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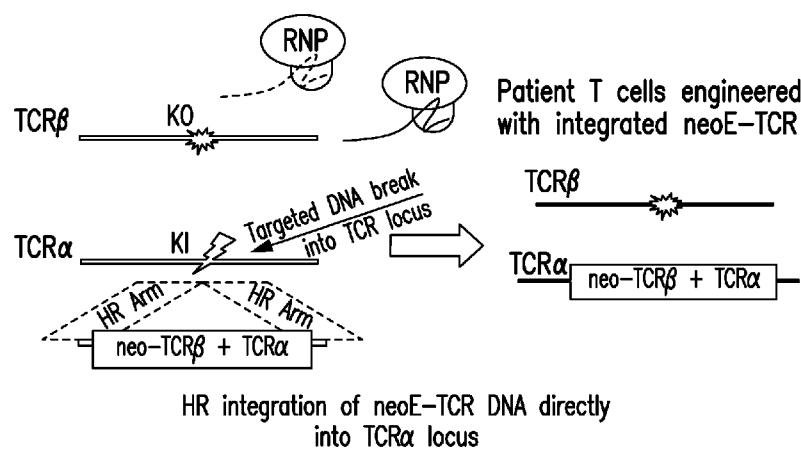


FIG. 1A

(57) Abstract: Compositions comprising and methods for the treatment of cancer using a NeoTCR based cell therapy with a secondary Payload in an expression construct.

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COMPOSITIONS AND METHODS FOR THE TREATMENT OF CANCER USING NEXT GENERATION ENGINEERED T CELL THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Application No. 63/106,122, filed on October 27, 2020, the content of which is incorporated in its entirety, and to which priority is claimed.

SEQUENCE LISTINGS

10 The instant application contains a Sequence Listings which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 26, 2021, is named 0875200225SL.txt and is 317,183 bytes in size.

BACKGROUND OF THE INVENTION

15 Human cancers are comprised of normal cells that have undergone a genetic or conversion to become abnormal cancer cells. In doing so, cancer cells begin to express proteins and other antigens that are distinct from those expressed by normal cells. These aberrant tumor antigens can be used by the body's innate immune system to specifically target and kill cancer cells. However, cancer cells employ various mechanisms to prevent immune cells from efficiently mounting an attack to effectively kill cancer cells and eradicate cancer. Nonetheless, 20 immune responses do occur and it is possible to isolate and detect individual T cells, for example, that recognize antigens on the cancer cells and have the ability to kill such cancer cells. Accordingly, the antigens on the cancer cells can be detected and T cells can be engineered to recognize and kill cells that express such cancer cells. However, simply engineering an immune cell to detect a cancer antigen (e.g., by engineering the immune cell to express a NeoTCR that is 25 specific to the cancer antigen) to elicit a cascade of events leading to the killing of the cancer antigen-expressing cell may not be sufficient for the treatment of cancers. In certain circumstances, in addition to the expression of the NeoTCR, it is also advantageous to knock in one or more additional genes into the immune cell. Furthermore, expression of NeoTCRs results in personalized therapies for each patient in need thereof. Specifically, each patient will 30 have a different cancer phenotype and thus the NeoTCRs need to be identified in each patient for a cell therapy that is designed specifically for such patient. In order to do this, viral gene-editing methods are not practical or feasible. Instead, non-viral gene-editing methods are needed to efficiently and cost-effectively engineer immune cells for personalized therapies.

Accordingly, there is a need to develop methods to express at least one NeoTCR and at least a Payload using non-viral methods.

SUMMARY OF THE INVENTION

The present disclosure provides cells comprising an exogenous TCR and at least one
5 Payload. In certain embodiments, the present disclosure provides a cell comprising an
exogenous polynucleotide comprising an exogenous enhancer, an insulator, a sequence encoding
an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous
polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the
sequence encoding an exogenous TCR is under control of an endogenous promoter and the
10 sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous
polynucleotide comprising an exogenous enhancer, a pause element, a sequence encoding an
exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous
polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the
15 sequence encoding an exogenous TCR is under control of an endogenous promoter and the
sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous
polynucleotide comprising a Woodchuck Hepatitis Virus Posttranscriptional Regulatory
Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least
20 one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within
the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an
endogenous promoter and the sequence encoding at least one Payload is under control of an
exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous
25 polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional
Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence
encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an
endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous
TCR is under control of an endogenous promoter and the sequence encoding at least one
30 Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous
polynucleotide comprising an insulator, a sequence encoding an exogenous TCR, and a
sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at

an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR and the first sequence encoding a Payload are under control of an endogenous promoter and the second sequence encoding a Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell and is under control of an endogenous promoter.

In certain embodiments, the insulator is an HS4 insulator or an IS2 insulator. In certain embodiments, the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19. In certain embodiments, the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19.

In certain embodiments, the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments, the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.

In certain embodiments, the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer. In certain embodiments, the exogenous enhancer is a TCR α enhancer. In certain embodiments, the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain

embodiments, the exogenous enhancer is a CMV enhancer. In certain embodiments, the CMV enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33.

5 In certain embodiments, the exogenous promoter is a constitutive promoter. In certain embodiments, the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter. In certain embodiments, the constitutive promoter is an MDN promoter. In certain embodiments, the constitutive promoter is an EF1 α promoter. In certain embodiments, the exogenous promoter is an inducible promoter. In certain
10 embodiments, the inducible promoter is an AP1 promoter, an NFAT promoter, an NF- κ B promoter, or an NR4A-responsive promoter.

In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence or a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence and a TCR β gene sequence. In certain
15 embodiments, the sequence encoding an exogenous TCR further comprises a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof. In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence, a TCR β gene sequence, a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage
20 peptide, or a combination thereof.

In certain embodiments, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, and a TCR α
25 gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR α gene sequence, and a poly-adenylation sequence. In certain embodiments, the sequence
30 encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence.

In certain embodiments, the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.

In certain embodiments, the 3' of the at least one Payload comprises a STOP codon. In certain embodiments, the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide. In certain embodiments, the 3' of the at least one Payload comprises a poly-adenylation sequence.

In certain embodiments, the at least one Payload is an inhibitory RNA molecule. In certain embodiments, the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster. In certain embodiments, the inhibitory RNA molecule is a miRNA. In certain embodiments, the miRNA comprises a first flanking sequence and a second flanking sequence. In certain embodiments, the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21. In certain embodiments, the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site. In certain embodiments, the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.

In certain embodiments, the sequences encoding a P2A peptide are codon diverged. In certain embodiments, the sequences encoding a protease cleavage peptide are codon diverged. In certain embodiments, the sequences encoding a signal sequence are codon diverged.

In certain embodiments, the exogenous TCR recognizes a cancer neoantigen. In certain embodiments, the neoantigen is a private neoantigen. In certain embodiments, the sequence encoding an exogenous TCR is obtained from a subject.

In certain embodiments, the cell is a primary cell. In certain embodiments, the cell is a patient-derived cell. In certain embodiments, the cell is a lymphocyte. In certain embodiments, the cell is a T cell. In certain embodiments, the cell is a young T cell. In certain embodiments, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain embodiments, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain embodiments, the cell is CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain embodiments, the endogenous locus within the genome of the cell is a TCR locus. In certain embodiments, the TCR locus is a TRAC locus or a TRBC locus. In certain embodiments, the TCR locus is a TRAC locus and a TRBC locus. In certain embodiments, the

endogenous promoter is a TRAC promoter. In certain embodiments, the endogenous promoter is a TRBC promoter.

In certain embodiments, the cell, the sequence encoding an exogenous TCR, and the sequence of the neoantigen are obtained from the same subject.

5 In certain embodiments, the present disclosure provides a polynucleotide comprising an exogenous enhancer, an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

10 In certain embodiments, the present disclosure provides a polynucleotide comprising an exogenous enhancer, a pause element, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

15 In certain embodiments, the present disclosure provides a polynucleotide comprising a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an
20 exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are
25 homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology
30 arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a

sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

5 In certain embodiments, the present disclosure provides a polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the second sequence encoding a Payload is under control of an exogenous promoter.

10 In certain embodiments, the present disclosure provides a polynucleotide comprising a sequence encoding a first homology arm, a second homology arm, an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus.

In certain embodiments, the insulator is an HS4 insulator or an IS2 insulator. In certain embodiments, the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19. In certain
15 embodiments, the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 18. In certain embodiments, the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments, the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.

20 In certain embodiments, the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer. In certain embodiments, the exogenous enhancer is a TCR α enhancer. In certain embodiments, the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain
25 embodiments, the exogenous enhancer is a CMV enhancer. In certain embodiments, the CMV enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33.

In certain embodiments, the exogenous promoter is a constitutive promoter. In certain
30 embodiments, the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter. In certain embodiments, the constitutive promoter is an MDN promoter. In certain embodiments, the constitutive promoter is an EF1 α promoter. In certain embodiments, the exogenous promoter is an inducible promoter. In certain

embodiments, the inducible promoter is an AP1 promoter, an NFAT promoter, an NF- κ B promoter, or an NR4A-responsive promoter.

In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence or a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence and a TCR β gene sequence. In certain
 5 embodiments, the sequence encoding an exogenous TCR further comprises a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof. In certain embodiments, the sequence encoding an exogenous TCR comprises a TCR α gene sequence, a TCR β gene sequence, a sequence encoding a P2A
 10 peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof.

In certain embodiments, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second
 15 sequence encoding a P2A peptide, a second sequence encoding a signal sequence, and a TCR α gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a
 20 second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR α gene sequence, and a poly-adenylation sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second
 25 sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence. In certain embodiments, the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.

30 In certain embodiments, the 3' of the at least one Payload comprises a STOP codon. In certain embodiments, the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide. In certain embodiments, the 3' of the at least one Payload comprises a poly-adenylation sequence.

In certain embodiments, the at least one Payload is an inhibitory RNA molecule. In certain embodiments, the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster. In certain embodiments, the inhibitory RNA molecule is a miRNA. In certain embodiments, the miRNA comprises a first flanking sequence and a second flanking sequence. In certain
5 embodiments, the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21. In certain embodiments, the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site. In certain
embodiments, the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.

10 In certain embodiments, the sequences encoding a P2A peptide are codon diverged. In certain embodiments, the sequences encoding a protease cleavage peptide are codon diverged. In certain embodiments, the sequences encoding a signal sequence are codon diverged. In certain embodiments, the exogenous TCR recognizes a cancer neoantigen. In certain
embodiments, the neoantigen is a private neoantigen. In certain embodiments, the sequence
15 encoding an exogenous TCR is obtained from a single subject.

In certain embodiments, the polynucleotide is a circular polynucleotide. In certain
embodiments, the circular polynucleotide is a plasmid or a nanoplasmid. In certain
embodiments, the polynucleotide is a linear polynucleotide.

20 In certain embodiments, the present disclosure further provides a vector comprising any one of the polynucleotide disclosed herein. In certain embodiments, the vector is a non-viral vector. In certain embodiments, the present disclosure provides a cell comprising any one of the
polynucleotides or vectors disclosed herein.

In certain embodiments, the present disclosure provides a method of modifying a cell. In
certain embodiments, the method comprises introducing into the cell any one of the
25 polynucleotides or vectors disclosed herein. In certain embodiments, the method further
comprises recombining the polynucleotide or vector into an endogenous locus of the cell.

In certain embodiments, the introducing occurs via electroporation. In certain
embodiments, the recombining comprises cleavage of the endogenous locus by a nuclease. In
certain embodiments, the recombining further comprises recombination of the polynucleotide
30 into the endogenous locus by homology-directed repair.

In certain embodiments, the nuclease is a Clustered Regularly Interspaced Short
Palindromic Repeats (CRISPR) family nuclease, or a functional fragment thereof. In certain
embodiments, the nuclease further comprises a gRNA.

In certain embodiments, the method further comprises culturing the cell in the presence of at least one cytokine. In certain embodiments, the at least one cytokine comprises IL2, IL7, IL15, or a combination thereof. In certain embodiments, the at least one cytokine comprises IL7 and IL15.

5 In certain embodiments, the cell is a primary cell. In certain embodiments, the cell is a patient-derived cell. In certain embodiments, the cell is a lymphocyte. In certain embodiments, the cell is a T cell. In certain embodiments, the cell is a young T cell. In certain embodiments, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain embodiments, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain embodiments, the
10 cell is CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain embodiments, the present disclosure also provides a cell modified by any one of the methods disclosed herein.

In certain embodiments, the present disclosure provides a composition comprising an effective amount of the any one of the cells disclosed herein. In certain embodiments, the
15 composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain embodiments, the composition is administered to a patient in need thereof for the treatment of cancer. In certain embodiments, the composition comprises a cryopreservation agent. In certain embodiments, the composition comprises serum albumin. In certain embodiments, the composition comprises Plasma-Lyte A, HSA, and CryoStor CS10.

20 In certain embodiments, the present disclosure provides methods of treating cancer in a subject in need thereof. In certain embodiments, the method comprises administering a therapeutically effective amount of any one of the cells disclosed herein. In certain embodiments, the method comprises administering a therapeutically effective amount of any one of the compositions disclosed herein. In certain embodiments, prior to administering, a non-
25 myeloablative lymphodepletion regimen is administered to the subject.

In certain embodiments, the cancer is a solid tumor or a liquid tumor. In certain embodiments, the solid tumor is selected from the group consisting of melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+
30 cancer, esophageal cancer, thyroid cancer, gastric cancer, hepatocellular cancer, cholangiocarcinomas, renal cell cancers, testicular cancer, sarcomas, and colorectal cancer. In certain embodiments, the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.

In certain embodiments, the present disclosure provides a kit comprising any one of the cells, the polynucleotides, the vectors, or the compositions disclosed herein. In certain embodiments, the kit further comprises written instructions for treating cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

5 Figures 1A-1C show an example of a NeoE TCR cassette and gene editing methods that can be used to make NeoTCR Products. Figure 1A shows a schematic representing the general targeting strategy used for integrating neoantigen-specific TCR constructs (NeoTCRs) into the TCR α locus. Figures 1B and 1C show a neoantigen-specific TCR construct design used for integrating a NeoTCR into the TCR α locus wherein the cassette is shown with signal sequences (“SS”), protease cleavage sites (“P”), and 2A peptides (“2A”). Figure 1B shows a target TCR α locus (endogenous TRAC, top panel) and its CRISPR Cas9 target site (horizontal stripes, cleavage site designated by the arrow), and the circular plasmid HR template (bottom panel) with the polynucleotide encoding the NeoTCR, which is located between left and right homology arms (“LHA” and “RHA” respectively) prior to integration. Figure 1C shows the integrated NeoTCR in the TCR α locus (top panel), the transcribed and spliced NeoTCR mRNA (middle panel), and translation and processing of the expressed NeoTCR (bottom panel).

15 Figures 2A and 2B illustrate representative Secondary Promoter Constructs. Figure 2A shows a diagram of Format 1 of the Secondary Promoter Constructs used to express a broad selection of knock-in (KI) targets. The aim of Format 1 constructs is to decouple the NeoTCR expression from a secondary KI gene (i.e., a Payload). The Format 1 constructs are modular such that the Payload, the TCR, the promoter, and insulators can be changed to suit the needs of the genetic engineering goals. As shown, the architecture of Format 1 constructs comprises a left homology arm, a linker sequence, a 2A sequence, a signal sequence, a full length TCR beta gene, a protease cleavage sequence, a linker, a 2A sequence, a signal sequence, a full length TCR alpha gene, a poly-A signal sequence, an insulator sequence, a promoter region, an optional Kozak sequence, a Payload of interest, and a right homology arm; all of which are retained within a backbone. Figure 2B shows an alternate modified version of Format 1 wherein a poly-A signal sequence is incorporated after the Payload. The abbreviations for the elements of Format 1 used in Figures 2A and 2B are 2A (2A sequence), SS (signal sequence), P (protease cleavage site), I (insulator).

25 Figures 3A and 3B illustrate representative Secondary Promoter Constructs. Figure 3A shows a diagram of an example of Format 1 of the Secondary Promoter Constructs used to express a broad selection of KI targets. As shown, this example of a Format 1 construct

comprises a left homology arm, a GSG linker, a P2A sequence, an HGH signal sequence, a full length TCR beta gene, a furin cleavage sequence, a GSG linker, a P2A sequence, an HGH signal sequence, a full length TCR alpha gene, a poly-A signal sequence, an insulator sequence, a promoter region, a Kozak sequence, a Payload of interest, and a right homology arm; all of which are retained within a backbone. Figure 3B shows an alternate example of Format 1 wherein a poly-A signal sequence is incorporated after the Payload. The abbreviations for the Insulator used in Format 1 used in Figures 3A and 3B is "I".

Figures 4A-4C illustrate representative Secondary Promoter Constructs. Figure 4A shows a diagram of Format 2 of the Secondary Promoter Constructs used to express a broad selection of KI targets. The aim of Format 2 constructs is to decouple the NeoTCR expression from a secondary KI gene (i.e., a Payload). The Format 2 constructs are modular such that the Payload, the TCR, the promoter, and insulators can be changed to suit the needs of the genetic engineering goals. As shown, the architecture of Format 2 constructs comprises a left homology arm, a linker sequence, a 2A sequence, a signal sequence, a full length TCR beta gene, a protease cleavage sequence, a linker sequence, a 2A sequence, a signal sequence, a full length TCR alpha gene, a poly-A signal sequence, an insulator sequence, a promoter region, a Kozak sequence (optional), a Payload of interest, a WPRE, a poly-A signal sequence, and a right homology arm; all of which are retained within a backbone. Figure 4B shows an alternate version of Format 2 wherein the post-transcriptional regulatory element is not included and instead an insulator is incorporated following the second poly-A signal sequence. Figure 4C shows an alternate version of Format 2 wherein the post-transcriptional regulatory element is not included and no insulator is incorporated following the second poly-A signal sequence. The abbreviations for the elements of Format 2 used in Figures 4A-4C are 2A (2A sequence), SS (signal sequence), P (protease cleavage site), I (insulator).

Figures 5A-5C illustrate representative Secondary Promoter Constructs. Figure 5A shows a diagram of an example of Format 2 of the Secondary Promoter Constructs used to express a broad selection of knock-in (KI) targets. As shown, the architecture of this example of Format 2 constructs comprise a left homology arm; GSG linker; P2A sequence; HGH signal sequence; full length TCR beta gene; Furin cleavage sequence; GSG linker; P2A sequence; HGH signal sequence; full length TCR alpha gene; poly-A signal sequence; insulator sequence; promoter region; Kozak sequence; Payload of interest; WPRE; poly-A signal sequence; right homology arm; all of which are retained within a backbone. Figure 5B shows an example of one of the alternate versions of Format 2 wherein the post-transcriptional regulatory element is

not included and instead an insulator is incorporated following the second poly-A signal sequence. Figure 5C shows an example of one of the alternate versions of Format 2 wherein the post-transcriptional regulatory element is not included and no insulator is incorporated following the second poly-A signal sequence. The abbreviation for the Insulator used in Format 1 used in
 5 Figures 5A-5C is "I".

Figure 6 shows a diagram of Format 3 of the Secondary Promoter Constructs used to express a broad selection of KI targets. The aim of Format 3 constructs is to decouple the NeoTCR expression from a secondary KI gene (i.e., a Payload). The Format 3 constructs are modular such that the Payload, the TCR, the promoter, and insulators can be changed to suit the
 10 needs of the genetic engineering goals. As shown, the architecture of Format 3 constructs comprises a left homology arm, a linker sequence, a 2A sequence, a signal sequence, a full length TCR beta gene, a protease cleavage sequence, a linker sequence, a 2A sequence, a signal sequence, a full length TCR alpha gene, a poly-A signal sequence, a WPRE, a Payload of interest, a Kozak sequence (optional), a promoter region, and a right homology arm; all of which
 15 are retained within a backbone. The abbreviations for the elements of Format 1 used in Figure 6 are 2A (2A sequence), SS (signal sequence), P (protease cleavage site), I (insulator).

Figure 7 shows a diagram of an example of Format 3 of the Secondary Promoter Constructs used to express a broad selection of KI targets. The aim of Format 3 constructs is to decouple the NeoTCR expression from a secondary KI gene (i.e., a Payload). The Format 3
 20 constructs are modular such that the Payload, the TCR, the promoter, and insulators can be changed to suit the needs of the genetic engineering goals. As shown, the architecture of this example of Format 3 constructs comprise a left homology arm, a GSG linker, a P2A sequence, an HGH signal sequence, a full length TCR beta gene, a Furin cleavage sequence, a GSG linker, a P2A sequence, an HGH signal sequence, a full length TCR alpha gene, a poly-A signal
 25 sequence, a WPRE, a Payload of interest, a Kozak sequence, a promoter region, and a right homology arm; all of which are retained within a backbone. The abbreviation for the Insulator used in Format 1 used in Figure 7 is "I".

Figure 8 shows a diagram of Format 4 of the Secondary Promoter Constructs used to express a broad selection of KI targets. The aim of Format 4 is to control the expression of the
 30 Payload and the TCR off of the endogenous TRAC or TRBC promoter. Unlike Formats 1-3 that only have the TCR controlled by the TRAC or TRBC promoter and have the Payloads controlled by a secondary promoter, Format 4 controls both the TCR and the Payload off of the single, endogenous TRAC or TRBC promoter.

Figures 9A and 9B illustrate the reduction of NeoTCR expression in cells transduced with Secondary Promoter Constructs including the indicated promoters. Figure 9A shows the reduced expression of NeoTCR in constructs comprising a secondary promoter as measured by dextramer labeling. Figure 9B shows tumor-killing induced by cells transduced with a construct with or without a secondary promoter.

Figures 10A-10C illustrate the effects of different genomic elements on NeoTCR expression in constructs comprising a secondary promoter. Figure 10A shows the effect of enhancer addition in constructs comprising a secondary promoter. Figure 10B shows the effect of replacement of STOP codon with a sequence encoding Furin and P2A after the Payload. Figure 10C shows the effects due to the addition of termination elements in constructs comprising a secondary promoter.

Figure 11 shows Secondary Promoter Constructs disclosed herein.

Figures 12A-12C illustrate the effect of Secondary Promoter Constructs on gene editing and exogenous TCR expression. Figure 12A shows the percentage of edited cells with the indicated different constructs. Figure 12B shows expression levels of NeoTCR induced by different Secondary Promoter Constructs. Figure 12C shows expression levels of mCherry induced by indicated Secondary Promoter Constructs. Details of the constructs can be found in the Example section.

Figures 13A-13D illustrate the effects of different Secondary Promoter Constructs on NeoTCR expression and Payload expression. Figures 13A and 13B show representative results of NeoTCR expression induced by different Secondary Promoter Constructs. Figures 13C and 13D show representative results of Payload (e.g., mCherry) expression induced by the indicated Secondary Promoter Constructs. Details of the constructs can be found in the Example section.

Figure 14 shows additional Secondary Promoter Constructs.

Figures 15A-15D illustrate gene expression and gene editing obtained by Secondary Promoter Constructs depicted in Figure 14. Figures 15A and 15B show NeoTCR gene expression levels in cells expressing the indicated Secondary Promoter Constructs. Figures 15C and 15D show Payload expression (e.g., mCherry) induced by different Secondary Promoter Constructs. Details of the constructs can be found in the Example section.

Figures 16A-16H show additional Secondary Promoter Constructs disclosed herein. Details of these Secondary Promoter Constructs can be found in Example 8. shRNA: inhibitory RNA molecule.

Figures 17A-17C illustrate the effects of secondary promoters upstream of inhibitory RNA molecules. Figure 17A shows representative Secondary Promoter Constructs disclosed herein. Figure 17B shows gene expression levels of CBLB in cells including different Secondary Promoter Constructs. Figure 17C shows the expression levels of NeoTCR.

- 5 MND>mirE(CBLB): MND promoter upstream of mir-E targeting CBLB (“mir-E(CBLB)”);
 MND>eSIBR(CBLB): MND promoter upstream of eSIBR targeting CBLB (“eSIBR(CBLB)”);
 MND>eSIBR(CBLB).mirE(A2AR): MND promoter upstream of eSIBR(CBLB) and mir-E
 targeting A2AR (“mir-E(A2AR)”); MND> mirE(A2AR).eSIBR(CBLB): MND promoter
 10 upstream of mir-E(A2AR) and eSIBR(CBLB); hPGK>mirE(CBLB): hPGk promoter upstream
 of mir-E(CBLB); U6>mir22(CBLB): U6 promoter upstream of mir-22 targeting CBLB.

- Figures 18A-18C illustrate Secondary Promoter Constructs including a Payload and an inhibitory RNA. Figure 18A shows representative Secondary Promoter Constructs disclosed herein. Figure 18B shows gene expression levels of CBLB in cells including different Secondary Promoter Constructs. Figure 18C shows gene expression levels of a Payload
 15 (mCherry) in cells including different Secondary Promoter Constructs. MND>mirE(CBLB):
 MND promoter upstream of mir-E targeting CBLB (“mir-E(CBLB)”);
 MND>mirE(CBLB).mCherry: MND promoter upstream of mir-E(CBLB) and mCherry as
 Payload; MND> mCherry.mirE(CBLB): MND promoter upstream of mCherry and mir-
 E(CBLB).

- 20 Figures 19A-19E illustrate Secondary Promoter Constructs including a promoter and an inhibitory RNA. Figure 19A shows representative Secondary Promoter Constructs disclosed herein. Figure 19B shows gene expression levels of CBLB in cells including different Secondary Promoter Constructs. Figure 19C shows the expression levels of NeoTCR. Figure 19D shows gene editing levels. Figure 19E shows the percentage of viability.
- 25 MND>mirE(CBLB): MND promoter upstream of mir-E targeting CBLB (“mir-E(CBLB)”);
 hPGK>mirE(CBLB): hPGk promoter upstream of mir-E(CBLB); U6>mir22(CBLB): U6
 promoter upstream of mir-22 targeting CBLB; MND>eSIBR(CBLB): MND promoter upstream
 of eSIBR targeting CBLB (“eSIBR(CBLB)”); 1: control; 2: MND promoter upstream of mir-E
 targeting CBLB (“mir-E(CBLB)”) and Payload (mCherry); 3: MND promoter upstream of mir-
 30 E(CBLB); 4: MND promoter upstream of Payload and mir-E(CBLB); 5: MND promoter
 upstream of splice donor site, mir-E(CBLB), and splice acceptor site; 6: MND promoter
 upstream of eSIBR targeting CBLB (“eSIBR(CBLB)”) and mir-E(CBLB); 7: MND promoter
 upstream of eSIBR(CBLB); 8: hPGK promoter upstream of mir-E(CBLB); 9: MND promoter

upstream of mir-E targeting A2AR and eSIBR(CBLB); 10: U6 promoter upstream of mir-22 targeting CBLB.

Figures 20A-20D illustrate Secondary Promoter Constructs including multiple Payloads or inhibitory RNA molecules. Figure 20A shows representative Secondary Promoter Constructs disclosed herein. Figure 20B shows gene expression levels of CBLB in cells including different Secondary Promoter Constructs. Figure 20C shows gene expression levels of A2AR. Figure 20D shows the expression levels of NeoTCR. MND>mirE(CBLB): MND promoter upstream of mir-E targeting CBLB ("mir-E(CBLB)"); MND>eSIBR(CBLB): MND promoter upstream of eSIBR targeting CBLB ("eSIBR(CBLB)"); MND>eSIBR(CBLB).mirE(A2AR): MND promoter upstream of eSIBR(CBLB) and mir-E targeting A2AR ("mir-E(A2AR)"); MND>mirE(A2AR).eSIBR(CBLB): MND promoter upstream of mir-E(A2AR) and eSIBR(CBLB).

Figures 21A-21C illustrate Secondary Promoter Constructs including miRNA clusters. Figure 21A shows representative Secondary Promoter Constructs disclosed herein. Figure 21B shows gene expression levels of TGFBR2 in cells including different Secondary Promoter Constructs. Figure 21C shows the expression levels of NeoTCR. TCR089(0.3kb): control; TCR089+TGFBR2KO: NeoTCR and TGFBR2 knockout; U6>mir22(TGFBR2).6T: U6 promoter upstream of mir-22 targeting TGFBR2 and termination sequence; MND>mir106(CBLB-A2AR-TGFBR2): MND promoter upstream of mir-106 targeting CBLB, A2AR, and TGFBR2; MND>mirE(A2AR): MND promoter upstream of mir-E targeting A2AR; U6>mirE(A2AR).6T: U6 promoter upstream of mir-E targeting A2AR and termination sequence.

Figures 22A-22C illustrate Secondary Promoter Constructs including splice acceptor site and splice donor site. Figure 22A shows representative Secondary Promoter Constructs disclosed herein. Figure 22B shows gene expression levels of CBLB in cells including different Secondary Promoter Constructs. Figure 21C shows the expression levels of NeoTCR. MND>mirE(CBLB): MND promoter upstream of mir-E targeting CBLB ("mir-E(CBLB)"); MND>SD.mirE(CBLB).SA: MND promoter upstream of splice donor site, mir-E(CBLB), and splice acceptor site.

Figure 23 shows representative Secondary Promoter Constructs disclosed herein.

DETAILED DESCRIPTION

The present disclosure provides adoptive cell therapies comprising a first and a second exogenous gene and having enhanced activity and efficacy against tumor cells. The present

disclosure is based, in part, on the ability of the inventors to decouple the gene expression of the first and second exogenous genes resulting in elevated and maintained expression levels of a sequence encoding an exogenous TCR (e.g., a NeoTCR) and a Payload that potentiates and improves the activity of the cells (e.g., cytotoxicity, cell proliferation, and/or cell persistence).

- 5 The present disclosure also provides methods for producing the cells, and compositions disclosed herein. Finally, the present disclosure provides methods of using such cells and composition for treating and/or preventing cancer.

Non-limiting embodiments of the present disclosure are described by the present description and examples. For purposes of clarity of disclosure and not by way of limitation, the
10 detailed description is divided into the following subsections:

1. Definitions;
2. NeoTCR Products;
3. Secondary Promoter Products;
4. Methods of Treatment;
- 15 5. Articles of Manufacture;
6. Therapeutic Compositions and Methods of Manufacturing;
7. Kits; and
8. Exemplary Embodiments.

20 1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art. The following references provide one of skill with a general definition of many of the terms used in the presently disclosed subject matter: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The
25 Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

It is understood that aspects and embodiments of the invention described herein include
30 "comprising," "consisting," and "consisting essentially of" aspects and embodiments. The terms "comprises" and "comprising" are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean "includes", "including" and the like.

As used herein, the term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, “about” can mean within 3 or more than 3 standard deviations, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, e.g., up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, e.g., within 5-fold or within 2-fold, of a value.

The term “antibody” as used herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific and tri-specific antibodies), and antibody fragments (e.g., bis-Fabs) so long as they exhibit the desired antigen-binding activity.

“Antibody Fragment” as used herein refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to bis-Fabs; Fv; Fab; Fab, Fab'-SH; F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); and multispecific antibodies formed from antibody fragments.

The terms “Cancer” and “Tumor” are used interchangeably herein. As used herein, the terms “Cancer” or “Tumor” refer to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms are further used to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Cancer can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Cancer includes cancers, such as sarcomas, carcinomas, or plasmacytomas (a malignant tumor of the plasma cells). Examples of cancer include, but are not limited to, those described herein. The terms “Cancer” or “Tumor” and “Proliferative Disorder” are not mutually exclusive as used herein.

As used herein, “sequence identity” or “identity” (in the context of two nucleic acid or polypeptide sequences) refers to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When the

percentage of sequence identity is used about proteins it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule.

5 When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have “sequence similarity” or “similarity.” Means for making this adjustment are well known to those of skill in the art.

As used herein, “percentage of sequence identity” refers to the value determined by
10 comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window can comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for the optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both
15 sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. Methods of alignment of sequences for comparison include, without any limitation, the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith et al. (1981) *Adv. Appl. Math.* 2:482; the homology
20 alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Computer implementations of these mathematical algorithms include, without any limitation, CLUSTAL,
25 CLUSTALW, CLUSTALOMEGA, ALIGN, ALIGN PLUS, GAP, BESTFIT, BLAST, FASTA, TFASTA, BLASTN, BLASTX, BLASTP, TBLASTN, and TBLASTX.

A “conservative substitution” or a “conservative amino acid,” refers to the substitution of amino acid with a chemically or functionally similar amino acid. Conservative substitution tables providing similar amino acids are well known in the art. In certain embodiments, acidic
30 amino acids D and E are conservative substitutions for one another; basic amino acids K, R, and H are conservative substitutions for one another; hydrophilic uncharged amino acids S, T, N, and Q are conservative substitutions for one another; aliphatic uncharged amino acids G, A, V, L, and I are conservative substitutions for one another; non-polar uncharged amino acids C, M, and

P are conservative substitutions for one another; aromatic amino acids F, Y, and W are conservative substitutions for one another; A, S, and T are conservative substitutions for one another; D and E are conservative substitutions for one another; N and Q are conservative substitutions for one another; R and K are conservative substitutions for one another; I, L, and M are conservative substitutions for one another; F, Y, and W are conservative substitutions for one another; A and G are conservative substitutions for one another; D and E are conservative substitutions for one another; N and Q are conservative substitutions for one another; R, K and H are conservative substitutions for one another; I, L, M, and V are conservative substitutions for one another; F, Y and W are conservative substitutions for one another; S and T are conservative substitutions for one another; and C and M are conservative substitutions for one another. Additional conservative substitutions may be found, for example, in Creighton, *Proteins: Structures and Molecular Properties* 2nd ed. (1993) W. H. Freeman & Co., New York, NY.

“Treat,” “Treatment,” and “treating” are used interchangeably and as used herein mean obtaining beneficial or desired results including clinical results. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In certain embodiments, the NeoTCR Product of the invention is used to delay the development of a proliferative disorder (e.g., cancer) or to slow the progression of such disease.

“Dextramer” as used herein means a multimerized neoepitope-HLA complex that specifically binds to its cognate NeoTCR.

The term “tumor antigen” as used herein refers to an antigen (e.g., a polypeptide) that is uniquely or differentially expressed on a tumor cell compared to a normal or non-neoplastic cell. In certain embodiments, a tumor antigen includes any polypeptide expressed by a tumor that is capable of activating or inducing an immune response via an antigen-recognizing receptor or capable of suppressing an immune response via receptor-ligand binding.

As used herein, the terms “neoantigen”, “neoepitope” or “neoE” refer to a newly formed antigenic determinant that arises, e.g., from a somatic mutation(s) and is recognized as “non-self.” A mutation giving rise to a “neoantigen”, “neoepitope” or “neoE” can include a frameshift or non-frameshift indel, missense or nonsense substitution, splice site alteration (e.g., alternatively spliced transcripts), genomic rearrangement or gene fusion, any genomic or

expression alterations, or any post-translational modifications. In certain embodiments, the neoantigen can be a private neoantigen. As used herein, the term “private neoantigen” refers to neoantigens that are exclusively expressed and present in a subject having certain cancer. For clarity, a private neoantigen is a neoantigen that cannot be used for another patient. In certain
 5 embodiments, the neoantigen can be a “public neoantigen”. The term “public neoantigen,” as used herein, refers to neoantigens that are shared by more than one subject.

“NeoTCR” and “NeoE TCR,” as used herein, mean a neoepitope-specific T cell receptor that is introduced into a T cell, e.g., by gene-editing methods. As used herein, the term “exogenous TCR” may be used in place of “NeoTCR”.

10 “NeoTCR Cells” as used herein means one or more cells precision-engineered to express one or more NeoTCRs. In certain embodiments, the cells are T cells. In certain embodiments, the T cells are CD8⁺ and/or CD4⁺ T cells. In certain embodiments, the CD8⁺ and/or CD4⁺ T cells are autologous cells from the patient for whom a NeoTCR Product will be administered. The terms “NeoTCR Cells” and “NeoTCR-P1 T cells” and “NeoTCR-P1 cells” are used
 15 interchangeably herein.

“NeoTCR Product” as used herein means a pharmaceutical formulation comprising one or more NeoTCR Cells. NeoTCR Product consists of autologous precision genome-engineered CD8⁺ and CD4⁺ T cells. Using a targeted DNA-mediated *non-viral* precision genome engineering approach, expression of the endogenous TCR is eliminated and replaced by a
 20 patient-specific NeoTCR isolated from peripheral CD8⁺ T cells targeting the tumor-exclusive neoepitope. In certain embodiments, the resulting engineered CD8⁺ or CD4⁺ T cells express NeoTCRs on their surface of native sequence, native expression levels, and native TCR function. The sequences of the NeoTCR external binding domain and cytoplasmic signaling domains are unmodified from the TCR isolated from native CD8⁺ T cells. Regulation of the
 25 NeoTCR gene expression is driven by the native endogenous TCR promoter positioned upstream of where the NeoTCR gene cassette is integrated into the genome. Through this approach, native levels of NeoTCR expression are observed in unstimulated and antigen-activated T cell states. The NeoTCR Product manufactured for each patient represents a defined dose of autologous CD8⁺ and/or CD4⁺ T cells that are precision genome engineered to express
 30 a single neoE-specific TCR cloned from neoE-specific CD8⁺ T cells individually isolated from the peripheral blood of that same patient.

“NeoTCR Viral Product” as used herein has the same definition of NeoTCR Product except that the genome engineering is performed using viral-mediated methods.

“Secondary Promoter Construct” as used herein refers to a construct comprising elements to express a NeoTCR and elements to express a Payload. Formats 1-4, along with the variations thereof described herein, are representative examples of Secondary Promoter Constructs. Additional information can be found in Section 3 below.

5 “Secondary Promoter Cell” as used herein refers to a NeoTCR Cell that is engineered to express a Payload under the control of a different promoter than the promoter used to express the NeoTCR.

“Secondary Promoter Product” as used herein refers to a NeoTCR Product that is engineered to express a Payload under the control of a different promoter than the promoter used
10 to express the NeoTCR.

“Payload” as used herein refers to a second or more gene, in addition to the NeoTCR, to be knocked into an immune cell using gene editing methods described herein. Examples of Payloads include but are not limited to the one disclosed in Section 3.1.1. below.

“Pharmaceutical Formulation” refers to a preparation that is in such form as to permit the
15 biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. For clarity, DMSO at quantities used in a NeoTCR Product is not considered unacceptably toxic.

“Promoter region” or “promoter” as used herein means the region of the construct that
20 encodes a promoter that controls the expression of the Payload in the Secondary Promoter Constructs.

As used herein, the term “enhancer” refers to a DNA control element that enhances the levels of expression of the gene when a specific transcription factor is bound. Unlike a promoter, an enhancer does not stimulate the expression of the gene on its own. Enhancers are frequently
25 found in the upstream (5') region of the gene.

A “subject,” “patient,” or an “individual” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

“TCR” as used herein means T cell receptor.

30 “2A” and “2A peptide” are used interchangeably herein and mean a class of 18-22 amino acid long, viral, self-cleaving peptides that are able to mediate cleavage of peptides during translation in eukaryotic cells. Four well-known members of the 2A peptide class are T2A, P2A, E2A, and F2A. The T2A peptide was first identified in the *Thosea asigna* virus 2A. The

P2A peptide was first identified in the porcine teschovirus-1 2A. The E2A peptide was first identified in the equine rhinitis A virus. The F2A peptide was first identified in the foot-and-mouth disease virus. The self-cleaving mechanism of the 2A peptides is a result of ribosome skipping the formation of a glycyl-prolyl peptide bond at the C-terminus of the 2A. Specifically, the 2A peptides have a C-terminal conserved sequence that is necessary for the creation of steric hindrance and ribosome skipping. The ribosome skipping can result in one of three options: 1) successful skipping and recommencement of translation resulting in two cleaved proteins (the upstream of the 2A protein which is attached to the complete 2A peptide except for the C-terminal proline and the downstream of the 2A protein which is attached to one proline at the N-terminal; 2) successful skipping but ribosome fall-off that results in discontinued translation and only the protein upstream of the 2A; or 3) unsuccessful skipping and continued translation (i.e., a fusion protein).

The term “endogenous” as used herein refers to a nucleic acid molecule or polypeptide that is normally expressed in a cell or tissue.

The term “exogenous” as used herein refers to a nucleic acid molecule or polypeptide that is not endogenously present in a cell. The term “exogenous” would therefore encompass any recombinant nucleic acid molecule or polypeptide expressed in a cell, such as foreign, heterologous, and over-expressed nucleic acid molecules and polypeptides. By “exogenous” nucleic acid is meant a nucleic acid not present in a native wild-type cell; for example, an exogenous nucleic acid may vary from an endogenous counterpart by sequence, by position/location, or both. For clarity, an exogenous nucleic acid may have the same or different sequence relative to its native endogenous counterpart; it may be introduced by genetic engineering into the cell itself or a progenitor thereof, and may optionally be linked to alternative control sequences, such as a non-native promoter or secretory sequence.

“Young” or “Younger” or “Young T cell” as it relates to T cells means memory stem cells (T_{MSC}) and central memory cells (T_{CM}). These cells have T cell proliferation upon specific activation and are competent for multiple cell divisions. They also have the ability to engraft after re-infusion, to rapidly differentiate into effector T cells upon exposure to their cognate antigen and target and kill tumor cells, as well as to persist for ongoing cancer surveillance and control.

“WPRE,” as used herein, refers to a woodchuck hepatitis virus post-transcriptional regulatory element that increases transgene expression.

As used herein, a “STOP codon” or “termination codon” is a nucleotide triplet within a messenger RNA that signals the termination of the translation process of a protein. Most codons in messenger RNA correspond to the addition of an amino acid to a growing polypeptide chain, which can ultimately become a protein; stop codons signal the termination of this process by binding release factors, which cause the ribosomal subunits to disassociate, releasing the amino acid chain.

“Kozak” or “Kozak sequence,” as used herein, refers to a nucleic acid motif that functions as the protein translation initiation site in certain eukaryotic mRNA transcripts.

“Insulator” or “transcriptional insulator,” as used herein, refer to a class of DNA sequence elements that possess a common ability to protect genes from inappropriate signals emanating from their surrounding environment. Insulators can be used to restrict the interaction of enhancers or silencers on promoters in a gene expression system. Insulators can set boundaries on the actions of enhancer and silencer elements and so partition the eukaryotic genome into regulatory domains. Physiologically, the transcriptional repressor CTCF (CCCTC-binding factor) binds through multiple zinc fingers (of which it has eleven) to a range of unrelated DNA sequences and functions as a transcriptional insulator, repressor, or activator, depending on the context of the binding site.

“Signal sequence” as used herein is a peptide that can be included at the N-terminus of a newly synthesized protein for the purpose of trafficking the newly synthesized protein to its intended and/or engineered location inside or outside of the cell.

2. NeoTCR Products

In certain embodiments, using the gene-editing technology and NeoTCR isolation technology described in PCT/US2020/17887 and PCT/US2019/025415, which are incorporated herein in their entireties, NeoTCRs are cloned in autologous CD8⁺ and CD4⁺ T cells from the same patient with cancer by precision genome engineered (using a DNA-mediated (non-viral) method as described in **Figures 1A-1C**) to express the NeoTCR. In other words, the NeoTCRs that are tumor-specific are identified in cancer patients, such NeoTCRs are then cloned, and then the cloned NeoTCRs are inserted into the cancer patient’s own T cells. Importantly, once expressed in the cells (e.g., CD8 T cells), the NeoTCR can recognize a private neoantigen. NeoTCR expressing T cells are then expanded in a manner that preserves a “young” T cell phenotypes, resulting in a NeoTCR-P1 product (i.e., a NeoTCR Product) in which the majority of the T cells exhibit T memory stem cell and T central memory phenotypes. These ‘young’ or ‘younger’ or less-differentiated T cell phenotypes are described to confer improved engraftment

potential and prolonged persistence post-infusion. Thus, the administration of NeoTCR Product, consisting significantly of ‘young’ T cell phenotypes, has the potential to benefit patients with cancer, through improved engraftment potential, prolonged persistence post-infusion, and rapid differentiation into effector T cells to eradicate tumor cells throughout the body.

5 *Ex vivo* mechanism-of-action studies were also performed with NeoTCR Products manufactured with T cells from patients with cancer. Comparable gene editing efficiencies and functional activities, as measured by antigen-specificity of T cell killing activity, proliferation, and cytokine production, were observed demonstrating that the manufacturing process described herein is successful in generating products with T cells from patients with cancer as starting
10 material.

 In certain embodiments, the NeoTCR Product manufacturing process involves electroporation of dual ribonucleoprotein species of CRISPR-Cas9 nucleases bound to guide RNA sequences, with each species targeting the genomic TCR α and the genomic TCR β loci. The specificity of targeting Cas9 nucleases to each genomic locus has been previously described
15 in the literature as being highly specific. Comprehensive testing of the NeoTCR Product was performed *in vitro* and *in silico* analyses to survey possible off-target genomic cleavage sites, using COSMID and GUIDE-seq, respectively. Multiple NeoTCR Products or comparable cell products from healthy donors were assessed for cleavage of the candidate off-target sites by deep sequencing, supporting the published evidence that the selected nucleases are highly
20 specific. Further aspects of the precision genome engineering process have been assessed for safety. No evidence of genomic instability following precision genome engineering was found in assessing multiple NeoTCR Products by targeted locus amplification (TLA) or standard FISH cytogenetics. No off-target integration anywhere into the genome of the NeoTCR sequence was detected. No evidence of residual Cas9 was found in the cell product.

25 The comprehensive assessment of the NeoTCR Product and precision genome engineering process indicates that the NeoTCR Product will be well tolerated following infusion back to the patient.

 The genome engineering approach described herein enables the highly efficient generation of bespoke NeoTCR Cells (i.e., NeoTCR Products) for personalized adoptive cell
30 therapy for patients with solid and liquid tumors. Furthermore, the engineering method is not restricted to the use in T cells and has also been applied successfully to other primary cell types, including natural killer and hematopoietic stem cells.

3. Secondary Promoter Products

In certain embodiments, the present disclosure provides Secondary Promoter Products. In certain embodiments, the NeoTCR Cells described can express a Payload under the control of a different promoter than the promoter used to express the NeoTCR to produce a Secondary Promoter Cell. In certain embodiments, the NeoTCR Cells described herein are further
5 engineered to express a Payload under the control of a different promoter than the promoter used to express the NeoTCR to produce a Secondary Promoter Cell. Such Secondary Promoter Cells can be formulated into Secondary Promoter Products for the treatment of an immune disease or disorder.

In certain embodiments, the immune disease or disorder is a primary immune disease or
10 disorder (i.e., a disease or disorder that a subject is born with). In certain embodiments, the immune disease or disorder is a secondary disorder (i.e., acquired disease or disorder). In certain embodiments, the immune disease selected from the group comprising cancers, autoimmune diseases and disorders, viral infections, bacterial infections, retroviral infections, and neurological diseases and disorders. In certain embodiments, the immune disease is cancer. In
15 certain embodiments, the immune disease is an autoimmune disease.

In certain embodiments, a single-step precision genome engineering method is performed in order for the NeoTCR insertion and the Payload insertion into a primary cell to occur in the same reaction. In certain embodiments, the NeoTCR and the Payload are encoded by the same polynucleotide. In certain embodiments, the primary cell is a T cell. In certain
20 embodiments, the primary cell is an NK cell. In certain embodiments, this single-step process reduces potential T cell heterogeneity that would otherwise be present if multiple reactions and multiple polynucleotides (e.g., DNA templates) were used. In certain embodiments, this single-step process reduces potential NK cell heterogeneity that would otherwise be present if multiple reactions and multiple polynucleotides (e.g., DNA templates) were used.

25 In certain embodiments, the single-step precision genome engineering method described herein allows for one or more Payloads to be engineered in and/or one or more removal (i.e., silencing) of one or more genes endogenous to the primary cell.

In certain embodiments, the Secondary Promoter Cells of the Secondary Promoter Products are designed to target molecules on the tumor. Non-limiting examples of molecules
30 expressed on tumors that the Secondary Promoter Cells and Secondary Promoter Products can target include but are not limited to antigens, neoantigens, private neoantigens, public neoantigens, receptors, cell surface molecules, cytokine receptors, and oncogenic pathway inhibitors or enhancers.

In certain embodiments, the Secondary Promoter Cells of the Secondary Promoter Products are designed to express a protein made by the Payload only upon activation of the Secondary Promoter Cells. In certain embodiments, the activated Secondary Promoter Cells are activated T cells.

5 In certain embodiments, the Secondary Promoter Cells of the Secondary Promoter Products are designed to secrete a protein made by the Payload only upon activation of the Secondary Promoter Cells. In certain embodiments, the activated Secondary Promoter Cells are activated T cells.

10 In certain embodiments, the Secondary Promoter Cells of the Secondary Promoter Products are designed to constitutively express a protein made by the Payload.

In certain embodiments, the Secondary Promoter Cells of the Secondary Promoter Products are designed to constitutively secrete a protein made by the Payload.

3.1. Secondary Promoter Constructs

15 Secondary Promoter Constructs disclosed herein (including but not limited to Formats 1-4 described herein and diagramed in the figures) are designed to allow for the dual expression of an exogenous TCR (e.g., a NeoTCR) and a Payload when transfected or transduced into a cell. In certain embodiments, the method of gene insertion is non-viral transfection. In certain embodiments, the non-viral transfection methods used are those described herein. In certain embodiments, the cell is a primary human cell. In certain embodiments, the primary human cell
20 is a T cell.

In certain embodiments, one or more of the following key elements are included in the Secondary Promoter Constructs: an element to promote translation of transcripts (e.g., enhancer), a poly-adenylation (poly-A) sequence, a promoter, a pause element, a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE), a scaffold/matrix attachment
25 region, and an insulator.

In certain embodiments, the Secondary Promoter Constructs are modular, as each element should be thought of as a broad and general category. For instance, any poly-A signal sequence known to one of skill in the art could be used in the modular construct. For instance, any element to promote translation of transcripts applicable to the intended cell and Payload
30 known to one of skill in the art could be used in the modular construct. For instance, any applicable promoter for the intended cell and Payload known to one of skill in the art could be used in the modular construct. For instance, any applicable insulator for the intended cell and Payload known to one of skill in the art could be used in the modular construct.

In certain embodiments, the Secondary Promoter Construct designs comprise a Payload, a regulatory element to increase the translation of the Payload transcript, a transcription stop signal, a promoter, and optionally one or more insulators. In certain embodiments, the Secondary Promoter Construct design is described in Figures 2A-8, 11, and 14. In certain
 5 embodiments, the Secondary Promoter Construct design is designated as “Format 1,” “Format 2,” “Format 3,” or “Format 4.”

3.1.1. Payloads

In certain embodiments, the Secondary Promoter Constructs disclosed herein include a Payload. In certain embodiments, the Payload of the Secondary Promoter Constructs is selected
 10 from the group comprising a cytokine receptors trap or ligand trap, an angiogenesis factors, an apoptotic factor, an inhibitory protein for various cell processes (e.g., inhibition of macrophage activation), an extracellular matrix modulator, a soluble TCRs, a soluble chimeric antigen receptor (CAR), a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, and a protein that binds to receptors and sterically hinders receptor
 15 function.

In certain embodiments, the Payload is a cytokine receptor trap or a ligand trap. In certain embodiments, the cytokine receptor trap or ligand trap selected as a Payload acts as a cytokine sink. Cytokine receptor traps recognize certain inflammatory cytokines with high affinity and specificity but are structurally incapable of signaling or presenting the agonist to
 20 signaling receptor complexes. They act as a molecular trap for the agonist and signaling receptor components. Non-limiting examples of cytokine receptor trap include TNFR1, TNFR2, TGFBR1, TGFBR2, TGFBR3, IL4, CSF1R, CXCR1, CXCR2, CCR2, and CCR5. In certain embodiments, the cytokine receptor trap is a TGFBR2. In certain embodiments, the TGFBR2 comprises an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%,
 25 about 96%, about 97%, about 98%, or about 99% identical to the amino sequence set forth in SEQ ID NO: 1. In certain embodiments, the TGFBR2 comprises the amino sequence set forth in SEQ ID NO: 1. In certain embodiments, the TGFBR2 consists of the amino sequence set forth in SEQ ID NO: 1. SEQ ID NO: 1 is provided below.

MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
 30 EKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAPKICIMKEKKKPGETFFMCSSSDECNDNIIFSEEYN
 TSNPDLLLIVIFQVTGISLLPPLGVAISVIIIFYCYRVNRQQKLSS [SEQ ID NO: 1]

In certain embodiments, the TGFBR2 is encoded by the nucleotide sequence set forth in SEQ ID NO: 2 provided below.

ATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCACC
 GCACGTTTCAGAAGTCGGTTAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACGTGTGTA
 AATTTTGTGATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGT
 GAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAAGATGACGAGAACATAACACTAGAGACAGTTTGCCATGA
 5 CCCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAAAAAAAAAAGC
 CTGGTGAGACTTTCTTCATGTGTTCTCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAAC
 ACCAGCAATCCTGACTTGTGCTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACCTGGGAGTTGCCAT
 ATCTGTTCATCATCATCTTCTACTGCTACCGCGTTAACCGGCAGCAGAAGCTGAGTTCA [SEQ ID NO: 2]

Additional information and examples of cytokine traps can be found in Economides et
 10 al., *Nature medicine* 9.1 (2003): 47-52.

In certain embodiments, the Payload is an angiogenesis factor. In certain embodiments,
 the angiogenesis factor selected as a Payload reshapes tumor vasculature. Non-limiting
 examples of angiogenesis factors include FGF1, FGF2, VEGF, PDGF, ANGPT1, ANGPT2,
 DLL4, MMP1, MMP2, MMP3, MMP47, MMP8, MMP9, MMP10, MMP11, MMP12, MMP13,
 15 MMP14, MMP15, MMP16, MMP17, MMP24, MMP25, MMP28, SEMA3A, SEMA3B,
 SEMA3C, SEMA3D, SEMA3E, SEMA3F, SEMA3G, urokinase, and tissue plasminogen
 activator.

In certain embodiments, the Payload is an apoptotic factor. In certain embodiments, the
 apoptotic factor selected as a Payload protects a Secondary Promoter Cell from apoptosis. In
 20 certain embodiments, the apoptotic factor selected as a Payload induces apoptosis in a target cell
 (e.g., a tumor cell). Non-limiting examples of apoptotic factors include FAS, FASL, TNF α ,
 XIAP, cIAP1, c-IAP2, NAIP, API4, Bcl-2, cFLIP, BNIP3, FADD, AKT, and NK- κ B.

In certain embodiments, the Payload is an inhibitory protein. In certain embodiments,
 the inhibitory protein selected as a Payload inhibits macrophage activity. Non-limiting examples
 25 of inhibitory proteins include anti-TIM3 antibodies, anti-PD1 antibodies, anti-CD32b antibodies,
 and anti-CD200R antibodies.

In certain embodiments, the Payload is an anti-PD1 antibody. In certain embodiment,
 the Payload is an anti-PD1 svFv. In certain embodiments, the anti-PD1 svFv comprises an
 amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%,
 30 about 97%, about 98%, or about 99% identical to the amino sequence set forth in SEQ ID NO:
 3. In certain embodiments, the anti-PD1 svFv comprises the amino sequence set forth in SEQ
 ID NO: 3. In certain embodiments, the anti-PD1 svFv consists of the amino sequence set forth
 in SEQ ID NO: 3. SEQ ID NO: 3 is provided below.

MYRMQLLSICIALSLALVTNSEIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATG
 35 IPARFSGSGSGTDFTLTITSSLEPEDFAVYYCQQSSNWPRFTFGQGTKVEIKGGGSGGGGSGGGGSGVQLVESGGGVV

QPGRSLRLDCKASGITFSNSGMHWVRQAPGKLEWVAVIWDGSKRYYADSVKGRFTISRDN SKNTLFLQMNSLRAE
DTAVYYCATNDDYWGQGLTVTVSS [SEQ ID NO: 3]

In certain embodiments, the anti-PD1 svFv is encoded by the nucleotide sequence set forth in SEQ ID NO: 4 provided below.

5 ATGTACAGGATGCAACTCCTGTCTTGCAATTGCACTAAGTCTTGCACTTGTACAAACAGTGAGATCGTGCTGACTCA
GTCCCCCTGCTACCCTGTCCCTGTCCCCGGTGAGCGTGCTACCCTGTCCGCGCTTCCCAGAGCGTGCTCCTCCT
ACCTGGCCTGGTATCAACAAAAGCCCGGCCAAGTCCCCGCTGCTGATCTACGACGCCTCCAACCGCGCTACTGGC
ATCCCTGCTCGCTTCTCCGGTTCCGGTTCCGGCACTGACTTCACTCTGACCATCTCCTCCCTGGAGCCCCGAGGATTT
CGCTGTGTACTACTGCCAGCAGTCCTCCAAGTGGCCCCGTACCTTCGGTCAGGGCACCAAGGTGGAGATCAAGGGTG
10 GAGGCGGTTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGAAGCCAGGTCCAGCTGGTGGAGAGCGGTGGTGGTGTGGTG
CAGCCCCGCTCGTTCCCTGCGTCTGGATTGCAAAGCCTCCGGCATCACCTTCTCCAACAGCGGCATGCACTGGGTGCG
TCAGGCTCCTGGTAAGGGCCTGGAGTGGGTGGCTGTGATCTGGTACGATGGCTCCAAGCGCTACTACGCCGACTCCG
TGAAGGGTCGTTTCACCATCTCCCGCGACAACAGCAAGAACACCCTGTTCCCTGCAGATGAACTCCCTGCGTGCCGAG
GACACCGCCGTCTACTACTGCGCCACCAACGACGACTACTGGGGTCAGGGCACTCTGGTGACCGTCTCCTCC [SEQ
15 ID NO: 4]

In certain embodiments, the Payload is an extracellular matrix modulator. In certain
embodiments, the extracellular matrix modulator selected as a Payload makes the tumor more
physically accessible to immune cells. Non-limiting examples of extracellular matrix modulator
include MATN1, MATN2, MATN3, MATN4, CTSB, CTSB, CEMIP, HPSE, HYAL1,
20 HYAL2, HYAL3, HYAL4, HYAL5, PLOD1, PLOD2, PLOD3, ELANE, PLG, SULF1, SULF2,
and TMPRSS6.

In certain embodiments, the Payload is a homing signal. This homing signal can guide
the Secondary Construct Cell towards the tumor core through chemotaxis mechanisms. In
certain embodiments, the homing signal is a cytokine or a chemokine. In certain embodiments,
25 the homing signal is a cytokine receptor or a chemokine receptor. Non-limiting examples of
homing signal include IFNGR2, IL6R+IL6ST, IL12RB1, IL12RB2, CXCR3, CXCR6, CCR2,
CCR5, CCR7, CCR1, CCR3, CCR4, CCR6, CCR8, CX3CR1, CXCR1, CXCR2, CXCR4,
CXCR6, XCR1, and CCR10.

In certain embodiments, the Payload is a modulator of reactive oxygen species (ROS).
30 In certain embodiments, the modulators of reactive oxygen species are selected as a Payload
because cancer cells typically have elevated ROS burden and neutrophils do create oxidative
bursts as a way of destroying cancer cells. Non-limiting examples of modulators of ROS
include nicotinamide adenine dinucleotide phosphate oxidase, nitric oxide synthase, and
xanthine oxidase.

In certain embodiments, the competitive ligand inhibitor selected as a Payload binds to receptors on tumor cells and prevents activation.

In certain embodiments, the Payload of the Secondary Promoter Constructs is a transcription factor, florescent proteins, cytokine/chemokine and their receptor, cytokine fusion, cytokine receptor fusion, switch receptor (an extracellular domain which binds an inhibitory signal and an intracellular stimulatory domain), costimulatory receptor and their ligand, dominant-negative and null mutant immune checkpoint receptor, immune checkpoint antagonist, antigen-binding receptor or fragment and their coreceptor, coreceptor fusion, metabolic regulator, antibody, transpeptidase, protease, sheddase, growth factor and growth regulator, scaffold protein, adaptor protein, kinase, phosphatase, ITAM containing receptor, methyltransferase loss of function or null mutant, methylcytosine loss of function or null mutant, toll-like receptor, glycosylase, glycosidase, glycosyltransferase, DNA repair protein, inducible caspase, Truncated LNGFR or EGFR, cytosolic DNA sensor, GPCR and their ligand, leucine zipper fusion, Cas protein, guide RNA, short hairpin RNA, innate immune agonist, or drug resistance marker.

3.1.2. Inhibitory RNA molecules

In certain embodiments, the Secondary Promoter Constructs disclosed herein include an inhibitory RNA molecule. In certain embodiments, the Payload is an inhibitory RNA molecule. As used here, the terms “inhibitory RNA” or “inhibitory RNA molecule” refer to an RNA molecule capable of interfering with, or suppressing, the expression of a gene (e.g., RNA interference or RNAi). In certain embodiments, the inhibitory RNA molecule is capable of interfering with, or suppressing, the expression of a single gene. In certain embodiments, the inhibitory RNA molecules are capable of interfering with, or suppressing, the expression of at least two or more, at least three or more, at least four or more, or at least five or more genes. In certain embodiments, the inhibitory RNA molecule comprises a small interference RNA (siRNA), a small hairpin RNA (shRNA), a microRNA (miRNA), or a combination thereof.

In certain embodiments, the inhibitory RNA molecules are positioned in the Secondary Promoter Construct in a series or multiplex arrangement such that multiple inhibitory RNA molecules are simultaneously expressed from a single polycistronic transcript. In certain embodiments, the inhibitory RNA molecules are positioned in the Payload in a series or multiplex arrangement such that multiple inhibitory RNA molecules are simultaneously expressed from a single polycistronic transcript. In certain embodiments, the inhibitory RNA molecules can be linked to each other by non-functional linker sequence(s).

In certain embodiments, the inhibitory RNA molecule comprises a small hairpin RNA (shRNA). In certain embodiments, the inhibitory RNA molecule comprises a microRNA (miRNA). A miRNA is an RNA molecule (artificial or naturally occurring) of about 22 nucleotides in length that can be used to silence target gene expression via RNA interference (RNAi).

In certain embodiments, the inhibitory RNA molecule is a naturally occurring miRNA. In certain embodiments, the inhibitory RNA molecule is an artificial miRNA. Artificial miRNAs can be produced by placing an RNA sequence directed against a target gene in a miRNA framework (e.g., microRNA sequences for processing and loop). In certain embodiments, the inhibitory RNA molecule comprises, from 5' to 3', a first miRNA flanking sequence, a first stem, a loop, a second stem that is partially or fully complementary to the first stem, and a second miRNA flanking sequence. In certain embodiments, the first and second miRNA flanking sequences are derived from a naturally occurring miRNA. For example, without any limitation, the first and second miRNA flanking sequences are derived from miR-155, miR-30, miR-17/92, miR-122, and miR-21. In certain embodiments, the first and second miRNA flanking sequences are derived from a miR-155. In certain embodiments, the first and second miRNA flanking sequences are derived from a miR-30. In certain embodiments, the first and second miRNA flanking sequences are derived from a miR-30a.

In certain embodiments, the inhibitory RNA molecule comprises a SIBR scaffold. In certain embodiments, the inhibitory RNA molecules comprise an eSIBR scaffold. Additional details on the eSIBR scaffold can be found in Fowler et al., *Nucleic acids research* 44.5 (2016): e48-e48, the content of which is incorporated by reference in its entirety.

In certain embodiments, the inhibitory RNA molecule comprises a miR-E scaffold. Additional details on the miR-E scaffold can be found in Fellmann et al., *Cell reports* 5.6 (2013): 1704-1713, the content of which is incorporated by reference in its entirety.

In certain embodiments, the inhibitory RNA molecules comprise a microRNA cluster. A miRNA cluster is a set of two or more miRNAs, which are transcribed from physically adjacent miRNA genes. miRNA genes can be found either in protein-coding or non-coding regions of transcription units (TUs). Typically, miRNAs in a cluster are transcribed in the same orientation and are not separated by a transcription unit or a miRNA in the opposite orientation. In certain embodiments, the miRNA cluster includes two miRNAs. In certain embodiments, the miRNA cluster includes three miRNAs. In certain embodiments, the miRNA cluster includes four miRNAs. In certain embodiments, the miRNA cluster includes five miRNAs. In certain

embodiments, the miRNA cluster includes six miRNAs. In certain embodiments, the miRNA cluster derives from naturally occurring miRNA clusters. Non-limiting examples of naturally occurring miRNA clusters include miR-17/92, miR-23b/27b/24, and miR-106.

In certain embodiments, the inhibitory RNA molecules target a gene selected from the group consisting of A2AR, CBLB, TGFBR2, or a combination thereof.

In certain embodiments, the inhibitory RNA molecule targets a CBLB transcript. In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 5. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 5. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 5.

In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 6. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 6. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 6.

In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 7. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 7. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 7. SEQ ID NOs: 5-7 are provided below.

TCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAAAGATGTCAAGATTGAGCC
TTTAGTGAAGCCACAGATGTAAAGGCTCAATCTTGACATCTTGTGCCTACTGCCTCGGACTTCAAGGGGCTACTTTA
GGA [SEQ ID NO: 5]
GTCGACTGGAGGCTTGCTTTGGGCTGTATGCTGAAGGCTCAATCTTGACATCTTGTGTTTGGCCTCTGACTGACAAGA
TTCAGATTGAGCCTTGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCCACCGTGGGAGGATGACAAC
TCGAG [SEQ ID NO: 6]
GATGTCAAGATTGAGCCTTGCCCTGACCCAGCAAGGCTCAATCTTGACATC [SEQ ID NO: 7]

In certain embodiments, the inhibitory RNA molecule targets a CBLB transcript and an A2AR transcript. In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%,

about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 8. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 8. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 8.

In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 9. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 9. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 9. SEQ ID NO: 8 and SEQ ID NO: 9 are provided below.

TGGAGGCTTGCTTTGGGCTGTATGCTGAAGGCTCAATCTTGACATCTTGTTTTGGCCTCTGACTGACAAGATTCAGATTGAGCCTTGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCCACCGTGGGAGGATGACAAGTCGACTCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCAGACCTTCCGCAAGATCATTTAGTGAAGCCACAGATGTAAATGATCTTGCGGAAGGTCTGGTGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGA [SEQ ID NO: 8]

TGCACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGCCAGACCTTCCGCAAGATCATTTAGTGAAGCCACAGATGTAAATGATCTTGCGGAAGGTCTGGTGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGTCGACTGGAGGCTTGCTTTGGGCTGTATGCTGAAGGCTCAATCTTGACATCTTGTTTTGGCCTCTGACTGACAAGATTCAGATTGAGCCTTGACACAAGGCCCTTTATCAGCACTCACATGGAACAAATGGCCACCGTGGGAGGATGACAA [SEQ ID NO: 9]

In certain embodiments, the inhibitory RNA molecule targets a CBLB transcript, an A2AR transcript, and a TGFBR2. In certain embodiments, the inhibitory RNA molecule comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 10. In certain embodiments, the inhibitory RNA molecule comprises the nucleotide sequence set forth in SEQ ID NO: 10. In certain embodiments, the inhibitory RNA molecule consists of the nucleotide sequence set forth in SEQ ID NO: 10. SEQ ID NO: 10 is provided below.

CTGACTACATCACAGCAGCATACGTGGAGATGAGGCGAGAGGCTTGGGCTAGTAAGGATGCCACCTATACTTCTGCCCGGACCCTGCTGGCTATCCTGCGCCTTTCCACTGCTCTGGTAAGTGCCCAAATTGCTGGAGGGCCATCTGTTTTGACCCTTAAAGGGGTAGCTCCTTACCGTGCTCTCATTTGCCGCCCTCCCACTCCCGCTCGAGCCCTGCCGGGGCGGATGTCAAGATTGAGCCTTGCTTTGTGTAGGCAAGGCTCAATCTTGACATCGCTCCAGCAGGGCACGCAATGCTTCGTGGAGGGAAAGGCCTTTTCCCCACTTCTTAACCTTCACTGAGAGGGTGGTTGGGGTCTGTTTCACTCCATGTGTCTTAGATCCTGTGTACAGACCTTCTTTCTGTCTCCCGTCTTGGAATTGAGTCTGGGGGCTGGAATGATCTTGCGGAAGGTCTGTGTGATTACCCAACCAGACCTCCGCAAGTATCATATATAGCCCCGGGTTCGGTTCTCTCTGCCAATTGTCTTCTTGGCTGAGCTCCCCAAGCTCCATCTGTCTGCTGGGAGCCAGTGGCGTTCAAAGGGTCTGGTCTCCCTCACAGGA

CAGCTGAACTCTAGAAGTCCTGGGGGCTCGATGAAGAAAGTCTCACCAGGCTGTGATTACCCAACGCCTGGTAGACT
 TTACTTCAGCGCGAGCCCCCGGGACACACCGCGGATGCTGGGGGGAGGGGGATTCCACTCCTGTTTTGTGAGTAGG
 CGACCCATGGGCTGCCCCAGCCTTAAAGCCAGAACAAGGGTGTCCCCTGACCTCGTTCCACTGCCCTCCTCCCGTTCC
 CATCTTTCCCCCTACCTTCCCCTTAGGCACGTCTGAGAATGGTGGATGTGGTGGAGAAAGAAGATGTGAATGAAGC
 5 CATCAGGCTAATGGAGATGTCAAAGGACTCTCTTCTAGGAGACAAGGGGCAGACA [SEQ ID NO: 10]

In certain embodiments, the inhibitory RNA molecule is flanked by a splice acceptor site. A “splice acceptor site” is a polynucleotide site at the 3’ of an intron and regulates the splicing of RNA. In certain embodiments, the inhibitory RNA molecule is flanked by a splice donor site. A “splice donor site” is a polynucleotide site at the 5’ of an intron and regulates the
 10 splicing of RNA. In certain embodiments, the inhibitory RNA molecule is flanked by a splice acceptor site and a splice donor site. In certain embodiments, the inhibitory RNA molecule is flanked at its 5’ by a splice acceptor site. In certain embodiments, the inhibitory RNA molecule is flanked at its 3’ by a splice acceptor site. In certain embodiments, the inhibitory RNA molecule is flanked at its 5’ by a splice donor site. In certain embodiments, the inhibitory RNA
 15 molecule is flanked at its 3’ by a splice donor site.

In certain embodiments, the splice acceptor site comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 11. In certain
 20 embodiments, the splice acceptor site comprises the nucleotide sequence set forth in SEQ ID NO: 11. In certain embodiments, the splice acceptor site consists of the nucleotide sequence set forth in SEQ ID NO: 11. SEQ ID NO: 11 is provided below.

TTCCCCTTAGGCACGTCTGAGAATGGTGGATGTGGTGGAGAAAGAAGATGTGAATGAAGCCATCAGGCTAATGGAGA
 TGTCAAAGGACTCTCTTCTAGGAGACAAGGGGCAGACA [SEQ ID NO: 11]

In certain embodiments, the splice acceptor site comprises a nucleotide sequence that is
 25 at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 12. In certain embodiments, the splice acceptor site comprises the nucleotide sequence set forth in SEQ ID NO: 12. In certain embodiments, the splice acceptor site consists of the nucleotide sequence set forth in SEQ ID NO: 12. SEQ ID NO: 12 is provided below.

30 TGTCCACAGATATCCAGAACCCCGACCCCGCGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTG
 CCTGTT [SEQ ID NO: 12]

In certain embodiments, the splice donor site comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 13. In certain

embodiments, the splice donor site comprises the nucleotide sequence set forth in SEQ ID NO: 13. In certain embodiments, the splice donor site consists of the nucleotide sequence set forth in SEQ ID NO: 13. SEQ ID NO: 13 is provided below.

CTGACTACATCACAGCAGCATACGTGGAGATGAGGCGAGAGGCTTGGGCTAGTAAGGATGCCACCTATACTTCTGCC
5 CGGACCCTGCTGGCTATCCTGCGCCTTTCCACTGCTCTGGTAAGTGCCC [SEQ ID NO: 13]

3.1.3. Poly-adenylation Sequence (Poly-A)

In certain embodiments, the Secondary Promoter Constructs disclosed herein include a poly-adenylation signal sequence (poly-A). The poly-A signal sequence is a long chain of adenine nucleotides that is added to a messenger RNA (mRNA) molecule during RNA
10 processing to increase the stability of the molecule. Typically, a poly-A signal sequence is between about 100 and about 250 residues long. The poly-A signal sequence makes the RNA molecule more stable and prevents its degradation. Additionally, the poly-A signal sequence allows the mature messenger RNA molecule to be exported from the nucleus and translated into a protein by ribosomes in the cytoplasm.

15 In certain embodiments, the poly-adenylation signal sequence is a type of DNA sequence that mediates the addition of a poly-A signal sequence to the 3' end of the mRNA transcript. In certain embodiments, the poly-A signal sequence acts as a transcription stop signal. In certain embodiments, the addition of a poly-A signal sequence is required for the production of mature mRNA. In certain embodiments, the poly-A signal sequence is important for nuclear export,
20 translation, and mRNA stability.

In certain embodiments, a poly-A signal sequence is only included after the TCR gene to decouple transcription of the TCR genes from the transcription of a Payload. In essence, adding the poly-A signal sequence at this position leads to the production of two separate transcripts. Accordingly, when combined with a secondary promoter, the poly-A signal sequence allows the
25 decoupling of the expression of the Payload from the expression of the TCR. In certain embodiments and as shown in Format 2, a second poly-A signal was added even though only the first poly-A signal sequence is required for the production of two distinct transcripts.

In certain embodiments, the Secondary Promoter Construct includes one poly-A signal sequence. In certain non-limiting embodiments, the poly-A signal sequence can be a simian
30 virus 40 (SV40) poly-A signal sequence, an SV40 poly-A signal sequence, a human growth hormone (hGH) poly-A signal sequence, a bovine growth hormone (BGH) poly-A signal sequence, or a rabbit beta-globin (rbGlob) poly-A signal sequence. In certain embodiments, the Secondary Promoter Construct includes two poly-A signal sequences. In certain embodiments,

the two poly-A signal sequences are the same. In certain embodiments, the two poly-A signal sequences are different.

In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs is the BGH poly-A signal sequence. In certain embodiments, the BGH poly-A signal sequence comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 14. In certain embodiments, the BGH poly-A signal sequence comprises the nucleotide sequence set forth in SEQ ID NO: 14. In certain embodiments, the BGH poly-A signal sequence consists of the nucleotide sequence set forth in SEQ ID NO: 14. SEQ ID NO: 14 is provided below.

TGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTTGACCCCTGGAAGGTGCCACTCCCACTGTCTCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGC [SEQ ID NO: 14]

In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs is the SV40 poly-A signal sequence. In certain embodiments, the SV40 poly-A signal sequence comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 15. In certain embodiments, the SV40 poly-A signal sequence comprises the nucleotide sequence set forth in SEQ ID NO: 15. In certain embodiments, the SV40 poly-A signal sequence consists of the nucleotide sequence set forth in SEQ ID NO: 15. SEQ ID NO: 15 is provided below.

GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGATGTGGGAGGTTTTTTAAAGC [SEQ ID NO: 15]

In certain embodiments, the SV40 poly-A signal sequence further comprises an SV40 upstream element. In certain embodiments, the SV40 upstream element comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 3. In certain embodiments, the SV40 upstream element comprises the nucleotide sequence set forth in SEQ ID NO: 16. In certain embodiments, the SV40 upstream element consists of the nucleotide sequence set forth in SEQ ID NO: 16. SEQ ID NO: 16 is provided below.

TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCAT [SEQ ID NO: 16]

In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs is a 6T sequence. In certain embodiments, the 6T sequence comprises a nucleotide

sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 17. In certain embodiments, the 6T poly-A signal sequence comprises the nucleotide sequence set forth in SEQ ID NO: 17. In certain embodiments, the 6T poly-A signal sequence consists of the nucleotide sequence set forth in SEQ ID NO: 17. SEQ ID NO: 17 is provided below.

TTTTTT [SEQ ID NO: 17]

In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2A** and **3A** is the BGH poly-A signal sequence. In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2A** and **3A** is the SV40 poly-A signal sequence.

In certain embodiments, the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2B** and **3B** is the BGH poly-A signal sequence and the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2B** and **3B** is the SV40 poly-A signal sequence. In certain embodiments, the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2B** and **3B** is the SV40 poly-A signal sequence and the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 2B** and **3B** is the BGH poly-A signal sequence.

In certain embodiments, the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 4A-4C** and **5A-5C** is the BGH poly-A signal sequence, and the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 4A-4C** and **5A-5C** is the SV40 poly-A signal sequence. In certain embodiments, the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 4A-4C** and **5A-5C** is the SV40 poly-A signal sequence, and the first poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 4A-4C** and **5A-5C** is the BGH poly-A signal sequence.

In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 6** and **7** is the BGH poly-A signal sequence. In certain embodiments, the poly-A signal sequence used in the Secondary Promoter Constructs shown in **Figures 6** and **7** is the SV40 poly-A signal sequence.

3.1.4. Insulators

In certain embodiments, the Secondary Promoter Constructs disclosed herein include a transcriptional insulator or insulator. Insulators are a type of DNA sequence that helps divide the genome into distinct “genetic neighborhoods.” Insulators can help prevent regulatory

elements designed to affect the expression of one gene from also affecting the expression of another nearby gene.

In certain embodiments, insulators can reduce promoter interference. Promoter interference is a molecular event characterized by the perturbation of one transcription unit by another. For example, without any limitation, promoter interference can occur between the transcription units of the genes of the Secondary Promoter Constructs (e.g., the sequence encoding an exogenous TCR and the Payload). Additional information on promoter interference can be found in Eszterhas et al., *Molecular and Cellular Biology* 22.2 (2002): 469-479.

In certain embodiments, promoter interference occurs when the expression of the Payload reduces the TCR expression. Accordingly, in certain embodiments, an insulator can be inserted into the Secondary Promoter Construct to prevent reduction in TCR expression.

In certain embodiments, insulators reduce promoter leakiness. Accordingly, in certain embodiments, insulators can be used to reduce transgene silencing.

In certain embodiments, an insulator is added to the construct between the TCR gene and the Payload. In certain embodiments, an insulator is added to the construct between the TCR genes and the promoter region.

In certain embodiments, the insulators used in the Secondary Promoter Constructs are HS4 or IS2. Additional examples of insulators encompassed by the present disclosure include, without any limitation, CTCF insulator, Cohesin insulator, TFIIC insulator, Condensin insulator, p68 insulator, PARP1 insulator, Bptf insulator, TGF- β insulator, and Kaiso insulator. Further information and examples of insulators can be found in Liu et al., *Nature biotechnology* 33.2 (2015): 198-203.

In certain embodiments, the insulator is an HS4 insulator. In certain embodiments, the HS4 insulator comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 18. In certain embodiments, the HS4 insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18. In certain embodiments, the HS4 insulator consists of the nucleotide sequence set forth in SEQ ID NO: 18. SEQ ID NO: 18 is provided below:

GAGCTCACGGGGACAGCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGA
GCCGCCCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGGGCAC
GGGGAAGGTGGCACGGGATCGCTTTCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGACACCTGGGGGGATAC
GGGGAAAAGCTT [SEQ ID NO: 18]

In certain embodiments, the insulator is an IS2 insulator. In certain embodiments, the IS2 insulator comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 19. In certain embodiments, the IS2 insulator comprises the nucleotide sequence set forth in SEQ ID NO: 19. In certain embodiments, the IS2 insulator consists of the nucleotide sequence set forth in SEQ ID NO: 19. SEQ ID NO: 19 is provided below:

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CGGGGACAGCCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCC
GGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGGCACGGGGAAAG
GTGGCACGGGATCGCTTTCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGACACCTGGGGGGATACGGGGAAA
ATGTGTCTGAGCCTGCATGTTTGATGGTGTCTGGATGCAAGCAGAAAGGGTGGAAGAGCTTGCCTGGAGAGATACAG
CTGGGTCACTAGGACTGGGACAGGCAGCTGGAGAATTGCCATGTAGATGTTTCATACAATCGTCAAATCATGAAGGCT
GGAAAAGCCCTCCAAGATCCCCAAGACCAACCCCAACCCACCCACCGTGCCCACTGGCCATGTCCCTCAGTGCCACA
TCCCCACAGTTCTTCATCACCTCCAGGGACGGTGACCCCCCACCTCCGTGGGCAGCTGTGCCACTGCAGCACCGCT
CTTTGGAGAAGGTAAATCTTGCTAAATCCAGCCCGACCTCCCCCTGGCACAACGTAAGGCCATTATCTCTCATCCAA
CTCCAGGACGGAGTCAGTGAGAATATTTAAATAAACTTATAAATTGTGAGAGAAATTAATGAATGTCTAAGTTAATG
CAGAAACGGAGGCTCCTCATTTATTTTTGAAGTTAAAGACTTAATATTGTGAAGGTATACCTTTCTTTAATAATAAGC
CTGCGCCCAATATGTTTACCCCCAAAAAGCTGTTTGTTAACTTGTC AACCTCATTTAAAATATATAAGAAACAGCCC
AAAGACAATAACAAAAGAATAATAAAAAAGAATGAAATATGTAATTCTTTCAGAGTAAAAATCACACCCATGACCTG
GCCACTGAGGGCTTGATCAATTCACCTTGAATTTGGCATTAAATACCATTAAAGGTATATTAAGTATTTTAAATAA
GATATATTC [SEQ ID NO: 19]

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In certain embodiments, the Secondary Promoter Construct includes one insulator. In certain embodiments, the Secondary Promoter Construct includes two insulators. In certain embodiments, the two insulators are the same. In certain embodiments, the two insulators are different.

In certain embodiments, the insulator used in Format 1 constructs is HS4 (**Figures 2A, 2B, 3A, and 3B**). In certain embodiments, the insulator used in Format 1 is IS2 (**Figures 2A, 2B, 3A, and 3B**).

In certain embodiments, the insulator used in Format 2 constructs that only use 1 insulator is HS4 (**Figure 4A, 4C, 5A, and 5C**). In certain embodiments, the insulator used in Format 2 constructs that only use 1 insulator is IS2 (**Figure 4A, 4C, 5A, and 5C**). In certain embodiments where Format 2 constructs use two insulators (**Figures 4B and 5B**), either both insulators are HS4, both insulators are IS2, the first insulator is HS4 and the second insulator is IS2, or the first insulator is IS2 and the second insulator is HS4.

In certain embodiments, while not shown in the figures, insulators can be inserted in any construct disclosed herein, and where constructs contain an insulator a second insulator can be added.

3.1.5. Additional Elements to Increase Translation of Transcripts

In mammalian cells, mRNA lacking in intronic regions do not get properly processed. This results in the production of abysmally low levels of proteins. Introns are ubiquitous in most eukaryotes, yet intron-free genes are commonly used for gene delivery as a method of significantly decreasing the Payload size and nucleic acid toxicity.

In certain embodiments, sequence elements that promote translation of transcripts are introduced into the Secondary Promoter Constructs. In certain embodiments, sequence elements that promote normal processing of intron-free transcripts are introduced into the Secondary Promoter Constructs.

In certain embodiments, the Secondary Promoter Construct includes an element to increase the translation of transcripts. In certain embodiments, the element to increase translation of transcripts is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). The WPRE can increase gene expression from a variety of vectors and is most effective when placed downstream of the gene, proximal to the polyadenylation signal.

In certain embodiments, the sequence element that promotes normal processing of intron-free transcripts and that is introduced into the Secondary Promoter Construct is a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE). In certain embodiments, WPREs are often used to promote normal processing of intron-free transcripts, resulting in normal levels of protein expression.

In certain embodiments, the WPRE is a WPRE3 element. In certain embodiments, the WPRE3 element comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments, the WPRE3 element comprises the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments, the WPRE3 element consists of the nucleotide sequence set forth in SEQ ID NO: 20. SEQ ID NO: 20 is provided below:

GATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACATATGTTGCTCCTTTTACGCTATG
TGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAAT
CCTGGTTAGTTCTTGCCACGGCGGAACATCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGC
ACTGACAATTCCGTGGTG [SEQ ID NO: 20]

In certain embodiments, the element to increase translation of transcripts is a transcriptional pause element. This “pause element” can regulate gene expression at the level of RNA synthesis in both prokaryotes and eukaryotes, serving to coordinate the appearance of RNA with its utilization in cellular functions, and to modulate the interaction of regulatory proteins with RNA polymerase (RNAP).

In certain embodiments, the pause element is a MAZ4 pause element. In certain embodiments, the MAZ4 pause element comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 21. In certain embodiments, the MAZ4 pause element comprises the nucleotide sequence set forth in SEQ ID NO: 21. In certain embodiments, the MAZ4 pause element consists of the nucleotide sequence set forth in SEQ ID NO: 21. SEQ ID NO: 21 is provided below:

CCTGGCCTTGGGGGAGGGGGAGGCCAGAATGAGAGCTCCTGGCCTTGGGGGAGGGGGAGGCCAGAATGACTCGACCT
GGCCTTGGGGGAGGGGGAGGCCAGAATGAGAGCTCCTGGCCTTGGGGGAGGGGGAGGCCAGAATGA [SEQ ID
NO: 21]

In certain embodiments, the Secondary Promoter Constructs described herein are designed to integrate into the genome in a manner such that the transcripts of the TCR and the Payload include intronic regions (*see, e.g.*, Format 1; **Figures 2A, 2B, 3A, and 3C**). In certain embodiments, the Secondary Promoter Constructs described herein are designed such that the transcript of the Payload lacks intronic regions (*see, e.g.*, Formats 2 and 3; **Figures 4A-4C, 5A-5C, 6, and 7**). In certain embodiments, in order to address the lack of intronic regions of the Payload transcript (*see, e.g.*, Formats 2 and 3; **Figures 4A-4C, 5A-5C, 6, and 7**), a sequence element that promotes translation of transcripts is introduced prior to the poly-A signal sequence in order to mediate proper processing prior to translation of the promoter transcript. In certain embodiments, the sequence element that promotes translation of transcripts is WPRE (*see, e.g.*, Formats 2 and 3; **Figures 4A, 5A, 6, and 7**).

In certain embodiments, the WPRE element shown in **Figures 4A, 5A, 6, and 7** as diagrams of Formats 2 and 3 can be substituted with wPRE3, HPRE, or wPRE-O.

In certain embodiments, the WPRE element shown in **Figures 4A, 5A, 6, and 7** as diagrams of Formats 2 and 3 is WPRE3 (i.e., WPRE element shown in the figures is replaced with WPRE3). In certain embodiments, the WPRE element shown in **Figures 4A, 5A, 6, and 7** as diagrams of Formats 2 and 3 is WPRE3 (i.e., WPRE element shown in the figures is replaced with WPRE3 as a preferred sequence element to promote translation of the transcript).

3.1.6. *Secondary Promoters*

In certain embodiments, the Secondary Promoter Construct includes a promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct can be a eukaryotic promoter, a mammalian promoter, a viral promoter, a synthetic promoter, a minimal promoter, a hybrid promoter, a tissue-specific promoter, an inducible promoter, or a constitutive promoter.

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct is a constitutive promoter (*i.e.*, a promoter that displays stable gene expression patterns over time). In certain embodiments, a constitutive promoter is selected in cases where the Payload of interest has a low toxicity profile and in cases where there is no clear benefit in linking protein expression to a specific time or place. In certain non-limiting examples, the constitutive promoter is the EF-1 α promoter, the hACTB promoter, the hPGK promoter, the MND promoter, or the U6 promoter.

In certain embodiments, the constitutive promoter is an EF-1 α promoter. In certain embodiments, the EF-1 α promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 22. In certain embodiments, the EF-1 α promoter comprises the nucleotide sequence set forth in SEQ ID NO: 22. In certain embodiments, the EF-1 α promoter consists of the nucleotide sequence set forth in SEQ ID NO:

