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(54) **Title:** COMPOSITIONS AND METHODS FOR MODULATING FORKHEAD BOX P3 (FOXP3) GENE EXPRESSION

(57) **Abstract:** The present invention provides agents and compositions for modulating expression (*e.g.*, enhanced or reduced expression) of a forkhead box P3 (FOXP3) gene by targeting a FOXP3 expression control region and methods of use thereof for treating a FOXP3 associated disorder, such as an autoimmune disease, *e.g.*, IPEX syndrome.

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COMPOSITIONS AND METHODS FOR MODULATING FORKHEAD BOX P3 (FOXP3) GENE EXPRESSION

RELATED APPLICATION

The instant application claims the benefit of priority to U.S. Provisional Application No. 62/988,044, filed on March 11, 2020, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 10, 2021, is named 131717-00420_SL.txt and is 1,166,669 bytes in size.

BACKGROUND OF THE INVENTION

A healthy immune system defends the body against disease and infection. But if the immune system malfunctions, it mistakenly attacks healthy cells, tissues, and organs. These attacks, characterizing autoimmune diseases or disorders, can affect any part of the body, weakening bodily function and even turning life-threatening. Some of the more common autoimmune diseases include IPEX syndrome (IPEX), type 1 diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

Collectively, these diseases affect more than 24 million people in the United States (*see* Progress in Autoimmune Diseases Research, https://www.niaid.nih.gov/sites/default/files/adccfinal.pdf). An additional eight million people have auto-antibodies, blood molecules that indicate a person's chance of developing an autoimmune disease. Autoimmune diseases are becoming more prevalent.

Treatment depends on the disease, but in most cases one important goal is to reduce inflammation. Corticosteroids or other drugs that reduce immune response are usually prescribed.

Regulatory T cells (Tregs) are a specialized subpopulation of T cells that act to suppress immune response, thereby maintaining homeostasis and self-tolerance. It has been shown that Tregs are able to inhibit T cell proliferation and cytokine production and play an important role in preventing or treating autoimmune disease. Forkhead box P3 (FOXP3) is a master transcription factor that controls the differentiation of naïve T-cells into regulatory T-cells (Tregs) and forced overexpression of FOXP3 has been shown to confer the Treg phenotype to T-cells.

In vitro generation of Tregs has been an important effort in the field of *ex vivo* therapy targeting autoimmune disorders. However, many of the strategies to produce Tregs either do not lead

to sustained expression of genes that lead to Tregs or give rise to Tregs that do not have suppression phenotype.

Accordingly, there is a need in the art for compositions and methods that treat an autoimmune disease, such as IPEX syndrome.

SUMMARY OF THE INVENTION

The present invention provides agents and compositions for modulating the expression (*e.g.*, enhancing or reducing the expression) of the Forkhead box P3 (FOXP3) gene by targeting a FOXP3 expression control region. The FOXP3 gene may be in a cell, *e.g.*, a mammalian cell, such as a mammalian somatic cell, *e.g.*, a human or mouse somatic cell, *e.g.*, a naïve T-cell. The present invention also provides methods of using the agents and compositions of the invention for modulating the expression of a FOXP3 gene in, or for treating, a subject who would benefit from modulating the expression of a FOXP3 gene, *e.g.*, a subject suffering or prone to suffering from a FOXP3-associated disease.

Accordingly, in one aspect, the present invention provides a site-specific forkhead box P3(FOXP3) disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region.

In one embodiment, the site-specific FOXP3 targeting moiety comprises a polymeric molecule. The polymeric molecule may comprise a polyamide, a polynucleotide, a polynucleotide encoding a DNA-binding domain, or fragment thereof, that specifically binds to the FOXP3 expression control region, or a peptide nucleic acid (PNA).

In yet another embodiment, the expression control region comprises a region upstream of a FOXP3 transcription start site (TSS).

In one embodiment, the expression control region comprises one or more FOXP3 associated anchor sequences within an anchor sequence-mediated conjunction comprising a first and a second FOXP3-associated anchor sequence.

In another embodiment, the FOXP3-associated anchor sequence comprises a CCCTC-binding factor (CTCF) binding motif.

In yet another embodiment, the FOXP3-associated anchor sequence-mediated conjunction comprises one or more transcriptional control elements internal to the conjunction. In one embodiment, the FOXP3-associated anchor sequence-mediated conjunction comprises one or more transcriptional control elements external to the conjunction.

In one embodiment, the FOXP3-associated anchor sequence is located within about 500 kb of the transcriptional control element. In another embodiment, the FOXP3-associated anchor sequence is located within about 300 kb of the transcriptional control element. In still another embodiment, the anchor sequence is located within 10 kb of the transcriptional control element.

In another embodiment, the expression control region comprises a FOXP3-specific transcriptional control element. In still another embodiment, the transcriptional control element comprises a FOXP3 promoter. In yet another embodiment, the transcriptional control element comprises a transcriptional enhancer. In still another embodiment, the transcriptional control element comprises a transcriptional repressor.

In one embodiment, the site-specific FOXP3 disrupting agent includes a nucleotide sequence having at least 85%,86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide identity to the entire nucleotide sequence of any one of the nucleotide sequences in Table 2.

In another embodiment, the site-specific FOXP3 disrupting agent includes a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

In one embodiment, the DNA-binding domain of the TALE or ZNF polypeptide comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences listed in Table 1B.

In still another embodiment, the site-specific FOXP3 disrupting agent includes a nucleotide modification, *e.g.*, a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, an abasic nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a nucleotide comprising a 5'-methylphosphonate group, a nucleotide comprising a 3'-phosphorothioate group, or a nucleotide comprising a 3'-methylphosphonate group.

In yet another embodiment, the polymeric molecule comprises a peptide nucleic acid (PNA).

In one aspect, the present invention provides a vector. The vector includes the site-specific FOXP3 disrupting agent of various embodiments of the above aspects or any other aspect of the invention delineated herein. In one embodiment, the vector is a viral expression vector.

In another aspect, the present invention provides a cell. The cell provides the site-specific FOXP3 disrupting agent or the vector of various embodiments of the above aspects or any other aspect of the invention delineated hterein.

In one embodiment, the site-specific FOXP3 disrupting agent is present in a composition. In another embodiment, the composition comprises a pharmaceutical composition. In still another embodiment, the pharmaceutical composition comprises a lipid formulation. In yet another embodiment, the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of

any of the foregoing. In one embodiment, the pharmaceutical composition comprises a lipid nanoparticle.

In another aspect, the present invention provides a site-specific FOXP3 disrupting agent. The site-specific FOXP3 disrupting agent includes a nucleic acid molecule encoding a fusion protein, the fusion protein comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region and an effector molecule.

In one embodiment, the site-specific FOXP3 targeting moiety comprises a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

In another embodiment, the effector molecule comprises a polypeptide or a nucleic acid molecule encoding a polypeptide. In still another embodiment, the fusion protein comprises a peptide-nucleic acid fusion.

In yet another embodiment, the effector is selected from the group consisting of a nuclease, a physical blocker, an epigenetic recruiter, and an epigenetic CpG modifier, and combinations of any of the foregoing.

In one embodiment, the effector comprises a CRISPR associated protein (Cas) polypeptide or nucleic acid molecule encoding the Cas polypeptide. In another embodiment, Cas polypeptide is an enzymatically inactive Cas polypeptide. In still another embodiment, the site-specific FOXP3 disrupting agent further includes a catalytically active domain of human exonuclease 1 (hEXO1).

In another embodiment, epigenetic recruiter comprises a transcriptional enhancer or a transcriptional repressor.

In one embodiment, the transcriptional enhancer is a VPR (VP64-p65-Rta).

In one embodiment, the VPR comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of

DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLSGGPKKKRK
VGSQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQP
YPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPV
LAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEF
QQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIA
DMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEGREVCQPKRLRPFHPPGSPWANRPLP
ASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVI
PQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHA
MHISTGLSIFDTSLF (SEQ ID NO: 64).

In one embodiment, the transcriptional enhancer comprises two, three, four, or five VPRs.

In one embodiment, the transcriptional enhancer is a p300.

In one embodiment, the p300 comprises an amino acid sequence having at least about 85% 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the entire amino acid sequence of IFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQY QEPWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSP QTLCCYGKQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRK NDTLDPELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRL GTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKA LFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLE YVKKLGYTTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYK DIFKQATEDRLTSAKELPYFEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKN AKKKNNKKTSKNKSSLSRGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANS LPPIVDPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQD (SEQ ID NO: 65).

In still another embodiment, the epigenetic CpG modifier comprises a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase.

In one embodiment, the effector molecule comprises a zinc finger polypeptide. In another embodiment, the effector molecule comprises a Transcription activator-like effector nuclease (TALEN) polypeptide.

In some embodiments, the site-specific FOXP3 disrupting agent further comprises a second nucleic acid molecule encoding a second fusion protein, wherein the second fusion comprises a second site-specific FOXP3 targeting moiety which targets a second FOXP3 expression control region and a second effector molecule, wherein the second FOXP3 expression control region is different than the FOXP3 expression control region.

In one embodiment, the second effector is different than the first effector.

In one embodiment, the second effector is the same as the first effector.

In one embodiment, the fusion protein and the second fusion protein are operably linked.

In one embodiment, the fusion protein and the second fusion protein comprise an amino acid sequence that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas9-P300, and dCas9-VPR.

In one embodiment, the fusion protein is encoded by a polynucleotide comprising a nucleotide sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide sequence identity to the entire nucleotide sequence

of a polynucleotide selected from the group consisting of dCas9-P300 mRNA, and dCas9-VPR mRNA.

In one aspect, the present invention provides a site-specific FOXP3 disrupting agent. The disrupting agent includes a nucleic acid molecule encoding a fusion protein, wherein the fusion protein comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas9-P300, and dCas9-VPR.

In one aspect, the present invention provides a site-specific FOXP3 disrupting agent. The site-specific FOXP3 disrupting agent comprises a polynucleotide encoding the amino acid sequence of dCas-P300 comprising the amino acid sequence of

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKK
NLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVE
EDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIE
GDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKK
NGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR
RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGAS
AQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN
EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQS
GKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGIL
QTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAAIVPQSFLKDDSIDNKVLTRSD
KARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLV
ETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHA
HDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFF
KTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESI
LPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERS
SFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS
QLGGDKRPAATKKAGQAKKKKGRAIFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLL
GIPDYFDIVKSPMDLSTIKRKLDTGQYQEPWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKL
SEVFEQEIDPVMQSLGYCCGRKLEFSPQTLCCYGKQLCTIPRDATYYSYQNRYHFCEKCFNEI
QGESVSLGDDPSQPQTTINKEQFSKRKNDTLDPELFVECTECGRKMHQICVLHHEIIWPAGFV
CDGCLKKSARTRKENKFSAKRLPSTRLGTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTV

EVKPGMKARFVDSGEMAESFPYRTKALFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYI
SYLDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGHIWACPPSEGDDYIFHCHPPDQKIPK
PKRLQEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAKELPYFEGDFWPNVLEESIKELE
QEEEERKREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLSRGNKKKPGMPNVSNDLSQ
KLYATMEKHKEVFFVIRLIAGPAANSLPPIVDPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRR
AQWSTMCMLVELHTQSQDSGGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 10).

In another aspect, the present invention provides a site-specific FOXP3 disrupting agent. The site-specific FOXP3 disrupting agent comprises a polynucleotide encoding the amino acid sequence of dCas-VPR comprising the amino acid sequence of

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKK
NLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVE
EDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIE
GDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKK
NGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNL
SDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYA
GYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILR
RQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGAS
AQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN
EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQS
GKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGIL
QTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVAAIVPQSFLKDDSIDNKVLTRSD
KARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLV
ETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHA
HDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFF
KTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESI
LPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERS
SFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS
QLGGDKRPAATKKAGQAKKKKGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLD
MLGSDALDDFDLDMLSGGPKKKRKVGSQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGP
TDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVL
PQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDED
LGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRPPD
PAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEG

REVCQPKRIRPFHPPGSPWANRPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASH LLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLN LDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLFSGGKRPAATKKAGQAKKKKGSYP YDVPDYA (SEQ ID NO: 11).

In one aspect, the present invention provides a vector. The vector includes a nucleic acid molecule encoding the site-specific FOXP3 disrupting agent of various embodiments of the above apsects or any other aspect of the invention delineated herein. In one embodiment, the vector is a viral expression vector.

In another aspect, the present invention provides a cell. The cell includes the site-specific FOXP3 disrupting agent or the vector of various embodiments of the above aspects or any other aspects of the invention delineated herein.

In one embodiment, the site-specific FOXP3 disrupting agent is present in a composition. In another embodiment, the composition comprises a pharmaceutical composition. In still another embodiment, the pharmaceutical composition comprises a lipid formulation. In yet another embodiment, the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of any of the foregoing. In one embodiment, the pharmaceutical composition comprises a lipid nanoparticle.

In one aspect, the present invention provides a method of modulating expression of forkhead box P3 (FOXP3) in a cell. The method includes contacting the cell with a site-specific FOXP3 disrupting agent, the disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, and an effector molecule, thereby modulating expression of FOXP3 in the cell.

In one embodiment, the modulation of expression is enhanced expression of FOXP3 in the cell. In another embodiment, the modulation of expression is reduced expression of FOXP3 in the cell. In still another embodiment, the site-specific FOXP3 targeting moiety comprises a polymeric molecule. In yet another embodiment, the polymeric molecule comprises a polyamide. In one embodiment, the polymeric molecule comprises a polynucleotide.

In another embodiment, the expression control region comprises a region upstream of FOXP3 transcription start site (TSS).

In still another embodiment, the expression control region comprises one or more FOXP3-associated anchor sequences within an anchor sequence-mediated conjunction comprising a first and a second FOXP3-associated anchor sequence. In yet another embodiment, the FOXP3-associated anchor sequence comprises a CCCTC-binding factor (CTCF) binding motif.

In one embodiment, the FOXP3-associated anchor sequence-mediated conjunction comprises one or more transcriptional control elements internal to the conjunction. In another embodiment, the

FOXP3-associated anchor sequence-mediated conjunction comprises one or more transcriptional control elements external to the conjunction.

In another embodiment, the anchor sequence is located within about 500 kb of the transcriptional control element. In another embodiment, the anchor sequence is located within about 300 kb of the transcriptional control element. In still another embodiment, the anchor sequence is located within 10 kb of the transcriptional control element.

In one embodiment, the expression control region comprises a FOXP3-specific transcriptional element. In another embodiment, the transcriptional element comprises a FOXP3 promoter. In still another embodiment, the transcriptional control element comprises transcriptional enhancer. In yet another embodiment, the transcriptional control element comprises a transcriptional repressor.

In another embodiment, the site-specific FOXP3 disrupting agent comprises a nucleotide sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide identity to the entire nucleotide sequence of any of the nucleotide sequences in Table 2.

In one embodiment, the site-specific FOXP3 disrupting agent comprises a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

In some embodiments, the DNA-binding domain of the TALE or ZNF comprises an amino acid sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of an amino acid sequence selected from the amino acid sequences listed in Table 1B.

In another embodiment, the site-specific FOXP3 disrupting agent comprises a nucleotide modification.

In still another embodiment, the polymeric molecule comprises a peptide nucleic acid (PNA).

In one embodiment, the effector molecule comprises a polypeptide. In another embodiment, the polypeptide comprises a fusion protein comprising the site-specific FOXP3 targeting moiety which targets a FOXP3 expression regulatory region, and the effector molecule. In still another embodiment, the fusion protein comprises a peptide-nucleic acid fusion molecule.

In another embodiment, the effector is selected from the group consisting of a nuclease, a physical blocker, an epigenetic recruiter, and an epigenetic CpG modifier, and combinations of any of the foregoing. In still another embodiment, the effector comprises a CRISPR associated protein (Cas) polypeptide or nucleic acid molecule encoding the Cas polypeptide. In yet another embodiment, the Cas polypeptide is an enzymatically inactive Cas polypeptide. In one embodiment, the effector further comprises a catalytically active domain of human exonuclease 1 (hEXO1).

In one embodiment, the epigenetic recruiter comprises a transcriptional enhancer or a transcriptional repressor.

In some embodiments, the transcriptional enhancer is a VPR.

In some embodiments, the VPR comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of

DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLSGGPKKKRK
VGSQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQP
YPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPV
LAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEF
QQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIA
DMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEGREVCQPKRLRPFHPPGSPWANRPLP
ASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVI
PQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHA
MHISTGLSIFDTSLF (SEQ ID NO: 64).

In some embodiments, the transcriptional enhancer comprises two, three, four, or five VPRs.

In some embodiments, the transcriptional enhancer is a p300.

In some embodiments, the p300 has an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of

IFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQY
QEPWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSP
QTLCCYGKQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRK
NDTLDPELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRL
GTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKA
LFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLE
YVKKLGYTTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYK
DIFKQATEDRLTSAKELPYFEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKN
AKKKNNKKTSKNKSSLSRGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANS
LPPIVDPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQD (SEQ ID NO: 65).

In another embodiment, the epigenetic CpG modifier comprises a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase.

In still another embodiment, the effector molecule comprises a zinc finger polypeptide.

In yet another embodiment, the effector molecule comprises a Transcription activator-like effector nuclease (TALEN) polypeptide.

In one embodiment, the fusion protein comprises an enzymatically inactive Cas polypeptide and an epigenetic recruiter polypeptide.

In another embodiment, the fusion protein comprises an enzymatically Cas polypeptide and an epigenetic CpG modifier polypeptide.

In some embodiments, the site-specific FOXP3 disrupting agent further comprises a second nucleic acid molecule encoding a second fusion protein, wherein the second fusion comprises a second site-specific FOXP3 targeting moiety which targets a second FOXP3 expression control region and a second effector molecule, wherein the second FOXP3 expression control region is different than the FOXP3 expression control region.

In one embodiment, the second effector is different than the first effector.

In one embodiment, the second effector is the same as the first effector.

In one embodiment, the fusion protein and the second fusion protein are operably linked.

In one embodiment, the fusion protein and the second fusion protein comprise an amino acid sequence that has at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas9-P300, and dCas9-VPR.

In one embodiment, the fusion protein is encoded by a polynucleotide comprising a nucleotide sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide sequence identity to the entire nucleotide sequence of a polynucleotide selected from the group consisting of dCas9-P300 mRNA, and dCas9-VPR mRNA.

In one aspect, the present invention provides a site-specific FOXP3 disrupting agent. The disrupting agent includes a nucleic acid molecule encoding a fusion protein, wherein the fusion protein comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas9-P300, and dCas9-VPR.

In one embodiment, the site-specific disrupting agent, the effector, or both the site-specific disrupting agent and the effector are present in a vector. In another embodiment, the site-specific disrupting agent and the effector are present in the same vector. In still another embodiment, the site-specific disrupting agent and the effector are present in different vectors. In yet another embodiment, the vector is a viral expression vector.

In one embodiment, the site-specific disrupting agent, the effector, or both the site-specific disrupting agent and the effector are present in a composition. In another embodiment, the site-specific disrupting agent and the effector are present in the same composition. In still another embodiment, the site-specific disrupting agent and the effector are present in different compositions. In yet another embodiment, the composition comprises a pharmaceutical composition. In one embodiment, the pharmaceutical composition comprises a lipid formulation. In another embodiment,

the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of any of the foregoing. In still another embodiment, the pharmaceutical composition comprises a lipid nanoparticle.

In another embodiment, the cell is a mammalian cell. In still another embodiment, the mammalian cell is a somatic cell. In yet another embodiment, the mammalian cell is a primary cell.

In one embodiment, the contacting is performed *in vitro*. In another embodiment, the contacting is performed *in vivo*. In still another embodiment, the contacting is performed *ex vivo*.

In one embodiment, the method further includes administering the cell to a subject.

In another embodiment, the cell is within a subject.

In still another embodiment, the subject has a FOXP3-associated disease. In yet another rembodiment, the FOXP3-associated disease is selected from the group consisting of IPEX syndrome (IPEX), type 1 diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

In one aspect, the present invention provides a method for treating a subject having a FOXP3-associated disease. The method includes administering to the subject a therapeutically effective amount of a site-specific FOXP3 disrupting agent, the disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, and an effector molecule, thereby treating the subject. In one embodiment, the FOXP3-associated disease is IPEX syndrome and the site-specific FOXP3 disrupting agent increases expression of FOXP3 in the subject. In another embodiment, the site-specific FOXP3 disrupting agent and the effector molecule are administered to the subject concurrently. In still another embodiment, the site-specific FOXP3 disrupting agent and the effector molecule are administered to the subject sequentially. In one embodiment, the effector molecule is administered to the subject prior to administration of the site-specific FOXP3 disrupting agent. In another embodiment, the site-specific FOXP3 disrupting agent is administered to the subject prior to administration of the effector molecule.

In various embodiments of the above aspects or any other aspects of the invention delineated herein, the cell is an immune cell. In one embodiment, the immune cell is a naïve T cell or a regulatory T cell (Treg). In another embodiment,

In one embodiment, the site-specific FOXP3 disrupting agent of the invention includes a first nucleotide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide identity to the entire nucleotide sequence of GD-28448, a second nucleotide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide identity to the entire nucleotide sequence of GD-28449, and a third nucleotide sequence having at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleotide identity to the entire nucleotide sequence of GD-28450.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are graphs depicting the activation of FOXP3 expression after contacting Jurkat cells with the indicated pools of site-specific FOXP3 targeting moieties and an effector molecule comprising dCas, dCas9 and p300, or dCas9 and VPR.

Figure 1A shows qPCR quantification of FOXP3 mRNA levels 48 hour after transfection with either dCas9 + sgRNA pools (1, 2, or 3) or with dCas9-p300 + sgRNA pools (1, 2, or 3) or with dCas9-VPR+ sgRNA pools (1, 2, or 3).

Figure 1B shows quantitation of a FACS experiment determining the percentage of Jurkat cells that were FOXP3 positive 72 hours post transfection. All transfections were carried out using Lipofectamine MessengerMax reagent (Thermofisher) following the manufacturer's instructions.

Figure 2 is graph depicting the activation of FOXP3 expression after contacting Jurkat cells with pools of site-specific FOXP3 targeting moieties and an effector molecule comprising dCas9 and p300, or dCas9 and VPR. Only the combination of sgRNA pool 2 and dCas9 + VPR showed significantly high FOXP3 activation of both mRNA level and protein level.

Figures 3A and 3B are graphs depicting activation of naïve T-cells after contacting the cells with the indicated pools of site-specific FOXP3 targeting moieties and an effector molecule comprising dCas9, dCas9 and p300, or dCas9 and VPR.

Figure 3A shows qPCR quantification of FOXP3 mRNA levels 58 hours after transfection with either dCas9 + sgRNA pool-2 or with dCas9-p300 + sgRNA pool-2 or with dCas9-VPR+ sgRNA pool-2. Figure 3B shows quantitation of the FACS experiment determining the percentage of naïve T-cells that were FOXP3 positive 72 hours post transfection. All transfections were carried out using the MaxCyte electroporation buffer and an ATx electroporation system according to the manufacturer's instructions. "Program T cell 2" and "Program T cell 3" are two of the electroporation settings on the instrument used for electroporating the T cells with mRNA+sgRNA for delivery into the cells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides agents and compositions for modulating the expression (*e.g.*, enhanced or reduced expression) of a forkhead box P3 (FOXP3) gene by targeting a FOXP3 expression control region. The FOXP3 gene may be in a cell, *e.g.*, a mammalian cell, such as a mammalian immune cell, *e.g.*, a mammalian naïve T cell, *e.g.*, a human or mouse naïve T cell. The present invention also provides methods of using the agents and compositions of the invention for modulating the expression of a FOXP3 gene in, and/or for treating, a subject who would benefit from modulating the expression of a FOXP3 gene, *e.g.*, a subject suffering or prone to suffering from an autoimmune disease.

The agents of the invention are referenced to herein as site-specific FOXP3 disrupting agents and are described in Section II, below.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element, *e.g.*, a plurality of elements, *e.g.*, a pool of elements, such as sgRNAs.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to". The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means ±10%. In certain embodiments, about means ±5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21 nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or integers, as logical from context, to zero. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range.

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 therefore be used in some embodiments herein to capture potential lack of completeness inherent in many biological and chemical phenomena.

As used herein, the term "forkhead box P3" or "FOXP3" refers to the gene that encodes the well known FOX protein family member that is a master transcription factor that controls the differentiation of naïve T-cells into regulatory T-cells (Tregs). FOX proteins belong to the forkhead/winged-helix family of transcriptional regulators and are believed to exert control via similar DNA binding interactions during transcription. In regulatory T cell model systems, the FOXP3 transcription factor occupies the promoters for genes involved in regulatory T-cell function, and may repress transcription of key genes following stimulation of T cell receptors. Defects in this gene's ability to function can cause immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (or IPEX), also known as X-linked autoimmunity-immunodeficiency syndrome, as well as numerous cancers. The nucleotide and amino acid sequence of FOXP3 is known and may be found in, for example, GenBank Accession Nos. NM_014009.4 and NM_001114377.2, the entire contents of each of which are incorporated herein by reference. The nucleotide sequence of the genomic region of the X Chromosome in human, which includes the endogenous promoters of FOXP3 and the FOXP3 coding sequence, is also known and may be found in, for example, NC_000023.11 (49250436-49264932). There are two common transcript variants for FOXP3 mRNA, the sequences of which can be found in GenBank Accession Nos. NM_014009.4 and NM_001114377.2. The entire contents of each of the foregoing GenBank Accession numbers are incorporated herein by reference as of the date of filing this application.

The term "site-specific FOXP3 disrupting agent," as used herein, refers to any agent that specifically binds to a target FOXP3 expression control region and, *e.g.,* modulates expression of a FOXP3 gene. Site-specific FOXP3 disruption agents of the invention may comprise a "site-specific FOXP3 targeting moiety."

As used herein, the term "site-specific FOXP3 targeting moiety" refers to a moiety that specifically binds to a FOXP3 expression control region, *e.g.,* a transcriptional control region of a FOXP3 gene, such as a DNA region around/proximally upstream of the transcription start site, a promoter, an enhancer, or a repressor; or a FOXP3-associated anchor sequence, such as, for example within a FOXP3-associated anchor sequence-mediated conjunction. Exemplary "site-specific FOXP3 targeting moieties" include, but are not limited to, polyamides, nucleic acid molecules, such as RNA, DNA, or modified RNA or DNA, polypeptides, protein nucleic acid molecules, and fusion proteins.

As used herein, the terms "specific binding" or "specifically binds" refer to an ability to discriminate between possible binding partners in the environment in which binding is to occur. In some embodiments, a disrupting agent that interacts, *e.g.,* preferentially interacts, with one particular target when other potential disrupting agents are present is said to "bind specifically" to the target (*i.e.,* the expression control region) with which it interacts. In some embodiments, specific binding is assessed by detecting or determining the degree of association between the disrupting agent and its target; in some embodiments, specific binding is assessed by detecting or determining degree of

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

As used herein, the term "expression control region" or expression control domain' refers to a region or domain present in a genomic DNA that modulates the expression of a target gene in a cell. A functionality associated with an expression control region may directly affect expression of a target gene, *e.g.*, by recruiting or blocking recruitment of a transcription factor that would stimulate expression of the gene. A functionality associated with an expression control region may indirectly affect expression of a target gene, *e.g.*, by introducing epigenetic modifications or recruiting other factors that introduce epigenetic modifications that induce a change in chromosomal topology that modulates expression of a target gene. Expression control regions may be upstream and/or downstream of the protein coding sequence of a gene and include, for example, transcriptional control elements, *e.g.*, DNA regions around/proximally upstream of the transcription start site, promoters, enhancers, or repressors; and anchor sequences, and anchor sequence-mediated conjunctions.

The term "transcriptional control element," as used herein, refers to a nucleic acid sequence that controls transcription of a gene. Transcriptional control elements include, for example, anchor sequences, anchor sequence-mediated conjunctions, DNA regions around/proximally upstream of the transcription start site, promoters, transcriptional enhancers, and transcriptional repressors.

A transcription start site (TSS) is the location where transcription starts at the 5'-end of a gene sequence. The DNA regions around/proximally upstream of the TSS can regulate the expression of a gene by, for example, recruiting a transcription factor. Alteration in the modification status of one or more nucleotides (*e.g.*, methylation) or one or more chromatin proteins (*e.g.*, acetylation) in the DNA regions around/proximally upstream of the TSS can regulate the expression of a gene.

A promoter is a region of DNA recognized by an RNA polymerase to initiate transcription of a particular gene and is generally located upstream of the 5'-end of the transcription start site of the gene.

A "transcriptional enhancer" increases gene transcription. A "transcriptional silencer" or "transcriptional repressor" decreases gene transcription. Enhancing and silencing sequences may be about 50-3500 base pairs in length and may influence gene transcription up to about 1 megabases away.

The term "gene," as used herein, refers to a sequence of nucleotides that encode a molecule, such as a protein, that has a function. A gene contains sequences that are transcribed (*e.g.*, a 3'UTR), sequences that are not transcribed (*e.g.*, a promoter), sequences that are translated (*e.g.*, an exon), and sequences that are not translated (*e.g.*, intron).

As used herein, the term "target gene" means a FOXP3 gene that is targeted for modulation, *e.g.*, increase or decrease, of expression. In some embodiments, a FOXP3 target gene is part of a

targeted genomic complex (*e.g.* a FOXP3 gene that has at least part of its genomic sequence as part of a target genomic complex, *e.g.* inside an anchor sequence-mediated conjunction), which genomic complex is targeted by one or more site-specific disrupting agents as described herein. In some embodiments, modulation comprises activation of expression of the target gene. In some embodiments, a FOXP3 gene is modulated by contacting the FOXP3 gene or a transcription control element operably linked to the FOXP3 gene with one or more site-specific disrupting agents as described herein. In some embodiments, a FOXP3 gene is aberrantly expressed (*e.g.*, over-expressed) in a cell, *e.g.*, a cell in a subject (*e.g.*, a subject having a FOXP3-associated disease or an autoimmune disease). In some embodiments, a FOXP3 gene is aberrantly expressed (*e.g.*, under-expressed) in a cell, *e.g.*, a cell in a subject (*e.g.*, a subject having a FOXP3-associated disease or an autoimmune disease).

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 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, 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 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 invention, 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 "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 other targeted anchor sequence is not modulated. In some embodiments, the anchor sequence comprises or is a nucleating polypeptide binding motif. In some embodiments, the anchor sequence is adjacent to a nucleating polypeptide binding motif.

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.

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 bind. In some embodiments, a genomic complex may comprise an anchor sequence-mediated conjunction. In some 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 region (*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 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 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, TFIIE, TFIIF, TFIIH, *etc.*), transcriptional regulators (*e.g.*, Mediator, P300, enhancer-binding proteins, repressor-binding proteins, histone modifiers, *etc.*), *etc.* In some embodiments, a genomic complex as 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 the stretch of genomic DNA does not adopt when the complex is not formed.

An "effector molecule," as used herein, refers to a molecule that is able to regulate a biological activity, such as enzymatic activity, gene expression, anchor sequence-mediated conjunction or cell signaling. Exemplary effectors are described in Section II, below, and in some embodiment include, for example, nucleases, physical blockers, epigenetic recruiters, *e.g.*, a transcriptional enhancer or a transcriptional repressor, and epigenetic CpG modifiers, *e.g.*, a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase, and combinations of any of the foregoing.

II. Site-Specific FOXP3 Disrupting Agents of the Invention

The present invention provides site-specific FOXP3 disrupting agents which, in one aspect of the invention, include a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region. In another aspect, the site-specific disrupting agents of the invention include a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region and an effector molecule. As will be appreciated by one of ordinary skill in the art, such disrupting agents are site-specific and, thus, specifically bind to a FOXP3 expression control region (*e.g.*, one or more transcriptional control elements and/or one or more target anchor sequences), *e.g.*, within a cell, and not to non-targeted expression control regions (*e.g.*, within the same cell).

FOXP3 is a master transcription factor that controls the differentiation of naïve T-cells into regulatory T-cells (Tregs) and forced overexpression of FOXP3 has been shown to confer the Treg phenotype to T-cells. The present invention features the use of effector molecules, *e.g.*, chromatin remodelers, that when fused to DNA-targeting moieties can induce epigenetic changes at specific genomic regions that lead to increased transcription of targeted genes, *e.g.*, FOXP3 gene. In certain embodiments, an effector molecule, p300-core or VPR, fused to dCas9, which is the DNA targeting moiety, is targeted to the FOXP3 locus using single guide RNAs (sgRNAs) complementary to the DNA region around/just upstream of the transcription start site (TSS) of a FOXP3 gene and provokes changes in histone acetylation. These epigenetic changes trigger mechanisms which ultimately result in activating FOXP3 in naïve T-cells and inducing differentiation to Tregs. These Tregs can be identified based on cell surface markers, such as CD127, and/or based on a suppression phenotype where Tregs kill effector T-cells incubated in a mixed culture.

In vitro generation of Tregs has been an important effort in the field of *ex vivo* therapy targeting auto-immune disorders. However, many of the strategies to produce Tregs either do not lead to sustained expression of genes that lead to Tregs or give Tregs that have a suppression phenotype. The present invention features methods to directly target the master regulator transcription factor in the Treg generation and maintenance pathway, FOXP3, using targeting moieties (*e.g.*, dCas9, TALEs or ZFP) to directly deliver an effector molecule (*e.g.*, an activator) to the site of action to increase activation of FOXP3 gene.

The site-specific FOXP3 disrupting agents of the invention comprise a site-specific FOXP3 targeting moiety targeting a FOXP3 expression control region. The expression control region targeted by the site-specific targeting moiety may be, for example, a transcriptional control element or an anchor sequence, such as an anchor sequence within an anchor-mediated conjunction.

Thus, site-specific FOXP3 disrupting agents of the invention may modulate expression of a gene, *i.e.*, FOXP3 , *e.g.*, by modulating expression of the gene from a DNA region around/proximally upstream of a transcription start site, an endogenous promoter, an enhancer, or an repressor; may alter

methylation of the control region; may alter acetylation of the chromatin protein, may introduce one or more mutations, *e.g.*, substitution, addition or deletion of nucleotide; may alter at least one anchor sequence; may alter at least one conjunction nucleating molecule binding site, such as by altering binding affinity for the conjunction nucleating molecule; may alter an orientation of at least one common nucleotide sequence, such as a CTCF binding motif by, *e.g.*, substitution, addition or deletion in at least one anchor sequence, such as a CTCF binding motif.

In certain embodiments, the site-specific disrupting agents and compositions described herein target an expression control region comprising one or more FOXP3-specific transcriptional control elements to modulate expression in a cell. FOXP3-specific transcriptional control elements that can be targeted include DNA region around or proximally upstream of FOXP3-transcription start site, FOXP3-specific promoter, FOXP3-specific enhancers, FOXP3-specific repressors, and FOXP3-associated anchor sequence. In one embodiment, FOXP3-specific transcriptional control element regulates expression in immune cells, *e.g.*, DNA region around or proximally upstream of FOXP3-transcription start site.

For example, a site-specific disrupting agent may include a site-specific targeting moiety, *e.g.*, a nucleic acid molecule encoding a DNA-binding domain of a Transcription activator-like effector (TALE) polypeptide or a zinc finger (ZNF) polypeptide, or fragment thereof, that specifically targets and binds to a FOXP3 expression control region, such as a FOXP3 endogenous promoter region, and an effector molecule, such as an effector molecule that includes a transcriptional enhancer or transcriptional repressor that modulates, e.g., enhances or represses, expression of a target gene from an endogenous promoter to modulate gene expression. In one embodiment, the disrupting agent is "bicistronic nucleic acid molecule," i.e., capable of making two fusion proteins from a single messenger RNA molecule, a first and a second site-specific targeting moiety, e.g., a nucleic acid molecule encoding a DNA-binding domain of a Transcription activator-like effector (TALE) polypeptide or a zinc finger (ZNF) polypeptide, or fragment thereof, that specifically targets and binds to a FOXP3 expression control region, such as a FOXP3 endogenous promoter region, and an effector molecule, such as an effector molecule that includes a transcriptional enhancer or transcriptional repressor that modulates, e.g., enhances or represses, expression of a target gene from an endogenous promoter to modulate gene expression.

In some embodiments of the invention, a site-specific disrupting agent may include a site-specific targeting moiety, *e.g.,* a nucleic acid molecule, such as a guide RNA targeting a FOXP3 endogenous DNA region around or proximally upstream of FOXP3 transcription starting site, and an effector molecule, such as an effector molecule that includes a transcriptional enhancer or transcriptional repressor that modulates, *e.g.,* enhances or represses, expression of a target gene from an endogenous promoter to modulate gene expression.

In certain embodiments of the invention, the site-specific disrupting agents and compositions described herein target an expression control region comprising one or more FOXP3-associated

anchor sequences, *e.g.,* within an anchor sequence-mediated conjunction, comprising a first and a second FOXP3-associated anchor sequence to alter a two-dimensional chromatin structure, *e.g.*, anchor sequence-mediated conjunctions in order to modulate expression in a cell, *e.g.,* a cell within a subject, *e.g.*, by modifying anchor sequence-mediated conjunctions in DNA, *e.g.*, genomic DNA.

In one aspect, the invention includes a site-specific FOXP3 disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region comprising one or more FOXP3-associated anchor sequences within an anchor sequence-mediated conjunction. The disrupting agent binds, *e.g.,* specifically binds, a specific anchor sequence-mediated conjunction to alter a topology of the anchor sequence-mediated conjunction, *e.g.*, an anchor sequence-mediated conjunction having a physical interaction of two or more DNA loci bound by a conjunction nucleating molecule.

The formation of an anchor sequence-mediated conjunction may force transcriptional control elements to interact with a FOXP3 gene or spatially constrain the activity of the transcriptional control elements. Altering anchor sequence- mediated conjunctions, therefore, allows for modulating FOXP3 expression without altering the coding sequences of the FOXP3 gene being modulated.

