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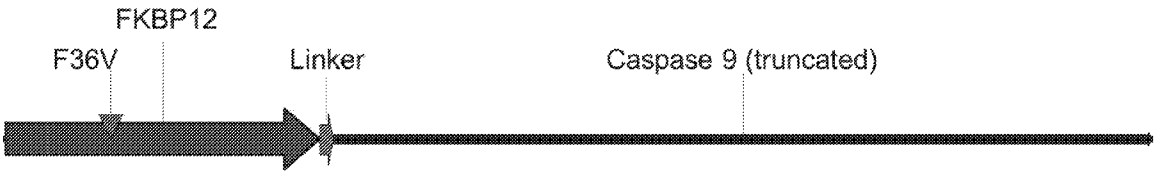
(54) Title: INDUCIBLE CASPASES AND METHODS FOR USE

FIGURE 1



(57) Abstract: The disclosure provides inducible caspase polypeptides, compositions comprising inducible caspase polypeptides and sequences encoding the same, cells modified to express the polypeptides and compositions of the disclosure, as well as methods of making and methods of using same for adoptive cell therapy.

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INDUCIBLE CASPASES AND METHODS FOR USE

RELATED APPLICATIONS

[01] This application claims the benefit of provisional application USSN 62/405,184 filed on 6 October 2016, the contents of which are herein incorporated by reference in their entirety.

INCORPORATION OF SEQUENCE LISTING

[02] The contents of the text filed named "POTH-011_001WO_SeqList.txt", which was created on 6 October 2017 and is 61 KB in size, are hereby incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[03] The disclosure is directed to molecular biology, and more, specifically, to compositions containing at least one sequences encoding an inducible caspase protein, as well as methods of making and using the same.

BACKGROUND

[04] There has been a long-felt but unmet need in the art for a method of selectively inducing apoptosis in genetically modified cells, and, in particular, those modified cells intended for administration to a subject as, for example an adoptive cell therapy. The disclosure provides a solution to this long-felt but unmet need.

SUMMARY

[05] The disclosure provides an inducible proapoptotic polypeptide operably linked to a ligand binding region that may be optimized to bind a chemical inducer of dimerization. When the ligand binding region specifically binds the induction agent, pro-apoptotic target molecules are cross-linked, and, consequently, activated to selectively induce apoptosis in a cell containing an inducible proapoptotic polypeptide of the disclosure. Preferred inducible proapoptotic polypeptides of the disclosure include, but are not limited to, inducible caspase polypeptides. Preferred inducible caspase polypeptides of the disclosure include, but are not

limited to, inducible caspase 9 polypeptides. Preferred inducible caspase 9 polypeptides of the disclosure may comprise a truncated caspase 9 polypeptide encoded by a truncated or modified amino acid and/or nucleic acid sequence encoding the truncated caspase 9 polypeptide.

[06] Inducible proapoptotic polypeptides of the disclosure are superior to existing inducible polypeptides because the inducible proapoptotic polypeptides of the disclosure are far less immunogenic. While inducible proapoptotic polypeptides of the disclosure are recombinant polypeptides, and, therefore, non-naturally occurring, the sequences that are recombined to produce the inducible proapoptotic polypeptides of the disclosure do not comprise non-human sequences that the host human immune system could recognize as "non-self" and, consequently, induce an immune response in the subject receiving an inducible proapoptotic polypeptide of the disclosure, a cell comprising the inducible proapoptotic polypeptide or a composition comprising the inducible proapoptotic polypeptide or the cell comprising the inducible proapoptotic polypeptide. Although the linker sequence is an artificial sequence, the linker sequence does not comprise a non-human sequence. For example, the linker sequence does not comprise a restriction site.

[07] The disclosure provides an inducible proapoptotic polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a proapoptotic polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region.

[08] The disclosure provides an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a caspase polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region.

[09] The disclosure provides an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the ligand binding region may be a multimeric ligand binding region.

[010] In certain embodiments of the inducible caspase polypeptide, the ligand binding region may specifically bind an induction agent and activate transcription of the proapoptotic polypeptide (e.g. caspase polypeptide) of the disclosure. For example, the ligand binding region will not bind a therapeutic agent. Induction agents specifically bound by the ligand binding region of the inducible polypeptides of the disclosure do not directly induce transcription of endogenous genes.

[011] Inducible proapoptotic (e.g. caspase) polypeptides of the disclosure may be under the control of one or more transcriptional regulatory elements, including, but not limited to, a promoter capable of initiating transcription of the caspase polypeptide in a cell modified to contain an inducible caspase polypeptide of the disclosure. For example, inducible caspase polypeptides of the disclosure may be under the control of one or more transcriptional regulatory elements, including, but not limited to, a mammalian promoter capable of initiating transcription of the caspase polypeptide in a mammalian cell modified to contain an inducible caspase polypeptide of the disclosure. For example, inducible caspase polypeptides of the disclosure may be under the control of one or more transcriptional regulatory elements, including, but not limited to, a heterologous or exogenous promoter capable of initiating transcription of the caspase polypeptide in a mammalian cell modified to contain an inducible caspase polypeptide of the disclosure. Preferred mammalian cells include, but are not limited to human cells.

[012] The disclosure provides an inducible caspase polypeptide comprising (a) a ligand binding region, (b) a linker, and (c) a truncated caspase 9 polypeptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the ligand binding region may be a multimeric ligand binding region.