22. SEQ ID NO: 22 is provided below:

```
CGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGC
AATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCC
CGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCCGCCAGAA
CACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCCTGCCTTGAATTACT
TCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGC
TTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCCGCTGCGAATCTGGTGGC
ACCTTCGCGCCTGTCTCGCTGCTTTGATAAGTCTCTAGCCATTTAAAATTTTGTGACCTGCTGCGACGCTTTTTT
TTCTGGCAAGATAGTCTTGTAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTTGGGGCCGCGGGCGGCGA
CGGGGCCCCGTGCGTCCAGCGCACATGTTTCGGCGAGGCGGGGCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGT
AGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTG
GCCCCGTGCGCACCAAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAATGGAGGAC
GCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCAT
GTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTTAGGT
TGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTT
GATGTAATTCTCCTTGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCACTCTCAAGCCTCAGACAGTGGTTCAAA
GTTTTTTTCTTCCATTTTCAGGTGTCGTGA [SEQ ID NO: 22]
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In certain embodiments, the EF-1 α promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 23. In certain embodiments, the EF-1 α promoter comprises the nucleotide sequence set forth in SEQ ID NO:

23. In certain embodiments, the EF-1 α promoter consists of the nucleotide sequence set forth in SEQ ID NO: 23. SEQ ID NO: 23 is provided below:

GAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGAGAAGTTGGGGGGAGGGGTCCGGCAAT
TGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTCCCGA
GGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACAC
AG [SEQ ID NO: 23]

In certain embodiments, the constitutive promoter is a hACTB promoter. In certain embodiments, the hACTB promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 24. In certain embodiments, the hACTB promoter comprises the nucleotide sequence set forth in SEQ ID NO: 24. In certain embodiments, the hACTB promoter consists of the nucleotide sequence set forth in SEQ ID NO: 24. SEQ ID NO: 24 is provided below:

ACTGCCTGGCCACTCCATGCCCTCCAAGAGCTCCTTCTGCAGGAGCGTACAGAACCCAGGGCCCTGGCACCCGTGCA
GACCCTGGCCACCCACCTGGGCGCTCAGTGCCCAAGAGATGTCCACACCTAGGATGTCCCGCGGTGGGTGGGGGG
CCCGAGAGACGGGCAGGCCGGGGGCAGGCCTGGCCATGCGGGGCCGAACCGGGCACTGCCAGCGTGGGGCGCGGGG
GCCACGGCGCGCGCCCCCAGCCCCGGGGCCAGCACCCCAAGCGGGCCAACGCCAAAACCTCTCCCTCCTCCTCTTCC
TCAATCTCGCTCTCGCTCTTTTTTTTTTTCGCAAAAAGGAGGGGAGAGGGGTAAAAAATGCTGCACTGTGCGGCGA
AGCCGGTGAGTGAGCGGCGGGGCCAATCAGCGTGCGCCGTTCCGAAAGTTGCCTTTTATGGCTCGAGCGGCCGCG
GCGGCGCCCTATAAAACCCAGCGGCGCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTC
GCCTTTGCCGA [SEQ ID NO: 24]

In certain embodiments, the constitutive promoter is an hPGK promoter. In certain embodiments, the hPGK promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 25. In certain embodiments, the hPGK promoter comprises the nucleotide sequence set forth in SEQ ID NO: 25. In certain embodiments, the hPGK promoter consists of the nucleotide sequence set forth in SEQ ID NO: 25. SEQ ID NO: 25 is provided below:

TCGAATTCACGGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCAGGGACGCGGCTGCTCTGGGCG
TGGTTCCGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCGTTTCGAGCGTCACCCGGATCT
TCGCCGCTACCCTTGTGGGCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGAAGGTTCTTGCAGTTTCGCG
GCGTGCCGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCTTCGAGACGGACAGCGCCAGGGAGCAATGGC

AGCGCGCCGACCGCGATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGCCGGGAAGGGG
CGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCTGCCCCGCGCGGTGTTCCGCATTCTGCAAGCCT
CCGGAGCGCACGTCCGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAG [SEQ ID NO:
25]

5 In certain embodiments, the constitutive promoter is an MND promoter. In certain
embodiments, the MND promoter comprises a nucleotide sequence that is at least about 80%,
about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical
to the nucleotide sequence set forth in SEQ ID NO: 26. In certain embodiments, the MND
promoter comprises the nucleotide sequence set forth in SEQ ID NO: 26. In certain
10 embodiments, the MND promoter consists of the nucleotide sequence set forth in SEQ ID NO:
26. SEQ ID NO: 26 is provided below:

GAACAGAGAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAA
CAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACA
GATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCT
15 GAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAG
CTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACTTCCATAG
AAG [SEQ ID NO: 26]

In certain embodiments, the constitutive promoter is a U6 promoter. In certain
embodiments, the U6 promoter comprises a nucleotide sequence that is at least about 80%,
20 about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical
to the nucleotide sequence set forth in SEQ ID NO: 27. In certain embodiments, the U6
promoter comprises the nucleotide sequence set forth in SEQ ID NO: 27. In certain
embodiments, the U6 promoter consists of the nucleotide sequence set forth in SEQ ID NO: 27.
SEQ ID NO: 27 is provided below:

25 GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGATAATTGGAATTAATTT
GACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAA
AATTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTGATTTCTTGGCTTTATATATCTTG
TGGAAGGACGAAACACC [SEQ ID NO: 27]

In certain embodiments, the promoter for the expression of the Payload of the Secondary
30 Promoter Construct is an inducible promoter (*i.e.*, a type of conditional promoter where the
promoter is in its “on” state only under certain conditions). In certain embodiments, the
inducible promoter is generally in its “off” state, unless and until it receives a signal that induces
switching into the “on” state. In certain embodiments, the inducible promoter allows for a basal
level of gene expression even if in the “off” state (*i.e.*, a phenomenon known as promoter
35 leakiness).

In certain embodiments, the inducible promoter is a TCR activation triggered inducible promoter. In certain embodiments, the TCR activation triggered inducible promoter works as follows: upon TCR engagement a transcription factor is activated (i.e. AP-1, NFAT, NF- κ B); these activated transcription factors will bind the inducible promoter and initiate a switch from the “off” state to the “on” state. In certain embodiments, the TCR activation triggered inducible promoter may contain either additional or a reduced number of response elements when compared to the wild-type promoter. In certain embodiments, the TCR activation triggered inducible promoter is used to localize a Payload of interest to the tumor site. In certain embodiments, the benefit of localizing a Payload of interest to the tumor site is to allow for high levels of the Payload to be predominantly expressed in response to TCR signaling at the tumor site. In certain embodiments, this is the site maximal TCR engagement. In certain embodiments, localizing a Payload of interest to the tumor site allows for the use of Payloads with high toxicity profiles because the use of an inducible promoter can limit the systemic availability of toxic Payloads. In certain embodiments, the TCR activation triggered inducible promoter comprises a sequence of at least one regulatory element of transcription factors induced by TCR activation. In certain embodiments, the TCR activation triggered inducible promoter is the AP-1 responsive, NFAT responsive, NF- κ B responsive promoter, or an NR4A-responsive promoter.

In certain embodiments, the TCR activation triggered inducible promoter is a promoter that can be activated by pathways associated with the TCR activation or T cell pathways or by induction with an activation agent (for example but not limited to a small molecule or polypeptide).

In certain embodiments, the inducible promoter is an AP-1 promoter. In certain embodiments, the AP-1 promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 28. In certain embodiments, the AP-1 promoter comprises the nucleotide sequence set forth in SEQ ID NO: 28. In certain embodiments, the AP-1 promoter consists of the nucleotide sequence set forth in SEQ ID NO: 28. SEQ ID NO: 28 is provided below:

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TGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAGGAATTCAGCTTGA
ATAAAATGAATATTAGAAGCTGTTAGAATAAGAGAAAATGACAGAGGAAAACCTGAAAGGGAGAACTGAAAGTGGGAA
ATTCCTCTGAGGCAGAAAGGACCATCCCTTATAAATAGCACAGGCCATGAAGGAAGATCATTCTCACTGCAGCCTTT
GACAGCCTTTGCCTCATCTTG      [SEQ ID NO: 28]

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In certain embodiments, the inducible promoter is an NFAT promoter. In certain embodiments, the NFAT promoter comprises a nucleotide sequence that is at least about 80%,

about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the NFAT promoter comprises the nucleotide sequence set forth in SEQ ID NO: 29. In certain embodiments, the NFAT promoter consists of the nucleotide sequence set forth in SEQ ID NO:

5 29. SEQ ID NO: 29 is provided below:

AGGAGGAAAACTGTTTCATACAGAAGGCGTCAATTAGGAGGAAAACTGTTTCATACAGAAGGCGTCAATTAGGAG
GAAAACTGTTTCATACAGAAGGCGTCAATTGGTCCCATCGAATTAGGAGGAAAACTGTTTCATACAGAAGGCGTC
AATTAGGAGGAAAACTGTTTCATACAGAAGGCGTCAATTAGGAGGAAAACTGTTTCATACAGAAGGCGTCAATTG
GTCCCGGGACATTTTGACACCCCCATAATATTTTCCAGAATTAACAGTATAAATTGCATCTCTTGTTCAAGAGTTC
10 CCTATCACTCTCTTTAATCACTACTCACAGTAACCTCAACTCCTG [SEQ ID NO: 29]

In certain embodiments, the inducible promoter is an NF- κ B promoter. In certain embodiments, the NF- κ B promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 30. In certain embodiments, the NF- κ B promoter comprises the nucleotide sequence set forth in SEQ ID NO: 30. In certain
15 embodiments, the NF- κ B promoter consists of the nucleotide sequence set forth in SEQ ID NO: 30. SEQ ID NO: 30 is provided below:

GGGACTTTCGCTTGGGGACTTTCGCTGGGGACTTTCGCTGGGGACTTTCGCTGGGGACTTTCGCAATTCAGCT
TGAATAAAATGAATATTAGAAGCTGTTAGAATAAGAGAAAAATGACAGAGGAAAACTGAAAGGGAGAAGTGAAGTGG
20 GAAATTCCTCTGAGGCAGAAAGGACCATCCCTTATAAATAGCACAGGCCATGAAGGAAGATCATTCTCACTGCAGCC
TTTGACAGCCTTTGCCTCATCTTG [SEQ ID NO: 30]

In certain embodiments, the inducible promoter is an NR4A-responsive promoter. In certain embodiments, the NR4A-responsive promoter comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or
25 about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the NR4A-responsive promoter comprises the nucleotide sequence set forth in SEQ ID NO: 31. In certain embodiments, the NR4A-responsive promoter consists of the nucleotide sequence set forth in SEQ ID NO: 31. SEQ ID NO: 31 is provided below:

CTCGAGAAAGGTCACCAATTAAAGGTCACCAATTAAAGGTCACCAATTAAAGGTCACCAATTAAAGGTCACCAATTA
30 AAGGTCACCAATTAAAGGTCACCAATTAAAGGTCACCAATT [SEQ ID NO: 31]

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct is an EF1a core (cEF1a) promoter, an hACTB promoter, an hPGK promoter, an MND promoter, an AP-1 responsive promoter, an NFAT responsive promoter, or an NF- κ B responsive promoter.

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct is a TRAC promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct is a TCR β promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figure 8**) is the TRAC promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figure 8**) is the TCR β promoter.

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the EF1a core promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the hACTB promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the hPGK promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the MND promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the AP-1 responsive promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the NFAT responsive promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 1 (**Figures 2A, 2B, 3A, and 3C**) is the NF- κ B promoter.

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the EF1a core promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the hACTB promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the hPGK promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the MND promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the AP-1 responsive promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the NFAT responsive promoter. In

certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 2 (**Figures 4A-4C and 5A-5C**) is the NF- κ B promoter.

In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the EF1a core promoter. In certain
 5 embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the hACTB promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the hPGK promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the MND promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the AP-1 responsive promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the NFAT responsive promoter. In certain embodiments, the promoter for the expression of the Payload of the Secondary Promoter Construct of Format 3 (**Figures 6 and 7**) is the NF- κ B promoter.

3.1.7. Enhancers

In certain embodiments, the Secondary Promoter Construct includes an enhancer. Enhancers are cis-acting elements of DNA, usually about 10 to about 300 bp in length, that act on a promoter to increase its transcription. Many enhancer sequences are now known from
 20 mammalian genes. For example, without any limitation, enhancer sequences are derived from the globin gene, the elastase gene, the albumin gene, the α -fetoprotein gene, and the insulin gene.

In certain embodiments, the enhancer is a T cell receptor (TCR) enhancer. In certain embodiments, the TCR enhancer can be a TCR α enhancer, a TCR β enhancer, a TCR γ enhancer,
 25 or a TCR δ enhancer. In certain embodiments, the TCR enhancer comprises a TCR α enhancer. In certain embodiments, the TCR α enhancer comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments, the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain
 30 embodiments, the TCR α enhancer consists of the nucleotide sequence set forth in SEQ ID NO: 32. SEQ ID NO: 32 is provided below:

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GGGCTGGGGCGGTCCCCTCCCATTTCCATGACGTCATGGTTACCAAGAGGGGCAAGTAGGGCACCCCTTTGAAGCTCT
CCCCGAGAAGCCACATCCTCTGGAAAGAAGAGTTTATAATACTGAGTTAGAGATAGCATCGCCCCAGGCCACGTGCC
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GAGGGGAGCAGGCTGGGCCGTTACACCACCCCCCAACCGCAGGTGCAGCAAGGCCAACATGCCAGGCTGGGAGGGGC
TGCCGGCCCCCTCGTTGAG [SEQ ID NO: 32]

In certain embodiments, the enhancer derives from a eukaryotic cell virus. Non-limiting examples of enhancers derived from eukaryotic cell virus include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

In certain embodiments, the enhancer is a cytomegalovirus (CMV) enhancer. In certain embodiments, the CMV comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments, the CMV enhancer consists of the nucleotide sequence set forth in SEQ ID NO: 33. SEQ ID NO: 33 is provided below:

CGTTACATAACTTACGTTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTCAATAATGATGT
ATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAACTGCCCACTTG
GCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTAT
GCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATG [SEQ
ID NO: 33]

In certain embodiments, the enhancer can be operably linked to a Kozak sequence. In certain embodiments, the Kozak sequence comprises the nucleotide sequence set forth in SEQ ID NO: 34. In certain embodiments, the Kozak sequence consists of the nucleotide sequence set forth in SEQ ID NO: 34. SEQ ID NO: 34 is provided below.

GCCACC [SEQ ID NO: 34]

3.1.8. Homology Recombination Templates

In certain embodiments, the Secondary Promoter Construct is a homologous recombination (HR) template nucleic acid sequence for insertion into an endogenous locus of a cell.

In certain embodiments, the present disclosure provides genome editing of a cell by introducing and recombining a homologous recombination (HR) template nucleic acid sequence into an endogenous locus of a cell. In certain embodiments, the HR template nucleic acid sequence is linear. In certain embodiments, the HR template nucleic acid sequence is circular. In certain embodiments, the circular HR template can be a plasmid, minicircle, or nanoplasmid. In certain embodiments, the HR template nucleic acid sequence comprises a first homology arm and second homology arm. In certain embodiments, the homology arms can be of about 300

bases to about 2,000 bases. For example, each homology arm can be 1,000 bases. In certain embodiments, the homology arms can be homologous to first and second endogenous sequences of the cell. In certain embodiments, the endogenous locus is a TCR locus. For example, the first and second endogenous sequences are within a TCR alpha locus or a TCR beta locus.

5 In certain embodiments, the first homology arm comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 35. In certain embodiments, the first homology arm comprises the nucleotide sequence set forth in SEQ ID NO: 35. In certain embodiments, the first homology arm consists of the nucleotide sequence set forth in SEQ ID NO: 35. In certain embodiments, the first homology arm comprises about 300 consecutive bases, about 400 consecutive bases, about 500 consecutive bases, about 600 consecutive bases, about 700 consecutive bases, about 800 consecutive bases, or about 900 consecutive bases of SEQ ID NO: 35. In certain embodiments, the first homology arm comprises about 300 consecutive bases of SEQ ID NO: 35. In certain embodiments, the first homology arm comprises about 400 consecutive bases of SEQ ID NO: 35. In certain embodiments, the first homology arm comprises about 600 consecutive bases of SEQ ID NO: 35.

In certain embodiments, the second homology arm comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 36. In certain embodiments, the second homology arm comprises the nucleotide sequence set forth in SEQ ID NO: 36. In certain embodiments, the second homology arm consists of the nucleotide sequence set forth in SEQ ID NO: 36. In certain embodiments, the second homology arm comprises about 300 consecutive bases, about 400 consecutive bases, about 500 consecutive bases, about 600 consecutive bases, about 700 consecutive bases, about 800 consecutive bases, or about 900 consecutive bases of SEQ ID NO: 36. In certain embodiments, the second homology arm comprises about 300 consecutive bases of SEQ ID NO: 36. In certain embodiments, the second homology arm comprises about 400 consecutive bases of SEQ ID NO: 36. In certain embodiments, the second homology arm comprises about 600 consecutive bases of SEQ ID NO: 36.

SEQ ID NO: 35 and SEQ ID NO: 36 are provided below.

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ACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAGCTCCTG
CTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTGGCAGGG
TCAGTGGCTCCAACTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCTCATTTTC
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TTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGATGTAAGG
 AGCTGCTGTGACTTGCTCAAGGCCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTGATTTAT
 AGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACATACCAT
 AAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCGAAGATGTACAGTTTGCTTTGCT
 5 GGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTAAATAAA
 AGAATAAGCAGTATTATTAAGTAGCCCTGCATTTTCAAGTTTCTTGTAGTGGCAGGCCAGGCCTGGCCGTGAACGTTT
 ACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGT
 TTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCAC
 TGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTGTCCCAC
 10 AGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTC
 [SEQ ID NO: 35]

ACCGATTTTGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGA
 CATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCT
 15 TCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGT
 TTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCT
 CGGCCTTATCCATTGCCACCAAAACCCTCTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGAC
 ACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACCTGAGTTCTT
 GCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCCTACTGCTCTTCTAGGCCTCATCTAAGCCCCCTTCTCCAAGTTGC
 20 CTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACC
 CACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAGTCAAGTCAAGTGGG
 TGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTGAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAA
 TGTGTTTTAACTCAGGGTTGAGAAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTAC
 TTGAAGATAACCAGCCCTACCAAGGGCAGGGAGAGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGA
 25 AGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAG
 [SEQ ID NO: 36]

In certain embodiments, the HR template comprises a TCR gene sequence. In non-
 limiting embodiments, the TCR gene sequence is a patient-specific TCR gene sequence. In non-
 limiting embodiments, the TCR gene sequence is tumor-specific. In non-limiting embodiments,
 30 the TCR gene sequence encodes a TCR recognizing a neoantigen. In certain embodiments, the
 neoantigen is a private neoantigen. In non-limiting embodiments, the TCR gene sequence can be
 identified and obtained using the methods described in PCT/US2020/017887, the content of
 which is herein incorporated by reference. In certain embodiments, the HR template comprises
 a TCR alpha gene sequence and a TCR beta gene sequence.

35 In certain embodiments, the HR template is a polycistronic polynucleotide. In certain
 embodiments, the HR template comprises sequences encoding for flexible polypeptide
 sequences (e.g., Gly-Ser-Gly sequence). In certain embodiments, the HR template comprises
 sequences encoding an internal ribosome entry site (IRES). In certain embodiments, the HR

template comprises a 2A peptide (e.g., P2A, T2A, E2A, and F2A). In certain embodiments, the HR template comprises a protease cleavage site. In certain embodiments, the HR template comprises a signal sequence.

In certain embodiments, the flexible polypeptide encodes a glycine-serine-glycine sequence. In certain embodiments, the flexible polypeptide is encoded by a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO: 37 or SEQ ID NO: 38. In certain embodiments, the flexible polypeptide is encoded by a nucleotide sequence consisting of the nucleotide sequence set forth in SEQ ID NO: 37 or SEQ ID NO: 38. SEQ ID NO: 37 and SEQ ID NO: 38 are provided below.

GGCAGCGGC [SEQ ID NO: 37]
GGCTCCGGA [SEQ ID NO: 38]

In certain embodiments, the 2A peptide is a P2A peptide. In certain embodiments, the P2A peptide comprises the amino acid sequence set forth in SEQ ID NO: 39. In certain embodiments, the P2A peptide consists of the amino acid sequence set forth in SEQ ID NO: 39. In certain embodiments, the P2A peptide is encoded by a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41. In certain embodiments, the P2A peptide is encoded by a nucleotide sequence consisting of the nucleotide sequence set forth in SEQ ID NO: 40 or SEQ ID NO: 41. SEQ ID NOs: 39-41 are provided below.

ATNFSLLKQAGDVEENPGP [SEQ ID NO: 39]

GCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCT [SEQ ID NO: 40]
GCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT [SEQ ID NO: 41]

In certain embodiments, the P2A peptide comprises, at its N-end, a flexible polypeptide comprising a glycine-serine-glycine sequence. In certain embodiments, the P2A peptide comprises, at its N-end, a flexible polypeptide consisting of a glycine-serine-glycine sequence.

In certain embodiments, the protease cleavage site is a Furin cleavage site. In certain embodiments, the Furin cleavage site is encoded by a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO: 42. In certain embodiments, the Furin cleavage site is encoded by a nucleotide sequence consisting of the nucleotide sequence set forth in SEQ ID NO: 42. SEQ ID NO: 42 is provided below.

CGGGCCAAGCGG [SEQ ID NO: 42]

In certain embodiments, the signal sequence is a human growth hormone (HGH) signal sequence. In certain embodiments, the HGH signal sequence comprises the amino acid sequence set forth in SEQ ID NO: 43. In certain embodiments, the HGH signal sequence

consists of the amino acid sequence set forth in SEQ ID NO: 43. In certain embodiments, the HGH signal sequence is encoded by a nucleotide sequence comprising the nucleotide sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45. In certain embodiments, the HGH signal sequence is encoded by a nucleotide sequence consisting of the nucleotide sequence set forth in SEQ ID NO: 44 or SEQ ID NO: 45. SEQ ID NOs: 43-45 are provided below.

MATGSRTSLLLAFGLLCLPWLQEGSA [SEQ ID NO: 43]

ATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCCTTGGCTGCAAGAGGGTTCCGC
C [SEQ ID NO: 44]

ATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGC
T [SEQ ID NO: 45]

In certain embodiments, the Secondary Promoter Constructs include multiple 2A peptides, flexible polypeptides, protease cleavage peptides, signal peptides, or combinations thereof. In certain embodiments, the multiple 2A peptides can have the same amino acid sequence. In certain embodiments, the multiple flexible polypeptides can have the same amino acid sequence. In certain embodiments, the multiple protease cleavage peptides can have the same amino acid sequence. In certain embodiments, the multiple signal peptides can have the same amino acid sequence. In certain embodiments, when the Secondary Promoter Constructs include multiple 2A peptides, flexible polypeptides, protease cleavage peptides, signal peptides, or combinations thereof, these amino acid sequences are encoded by codon diverged nucleotide sequences. Additional information on the HR template nucleic acids and methods of modifying a cell thereof can be found in International Patent Application no. PCT/US2018/058230, the content of which is herein incorporated by reference.

3.1.9. Exemplified Secondary Promoter Constructs

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the Secondary Promoter Construct further comprises a promoter and an insulator. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a

second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, an insulator, a promoter, a Payload, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence
5 encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a first poly-A signal sequence and a second poly-A signal sequence. In certain embodiments, the
10 Secondary Promoter Construct further comprises a promoter, an insulator, and a WPRE element. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal
15 sequence peptide, a TRC α gene sequence, a first poly-A signal sequence, an insulator, a promoter, a Payload, a WPRE element, a second poly-A signal sequence, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a
20 protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a first poly-A signal sequence, a first insulator, a promoter, a Payload, a second poly-A signal sequence, a second insulator, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a
25 protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a first poly-A signal sequence, an insulator, a promoter, a Payload, a second poly-A signal sequence, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence
30 encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a

poly-A signal sequence. In certain embodiments, the Secondary Promoter Construct further comprises a promoter and a WPRE element. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a WPRE element, a Payload, a promoter, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a Payload, a protease cleavage site, a second 2A peptide, a second signal sequence, a TCR β gene sequence, a second protease cleavage site, a third 2A peptide, a third signal sequence peptide, and a TRC α gene sequence.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence and an insulator. In certain embodiments, the 3' of the at least one Payload comprises a STOP codon. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, an insulator, a promoter, a Payload having at its 3' a termination codon, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the 3' of the at least one Payload comprises a protease cleavage site and a 2A peptide. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a Payload, a second protease cleavage site, a third 2A peptide, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct comprises an enhancer. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence and a pause element. In certain embodiments, the 3' of the at least one Payload comprises protease cleavage site and a 2A peptide. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, an enhancer, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a pause element, a promoter, a Payload, a second protease cleavage site, a third 2A peptide, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence

encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct comprises an enhancer. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence and an insulator. In certain embodiments, the 3' of the at least one Payload comprises a protease cleavage site and a 2A peptide. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, an enhancer, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, an insulator, a promoter, a Payload, a second protease cleavage site, a third 2A peptide, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence, an enhancer, and an insulator. In certain embodiments, the 3' of the at least Payload comprises a STOP codon. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, an enhancer, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, an insulator, a promoter, a Payload having at its 3' a termination codon, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide

and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, an inhibitory RNA molecule, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence and a termination sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, an inhibitory RNA molecule, a termination sequence, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the 3' of the at least one Payload comprises

a protease cleavage site and a 2A peptide. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a first Payload, a second Payload, a second protease cleavage site, a third 2A peptide, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the at least one Payload comprises with a protease cleavage site and a 2A peptide. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a first Payload, a second protease cleavage site, a third 2A peptide, a second Payload, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the inhibitory RNA molecule is flanked by a splice acceptor site, a splice donor site, or a combination thereof. In certain embodiments, the

Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a splice donor site, an inhibitory RNA molecule, a splice acceptor site, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the Secondary Promoter Construct further comprises a splice acceptor site. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, an inhibitory RNA molecule, a splice acceptor site, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, an inhibitory RNA molecule, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises an inhibitory RNA molecule. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a first Payload, a second Payload, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the at least one Payload comprises a first inhibitory RNA molecule. In certain embodiments, the at least one Payload comprises a second inhibitory RNA molecule. In certain embodiments, the first and second inhibitory RNA molecules knock down the gene expression of a same gene. In certain embodiments, the first and second inhibitory RNA molecules knock down the gene expression of different genes. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a first inhibitory RNA molecule, a second inhibitory RNA molecule, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least one Payload. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the Payload is a miRNA cluster. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a miRNA cluster, and a second homology arm.

In certain embodiments, the Secondary Promoter Construct comprises a sequence encoding an exogenous TCR and at least two Payloads. In certain embodiments, the sequence encoding an exogenous TCR encodes a TRC α gene sequence and a TCR β gene sequence. In certain embodiments, the sequence encoding an exogenous TCR comprises a first 2A peptide and a second 2A peptide, a first signal peptide and a second signal peptide, and a protease cleavage site. In certain embodiments, the Secondary Promoter Construct further comprises a poly-A signal sequence. In certain embodiments, the first Payload is an inhibitory RNA molecule. In certain embodiments, the inhibitory RNA molecule is flanked by a splice acceptor site, a splice donor site, or a combination thereof. In certain embodiments, the Secondary Promoter Construct further comprises a promoter. In certain embodiments, the Secondary Promoter Construct further comprises a first homology arm and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, a first Payload, a splice donor site, an inhibitory RNA molecule, a splice acceptor site, and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first Payload, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal

sequence, a promoter, a splice donor site, an inhibitory RNA molecule, a splice acceptor site, and a second homology arm. In certain embodiments, the Secondary Promoter Construct comprises, from 5' to 3', a first homology arm, a first 2A peptide, a first signal sequence peptide, a TCR β gene sequence, a protease cleavage site, a second 2A peptide, a second signal sequence peptide, a TRC α gene sequence, a poly-A signal sequence, a promoter, an inhibitory RNA molecule, and a second homology arm.

In certain embodiments, when a Secondary Promoter Construct includes two or more sequences encoding a 2A peptide, the sequences encode for the same 2A peptide and can be codon diverged. For example, without any limitation, two sequences can encode for a P2A and be codon diverged. In certain embodiments, when a Secondary Promoter Construct includes a sequence encoding a 2A peptide, the Secondary Promoter Construct can include a sequence encoding for a GSG amino acid at the 5' of the sequence encoding a 2A peptide. In certain embodiments, when a Secondary Promoter Construct includes two or more sequences encoding a signal peptide, the sequences encode for the same signal peptide and can be codon diverged. For example, without any limitation, two sequences can encode for a HGH signal peptide and be codon diverged.

In certain embodiments, the Secondary Promoter Construct comprises a TCR β gene sequence comprising a nucleotide sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 46. In certain embodiments, the TCR β gene sequence comprises the nucleotide sequence set forth in SEQ ID NO: 46. In certain embodiments, TCR β gene sequence consists of the nucleotide sequence set forth in SEQ ID NO: 46.

In certain embodiments, the TCR β gene sequence encodes an amino acid sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% identical to the amino acid sequence set forth in SEQ ID NO: 47. In certain embodiments, the TCR β gene sequence encodes the amino acid sequence set forth in SEQ ID NO: 47. SEQ ID NOs: 46 and 47 are provided below.

GATGCTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTCACCAGAC
TTGGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTG
CTGATATTACAGATAAAGGAGAAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACT
CTGGAGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCCG
GCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTG
AGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTCT
TCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCT
GAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCA

GATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATC
GTGTCTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCAC
CATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGCTGATGGCTATGG
TCTCCCGGGAGCGCATCCCCGAGGCC [SEQ ID NO: 46]

5 DAGITQSPRYKITETGRQVTLMCHQTWSHSYMFYRQDLGHGLRLIYYSAAADITDKGEVSDGYVVSRSKTENFPLT
LESATRSQTSVYFCASSEDSEYQYFGPGTRLTVTEDLKNVFPPKVAVFEPSEAEISHTQKATLVCLATGFYDPHVEL
SWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVTQI
VSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVLSALVLMAMVSRERIPEA [SEQ ID NO: 47]

In certain non-limiting embodiments, the Secondary Promoter Construct comprises a
10 Payload encoding an mCherry protein. In certain embodiments, the Secondary Promoter
Construct comprises a nucleotide sequence that is at least about 80%, about 85%, about 90%,
about 95%, about 96%, about 97%, about 98%, about 99% identical to the nucleotide sequence
set forth in SEQ ID NOs: 48-64. In certain embodiments, the Secondary Promoter Construct
comprises the nucleotide sequence set forth in SEQ ID NOs: 48-64. In certain embodiments, the
15 Secondary Promoter Construct consists of the nucleotide sequence set forth in SEQ ID NOs: 48-
64.

In certain embodiments, the at least one Payload (e.g., mCherry protein) of any one of
SEQ ID NOs: 48-64 can be replaced by any Payload disclosed herein. For example, but without
any limitation, the at least one Payload can be replaced by a sequence encoding a cytokine
20 receptors trap or ligand trap, an angiogenesis factors, an apoptotic factor, an inhibitory protein
for various cell processes (e.g., inhibition of macrophage activation), an extracellular matrix
modulator, a soluble TCRs, a soluble chimeric antigen receptor (CAR), a homing signal, an
enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that
binds to receptors and sterically hinders receptor function, or an inhibitory RNA molecule.

25 In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
3794 of SEQ ID NO: 48. In certain embodiments, the Secondary Promoter Construct comprises
nucleotides 4503 to 7046 of SEQ ID NO: 48. In certain embodiments, the Secondary Promoter
Construct comprises nucleotides 1 to 3821 and nucleotides 4503 to 7046 of SEQ ID NO: 48.
SEQ ID NO: 48 is provided below.

30 GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
GCAGGGTCAGTGCTCCAACCTAATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
35 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
TACCATAAACCTCCCATTTCTGCTAATGCCAGCCTAAGTTGGGGAGACCACTCCAGATTTCCAAGATGTACAGTTTGC

TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
AATAAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGGCCAAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
5 CATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT
ATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGC
TGATGCTGGAATCACCCAGAGCCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTCAACCAGA
10 CTTGGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCT
GCTGATATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCAC
TCTGGAGTCAGCTACCCGCTCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCG
GGCCGGGCACCAAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCT
GAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACCT
15 GTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCAGCCTCTGAAAGAACAGCCCGCTC
TGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCAGAAACCACTTC
AGATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGAT
CGTGTCTGCCAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCA
CCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGATGGCTATG
20 GTCTCCCGGGAGCGCATCCCCGAGGCCCGGGCCAAAGCGGGGCAGCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGC
CGGCGACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGT
GTCTGCCTTGGCTGCAAGAGGGTTCCGCGGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGA
GCCTCACTGGAGTTGAGATGTAACCTATTCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCA
AGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTA
25 AGAGGAGTCAATCTTCCTTCAATCTGAGGAAACCCCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTG
GGTGAATTGGACACAGGCTTTTCAGAACTTGTATTTGGAACCTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAA
CCCCGATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCCGACTTCGACA
GCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATG
GACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTCGCCTGCGCCAACGCCTTCAACAACAGCAT
30 TATCCCCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAG
ACACCAACCTGAACCTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTG
CTGATGACCTGAGACTGTGGTCCAGCTGATTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTTCT
AGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAA
CAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTTAAAGCGTCGACGAGCTCACGGGGA
35 CAGCCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCCGGGGCT
CCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCGGGCACGGGGAAGGTGGCA
CGGGATCGCTTTCTCTGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTT
CTCGAGGAACAGAGAAACAGGAGAATATGGGCCAAAACAGGATATCTGTGGTAAGCAGTTCTTGCCCCGGCTCAGGGC
CAAGAACAGTTGGAACAGCAGAATATGGGCCAAAACAGGATATCTGTGGTAAGCAGTTCTTGCCCCGGCTCAGGGCCA
40 AGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAA
GGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTC

CCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACTT
CCATAGAAGGGATCCGCCACCATGGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAGAGTTCATGCGGTT
CAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGG
GAACACAGACCGCCAAGCTGAAAGTGACCAAAGGCGGCCCTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTT
5 ATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGATATTCCCAGCTACCTGAAGCTGAGCTTCCCCGAGGGCTT
CAAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCG
AGTTCATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGC
TGGGAAGCCAGCAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAAACTGAA
GGATGGCGGCCACTACGACGCCGAAGTGAAAACCACTACAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACA
10 ACGTGAACATCAAGCTGGACATCACCAGCCACAACGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGC
AGACACAGCACAGGCGGAATGGACGAGCTGTACAAGTGACATATGACCGATTTTGTATTCTCAAACAAATGTGTCACA
AAGTAAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTG
CTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAGACACCTTC
TTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCC
15 AGAGCTCTGGTCAATGATGTCTAAAACCTCTGATTGGTGGTCTCGGCCCTTATCCATTGCCACCAAAACCCCTCTTT
TTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCA
GGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACCTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCC
TTACTGCTCTTCTAGGCCTCATTCTAAGCCCCCTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAAT
CTTTCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATG
20 CACCAGGTGTTGAAGTGGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGA
GCCCATCTGTGAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTA
CCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAG
AGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGC
AGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCT
25 CCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACC
AGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCCTTT
CTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGCTCCAA
GCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC
CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
30 ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCC
AGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTT
GCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAAACTCATCGAGCATCA
AATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAA
AACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACA
35 ACCTATTAATTTCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGA
ATGGCAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAATCACTCGCA
TCAACCAAACCGTTATTCATTCTGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACA
AACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTT
CTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGC
40 TTGATGGTCGGAAGAGGCATAAATTCGTGAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCT
ACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGCC

CGACATTATCGCGAGCCCATTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCC
CGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATT
TTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 48]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
5 3837 of SEQ ID NO: 49. In certain embodiments, the Secondary Promoter Construct comprises
nucleotides 4546 to 7520 of SEQ ID NO: 49. In certain embodiments, the Secondary Promoter
Construct comprises nucleotides 1 to 3837 and nucleotides 4546 to 7520 of SEQ ID NO: 49.
SEQ ID NO: 49 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
10 CTCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
GCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
GTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
15 TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCGAAGATGTACAGTTTGC
TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
AATAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAAGTTTCTTGTAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAAGTGGCAGCCCCACAGAGCCCCGCCCTTGTC
20 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCTAACCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCTGAATTCGGCTCCGGAGCCACTAAGTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCTCT
ATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCCTCCCATGGCTCCAAGAAGGATCTGC
TGATGCTGGAATCACCCAGAGCCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTCAACAGA
25 CTTGGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCT
GCTGATATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCAC
TCTGGAGTCAGTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCG
GGCCGGGCACAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTGAGCCTTCT
GAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCCGATCACGTGGAAGT
30 GTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCCGCTC
TGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTC
AGATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGAT
CGTGTCTGCCAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCA
CCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGATGGCTATG
35 GTCTCCCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGC
CGGCGACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGT
GTCTGCCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGA
GCCTCACTGGAGTTGAGATGTAACCTATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCA
AGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTA

AGAGGAGTCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTG
GGTGAATTGGACACAGGCTTTCAGAACTTGTATTTGGAAGTGGCACCCGACTTCTGGTCAGTCCAAATATTAGAA
CCCCGATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCCGACTTCGACA
GCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATG
5 GACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTCGCCTGCGCCAACGCCTTCAACAACAGCAT
TATCCCCGAGGACACATTCTTCCCAAGTCTGTAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAG
ACACCAACCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTG
CTGATGACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGC
CTTCCTTGACCCTGGAAGGTGCCACTCCCCTGTCTTTTCTTAATAAAATGAGGAAATTGCATCGCATTTGTCTGAGT
10 AGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGC
TGGGGATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCCAAAGCCCCAGGGATGTAATTA
CGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGA
GCCGGCAGCGTGCGGGGACAGCCCGGGCACGGGGAAGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCT
CTTTGAGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGAGGAACAGAGAAACAGGAGAATATGGGCCA
15 AACAGGATATCTGTGGTAAGCAGTTTCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAA
CAGGATATCTGTGGTAAGCAGTTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTC
AGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGGCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTA
ACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGA
ACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACTTCCATAGAAGGGATCCGCCACCATGGTGTCCAAGG
20 GCGAAGAGGACAACATGGCCATCATCAAAGAGTTTATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCAC
GAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACCAAAGG
CGGCCCTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCCTACGTGAAGCACCCCCG
CCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAACTTCGAGGACGGC
GGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTTATCTACAAAGTGAAGCTGCGGGGCACCAA
25 CTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCCTGAAG
ATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAACTGAAGGATGGCGGCCACTACGACGCCGAAGTGAAGAAC
ACCTACAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCACAA
CGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGTACA
AGTGACATATGGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTTCTTAACATATGTTGCTCCT
30 TTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTTATTTCTCCTC
CTTGATATAAATCCTGGTTAGTTCTTGCCACGGCGGAACATCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTC
GGCTGTTGGGCACTGACAATTCCGTGGTGTATTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTTCTA
GCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTTATAAGCTGCAATAAACAAGTTAACAACAAC
AATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTAAAGCACCGATTTTGATTCTCAAAC
35 AAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCA
AGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCA
GAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGC
CAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACC
AAAACCTCTTTTTACTAAGAAACAGTGAGCCTTGTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAA
40 GAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCCAACTGAGTTTCTGCCTGCCTGCCTTTGCTCAG
ACTGTTTGCCCTTACTGCTCTTCTAGGCCCTATTCTAAGCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGT

CTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCC
 GGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTAAGTTCAGATGAGGGGTGTGCCAGAGGAAGCACCATT
 CTAGTTGGGGGAGCCCCTGTGTGAGTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTAACTCAGGGTTG
 AGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACC
 5 AAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTG
 AATGAAGGAGGCAGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTT
 TTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC
 TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCCTGCCGCTTACCGGATAC
 CTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGGTGTAGGT
 10 CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCGACCGCTGCGCCTTATCCGGTAACCTATCGTC
 TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTAT
 GTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGC
 TCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTG
 GTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACT
 15 CATCGAGCATCAAATGAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGT
 AATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCC
 AACATCAATACAACCTATTAATTTCCCCCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTG
 AATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCACAGGCCAGCCATTACGCTCGTCATCA
 AAATCACTCGCATCAACCAAACCGTTATTCATTCTGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAA
 20 AGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAAT
 CAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTA
 CGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATTCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATC
 ATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCG
 CACCTGATTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGC
 25 CTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTATTGTTCA
 TGATGATATATTTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 49]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 3402 of SEQ ID NO: 50. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 4111 to 7051 of SEQ ID NO: 50. In certain embodiments, the Secondary Promoter
 30 Construct comprises nucleotides 1 to 3402 and nucleotides 4111 to 7051 of SEQ ID NO: 50.
 SEQ ID NO: 50 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 GCAGGGTCAGTGGCTCCAACATAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 35 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGC
 TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA

AATAAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
CATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCCTGATCCTCTTG
5 TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCCT
ATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGC
TGATGCTGGAATCACCCAGAGCCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTCACCAGA
CTTGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCT
10 GCTGATATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCAC
TCTGGAGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCG
GGCCGGGACACAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCT
GAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAC
GTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCAGCCTCTGAAAGAACAGCCCCGCTC
15 TGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCAAGAACCACTTC
AGATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGAT
CGTGTCTGCCAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCA
CCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGCTGATGGCTATG
GTCTCCCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGC
20 CGGCGACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGT
GTCTGCCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGA
GCCTCACTGGAGTTGAGATGTAATCTATGTTGAGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCA
AGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTA
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25 GGTGAATTGGACACAGGCTTTTCAAGAACTTGTATTTGGAACCTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAA
CCCCGATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACA
GCCAGACCAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATG
GACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTTCGCCCTGCGCCAACGCCTTCAACAACAGCAT
TATCCCCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAG
30 ACACCAACCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTG
CTGATGACCCTGAGACTGTGGTCCAGCTGAGTCGACGCTTTAAAAAACCTCCACATCTCCCCCTGAACCTGAAACA
TAAAATGAATGCAATTGTTGTTGTTAACTTGTATTATGTCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA
ATTTTCAAAATAAAGCTAGAATGGTTACAAATAAAGCAATAGCATCACAAATTTTCAAAATAAACACCACGGAATTG
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35 CCAGGATTTATACAAGGAGGAGAAAAATGAAAGCCATACGGGAAGCAATAGCATGATACAAAGGCATTAAAGCAGCGT
ATCCACATAGCGTAAAAGGAGCAACATAGTTAAGAATACCAGTCAATCTTTTCAAAATTTTGTAAATCCAGAGGTTGA
TTATCCATATGTCACTTGTACAGCTCGTCCATTCCGCCGTGTGCTGTGCTGCTGCTTCCGGCTCTCTCGTACTGTTCCAC
GATGGTGTAGTCCTCGTTGTGGCTGGTGATGTCCAGCTTGATGTTTACGTTGTAGGCGCCAGGCAGCTGCACGGGTT
TCTTGGCCTTGTAGGTGGTTTTTCACTTCGGCGTCGTAGTGGCCGCCATCCTTCAGTTTTCAGCCGCTGCTTGATCTCG
40 CCCTTCAGGGCGCCATCTTCAGGGTACATTCTCTCGCTGCTGGCTTCCCAGCCCATTTGTCTTTTTTCTGCATCACGGG
GCCATCAGAGGGAAAGTTGGTGCCCCGAGCTTCACTTTGTAGATGAACTCGCCGTCCTGCAGAGAGCTATCTTGAG

TCACGGTGACCACGCCGCCGTCCTCGAAGTTCATCACTCTCTCCCACTTGAAGCCCTCGGGGAAGCTCAGCTTCAGG
 TAGTCGGGAATATCGGCGGGGTGCTTCACGTAGGCCCTTGCTGCCGTACATAAACTGAGGGCTCAGAATGTCCCAGGC
 AAAAGGCAGAGGGGCCGCTTTGGTCACTTTAGCTTGGCGGTCTGTGTTCCCTCGTAAGGTCTGCCCTCGCCTTCGC
 CTTCAATCTCGAACTCGTGGCCGTTACAGCTGCCTTCCATGTGCACCTTGAACCGCATGAACTCTTTGATGATGGCC
 5 ATGTTGTCCTCTTCGCCCTTGACACCATGGTGGCGGATCCCTTCTATGGAAGTCAAACAGCGTGGATGGCGTCTC
 CAGGCGATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGAGCTCGGGGAGCAGAAGCGCGCAACAGAAGCGAG
 AAGCGAACTGATTGGTTAGTTCAAATAAGGCACAGGGTCATTTAGGTCCCTGGGGCACCTGGAAACATCTGATGG
 TTCTCTAGAACTGCTGAGGGCGGGACCGCATCTGGGGACCATCTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCT
 TACCACAGATATCCTGTTTGGCCCATATTCTGCTGTTCCAACTGTTCTTGGCCCTGAGCCGGGGCAGGAACTGCTTA
 10 CCACAGATATCCTGTTTGGCCCATATTCTCCTGTTTCTCTGTTCCCTCGAGACCGATTTTGATTCTCAAACAAATGTG
 TCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAA
 CAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCCTCAACAACAGCATTATTCAGAAGACA
 CCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTT
 TGCCAGAGCTCTGGTCAATGATGTCTAAAACCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAACCC
 15 TCTTTTTACTAAGAAACAGTGAGCCTTGTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGG
 TGGCAGGAGAGGGCACGTGGCCAGCCTCAGTCTCTCCAACTGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTT
 GCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAA
 AAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACAT
 GAATGCACCAGGTGTTGAAGTGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTG
 20 GGGGAGCCCATCTGTGAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAAC
 AGCTACCTTCAGGACAAAAGTCAGGGAAAGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCA
 GGGAGAGGACCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAG
 GAGGCAGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCAT
 AGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG
 25 ATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCG
 CCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGC
 TCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTC
 CAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCG
 GTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTG
 30 AAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTT
 TGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAAACTCATCGAG
 CATCAAATGAACTGCAATTTATTATATCAGGATTATCAATACCATATTTTGA AAAAGCCGTTTCTGTAATGAAG
 GAGAAAACTACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCA
 ATACAACCTATTAATTTCCCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGG
 35 TGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAATCAC
 TCGCATCAACCAAACCGTTATTTCATTTCGTGATTGCGCTGAGCCAGACGAAATACGCGATCGCTGTTAAAGGACAA
 TTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATA
 TTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAA
 AATGCTTGATGGTGGGAAGAGGCATAAATTCGCTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCA
 40 ACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGA
 TTGCCCCGACATTATCGCGAGCCCATTTATACCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGACG

TTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGAT
ATATTTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 50]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 3931 of SEQ ID NO: 51. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 4640 to 7258 of SEQ ID NO: 51. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 4086 and nucleotides 4795 to 7413 of SEQ ID NO: 51. SEQ ID NO: 51 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
10 GCAGGGTCAGTGGCTCCAACATAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
TACCATAAACCTCCCATTTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGC
15 TTTGCTGGGCCTTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
AATAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCTTAACCTGATCCTCTTG
20 TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCGGGCTGGGGCGGTCCCCCTCCCATTTCCATGACGTCATGGTTACCAAGAGGGGCAAGTAGGGCACCC
TTTGAAGCTCTCCCGCAGAAGCCACATCCTCTGGAAAGAAAGTTTATAATACTGAGTTAGAGATAGCATCGCCCCA
GGCCACGTGCCGAGGGGAGCAGGCTGGGCCGTACACCACCCCCCAACCGCAGGTGCAGCAAGGCCAACATGCCAGG
CTGGGAGGGGCTGCCGGCCCCCTCGTTGAGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTT
25 GAAGAAAACCCCGGTCCTATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATG
GCTCCAAGAAGGATCTGCTGATGCTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGA
CCTTGATGTGTACACGACTTTGGAGCCACAGCTATATGTTCTGTTATCGACAAGACCTGGGACATGGGCTGAGGCTG
ATCTATTACTCAGCAGCTGCTGATATTACAGATAAAGGAGAAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGAC
AGAGAATTTCCCCCTCACTCTGGAGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACA
30 GTTACGAGCAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTG
GCCGTGTTTCGAGCCTTCTGAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTA
CCCCGATCACGTGGAACCTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTC
TGAAAGAACAGCCCCTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAG
AACCCACAGAAACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGC
35 CAAGCCTGTGACACAGATCGTGTCTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGC
AGGGCGTGCTGTCTGCCACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCT
CTGGTGCTGATGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGAGCGGCGCCACCAACTT
CAGCCTGCTGAAGCAGGCCGGCGACGTGGAGGAGAAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGC
TGGCCTTCGGACTGCTGTGTCTGCCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCAC

ATCACTGTCTCTGAAGGAGCCTCACTGGAGTTGAGATGTAACCTATTCCTATGGGGCAACACCTTATCTCTTCTGGTA
TGTCCAGTCCCCCGGCCAAGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAG
GCTTTGAGGCTGAATTTAAGAGGAGTCAATCTTCCTTCAATCTGAGGAAACCCTCTGTGCATTGGAGTGATGCTGCT
GAGTACTTCTGTGCTGTGGGTGAATTGGACACAGGCTTTTCAGAACTTGTATTTGGAACCTGGCACCCTGACTTCTGGT
5 CAGTCCAAATATTTCAGAACCCCGATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCC
TGTTACCCGACTTCGACAGCCAGACCAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTG
CTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTTCGCTGCGCCAA
CGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGG
AAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAA
10 GTGGCCGGCTTCAACCTGCTGATGACCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTG
TTTGCCCCCTCCCCCGTGCCTTCTTGACCTGGAAGGTGCCACTCCCCTGTCTTTTCTAATAAAATGAGGAAATT
GCATCGCATTTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGA
AGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCCAAAG
CCCCCAGGGATGTAATTACGTCCCTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCCGGGGCTCCGCTCCGGTCCGGCG
15 CTCCCCCGCATCCCCGAGCCGGCAGCGTGCGGGGACAGCCCGGGCACGGGGAAGGTGGCAGGGATCGCTTCTCTC
TGAACGCTTCTCGCTGCTCTTTGAGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGACGAACAGAGAA
ACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAAC
AGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCC
AGATGCGGTCCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGTGCCCCAAGGACCTGAAATGACCC
20 TGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCTATATAA
GCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACTTCCATAGAAGGGATCCG
CCACCATGGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAGAGTTCATGCGGTTCAAGGTGCACATGGAA
GGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAA
GCTGAAAGTGACCAAAGGCGGCCCTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGG
25 CCTACGTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTG
ATGAACTTCGAGGACGGCGGCGTGGTCAACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCATCTACAAAGT
GAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCG
AGAGAATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAACTGAAGGATGGCGGCCACTAC
GACGCCGAAGTGAAAACACCTACAAGGCCAAGAAACCCGTGCAGCTGCCCTGGCGCTACAACGTGAACATCAAGCT
30 GGACATCACAGCCACAACGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCG
GAATGGACGAGCTGTACAAGAGAGCCAAACGGGGTCCGGCGCTACCAATTTAGTTTGTCTCAAACAGGCCGGAGAC
GTCGAGGAAAATCCTGGCCCCCATATGACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGATGT
GTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACA
AATCTGACTTTGCATGTGCAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTTCTTCCCCAGCCCAGGTAAG
35 GGCAGCTTTGGTGCCTTCGACGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGAT
GTCTAAACTCCTCTGATTGGTGGTCTCGGCTTATCCATTGCCACCAAAACCCCTCTTTTTACTAAGAAACAGTGAG
CCTTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCC
AGCCTCAGTCTCTCAACTGAGTTCTTGCTGCTGCTGCTTTGCTCAGACTGTTTGCCCCCTTACTGCTCTTCTAGGCC
TCATTCTAAGCCCCCTTCTCCAAGTTGCTCTCCTTATTTCTCCCTGTCTGCCAAAAATCTTTCCAGCTCACTAAG
40 TCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGG
AGGAATTAAAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGG

AAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCA
 GGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCCTATAGAGGCCT
 GGGACAGGAGCTCAATGAGAAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTACAGGGC
 CTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCA
 5 TCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAA
 GCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTG
 GCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGA
 ACCCCCCGTTACGCCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTAT
 CGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG
 10 TGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAG
 AGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGC
 GCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACTCATCGAGCATCAAATGAACTGCAATTTAT
 TCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACCTCACCGAGGCAGTTC
 CATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTC
 15 GTCAAAAATAAGGTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCA
 TTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTC
 ATTCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAA
 CCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTG
 TTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGC
 20 ATAAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAG
 AAACAACCTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGCCCCGACATTATCGCGAGCCC
 ATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATA
 ACACCCCTTGATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTA
 ACATCAGAGATTTTGAGACAC [SEQ ID NO: 51]

25 In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 4140 of SEQ ID NO: 52. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 4849 to 7467 of SEQ ID NO: 52. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 4140 and nucleotides 4849 to 7467 of SEQ ID NO: 52.
 SEQ ID NO: 52 is provided below.

30 GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 GCAGGGTCAGTGGCTCCAACCTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 35 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTTCTGCTAATGCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGC
 TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
 AATAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
 ACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA

GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGA CTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
CATCACTGGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCTTAACCCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCCGTTACATAACTTACGTTAAATGGCCCCGCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC
5 AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTTATGGC
CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
10 CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
AGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGA
TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
AGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCG
GGCACAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGC
15 CGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTT
GGTGGGTCAACGGCAAAGAGGTGCACAGCGGCTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAAC
GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
CCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGT
CTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATC
20 CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTGATGGCTATGGTCTC
CCGGGAGCGCATCCCCGAGGCCCGGGCCAAAGCGGGGCGAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCG
ACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTG
CCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTC
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25 TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
AGTCAATCTTCCTTCAATCTGAGGAAACCCCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGA
ATTGGACACAGGCTTTTCAGAACTTGTATTTGGAAGTGGCACCCGACTTCTGGTCAGTCCAAATATTAGAACCCCG
ATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCCGACTTCGACAGCCAG
ACCAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTT
30 CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTTCGCTGCGCCAACGCCTTCAACAACAGCATTATCC
CCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACC
AACCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCACTGTCTTTCTTAATAAAATGAGGAAATGCATCGCATTGTCTGAGTAGGTG
35 TCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG
ATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCC
CTCCCCGCTAGGGGGCAGCAGCGAGCCCGCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
CAGCGTGCGGGGACAGCCCGGGCACGGGGAAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTG
AGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGAGGAACAGAGAAACAGGAGAATATGGGCCAAACAG
40 GATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGA
TATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAG

TTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCGTGTGCCTTATTTGAAC TAACCAA
TCAGTTCGCTTCTCGCTTCTGTTTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTT TAGTGAACCGT
CAGATCGCCTGGAGACGCCATCCACGCTGTTTTGACTTCCATAGAAGGGATCCGCCACCATGGTGTCCAAGGGCGAA
GAGGACAACATGGCCATCATCAAAGAGTTTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTT
5 CGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACCAAAGGCGGCC
CTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGAT
ATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAAC TTCGAGGACGGCGGCGT
GGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTTCATCTACAAAGTGAAGCTGCGGGGCACCAACTTTC
CCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCCTGAAGATGGC
10 GCCCTGAAGGGCGAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCACTACGACGCCGAAGTGAAAACCACTA
CAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCACAACGAGG
ACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGTACAAGAGA
GCCAAACGGGGGTCCGGCGCTACCAATTT CAGTTTGCTCAAACAGGCCGGAGACGTGAGGAAAATCCTGGCCCCCA
TATGACCGATTTTGATTCTCAAACAAATGTGTCAAAAGTAAGGATTCTGATGTGTATATCACAGACAAAAC TGTC
15 TAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAAC
GCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGG
CTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTG
GTCTCGGCCTTATCCATTGCCACCAAAACCCCTCTTTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAA
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20 TCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCCTACTGCTCTTCTAGGCCTCATTC TAAGCCCCCTCTCCAAG
TTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATT
AACCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTA AAAAGTCAGATGAGG
GGTGTGCCCAGAGGAAGCACCATCTAGTTGGGGGAGCCCATCTGT CAGCTGGGAAAAGTCCAAATAACTTCAGATT
GGAATGTGTTTTAACTCAGGGTTGAGAAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATG
25 CTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAG
GAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTCACAGGGCCTTCTAGGCCATGAGAGGGTAGA
CAGGCTAGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTC
AGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTT
CCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTG
30 TAGGTATCTCAGTTCGGTG TAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT CAGCCGACCGCT
GCGCCTTATCCGGTA ACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGT
AACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG
AAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCA
AACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAA
35 GATCCTTTGATCTTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCA
TATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCAGGCAGTTCCATAGGATGGCAAGATCCTGGTA
TCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCCTCGTCAAAAATAAGGTTATCAAGTG
AGAAATCACCATGAGTGACGACTGAATCCGGTGAGAAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACA
GGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTCGTGATTGCGCCTGAGCCAG
40 ACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCG
CATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTG

AGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGGTCAGCCAGTTTAG
 TCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCT
 TCCCATAACAAGCGATAGATTGTCGCACCTGATTGCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCA
 TCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTAT
 5 GTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC
 [SEQ ID NO: 52]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 4004 of SEQ ID NO: 53. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 4713 to 7331 of SEQ ID NO: 53. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 4004 and nucleotides 4713 to 7331 of SEQ ID NO: 53
 10 SEQ ID NO: 53 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCTTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 GCAGGGTCAGTGCTCCAACCTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 15 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTCTGCTAATGCCAGCCTAAGTTGGGGAGACCACTCCAGATTTCCAAGATGTACAGTTTGC
 TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
 20 AATAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
 ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
 GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTG
 TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
 25 TATTCGAATTCGGTTACATAACTTACGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC
 AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
 CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
 CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
 ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGC
 30 CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
 CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
 AGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGA
 TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
 AGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCG
 35 GGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGC
 CGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTT
 GGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAAC
 GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
 CCAGGTCCAGTTCTACGGCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGT

CTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATC
CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGATGGCTATGGTCTC
CCGGGAGCGCATCCCCGAGGCCCGGGCCAAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCG
ACGTGGAGGAGAAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTG
5 CCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTC
ACTGGAGTTGAGATGTAACATATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCC
TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
AGTCAATCTTCCTTCAATCTGAGGAAACCCCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGA
ATTGGACACAGGCTTTTCAGAACTTGTATTTGGAACGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCG
10 ATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAG
ACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTT
CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCTGCGCCAACGCCTTCAACAACAGCATTATCC
CCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACC
AACCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
15 GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCCTGTCTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
TCATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG
ATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCC
CTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
20 CAGCGTGCGGGGACAGCCCGGGCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTG
AGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGAGTGAGTCAGTGACTCAGTGAGTCAGTGACTCAGT
GAGTCACTGACTCACTGAGTCAGTGACTCAGGAATTGAGCTTGAATAAAATGAATATTAGAAGCTGTTAGAATAAGA
GAAAATGACAGAGGAAAACTGAAAGGGAGAACTGAAAGTGGGAAATTCCTCTGAGGCAGAAAGGACCATCCCTTATA
AATAGCACAGGCCATGAAGGAAGATCATTCTCACTGCAGCCTTTGACAGCCTTTGCCTCATCTTGGGATCCGCCACC
25 ATGGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAGAGTTCATGCGGTTCAAGGTGCACATGGAAGGCAG
CGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGA
AAGTGACCAAAGGCGGCCCTCTGCCTTTTGCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCTAC
GTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAA
CTTCGAGGACGGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCATCTACAAAGTGAAGC
30 TGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGA
ATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGTGAAACTGAAGGATGGCGGCCACTACGACGC
CGAAGTGAAAACACCTACAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACA
TCACCAGCCACAACGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATG
GACGAGCTGTACAAGAGAGCCAAACGGGGGTCCGGCGCTACCAATTTAGTTTGCTCAAACAGGCCGGAGACGTGCA
35 GGAAAATCCTGGCCCCCATATGACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGTATA
TCACAGACAAAATGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCT
GACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAG
CTTTGGTGCTTCGACAGGCTGTTTCTTGTCTTCAAGGAATGGCCAGGTTCTGCCAGAGCTCTGGTCAATGATGTCTA
AAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCCTCTTTTTACTAAGAAACAGTGAGCCTTG
40 TTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCAGCCT
CAGTCTCTCCAACCTGAGTTCTGCTGCCTGCCTTGTCTCAGACTGTTTGCCCCCTTACTGCTCTTCTAGGCCTCAT

CTAAGCCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAATCTTTCCCAGCTCACTAAGTCAGT
 CTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAA
 TTAAAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAG
 TCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAA
 5 GGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGAGGCCTGGGAC
 AGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTACAGGGCCTTCT
 AGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACA
 AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGCTCC
 CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGAAGCGTGGCGCT
 10 TTCTCATAGCTCAGCGTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCC
 CCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA
 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC
 TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTG
 GTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGA
 15 AAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAAACTCATCGAGCATCAAATGAACTGCAATTTATTCATA
 TCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAG
 GATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCCTCGTCAA
 AAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCT
 TTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCTG
 20 TGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGC
 GCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTT
 CCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAA
 TTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACA
 ACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCCGACATTATCGCGAGCCCATTTA
 25 TACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATAACACC
 CCTTGATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATC
 AGAGATTTTGAGACAC [SEQ ID NO: 53]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 4938 of SEQ ID NO: 54. In certain embodiments, the Secondary Promoter Construct comprises
 30 nucleotides 5647 to 8265 of SEQ ID NO: 54. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 4938 and nucleotides 5647 to 8265 of SEQ ID NO: 54
 SEQ ID NO: 54 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 35 GCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCCAAGATGTACAGTTTGC

TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
AATAAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACAGAGCA
GCTGGTTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
5 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCGGTTACATAACTTACGTTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTC
AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
10 CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGC
CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
AGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGA
15 TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
AGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCG
GGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGC
CGAGATCAGCCACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACGTCTT
GGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAAC
20 GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
CCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGT
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CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTGATGGCTATGGTCTC
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25 ACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTG
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TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
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30 ATTGGACACAGGCTTTCAGAACTTGTATTTGGAACCTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCG
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ACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTT
CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTCCGCTGCGCCAACGCCTTCAACAACAGCATTATCC
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35 AACCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCCTGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
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ATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCAAAGCCCCAGGGATGTAATTACGTCC
40 CTCCCCGCTAGGGGGCAGCAGCGAGCCGCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
CAGCGTGCGGGGACAGCCGGGCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTG

AGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTGCGGCCGCCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGA
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5 GCCTGGCCTCTTTACGGGTTATGGCCCTTGCCTGCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATC
CCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCCTTAAGGAGCCCCCTTCGCCTCGTGCTTGAGTT
GAGGCCTGGCCTGGGCGCTGGGGCCGCCGCTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAA
GTCTCTAGCCATTTAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCC
AAGATCTGCACACTGGTATTTTCGGTTTTTTGGGGCCGCCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTTCG
10 GCGAGGCGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCC
TGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGTTCGGCACCAAGTTGCGTGAGCGGAAA
GATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCA
CCCACACAAAGGAAAAGGGCCTTTCGCTCCTCAGCCGTGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG
GCACCTCGATTAGTTCTCGAGCTTTTGAGTACGTCTTCTTAGGTTGGGGGAGGGTTTTATGCGATGGAGTTTC
15 CCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTTCTCCTTGAATTTGCCCTTTTT
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TGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACC
GCCAAGCTGAAAGTGACCAAAGGCGGCCCTCTGCCTTTTGCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAG
20 CAAGGCCTACGTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGA
GAGTGATGAACTTCGAGGACGGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCATCTAC
AAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAG
CAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAACTGAAGGATGGCGGCC
ACTACGACGCCGAAGTGAAAACCACTACAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATC
25 AAGCTGGACATCACCAGCCACAACGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCAC
AGGCGGAATGGACGAGCTGTACAAGAGAGCCAAACGGGGGTCCGGCGCTACCAATTTAGTTTGTCAAACAGGCCG
GAGACGTGAGGAAAATCCTGGCCCCATATGACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCT
GATGTGTATATCACAGACAAAACGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAG
CAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTTCTTCCCCAGCCCAG
30 GTAAGGGCAGCTTTGGTGCCCTTCGAGGCTGTTTCTTGTCTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCA
ATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAACCCCTCTTTTTACTAAGAAACA
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AGGCCTCATTCTAAGCCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCA
35 CTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGA
AGTGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTACG
CTGGGAAAAGTCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAA
AGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCTATAGA
GGCCTGGGACAGGAGCTCAATGAGAAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTAC
40 AGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTTCCATAGGCTCCGCCCCCTGAC
GAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCC

TGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAA
 GCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTG
 CACGAACCCCCCGTTACGCCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGA
 CTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGA
 5 AGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGA
 AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGAT
 TACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACTCATCGAGCATCAAATGAAACTGCAA
 TTTATTTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACCTACCGAGGC
 AGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTT
 10 CCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTT
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 TATTCATTCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAA
 TGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTTCTTAATACCTGGAA
 TGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAA
 15 GAGGCATAAATTCCGTAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGT
 TTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCCGACATTATCGCG
 AGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGACGTTTCCCGTTGAATATGGC
 TCATAACACCCCTTGATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTCGA
 ATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 54]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 4302 of SEQ ID NO: 55. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 5011 to 7629 of SEQ ID NO: 55. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 4302 and nucleotides 5011 to 7629 of SEQ ID NO: 55. SEQ ID NO: 55 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 GCAGGGTCAGTGGCTCCAACCTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 30 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTTCTGCTAATGCCAGCCTAAGTTGGGGAGACCACTCCAGATTTCCAAGATGTACAGTTTGC
 TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
 AATAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
 ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
 35 GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTG
 TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
 TATTCGAATTCGGTTACATAACTTACGTTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC
 AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAA

CTGCCCCACTTGCGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCG
CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCTATGGC
CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
5 CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
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10 CGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTT
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15 CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTGATGGCTATGGTCTC
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20 TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
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ATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAG
ACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTT
25 CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCGCTGCGCCAACGCCTTCAACAACAGCATTATCC
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30 TCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG
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35 CTTCTGCAGGAGCGTACAGAACCCAGGGCCCTGGCACCCGTGCAGACCCTGGCCCACCCACCTGGGCGCTCAGTGC
CCAAGAGATGTCCACACCTAGGATGTCCCGCGGTGGGTGGGGGGCCCCGAGAGACGGGCAGGCCGGGGGCAGGCCTGG
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CACCCCAAGGCGGCCAACGCCAAAACCTCTCCCTCCTCCTCTTCTCAATCTCGCTCTCGCTCTTTTTTTTTTTCGCA
AAAGGAGGGGAGAGGGGTAAAAAAATGCTGCACTGTGCGGCGAAGCCGGTGAGTGAGCGGCGCGGGGCCAATCAGC
40 GTGCGCCGTTCCGAAAGTTGCCTTTTATGGCTCGAGCGGCCGCGGCGGCCCTATAAAACCCAGCGGCGCGACGCG
CCACCACCGCCGAGACCGGTCCGCCCGCGAGCACAGAGCCTCGCCTTTGCCGAGGATCCGCCACCATGGTGTCCA

AGGGCGAAGAGGACAACATGGCCATCATCAAAGAGTTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGC
CACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACCAA
AGGGCGCCCTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCTACGTGAAGCACC
CCGCCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAACTTCGAGGAC
5 GGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCATCTACAAAGTGAAGCTGCGGGGCAC
CAACTTTCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCCTG
AAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCACTACGACGCCGAAGTGAAA
ACCACCTACAAGGCCAAGAAACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCA
CAACGAGGACTACACCATCGTGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGT
10 ACAAGAGAGCCAAACGGGGGTCCGGCGCTACCAATTTTCAAGTTTGTCTCAAACAGGCCGGAGACGTGAGGAAAATCCT
GGCCCCCATATGACCGATTTTGTATTCTCAAACAAATGTGTACAAAAGTAAGGATTCTGATGTGTATATCACAGACAA
AACTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCAT
GTGCAAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTTCTTCCCAGCCCAGGTAAGGGCAGCTTTGGTGCC
TTCGCAGGCTGTTTCTTGTCTCAGGAATGGCCAGGTTCTGCCAGAGCTCTGGTCAATGATGTCTAAAACCTCCTCT
15 GATTGGTGGTCTCGGCCTTATCCATTGCCACCAAAAACCTCTTTTACTAAGAAACAGTGAGCCTTGTCTGGCAGT
CCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAGCCTCAGTCTCTCC
AACTGAGTTCTCTGCCTGCCTGCCTTTGCTCAGACTGTTTGCCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCT
TCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCCAGCTCACTAAGTCAGTCTCACGCAGT
CACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAGGAATTA AAAAGTC
20 AGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAAAAGTCCAAATAAC
TTCAGATTGGAATGTGTTTAACTCAGGGTTGAGAAAAACAGTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTG
AAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCATAGAGGCCTGGGACAGGAGCTCAA
TGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTACAGGGCCTTCTAGGCCATGAG
AGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACG
25 CTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT
CTCCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGC
TCACGCTGTAGGTATCTCAGTTCGGTGATAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCC
CGACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAG
CCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGC
30 TACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTG
ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGAT
CTCAAGAAGATCCTTTGATCTTTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTAT
CAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGA
TCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTT
35 ATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTT
GTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTCGTGATTGCGCC
TGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACAC
TGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCG
CAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGCTCAGC
40 CAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGC
ATCGGGCTTCCCATAACAAGCGATAGATTGTGCGACCTGATTGCCCGACATTATCGCGAGCCATTTATACCCATATA

AATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTA
CTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTATCTTGTGCAATGTAACATCAGAGATTTTG
AGACAC [SEQ ID NO: 55]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
5 4274 of SEQ ID NO: 56. In certain embodiments, the Secondary Promoter Construct comprises
nucleotides 4983 to 7601 of SEQ ID NO: 56. In certain embodiments, the Secondary Promoter
Construct comprises nucleotides 1 to 4274 and nucleotides 4983 to 7601 of SEQ ID NO: 56
SEQ ID NO: 56 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
10 CTCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
GCAGGGTCAGTGGCTCCAACTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
GTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
15 TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTCGAAGATGTACAGTTTGC
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AATAAAAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTCAAGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGAAGTGGCAGCCCCACAGAGCCCCGCCCTTGTG
20 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCTAACCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTGCAATTCCGTTACATAACTTACGTTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACGTC
AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCG
25 CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGC
CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
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30 TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
AGTCAGTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCCGGGCCG
GGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTGAGCCTTCTGAGGC
CGAGATCAGCCACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACCTGTCTT
GGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCGCTCTGAAC
35 GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
CCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGT
CTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATC
CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTGATGGCTATGGTCTC
CCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCG

ACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTG
CCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTC
ACTGGAGTTGAGATGTAACCTATTCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCC
TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
5 AGTCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGA
ATTGGACACAGGCTTTTCAAAACTTGTATTTGGAAGTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCG
ATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAG
ACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTT
CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTCCGCTGCGCCAACGCCTTCAACAACAGCATTATCC
10 CCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACC
AACCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCC
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15 ATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCAAAGCCCCCAGGGATGTAATTACGTCC
CTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
CAGCGTGCGGGGACAGCCGGGCACGGGGAAGGTGGCACGGGATCGCTTTCCTCTGAACGCTTCTCGCTGCTCTTTG
AGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGAGTCGAATTCCACGGGGTTGGGGTTGCGCCTTTTC
CAAGGCAGCCCTGGGTTTTCGCGAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAACGCAGCGGCGCCGACCCTGG
20 GTCTCGCACATTCTTCACGTCCGTTTCGACGCGTCACCCGGATCTTCGCGCTACCCCTTGTGGGCCCCCGCGACGC
TTCCTGCTCCGCCCCCTAAGTCGGGAAGGTTCCCTTGCGGTTTCGCGCGTGCCTGGACGTGACAAACGGAAGCCGCACGT
CTCACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGTGGCCAATAG
CGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGT
GGGCCCTGTTCTGCCCCGCGGGTGTTCGCATTCTGCAAGCCTCCGGAGCGCACGTCCGGCAGTCCGCTCCCTCGTT
25 GACCGAATCACCGACCTCTCTCCCCAGGGATCCGCCACCATGGTGTCCAAGGCGAAGAGGACAACATGGCCATCAT
CAAAGAGTTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCG
AGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACCAAAGCGGGCCCTCTGCCTTTTGCCTGGGAC
ATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCT
GAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCTGGTCAACGTGACTCAAGATA
30 GCTCTCTGCAGGACGGCGAGTTTATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATG
CAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAA
GCAGCGGCTGAAACTGAAGGATGGCGGCCACTACGACGCCGAAGTAAAAACCACCTACAAGGCCAAGAAACCCGTGC
AGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCGCCACAACGAGGACTACACCATCGTGGAACAG
TACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGTACAAGAGAGCCAAACGGGGGTCCGGCGC
35 TACCAATTTTCAAGTTTGTCTCAAACAGGCCGGAGACGTGAGGAAAATCCTGGCCCCCATATGACCGATTTTGATTCTC
AAACAAATGTGTACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAATGTGCTAGACATGAGGTCTATGGAC
TTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTAT
TCCAGAAGACACCTTCTTCCCCAGCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCTTGTCTCAGGAA
TGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAATCCTCTGATTGGTGGTCTCGGCCTTATCCATTGC
40 CACCAAAACCTCTTTTTACTAAGAAACAGTGAGCCTTGTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGA
TGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCAGCCTCAGTCTCTCAACTGAGTTTCTGCCTGCCTGCCTTTGC

TCAGACTGTTTGCCCCCTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCC
 CTGTCTGCCAAAAAATCTTTCCAGCTCACTAAGTCAGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTG
 TGCCGGCACATGAATGCACCAGGTGTTGAAAGTGGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCAC
 CATTCTAGTTGGGGGAGCCCATCTGTGAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGG
 5 GTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCC
 TACCAAGGGCAGGGAGAGGACCCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGA
 GTTGAATGAAGGAGGCAGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGG
 CGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACA
 GGACTATAAAGATAACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGG
 10 ATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT
 AGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTAGCCCCGACCGCTGCGCCTTATCCGGTAACTAT
 CGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG
 GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCT
 GCGCTCTGCTGAAGCCAGTTACCTTCGGA AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGC
 15 GGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAA
 AACTCATCGAGCATCAAATGAAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTT
 CTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTC
 GTCCAACATCAATACAACCTATTAATTTCCCTCGTCAAAAAATAAGTTATCAAGTGAGAAATCACCATGAGTGACG
 ACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTC
 20 ATCAAAATCACTCGCATCAACCAAACCGTTATTCATTTCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGT
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 GAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGG
 AGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAA
 CATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATT
 25 GTCGCACCTGATTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCG
 CGGCCTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTTATTACTGTTTATGTAAGCAGACAGTTTATTG
 TTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 56]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 4105 of SEQ ID NO: 57. In certain embodiments, the Secondary Promoter Construct comprises
 30 nucleotides 4814 to 7432 of SEQ ID NO: 57. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 4105 and nucleotides 4814 to 7432 of SEQ ID NO: 57
 SEQ ID NO: 57 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 35 GCAGGGTCAGTGGCTCCAACCTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 TACCATAAACCTCCCATTTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTTCAAGATGTACAGTTTGC

TTTGCTGGGCCTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
AATAAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
GCTGGTTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
5 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCCTGATCCTCTTG
TCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
TATTCGAATTCGGTTACATAACTTACGTTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTC
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CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
10 CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
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15 TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
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GGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGC
CGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACGTCTT
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20 GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
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30 ATTGGACACAGGCTTTCAGAACTTGTATTTGGAACCTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCG
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ACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTT
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35 AACCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCCTGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
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40 CTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
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5 TTCCAGAATTAACAGTATAAATTGCATCTCTTGTTCAGAGTTCCCTATCACTCTCTTTAATCACTACTCACAGTAA
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GTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACG
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10 CTTCAAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACG
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GGCTGGGAAGCCAGCAGCGAGAGAATGTACCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAAACT
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15 GGCAGACACAGCACAGGCGGAATGGACGAGCTGTACAAGAGAGCCAAACGGGGGTCCGGCGCTACCAATTTAGTTT
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GCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTT
CTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCC
20 CAGAGCTCTGGTCAATGATGTCTAAAACTCCTCTGATTGGTGGTCTCGGCCTTATCCATTGCCACCAAACCCCTCTT
TTTACTAAGAAACAGTGAGCCTTGTTCTGGCAGTCCAGAGAAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGC
AGGAGAGGGCACGTGGCCAGCCTCAGTCTCTCAAATGAGTTCTGCTGCCTGCCTGCTTTGCTCAGACTGTTTGCCC
CTTACTGCTCTTCTAGGCCTCATTCTAAGCCCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAA
TCTTTCCCAGCTCACTAAGTCAGTCTCACGCGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAAT
25 GCACCAGGTGTTGAAGTGGAGGAATTA AAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGG
AGCCCATCTGTGAGCTGGGAAAAGTCCAAATAACTTCAGATTGGAATGTGTTTTAACTCAGGGTTGAGAAAACAGCT
ACCTTCAGGACAAAAGTCAGGGAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGA
GAGGACCCTATAGAGGCCTGGGACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGG
CAGGGCCGGGTACAGGGCCTTCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGC
30 TCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATAC
CAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTT
TCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCA
AGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAGTCCAAC
CCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGC
35 TACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGC
CAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGT
TGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACTCATCGAGCATC
AAATGAACTGCAATTTATTATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGA
AACTACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATAC
40 AACCTATTAATTTCCCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAG
AATGGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAATCACTCGC

ATCAACCAAACCGTTATTTCATTCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTAAAAGGACAATTAC
 AAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCT
 TCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATG
 CTTGATGGTCGGAAGAGGCATAAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGC
 5 TACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGC
 CCGACATTATCGCGAGCCCATTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGACGTTTC
 CCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATAT
 TTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 57]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 10 4007 of SEQ ID NO: 58. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 4716 to 7334 of SEQ ID NO: 58. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 4007 and nucleotides 4716 to 7334 of SEQ ID NO: 58
 SEQ ID NO: 58 is provided below.

GGTACCACATTAAAAACACAAAATCCTACGGAAATACTGAAGAATGAGTCTCAGCACTAAGGAAAAGCCTCCAGCAG
 15 CTCCTGCTTTCTGAGGGTGAAGGATAGACGCTGTGGCTCTGCATGACTCACTAGCACTCTATCACGGCCATATTCTG
 GCAGGGTCAGTGGCTCCAACCTAACATTTGTTTGGTACTTTACAGTTTATTAAATAGATGTTTATATGGAGAAGCTCT
 CATTTCTTTCTCAGAAGAGCCTGGCTAGGAAGGTGGATGAGGCACCATATTCATTTTGCAGGTGAAATTCCTGAGAT
 GTAAGGAGCTGCTGTGACTTGCTCAAGGCCTTATATCGAGTAAACGGTAGTGCTGGGGCTTAGACGCAGGTGTTCTG
 ATTTATAGTTCAAAACCTCTATCAATGAGAGAGCAATCTCCTGGTAATGTGATAGATTTCCCAACTTAATGCCAACA
 20 TACCATAAACCTCCCATTCTGCTAATGCCCAGCCTAAGTTGGGGAGACCACTCCAGATTTCCAAGATGTACAGTTTGC
 TTTGCTGGGCCTTTTTTCCCATGCCTGCCTTTACTCTGCCAGAGTTATATTGCTGGGGTTTTGAAGAAGATCCTATTA
 AATAAAGAATAAGCAGTATTATTAAAGTAGCCCTGCATTTACAGTTTCCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
 ACGTTCACTGAAATCATGGCCTCTTGCCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
 GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
 25 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTG
 TCCCACAGATATCCAGAACCCTGACCCTGCCGTGACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
 TATTTCGAATTCCGTTACATAACTTACGTTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTC
 AATAATGATGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAA
 CTGCCCCTTGGCAGTACATCAAGTGTATCATATCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG
 30 CCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATT
 ACCATGGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTTATGGC
 CACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATG
 CTGGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGG
 AGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGA
 35 TATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGG
 AGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCG
 GGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGC
 CGAGATCAGCCACACACAGAAAGCCACACTCGTGTGCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTT
 GGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAAC

GACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATG
CCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGT
CTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATC
CTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGCTGATGGCTATGGTCTC
5 CCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCG
ACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTG
CCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTC
ACTGGAGTTGAGATGTAACATATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCC
TCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGG
10 AGTCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGA
ATTGGACACAGGCTTTTCAGAACTTGTATTTGGAAGTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCG
ATCCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAG
ACCAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTT
CAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCGCTGCGCCAACGCCTTCAACAACAGCATTATCC
15 CCGAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACC
AACCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGAT
GACCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCC
TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCTTAATAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTG
TCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG
20 ATGCGGTGGGCTCTATGGCGTCGACGAGCTCACGGGGACAGCCCCCCCCCAAAGCCCCAGGGATGTAATTACGTCC
CTCCCCCGCTAGGGGGCAGCAGCGAGCCGCCCGGGGCTCCGCTCCGGTCCGGCGCTCCCCCGCATCCCCGAGCCGG
CAGCGTGCGGGGACAGCCCGGGCACGGGGAAGGTGGCACGGGATCGCTTTCTCTGAACGCTTCTCGCTGCTCTTTG
AGCCTGCAGACACCTGGGGGGATACGGGGAAAAAGCTTCTCGAGGGGACTTTCCGCTTGGGGACTTTCCGCTGGGGA
CTTTCCGCTGGGGACTTTCCGCTGGGGACTTTCCGAATTCAAGCTTGAATAAAAATGAATATTAGAAGCTGTTAGAATA
25 AGAGAAAATGACAGAGGAAAACCTGAAAGGGAGAACTGAAAGTGGGAAATTCTCTGAGGCAGAAAGGACCATCCCTT
ATAAATAGCACAGGCCATGAAGGAAGATCATTTCTCACTGCAGCCTTTGACAGCCTTTGCCTCATCTTGGGATCCGCC
ACCATGGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATCAAAGAGTTTCATGCGGTTCAAGGTGCACATGGAAGG
CAGCGTGAACGGCCACGAGTTTCGAGATTGAAGGCGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGC
TGAAAGTGACCAAAGGCGGCCCTCTGCCTTTTGCCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCC
30 TACGTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGAT
GAACTTCGAGGACGGCGGCGTGGTCACCGTGACTCAAGATAGCTCTCTGCAGGACGGCGAGTTCATCTACAAAGTGA
AGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAG
AGAATGTACCCTGAAGATGGCGCCCTGAAGGGCGAGATCAAGCAGCGGCTGAACTGAAGGATGGCGGCCACTACGA
CGCCGAAGTGAAAACCACTACAAGGCCAAGAAAACCGTGCAGCTGCCCTGGCGCCTACAACGTGAACATCAAGCTGG
35 ACATCACCAGCCACAACGAGGACTACACCATCGTGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGA
ATGGACGAGCTGTACAAGAGAGCCAAACGGGGGTCCGGCGCTACCAATTTAGTTTGTCTCAAACAGGCCGGAGACGT
CGAGGAAAATCCTGGCCCCCATATGACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGT
ATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAA
TCTGACTTTGCATGTGCAACGCCTTCAACAACAGCATTATTCAGAAAGACACCTTCTTCCCCAGCCAGGTAAGGG
40 CAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGT
CTAAAACTCCTCTGATTGGTGGTCTCGGCCCTATCCATTGCCACCAAAACCTCTTTTTACTAAGAAACAGTGAGCC

TTGTTCTGGCAGTCCAGAGAATGACACGGGAAAAAAGCAGATGAAGAGAAGGTGGCAGGAGAGGGCACGTGGCCCAG
 CCTCAGTCTCTCCAAGTGGAGTTCCTGCCTGCCTGCCTTTGCTCAGACTGTTTGGCCCTTACTGCTCTTCTAGGCCTC
 ATTCTAAGCCCTTCTCCAAGTTGCCTCTCCTTATTTCTCCCTGTCTGCCAAAAAATCTTTCCAGCTCACTAAGTC
 AGTCTCACGCAGTCACTCATTAACCCACCAATCACTGATTGTGCCGGCACATGAATGCACCAGGTGTTGAAGTGGAG
 5 GAATTAAAAAGTCAGATGAGGGGTGTGCCAGAGGAAGCACCATTCTAGTTGGGGGAGCCCATCTGTCAGCTGGGAA
 AAGTCCAAATAACTTCAGATTGGAATGTGTTTAACTCAGGGTTGAGAAAACAGCTACCTTCAGGACAAAAGTCAGG
 GAAGGGCTCTCTGAAGAAATGCTACTTGAAGATACCAGCCCTACCAAGGGCAGGGAGAGGACCCATATAGAGGCCTGG
 GACAGGAGCTCAATGAGAAAGGAGAAGAGCAGCAGGCATGAGTTGAATGAAGGAGGCAGGGCCGGGTACAGGGCCT
 TCTAGGCCATGAGAGGGTAGACAGGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATC
 10 ACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTGGAAGC
 TCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGC
 GCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAAC
 CCCCCGTTGAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCG
 CCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTG
 15 GCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGGAGAG
 TTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGC
 AGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAAACTCATCGAGCATCAAATGAACTGCAATTTATTC
 ATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACACCCGAGGCAGTTCCA
 TAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCTCGT
 20 CAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATT
 TCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTCAT
 TCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACC
 GGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTT
 TTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCAT
 25 AAATTCCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAA
 ACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTGCGACCTGATTGCCCCGACATTATCGCGAGCCCAT
 TTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGACGTTTCCCGTTGAATATGGCTCATAAC
 ACCCCTTGATTACTGTTTATGTAAGCAGACAGTTTTATTTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAAC
 ATCAGAGATTTTGAGACAC [SEQ ID NO: 58]

30 In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2881 of SEQ ID NO: 59. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 3759 to 5679 of SEQ ID NO: 59. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2881 and nucleotides 3759 to 5679 of SEQ ID NO: 59. SEQ ID NO: 59 is provided below.

35 GGTACCTCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTT
 TCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACT
 GGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCACA
 GATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTTCGA
 ATTCCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGCCA

CCGGCTCTAGAACAAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATGCT
GGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGGAG
CCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATA
TTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG
5 TCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCGGG
CACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGCCG
AGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACGTCTTGG
TGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAACGA
CAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATGCC
10 AGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCT
GCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATCCT
GTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGATGGCTATGGTCTCCC
GGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGAC
GTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCC
15 TTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCAC
TGGAGTTGAGATGTAACCTATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTC
CAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGGAG
TCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGAAT
TGGACACAGGCTTTTCAGAACTTGTATTTGGAACGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCGAT
20 CCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAGAC
CAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTTCA
AGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCGCTGCGCCAACGCCTTCAACAACAGCATTTATCCCC
GAGGACACATTCTTCCCAAGTCCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAA
CCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGATGA
25 CCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCCTGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCAAGCTTGAACAGAGAAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
CTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCT
30 GCCCCGGCTCAGGGCCAAGAACAGATGGTCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATG
TTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTTCGCTTCTCGCTTCTG
TTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT
CCACGCTGTTTTGACTTCCATAGAAGGGATCCTCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGT
TGACAGTGAGCGAAAGATGTCAAGATTGAGCCTTTAGTGAAGCCACAGATGTAAAGGCTCAATCTTGACATCTTGTG
35 CCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGTCGACGCCACCATGGTGTCCAAGGGCGAAGAGGACAACATG
GCCATCATCAAAGAGTTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGG
CGAAGGCGAGGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAGTGACCAAAGGCGGCCCTCTGCCTTTTG
CCTGGGACATTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCACGTGAAGCACCCCGCCGATATTCCCAGCTAC
CTGAAGCTGAGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTGGTCACCGTGAC
40 TCAAGATAGCTCTCTGCAGGACGGCGAGTTTCATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCC
CCGTGATGCAGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCCTGAAGATGGCGCCCTGAAGGGC

GAGATCAAGCAGCGGCTGAAACTGAAGGATGGCGGCCACTACGACGCCGAAGTGAAAACCACCTACAAGGCCAAGAA
 ACCCGTGCAGCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCACAACGAGGACTACACCATCG
 TGGAACAGTACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGTACAAGCGGGCTAAGAGAGGC
 TCTGGAGCCACAACTTTAGCCTCCTGAAACAAGCAGGGGATGTGGAAGAGAATCCCGGACCTGCGGCCGCACCGAT
 5 TTTGATTCTCAAACAAATGTGTCAAAAGTAAGGATTCTGATGTGTATATCACAGACAAACTGTGCTAGACATGAG
 GTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACA
 ACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTT
 GCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAACTCCTCTGATTGCTAGCCGCGTTGC
 TGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG
 10 ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTAC
 CGGATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGG
 TGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAAC
 TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC
 GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTA
 15 TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGT
 AGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAG
 AAAAATCATCGAGCATCAAATGAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCG
 TTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGA
 CTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAAATAAGGTATCAAGTGAGAAATCACCATGAGTG
 20 ACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTC
 GTCATCAAAATCACTCGCATCAACCAAACCGTTATTCATTCTGATGTCGCTGAGCCAGACGAAATACGCGATCGC
 TGTATAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCA
 CCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATC
 AGGAGTACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATCCGTCAGCCAGTTTAGTCTGACCATCTCATCTG
 25 TAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAG
 ATTGTGCGACCTGATTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAA
 TCGCGGCTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTTA
 TTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 59]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 30 2887 of SEQ ID NO: 60. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 3837 to 5691 of SEQ ID NO: 60. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 2887 and nucleotides 3837 to 5691 of SEQ ID NO: 60.
 SEQ ID NO: 60 is provided below.

GGTACCTCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTT
 35 TCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACT
 GGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCTGATCCTCTTGTCCACAA
 GATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCATTTTCA
 ATTTCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGCCA
 CCGGCTCTAGAACAAGCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATGCT

GGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGAG
CCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATA
TTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG
TCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCGGG
5 CACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGCCG
AGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTTGG
TGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCGCTCTGAACGA
CAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATGCC
AGGTCCAGTTCTACGGCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCT
10 GCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATCCT
GTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGCTGATGGCTATGGTCTCCC
GGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGAC
GTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCC
TTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCAC
15 TGGAGTTGAGATGTAACATATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCGGCCAAGGCCTC
CAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGGAG
TCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGAAT
TGGACACAGGCTTTCAGAACTTGTATTTGAACTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCGAT
CCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTCCTGTTACCGACTTCGACAGCCAGAC
20 CAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTTCA
AGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTCCGCTGCGCCAACGCCTTCAACAACAGCATATCCCC
GAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAA
CCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGATGA
CCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCTT
25 GACCCTGGAAGGTGCCACTCCCCTGTCTTTCCCTAATAAAATGAGGAAATTGCATCGCATTTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCAAGCTTGAACAGAGAAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
CTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCT
GCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATG
30 TTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTG
TTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT
CCACGCTGTTTTGACTTCCATAGAAGGGATCCGCCACCATGGTGTCCAAGGGCGAAGAGGACAACATGGCCATCATC
AAAGAGTTCATGCGGTTCAAGGTGCACATGGAAGGCAGCGTGAACGGCCACGAGTTCGAGATTGAAGGCGAAGGCGA
GGGCAGACCTTACGAGGGAACACAGACCGCCAAGCTGAAAAGTGACCAAAGGCGGCCCTCTGCCTTTTGCCTGGGACA
35 TTCTGAGCCCTCAGTTTATGTACGGCAGCAAGGCCTACGTGAAGCACCCCGCCGATATTCCCGACTACCTGAAGCTG
AGCTTCCCCGAGGGCTTCAAGTGGGAGAGAGTGATGAACTTCGAGGACGGCGGCGTGGTACCCTGACTCAAGATAG
CTCTCTGCAGGACGGCGAGTTCATCTACAAAGTGAAGCTGCGGGGCACCAACTTTCCCTCTGATGGCCCCGTGATGC
AGAAAAAGACAATGGGCTGGGAAGCCAGCAGCGAGAGAATGTACCTGAAGATGGCGCCCTGAAGGGCGAGATCAAG
CAGCGGCTGAACTGAAGGATGGCGGCCACTACGACGCCGAAAGTGAAGAACCACTACAAGGCCAAGAAACCCGTGCA
40 GCTGCCTGGCGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCACAACGAGGACTACACCATCGTGGAACAGT
ACGAGAGAGCCGAAGGCAGACACAGCACAGGCGGAATGGACGAGCTGTACAAGCGGGCTAAGAGAGGCTCTGGAGCC

ACAAACCTTTAGCCTCCTGAAACAAGCAGGGGATGTGGAAGAGAATCCCGGACCTACTAGTTCGACTTCTTAACCCAA
 CAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAAAGATGTCAAGATTGAGCCTTTAGTGAAGCCACAGA
 TGTAAGGCTCAATCTTGACATCTTGTGCCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGTCGACTGCGATGC
 GGCCGCACCGATTTTATTCTCAAACAAATGTGTACAAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACGT
 5 GCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAA
 ACGCCTTCAACAACAGCATTATTCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCA
 GGCTGTTTCTTGTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACCTCCTCTGATTGC
 TAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG
 TGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGAC
 10 CCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGT
 ATCTCAGTTCGGTGTAGGTCTGTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCCAGCCGACCGCTGCGCC
 TTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAG
 GATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA
 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAA
 15 ACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCC
 TTTGATCTTTAGAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTTCATATCAGGATTATCAATACCATATTT
 TTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGT
 CTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAAATAAGTTATCAAGTGAGAAA
 TCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCA
 20 GCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTGCTGATTGCGCCTGAGCCAGACGAA
 ATACGCGATCGCTGTTAAAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCA
 ACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAA
 CCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAAGAGGCATAAATTCCTGTCAGCCAGTTTAGTCTGA
 CCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCA
 25 TACAAGCGATAGATTGTGCGACCTGATTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCAT
 GTTGGAATTTAATCGCGGCGCTCGACGTTTTCCCGTTGAATATGGCTCATAACACCCCTTGATTACTGTTTATGTAAG
 CAGACAGTTTTTATTGTTTCATGATGATATATTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ
 ID NO: 60]

30 In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 3007 of SEQ ID NO: 61. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 3165 to 5134 of SEQ ID NO: 61. In certain embodiments, the Secondary Promoter
 Construct comprises nucleotides 1 to 3007 and nucleotides 3165 to 5134 of SEQ ID NO: 61.
 SEQ ID NO: 61 is provided below.

35 GGTACCTCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTT
 TCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTTCCATCACT
 GGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTGTCCACA
 GATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTTCGA
 ATTCCGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGCCA

CCGGCTCTAGAACAAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATGCT
GGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGGAG
CCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATA
TTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG
5 TCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCGGG
CACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGCCG
AGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACGTCTTGG
TGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCCGCTCTGAACGA
CAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATGCC
10 AGGTCCAGTTCTACGGCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCT
GCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATCCT
GTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGATGGCTATGGTCTCCC
GGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGAC
GTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCC
15 TTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCAC
TGGAGTTGAGATGTAACCTATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTC
CAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGGAG
TCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGAAT
TGGACACAGGCTTTTCAGAACTTGTATTTGGAACGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCGAT
20 CCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAGAC
CAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTTCA
AGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCGCTGCGCCAACGCCTTCAACAACAGCATTTATCCCC
GAGGACACATTCTTCCCAAGTCCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAA
CCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGATGA
25 CCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCCTGTCTTTCTTAATAAAATGAGGAAATTGCATCGCATTTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCAAGCTTGAACAGAGAAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
CTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCT
30 GCCCCGGCTCAGGGCCAAGAACAGATGGTCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATG
TTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTG
TTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT
CCACGCTGTTTTGACTTCCATAGAAGGGATCCCTGACTACATCACAGCAGCATACGTGGAGATGAGGCGAGAGGCTT
GGGCTAGTAAGGATGCCACCTATACTTCTGCCCGGACCCTGCTGGCTATCCTGCGCCTTTCCACTGCTCTGGTAAGT
35 GCCCTCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAAAGATGTCAAGATTG
AGCCTTTAGTGAAGCCACAGATGTAAAGGCTCAATCTTGACATCTTGTGCCTACTGCCTCGGACTTCAAGGGGCTAC
TTTAGGATTCCCCTTAGGCACGTCTGAGAATGGTGGATGTGGTGGAGAAAGAAGATGTGAATGAAGCCATCAGGCTA
ATGGAGATGTCAAAGGACTCTCTTCTAGGAGACAAGGGGCAGACAGTCGACTGCGATGCGGCCGCACCGATTTTGAT
TCTCAAACAAATGTGTCAAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACGTGTGCTAGACATGAGGTCTAT
40 GGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCA
TTATTCCAGAAGACACCTTCTTCCCCAGCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCTTGCTTCA

GGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAACTCCTCTGATTGCTAGCCGCGTTGCTGGCGT
 TTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGA
 CTATAAAGATAACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATA
 CCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGG
 5 TCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGT
 CTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
 TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCG
 CTCTGCTGAAGCCAGTTACCTTCGGA AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGT
 GGTTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAAAC
 10 TCATCGAGCATCAAATGAACTGCAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTG
 TAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTC
 CAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACT
 GAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATC
 AAAATCACTCGCATCAACCAACCGTTATTCATTCTGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAA
 15 AAGGACAATTACAAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAA
 TCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGT
 ACGGATAAAATGCTTGATGGTTCGGAAGAGGCATAAATTCGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACAT
 CATTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTC
 GCACCTGATTGCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGG
 20 CCTCGACGTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTT
 ATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 61]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to
 312 of SEQ ID NO: 62. In certain embodiments, the Secondary Promoter Construct comprises
 nucleotides 470 to 4001 of SEQ ID NO: 62. In certain embodiments, the Secondary Promoter
 25 Construct comprises nucleotides 1 to 312 and nucleotides 470 to 4001 of SEQ ID NO: 62. SEQ
 ID NO: 62 is provided below.

GGTACCAGAATAAGCAGTATTATTAAGTAGCCCTGCATTTTCAGGTTTCCTTGAGTGGCAGGCCAGGCCTGGCCGTGA
 ACGTTCACTGAAATCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCA
 GCTGGTTTCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTC
 30 CATCACTGGCATCTGGACTCCAGCCTGGGTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCCCTGATCCTCTGA
 ATTCTCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCGAAAGATGTCAAGATTG
 AGCCTTTAGTGAAGCCACAGATGTAAAGGCTCAATCTTGACATCTTGTGCCTACTGCCTCGGACTTCAAGGGGCTAC
 TTTAGGAGTGCAGTGTCCACAGATATCCAGAACCCCGACCCCGCCGTGTACCAGCTGCGGGACAGCAAGAGCAGCG
 ACAAGAGCGTGTGCCTGTTGGCTCCGGAGGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACC
 35 CCGGTCCTATGGCCACCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAA
 GGATCTGCTGATGCTGGAATCACCCAGAGCCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTG
 TCACCAGACTTGAGCCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACT
 CAGCAGCTGCTGATATTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTT
 CCCCTCACTCTGGAGTCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCA

GTACTTCGGGCGGGGCACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCG
AGCCTTCTGAGGCCGAGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCAC
GTGGAACGTGTCTTGGTGGGTCAACGGCAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACA
GCCCCGTCTGAACGACAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCAGAA
5 ACCACTTCAGATGCCAGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTG
ACACAGATCGTGTCTGCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCT
GTCTGCCACCATCCTGTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTCTGA
TGGCTATGGTCTCCCGGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTG
AAGCAGGCCGGCGACGTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGG
10 ACTGCTGTGTCTGCCTTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCT
CTGAAGGAGCCTCACTGGAGTTGAGATGTAACATATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCC
CCCGGCCAAGGCCTCCAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGC
TGAATTTAAGAGGAGTCAATCTTCCTTCAATCTGAGGAAACCCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCT
GTGCTGTGGGTGAATTGGACACAGGCTTTTCAGAACTTGTATTTGAACTGGCACCCGACTTCTGGTCAGTCCAAAT
15 ATTCAGAACCCCGATCCTGCTGTGTATCAGCTGCGCGACAGCAAGAGCAGCGACAAGAGCGTGTGTTTGTTCACCGA
TTTTGATTCTCAAACAAATGTGTCAAAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACGTGCTAGACATGA
GGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAAC
AACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCCT
TGCTTCAGGAATGGCCAGGTTCTGCCCAGAGCTCTGGTCAATGATGTCTAAACCTCCTCTGATTGGCGGCCGCTGCG
20 ATGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCA
GAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTT
CGACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGGAAGCGTGGCGCTTTTCTCATAGCTCACGCTGT
AGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTACGCCCCAGCCGTG
CGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTA
25 ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGA
AGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAA
ACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAG
ATCCTTTGATCTTTAGAAAAACTCATCGAGCATCAAATGAACTGCAATTTATTCATATCAGGATTATCAATACCAT
ATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTAT
30 CGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCTCGTCAAAAATAAGGTTATCAAGTGA
GAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCACAG
GCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACCGTTATTTCATTGCTGATTGCGCCTGAGCCAGA
CGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCGAATGCAACGGGCGCAGGAACACTGCCAGCGC
ATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGA
35 GTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTGGGAAGAGGCATAAATTCGTCAGCCAGTTTAGT
CTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTT
CCCATAACAAGCGATAGATTGTGCGACCTGATTGCCCCGACATTATCGCGAGCCCATTATACCCATATAAATCAGCAT
CCATGTTGGAATTTAATCGCGGCCCTCGACGTTTTCCCGTTGAATATGGCTCATAACACCCCTTGTATTACTGTTTATG
TAAGCAGACAGTTTTTATTGTTTCATGATGATATATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGGAGACAC
40 [SEQ ID NO: 62]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2881 of SEQ ID NO: 63. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 3039 to 4893 of SEQ ID NO: 63. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2881 and nucleotides 3039 to 4893 of SEQ ID NO: 63.

5 SEQ ID NO: 63 is provided below.

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GGTACCTCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTT
TCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACT
GGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCCTAACCTGATCCTCTTGTCCCACA
GATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCTGA
10 ATTTCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGTCTATGGCCA
CCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATGCT
GGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGGAG
CCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATA
TTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG
15 TCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCGGG
CACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGCCG
AGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAAGTGTCTTGG
TGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCGCTCTGAACGA
CAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATGCC
20 AGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCT
GCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATCCT
GTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGTGATGGCTATGGTCTCCC
GGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCGAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGAC
GTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCC
25 TTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCAC
TGGAGTTGAGATGTAACATATTCCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTC
CAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGGCTTTGAGGCTGAATTTAAGAGGAG
TCAATCTTCCTTCAATCTGAGGAAACCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGAAT
TGGACACAGGCTTTCAGAACTTGTATTTGGAAGTGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCGAT
30 CCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCCGACTTCGACAGCCAGAC
CAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGCTGGACATGCGGAGCATGGACTTCA
AGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTGCGCTGCGCCAACGCCTTCAACAACAGCATTATCCCC
GAGGACACATTCTTCCCAAGTCCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAA
CCTGAACTTCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGATGA
35 CCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCCTGTCTTTCTTAATAAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCAAGCTTGAACAGAGAAACAGGAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTC
CTGCCCCGGCTCAGGGCCAAGAACAGTTGGAACAGCAGAAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCCT

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GCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCGGTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATG
 TTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTCGCTTCTCGCTTCTG
 TTCGCGCGCTTCTGCTCCCCGAGCTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT
 CCACGCTGTTTTGACTTCCATAGAAGGGATCCTCGACTTCTTAACCCAACAGAAGGCTCGAGAAGGTATATTGCTGT
 5 TGACAGTGAGCGAAAGATGTCAAGATTGAGCCTTTAGTGAAGCCACAGATGTAAAGGCTCAATCTTGACATCTTGTG
 CCTACTGCCTCGGACTTCAAGGGGCTACTTTAGGAGTCGACTGCGATGCGGCCGCACCGATTTTGATTCTCAAACAA
 ATGTGTACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAAG
 AGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTTATCCAGA
 AGACACCTTCTTCCCCAGCCAGGTAAGGGCAGCTTTGGTGCCCTTCGCAGGCTGTTTCCTTGCTTCAGGAATGGCCA
 10 GGTCTGCCCAGAGCTCTGGTCAATGATGTCTAAAACCTCCTCTGATTGCTAGCCGCGTTGCTGGCGTTTTTCCATAG
 GCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGAT
 ACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCC
 TTTCTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGTTGCTC
 CAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACCTATCGTCTTGAGTCCA
 15 ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGT
 GCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA
 GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTG
 TTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACTCATCGAGCA
 TCAAATGAACTGCAATTTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGA
 20 GAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAAT
 ACAACCTATTAATTTCCCCCTCGTCAAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTG
 AGAATGGCAAAAGTTTATGCATTTCTTTCCAGACTTGTTCAACAGGCCAGCCATTACGCTCGTCATCAAAATCACTC
 GCATCAACCAAACCGTTATTTCATTCTGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAGGACAATT
 ACAACAGGAATCGAATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATT
 25 CTTCTAATACCTGGAATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAA
 TGCTTGATGGTTCGGAAGAGGCATAAATTCGGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAAC
 GCTACCTTTGCCATGTTTCAGAAACAACCTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATT
 GCCCCGACATTATCGCGAGCCCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCCTCGACGTT
 TCCCGTTGAATATGGCTCATAACACCCCTTGTTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATAT
 30 ATTTTTATCTTGTGCAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 63]

In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2736 of SEQ ID NO: 64. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 2788 to 4648 of SEQ ID NO: 64. In certain embodiments, the Secondary Promoter Construct comprises nucleotides 1 to 2736 and nucleotides 2788 to 4648 of SEQ ID NO: 64.

35 SEQ ID NO: 64 is provided below.

GGTACCTCATGGCCTCTTGGCCAAGATTGATAGCTTGTGCCTGTCCCTGAGTCCCAGTCCATCACGAGCAGCTGGTT
 TCTAAGATGCTATTTCCCGTATAAAGCATGAGACCGTGACTTGCCAGCCCCACAGAGCCCCGCCCTTGTCCATCACT
 GGCATCTGGACTCCAGCCTGGGTTGGGGCAAAGAGGGAAATGAGATCATGTCTAACCCTGATCCTCTTGTCCACA
 GATATCCAGAACCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCTGA

ATTTCGGCTCCGGAGCCACTAACTTCTCCCTGTTGAAACAGGCTGGCGATGTTGAAGAAAACCCCGGTCTATGGCCA
CCGGCTCTAGAACAAGCCTGCTGCTCGCTTTTGGCTGCTCTGCCTCCCATGGCTCCAAGAAGGATCTGCTGATGCT
GGAATCACCCAGAGCCCAAGATACAAGATCACAGAGACAGGAAGGCAGGTGACCTTGATGTGTACCAGACTTGGAG
CCACAGCTATATGTTCTGGTATCGACAAGACCTGGGACATGGGCTGAGGCTGATCTATTACTCAGCAGCTGCTGATA
5 TTACAGATAAAGGAGAAGTCTCCGATGGCTATGTTGTCTCCAGATCCAAGACAGAGAATTTCCCCCTCACTCTGGAG
TCAGCTACCCGCTCCCAGACATCTGTGTATTTCTGCGCCAGCAGTGAGGACAGTTACGAGCAGTACTTCGGGCCGGG
CACCAGGCTCACGGTCACAGAGGACCTGAAAAACGTGTTCCCTCCAAAAGTGGCCGTGTTTCGAGCCTTCTGAGGCCG
AGATCAGCCACACACAGAAAGCCACACTCGTGTGTCTGGCTACCGGCTTCTACCCCGATCACGTGGAACGTCTTGG
TGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCAGCACAGATCCCCAGCCTCTGAAAGAACAGCCGCTCTGAACGA
10 CAGCCGCTACTGCCTGTCTAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCAGAAACCACTTCAGATGCC
AGGTCCAGTTCTACGGCCTGAGCGAGAACGATGAGTGGACCCAGGACAGAGCCAAGCCTGTGACACAGATCGTGTCT
GCCGAAGCCTGGGGCAGAGCCGATTGTGGCTTTACCAGCGAGTCATACCAGCAGGGCGTGCTGTCTGCCACCATCCT
GTATGAGATCCTGCTCGGCAAGGCCACACTGTACGCTGTGCTGGTGTCTGCTCTGGTGCTGATGGCTATGGTCTCCC
GGGAGCGCATCCCCGAGGCCCGGGCCAAGCGGGGCAGCGGCCACCAACTTCAGCCTGCTGAAGCAGGCCGGCGAC
15 GTGGAGGAGAACCCCGGCCCTATGGCCACAGGCAGCAGAACATCTCTGCTGCTGGCCTTCGGACTGCTGTGTCTGCC
TTGGCTGCAAGAGGGTTCCGCCGCCAGTCAGTGACCCAGCCTGACATCCACATCACTGTCTCTGAAGGAGCCTCAC
TGGAGTTGAGATGTAACATATTCTATGGGGCAACACCTTATCTCTTCTGGTATGTCCAGTCCCCCGGCCAAGGCCTC
CAGCTGCTCCTGAAGTACTTTTCAGGAGACACTCTGGTTCAAGGCATTAAAGCCTTTGAGGCTGAATTTAAGAGGAG
TCAATCTTCCTTCAATCTGAGGAAACCCCTCTGTGCATTGGAGTGATGCTGCTGAGTACTTCTGTGCTGTGGGTGAAT
20 TGGACACAGGCTTTTCAGAACTTGTATTTGGAACGGCACCCGACTTCTGGTCAGTCCAAATATTCAGAACCCCGAT
CCTGCCGTGTACCAGCTGAGAGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACAGCCAGAC
CAACGTGTCCAGAGCAAGGACAGCGACGTGTACATCACCGACAAGACCGTGTGGACATGCGGAGCATGGACTTCA
AGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGATTTTCGCCTGCGCCAACGCCTTCAACAACAGCATTATCCCC
GAGGACACATTCTTCCCAAGTCTGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAA
25 CCTGAACTTCCAGAACCTGTCCGTGATCGGCTTCCGCATCCTGCTGCTGAAAGTGGCCGGCTTCAACCTGCTGATGA
CCCTGAGACTGTGGTCCAGCTGATGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCCTCCCCCGTGCCTTCCTT
GACCCTGGAAGGTGCCACTCCCCTGTCTTTCTAATAAAAAAGAGGAAATTGCATCGCATTTGTCTGAGTAGGTGTC
ATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGAT
GCGGTGGGCTCTATGGCAAGCTTGAGGGCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTT
30 AGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGACGTAGAAAAGTAATAATT
TCTTGGGTAGTTTGCAGTTTTAAATATATGTTTTAAATGGAATCATATGCTTACCGTAACTTGAAAGTATTTTCG
ATTTCTTGGCTTTTATATATCTTGTGGAAAGGACGAAACACCGATGTCAAGATTGAGCCTTGCCCTGACCCAGCAAGG
CTCAATCTTGACATCTTTTTTGTGCGACTGCGATGCGGCCGCACCGATTTTGATTCTCAAACAAATGTGTCACAAAGT
AAGGATTCTGATGTGTATATCACAGACAAAACGTGCTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGT
35 GGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCAGAAGACACCTTCTTCC
CCAGCCCAGGTAAGGGCAGCTTTGGTGCCTTCGCAGGCTGTTTCTTGTTCAGGAATGGCCAGGTTCTGCCCAGAG
CTCTGGTCAATGATGTCTAAACTCCTCTGATTGCTAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTG
ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCC
CCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTTCTCCCTTCGGG
40 AAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTG
TGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACAC

GACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT
 GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCG
 GAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAG
 ATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTAGAAAACTCATCGAGCATCAAATGAACTGC
 5 AATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACACCCGAG
 GCAGTTCCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATT
 TCCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGT
 TTATGCATTTCTTTCCAGACTTGTTCACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAACC
 GTTATTTCATTCGTGATTGCGCCTGAGCCAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCG
 10 AATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTCACCTGAATCAGGATATTCTTCTAATACCTGG
 AATGCTGTTTTTCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGG
 AAGAGGCATAAATTCGGTCAGCCAGTTTAGTCTGACCATCTCATCTGTAACATCATTTGGCAACGCTACCTTTGCCAT
 GTTTCAGAAACAACTCTGGCGCATCGGGCTTCCCATACAAGCGATAGATTGTCGCACCTGATTGCCCCGACATTATCG
 CGAGCCCATTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCCTCGACGTTTCCCGTTGAATATG
 15 GCTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTTGTG
 CAATGTAACATCAGAGATTTTGAGACAC [SEQ ID NO: 64]

In certain embodiments, the Secondary Promoter Construct comprises a nucleotide
 sequence that is at least about 80%, about 85%, about 90%, about 95%, about 96%, about 97%,
 about 98%, about 99% identical to the nucleotide sequence set forth in SEQ ID NOs: 65-98. In
 20 certain embodiments, the Secondary Promoter Construct comprises the nucleotide sequence set
 forth in SEQ ID NOs: 65-98. In certain embodiments, the Secondary Promoter Construct
 comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 65, 67, 69,
 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, and 97. In certain embodiments, the Secondary
 Promoter Construct comprises a nucleotide sequence selected from the group consisting of SEQ
 25 ID NOs: 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98. In certain
 embodiments, the Secondary Promoter Construct comprises a first nucleotide sequence selected
 from the group consisting of SEQ ID NOs: 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91,
 93, 95, and 97, and a second nucleotide sequence selected from the group consisting of SEQ ID
 NOs: 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98.

30

3.2. *Methods Of Producing Secondary Promoter Products With A Young Phenotype*

In certain embodiments, the present disclosure relates, in part, to the production of
 engineered “young” T cells comprising the Secondary Promoter Constructs disclosed herein. In
 certain embodiments, the present disclosure comprises methods for producing antigen-specific
 35 cells, e.g., T cells, *ex vivo*, comprising activating, engineering, and expanding antigen-specific
 cells originally obtained from a subject or isolated from such sample.

In certain embodiments, the methods for activating cells comprise the steps of activating the TCR/CD3 complex. For example, without limitation, the T cells can be incubated and/or cultured with CD3 agonists, CD28 agonists, or a combination thereof.

In certain embodiments, engineered activated antigen-specific cells, e.g., engineered activated T cells, can be expanded by culturing the engineered activated antigen-specific cells, e.g., T cells, with cytokines, chemokine, soluble peptides, or combination thereof. In certain embodiments, the engineered activated antigen-specific cells, e.g., engineered activated T cells, can be cultured with one or more cytokines. In certain embodiments, the cytokines can be IL2, IL7, IL15, or combinations thereof. For example, engineered activated antigen-specific cells, e.g., engineered activated T cells, can be cultured with IL7 and IL15. In certain embodiments, the cytokine used in connection with the engineered activated antigen-specific cell, e.g., engineered activated T cell, culture can be present at a concentration from about 1 pg/ml to about 1 g/ml, from about 1 ng/ml to about 1 g/ml, from about 1 µg/ml to about 1 g/ml, or from about 1 mg/ml to about 1g/ml, and any values in between.

3.3. *Gene-Editing Methods*

In certain embodiments, the present disclosure involves, in part, methods of engineering human cells, e.g., engineered T cells or engineered human stem cells, comprising the Secondary Promoter Constructs disclosed herein. In certain embodiments, the present disclosure involves, in part, methods of engineering human cells, e.g., NK cells, NKT cells, macrophages, hematopoietic stem cells (HSCs), cells derived from HSCs, or dendritic/antigen-presenting cells. In certain embodiments, such engineering involves genome editing. For example, but not by way of limitation, such genome editing can be accomplished with nucleases targeting one or more endogenous loci, e.g., TCR alpha (TCR α) locus and TCR beta (TCR β) locus. In certain embodiments, the nucleases can generate single-stranded DNA nicks or double-stranded DNA breaks in an endogenous target sequence. In certain embodiments, the nuclease can target coding or non-coding portions of the genome, e.g., exons, introns. In certain embodiments, the nucleases contemplated herein comprise homing endonuclease, meganuclease, megaTAL nuclease, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas nuclease. In certain embodiments, the nucleases can themselves be engineered, e.g., via the introduction of amino acid substitutions and/or deletions, to increase the efficiency of the cutting activity.

In certain embodiments, a CRISPR/Cas nuclease system is used to engineer human cells. In certain embodiments, the CRISPR/Cas nuclease system comprises a Cas nuclease and one or

more RNAs that recruit the Cas nuclease to the endogenous target sequence, *e.g.*, single guide RNA. In certain embodiments, the Cas nuclease and the RNA are introduced in the cell separately, *e.g.* using different vectors or compositions, or together, *e.g.*, in a polycistronic construct or a single protein-RNA complex. In certain embodiments, the Cas nuclease is Cas9 or Cas12a. In certain embodiments, the Cas9 polypeptide is obtained from a bacterial species including, without limitation, *Streptococcus pyogenes* or *Neisseria meningitidis*. Additional examples of CRISPR/Cas systems are known in the art. See Adli, Mazhar. "The CRISPR tool kit for genome editing and beyond." *Nature communications* vol. 9,1 1911 (2018), herein incorporated by reference for all that it teaches.

In certain embodiments, genome editing occurs at one or more genome loci that regulate immunological responses. In certain embodiments, the loci include, without limitation, TCR alpha (TCR α) locus, TCR beta (TCR β) locus, TCR gamma (TCR γ), and TCR delta (TCR δ). In certain embodiments, the locus for inserting a Secondary Promoter Construct is anywhere in the genome. In certain embodiments, the locus for inserting a Secondary Promoter Construct is the TRAC locus. In certain embodiments, the locus for inserting a Secondary Promoter Construct is one of the two TRBC loci. In certain embodiments, the locus for inserting a Secondary Promoter Construct is a locus other than the TRAC locus or TRAB loci. In certain embodiments, the Secondary Promoter Construct is inserted into a gene locus wherein such gene is knocked out.

In certain embodiments, genome editing is performed by using non-viral delivery systems. For example, a nucleic acid molecule can be introduced into a cell by administering the nucleic acid in the presence of lipofection (Feigner et al., *Proc. Natl. Acad. Sci. U.S.A.* 84:7413, 1987; Ono et al., *Neuroscience Letters* 17:259, 1990; Brigham et al., *Am. J. Med. Sci.* 298:278, 1989; Staubinger et al., *Methods in Enzymology* 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu et al., *Journal of Biological Chemistry* 263:14621, 1988; Wu et al., *Journal of Biological Chemistry* 264:16985, 1989), or by micro-injection under surgical conditions (Wolff et al., *Science* 247:1465, 1990). Other non-viral means for gene transfer include transfection in vitro using calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for the delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type *ex vivo* (*e.g.*, an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically.

In certain embodiments, genome editing is performed by using viral delivery systems. In certain embodiments, the viral methods include targeted integration (including but not limited to AAV) and random integration (including but not limited to lentiviral approaches). In certain embodiments, the viral delivery would be accomplished without integration of the nuclease. In such embodiments, the viral delivery system can be Lentiflash or another similar delivery system.

3.4. *Compositions and Vectors*

The presently disclosed subject matter provides compositions comprising cells (e.g., Secondary Promoter Cells) disclosed herein.

In certain embodiments, the presently disclosed subject matter provides nucleic acid compositions comprising a polynucleotide encoding the NeoTCR disclosed herein. In certain embodiments, the nucleic acid compositions disclosed herein comprise a polynucleotide encoding a Secondary Promoter Construct disclosed herein. Also provided are cells comprising such nucleic acid compositions.

In certain embodiments, the nucleic acid composition further comprises a promoter that is operably linked to the NeoTCR disclosed herein. In certain embodiments, the nucleic acid composition further comprises a promoter that is operably linked to the Secondary Promoter Construct disclosed herein.

In certain embodiments, the promoter is endogenous or exogenous. In certain embodiments, the exogenous promoter is selected from the group consisting of an elongation factor (EF)-1 promoter, a CMV promoter, an SV40 promoter, a PGK promoter, a long terminal repeat (LTR) promoter, and a metallothionein promoter. In certain embodiments, the promoter is an inducible promoter. In certain embodiments, the inducible promoter is selected from the group consisting of an NFAT transcriptional response element (TRE) promoter, a CD69 promoter, a CD25 promoter, an IL-2 promoter, an IL-12 promoter, a p40 promoter, and a Bcl-xL promoter.

The compositions and nucleic acid compositions can be administered to subjects or and/delivered into cells by art-known methods or as described herein. Genetic modification of a cell (e.g., a T cell) can be accomplished by transducing a substantially homogeneous cell composition with a recombinant DNA construct. In certain embodiments, a retroviral vector (either a gamma-retroviral vector or a lentiviral vector) is employed for the introduction of the DNA construct into the cell. Non-viral vectors may be used as well.

Possible methods of transduction also include direct co-culture of the cells with producer cells, e.g., by the method of Bregni, *et al.* (1992) *Blood* 80:1418-1422, or culturing with viral supernatant alone or concentrated vector stocks with or without appropriate growth factors and polycations, e.g., by the method of Xu, *et al.* (1994) *Exp. Hemat.* 22:223-230; and Hughes, *et al.* (1992) *J. Clin. Invest.* 89:1817.

Other transducing viral vectors can be used to modify a cell. In certain embodiments, the chosen vector exhibits high efficiency of infection and stable integration and expression (see, e.g., Cayouette *et al.*, *Human Gene Therapy* 8:423-430, 1997; Kido *et al.*, *Current Eye Research* 15:833-844, 1996; Bloomer *et al.*, *Journal of Virology* 71:6641-6649, 1997; Naldini *et al.*, *Science* 272:263-267, 1996; and Miyoshi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 94:10319, 1997). Other viral vectors that can be used include, for example, adenoviral, lentiviral, and adeno-associated viral vectors, vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, *Human Gene Therapy* 15-14, 1990; Friedman, *Science* 244:1275-1281, 1989; Eglitis *et al.*, *BioTechniques* 6:608-614, 1988; Tolstoshev *et al.*, *Current Opinion in Biotechnology* 1:55-61, 1990; Sharp, *The Lancet* 337:1277-1278, 1991; Cornetta *et al.*, *Nucleic Acid Research and Molecular Biology* 36:311-322, 1987; Anderson, *Science* 226:401-409, 1984; Moen, *Blood Cells* 17:407-416, 1991; Miller *et al.*, *Biotechnology* 7:980-990, 1989; LeGal La Salle *et al.*, *Science* 259:988-990, 1993; and Johnson, *Chest* 107:77S- 83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg *et al.*, *N. Engl. J. Med* 323:370, 1990; Anderson *et al.*, U.S. Pat. No. 5,399,346).

Non-viral approaches can also be employed for genetic modification of a cell. For example, a nucleic acid molecule can be introduced into a cell by administering the nucleic acid in the presence of lipofection (Feigner *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 84:7413, 1987; Ono *et al.*, *Neuroscience Letters* 17:259, 1990; Brigham *et al.*, *Am. J. Med. Sci.* 298:278, 1989; Staubinger *et al.*, *Methods in Enzymology* 101:512, 1983), asialoorosomucoid-polylysine conjugation (Wu *et al.*, *Journal of Biological Chemistry* 263:14621, 1988; Wu *et al.*, *Journal of Biological Chemistry* 264:16985, 1989), or by micro-injection under surgical conditions (Wolff *et al.*, *Science* 247:1465, 1990). Other non-viral means for gene transfer include transfection *in vitro* using calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for the delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type *ex vivo* (e.g., an autologous or

heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically.

Polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element or intron (e.g. the elongation factor 1a enhancer/promoter/intron structure). For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.

The resulting cells can be grown under conditions similar to those for unmodified cells, whereby the modified cells can be expanded and used for a variety of purposes.

3.5. Cells

The presently disclosed subject matter provides cells comprising a presently disclosed Secondary Promoter Constructs. In certain embodiments, the cell is an immune cell (e.g., a lymphocyte). For example, but without any limitation, the cells can be T cells, Natural Killer (NK) cells, B cells, dendritic cells, hematopoietic stem cells, or pluripotent stem cells.

In certain embodiments, the cell is a T cell. Non-limiting examples of T cells encompassed by the present disclosure include helper T cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: e.g., TEM cells and TEMRA cells, Regulatory T cells (also known as suppressor T cells), tumor-infiltrating lymphocyte (TIL), Natural killer T cells, Mucosal associated invariant T cells, and $\gamma\delta$ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. In certain embodiments, the T cell is a CD4⁺ T cell. In certain embodiments, the T cell is a CD8⁺ T cell.

In certain embodiments, the T cell is a peripheral T cell. Peripheral T cells are differentiated T cells that have undergone the maturation process. Peripheral T cells can be found in peripheral blood. In certain embodiments, the T cell is not a naïve T cell. Naïve T cells are precursors for effector and memory T cell subsets. Phenotypically, naïve T cells are small cells with little cytoplasm; they express surface markers, such as CD45RA, CCR7,

CD62L, CD127, and CD132. Naïve T cells lack expression of markers of previous activation, such as CD25, CD44, CD69, CD45RO, or HLA-DR.

In certain embodiments, the cell is a NK cell. Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response.

5 NK cells do not require prior activation to perform their cytotoxic effect on target cells.

In certain embodiments, the cells are autologous. In certain embodiments, the cells can be transduced with the presently disclosed Secondary Promoter Constructs to express an exogenous TCR (e.g., NeoTCR) and a Payload.

3.6. *Pharmaceutical Formulations.*

10 In certain embodiments, the present disclosure provides a pharmaceutical formulation comprising the Secondary Promoter Cells disclosed herein. In certain embodiments, pharmaceutical formulations of the Secondary Promoter Products are prepared by combining the Secondary Promoter Cells in a solution that can preserve the ‘young’ phenotype of the cells in a cryopreserved state.

15 In certain embodiments, pharmaceutically acceptable carriers, buffers, stabilizers, and/or preservatives can be added to the cryopreservation solution. Any cryopreservation agent and/or media can be used to cryopreserve the Secondary Promoter Product, including but not limited to CryoStor, CryoStor CS5, CELLBANKER, and custom cryopreservation media that optionally include DMSO.

20 **4. Methods of Treatment**

The presently disclosed subject matter provides methods for inducing and/or increasing an immune response in a subject in need thereof. The Secondary Promoter Products can be used for treating and/or preventing a cancer in a subject. The Secondary Promoter Products can be used for prolonging the survival of a subject suffering from a cancer. The Secondary Promoter
25 Products can also be used for treating and/or preventing a cancer in a subject. The Secondary Promoter Products can also be used for reducing tumor burden in a subject. Such methods comprise administering the Secondary Promoter Products in an amount effective or a composition (e.g., a pharmaceutical composition or formulation disclosed herein) comprising thereof to achieve the desired effect, be it palliation of an existing condition or prevention of
30 recurrence. For treatment, the amount administered is an amount effective in producing the desired effect. An effective amount can be provided in one or a series of administrations. An effective amount can be provided in a bolus or by continuous perfusion.

In certain embodiments, the Secondary Promoter Products can be used for treating viral or bacterial diseases. In certain embodiments, the Secondary Promoter Products can be used for treating autoimmune diseases.

In certain embodiments, an effective amount of the Secondary Promoter Products are delivered through intravenous (IV) administration. In certain embodiments, the Secondary Promoter Products are delivered through intravenous administration in a single administration. In certain embodiments, the Secondary Promoter Products are delivered through intravenous administration in multiple administrations. In certain embodiments, the Secondary Promoter Products are delivered through intravenous administration in two or more administrations. In certain embodiments, the Secondary Promoter Products are delivered through intravenous administration in two administrations. In certain embodiments, the Secondary Promoter Products are delivered through intravenous administration in three administrations.

The presently disclosed subject matter provides methods for treating and/or preventing cancer in a subject. In certain embodiments, the method comprises administering an effective amount of the Secondary Promoter Products to a subject having cancer.

Non-limiting examples of cancer include blood cancers (e.g. leukemias, lymphomas, and myelomas), ovarian cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, throat cancer, melanoma, neuroblastoma, adenocarcinoma, glioma, soft tissue sarcoma, and various carcinomas (including prostate and small cell lung cancer). Suitable carcinomas further include any known in the field of oncology, including, but not limited to, astrocytoma, fibrosarcoma, myxosarcoma, liposarcoma, oligodendroglioma, ependymoma, medulloblastoma, primitive neural ectodermal tumor (PNET), chondrosarcoma, osteogenic sarcoma, pancreatic ductal adenocarcinoma, small and large cell lung adenocarcinomas, chordoma, angiosarcoma, endotheliosarcoma, squamous cell carcinoma, bronchoalveolarcarcinoma, epithelial adenocarcinoma, and liver metastases thereof, lymphangiosarcoma, lymphangioendotheliosarcoma, hepatoma, cholangiocarcinoma, synovioma, mesothelioma, Ewing's tumor, rhabdomyosarcoma, colon carcinoma, basal cell carcinoma, sweat gland carcinoma, papillary carcinoma, sebaceous gland carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, testicular tumor, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma,

retinoblastoma, leukemia, multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease, breast tumors such as ductal and lobular adenocarcinoma, squamous and adenocarcinomas of the uterine cervix, uterine and ovarian epithelial carcinomas, prostatic adenocarcinomas, transitional squamous cell carcinoma of the bladder, B and T cell lymphomas (nodular and diffuse) plasmacytoma, acute and chronic leukemias, malignant melanoma, soft tissue sarcomas and leiomyosarcomas. In certain embodiments, the neoplasia is selected from the group consisting of blood cancers (e.g. leukemias, lymphomas, and myelomas), ovarian cancer, prostate cancer, breast cancer, bladder cancer, brain cancer, colon cancer, intestinal cancer, liver cancer, lung cancer, pancreatic cancer, prostate cancer, skin cancer, stomach cancer, glioblastoma, and throat cancer. In certain embodiments, the presently disclosed Secondary Promoter Products comprising young T cells can be used for treating and/or preventing blood cancers (e.g., leukemias, lymphomas, and myelomas) or ovarian cancer, which are not amenable to conventional therapeutic interventions.

In certain embodiments, the neoplasia is a solid cancer or a solid tumor. In certain embodiments, the solid tumor or solid cancer is selected from the group consisting of glioblastoma, prostate adenocarcinoma, kidney papillary cell carcinoma, sarcoma, ovarian cancer, pancreatic adenocarcinoma, rectum adenocarcinoma, colon adenocarcinoma, esophageal carcinoma, uterine corpus endometrioid carcinoma, breast cancer, skin cutaneous melanoma, lung adenocarcinoma, stomach adenocarcinoma, cervical and endocervical cancer, kidney clear cell carcinoma, testicular germ cell tumors, and aggressive B-cell lymphomas.

The subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. The subjects can have a history of the condition, for which they have already been treated, in which case the therapeutic objective will typically include a decrease or delay in the risk of recurrence.

Suitable human subjects for therapy typically comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor can be detected based on tumor mass (e.g., by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). A pharmaceutical composition is administered to these subjects to elicit an anti-tumor response, to palliate their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical

improvement constitutes a benefit. Clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor.

5. Articles of Manufacture

The Secondary Promoter Products can be used in combination with articles of manufacture. Such articles of manufacture can be useful for the prevention or treatment of proliferative disorders (e.g., cancer). Examples of articles of manufacture include but are not limited to containers (e.g., infusion bags, bottles, storage containers, flasks, vials, syringes, tubes, and IV solution bags) and a label or package insert on or associated with the container. The containers may be made of any material that is acceptable for the storage and preservation of the Secondary Promoter Cells within the Secondary Promoter Products. In certain embodiments, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle. For example, the container may be a CryoMACS freezing bag. The label or package insert indicates that the Secondary Promoter Products are used for treating the condition of choice and the patient of origin. The patient is identified on the container of the Secondary Promoter Product because the Secondary Promoter Products is made from autologous cells and engineered as a patient-specific and individualized treatment.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; and 2) a second container with the same Secondary Promoter Product as the first container contained therein. Optionally, additional containers with the same Secondary Promoter Product as the first and second containers can be prepared and made. Optionally, additional containers containing a composition comprising a different cytotoxic or otherwise therapeutic agent may also be combined with the containers described above.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; and 2) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

In certain embodiments, the article of manufacture can comprise: 1) a first container with two Secondary Promoter Products contained therein; and 2) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; and 3) optionally a third container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In certain embodiments, the first and second Secondary Promoter Products are different Secondary Promoter Products. In certain embodiments, the first and second Secondary Promoter Products are the same Secondary Promoter Products.

In certain embodiments, the article of manufacture can comprise: 1) a first container with three Secondary Promoter Products contained therein; and 2) optionally a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein; and 4) optionally a fourth container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In certain embodiments, the first, second, and third Secondary Promoter Products are different Secondary Promoter Products. In certain embodiments, the first, second, and third Secondary Promoter Products are the same Secondary Promoter Products. In certain embodiments, two of the first, second, and third Secondary Promoter Products are the same Secondary Promoter Products.

In certain embodiments, the article of manufacture can comprise: 1) a first container with four Secondary Promoter Products contained therein; and 2) optionally a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein; 4) a fourth container with a fourth Secondary Promoter Product contained therein; and 5) optionally a fifth container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In certain embodiments, the first, second, third, and fourth Secondary Promoter Products are different Secondary Promoter Products. In certain embodiments, the first, second, third, and fourth

Secondary Promoter Products are the same NeoTCR Products. In certain embodiments, two of the first, second, third, and fourth Secondary Promoter Products are the same NeoTCR Products. In certain embodiments, three of the first, second, third, and fourth Secondary Promoter Products are the same Secondary Promoter Products.

5 In certain embodiments, the article of manufacture can comprise: 1) a first container with five or more Secondary Promoter Products contained therein; and 2) optionally a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent.

10 In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein; 4) a fourth container with a fourth Secondary Promoter Product contained therein; 5) a fifth container with a fifth Secondary Promoter Product contained therein; 6) optionally a sixth or more additional container with a sixth or more Secondary
15 Promoter Product contained therein; and 7) optionally an additional container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. In certain embodiments, all of the containers of Secondary Promoter Products are different Secondary Promoter Products. In certain embodiments, all of the containers of Secondary Promoter Products are the same Secondary Promoter Products. In
20 certain embodiments, there can be any combination of same or different Secondary Promoter Products in the five or more containers based on the availability of detectable Secondary Promoters in a patient's tumor sample(s), the need and/or desire to have multiple Secondary Promoter Products for the patient, and the availability of any Secondary Promoter Product that may require or benefit from one or more container.

25 In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein.

30 In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein; 4) optionally a fourth container with a fourth Secondary Promoter Product contained therein.

In certain embodiments, the article of manufacture can comprise: 1) a first container with a Secondary Promoter Product contained therein; 2) a second container with a second Secondary Promoter Product contained therein; 3) a third container with a third Secondary Promoter Product contained therein; 4) a fourth container with a fourth Secondary Promoter Product contained therein; 5) optionally a fifth container with a fourth Secondary Promoter Product contained therein.

In certain embodiments, the article of manufacture can comprise a container with one Secondary Promoter Product contained therein. The article of manufacture may comprise a container with two Secondary Promoter Products contained therein. The article of manufacture can comprise a container with three Secondary Promoter Products contained therein. The article of manufacture can comprise a container with four Secondary Promoter Products contained therein. The article of manufacture can comprise a container with five Secondary Promoter Products contained therein.

In certain embodiments, the article of manufacture can comprise 1) a first container with one Secondary Promoter Product contained therein, and 2) a second container with two Secondary Promoter Products contained therein. In certain embodiments, the article of manufacture can comprise 1) a first container with two Secondary Promoter Products contained therein, and 2) a second container with one Secondary Promoter Product contained therein. In the examples above, a third and/or fourth container comprising one or more additional Secondary Promoter Products may be included in the article of manufacture. Additionally, a fifth container comprising one or more additional Secondary Promoter Products may be included in the article of manufacture.

Furthermore, any container of Secondary Promoter Product described herein can be split into two, three, or four separate containers for multiple time points of administration and/or based on the appropriate dose for the patient.

In certain embodiments, the Secondary Promoter Products are provided in a kit. The kit can, by means of non-limiting examples, contain package insert(s), labels, instructions for using the Secondary Promoter Product(s), syringes, disposal instructions, administration instructions, tubing, needles, and anything else a clinician would need in order to properly administer the Secondary Promoter Product(s).

6. Therapeutic Compositions and Methods of Manufacturing

As described herein, plasmid DNA-mediated precision genome engineering process for Good Manufacturing Practice (GMP) manufacturing of Secondary Promoter Products was

developed. Targeted integration of the patient-specific NeoTCR was accomplished by electroporating CRISPR endonuclease ribonucleoproteins (RNPs) together with the personalized NeoTCR gene cassette, encoded by the plasmid DNA. In addition to the NeoTCR, the Secondary Promoter Constructs were inserted by incorporating them into the NeoTCR vector and then electroporating with CRISPR endonuclease ribonucleoproteins (RNPs) as described above.

In certain embodiments, the Secondary Promoter Products can be formulated into a drug product using the clinical manufacturing process. Under this process, the Secondary Promoter Products are cryopreserved in CryoMACS Freezing Bags. One or more bags may be shipped to the site for each patient depending on patient needs. In certain embodiments, the product is composed of apheresis-derived, patient-autologous, and CD4/CD8 T cells that have been precision genome engineered to express one or more autologous NeoTCRs targeting a neoepitope (e.g., against a private neoantigen) complexed to one of the endogenous HLA receptors presented exclusively on the surface of that patient's tumor cells and a Payload. In certain embodiments, the cell includes a Secondary Promoter Construct disclosed herein.

In certain embodiments, the final product contains about 5% dimethyl sulfoxide (DMSO), human serum albumin, and Plasma-Lyte. In certain embodiments, the final cell product contains the list of components provided in **Table 1**.

Table 1: Composition of the Secondary Promoter Product

Component	Specification/Grade
Total nucleated NeoTCR Cells	cGMP manufactured
Plasma-Lyte A	USP
Human Serum Albumin in 0.02 - 0.08 M sodium caprylate and sodium tryptophanate	USP
CryoStor CS10	cGMP manufactured with USP grade materials

7. Kits

The presently disclosed subject matter provides kits for inducing and/or enhancing immune response and/or treating and/or preventing cancer or a pathogen infection in a subject.

In certain embodiments, the kit comprises an effective amount of presently disclosed cells (e.g. Secondary Promoter Cells) or a pharmaceutical composition comprising thereof (e.g., Secondary Promoter Products). In certain embodiments, the kit comprises a sterile container; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other

suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments. In certain non-limiting embodiments, the kit includes an isolated nucleic acid molecule encoding a presently disclosed HR template.

5 If desired, the cells and/or nucleic acid molecules are provided together with instructions for administering the cells or nucleic acid molecules to a subject having or at risk of developing cancer or pathogen, or immune disorder. The instructions generally include information about the use of the composition for the treatment and/or prevention of cancer or a pathogen infection. In certain embodiments, the instructions include at least one of the following: description of the
10 therapeutic agent; dosage schedule and administration for treatment or prevention of a neoplasia, pathogen infection, or immune disorder or symptoms thereof; precautions; warnings; indications; counter-indications; over-dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet,
15 pamphlet, card, or folder supplied in or with the container. The resulting cells can be grown under conditions similar to those for unmodified cells, whereby the modified cells can be expanded and used for a variety of purposes.

8. Exemplary Embodiments

20 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising an exogenous enhancer, an insulator, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

25 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising an exogenous enhancer, a pause element, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the
30 sequence encoding at least one Payload is under control of an exogenous promoter.

 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least

one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

5 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous
10 TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising an insulator, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at
15 an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at
20 least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

 In certain embodiments, the present disclosure provides a cell comprising an exogenous
25 polynucleotide comprising a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR and the first sequence encoding a Payload are under control of an endogenous promoter and the second sequence encoding a Payload is under control of an
30 exogenous promoter.

 In certain embodiments, the present disclosure provides a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at

least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell and is under control of an endogenous promoter.

In certain embodiments of the cells disclosed herein, the insulator is an HS4 insulator or an IS2 insulator. In certain embodiments of the cells disclosed herein, the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19. In certain embodiments of the cells disclosed herein, the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19.

In certain embodiments of the cells disclosed herein, the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments of the cells disclosed herein, the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.

In certain embodiments of the cells disclosed herein, the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer. In certain embodiments of the cells disclosed herein, the exogenous enhancer is a TCR α enhancer. In certain embodiments of the cells disclosed herein, the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments of the cells disclosed herein, the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments of the cells disclosed herein, the exogenous enhancer is a CMV enhancer. In certain embodiments of the cells disclosed herein, the CMV enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 33. In certain embodiments of the cells disclosed herein, the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33.

In certain embodiments of the cells disclosed herein, the exogenous promoter is a constitutive promoter. In certain embodiments of the cells disclosed herein, the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter. In certain embodiments of the cells disclosed herein, the constitutive promoter is an MDN promoter. In certain embodiments of the cells disclosed herein, the constitutive promoter is an EF1 α promoter. In certain embodiments of the cells disclosed herein, the exogenous promoter is an inducible promoter. In certain embodiments of the cells disclosed herein, the inducible promoter is an AP1 promoter, an NFAT promoter, an NF- κ B promoter, or an NR4A-responsive promoter.

In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises a TCR α gene sequence or a TCR β gene sequence. In certain

embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises a TCR α gene sequence and a TCR β gene sequence. In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR further comprises a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof. In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises a TCR α gene sequence, a TCR β gene sequence, a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof.

In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, and a TCR α gene sequence. In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR α gene sequence, and a poly-adenylation sequence. In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence.