In some embodiments, the site-specific disrupting agents and compositions of the invention modulate expression of a FOXP3 gene associated with an anchor sequence-mediated conjunction by physically interfering between one or more anchor sequences and a conjunction nucleating molecule. For example, a DNA binding small molecule (*e.g.*, minor or major groove binders), peptide (*e.g.*, zinc finger, TALE, novel or modified peptide), protein (*e.g.*, CTCF, modified CTCF with impaired CTCF binding and/or cohesion binding affinity), or nucleic acids (*e.g.*, ssDNA, modified DNA or RNA, peptide oligonucleotide conjugates, locked nucleic acids, bridged nucleic acids, polyamides, and/or triplex forming oligonucleotides) may physically prevent a conjunction nucleating molecule from interacting with one or more anchor sequences to modulate FOXP3 gene expression.

In some embodiments, the site-specific disrupting agents and compositions of the invention modulate expression of a FOXP3 gene associated with an anchor sequence-mediated conjunction by modification of an anchor sequence, *e.g.*, epigenetic modifications, *e.g.,* histone protein modifications, or genomic editing modifications. For example, one or more anchor sequences associated with an anchor sequence-mediated conjunction comprising a FOXP3 gene may be targeted for genome editing, *e.g.*, Cas9-mediated genome editing.

In some embodiments, the site-specific disrupting agents and compositions of the invention modulate expression of a FOXP3 gene associated with an anchor sequence-mediated conjunction, *e.g.*, activate or represses transcription, *e.g.*, induces epigenetic changes to chromatin or genome editing.

In some embodiments, an anchor sequence-mediated conjunction includes one or more anchor sequences, a FOXP3 gene, and one or more transcriptional control elements, such as an enhancing or

silencing element. In some embodiments, the transcriptional control element is within, partially within, or outside the anchor sequence-mediated conjunction.

In one embodiment, the anchor sequence-mediated conjunction comprises a loop, such as an intra-chromosomal loop. In certain embodiments, the anchor sequence-mediated conjunction has a plurality of loops. One or more loops may include a first anchor sequence, a nucleic acid sequence, a transcriptional control element, and a second anchor sequence. In another embodiment, at least one loop includes, in order, a first anchor sequence, a transcriptional control element, and a second anchor sequence; or a first anchor sequence, a nucleic acid sequence, and a second anchor sequence. In yet another embodiment, either one or both of the nucleic acid sequences and the transcriptional control element is located within or outside the loop. In still another embodiment, one or more of the loops comprises a transcriptional control element.

In some embodiments, the anchor sequence-mediated conjunction includes a TATA box, a CAAT box, a GC box, or a CAP site.

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

In one aspect, the site-specific disrupting agents and compositions of the invention may introduce a targeted alteration to an anchor sequence-mediated conjunction to modulate expression of a nucleic acid sequence with a disrupting agent that binds the anchor sequence. In some embodiments, the anchor sequence-mediated conjunction is altered by targeting one or more nucleotides within the anchor sequence-mediated conjunction for substitution, addition or deletion.

In some embodiments, expression, *e.g.,* transcription, is activated by inclusion of an activating loop or exclusion of a repressive loop. In one such embodiment, the anchor sequence-mediated conjunction comprises a transcriptional control sequence that increases transcription of a nucleic acid sequence, *e.g.,* such a FOXP3 encoding nucleic acid. In another such embodiment, the anchor sequence-mediated conjunction excludes a transcriptional control element that decreases expression, *e.g.,* transcription, of a nucleic acid sequence, *e.g.,* such a FOXP3 encoding nucleic acid.

In some embodiments, expression, *e.g.,* transcription, is repressed by inclusion of a repressive loop or exclusion of an activating loop. In one such embodiment, the anchor sequence-mediated conjunction includes a transcriptional control element that decreases expression, *e.g.,* transcription, of a nucleic acid sequence, *e.g.,* such a FOXP3 encoding nucleic acid sequence. In another such embodiment, the anchor sequence-mediated conjunction excludes a transcriptional control sequence that increases transcription of a nucleic acid sequence, *e.g.,* such a FOXP3 encoding nucleic acid.

Each anchor sequence-mediated conjunction comprises one or more anchor sequences, *e.g.*, a plurality. Anchor sequences can be manipulated or altered to disrupt naturally occurring loops or form new loops (*e.g.*, to form exogenous loops or to form non-naturally occurring loops with exogenous or

altered anchor sequences). Such alterations modulate FOXP3 gene expression by changing the 2-dimensional structure of DNA containing all or a portion of a FOXP3 gene, *e.g.*, by thereby modulating the ability of the FOXP3 gene to interact with transcriptional control elements (*e.g.*, enhancing and silencing/repressive sequences). In some embodiments, the chromatin structure is modified by substituting, adding or deleting one or more nucleotides within an anchor sequence of the anchor sequence-mediated conjunction.

The anchor sequences may be non-contiguous with one another. In embodiments with noncontiguous anchor sequences, the first anchor sequence may be separated from the second anchor sequence by about 500bp to about 500Mb, about 750bp to about 200Mb, about lkb to about 100Mb, about 25kb to about 50Mb, about 50kb to about 1Mb, about l00kb to about 750kb, about 150kb to about 500kb, or about 175kb to about 500kb. In some embodiments, the first anchor sequence is separated from the second anchor sequence by about 500bp, 600bp, 700bp, 800bp, 900bp, lkb, 5kb, l0kb, 15kb, 20kb, 25kb, 30kb, 35kb, 40kb, 45kb, 50kb, 55kb, 60kb, 65kb, 70kb, 75kb, 80kb, 85kb, 90kb, 95kb, l00kb, 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.

In one embodiment, the 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 the 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 one embodiment, the anchor sequence comprises SEQ ID NO: 1 or SEQ ID NO:2 or a nucleotide sequence at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, 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, the anchor sequence-mediated conjunction comprises at least a first anchor sequence and a second anchor sequence. The first anchor sequence and second anchor sequence may each comprise a common nucleotide sequence, *e.g.*, each comprises a CTCF binding motif. In some embodiments, the first anchor sequence and second anchor sequence comprise different sequences, *e.g.*, the first anchor sequence comprises a CTCF binding motif and the second anchor sequence comprises an anchor sequence other than a CTCF binding motif. In some embodiments, each anchor sequence comprises a common nucleotide sequence and one or more flanking nucleotides on one or both sides of the common nucleotide sequence.

Two CTCF-binding motifs (*e.g.*, contiguous or non-contiguous CTCF binding motifs) that can form a conjunction may be present in the 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 the other comprises SEQ ID NO:2. CTCFBSDB 2.0: Database For CTCF binding motifs And Genome Organization (http ://insulatordb .uthsc . edu/) can be used to identify CTCF binding motifs associated with a target gene, *e.g.,* FOXP3.

In some embodiments, the anchor sequence-mediated conjunction is altered by changing an orientation of at least one common nucleotide sequence, *e.g.*, a conjunction nucleating molecule binding site.

In some embodiments, the anchor sequence comprises a conjunction nucleating molecule binding site, *e.g.*, CTCF binding motif, and site-specific disrupting agent of the invention introduces an alteration in at least one conjunction nucleating molecule binding site, *e.g.* altering binding affinity for the conjunction nucleating molecule.

In some embodiments, the anchor sequence-mediated conjunction is altered by introducing an exogenous anchor sequence. Addition of a non-naturally occurring or exogenous anchor sequence to form or disrupt a naturally occurring anchor sequence-mediated conjunction, *e.g.*, by inducing a non-naturally occurring loop to form that alters transcription of the nucleic acid sequence.

In some embodiments, the anchor sequence-mediated conjunction comprises a FOXP3 gene, and one or more, *e.g.*, 2, 3, 4, 5, or other genes other than the FOXP3 gene.

In some embodiments, the anchor sequence-mediated conjunction is associated with one or more, *e.g.*, 2, 3, 4, 5, or more, transcriptional control elements. In some embodiments, the FOXP3 gene is noncontiguous with one or more of the transcriptional control elements. In some embodiments where the FOXP3 gene is non-contiguous with the transcriptional control element, the gene may be separated from one or more transcriptional control elements by about l00bp to about 500Mb, about 500bp to about 200Mb, about lkb 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, the gene is separated from the transcriptional control element by about l00bp, 300bp, 500bp, 600bp, 700bp, 800bp, 900bp, lkb, 5kb, l0kb, 15kb, 20kb, 25kb, 30kb, 35kb, 40kb, 45kb, 50kb, 55kb, 60kb, 65kb, 70kb, 75kb, 80kb, 85kb, 90kb, 95kb, l00kb, 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.

In some embodiments, the type of anchor sequence-mediated conjunction may help to determine how to modulate gene expression, *e.g.*, choice of site-specific targeting moiety, by altering the anchor sequence- mediated conjunction. For example, some types of anchor sequence-mediated conjunctions comprise one or more transcription control elements within the anchor sequence -

mediated conjunction. Disruption of such an anchor sequence-mediated conjunction by disrupting the formation of the anchor sequence- mediated conjunction, *e.g.*, altering one or more anchor sequences, is likely to decrease transcription of a FOXP3 gene within the anchor sequence-mediated conjunction.

In some embodiments, expression of the FOXP3 gene is regulated, modulated, or influenced by one or more transcriptional control elements associated with the anchor sequence-mediated conjunction. In some embodiments, the anchor sequence-mediated conjunction comprises a FOXP3 gene and one or more transcriptional control elements. For example, the FOXP3 gene and one or more transcriptional control sequences are located within, at least partially, an anchor sequence-mediated conjunction, *e.g.*, a Type 1 anchor sequence-mediated conjunction. The anchor sequence-mediated conjunction may also be referred to as a "Type 1, EP subtype." In some embodiments, the FOXP3 gene has a defined state of expression, *e.g.*, in its native state, *e.g.*, in a diseased state. For example, the FOXP3 gene may have a high level of expression. By disrupting the anchor sequence-mediated conjunction, expression of the FOXP3 gene may be decreased, *e.g.*, decreased transcription due to conformational changes of the DNA previously open to transcription within the anchor sequence-mediated conjunction, *e.g.*, decreased transcription due to conformational changes of the DNA creating additional distance between the FOXP3 gene and the enhancing sequences. In one embodiment, both theFOXP3 gene associated and one or more transcriptional control sequences, *e.g.*, enhancing sequences, reside inside the anchor sequence-mediated conjunction. Disruption of the anchor sequence-mediated conjunction decreases expression of the FOXP3 gene. In one embodiment, the FOXP3 gene associated with the anchor sequence-mediated conjunction is accessible to one or more transcriptional control elements that reside inside, at least partially, the anchor sequence-mediated conjunction.

In some embodiments, expression of the FOXP3 gene is regulated, modulated, or influenced by one or more transcriptional control elements associated with, but inaccessible due to the anchor sequence- mediated conjunction. For example, the anchor sequence-mediated conjunction associated with a FOXP3 gene disrupts the ability of one or more transcriptional control elements to regulate, modulate, or influence expression of the FOXP3 gene. The transcriptional control sequences may be separated from the FOXP3 gene, *e.g.*, reside on the opposite side, at least partially, *e.g.*, inside or outside, of the anchor sequence-mediated conjunction as the FOXP3 gene, *e.g.*, the FOXP3 gene is inaccessible to the transcriptional control elements due to proximity of the anchor sequence-mediated conjunction. In some embodiments, one or more enhancing sequences are separated from the FOXP3 gene by the anchor sequence-mediated conjunction, *e.g.*, a Type 2 anchor sequence-mediated conjunction.

In some embodiments, the FOXP3 gene is inaccessible to one or more transcriptional control elements due to the anchor sequence-mediated conjunction, and disruption of the anchor sequence-mediated conjunction allows the transcriptional control element to regulate, modulate, or influence

expression of the FOXP3 gene. In one embodiment, the FOXP3 gene is inside and outside the anchor sequence-mediated conjunction and inaccessible to the one or more transcriptional control elements. Disruption of the anchor sequence- mediated conjunction increases access of the transcriptional control elements to regulate, modulate, or influence expression of the FOXP3 gene, *e.g.*, the transcriptional control elements increase expression of the FOXP3 gene. In one embodiment, the FOXP3 gene is inside the anchor sequence-mediated conjunction and inaccessible to the one or more transcriptional control elements residing outside, at least partially, the anchor sequence-mediated conjunction. Disruption of the anchor sequence-mediated conjunction increases expression of the FOXP3 gene. In one embodiment, the FOXP3 gene is outside, at least partially, the anchor sequence- mediated conjunction and inaccessible to the one or more transcriptional control elements residing inside the anchor sequence-mediated conjunction. Disruption of the anchor sequence - mediated conjunction increases expression of the FOXP3 gene.

A. FOXP3 Site-Specific Targeting Moieties

The site-specific FOXP3 targeting moieties of the invention target a FOXP3 expression control region and may comprise a polymer or polymeric molecule, such as a polyamide (*i.e.*, a molecule of repeating units linked by amide binds, *e.g.*, a polypeptide), a polymer of nucleotides (such as a guide RNA, a nucleic acid molecule encoding a TALE polypeptide or a zinc finger polypeptides), a peptide nucleic acid (PNA), or a polymer of amino acids, such as a peptide or polypeptide, *e.g.*, a fusion protein, *etc.* Suitable site-specific FOXP3 targeting moieties, compositions, and methods of use of such agents and compositions are described below and in PCT Publication WO 2018/049073, the entire contents of which are expressly incorporated herein by reference.

In one embodiment, a site-specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, such as a guide RNA (or gRNA) or a guide RNA and an effector, or fragment thereof, or nucleic acid molecule encoding an effector, or fragment thereof.

In another embodiment, a site specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule encoding a polypeptide, such as a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (i.e., a TALE DNA binding domain, or TALE) polypeptide, that is engineered to specifically target a FOXP3 expression control region to modulate expression of a FOXP3 gene.

In another embodiment, a site-specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a polynucleotide, such as a PNA, *e.g.*, a nucleic acid gRNA linked to an effector polypeptide, or fragment thereof.

In another embodiment, a site-specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a fusion molecule, such as a nucleic acid molecule

encoding a DNA-binding domain, of a Transcription activator-like effector (TALE) polypeptide or a zinc finger (ZNF) polypeptide, or fragment thereof, and an effector.

In one embodiment, such site-specific disrupting agents comprise a second fusion protein, wherein the second fusion protein comprises a second site-specific FOXP3 targeting moiety which targets a second FOXP3 expression control region and a second effector molecule, wherein the second FOXP3 expression control region is different than the FOXP3 expression control region.

In another embodiment, a site-specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a fusion molecule, such as a nucleic acid molecule encoding a fusion protein comprising a Cas polypeptide and, *e.g.*, an epigenetic recruiter or an epigenetic CpG modifier.

In yet, another embodiment, a site-specific disrupting agent of the invention comprises a site-specific FOXP3 targeting moiety comprising a fusion molecule, such as fusion protein comprising a Cas polypeptide and, *e.g.*, an epigenetic recruiter or an epigenetic CpG modifier.

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 a polynucleotide 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 embodiments, "nucleic acid" refers to a polynucleotide 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 a "mixmer" comprising locked nucleic acid molecules and deoxynucleic acid molecules. 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, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 20, 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.

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.

In certain embodiments, a polypeptide is or may comprise a chimeric or "fusion protein." As used herein, a "chimeric protein" or "fusion protein" comprises all or part (preferably a biologically active part) of a first protein operably linked to a heterologous second polypeptide (*i.e.*, a polypeptide other than the first protein). Within the fusion protein, the term "operably linked" is intended to indicate that the first protein or segment thereof and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the amino-terminus or the carboxyl-terminus of the first protein or segment.

A "polyamide" is a polymeric molecule with repeating units linked by amide binds. Proteins are examples of naturally occurring polyamides. In some embodiments, a polyamide comprises a peptide nucleic acid (PNA).

A "peptide nucleic acid" ("PNA") is a molecule in which one or more amino acid units in the PNA have an amide containing backbone, *e.g.*, aminoethyl-glycine, similar to a peptide backbone,

with a nucleic acid side chain in place of the amino acid side chain. Peptide nucleic acids (PNA) are known to hybridize complementary DNA and RNA with higher affinity than their oligonucleotide counterparts. This character of PNA not only makes them a stable hybrid with the nucleic acid side chains, but at the same time, the neutral backbone and hydrophobic side chains result in a hydrophobic unit within the polypeptide. The nucleic acid side chain includes, but is not limited to, a purine or a pyrimidine side chain such as adenine, cytosine, guanine, thymine and uracil. In one embodiment, the nucleic acid side chain includes a nucleoside analog as described herein.

In one embodiment, a site-specific FOXP3 targeting moiety of the invention comprises a polyamide. Suitable polyamides for use in the agents and compositions of the invention are known in the art.

In one embodiment, a site-specific FOXP3 targeting moiety of the invention comprises a polynucleotide. In some embodiments, the nucleotide sequence of the polynucleotide encodes a FOXP3 gene or a FOXP3 expression product. In some embodiments, the nucleotide sequence of the polynucleotide does not include a FOXP3 coding sequence or a FOXP3 expression product. For example, in some embodiments, a site-specific FOXP3 targeting moiety of the invention comprises a polynucleotide that hybridizes to a target expression control region, *e.g.,* a promoter, an anchor sequence, or a DNA region around or proximally upstream of the transcription starting site. In some embodiments, the nucleotide sequence of the polynucleotide is a complement of a target DNA region around or proximally upstream of the transcription starting site, or has a sequence that is at least 80%, at least 85%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to a complement of the target sequence.

The polynucleotides of the invention may include deoxynucleotides, ribonucleotides, modified deoxynucleotides, modified ribonucleotides (*e.g.*, chemical modifications, such as modifications that alter the backbone linkages, sugar molecules, and/or nucleic acid bases), and artificial nucleic acids. In some embodiments, the polynucleotide 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, mPvNA, rPvNA, modified RNA, miRNA, gRNA, and siRNA or other RNA or DNA molecules.

In some embodiments, the polynucleotides of the invention have a length from about 2 to about 5000 nts, about 10 to about 100 nts, about 50 to about 150 nts, about 100 to about 200 nts, about 150 to about 250 nts, about 200 to about 300 nts, about 250 to about 350 nts, about 300 to about 500 nts, about 10 to about 1000 nts, about 50 to about 1000 nts, about 100 to about 1000 nts, about 1000 to about 2000 nts, about 2000 to about 3000 nts, about 3000 to about 4000 nts, about 4000 to about 5000 nts, or any range therebetween.

The polynucleotides of the invention may include nucleosides, *e.g.*, purines or pyrimidines, *e.g.*, adenine, cytosine, guanine, thymine and uracil. In some embodiments, the polynucleotides include one or more nucleoside analogs. The nucleoside analog includes, but is not limited to, a nucleoside analog, such as 5-fluorouracil; 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 4- methylbenzimidazole, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5- carboxymethylaminomethyluracil, dihydrouracil, dihydrouridine, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2- methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5- methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'- methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4- thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2- thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, 3-nitropyrrole, inosine, thiouridine, queuosine, wyosine, diaminopurine, isoguanine, isocytosine, diaminopyrimidine, 2,4- difluorotoluene, isoquinoline, pyrrolo[2,3-]pyridine, and any others that can base pair with a purine or a pyrimidine side chain.

In some embodiments, the site-specific FOXP3 targeting moieties of the invention comprising a polynucleotide encoding a polypeptide that comprises a DNA-binding domain (DBD), or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide, that is engineered to specifically target a FOXP3 expression control region to modulate expression of a FOXP3 gene.

The design and preparation of such zinc finger polypeptides which specifically bind to a DNA target region of interest, such as a FOXP3 expression control region, is well known in the art. For example, zinc finger (ZNF) proteins contain a DNA binding motif that specifically binds a triplet of nucleotides. Thus to design and prepare the site-specific FOXP3 targeting moieties of the invention, a modular assembly process which includes combining separate zinc finger DNA binding domains that can each recognize a specific 3-basepair DNA sequence to generate 3-finger, 4-, 5-, 6-, 6-, or 8- zinc finger polypeptide that recognizes specific target sites ranging from 9 basepairs to 24 basepairs in length may be used. Another suitable method may include 2-finger modules to generate ZNF polynucleotides with up to six individual zinc fingers. See, *e.g.*, Shukla VK, *et al., Nature*. 459 (7245) 2009: 437–41; Dreier B, *et al., JBC*. 280 (42) 2005: 35588–97; Dreier B, *et al, JBC* 276 (31) 2001: 29466–78; Bae KH, et al., Nature Biotechnology. 21 (3) 2003: 275–80.

In some embodiments, a site-specific FOXP3 targeting moiety of the invention comprises a polynucleotide encoding a polypeptide that comprises a DNA-binding domain (DBD), or fragment thereof, of a zinc finger, that is engineered to specifically target a FOXP3 expression control region to modulate expression of a FOXP3 gene. Exemplary amino acid sequences encoding a zinc finger that

binds to a nucleotide triplet suitable for use in the present invention are provide in Table 1A below. (See, e.g., Gersbach et al., Synthetic Zinc Finger Proteins: The Advent of Targeted Gene Regulation and Genome Modification Technologies).

Table 1A.

Amino Acid Sequence of Zing Finger DNA Binding Domain (Finger)	Nucleotide Triplet	SEQ ID NO:
RKDALRG	TTG	12
TTGALTE	CTT	13
QRHHLVE	CTC	14
QNSTLTE	CTA	15
RNDALTE	CTG	16
HKNALQN	ATT	17
RRSACRR	ATC	18
QKSSLIA	ATA	19
RRDELNV	ATG	20
TSGSLVR	GTT	21
DPGALVR	GTC	22
QSSSLVR	GTA	23
RSDELVR	GTG	24
RLRDIQF	TCT	25
RSDERKR	TCC	26
RSDHLTT	TCA	27
RLRALDR	TCG	28
TKNSLTE	CCT	29
SKKHLAE	CCC	30
TSHSLTE	CCA	31
RNDTLTE	CCG	32
THLDLIR	ACT	33
DKKDLTR	ACC	34
SPADLTR	ACA	35
RTDTLRD	ACG	36
TSGELVR	GCT	37
DCRDLAR	GCC	38
QSGDLRR	GCA	39
RSDDLVR	GCG	40
ARGNLRT	TAT	41
SRGNLKS	TAC	42
QASNLIS	TAA	43
REDNLHT	TAG	44
TSGNLTE	CAT	45
SKKALTE	CAC	46
QSGNLTE	CAA	47
RADNLTE	CAG	48
TTGNLTV	AAT	49
DSGNLRV	AAC	50
QRANLRA	AAA	51
RKDNLKN	AAG	52

Amino Acid Sequence of Zing Finger DNA Binding Domain (Finger)	Nucleotide Triplet	SEQ ID NO:
TSGNLVR	GAT	53
DPGNLVR	GAC	54
QSSNLVR	GAA	55
RSDNLVR	GAG	56
APKALGW	TGC	57
QAGHLAS	TGA	58
RSDHLTT	TGG	27
SRRTCRA	CGT	59
HTGHLLE	CGC	60
QSGHLTE	CGA	66
RSDKLTE	CGG	67
HRTTLTN	AGT	68
ERSHLRE	AGC	69
QLAHLRA	AGA	70
RSDHLTN	AGG	71
TSGHLVR	GGT	72
DPGHLVR	GGC	73
QRAHLER	GGA	74
RSDKLVR	GGG	75

A zinc finger DNA binding domain comrpises an N-terminal region and a C-terminal region with the "fingers" that bind to the target DNA sequence in between. The N-terminal region generally is 7 amino acids in length. The C-terminal region is generally 6 amino acids in length. Thus, the N-terminal region generally comprises the amino acid sequence of $X_1X_2X_3X_4X_5X_6X_7$. "X" can be any amino acid. In some embodiments, the N-terminal region comprises the exemplary amino acid sequence of LEPGEKP (SEQ ID NO: 76). "X" can be any amino acid. The C-terminal region generally comprises the amino acid sequence of $X_{25}X_{26}X_{27}X_{28}X_{29}X_{30}$. In certain embodiments, the C-terminal region comprises the exemplary amino acid sequence of TGKKTS (SEQ ID NO: 77).

Each finger in the DNA binding domain is flanked by a N-terminal backbone located to the N-terminus of the finger and a C-terminal backbone located to the C-terminus of the finger. The N-terminal backbone of the finger generally is 11 amino acids long with two conservative cysteines (C) locate at 3rd and 6th positions. Thus, the N-terminal backbone of the finger generally comprises the amino acid sequence of $X_8X_9CX_{10}X_{11}CX_{12}X_{13}X_{14}X_{15}X_{16}$. "X" can be any amino acid. The C-terminal backbone of the finger generally is 5 amino acids long with two conservative histines (H) located at 1st and 5th positions. Thus, the C-terminal backbone of the finger generally comprises the amino acid sequence of $HX_{17}X_{18}X_{19}H$. "X" can be any amino acid. In some embodiments, the N-terminal backbone comprises the exemplary amino acid sequence of YKCPECGKSFS (SEQ ID No: 61) and the C-terminal backbone comprises the exemplary amino acid sequence of HQRTH (SEQ ID No: 62). Two "fingers" are linked through a linker. A linker generally is 5 amino acids in length and

comprises the amino acidsequence of $X_{20}X_{21}X_{22}X_{23}X_{24}$. "X" can be any amino acid. In certain embodiments, the linker comprises the exemplary amino acid sequence of TGEKP (SEQ ID No: 63). Thus, the zinc finger of a site specific FOXP3 site-specific disrupting agent has a structure as follows: (N-terminal backbone – finger – C-terminal backbone – linker)$_n$ and the zinc finger DNA binding domain of a site specific FOXP3 site-specific disrupting agent has a structure as follows: [N-terminal region (N-terminal backbone – finger – C-terminal backbone – linker)$_n$– C-terminal region]. "N" represents the number of triplets of nucleotides to which the zinc finger DNA binding domain and, thus, to which the FOXP3 site-specific disrupting agent binds.

The "finger" amino acid sequences of four nucleotide triplets are unknown, however, if such a triplet is identified in a target area of interest, two "linker span sequences" – linker span 1 and linker span 2 – are useful to circumvent the issue. Linker span 1 is used to skip one base pair if a "finger" amino acid sequence of a triplet is not available. Linker span 2 is used to skip 2 base pairs if a "finger" amino acid sequence of a triplet is not available. Linker span 1 is generally 12 amino acids long. Linker span 2 is generally 16 amino acids long. Thus, linker span 1 generally comprises the amino acid sequence of $X_{31}X_{32}X_{33}X_{34}X_{35}X_{36}X_{37}X_{38}X_{39}X_{40}X_{41}X_{42}$. Linker span 2 generally comprises the amino acid sequence of $X_{43}X_{44}X_{45}X_{46}X_{47}X_{48}X_{49}X_{50}X_{51}X_{52}X_{53}X_{54}X_{55}X_{56}X_{57}X_{58}$. In some embodiments, linker span 1 comprises the amino acid sequence of THPRAPIPKPFQ (SEQ ID NO: 78). In certain embodiments, linker span 2 comprises the amino acid sequence of TPNPHRRTDPSHKPFQ (SEQ ID NO: 79). When linker span 1 and/or linker span 2 is used, the finger – linker span 1/ span 2 – finger comprises the structure as follows: N-terminal back bone – finger – C-terminal backbone – linker span 1 /span 2 – N-terminal backbone – finger – C-terminal backbone – linker.

Table 1B provides the amino acid sequence of exemplary zinc finger DNA binding domains for use in the present invention and their corresponding target regions.

In some embodiments, a zinc finger DNA binding domain suitable for use in the disrupting agents of the invention comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% amino acid identity to the entire amino acid sequence of any one of the zinc finger DNA binding domains provided in Table 1B.

Table 1B.

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cacTcaccttg gtgaagtggac	80	LEPGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSTTGALTEHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGKKTS	609
+	accTtggtgaa gtggactgaca	81	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECG KSFSQRAHLERHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQAGHLASHQR THTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	610
+	ccttggtgaagt ggactgaca	82	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECG KSFSQRAHLERHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQAGHLASHQR THTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	611
+	tggtgaagtgga ctgacagaa	83	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSHRTTLTNHQR THTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGKKTS	612
+	tgaagtggactg acagaaaag	84	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQAGHLASHQR THTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	613
+	agtggactgaca gaaaaggat	85	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRAHLERHQR THTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGKKTS	614
+	ggactgacaga aaaggatcag	86	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECG KSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRAHLERHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGKKTS	615
+	ctgacagaaaa ggatcagcct	87	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	616
+	acagaaaagga tcagcctggc	88	LEPGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQR THTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGKKTS	617
+	gaaaaggatca gcctggcTTgt g	89	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPNPHRRTDPSHKPFQYKCPECGKSFSFSDPGHLVRHQRTHT GEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSF STSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHT GKKTS	618
+	aaggatcagcct ggcTTgtggg a	90	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSF SRADNLTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTH TGKKTS	619

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	gatcagcctggcTTgtgggaaac	91	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSKSFSRSDELVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGKKTS	620
+	cagcctggcTTgtgggaaacTgtc	92	LEPGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	621
+	cctggcTTgtgggaaacTgtcacg	93	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	622
+	gtgaagtggactgacagaaaa	94	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	623
+	aagtggactgacagaaaagga	142	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDHLTTHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	624
+	tggactgacagaaaaggaTcag	143	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGKKTS	625
+	actgacagaaaaggaTcagcct	144	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGKKTS	626
+	gacagaaaaggaTcagcctggc	145	LEPGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGKKTS	627
+	agaaaaggaTcagcctggcTTgtg	146	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQLAHLRAHQRTHTGKKTS	628
+	aaaggaTcagcctggcTTgtggga	147	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGKKTS	629
+	ggaTcagcctg	148	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECG	630

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	gcTTgtggga aac		KSFSRSDELVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFS TKNSLTEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERH QRTHTGKKTS	
+	agcctggctTgt gggaaacTgtc	149	LEPGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPY KCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKS FSTSGELVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSERSHLREHQRTH TGKKTS	631
+	ctggctTgtgg gaaacTgtcac g	150	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQY KCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDE LVRHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTH TGKKTS	632
+	gcctggcttgtg ggaaacTgtc	151	LEPGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPY KCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTTGA LTEHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGKKTS	633
+	tggcttgtggga aacTgtcacg	152	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQY KCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDE LVRHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGKKTS	634
+	gggaaactgTc acgtaTcaaaa a	153	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKS FSRNDALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRT HTGKKTS	635
+	aaactgTcacg taTcaaaaaca a	154	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSSKKA LTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRT HTGKKTS	636
+	ctgTcacgtaT caaaaacaactt	155	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSS LVRHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPY KCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	637
+	cacgtaTcaaa aacaacttTgct	156	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTGEKPY KCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGN LTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTH TGKKTS	638
+	cttTataccga gaagaaaaacc	157	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRNDTLTEHQR THTGEKPYKCPECGKSFSQKSSLIAHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTG KKTS	639

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	ataccgagaag aaaaaccacg	158	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSSLIAHQRTHTGKKTS	640
+	ccgagaagaaa aaccacgctg	159	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGKKTS	641
+	agaagaaaaac cacgctgTacg	160	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTGEKPY KCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRAN LRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKT S	642
+	agaaaaaccac gctgTacggtg	161	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQY KCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSQRAN LTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKT S	643
+	aaaaccacgct gTacggtgtgg	162	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQRAN LRDHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKT S	644
+	accacgctgTa cggtgtggaag	163	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSRNDA LTEHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	645
+	acgctgTacgg tgtggaagccg	164	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRTDTLRDHQR THTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGKKTS	646
+	ctgTacggtgt ggaagccgcag	165	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECG KSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQR THTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTGKKTS	647
+	accgagaagaa aaaccacgct	166	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQ RTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	648
+	gagaagaaaaa ccacgctgta	167	LEPGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGK SFSSKKALTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRT HTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	649
+	aagaaaaacca cgctgtacgg	168	LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGK SFSTSGELVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRT HTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	650
+	aaaaaccacgct	169	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTGEKPY	651

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	gtacggTgtg		KCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSSKKAL	
			TEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	
			LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY	
	aaccacgctgta		KCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSTSGEL	
+	cggTgtggaa	170	VRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKTS	652
			LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG	
	cacgctgtacgg		KSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSL	
+	Tgtggaagcc	171	VRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGKKTS	653
			LEPGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECG	
	gctgtacggTg		KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDK	
+	tggaagccgca	172	LTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGKKTS	654
			LEPGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECG	
	gtacggTgtgg		KSFSDCRDLARHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQR	
+	aagccgcagac	173	THTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGKKTS	655
			LEPGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSHTGHLLEHQRTHTGEKPY	
	cgagaagaaaa		KCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNL	
+	accacgcTgta	174	VRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGKKTS	656
			LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQY	
	gaagaaaaacc		KCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANL	
+	acgcTgtacgg	175	RAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	657
			LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTGEKPY	
	gaaaaacccacg		KCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKS	
	cTgtacggTgt		FSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTH	
+	g	176	TGKKTS	658
			LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY	
	aaaccacgcTg		KCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKS	
	tacggTgtgga		FSHTGHLLEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTH	
+	a	177	TGKKTS	659
			LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG	
	ccacgcTgtac		KSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQSSSL	
	ggTgtggaag		VRHQRTHTHPRAPIPKPFQYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHT	
+	cc	178	GKKTS	660
			LEPGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECG	
	cgcTgtacgg		KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDK	
	Tgtggaagccg		LTEHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSHTGHLLEHQRTH	
+	ca	179	TGKKTS	661
+	acggtggaa	180	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG	662

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	gccgcagacc		KSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGKKTS	
+	cgatgagtgTg tgcgctgaTaat	181	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTGEKPY KCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKS FSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTH TGKKTS	663
+	tgagtgTgtgc gctgaTaatca c	182	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTHPRAPIPKPFQY KCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRSDE LVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRT HTGKKTS	664
+	gtgTgtgcgct gaTaatcacgg g	183	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSHTGH LLEHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTH TGKKTS	665
+	gatgagTgtgT gcgctgataAT cac	184	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTG EKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTHPRAPIPKPFQYKCPE CGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTS GNLVRHQRTHTGKKTS	666
+	gagTgtgTgc gctgataATca cggg	185	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFS RSDDLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRS DNLVRHQRTHTGKKTS	667
+	atgagtgtgTg cgctgataATc ac	186	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTG EKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTHPRAPIPKPFQYKCPE CGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRRDELNVH QRTHTGKKTS	668
+	agtgtgTgcgc tgataATcacg gg	187	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFS RSDDLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSHRTTLTNH QRTHTGKKTS	669
+	gtgTgcgctga taATcacggg gtg	188	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFS RNDALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRH QRTHTGKKTS	670
+	gtgcgctgaTa atcacggggtg	189	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGH LASHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	671

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cgctgaTaatc acggggtgggg	190	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGKKT S	672
+	tgaTaatcacg gggtgggggg g	191	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSTTGNLTVHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTGKKT S	673
+	gcgctgataAT cacgggtggg g	192	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTG KKTS	674
+	ctgataATcac ggggtggggg gg	193	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTG KKTS	675
+	ataATcacgg ggtgggggg ggt	194	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTG KKTS	676
+	gctgataatcac ggggtgggg	195	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGKKTS	677
+	gataatcacggg gtgggggggg	196	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGKKTS	678
+	aatcacgggt gggggggggt	197	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGKKTS	679
+	acgggtgggg ggggttCTc at	198	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTG EKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFS RSDHLTTHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTG KKTS	680
+	gggtgggggg gggttCTcata gt	199	LEPGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFS SDKLVRHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGK KTS	681

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cttTTcttgatT atgagacttaaa	200	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGN LVRHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTTGALTE HQRTHTGKKTS	682
+	cttgatTatgag acttaaacgg	201	LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSTTGALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGKKTS	683
+	gatTatgagact taaacggaaa	202	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTGKKTS	684
+	tgaTTatgaga cttaaacggaaa	203	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSRRDELNVHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQAGHLASHQRTHT GKKTS	685
+	attatgagactta aacggaaa	204	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGKKTS	686
+	aacggaaaatTT tgaaatTTtgg gtt	205	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQ YKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSDSG NLRVHQRTHTGKKTS	687
+	acggaaattTtg aaatTTtgggtt	206	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPE CGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRTDTLRDH QRTHTGKKTS	688
+	cttTgccttTa cgagtcatctg	207	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG KSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGA LTEHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTH TGKKTS	689
+	gccctTacga gtcatctgaaa	208	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRTDTLRDHQR THTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGKKTS	690
+	cttTacgagtca tctgaaaata	209	LEPGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSHRTTLTNHQR THTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTGKKTS	691
+	cctTTacgagt catctgaaaata	210	LEPGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSHRTTLTNHQR	692

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			THTGEKPYKCPECGKSFSRTDTLRDHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHT GKKTS	
+	acgagtcatctg aaaatatga	211	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQR THTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTGKKTS	693
+	tgaaaaTatgat tTcttcccCTc ac	212	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHT GEKPYKCPECGKSFSTTGALTEHQRTHTHIPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKC PECGKSFSRRDELNVHQRTHTHIPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFS QAGHLASHQRTHTGKKTS	694
+	aaaTatgattTc ttcccCTcacc ac	213	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTHIPRAPIPKPFQYKCPE CGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTHIPRAPIPKPFQYKCPECGKSFSQ RANLRAHQRTHTGKKTS	695
+	gaaaatatgatt TcttcccCTca c	214	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHT GEKPYKCPECGKSFSTTGALTEHQRTHTHIPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKC PECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQSSNLV RHQRTHTGKKTS	696
+	aatatgattTctt cccCTcacca c	215	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTHIPRAPIPKPFQYKCPE CGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTTGNLTV HQRTHTGKKTS	697
+	atgattTcttccc CTcaccacag a	216	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSKKHLAEHQRTHTGEKPYKCPECGKSFS TTGALTEHQRTHTHIPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRRDELNV HQRTHTGKKTS	698
+	attTcttcccCT caccacagagg t	217	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFS SKKHLAEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSG HQRTHTGKKTS	699
+	gatTTcttccc CTcaccacag aggt	218	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFS SKKHLAEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSG NLVRHQRTHTGKKTS	700
+	cttcccCTcac cacagaggtga g	219	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHT GKKTS	701

