector moiety comprises an effector chosen from DNMT3a/3l, MQ1, KRAB, G9A, HDAC8, or EZH2.

24. The site-specific disrupting agent of claim 23, wherein the targeting domain comprises a CRISPR/Cas molecule, e.g., a catalytically inactive CRISPR/ Cas protein, and a gRNA, e.g., a gRNA that binds a genomic locus comprising at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62, e.g., wherein the gRNA comprises a sequence that comprises at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62, the first effector moiety comprises an effector chosen from DNMT3a/3l, MQ1, KRAB, G9A, HDAC8, or EZH2, and the second effector moiety comprises an effector chosen from DNMT3a/3l, MQ1, KRAB, G9A, HDAC8, or EZH2.

25. The site-specific disrupting agent of any of claims 2-24, wherein the targeting domain binds a genomic locus comprising at least 14, 15, 16, 17, 18, 19, or 20 nucleotides of the sequence of any of SEQ ID NOs: 20-62.

26. The site-specific disrupting agent of any of claims 2-25, wherein the targeting domain binds a genomic locus that is within 50 nucleotides (e.g., upstream or downstream) of the sequence of any of SEQ ID NOs: 20-62.

27. The site-specific disrupting agent of any of claims 2-26, wherein the targeting domain binds a genomic locus that is within 100 nucleotides (e.g., upstream or downstream) of the sequence of any of SEQ ID NOs: 20-62.
28. The site-specific disrupting agent of any of claims 2-27, wherein the targeting domain binds a genomic locus that is within 200 (e.g., upstream or downstream) nucleotides of the sequence of any of SEQ ID NOs: 20-62.
29. The site-specific disrupting agent of any of claims 2-28, wherein the targeting domain binds a genomic locus that is within 300 nucleotides (e.g., upstream or downstream) of the sequence of any of SEQ ID NOs: 20-62.
30. The site-specific disrupting agent of any of claims 2-29, which: (i) comprises one or more nuclear localization signal sequences (NLS), or (ii) does not comprise an NLS, optionally wherein the NLS comprises an amino acid sequence of SEQ ID NO: 63 and/or 64.
31. The site-specific disrupting agent of any of claims 18-30, wherein the first and/or second effector moiety comprises a DNA methyltransferase, a histone methyltransferase, a histone deacetylase, a histone demethylase, or a recruiter of a histone modifying complex.
32. The site-specific disrupting agent of claim 2-31, wherein the ASMC comprises two loops.
33. The site-specific disrupting agent of any of claims 2-32 or the method of claim 1, wherein the first gene is situated in a first loop of the ASMC, and the second gene is situated in a second loop of the ASMC.
34. The site-specific disrupting agent or method of claim 33, wherein the first anchor sequence is situated between the first and second loops.
35. A nucleic acid encoding a site-specific disrupting agent of any of claims 2-34.
36. The method of claim 1 or site-specific disrupting agent of any of claims 2-36, wherein the anchor sequence-mediated conjunction further comprises a third gene, and optionally wherein the method results in decreased expression of the third gene.

37. The method or site-specific disrupting agent of claim 36, wherein the anchor sequence-mediated conjunction further comprises a fourth gene, and optionally wherein the method results in decreased expression of the fourth gene.

38. The method or site-specific disrupting agent of claim 37, wherein the anchor sequence-mediated conjunction further comprises a fifth gene, and optionally wherein the method results in decreased expression of the fifth gene.

39. The method or site-specific disrupting agent of claim 38, wherein the anchor sequence-mediated conjunction further comprises a sixth gene, and optionally wherein the method results in decreased expression of the sixth gene.

40. The method or site-specific disrupting agent of claim 39, wherein the anchor sequence-mediated conjunction further comprises a seventh gene, and optionally wherein the method results in decreased expression of the seventh gene.

41. The method or site-specific disrupting agent of claim 40, wherein the anchor sequence-mediated conjunction further comprises an eighth gene, and optionally wherein the method results in decreased expression of the eighth gene.

42. A human cell having decreased expression of a first gene and a second gene, wherein the first gene and the second gene are proinflammatory genes,
wherein the cell comprises a disrupted (e.g., fully disrupted) anchor sequence-mediated conjunction that comprises the first and second genes.

43. The human cell of claim 42, which has reduced CTCF binding to an anchor sequence that is comprised by the anchor sequence-mediated conjunction, e.g., reduced by at least 20, 30, 40, 50, 60, 70, 80, 90, or 100%.

44. The human cell of either of claim 42 or 43, wherein the human cell has decreased expression of a third proinflammatory gene.

45. The human cell of claim 44, wherein the human cell has decreased expression of a fourth proinflammatory gene.

46. The human cell of claim 45, wherein the human cell has decreased expression of a fifth proinflammatory gene.

47. The human cell of claim 46, wherein the human cell has decreased expression of a sixth proinflammatory gene.

48. The human cell of claim 47, wherein the human cell has decreased expression of a seventh proinflammatory gene.

49. The human cell of claim 48, wherein the human cell has decreased expression of an eighth proinflammatory gene.

50. The human cell of any of claims 42-49, wherein the human cell comprises a mutation at chr4:74595464-74595486, chr4:74595457-74595479, chr4:74595460-74595482, chr4:74595472-74595494, chr4:75000088-75000110, chr4:75000091-75000113, chr4:75000085-75000107, chr4:75000157-75000179, chr4:75000156-75000178, chr4:74595215-74595237, chr4:74595370-74595392, chr4:74595560-74595582, chr4:74595642-74595664, chr4:74595787-74595809, chr4:74528428-74528450, chr4:74528567-74528589, chr4:74528609-74528631, chr4:74789132-74789154, chr4:74789250-74789272, chr4:74789312-74789334, chr4:74964853-74964875, chr4:74964906-74964928, chr4:74965538-74965560, chr4:74965737-74965759, chr4:75000031-75000053, chr4:75000115-75000137, chr4:75000231-75000253, chr4:74975146-74975168, chr4:74975369-74975391, chr4:74976318-74976340, chr4:74570348-74570370, chr4:74570503-74570525, or chr4:74570526-74570548, or within 5, 10, 15, 20, 30, 40, or 50 nucleotides of said region.

51. A human cell comprising a mutation at chr4:74595464-74595486, chr4:74595457-74595479, chr4:74595460-74595482, chr4:74595472-74595494, chr4:75000088-75000110, chr4:75000091-75000113, chr4:75000085-75000107, chr4:75000157-75000179, chr4:75000156-75000178, chr4:74595215-74595237, chr4:74595370-74595392, chr4:74595560-74595582, chr4:74595642-

74595664, chr4:74595787-74595809, chr4:74528428-74528450, chr4:74528567-74528589, chr4:74528609-74528631, chr4:74789132-74789154, chr4:74789250-74789272, chr4:74789312-74789334, chr4:74964853-74964875, chr4:74964906-74964928, chr4:74965538-74965560, chr4:74965737-74965759, chr4:75000031-75000053, chr4:75000115-75000137, chr4:75000231-75000253, chr4:74975146-74975168, chr4:74975369-74975391, chr4:74976318-74976340, chr4:74570348-74570370, chr4:74570503-74570525, or chr4:74570526-74570548, or within 5, 10, 15, 20, 30, 40, or 50 nucleotides of said region.

52. The human cell of either of claims 27 or 28, wherein the mutation comprises a deletion, substitution, or insertion (e.g., of 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 nucleotides).

53. The human cell of any of claims 50-52, which has reduced CTCF binding to the mutation, e.g., reduced by at least 20, 30, 40, 50, 60, 70, 80, 90, or 100% compared to a human cell with an undisrupted ASMC.

54. The human cell of any of claims 42-53, wherein expression of the first and second genes is reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to a human cell with an undisrupted ASMC.

55. A system comprising:

a first site-specific disrupting agent comprising a first targeting moiety and optionally a first effector moiety, wherein the first site-specific disrupting agent binds specifically to a first anchor sequence of an anchor sequence mediated conjunction (ASMC), wherein the ASMC comprises a first gene and a second gene, and

a second site-specific disrupting agent comprising a second targeting moiety and optionally a second effector moiety, wherein the second site-specific disrupting agent binds to a second anchor sequence of the ASMC.

56. The system of claim 55, wherein the first anchor sequence is between IL-8 and RASSF6; between the IL-8 enhancer and RASSF6; between CXCL1 and CXCL4; between CXCL2 and EPGN; or between the E2 enhancer and EPGN.

57. The system of claim 55 or 56, wherein the second anchor sequence is between IL-8 and RASSF6; between the IL-8 enhancer and RASSF6; between CXCL1 and CXCL4; between CXCL2 and EPGN; or between the E2 enhancer and EPGN.

58. The system of any of claims 55-57, wherein the first anchor sequence is between the IL-8 enhancer and RASSF6 and the second anchor sequence is between CXCL1 and CXCL4.

59. The system of any of claims 55-58, wherein the first anchor sequence is between the IL-8 enhancer and RASSF6 and the second anchor sequence is between the E2 enhancer and EPGN.

60. The system of any of claims 55-59, wherein the first anchor sequence is between CXCL1 and CXCL4 and the second anchor sequence is between the E2 enhancer and EPGN.

61. The system of any of claims 55-60, wherein the first site-specific disrupting agent is a site-specific disrupting agent described herein, e.g., a site-specific disrupting agent of any of claims 2-9.

62. The system of any of claims 55-61, wherein the second site-specific disrupting agent is a site-specific disrupting agent described herein, e.g., a site-specific disrupting agent of any of claims 2-9.

63. The system of any of claims 55-62, wherein the first targeting moiety and the second targeting moiety each independent comprises a TAL effector molecule, a CRISPR/Cas molecule, a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide.

64. The system of any of claims 55-63, wherein the first effector and the second effector each independently comprises an effector described herein, e.g., MQ1, EZH2, HDAC8, KRAB, G9A, or DNMT3a/3l, or a functional variant or fragment of any thereof.

65. The system of any of claims 55-62, wherein the first effector and the second effector each independently comprises a protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, or a functional variant or fragment of any thereof.

66. The system of any of claims 55-65, wherein the first effector and the second effector each independently comprises a protein chosen from HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6,

HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, or a functional variant or fragment of any thereof.

67. The system of any of claims 55-43, wherein the first effector and the second effector each independently comprises a protein chosen from MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, DNMT3a/3l, or a functional variant or fragment of any thereof.

68. The system of any of claims 55-67, wherein the first effector and the second effector each independently comprises a protein chosen from KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment of any thereof.

69. The system of any of claims 55-68, wherein the first effector and the second effector each independently comprises a polymer e.g., an oligonucleotide.

70. The system of any of claims 55-69, wherein the first oligonucleotide and the second oligonucleotide are identical.

71. The system of any of claims 55-70, wherein the first oligonucleotide and the second oligonucleotide are different.

72. The system of any of claims 55-71, wherein the first oligonucleotide has a sequence that comprises a complement of the first anchor sequence or to a sequence proximal to the first anchor sequence and the second oligonucleotide has a sequence that comprises a complement of the second anchor sequence or to a sequence proximal to the second anchor sequence.

73. The system of any of claims 55-72, wherein the anchor sequence-mediated conjunction further comprises a third gene.

74. The system of any of claims 55-73, wherein the anchor sequence-mediated conjunction further comprises a fourth gene.

75. The system of any of claims 55-74, wherein the anchor sequence-mediated conjunction further comprises a fifth gene.

76. The system of any of claims 55-75, wherein the anchor sequence-mediated conjunction further comprises a sixth gene.

77. The system of any of claims 55-76, wherein the anchor sequence-mediated conjunction further comprises a seventh gene.

78. The system of any of claims 55-77, wherein the anchor sequence-mediated conjunction further comprises an eighth gene.

79. The system of any of claims 55-78, wherein the ASMC comprises two loops.

80. A nucleic acid composition encoding the system of any of claims 55-79.

81. The nucleic acid of claim 80, wherein a single nucleic acid encodes both of the first site-specific disrupting agent and the second site-specific disrupting agent.

82. The nucleic acid of claim 81, wherein a first nucleic acid encodes the first site-specific disrupting agent and a second nucleic acid encodes the second site-specific disrupting agent.

83. A method of decreasing expression of a first gene and a second gene in a cell, comprising contacting the cell with a system according to any of claims 55-79 of a nucleic acid composition according to any of claims 80-82.

84. The method of claim 83, wherein the cell is simultaneously contacted with the first site-specific disrupting agent and the second site-specific disrupting agent.

85. The method of claim 83, wherein the cell is sequentially contacted with the first site-specific disrupting agent and the second site-specific disrupting agent.

86. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL2.

87. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL3.
88. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is IL-8.
89. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL4.
90. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL5.
91. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL6.
92. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL1 and the second gene is CXCL7.
93. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL3.
94. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is IL-8.
95. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL4.
96. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL4.
97. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL5.

98. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL6.
99. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL2 and the second gene is CXCL7.
100. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL3 and the second gene is IL-8.
101. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL3 and the second gene is CXCL4.
102. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL3 and the second gene is CXCL5.
103. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL3 and the second gene is CXCL6.
104. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL3 and the second gene is CXCL7.
105. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL4 and the second gene is CXCL5.
106. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL4 and the second gene is CXCL6.
107. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL4 and the second gene is CXCL7.
108. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL4 and the second gene is IL-8.

109. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL5 and the second gene is CXCL6.
110. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL5 and the second gene is CXCL7.
111. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL5 and the second gene is IL-8.
112. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL6 and the second gene is CXCL7.
113. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL6 and the second gene is IL-8.
114. The method, human cell, site-specific disrupting agent, or system of any of claims 1-85, wherein the first gene is CXCL7 and the second gene is IL-8.
115. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first gene is CXCL1, the second gene is CXCL2, and the third gene is CXCL3.
116. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, and third genes are chosen from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.
117. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, third, and fourth genes are chosen from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.
118. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, third, fourth, and fifth genes are chosen from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.

119. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, third, fourth, fifth, and sixth genes are chosen from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.

120. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, third, fourth, fifth, sixth, and seventh genes are chosen from CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.

121. The method, human cell, site-specific disrupting agent, or system of any of claims 36-85, wherein the first, second, third, fourth, fifth, sixth, seventh, and eighth genes are CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, or IL-8.

122. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the first gene is a cytokine.

123. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the second gene is a cytokine.

124. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the third gene is a cytokine.

125. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the fourth gene is a cytokine.

126. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the fifth gene is a cytokine.

127. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the sixth gene is a cytokine.

128. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the seventh gene is a cytokine.

129. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the eighth gene is a cytokine.

130. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the anchor sequence-mediated conjunction comprises 3, 4, or 5 proinflammatory genes.

131. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent comprises a nucleic acid (e.g., DNA or RNA) comprising a nucleotide sequence chosen from SEQ ID NOs: 20-62, or a sequence having at least 90%, 95%, 98%, or 99% identity thereto, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

132. The method, site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises a nucleic acid (e.g., DNA or RNA) comprising a nucleotide sequence chosen from SEQ ID NOs: 21, 22, 24, 40, or a sequence having at least 90%, 95%, 98%, or 99% identity thereto, or differing at no more than 1, 2, 3, 4, or 5 positions relative thereto.

133. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent binds to a sequence at least partially overlapping with the region having genomic coordinates chosen from Table 4 5, 6, 7, or a sequence that is within 5, 10, 15, 20, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides of said region.

134. The method of any of the preceding claims, which results in a decrease in a level of a cytokine, e.g., a chemokine, e.g., upon stimulation of the cell with TNF-alpha, e.g., measured as described in Examples 2-11.

135. The method or human cell of any of the preceding claims, wherein a level of a cytokine (e.g., a chemokine) is decreased, e.g., upon stimulation of the cell with TNF-alpha, e.g., measured as described in Examples 2-11.

136. The method or human cell of any of the preceding claims, wherein the transcript level of one or more of (e.g., 2, 3, or all of) CXCL1, CXCL2, CXCL3, and IL8 is decreased, e.g., upon stimulation of the cell with TNF-alpha, e.g., measured as described in Examples 2 or 4-11.

137. The method or human cell of any of the preceding claims, wherein the transcript level of one or more of (e.g., 2, 3, or all of) CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and IL8 is decreased, e.g., upon stimulation of the cell with TNF-alpha.

138. The method or human cell of any of claims 132-137, wherein the decrease is a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to pre-treatment levels or to a human cell with an undisrupted ASMC.

139. The method or human cell of any of the preceding claims, wherein the protein level (e.g., secreted protein level) of one or more of (e.g., 2, 3, or all of) of CXCL1, CXCL2, CXCL3, and IL8 is decreased, e.g., upon stimulation of the cell with TNF-alpha, e.g., measured as described Example 3.

140. The method or human cell of any of the preceding claims, wherein the protein level (e.g., secreted protein level) of one or more of (e.g., 2, 3, or all of) CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, and IL8 is decreased, e.g., upon stimulation of the cell with TNF-alpha.