In certain embodiments of the cells disclosed herein, the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.

In certain embodiments of the cells disclosed herein, the 3' of the at least one Payload comprises a STOP codon. In certain embodiments of the cells disclosed herein, the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide. In certain embodiments of the cells disclosed herein, the 3' of the at least one Payload comprises a poly-adenylation sequence.

In certain embodiments of the cells disclosed herein, the at least one Payload is an inhibitory RNA molecule. In certain embodiments of the cells disclosed herein, the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster. In certain embodiments of the cells disclosed herein, the inhibitory RNA molecule is a miRNA. In certain embodiments of the cells disclosed herein, the miRNA comprises a first flanking sequence and a second flanking sequence. In certain embodiments of the cells disclosed herein, the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21. In certain embodiments of the cells disclosed herein, the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site. In certain embodiments of the cells disclosed herein, the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.

In certain embodiments of the cells disclosed herein, the sequences encoding a P2A peptide are codon diverged. In certain embodiments of the cells disclosed herein, the sequences encoding a protease cleavage peptide are codon diverged. In certain embodiments of the cells disclosed herein, the sequences encoding a signal sequence are codon diverged.

In certain embodiments of the cells disclosed herein, the exogenous TCR recognizes a cancer neoantigen. In certain embodiments of the cells disclosed herein, the neoantigen is a private neoantigen. In certain embodiments of the cells disclosed herein, the sequence encoding an exogenous TCR is obtained from a subject.

In certain embodiments of the cells disclosed herein, the cell is a primary cell. In certain embodiments of the cells disclosed herein, the cell is a patient-derived cell. In certain embodiments of the cells disclosed herein, the cell is a lymphocyte. In certain embodiments of the cells disclosed herein, the cell is a T cell. In certain embodiments of the cells disclosed herein, the cell is a young T cell. In certain embodiments of the cells disclosed herein, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain embodiments of the cells disclosed herein, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain embodiments of the cells disclosed herein, the cell is CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain embodiments of the cells disclosed herein, the endogenous locus within the genome of the cell is a TCR locus. In certain embodiments of the cells disclosed herein, the TCR locus is a TRAC locus or a TRBC locus. In certain embodiments of the cells disclosed herein, the TCR locus is a TRAC locus and a TRBC locus. In certain embodiments of the cells

disclosed herein, the endogenous promoter is a TRAC promoter. In certain embodiments of the cells disclosed herein, the endogenous promoter is a TRBC promoter.

In certain embodiments of the cells disclosed herein, the cell, the sequence encoding an exogenous TCR, and the sequence of the neoantigen are obtained from the same subject.

5 In certain embodiments of the polynucleotides disclosed herein, the present disclosure provides a polynucleotide comprising an exogenous enhancer, an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an
10 exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising an exogenous enhancer, a pause element, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the
15 sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or
20 TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a
25 sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous
30 TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the second sequence encoding a Payload is under control of an exogenous promoter.

In certain embodiments, the present disclosure provides a polynucleotide comprising a sequence encoding a first homology arm, a second homology arm, an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus.

In certain embodiments of the polynucleotides disclosed herein, the insulator is an HS4 insulator or an IS2 insulator. In certain embodiments of the polynucleotides disclosed herein, the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19. In certain embodiments of the polynucleotides disclosed herein, the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 18. In certain embodiments of the polynucleotides disclosed herein, the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20. In certain embodiments of the polynucleotides disclosed herein, the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.

In certain embodiments of the polynucleotides disclosed herein, the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer. In certain embodiments of the polynucleotides disclosed herein, the exogenous enhancer is a TCR α enhancer. In certain embodiments of the polynucleotides disclosed herein, the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments of the polynucleotides disclosed herein, the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32. In certain embodiments of the polynucleotides disclosed herein, the exogenous enhancer is a CMV enhancer. In certain embodiments of the polynucleotides disclosed herein, the CMV enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence

set forth in SEQ ID NO: 33. In certain embodiments of the polynucleotides disclosed herein, the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33.

In certain embodiments of the polynucleotides disclosed herein, the exogenous promoter is a constitutive promoter. In certain embodiments of the polynucleotides disclosed herein, the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter. In certain embodiments of the polynucleotides disclosed herein, the constitutive promoter is an MDN promoter. In certain embodiments of the polynucleotides disclosed herein, the constitutive promoter is an EF1 α promoter. In certain embodiments of the polynucleotides disclosed herein, the exogenous promoter is an inducible promoter. In certain
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first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence. In certain embodiments of the polynucleotides disclosed herein, the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.

In certain embodiments of the polynucleotides disclosed herein, the 3' of the at least one Payload comprises a STOP codon. In certain embodiments of the polynucleotides disclosed herein, the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide. In certain embodiments of the polynucleotides disclosed herein, the 3' of the at least one Payload comprises a poly-adenylation sequence.

In certain embodiments of the polynucleotides disclosed herein, the at least one Payload is an inhibitory RNA molecule. In certain embodiments of the polynucleotides disclosed herein, the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster. In certain embodiments of the polynucleotides disclosed herein, the inhibitory RNA molecule is a miRNA. In certain embodiments of the polynucleotides disclosed herein, the miRNA comprises a first flanking sequence and a second flanking sequence. In certain embodiments of the polynucleotides disclosed herein, the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21. In certain embodiments of the polynucleotides disclosed herein, the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site. In certain embodiments of the polynucleotides disclosed herein, the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.

In certain embodiments of the polynucleotides disclosed herein, the sequences encoding a P2A peptide are codon diverged. In certain embodiments of the polynucleotides disclosed herein, the sequences encoding a protease cleavage peptide are codon diverged. In certain embodiments of the polynucleotides disclosed herein, the sequences encoding a signal sequence are codon diverged. In certain embodiments of the polynucleotides disclosed herein, the exogenous TCR recognizes a cancer neoantigen. In certain embodiments of the polynucleotides disclosed herein, the neoantigen is a private neoantigen. In certain embodiments of the

polynucleotides disclosed herein, the sequence encoding an exogenous TCR is obtained from a single subject.

In certain embodiments of the polynucleotides disclosed herein, the polynucleotide is a circular polynucleotide. In certain embodiments of the polynucleotides disclosed herein, the circular polynucleotide is a plasmid or a nanoplasmid. In certain embodiments of the polynucleotides disclosed herein, the polynucleotide is a linear polynucleotide.

In certain embodiments, the present disclosure further provides a vector comprising any one of the polynucleotide disclosed herein. In certain embodiments of the vectors disclosed herein, the vector is a non-viral vector. In certain embodiments, the present disclosure provides a cell comprising any one of the polynucleotides or vectors disclosed herein.

In certain embodiments, the present disclosure provides a method of modifying a cell. In certain embodiments of the methods disclosed herein, the method comprises introducing into the cell any one of the polynucleotides or vectors disclosed herein. In certain embodiments of the methods disclosed herein, the method further comprises recombining the polynucleotide or vector into an endogenous locus of the cell.

In certain embodiments of the methods disclosed herein, the introducing occurs via electroporation. In certain embodiments of the methods disclosed herein, the recombining comprises cleavage of the endogenous locus by a nuclease. In certain embodiments of the methods disclosed herein, the recombining further comprises recombination of the polynucleotide into the endogenous locus by homology-directed repair.

In certain embodiments of the methods disclosed herein, the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease, or a functional fragment thereof. In certain embodiments of the methods disclosed herein, the nuclease further comprises a gRNA.

In certain embodiments of the methods disclosed herein, the method further comprises culturing the cell in the presence of at least one cytokine. In certain embodiments of the methods disclosed herein, the at least one cytokine comprises IL2, IL7, IL15, or a combination thereof. In certain embodiments of the methods disclosed herein, the at least one cytokine comprises IL7 and IL15.

In certain embodiments of the methods disclosed herein, the cell is a primary cell. In certain embodiments of the methods disclosed herein, the cell is a patient-derived cell. In certain embodiments of the methods disclosed herein, the cell is a lymphocyte. In certain embodiments of the methods disclosed herein, the cell is a T cell. In certain embodiments of the

methods disclosed herein, the cell is a young T cell. In certain embodiments of the methods disclosed herein, the cell is CD45RA+, CD62L+, CD28+, CD95-, CCR7+, and CD27+. In certain embodiments of the methods disclosed herein, the cell is CD45RA+, CD62L+, CD28+, CD95+, CD27+, CCR7+. In certain embodiments of the methods disclosed herein, the cell is
5 CD45RO+, CD62L+, CD28+, CD95+, CCR7+, CD27+, CD127+.

In certain embodiments, the present disclosure also provides a cell modified by any one of the methods disclosed herein.

In certain embodiments, the present disclosure provides a composition comprising an effective amount of the any one of the cells disclosed herein. In certain embodiments of the
10 compositions disclosed herein, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain embodiments of the compositions disclosed herein, the composition is administered to a patient in need thereof for the treatment of cancer. In certain embodiments of the compositions disclosed herein, the composition
15 comprises a cryopreservation agent. In certain embodiments of the compositions disclosed herein, the composition comprises serum albumin. In certain embodiments of the compositions disclosed herein, the composition comprises Plasma-Lyte A, HSA, and CryoStor CS10.

In certain embodiments, the present disclosure provides methods of treating cancer in a subject in need thereof. In certain embodiments of the methods disclosed herein, the method
20 comprises administering a therapeutically effective amount of any one of the cells disclosed herein. In certain embodiments of the methods disclosed herein, the method comprises administering a therapeutically effective amount of any one of the compositions disclosed herein. In certain embodiments of the methods disclosed herein, prior to administering, a non-myeloablative lymphodepletion regimen is administered to the subject.

In certain embodiments of the methods disclosed herein, the cancer is a solid tumor or a
25 liquid tumor. In certain embodiments of the methods disclosed herein, the solid tumor is selected from the group consisting of melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+ cancer, esophageal cancer, thyroid cancer, gastric cancer, hepatocellular cancer, cholangiocarcinomas, renal cell cancers, testicular
30 cancer, sarcomas, and colorectal cancer. In certain embodiments of the methods disclosed herein, the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.

In certain embodiments, the present disclosure provides a kit comprising any one of the cells, the polynucleotides, the vectors, or the compositions disclosed herein. In certain embodiments of the kits disclosed herein, the kit further comprises written instructions for treating cancer.

5 In certain embodiments, the present disclosure provides a method of modifying a cell. In certain embodiments of the methods disclosed herein, the method comprises introducing into the cell a homologous recombination (HR) template nucleic acid sequence, wherein the HR template comprises a Secondary Promoter Construct, and recombining the HR template nucleic acid into an endogenous locus of the cell. In certain embodiments of the methods disclosed
10 herein, the Secondary Promoter Construct comprises Format 1, 2, or 3. In certain embodiments of the methods disclosed herein, the Secondary Promoter Construct comprises Format 4.

 In certain embodiments, the present disclosure provides a cell modified by any of the methods disclosed herein. In certain embodiments, the present disclosure provides a composition comprising a Secondary Cell Product made according to any of the methods
15 disclosed herein. In certain embodiments of the compositions disclosed herein, the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient. In certain embodiments of the compositions disclosed herein, the composition is administered to a patient in need thereof for the treatment of cancer.

 In certain embodiments, the present disclosure provides a method of treating cancer in a
20 subject in need thereof, the method comprising administering a therapeutically effective amount of any one of the cells or compositions disclosed herein. In certain embodiments of the methods disclosed herein, prior to administering, a non-myeloablative lymphodepletion regimen is administered to the subject. In certain embodiments of the methods disclosed herein, the cancer is a solid tumor. In certain embodiments of the methods disclosed herein, the cancer is liquid
25 tumor. In certain embodiments of the methods disclosed herein, the solid tumor is selected from the group consisting of melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+ cancer, esophageal cancer, thyroid cancer, gastric cancer, hepatocellular cancer, cholangiocarcinomas, renal cell cancers, testicular cancer,
30 sarcomas, and colorectal cancer. In certain embodiments of the methods disclosed herein, the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.

In certain embodiments, the present disclosure provides a composition or a method of any of the embodiments described herein.

EXAMPLES

5 The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1. Generation of NeoTCR Products

10 Neoepitope-specific TCRs identified by the impACT Isolation Technology described in PCT/US2020/17887 (which is herein incorporated by reference in its entirety) were used to generate homologous recombination (HR) DNA templates. These HR templates were transfected into primary human T cells in tandem with site-specific nucleases (*see Figures 1A-1C*). The single-step non-viral precision genome engineering resulted in the seamless replacement of the endogenous TCR with the patient's neoepitope-specific TCR, expressed by
15 the endogenous promoter. The TCR expressed on the surface is entirely native in sequence.

 The precision of NeoTCR-T cell genome engineering was evaluated by Targeted Locus Amplification (TLA) for off-target integration hot spots or translocations, and by next-generation sequencing based off-target cleavage assays and found to lack evidence of unintended outcomes.

20 As shown in **Figures 1A-1C**, constructs containing genes of interest were inserted into endogenous loci. This was accomplished with the use of homologous repair templates containing the coding sequence of the gene of interest flanked by left and right HR arms. In addition to the HR arms, the gene of interest was sandwiched between 2A peptides, a protease cleavage site that is upstream of the 2A peptide to remove the 2A peptide from the upstream
25 translated gene of interest, and signal sequences (**Figure 1B**). Once integrated into the genome, the gene of interest expression gene cassette was transcribed as single messenger RNA. During the translation of this gene of interest in messenger RNA, the flanking regions were unlinked from the gene of interest by the self-cleaving 2A peptide and the protease cleavage site was cleaved for the removal of the 2A peptide upstream from the translated gene of interest
30 (**Figure 1C**). In addition to the 2A peptide and protease cleavage site, a Gly-Ser-Gly (GSG) linker was inserted before each 2A peptide to further enhance the separation of the gene of interest from the other elements in the expression cassette.

It was determined that P2A peptides were superior to other 2A peptides for Cell Products because of their efficient cleavage. Accordingly, two (2) P2A peptides and codon divergence were used to express the gene of interest without introducing any exogenous epitopes from the remaining amino acids on either end of the gene of interest from the P2A peptide. The benefit of the gene-edited cell having no exogenous epitopes (i.e., no flanking P2A peptide amino acids on either side of the gene of interest) is that immunogenicity is drastically decreased and there is less likelihood of a patient infused with a Cell Product containing the gene-edited cell to have an immune reaction against the gene-edited cell.

As described in PCT/US/2018/058230, NeoTCRs were integrated into the TCR α locus of T cells. Specifically, a homologous repair template containing a NeoTCR coding sequence flanked by left and right HR Arms was used. In addition, the endogenous TCR β locus was disrupted leading to the expression of only TCR sequences encoded by the NeoTCR construct. The general strategy was applied using circular HR templates as well as linear templates.

The target TCR α locus (C α) is shown along with the plasmid HR template, and the resulting edited sequence and downstream mRNA/protein products in **Figures 1B and 1C**. The target TCR α locus (endogenous TRAC) and its CRISPR Cas9 target site (horizontal stripe, cleavage site designated by arrow) are shown (**Figures 1A-1C**). The circular plasmid HR template with the polynucleotide encoding the NeoTCR is located between left and right homology arms ("LHA" and "RHA" respectively). The region of the TRAC introduced by the HR template that was codon-optimized is shown (vertical stripe). The TCR β constant domain was derived from TRBC2, which is indicated as being functionally equivalent to TRBC1. Other elements in the NeoTCR cassette include 2A = 2A ribosome skipping element (by way of non-limiting example, the 2A peptides used in the cassette are both P2A sequences that are used in combination with codon divergence to eliminate any otherwise occurring non-endogenous epitopes in the translated product); P = protease cleavage site upstream of 2A that removes the 2A tag from the upstream TCR β protein (by way of non-limiting example the protease cleavage site can be a furin protease cleavage site); SS = signal sequences (by way of non-limited example the protease cleavage site can be a human growth hormone signal sequence). The HR template of the NeoTCR expression gene cassette includes two flanking homology arms to direct insertion into the TCR α genomic locus targeted by the CRISPR Cas9 nuclease RNP with the TCR α guide RNA. These homology arms (LHA and RHA) flank the neoE-specific TCR sequences of the NeoTCR expression gene cassette. While the protease cleavage site used in this example was a furin protease cleavage site, any appropriate protease cleavage site known to

one of skill in the art could be used. Similarly, while HGH was the signal sequence chosen for this example, any signal sequence known to one of skill in the art could be selected based on the desired trafficking and used.

Once integrated into the genome (**Figure 1C**), the NeoTCR expression gene cassette is transcribed as a single messenger RNA from the endogenous TCR α promoter, which still includes a portion of the endogenous TCR α polypeptide from that individual T cell (**Figure 1C**). During ribosomal polypeptide translation of this single NeoTCR messenger RNA, the NeoTCR sequences are unlinked from the endogenous, CRISPR-disrupted TCR α polypeptide by self-cleavage at a P2A peptide (**Figure 1C**). The encoded NeoTCR α and NeoTCR β polypeptides are also unlinked from each other through cleavage by the endogenous cellular human furin protease and a second self-cleaving P2A sequence motifs included in the NeoTCR expression gene cassette (**Figure 1C**). The NeoTCR α and NeoTCR β polypeptides are separately targeted by signal leader sequences (derived from the human growth hormone, HGH) to the endoplasmic reticulum for multimer assembly and trafficking of the NeoTCR protein complexes to the T cell surface. The inclusion of the furin protease cleavage site facilitates the removal of the 2A sequence from the upstream TCR β chain to reduce potential interference with TCR β function. The inclusion of a Gly-Ser-Gly linker before each 2A (not shown) further enhances the separation of the three polypeptides.

Additionally, three repeated protein sequences are codon diverged within the HR template to promote genomic stability. The two P2A are codon diverged relative to each other, as well as the two HGH signal sequences relative to each other, within the TCR gene cassette to promote stability of the introduced NeoTCR cassette sequences within the genome of the *ex vivo* engineered T cells. Similarly, the re-introduced 5' end of TRAC exon 1 (vertical stripe) reduces the likelihood of the entire cassette being lost over time through the removal of an intervening sequence of two direct repeats.

In addition to NeoTCR Products, this method can be used for any Secondary Promoter Product.

In-Out PCR was used to confirm the precise target integration of the NeoE TCR cassette. Agarose gels show the results of a PCR using primers specific to the integration cassette and site generate products of the expected size only for cells treated with both nuclease and DNA template (KOKI and KOKIKO), demonstrating site-specific and precise integration.

Furthermore, Targeted Locus Amplification (TLA) was used to confirm the specificity of targeted integration. Crosslinking, ligation, and use of primers specific to the NeoTCR insert

were used to obtain sequences around the site(s) of integration. The reads mapped to the genome are binned in 10 kb intervals. Significant read depths were obtained only around the intended site the integration site on chromosome 14, showing no evidence of common off-target insertion sites.

Antibody staining for endogenous TCR and peptide-HLA staining for NeoTCR revealed that the engineering results in high frequency knock-in of the NeoTCR, with some TCR- cells and few WT T cells remaining. Knock-in is evidenced by NeoTCR expression in the absence of an exogenous promoter. Engineering was carried out multiple times using the same NeoTCR with similar results. Therefore, efficient and consistent expression of the NeoTCR and knockout of the endogenous TCR in engineered T cells was achieved.

Example 2. Generation of Secondary Promoter Constructs

Format 1. Format 1 of the modular Secondary Promoter Constructs is exemplified in **Figures 2A, 2B, 3A, and 3B**. Format 1 is designed so that the secondary transcript (i.e., the transcript of the Payload) appears in the same orientation as the TCR transcript. Another important feature of Format 1 is that the secondary transcript (i.e., the transcript of the Payload) uses the endogenous poly-A. In this format, the TCR transcript and secondary transcript both start at the 5' end of the sense strand. It is shown that the promoter region is followed by a Payload of interest and the right homology arm.

The 2A sequences of Format 1 can be two P2As, one P2A, and one T2A, or two T2As. However, the preferred format used to design the construct and make mCherry Payload Secondary Promoters and resulting Secondary Promoter Cells and Products used two P2As.

In Format 1, each of the 2A sequences is preceded by a GSG linker or functionally equivalent linker and the Secondary Promoter region may be followed by a Kozak sequence.

As shown in **Figure 3A**, an exemplary Secondary Promoter Construct was designed and made into Secondary Promoter Cells and Products as follows (listed in order of each element): Left homology arm; GSG linker; P2A sequence; HGH signal sequence; full length TCR beta gene; Furin cleavage sequence; GSG linker; P2A sequence; HGH signal sequence; full length TCR alpha gene; poly-A signal sequence; insulator sequence; promoter region; Kozak sequence; Payload of interest; right homology arm— all retained within a backbone.

As shown in **Figure 2A**, as a modular construct, one of skill in the art can select the appropriate element for each modular component (i.e., which 2A sequence, which promoter, which signal sequence, which protease cleavage site, etc.) based on the expression needs of the Secondary Promoter Construct which will be dependent on the Payload.

Furthermore, **Figures 2B** and **3B** provide an alternate version of Format 1 with modular optionality (**Figure 2B**) and by example (**Figure 3B**).

Format 2. Format 2 of the modular Secondary Promoter Constructs is exemplified in **Figures 4A-C**, and **5A-5C**. Format 2 is designed so that the secondary transcript (i.e., the transcript of the Payload) appears in the same orientation as the TCR transcript much like in Format 1. However, unlike in Format 1, the secondary transcript uses its own poly-A rather than the endogenous poly-A.

The 2A sequences of Format 2 can be two P2As, one P2A, and one T2A, or two T2As. However, the preferred format used to design the construct and make mCherry Payload Secondary Promoters and resulting Secondary Promoter Cells and Products used two P2As.

In Format 2, each of the 2A sequences is preceded by a GSG linker or functionally equivalent linker and the Secondary Promoter region may be followed by a Kozak sequence.

As shown in **Figure 5A**, an exemplary Secondary Promoter Construct was designed and made into Secondary Promoter Cells and Products as follows (listed in order of each element):

Left homology arm; GSG linker; P2A sequence; HGH signal sequence; full length TCR beta gene; Furin cleavage sequence; GSG linker; P2A sequence; HGH signal sequence; full length TCR alpha gene; poly-A signal sequence; insulator sequence; promoter region; Kozak sequence; Payload of interest; WPRE; poly-A signal sequence; right homology arm—all retained within a backbone.

As shown in **Figure 4A**, as a modular construct, one of skill in the art can select the appropriate element for each modular component (i.e., which 2A sequence, which promoter, which signal sequence, which protease cleavage site, etc.) based on the expression needs of the Secondary Promoter Construct which will be dependent on the Payload.

Furthermore, **Figures 4B**, **4C**, **5B**, and **5C** provide an alternate version of Format 1 with modular optionality (**Figure 4B** and **4C**) and by example (**Figure 5B** and **5C**).

Format 3. Format 3 of the modular Secondary Promoter Constructs is exemplified in **Figures 6** and **7**. Format 3 is designed so that the secondary transcript (i.e., the transcript of the Payload) appears in the reversed orientation as compared to the TCR transcript. Much like in Format 2, the secondary transcript does not use the endogenous poly-A. Instead, the secondary transcript and TCR transcript share the same poly-A (e.g., a bidirectional poly-A such as SV40).

The 2A sequences of Format 3 can be two P2As, one P2A, and one T2A, or two T2As. However, the preferred format used to design the construct and make mCherry Payload Secondary Promoters and resulting Secondary Promoter Cells and Products used two P2As.

In Format 3, each of the 2A sequences is preceded by a GSG linker or functionally equivalent linker and the Secondary Promoter region may be followed by a Kozak sequence.

As shown in **Figure 7**, an exemplary Secondary Promoter Construct was designed and made into Secondary Promoter Cells and Products as follows (listed in order of each element):

- 5 Left homology arm; GSG linker; P2A sequence; HGH signal sequence; full length TCR beta gene; Furin cleavage sequence; GSG linker; P2A sequence; HGH signal sequence; full length TCR alpha gene; poly-A signal sequence; WPRE; Payload of interest; Kozak sequence; promoter region; right homology arm— all retained within a backbone.

- 10 As shown in **Figure 6**, as a modular construct, one of skill in the art can select the appropriate element for each modular component (i.e., which 2A sequence, which promoter, which signal sequence, which protease cleavage site, etc.) based on the expression needs of the Secondary Promoter Construct which will be dependent on the Payload.

Example 3. Generation of Secondary Promoter Products

- 15 *T cell Isolation and Editing.* CD4 and CD8 T cells were isolated from healthy donor PBMCs using the Miltenyi Prodigy or Miltenyi MACS separation columns according to the manufacturers' instructions. Positively-selected CD4 and CD8 T cells were used fresh or cryopreserved in 1% human serum albumin (Gemini), 49% Plasmalyte (Baxter), and 50% CS10 (Sigma). Cryopreserved cells were thawed, washed in media, and seeded at a density of 2×10^6
- 20 cells per mL culture medium. One day after thaw, or immediately if used fresh, the cells were washed and re-seeded at a density of 1.46×10^6 cells per mL in culture medium + 12.5 ng/mL IL7 + 12.5 ng/mL IL15 + a T cell activation reagent by volume. Two days after activation, T cells were electroporated with a plasmid (containing a Secondary Promoter Construct) for the production of Secondary Promoter Cells and a Secondary Promoter Product. T cells were
- 25 electroporated expanded in culture medium supplemented with 12.5 ng/mL IL7 + 12.5 ng/mL IL15. Supplemented medium was exchanged every 2-3 days or as needed until the end of the study, 13 days after activation.

- comPACT and comPACT-Dextramer preparation.* Neoantigen-specific peptide-HLA complex polypeptides (each a "comPACT") were prepared according to the method as
- 30 described in PCT/US2019/025415, hereby incorporated by reference in its entirety. A comPACT-dextramer complex was made for the labeling of NeoTCR expressing T cells. Biotinylated comPACT protein was incubated with a streptavidin-conjugated fluorophore for 10

min at room temperature (RT). Biotin-40-dextran (NANOCS) was added to the mixture and incubated at RT for an additional 10 minutes. The comPACT-Dextramer was stored at 4°C.

Confirmation of comPACT binding to NeoTCR edited T cells. T cells were stained for flow cytometry. Cells were first stained with viability dye for 20 minutes at 4°C, then washed and stained with the comPACT-dextramer for 10 minutes at 4°C. Surface antibodies (anti-CD8 α , anti-CD8 β , anti-CD4) were added to the suspension of cells and comPACT-dextramer, and the cells are incubated for an additional 20 minutes at 4°C. Cells were then washed and fixed in intracellular fixation buffer (BD Biosciences). All cells were acquired on an Attune NxT Flow Cytometer (ThermoFisher Scientific) and data were analyzed with either FCS Express or FlowJo.

Cytometric Bead Array (CBA). Streptavidin-coated plates (Eagle Biosciences) were washed 3 times with wash buffer (PBS supplemented with 1% BSA and 0.05% tween20) and then coated with comPACTs at different concentrations ranging from 100-0.01 ng/well. Wells with no comPACT and wells coated with mismatched comPACT were used as controls. The plates were incubated for 2 hr at room temperature, washed three times with wash buffer, and then washed three times with TexMACS supplemented with 3% human AB serum to remove the tween20. T cells were given two washes with TexMACS supplemented with 3% human AB serum and resuspended at 1 million cells/mL in TexMACS supplemented with 3% human AB serum and 1X penicillin-streptomycin solution. T cells were plated onto the comPACT coated plate at 100 μ L/well and incubated at 37°C, 5% CO₂. After 24h the supernatant was collected, and the cytokine concentrations were analyzed using the BD Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit II (Catalog No. 551809) following the manufacturer's protocol. Capture beads were mixed with culture supernatant, incubated with the detection reagent for 3 hr at RT protected from light, washed, and resuspended in wash buffer. Samples were assayed on an Attune NxT Flow Cytometer and data analyzed with FlowJo. The EC₅₀ represents the concentration of cognate comPACT that elicits 50% of the maximum response and is calculated utilizing a least-squares fit of IFN γ secretion over a range of comPACT concentrations.

Intracellular Staining. T cells were stained for flow cytometry on the indicated days. T cells are first stained with viability dye for 20 minutes at 4°C, then washed and incubated with surface antibodies (anti-CD8 α , anti-CD8 β , anti-CD4) for an additional 20 minutes at 4°C. T cells are then washed and permeabilized for intracellular staining. T cells are stained with anti-2A peptide or with anti-IFN γ , anti-TNF, or anti-IL2 in permeabilization buffer for 20 minutes at 4°C. T cells are fixed in intracellular fixation buffer (BD Biosciences). Samples are assayed on

an Attune NxT Flow Cytometer (ThermoFisher Scientific) and data analyzed with either FCS Express or FlowJo.

T cell Proliferation Assay. Edited CD4 and CD8 T cells are labeled with the e450 proliferation dye (eBioscience) according to the manufacturer's instructions. Labeled cells were stimulated on comPACT coated plates with a range of concentrations as described above. T cells were harvested over 48-96 hours and analyzed for proliferation as measured by dilution of the e450 dye.

T cell Killing Assay. HLA-matched cell lines were pulsed with the cognate neoantigen peptide or mismatched peptide for 1h at 37°C, 5% CO₂. The cells were washed 3 times with media to remove any unbound peptide and then co-cultured with edited CD4 and CD8 T cells that are labeled with the e450 proliferation dye described above. Co-cultures were incubated for 48h at 37°C with 5% CO₂ before harvest. Cells were washed and stained with a fixable viability dye to determine killing efficiency. The e450 proliferation dye was used to distinguish edited T cells from target cells.

Example 4. Decoupling of NeoTCR Expression from a Secondary Payload

The present example provides several Secondary Promoter Constructs in order to decouple the expression of exogenous TCRs (e.g., NeoTCR) from the expression of a secondary Payload. It was observed that the introduction of a second promoter to express a Payload (i.e., a Secondary Promoter Construct) had a negative impact on the expression of the exogenous TCR (*see* Figure 9A) and on the killing activity against tumor cells (*see* Figure 9B). Thus, studies were performed to identify genomic elements that could improve the expression of NeoTCR in edited cells.

Figure 10A shows that the use of a secondary promoter significantly reduced the expression of the NeoTCR and that the addition of the TCR α enhancer or a CMV-derived enhancer mitigated the reduction in NeoTCR expression. In addition, it was determined that the replacement of a STOP codon with a sequence encoding Furin and P2A peptides after the Payload improved the expression of NeoTCR (*see* Figure 10B). Notably, the removal of an HS4 insulator between the TCR gene sequence and the Payload has little to no effect on the NeoTCR expression. Finally, it was observed that the insertion of a MAZ4 pause element after the poly-A signal sequence increased the NeoTCR expression (*see* Figure 10C).

Example 5. Design of Secondary Promoter Constructs

Based on the observations from Example 4, several Secondary Promoter Constructs were designed in order to decouple the expression of the NeoTCR from the Payload. The used constructs are depicted in Figure 11.

The “base construct” encodes for a T cell receptor and includes a left homology arm, a sequence encoding a GSG linker, a sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a polyadenylation sequence, and a right homology arm.

The “original MND” Secondary Promoter Construct encodes for a T cell receptor and a Payload and includes a left homology arm, a sequence encoding a GSG linker, a sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a polyadenylation sequence, an insulator, an MND promoter, a Payload, a STOP codon, and a right homology arm. The original MND Secondary Promoter Construct encoding mCherry as Payload is also identified as “TCR-HS4-MND>mCherry-STOP.”

The “second generation MND” Secondary Promoter Construct encodes for a T cell receptor and a Payload and includes a left homology arm, a sequence encoding a GSG linker, a sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a polyadenylation sequence, an MND promoter, a Payload, a sequence encoding a second Furin cleavage sequence, a sequence encoding a third GSG linker, a sequence encoding a third P2A sequence, and a right homology arm. The second-generation MND Secondary Promoter Construct encoding mCherry as Payload is also identified as “TCR-MND>mCherry-2A.”

The “alternative MND” Secondary Promoter Construct encodes for a T cell receptor and a Payload and includes a left homology arm, a TCR gene enhancer, a sequence encoding a GSG linker, a sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a poly-adenylation sequence, a pause element, an MND promoter, a Payload, a

sequence encoding a second Furin cleavage sequence, a sequence encoding a third GSG linker, a sequence encoding a third P2A sequence, and a right homology arm. The alternative MND Secondary Promoter Construct encoding mCherry as Payload is also identified as “3E-TCR-HS4-MND>mCherry-2A.”

5 The “original EF-1 α ” Secondary Promoter Construct encodes for a T cell receptor and a Payload and includes a left homology arm, a sequence encoding a GSG linker, a sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a
10 polyadenylation sequence, an insulator, an EF-1 α promoter, a Payload, a STOP codon, and a right homology arm. The original EF-1 α Secondary Promoter Construct encoding mCherry as Payload is also identified as “TCR-HS4-cEF1 α >mCherry-STOP.”

 The “second generation EF-1 α ” Secondary Promoter Construct encodes for a T cell receptor and a Payload and includes a left homology arm, a sequence encoding a GSG linker, a
15 sequence encoding a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a polyadenylation sequence, an EF-1 α promoter, a Payload, a sequence encoding a second Furin cleavage sequence, a sequence encoding a third GSG linker, a sequence encoding a
20 third P2A sequence, and a right homology arm. The second generation EF-1 α Secondary Promoter Construct encoding mCherry as Payload is also identified as “TCR-cEF1 α >mCherry-2A.”

 Alternatively, the second generation EF-1 α Secondary Promoter Construct includes a left homology arm, a TCR α gene enhancer, a sequence encoding a GSG linker, a sequence encoding
25 a P2A sequence, a sequence encoding an HGH signal sequence, a full length TCR β gene sequence, a sequence encoding a Furin cleavage sequence, a sequence encoding a second GSG linker, a sequence encoding a second P2A sequence, a full length TCR α gene sequence, a polyadenylation sequence, an insulator, an EF-1 α promoter, a Payload, a STOP codon, and a right homology arm. The second generation EF-1 α Secondary Promoter Construct encoding
30 mCherry as Payload and including enhancer and insulator is also identified as “3E-TCR-HS4-cEF1 α >mCherry-STOP.”

Example 6. Effects of Secondary Promoter Constructs on Gene Expression Decoupling

The Secondary Promoter Constructs described in Example 5 were used to determine the decoupling of gene expression of NeoTCR and Payload. As seen in Figure 12A, cells transduced with the alternative MND Secondary Promoter Construct had a low gene editing efficacy. To confirm whether this effect was related to other features (e.g., the backbone of the plasmid) rather than the construct, the expression levels of the NeoTCR and the mCherry Payload were determined. Surprisingly, these constructs had similar NeoTCR expression levels and reduced mCherry expression. These results are in contrast to the TCR-cEF1 α >mCherry-2A construct, which had improved NeoTCR expression and no significant reduction in mCherry. See Figures 12B and 12C.

Next, experiments were conducted to confirm whether the TCR α enhancer (e.g., the 3' portion of the protein coding sequence) could increase the expression of NeoTCR when using an EF-1 α promoter. As shown in Figures 13A and 13B, the Secondary Promoter Constructs including the TCR α enhancer had increased NeoTCR expression levels in presence of the EF-1 α promoter. Notably, the TCR α enhancer in presence of MND promoters did not improve NeoTCR expression beyond the addition of P2A alone. In addition, it was observed that Secondary Promoter Constructs including a sequence encoding a P2A after the Payload significantly increased the expression of the Payload mCherry (*see* Figures 13C and 13D).

These results show that removal of STOP codon improves the expression of TCR genes (e.g., NeoTCR) and Payload in all tested constructs. Further, the addition of enhancers did not improve the NeoTCR expression in presence of an MND promoter.

Example 7. Effects of Secondary Promoter Constructs including an Enhancer on Gene Expression Decoupling

Since Secondary Promoter Constructs including an enhancer had improved NeoTCR expression, new constructs were designed to determine the relationship between enhancers and promoters. As seen in Figure 14, three new Secondary Promoter Constructs were designed. The “construct 1” or “3E-TCR-HS4-MND>mCherry-2A” included a TCR enhancer and the MND promoter; “construct 2” or “CMVE-TCR-HS4-MND>mCherry-2A” included a CMV enhancer and the MND promoter; and “construct 3” or “3E-TCR-HS4-cEF1 α >mCherry-2A” included a TCR enhancer and the EF-1 α promoter. All the tested constructs included an insulator after the polyadenylation sequence. As seen in Figures 15A-15B, constructs with CMV enhancers and insulators showed comparable NeoTCR expression to the base constructs. In particular, the data in Figures 15A and 15B show that the inclusion of insulators in Secondary Promoter Constructs