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cccCTcacca cagaggtgaga gg	220	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHT GKKTS	702
+	cccTcaccaca gaggtgagagg	221	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGKKTS	703
+	cctcaccacaga ggtgagagg	222	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	704
+	accacagaggt gagaggtATc aa	223	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSH EKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFS RSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTG KKTS	705
+	acagaggtgag aggtATcaatg a	224	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KPFQYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSF SRSDELVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHT GKKTS	706
+	gaggtgagagg tATcaatgaga t	225	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFS QLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHT GKKTS	707
+	gtgagaggtA Tcaatgagata at	226	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHT STSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHT GKKTS	708
+	agaggtATca atgagataatag g	227	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHT GKKTS	709
+	ggtATcaatga gataatatggct	228	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGNLVRHQR THTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGHLVRHQRTHT GKKTS	710
+	ccacagaggtg agaggtaTcaa	229	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGEKPY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDH LTNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGKKTS	711

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cagaggtgaga ggtaTcaatga	230	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQAGH LASHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	712
+	aggtgagaggt aTcaatgagat	231	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNL VRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	713
+	tgaggaggtaTc aatgagataat	232	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKT S	714
+	gaggtaTcaat gagataatagg	233	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	715
+	gtaTcaatgag ataatagggct	234	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVRHQRTHTGKKTS	716
+	caatgagataat agggctcat	235	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQR THTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS	717
+	tgagataatagg gctcatgag	236	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG KSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	718
+	gataatagggct catgagaaa	237	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRT HTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGKKTS	719
+	aatagggctcat gagaaacca	238	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQR THTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTSGNLVTVHQRTHTGKKTS	720
+	agggctcatga gaaaccacag	239	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQR THTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	721
+	aatgagataata gggCTcatga g	240	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG PFQYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQ KSSLIAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGK KTS	722
+	gagataatagg gCTcatgaga	241	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFS	723

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	aa		QKSSLIAHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGK KTS	
+	ataataggaCT catgagaaacc a	242	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFS RSDKLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGK KTS	724
+	ataggCTcat gagaaaccaca g	243	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTG KKTS	725
+	atgagaTaata gggctcatgag	244	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG KSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTHPRAPIPKPFQYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGKKT S	726
+	agaTaataggg ctcatgagaaa	245	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRT HTGEKPYKCPECGKSFSTTGNLTVHQRTHTHPRAPIPKPFQYKCPECGKSFSQLAHLRAHQRTHTGKKTS	727
+	tagggcTcatg agaaaccacag	246	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGNLTEHQR THTHPRAPIPKPFQYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGKKT S	728
+	aaaacaaaagt gTatagagTTt ga	247	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRSDNLVRHQRTHT GEKPYKCPECGKSFSQKSSLIAHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTHTGEKPYKCP ECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRANLRA HQRTHTGKKTS	729
+	acaaaagtgTa tagagTTtgaa aa	248	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTHPRAPIPKPFQYKCP ECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSPADLTR HQRTHTGKKTS	730
+	aaagtgTatag agTTtgaaaaa aa	249	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKS FSQKSSLIAHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQRANLR AHQRTHTGKKTS	731
+	gtgTatagagT Ttgaaaaaaaa aa	250	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKS FSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVR HQRTHTGKKTS	732

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	aaacaaaaagTg tatagagtTtga	251	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPY KCPECGKSFSREDNLHTHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKS FSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS HTGKKTS	733
+	caaaagTgtat agagtTtgaaa a	252	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQ YKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGEKPYKCPECGKSFSQSSS LVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRT HTGKKTS	734
+	aagTgtataga gtTtgaaaaaa a	253	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRED NLHTHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRKDNLKNHQR THTGKKTS	735
+	aacaaaagtgta tagagtTtga	254	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRED NLHTHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRKDNLKNHQR TNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKTS	736
+	aaaagtgtatag agtTtgaaaa	255	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQ YKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGEKPYKCPECGKSFSQSSS LVRHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKT S	737
+	agtgtatagagt Ttgaaaaaaa	256	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRED NLHTHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRKDNLKNHQR TTLTNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKT S	738
+	gtatagagtTtg aaaaaaaaaa	257	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHR TTLTNHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGEKPYKCPECGKSFSQSSSLVRHQRTHTGKK TS	739
+	tagagtTtgaaa aaaaaaaaac	258	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQ RTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGKKT S	740
+	agtTtgaaaaa aaaaaaacaag	259	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSQAGHLASHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGKK TS	741
+	atagagTTtga aaaaaaaaaaa	260	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQ	742

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	c		RTHTPNPHRRTDPSHKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHT GKKTS	
+	gagTTtgaaaa aaaaaaaacaa g	261	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTH TGKKTS	743
+	agagtttgaaaa aaaaaaaac	262	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGKKTS	744
+	gtttgaaaaaaa aaaaacaag	263	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	745
+	tgaaaaaaaaaa aacaaggga	264	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	746
+	aaaaaaaaaaaa caagggaaaa	265	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECG KSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	747
+	aaaaaaaaacaa gggaaaagaa	266	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSDSGNLRVHQ RTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	748
+	aaaaacaaggg aaaagaacta	267	LEPGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	749
+	aacaaggaaa agaactaaaa	268	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKP YKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRA NLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKK TS	750
+	aagggaaaaga actaaaaTaaa	269	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTHPRAP IPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKS FSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTH TGKKTS	751
+	ggaaaagaact aaaaTaaaTca c	270	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGK	752
+	aaagaactaaaa TaaaTcacag	271	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGK	753

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	g		SFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRT HTGKKTS	
+	gaactaaaaTa aaTcacagggc c	272	LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGK SFSQRANLRAHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRT HTGKKTS	754
+	ctaaaaTaaaT cacagggccaa c	273	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAN LRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRT HTGKKTS	755
+	aaaTaaaTcac agggccaaccc g	274	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRT HTGKKTS	756
+	gaaaaaaaaaa aacaagggaa	275	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	757
+	aaaaaaaaaac aagggaaaag	276	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	758
+	aaaaaaaacaag ggaaaagaac	277	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSPADLTRHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	759
+	aaaacaaggga aaagaacTaaa	278	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKP YKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSD HLTNHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKK TS	760
+	acaaggaaaa gaacTaaaata	279	LEPGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQY KCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSN LVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSPADLTRHQRTHTGKKTS	761
+	agggaaaagaa cTaaaataaat	280	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGK SFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSRKDN LKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	762
+	gaaaagaacTa aaataaatcac	281	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGN LRVHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKT S	763

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	aagaacTaaaa taaatcacagg	282	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKT S	764
+	aacTaaaataa atcacagggcc	283	LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHILTNHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRT HTGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGKKTS	765
+	aaaaaaaaaaa acaagggaaa	284	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	766
+	aaaaaaaaaca agggaaaaga	285	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	767
+	aaaaaaacaagg gaaaagaact	286	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	768
+	aaacaagggaa aagaactaaa	287	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQ RTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	769
+	caagggaaaag aactaaaata	288	LEPGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS	770
+	gggaaaagaac taaaataaat	289	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGK SFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRT HTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGKKTS	771
+	aaaagaactaaa ataaatcac	290	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRT HTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	772
+	agaactaaaata aatcacagg	291	LEPGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	773
+	actaaaataaat cacagggcc	292	LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHILTNHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRT HTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGKKTS	774
+	aaaataaatcac agggccaac	293	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	775

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	ataaatcacagg gccaacccg	294	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGKKTS	776
+	aatcacagggc caacccgagg	295	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGKKTS	777
+	cacagggccaa cccgaggcag	296	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQR THTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGKKTS	778
+	agggccaaccc gaggcaggca	297	LEPGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQR THTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	779
+	gccaacccgag gcaggcagag	298	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRNDTLTEHQR THTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGKKTS	780
+	aacccgaggca ggcagagaca	299	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQR THTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKTS	781
+	ccgaggcaggc agagacacca	300	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGK SFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRT HTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGKKTS	782
+	aggcaggcaga gacaccaTTct g	301	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSHSLTEHQRTHTG EKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFS QSGDLRRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHT GKKTS	783
+	caggcagagac accaTTctgtg a	302	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFS RSDNLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHT GKKTS	784
+	gcagagacacc aTTctgtgagt g	303	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFS SPADLTRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTG KKTS	785
+	gagacaccaT Tctgtgagtga ga	304	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSF STSHSLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHT GKKTS	786

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	acaccaTTctg tgagtgagagg a	305	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTG KKTS	787
+	ccaTTctgtga gtgagaggaTa tt	306	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKP YKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAG HLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSHSLTE HQRTHTGKKTS	788
+	aataaaTcaca gggccaacccg	307	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSSKKALTEHQR THTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGKKT S	789
+	aaaTcacaggg ccaacccgagg	308	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDHLTNHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGKKT S	790
+	acagggccaac ccgaggcagg	309	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECG KSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSTSHSLTEHQR THTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGKKTS	791
+	gggccaacccg aggcaggcag	310	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECG KSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQR THTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGKKTS	792
+	ccaacccgagg caggcagaga	311	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGHLTEHQR THTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGKKTS	793
+	acccgaggcag gcagagacac	312	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSDPGHLVRHQR THTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	794
+	cgaggcaggca gagacaccat	313	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQR THTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGKKTS	795
+	ggcaggcagag acaccatTctg	314	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGNLTEHQRTHTGEKPY KCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADN LTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGKKTS	796
+	aggcagagaca ccatTctgtga	315	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	797

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	cagagacacca tTctgtgagtg	316	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSSKKA LTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	798
+	agacaccatTct gtgagtgaga	317	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS LTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	799
+	caccatTctgtg agtgagagga	318	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQR THTHPRAPIPKPFQYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGKKTS	800
+	catTctgtgagt gagaggaTatt	319	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKP YKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAG HLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGNLTEHQRT HTGKKTS	801
+	cagggccaacc cgaggcaggc	320	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	802
+	ggccaacccga ggcaggcaga	321	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECG KSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQR THTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGKKTS	803
+	caacccgaggc aggcagagac	322	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECG KSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQR THTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS	804
+	cccgaggcagg cagagcacc	323	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQR THTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGKKTS	805
+	gaggcaggcag agacaccatt	324	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPEC GKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRH QRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	806
+	gcaggcagaga caccattctg	325	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGKKTS	807
+	ggcagagacac cattctgtga	326	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECG KSFSHKNALQNHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSDPGNLVRHQ RTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGKKTS	808
+	agagacaccatt ctgtgagtg	327	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSDKKDLTRHQ RTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	809

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	gacaccattctgt gagtgaga	328	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSHKNALQNHQ RTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGKKTS	810
+	accattctgtga gtgagagga	329	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	811
+	attctgtgagtga gaggaTatt	330	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKP YKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAG HLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGKK TS	812
+	ctgtgagtgaga ggaTatttga	331	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQ YKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSD ELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKK TS	813
+	tgagtgagagg aTattgaggg	332	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSQLA HLRAHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKK TS	814
+	gtgagtgagag gataTTtgagg g	333	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFS RSDNLVRHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTG KKTS	815
+	gagtgagagga tattgaggg	334	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQ RTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	816
+	gagggtCTct gggaaagaaa ga	335	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHT GKKTS	817
+	ggtCTctggg gaaagaaagag aa	336	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGHLVRHQRTHT GKKTS	818
+	agggtcTctgg ggaaagaaaga	337	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQR THTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	819
+	gtcTctgggga	338	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG	820

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	aagaaagagaa		KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGKKT S	
+	ctgggaaaga aagagaaTctg	339	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSNLVRHQRTHTGEKPY KCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRAN LRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKT S	821
+	gggaaaagaag agaaTctgaag	340	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQ YKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSS NLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGKK TS	822
+	aaagaaagaga aTctgaagCT cta	341	LEPGEKPYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHT GEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCP ECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRA HQRTHTGKKTS	823
+	gaaagagaaTc tgaagCTctaT gtg	342	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPK PFQYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFS QSSNLVRHQRTHTGKKTS	824
+	agagaaTctga agCTctaTgt gtgg	343	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY KCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCP ECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQ LAHLRAHQRTHTGKKTS	825
+	gaaTctgaag CTctaTgtgtg gatg	344	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPE CGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQS SNLVRHQRTHTGKKTS	826
+	tggggaaagaa agagaatctg	345	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQ RTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGKKTS	827
+	ggaaagaaaga gaatctgaag	346	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGKKTS	828
+	aagaaagagaa tctgaagCTct a	347	LEPGEKPYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHT GEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCP SRSDNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTH TGKKTS	829

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
+	aaagagaatctg aagCTctaTgt g	348	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCP ECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQRANLRA HQRTHTGKKTS	830
+	gagaatctgaag CTctaTgtgtg g	349	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY KCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCP ECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSRSDNLVR HQRTHTGKKTS	831
+	aatctgaagCT ctaTgtgtggat g	350	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECG KSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPE CGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTTGNLTVH QRTHTGKKTS	832
+	ctgaagCTcta Tgtgtggatgg ga	351	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNST LTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTE HQRTHTGKKTS	833
+	aagCTctaTgt gtggatgggaa at	352	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECG KSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQR THTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKN HQRTHTGKKTS	834
+	ggggaaagaaa gagaATctga ag	353	LEPGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKS FSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTH TGKKTS	835
+	gaaagaaagag aATctgaagC Tcta	354	LEPGEKPYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHT GEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQLAHLRAHQRTHTGEK PYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQS SNLVRHQRTHTGKKTS	836
+	agaaagagaA TctgaagCTct aTgtg	355	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTHPRAPIPKPFQY RTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTD PSHKPFQYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPEC GKSFSQLAHLRAHQRTHTGKKTS	837
+	aagagaATct gaagCTctaT gtgtgg	356	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY KCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCP ECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRKDNLKNHQRTHTGKKTS	838
+	agaATctgaa	357	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECG	839

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	gCTctaTgtgt ggatg		KSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTPNPHRRTDPSHKPFQYKCPE CGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGK SFSQLAHLRAHQRTHTGKKTS	
+	tgaagcTctaT gtgtggatggg a	358	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNST LTEHQRTHTHPRAPIPKPFQYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTH TGKKTS	840
+	agcTctaTgtg tggatgggaaat	359	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECG KSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQR THTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSERSHLREHQRTH TGKKTS	841
+	gaagctctaTgt gtggatggga	360	LEPGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNST LTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	842
+	gctctaTgtgtg gatgggaaat	361	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECG KSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDELVRHQR THTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGKKTS	843
+	ctaTgtgtggat gggaaatgcc	362	LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTGKKTS	844
+	atgTgtggatg ggaaaTgcca gg	363	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTHPRAPIPKPFQY KCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSTSGN LVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRRDELNVHQRT HTGKKTS	845
-	attTcccATcc acacatagagct t	364	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNH QRTHTGKKTS	846
-	cccATccaca catagagcttca g	365	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT HTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTG KKTS	847
-	ccaTccacaca tagagcttcag	366	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT HTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSHSLTEHQRTHTGKKTS	848
-	catccacacata gagcttcag	367	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT	849

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			HTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGKKTS	
-	ccacacataga gcttcagatt	368	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRT HTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGKKTS	850
-	cacatagagctt cagattCTctt	369	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSF SRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHT GKKTS	851
-	atagagcttcag attCTcttTctt	370	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCP ECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQKSSLIAH QRTHTGKKTS	852
-	gagcttcagatt CTcttTcttTc cc	371	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTHPRAPI PKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGE KPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSR SDNLVRHQRTHTGKKTS	853
-	cttcagattCTc ttTcttTcccca g	372	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQY KCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPF QYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTT GALTEHQRTHTGKKTS	854
-	cagattCTctt TcttTccccag aga	373	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKS FSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSR ADNLTEHQRTHTGKKTS	855
-	attCTcttTctt Tccccagagac cc	374	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGA LTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSH KNALQNHQRTHTGKKTS	856
-	cacacatagag cTTcagattC Tctt	375	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHR GEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSERSHLREHQRTHTHTGEK PYKCPECGKSFSRSREDNLHTHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSK KALTEHQRTHTGKKTS	857
-	acatagagcTT cagattCTctt Tctt	376	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTD PSHKPFQYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGKKTS	858
-	tagagcTTcag	377	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTHPRAPI	859

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	attCTcttTctt Tccc		PKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGE KPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSERSHLREHQRTHTGEKPY KCPECGKSFSREDNLHTHQRTHTGKKTS	
-	agcTTcagatt CTcttTcttTc cccag	378	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQY KCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFF QYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQY KCPECGKSFSERSHLREHQRTHTGKKTS	860
-	acacatagagct TcagattCTct t	379	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCP ECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSSPADLTR HQRTHTGKKTS	861
-	catagagctTc agattCTcttT ctt	380	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPK PFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFS TSGNLTEHQRTHTGKKTS	862
-	agagctTcaga ttCTcttTcttT ccc	381	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTHPRAPI PKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGE KPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPEC GKSFSQLAHLRAHQRTHTGKKTS	863
-	gctTcagattC TcttTcttTccc cag	382	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQY KCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPF QYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPEC GKSFSTSGELVRHQRTHTGKKTS	864
-	cttTcttTcccc agagacccTca a	383	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGEKPY KCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKH LAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALT EHQRTHTGKKTS	865
-	cttTcccccaga gacccTcaaat a	384	LEPGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYK CPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNL TEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTTGALTEHQRTHT GKKTS	866
-	ccccagagacc cTcaaataTcc t	385	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYK CPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSF SQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTH TGKKTS	867
-	cagagacccTc aaataTcctCT	386	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTH PRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPF	868

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	cac		QYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRA DNLTEHQRTHTGKKTS	
-	agacccTcaaa taTcctCTcac Tcac	387	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEK PYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGKKTS	869
-	cccTcaaataT cctCTcacTc acaga	388	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPF QYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECG KSFSSKKHLAEHQRTHTGKKTS	870
-	cccagagaccc tcaaataTcct	389	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYK CPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGNLV RHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	871
-	agagaccctca aataTcctCTc ac	390	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTH PRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPEC GKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGKKTS	872
-	gaccctcaaata TcctCTcacT cac	391	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEK PYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSDPG NLVRHQRTHTGKKTS	873
-	cctcaaataTcc tCTcacTcac aga	392	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTKN SLTEHQRTHTGKKTS	874
-	caaataTcctC TcacTcacag aatg	393	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCP ECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQS GNLTEHQRTHTGKKTS	875
-	ataTcctCTca cTcacagaatg gtg	394	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKA LTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQ KSSLIAHQRTHTGKKTS	876
-	ccagagaccC TcaaataTcct CTcac	395	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTH PRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPS HKPFQYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKS FSTSHSLTEHQRTHTGKKTS	877

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	gagaccCTca aataTcctCTc acTcac	396	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTPRAPIPKPFQYKCPECGKSFSQKSSLIAHQRTHTGEK PYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDKKDLTRHQRTHTGEKPYK CPECGKSFSRSDNLVRHQRTHTGKKTS	878
-	accCTcaaata TcctCTcacT cacaga	397	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTHPRAPIPKPF QYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKC PECGKSFSDKKDLTRHQRTHTGKKTS	879
-	aatATcctCT cacTcacagaa tggtg	398	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKA LTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGK SFSTTGNLTVHQRTHTGKKTS	880
-	acagaatggTg tcTctgcctgcc	399	LEPGEKPYKCPECGKSFSRSDCRDLARHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGK SFSRSDHLTTHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTH TGKKTS	881
-	gaatggTgtcT ctgcctgccTc gg	400	LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRTHTGEKPY KCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKS FSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSQSSNLV RHQRTHTGKKTS	882
-	tggTgtcTctg cctgccTcggg tt	401	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSTKNSL TEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLT THQRTHTGKKTS	883
-	cagaatggtgtc Tctgcctgcc	402	LEPGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGEKPYKCPECGKSFSTSGH LVRHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	884
-	aatggtgtcTct gcctgccTcgg	403	LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPY KCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKS FSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTH TGKKTS	885
-	ggtgtcTctgcc tgccTcgggtt	404	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRNDA LTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTH TGKKTS	886
-	gtcTctgcctgc cTcgggttggc	405	LEPGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECG KSFSRSDKLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTKNS	887

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			LTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRT HTGKKTS	
-	ctgcctgccTc gggttggccct	406	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECG KSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDCRD LARHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	888
-	cctgccTcggg ttggccctgtg	407	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQR THTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	889
-	gccTcgggttg gccctgtgatt	408	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQR THTGEKPYKCPECGKSFSRSDKLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRTHTGKKTS	890
-	gccTgccTcg ggttggccctgt g	409	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQR THTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRTHTHPRAPIPKPFQYKCPECGKSFSDCRDLARHQRT HTGKKTS	891
-	ctgcctcgggtt ggccctgtg	410	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQR THTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	892
-	cctcgggttggc cctgtgatt	411	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQR THTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	893
-	cgggttggccct gtgattTatt	412	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKP YKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPG HLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGKKT S	894
-	gttggccctgtg attTatTTag	413	LEPGEKPYKCPECGKSFSREDNLHTHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAP IPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGK SFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRT HTGKKTS	895
-	ggccctgtgatt TattTtagTTc tt	414	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSREDNLHTHQRTHTH PRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGE KPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDP GHLVRHQRTHTGKKTS	896
-	cctgtgatTatt TtagTTcttT Tccc	415	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSREDNLHTHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQR THTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKP YKCPECGKSFSTKNSLTEHQRTHTGKKTS	897

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	gtgattTattTt agTTcttTTc ccTTgtt	416	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSKKHLAEHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSREDNLHT HQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQN HQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	898
-	gggTtggccc TgtgattTattT tag	417	LEPGEKPYKCPECGKSFSREDNLHTHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAP IPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYK CPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTHPRAPIPKPFQYKCPECGKSF SRSDKLVRHQRTHTGKKTS	899
-	ggttggcccTg tgattTattTta g	418	LEPGEKPYKCPECGKSFSREDNLHTHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAP IPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYK CPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTHPRAPIPKPFQYKCPECGKSF SRSDKLVRHQRTHTGKKTS	900
-	tggcccTgtgat tTattTagTT ctt	419	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSREDNLHTHQRTHTH PRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGE KPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPEC GKSFSRSDHLTTHQRTHTGKKTS	901
-	cccTgtgattT attTagTTctt TTccc	420	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSREDNLHTHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQR THTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRA PIPKPFQYKCPECGKSFSSKKHLAEHQRTHTGKKTS	902
-	caaactctaTac acttTTgttTT aaa	421	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSREDNLHT HQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDELVRH QRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGKKTS	903
-	actctaTacact tTTgttTTaaa aac	422	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTGEKP YKCPECGKSFSSPADLTRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGK SFSTHLDLIRHQRTHTGKKTS	904
-	ctaTacactT TgttTTaaaaa cTgtg	423	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPY KCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTD PSHKPFQYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTHPRAPIPKPFQY KCPECGKSFSQNSTLTEHQRTHTGKKTS	905
-	aaaCTctaTa cacttTTgttT Taaaaac	424	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTGEKP YKCPECGKSFSSPADLTRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGK SFSQRANLRAHQRTHTGKKTS	906
-	aacTctaTaca	425	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSH	907

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	cttTTgttTTaaaaac		KPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTGEKP YKCPECGKSFSSPADLTRHQRTHTHPRAPIPKPFQYKCPECGKSFSQNSTLTEHQRTHTHPRAPIPKPFQYK CPECGKSFSDSGNLRVHQRTHTGKKTS	
-	acactTTgttTTaaaaacTgtggtt	426	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTHPRAPIPKPFQY KCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTPNPHRRTDPSHKPFQYKCP ECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGK SFSSPADLTRHQRTHTTGKKTS	908
-	atacctCTcacCTctgtggtgaggg	427	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFS SKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSQKSS LIAHQRTHTTGKKTS	909
-	cctCTcacCTctgtggtgaggaa	428	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSFSSKKALTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTKN SLTEHQRTHTTGKKTS	910
-	cacCTctgtggtgagggaaaga	429	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTTPNPHRRTDPSHKPFQYKCPECGKSFSSKKALTEHQRTHT GKKTS	911
-	accTctgtggtgagggaagaa	430	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTTHPRAPIPKPFQYKCPECGKSFSDKKDLTRHQRTHTGKKT S	912
-	cctctgtggtgaggggaagaa	431	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQR THTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGKKTS	913
-	ctgtggtgaggggaagaATcat	432	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGNLTEHQRTHTGEKP YKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHL ASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	914
-	tggtgagggagaagaATcatatt	433	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSF SRSDKLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHT GKKTS	915
-	tgagggaagaaATcatattTTcag	434	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQSSNLVRHQRTHTGEKP YKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQAG	916

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			HLASHQRTHTGKKTS	
-	ggggaagaaA TcatattTTca gatg	435	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPF QYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSRS DKLVRHQRTHTGKKTS	917
-	gaagaaATca tatTTcagatg act	436	LEPGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSS NLVRHQRTHTGKKTS	918
-	gaaATcatatt TTcagatgact cgt	437	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGK SFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHKPFQYKCPECGKSFS HKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQSS NLVRHQRTHTGKKTS	919
-	gtggtgagggg aagaaatcat	438	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDHLTNHQR THTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	920
-	gtgagggaag aaatcatatt	439	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECG KSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSF SQLAHLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTH THTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	921
-	aggggaagaaa tcatattTTcag	440	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSF TTGNLTVHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSF SQLAHLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTH TGKKTS	922
-	ggaagaaatcat attTTcagatg	441	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSF STTGNLTVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHT GKKTS	923
-	agaaatcatatt TTcagatgact	442	LEPGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHT GKKTS	924
-	aatcatattTTc agatgactcgt	443	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGK SFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFS HKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHT GKKTS	925
-	catattTTcaga tgactcgtaaa	444	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECG KSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQR	926

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			THTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHT GKKTS	
-	attTTcagatg actcgtaaaggg	445	LEPGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRT HTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTG KKTS	927
-	ggtgagggaa gaaaTcatatt	446	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGKK TS	928
-	gaggggaagaa aTcatattTTc ag	447	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCP ECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSRSDNLVR HQRTHTGKKTS	929
-	gggaagaaaT catattTTcaga tg	448	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCP ECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSRSDKLV RHQRTHTGKKTS	930
-	aagaaaTcatat tTTcagatgac t	449	LEPGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLK NHQRTHTGKKTS	931
-	aaaTcatattT Tcagatgactc gt	450	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGK SFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSQRANLRA HKNALQNHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSQRANLRA HQRTHTGKKTS	932
-	cagatgactcgt aaagggcaa	451	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQR THTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	933
-	atgactcgtaaa gggcaaaga	452	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQR THTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGKKTS	934
-	actcgtaaaggg caaagaaaa	453	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGKKTS	935
-	cgtaaagggca aagaaaaaaa	454	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQ	936

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			RTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGKKTS	
-	aaagggcaaag aaaaaaaccc	455	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDKLVRHQR THTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	937
-	gggcaaagaaa aaaacccaaa	456	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGKKTS	938
-	caaagaaaaaa acccaaaatt	457	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQRANLRAH QRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS	939
-	agaaaaaaacc caaaattTcaa	458	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPY KCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQRAN LRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKT S	940
-	aaaaaacccaa aattTcaaaat	459	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSKK HLAEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKK TS	941
-	aaacccaaaatt TcaaaatTTcc g	460	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGNLTVHQRTHTG EKPYKCPECGKSFSQSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCP ECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQRANLR AHQRTHTGKKTS	942
-	agatgaCTcgt aaagggcaaag a	461	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQR THTPNPHRRTDPSHKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHT GKKTS	943
-	tgaCTcgtaaa gggcaaagaaa a	462	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSSRRTCRAHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQAGHLASHQRTHT GKKTS	944
-	gatgacTcgta aagggcaaaga	463	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSSRRTCRAHQR THTHPRAPIPKPFQYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGKKTS	945
-	gacTcgtaaag ggcaaagaaaa	464	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSRSDKLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSSRRTCRAHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGNLVRHQRTHTGKKTS	946
-	gtaaagggcaa	465	LEPGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG	947

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	agaaaaaaac		KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQR THTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	
-	aagggcaaaga aaaaaaccca	466	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	948
-	ggcaaagaaaa aaacccaaaa	467	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSDPGHLVRHQRTHTGKKTS	949
-	agggcaaagaa aaaaacccaa	468	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQ RTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	950
-	gcaaagaaaaa aacccaaaat	469	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQ RTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGKKTS	951
-	aagaaaaaaac ccaaaatTTca a	470	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGNLTVHQRTHTG EKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFS QRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHT GKKTS	952
-	aaaaaaaccca aaatTTcaaaa t	471	LEPGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSF SDKKDLTRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTH TGKKTS	953
-	aaaacccaaaat TTcaaaatTT ccg	472	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTTGNLTVHQRTHTG EKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKP YKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQRA NLRAHQRTHTGKKTS	954
-	agtCTcataat caagaaaagga g	473	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHRTTLTNHQRTHTG KKTS	955
-	gtcTcataatca agaaaaggag	474	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQR THTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGKKTS	956
-	cataatcaagaa aaggagaaa	475	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQR THTGEKPYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGKKTS	957
-	aatcaagaaaaa	476	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG	958

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	ggagaaacac		KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGKKTS	
-	caagaaaagga gaaacacaga	477	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQ RTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGKKTS	959
-	gaaaaggagaa acacagagag	478	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQSRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	960
-	aaggagaaaca cagaggaga	479	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	961
-	gagaaacacag agagagagaa	480	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	962
-	aaacacagaga gagagaaaaa	481	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	963
-	cacagagagag agaaaaaaaa	482	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQ RTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGKKTS	964
-	agagagagaga aaaaaaaaac	483	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	965
-	gagagagaaaa aaaaaacTatg	484	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPY KCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSN LVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKT S	966
-	agagaaaaaaa aaacTatgaga	485	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRA NLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKK TS	967
-	gaaaaaaaaaa cTatgagaacc	486	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRRDELNVHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRA NLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKK TS	968
-	aaaaaaaaacTa tgagaaccccc	487	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGN	969

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			LRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKT S	
-	aaaaacTatga gaaccccccc	488	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQ RTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKK TS	970
-	aacTatgagaa ccccccccccac	489	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSRRDELNVHQRTHTHPRAPIPKPFQYKCPECGKSFSDSGNLRVHQRTHTGKK TS	971
-	ataATcaagaa aaggagaaaca c	490	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSQSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQKSSLIAHQRTHTG KKTS	972
-	aagaaaaggag aaacacagag	491	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDHLTNHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGKKTS	973
-	aaaaggagaaa cacagagaga	492	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	974
-	aggagaaacac agagagagag	493	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDHLTNHQRTHTGKKTS	975
-	agaaacacaga gagagagaaa	494	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQR THTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	976
-	aacacagagag agagaaaaaa	495	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQ RTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKTS	977
-	acagagagaga gaaaaaaaaa	496	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAH QRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGKKTS	978
-	gagagagagaa aaaaaaaact	497	LEPGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQ RTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	979
-	agagagaaaaa aaaaactatg	498	LEPGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQ	980

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			RTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	
-	gagaaaaaaaa aactatgaga	499	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECG KSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	981
-	aaaaaaaaaact atgagaacc	500	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	982
-	aaaaaaactatg agaaccccc	501	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	983
-	aaaactatgaga accccccccc	502	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRRDELNVHQ RTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	984
-	actatgagaacc ccccccccac	503	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQ RTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGKKTS	985
-	atgagaacccc ccccaccccc	504	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR TTHTGEKPYKCPECGKSFSRRDELNVHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGKKTS	986
-	agaaccccccc ccacccgtg	505	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR TTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	987
-	acccccccccca ccccgtgatt	506	LEPGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGKKTS	988
-	ccccccccaccc cgtgattATca g	507	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHT GEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSF SSKKALTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHT GKKTS	989
-	ccccaccccgt gattATcagcg c	508	LEPGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGEKPYKCPECGKSF SSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHT GKKTS	990
-	cacccgtgatt ATcagcgcac a	509	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSF SRSDELVRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHT GKKTS	991

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	cccgtgattATcagcgcacac	510	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	992
-	gtgattATcagcgcacacTcat	511	LEPGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	993
-	attATcagcgcacacTcatcga	512	LEPGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHKNALQNHQRTHTGKKTS	994
-	agaaaaggagaaacagagaga	513	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	995
-	aaaggagaaacacagagagag	514	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGKKTS	996
-	ggagaaacacagagagaga	515	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQRAHLERHQRTHTGKKTS	997
-	gaaacacagagagagaaaaa	516	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	998
-	acacagagagagagaaaaaa	517	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGKKTS	999
-	cagagagagagaaaaaaaaa	518	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	1000
-	agagagagaaaaaaaacta	519	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	1001
-	gagagaaaaaaaaactatga	520	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	1002
-	agaaaaaaaaa	521	LEPGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG	1003

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	actatgagaa		KSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	
-	aaaaaaaaaacta tgagaaccc	522	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	1004
-	aaaaaactatga gaaccccccc	523	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQNSTLTEHQR THTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	1005
-	aaactatgagaa ccccccccc	524	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECGKSFSQAGHLASHQR THTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	1006
-	ctatgagaaccc ccccccacc	525	LEPGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGKKTS	1007
-	tgagaaccccc ccccaccccg	526	LEPGEKPYKCPECGKSFSRNDTLTEHQRTHTGEKPYKCPECGKSFSDKKDLTRHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQR THTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGKKTS	1008
-	gaaccccccccc cacccgtga	527	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGEKPYKCPECG KSFSDKKDLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQR THTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKKTS	1009
-	gagaaccccccc cccaccccgt	528	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECG KSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQR THTGEKPYKCPECGKSFSDSGNLRVHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	1010
-	aaccccccccc acccgtgat	529	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECG KSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQR THTGEKPYKCPECGKSFSSKKHLAEHQRTHTGEKPYKCPECGKSFSDSGNLRVHQRTHTGKKTS	1011
-	cagcgcacaca cTcatcgaaaa	530	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSPADL TRHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGKKTS	1012
-	cgcacacacTc atcgaaaaaaa	531	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKK ALTEHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSHTGHLLEHQRTHTGKKT S	1013
-	acacacTcatc gaaaaaaaTTt gg	532	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQRANLRAHQRTHT GEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSPADLTR HQRTHTGKKTS	1014

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	cacTcatcgaa aaaaaTTtgga tt	533	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKS FSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPRAPIPKPFQYKCPECGKSFSSKKALT EHQRTHTGKKTS	1015
-	agcgcacacac tcatcgaaaa	534	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECG KSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT HTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGKKTS	1016
-	gcacacactcat cgaaaaaaa	535	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTHLDLIRHQR THTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGKKTS	1017
-	cacactcatcga aaaaaaTTtgg	536	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQRANLRAHQRTHT GEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTG KKTS	1018
-	actcatcgaaaa aaaTTtggatt	537	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKS FSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTHT GKKTS	1019
-	catcgaaaaaaa TTtggattatt	538	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPEC GKSFSRSDHLTTHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKS FSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTH TGKKTS	1020
-	cgaaaaaaaT Ttggattattag a	539	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPEC GKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRT FSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRT HTGKKTS	1021
-	aaaaaaTTtgg attattagaaga	540	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQ RTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQSGHLTEHQRTH TGKKTS	1022
-	aaaTTtggatt attagaagagag	541	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSF SSPADLTRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHT	1023
-	gcgcacacaC Tcatcgaaaaa aa	542	LEPGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSF SSPADLTRHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHT	1024

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			GKKTS	
-	cacacaCTcat cgaaaaaaaT Ttgg	543	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSQRANLRAHQRTHT GEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSGHLTEHQRTHTGEKPYKCPECGKSF STSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKK ALTEHQRTHTGKKTS	1025
-	acaCTcatcga aaaaaaTTtgg att	544	LEPGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTPNPHRRTDPSH KPFQYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKS FSQSGHLTEHQRTHTGEKPYKCPECGKSFSTSGNLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSP ADLTRHQRTHTGKKTS	1026
-	gaaaaaaaaTt ggattattaga	545	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPEC GKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTHPRAPIPKPFQYKCPECGKSFSTTG NLTVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQSSNLVRHQRTHTGKK TS	1027
-	aaaaatTtggat tattagaaga	546	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQ RTHTHPRAPIPKPFQYKCPECGKSFSTTGNLTVHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKK TS	1028
-	aatTtggattatt agaagagag	547	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQ RTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSTTGNLTVHQRTHTGKKT S	1029
-	aaaaaaaatttgg attattaga	548	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPEC GKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	1030
-	aaaatttggatta ttagaaga	549	LEPGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECG KSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQ RTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGKKTS	1031
-	attggattatta gaagagag	550	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQ RTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGKKTS	1032
-	tggattattagaa gagagagg	551	LEPGEKPYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECG KSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQ RTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSRSDHLTTHQRTHTGKKTS	1033
-	attattagaaga gagaggTctg	552	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGEKPY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGKKT S	1034

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	attagaagagag aggTctgcgg	553	LEPGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQY KCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTGKKT S	1035
-	agaagagagag gTctgcggctt	554	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDN LVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKT S	1036
-	agagagaggT ctgcggctcca	555	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGK SFSRSDKLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHL TNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	1037
-	gagaggTctgc ggcttccacac	556	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGK SFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRT HTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	1038
-	aggTctgcggc ttccacaccgt	557	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRT HTGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGKKTS	1039
-	ggaTTattaga agagagaggT ctg	558	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGEKPY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSQRAHLER HQRTHTGKKTS	1040
-	gatTattagaag agagaggTctg	559	LEPGEKPYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDHLTNHQRTHTGEKPY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSHKNALQNHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGNLVRHQRT HTGKKTS	1041
-	tagaagagaga ggtcTgcggct	560	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTHPRAPIPKPFQY KCPECGKSFSDPGALVRHQRTHTGEKPYKCPECGKSFSQLAH LRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGKKT S	1042
-	aagagagaggt cTgcggctTcc a	561	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYK CPECGKSFSRSDDLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTHTGEKPYKCPECGKSF SRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRKDNLKNHQRTH TGKKTS	1043
-	agagaggtcTg cggctTccaca c	562	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQY KCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSDDLVRHQRTHTHPRAPIPKPFQYKCPECGKS FSDPGALVRHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRT HTGKKTS	1044