141. The method or human cell of claim 140, wherein the decrease is a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to pre-treatment levels or to a human cell with an undisrupted ASMC.

142. The method of any of the preceding claims, which results in decrease in binding of CTCF to the first anchor sequence, e.g., a complete loss of binding or a loss of at least 20, 30, 40, 50, 60, 70, 80, 90, or 100% compared to a human cell with an undisrupted ASMC, e.g., as measured by ChIP and quantitative PCR.

143. The method of any of the preceding claims, which results in disruption of the anchor sequence-mediated conjunction.

144. The method of any of the preceding claims, wherein a population of the cells is contacted with the site-specific disrupting agent, and wherein the first anchor sequence is edited in at least 50%, 60%, 70%, 80%, 90%, or 95% of cells in the population.

145. The method of any of the preceding claims, wherein the effect (e.g., the decrease in cytokine levels) is additive or synergistic compared to the effect of inhibiting the first gene or the second gene individually.

146. The method of any of the preceding claims, wherein expression is decreased for at least 1, 2, 3, 4, 5, 6, 7, 10, or 14 days, or at least 1, 2, 3, 4, or 5 weeks.

147. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a cell of a subject having an inflammatory disease, e.g., an immune mediated inflammatory disease.

148. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the inflammatory disease is an autoimmune disorder, e.g., rheumatoid arthritis.

149. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the inflammatory disease is associated with a pathogenic infection, e.g., viral infection, e.g., SARS-CoV2 infection.

150. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the inflammatory disease is associated with a superinfection, e.g., infection caused by two or more pathogenic agents, e.g., by a virus and a bacterium, (e.g., by SARS-CoV2 and Streptococcus pneumoniae), e.g., by a virus and a fungus, (e.g., by SARS-CoV2 and mucormycosis).

151. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a cell of a subject having rheumatoid arthritis, inflammatory arthritis, gout, asthma, , neutrophilic asthma, neutrophilic dermatosis, paw edema, acute respiratory disease syndrome (ARDS), COVID-19, psoriasis, inflammatory bowel disease, infection (e.g., by a pathogen, e.g., a bacteria, a viruses, or a fungus), external injury (e.g., scrapes or foreign objects), effects of radiation or chemical injury, osteoarthritis, osteoarthritic joint pain, joint pain, inflammatory pain, acute pain, chronic pain, cystitis, bronchitis, dermatitis, dermatosis, cardiovascular disease, neurodegenerative disease, liver disease, lung disease, kidney disease, pain, swelling, stiffness, tenderness, redness, warmth, or elevated biomarkers related to disease states (e.g., cytokines, chemokines, growth factors, immune receptors, infection markers, or inflammatory markers).

152. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a cell of a subject having rheumatoid arthritis, psoriasis, or inflammatory bowel disease.

153. The method, human cell, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a cell of a subject having rheumatoid arthritis, gout, neutrophilic asthma, neutrophilic dermatosis, acute respiratory disease syndrome (ARDS), or COVID-19.

154. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the anchor sequence-mediated conjunction comprises an internal enhancing sequence.

155. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the second gene (and optionally the third, fourth, fifth, sixth, seventh, or eighth genes) is transcribed in the same direction as the first gene.

156. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the first anchor sequence comprises a binding motif selected from a CTCF binding motif, USF1 binding motif, YY1 binding motif, TAF3 binding motif, or ZNF143 binding motif.

157. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the first anchor sequence comprises a CTCF binding motif.

158. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent binds specifically to or proximal to the first anchor sequence with sufficient affinity that it competes with binding of an endogenous nucleating polypeptide (e.g., CTCF, USF1, YY1, TAF3, or ZNF143) within the cell.

159. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent adds, deletes, or substitutes one or more nucleotides within or proximal to the first anchor sequence.

160. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises a targeting moiety or effector moiety comprising a first CRISPR/Cas molecule comprising a first CRISPR/Cas protein and first guide RNA.

161. The method or system of any of the preceding claims, wherein the first site-specific disrupting agent comprises a first targeting moiety or first effector moiety comprising a first CRISPR/Cas molecule comprising a first CRISPR/Cas protein and first guide RNA and the second site-specific disrupting agent comprises a second targeting moiety or second effector moiety comprising a second CRISPR/Cas molecule comprising a second CRISPR/Cas protein and second guide RNA.

162. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises a targeting moiety or effector moiety comprising TAL effector molecule, a CRISPR/Cas molecule, a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide.

163. The method or system of any of the preceding claims, wherein the first site-specific disrupting agent comprises a first targeting moiety or first effector moiety comprising TAL effector molecule, a CRISPR/Cas molecule, a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide and the second site-specific disrupting agent comprises a second targeting moiety or second effector moiety comprising TAL effector molecule, a CRISPR/Cas molecule, a zinc finger domain, a tetR domain, a meganuclease, or an oligonucleotide.

164. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises an effector moiety comprising a histone modifying functionality, e.g., a histone methyltransferase, histone demethylase, or histone deacetylase activity.

165. The method or system of any of the preceding claims, wherein the first and/or the second site-specific disrupting agent comprises an effector moiety comprising a histone modifying functionality, e.g., a histone methyltransferase, histone demethylase, or histone deacetylase activity.

166. The method, site-specific disrupting agent, or system of claim 164 or 165, wherein the effector moiety comprises a protein chosen from SETDB1, SETDB2, EHMT2 (i.e., G9A), EHMT1 (i.e., GLP), SUV39H1, EZH2, EZH1, SUV39H2, SETD8, SUV420H1, SUV420H2, or a functional variant or fragment of any thereof.

167. The method, site-specific disrupting agent, or system of claim 164 or 165, wherein the effector moiety comprises a protein chosen from HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC6,

HDAC7, HDAC8, HDAC9, HDAC10, HDAC11, SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, SIRT7, SIRT8, SIRT9, or a functional variant or fragment of any thereof.

168. The method, site-specific disrupting agent, or system of claim 164 or 165,, wherein the effector moiety comprises EZH2 or a functional variant or fragment of any thereof.

169. The method, site-specific disrupting agent, or system of claim 164 or 165,, wherein the effector moiety comprises HDAC8 or a functional variant or fragment of any thereof.

170. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises an effector moiety comprising a DNA modifying functionality, e.g., a DNA methyltransferase.

171. The method or system of any of the preceding claims, wherein the first and/or the second site-specific disrupting agent comprises an effector moiety comprising a DNA modifying functionality, e.g., a DNA methyltransferase.

172. The method, site-specific disrupting agent, or system of claim 170 or 171, wherein the effector moiety comprises a protein chosen from MQ1, DNMT1, DNMT3A1, DNMT3A2, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, DNMT3B6, DNMT3L, DNMT3a/3l, or a functional variant or fragment of any thereof.

173. The method, site-specific disrupting agent, or system of claim 170 or 171, wherein the effector moiety comprises MQ1 or a functional variant or fragment of any thereof.

174. The method, site-specific disrupting agent, or system of claim 170 or 171, wherein the effector moiety comprises DNMT3 (e.g., DNMT3a, DNMT3L, DNMT3a/3l, DNMT3B1, DNMT3B2, DNMT3B3, DNMT3B4, DNMT3B5, or DNMT3B6) or a functional variant or fragment of any thereof.

175. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises an effector moiety comprising a transcriptional repressor.

176. The method or site-specific disrupting agent of any of the preceding claims, wherein the first and/or the second site-specific disrupting agent comprises an effector moiety comprising a transcriptional repressor.

177. The method, site-specific disrupting agent, or system of claim 175 or 176, wherein the effector moiety comprises a protein chosen from KRAB, MeCP2, HP1, RBBP4, REST, FOG1, SUZ12, or a functional variant or fragment of any thereof.

178. The method, site-specific disrupting agent, or system of claim 177, wherein the effector moiety comprises KRAB or a functional variant or fragment of any thereof.

179. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises a polymer.

180. The method or system of any of the preceding claims, wherein the first and/or the second site-specific disrupting agent comprises a polymer.

181. The method, site-specific disrupting agent, or system of claim 179 or 180, wherein the polymer comprises a polyamide.

182. The method, site-specific disrupting agent, or system of claim 179 or 180, wherein the polymer is an oligonucleotide.

183. The method, site-specific disrupting agent, or system of claim 182, wherein the oligonucleotide has a sequence that comprises a complement of the first anchor sequence or to a sequence proximal to the first anchor sequence.

184. The method, site-specific disrupting agent, or system of claim 182, wherein the oligonucleotide has a sequence that comprises a complement of the second anchor sequence or to a sequence proximal to the second anchor sequence.

185. The method, site-specific disrupting agent, or system of any of claims 182-184, wherein the oligonucleotide comprises a chemical modification.

186. The method or site-specific disrupting agent, or system of claim 179 or 180, wherein the polymer is a peptide nucleic acid.
187. The method, site-specific disrupting agent, or system of any preceding claim, wherein the site-specific disrupting agent comprises a peptide-nucleic acid mixmer.
188. The method, site-specific disrupting agent, or system of any preceding claim wherein the site-specific disrupting agent (e.g., a targeting moiety or effector moiety of the site-specific disrupting agent) comprises a peptide or polypeptide.
189. The method, site-specific disrupting agent, or system of claim 188, wherein the polypeptide is a zinc finger polypeptide.
190. The method, site-specific disrupting agent, or system of claim 188, wherein the polypeptide is or comprises a Transcription activator-like effector nuclease (TALEN) polypeptide.
191. The method or site-specific disrupting agent of any preceding claim, wherein the site-specific disrupting agent comprises a small molecule.
192. The method or system of any preceding claim, wherein the first and/or the second site-specific disrupting agent comprises a small molecule.
193. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent further comprises an effector moiety, e.g., an epigenetic modifying agent, e.g., a DNA methyltransferase, histone deacetylase, or a histone methyltransferase.
194. The method or system of any preceding claim, wherein the first and/or the second site-specific disrupting agent further comprises an effector moiety, e.g., an epigenetic modifying agent, e.g., a DNA methyltransferase, histone deacetylase, or a histone methyltransferase.
195. The method or site-specific disrupting agent of any of the preceding claims, wherein the site-specific disrupting agent comprises a fusion molecule.

196. The method or system of any preceding claims, wherein the first and/or the second site-specific disrupting agent comprises a fusion molecule.

197. The method or site-specific disrupting agent of any preceding claims wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule and an effector moiety comprising a transcriptional repressor, e.g., as a fusion molecule.

198. The method or system of any preceding claim, wherein the first and/or the second site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule and an effector moiety comprising a transcriptional repressor, e.g., as a fusion molecule.

199. The method or site-specific disrupting agent of claim 198, wherein the targeting moiety comprises dCas9 and the effector moiety KRAB or a functional variant or portion thereof.

200. The method or system of any preceding claim, wherein the first and/or the second targeting moiety comprises dCas9 and the effector moiety KRAB or a functional variant or portion thereof.

201. The method or site-specific disrupting agent of any claims 1-177, wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule and an effector moiety comprising a histone methyltransferase, e.g., as a fusion molecule.

202. The method or system of any preceding claim, wherein the first and/or the second site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule and an effector moiety comprising a histone methyltransferase, e.g., as a fusion molecule.

203. The method, site-specific disrupting agent, or system of claim 201, wherein the targeting moiety comprises dCas9 and the effector moiety comprises EZH2 or a functional variant or portion thereof.

204. The method, site-specific disrupting agent, or system of any of claims 1-196, wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule and an effector moiety comprising a DNA methyltransferase, e.g., as a fusion molecule.

205. The method, site-specific disrupting agent, or system of claim 204, wherein the targeting moiety comprises dCas9 and the effector moiety comprises MQ1 or a functional variant or portion thereof.

206. The method, site-specific disrupting agent, or system of claim 203, wherein the targeting moiety comprises dCas9 and the effector moiety comprises DNMT3, e.g., DNMT3a/3l or a functional variant or portion thereof.

207. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule, a first effector moiety comprising a histone methyltransferase, and a second effector moiety comprising a transcriptional repressor, e.g., as a fusion molecule.

208. The method, site-specific disrupting agent, or system of claim 207, wherein the targeting moiety comprises dCas9, the first effector moiety comprises EZH2 or a functional variant or portion thereof, and the second effector moiety comprises KRAB or a functional variant or portion thereof.

209. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule, and an effector moiety comprising a histone deacetylase, e.g., as a fusion molecule.

210. The method, site-specific disrupting agent, or system of claim 209, wherein the targeting moiety comprises dCas9 and the effector moiety comprises HDAC8 or a functional variant or portion thereof.

211. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the site-specific disrupting agent comprises a targeting moiety comprising a CRISPR/Cas molecule, a first effector moiety comprising a histone methyltransferase, and a second effector moiety comprising a histone deacetylase, e.g., as a fusion molecule.

212. The method, site-specific disrupting agent, or system of claim 211, wherein the targeting moiety comprises dCas9, the first effector moiety comprises EZH2 or a functional variant or portion thereof, and the second effector moiety comprises HDAC8 or a functional variant or portion thereof.

213. The method, site-specific disrupting agent, or system of any of claims 195-212, wherein the site-specific disrupting agent comprises an amino acid sequence encoded by a nucleic acid sequence chosen from SEQ ID NOs: 69, 71, 85, 201, 202, 204, 205, 207, 209, 211, 213, 215, 217, or 219-242, a

complementary or reverse complementary sequence of any thereof, or comprises a sequence with at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any thereof.

214. The method, site-specific disrupting agent, or system of any of claims 195-213, wherein the site-specific disrupting agent comprises an amino acid sequence chosen from any one of SEQ ID NOs:70, 72, 82, 84, 86, 203, 206, 208, 210, 212, 214, 216, or 218, or encoded by a sequence chosen from any one of SEQ ID NOs: 219-242, or comprises a sequence with at least 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to any thereof.

215. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is situated in a subject.

216. The method, site-specific disrupting agent of any of claims 1-215, wherein the cell is *ex vivo*.

217. The method or site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a mammalian cell, e.g., a human cell.

218. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a somatic cell.

219. The method, site-specific disrupting agent, or system of any of the preceding claims, wherein the cell is a primary cell.

220. The method of any of the preceding claims, wherein the step of contacting is performed *ex vivo*.

221. The method of claim 220, further comprising, prior to the step of contacting, a step of removing the cell (e.g., mammalian cell) from a subject.

222. The method of either of claims 220 or 221, wherein further comprising, after the step of contacting, a step of (b) administering the cells (e.g., mammalian cells) to a subject.

223. The method of any of claims 1-222, wherein the step of contacting comprises administering a composition comprising the site-specific disrupting agent to a subject.

224. The method of claim 223, wherein the site-specific disrupting agent is administered as a monotherapy.
225. The method of claim 223, wherein the site-specific disrupting agent is administered in combination with a second therapeutic agent.
226. A reaction mixture comprising a cell (e.g., a human cell, e.g., a primary human cell) and a site-specific disrupting agent, or system of any of preceding claims.
227. A method of treating a subject having an inflammatory disorder, comprising:
administering to the subject a site-specific disrupting agent, system or reaction mixture of any preceding claims in an amount sufficient to treat the inflammatory disorder,
thereby treating the inflammatory disorder.
228. The method of claim 227, wherein the inflammatory disorder is rheumatoid arthritis, psoriasis, or inflammatory bowel disease.
229. The method of claim 227 or 228, wherein the inflammatory disorder is rheumatoid arthritis, gout, neutrophilic asthma, neutrophilic dermatosis, acute respiratory disease syndrome (ARDS), or COVID-19.
230. The method of any of claims 227-229, wherein the inflammatory disorder is an autoimmune disorder, e.g., rheumatoid arthritis.
231. The method of any of claims 227-229, wherein the inflammatory disease is associated with a pathogenic infection, e.g., viral infection, e.g., SARS-CoV2 infection.
232. The method of any of claims 227-229, wherein the inflammatory disease is associated with a superinfection, e.g., infection caused by two or more pathogenic agents, e.g., by a virus and a bacterium, (e.g., by SARS-CoV2 and *Streptococcus pneumoniae*), e.g., by a virus and a fungus, (e.g., by SARS-CoV2 and mucormycosis).