[013] In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the amino acid sequence of the ligand binding region that comprise a FK506 binding protein 12 (FKBP12) polypeptide may comprise a modification at position 36 of the sequence. The modification may be a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, the FKBP12 polypeptide is encoded by an amino acid sequence comprising

GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 3). In certain embodiments, the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGG GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAACTGCTGAAGCTGGAG (SEQ ID NO: 4). In certain embodiments, the induction agent specific for the ligand binding region may comprise a FK506 binding protein 12 (FKBP12) polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V) comprises AP20187 and/or AP1903 (Rimiducid), both synthetic drugs.

[014] In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the linker region is encoded by an amino acid comprising GGGGS (SEQ ID NO: 5) or a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 6). In certain embodiments, the nucleic acid sequence encoding the linker does not comprise a restriction site.

[015] In certain embodiments of the truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence. Alternatively, or in addition, in certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence. In certain embodiments of the inducible proapoptotic polypeptides, inducible caspase polypeptides or truncated caspase 9 polypeptides of the disclosure, the truncated caspase 9 polypeptide is encoded by an amino acid comprising GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRR RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG AVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE

SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID
NO: 7) or a nucleic acid sequence comprising
TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTAC
ATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAACAATGTGAACTTCT
GCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGC
TGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGAC
CGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGC
TCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTG
CAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAG
ATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
TGTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGG
CCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAA
CTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCT
GCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCAT
GGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTG
AACAGTGGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACG
CTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAG
AAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 8).

[016] I n certain embodiments of the inducible proapoptotic polypeptides, wherein the polypeptide comprises a truncated caspase 9 polypeptide, the inducible proapoptotic polypeptide is encoded by an amino acid sequence comprising GVQVETISPGDGRTFPKR
GQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVIRGWEEGVAQMSVGQRA
KLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGSGFGDVGALESLRGNADL
AYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRRRFSSLHFMVEVKGDLTA
KKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPGAVYGTDGCPVSVEKIVN
IFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDESPGSNPEPDATPFQEGLR
TFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVETLDDIFEQWAHSEDLQSL
LLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID NO: 9) or the nucleic acid sequence comprising
GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGG

GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAACTGCTGAAGCTGGAGGGAG
GAGGAGGATCCGGATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATG
CCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATAT
TGACTGTGAGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTG
AAAGGGGATCTGACCGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAG
CAGGACCATGGAGCTCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCC
AGGCTTCTCATCTGCAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGT
CAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGG
GGAAAGCCAAAACTGTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCAC
GGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCT
GAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGAT
GCTATCTCAAGCCTGCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCC
AGGCTTTGTCTCATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACT
GGACGACATCTTTGAACAGTGGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCT
GCGAGTGGCAAACGCTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTG
CTTCAATTTTCTGAGAAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 10).

[017] The disclosure provides a composition comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure.

[018] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure.

[019] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments of the transposons of the disclosure, the transposon further comprises a sequence encoding a therapeutic protein.

In certain embodiments, the therapeutic protein is naturally-occurring. In certain embodiments, the therapeutic protein is an endogenous protein. In certain embodiments, the therapeutic protein is an exogenous protein.

[020] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments of the transposons of the disclosure, the transposon further comprises a sequence encoding a therapeutic protein. In certain embodiments, the therapeutic protein is not naturally-occurring. In certain embodiments, the therapeutic protein is an endogenous protein. In certain embodiments, the therapeutic protein is an exogenous protein. In certain embodiments, the therapeutic protein is a synthetic protein. In certain embodiments, the therapeutic protein is a chimeric or a recombinant protein. In certain embodiments, the therapeutic protein is a fusion protein. In certain embodiments, the therapeutic protein is a human protein, a wild type protein or a sequence variant thereof.

[021] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments of the transposons of the disclosure, the transposon further comprises a sequence encoding a therapeutic protein. In certain embodiments, the therapeutic protein comprises a cell surface protein, a membrane-bound protein, an extracellular membrane-bound protein, an intracellular membrane-bound protein, an intracellular protein, a nuclear localized protein, a nuclear protein, a cytoplasmic protein, a cytosolic protein, a secreted protein, a lysosomal protein, an endosomal protein, a vesicle-associated protein, a mitochondrial protein, an endoplasmic reticulum protein, a cytoskeletal protein, a protein involved in intracellular signaling and/or a protein involved in extracellular signaling.

[022] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments of the transposons of the disclosure, the transposon further comprises a sequence encoding a therapeutic protein. In certain embodiments, the therapeutic protein comprises an antigen receptor. In certain embodiments, antigen receptor comprises a T-cell receptor. In certain embodiments, antigen

receptor comprises a receptor isolated or derived from a T-cell receptor. In certain embodiments, the antigen receptor comprises one or more sequence variation(s) and/or mutation(s) compared to a wild-type T-cell receptor. In certain embodiments, the T-cell receptor is a recombinant T-cell receptor.

[023] The disclosure provides a transposon comprising an inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments of the transposons of the disclosure, the transposon further comprises a sequence encoding a therapeutic protein. In certain embodiments, wherein the antigen receptor is a Chimeric Antigen Receptor (CAR). In certain embodiments, the CAR comprises one or more Centyrin sequence(s). In certain embodiments, the CAR is a CARTyrin. In certain embodiments, the CAR comprises one or more VHH sequence(s). In certain embodiments, the CAR is a VCAR.