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
-	gaggtcTgcg gctTccacacc gt	563	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDDL VRHQRTHTHPRAPIPKPFQYKCPECGKSFSDPGALVRHQRTH TGKKTS	1045
-	gtcTgcggctT ccacaccgtaca	564	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGEL VRHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTH TGKKTS	1046
-	gaagagagagg tctgcggctt	565	LEPGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECG KSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQR THTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	1047
-	gagagaggtct gcggcttcca	566	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGK SFSRSDKLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRT HTGEKPYKCPECGKSFSQLAHLRAHQRTHTGEKPYKCPECGKSFSRSDNLVRHQRTHTGKKTS	1048
-	agaggtctgcg gcttccacac	567	LEPGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGK SFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRT HTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQLAHLRAHQRTHTGKKTS	1049
-	ggtctgcggctt ccacaccgt	568	LEPGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRT HTGEKPYKCPECGKSFSRNDALTEHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGKKTS	1050
-	ctgcggcttcca caccgtaca	569	LEPGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRT HTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRNDALTEHQRTHTGKKTS	1051
-	cggcttccacac cgtacagcg	570	LEPGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECG KSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSRSDKLTEHQRTHTGKKTS	1052
-	cttccacaccgt acagcgtgg	571	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGKKTS	1053
-	gcggcttccac accgtacagcg	572	LEPGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECG KSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGKKTS	1054
-	gctTccacacc gtacagcgtgg	573	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT HTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGELVRHQRTHTGKKTS	1055
-	ggcTTccacaccgtacagcgtg	574	LEPGEKPYKCPECGKSFSRSDHLTTHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSSKKALTEHQRT	1056

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	g		HTGEKPYKCPECGKSFSTSHSLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSDPGHLVRHQRTHTG KKTS	
-	cttCTcggTat aaaagcaaagT Tgtt	575	LEPGEKPYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHT GEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSF SQKSSLIAHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDKLTEHQRTHTPNPHRRTDPSHKPFQYKCPECGK SFSTTGALTEHQRTHTGKKTS	1057
-	cggTataaaag caaagTTgttT Ttga	576	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKP YKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTHPRAPIPKPFQYKCPECGK SFSRSDKLTEHQRTHTGKKTS	1058
-	ggtataaaagca aagTTgttTTt ga	577	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTP NPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKP YKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGEKPYKCPECGKSFSTSG HLVRHQRTHTGKKTS	1059
-	ataaaagcaaa gTTgttTTg aTacg	578	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQR THTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPEC GKSFSQKSSLIAHQRTHTGKKTS	1060
-	aaagcaaagT TgttTTgaTa cgtga	579	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQY KCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTPNPHRRTDP SHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECGKSFSQSGDLRRHQRTHTGEKPYKCPECG KSFSQRANLRAHQRTHTGKKTS	1061
-	gcaaagTTgtt TTgaTacgtg acag	580	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCP ECGKSFSTSGSLVRHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSRKDNLKNHQRTHTGEKPYKCPECG KSFSQSGDLRRHQRTHTGKKTS	1062
-	gtaTaaaagca aagttgttTTg a	581	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHT GEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSF SERSHLREHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTHPRAPIPKPFQYKCPECGKSFSQSSSLVR HQRTHTGKKTS	1063
-	aaaagcaaagtt gttTTgaTac g	582	LEPGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTPNPHR RTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPE CGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSQRANLRAH QRTHTGKKTS	1064
-	agcaaagttgtt TTgaTacgtg a	583	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQY KCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCP ECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSQRANLRAHQRTHTGEKPYKCPECGKSFSERSHLRE	1065

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			HQRTHTGKKTS	
-	aaagttgttTTt gaTacgtgaca g	584	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCP ECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSQRANLRA HQRTHTGKKTS	1066
-	aagcaaagtTg ttTTtgaTacg tga	585	LEPGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECGKSFSRTDTLRDHQRTHTHPRAPIPKPFQY KCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGSLVRHQRTHTHPRAPIPKP FQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSR KDNLKNHQRTHTGKKTS	1067
-	caaagtTgttT TtgaTacgtga cag	586	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSQAGHLASHQRTHTGEKPYKCPECG KSFSRTDTLRDHQRTHTHPRAPIPKPFQYKCPECGKSFSQAGHLASHQRTHTPNPHRRTDPSHKPFQYKCP ECGKSFSTSGSLVRHQRTHTHPRAPIPKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSQ SGNLTEHQRTHTGKKTS	1068
-	atacgtgacagt TTcccacaag c	587	LEPGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGK SFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSD PGNLVRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTGEKPYKCPECGKSFSQKSSLIAHQRTHTGK KTS	1069
-	cgtgacagtTT cccacaagcca g	588	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFS HRTTLTNHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTGEKPYKCPECGKSFSSRRTCRAHQRTHTG KKTS	1070
-	gacagtTTccc acaagccaggc t	589	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSERSHLREHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRT HTPNPHRRTDPSHKPFQYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSDPGNLVRHQRTHTG KKTS	1071
-	agtTTcccaca agccaggctgat	590	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSSPADLTRHQRT HTGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSHRTTLTNHQRTHTG KKTS	1072
-	gtgacagttTcc cacaagccag	591	LEPGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECG KSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGSL VRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSRSDELVRHQRTHTGKKTS	1073
-	acagttTcccac aagccaggct	592	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECG KSFSERSHLREHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRT HTHPRAPIPKPFQYKCPECGKSFSTSGSLVRHQRTHTGEKPYKCPECGKSFSSPADLTRHQRTHTGKKTS	1074
-	gttTcccacaa gccaggctgat	593	LEPGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECG KSFSRADNLTEHQRTHTGEKPYKCPECGKSFSERSHLREHQRTHTGEKPYKCPECGKSFSSPADLTRHQRT	1075

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
			HTGEKPYKCPECGKSFSSKKHLAEHQRTHTHPRAPIPKPFQYKCPECGKSFSTSGSLVRHQRTHTGKKTS	
-	cccacaagcca ggctgatcct	594	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSTSGNLVRHQRTHTGEKPYKCPECG KSFSTSGELVRHQRTHTGEKPYKCPECGKSFSRADNLTEHQRTHTGEKPYKCPECGKSFSERSHLREHQR THTGEKPYKCPECGKSFSSPADLTRHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTGKKTS	1076
-	cttTTctgTca gTccacttcacc aa	595	LEPGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECG KSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRADNL TEHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTHTHPRAPIPKPFQYKCPECGKSFSTT GALTEHQRTHTGKKTS	1077
-	ctgTcagTcca cttcaccaaggt	596	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTHPRAPIPKPFQYKCPECGKSFSRADNLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRNDALTEHQRTH TGKKTS	1078
-	gtcagtccacttc accaaggt	597	LEPGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECG KSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRT HTGEKPYKCPECGKSFSHRTTLTNHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTGKKTS	1079
-	agtccacttcac caaggtgag	598	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQR THTGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSHRTTLTNHQRTHTGKKTS	1080
-	ccacttcaccaa ggtgagTgtc	599	LEPGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPY KCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSTTGEKPYKCPECGKSFSSKKA LTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGEKPYKCPECGKSFSTSHSLTEHQRTHTGKKTS	1081
-	cttcaccaaggt gagTgtccct	600	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGN LTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQRTHTGKKTS	1082
-	caccaaggtga gTgtccctgct	601	LEPGEKPYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECG KSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGH LVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGKKTS	1083
-	caaggtgagTg tcctgctCTc cc	602	LEPGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHKPPFQYKCPECGKSFSTSGELVRHQRTHTG EKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPE CGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEH QRTHTGKKTS	1084
-	ggtgagTgtcc ctgctCTcccc ta	603	LEPGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGKSFSSKKHLAEHQRTHTPNPHRRTDPSHK PFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSD PGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRH QRTHTGKKTS	1085
-	gagTgtccctg ctCTcccctac	604	LEPGEKPYKCPECGKSFSTSHSLTEHQRTHTGEKPYKCPECGKSFSQNSTLTEHQRTHTGEKPYKCPECGK SFSSKKHLAEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSTSGELVRHQRTHTGEKPYKCPECGKSFST	1086

Strand	Long Target	SEQ ID NO:	ZF amino acid sequence	SEQ ID NO:
	ca		KNSLTEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRH QRTHTGKKTS	
-	cagTccacttca ccaaggtgag	605	LEPGEKPYKCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECG KSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTTGALTEHQR THTGEKPYKCPECGKSFSTSHSLTEHQRTHTHPRAPIPKPFQYKCPECGKSFSRADNLTEHQRTHTGKKTS	1087
-	gtccacTTcac caaggtgagTg tc	606	LEPGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQYKCPECGKSFSRSDNLVRHQRTHTGEKPY KCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGNLTEHQRTHTGEKPYKCPECGKSFSSKKA LTEHQRTHTPNPHRRTDPSHKPFQYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSDPGALVR HQRTHTGKKTS	1088
-	cacTTcaccaa ggtgagTgtcc ct	607	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGN LTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSSKKALTEH QRTHTGKKTS	1089
-	actTcaccaag gtgagTgtcct	608	LEPGEKPYKCPECGKSFSTKNSLTEHQRTHTGEKPYKCPECGKSFSDPGALVRHQRTHTHPRAPIPKPFQY KCPECGKSFSRSDNLVRHQRTHTGEKPYKCPECGKSFSTSGHLVRHQRTHTGEKPYKCPECGKSFSQSGN LTEHQRTHTGEKPYKCPECGKSFSSKKALTEHQRTHTGEKPYKCPECGKSFSTHLDLIRHQRTH TGKKTS	1090

Similarly, the design and preparation of such TALE polypeptides which specifically bind to a DNA target region of interest, such as a FOXP3 expression control region, is well known in the art. For example, the TALE DNA binding domain contains a repeated highly conserved 33–34 amino acid sequence with divergent 12th and 13th amino acids. These two positions, referred to as the Repeat Variable Diresidue (RVD), are highly variable and show a strong correlation with specific nucleotide recognition. This straightforward relationship between amino acid sequence and DNA recognition has allowed for the engineering of specific DNA-binding domains by selecting a combination of repeat segments containing the appropriate RVDs. See, *e.g.*, Boch J *Nature Biotechnology*. 29 (2) 2011: 135–6; Boch J, *et al., Science*. 326 (5959) 2009: 1509–12; Moscou MJ & Bogdanove AJ *Science*. 326 (5959) 2009: 1501.

In some embodiments, the site-specific FOXP3 targeting moieties of the invention comprising a polynucleotide comprise a guide RNA (or gRNA) or nucleic acid encoding a guide RNA. A gRNA is a short synthetic RNA molecule comprising a "scaffold" sequence necessary for, *e.g.,* directing an effector to a FOXP3 expression control element which may, *e.g.,* include an about 20 nucleotide site-specific sequence targeting a genomic target sequence comprising the FOXP3 expression control element.

Generally, guide RNA sequences are designed to have a length of between about 17 to about 24 nucleotides (*e.g.*, 19, 20, or 21 nucleotides) and are complementary to the target sequence. 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 sgNAs have also been demonstrated to be effective in genome editing; see, for example, Hendel *et al.* (2015) *Nature Biotechnol.*, 985 - 991.

In certain embodiments, the site-specific FOXP3 targeting moieties of the invention comprise a guide RNA (or gRNA) or nucleic acid encoding a guide RNA and a protein or a peptide. In some embodiment, the protein or the peptide comprises a CRISPR associated protein (Cas) polypeptide, or fragment thereof (*e.g.*, a Cas9 polypeptide, or fragment thereof). In one embodiment, a suitable Cas polypeptide is an enzymatically inactive Cas polypeptide, *e.g.*, a “dead Cas polypeptide” or “dCas” polypeptide.

Exemplary site-specific FOXP3 targeting moieties comprising a polynucleotide, *e.g.,* gRNA, are provided in Table 2, below. In some embodiments, the polynucleotide comprises a nucleotide sequence at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99% identical to the entire nucleotide sequence of any one of the nucleotide sequences in Table 2.

It will be understood that, although the sequences in Table 2 are described as modified (or unmodified), the nucleic acid molecules encompassed by the invention, *e.g.*, a site-specific disrupting agent, may comprise any one of the sequences set forth in Table 2 that is unmodified or modified differently than described therein. It will also be understood that although some of the sequences in Table 2 have "Ts", when used as an RNA molecule, such as a guide RNA, in the site-specific targeting moieties of the invention, the "Ts" may be replaced with "Us."

In some embodiments, a site-specific FOXP3 targeting moiety comprising a polynucleotide, *e.g.*, gRNA, comprises a nucleotide sequence complementary to an anchor sequence. In one embodiment, the anchor sequence comprises a CTCF-binding motif or consensus sequence: 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 or consensus sequence may also be in the 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, the nucleic acid sequence comprises a sequence complementary to a CTCF-binding motif or consensus sequence.

In some embodiments, the polynucleotide comprises a nucleotide sequence at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% complementary to an anchor sequence.

In some embodiments, the polynucleotide comprises a nucleotide sequence at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% complementary to a CTCF-binding motif or consensus sequence. In some embodiments, the polynucleotide is selected from the group consisting of a gRNA, and a sequence complementary or a sequence comprising at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% complementary sequence to an anchor sequence.

In some embodiments, a site-specific FOXP3 targeting moiety comprising a polynucleotide of the invention is an RNAi molecule. RNAi molecules comprise RNA or RNA-like structures typically containing 15-50 base pairs (such as about 18-25 base pairs) and having a nucleobase sequence identical (complementary) or nearly identical (substantially complementary) to a coding sequence in an expressed target gene within the cell. RNAi molecules include, but are not limited to: short interfering RNAs

(siRNAs), double-strand RNAs (dsRNA), micro RNAs (miRNAs), short hairpin RNAs (shRNA), meroduplexes, and dicer substrates (U.S. Patent Nos. 8,084,599, 8,349,809, and 8,513,207). In one embodiment, the invention includes a composition to inhibit expression of a gene encoding a polypeptide described herein, *e.g.*, a conjunction nucleating molecule.

RNAi molecules comprise a sequence substantially complementary, or fully complementary, to all or a fragment of a target gene. RNAi molecules may complement sequences at the boundary between introns and exons to prevent the maturation of newly-generated nuclear RNA transcripts of specific genes into mRNA for transcription. RNAi molecules complementary to specific genes can hybridize with the mRNA for that gene and prevent its translation. The antisense molecule can be DNA, RNA, or a derivative or hybrid thereof. Examples of such derivative molecules include, but are not limited to, peptide nucleic acid (PNA) and phosphorothioate-based molecules such as deoxyribonucleic guanidine (DNG) or ribonucleic guanidine (R G).

RNAi molecules can be provided to the cell as "ready-to-use" RNA synthesized in vitro or as an antisense gene transfected into cells which will yield RNAi molecules upon transcription. Hybridization with mRNA results in degradation of the hybridized molecule by RNAse H and/or inhibition of the formation of translation complexes. Both result in a failure to produce the product of the original gene.

The length of the RNAi molecule that hybridizes to the transcript of interest should be around 10 nucleotides, between about 15 or 30 nucleotides, or about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more nucleotides. The degree of identity of the antisense sequence to the targeted transcript should be at least 75%, at least 80%, at least 85%, at least 90%, or at least 95.

RNAi molecules may also comprise overhangs, i.e. typically unpaired, overhanging nucleotides which are not directly involved in the double helical structure normally formed by the core sequences of the herein defined pair of sense strand and antisense strand. RNAi molecules may contain 3' and/or 5' overhangs of about 1-5 bases independently on each of the sense strands and antisense strands. In one embodiment, both the sense strand and the antisense strand contain 3' and 5' overhangs. In one embodiment, one or more of the 3' overhang nucleotides of one strand base pairs with one or more 5' overhang nucleotides of the other strand. In another embodiment, the one or more of the 3' overhang nucleotides of one strand base do not pair with the one or more 5' overhang nucleotides of the other strand. The sense and antisense strands of an RNAi molecule may or may not contain the same number of nucleotide bases. The antisense and sense strands may form a duplex wherein the 5' end only has a blunt end, the 3' end only has a blunt end, both the 5' and 3' ends are blunt ended, or neither the 5' end nor the 3' end are blunt ended. In another embodiment, one or more of the nucleotides in the overhang contains a thiophosphate, phosphorothioate, deoxynucleotide inverted (3' to 3' linked) nucleotide or is a modified ribonucleotide or deoxynucleotide.

Small interfering RNA (siRNA) molecules comprise a nucleotide sequence that is identical to about 15 to about 25 contiguous nucleotides of the target mRNA. In some embodiments, the siRNA sequence commences with the dinucleotide AA, comprises a GC -content of about 30-70% (about 50-60%, about 40-60%, or about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other than the target in the genome of the mammal in which it is to be introduced, for example as determined by standard BLAST search.

siRNAs and shRNAs resemble intermediates in the processing pathway of the endogenous microRNA (miRNA) genes (Bartel, Cell 116:281-297, 2004). In some embodiments, siRNAs can function as miRNAs and vice versa (Zeng et al., Mol Cell 9: 1327-1333, 2002; Doench et al., Genes Dev 17:438-442, 2003). MicroRNAs, like siRNAs, use RISC to downregulate target genes, but unlike siRNAs, most animal miRNAs do not cleave the mRNA. Instead, miRNAs reduce protein output through translational suppression or polyA removal and mRNA degradation (Wu et al., Proc Natl Acad Sci USA 103 :4034-4039, 2006). Known miRNA binding sites are within mRNA 3' UTRs; miRNAs seem to target sites with near-perfect complementarity to nucleotides 2-8 from the miRNA's 5' end (Rajewsky, Nat Genet 38 Suppl: S8- 13, 2006; Lim et al, Nature 433 :769-773, 2005). This region is known as the seed region. Because siRNAs and miRNAs are interchangeable, exogenous siRNAs downregulate mRNAs with seed complementarity to the siRNA (Birmingham et al., Nat Methods 3 : 199-204, 2006. Multiple target sites within a 3' UTR give stronger downregulation (Doench et al., Genes Dev 17:438-442, 2003).

Lists of known miRNA sequences can be found in databases maintained by research organizations, such as Wellcome Trust Sanger Institute, Perm Center for Bioinformatics, Memorial Sloan Kettering Cancer Center, and European Molecule Biology Laboratory, among others. Known effective siRNA sequences and cognate binding sites are also well represented in the relevant literature. RNAi molecules are readily designed and produced by technologies known in the art. In addition, there are computational tools that increase the chance of finding effective and specific sequence motifs (Pei et al. 2006, Reynolds et al. 2004, Khvorova et al. 2003, Schwarz et al. 2003, Ui-Tei et al. 2004, Heale et al. 2005, Chalk et al. 2004, Amarzguioui et al. 2004).

An RNAi molecule modulates expression of RNA encoded by a gene. Because multiple genes can share some degree of sequence homology with each other, in some embodiments, the RNAi molecule can be designed to target a class of genes with sufficient sequence homology. In some embodiments, the RNAi molecule can contain a sequence that has complementarity to sequences that are shared amongst different gene targets or are unique for a specific gene target. In some embodiments, the RNAi molecule can be designed to target conserved regions of an RNA sequence having homology between several genes thereby targeting several genes in a gene family (e.g., different gene isoforms, splice variants, mutant

genes, etc.). In some embodiments, the RNAi molecule can be designed to target a sequence that is unique to a specific RNA sequence of a single gene.

In some embodiments, the RNAi molecule targets a sequence in a conjunction nucleating molecule, e.g., CTCF, cohesin, USF 1, YY1, TATA-box binding protein associated factor 3 (TAF3), ZNF 143, or another polypeptide that promotes the formation of an anchor sequence-mediated conjunction, or an epigenetic modifying agent, e.g., an enzyme involved in post-translational modifications including, but are not limited to, DNA methylases (e.g., DNMT3a, DNMT3b, DNMTL), DNA demethylation (e.g., the TET family enzymes catalyze oxidation of 5-methylcytosine to 5- hydroxymethylcytosine and higher oxidative derivatives), histone methyltransferases, histone deacetylase (e.g., HDACl, HDAC2, HDAC3), sirtuin 1, 2, 3, 4, 5, 6, or 7, lysine-specific histone demethylase 1 (LSD1), histone-lysine-N-methyltransferase (Setdbl), 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), protein-lysine N-methyltransferase (SMYD2), and others. In one embodiment, the RNAi molecule targets a protein deacetylase, e.g., sirtuin 1, 2, 3, 4, 5, 6, or 7. In one embodiment, the invention includes a composition comprising an RNAi that targets a conjunction nucleating molecule, e.g., CTCF.

In some embodiments, the site-specific FOXP3 targeting moiety comprises a peptide or protein moiety. In some embodiments, a site-specific disrupting agent comprises a fusion protein. In some embodiments, an effector is a peptide or protein moiety. The peptide or protein moieties may include, but is not limited to, a peptide ligand, antibody fragment, or targeting aptamer that binds a receptor such as an extracellular receptor, neuropeptide, hormone peptide, peptide drug, toxic peptide, viral or microbial peptide, synthetic peptide, and agonist or antagonist peptide.

Exemplary peptides or protein include a DNA- binding protein, a CRISPR component protein, a conjunction nucleating molecule, a dominant negative conjunction nucleating molecule, an epigenetic modifying agent, or any combination thereof. In some embodiments, the peptide comprises a nuclease, a physical blocker, an epigenetic recruiter, and an epigenetic CpG modifier, and fragments and combinations of any of the foregoing. In some embodiments, the peptide comprises a DNA-binding domain of a protein, such as a helix-turn-helix motif, a leucine zipper, a Zn-finger, a TATA box binding proteins, a transcription factor.

Peptides or proteins may be linear or branched. The peptide or protein moiety may have a length from about 5 to about 200 amino acids, about 15 to about 150 amino acids, about 20 to about 125 amino acids, about 25 to about 100 amino acids, about 20-70 amino acids, about 20-80 amino acids, about 20-90 amino acids, about 30-100 amino acids, about 30-60 amino acids, about 30-80 amino acids, about 35-85 amino acids, about 40-100 amino acids, or about 50-125 amino acids or any range therebetween.

As indicated above, in some embodiments, the site-specific FOXP3 targeting moieties of the invention comprise a fusion protein.

In some embodiments, the fusion proteins of the invention include a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region and an effector molecule. In other embodiments, a fusion protein of the invention comprises an effector molecule. Exemplary effector molecules are described below and in some embodiments include, for example, nucleases, physical blockers, epigenetic recruiters, *e.g.,* a transcriptional enhancer or a transcriptional repressor, and epigenetic CpG modifiers, *e.g.,* a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase, and combinations of any of the foregoing.

For example, a site-specific targeting moiety may comprise a gRNA and an effector, such as a nuclease, *e.g.*, a Cas9, *e.g.*, a wild type Cas9, a nickase Cas9 (*e.g.*, Cas9 D10A), a dead Cas9 (dCas9), eSpCas9, Cpfl, C2C1, or C2C3, or a nucleic acid encoding such a nuclease. The choice of nuclease and gRNA(s) is determined by whether the targeted mutation is a deletion, substitution, or addition of nucleotides, *e.g.*, a deletion, substitution, or addition of nucleotides to a target sequence. Fusions of a catalytically inactive endonuclease *e.g.*, a dead Cas9 (dCas9, *e.g.*, D10A; H840A) tethered with all or a portion of (*e.g.*, biologically active portion of) an (one or more) effector domain create chimeric proteins that can be linked to the polypeptide to guide the composition to specific DNA sites by one or more RNA sequences (*e.g.*, DNA recognition elements including, but not restricted to zinc finger arrays, sgRNA, TAL arrays, peptide nucleic acids described herein) to modulate activity and/or expression of one or more target nucleic acids sequences (*e.g.*, to methylate or demethylate a DNA sequence).

In one embodiment, a fusion protein of the invention may comprise an effector molecule comprising, for example, a CRISPR associated protein (Cas) polypeptide, or fragment thereof, (*e.g.,* a Cas9 polypeptide, or fragment thereof) and an epigenetic recruiter or an epigenetic CpG modifier.

In one embodiment, a suitable Cas polypeptide is an enzymatically inactive Cas polypeptide, *e.g.,* a "dead Cas polypeptide" or "dCas" polypeptide

Exemplary Cas polypeptides that are adaptable to the methods and compositions described herein are described below. Using methods known in the art, a Cas polypeptide can be fused to any of a variety of agents and/or molecules as described herein; such resulting fusion molecules can be useful in various disclosed methods.

In one aspect, the invention includes a composition comprising a protein comprising a domain, *e.g.*, an effector, that acts on DNA (*e.g.*, a nuclease domain, *e.g.*, a Cas9 domain, *e.g.*, a dCas9 domain; a DNA methyltransferase, a demethylase, a deaminase), in combination with at least one guide RNA (gRNA) or antisense DNA oligonucleotide that targets the protein to site-specific target sequence, wherein the composition is effective to alter, in a human cell, the expression of a target gene. In some

embodiments, the enzyme domain is a Cas9 or a dCas9. In some embodiments, the protein comprises two enzyme domains, *e.g.*, a dCas9 and a methylase or demethylase domain.

In one aspect, the invention includes a composition comprising a protein comprising a domain, *e.g.*, an effector, that comprises a transcriptional control element (*e.g.*, a nuclease domain, *e.g.*, a Cas9 domain, *e.g.*, a dCas9 domain; a transcriptional enhancer; a transcriptional repressor), in combination with at least one guide RNA (gRNA) or antisense DNA oligonucleotide that targets the protein to a site-specific target sequence, wherein the composition is effective to alter, in a human cell, the expression of a target gene. In some embodiments, the enzyme domain is a Cas9 or a dCas9. In some embodiments, the protein comprises two enzyme domains, *e.g.*, a dCas9 and a transcriptional enhancer or transcriptional repressor domain.

As used herein, a "biologically active portion of an effector domain" is a portion that maintains the function (*e.g.* completely, partially, minimally) of an effector domain (*e.g.*, a "minimal" or "core" domain).

The chimeric proteins described herein may also comprise a linker, *e.g.*, an amino acid linker. In some aspects, a linker comprises 2 or more amino acids, *e.g.*, one or more GS sequences. In some aspects, fusion of Cas9 (*e.g.*, dCas9) with two or more effector domains (*e.g.*, of a DNA methylase or enzyme with a role in DNA demethylation or protein acetyl transferase or deacetylase) comprises one or more interspersed linkers (*e.g.*, GS linkers) between the domains. In some aspects, dCas9 is fused with 2- 5 effector domains with interspersed linkers.

In some embodiments, a site-specific FOXP3 targeting moiety comprises a conjunction nucleating molecule, a nucleic acid encoding a conjunction nucleating molecule, or a combination thereof. In some embodiments, an anchor sequence-mediated conjunction is mediated by a first conjunction nucleating molecule bound to the first anchor sequence, a second conjunction nucleating molecule bound to the noncontiguous second anchor sequence, and an association between the first and second conjunction nucleating molecules. In some embodiments, a conjunction nucleating molecule may disrupt, *e.g.*, by competitive binding, the binding of an endogenous conjunction nucleating molecule to its binding site.

The conjunction nucleating molecule may be, *e.g.*, CTCF, cohesin, USF1, YY1, TATA-box binding protein associated factor 3 (TAF3), ZNF143 binding motif, or another polypeptide that promotes the formation of an anchor sequence-mediated conjunction. The conjunction nucleating molecule may be an endogenous polypeptide or other protein, such as a transcription factor, *e.g.*, autoimmune regulator (AIRE), another factor, *e.g.*, X-inactivation specific transcript (XIST), or an engineered polypeptide that is engineered to recognize a specific DNA sequence of interest, *e.g.*, having a zinc finger, leucine zipper or bHLH domain for sequence recognition. The conjunction nucleating molecule may modulate DNA

interactions within or around the anchor sequence -mediated conjunction. For example, the conjunction nucleating molecule can recruit other factors to the anchor sequence that alters an anchor sequence-mediated conjunction formation or disruption.

The conjunction nucleating molecule may also have a dimerization domain for homo- or heterodimerization. One or more conjunction nucleating molecules, *e.g.*, endogenous and engineered, may interact to form the anchor sequence-mediated conjunction. In some embodiments, the conjunction nucleating molecule is engineered to further include a stabilization domain, *e.g.*, cohesion interaction domain, to stabilize the anchor sequence-mediated conjunction. In some embodiments, the conjunction nucleating molecule is engineered to bind a target sequence, *e.g.*, target sequence binding affinity is modulated. In some embodiments, the conjunction nucleating molecule is selected or engineered with a selected binding affinity for an anchor sequence within the anchor sequence-mediated conjunction. Conjunction nucleating molecules and their corresponding anchor sequences may be identified through the use of cells that harbor inactivating mutations in CTCF and Chromosome Conformation Capture or 3C-based methods, *e.g.*, Hi-C or high-throughput sequencing, to examine topologically associated domains, *e.g.*, topological interactions between distal DNA regions or loci, in the absence of CTCF. Long-range DNA interactions may also be identified. Additional analyses may include ChIA- PET analysis using a bait, such as Cohesin, YY1 or USF1, ZNF143 binding motif, and MS to identify complexes that are associated with the bait.

B. Effector Molecules

Effector molecules for use in the compositions and methods of the invention include those that modulate a biological activity, for example increasing or decreasing enzymatic activity, gene expression, cell signaling, and cellular or organ function. Preferred effector molecules of the invention are nucleases, physical blockers, epigenetic recruiters, *e.g.*, a transcriptional enhancer or a transcriptional repressor, and epigenetic CpG modifiers, *e.g.*, a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase, and combinations of any of the foregoing.

Additional effector activities may also include binding regulatory proteins to modulate activity of the regulator, such as transcription or translation. Effector molecules also may include activator or inhibitor (or "negative effector") functions as described herein. In another example, the effector molecule may inhibit substrate binding to a receptor and inhibit its activation, *e.g.*, naltrexone and naloxone bind opioid receptors without activating them and block the receptors' ability to bind opioids. Effector molecules may also modulate protein stability/degradation and/or transcript stability /degradation. For example, proteins may be targeted for degradation by the polypeptide co-factor, ubiquitin, onto proteins to mark them for degradation. In another example, an effector molecule inhibits enzymatic activity by

blocking the enzyme's active site, *e.g.*, methotrexate is a structural analog of tetrahydrofolate, a coenzyme for the enzyme dihydrofolate reductase that binds to dihydrofolate reductase 1000-fold more tightly than the natural substrate and inhibits nucleotide base synthesis.

In some embodiments, the effector molecule is a chemical, *e.g.*, a chemical that modulates a cytosine (C) or an adenine(A) (*e.g.*, Na bisulfite, ammonium bisulfite). In some embodiments, the effector molecule has enzymatic activity (methyl transferase, demethylase, nuclease (*e.g.*, Cas9), a deaminase). In some embodiments, the effector molecule sterically hinders formation of an anchor sequence-mediated conjunction or binding of an RNA polymerase to a promoter.

The effector molecule with effector activity may be any one of the small molecules, peptides, fusion proteins, nucleic acids, nanoparticle, aptamers, or pharmacoagents with poor PK/PD described herein.

In some embodiments, the effector molecule is an inhibitor or "negative effector molecule". In the context of a negative effector molecule that modulates formation of an anchor sequence-mediated conjunction, in some embodiments, the negative effector molecule is characterized in that dimerization of an endogenous nucleating polypeptide is reduced when the negative effector molecule is present as compared with when it is absent. For example, in some embodiments, the negative effector molecule is or comprises a variant of the endogenous nucleating polypeptide's dimerization domain, or a dimerizing portion thereof.

For example, in certain embodiments, an anchor sequence-mediated conjunction is altered (*e.g.*, disrupted) by use of a dominant negative effector, *e.g.*, a protein that recognizes and binds an anchor sequence, (*e.g.*, a CTCF binding motif), but with an inactive (*e.g.*, mutated) dimerization domain, *e.g.*, a dimerization domain that is unable to form a functional anchor sequence-mediated conjunction. For example, the Zinc Finger domain of CTCF can be altered so that it binds a specific anchor sequence (by adding zinc fingers that recognize flanking nucleic acids), while the homo-dimerization domain is altered to prevent the interaction between the engineered CTCF and endogenous forms of CTCF.

In some embodiments, the effector molecule comprises a synthetic conjunction nucleating molecule with a selected binding affinity for an anchor sequence within a target anchor sequence-mediated conjunction, (the binding affinity may be at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or higher or lower than the affinity of an endogenous conjunction nucleating molecule that associates with the target anchor sequence. The synthetic conjunction nucleating molecule may have between 30-90%, 30-85%, 30-80%, 30-70%, 50-80%, 50-90% amino acid sequence identity to the endogenous conjunction nucleating molecule). The conjunction nucleating molecule may disrupt, such as through competitive binding, the binding of an endogenous conjunction nucleating molecule to its anchor sequence. In some more embodiments, the

conjunction nucleating molecule is engineered to bind a novel anchor sequence within the anchor sequence-mediated conjunction.

In some embodiments, a dominant negative effector molecule has a domain that recognizes specific DNA sequences (*e.g.*, an anchor sequence, a CTCF anchor sequence, flanked by sequences that confer sequence specificity), and a second domain that provides a steric presence in the vicinity of the anchoring sequence. The second domain may include a dominant negative conjunction nucleating molecule or fragment thereof, a polypeptide that interferes with conjunction nucleating molecule sequence recognition (*e.g.*, the amino acid backbone of a peptide/nucleic acid or PNA), a nucleic acid sequence ligated to a small molecule that imparts steric interference, or any other combination of DNA recognition elements and a steric blocker.

In some embodiments, the effector molecule is an epigenetic modifying agent. Epigenetic modifying agents useful in the methods and compositions described herein include agents that affect, *e.g.*, DNA methylation / demethylation, histone acetylation / deacetylation, and RNA-associated silencing. In some embodiments, the effectors sequence-specifically target an epigenetic enzyme (*e.g.*, an enzyme that generates or removes epigenetic marks, *e.g.*, acetylation and/or methylation). Exemplary epigenetic effectors may target an expression control region comprising, *e.g.*, a transcriptional control element or an anchor sequence, by a site-specific disrupting agent comprising a site-specific targeting moiety.

In some embodiments, an effector molecule comprises one or more components of a gene editing system. Components of gene editing systems may be used in a variety of contexts including but not limited to gene editing. For example, such components may be used to target agents that physically modify, genetically modify, and/or epigenetically modify FOXP3 sequences.

Exemplary gene editing systems include the clustered regulatory interspaced short palindromic repeat (CRISPR) system, zinc finger nucleases (ZFNs), and Transcription Activator-Like Effector-based Nucleases (TALEN). ZFNs, TALENs, and CRISPR-based methods are described, *e.g.*, in Gaj et al. Trends Biotechnol. 31.7(2013):397-405; CRISPR methods of gene editing are described, *e.g.*, in Guan et al, Application of CRISPR-Cas system in gene therapy: Pre-clinical progress in animal model. DNA Repair 2016 July 30 [Epub ahead of print]; Zheng et al, Precise gene deletion and replacement using the CRISPR/Cas9 system in human cells. BioTechniques, Vol. 57, No. 3, September 2014, pp. 115-124; .

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 Cpfl) to cleave foreign DNA. In a typical CRISPR/Cas system, an endonuclease is 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. The 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. The crRNA/tracrRNA hybrid then directs the Cas9 endonuclease to recognize and cleave the target DNA sequence. The 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 meningiditis). Some endonucleases, e. g., Cas9 endonucleases, are associated with G-rich PAM sites, e. g., 5'-NGG, 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 Cpfl, which is smaller than Cas9; examples include AsCpfl (from Acidaminococcus sp.) and LbCpfl (from Lachnospiraceae sp.). Cpf 1 -associated CRISPR arrays are processed into mature crRNAs without the requirement of a tracrRNA; in other words a Cpfl system requires only the Cpfl nuclease and a crRNA to cleave the target DNA sequence. Cpfl endonucleases, are associated with T-rich PAM sites, e. g., 5'-TTN. Cpfl can also recognize a 5'-CTA PAM motif. Cpfl cleaves the 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 the 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.

A variety of CRISPR associated (Cas) genes or proteins can be used in the present invention 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 Casl, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, CaslO, Cpfl, 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, the site-specific targeting moiety includes a sequence targeting polypeptide, such as an enzyme, *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, a S. thermophilus) a Crptococcus, a Corynebacterium, a Haemophilus, a Eubacterium, a Pasteurella, a Prevotella, a Veillonella, or a Marinobacter. In some embodiments nucleic acids encoding two or more different Cas proteins, or two or more Cas proteins, may be introduced into a cell, zygote, embryo, or animal, *e.g.*, to allow for recognition and modification of sites comprising the same, similar or different PAM motifs. In some embodiments, the Cas protein is modified to deactivate the nuclease, *e.g.*, nuclease-deficient Cas9, and to recruit transcription activators or repressors, *e.g.*, the co-subunit of the E. coli Pol, VP64, the activation domain of p65, KRAB, or SID4X, to induce epigenetic modifications, *e.g.*, histone acetyltransferase, histone methyltransferase and demethylase, DNA methyltransferase and enzyme with a role in DNA demethylation (*e.g.*, the TET family enzymes catalyze oxidation of 5-methylcytosine to 5-hydroxymethylcytosine and higher oxidative derivatives).

For the purposes of gene editing, CRISPR arrays can be designed to contain one or multiple guide RNA sequences corresponding to a desired target DNA sequence; see, for example, Cong et al. (2013) Science, 339:819-823; Ran et al. (2013) Nature Protocols, 8:2281 - 2308. At least about 16 or 17 nucleotides of gRNA sequence are required by Cas9 for DNA cleavage to occur; for Cpfl at least about 16 nucleotides of gRNA sequence is needed to achieve detectable DNA cleavage.

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 the target DNA but interferes with transcription by steric hindrance. dCas9 can further be fused with a heterologous effector to repress (CRISPRi) or activate (CRISPRa) expression of a target gene. For example, Cas9 can be fused to a transcriptional silencer (*e.g.*, a KRAB domain) or a transcriptional activator (*e.g.*, a dCas9-VP64 fusion). A catalytically inactive Cas9 (dCas9) fused to FokI nuclease ("dCas9-FokI") can be used to generate DSBs at target sequences homologous to two gRNAs. See, e. g., the numerous CRISPR/Cas9 plasmids disclosed in and publicly available from the Addgene repository (Addgene, 75 Sidney St., Suite 550A, Cambridge, MA 02139; addgene.org/crispr). A "double nickase" Cas9 that introduces two separate double-strand breaks, each directed by a separate guide RNA, is described as achieving more accurate genome editing by Ran et al. (2013) Cell, 154: 1380 - 1389.