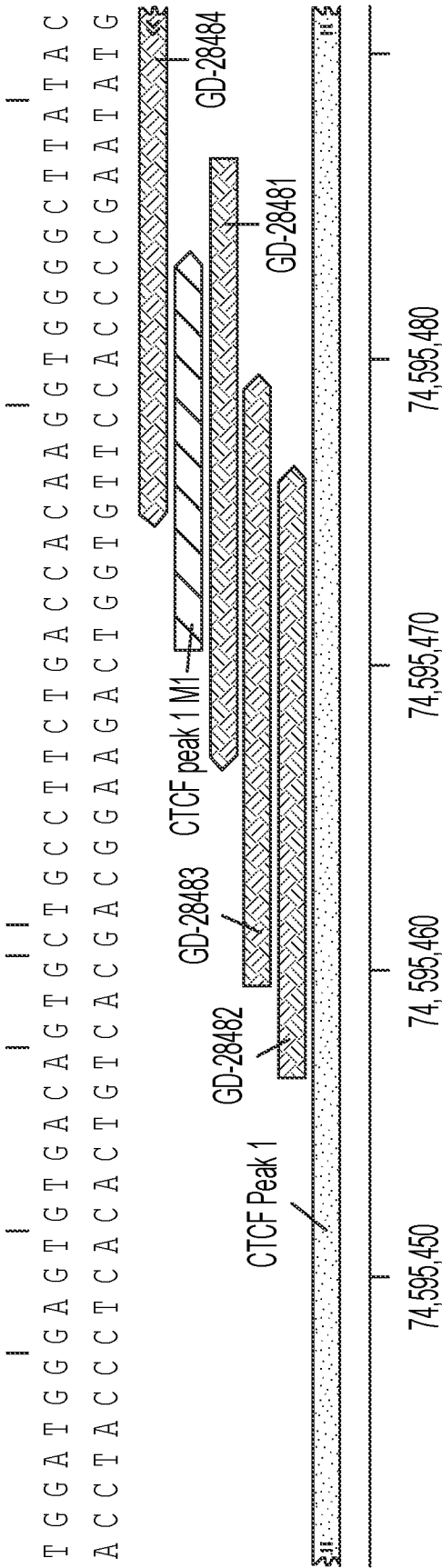


FIG. 1

FIG. 2

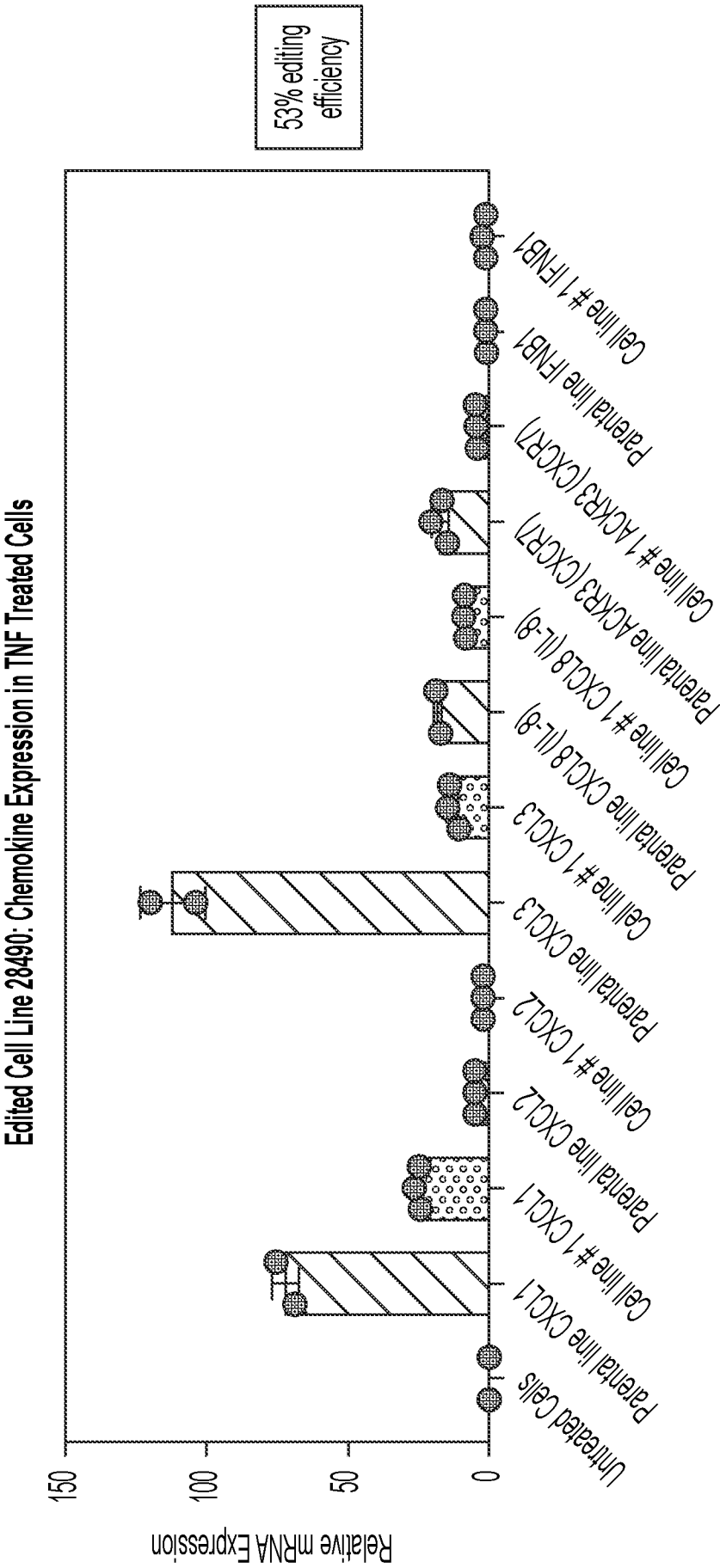


FIG. 3

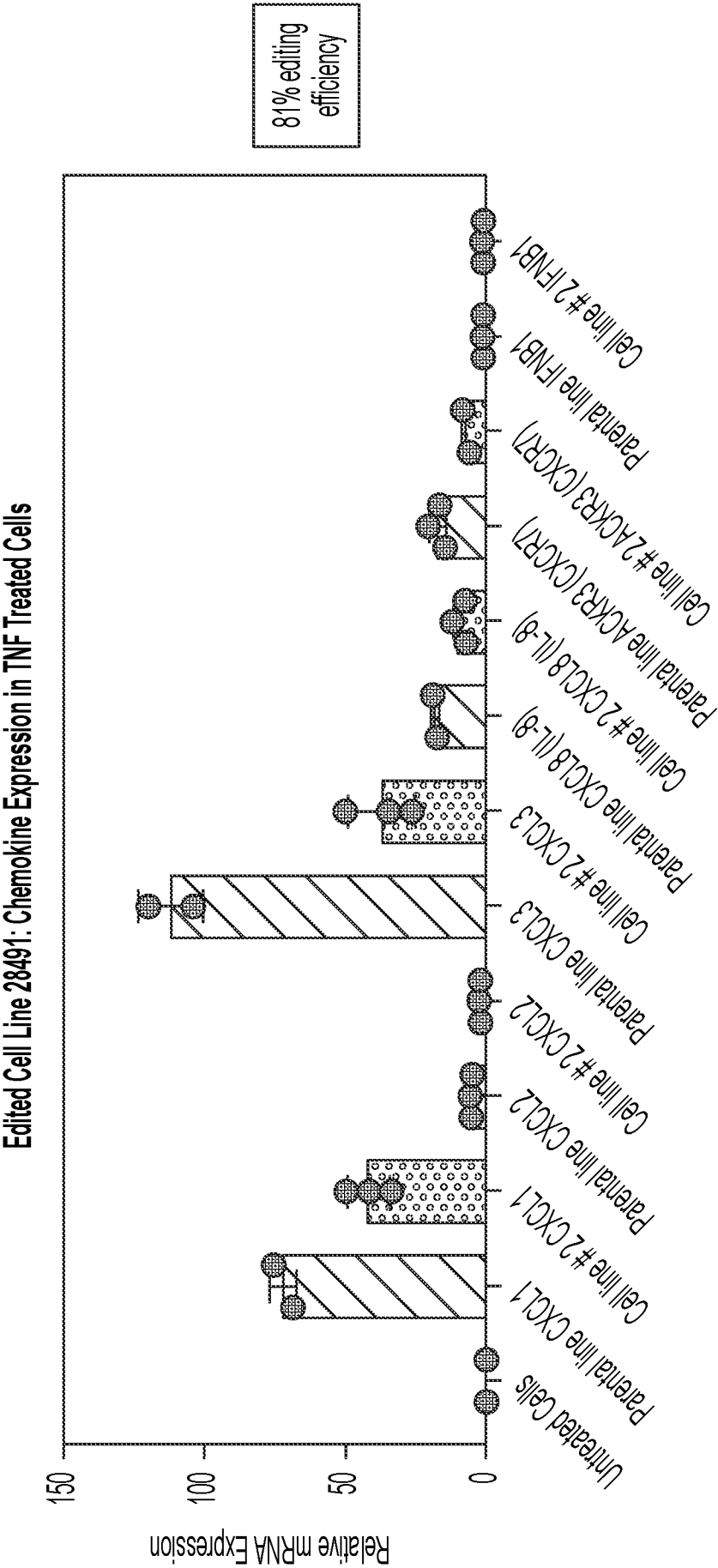


FIG. 4

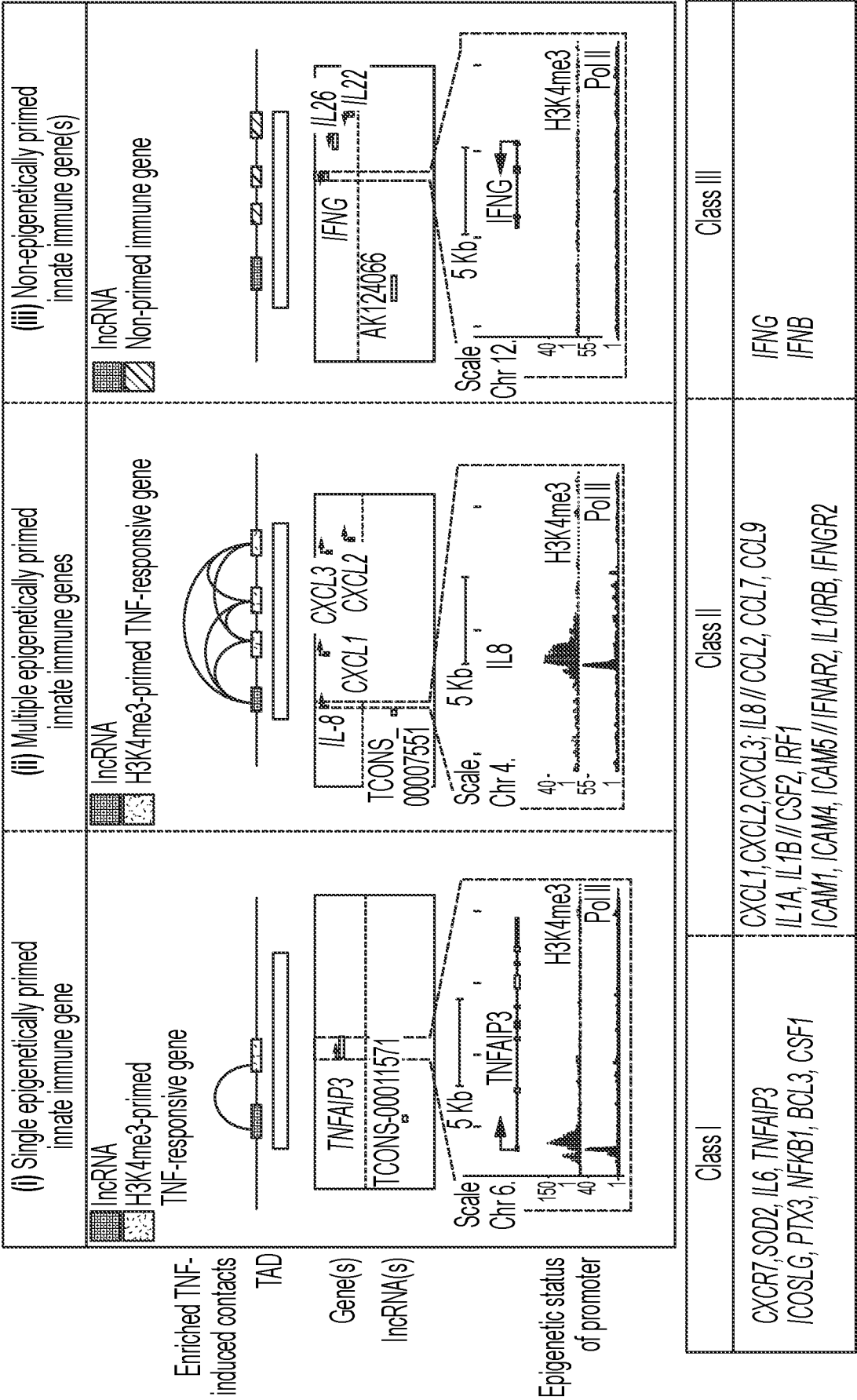


FIG. 5

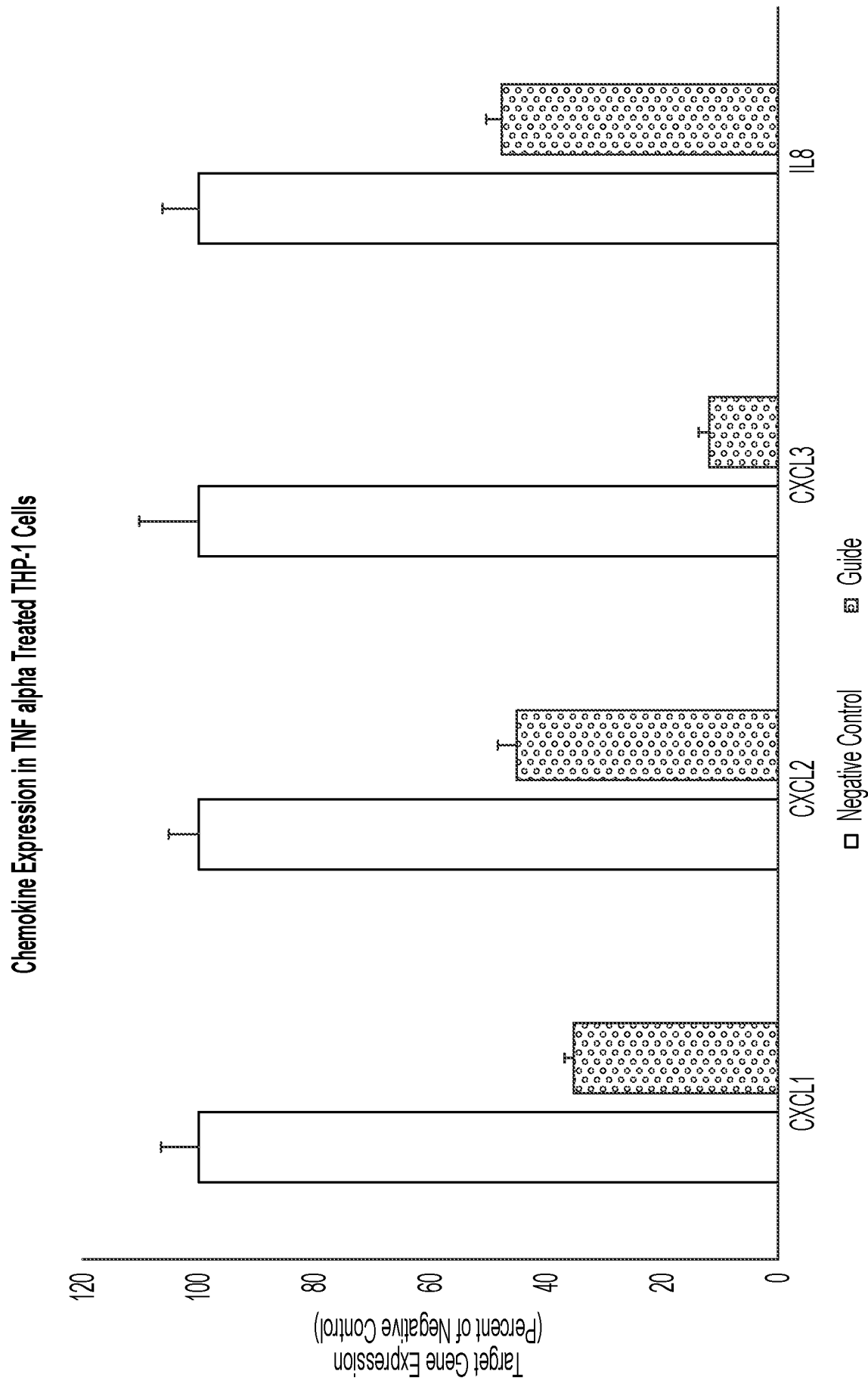


FIG. 6