[024] In certain embodiments, a transposon of the disclosure may further comprise at least one self-cleaving peptide. In certain embodiments, a transposon of the disclosure may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure and another sequence in the transposon. In certain embodiments, a transposon of the disclosure may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located upstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure and a second self-cleaving peptide is located downstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments, a transposon of the disclosure may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located immediately upstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure and a second self-cleaving peptide is located immediately downstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. The at least one self-cleaving peptide may comprise a T2A peptide, GSG-T2A

peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. In certain embodiments, the T2A peptide comprises an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 11). In certain embodiments, the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 12). In certain embodiments, the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 13). In certain embodiments, the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 14). In certain embodiments, the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 15). In certain embodiments, the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 16). In certain embodiments, the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 17). In certain embodiments, the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 18).

[025] The disclosure provides a composition comprising a transposon of the disclosure. In certain embodiments of the compositions comprising a transposon, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence. In certain embodiments, the transposon is a piggyBac transposon. In certain embodiments, the transposon is a piggyBac transposon and the transposase is a Super piggyBac transposase.

[026] Transposons of the disclosure may comprise piggyBac transposons. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. Transposase enzymes of the disclosure may include piggyBac transposases or compatible enzymes. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBac™ or a Super piggyBac™ (SPB) transposase. In certain embodiments, and, in particular, those embodiments wherein the transposase is a Super piggyBac™ (SPB) transposase, the sequence encoding the transposase enzyme is an mRNA sequence.

[027] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme. The piggyBac (PB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

```
  1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEI SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG
 61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG
121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTGATFRD TNEDEIYAFF
181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV
241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RMYIPNKPSK YGIKILMMCD
301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ
361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC
421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN
481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPNEV
541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO:
1).
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[028] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at one or more of positions 30, 165, 282, or 538 of the sequence:

```
  1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEI SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG
 61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG
121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTGATFRD TNEDEIYAFF
181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV
241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RMYIPNKPSK YGIKILMMCD
301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ
361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC
421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN
481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPNEV
541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO:
1).
```

[029] In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid

substitution at two or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at three or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at each of the following positions 30, 165, 282, and 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the amino acid substitution at position 30 of the sequence of SEQ ID NO: 1 is a substitution of a valine (V) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 165 of the sequence of SEQ ID NO: 1 is a substitution of a serine (S) for a glycine (G). In certain embodiments, the amino acid substitution at position 282 of the sequence of SEQ ID NO: 1 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 538 of the sequence of SEQ ID NO: 1 is a substitution of a lysine (K) for an asparagine (N).

[030] In certain embodiments of the methods of the disclosure, the transposase enzyme is a Super piggyBac™ (SPB) transposase enzyme. In certain embodiments, the Super piggyBac™ (SPB) transposase enzymes of the disclosure may comprise or consist of the amino acid sequence of the sequence of SEQ ID NO: 1 wherein the amino acid substitution at position 30 is a substitution of a valine (V) for an isoleucine (I), the amino acid substitution at position 165 is a substitution of a serine (S) for a glycine (G), the amino acid substitution at position 282 is a substitution of a valine (V) for a methionine (M), and the amino acid substitution at position 538 is a substitution of a lysine (K) for an asparagine (N). In certain embodiments, the Super piggyBac™ (SPB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

```
  1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEV SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG
 61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG
121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTSATFRD TNEDEIYAFF
181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV
241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RVYIPNKPSK YGIKILMMCD
301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ
361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC
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421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN

481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPKEV

541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO: 2).

[031] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 3, 46, 82, 103, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 258, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 486, 503, 552, 570 and 591 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 46, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 485, 503, 552 and 570. In certain embodiments, the amino acid substitution at position 3 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an asparagine (N) for a serine (S). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an alanine (A). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 82 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 119 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for an arginine (R). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) a cysteine (C). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution

of a histidine (H) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 185 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 187 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for an alanine (A). In certain embodiments, the amino acid substitution at position 200 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a phenylalanine (F).In certain embodiments, the amino acid substitution at position 207 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a valine (V). In certain embodiments, the amino acid substitution at position 209 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a valine (V). In certain embodiments, the amino acid substitution at position 226 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a methionine (M). In certain embodiments, the amino acid substitution at position 235 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a leucine (L). In certain embodiments, the amino acid substitution at position 240 of SEQ ID NO: 1 or SEQ ID NO: 1 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 241 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 243 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a proline (P). In certain embodiments, the amino acid substitution at position 258 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1 or SEQ ID NO: 2

is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a proline (P). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine for a proline (P). In certain embodiments, the amino acid substitution at position 315 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for an arginine (R).In certain embodiments, the amino acid substitution at position 319 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for a threonine (T). In certain embodiments, the amino acid substitution at position 327 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 328 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for a cysteine (C). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 421 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a histidine (H) for the aspartic acid (D). In certain embodiments, the amino acid substitution at position 436 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a valine (V). In certain embodiments, the amino acid substitution at position 456 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a methionine (M). In certain embodiments, the amino acid substitution at position 470 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 485 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a serine (S). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a methionine (M). In certain embodiments, the amino acid substitution at position 552 of SEQ

ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a glutamine (Q). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a glutamine (Q).