CRISPR technology for editing the genes of eukaryotes is disclosed in US Patent Application Publications 2016/0138008A1 and US2015/0344912A1, and in US Patents 8,697,359, 8,771,945, 8,945,839, 8,999,641, 8,993,233, 8,895,308, 8,865,406, 8,889,418, 8,871,445, 8,889,356, 8,932,814, 8,795,965, and 8,906,616. Cpfl endonuclease and corresponding guide RNAs and PAM sites are disclosed in US Patent Application Publication 2016/0208243 Al .

In some embodiments, an effector comprises one or more components of a CRISPR system described hereinabove.

In some embodiments, suitable effectors for use in the agents, compositions, and methods of the invention include, for example, nucleases, physical blockers, epigenetic recruiters, *e.g.*, a transcriptional enhancer or a transcriptional repressor, and epigenetic CpG modifiers, *e.g.*, a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase, and combinations of any of the foregoing.

Suitable effectors include a polypeptide or its variant. The term "variant," as used herein, refers to a polypeptide that is derived by incorporation of one or more amino acid insertions, substitutions, or deletions in a precursor polypeptide (e.g., "parent" polypeptide). In certain embodiments, a variant polypeptide has at least about 85% amino acid sequence identity, e.g., about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%, amino acid sequence identity to the entire amino acid sequence of a parent polypeptide.

The term "sequence identity," as used herein, refers to a comparison between pairs of nucleic acid or amino acid molecules, i.e., the relatedness between two amino acid sequences or between two nucleotide sequences. In general, the sequences are aligned so that the highest order match is obtained. Methods for determining sequence identity are known and can be determined by commercially available computer programs that can calculate the percentage of identity between two or more sequences. A typical example of such a computer program is CLUSTAL.

Exemplary effectors include 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, DNMTL), protein methyltransferases (*e.g.*, viral lysine methyltransferase (vSET), protein-lysine N- methyltransferase (SMYD2), deaminases (*e.g.*, FOXP3 EC, UG1), histone methyltransferases such as enhancer of zeste homolog 2 (EZH2), PRMT1, histone-lysine-N-methyltransferase (Setdbl), histone methyltransferase (SET2), euchromatic histone-lysine N-methyltransferase 2 (G9a), histone-lysine N- methyltransferase (SUV39H1), and G9a), histone deacetylase (*e.g.*, HDACl, 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 KDMIA and lysine-specific histone demethylase 1 (LSD1), helicases such as DHX9, acetyltransferases, 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-naphthylamide or quinoline derivatives, nuclear receptor activators and inhibitors, proteasome inhibitors, competitive inhibitors for enzymes such as those involved in lysosomal storage

diseases, zinc finger proteins, TALENs, specific domains from proteins, such as a KRAB domain, a VP64 domain, a p300 domain (*e.g.*, p300 core domain), an MeCP2 domain, an MQ1 domain, a DNMT3a-3L domain, a TET1 domain, and/or TET2 domain, protein synthesis inhibitors, nucleases (*e.g.*, Cpfl, Cas9, zinc finger nuclease), fusions of one or more thereof (*e.g.*, dCas9-DNMT, dCas9-FOXP3 EC, dCas9-UGl, dCas9-VP64, dCas9-p300 core, dCas9-KRAB, dCas9-KRAB-MeCP2, dCas9-MQ1, dCas9-DNMT3a-3L, dCas9-TET1/TET2, and dCas9-MC/MN).

In some embodiments, a suitable nuclease for use in the agent, compositions, and methods of the invention comprises a Cas9 polypeptide, or enzymatically active portion thereof. In one embodiment, the Cas9 polypeptide, or enzymatically active portion thereof, further comprises a catalytically active domain of human exonuclease 1 (hEXO1), *e.g.*, 5' to 3' exonuclease activity and/or an RNase H activity. In other embodiments, a suitable nuclease comprises a transcription activator like effector nuclease (TALEN). In yet other embodiments, a suitable nuclease comprises a zinc finger protein.

The term TALEN, as used herein, is broad and includes a monomeric TALEN that can cleave double stranded DNA without assistance from another TALEN. The term TALEN is also used to refer to one or both members of a pair of TALENs that are engineered to work together to cleave DNA at the same site. TALENs that work together may be referred to as a left-TALEN and a right-TALEN, which references the handedness of DNA. See USSN 12/965,590; USSN 13/426,991 (US 8,450,471); USSN 13/427,040 (US 8,440,431); USSN 13/427,137 (US 8, 440,432); and USSN 13/738,381 , all of which are incorporated by reference herein in their entirety.

TAL effectors (TALE) are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a highly conserved 33-34 amino acid sequence with the exception of the 12th and 13th amino acids. These two locations are highly variable (Repeat Variable Diresidue (RVD)) and show a strong correlation with specific nucleotide recognition. This simple relationship between amino acid sequence and DNA recognition has allowed for the engineering of specific DNA binding domains by selecting a combination of repeat segments containing the appropriate RVDs.

The non-specific DNA cleavage domain from the end of the FokI endonuclease can be used to construct hybrid nucleases that are active in a yeast assay. These reagents are also active in plant cells and in animal cells. Initial TALEN studies used the wild-type FokI cleavage domain, but some subsequent TALEN studies also used FokI cleavage domain variants with mutations designed to improve cleavage specificity and cleavage activity. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALE binding sites are parameters for achieving high levels of activity. The number of amino acid residues between the TALE DNA binding domain and

the FokI cleavage domain may be modified by introduction of a spacer (distinct from the spacer sequence) between the plurality of TAL effector repeat sequences and the FokI endonuclease domain. The spacer sequence may be 12 to 30 nucleotides, *e.g.*, 12-15, 12-20, 20-25, or 15-30 nucleotides.

The relationship between amino acid sequence and DNA recognition of the TALE binding domain allows for designable proteins. In this case artificial gene synthesis is problematic because of improper annealing of the repetitive sequence found in the TALE binding domain. One solution to this is to use a publicly available software program (DNAWorks) to calculate oligonucleotides suitable for assembly in a two step PCR; oligonucleotide assembly followed by whole gene amplification. A number of modular assembly schemes for generating engineered TALE constructs have also been reported. Both methods offer a systematic approach to engineering DNA binding domains that is conceptually similar to the modular assembly method for generating zinc finger DNA recognition domains.

Once the TALEN genes have been assembled they are inserted into plasmids; the plasmids are then used to transfect the target cell where the gene products are expressed and enter the nucleus to access the genome. TALENs can be used to edit genomes by inducing double-strand breaks (DSB), which cells respond to with repair mechanisms. In this manner, they can be used to correct mutations in the genome which, for example, cause disease.

As used herein, a "zinc finger polypeptide" or "zinc finger protein" is a protein that binds to DNA, RNA and/or protein, in a sequence-specific manner, by virtue of a metal stabilized domain known as a zinc finger. Zinc finger proteins are nucleases having a DNA cleavage domain and a DNA binding zinc finger domain. Zinc finger polypeptides may be made by fusing the nonspecific DNA. cleavage domain of an endonuclease with site-specific DNA binding zinc finger domains. Such nucleases are powerful tools for gene editing and can be assembled to induce double strand breaks (DSBs) site-specifically into genomic DNA. ZFNs allow specific gene disruption as during DNA repair, the targeted genes can be disrupted via mutagenic non-homologous end joint (NHEJ) or modified via homologous recombination (HR) if a closely related DNA template is supplied.

Zinc finger nucleases are chimeric enzymes made by fusing the nonspecific DNA. cleavage domain of the endonuclease FokI with site-specific DNA binding zinc finger domains. Due to the flexible nature of zinc finger proteins (ZFPs), ZFNs can be assembled that induce double strand breaks (DSBs) site-specifically into genomic DNA. ZFNs allow specific gene disruption as during DNA repair, the targeted genes can be disrupted via mutagenic non-homologous end joint (NHEJ) or modified via homologous recombination (HR) if a closely related DNA template is supplied.

In some embodiments, a suitable physical blocker for use in the agent, compositions, and methods of the invention comprises a gRNA, antisense DNA, or triplex forming oligonucleotide (which may target an expression control unit) steric block a transcriptional control element or anchoring sequence.

The gRNA recognizes specific DNA sequences and further includes sequences that interfere with, *e.g.*, a conjunction nucleating molecule sequence to act as a steric blocker. In some embodiments, the gRNA is combined with one or more peptides, *e.g.*, S-adenosyl methionine (SAM), that acts as a steric presence. In other embodiments, a physical blocker comprises an enzymatically inactive Cas9 polypeptide, or fragment thereof (*e.g.*, dCas9).

In one embodiment, an epigenetic recruiter activates or enhances transcription of a target gene. In some embodiments, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises a VP64 domain or a p300 core domain.

In one embodiment, an epigenetic recruiter silences or represses transcription of a target gene. In some embodiments, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises a KRAB domain, or an MeCP2 domain.

In one embodiment, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises dCas9-VP64 fusion, a dCas9-p300 core fusion, a dCas9-KRAB fusion, or a dCas9-KRAB-MeCP2 fusion.

As used herein, "VP64" is a transcriptional activator composed of four tandem copies of VP16 (Herpes Simplex Viral Protein 16, amino acids 437-447*: DALDDFDLDML (SEQ ID NO: 95)) connected with glycine-serine (GS) linkers. In one embodiment, the VP64 further comprises the transcription factors p65 and Rta at the C terminus. The VP64 that comprises p65 and Rta is sometimes referred to as "VPR," or "VP64-p65-Rta." The VP64-p65-Rta, or VPR, was created by adding the transcription factors p65 and Rta to the Vp64 at the C terminus. Therefore, all three transcription factors can be targeted to the same gene. The use of three transcription factors, as opposed to solely Vp64, can result in increased expression of targeted genes. The GenBank Accession number of VP64 is ADD60007.1, the GenBank Accession number of p65 is NP_001138610.1, and the GenBank Accession number of Rta is AAA66528.1.

An exemplary amino acid sequence of a VPR is as follows:

DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLSGGPKKKRKVGS
QYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSS
LSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQA
VAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVA
PHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSG
SRDSREGMFLPKPEAGSAISDVFEGREVCQPKRLRPFHPPGSPWANRPLPASLAPTPTGPVHEPVG
SLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPP
PRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO.: 64).

As used herein, "p300 core domain" refers to the catalytic core of the human acetyltransferase p300. The GenBank Accession number for the protein comprising p300 is NP_001420.2.

An exemplary amino acid sequence of a p300 is as follows:

IFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQE
PWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLC
CYGKQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTLDP
ELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRLGTFLENRV
NDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKALFAFEEIDGV
DLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGH
IWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAK
ELPYFEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLS
RGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANSLPPIVDPDPLIPCDLMDGRDA
FLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQD (SEQ ID NO.: 65).

As used herein, "KRAB" refers to a Krüppel associated box (KRAB) transcriptional repression domain present in human zinc finger protein-based transcription factors (KRAB zinc finger proteins).

As used herein, MeCp2" refers to methyl CpG binding protein 2 which represses transcription, *e.g.*, by binding to a promoter comprising methylated DNA.

In one embodiment, an epigenetic CpG modifier methylates DNA and inactivates or represses transcription. In some embodiments, a suitable epigenetic CpG modifier for use in the agent, compositions, and methods of the invention comprises a MQ1 domain or a DNMT3a-3L domain.

In one embodiment, an epigenetic CpG modifier demethylates DNA and activates or stimulates transcription. In some embodiments, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises a TET1 or TET2 domain.

As used herein "MQ1" refers to a prokaryotic DNA methyltransferase.

As used herein "DNMT3a-3L" refers to a fusion of a DNA methyltransferase, Dnmt3a and a Dnmt3L which is catalytically inactive, but directly interacts with the catalytic domains of Dnmt3a.

As used herein "TET1" refers to "ten-eleven translocation methylcytosine dioxygenase 1," a member of the TET family of enzymes, encoded by the TET1 gene. TET1 is a dioxygenase that catalyzes the conversion of the modified DNA base 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) by oxidation of 5-mC in an iron and alpha-ketoglutarate dependent manner, the initial step of active DNA demethylation in mammals. Methylation at the C5 position of cytosine bases is an epigenetic modification of the mammalian genome which plays an important role in transcriptional regulation. In addition to its role in DNA demethylation, plays a more general role in chromatin regulation. Preferentially binds to CpG-rich sequences at promoters of both transcriptionally active and Polycomb-

repressed genes. Involved in the recruitment of the O-GlcNAc transferase OGT to CpG-rich transcription start sites of active genes, thereby promoting histone H2B GlcNAcylation by OGT. Exemplary TET1 nucleotide and amino acid sequence can be found at GenBank Accession Nos.: NM_030625.3, NP_085128.2

As used herein, "TET2" refers to "ten-eleven translocation 2 (TET2)," a member of the TET family of enzymes, encoded by the TET1 gene. Similarly to TET1, TET2 is a dioxygenase that catalyzes the conversion of the modified genomic base 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC) and plays a key role in active DNA demethylation. TET2 a preference for 5-hydroxymethylcytosine in CpG motifs. TET2 also mediates subsequent conversion of 5hmC into 5-formylcytosine (5fC), and conversion of 5fC to 5-carboxylcytosine (5caC). The conversion of 5mC into 5hmC, 5fC and 5caC probably constitutes the first step in cytosine demethylation. Methylation at the C5 position of cytosine bases is an epigenetic modification of the mammalian genome which plays an important role in transcriptional regulation. In addition to its role in DNA demethylation, also involved in the recruitment of the O-GlcNAc transferase OGT to CpG-rich transcription start sites of active genes, thereby promoting histone H2B GlcNAcylation by OGT. Exemplary nucleotide and amino acid sequence can be found at Genbank Accession No.: NM_001127208.2, NP_001120680.1

In some embodiments, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises a MQ1 domain, a DNMT3a-3L, a TET1 or TET2 domain. In one embodiment, a suitable epigenetic recruiter for use in the agent, compositions, and methods of the invention comprises a dCas9-MQ1 fusion, a dCas9-DNMT3a-3L fusion, or a dCas9-TET1 fusion or - dCase9-TET2 fusion.

III. Delivery of a Site-Specific FOXP3 Disrupting Agent of the Invention and Compositions Comprising a Site-Specific a FOXP3 Disrupting Agents of the Invention

The delivery of the disrupting agents of the invention to a cell *e.g.,* a cell within a subject, such as a human subject (*e.g.,* a subject in need thereof, such as a subject having a FOXP3-associated disorder, *e.g.,* an autoimmune disease, such as IPEX syndrome) may be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with a disrupting agent of the invention either *in vitro*, *ex vivo*, or *in vivo*. *In vivo* delivery may be performed directly by administering a composition, such as a lipid composition, comprising a disrupting agent to a subject. Alternatively, *in vivo* delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the disrupting agent in a cell of a subject. These alternatives are discussed further below. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below and/or are known in the art.

In some embodiments, the disrupting agent comprises a nucleic acid molecule encoding a fusion protein, the fusion protein comprising a site-specific FOXP3 targeting moiety, such as a polynucleotide encoding a DNA-binding domain of a Transcription activator-like effector (TALE) polypeptide or a zinc finger (ZNF) polypeptide, or fragment thereof, that specifically targets and binds to the FOXP3 expression control region and an effector molecule., such as a VPR.

In other embodiments, the disrupting agent comprises a guide RNA and an mRNA encoding an effector molecule. The ratio of guide RNA to mRNA may be about 100:1 to about 1:100 (wt:wt).

In general, any method of delivery of a site-specific FOXP3 disrupting agent of the invention (*in vitro*, *ex vivo*, or *in vivo*) may be adapted for use with the disrupting agents of the invention (see *e.g.*, Akhtar S. and Julian RL., (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For *in vivo* delivery, factors to be considered for delivering a site-specific FOXP3 disrupting agent of the invention include, for example, biological stability of the disrupting agent, prevention of non-specific effects, and accumulation of the disrupting agent in the target tissue. The non-specific effects of a disrupting agent can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering a composition comprising the disrupting agent. Local administration to a treatment site maximizes local concentration of the disrupting agent, limits the exposure of the disrupting agent to systemic tissues that can otherwise be harmed by the disrupting agent or that can degrade the disrupting agent, and permits a lower total dose of the disrupting agent to be administered.

For administering a site-specific FOXP3 disrupting agent systemically for the treatment of a disease, such as a FOXP3-associate disease, the disrupting agent, *e.g.*, a disrupting agent comprising a site-specific targeting moiety comprising a nucleic acid molecule, can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of a site-specific targeting moiety comprising a nucleic acid molecule by endo- and exo-nucleases *in vivo*. Modification of a disrupting agent comprising a site-specific targeting moiety comprising a nucleic acid molecule or a pharmaceutical carrier also permits targeting of the disrupting agent to a target tissue and avoidance of undesirable off-target effects. For example, a disrupting agent of the invention may be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation.

Alternatively, a disrupting agent of the invention may be delivered using a drug delivery system such as a nanoparticle, a dendrimer, a polymer, a liposome, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of disrupting agent (*e.g.*, negatively charged molecule) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of a disrupting agent by the cell. Cationic lipids, dendrimers, or polymers can either be bound to a

disrupting agent, or induced to form a vesicle or micelle (see *e.g.*, Kim SH. *et al.*, (2008) *Journal of Controlled Release* 129(2):107-116) that encases the disrupting agent. The formation of vesicles or micelles further prevents degradation of the disrupting agent when administered systemically. Methods for making and administering cationic complexes are well within the abilities of one skilled in the art (see *e.g.*, Sorensen, DR., *et al.* (2003) *J. Mol. Biol* 327:761-766; Verma, UN. *et al.*, (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, AS *et al.* (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of a distrupting agent of the invention include DOTAP (Sorensen, DR., et al (2003), supra; Verma, UN. *et al.*, (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, TS. *et al.*, (2006) *Nature* 441:111-114), cardiolipin (Chien, PY. *et al.*, (2005) *Cancer Gene Ther.* 12:321-328; Pal, A. *et al.*, (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet ME. *et al.*, (2008) *Pharm. Res.* Aug 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, DA. *et al.*, (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H. *et al.*, (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, a disrupting agent (*e.g.*, gRNA, or mRNA) forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions comprising cyclodextrins may be found in U.S. Patent No. 7,427,605, the entire contents of which are incorporated herein by reference.

The disrupting agents of the invention may be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically include one or more species of disrupting agent and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral, or parenteral. Parenteral administration includes intravenous drip, subcutaneous, intraperitoneal or intramuscular injection, or intrathecal or intraventricular administration.

The route and site of administration may be chosen to enhance delivery or targeting of the disrupting agent comprising a site-specific targeting moiety to a particular location. For example, to target

liver cells, intravenous injection may be used. Lung cells may be targeted by administering the disrupting agent in aerosol form. Jejunum cells may be targeted by anal administration.

Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids, and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water, syrups, elixirs or non-aqueous media, tablets, capsules, lozenges, or troches. In the case of tablets, carriers that can be used include lactose, sodium citrate and salts of phosphoric acid. Various disintegrants such as starch, and lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc, are commonly used in tablets. For oral administration in capsule form, useful diluents are lactose and high molecular weight polyethylene glycols. When aqueous suspensions are required for oral use, the nucleic acid compositions can be combined with emulsifying and suspending agents. If desired, certain sweetening or flavoring agents can be added.

Compositions for intravenous administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives.

Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, diluents, and other suitable additives. For intravenous use, the total concentration of solutes may be controlled to render the preparation isotonic.

In one embodiment, the administration of a disrupting agent composition of the invention is parenteral, *e.g.,* intravenous (*e.g.*, as a bolus or as a diffusible infusion), intradermal, intraperitoneal, intramuscular, intrathecal, intraventricular, intracranial, subcutaneous, transmucosal, buccal, sublingual, endoscopic, rectal, oral, vaginal, topical, pulmonary, intranasal, urethral, or ocular. Administration can be provided by the subject or by another person, *e.g.*, a health care provider. The composition may be provided in measured doses or in a dispenser which delivers a metered dose. Selected modes of delivery are discussed in more detail below.

In certain embodiments, the disrupting agents of the invention are polynucleotides, such as mRNAs, and are formulated in lipid nanoparticles (LNPs).

A. Compositions Comprising a Site-Specific a FOXP3 Disrupting Agent of the Invention

The site-specific FOXP3 disrupting agents of the invention may be formulated into compositions, such as pharmaceutical compositions, using one or more excipients to: (1) increase stability; (2) increase cell transfection; (3) permit sustained or delayed release (*e.g.*, from a depot formulation); (4) alter the biodistribution (*e.g.*, target the disrupting agent to specific tissues or cell types); (5) increase the

translation of an encoded protein *in vivo*; and/or (6) alter the release profile of an encoded protein *in vivo*. In addition to traditional excipients, such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, excipients for use in the compositions of the invention may include, without limitation, lipidoids, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with nucleic acid molecules, modified nucleic acid molecules, or RNA (*e.g.*, for transplantation into a subject), hyaluronidase, nanoparticle mimics and combinations thereof. Accordingly, the pharmaceutical compositions of the invention can include one or more excipients, each in an amount that together increases the stability of the disrupting agent, increases cell transfection by the disrupting agent, increases the expression of modified nucleic acid, or mRNA encoded protein, and/or alters the release profile of a disrupting agent. Further, the disrupting agents of the present invention may be formulated using self-assembled nucleic acid nanoparticles (see, *e.g.,* U.S. Patent Publication No. 2016/0038612A1, which is incorporated herein by reference in its entirety.

i. Lipidoid

The synthesis of lipidoids has been extensively described and formulations containing these compounds are particularly suited for delivery of a disrupting agent of the invention, such as a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, *e.g.,* comprising modified nucleic acid molecules or mRNA (see Mahon *et al.*, Bioconjug Chem. 2010 21:1448-1454; Schroeder *et al.*, J Intern Med. 2010 267:9-21; Akinc *et al.*, Nat Biotechnol. 2008 26:561-569; Love *et al.*, Proc Natl Acad Sci USA. 201 0 107: 1864-1869; Siegwart *et al.*, Proc Natl Acad Sci USA. 2011108:12996-3001; the contents of all of which are incorporated herein in their entireties).

For example, lipidoids have been used to effectively deliver double stranded small interfering RNA molecules, single stranded nucleic acid molecules, modified nucleic acid molecules or modified mRNA. (*See*, *e.g.*, US Patent Publication 2016/0038612A1). Complexes, micelles, liposomes or particles can be prepared containing these lipidoids and, therefore, provide effective delivery of a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, as judged by the production of an encoded protein, following the administration of a lipidoid formulation, *e.g., via* localized and/or systemic administration. Lipidoid complexes of can be administered by various means including, but not limited to, intravenous, intramuscular, intradermal, intraperitoneal or subcutaneous routes.

In vivo delivery of a site-specific FOXP3 targeting moiety comprising, *e.g.,* a nucleic acid molecule, may be affected by many parameters, including, but not limited to, the formulation composition, nature of particle PEGylation, degree of loading, polynucleotide to lipid ratio, and biophysical parameters such as, but not limited to, particle size (Akinc *et al.*, Mol Ther. 2009 17:872-879; herein incorporated by reference in its entirety). As an example, small changes in the anchor chain length of poly(ethylene

glycol) (PEG) lipids may result in significant effects on *in vivo* efficacy. Formulations with different lipidoids, including, but not limited to penta[3 -(1-laury laminopropiony I)]-triethy lenetetramine hydrochloride (TETA-5LAP; aka 98NI2-5, see Murugaiah *et al.*, *Analytical Biochemistry*, 401:61 (2010); the contents of which are herein incorporated by reference in its entirety), C12-200 (including derivatives and variants), and MDl, may be used.

In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising, *e.g.,* a nucleic acid molecule, is formulated with a lipidoid for systemic intravenous administration to target cells of the liver. For example, a final optimized intravenous formulation comprising a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, and a lipid molar composition of 42% 98NI2-5, 48% cholesterol, and 10% PEG-lipid with a final weight ratio of about 7.5 to 1 total lipid to nucleic acid molecule, and a C14 alkyl chain length on the PEG lipid, with a mean particle size of roughly 50-60 nm, can result in the distribution of the formulation to be greater than 90% to the liver (see, Akinc *et al.*, Mol Ther. 2009 17:872-879; the contents of which is herein incorporated by reference in its entirety). In another example, an intravenous formulation using a C12-200 lipidoid (see, *e.g.,* PCT Publication No. WO 2010/129709, which is herein incorporated by reference in their entirety) having a molar ratio of 50/10/38.5/1.5 of C12-200/disteroylphosphatidyl choline/cholesteroI/PEG-DMG, with a weight ratio of 7 to 1 total lipid to nucleic acid molecule, and a mean particle size of 80 nm may be used to deliver a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, to hepatocytes (see, Love *et al.*, Proc Natl Acad Sci USA. 2010 107:1864-1869; the contents of which are herein incorporated by reference in its entirety). In another embodiment, an MDl lipidoid-containing formulation may be used to effectively deliver a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule, to hepatocytes *in vivo*. The characteristics of optimized lipidoid formulations for intramuscular or subcutaneous routes may vary significantly depending on the target cell type and the ability of formulations to diffuse through the extracellular matrix into the blood stream. While a particle size of less than 150 nm may be desired for effective hepatocyte delivery due to the size of the endothelial fenestrae (see, Akinc *et al.*, Mol Ther. 2009 17:872-879; the contents of which are herein incorporated by reference in their entirety), use of lipidoid-formulated nucleic acid molecules to deliver the formulation to other cells types including, but not limited to, endothelial cells, myeloid cells, and muscle cells may not be similarly size-limited. Use of lipidoid formulations to deliver siRNA in vivo to other non-hepatocyte cells such as myeloid cells and endothelium has been reported (see Akinc *et al.*, Nat Biotechnol. 200826:561-569; Leuschner *et al.*, Nat Biotechnol. 2011 29: 1005-101 0; Cho *et al.* Adv. Funct. Mater. 2009 19 :3112-3118; 8th International Judah Folkman Conference, Cambridge, Mass. Oct. 8-9, 2010; the contents of each of which are herein incorporated by reference in their entirety). For

delivery to myeloid cells, such as monocytes, lipidoid formulations may have a similar component molar ratio. Different ratios of lipidoids and other components including, but not limited to, disteroylphosphatidyl choline, cholesterol and PEG-DMG, may be used to optimize the formulation for delivery to different cell types including, but not limited to, hepatocytes, myeloid cells, muscle cells, *etc.* For example, the component molar ratio may include, but is not limited to, 50% CI2-200, 10% disteroylphosphatidyl choline, 38.5% cholesterol, and 1.5% PEG-DMG (see Leuschner *et al.*, Nat Biotechnol 2011 29: 1005-101 0; the contents of which are herein incorporated by reference in their entirety). The use of lipidoid formulations for the localized delivery to cells (such as, but not limited to, adipose cells and muscle cells) via either subcutaneous, intradermal or intramuscular delivery, may not require all of the formulation components desired for systemic delivery and, as such, may comprise only the lipidoid and a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising, *e.g.*, a nucleic acid molecule, as described herein.

Combinations of different lipidoids may be used to improve the efficacy of the formulations by increasing cell transfection and/or increasing the translation of encoded protein contained therein(see Whitehead *et al.*, Mol. Ther. 2011,19:1688-1694, the contents of which are herein incorporated by reference in their entirety).

In one embodiment, the lipidoid may be prepared from the conjugate addition of alklamines to acrylates. As a non-limiting example, a lipidoid may be prepared by the methods described in PCT Patent Publication No. WO 2014/028487, the contents of which are herein incorporated by reference in its entirety. In one embodiment, the lipidoid may comprise a compound having formula (I), formula (II), formula (III), formula (IV) or formula (V) as described in PCT Patent Publication No. WO 2014/028487, the contents of which are herein incorporated by reference in their entirety. In one embodiment, the lipidoid may be biodegradable.

ii. Liposomes, Lipoplexes, and Lipid Nanoparticles

A disrupting agent of the invention may be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one embodiment, pharmaceutical compositions of the invention include liposomes. Liposomes are artificially-prepared vesicles which are primarily composed of a lipid bilayer and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes may be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to, endocytosis.

Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations. The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

As a non-limiting example, liposomes, such as synthetic membrane vesicles, may be prepared by the methods, apparatus and devices described in U.S. Patent Publication Nos. 2013/0177638, 2013/0177637, 2013/0177636, 201/30177635, 2013/0177634, 2013/0177633, 2013/0183375, 2013/0183373, 2013/0183372 and 2016/0038612) and PCT Patent Publication No WO 2008/042973, the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, a pharmaceutical composition described herein may include, without limitation, liposomes such as those formed from 1 ,2-dioleyloxy-N,N -dimethyl ami - nopropane (DODMA) liposomes, DiLa2 liposomes from Marina Biotech (Bothell, Wash.), 1,2-dilinoleyloxy-3-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), and MC3 (US20100324120; herein incorporated by reference in its entirety) and liposomes which may deliver small molecule drugs such as, but not limited to, DOXIL® from Janssen Biotech, Inc. (Horsham, Pa.). In one embodiment, a pharmaceutical composition described herein may include, without limitation, liposomes such as those formed from the synthesis of stabilized plasmid-lipid particles (SPLP) or stabilized nucleic acid lipid particle (SNALP) that have been previously described and shown to be suitable for oligonucleotide delivery *in vitro* and *in vivo* (*see* Wheeler *et al.* Gene Therapy. 1999 6:271-281; Zhang *et al.* Gene Therapy. 19996:1438-1447; Jeffs *et al.* Pharm Res. 2005 22:362-372; Morrissey *et al.*, Nat Biotechnol. 2005 2:1002-1007; Zimmermann *et al.*, Nature. 2006 441:111-114; Heyes *et al.* J Contr Rel. 2005 107 :276-287; Semple *et al.* Nature Biotech. 2010 28:172-176; Judge *et al.* J Clin Invest. 2009 119:661-673; deFougerolles Hum Gene Ther. 2008 19:125-132; U.S. Patent Publication Nos 2013/0122104, 2013/0303587, and 2016/0038612; the contents of each of which are incorporated herein in their entireties). The original manufacturing method of Wheeler *et al.* was a detergent dialysis method, which was later improved by Jeffs *et al.* and is referred to as the spontaneous vesicle formation method. The liposome formulations of the invention may be composed of 3 to 4 lipid components in addition a disrupting agent comprising a site-specific FOXP3 targeting moiety. As an example a liposome of the invention can contain, but is not limited to, 55% cholesterol, 20% disteroylphosphatidyl choline (DSPC), 10% PEG-SDSG, and 15% 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), as described by Jeffs

et al. As another example, liposome formulations of the invention may contain, but are not limited to, 48% cholesterol, 20% DSPC, 2% PEG-c-DMA, and 30% cationic lipid, where the cationic lipid can be 1,2-distearloxy- N,N-dimethylaminopropane (DSDMA), DODMA, DLin-DMA, or 1 ,2-dilinolenyloxy -3 -dimethy laminopropane (DLenDMA), as described by Heyes *et al*. In some embodiments, liposome formulations may comprise from about 25.0% cholesterol to about 40.0% cholesterol, from about 30.0% cholesterol to about 45.0% cholesterol, from about 35.0% cholesterol to about 50.0% cholesterol and/or from about 48.5% cholesterol to about 60% cholesterol. In another embodiment, formulations of the invention may comprise a percentage of cholesterol selected from the group consisting of 28.5%, 31.5%, 33.5%, 36.5%, 37.0%, 38.5%, 39.0% and 43.5%. In some embodiments, liposome formulations of the invention may comprise from about 5.0% to about 10.0% DSPC and/or from about 7.0% to about 15.0% DSPC.

In one embodiment, a pharmaceutical composition may include liposomes which may be formed to deliver a disrupting agent of the invention. The disrupting agent comprising a site-specific FOXP3 targeting moiety comprising may be encapsulated by the liposome and/or it may be contained in an aqueous core which may then be encapsulated by the liposome (see, *e.g.*, PCT Patent Publication Nos. WO 2012/031046, WO 2012/031043, WO 2012/030901 and WO 2012/006378 and U.S. Patent Publication Nos. 2013/0189351, 2013/0195969 and 201/30202684, the contents of each of which are herein incorporated by reference in their entirety).

In another embodiment, liposomes for use in the present invention may be formulated for targeted delivery. As a non-limiting example, the liposome may be formulated for targeted delivery to the liver. Such a liposome may include, but is not limited to, a liposome described in U.S. Patent Publication No. 2013/0195967, the contents of which are herein incorporated by reference their its entirety.

In one embodiment, formulations comprising liposomes and a disrupting agent may be administered intramuscularly, intrademrally, or intravenously.

In another embodiment, a lipid formulation of the invention may include at least one cationic lipid, a lipid which enhances transfection and a least one lipid which contains a hydrophilic head group linked to a lipid moiety (International Pub. No. WO2011076807 and U.S. Pub. No. 20110200582; the entire contents of each of which is herein incorporated by reference in their entirety). In another embodiment, a lipid formulation of the invention is a lipid vesicle which may have crosslinks between functionalized lipid bilayers (see U.S Patent Publication No. 2012/0177724, the contents of which are herein incorporated by reference in their entirety).

In one embodiment, a formulation comprising a disrupting agent is a lipid nanoparticle (LNP) which may comprise at least one lipid. The lipid may be selected from, but is not limited to, DLin-DMA, DLin-K-DMA, 98NI2-5, CI2-200, DLin-MC3-DMA, DLin-KC2-DMA, DODMA, PLGA, PEG, PEG-

DMG, PEGylated lipids and amino alcohol lipids. In another aspect, the lipid may be a cationic lipid such as, but not limited to, DLin-DMA, DLin-D-DMA, DLin-MC3- DMA, DLin-KC2-DMA, DODMA and amino alcohol lipids. The amino alcohol cationic lipid may be the lipids described in and/or made by the methods described in U.S. Patent Publication No. 2013/0150625.

In one embodiment, the cationic lipid may be selected from, but not limited to, a cationic lipid described in PCT Publication Nos. WO 2012/040184, WO 2011/153120, WO 2011/149733, WO 2011/090965, WO 2011/043913, WO 2011/022460, WO 2012/061259, WO 2012/054365, WO 2012/044638, WO 2010/080724, WO 2010/21865, WO 2008/103276, WO 2013/086373 and WO 2013/086354, U.S. Patent Nos. 7,893,302, 7,404,969, 8,283, 333, 8,466,122 and 8,569,256, and U.S. Patent Publication Nos. 2010/0036115, 2012/0202871, 2013/0064894, 2013/0129785, 2013/0150625, 2013/0178541, 2013/0225836 and 2014/0039032; the contents of each of which are herein incorporated by reference in their entirety. In another embodiment, the cationic lipid may be selected from, but not limited to, formula A described in PCT Publication Nos. WO 2012/040184, WO 0111/53120, WO 2011/149733, WO 2011/090965, WO 2011/043913, WO 2011/022460, WO 2012/061259, WO 2012/054365, WO 2012/044638 and WO 2013/116126 or U.S. Patent Publication Nos. 2013/0178541 and 2013/0225836; the contents of each of which is herein incorporated by reference in their entirety. In yet another embodiment, the cationic lipid may be selected from, but not limited to, formula CLI-CLXXIX of PCT Publication No. WO 2008/103276, formula CLICLXXIX of U.S Patent No. 7,893,302, formula CLICLXXXXII of U.S. Patent No. 7,404,969 and formula I-VI of us Patent Publication No. 2010/0036115, formula I of U.S. Patent Publication No 2013/0123338; each of which is herein incorporated by reference in their entirety.

In one embodiment, the cationic lipid may be synthesized by methods known in the art and/or as described in PCT Publication Nos. WO 2012/040184 WO 2011/153120, WO 2011/149733, WO 2011/090965: WO 2011/043913, WO 2011/022460, WO 2012/061259, WO 2012/054365, WO 2012/044638, WO 2010/080724, WO 2010/21865, WO 2013/126803, WO 2013/086373, and WO 2013/086354; the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, the lipids which may be used in the formulations and/or for delivery of the disrupting agents described herein may be a cleavable lipid. As a non-limiting example, a cleavable lipid and/or pharmaceutical compositions comprising cleavable lipids include those described in PCT Patent Publication No. WO 2012/170889, the contents of which are herein incorporated by reference in their entirety. As another non-limiting example, the cleavable lipid may be HGT4001, HGT4002, HGT4003, HGT4004 and/or HGT4005 as described in PCT Patent Publication No. WO 2012/170889, the contents of which are herein incorporated by reference in their entirety.

In one embodiment, polymers which may be used in the formulation and/or delivery of the disrupting agents described herein may include, but is not limited to, poly(ethylene) glycol (PEG), polyethylenimine (PEI), dithiobis(succinimidylpropionate) (DSP), Dimethy 1-3,3' - dithiobispropionimidate (DTBP), poly(ethylene imine) biscarbamate (PEIC), poly(L-lysine) (PLL), histidine modified PLL, poly(N-vinylpyrrohdone) (PVP), poly(propylenimine (PPI), poly(amidoamine) (PAMAM), poly(amido ethylenimine) (SS-PAEI), triehtylenetetramine (TETA), poly(β-aminoester), poly(4- hydroxy-L-proine ester) (PHP), poly(allylamine), poly(α-[4- aminobutyl]-L-glycolic acid (PAGA), Poly(D,L-lactic-coglycolid acid (PLGA), Poly(N-ethyl-4-vinylpyridinium bromide), poly(phosphazene)s (PPZ), poly(phosphoester)s (PPE), poly(phosphoramidate)s (PPA), poly(N-2-hydroxypropylmethacrylamide) (pHPMA), poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA), poly(2-aminoethyl propylene phosphate) PPE_EA), Chitosan, galactosylated chitosan, N-dodecylated chitosan, histone, collagen and dextran-spermine. In one embodiment, the polymer may be an inert polymer such as, but not limited to, PEG. In one embodiment, the polymer may be a cationic polymer such as, but not limited to, PEl, PLL, TETA, poly(allylamine), Poly(N -ethyl-4-vinylpyridinium bromide), pHPMA and pDMAEMA. In one embodiment, the polymer may be a biodegradable PEl such as, but not limited to, DSP, DTBP and PEIC. In one embodiment, the polymer may be biodegradable such as, but not limited to, histine modified PLL SSPAEI, poly(β-aminoester), PHP, PAGA, PLGA, PPZ, PPE, PPA and PPE-EA.