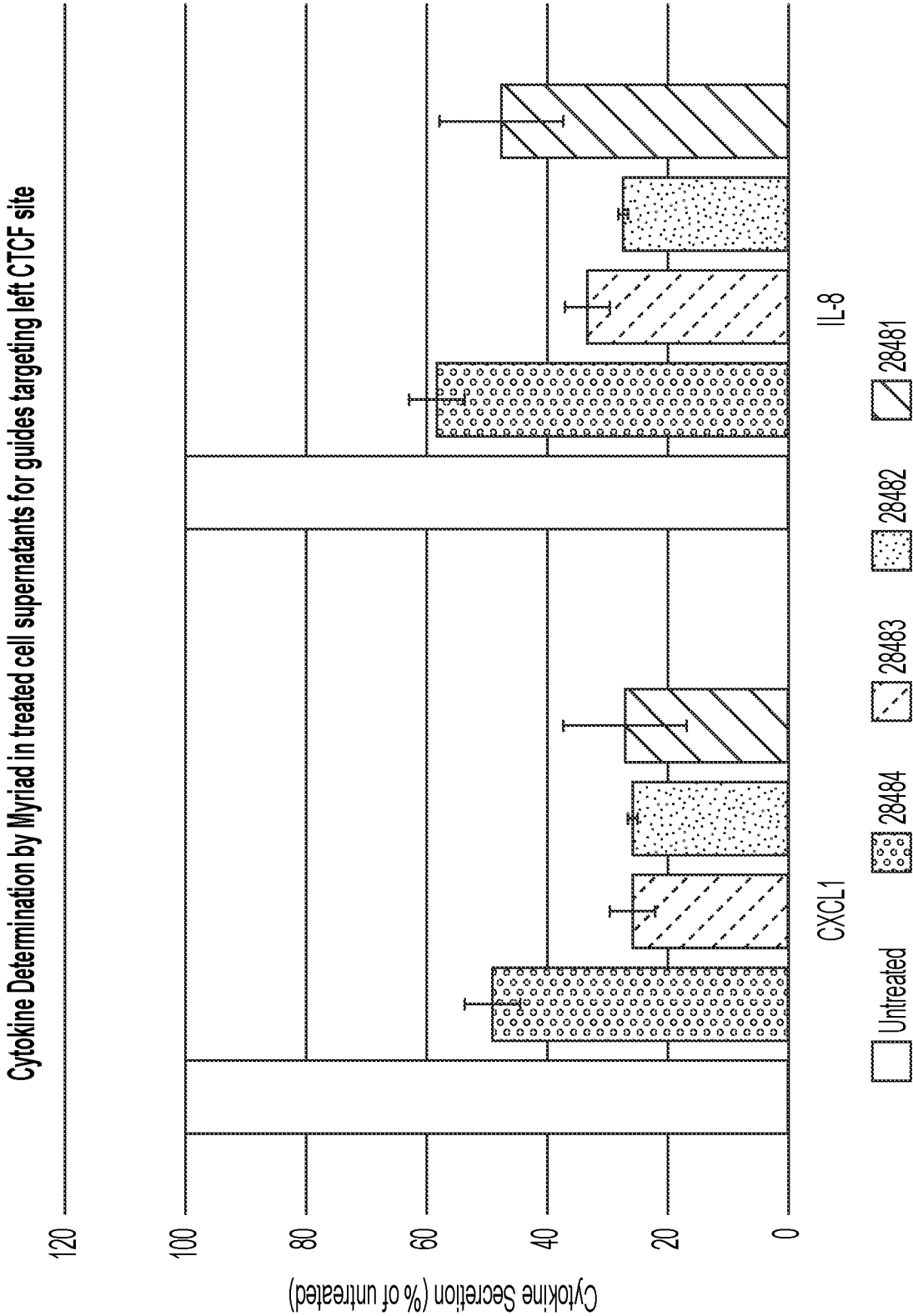


FIG. 7

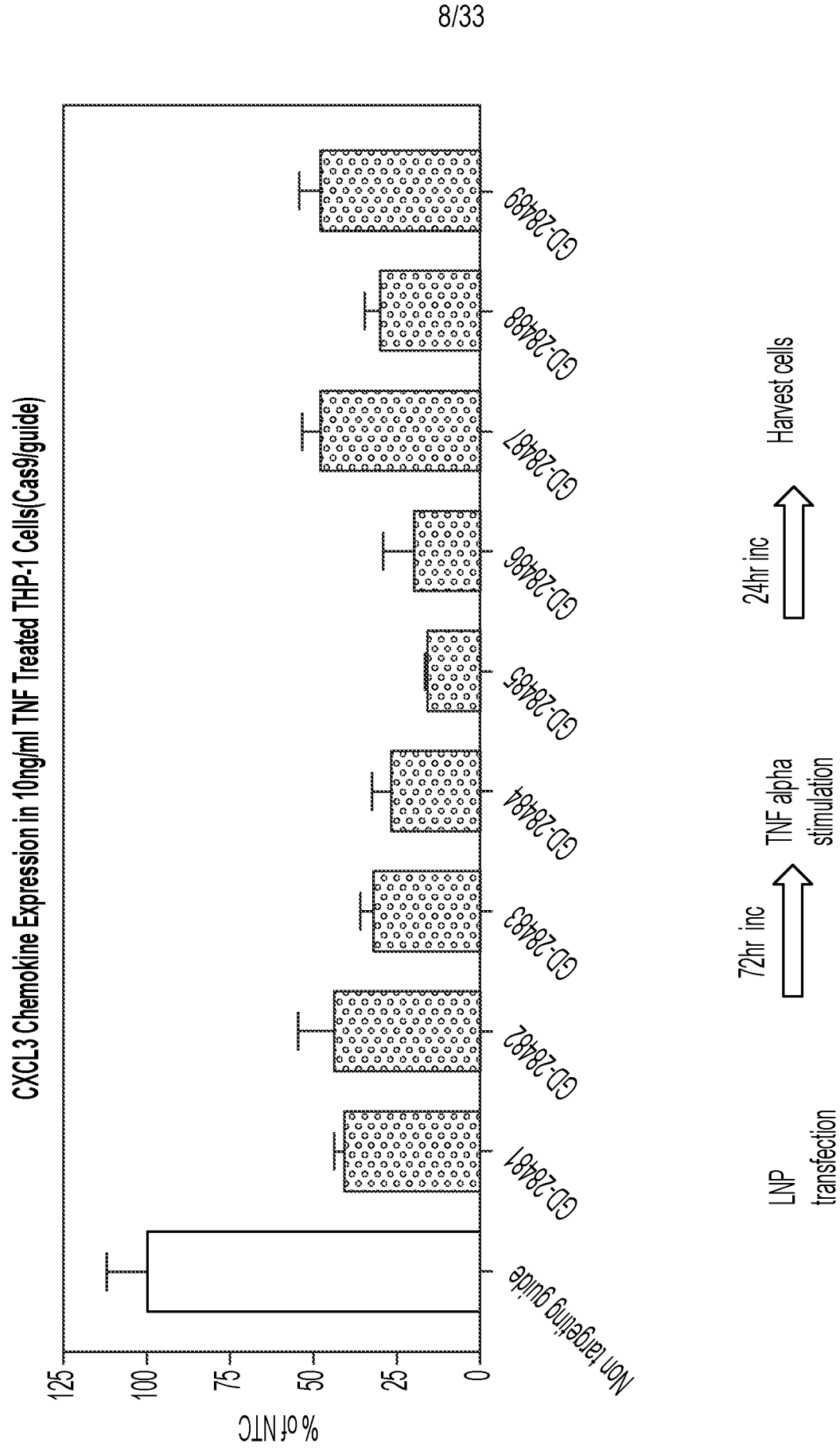


FIG. 8

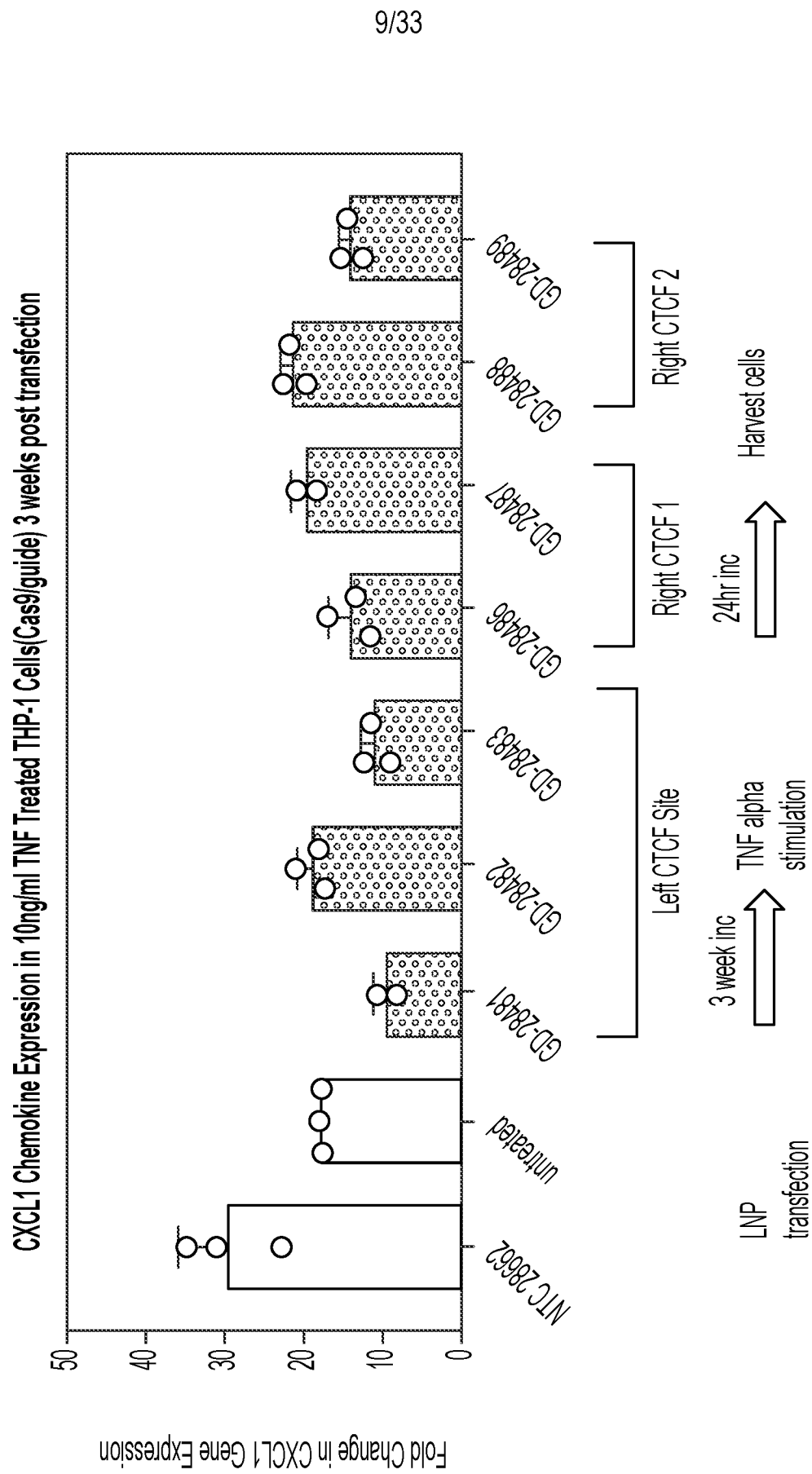


FIG. 9A

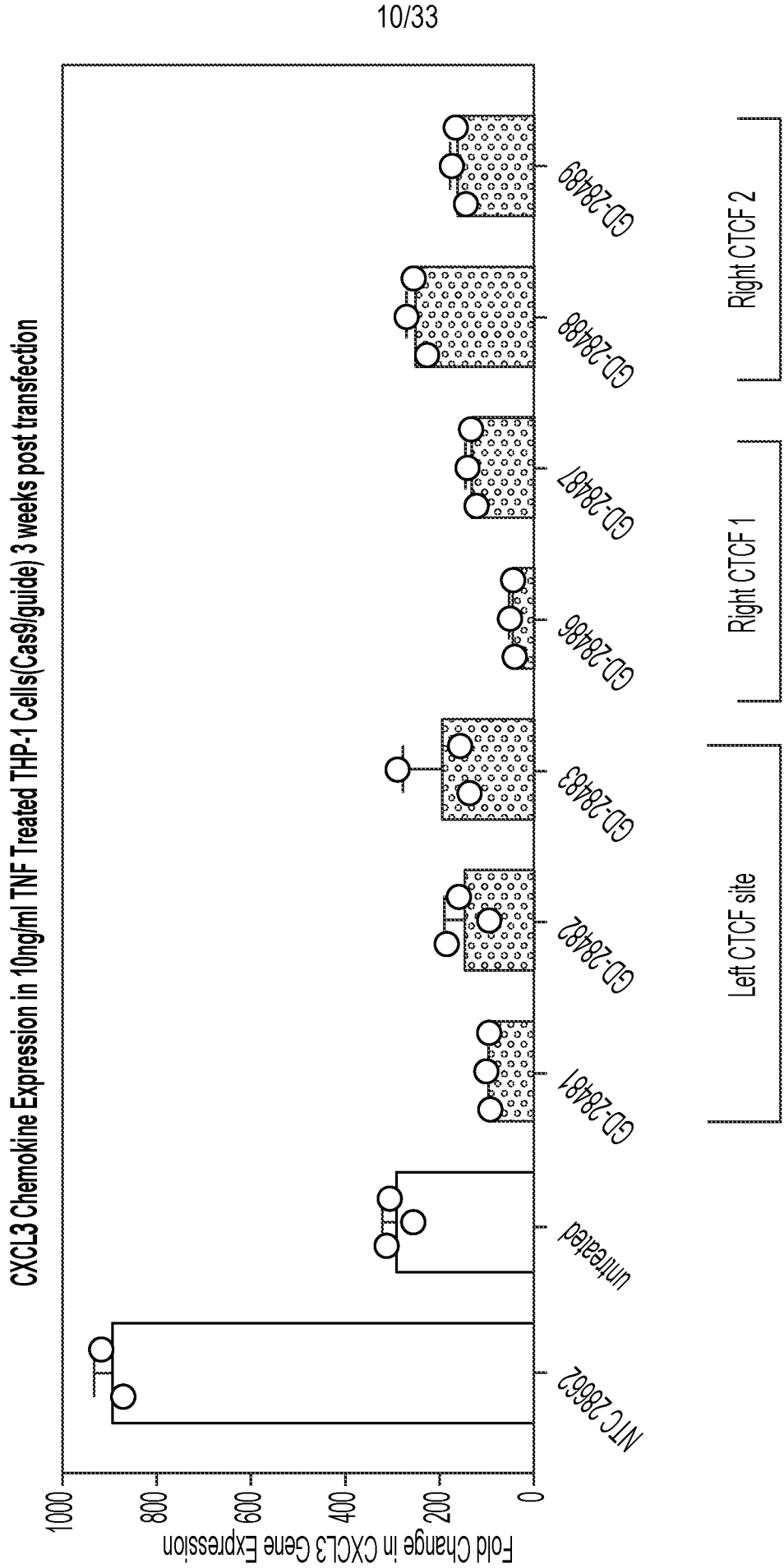


FIG. 9B

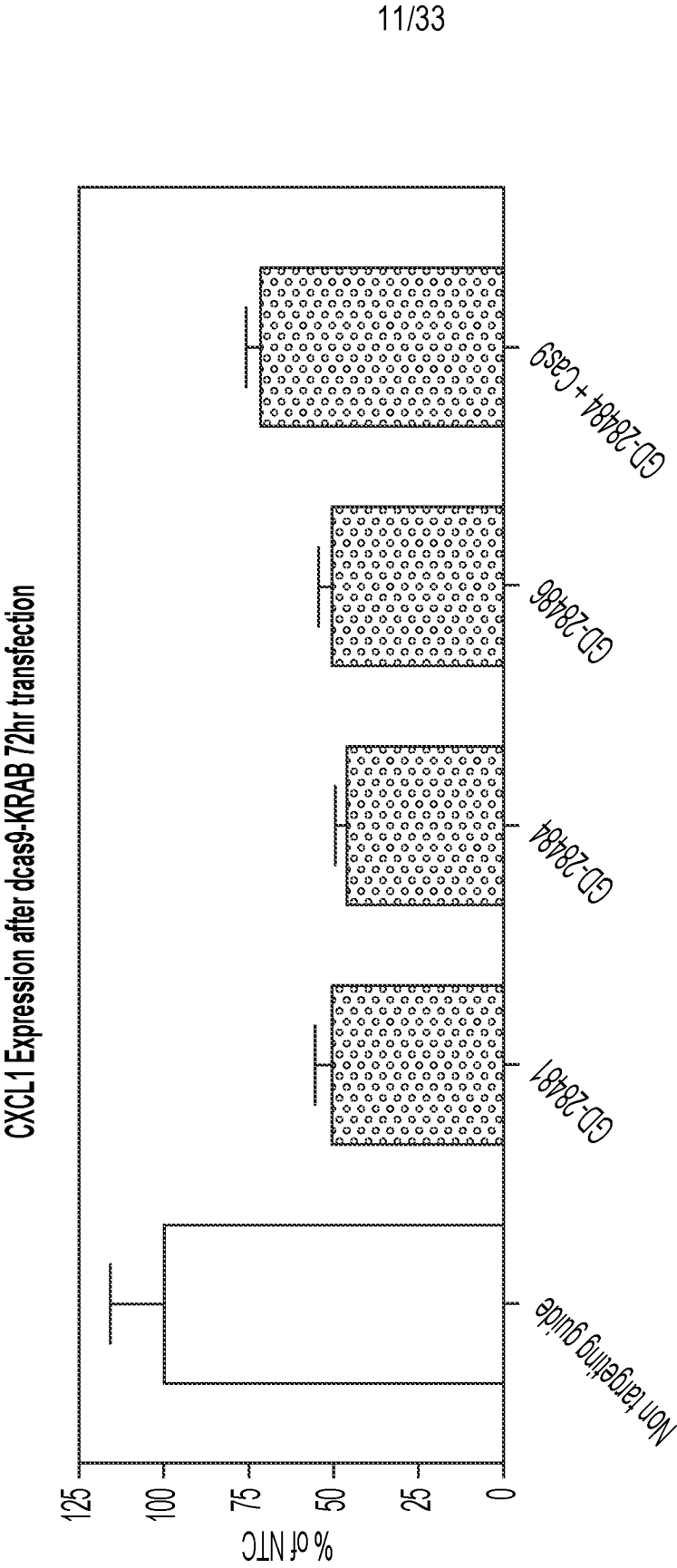


FIG. 10

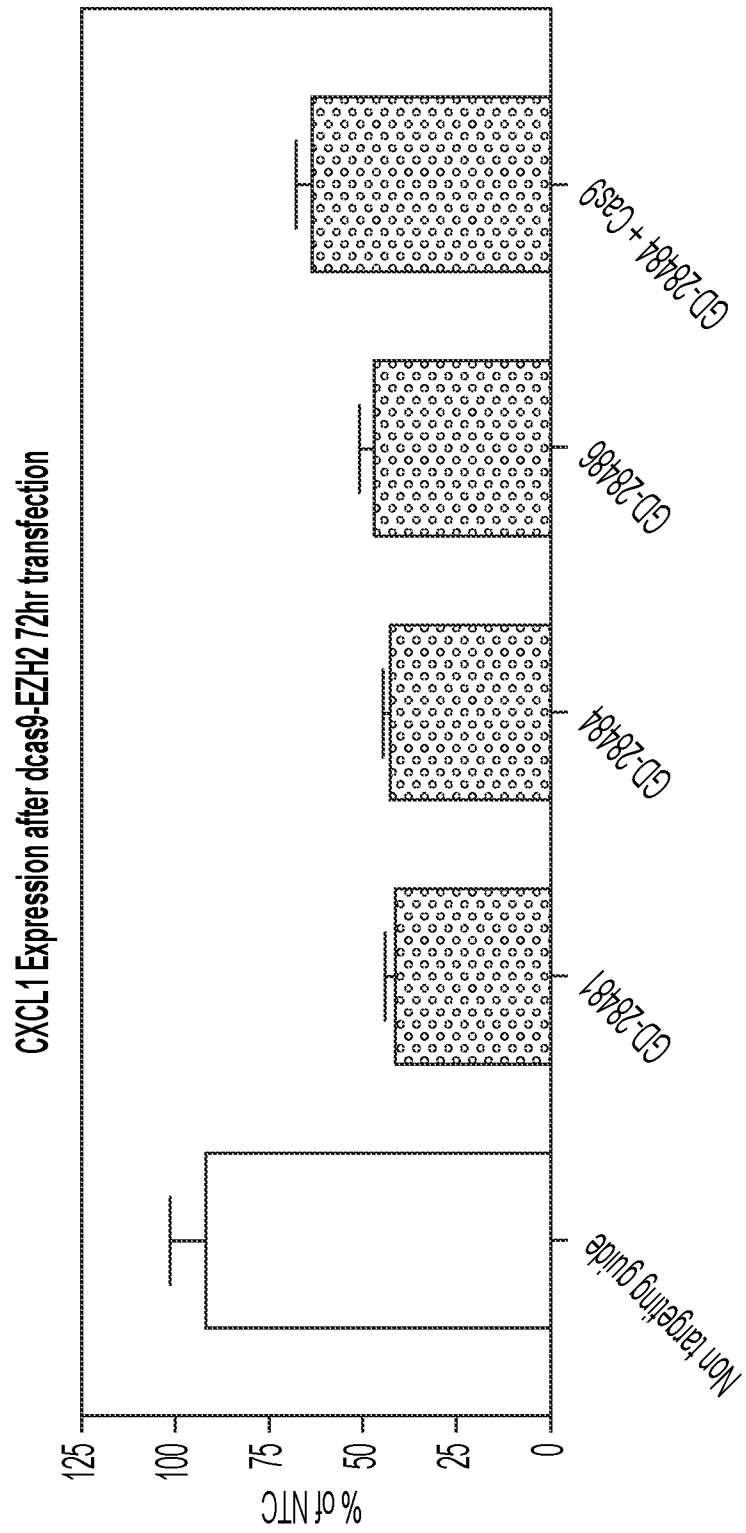