with an MND promoter enhanced the NeoTCR expression and that the CMV enhancer induced significantly elevated NeoTCR expression levels (i.e., similar to the base constructs). Finally, the addition of P2A sequence in constructs with EF-1 α did not improve the NeoTCR expression as compared to constructs having an enhancer (*see* Figures 15C and 15D).

5 These data show that Secondary Promoter Constructs including an MND promoter perform best when in presence of a CMV enhancer, an HS4 insulator, and P2A peptides.

Example 8. Secondary Promoter Construct including inhibitory RNA molecules.

10 The present example discloses additional Secondary Promoter Constructs including an inhibitory RNA molecule disclosed herein. Figure 16A shows a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second
15 sequence encoding a signal sequence, a TCR α gene sequence, a BGH polyadenylation signal, a secondary promoter, an inhibitory RNA, a second partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

 Figure 16B shows a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in
20 the TRAC locus), a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a BGH polyadenylation signal, a secondary promoter, an inhibitory RNA, a termination signal or a polyadenylation site, a second partial gRNA target sequence (e.g., used
25 for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

 Figures 16C and 16D show a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a
30 second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a BGH polyadenylation signal, a secondary promoter, an inhibitory RNA, a second Payload, a second sequence encoding a furin cleavage site, a third

sequence encoding GSG and P2A peptides, a second partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

Figure 16E shows a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a BGH polyadenylation signal, a secondary promoter, a second Payload, a second sequence encoding a furin cleavage site, a third sequence encoding GSG and P2A peptides, an inhibitory RNA, a second partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

Figure 16F shows a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a BGH polyadenylation signal, a secondary promoter, a splice donor site, an inhibitory RNA, a splice acceptor site, a second partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

Figure 16G shows a Secondary Promoter Construct including a left homology arm for TRAC locus, an inhibitory RNA, a splice acceptor site, a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

Figure 16H shows a Secondary Promoter Construct including a left homology arm for TRAC locus, a first partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), an inhibitory RNA, a first sequence encoding GSG and P2A peptides, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a furin cleavage site, a second sequence encoding GSG and P2A peptides, a second sequence encoding a signal sequence, a TCR α gene sequence, a second partial gRNA target sequence (e.g., used for integration of the construct in the TRAC locus), and a right homology arm for TRAC locus.

Example 9. Effects of Secondary Promoter Constructs including an inhibitory RNA on Gene Expression Decoupling

The present example describes several approaches to include an inhibitory RNA in a Secondary Promoter Construct. CBLB was used as target gene for knockdown analysis.

Initially, it was determined whether NeoTCR expression could be improved in Secondary Promoter Constructs including an inhibitory RNA molecule as a Payload. Figure 17A shows the construct having different inhibitory RNA scaffolds and used in this example. Gene expression profile analysis was performed by droplet digital PCR (ddPCR) and showed that both scaffolds were able to knock down the expression of the gene of interest CBLB. Here it is knocking down the expression of a gene called CBLB (Figure 17B). Further, it was determined whether these constructs would lead to a reduction in NeoTCR. As seen in Figure 17C, Secondary Promoter Constructs including an inhibitory RNA having mir-E scaffold did not reduce the expression of NeoTCR. Thus, use of inhibitory RNA molecules including the mir-E scaffold improves the NeoTCR expression.

Next, it was verified whether addition of a second Payload could be achieved. Figure 18A shows the constructs used for this aim. As seen in Figures 18B and 18C, Secondary Promoter Constructs including an inhibitory RNA positioned before the Payload (mCherry) had knockdown of the CBLB gene as well as expression of the Payload (mCherry).

Next, it was tested whether addition of certain secondary promoters upstream of an inhibitory RNA molecules would increase the knockdown of the CBLB target gene. Analysis of gene expression levels confirmed that U6 promoters significantly reduced the expression of the CBLB target gene without negatively impacting the expression of the NeoTCRs (Figures 19A-19E).

Further, Secondary Promoter Constructs including multiple inhibitory RNA molecules were designed and tested to evaluate the knockdown of the multiple target genes such as CBLB and A2AR (Figure 20A). Using ddPCR, it was observed that miRNA were effective in knocking down the gene expression of the targets with relatively minimal effect on the NeoTCR expression (Figures 20B-20D). These data demonstrated that Secondary Promoter Constructs including inhibitory RNA molecules can reduce gene expression of a target without significantly alter the expression of NeoTCRs.

Finally, it was determined whether miRNA clusters could target multiple genes. Cells were transfected with the constructs depicted in Figure 21A including a miRNA cluster based on

mir-106 targeting TGFB2R, A2AR, and CBLB. Analysis of expression levels of TGFB2R and NeoTCR demonstrated that the use of this specific miRNA cluster reduced the expression levels of the NeoTCR.

Collectively, these data demonstrated that the inclusion of promoters upstream of
5 Payloads improved the knockdown of target genes induced by inhibitory RNA molecules without altering the expression of NeoTCRs.

Example 10. Effects of Secondary Promoter Constructs including splice acceptor sites and splice donor site

10 Next, it was hypothesized that flanking inhibitory RNA molecules by splice acceptor sites (SA) and/or splice donor sites (SD) could improve the knockdown of target genes. Figure 22A shows the Secondary Promoter Constructs used in a first experiment. Notably, addition of SA and SD sites had no impact on the knockdown of the target genes nor on the expression of the NeoTCR (Figures 22B and 22C). These data showed that addition of splice donor site and
15 splice acceptor site can be useful for Secondary Promoter Constructs that include additional Payloads without impacting on the expression of NeoTCRs and knockdown of target genes.

* * * * *

While the present invention has been described at some length and with some
20 particularity with respect to the several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope of the invention.

25 All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, section headings, the materials, methods, and examples are illustrative only and not intended to be limiting.

WHAT IS CLAIMED IS:

1. A cell comprising an exogenous polynucleotide comprising an exogenous enhancer, an insulator, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus
5 within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.
2. A cell comprising an exogenous polynucleotide comprising an exogenous enhancer, a pause element, a sequence encoding an exogenous TCR, and a sequence encoding at
10 least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.
3. A cell comprising an exogenous polynucleotide comprising a Woodchuck Hepatitis
15 Virus Posttranscriptional Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an
20 exogenous promoter.
4. A cell comprising an exogenous polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell,
25 wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.
5. A cell comprising an exogenous polynucleotide comprising an insulator, a sequence
30 encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.

6. A cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter.
7. A cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR and the first sequence encoding a Payload are under control of an endogenous promoter and the second sequence encoding a Payload is under control of an exogenous promoter.
8. A cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell and is under control of an endogenous promoter.
9. The cell of claim 1, 4 or 5, wherein the insulator is an HS4 insulator or an IS2 insulator.
10. The cell of claim 10, wherein the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19.
11. The cell of claim 10 or 11, wherein the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19.
12. The cell of claim 3 or 4, wherein the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20.
13. The cell of claim 12, wherein the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.
14. The cell of claim 1 or 2, wherein the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer.
15. The cell of claim 14, wherein the exogenous enhancer is a TCR α enhancer.
16. The cell of claim 14 or 15, wherein the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32.

17. The cell of any one of claims 14-16, wherein the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32.
18. The cell of claim 14, wherein the exogenous enhancer is a CMV enhancer.
19. The cell of claim 14 or 18, wherein the CMV enhancer comprises a nucleotide sequence
5 that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 33.
20. The cell of any one of claims 14, 18 or 19, wherein the CMV enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 33.
21. The cell of any one of claims 1-7, wherein the exogenous promoter is a constitutive
10 promoter.
22. The cell of claim 21, wherein the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter.
23. The cell of claim 21 or 22, wherein the constitutive promoter is an MDN promoter.
24. The cell of claim 22 or 23, wherein the constitutive promoter is an EF1 α promoter.
- 15 25. The cell of any one of claims 1-7, wherein the exogenous promoter is an inducible promoter.
26. The cell of claim 25, wherein the inducible promoter is an AP1 promoter, an NFAT promoter, an NF- κ B promoter, or an NR4A-responsive promoter.
27. The cell of any one of claims 1-26, wherein the sequence encoding an exogenous TCR
20 comprises a TCR α gene sequence or a TCR β gene sequence.
28. The cell of claim 27, wherein the sequence encoding an exogenous TCR comprises a TCR α gene sequence and a TCR β gene sequence.
29. The cell of any one of claims 1-28, wherein the sequence encoding an exogenous TCR further comprises a sequence encoding a P2A peptide, a sequence encoding a signal
25 sequence, a sequence encoding a protease cleavage peptide, or a combination thereof.
30. The cell of any one of claims 1-29, wherein the sequence encoding an exogenous TCR comprises a TCR α gene sequence, a TCR β gene sequence, a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof.
- 30 31. The cell of any one of claims 1-30, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a

protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, and a TCR α gene sequence.

32. The cell of any one of claims 1-31, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR α gene sequence, and a poly-adenylation sequence.

33. The cell of any one of claims 1-31, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence.

34. The cell of any one of claims 1-33, wherein the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.

35. The cell of any one of claims 1-34, wherein the 3' of the at least one Payload comprises a STOP codon.

36. The cell of any one of claims 1-34, wherein the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide.

37. The cell of any one of claims 1-34, wherein the 3' of the at least one Payload comprises a poly-adenylation sequence.

38. The cell of claim 1-37, wherein the at least one Payload is an inhibitory RNA molecule.

39. The cell of claim 38, wherein the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster.

40. The cell of claim 38 or 39, wherein the inhibitory RNA molecule is a miRNA.

41. The cell of claim 39 or 40, wherein the miRNA comprises a first flanking sequence and a second flanking sequence.

42. The cell of claim 41, wherein the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21.
43. The cell of any one of claims 38-42, wherein the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site.
- 5 44. The cell of any one of claims 38-43, wherein the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.
45. The cell of any one of claims 29-44, wherein the sequences encoding a P2A peptide are codon diverged.
46. The cell of any one of claims 29-45, wherein the sequences encoding a protease cleavage peptide are codon diverged.
- 10 47. The cell of any one of claims 29-43, wherein the sequences encoding a signal sequence are codon diverged.
48. The cell of any one of claims 1-47, wherein the exogenous TCR recognizes a cancer neoantigen.
- 15 49. The cell of claim 48, wherein the neoantigen is a private neoantigen.
50. The cell of any one of claims 1-49, wherein the sequence encoding an exogenous TCR is obtained from a subject.
51. The cell of any one of claims 1-50, wherein the cell is a primary cell.
52. The cell of any one of claims 1-51, wherein the cell is a patient-derived cell.
- 20 53. The cell of any one of claims 1-52, wherein the cell is a lymphocyte.
54. The cell of any one of claims 1-53, wherein the cell is a T cell.
55. The cell of any one of claims 1-54, wherein the cell is a young T cell.
56. The cell of claim 55, wherein the cell is CD45RA⁺, CD62L⁺, CD28⁺, CD95⁺, CCR7⁺, and CD27⁺.
- 25 57. The cell of claim 55, wherein the cell is CD45RA⁺, CD62L⁺, CD28⁺, CD95⁺, CD27⁺, CCR7⁺.
58. The cell of claim 55, wherein the cell is CD45RO⁺, CD62L⁺, CD28⁺, CD95⁺, CCR7⁺, CD27⁺, CD127⁺.
59. The cell of any one of claims 1-58, wherein the endogenous locus within the genome of the cell is a TCR locus.
- 30 60. The cell of claim 59, wherein the TCR locus is a TRAC locus or a TRBC locus.
61. The cell of claim 59, wherein the TCR locus is a TRAC locus and a TRBC locus.

62. The cell of any one of claims 1-61, wherein the endogenous promoter is a TRAC promoter.
63. The cell of any one of claims 1-61, wherein the endogenous promoter is a TRBC promoter
- 5 64. The cell of any one of claims 1-63, wherein the cell, the sequence encoding an exogenous TCR, and the sequence of the neoantigen are obtained from the same subject.
65. A polynucleotide comprising an exogenous enhancer, an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are
- 10 homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.
66. A polynucleotide comprising an exogenous enhancer, a pause element, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are
- 15 homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.
67. A polynucleotide comprising a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the
- 20 first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.
68. A polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at
- 25 least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.
69. A polynucleotide comprising an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one
- 30 Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.

70. A polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter.
- 5 71. A polynucleotide comprising a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the second sequence encoding a Payload is under control of an exogenous promoter.
- 10 72. A polynucleotide comprising a sequence encoding a first homology arm, a second homology arm, an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus.
73. The polynucleotide of claim 65, 68 or 69, wherein the insulator is an HS4 insulator or an IS2 insulator.
- 15 74. The polynucleotide of claim 73, wherein the insulator comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 19.
75. The polynucleotide of claim 73 or 74, wherein the insulator comprises the nucleotide sequence set forth in SEQ ID NO: 18 or SEQ ID NO: 18.
- 20 76. The polynucleotide of claim 67 or 68, wherein the WPRE comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 20.
77. The polynucleotide of claim 76, wherein the WPRE comprises the nucleotide sequence set forth in SEQ ID NO: 20.
- 25 78. The polynucleotide of claim 65 or 66, wherein the exogenous enhancer is a CMV enhancer, a TCR α enhancer, or a TCR β enhancer.
79. The polynucleotide of claim 78, wherein the exogenous enhancer is a TCR α enhancer.
80. The polynucleotide of claim 78 or 79, wherein the TCR α enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 32.
- 30 81. The polynucleotide of any one of claims 78-80, wherein the TCR α enhancer comprises the nucleotide sequence set forth in SEQ ID NO: 32.
82. The polynucleotide of claim 78, wherein the exogenous enhancer is a CMV enhancer.

83. The polynucleotide of claim 78 or 82, wherein the CMV enhancer comprises a nucleotide sequence that is at least about 80% identical to the nucleotide sequence set forth in SEQ ID NO: 33.
84. The polynucleotide of any one of claims 78, 82, or 83, wherein the CMV enhancer
5 comprises the nucleotide sequence set forth in SEQ ID NO: 33.
85. The polynucleotide of any one of claims 65-84, wherein the exogenous promoter is a constitutive promoter.
86. The polynucleotide of claim 85, wherein the constitutive promoter is an MDN promoter, an EF1 α promoter, an ACTB promoter, a PGK promoter, or a U6 promoter.
- 10 87. The polynucleotide of claim 85 or 86, wherein the constitutive promoter is an MDN promoter.
88. The polynucleotide of claim 85 or 86, wherein the constitutive promoter is an EF1 α promoter.
89. The polynucleotide of any one of claims 65-84, wherein the exogenous promoter is an
15 inducible promoter.
90. The polynucleotide of claim 89, wherein the inducible promoter is an AP1 promoter, an NFAT promoter, an NF- κ B promoter, or an NR4A-responsive promoter.
91. The polynucleotide of any one of claims 65-90, wherein the sequence encoding an exogenous TCR comprises a TCR α gene sequence or a TCR β gene sequence.
- 20 92. The polynucleotide of claim 91, wherein the sequence encoding an exogenous TCR comprises a TCR α gene sequence and a TCR β gene sequence.
93. The polynucleotide of any one of claims 65-92, wherein the sequence encoding an exogenous TCR further comprises a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a
25 combination thereof.
94. The polynucleotide of any one of claims 65-93, wherein the sequence encoding an exogenous TCR comprises a TCR α gene sequence, a TCR β gene sequence, a sequence encoding a P2A peptide, a sequence encoding a signal sequence, a sequence encoding a protease cleavage peptide, or a combination thereof.
- 30 95. The polynucleotide of any one of claims 65-94, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence

encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, and a TCR α gene sequence.

96. The polynucleotide of any one of claims 65-95, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR β gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR α gene sequence, and a poly-adenylation sequence.
97. The polynucleotide of any one of claims 65-96, wherein the sequence encoding an exogenous TCR comprises, from 5' end to 3' end, a first sequence encoding a P2A peptide, a first sequence encoding a signal sequence, a TCR α gene sequence, a sequence encoding a protease cleavage peptide, a second sequence encoding a P2A peptide, a second sequence encoding a signal sequence, a TCR β gene sequence, and a poly-adenylation sequence.
98. The polynucleotide of any one of claims 65-97, wherein the at least one Payload is selected from the group consisting of a cytokine receptors trap, a ligand trap, an angiogenesis factor, an apoptotic factor, an inhibitory protein, an extracellular matrix modulator, a soluble TCR, a homing signal, an enzyme, a modulator of reactive oxygen species, a competitive ligand inhibitor, a protein that binds to receptors and sterically hinders receptor function, and an inhibitory RNA molecule.
99. The polynucleotide of any one of claims 65-98, wherein the 3' of the at least one Payload comprises a STOP codon.
100. The polynucleotide of any one of claims 65-98, wherein the 3' of the at least one Payload comprises a sequence encoding a 2A peptide and a sequence encoding a protease cleavage peptide.
101. The polynucleotide of any one of claims 65-98, wherein the 3' of the at least one Payload comprises a poly-adenylation sequence.
102. The polynucleotide of claim 65-101, wherein the at least one Payload is an inhibitory RNA molecule.
103. The polynucleotide of claim 102, wherein the inhibitory RNA molecule is a shRNA, a miRNA, or a miRNA cluster.
104. The polynucleotide of claim 102 or 103, wherein the inhibitory RNA molecule is a miRNA.

105. The polynucleotide of claim 103 or 104, wherein the miRNA comprises a first flanking sequence and a second flanking sequence.
106. The polynucleotide of claim 105, wherein the first flanking sequence and the second flanking sequence are derived from miR-155, miR-30, miR-17/92, miR-122, or miR-21.
107. The polynucleotide of any one of claims 102-106, wherein the inhibitory RNA molecule is flanked by a splice donor site or a splice acceptor site.
108. The polynucleotide of any one of claims 102-107, wherein the inhibitory RNA molecule is flanked by a splice donor site and a splice acceptor site.
109. The polynucleotide of any one of claims 93-108, wherein the sequences encoding a P2A peptide are codon diverged.
110. The polynucleotide of any one of claims 93-109, wherein the sequences encoding a protease cleavage peptide are codon diverged.
111. The polynucleotide of any one of claims 93-110, wherein the sequences encoding a signal sequence are codon diverged.
112. The polynucleotide of any of one of claims 65-111, wherein the exogenous TCR recognizes a cancer neoantigen.
113. The polynucleotide of claim 112, wherein the neoantigen is a private neoantigen.
114. The polynucleotide of any one of claims 65-113, wherein the sequence encoding an exogenous TCR is obtained from a single subject.
115. The polynucleotide of any one of claims 65-114, wherein the polynucleotide is a circular polynucleotide.
116. The polynucleotide of claim 115, wherein the circular polynucleotide is a plasmid or a nanoplasmid.
117. The polynucleotide of any one of claims 65-114, wherein the polynucleotide is a linear polynucleotide.
118. A vector comprising the polynucleotide of any one of claims 65-117.
119. The vector of claim 118, wherein the vector is a non-viral vector.
120. A cell comprising the polynucleotide of any one of claims 65-117 or the vector of claim 118 or 119.
121. A method of modifying a cell, the method comprising:
- a) introducing into the cell the polynucleotide of any one of claims 65-117 or the vector of claim 118 or 119; and

- b) recombining the polynucleotide or vector into an endogenous locus of the cell.
122. The method of claim 121, wherein the introducing occurs via electroporation.
123. The method of claim 121 or 122, wherein the recombining comprises:
- 5 a) cleavage of the endogenous locus by a nuclease; and
- b) recombination of the polynucleotide into the endogenous locus by homology-directed repair.
124. The method of claim 123, wherein the nuclease is a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) family nuclease, or a functional fragment thereof.
- 10 125. The method of claim 124, further comprising a gRNA.
126. The method of any one of claims 121-125, wherein the method further comprises culturing the cell in the presence of at least one cytokine.
127. The method of claim 126, wherein the at least one cytokine comprises IL2, IL7, IL15, or a combination thereof.
- 15 128. The method of claim 126 or 127, wherein the at least one cytokine comprises IL7 and IL15.
129. The method of any one of claims 121-128, wherein the cell is a primary cell.
130. The method of any one of claims 121-129, wherein the cell is a patient-derived cell.
- 20 131. The method of any one of claims 121-130, wherein the cell is a lymphocyte.
132. The method of any one of claims 121-129, wherein the cell is a T cell.
133. The method of any one of claims 121-130, wherein the cell is a young T cell.
134. The method of claim 133, wherein the cell is CD45RA⁺, CD62L⁺, CD28⁺, CD95⁻, CCR7⁺, and CD27⁺.
- 25 135. The method of claim 133, wherein the cell is CD45RA⁺, CD62L⁺, CD28⁺, CD95⁺, CD27⁺, CCR7⁺.
136. The method of claim 133, wherein the cell is CD45RO⁺, CD62L⁺, CD28⁺, CD95⁺, CCR7⁺, CD27⁺, CD127⁺.
137. A cell modified by the method of any one of claims 121-136.
- 30 138. A composition comprising an effective amount of the cell of any one of claims 1-64, claim 120, or claim 137.
139. The composition of claim 138, wherein the composition is a pharmaceutical composition that further comprises a pharmaceutically acceptable excipient.

140. The composition of claim 138 or 139, wherein the composition is administered to a patient in need thereof for the treatment of cancer.
141. The composition of any one of claims 138-140, wherein the composition comprises a cryopreservation agent.
- 5 142. The composition of any one of claims 138-141, wherein the composition comprises serum albumin.
143. The composition of any one of claims 138-142, wherein the composition comprises Plasma-Lyte A, HSA, and CryoStor CS10.
- 10 144. A method of treating cancer in a subject in need thereof, the method comprising administering a therapeutically effective amount of the cell of any one of claims 1-64, 120 or 137, or the composition of any one of claims 138-143.
145. The method of claim 144, wherein prior to administering, a non-myeloablative lymphodepletion regimen is administered to the subject.
146. The method of claim 144 or 145, wherein the cancer is a solid tumor or a liquid
15 tumor.
147. The method of claim 146, wherein the solid tumor is selected from the group consisting of melanoma, thoracic cancer, lung cancer, ovarian cancer, breast cancer, pancreatic cancer, head and neck cancer, prostate cancer, gynecological cancer, central nervous system cancer, cutaneous cancer, HPV+ cancer, esophageal cancer, thyroid
20 cancer, gastric cancer, hepatocellular cancer, cholangiocarcinomas, renal cell cancers, testicular cancer, sarcomas, and colorectal cancer.
148. The method of claim 146, wherein the liquid tumor is selected from the group consisting of follicular lymphoma, leukemia, and multiple myeloma.
149. A kit comprising the cell of any one of claims 1-64, 120 or 137, the polynucleotide
25 of any one of claims 65-117, the vector of claim 118 or 119, or the composition of any one of claims 138-143.
150. The kit of claim 149, wherein the kit further comprises written instructions for treating cancer.

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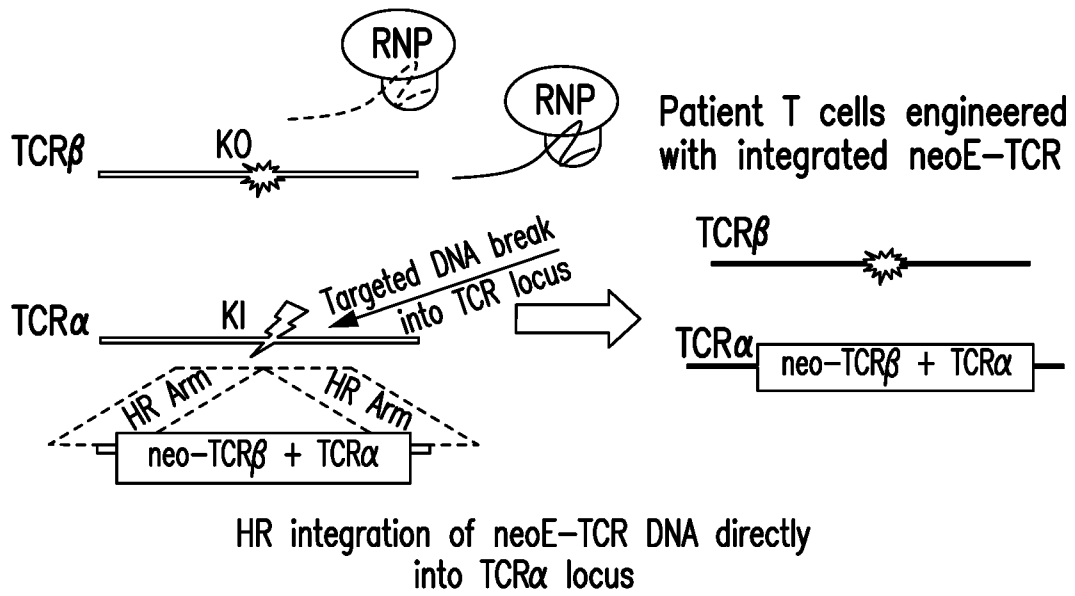
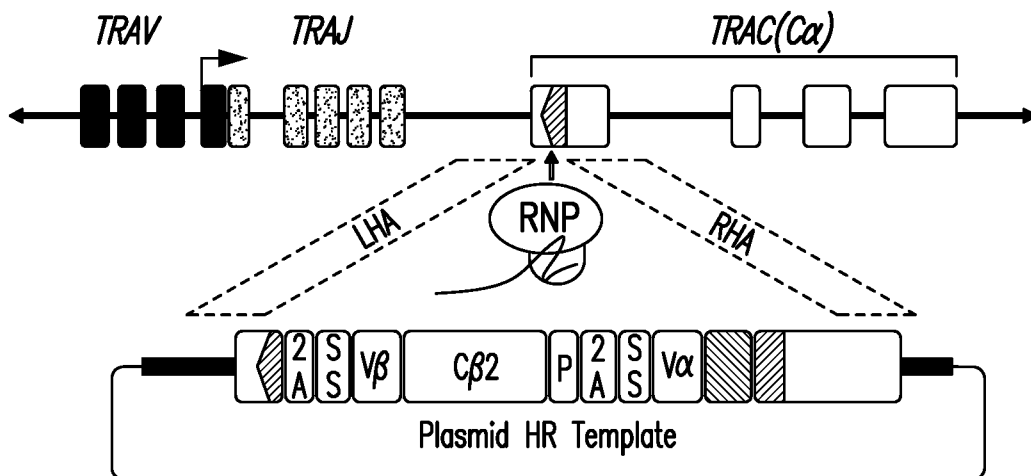


FIG. 1A

Rearranged TCR α Locus

= gRNA target sequence = codon optimized TRAC sequence

FIG. 1B

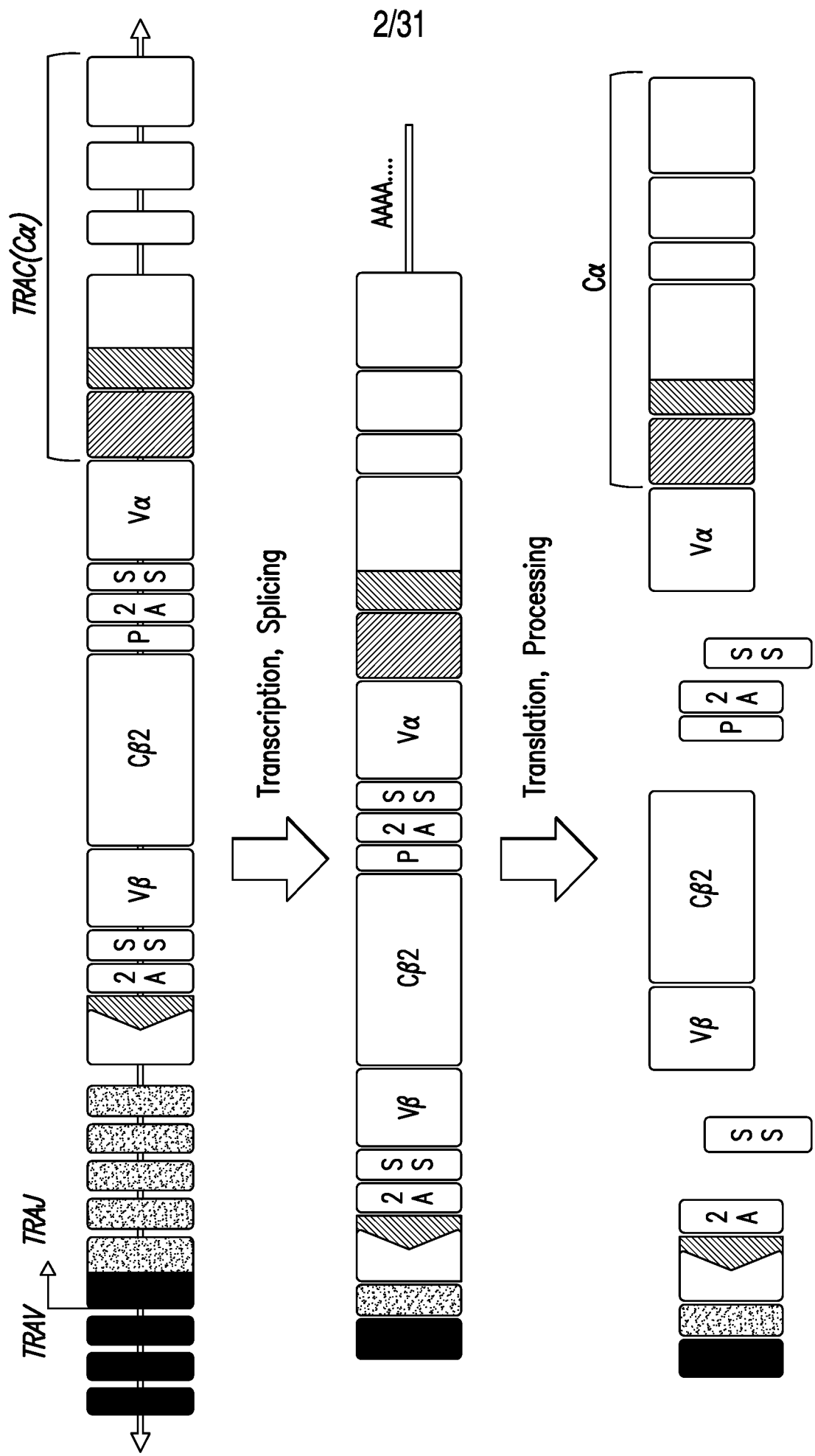


FIG. 1C

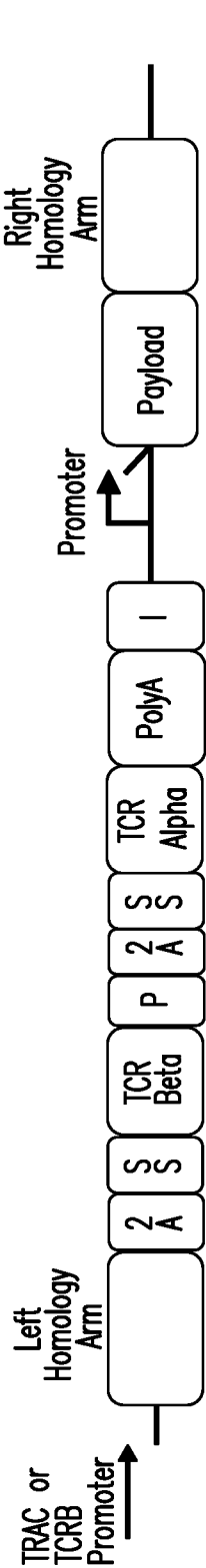


FIG. 2A

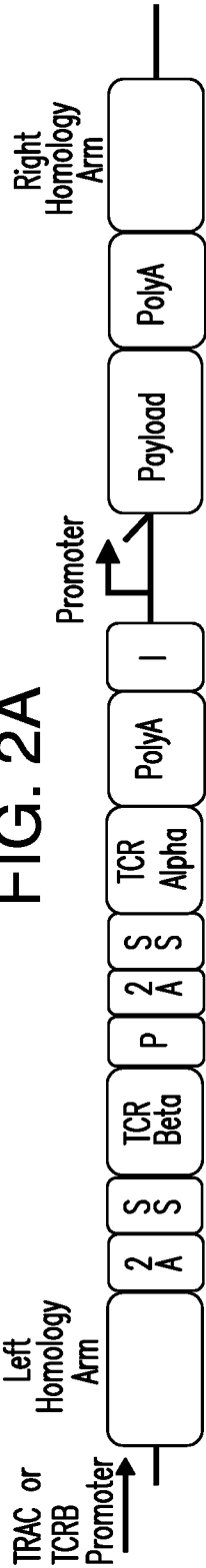


FIG. 2B

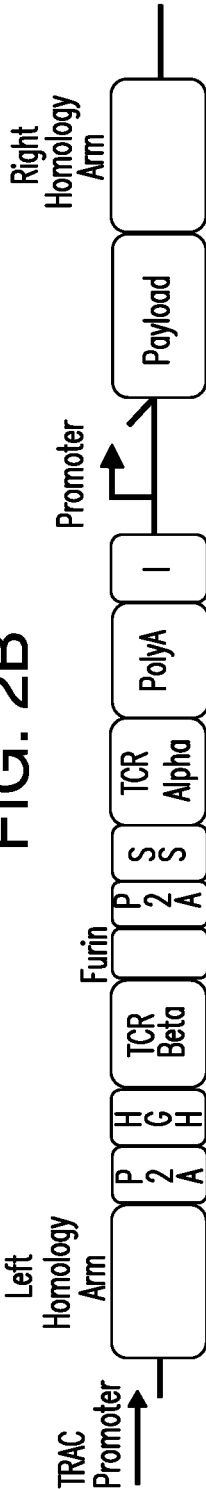


FIG. 3A

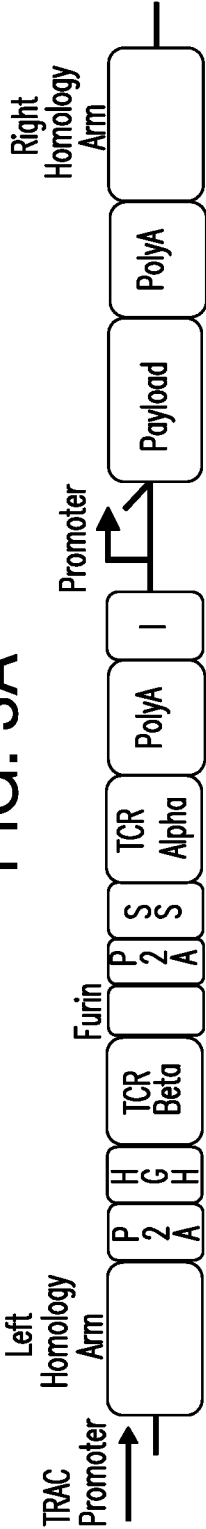


FIG. 3B

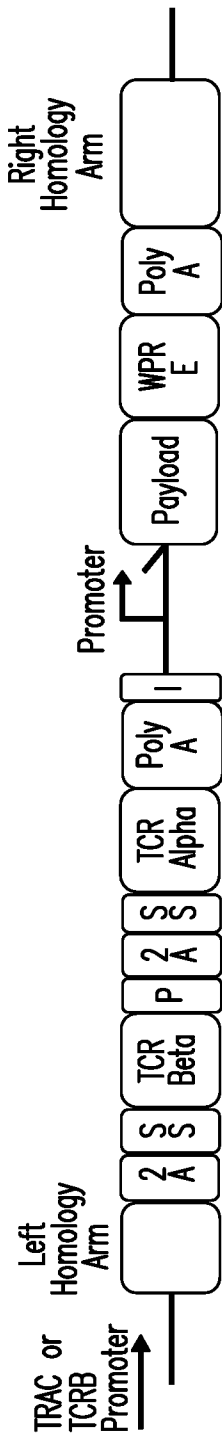


FIG. 4A

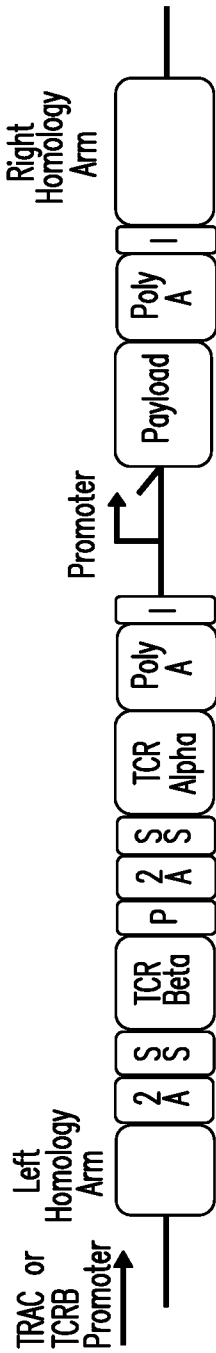


FIG. 4B

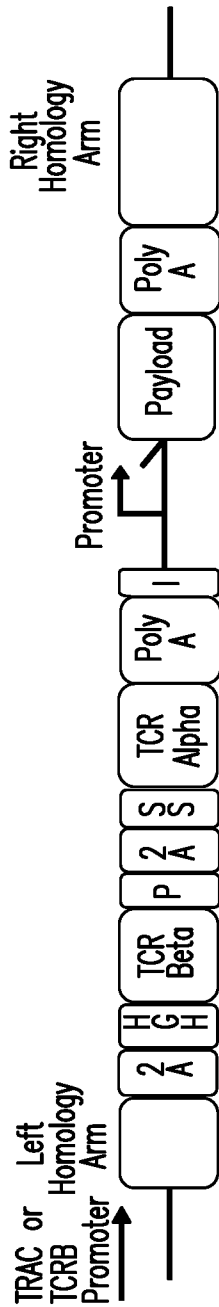


FIG. 4C

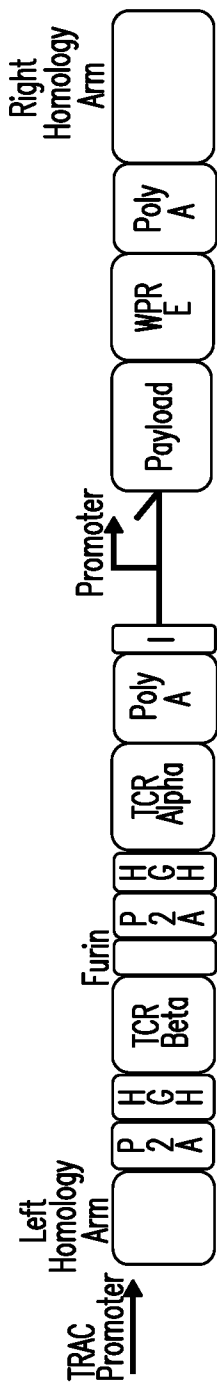


FIG. 5A

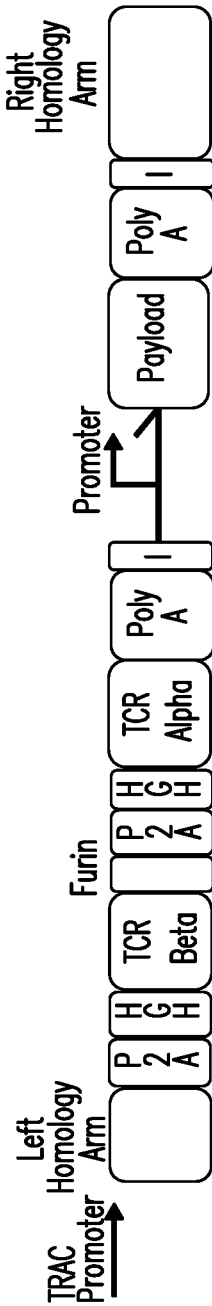


FIG. 5B

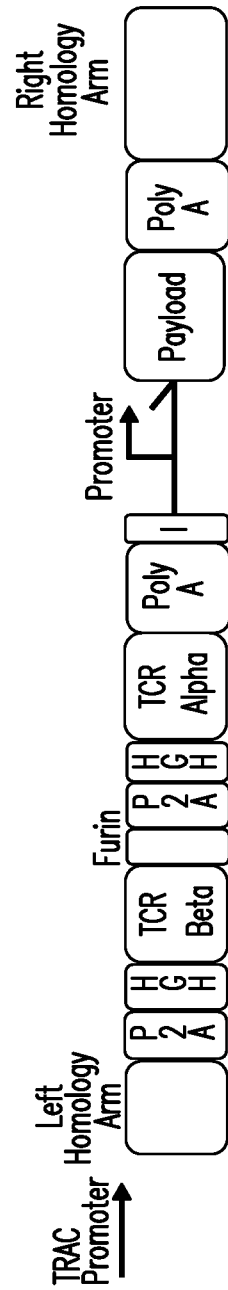


FIG. 5C

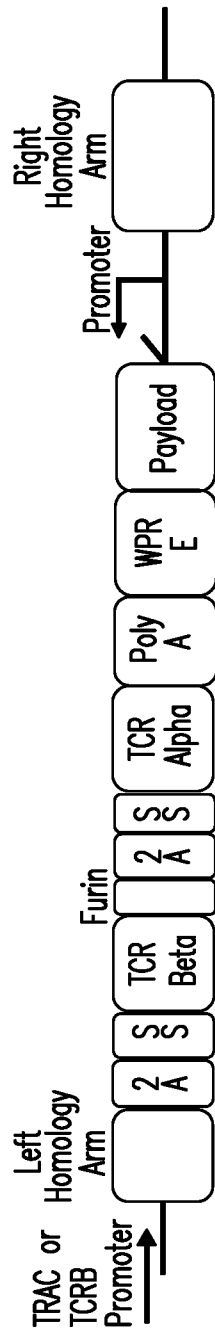


FIG. 6

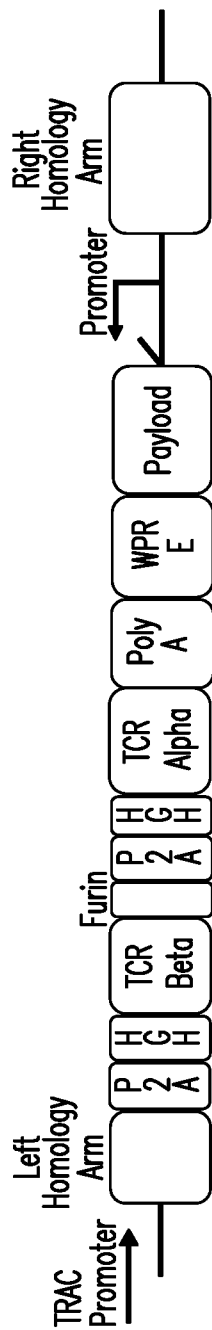


FIG. 7

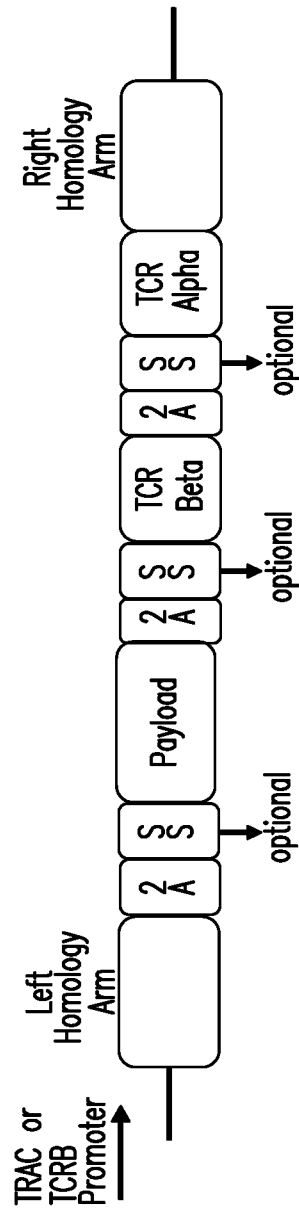


FIG. 8

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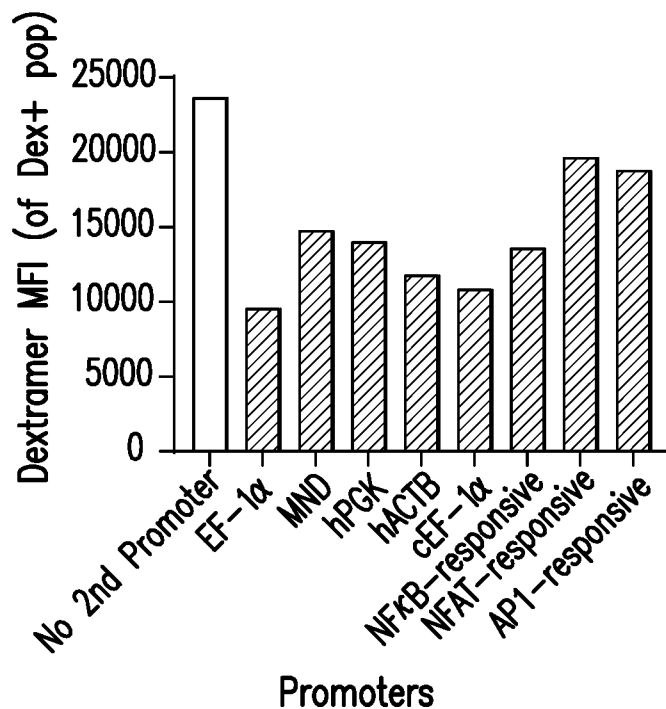


FIG. 9A

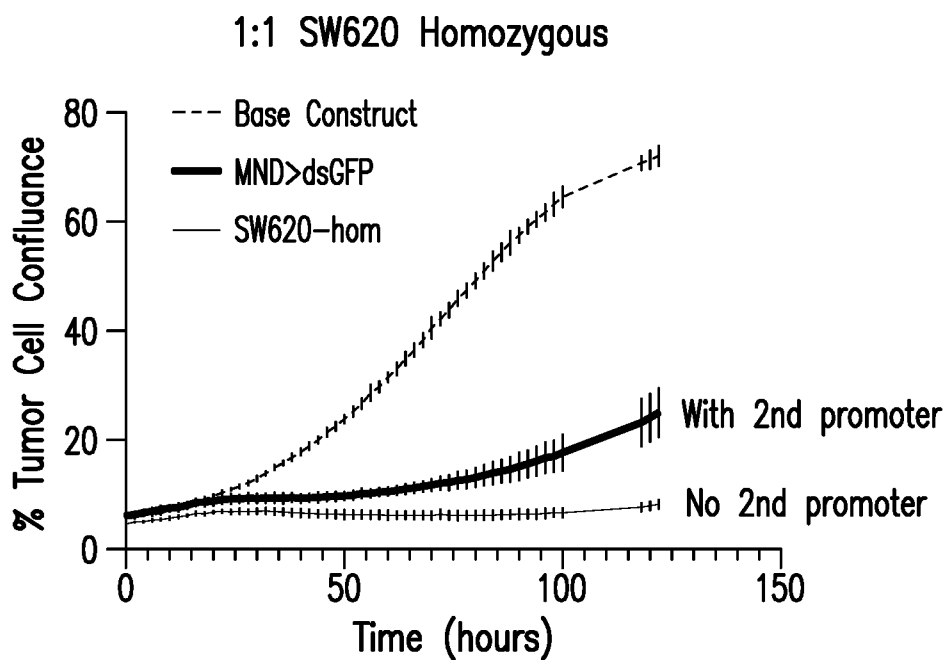


FIG. 9B

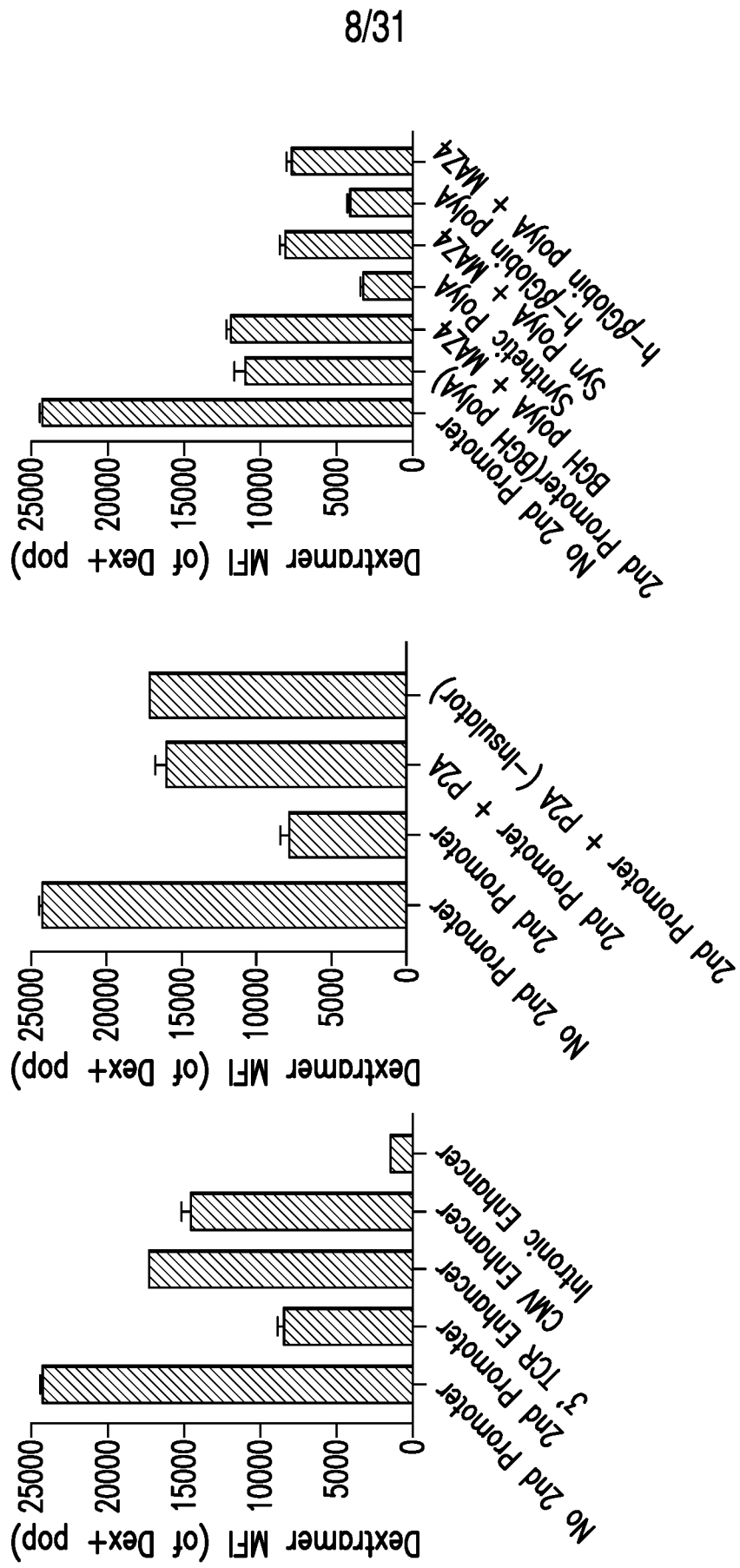


FIG. 10C

FIG. 10B

FIG. 10A

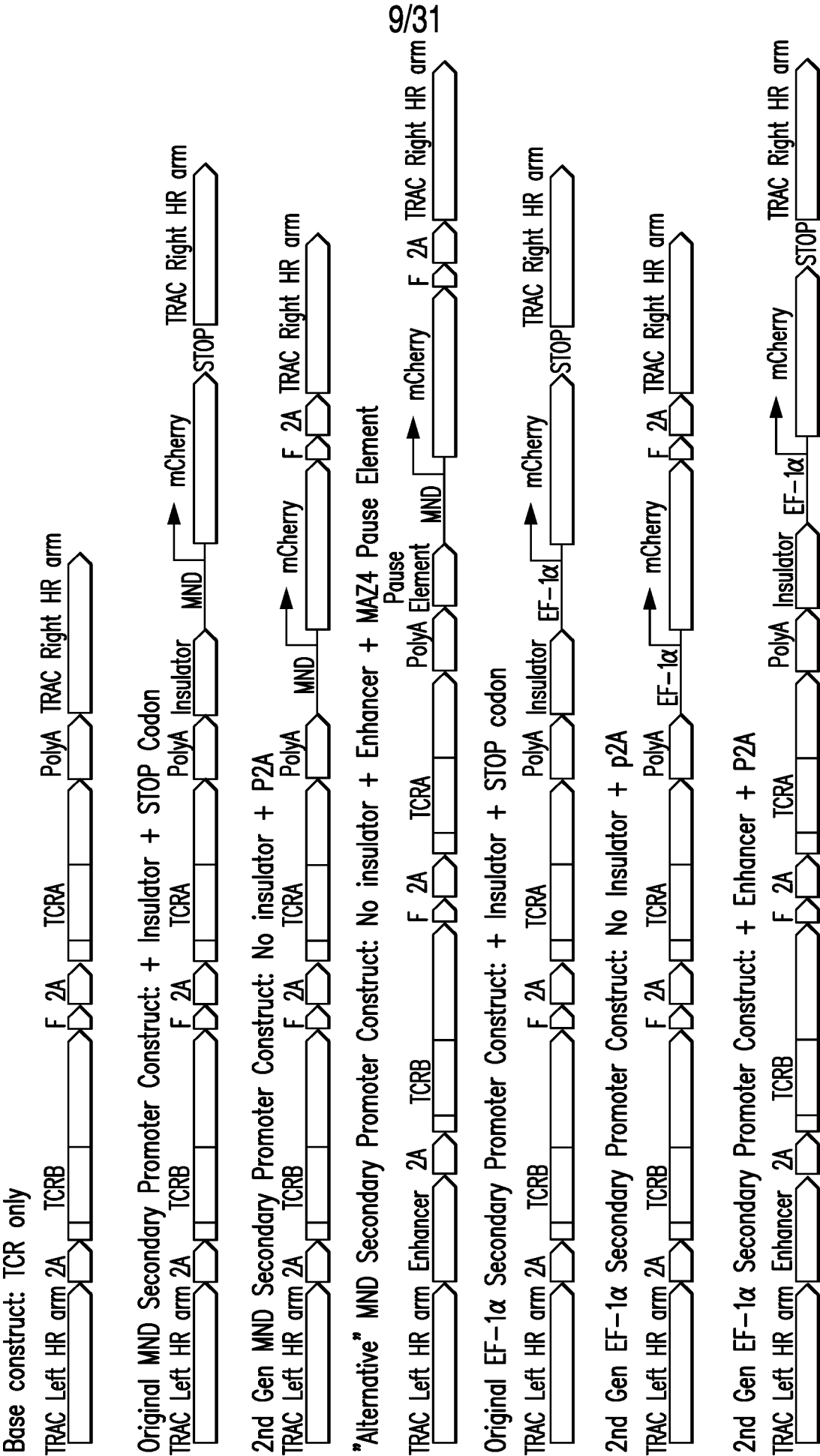


FIG. 11

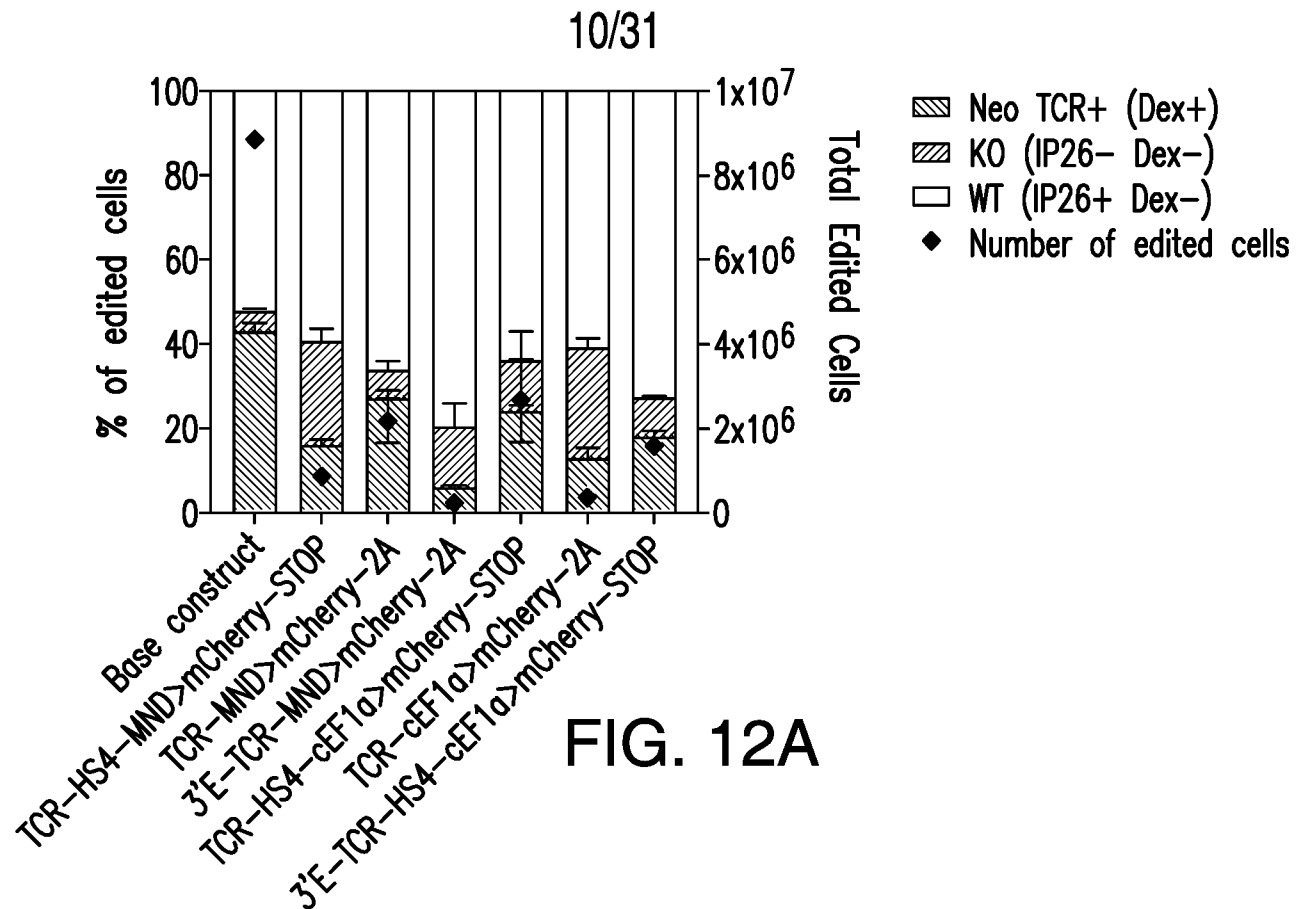


FIG. 12A

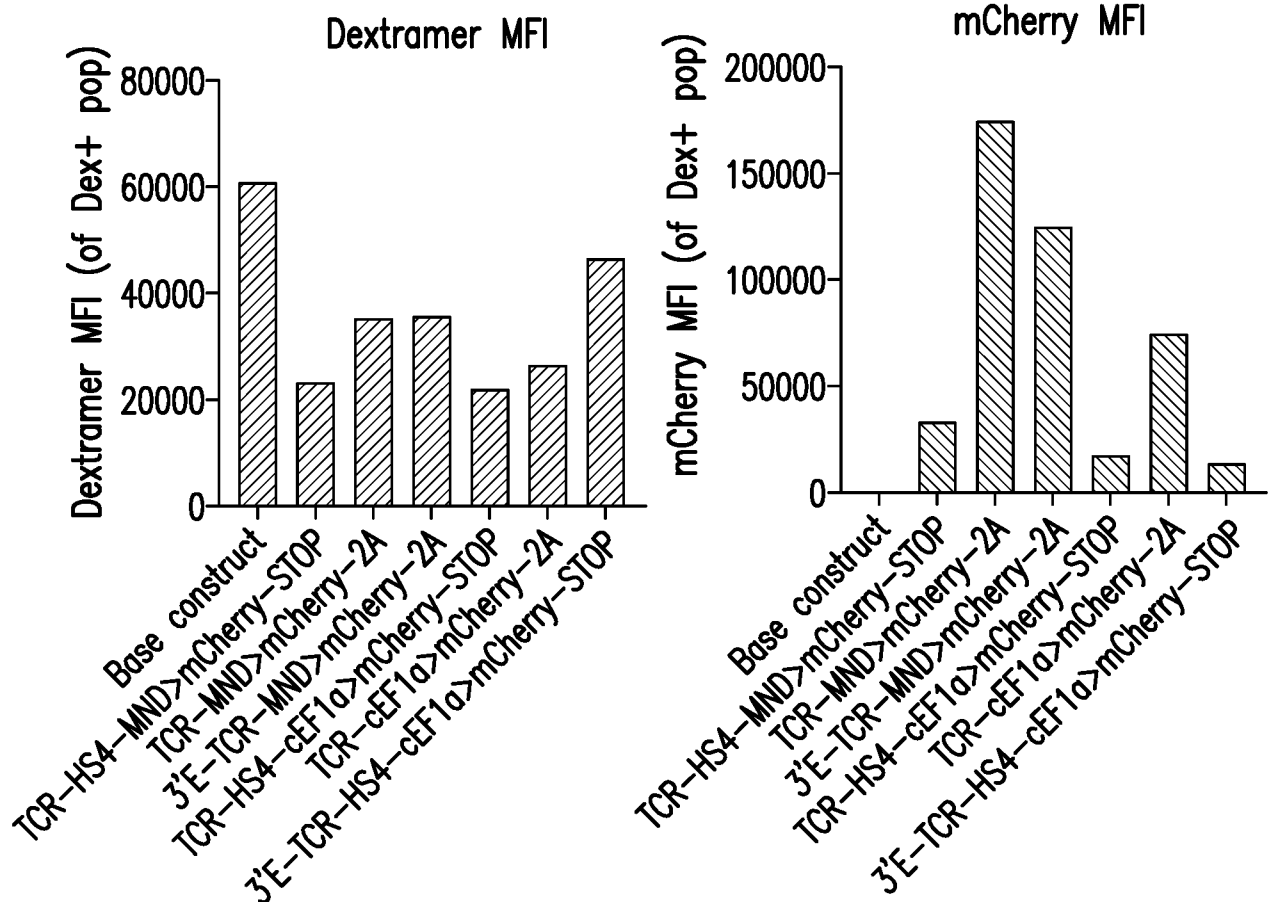
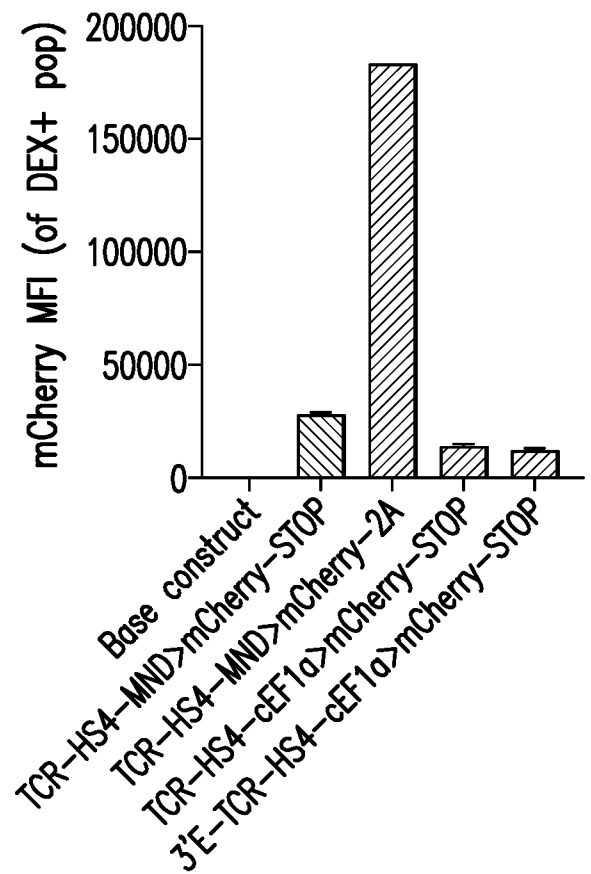
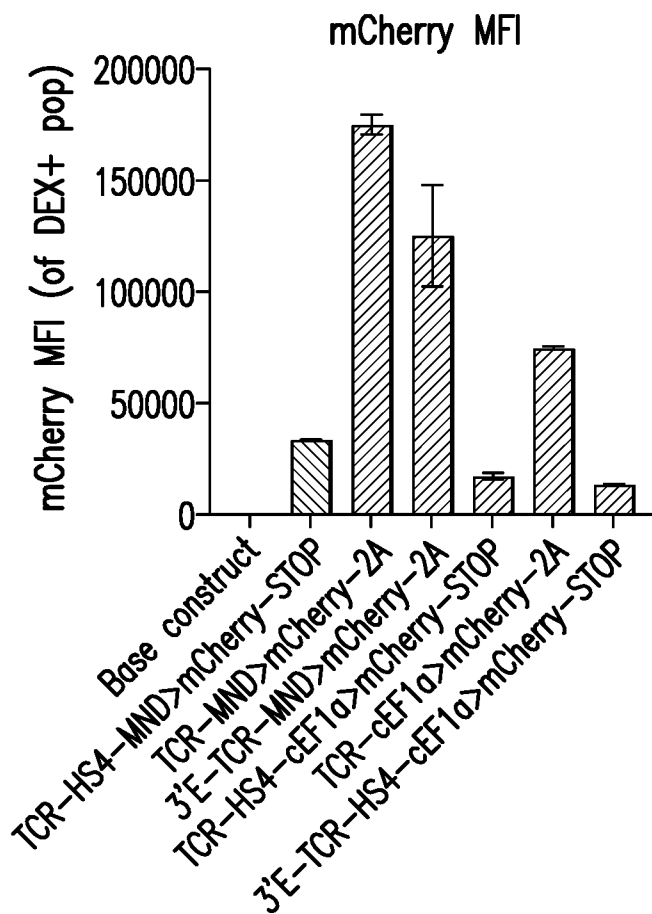
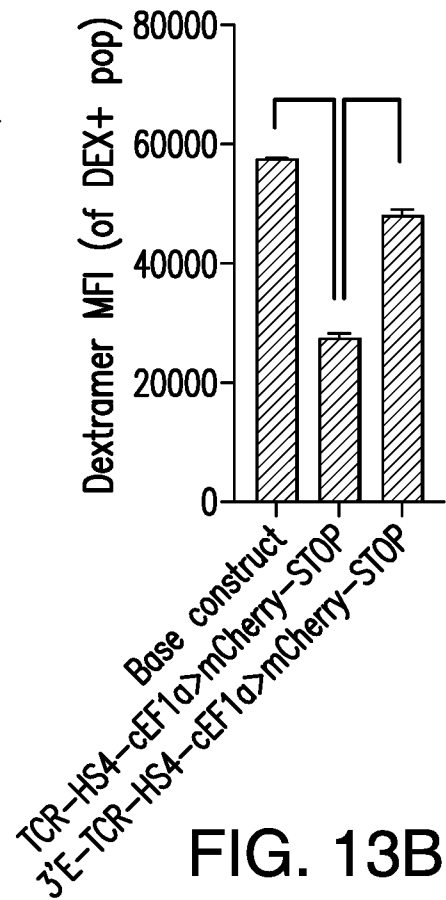
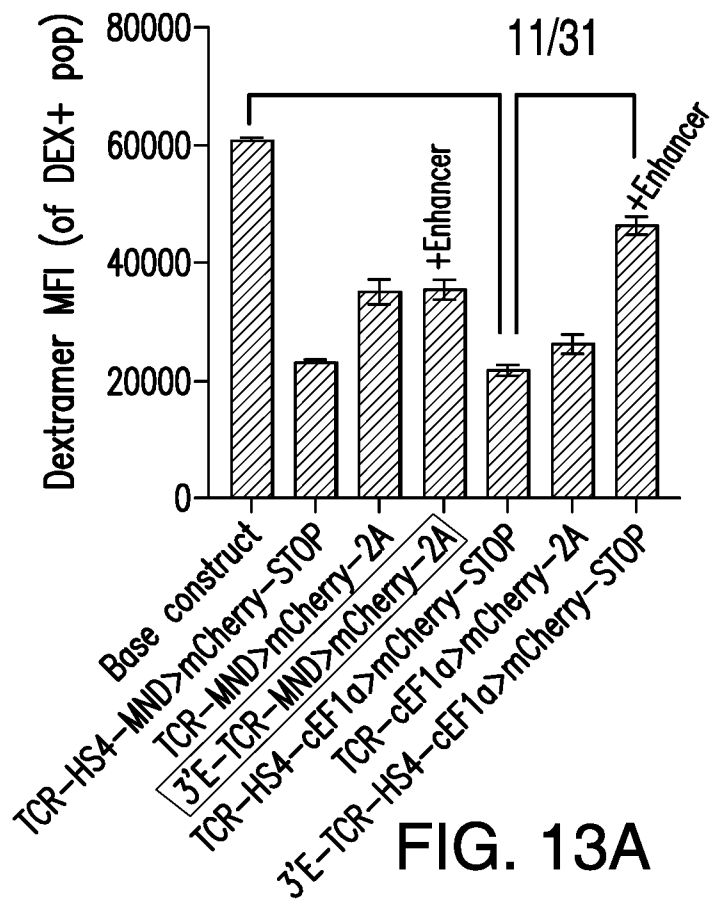


FIG. 12B

FIG. 12C



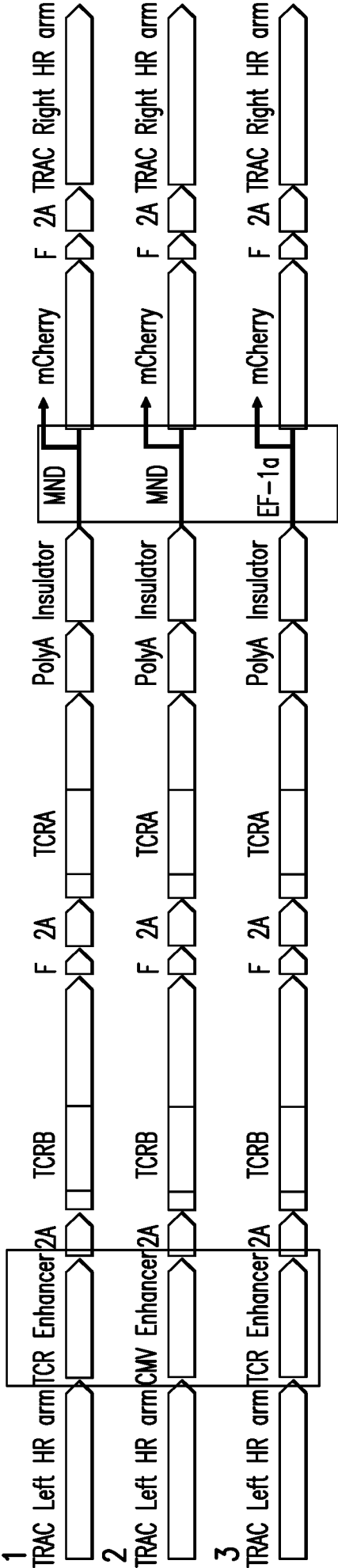
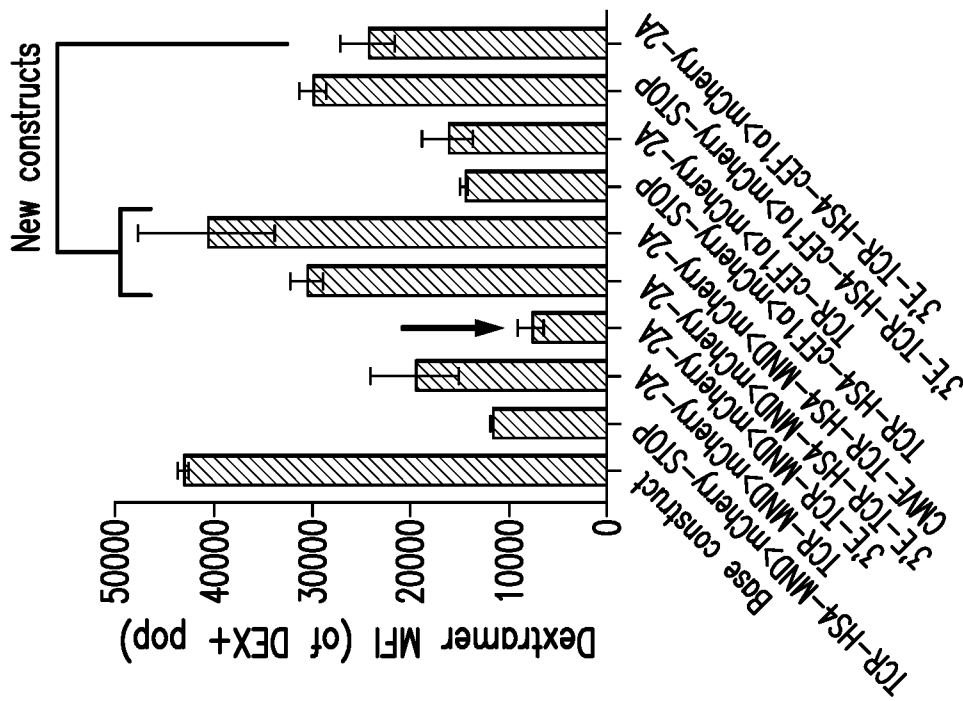
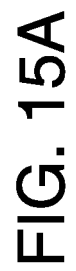


FIG. 14



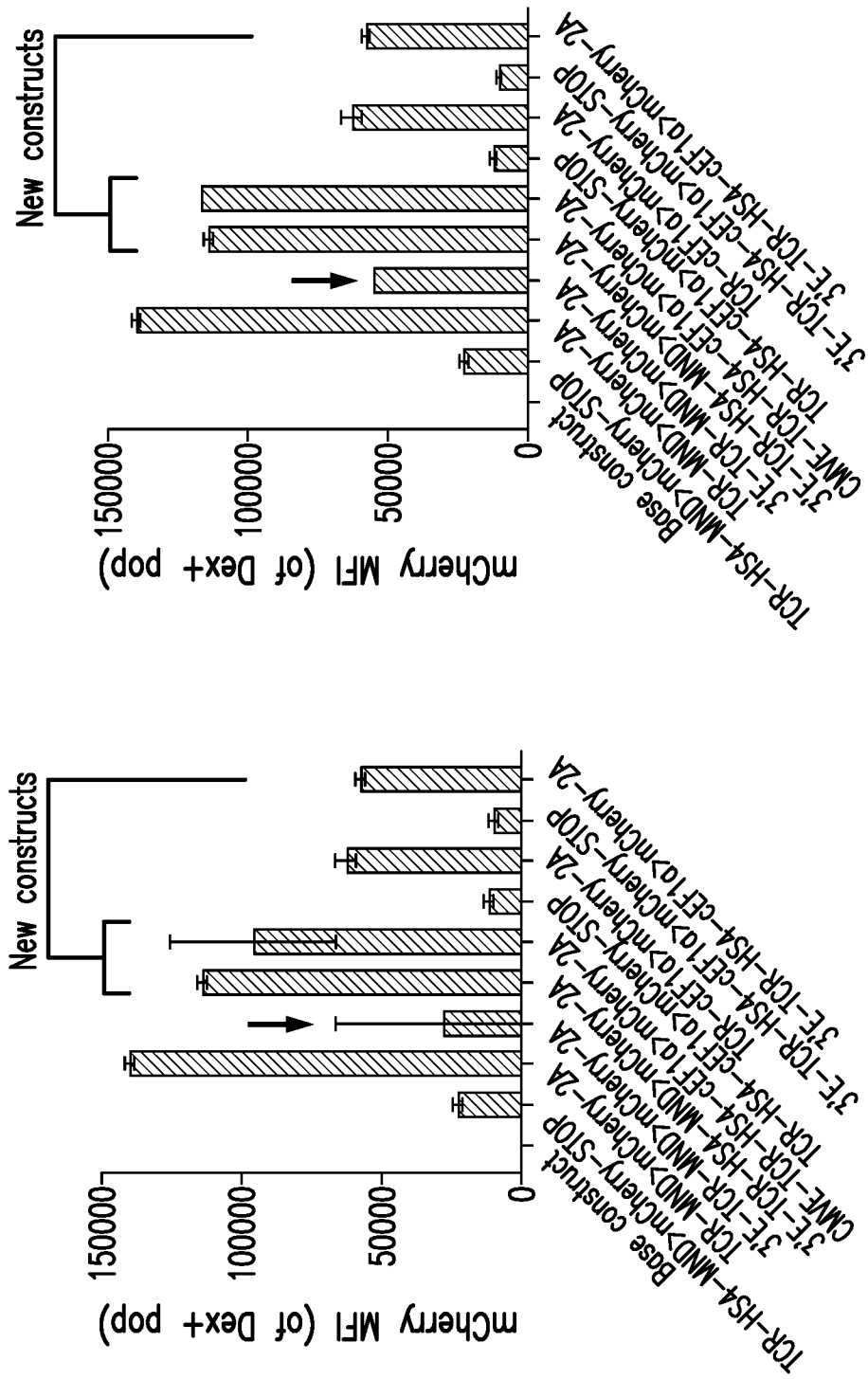
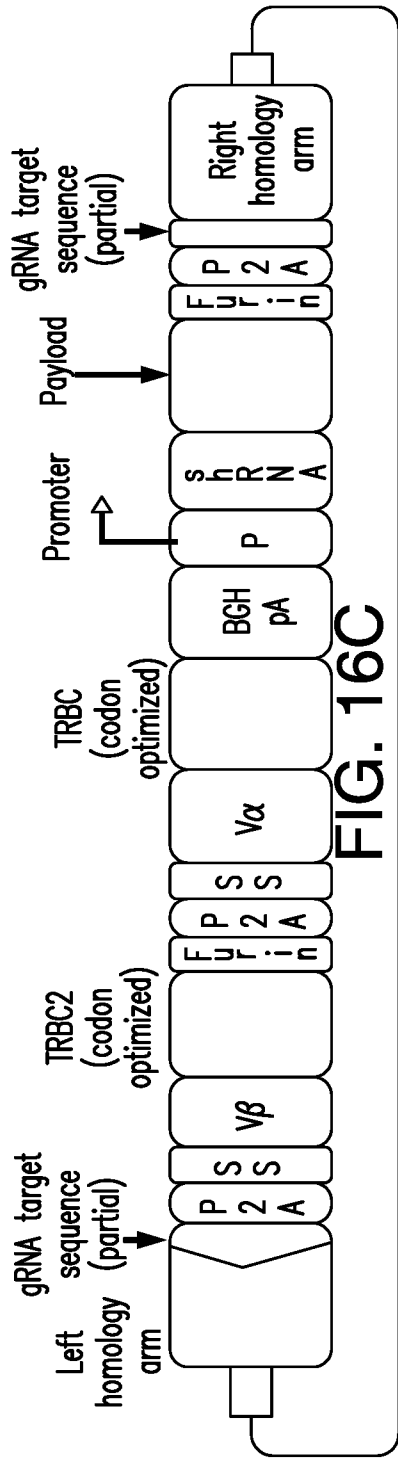
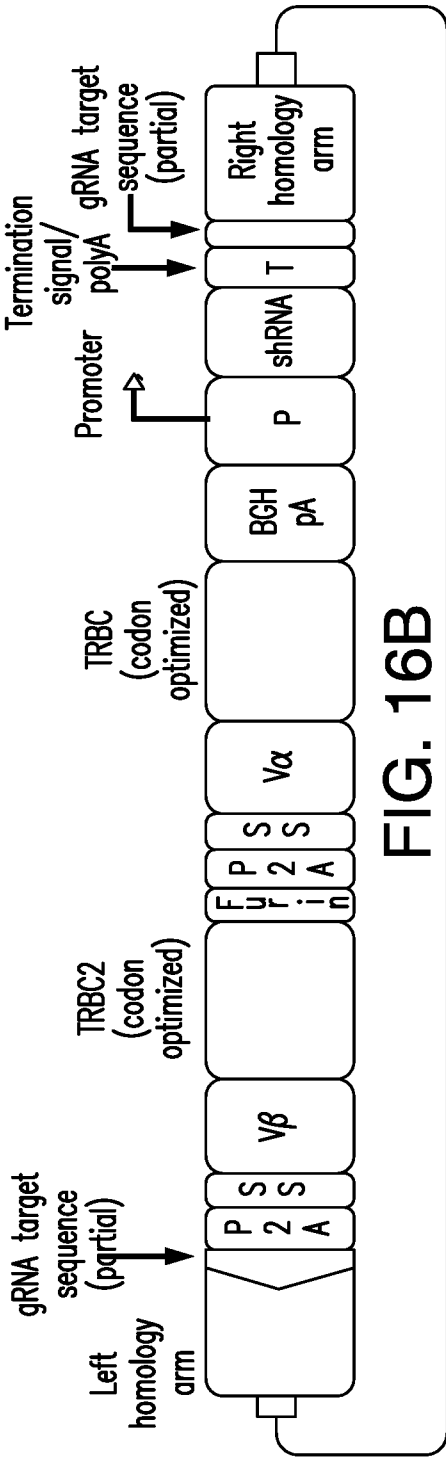
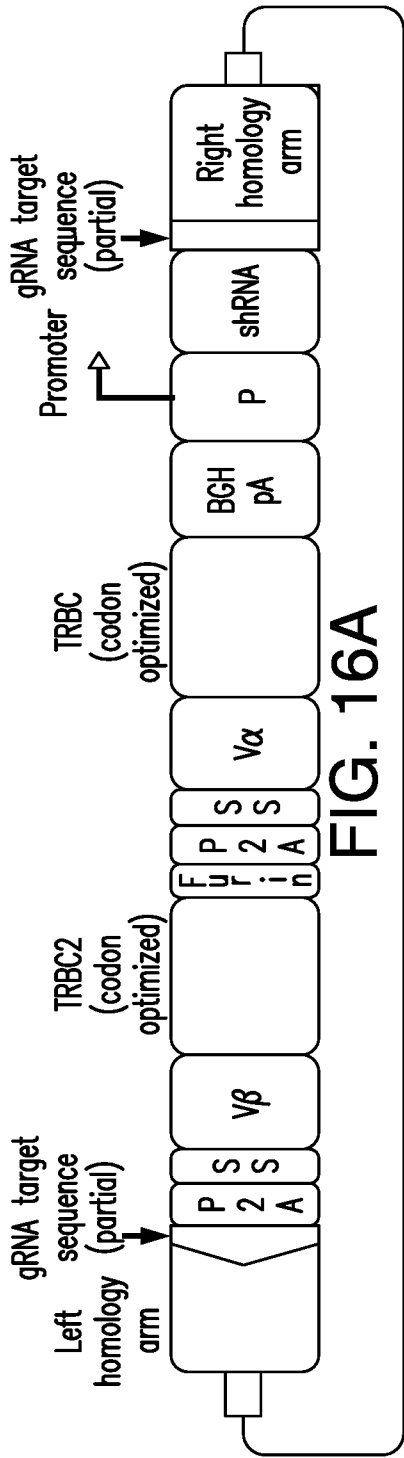


FIG. 15D



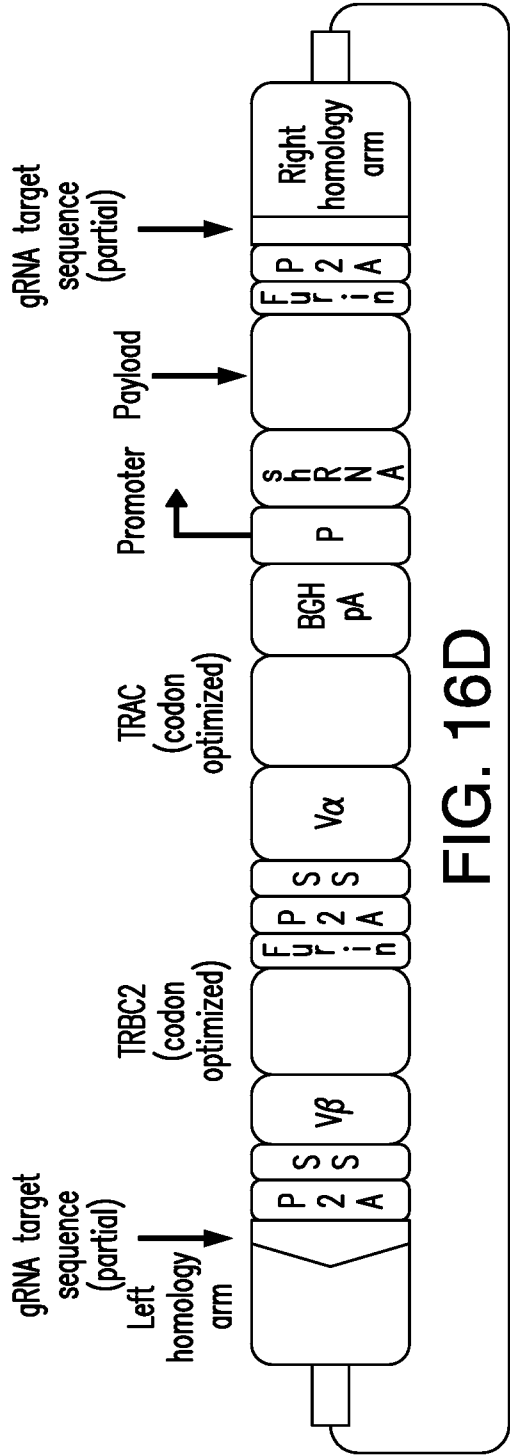


FIG. 16D

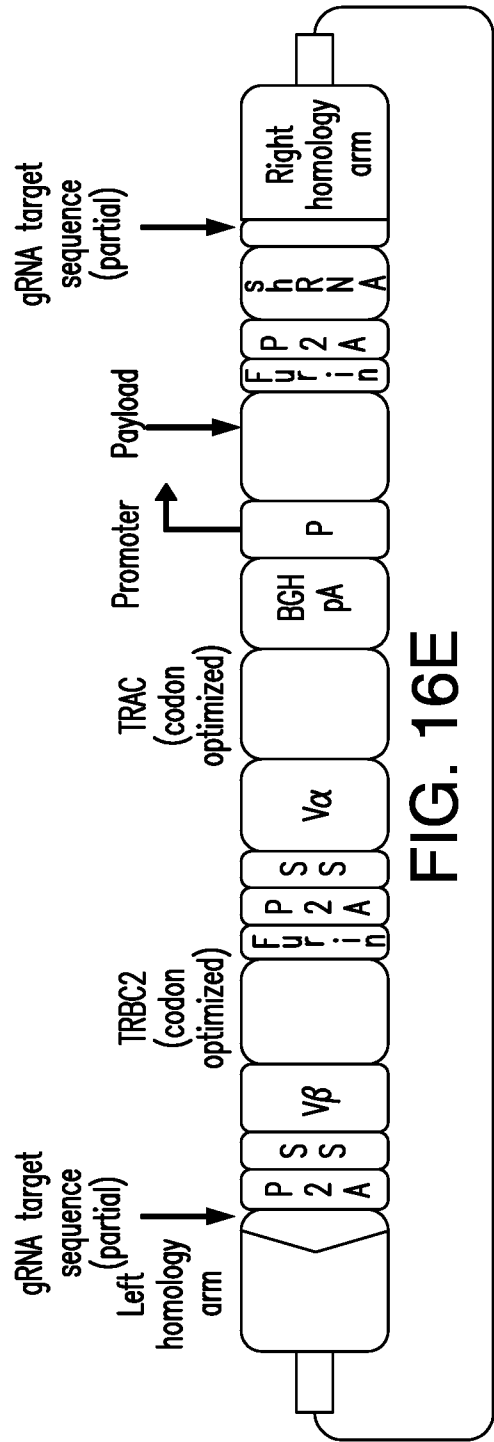
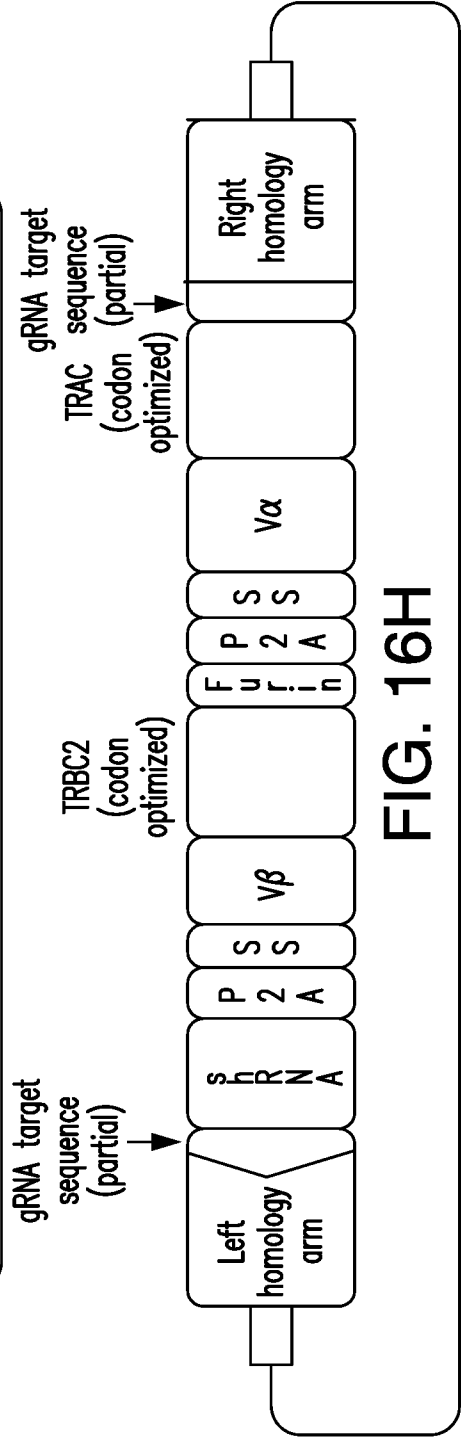
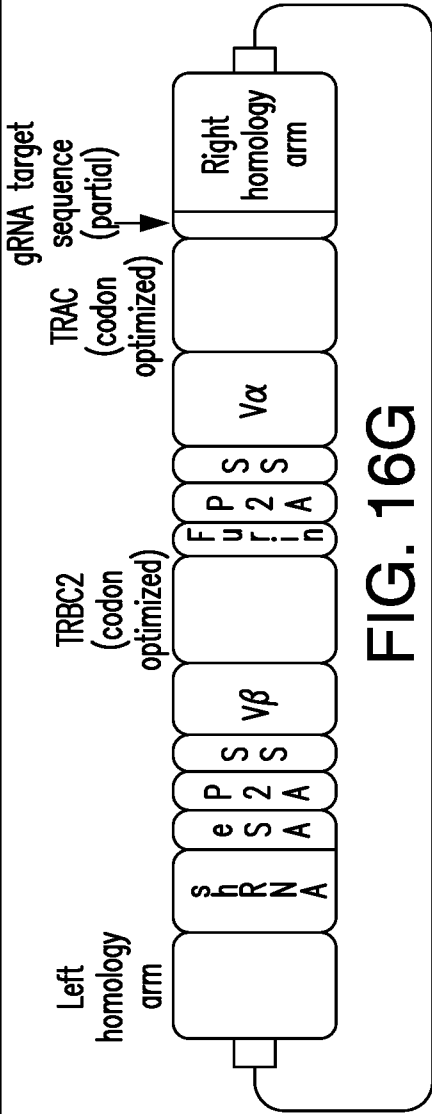
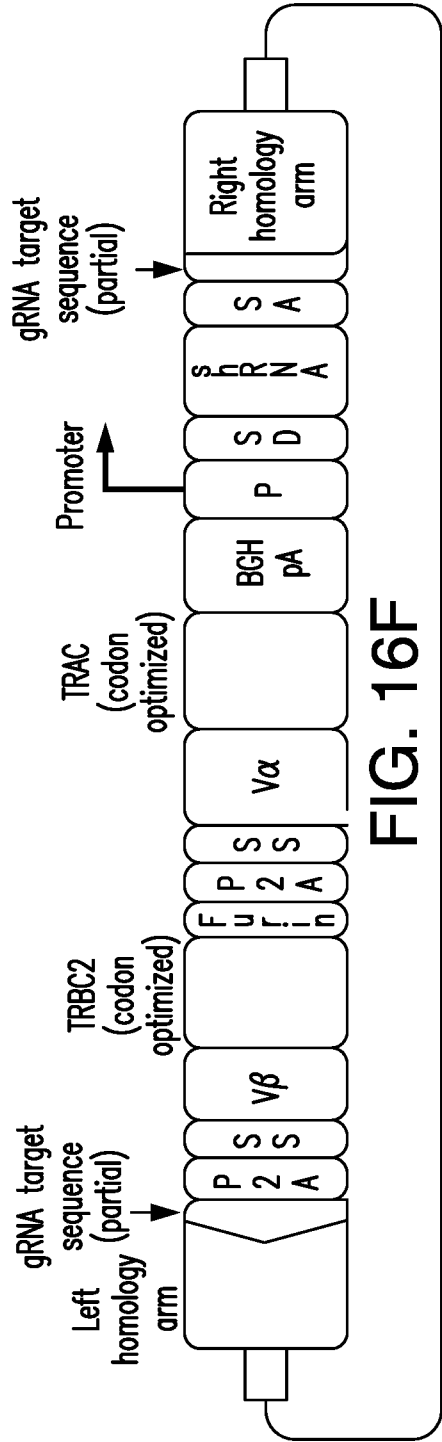


FIG. 16E



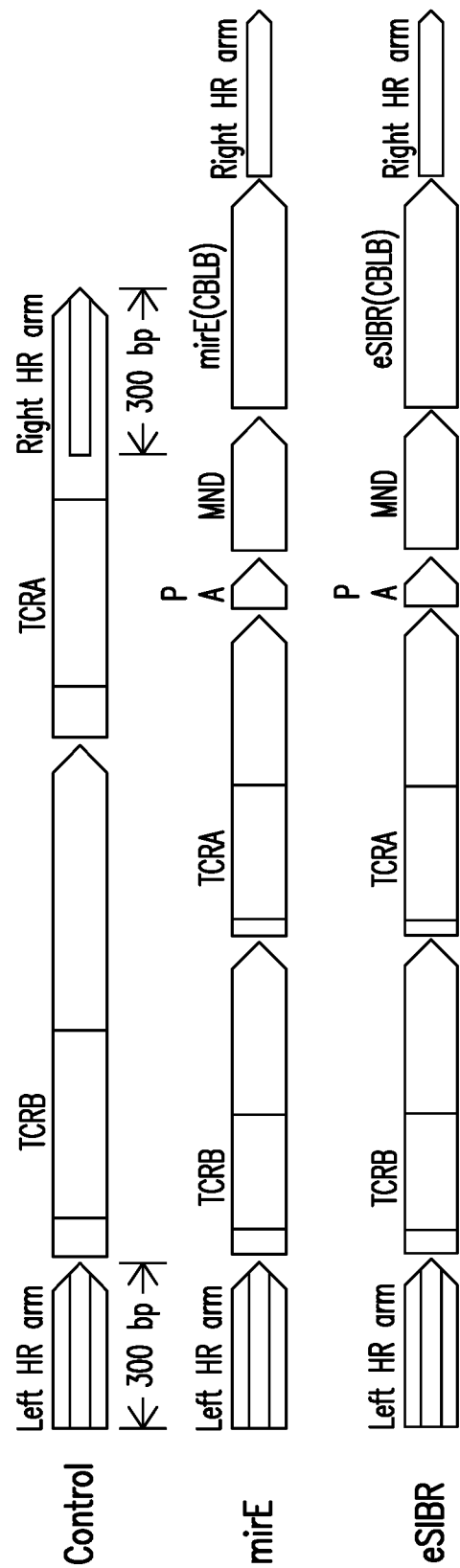


FIG. 17A

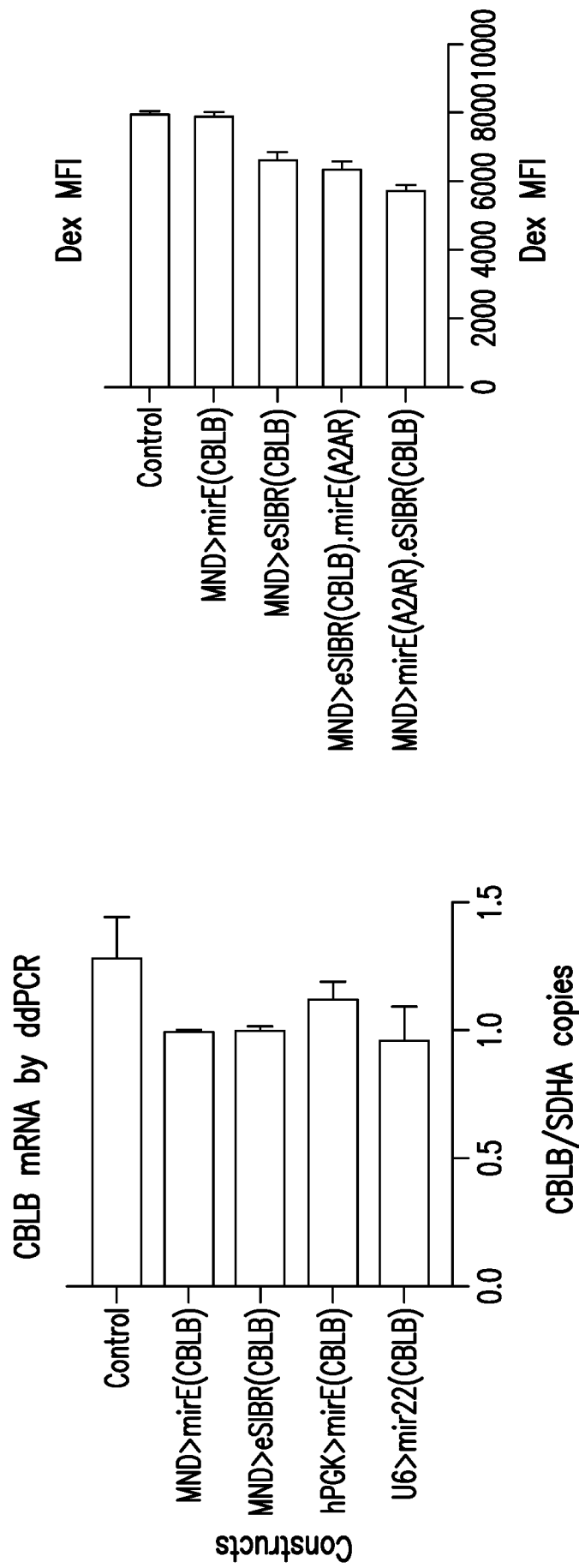


FIG. 17B

FIG. 17C

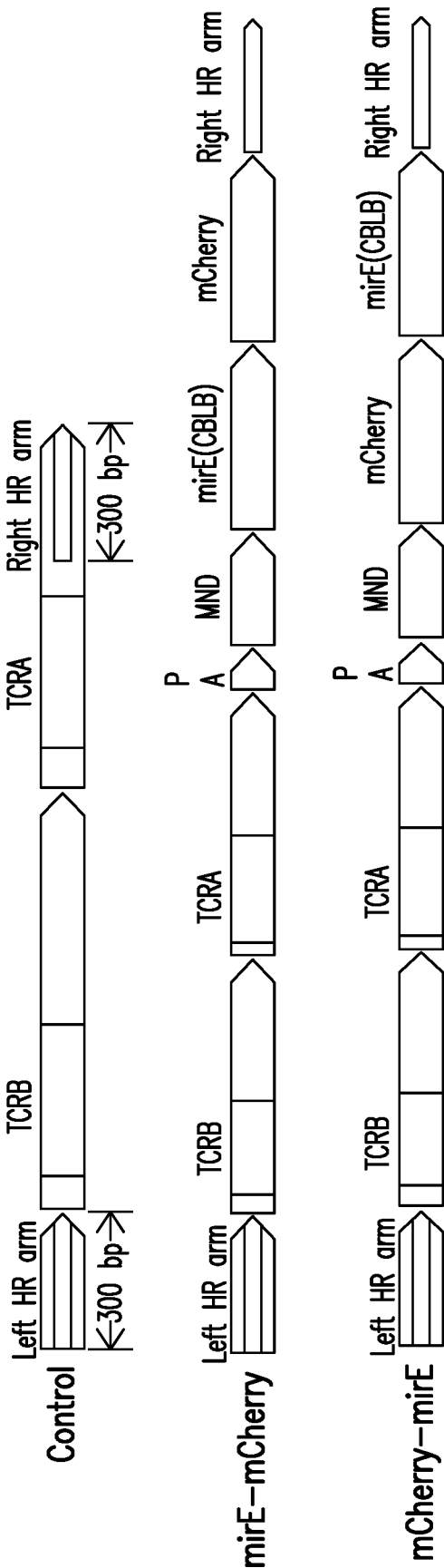


FIG. 18A

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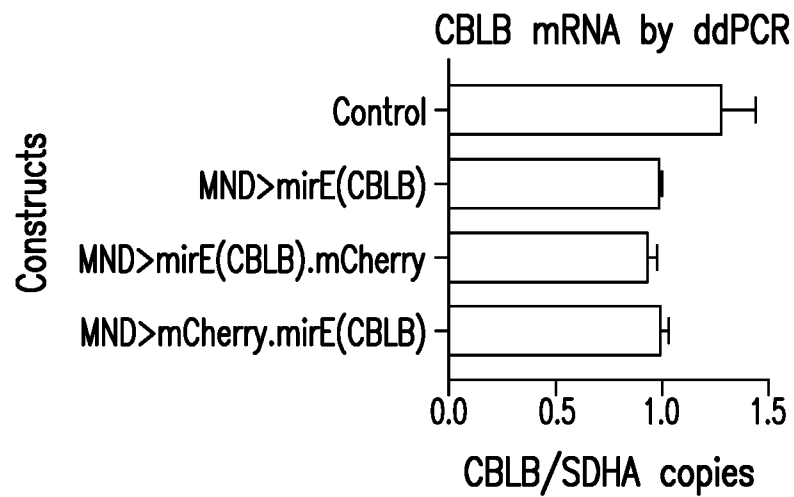


FIG. 18B

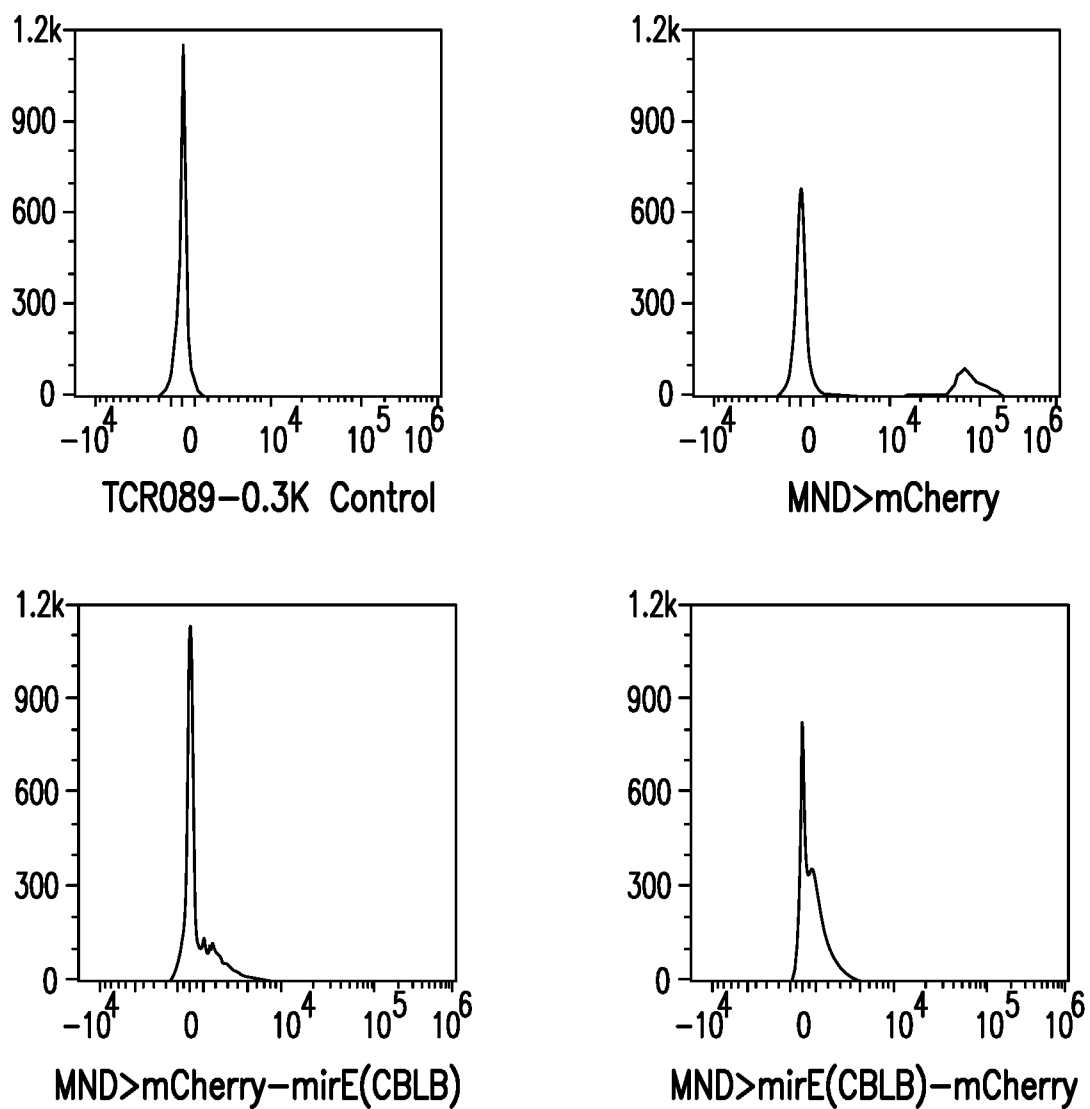


FIG. 18C

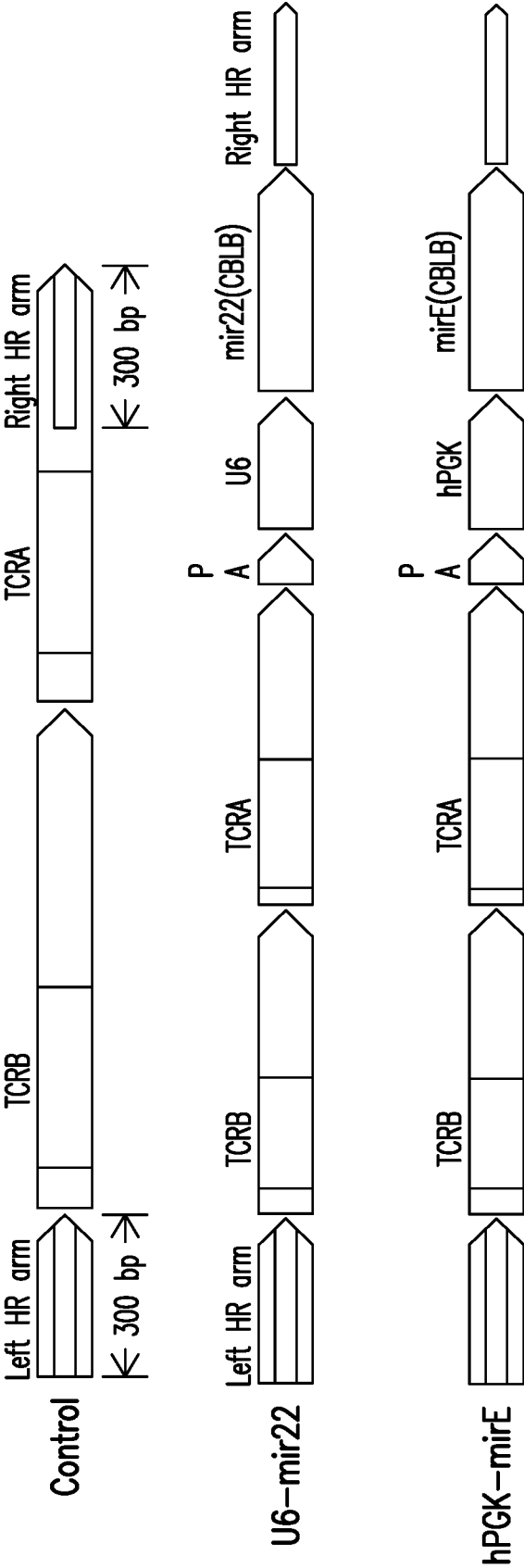


FIG. 19A

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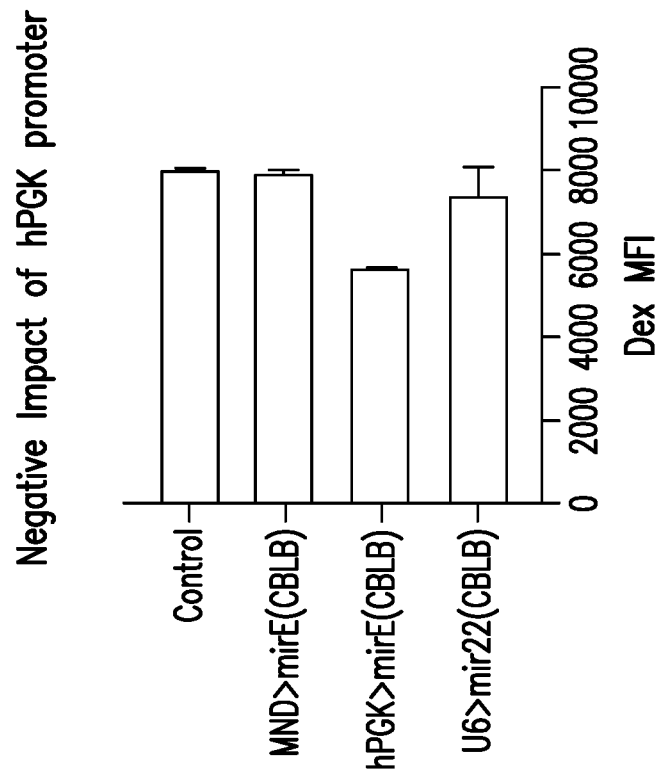


FIG. 19C

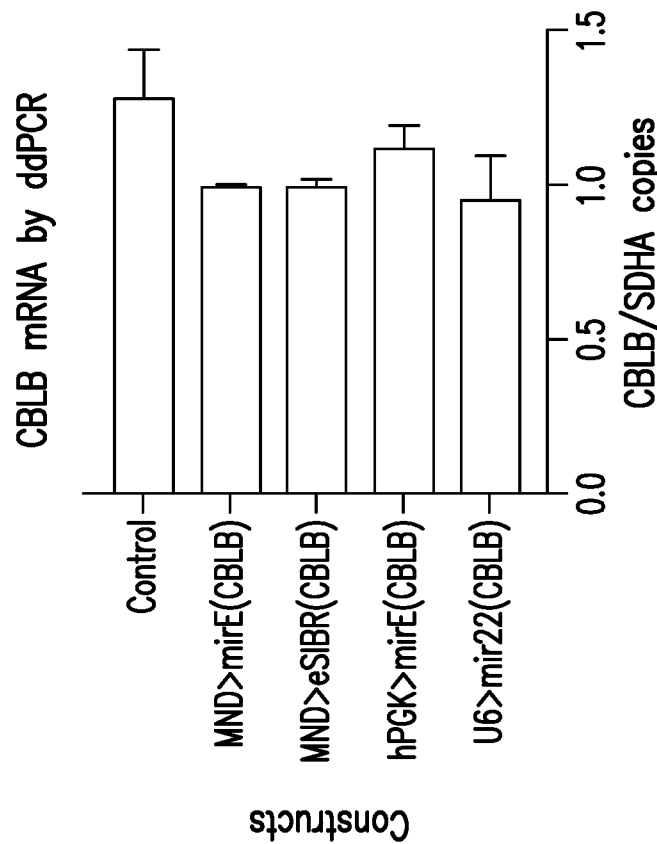


FIG. 19B

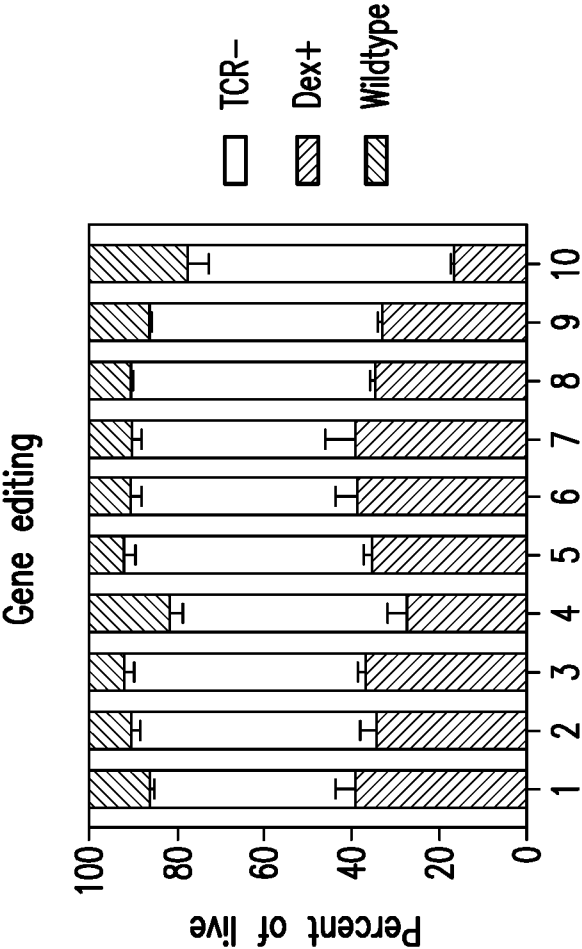


FIG. 19D

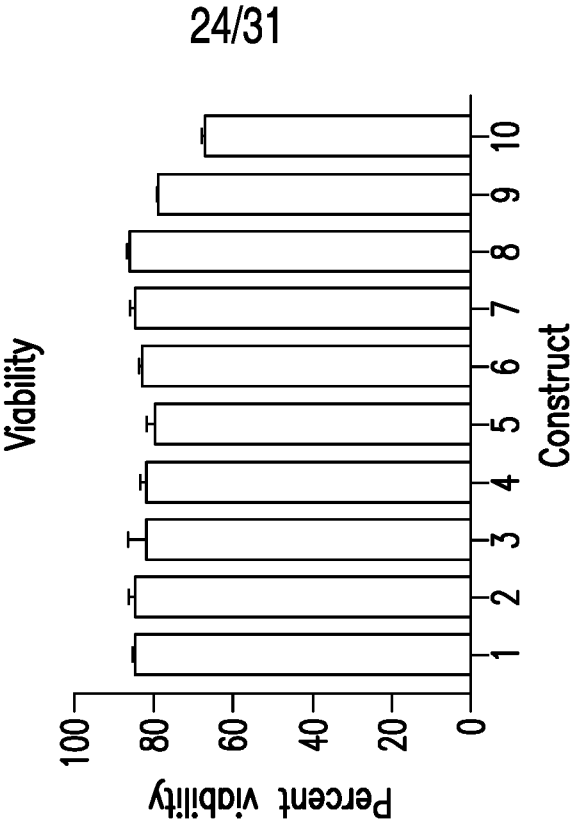


FIG. 19E

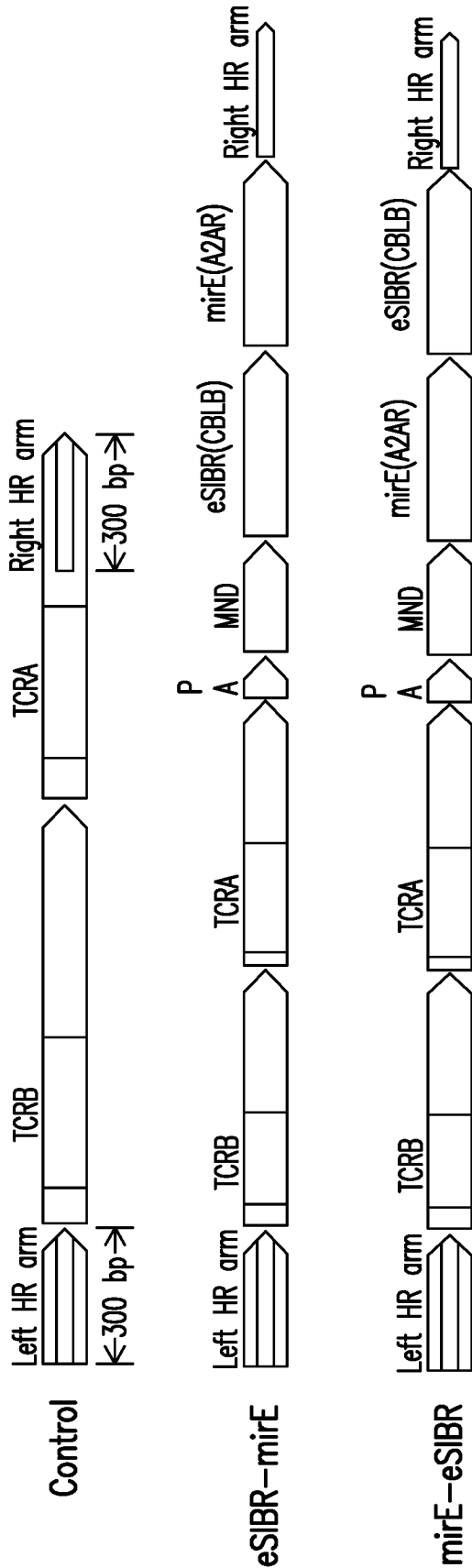


FIG. 20A

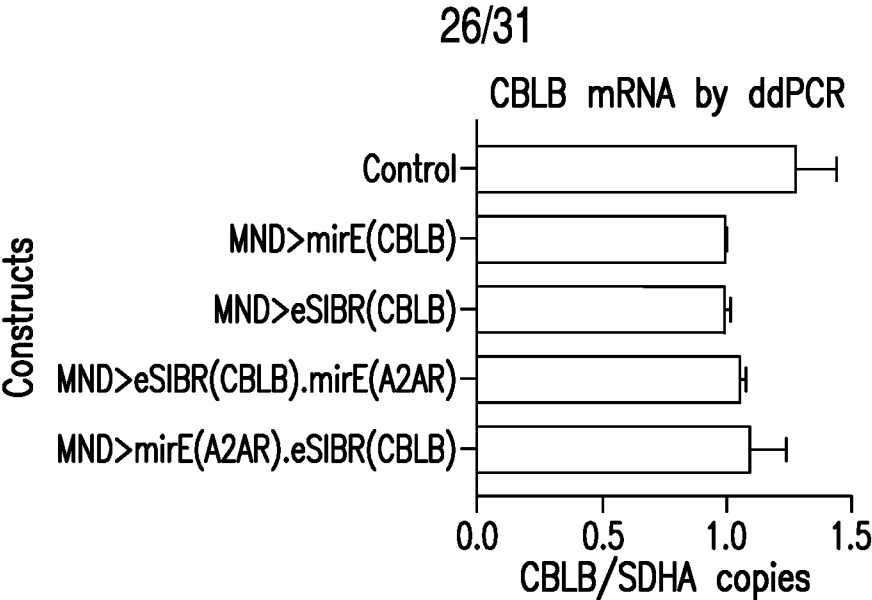


FIG. 20B

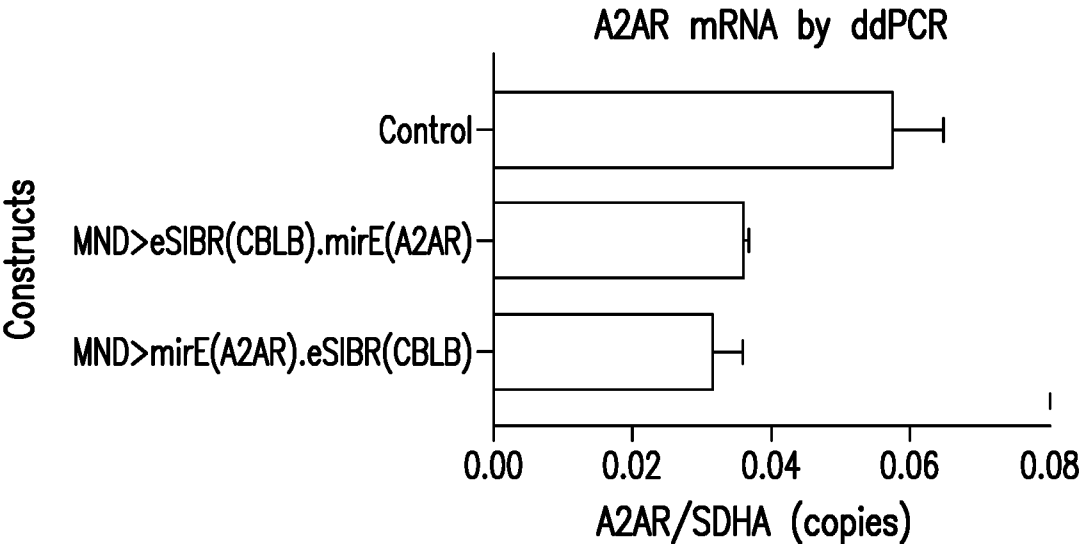


FIG. 20C

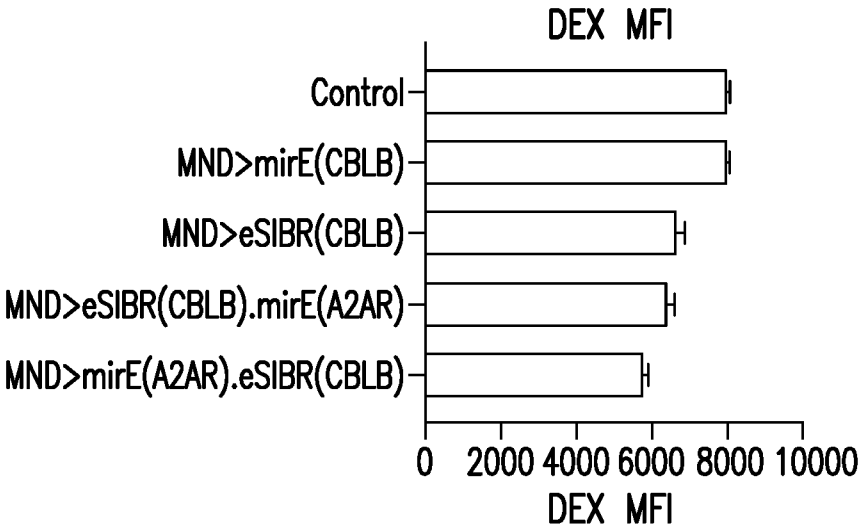


FIG. 20D

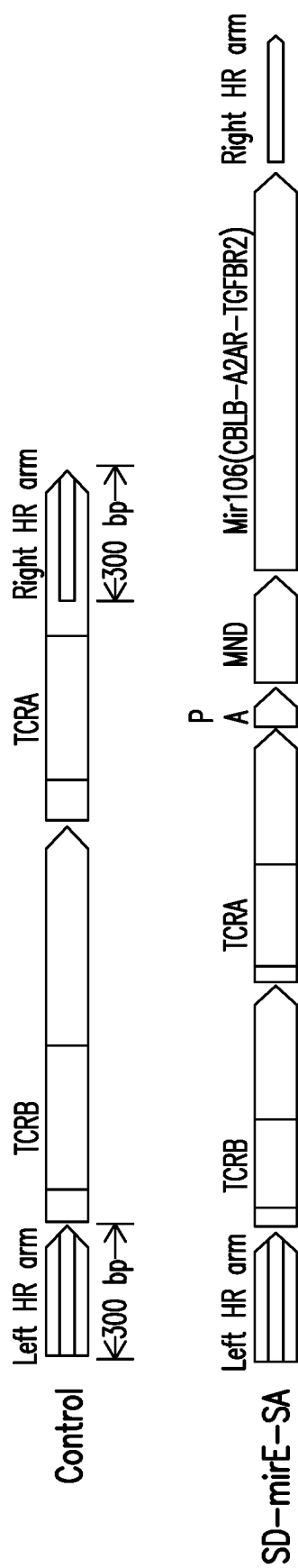


FIG. 21A

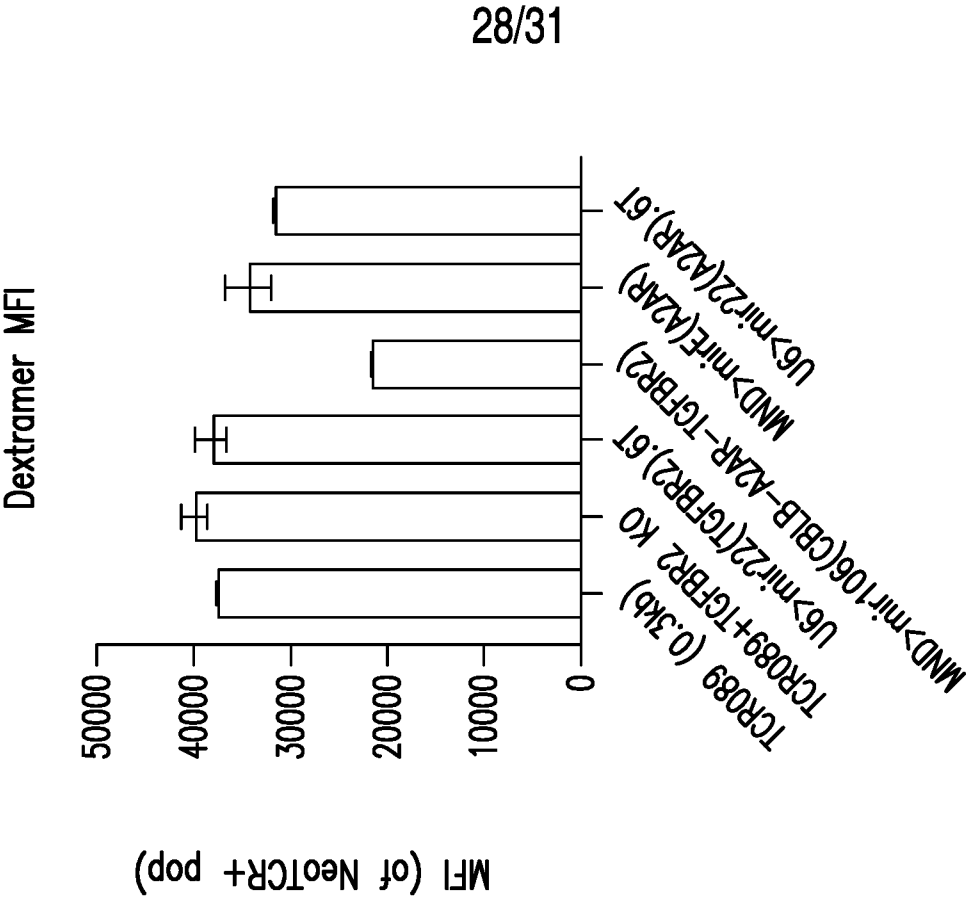


FIG. 21C

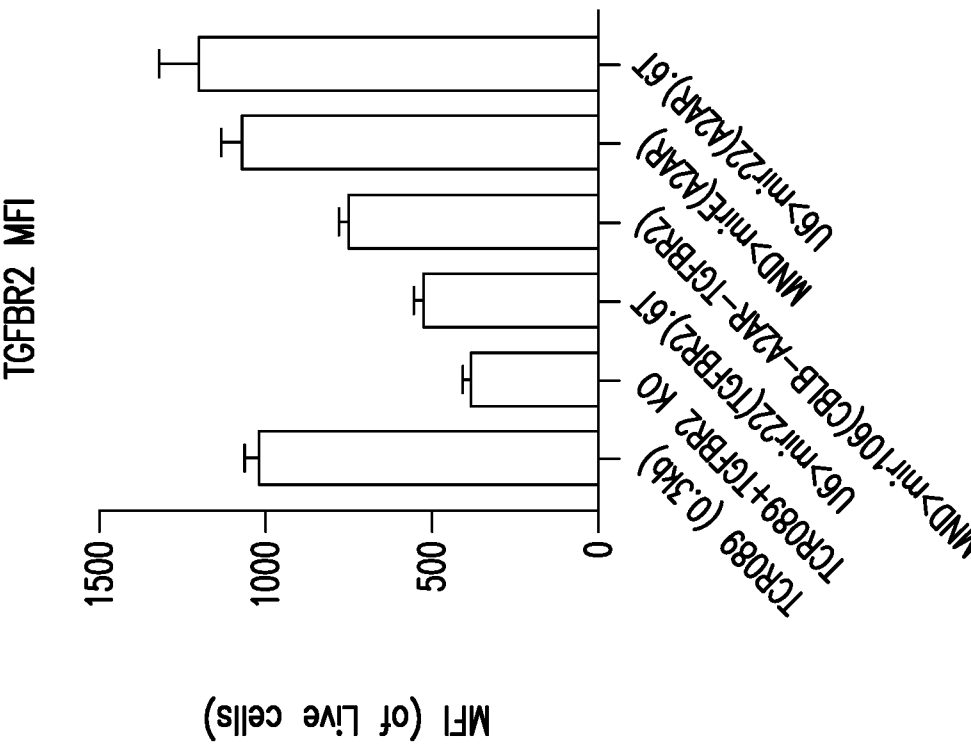


FIG. 21B

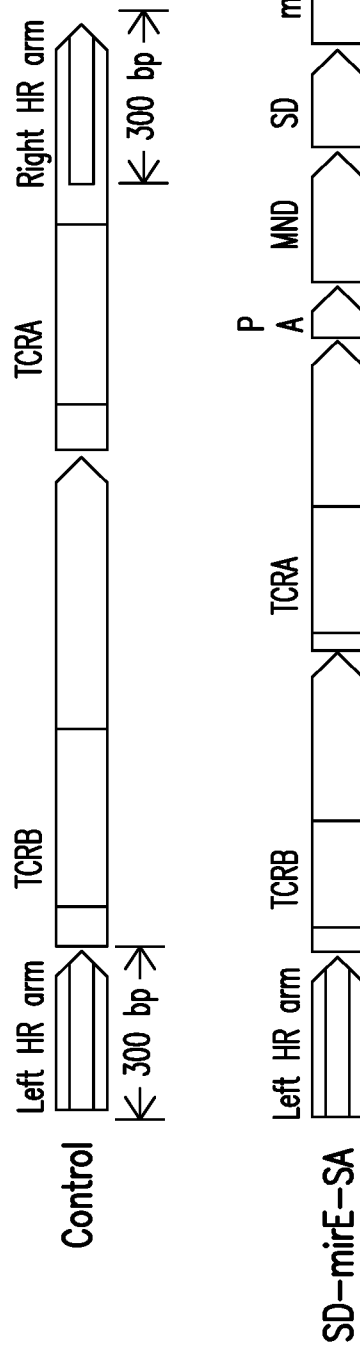


FIG. 22A

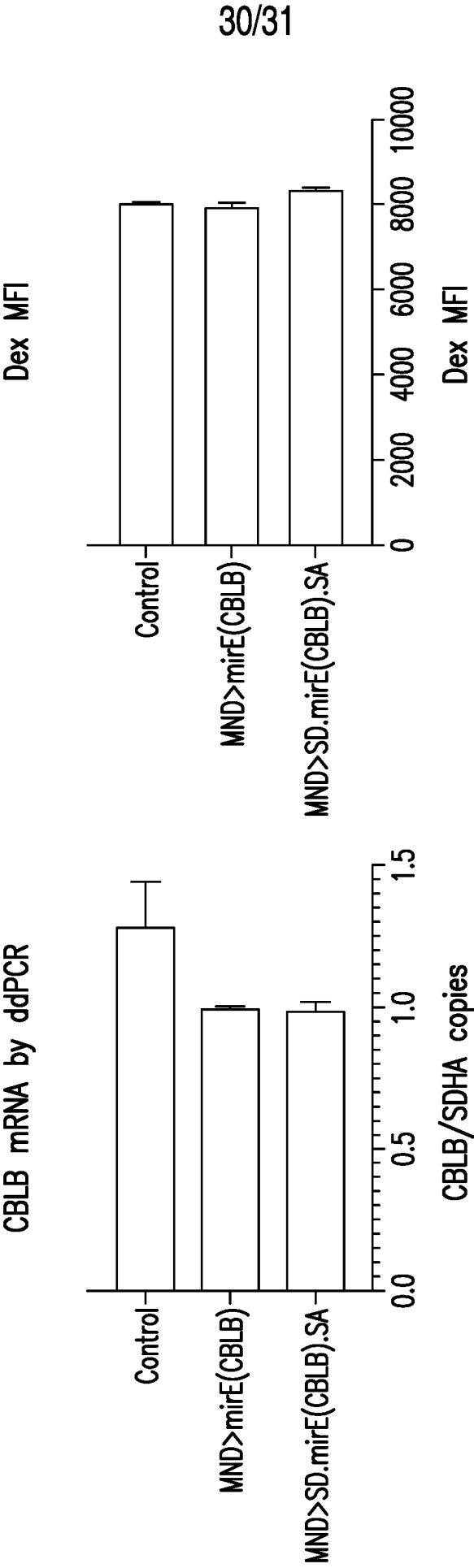


FIG. 22C

FIG. 22B

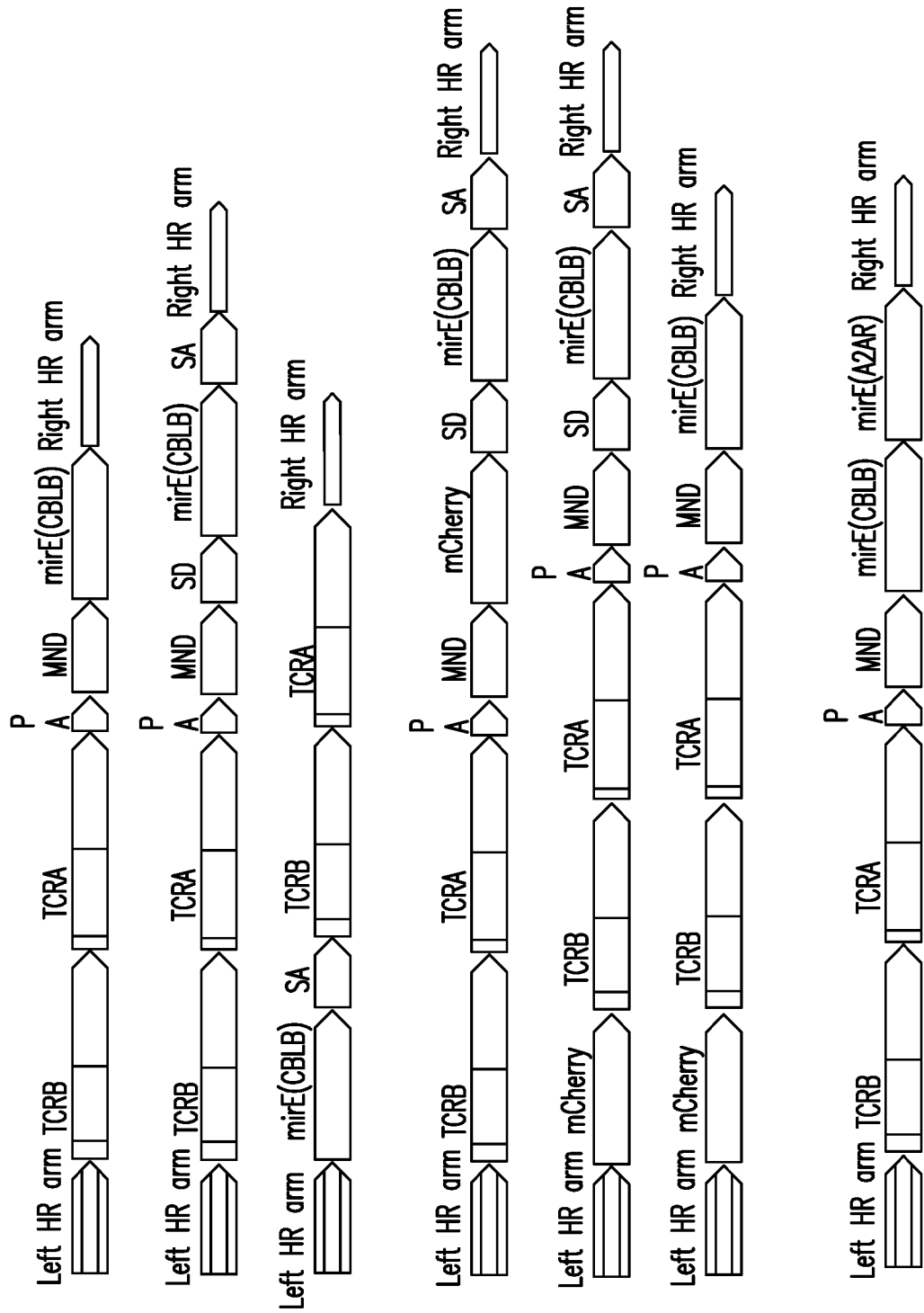


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/56737

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.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 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.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/US21/56737

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.: 16-17, 19-20, 23-24, 27-64, 75, 80-81, 83-150
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:
-***-Please See Supplemental Page-***-

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 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.:
Groups I+, Claims 1-15, 18, 21-23, 25-26, 65-74, 76-79, 82 and SEQ ID NO: 18 (insulator sequence)

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/56737

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 14/725; C12N 15/85; C12N 5/0783; C12N 5/10 (2021.01)

CPC - C07K 14/7051; C12N 15/85; C12N 5/0636; C12N 2800/107

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)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2017/023803 A1 (REGENTS OF THE UNIVERSITY OF MINNESOTA, ET AL.) 09 February 2017; paragraphs [0005], [0009], [0016]-[0017], [0050], [00250], [00256], [00259]-[00261], [00279], [00361]-[00363], [00421]	1, 3-6, 8, 21/1, 21/3-6, 22/21/1, 22/21/3-6, 25/1, 25/3-6, 65, 67-70, 72 --- 2, 7, 9-18, 21/2, 21/7, 22/21/2, 22/22/1/7, 25/2, 25/7, 26, 66, 71, 73-74, 76-77, 78-79, 82
Y	(TIAN, J ET AL.). "Independent and high-level dual-gene expression in adult stem-progenitor cells from a single lentiviral vector." pages 874-884. Gene Therapy. Vol. 16, No. 7. July 2009; page 3, paragraphs 3-4; DOI: 10.1038/gt.2009.46; DOI: 10.1038/gt.2009.46	2, 14/2, 15/14/2, 18/14/2, 21/2, 22/21/2, 25/2, 26/25/2, 66, 78/66, 79/78/66, 82/78/66
Y	WO 2019/210281 A1 (CASEBIA THERAPEUTICS LIMITED LIABILITY PARTNERSHIP) 31 October 2019; paragraphs [0261], [0292]	7, 21/7, 22/21/7, 25/7, 26/25/7, 71
Y	WO 2015/157579 A2 (DNA2.0, INC.) 15 October 2015; paragraph [00191]	9-11, 14, 18, 73-74, 78, 82
Y	WO 2020/210552 A1 (CALIFORNIA INSTITUTE OF TECHNOLOGY) 15 October 2020; paragraphs [0111], [0130]; Fig. 3A-B	12-13, 76-77
Y	WO 2019/210042 A1 (SEATTLE CHILDREN'S HOSPITAL) 31 October 2019; paragraph [0036]	14-15, 78-79

☒ Further documents are listed in the continuation of Box C.☐ 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

"D" document cited by the applicant in the international application

"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

03 March 2022 (03.03.2022)

Date of mailing of the international search report

MAR 30 2022

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/56737

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/133175 A1 (NANJING LEGEND BIOTECH CO., LTD.) 10 August 2017; paragraph [0251]	26

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/56737

-***-Continued From Box No. III: Observations where unity of invention is lacking-***-

Groups I+, Claims 1-15, 18, 21-23, 25-26, 65-74, 76-79, 82 and SEQ ID NO: 18 (insulator sequence) are directed towards cells and polynucleotides comprising exogenous TCR.

The cells and polynucleotides of Claims 1-9, 10-11 (each in-part), 12-15, 18, 21-23, 25-26, 65-73, 74 (in-part), 76-79, 82 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass SEQ ID NO: 18 (first exemplary insulator sequence).

Applicant is invited to elect additional insulator sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO: such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and where available as an option within at least one searchable claim, to be searched. Additional insulator sequence(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected insulator sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 19 (insulator sequence).

Groups I+ share the technical features including: a cell comprising an exogenous polynucleotide comprising an exogenous enhancer, an insulator, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter; a cell comprising an exogenous polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter and the sequence encoding at least one Payload is under control of an exogenous promoter; a cell comprising an exogenous polynucleotide comprising a sequence encoding an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell, wherein the sequence encoding an exogenous TCR and the first sequence encoding a Payload are under control of an endogenous promoter and the second sequence encoding a Payload is under control of an exogenous promoter; a polynucleotide comprising an exogenous enhancer, an insulator, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter; a polynucleotide comprising an exogenous enhancer, a pause element, a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter; a polynucleotide comprising an insulator, a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), a first homology arm, a second homology arm, a sequence encoding an exogenous TCR, and a sequence encoding at least one Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the sequence encoding at least one Payload is under control of an exogenous promoter; a polynucleotide comprising an exogenous TCR, a first sequence encoding a Payload, and a second sequence encoding a Payload, wherein the first and second homology arms are homologous to a TRAC or TRBC locus and wherein the second sequence encoding a Payload is under control of an exogenous promoter; these shared technical features are previously disclosed by WO 2017/023803 A1 (REGENTS OF THE UNIVERSITY OF MINNESOTA) (hereinafter 'Minnesota') in view of the publication entitled 'Independent and high-level dual-gene expression in adult stem-progenitor cells from a single lentiviral vector' by Tian et al. (hereinafter 'Tian') and further in view of WO 2019/210281 A1 (CASEBIA THERAPEUTICS LIMITED LIABILITY PARTNERSHIP) (hereinafter 'Casebia').

Minnesota discloses a cell (a cell; paragraph [0005]) comprising an exogenous polynucleotide (comprising an exogenous polynucleic acid; paragraphs [0005], [00261]) comprising an exogenous enhancer (an exogenous enhancer; paragraphs [0007], [0009], [00363]), an insulator (an insulator; paragraph [00363]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR transgene; paragraphs [0009], [0050]), and a sequence encoding at least one Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell (the transgenes are inserted into a locus in the genome of the cell; paragraphs [00250], [00256]), wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter (the transgene comprising an exogenous TCR is under control of an endogenous promoter; paragraphs [0009], [0050], [00250], [00256]) and the sequence encoding at least one Payload is under control of an exogenous promoter (the transgene comprising the anti-DNA sensing protein transgene is under control of its own promoter (exogenous promoter); paragraphs [0050], [00250]); a cell (a cell; paragraph [0005]) comprising an exogenous polynucleotide (comprising an exogenous polynucleic acid; paragraphs [0005], [00261]) comprising an exogenous enhancer (an exogenous enhancer; paragraphs [0007], [0009], [00363]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), and a sequence encoding at least one Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell (the transgenes are inserted into a locus in the genome of the cell; paragraphs [00250], [00256]), wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter (the transgene comprising an exogenous TCR is under control of an endogenous promoter; paragraphs [0009], [0050], [00250], [00256]) and the sequence encoding at least one Payload is under control of an exogenous promoter (the transgene comprising the anti-DNA sensing protein transgene is under control of its own promoter (exogenous promoter); paragraphs [0050], [00250]); a cell (a cell; paragraph [0005]) comprising an exogenous polynucleotide (comprising an exogenous polynucleic acid; paragraphs [0005], [00261]) comprising an insulator (an insulator; paragraph [00363]), a Woodchuck Hepatitis Virus Posttranscriptional

-***-Continued Within the Next Supplemental Box-***-

-Continued from previous Supplemental Box-

Regulatory Element "WPRE" (the nucleic acid sequence comprises a woodchuck hepatitis virus post-transcriptional regulatory element; paragraph [00247]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), and a sequence encoding at least one Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell (the transgenes are inserted into a locus in the genome of the cell; paragraphs [00250], [00256]), wherein the sequence encoding an exogenous TCR is under control of an endogenous promoter (the transgene comprising an exogenous TCR is under control of an endogenous promoter; paragraphs [0009], [0050], [00250], [00256]) and the sequence encoding at least one Payload is under control of an exogenous promoter (the transgene comprising the anti-DNA sensing protein transgene is under control of its own promoter (exogenous promoter); paragraphs [0050], [00250]); a cell (a cell; paragraph [0005]) comprising an exogenous polynucleotide (comprising an exogenous polynucleic acid; paragraphs [0005], [00261]) comprising a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), a first sequence encoding a Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), wherein the exogenous polynucleotide is integrated at an endogenous locus within the genome of the cell (the transgenes are inserted into a locus in the genome of the cell; paragraphs [00250], [00256]), wherein the sequence encoding an exogenous TCR and the first sequence encoding a Payload are under control of an endogenous promoter (the transgenes comprising an exogenous TCR and an anti-DNA sensing protein are under control of an endogenous promoter; paragraphs [0009], [0050], [00250], [00256]); a polynucleotide (a polynucleotide; paragraphs [00421]) comprising an exogenous enhancer (an exogenous enhancer; paragraphs [0007], [0009], [00363]), an insulator (an insulator; paragraph [00363]), a first homology arm, a second homology arm (a pair of recombination or homology arms; paragraphs [0008]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), and a sequence encoding at least one Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), and wherein the sequence encoding at least one Payload is under control of an exogenous promoter (the transgene comprising the anti-DNA sensing protein transgene is under control of its own promoter (exogenous promoter); paragraphs [0050], [00250]); a polynucleotide (a polynucleotide; paragraphs [00421]) comprising an insulator (an insulator; paragraph [00363]), a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element "WPRE", a first homology arm, a second homology arm (a pair of recombination or homology arms; paragraphs [0008]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), and a sequence encoding at least one Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]), and wherein the sequence encoding at least one Payload is under control of an exogenous promoter (the transgene comprising the anti-DNA sensing protein transgene is under control of its own promoter (exogenous promoter); paragraphs [0050], [00250]); a polynucleotide (a polynucleotide; paragraphs [00421]) comprising a first homology arm, a second homology arm (a pair of recombination or homology arms; paragraphs [0008]), a sequence encoding an exogenous TCR (a sequence encoding an exogenous TCR; paragraph [0009]), a first sequence encoding a Payload (a sequence encoding an anti-DNA sensing protein transgene, where the TCR transgene and anti-DNA sensing protein transgene are carried into the cell on the same vector; paragraphs [0050], [00421]).

Minnesota does not disclose a pause element; a second sequence encoding a Payload; the second sequence encoding a Payload is under control of an exogenous promoter; wherein the first and second homology arms are homologous to a TRAC or TRBC locus.

Tian discloses a pause element (a pause element; page 3, third paragraph).

Casebia discloses a second sequence encoding a Payload (a polycistronic cassette comprising a nucleic acid encoding a first CISC component, and a nucleic acid encoding a second CISC component (a second sequence encoding a Payload); paragraph [0261]); the second sequence encoding a Payload is under control of an exogenous promoter (where the polycistronic cassette comprising a first and second CISC component is under control of an exogenous promoter; paragraphs [0261], [0292]); first and second homology arms are homologous to a TRAC or TRBC locus (homology arms are homologous to a target DNA sequence, where the homology arms target a TRAC gene; paragraphs [0015], [0349], [0406]).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the cell comprising an exogenous polynucleotide and a polynucleotide, as previously disclosed by Minnesota, with a pause element, as previously disclosed by Tian, for a superior polynucleotide which provides the benefit of including a pause element which enhances gene expression where two genes are included in a cassette (Tian reference; page 3, third-fourth paragraphs). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the cell comprising an exogenous polynucleotide and a polynucleotide and homology arms, as previously disclosed by Minnesota, with a second sequence encoding a Payload which is under control of an exogenous promoter and homology arms homologous to a TRAC locus, as previously disclosed by Casebia, to provide the benefit of including another sequence encoding a payload to provide a secondary payload that encodes another gene or a copy of the first payload to increase gene expression within a cell, and further provides the benefit of using homology arms to insert an exogenous TCR into a subunit gene in a cell encoding for the TCR to provide a modified TCR expression.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Minnesota, Tian, and Casebia references, unity of invention is lacking.