In one embodiment, an LNP formulation of the invention may be prepared according to the methods described in PCT Publication Nos. WO 2011/127255 or WO 2008/103276, the contents of each of which are herein incorporated by reference in their entirety. As a non-limiting example, a disrupting agent comprising a site-specific FOXP3 targeting moiety may be encapsulated in an LNP formulation as described in PCT Publication Nos. WO 2011/127255 and/or WO 2008/103276; the contents of each of which are herein incorporated by reference in their entirety. As another non-limiting example, a disrupting agent comprising a site-specific FOXP3 targeting moiety as described herein, may be formulated in a nanoparticle to be delivered by a parenteral route as described in U.S. Patent Publication No. 2012/0207845 and PCT Publication No. WO 2014/008334; the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, LNP formulations described herein may be administered intramusculary. The LNP formulation may comprise a cationic lipid described herein, such as, but not limited to, DLin-DMA, DLin-KC2-DMA, DLin-MC3-DMA, DODMA and C12-200.

In one embodiment, LNP formulations described herein comprising a disrupting agent as described herein, may be administered intradermally. The LNP formulation may comprise a cationic lipid

described herein, such as, but not limited to, DLin-DMA, DLin-KC2-DMA, DLin-MC3-DMA, DODMA and C12-200.

The nanoparticle formulations may comprise conjugate, such as a phosphate conjugate, a polymer conjugates, a conjugate that enhances the delivery of nanoparticle as described in US Patent Publication No. US20160038612 A1.

In one embodiment, the lipid nanoparticle formulation comprises DLin-MC3-DMA as described in US Patent Publication No. US20100324120.

In one embodiment, the lipid nanoparticle comprises a lipid compound, or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, or a lipid nanoparticle formulation, as described in US Patent No.: US10723692B2, US Patent Publication Nos. US20200172472A1, US20200163878A1, US20200046838A1, US20190359556A1, US20190314524A1, US20190274968A1, US20190022247A1, US20180303925A1, US20180185516A1, US20160317676A1, International Patent Publication No.: WO20200146805A1, WO2020081938A1, WO2019089828A1, WO2019036030A1, WO2019036028A1, WO2019036008A1, WO 2018200943A1, WO2018191719A1, WO2018107026A1, WO2018081480A1, the contents of each of which are herein incorporated by reference in their entirety (Acuitas Therapeutics, Inc.).

In one embodiment, the lipid nanoparticle comprises an amino lipid, or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, or a lipid nanoparticle formulation, described by Tekmira Pharmaceuticals Corp. in US9139554B2, US9051567B2, US8883203B2, US Patent Publication US20110117125A1, the contents of each of which are herein incorporated by reference in their entirety. In one particular example, the compound described in US9139554B2 is DLin-kC2-DMA.

In one embodiment, the lipid nanoparticle comprises an amino lipid, or a pharmaceutically acceptable salt, tautomer or stereoisomer thereof, or a lipid nanoparticle formulation, described by Arbutus Biopharma Corp. in US10561732B2, US9938236B2, US9687550B2, US Patent Publication US20190240354A1, US20170027658A1, WO2020097493A1, WO2020097520A1, WO2020097540A1, WO2020097548A1, the contents of each of which are herein incorporated by reference in their entirety.

Lipid nanoparticles may be engineered to alter the surface properties of particles so the lipid nanoparticles may penetrate the mucosal barrier. Mucus is located on mucosal tissue such as, but not limited to, oral (*e.g.*, the buccal and esophageal membranes and tonsil tissue), ophthalmic, gastrointestinal (*e.g.*, stomach, small intestine, large intestine, colon, rectum), nasal, respiratory (*e.g.*, nasal, pharyngeal, tracheal and bronchial membranes), genital (*e.g.*, vaginal, cervical and urethral membranes). Nanoparticles larger than 10-200 nm which are preferred for higher drug encapsulation efficiency and the ability to provide the sustained delivery of a wide array of drugs have been thought to be too large to rapidly diffuse through mucosal barriers. Mucus is continuously secreted, shed, discarded or digested and

recycled so most of the trapped particles may be removed from the mucosla tissue within seconds or within a few hours. Large polymeric nanoparticles (200 nm-500 nm in diameter) which have been coated densely with a low molecular weight polyethylene glycol (PEG) diffused through mucus only 4 to 6-fold lower than the same particles diffusing in water (Lai *et al.* PNAS 2007 104(5): 1482-487; Lai *et al.* Adv Drug Deliv Rev. 200961(2): 158-171; the contents of each of which are herein incorporated by reference in their entirety). The transport of nanoparticles may be determined using rates of permeation and/or fluorescent microscopy techniques including, but not limited to, fluorescence recovery after photobleaching (FRAP) and high resolution multiple particle tracking (MPT). As a non-limiting example, compositions which can penetrate a mucosal barrier may be made as described in US Pat. No. 8,241,670 or International Patent Publication No. WO2013110028, the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety as described herein, is formulated as a lipoplex, such as, without limitation, the ATUPLEX™ system, the DACC system, the DBTC system and other siRNAlipoplex technology from Silence Therapeutics (London, United Kingdom), STEMFECFM from STEMGENT® (Cambridge, Mass.), and polyethylenimine (PEI) or protamine- based targeted and non-targeted delivery of nucleic acids (Aleku *et al.* Cancer Res. 2008 68:9788-9798; Strumberg *et al.* Int J Clin Pharmacol Ther 2012 50:76-78; Santel *et al.*, Gene Ther 2006 13:1222-1234; Santel et al., Gene Ther 200613:1360-1370; Gutbier *et al.*, PulmPharmacol. Ther. 201023:334-344; Kaufmann *et al.* Microvasc Res 2010 80:286-293; Weide *et al.* J Immunother. 2009 32:498- 507; Weide *et al.* J Immnnother. 2008 31:180-188; Pascolo Expert Opin. Biol. Ther. 4:1285-1294; Fotin-Mleczek *et al.*, 2011 J. Immunother. 34: 1-15; Song *et al.*, Nature Biotechnol. 2005,23:709-717; Peer *et al.*, Proc NatlAcad Sci USA. 2007 6; 104:4095-4100; deFougerolles *Hum Gene Ther. 2008* 19: 125-132; all of which are incorporated herein by reference in their entirety).

In one embodiment such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo*, including but not limited to hepatocytes, immune cells, tumor cells, endothelial cells, antigen presenting cells, and leukocytes (Akinc *et al.* Mol Ther. 2010 18:1357-1364; Song *et al.*, Nat Biotechnol. 2005 23:709-717; Judge *et al.*, J Clinlnvest. 2009 119:661-673; Kaufmann *et al.*, Microvasc Res 2010 80:286- 293; Santel *et al.*, Gene Ther 200613:1222-1234; Santel *et al.*, Gene Ther 2006 13: 1360-1370; Gutbier *et al.*, Pulm Pharmacol. Ther. 2010 23:334-344; Basha *et al.*, Mol. Ther. 2011 19:2186-2200; Fenske and Cullis, Expert Opin Drug Deliv. 20085:25-44; Peer *et al.*, Science. 2008 319:627-630; Peer and Lieberman, Gene Ther. 2011 18: 1127-1133; all of which are incorporated herein by reference in its entirety). One example of passive targeting of formulations to liver cells includes the DLin-DMA, DLin-KC2-DMA and DLin-MC3-DMA-based lipid nanoparticle formulations which have been shown to bind to apolipoprotein E and promote

binding and uptake of these formulations into hepatocytes *in vivo* (Akinc *et al.* Mol Ther. 2010 18: 1357 - 1364; the contents of which are herein incorporated by reference in its entirety). Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GaINAc), and antibody targeted approaches (Kolhatkar *et al.*, Curr Drug Discov Technol. 2011 8: 197 -206; Musacchio and Torchilin, Front Biosci. 201116: 1388-1412; Yu *et al.*, Mol Membr BioI. 2010 27:286-298; Patil *et al.*, Crit Rev Ther Drug Carrier Syst. 2008 25: 1-61; Benoit *et al.*, Biomacromolecules. 2011 12:2708-2714; Zhao *et al.*, Expert Opin Drug Deliv. 2008 5:309-319;Akinc *et al.*, Mol Ther. 2010 18:1357-1364; Srinivasan *et al.*, Methods Mol BioI. 2012 820: 105-116; Ben-Arie *et al.*, Methods Mol Biol. 2012 757:497-507; Peer 2010 J Control Release. 20:63-68; Peer *et al.*, Proc Natl Acad Sci USA. 2007 104:4095-4100; Kim *et al.*, Methods Mol BioI. 2011 721:339-353; Subramanya et al., Mol Ther. 2010 18:2028-2037; Song et al., Nat Biotechnol. 2005 23:709-717; Peer *et al.*, Science. 2008 319:627 -630; Peer and Lieberman, Gene Ther. 2011 18:1127-1133; the contents of all of which are incorporated herein by reference in its entirety).

In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety of the invention, may be formulated as a solid lipid nanoparticle. A solid lipid nanoparticle (SLN) may be spherical with an average diameter between 10 to 1000 nm. SLN possess a solid lipid core matrix that can solubilize lipophilic molecules and may be stabilized with surfactants and/or emulsifiers. In a further embodiment, the lipid nanoparticle may be a self-assembly lipid-polymer nanoparticle (see Zhang *et al.*, ACS Nano, 2008, 2 (8), pp 1696-1702; herein incorporated by reference in its entirety). As a non-limiting example, the SLN may be the SLN described in PCT Publication No. WO2013/105101, the contents of which are herein incorporated by reference in their entirety. As another non-limiting example, the SLN may be made by the methods or processes described in PCT Publication No. WO 2013/105101, the contents of which are herein incorporated by reference in their entirety.

Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising, *e.g.,* a nucleic acid molecule, to direct protein production as these formulations may be able to increase cell transfection by a nucleic acid molecule; and/or increase the translation of encoded protein (*e.g.*, an effector of the invention). One such example involves the use of lipid encapsulation to enable the effective systemic delivery of polyplex plasmid DNA (Heyes *et al.*, Mol Ther. 2007 15:713- 720; the contents of which are herein incorporated by reference in its entirety). The liposomes, lipoplexes, or lipid nanoparticles of the invention may also increase the stability of a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising, *e.g.,* a nucleic acid molecule. Liposomes, lipoplexes, or lipid nanoparticles are described in U.S. Patent Publication No. 2016/0038612, the contents of which are incorporated herein by reference in their entirety.

In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising may be formulated for controlled release and/or targeted delivery. As used herein, "controlled release" refers to a pharmaceutical composition or compound release profile that conforms to a particular pattern of release to effect a therapeutic outcome. In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety, as described herein, may be encapsulated into a delivery agent described herein and/or known in the art for controlled release and/or targeted delivery. As used herein, the term "encapsulate" means to enclose, surround or encase. As it relates to the formulation of the compounds of the invention, encapsulation may be substantial, complete or partial. The term "substantially encapsulated" means that at least greater than 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.9 or greater than 99.999% of the pharmaceutical composition or disrupting agent of the invention may be enclosed, surrounded or encased within the delivery agent. "Partial encapsulation" or "partially encapsulated" means that less than 10, 10, 20, 30, 40 50 or less of the pharmaceutical composition or disrupting agent of the invention may be enclosed, surrounded or encased within the delivery agent. Advantageously, encapsulation may be determined by measuring the escape or the activity of the pharmaceutical composition or compound of the invention using fluorescence and/or electron micrograph. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the pharmaceutical composition or disrupting agent of the invention are encapsulated in the delivery agent.

In one embodiment, a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising as described herein, may be encapsulated in a therapeutic nanoparticle. Therapeutic nanoparticles may be formulated by methods described herein and known in the art such as, but not limited to, PCT Publication Nos. WO 2010/005740, WO 2010/030763, WO 2010/005721, WO 2010/005723, WO 2012/054923, U.S. Patent Publication Nos. 2201/10262491, 2010/0104645, 2010/0087337, 2010/0068285, 2011/0274759, 2010/0068286, 2012/0288541, 2013/0123351, 2013/0230567, 2013/0236500, 2013/0302433, 2013/0302432, 1013/0280339 and 2013/0251757, and U.S. Patent Nos. 8,206,747, 8,293,276 8,318,208, 8,318,211, 8,623,417, 8,617,608, 8,613,954, 8,613,951, 8,609,142, 8,603,534 and 8,563,041; the contents of each of which is herein incorporated by reference in their entirety. In another embodiment, therapeutic polymer nanoparticles may be prepared by the methods described in U.S. Patent Publication No. 2012/0140790, herein incorporated by reference in its entirety. As a non-limiting example, the therapeutic nanoparticle may comprise about 4 to about 25 weight percent of a disrupting agent and about 10 to about 99 weight percent of a diblock poly (lactic) acid-poly (ethylene)glycol copolymer comprising poly(lactic) acid as described in US Patent Publication No. 2013/0236500, the contents of which are herein incorporated by reference in its entirety. As another non-limiting example, the nanoparticle may comprise about 0.2 to about 35 weight percent of a disrupting

agent and about 10 to about 99 weight percent of a diblock poly(lactic) acid-poly(ethylene)glycol copolymer as described in U.S. Patent Publication Nos. 2013/0280339 and 2010251757 and U.S. Patent No. 8,652,528, the contents of each of which are herein incorporated by reference in their entirety.

In one embodiment, a disrupting agent formulated in therapeutic nanoparticles may be administered intramuscularly, intradermally, or intravenously.

In one embodiment, a disrupting agent may be delivered in therapeutic nanoparticles having a high glass transition temperature such as, but not limited to, the nanoparticles described in US Patent Publication Nos. 2014/0030351 and 2011/0294717, the entire contents of each of which are incorporated herein by reference.

In one embodiment, the therapeutic nanoparticle may be formulated for sustained release. As used herein, "sustained release" refers to a pharmaceutical composition or compound that conforms to a release rate over a specific period of time. The period of time may include, but is not limited to, hours, days, weeks, months and years. As a nonlimiting example, the sustained release nanoparticle may comprise a polymer and a disrupting agent of the present invention (see PCT Publication No. WO2010075072 and U.S. Patent Publication Nos. 2010/0216804, 2011/0217377, 2012/0201859, 2013/0243848 and 2013/0243827, each of which is herein incorporated by reference in their entirety).

In one embodiment, a disrupting agent of the invention may be encapsulated in, linked to and/or associated with synthetic nanocarriers. Synthetic nanocarriers include, but are not limited to, those described in PCT Publication . Nos. WO 2010/005740, WO 2010/030763, WO 2012/13501, WO 2012/149252, WO 2012149255, WO 2012149259, WO 2012149265, WO 2012149268, WO 2012149282, WO 2012149301, WO 2012149393, WO 2012149405, WO 2012149411 and WO 20l2149454 and US Patent Publication Nos. 20l10262491, 20100104645, 20100087337, 20120244222 and US20130236533, and U.S. Patent No. 8,652,487, the contents of each of which is herein incorporated by reference in their entirety. The synthetic nanocarriers may be formulated using methods known in the art and/or described herein. As a nonlimiting example, the synthetic nanocarriers may be formulated by the methods described in PCT Publication Nos. WO 2010005740, WO 2010030763 and WO 201213501 and US Patent Publication Nos. 20110262491, 20100104645, 20100087337 and 20120244222, each of which is herein incorporated by reference in their entirety. In another embodiment, the synthetic nanocarrier formulations may be lyophilized by methods described in PCT Publication No. WO 2011072218 and U.S. Patent No. 8,211,473; each of which is herein incorporated by reference in their entirety. In yet another embodiment, formulations of the present invention, including, but not limited to, synthetic nanocarriers, may be lyophilized or reconstituted by the methods described in US Patent Publication No. 20130230568, the contents of which are herein incorporated by reference in its entirety.

In one embodiment, synthetic nanocarriers comprising a disrupting agent may be administered intramuscularly, intradermally, or intravenously.

In some embodiments, a disrupting agent may be formulated for delivery using smaller LNPs. Such particles may comprise a diameter from below 0.1 μm up to 1000 μm such as, but not limited to, less than 0.1 μm, less than 1.0 μm, less than 5 μm, less than 10 μm, less than 15 μm, less than 20 μm, less than 25 μm, less than 30 μm, less than 35 μm, less than 40 μm, less than 50 μm, less than 55 μm, less than 60 μm, less than 65 μm, less than 70 μm, less than 75 μm, less than 80 μm, less than 85 μm, less than 90 μm, less than 95 μm, less than 100 μm, less than 125 μm, less than 150 μm, less than 175 μm, less than 200 μm, less than 225 μm, less than 250 μm, less than 275 μm, less than 300 μm, less than 325 μm, less than 350 μm, less than 375 μm, less than 400 μm, less than 425 μm, less than 450 μm, less than 475 μm, less than 500 μm, less than 525 μm, less than 550 μm, less than 575 μm, less than 600 μm, less than 625 μm, less than 650 μm, less than 675 μm, less than 700 μm, less than 725 μm, less than 750 μm, less than 775 μm, less than 800 μm, less than 825 μm, less than 850 μm, less than 875 μm, less than 900 μm, less than 925 μm, less than 950 μm, less than 975 μm.

In another embodiment, a disrupting agent may be formulated for delivery using smaller LNPs which may comprise a diameter from about 1 nm to about 100 nm, from about 1 nm to about 10 nm, about 1 nm to about 20 nm, from about 1 nm to about 30 nm, from about 1 nm to about 40 nm, from about 1 nm to about 50 nm, from about 1 nm to about 60 nm, from about 1 nm to about 70 nm, from about 1 nm to about 80 nm, from about 1 nm to about 90 nm, from about 5 nm to about from 100 nm, from about 5 nm to about 10 nm, about 5 nm to about 20 nm, from about 5 nm to about 30 nm, from about 5 nm to about 40 nm, from about 5 nm to about 50 nm, from about 5 nm to about 60 nm, from about 5 nm to about 70 nm, from about 5 nm to about 80 nm, from about 5 nm to about 90 nm, about 10 to about 50 nm, from about 20 to about 50 nm, from about 30 to about 50 nm, from about 40 to about 50 nm, from about 20 to about 60 nm, from about 30 to about 60 nm, from about 40 to about 60 nm, from about 20 to about 70 nm, from about 30 to about 70 nm, from about 40 to about 70 nm, from about 50 to about 70 nm, from about 60 to about 70 nm, from about 20 to about 80 nm, from about 30 to about 80 nm, from about 40 to about 80 nm, from about 50 to about 80 nm, from about 60 to about 80 nm, from about 20 to about 90 nm, from about 30 to about 90 nm, from about 40 to about 90 nm, from about 50 to about 90 nm, from about 60 to about 90 nm and/or from about 70 to about 90 nm.

In one embodiment, a disrupting agent may be formulated in smaller LNPs and may be administered intramuscularly, intrademrally, or intravenously.

In one embodiment, a disrupting agent may be formulated for delivery using the drug encapsulating microspheres described in PCT Patent Publication No. WO 2013063468 or U.S. Patent No. 8,440,614, each of which is herein incorporated by reference in its entirety. In another aspect, the amino

acid, peptide, polypeptide, lipids (APPL) are useful in delivering the disrupting agents of the invention to cells (see PCT Patent Publication No. WO 2013063468, herein incorporated by reference in its entirety).

In one aspect, the lipid nanoparticle may be a limit size lipid nanoparticle described in PCT Patent Publication No. WO 2013059922, herein incorporated by reference in its entirety. The limit size lipid nanoparticle may comprise a lipid bilayer surrounding an aqueous core or a hydrophobic core; where the lipid bilayer may comprise a phospholipid such as, but not limited to, diacylphosphatidylcholine, a diacylphosphatidylethanolamine, a ceramide, a sphingomyelin, a dihydrosphingomyelin, a cephalin, a cerebroside, a C8-C20 fatty acid diacylphophatidylcholine, and I-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In another aspect the limit size lipid nanoparticle may comprise a polyethylene glycol-lipid such as, but not limited to, DLPEPEG, DMPE-PEG, DPPC-PEG and DSPE-PEG.

In one embodiment, a disrupting agent of the invention may be delivered, localized and/or concentrated in a specific location using the delivery methods described in PCT Patent Publication No. WO 2013063530, the contents of which are herein incorporated by reference in its entirety. As a non-limiting example, a subject may be administered an empty polymeric particle prior to, simultaneously with or after delivering the disrupting agent to the subject. The empty polymeric particle undergoes a change in volume once in contact with the subject and becomes lodged, embedded, immobilized or entrapped at a specific location in the subject.

In one embodiment, a disrupting agent may be formulated in an active substance release system (See *e.g.*, US Patent Publication No. 20130102545, herein incorporated by reference in its entirety). The active substance release system may comprise 1) at least one nanoparticle bonded to an oligonucleotide inhibitor strand which is hybridized with a catalytically active nucleic acid and 2) a compound bonded to at least one substrate molecule bonded to a therapeutically active substance (*e.g.*, a disrupting agent of the invention), where the therapeutically active substance is released by the cleavage of the substrate molecule by the catalytically active nucleic acid.

In one embodiment, the nanoparticles of the present invention may be water soluble nanoparticles such as, but not limited to, those described in PCT Publication No. WO 2013090601, the contents of which are herein incorporated by reference in its entirety. The nanoparticles may be inorganic nanoparticles which have a compact and zwitterionic ligand in order to exhibit good water solubility. The nanoparticles may also have small hydrodynamic diameters (HD), stability with respect to time, pH, and salinity and a low level of non-specific protein binding.

In one embodiment, the nanoparticles of the present invention are stealth nanoparticles or target-specific stealth nanoparticles such as, but not limited to, those described in U.S. Patent Publication Nos. 20130172406 (Bind), US20130251817 (Bind), 2013251816 (Bind) and 20130251766 (Bind), the contents of each of which are herein incorporated by reference in its entirety. The stealth nanoparticles may

comprise a diblock copolymer and a chemotherapeutic agent. These stealth nanoparticles may be made by the methods described in us Patent Publication Nos. 20130172406, 20130251817, 2013251816 and 20130251766, the contents of each of which are herein incorporated by reference in its entirety. As a non-limiting example, the stealth nanoparticles may target cancer cells such as the nanoparticles described in US Patent Publication Nos. 20130172406, 20130251817, 2013251816 and 20130251766, the contents of each of which are herein incorporated by reference in its entirety.

In one embodiment, stealth nanoparticles comprising a disrupting agent of the invention may be administered intramuscularly, intradermally, or intravenously.

In one embodiment, a disrupting agent of the invention may be formulated in and/or delivered in a lipid nanoparticle comprising a plurality of cationic lipids such as, but not limited to, the lipid nanoparticles described in US Patent Publication No. 20130017223, the contents of which are herein incorporated by reference in its entirety. As a non-limiting example, the LNP formulation may comprise a first cationic lipid and a second cationic lipid. As another non-limiting example, the LNP formulation may comprise DLin-MC2-DMA and DLinMC4- DMA. As yet another non-limiting example, the LNP formulation may comprise DLin-MC3-DMA and CI2-200. In one embodiment, the LNP formulations comprising a plurality of cationic lipids (such as, but not limited to, those described in US Patent Publication No. US20130017223, the contents of which are herein incorporated by reference in its entirety) and may be administered intramuscularly, intradermally, or intravenously.

In one embodiment, a disrupting agent as described herein, may be formulated in and/or delivered in a lipid nanoparticle comprising the cationic lipid DLin-MC3-DMA and the neutral lipid DOPE. The lipid nanoparticle may also comprise a PEG based lipid and a cholesterol or antioxidant. These lipid nanoparticle formulations comprising DLin-MC3-DMA and DOPE and a disrupting agent may be administered intramuscularly, intradermally, or intravenously.

In one embodiment, the lipid nanoparticle comprising DLin-MC3-DMA and DOPE may comprise a PEG lipid such as, but not limited to, pentaerythritol PEG ester tetrasuccinimidyl and pentaerythritol PEG ether tetra-thiol, PEGc- DOMG, PEG-DMG (1,2-Dimyristoyl-sn-glycerol, methoxypolyethylene Glycol), PEG-DSG (1,2-Distearoyl-snglycerol, methoxypolyethylene Glycol), PEG-DPG (1,2- Dipalmitoyl-sn-glycerol, methoxypolyethylene glycol), PEG-DSA (PEG coupled to 1,2-distearyloxypropyl-3- amine), PEG-DMA (PEG coupled to 1,2-dimyristyloxypropyl- 3-amine, PEG-c-DNA, PEG-c-DMA, PEG-S-DSG, PEG-c-DMA, PEG-DPG, PEG-DMG 2000 and those described herein and/or known in the art.

In one embodiment, the lipid nanoparticle comprising DLin-MC3-DMA and DOPE may include 0.5% to about 3.0%, from about 1.0% to about 3.5%, from about 1.5% to about 4.0%, from about 2.0% to

about 4.5%, from about 2.5% to about 5.0% and/or from about 3.0% to about 6.0% of the lipid molar ratio of a PEG lipid.

In one embodiment, the lipid nanoparticle comprising DLin-MC3-DMA and DOPE may include 25.0% cholesterol to about 50.0% cholesterol, from about 30.0% cholesterol to about 45.0% cholesterol, from about 35.0% cholesterol to about 50.0% cholesterol and/or from about 48.5% cholesterol to about 60% cholesterol. In one embodiment, formulations may comprise a percentage of cholesterol selected from the group consisting of 28.5%, 31.5%, 33.5%, 36.5%, 37.0%, 38.5%, 39.0%, 43.5% and 48.5%.

In one embodiment, the lipid nanoparticle comprising DLin-MC3-DMA and DOPE may include 25.0% antioxidant to about 50.0% antioxidant, from about 30.0% antioxidant to about 45.0% antioxidant, from about 35.0% antioxidant to about 50.0% antioxidant and/or from about 48.5% antioxidant to about 60% antioxidant. In one embodiment, formulations may comprise a percentage of antioxidant selected from the group consisting of 28.5%, 31.5%, 33.5%, 36.5%, 37.0%, 38.5%, 39.0%, 43.5% and 48.5%.

The disrupting agent of the invention can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to, DYNAMIC POLYCONJUGATE ® (Arrowhead Research Corp., Pasadena, Calif.) formulations from MIRUS® Bio (Madison, Wis.) and Roche Madison (Madison, Wis.), PHASERXTM polymer formulations such as, without limitation, SMARTT POLYMER TECHNOLOGYTM (Seattle, Wash.), DMRIIDOPE, poloxamer, VAXFECTIN® adjuvant from Vical (San Diego, Calif.), chitosan, cyclodextrin from Calando Pharmaceuticals (Pasadena, Calif.), dendrimers and poly(lactic-co-glycolic acid) (PLGA) polymers, RONDELTM (RNAi/Oligonucleotide Nanoparticle Delivery) polymers (Arrowhead Research Corporation, Pasadena, Calif.) and pH responsive co-block polymers such as, but not limited to, PHASERXTM (Seattle, Wash.).

The polymer formulations may permit the sustained or delayed release of a disrupting agent (*e.g.*, following intramuscular, intradermal or subcutaneous injection). The altered release profile of the disrupting agent can result in, for example, translation of an encoded protein over an extended period of time. The polymer formulation may also be used to increase the stability of the disrupting agent. For example, biodegradable polymers have been previously used to protect nucleic acids other than modified mRNA from degradation and been shown to result in sustained release of payloads in vivo (Rozema *et al.*, Proc Natl Acad Sci USA. 2007 104:12982-12887; Sullivan *et al.*, Expert Opin Drug Deliv. 2010 7:1433-1446; Convertine *et al.*, Biomacromolecules. 2010 Oct. 1; Chu *et al.*, Acc Chern Res. 2012 Jan. 13; Manganiello *et al.*, Biomaterials. 2012 33:2301-2309; Benoit et a!., Biomacromolecules. 2011 12:2708-2714; Singha et al., Nucleic Acid Ther. 2011 2: 133- 147; deFougerolles Hum Gene Ther. 2008 19:125-132; Schaffert and Wagner, Gene Ther. 2008 16:1131-1138; Chaturvedi *et al.*, Expert Opin Drug Deliv.

2011 8: 1455- 1468; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 201 0464: 1067 -1070; each of which is herein incorporated by reference in its entirety).

In one embodiment, the pharmaceutical compositions may be sustained release formulations. In a further embodiment, the sustained release formulations may be for subcutaneous delivery. Sustained release formulations may include, but are not limited to, PLGA microspheres, ethylene vinyl acetate (EVAc), poloxamer, GELSITE® (Nanotherapeutics, Inc. Alachua, Fla.), HYLENEX® (Halozyme Therapeutics, San Diego Calif.), surgical sealants such as fibrinogen polymers (Ethic on Inc. Cornelia, Ga.), TISSELL® (Baxter International, Inc Deerfield, Ill.), PEG-based sealants, and COSEAL® (Baxter International, Inc Deerfield, Ill.).

B. Vector Encoded Site-Specific FOXP3 Disrupting Agents of the Invention

Disrupting agents comprising a site-specific FOXP3 targeting moiety, *e.g.,* comprising a nucleic acid molecule, may be expressed from transcription units inserted into DNA or RNA vectors (see, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; WO 00/22113, WO 00/22114, and US 6,054,299). In some embodiment, expression is sustained (months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* 92:1292). Different components of the disrupting agent, e.g., gRNA and effector, can be located on separate expression vectors that can be co-introduced (e.g., by transfection or infection) into a target cell. Alternatively, each individual component can be transcribed by promoters both of which are located on the same expression plasmid.

Delivery of a disrupting agent expressing vector can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

In certain embodiment, the nucleic acids described herein or the nucleic acids encoding a protein described herein, e.g., an effector, are incorporated into a vector, e.g., a viral vector.

The individual strand or strands of a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule can be transcribed from a promoter in an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (*e.g.*, by transfection or infection) into a target cell. Alternatively, each individual strand of a nucleic acid molecule can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a nucleic acid molecule is expressed as

inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the nucleic acid molecule has a stem and loop structure.

Expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of a disrupting agent as described herein.

Constructs for the recombinant expression of a disrupting agent will generally require regulatory elements, e.g., promoters, enhancers, etc., to ensure the expression of the disrupting agent in target cells.

Expression of natural or synthetic nucleic acids is typically achieved by operably linking a nucleic acid encoding the nucleic acid of interest to a regulatory region, such as a promoter, and incorporating the construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes.

Regulatory regions, such as a promoter, suitable for operable linking to a nucleic acid molecules can be operably linked to a regulatory region such as a promoter. can be from any species. Any type of promoter can be operably linked to a nucleic acid sequence. Examples of promoters include, without limitation, tissue-specific promoters, constitutive promoters, and promoters responsive or unresponsive to a particular stimulus (e.g., inducible promoters). Additional promoter elements, e.g., enhancing sequences, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, individual elements 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. Another example of a suitable promoter is Elongation Growth Factor- la (EF-la). 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, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter.

Further, the present invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a

molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

Additional regulatory regions that may be useful in nucleic acid constructs, include, but are not limited to, transcription and translation terminators, initiation sequences, polyadenylation sequences, translation control sequences (e.g., an internal ribosome entry segment, IRES), enhancers, inducible elements, or introns. Such regulatory regions may not be necessary, although they may increase expression by affecting transcription, stability of the mRNA, translational efficiency, or the like. Such regulatory regions can be included in a nucleic acid construct as desired to obtain optimal expression of the nucleic acids in the cell(s). Sufficient expression, however, can sometimes be obtained without such additional elements.

The 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 other aspects, the 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 include, for example, antibiotic -resistance genes, such as neo and the like. Non-limiting examples of selectable markers include puromycin, ganciclovir, adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo, G418, APH), dihydrofolate reductase (DHFR), hygromycin-B-phosphtransferase, thymidine kinase (TK), and xanthin-guanine phosphoribosyltransferase (XGPRT). Such markers are useful for selecting stable transformants in culture. Other selectable markers include fluorescent polypeptides, such as green fluorescent protein or yellow fluorescent protein.

Signal peptides may also be included and can be used such that an encoded polypeptide is directed to a particular cellular location (e.g., the cell surface).

Reporter genes may be used for identifying potentially transfected cells and for evaluating the functionality of transcriptional control sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient source and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the 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, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

Other aspects to consider for vectors and constructs are known in the art.

In some embodiments, a vector, e.g., a viral vector comprises a disrupting agent comprising a site-specific FOXP3 targeting moiety comprising a nucleic acid molecule.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors (e.g., an Ad5/F35 vector); (b) retrovirus vectors, including but not limited to lentiviral vectors (including integration competent or integration-defective lentiviral vectors), moloney murine leukemia virus, *etc.*; (c) adeno- associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, *e.g.*, vaccinia virus vectors or avipox, *e.g.* canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, *e.g.* EPV and EBV vectors. See, e.g.,, U.S. Patent Nos. 6,534,261; 6,607,882; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the entire contents of each of which is incorporated by reference herein.

Vectors, including those derived from retroviruses such as lentivinis, 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. The expression vector may be 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.

In one embodiment, a suitable viral vector for use in the present invention is an adeno-associated viral vector, such as a recombinant adeno-associate viral vector.

Recombinant adeno-associated virus vectors (rAAV) are gene delivery systems based on the defective and nonpathogenic parvovirus adeno-associated type 2 virus. All vectors are derived from a plasmid that retains only the AAV 145 bp inverted terminal repeats flanking the transgene expression cassette. Efficient gene transfer and stable transgene delivery due to integration into the genomes of the transduced cell are key features for this vector system. (Wagner et al., Lancet 351:9117 1702-3 (1998), Kearns et al., Gene Ther. 9:748-55 (1996)). AAV serotypes, including AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV9, can be used in accordance with the present invention.

Replication-deficient recombinant adenoviral vectors (Ad) can be produced at high titer and readily infect a number of different cell types. Most adenovirus vectors are engineered such that a transgene replaces the Ad E1a, E1b, and/or E3 genes; subsequently the replication defective vector is propagated in human 293 cells that supply deleted gene function in trans. Ad vectors can transduce multiple types of tissues in vivo, including nondividing, differentiated cells such as those found in liver, kidney and muscle. Conventional Ad vectors have a large carrying capacity. An example of the use of an Ad vector in a clinical trial involved polynucleotide therapy for antitumor immunization with intramuscular injection (Sterman et al., Hum. Gene Ther. 7:1083-9 (1998)). Additional examples of the use of adenovirus vectors for gene transfer in clinical trials include Rosenecker et al., Infection 24:1 5-10 (1996); Sterman et al., Hum. Gene Ther. 9:7 1083-1089 (1998); Welsh et al., Hum. Gene Ther. 2:205-18 (1995); Alvarez et al., Hum. Gene Ther. 5:597-613 (1997); Topf et al., Gene Ther. 5:507-513 (1998); Sterman et al., Hum. Gene Ther. 7:1083-1089 (1998).

Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and ψ2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by a producer cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host (if applicable), other viral sequences being replaced by an expression cassette encoding the protein to be expressed. The missing viral functions are supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess inverted terminal repeat (ITR) sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

IV. Methods of the Invention

A. Modulation of Expression of FOXP3 in a Cell

The present invention also provides methods of use of the agents and compositions described herein to modulate expression of forkhead box P3 (FOXP3) in a cell. The methods include contacting the cell, *e.g.*, a naïve T cell, with a site-specific FOXP3 disrupting agent, the disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, and an effector

molecule, thereby modulating expression of FOXP3 in the cell. The site-specific disrupting agent, the effector, or both the site-specific disrupting agent and the effector may be present in a composition, such as a composition described above. In some embodiments, the site-specific disrupting agent and the effector are present in the same compositions. In other embodiments, the site-specific disrupting agent and the effector are present in different compositions. In some embodiments, the methods of the invention include contacting a cell with two site-specific FOXP3 disrupting agents (a first and a second agent). The two site specific FOXP3 disrupting agents may be present in the same composition, e.g., pharmaceutical composition, e.g., pharmaceutical composition comprising an LNP, or in seprate compositions , e.g., pharmaceutical composition, e.g., pharmaceutical composition comprising an LNP. The cell may be contacted with the first site specific FOXP3 disrupting agent at one time and contacted with the second site specific FOXP3 disrupting agent at a second time, or the cell may be contacted with both agents at the same time.

Expression of FOXP3 may be enhanced or reduced as compared to, for example, a cell that was not contacted with the site-specific FOXP3 disrupting agent. Modulation in gene expression can be assessed by any methods known in the art. For example, a modulation in the expression may be determined by determining the mRNA expression level of a gene, *e.g.*, in a cell, a plurality of cells, and/or a tissue sample, using methods routine to one of ordinary skill in the art, *e.g.*, northern blotting, qRT-PCR; by determining the protein level of a gene using methods routine to one of ordinary skill in the art, such as western blotting, immunological techniques.

The term "reduced" in the context of the level of FOXP3 gene expression or FOXP3 protein production in a subject, or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection for the detection method. In certain embodiments, the expression of the target is normalized, *i.e.*, decreased towards or to a level accepted as within the range of normal for an individual without such disorder. As used here, "lower" in a subject can refer to lowering of gene expression or protein production in a cell in a subject does not require lowering of expression in all cells or tissues of a subject. For example, as used herein, lowering in a subject can include lowering of gene expression or protein production in the liver of a subject.

The term "reduced" can also be used in association with normalizing a symptom of a disease or condition, *i.e.* decreasing the difference between a level in a subject suffering from an autoimmune disease or a FOXP3-associated disease towards or to a level in a normal subject not suffering from an autoimmune disease or a FOXP3-associated disease. As used herein, if a disease is associated with an elevated value for a symptom, "normal" is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, "normal" is considered to be the lower limit of normal.

The term "enhanced" in the context of the level of FOXP3 gene expression or FOXP3 protein production in a subject, or a disease marker or symptom refers to a statistically significant increase in such level. The increase can be, for example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or above the level of detection for the detection method. In certain embodiments, the expression of the target is normalized, *i.e.*, increase towards or to a level accepted as within the range of normal for an individual without such disorder. As used here, "higher" in a subject can refer to increasing gene expression or protein production in a cell in a subject does not require increasing expression in all cells or tissues of a subject. For example, as used herein, increasing in a subject can include increasing gene expression or protein production in the liver of a subject.