FIG. 11

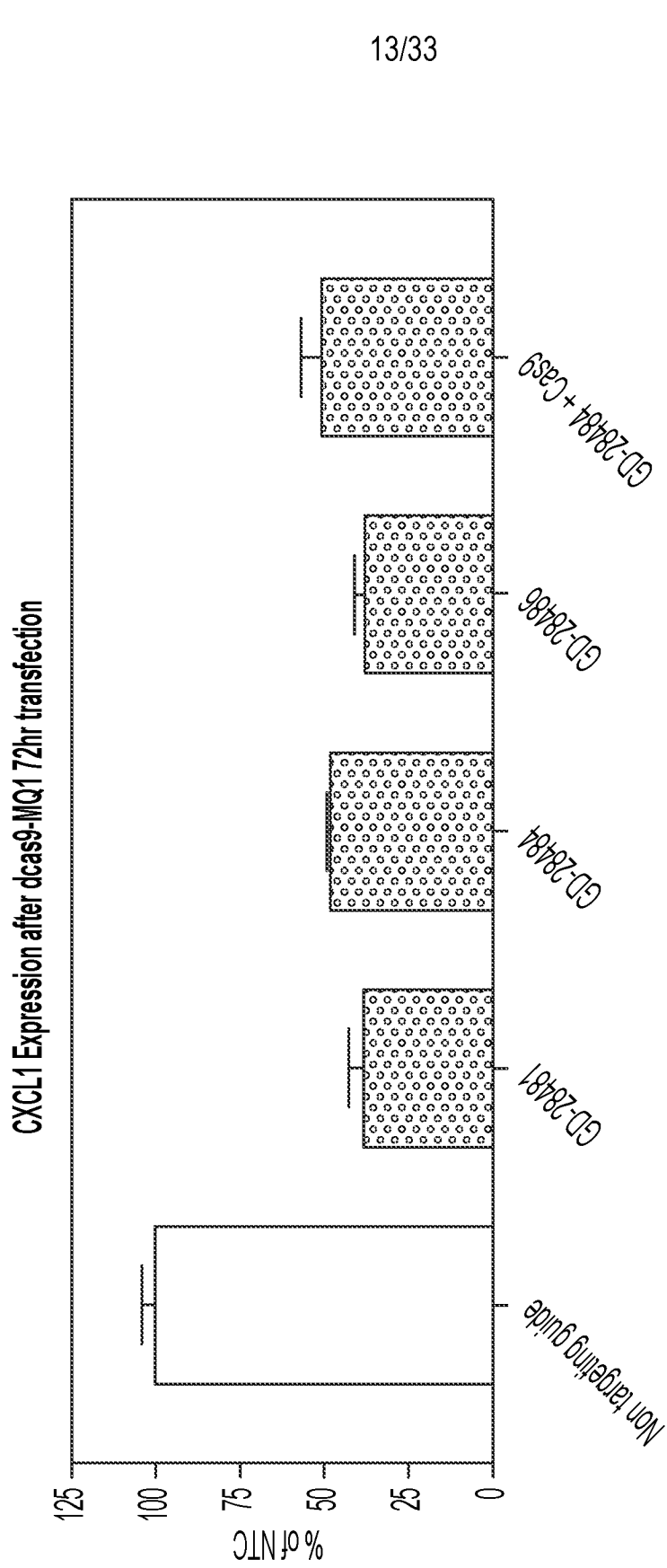


FIG. 12

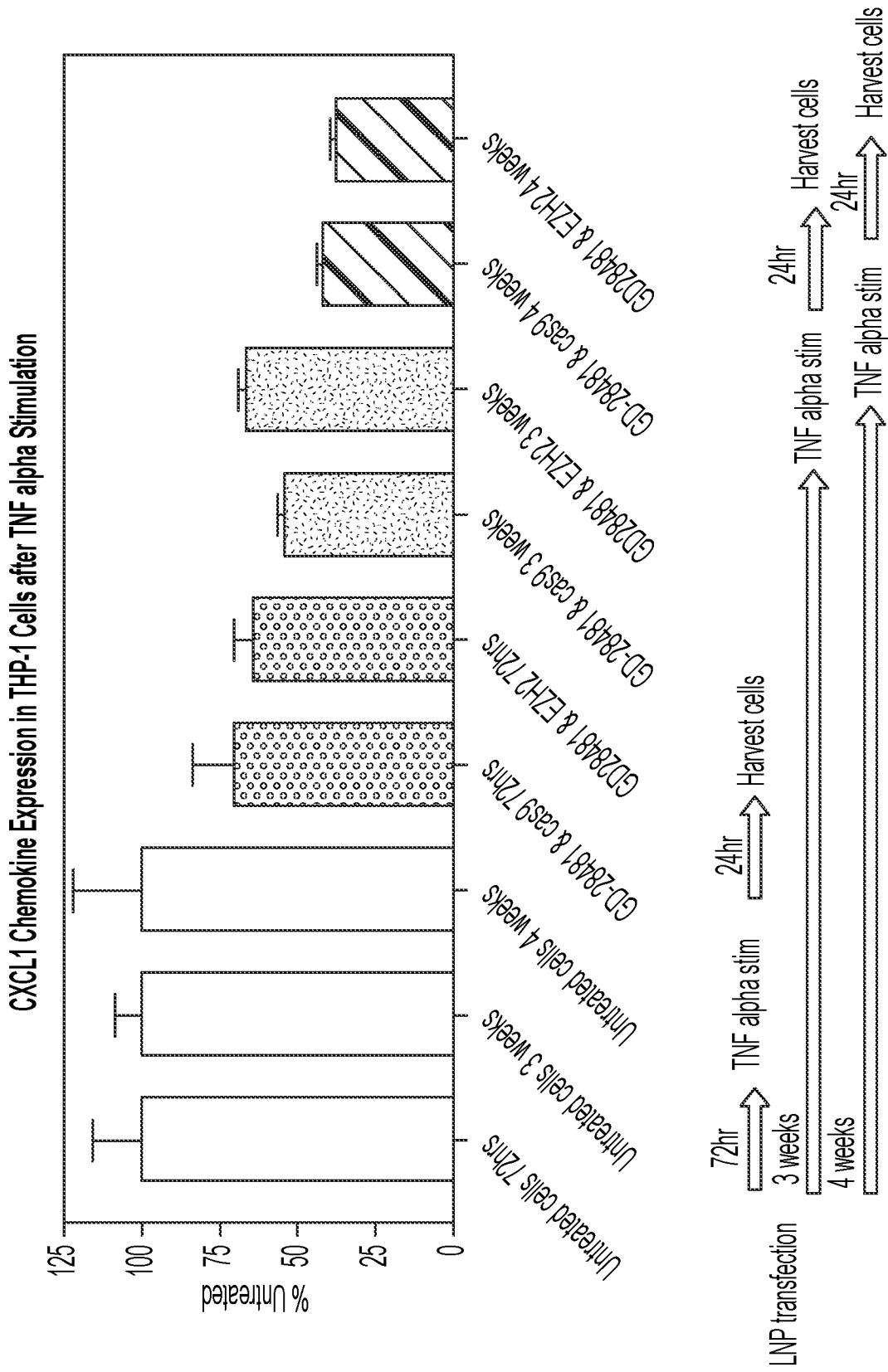
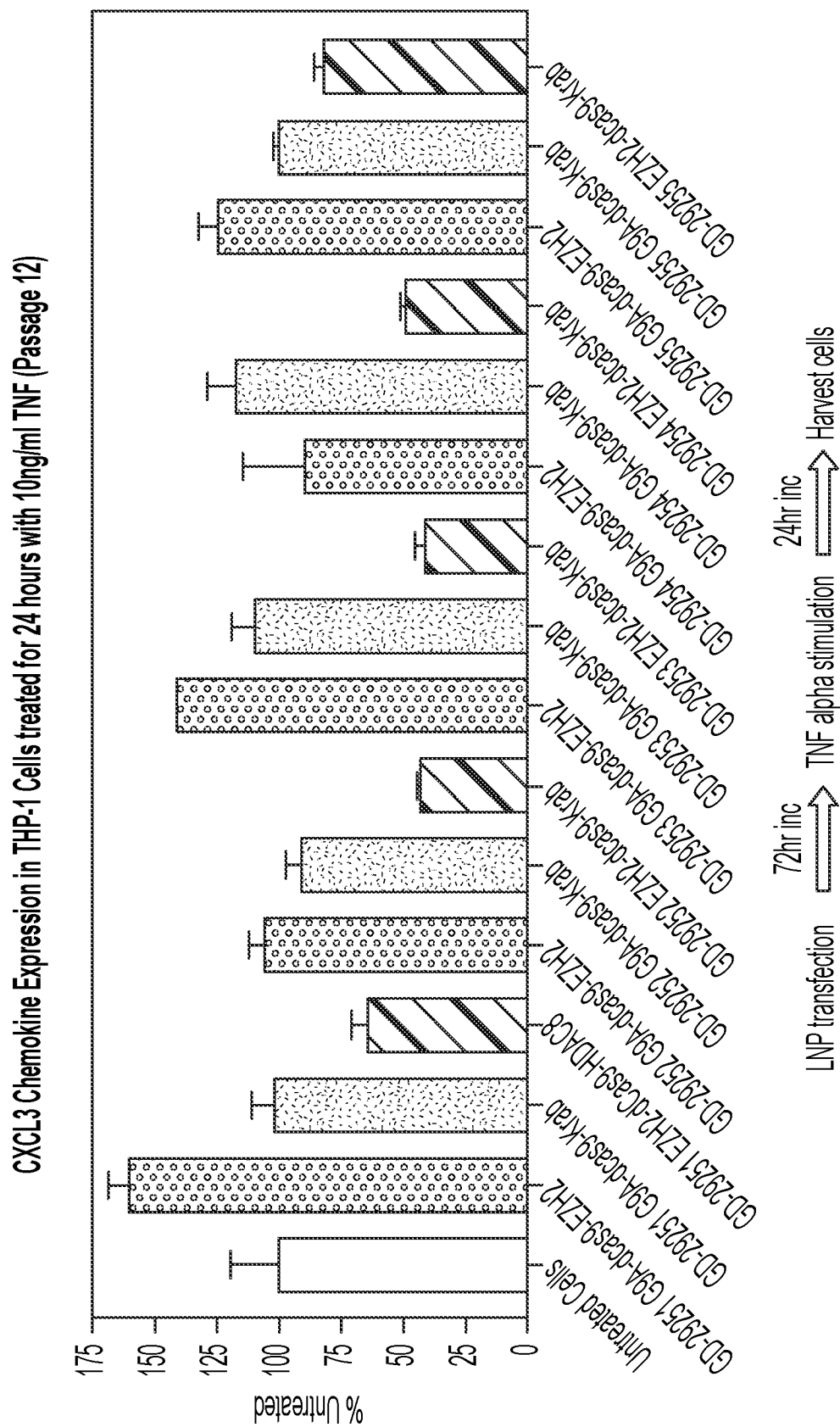


FIG. 13



CXCL1 Chemokine Expression in THP-1 Cells treated for 24 hours with 10ng/ml TNF (Passage 11) 72 hours post