[031] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at two, three, four, five, six or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 194 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 372 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for an arginine (R). In certain embodiments, the amino acid substitution at position 375 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for a lysine (K). In certain embodiments, the amino acid substitution at position 450 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an asparagine (N) for an aspartic acid (D). In certain embodiments, the amino acid substitution at position 509 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution

of a glycine (G) for a serine (S). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1. In certain embodiments, including those embodiments wherein the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, the piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 372, 375 and 450 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 1, and a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 1. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 1, a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 1 and a substitution of an asparagine (N) for an aspartic acid (D) at position 450 of SEQ ID NO: 1.

[032] The disclosure provides a composition comprising a transposon of the disclosure. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments of the compositions comprising a transposon, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence. In certain embodiments, the transposon is a Sleeping Beauty transposon. In certain embodiments, the transposon is a Sleeping Beauty transposon and the transposase is a Sleeping Beauty transposase or a hyperactive Sleeping Beauty (SB100X) transposase.

[033] Transposons of the disclosure may comprise Sleeping Beauty transposons. In certain embodiments, and, in particular, those embodiments wherein the transposon is a Sleeping Beauty transposon, the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme. In certain embodiments, the sequence encoding the transposase enzyme is a sequence encoding a Sleeping Beauty transposase or a hyperactive

Sleeping Beauty (SB100X) transposase. In certain embodiments, the sequence encoding the transposase enzyme is an mRNA sequence.

[034] In certain embodiments of the methods of the disclosure, the Sleeping Beauty transposase enzyme comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGKSKEISQD LRKKIVDLHK SGSSLGAISK RLKVPRSSVQ TIVRKYKHHG TTQPSYRSGR
 61 RRVLSPRDER TLVRKVQINP RTTAKDLVKM LEETGTKVSI STVKRVLYRH NLKGRSARKK
121 PLLQNRHKKA RLRFATAHGD KDRTFWRNVL WSDETKIELF GHNDHRYVWR KKGEACKPKN
181 TIPTVKHGGG SIMLWGCFAA GGTGALHKID GIMRKENYVD ILKQHLKTSV RKLKLGRKWV
241 FQMDNDPKHT SKVVAKWLKD NKVKVLEWPS QSPDLNPIEN LWAELKKRVR ARRPTNLTQL
301 HQLCQEEWAK IHPTYCGKLV EGYPKRLTQV KQFKGNATKY (SEQ ID NO: 19).
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[035] In certain embodiments of the methods of the disclosure, the hyperactive Sleeping Beauty (SB100X) transposase enzyme comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGKSKEISQD LRKRIVDLHK SGSSLGAISK RLAVPRSSVQ TIVRKYKHHG TTQPSYRSGR
 61 RRVLSPRDER TLVRKVQINP RTTAKDLVKM LEETGTKVSI STVKRVLYRH NLKGHSARKK
121 PLLQNRHKKA RLRFATAHGD KDRTFWRNVL WSDETKIELF GHNDHRYVWR KKGEACKPKN
181 TIPTVKHGGG SIMLWGCFAA GGTGALHKID GIMDAVQYVD ILKQHLKTSV RKLKLGRKWV
241 FQHDNDPKHT SKVVAKWLKD NKVKVLEWPS QSPDLNPIEN LWAELKKRVR ARRPTNLTQL
301 HQLCQEEWAK IHPNYCGKLV EGYPKRLTQV KQFKGNATKY (SEQ ID NO: 20).
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[036] The disclosure provides a composition comprising a transposon of the disclosure. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments of the compositions comprising a transposon, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence. In certain embodiments, the transposon is a Helraiser transposon. In certain embodiments, the transposon is a Helraiser transposon and the transposase is a Helitron transposase.

[037] Transposons of the disclosure may comprise Helraiser transposons. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments of this method, the transposon is a Helraiser transposon. In certain embodiments, and, in particular, those embodiments wherein the

transposon is a Helraiser transposon, the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme. In certain embodiments, the sequence encoding the transposase enzyme comprises a sequence encoding a Helitron transposase. In certain embodiments, the sequence encoding the transposase enzyme is an mRNA sequence.

[038] In certain embodiments, the transposase is a Helitron transposase. Helitron transposases mobilize the Helraiser transposon, an ancient element from the bat genome that was active about 30 to 36 million years ago. An exemplary Helraiser transposon of the disclosure includes Helibat1, which comprises a nucleic acid sequence comprising:

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   1 TCCTATATAA TAAAAGAGAA ACATGCAAAT TGACCATCCC TCCGCTACGC TCAAGCCACG
  61 CCCACCAGCC AATCAGAAGT GACTATGCAA ATTAACCCAA CAAAGATGGC AGTTAAATTT
 121 GCATACGCAG GTGTCAAGCG CCCCAGGAGG CAACGGCGGC CGCGGGCTCC CAGGACCTTC
 181 GCTGGCCCCG GGAGGCGAGG CCGGCCGCGC CTAGCCACAC CCGCGGGCTC CCGGGACCTT
 241 CGCCAGCAGA GAGCAGAGCG GGAGAGCGGG CGGAGAGCGG GAGGTTTGGA GGACTTGGCA
 301 GAGCAGGAGG CCGCTGGACA TAGAGCAGAG CGAGAGAGAG GGTGGCTTGG AGGGCGTGGC
 361 TCCCTCTGTC ACCCCAGCTT CCTCATCACA GCTGTGGAAA CTGACAGCAG GGAGGAGGAA
 421 GTCCCACCCC CACAGAATCA GCCAGAATCA GCCGTTGGTC AGACAGCTCT CAGCGGCCTG
 481 ACAGCCAGGA CTCTCATTCA CCTGCATCTC AGACCGTGAC AGTAGAGAGG TGGGACTATG
 541 TCTAAAGAAC AACTGTTGAT ACAACGTAGC TCTGCAGCCG AAAGATGCCG GCGTTATCGA
 601 CAGAAAATGT CTGCAGAGCA ACGTGCGTCT GATCTTGAAA GAAGGCGGCG CCTGCAACAG
 661 AATGTATCTG AAGAGCAGCT ACTGGAAAAA CGTCGCTCTG AAGCCGAAAA ACAGCGGCGT
 721 CATCGACAGA AAATGTCTAA AGACCAACGT GCCTTTGAAG TTGAAAGAAG GCGGTGGCGA
 781 CGACAGAATA TGTCTAGAGA ACAGTCATCA ACAAGTACTA CCAATACCGG TAGGAACTGC
 841 CTTCTCAGCA AAAATGGAGT ACATGAGGAT GCAATTCTCG AACATAGTTG TGGTGGAATG
 901 ACTGTTCGAT GTGAATTTTG CCTATCACTA AATTTCTCTG ATGAAAAACC ATCCGATGGG
 961 AAATTTACTC GATGTTGTAG CAAAGGGAAA GTCTGTCCAA ATGATATACA TTTTCCAGAT
1021 TACCCGGCAT ATTTAAAAAG ATTAATGACA AACGAAGATT CTGACAGTAA AAATTTCATG
1081 GAAAATATTC GTTCCATAAA TAGTTCTTTT GCTTTTGCTT CCATGGGTGC AAATATTGCA
1141 TCGCCATCAG GATATGGGCC ATACTGTTTT AGAATACACG GACAAGTTTA TCACCGTACT
1201 GGAACTTTAC ATCCTTCGGA TGGTGTTTCT CGGAAGTTTG CTCAACTCTA TATTTTGGAT
1261 ACAGCCGAAG CTACAAGTAA AAGATTAGCA ATGCCAGAAA ACCAGGGCTG CTCAGAAAGA
1321 CTCATGATCA ACATCAACAA CCTCATGCAT GAAATAAATG AATTAACAAA ATCGTACAAG
1381 ATGCTACATG AGGTAGAAAA GGAAGCCCAA TCTGAAGCAG CAGCAAAAGG TATTGCTCCC
1441 ACAGAAGTAA CAATGGCGAT TAAATACGAT CGTAACAGTG ACCCAGGTAG ATATAATTCT
1501 CCCCGTGTAA CCGAGGTTGC TGTCATATTC AGAAACGAAG ATGGAGAACC TCCTTTTGAA
1561 AGGGACTTGC TCATTCATTG TAAACCAGAT CCCAATAATC CAAATGCCAC TAAAATGAAA
1621 CAAATCAGTA TCCTGTTTCC TACATTAGAT GCAATGACAT ATCCTATTCT TTTTCCACAT
1681 GGTGAAAAAG GCTGGGGAAC AGATATTGCA TTAAGACTCA GAGACAACAG TGTAATCGAC
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1741 AATAATACTA GACAAAATGT AAGGACACGA GTCACACAAA TGCAGTATTA TGGATTTCAT
1801 CTCTCTGTGC GGGACACGTT CAATCCTATT TTAAATGCAG GAAAATTAAC TCAACAGTTT
1861 ATTGTGGATT CATATTCAAA AATGGAGGCC AATCGGATAA ATTTCATCAA AGCAAACCAA
1921 TCTAAGTTGA GAGTTGAAAA ATATAGTGGT TTGATGGATT ATCTCAAATC TAGATCTGAA
1981 AATGACAATG TGCCGATTGG TAAAATGATA ATACTTCCAT CATCTTTTGA GGGTAGTCCC
2041 AGAAATATGC AGCAGCGATA TCAGGATGCT ATGGCAATTG TAACGAAGTA TGGCAAGCCC
2101 GATTTATTCA TAACCATGAC ATGCAACCCC AAATGGGCAG ATATTACAAA CAATTTACAA
2161 CGCTGGCAAA AAGTTGAAAA CAGACCTGAC TTGGTAGCCA GAGTTTTTAA TATTAAGCTG
2221 AATGCTCTTT TAAATGATAT ATGTAAATTC CATTTATTTG GCAAAGTAAT AGCTAAAATT
2281 CATGTCATTG AATTTCAGAA ACGCGGACTG CCTCACGCTC ACATATTATT GATATTAGAT
2341 AGTGAGTCCA AATTACGTTC AGAAGATGAC ATTGACCGTA TAGTTAAGGC AGAAATTCCA
2401 GATGAAGACC AGTGTCCTCG ACTTTTTCAA ATTGTAAAAT CAAATATGGT ACATGGACCA
2461 TGTGGAATAC AAAATCCAAA TAGTCCATGT ATGGAAAATG GAAAATGTTC AAAGGGATAT
2521 CCAAAAGAAT TTCAAAATGC GACCATTGGA AATATTGATG GATATCCCAA ATACAAACGA
2581 AGATCTGGTA GCACCATGTC TATTGGAAAT AAAGTTGTCG ATAACACTTG GATTGTCCCT
2641 TATAACCCGT ATTTGTGCCT TAAATATAAC TGTCATATAA ATGTTGAAGT CTGTGCATCA
2701 ATTAAAAGTG TCAAATATTT ATTTAAATAC ATCTATAAAG GGCACGATTG TGCAAATATT
2761 CAAATTTCTG AAAAAAATAT TATCAATCAT GACGAAGTAC AGGACTTCAT TGACTCCAGG
2821 TATGTGAGCG CTCCTGAGGC TGTTTGGAGA CTTTTTGCAA TGCGAATGCA TGACCAATCT
2881 CATGCAATCA CAAGATTAGC TATTCATTTG CCAAATGATC AGAATTTGTA TTTTCATACC
2941 GATGATTTTG CTGAAGTTTT AGATAGGGCT AAAAGGCATA ACTCGACTTT GATGGCTTGG
3001 TTCTTATTGA ATAGAGAAGA TTCTGATGCA CGTAATTATT ATTATTGGGA GATTCCACAG
3061 CATTATGTGT TTAATAATTC TTTGTGGACA AAACGCCGAA AGGGTGGGAA TAAAGTATTA
3121 GGTAGACTGT TCACTGTGAG CTTTAGAGAA CCAGAACGAT ATTACCTTAG ACTTTTGCTT
3181 CTGCATGTAA AAGGTGCGAT AAGTTTTGAG GATCTGCGAA CTGTAGGAGG TGTAACTTAT
3241 GATACATTTC ATGAAGCTGC TAAACACCGA GGATTATTAC TTGATGACAC TATCTGGAAA
3301 GATACGATTG ACGATGCAAT CATCCTTAAT ATGCCCAAAC AACTACGGCA ACTTTTTGCA
3361 TATATATGTG TGTTTGGATG TCCTTCTGCT GCAGACAAAT TATGGGATGA GAATAAATCT
3421 CATTTTATTG AAGATTTCTG TTGGAAATTA CACCGAAGAG AAGGTGCCTG TGTGAACTGT
3481 GAAATGCATG CCCTTAACGA AATTCAGGAG GTATTCACAT TGCATGGAAT GAAATGTTCA
3541 CATTTCAAAC TTCCGGACTA TCCTTTATTA ATGAATGCAA ATACATGTGA TCAATTGTAC
3601 GAGCAACAAC AGGCAGAGGT TTTGATAAAT TCTCTGAATG ATGAACAGTT GGCAGCCTTT
3661 CAGACTATAA CTTCAGCCAT CGAAGATCAA ACTGTACACC CCAAATGCTT TTTCTTGGAT
3721 GGTCCAGGTG GTAGTGGAAA AACATATCTG TATAAAGTTT TAACACATTA TATTAGAGGT
3781 CGTGGTGGTA CTGTTTTACC CACAGCATCT ACAGGAATTG CTGCAAATTT ACTTCTTGGT
3841 GGAAGAACCT TTCATTCCCA ATATAAATTA CCAATTCCAT TAAATGAAAC TTCAATTTCT
3901 AGACTCGATA TAAAGAGTGA AGTTGCTAAA ACCATTAAAA AGGCCCAACT TCTCATTATT
3961 GATGAATGCA CCATGGCATC CAGTCATGCT ATAAACGCCA TAGATAGATT ACTAAGAGAA