The term "enhanced" can also be used in association with normalizing a symptom of a disease or condition, *i.e.* increasing the difference between a level in a subject suffering from a FOXP3-associated disease or an autoimmune disease towards or to a level in a normal subject not suffering from a FOXP3-associated disease or an autoimmune disease. As used herein, if a disease is associated with an elevated value for a symptom, "normal" is considered to be the upper limit of normal. If a disease is associated with a decreased value for a symptom, "normal" is considered to be the lower limit of normal.

In some embodiments, a suitable cell for use in the methods of the invention is a mammalian cell. In some embodiments, the cell is a somatic cell. In some embodiments, the cell is a primary cell. For example, in some embodiments, the cell is a mammalian somatic cell. In some embodiments, the mammalian somatic cell is a primary cell. In some embodiments, the mammalian somatic cell is a non-embryonic cell.

B. *In vitro* Generation of Immune Cells

The step of contacting may be performed *in vitro, in vivo* (*i.e.*, the cell may be within a subject), or *ex vivo*. In some embodiments, contacting a cell is performed *ex vivo* and the methods further include, prior to the step of contacting, a step of removing the cell (*e.g.*, a mammalian cell) from a subject. In some embodiments, the methods further comprise, after the step of contacting, a step of (b) administering the cell (*e.g.*, mammalian cells) to a subject.

The present invention provides methods of generating immune cells, *e.g.*, Tregs, which, in one aspect of the invention include a site-specific FOXP3 disrupting agent of the invention. The FOXP3 disrupting agent may modulate, *e.g.*, increase, the expression of FOXP3 gene for a period of time that is sufficient to direct the immune cells to a differentiation pathway or alter the activation status, for example, to induce a naïve T cell to differentiate into a Treg cell or to activate a Treg cell.

Methods for the Manipulation of Immune Cells

In one embodiment, the instant invention provides a method for manipulating cells, *e.g.*, immune cells or a sub-population thereof (*e.g.*, Tregs or naïve T cells). In this context, the term "manipulation" includes, for example, activation, division, differentiation, growth, expansion, reprogramming, anergy, quiescence, senescence, apoptosis or death of the target cells.

A variety of cells, *e.g.*, immune cells, may be manipulated, including, fresh samples derived from subjects, primary cultured cells, immortalized cells, cell-lines, hybridomas, *etc*. The cells to be manipulated may also include stem cells, such as embryonic stem cells, induced pluripotent stem cells, mobilized peripheral blood stem cells. The manipulated cells may be used for various immunotherapeutic applications as well as for research.

In certain embodiments of the invention, the cells may be manipulated *ex vivo* by culturing a sample containing immune cells, such as a sample obtained from a subject, such as a subject that would benefit from modulating the expression of FOXP3, with the FOXP3 disrupting agent of the invention.

In certain embodiments, the immune cells to be manipulated may be naïve T cells isolated from cord blood or peripheral blood. The naïve T cells may be manipulated (*e.g.*, differentiated and/or activated) by contacting the cells with a FOXP3 disrupting agent of the invention. In some embodiments, the naïve T cells may further be contacted with an antigen or an antigen presenting cell to differentiate into antigen specific Tregs. In some embodiment, the immune cells to be manipulated may be Treg cells. Tregs may be manipulated (*e.g.*, activated) by contacting the cells with the FOXP3 disrupting agent of the invention.

Methods to isolate the foregoing T cells from a sample, such as a sample derived from a subject, are known in the art and described below.

As used herein, the term "regulatory T cells," "Treg cells," or "Tregs," also known as "suppressor T cells," refers to a population of T cells which modulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disease. Tregs are immunosuppressive and generally suppress or downregulate induction and proliferation of effector T cells. Tregs express the biomarkers CD4, FOXP3, and CD25 and are thought to be derived from the same lineage as naïve CD4 cells.

As used herein, the term "naïve T cells," refers to a population of T cells that has differentiated in bone marrow, and successfully undergone the positive and negative processes of central selection in the thymus. Among these are the naïve forms of helper T cells (CD4+) and cytotoxic T cells (CD8+). A naïve T cell is considered mature and, unlike activated or memory T cells, has not encountered its cognate antigen within the periphery.

Expansion of T Cell Population

In a related embodiment, the present invention further relates to methods for expanding certain immune cells, such as naïve T cells or Tregs from a population of immune cells, *e.g.*, expanding Tregs or naïve T cells contained in sample containing B-cells, dendritic cells, macrophages, plasma cells, and the like. In another embodiment, the present invention also relates to methods for expanding a specific population of T-cells, *e.g.*, expanding differentiated / activated Tregs.

In one embodiment, the immune cells, *e.g.*, Tregs, are expanded (*e.g.*, grown or differentiated) *ex vivo* by culturing a sample containing immune cells with the FOXP3 disrupting agent of the invention. In one embodiment, *ex vivo* T cell expansion can be performed by first isolating Tregs or naïve T cells from a sample and subsequently stimulating T-cells by contacting them with the FOXP3 disrupting agent of the invention, such that the Tregs are activated, and/or expanded.

In one embodiment of the invention, the T cells are primary T-cells obtained from a subject. T-cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, spleen tissue, and tumors. In certain embodiments of the present invention, any number of primary T-cells and/or T-cell lines available in the art, may be used.

Studies on whole blood counts reveal that the number of T-cells in whole blood is very low. For example, according to the product catalog published by Stem Cell Technologies, Vancouver, BC, CANADA (Document #23629, VERSION 2.1.0), the leukocyte population in whole blood is about 0.1-0.2% (due to predominance of erythrocytes), of which T-cells make up about 7-24% of the overall leukocyte population. Among T-cells, CD4+ T-cells make up about 4-20% of the overall leukocyte population (translating to less than 0.04% of the overall cell population in whole blood) and CD8+ T-cells make up about 2-11% of the overall leukocyte population (translating to less than 0.022% of the overall cell population in whole blood). Thus, in certain embodiments of the present invention, methods of the invention may be coupled with other art-known techniques for enrichment of immune cells, *e.g.*, naïve T cells or Tregs. The enrichment step may be carried out prior to contacting the sample with the FOXP3 disrupting agent of the instant invention. In another embodiment, the enrichment step may be carried out after the sample has been contacted with the FOXP3 disrupting agent of the present invention.

In one embodiment, the Tregs population may be enriched using FICOLL separation. In one embodiment, cells from the circulating blood of an individual are obtained by apheresis or leukapheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. The cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for

subsequent processing steps. The cells are then washed with phosphate buffered saline (PBS). Alternately, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations. A semi-automated "flow-through" centrifuge may also be used according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

In another embodiment, peripheral or whole blood T cells may be enriched by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient. A specific subpopulation of T cells, such as CD28+, CD4+, CD8+, CD45RA+, and CD45RO+T cells, can be further isolated by positive or negative selection techniques.

In accordance with the present invention, various sorting techniques may be optionally employed. For example, the expanded or manipulated T cell population may be further sorted using a combination of antibodies directed to surface markers unique to the cells. A preferred method is cell sorting and/or selection via magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells selected. For example, to enrich Tregs, it may be desirable to select regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+.

For isolation of a desired population of cells, the concentration of cells and scaffold surface can be varied. In certain embodiments, it may be desirable to significantly decrease the volume in which the FOXP3 disrupting agent and T cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and the FOXP3 disrupting agent. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest.

In one embodiment, the instant invention may include art-known procedures for sample preparation. For example, T cells may be frozen after the washing step and thawed prior to use. Freezing and subsequent thawing provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human

serum albumin, or other suitable cell freezing media containing for example, HESPAN and PLASMALYTE A, the cells then are frozen to −80° C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing may be used as well as uncontrolled freezing immediately at −20° C or in liquid nitrogen.

Also contemplated in the context of the invention is the collection of blood samples or leukapheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein. In one embodiment a blood sample or a leukapheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or a leukapheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells may be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments.

In a related embodiment, the instant invention relates to methods for obtaining a polyclonal population of CD4+/FOXP3+ or CD4+/FOXP3- cells. The method comprises contacting the FOXP3 disrupting agent of the invention with a subject's biological sample, thereby activating, and optionally expanding a population of T-cells present within the sample; contacting the T-cells in the sample with a reagent for detection of CD4+ cells; further contacting the T-cells with a reagent for detection of FOXP3+ cells; and isolating a sub-population of detected CD4+/FOXP3+ or CD4+/FOXP3- T-cells from the sample. In these embodiments, the reagent for the detection and/or isolation of CD4+ and/or FOXP3+ T-cells is preferably an antibody or antigen-binding fragment thereof which specifically binds to CD4+ and FOXP3 markers.

In yet another embodiment, the present invention relates to methods for obtaining a population of naïve T cells. The method for isolating naïve T cells are known in the art, for example, using commercially available kits such as EasySep™ Human Naïve CD4+ T Cell Isolation Kit of STEMCELL Technologies.

In certain embodiments, the immune cells that have been differentiated / activated may be further expanded. For example, the activated Tregs may be cultured in the presence of certain cytokines, *e.g.*, IL-2, to be further expanded.

Accordingly, in another aspect, the present invention provides immune cells which include the FOXP3 disrupting agent. In some embodiments, the FOXP3 disrupting agent may be present in the immune cells for a period of time that is long enough to induce the immune cells, *e.g.*, naïve T cells, to

differentiate into Tregs or to activate the Tregs. In certain embodiments, the immune cells may contain one or more genetic modifications that modulate, *e.g.*, activate, the expression of the FOXP3 gene. Such a genetic modification may be present in the cells after the FOXP3 disrupting agent disappears from the cells or remains in the cell at very low level. Accordingly, the expression of the FOXP3 gene may remain activated even after the FOXP3 disrupting agent stops functioning.

In such context, the genetic modification may be introduced by the site-specific FOXP3 disrupting agent. The genetic modification includes addition, deletion, or substitution of one or more nucleotides to a target sequence, *e.g.*, DNA regions around/proximally upstream of the TSS of FOXP3 gene. The genetic modification may be an epigenetic modification of one or more nucleotides of the target sequence (*e.g.*, methylation /demethylation) or an epigenetic modification of one or more chromatin proteins (*e.g.*, acetylation / deacetylation) at the target sequence, *e.g.*, DNA regions around/proximally upstream of the TSS of FOXP3 gene.

C. *In vivo* Methods of the Invention

The *in vivo* methods of the invention may include administering to a subject an agent, a composition, or cells of the invention.

In one embodiment, immune cells, *e.g.,* naïve T cell or Tregs, are manipulated (*e.g.,* activated) *in vivo* by providing the FOXP3 disrupting agent of the invention such that the immune cells, *e.g.*, naïve T cells or Tregs, come into contact with the disrupting agent. In order to facilitate the contact, the FOXP3 disrupting agent may be administered in a subject, *e.g.,* subcutaneously or intravenously.

The term "subject," as used herein refers to an organism, for example, a mammal (*e.g.*, a human, a non-human mammal, a non-human primate, a primate, a laboratory animal, a mouse, a rat, a hamster, a gerbil, a cat, or a dog). In some embodiments a human subject is an adult, adolescent, or pediatric subject. In some embodiments, a subject had a disease or a condition. In some embodiments, the subject is suffering from a disease, disorder or condition, *e.g.*, a disease, disorder or condition that can be treated as provided herein. In some embodiments, a subject is susceptible to a disease, disorder, or condition; in some embodiments, a susceptible subject is predisposed to and/or shows an increased risk (as compared to the average risk observed in a reference subject or population) of developing the disease, disorder or condition. In some embodiments, a subject displays one or more symptoms of a disease, disorder or condition. In some embodiments, a subject does not display a particular symptom (e.g.,. clinical manifestation of disease) or characteristic of a disease, disorder, or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some

embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

Subjects that would benefit from the methods of the invention include subjects having an autoimmune disease, a subject at risk of an autoimmune disease, "FOXP3-associated disease," or a subject at risk of an "FOXP3-associated disease."

Thus, the present invention further provides methods of treatment of a subject in need thereof. The treatment methods of the invention include administering an agent, a composition, or cells of the invention to a subject, *e.g.*, a subject that would benefit from a modulation of FOXP3 expression, such as a subject having an autoimmune disease or a FOXP3-associated disease, in a therapeutically effective amount. In some embodiments, the methods of the invention include the subject may be administered two site-specific FOX3P disrupting agents (a first and a second agent). The two site specific FOX3P disrupting agents may be present in the same composition, e.g., pharmaceutical composition, e.g., pharmaceutical composition comprising an LNP, or in seprate compositions, e.g., pharmaceutical composition, e.g., pharmaceutical composition comprising an LNP. The subject may be administered the first site specific FOX3P disrupting agent at one time and administered the second site specific FOX3P disrupting agent at a second time, or the subject may be administered both agents at the same time.

In addition, the present invention provides methods for preventing at least one symptom in a subject that would benefit from a modulation of FOXP3 expression, such as a subject having an autoimmune disease or a FOXP3-associated disease, by administering to the subject an agent, a composition, or cells of the invention in a prophylactically effective amount.

"Therapeutically effective amount," as used herein, is intended to include the amount of an agent or composition or cells that, when administered to a patient for treating a subject having an autoimmune disease or a FOXP3-associated disease, is sufficient to effect treatment of the disease (*e.g.*, by diminishing, ameliorating, or maintaining the existing disease or one or more symptoms of disease or its related comorbidities). The "therapeutically effective amount" may vary depending on the agent or composition or cells, how it is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by FOXP3 gene expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

"Prophylactically effective amount," as used herein, is intended to include the amount of an agent or composition or cells that, when administered to a subject who does not yet experience or display symptoms of a FOXP3-associated disease, but who may be predisposed to a FOXP3-associated disease, is sufficient to prevent or delay the development or progression of the disease or one or more symptoms of the disease for a clinically significant period of time. The "prophylactically effective amount" may vary

depending on the agent or composition, how it is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder or condition thereof, that would benefit from an activation in expression of a FOXP3 gene or production of FOXP3 protein, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, *e.g.*, a sign or symptom of Tregs or FOXP3 gene dysfunction.

A "therapeutically-effective amount" or "prophylactically effective amount" also includes an amount of an agent or composition or cells that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. Agents and compositions or cells employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment. In some embodiments, a therapeutically effective amount or prophylactically effect amount is administered in a single dose; in some embodiments, multiple unit doses are required to deliver a therapeutically or prophylactically effective amount.

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 symptom is considered one form of reducing the frequency of that symptom.

When the subject to be treated is a mammal such as a human, the composition or cells can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intracranial (*e.g.*, intraventricular, intraparenchymal, and intrathecal), intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection.

As used herein, the term " FOXP3-associated disease," includes a disease, disorder or condition that would benefit from a modulation, *e.g.*, an increase, in FOXP3 gene expression, replication, or protein activity, such as an autoimmune disease or a disease that is associated with a Treg dysfunction. Non-limiting examples of FOXP3-associated diseases include autoimmune diseases, for example, IPEX syndrome (IPEX), Type 1 diabetes, Multiple sclerosis, Systemic lupus erythematosus (SLE), Rheumatoid arthritis (RA), Achalasia, Addison's disease, Adult Still's disease, Agammaglobulinemia, Alopecia areata, Amyloidosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Antiphospholipid syndrome, Autoimmune angioedema, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune myocarditis, Autoimmune oophoritis,

Autoimmune orchitis, Autoimmune pancreatitis, Autoimmune retinopathy, Autoimmune urticaria, Axonal & neuronal neuropathy (AMAN), Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA), Cicatricial pemphigoid, Cogan's syndrome, Cold agglutinin disease, Congenital heart block, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Dermatitis herpetiformis, Dermatomyositis, Devic's disease (neuromyelitis optica), Discoid lupus, Dressler's syndrome, Endometriosis, Eosinophilic esophagitis (EoE), Eosinophilic fasciitis, Erythema nodosum, Essential mixed cryoglobulinemia, Evans syndrome, Fibromyalgia, Fibrosing alveolitis, Giant cell arteritis (temporal arteritis), Giant cell myocarditis, Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, Hemolytic anemia, Henoch-Schonlein purpura (HSP), Herpes gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS) (Acne Inversa), Hypogammalglobulinemia, IgA Nephropathy, IgG4-related sclerosing disease, Immune thrombocytopenic purpura (ITP), Inclusion body myositis (IBM), Interstitial cystitis (IC), Juvenile arthritis, Juvenile diabetes (Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous conjunctivitis, Linear IgA disease (LAD), Lupus, Lyme disease chronic, Meniere's disease, Microscopic polyangiitis (MPA), Mixed connective tissue disease (MCTD), Mooren's ulcer, Mucha-Habermann disease, Multifocal Motor Neuropathy (MMN) or MMNCB, Myasthenia gravis, Myositis, Narcolepsy, Neonatal Lupus, Neuromyelitis optica, Neutropenia, Ocular cicatricial pemphigoid, Optic neuritis, Palindromic rheumatism (PR), PANDAS, Paraneoplastic cerebellar degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Pars planitis (peripheral uveitis), Parsonage-Turner syndrome, Pemphigus, Peripheral neuropathy, Perivenous encephalomyelitis, Pernicious anemia (PA), POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III, Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome, Postpericardiotomy syndrome, Primary biliary cirrhosis, Primary sclerosing cholangitis, Progesterone dermatitis, Psoriasis, Psoriatic arthritis, Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Raynaud's phenomenon, Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever, Sarcoidosis, Schmidt syndrome, Scleritis, Scleroderma, Sjögren's syndrome, Sperm & testicular autoimmunity, Stiff person syndrome (SPS), Subacute bacterial endocarditis (SBE), Susac's syndrome, Sympathetic ophthalmia (SO), Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenic purpura (TTP), Tolosa-Hunt syndrome (THS), Transverse myelitis, Ulcerative colitis (UC), Undifferentiated connective tissue disease (UCTD), Uveitis, Vasculitis, Vitiligo, Vogt-Koyanagi-Harada Disease.

In one embodiment, a FOXP3-associated disease is selected from the group consisting of IPEX syndrome (IPEX), Type 1 diabetes, Multiple sclerosis, Systemic lupus erythematosus (SLE), and Rheumatoid arthritis (RA).

Further details regarding signs and symptoms of the various diseases or conditions are provided herein and are well known in the art (see, *e.g.*, ghr.nlm.nih.gov)

Administration of the agents or compositions or cells of the invention according to the methods of the invention may result in a reduction of the severity, signs, symptoms, or markers of a FOXP3-associated disease or disorder in a patient with a FOXP3-associated disease or disorder. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction (absolute reduction or reduction of the difference between the elevated level in the subject and a normal level) can be, for example, at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay used.

Administration of the agents or compositions or cells according to the methods of the invention may stably or transiently modulating expression of a target gene, or may stably or transiently increase the amount or the activation level of Tregs. In some embodiments, a modulation of expression 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, 1 1 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 other embodiments, a modulation of expression persists for no more than about 30 mins to about 7 days, or no more than about 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 7 hrs, 8 hrs, 9 hrs, 10 hrs, 11 hrs, 12 hrs, 13 hrs, 14 hrs, 15 hrs, 16 hrs, 17 hrs, 18 hrs, 19 hrs, 20 hrs, 21 hrs, 22 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 4 days, 5 days, 6 days, 7 days, or any time therebetween. In certain embodiments, the amount of Tregs may increase by about at least 5% to about 10 fold, or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, or more or any time therebetween. In certain embodiment, the percentage of activated Tregs, *e.g.*, Tregs characterized by increased expression of FOXP3, may increase by about at least 5% to about 10 fold, or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, or more or any time therebetween. In some embodiments, the expression of FOXP3 in a Tregs, or in a population of Tregs, may increase by about at least 5% to about 10 fold, or at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, or more or any time therebetween.

The agents or compositions or cells may be administered once to the subject or, alternatively, multiple administrations may be performed over a period of time. For example, two, three, four, five, or

more administrations may be given to the subject during one treatment or over a period of time. In some embodiments, six, eight, ten, 12, 15 or 20 or more administrations may be given to the subject during one 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.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker, or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. As discussed herein, the specific parameters to be measured depend on the autoimmune disease or FOXP3-associated disease that the subject is suffering from.

Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an agent or composition, "effective against" an autoimmune disease or a FOXP3-associated disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating FOXP3-associated disorders.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given agent or composition can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading

scale. Any positive change resulting in *e.g.*, lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an agent or composition as described herein.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more signs or symptoms associated with an autoimmune disease or reduction in FOXP3 gene expression or FOXP3 protein production. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

D. Combination Methods

The present invention further provides combination methods to activate Treg cells. In certain embodiments, *in vitro* or *ex vivo* differentiation or activation of Tregs by FOXP3 activation can be combined with stimulation by TGFβ, for example, by including TGFβ in the growth medium used when culturing cells *in vitro* or *ex vivo*, as described herein. TGFβ is an important growth factor in T-cell differentiation and known for triggering FOXP3 activation.

The present invention is next described by means of the following examples. However, the use of these and other examples anywhere in the specification is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified form. The invention is not limited to any particular preferred embodiments described herein. Many modifications and variations of the invention may be apparent to those skilled in the art and can be made without departing from its spirit and scope. The contents of all references, patents and published patent applications cited throughout this application, including the figures, are incorporated herein by reference.

EXAMPLES

Example 1. FOXP3 activation in Jurkat Cells

This example describes activation of FOXP3 expression in Jurkat cells, as measured by the increase of FOXP3 mRNA level and protein level, using a site-specific FOXP3 disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, *i.e.*, a sgRNA, and an effector comprising dCas9, dCas9 and p300, or dCas9 and VPR.

Towards understanding the effect of the activators of the invention on FOXP3 gene expression, Jurkat cells that are human leukemic T-cell line, were transfected with either dCas9- or dCas9-p300- or dCas9-VPR- encoding mRNA along with sgRNA targeting different regions around and upstream of the transcription start site (TSS) with Lipofectamine Messeger Max according to manufacturer's recommendations. Pools of three guide RNAs were used and one pool was found to activate FOXP3 mRNA with both p300 (9 fold) and VPR (100 fold) (Figure 1A). A pool of sgRNAs was found to induce FOXP3 protein production in combination with a VPR protein. Eight percent (8%) of the cells turned FOXP3 positive when assayed by FACS after treatment with a combination of sgRNAs (Pool2) and mRNA encoding dCas9-VPR (Figure 1B).

The activation response by VPR can be achieved when using guide pools or individual guides. Without wishing to be bound by theory, it is believed that the activation observed in this experiment is the result of multiple effectors/ activators recruiting more of the activation machinery to the target site (Figure 2). The guide pools used in Examples 1 and 2 are summarized in Table 2.

Example 2. FOXP3 activation in naïve T-cells

This example describes activation of FOXP3 expression in naïve T-cells, as measured by the increase of FOXP3 mRNA level and protein level, using a site-specific FOXP3 disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, *i.e.*, a sgRNA, and an effector comprising dCas9, dCas9 and p300, or dCas9 and VPR.

For activation in naïve T-cells, electroporation with MaxCyte ATx using manufacturer's recommended electroporation settings was used to transfect the same mRNA and 3 sgRNA guide combination (Pool 2) as optimized in the Jurkat experiment detailed above to compare the effect of dCas9 alone with dCas9-p300 or dCas9-VPR activator fusions. As in Jurkat cells, VPR was found to elicit the greatest response in terms of FOXP3 mRNA expression (up to 600-fold compared to dCas9 alone) as determined by qPCR with 10 to 14% of the cells determined to be $FOXP3^+$ cells as assayed by FACS analysis (Figure 3).

Table 2: Site-Specific FOXP3 Targeting Moieties – The first 20 nucleotides in each moiety below comprise the targeting portion of the moiety.

Identifier	Nucleotide Sequence 5'-3'
GD-28445	mUs;mCs;mUs;rG;rU;rC;rA;rG;rU;rC;rC;rA;rC;rU;rU;rC;rA;rC;rC;rA;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 96)
	TCTGTCAGTCCACTTCACCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 97)
GD-28446	mGs;mUs;mGs;rA;rC;rA;rG;rU;rU;rU;rC;rC;rC;rA;rC;rA;rA;rG;rC;rC;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 98)
	GTGACAGTTTCCCACAAGCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 99)
GD-28447	mAs;mAs;mAs;rA;rA;rC;rC;rA;rC;rG;rC;rU;rG;rU;rA;rC;rG;rG;rU;rG;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 100)
	AAAAACCACGCTGTACGGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT` (SEQ ID NO: 101)
GD-28448	mUs;mGs;mUs;rG;rU;rG;rC;rG;rC;rU;rG;rA;rU;rA;rA;rU;rC;rA;rC;rG;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 102)
	TGTGTGCGCTGATAATCACGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 103)
GD-28449	mUs;mAs;mAs;rG;rU;rC;rU;rC;rA;rU;rA;rA;rU;rC;rA;rA;rG;rA;rA;rA;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 104)
	TAAGTCTCATAATCAAGAAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 105)
GD-28450	mUs;mAs;mUs;rU;rU;rU;rC;rA;rG;rA;rU;rG;rA;rC;rU;rC;rG;rU;rA;rA;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 106)
	TATTTTCAGATGACTCGTAAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 107)
GD-28451	mUs;mAs;mAs;rA;rU;rC;rA;rC;rA;rG;rG;rG;rC;rC;rA;rA;rC;rC;rC;rG;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 108)
	TAAATCACAGGGCCAACCCGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 109)
GD-28452	mGs;mAs;mCs;rA;rC;rC;rA;rU;rU;rC;rU;rG;rU;rG;rA;rG;rU;rG;rA;rG;rG;rU;rU;rU;rU;rA;rG;rA;rG;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 110)
	GACACCATTCTGTGAGTGAGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 111)
GD-28453	mAs;mGs;mAs;rA;rU;rC;rU;rG;rA;rA;rG;rC;rU;rC;rU;rA;rU;rG;rU;rG;rG;rU;rU;rU;rU;rA;rG;rA;r

	G;rC;rU;rA;rG;rA;rA;rA;rU;rA;rG;rC;rA;rA;rG;rU;rU;rA;rA;rA;rA;rU;rA;rA;rG;rG;rC;rU;rA;rG;rU;rC;rC;rG;rU;rU;rA;rU;rC;rA;rA;rC;rU;rU;rG;rA;rA;rA;rA;rG;rU;rG;rG;rC;rA;rC;rC;rG;rA;rG;rU;rC;rG;rG;rU;rG;rC;rUs;mUs;mUs;mU (SEQ ID NO: 112)
	AGAATCTGAAGCTCTATGTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT (SEQ ID NO: 113)

Table 3: Site-Specific FOXP3 Targeting Moieties – Nucleotide Sequences of FOXP3 Guides Used in Examples 1 and 2 and Complementary Target Sequence in the Genome

Pool#	Effector ID	Guide NT sequence (20 nt targeting region only)* 5'-3'	Complementary target sequence 5'->3'
1	GD-28445	TCTGTCAGTCCACTTCACCA (SEQ ID NO: 114)	TGGTGAAGTGGACTGACAGA (SEQ ID NO: 115)
	GD-28446	GTGACAGTTTCCCACAAGCC (SEQ ID NO: 116)	GGCTTGTGGGAAACTGTCAC (SEQ ID NO: 117)
	GD-28447	AAAAACCACGCTGTACGGTG (SEQ ID NO: 118)	CACCGTACAGCGTGGTTTTT (SEQ ID NO: 119)
2**	GD-28448	TGTGTGCGCTGATAATCACG (SEQ ID NO: 120)	CGTGATTATCAGCGCACACA (SEQ ID NO: 121)
	GD-28449	TAAGTCTCATAATCAAGAAA (SEQ ID NO: 122)	TTTCTTGATTATGAGACTTA (SEQ ID NO: 123)
	GD-28450	TATTTTCAGATGACTCGTAA (SEQ ID NO: 124)	TTACGAGTCATCTGAAAATA (SEQ ID NO: 125)
3	GD-28451	TAAATCACAGGGCCAACCCG (SEQ ID NO: 126)	CGGGTTGGCCCTGTGATTTA (SEQ ID NO: 127)
	GD-28452	GACACCATTCTGTGAGTGAG (SEQ ID NO: 128)	CTCACTCACAGAATGGTGTC (SEQ ID NO: 129)
	GD-28453	AGAATCTGAAGCTCTATGTG (SEQ ID NO: 130)	CACATAGAGCTTCAGATTCT (SEQ ID NO: 131)

Note:

*: All single guides were 100 nucleotide long. The indicated 20mer targeting region was part of the SpCas9 PAM single guide RNA having the following 80 nt sequence for dCas9 binding: 5'-GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT-3' (SEQ ID NO: 132)

#: Pool numbers are the same as those in Figures 1A and 1B.

**: Pool 2 strongly induced upregulation response in Jurkat cells and was successfully used to activate FOXP3 in naïve T-cells.

Table 4: Site-Specific FOXP3 Targeting Moieties

Effector ID	Target Sequence	Strand	Genome	Species	PAM Sequence	Length (NTs)
GD-28445	TCTGTCAGTCCACTTCACCA (SEQ ID NO: 133)	-	GRCh37: chrX:49121122-49121144	Human	AGG	100

GD-28446	GTGACAGTTTCCCACAAGCC (SEQ ID NO: 134)	-	GRCh37: chrX:49121155-49121177	Human	AGG	100
GD-28447	AAAAACCACGCTGTACGGTG (SEQ ID NO: 135)	+	GRCh37: chrX:49121212-49121234	Human	TGG	100
GD-28448	TGTGTGCGCTGATAATCACG (SEQ ID NO: 136)	+	GRCh37: chrX:49121282-49121304	Human	GGG	100
GD-28449	TAAGTCTCATAATCAAGAAA (SEQ ID NO: 137)	-	GRCh37: chrX:49121352-49121374	Human	AGG	100
GD-28450	TATTTTCAGATGACTCGTAA (SEQ ID NO: 138)	-	GRCh37: chrX:49121411-49121433	Human	AGG	100
GD-28451	TAAATCACAGGGCCAACCCG (SEQ ID NO: 139)	+	GRCh37: chrX:49121561-49121583	Human	AGG	100
GD-28452	GACACCATTCTGTGAGTGAG (SEQ ID NO: 140)	+	GRCh37: chrX:49121592-49121614	Human	AGG	100
GD-28453	AGAATCTGAAGCTCTATGTG (SEQ ID NO: 141)	+	GRCh37: chrX:49121643-49121665	Human	TGG	100

Table 5. Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation	**Nucleotide(s)**
A	Adenosine-3'-phosphate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gs	guanosine-3'-phosphorothioate
U	Uridine-3'-phosphate
Us	uridine -3'-phosphorothioate
N	any nucleotide, modified or unmodified
mA	2'-O-methyladenosine-3'-phosphate
mAs	2'-O-methyladenosine-3'- phosphorothioate
mC	2'-O-methylcytidine-3'-phosphate
mCs	2'-O-methylcytidine-3'- phosphorothioate
mG	2'-O-methylguanosine-3'-phosphate
mGs	2'-O-methylguanosine-3'- phosphorothioate
mU	2'-O-methyluridine-3'-phosphate
mUs	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
r	ribonucleotide

We claim:

1. A site-specific forkhead box P3(FOXP3) disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region.

2. The site-specific FOXP3 disrupting agent of claim 1, wherein the site-specific FOXP3 targeting moiety comprises a polymeric molecule.

3. The site-specific FOXP3 disrupting agent of claim 2, wherein the polymeric molecule comprises a polyamide.

4. The site-specific FOXP3 disrupting agent of claim 2, wherein the polymeric molecule comprises a polynucleotide.

5. The site-specific FOXP3 disrupting agent of claim 1, wherein the expression control region comprises a region upstream of a FOXP3 transcription start site (TSS).

6. The site-specific FOXP3 disrupting agent of claim 1, wherein the expression control region comprises one or more FOXP3-associated anchor sequences within an anchor sequence-mediated conjunction comprising a first and a second FOXP3-associated anchor sequence.

7. The site-specific FOXP3 disrupting agent of claim 6, wherein the anchor sequence comprises a CCCTC-binding factor (CTCF) binding motif.

8. The site-specific FOXP3 disrupting agent of claim 6 or 7, wherein the anchor sequence-mediated conjunction comprises one or more transcriptional control elements internal to the conjunction.

9. The site-specific FOXP3 disrupting agent of claim 6 or 7, wherein the anchor sequence-mediated conjunction comprises one or more transcriptional control elements external to the conjunction.

10. The site-specific FOXP3 disrupting agent of any one of claims 6-9, wherein the first and/or the second anchor sequence is located within about 500 kb of the transcriptional control element.

11. The site-specific FOXP3 disrupting agent of claim 10, wherein the first and/or the second anchor sequence is located within about 300 kb of the transcriptional control element.

12. The site-specific FOXP3 disrupting agent of claim 11, wherein the first and/or the second anchor sequence is located within 10 kb of the transcriptional control element.

13. The site-specific FOXP3 disrupting agent of claim 1, wherein the expression control region comprises a FOXP3-specific transcriptional control element.

14. The site-specific FOXP3 disrupting agent of claim 13, wherein the transcriptional control element comprises a FOXP3 promoter.

15. The site-specific FOXP3 disrupting agent of claim 13, wherein the transcriptional control element comprises a transcriptional enhancer.

16. The site-specific FOXP3 disrupting agent of claim 13, wherein the transcriptional control element comprises a transcriptional repressor.

17. The site-specific FOXP3 disrupting agent of any one of claims 1-16, comprising a nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of any one of the nucleotide sequences in Table 2.

18. The site-specific FOXP3 disrupting agent of claim 17, comprising a first nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of GD-28448, a second nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of GD-28449, and a third nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of GD-28450.

19. The site-specific FOXP3 disrupting agent of claim 2, wherein the polymeric molecule comprises a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

20. The site-specific FOXP3 disrupting agent of claim 19, wherein the DNA-binding domain of the TALE or ZNF polypeptide comprises an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of any one of the amino acid sequences listed in Table 1B.

21. The site-specific FOXP3 disrupting agent of any one of claims 1-20, comprising a nucleotide modification.

22. The site-specific FOXP3 disrupting agent of claim 2, wherein the polymeric molecule comprises a peptide nucleic acid (PNA).

23. A vector comprising the site-specific FOXP3 disrupting agent of any one of claims 1-22.

24. The vector of claim 23, wherein the vector is a viral expression vector.

25. A cell comprising the site-specific FOXP3 disrupting agent of any one of claims 1-22 or the vector of claim 23 or 24.

26. The cell of claim 25, wherein the cell is an immune cell.

27. The cell of claim 26, wherein the immune cell is a naïve T cell or a regulatory T cell (Treg).

28. The site-specific FOXP3 disrupting agent of any one of claims 1-22, wherein the site-specific FOXP3 disrupting agent is present in a composition.

29. The site-specific FOXP3 disrupting agent claim 28, wherein the composition comprises a pharmaceutical composition.

30. The site-specific FOXP3 disrupting agent claim 29, wherein the pharmaceutical composition comprises a lipid formulation.

31. The site-specific FOXP3 disrupting agent claim 30, wherein the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of any of the foregoing.

32. The site-specific FOXP3 disrupting agent claim 30, wherein the pharmaceutical composition comprises a lipid nanoparticle.

33. A site-specific FOXP3 disrupting agent, comprising a nucleic acid molecule encoding a fusion protein, the fusion protein comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region and an effector molecule.

34. The site-specific FOXP3 disrupting agent of claim 30, wherein the site-specific FOXP3 targeting moiety comprises a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

35. The site-specific FOXP3 disrupting agent of claim 34, wherein the DNA-binding domain of the TALE or zinc finger polypeptide comprises an amino acid sequence having at least 85% amino acid identity to the entire amino acid sequence of an amino acid seqeunce selected from the amino acid sequences listed in Table 1B.

36. The site-specific FOXP3 disrupting agent of claim 33, wherein the effector molecule comprises a nucleic acid molecule encoding a polypeptide.

37. The site-specific FOXP3 disrupting agent of claim 33, wherein the fusion protein comprises a peptide-nucleic acid fusion.

38. The site-specific FOXP3 disrupting agent of any one of claims 33-37, wherein the effector is selected from the group consisting of a nuclease, a physical blocker, an epigenetic recruiter, and an epigenetic CpG modifier, and combinations of any of the foregoing.

39. The site-specific FOXP3 disrupting agent of claim 38, wherein the effector comprises a CRISPR associated protein (Cas) polypeptide or nucleic acid molecule encoding the Cas polypeptide.

40. The site-specific FOXP3 disrupting agent of claim 39, wherein the Cas polypeptide is an enzymatically inactive Cas polypeptide.

41. The site-specific FOXP3 disrupting agent of claim 39, further comprising a catalytically active domain of human exonuclease 1 (hEXO1).

42. The site-specific FOXP3 disrupting agent of claim 38, wherein the epigenetic recruiter comprises a transcriptional enhancer or a transcriptional repressor.

43. The site-specific FOXP3 disrupting agent of claim 42, wherein the transcriptional enhancer is a VPR (VP64-p65-Rta).

44. The site-specific FOXP3 disrupting agent of claim 43, wherein the VPR comprises an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLSGGPKKKRKVGS QYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSS LSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQA VAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVA PHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSG SRDSREGMFLPKPEAGSAISDVFEGREVCQPKRLRPFHPPGSPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPP PRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO: 64).

45. The site-specific FOXP3 disrupting agent of claim 43 or 44, wherein the transcriptional enhancer comprises two, three, four, or five VPRs.

46. The site-specific FOXP3 disrupting agent of 42, wherein the transcriptional enhancer is a p300.

47. The site-specific FOXP3 disrupting agent of claim 46, wherein the p300 comprises an amino acid sequence having at least about 85% identity to the entire amino acid sequence of IFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQE PWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLC CYGKQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTLDP

ELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRLGTFLENRV
NDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKALFAFEEIDGV
DLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGH
IWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAK
ELPYFEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLS
RGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANSLPPIVDPDPLIPCDLMDGRDA
FLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQD (SEQ ID NO: 65).

48. The site-specific FOXP3 disrupting agent of claim 38, wherein the epigenetic CpG modifier comprises a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase.

49. The site-specific FOXP3 disrupting agent of any one of claims 33-38, wherein the effector molecule comprises a zinc finger polypeptide.