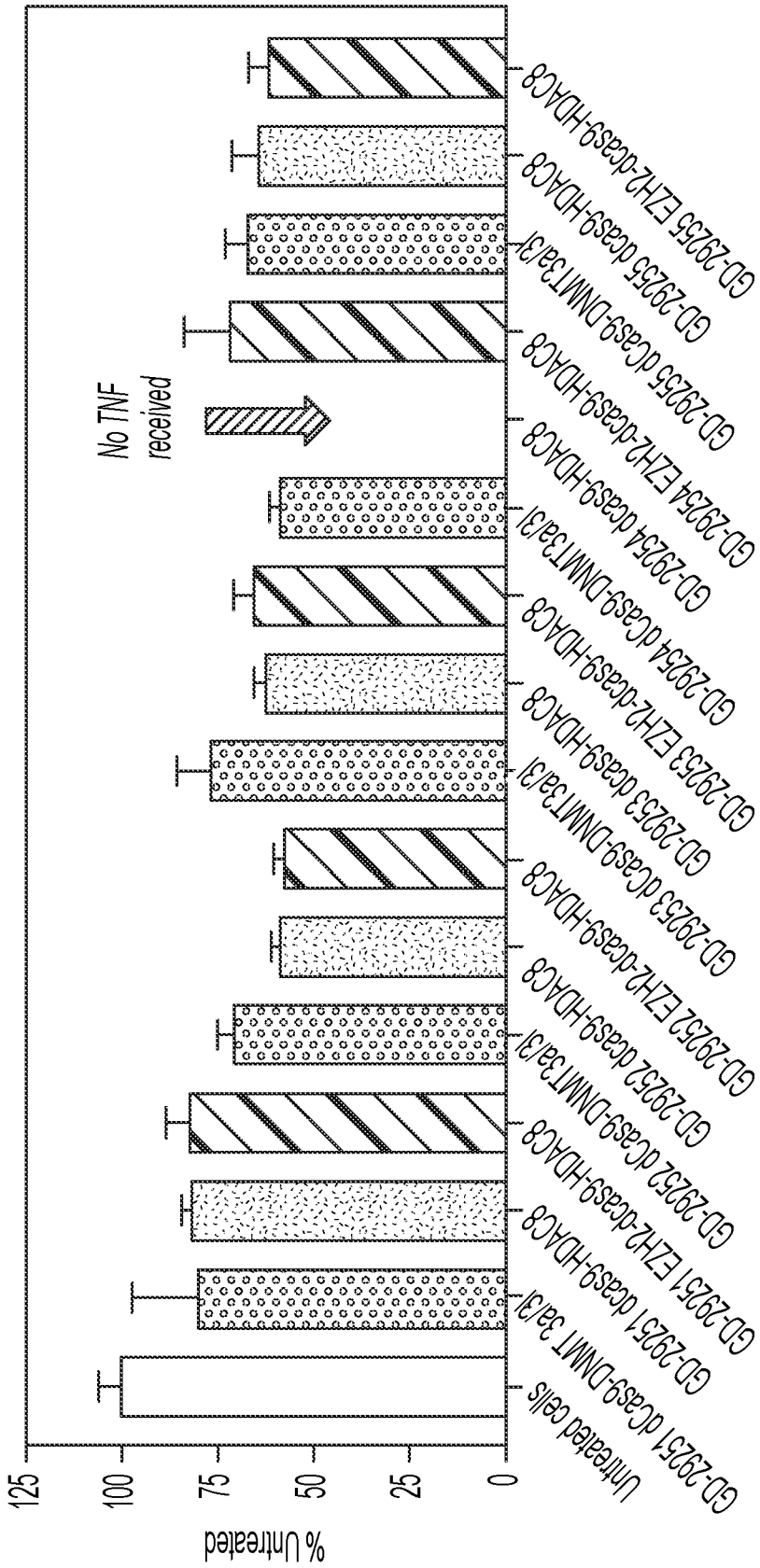


FIG. 15

Human CXCL IGD and gene cluster organization

CXCL8 □ CXCL6 □ CXCL4 □ CXCL3
CXCL5 □ CXCL7 □ CXCL2 □

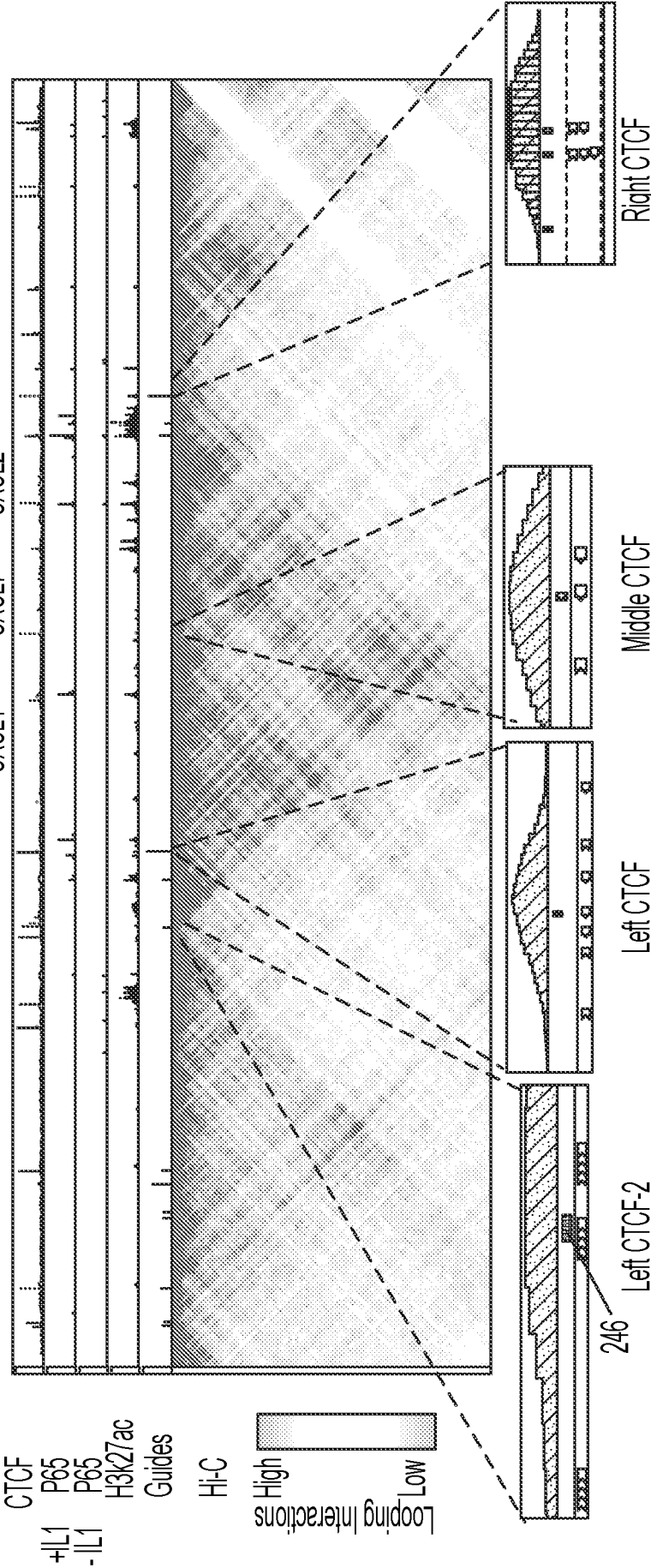
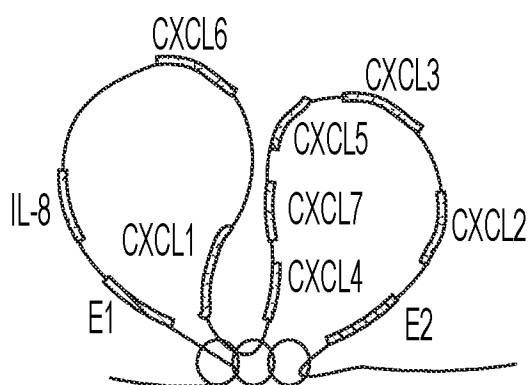