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4021 ATTATGAATT TGAATGTTGC ATTTGGTGGG AAAGTTCTCC TTCTCGGAGG GGATTTTCGA
4081 CAATGTCTCA GTATTGTACC ACATGCTATG CGATCGGCCA TAGTACAAAC GAGTTTAAAG
4141 TACTGTAATG TTTGGGGATG TTTCAGAAAG TTGTCTCTTA AAACAAATAT GAGATCAGAG
4201 GATTCTGCTT ATAGTGAATG GTTAGTAAAA CTTGGAGATG GCAAACTTGA TAGCAGTTTT
4261 CATTTAGGAA TGGATATTAT TGAAATCCCC CATGAAATGA TTTGTAACGG ATCTATTATT
4321 GAAGCTACCT TTGGAAATAG TATATCTATA GATAATATTA AAAATATATC TAAACGTGCA
4381 ATTCTTTGTC CAAAAAATGA GCATGTTCAA AAATTAAATG AAGAAATTTT GGATATACTT
4441 GATGGAGATT TTCACACATA TTTGAGTGAT GATTCCATTG ATTCAACAGA TGATGCTGAA
4501 AAGGAAAATT TTCCCATCGA ATTTCTTAAT AGTATTACTC CTTCGGGAAT GCCGTGTCAT
4561 AAATTAAAAT TGAAAGTGGG TGCAATCATC ATGCTATTGA GAAATCTTAA TAGTAAATGG
4621 GGTCTTTGTA ATGGTACTAG ATTTATTATC AAAAGATTAC GACCTAACAT TATCGAAGCT
4681 GAAGTATTAA CAGGATCTGC AGAGGGAGAG GTTGTTCTGA TTCCAAGAAT TGATTTGTCC
4741 CCATCTGACA CTGGCCTCCC ATTTAAATTA ATTCGAAGAC AGTTTCCCGT GATGCCAGCA
4801 TTTGCGATGA CTATTAATAA ATCACAAGGA CAAACTCTAG ACAGAGTAGG AATATTCCTA
4861 CCTGAACCCG TTTTCGCACA TGGTCAGTTA TATGTTGCTT TCTCTCGAGT TCGAAGAGCA
4921 TGTGACGTTA AAGTTAAAGT TGTAAATACT TCATCACAAG GGAAATTAGT CAAGCACTCT
4981 GAAAGTGTTT TTACTCTTAA TGTGGTATAC AGGGAGATAT TAGAATAAGT TTAATCACTT
5041 TATCAGTCAT TGTTTGCATC AATGTTGTTT TTATATCATG TTTTTGTTGT TTTTATATCA
5101 TGTCTTTGTT GTTGTTATAT CATGTTGTTA TTGTTTATTT ATTAATAAAT TTATGTATTA
5161 TTTTCATATA CATTTTACTC ATTTCCTTTC ATCTCTCACA CTTCTATTAT AGAGAAAGGG
5221 CAAATAGCAA TATTAAAATA TTTCCTCTAA TTAATTCCCT TTCAATGTGC ACGAATTTCG
5281 TGCACCGGGC CACTAG (SEQ ID NO: 21).
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[039] Unlike other transposases, the Helitron transposase does not contain an RNase-H like catalytic domain, but instead comprises a RepHel motif made up of a replication initiator domain (Rep) and a DNA helicase domain. The Rep domain is a nuclease domain of the HUH superfamily of nucleases.