50. The site-specific FOXP3 disrupting agent of any one of claims 33-38, wherein the effector molecule comprises a Transcription activator-like effector nuclease (TALEN) polypeptide.

51. The site-specific FOXP3 disrupting agent of any one of claims 33-50, further comprising a second nucleic acid molecule encoding a second fusion protein, wherein the second fusion comprises a second site-specific FOXP3 targeting moiety which targets a second FOXP3 expression control region and a second effector molecule, wherein the second FOXP3 expression control region is different than the FOXP3 expression control region.

52. The site-specific FOXP3 disrupting agent of claim 51, wherein the second effector is different than the effector.

53. The site-specific FOXP3 disrupting agent of claim 51, wherein the second effector is the same as the effector.

54. The site-specific FOXP3 disrupting agent of any one of claims 51-53, wherein the fusion protein and the second fusion protein are operably linked.

55. The site-specific FOXP3 disrupting agent of claim 52, wherein the fusion protein and the second fusion protein comprise an amino acid sequence that has at least about 85% amino acid sequence identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas-P300 (SEQ ID NO: 10) and dCas-VPR (SEQ ID NO: 11).

56. The site-specific FOXP3 disrupting agent of claim 52, wherein the fusion protein is encoded by a polynucleotide comprising a nucleotide sequence that has at least about 85% amino acid sequence identity to the entire nucleotide sequence of a polynucleotide selected from the group consisting of dCas-P300 mRNA (SEQ ID NO: 7) and dCas-VPR mRNA (SEQ ID NO: 8).

57. A site-specific FOXP3 disrupting agent, comprising a nucleic acid molecule encoding a fusion protein, wherein the fusion protein comprises an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of a polypeptide selected from the group consisting of dCas-P300 (SEQ ID NO: 10) and dCas-VPR (SEQ ID NO: 11).

58. A site-specific FOXP3 disrupting agent, comprising a polynucleotide encoding the amino acid sequence of dCas-P300 comprising the amino acid sequence of

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEK
ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT
YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL
TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI
AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG

KSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILAD
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKGRAIFKPEELRQALMPTLEALYRQDPESLPF
RQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQEPWQYVDDIWLMFNNAWLYNRKTSRV
YKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLCCYGKQLCTIPRDATYYSYQNRYHFCEK
CFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTLDPELFVECTECGRKMHQICVLHHEIIWPAG
FVCDGCLKKSARTRKENKFSAKRLPSTRLGTFLENRVNDFLRRQNHPESGEVTVRVVHASDKTV
EVKPGMKARFVDSGEMAESFPYRTKALFAFEEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYISY
LDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRL
QEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAKELPYFEGDFWPNVLEESIKELEQEEEER
KREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLSRGNKKKPGMPNVSNDLSQKLYATME
KHKEVFFVIRLIAGPAANSLPPIVDPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRRAQWSTMCM
LVELHTQSQDSGGKRPAATKKAGQAKKKKGSYPYDVPDYA (SEQ ID NO: 10).

59. A site-specific FOXP3 disrupting agent, comprising a polynucleotide encoding the amino acid sequence of dCas-VPR comprising the amino acid sequence of

MAPKKKRKVGIHGVPAADKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLI
GALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS
DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALS
LGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKF
IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEK
ILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKV
LPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFK
KIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKT
YAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSL
TFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVAAIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQLLNAKLITQ
RKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI
AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS

MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG
KSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG
ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILAD
ANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKGRADALDDFDLDMLGSDALDDFDLDMLGS
DALDDFDLDMLGSDALDDFDLDMLSGGPKKKRKVGSQYLPDTDDRHRIEEKRKRTYETFKSIM
KKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAP
APPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQF
DDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRP
PDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEG
REVCQPKRIRPFHPPGSPWANRPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPEASHLL
EDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPPPRGHLDELTTTLESMTEDLNLDSPL
TPELNEILDTFLNDECLLHAMHISTGLSIFDTSLFSGGKRPAATKKAGQAKKKKGSYPYDVPDYA
(SEQ ID NO: 11).

60. A vector comprising a nucleic acid molecule encoding the site-specific FOXP3 disrupting agent of any one of claims 33-59.

61. The vector of claim 60, wherein the vector is a viral expression vector.

62. A cell comprising the site-specific FOXP3 disrupting agent of any one of claims 33-59 or the vector of claim 60 or 61.

63. The cell of claim 62, wherein the cell is an immune cell.

64. The cell of claim 63, wherein the immune cell is a naïve T cell or a regulatory T cell (Treg).

65. The site-specific FOXP3 disrupting agent of any one of claims 33-59, wherein the site-specific FOXP3 disrupting agent is present in a composition.

66. The site-specific FOXP3 disrupting agent claim of 65, wherein the composition comprises a pharmaceutical composition.

67. The site-specific FOXP3 disrupting agent of claim 66, wherein the pharmaceutical composition comprises a lipid formulation.

68. The site-specific FOXP3 disrupting agent of claim 67, wherein the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of any of the foregoing.

69. The site-specific FOXP3 disrupting agent claim 66, wherein the pharmaceutical composition comprises a lipid nanoparticle.

70. A method of modulating expression of forkhead box P3 (FOXP3) in a cell, the method comprising contacting the cell with a site-specific FOXP3 disrupting agent, the disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, and an effector molecule, thereby modulating expression of FOXP3 in the cell.

71. The method of claim 70, wherein the modulation of expression is enhanced expression of FOXP3 in the cell.

72. The method of claim 70, wherein the modulation of expression is reduced expression of FOXP3 in the cell.

73. The method of claim 70, wherein the site-specific FOXP3 targeting moiety comprises a polymeric molecule.

74. The method of claim 73, wherein the polymeric molecule comprises a polyamide.

75. The method of claim 73, wherein the polymeric molecule comprises a polynucleotide.

76. The method of claim 70, wherein the expression control region comprises a region upstream of FOXP3 transcription start site (TSS).

77. The method of claim 70, wherein the expression control region comprises one or more FOXP3-associated anchor sequences within an anchor sequence-mediated conjunction comprising a first and a second FOXP3-associated anchor sequence.

78. The method of claim 77, wherein the anchor sequence comprises a CCCTC-binding factor (CTCF) binding motif.

79. The method of claim 77, wherein the anchor sequence-mediated conjunction comprises one or more transcriptional control elements internal to the conjunction.

80. The method of claim 77, wherein the anchor sequence-mediated conjunction comprises one or more transcriptional control elements external to the conjunction.

81. The method of any one of claims 77-80, wherein the anchor sequence is located within about 500 kb of the transcriptional control element.

82. The method of claim 81, wherein the anchor sequence is located within about 300 kb of the transcriptional control element.

83. The method of claim 82, wherein the anchor sequence is located within 10 kb of the transcriptional control element.

84. The method of claim 70, wherein the expression control region comprises a FOXP3-specific transcriptional control element.

85. The method of claim 84, wherein the transcriptional control element comprises a FOXP3 promoter.

86. The method of claim 84, wherein the transcriptional control element comprises transcriptional enhancer.

87. The method of claim 84, wherein the transcriptional control element comprises a transcriptional repressor.

88. The method of any one of claims 70-87, wherein the site-specific FOXP3 disrupting agent comprises a nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of any of the nucleotide sequences in Table 2.

89. The method of claim 70, wherein the site-specific FOXP3 disrupting agent comprises a polynucleotide encoding a DNA-binding domain, or fragment thereof, of a zinc finger polypeptide (ZNF) or a transcription activator-like effector (TALE) polypeptide that specifically binds to the FOXP3 expression control region.

90. The method of claim 89, wherein the DNA-binding domain of the TALE or ZNF comprises an amino acid sequence having at least 85% amino acid identity to the entire amino acid sequence of an amino acid sequence selected from the amino acid sequences listed in Table 1.

91. The method of any one of claims 70-90, wherein the site-specific FOXP3 disrupting agent comprises a nucleotide modification.

92. The method of claim 70, wherein the polymeric molecule comprises a peptide nucleic acid (PNA).

93. The method of claim 70, wherein the effector molecule comprises a polypeptide.

94. The method of claim 93, wherein the polypeptide comprises a fusion protein comprising the site-specific FOXP3 targeting moiety which targets a FOXP3 expression regulatory region, and the effector molecule.

95. The method of claim 94, wherein the fusion protein comprises a peptide-nucleic acid fusion molecule.

96. The method of claim 94, wherein the effector is selected from the group consisting of a nuclease, a physical blocker, an epigenetic recruiter, and an epigenetic CpG modifier, and combinations of any of the foregoing.

97. The method of claim 96, wherein the effector comprises a CRISPR associated protein (Cas) polypeptide or nucleic acid molecule encoding the Cas polypeptide.

98. The method of claim 97, wherein the Cas polypeptide is an enzymatically inactive Cas polypeptide.

99. The method of claim 97, wherein the effector further comprises a catalytically active domain of human exonuclease 1 (hEXO1).

100. The method of claim 96, wherein the epigenetic recruiter comprises a transcriptional enhancer or a transcriptional repressor.

101. The method of claim 100, wherein the transcriptional enhancer is a VPR.

102. The method of claim 101, wherein the VPR comprises an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of DALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLSGGPKKKRKVGS QYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPPRRIAVPSRSSASVPKPAPQPYPFTSS LSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAPAPAPAPAMVSALAQAPAPVPVLAPGPPQA VAPPAPKPTQAGEGTLSEALLQLQFDDEDLGALLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVA PHTTEPMLMEYPEAITRLVTGAQRPPDPAPAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSG SRDSREGMFLPKPEAGSAISDVFEGREVCQPKRLRPFHPPGSPWANRPLPASLAPTPTGPVHEPVG SLTPAPVPQPLDPAPAVTPEASHLLEDPDEETSQAVKALREMADTVIPQKEEAAICGQMDLSHPP PRGHLDELTTTLESMTEDLNLDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLF (SEQ ID NO: 64).

103. The method of claim 101 or 102, wherein the transcriptional enhancer comprises two, three, four, or five VPRs.

104. The method of claim 100, wherein the transcriptional enhancer is a p300.

105. The method of claim 104, wherein the p300 has an amino acid sequence having at least about 85% amino acid identity to the entire amino acid sequence of IFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQE PWQYVDDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLC CYGKQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTLDP ELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRLGTFLENRV NDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKALFAFEEIDGV DLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLEYVKKLGYTTGH IWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYKKMLDKAVSERIVHDYKDIFKQATEDRLTSAK

ELPYFEGDFWPNVLEESIKELEQEEEERKREENTSNESTDVTKGDSKNAKKKNNKKTSKNKSSLS
RGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANSLPPIVDPDPLIPCDLMDGRDA
FLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQD (SEQ ID NO: 65).

106. The method of claim 96, wherein the epigenetic CpG modifier comprises a DNA methylase, a DNA demethylase, a histone modifying agent, a histone transacetylase, or a histone deacetylase.

107. The method of claim 94, wherein the effector molecule comprises a zinc finger polypeptide.

108. The method of claim 94, wherein the effector molecule comprises a Transcription activator-like effector nuclease (TALEN) polypeptide.

109. The method of claim 94, wherein the fusion protein comprises an enzymatically inactive Cas polypeptide and an epigenetic recruiter polypeptide.

110. The method of claim 94, wherein the fusion protein comprises an enzymatically Cas polypeptide and an epigenetic CpG modifier polypeptide.

111. The method of any one of claims 70-110, wherein the site-specific FOXP3 disrupting agent comprises a second nucleic acid molecule encoding a second fusion protein, wherein the second fusion protein comprises a second site-specific FOXP3 targeting moiety which targets a second FOXP3 expression control region and a second effector molecule, wherein the second FOXP3 expression control region is different than the FOXP3 expression control region.

112. The method of claim 111 ,wherein the second effector is different than the effector.

113. The method of claim 111, wherein the second effector is the same as the effector.

114. The method of any one of claim 111-113, wherein the fusion protein and the second fusion protein are operably linked.

115. The method of claim 111, wherein the fusion protein and the second fusion protein comprise an amino acid sequence that has at least about 85% sequence identity to the entire amino acid

sequence of a polypeptide selected from the group consisting of dCas-P300 (SEQ ID NO: 10) and dCas-VPR (SEQ ID NO: 11).

116. The method of any one of claim 111-115, wherein the administration of the site-specific FOXP3 disrupting agent and the second site-specific FOXP3 disrupting agent has a synergistic effect in modulating the expression of FOXP3.

117. The method of any one of claims 70-116, wherein the site-specific disrupting agent, the effector, or both the site-specific disrupting agent and the effector are present in a vector.

118. The method of claim 117, wherein the site-specific disrupting agent and the effector are present in the same vector.

119. The method of claim 117, wherein the site-specific disrupting agent and the effector are present in different vectors.

120. The method of any one of claims 117-119, wherein the vector is a viral expression vector.

121. The method of any one of claims 70-120, wherein the site-specific disrupting agent, the effector, or both the site-specific disrupting agent and the effector are present in a composition.

122. The method of claim 121, wherein the site-specific disrupting agent and the effector are present in the same composition.

123. The method of claim 121, wherein the site-specific disrupting agent and the effector are present in different compositions.

124. The method of any one of claims 121-123, wherein the composition comprises a pharmaceutical composition.

125. The method of claim 124, wherein the pharmaceutical composition comprises a lipid formulation.

126. The method of claim 125, wherein the lipid formulation comprises one or more cationic lipids, one or more non-cationic lipids, one or more cholesterol-based lipids, or one or more PEG-modified lipids, or combinations of any of the foregoing.

127. The method of claim 124, wherein the pharmaceutical composition comprises a lipid nanoparticle.

128. The method of claim 70, wherein the cell is a mammalian cell.

129. The method of claim 128, wherein the mammalian cell is a somatic cell.

130. The method of claim 128, wherein the mammalian cell is a primary cell.

131. The method of claim 70, wherein the cell is an immune cell.

132. The method of claim 131, wherein the immune cell is a naïve T cell or a regulatory T cell (Treg).

133. The method of claim 70, wherein the contacting is performed *in vitro*.

134. The method of claim 70, wherein the contacting is performed *in vivo*.

135. The method of claim 70, wherein the contacting is performed *ex vivo*.

136. The method of claim 135, further comprising administering the cell to a subject.

137. The method of claim 70, wherein the cell is within a subject.

138. The method of claim 136 or 137, wherein the subject has a FOXP3-associated disease.

139. The method of claim 138, wherein the FOXP3-associated disease is selected from the group consisting of IPEX syndrome (IPEX), type 1 diabetes, multiple sclerosis, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA).

140. A method for treating a subject having a FOXP3-associated disease, comprising administering to the subject

a therapeutically effective amount of a site-specific FOXP3 disrupting agent, the disrupting agent comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, and

an effector molecule, thereby treating the subject.

141. The method of claim 140, wherein the FOXP3-associated disease is IPEX syndrome and the site-specific FOXP3 disrupting agent increases expression of FOXP3 in the subject.

142. The method of claim 140 or 141, wherein the site-specific FOXP3 disrupting agent and the effector molecule are administered to the subject concurrently.

143. The method of claim 140 or 141, wherein the site-specific FOXP3 disrupting agent and the effector molecule are administered to the subject sequentially.

144. The method of claim 143, wherein the effector molecule is administered to the subject prior to administration of the site-specific FOXP3 disrupting agent.

145. The method of claim 143, wherein the site-specific FOXP3 disrupting agent is administered to the subject prior to administration of the effector molecule.

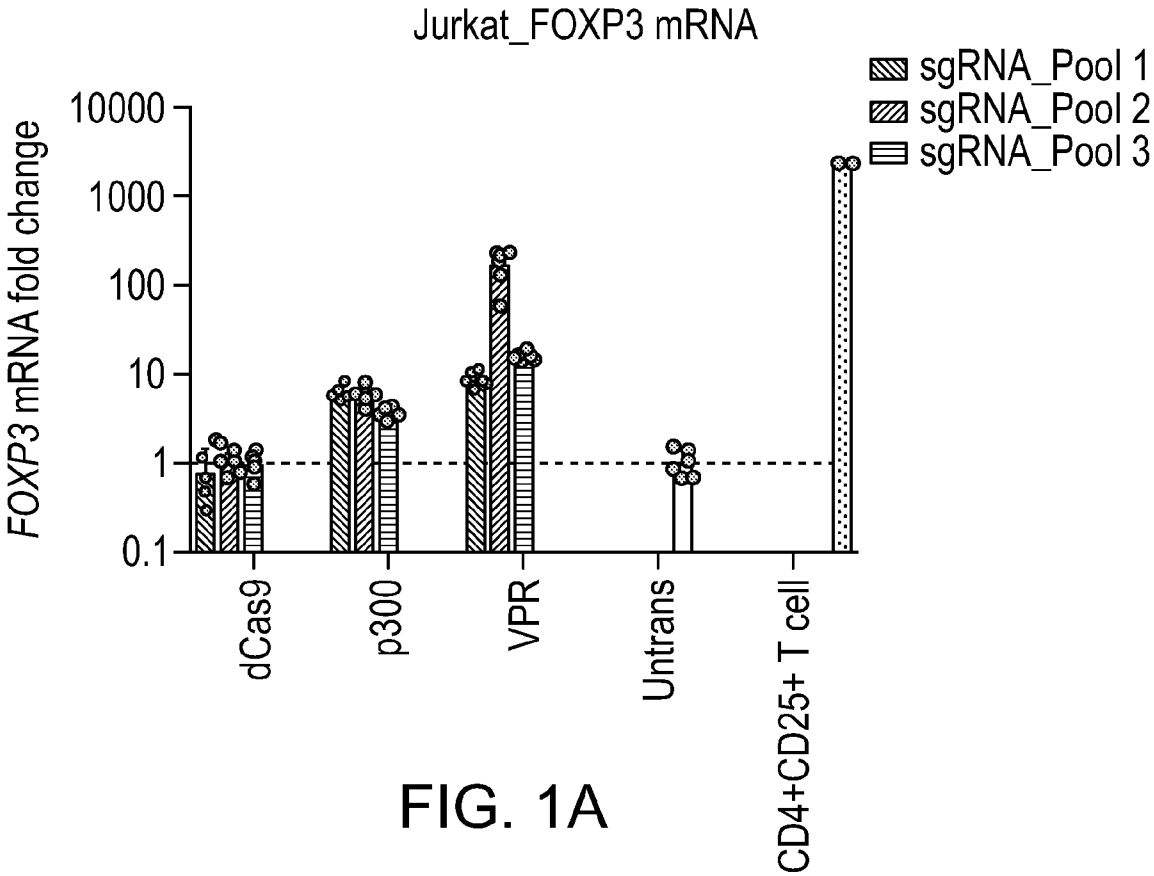
Jurkat_FOXP3 mRNA
sgRNA_Pool 1
sgRNA_Pool 2
sgRNA_Pool 3
FOXP3 mRNA fold change
10000
1000
100
10
1
0.1
dCas9
p300
VPR
Untrans
CD4+CD25+ T cell

FIG. 1A

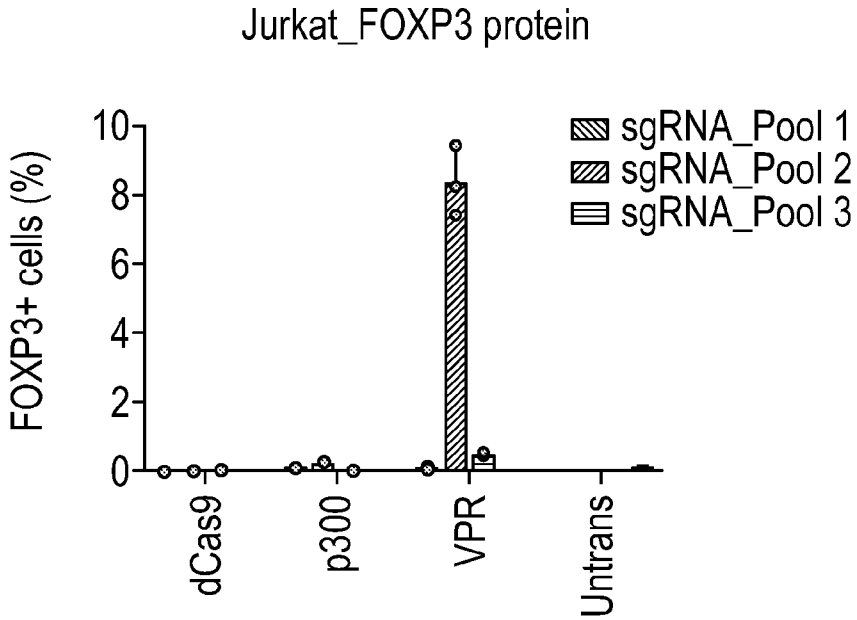
Jurkat_FOXP3 protein
sgRNA_Pool 1
sgRNA_Pool 2
sgRNA_Pool 3
FOXP3+ cells (%)
10
8
6
4
2
0
dCas9
p300
VPR
Untrans

FIG. 1B

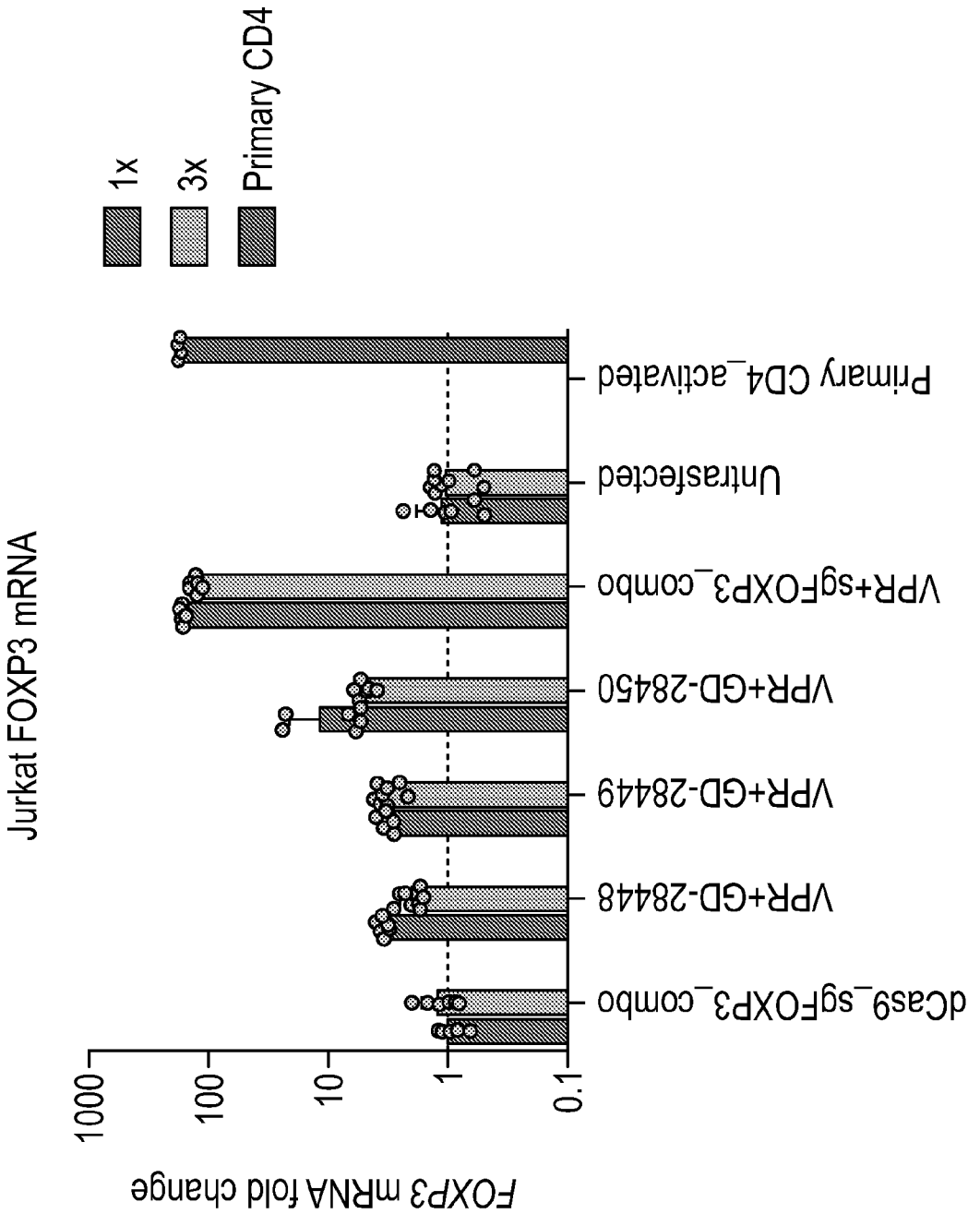
Jurkat FOXP3 mRNA
FOXP3 mRNA fold change
1000
100
10
1
0.1
1x
3x
Primary CD4
dCas9_sgFOXP3_combo
VPR+GD-28448
VPR+GD-28449
VPR+GD-28450
VPR+sgFOXP3_combo
Untrasfected
Primary CD4_activated

FIG. 2

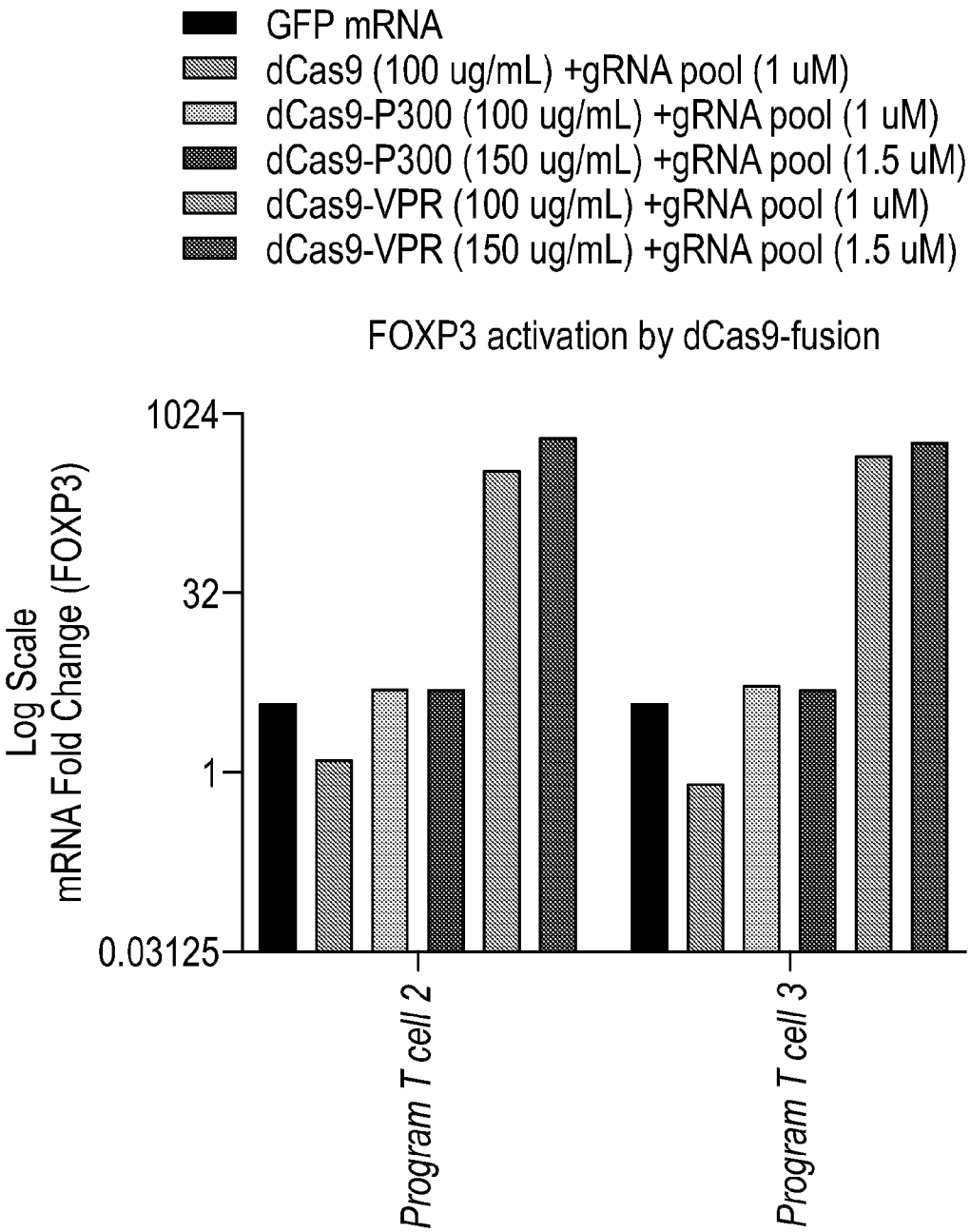
GFP mRNA
dCas9 (100 ug/mL) +gRNA pool (1 uM)
dCas9-P300 (100 ug/mL) +gRNA pool (1 uM)
dCas9-P300 (150 ug/mL) +gRNA pool (1.5 uM)
dCas9-VPR (100 ug/mL) +gRNA pool (1 uM)
dCas9-VPR (150 ug/mL) +gRNA pool (1.5 uM)
FOXP3 activation by dCas9-fusion
Log Scale
mRNA Fold Change (FOXP3)
1024
32
1
0.03125
Program T cell 2
Program T cell 3

FIG. 3A

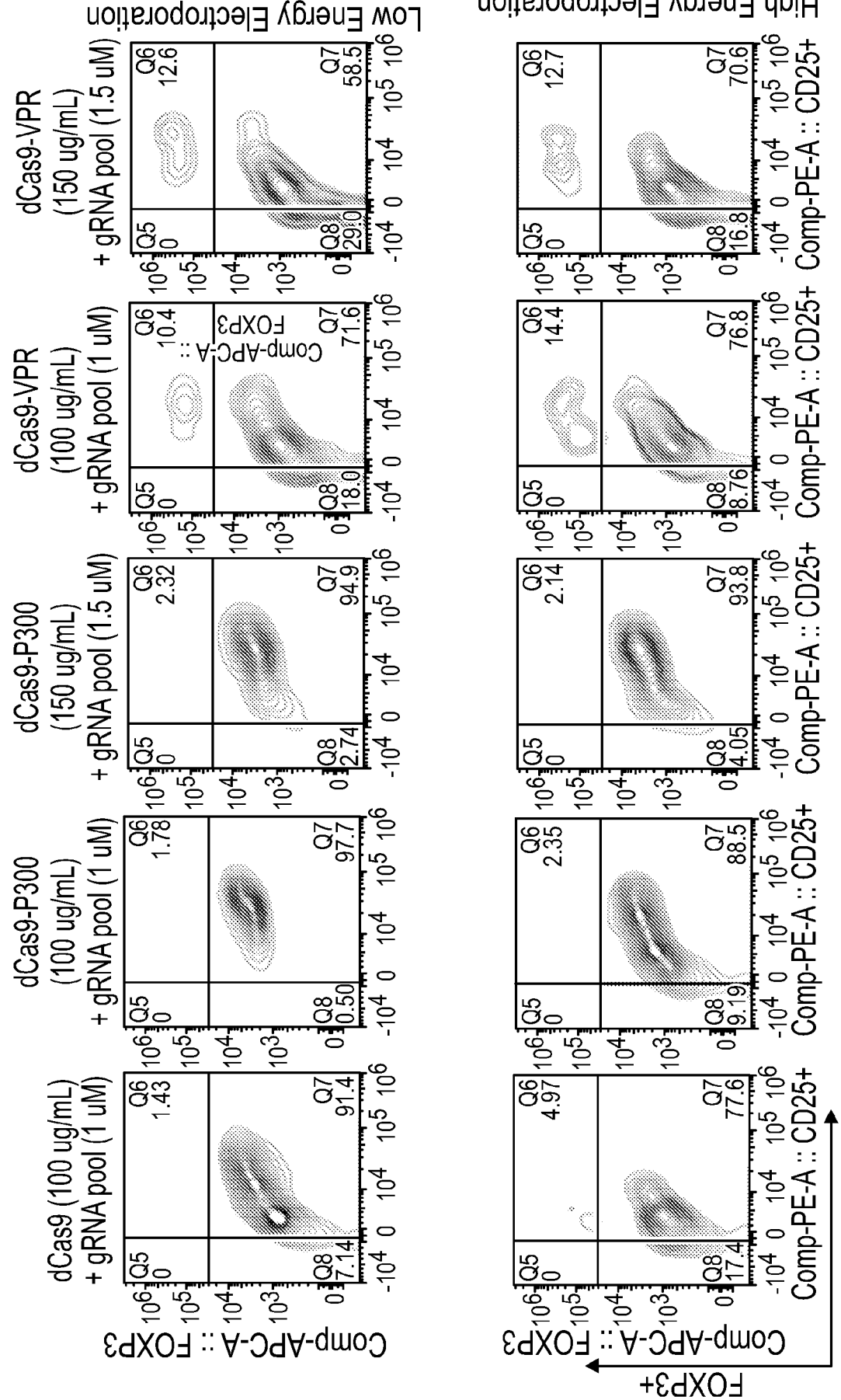
Low Energy Electroporation
High Energy Electroporation
dCas9 (100 ug/mL) + gRNA pool (1 uM)
dCas9-P300 (100 ug/mL) + gRNA pool (1 uM)
dCas9-P300 (150 ug/mL) + gRNA pool (1.5 uM)
dCas9-VPR (100 ug/mL) + gRNA pool (1 uM)
dCas9-VPR (150 ug/mL) + gRNA pool (1.5 uM)
Comp-APC-A :: FOXP3
Comp-PE-A :: CD25+
FOXP3+
CD25+

FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/021825

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 C12N9/22 A61K31/7088
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K C40B

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2018/031762 A1 (UNIV DUKE [US]) 15 February 2018 (2018-02-15) example 6; sequences 3,4 -----	1-32, 70-145
Y	FORSTNERIC VIDA ET AL: "CRISPRa-mediated FOXP3 gene upregulation in mammalian cells", CELL & BIOSCIENCE, vol. 9, no. 1, 21 November 2019 (2019-11-21), XP055804187, DOI: 10.1186/s13578-019-0357-0 Retrieved from the Internet: URL:https://cellandbioscience.biomedcentral.com/track/pdf/10.1186/s13578-019-0357-0.pdf> figures 1-4; table 1 ----- -/--	1-32, 70-145

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 June 2021	30/08/2021

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MASAHIRO OKADA ET AL: "Stabilization of Foxp3 expression by CRISPR-dCas9-based epigenome editing in mouse primary T cells", EPIGENETICS & CHROMATIN, BIOMED CENTRAL LTD, LONDON, UK, vol. 10, 8 May 2017 (2017-05-08), pages 1-17, XP002773366, ISSN: 1756-8935, DOI: 10.1186/S13072-017-0129-1 figures 2-4,7	1-32, 70-145
A	WO 2015/162422 A1 (MINA THERAPEUTICS LTD [GB]) 29 October 2015 (2015-10-29) page 126 - page 130	1-32, 70-145
A	KARL MARTIN ET AL: "Recruitment of Histone Methyltransferase Ehmt1 to Foxp3 TSDR Counteracts Differentiation of Induced Regulatory T Cells", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 431, no. 19, 27 July 2019 (2019-07-27), pages 3606-3625, XP085804069, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2019.07.031 [retrieved on 2019-07-27] figures 1,2,4	1-32, 70-145
A	JEFFRIES MATLOCK A ED - HEDRICH CHRISTIAN: "Epigenetic editing: How cutting-edge targeted epigenetic modification might provide novel avenues for autoimmune disease therapy", CLINICAL IMMUNOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 196, 6 February 2018 (2018-02-06), pages 49-58, XP085557478, ISSN: 1521-6616, DOI: 10.1016/J.CLIM.2018.02.001 figures 2,3	1-32, 70-145
A	GEORGIEV PETER ET AL: "Regulatory T Cells: the Many Faces of Foxp3", JOURNAL OF CLINICAL IMMUNOLOGY, KLUWER ACADEMIC PUBLISHERS, NEW YORK, vol. 39, no. 7, 2 September 2019 (2019-09-02), pages 623-640, XP036887261, ISSN: 0271-9142, DOI: 10.1007/S10875-019-00684-7 [retrieved on 2019-09-02] the whole document	1-32, 70-145

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1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JIA HAO ET AL: "The expression of FOXP3 and its role in human cancers", BBA - REVIEWS ON CANCER, vol. 1871, no. 1, 7 January 2019 (2019-01-07), pages 170-178, XP085582038, ISSN: 0304-419X, DOI: 10.1016/J.BBCAN.2018.12.004 the whole document -----	1-32, 70-145
A	MARUYAMA TAKASHI ET AL: "The molecular mechanisms of Foxp3 gene regulation", SEMINARS IN IMMUNOLOGY, vol. 23, no. 6, 12 July 2011 (2011-07-12), pages 418-423, XP055806168, US ISSN: 1044-5323, DOI: 10.1016/j.smim.2011.06.005 Retrieved from the Internet: URL:https://www.sciencedirect.com/science/article/pii/S1044532311000546/pdfft?md5=283b046999754aba7730568d27985bd7&pid=1-s2.0-S1044532311000546-main.pdf> figure 1 -----	1-32, 70-145

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/021825

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

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

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

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

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

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

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

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

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

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/021825

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-32, 70-145(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-32, 70-145(all partially)

A site-specific forkhead box P3(FOXP3) disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, wherein the agent comprises a nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of the first nucleotide sequence in Table 2 (designated GD-28445).

2-9. claims: 1-32, 70-145(all partially)

A site-specific forkhead box P3(FOXP3) disrupting agent, comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region, wherein in each separate invention the agent comprises a nucleotide sequence having at least 85% nucleotide identity to the entire nucleotide sequence of any one of the nucleotide sequences in Table 2.

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10. claims: 33-69(completely); 70-145(partially)

A site-specific FOXP3 disrupting agent, comprising a nucleic acid molecule encoding a fusion protein, the fusion protein comprising a site-specific FOXP3 targeting moiety which targets a FOXP3 expression control region and an effector molecule.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/021825

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018031762 A1	15-02-2018	EP 3497221 A1	19-06-2019
		US 2019194633 A1	27-06-2019
		WO 2018031762 A1	15-02-2018
WO 2015162422 A1	29-10-2015	US 2017044540 A1	16-02-2017
		US 2018258429 A1	13-09-2018
		WO 2015162422 A1	29-10-2015