FIG. 16A

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E1 : enhancer 1

E2 : enhancer 2

FIG. 16B

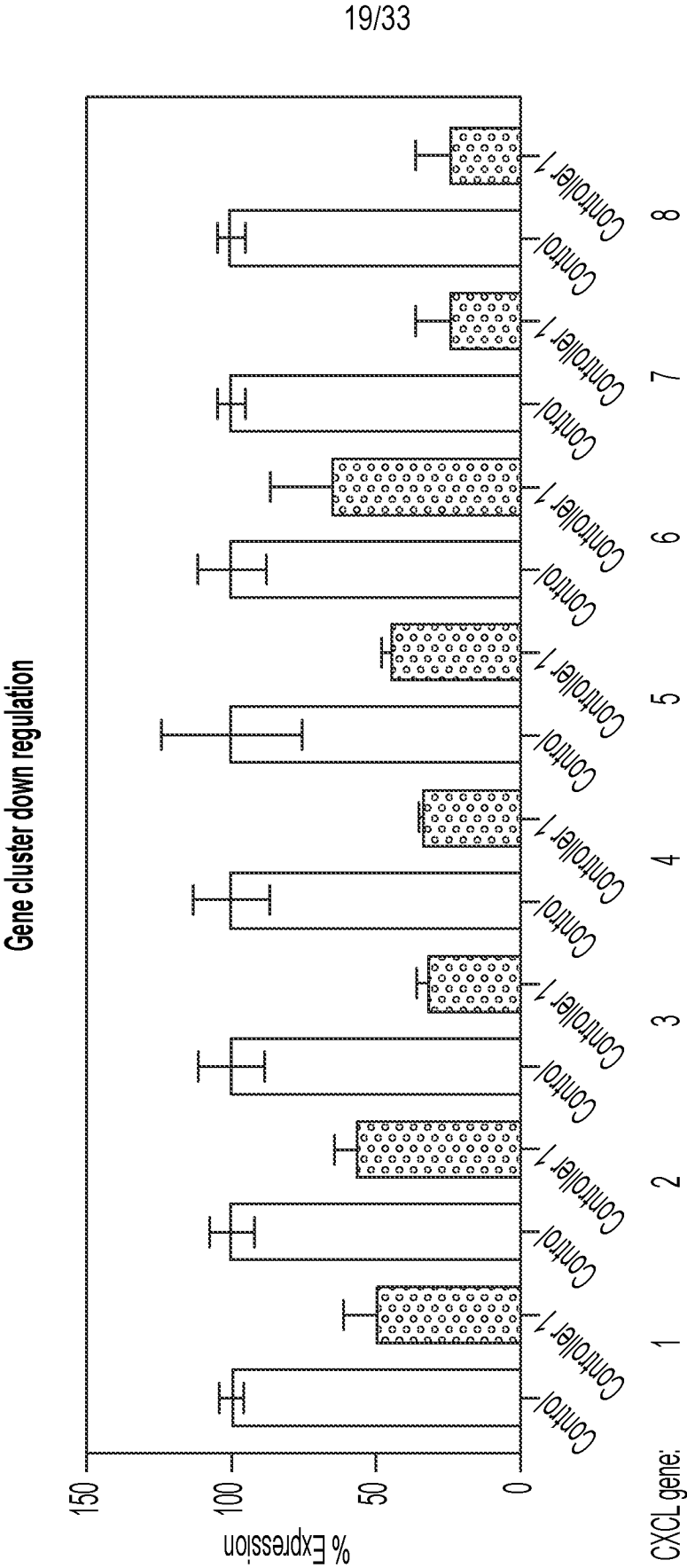


FIG. 17

IMR90 CXCL gene cluster down regulation using middle GD-30183 and EZH2-dCas9

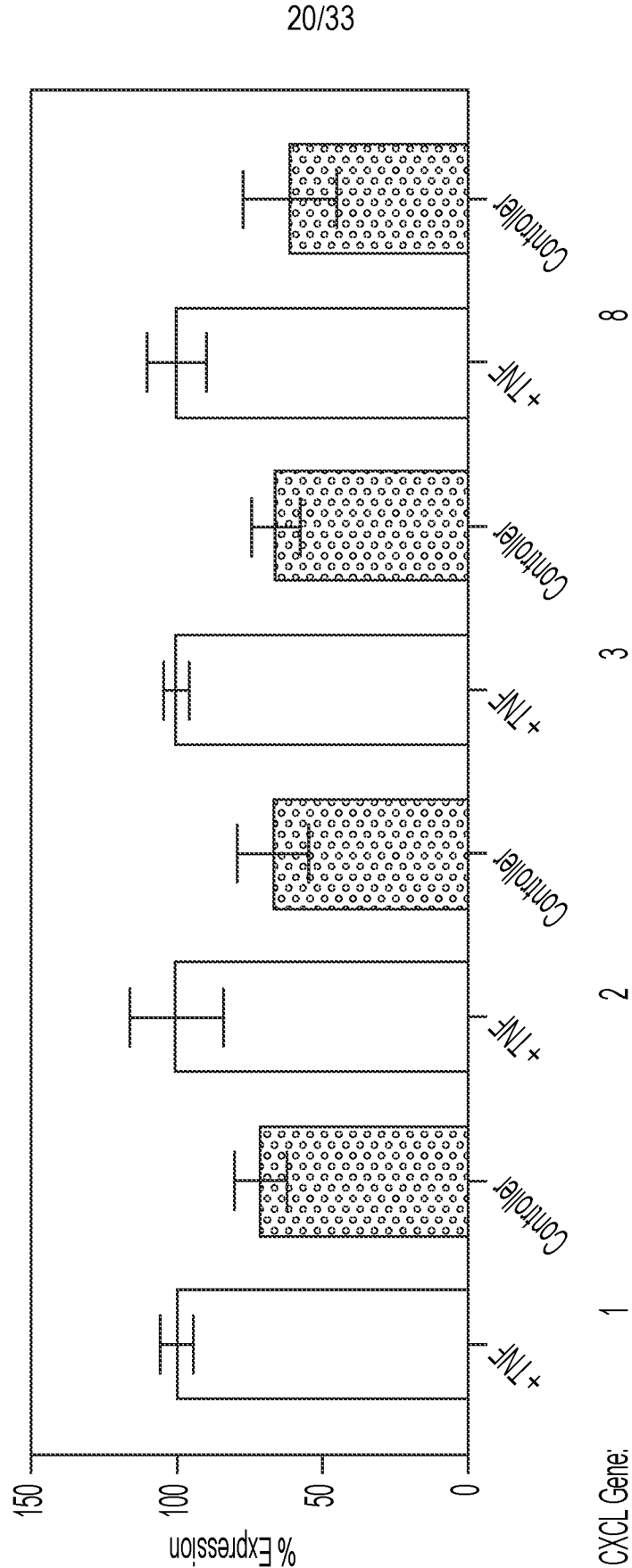


FIG. 18

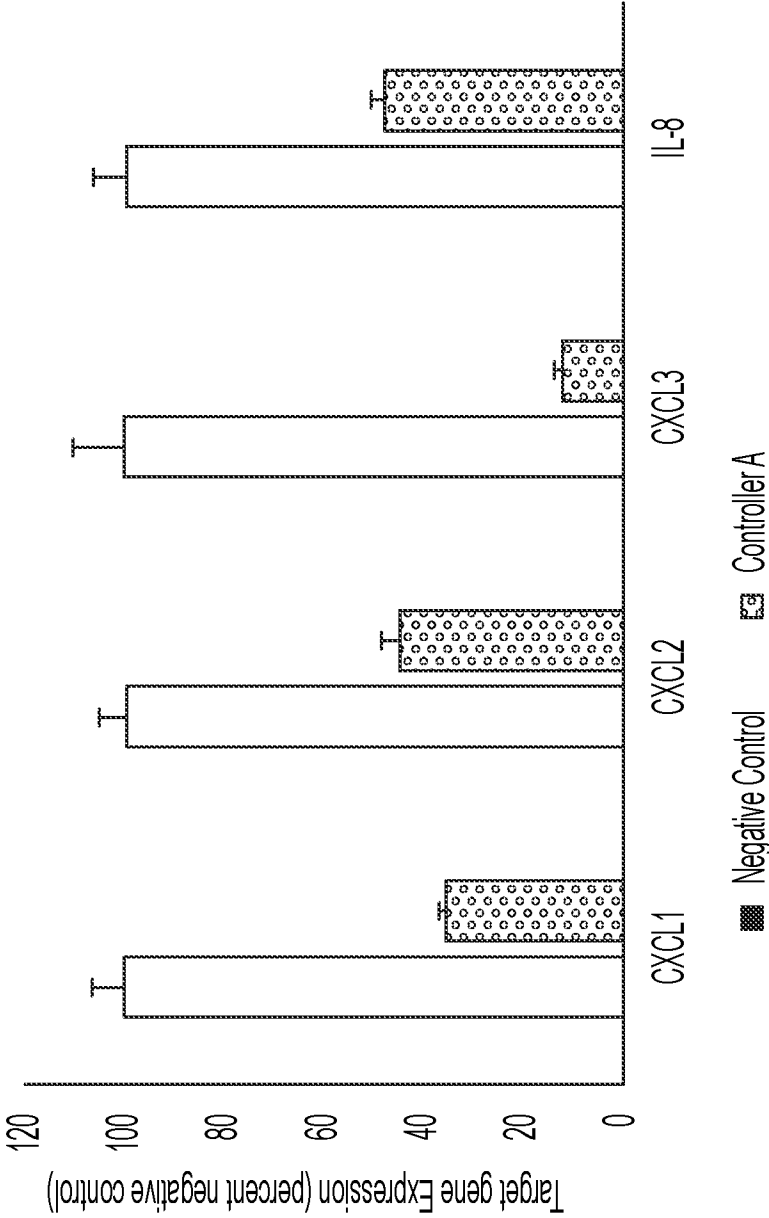
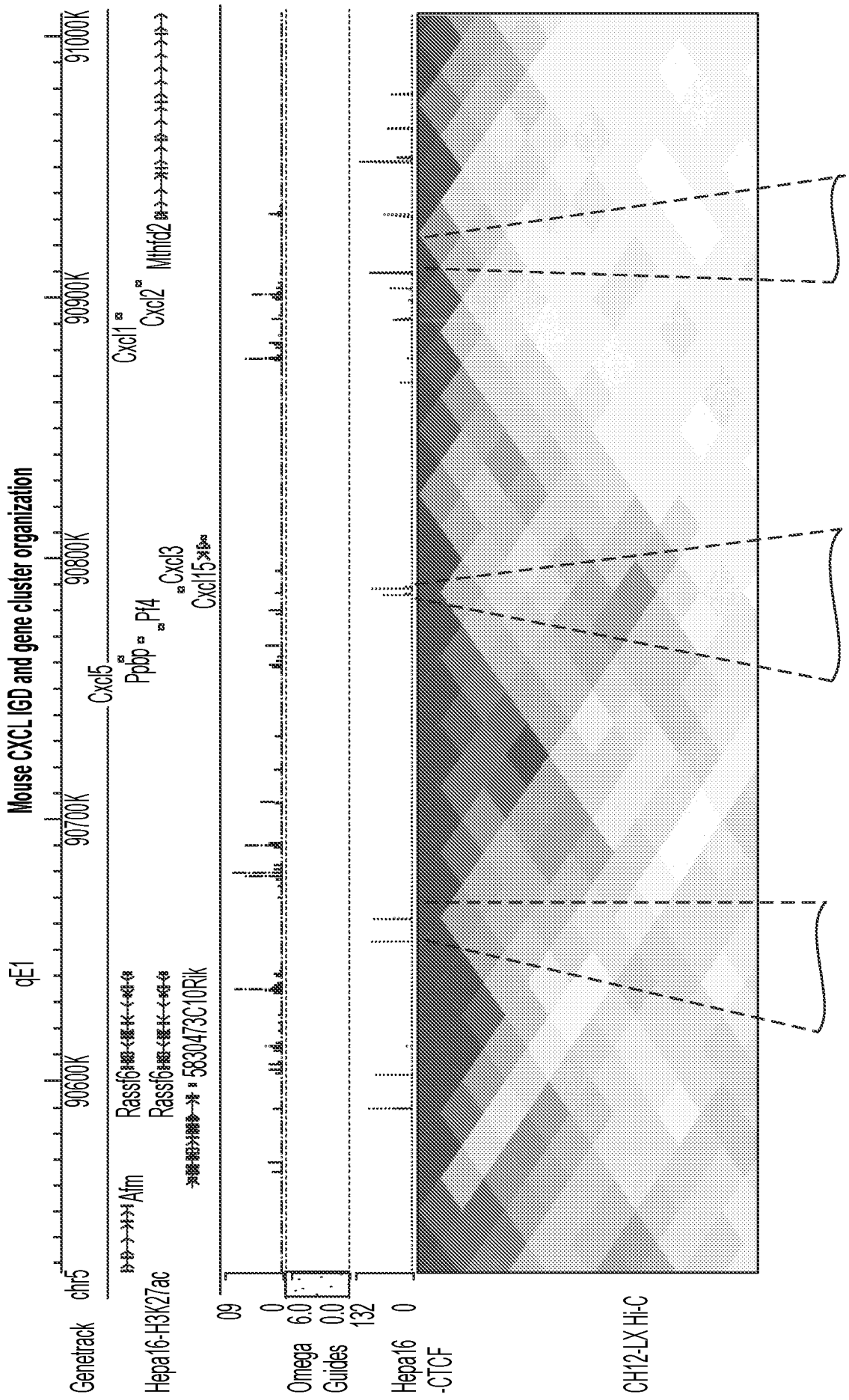


FIG. 19



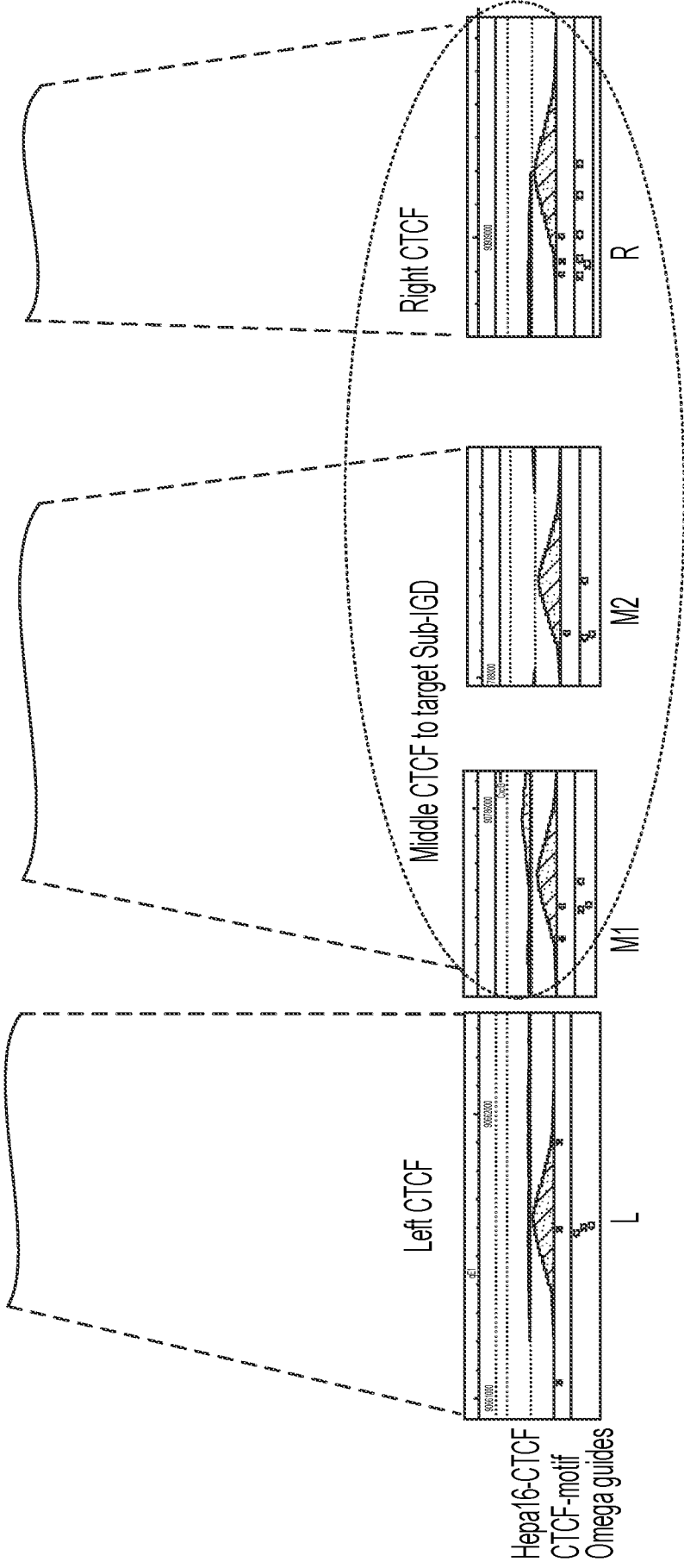


FIG. 20A
CONTINUED

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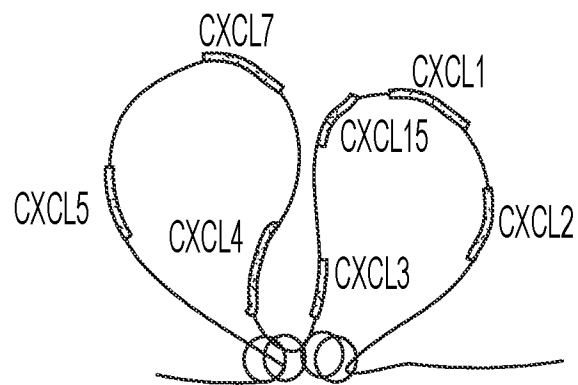


FIG. 20B

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SSOP LNP sparks formulation
Effector: dCas9-MQ1
Guide location:
Right CTCF GD-30594
Middle CTCF GD-30592 & GD-30593

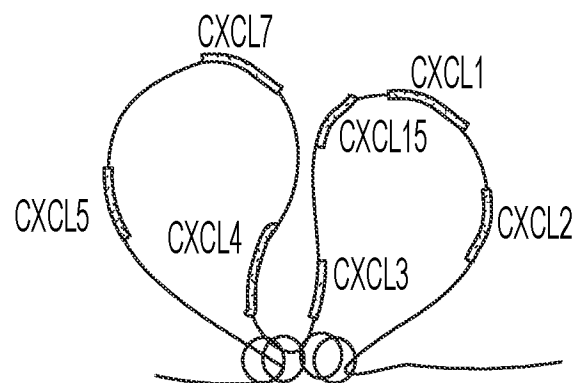
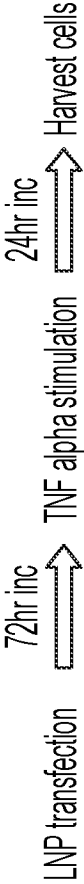
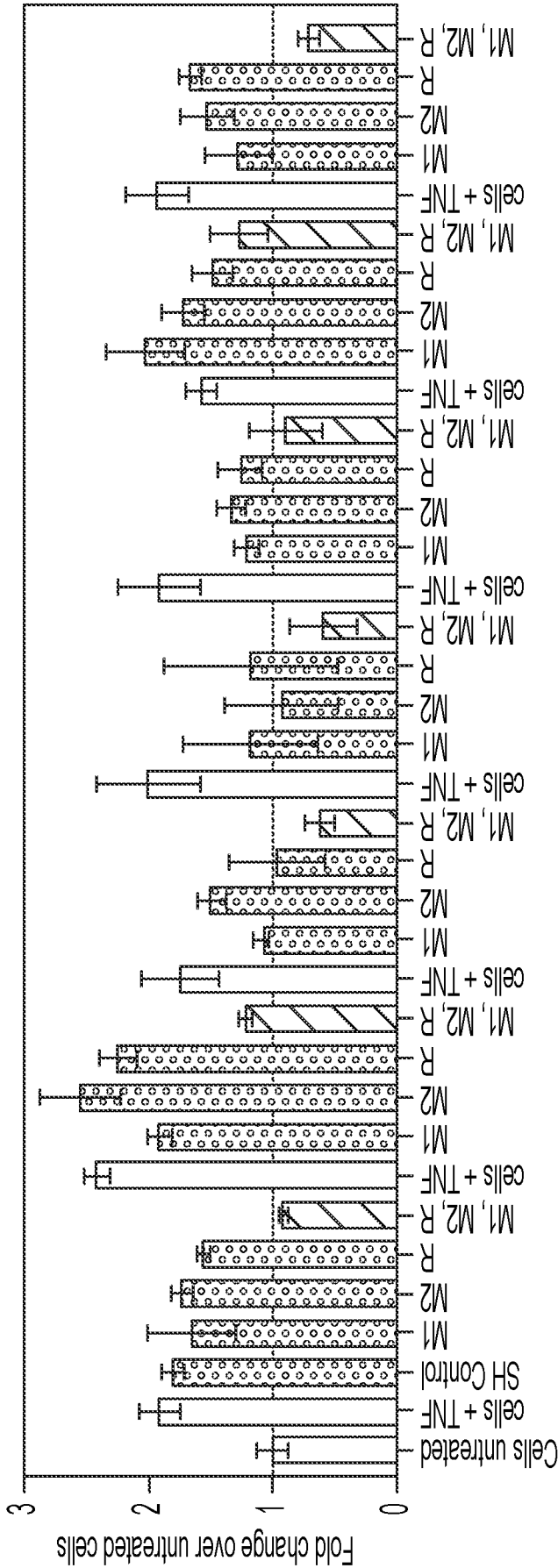


FIG. 21A



CXCL Chemokine Expression in HEPA 1-6 Cells After 24hr TNF stimulation



CXCL Gene:

1

2

3

4

5

7

15

FIG. 21B

Systemic administration of an Omega controller demonstrates a significant decrease in leukocyte infiltration in the inflamed lungs