[040] An exemplary Helitron transposase of the disclosure comprises an amino acid sequence comprising:

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   1 MSKEQLLIQR SSAAERCRRY RQKMSAEQRA SDLERRRRLQ QNVSEEQLLE KRRSEAEKQR
  61 RHRQKMSKDQ RAFEVERRRW RRQNMSREQS STSTTNTGRN CLLSKNGVHE DAILEHSCGG
 121 MTVRCEFCLS LNFSDEKPSD GKFTRCCSKG KVCPNDIHFP DYPAYLKRLM TNEDSDSKNF
 181 MENIRSINSS FAFASMGANI ASPSGYGPYC FRIHGQVYHR TGTLHPSDGV SRKFAQLYIL
 241 DTAEATSKRL AMPENQGCSE RLMININNLM HEINELTKSY KMLHEVEKEA QSEAAAKGIA
 301 PTEVTMAIKY DRNSDPGRYN SPRVTEVAVI FRNEDGEPPF ERDLLIHCKP DPNNPNATKM
 361 KQISILFPTL DAMTYPILFP HGEKGWGTDI ALRLRDNSVI DNNTRQNVRT RVTQMQYYGF
 421 HLSVRDTFNP ILNAGKLTQQ FIVDSYSKME ANRINFIKAN QSKLRVEKYS GLMDYLKSRS
 481 ENDNVPIGKM IILPSSFEGS PRNMQQRYQD AMAIVTKYGK PDLFITMTCN PKWADITNNL
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541 QRWQKVENRP DLVARVFNIK LNALLNDICK FHLFGKVIAK IHVIEFQKRG LPHAHILLIL
601 DSESKLRSED DIDRIVKAEI PDEDQCPRLF QIVKSNMVHG PCGIQNPNSP CMENGKCSKG
661 YPKEFQNATI GNIDGYPKYK RRSGSTMSIG NKVVDNTWIV PYNPYLCLKY NCHINVEVCA
721 SIKSVKYLFK YIYKGHDCAN IQISEKNIIN HDEVQDFIDS RYVSAPEAVW RLFAMPMHDQ
781 SHAITRLAIH LPNDQNLYFH TDDFAEVLDR AKRHNSTLMA WFLLNREDSD ARNYYYWEIP
841 QHYVFNNSLW TKRRKGGNKV LGRLFTVSFR EPERYYLRLL LLHVKGAISF EDLRTVGGVT
901 YDTFHEAAKH RGLLLDDTIW KDTIDDAIIL NMPKQLRQLF AYICVFGCPS AADKLWDENK
961 SHFIEDFCWK LHRREGACVN CEMHALNEIQ EVFTLHGMKC SHFKLPDYPL LMNANTCDQL
1021 YEQQQAEVLI NSLNDEQLAA FQTITSAIED QTVHPKCFFL DGPGGSGKTY LYKVLTHYIR
1081 GRGGTVLPTA STGIAANLLL GGRTFHSQYK LPIPLNETSI SRLDIKSEVA KTIKKAQLLI
1141 IDECTMASSH AINAIDRLLR EIMNLNVAFG GKVLLLGGDF RQCLSIVPHA MRSAIVQTSL
1201 KYCNVWGCFR KLSLKTNMRS EDSAYSEWLV KLGDGKLDSS FHLGMDIIEI PHEMICNGSI
1261 IEATFGNSIS IDNIKNISKR AILCPKNEHV QKLNEEILDI LDGDFHTYLS DDSIDSTDDA
1321 EKENFPIEFL NSITPSGMPC HKLKLKVGAI IMLLRNLNSK WGLCNGTRFI IKRLRPNIIE
1381 AEVLTGSAEG EVVLIPRIDL SPSDTGLPFK LIRRQFPVMP AFAMTINKSQ GQTLDRVGIF
1441 LPEPVFAHGQ LYVAFSRVRR ACDVKVKVVN TSSQGKLVKH SESVFTLNVV YREILE (SEQ ID NO: 22).