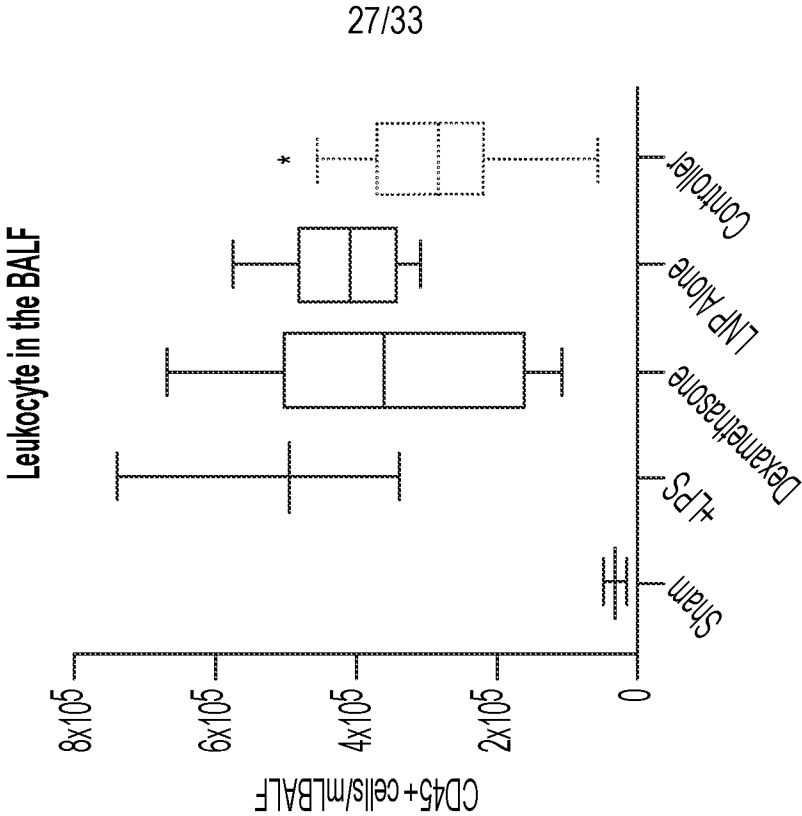


FIG. 22B

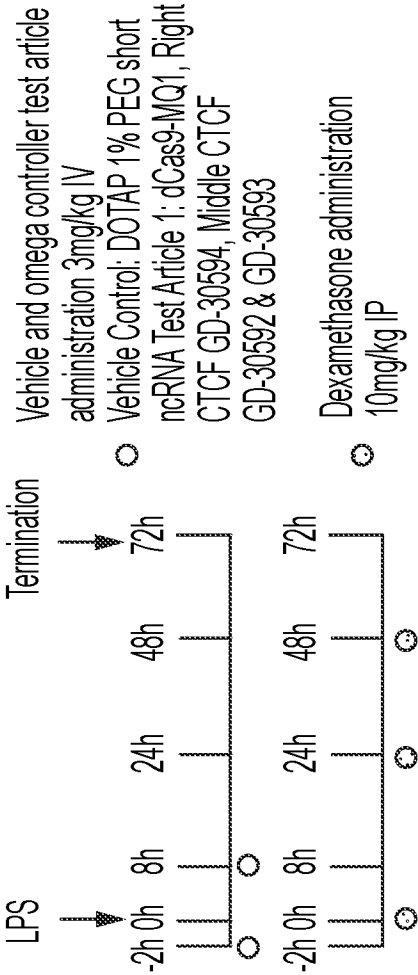


FIG. 22A

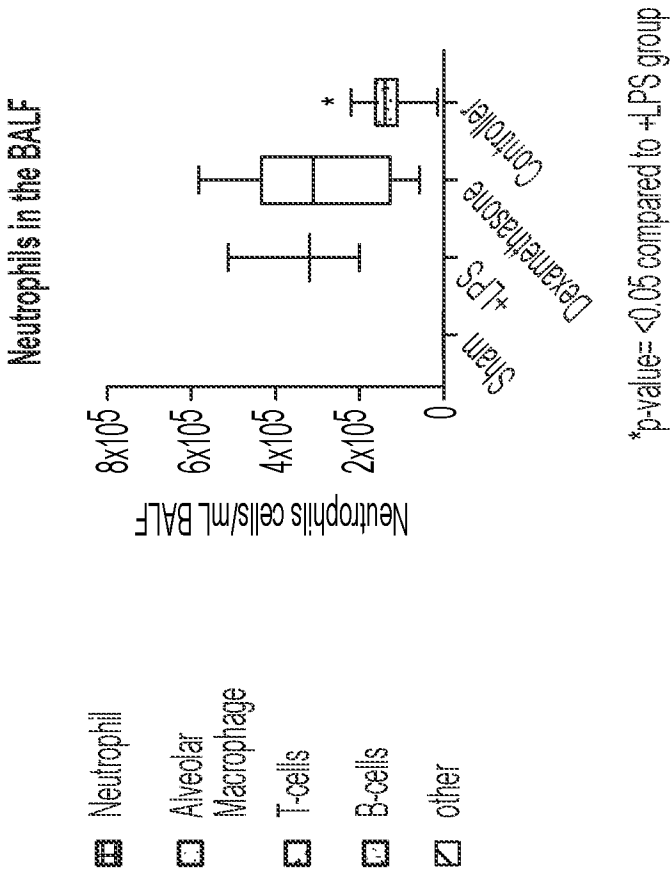


FIG. 23B

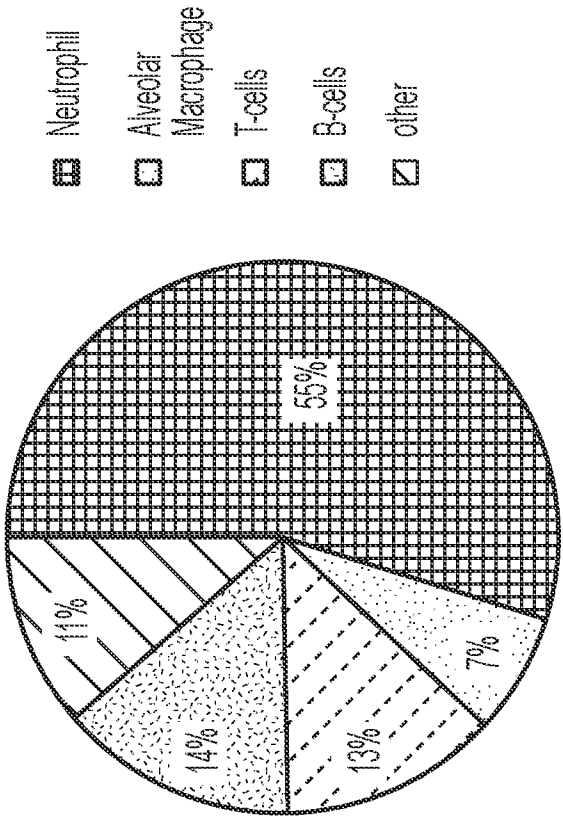
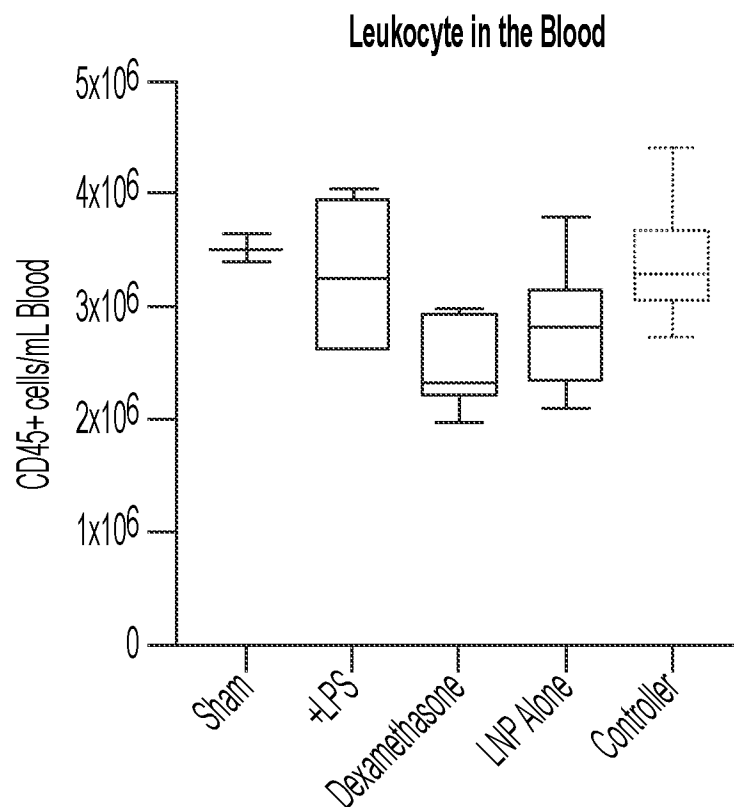


FIG. 23A

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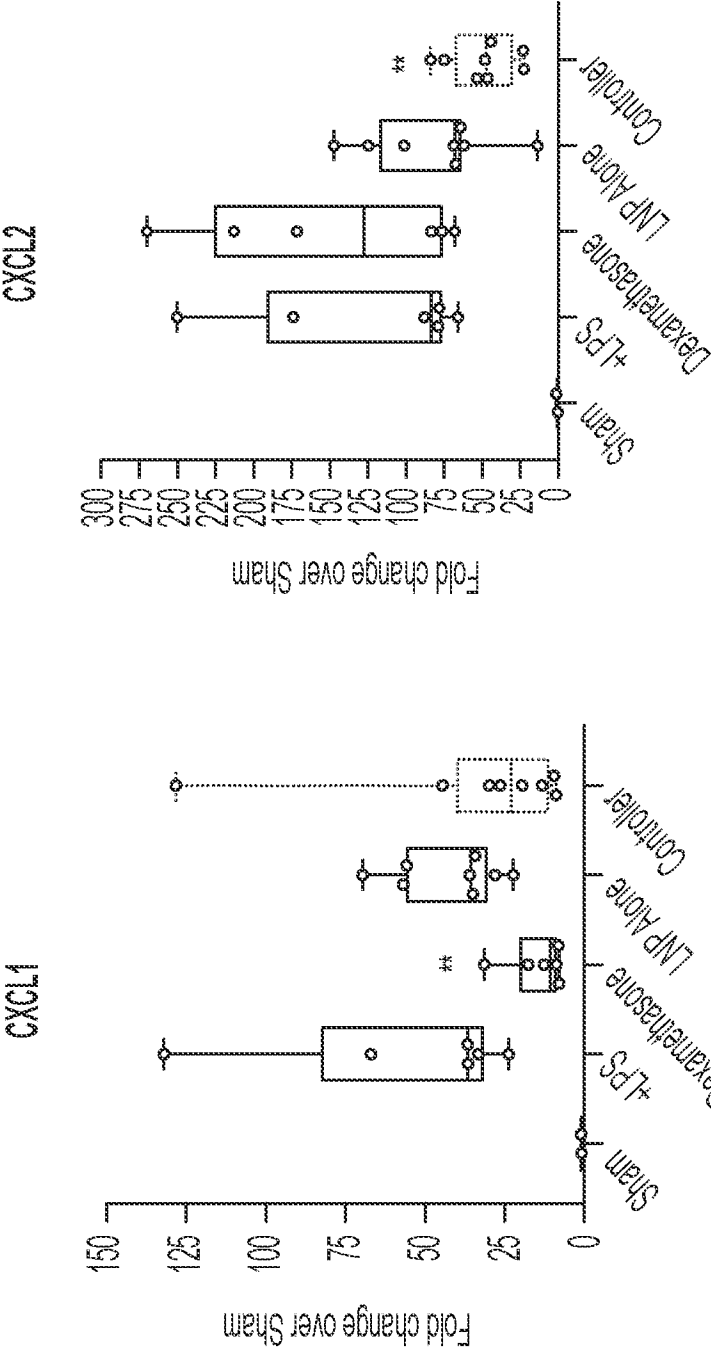
The decrease of leukocyte cells in the BALF is lung specific and not due to the decrease of white blood cells in the peripheral blood



- Peripheral blood showed similar levels of hematopoietic cells present in each group indicating that the drop in leukocyte count in the lung was a specific effect from the controller

FIG. 24

CXCL gene expression is decreased in the lung tissue; Side 12 04/08/21 (new, not present in 02/21 deck)



** p value < 0.01 compared to +LPS group

FIG. 25A

FIG. 25B

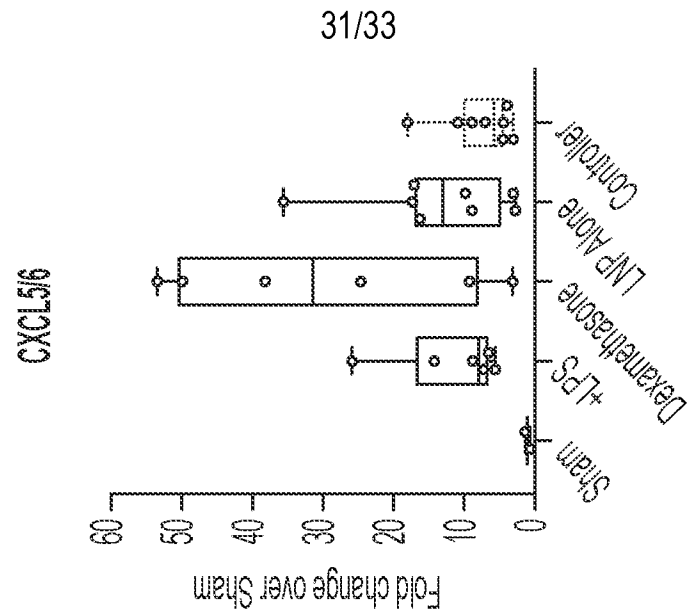


FIG. 25E

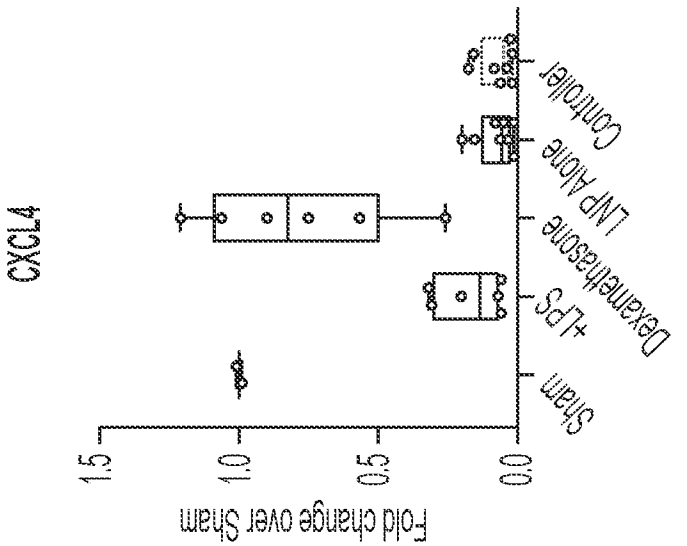


FIG. 25D

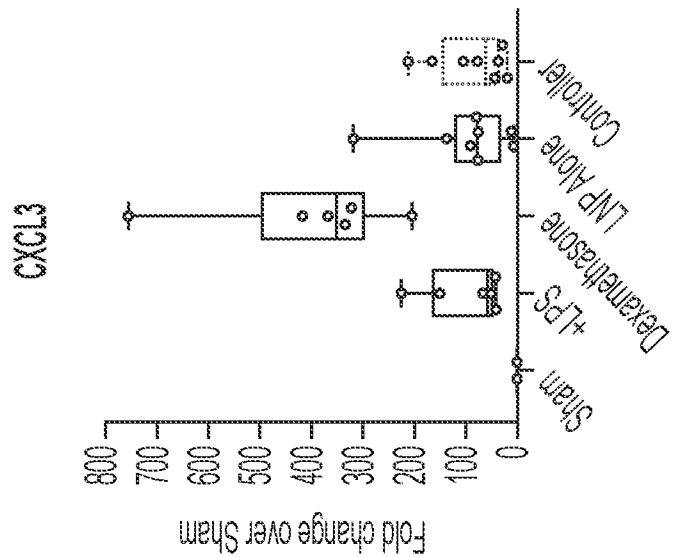


FIG. 25C

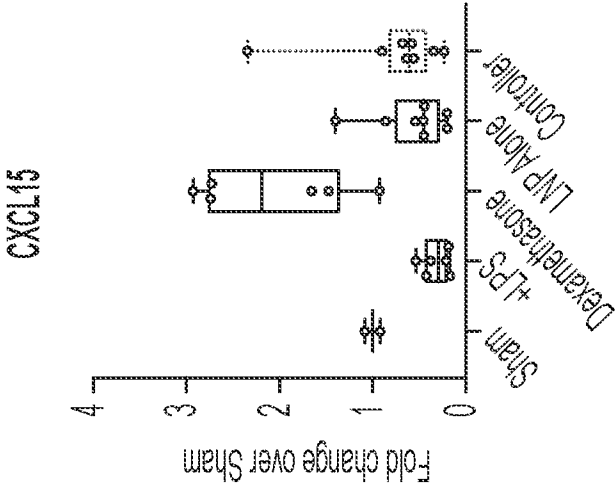


FIG. 25G

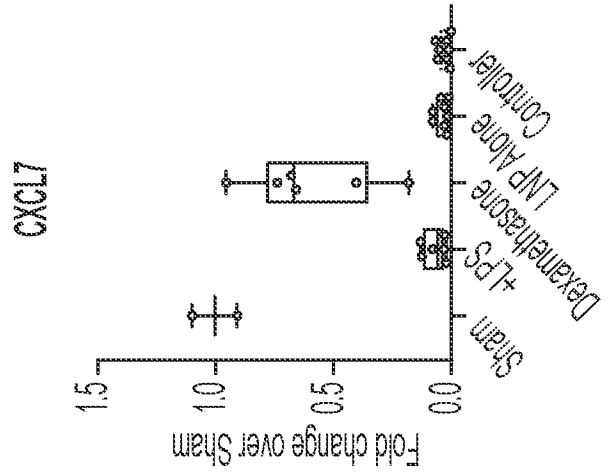


FIG. 25F

Decreasing CXCL expression and cellular recruitment to the site of inflammation has a beneficial downstream effect of decreasing the presence of other cytokines

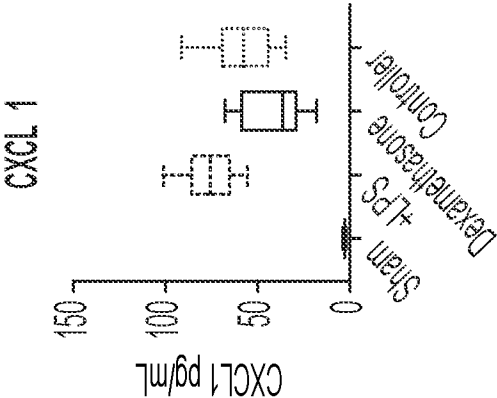


FIG. 26A

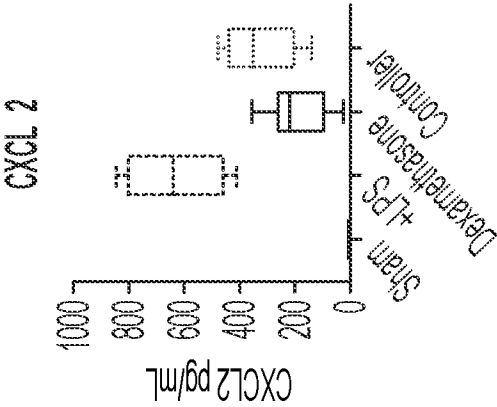


FIG. 26B

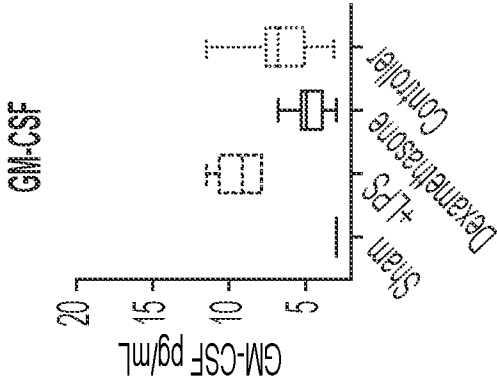


FIG. 26C

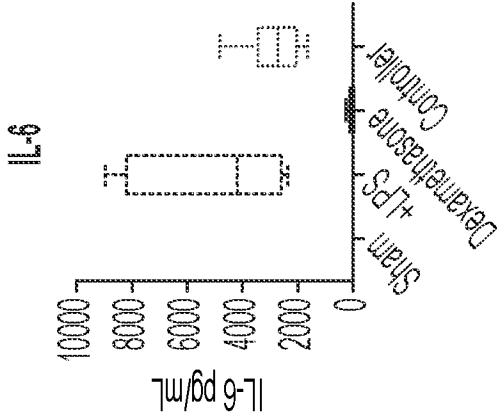


FIG. 26D