[041] In Helitron transpositions, a hairpin close to the 3' end of the transposon functions as a terminator. However, this hairpin can be bypassed by the transposase, resulting in the transduction of flanking sequences. In addition, Helraiser transposition generates covalently closed circular intermediates. Furthermore, Helitron transpositions can lack target site duplications. In the Helraiser sequence, the transposase is flanked by left and right terminal sequences termed LTS and RTS. These sequences terminate with a conserved 5'-TC/CTAG-3' motif. A 19 bp palindromic sequence with the potential to form the hairpin termination structure is located 11 nucleotides upstream of the RTS and consists of the sequence GTGCACGAATTTCGTGCACCGGGCCACTAG (SEQ ID NO: 23).

[042] The disclosure provides a composition comprising a transposon of the disclosure. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments of the compositions comprising a transposon, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence. In certain embodiments, the transposon is a Tol2 transposon. In certain embodiments, the transposon is a Tol2 transposon and the transposase is a Tol2 transposase.

[043] Transposons of the disclosure may comprise Tol2 transposons. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments of this method, the transposon is a Tol2 transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a Tol2 transposon, the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme. In certain embodiments, the sequence encoding the transposase enzyme comprises a sequence encoding a Tol2 transposase. In certain embodiments, the sequence encoding the transposase enzyme is an mRNA sequence.

[044] Tol2 transposons may be isolated or derived from the genome of the medaka fish, and may be similar to transposons of the hAT family. Exemplary Tol2 transposons of the disclosure are encoded by a sequence comprising about 4.7 kilobases and contain a gene encoding the Tol2 transposase, which contains four exons. An exemplary Tol2 transposase of the disclosure comprises an amino acid sequence comprising the following:

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  1 MEEVCDSSAA ASSTVQNQPQ DQEHPWPYLR EFFSLSGVNK DSFKMKCVLC LPLNKEISAF
 61 KSSPSNLRKH IERMHPNYLK NYSKLTAQKR KIGTSTHASS SKQLKVDSVF PVKHVSPVTV
121 NKAILRYIIQ GLHPFSTVDL PSFKELISTL QPGISVITRP TLRSKIAEAA LIMKQKVTAA
181 MSEVEWIATT TDCWTARRKS FIGVTAHWIN PGSLERHSAA LACKRLMGSH TFEVLASAMN
241 DIHSEYEIRD KVVCTTTDSG SNFMKAFRVF GVENNDIETE ARRCESDDTD SEGCGEGSDG
301 VEFQDASRVL DQDDGFEFQL PKHQKCACHL LNLVSSVDAQ KALSNEHYKK LYRSVFGKCQ
361 ALWNKSSRSA LAAEAVESES RLQLLRPNQT RWNSTFMAVD RILQICKEAG EGALRNICTS
421 LEVPMFNPAE MLFLTEWANT MRPVAKVLDI LQAETNTQLG WLLPSVHQLS LKLQRLHHSL
481 RYCDPLVDAL QQGIQTRFKH MFEDPEIIAA AILLPKFRTS WTNDETIIKR GMDYIRVHLE
541 PLDHKKELAN SSSDDEDFFA SLKPTTHEAS KELDGYLACV SDTRESLLTF PAICSLSIKT
601 NTPLPASAAC ERLFSTAGLL FSPKPARLDT NNFENQLLLK LNLRFYNFE
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(SEQ ID NO: 24).

[045] An exemplary Tol2 transposon of the disclosure, including inverted repeats, subterminal sequences and the Tol2 transposase, is encoded by a nucleic acid sequence comprising the following:

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  1 CAGAGGTGTA AAGTACTTGA GTAATTTTAC TTGATTACTG TACTTAAGTA TTATTTTTGG
 61 GGATTTTTAC TTTACTTGAG TACAATTAAA AATCAATACT TTTACTTTTA CTTAATTACA
121 TTTTTTTAGA AAAAAAAGTA CTTTTTACTC CTTACAATTT TATTTACAGT CAAAAAGTAC
181 TTATTTTTTG GAGATCACTT CATTCTATTT TCCCTTGCTA TTACCAAACC AATTGAATTG
241 CGCTGATGCC CAGTTTAATT TAAATGTTAT TTATTCTGCC TATGAAAATC GTTTTCACAT
301 TATATGAAAT TGGTCAGACA TGTTCATTGG TCCTTTGGAA GTGACGTCAT GTCACATCTA
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 361 TTACCACAAT GCACAGCACC TTGACCTGGA AATTAGGGAA ATTATAACAG TCAATCAGTG
 421 GAAGAAAATG GAGGAAGTAT GTGATTCATC AGCAGCTGCG AGCAGCACAG TCCAAAATCA
 481 GCCACAGGAT CAAGAGCACC CGTGGCCGTA TCTTCGCGAA TTCTTTTCTT TAAGTGGTGT
 541 AAATAAAGAT TCATTCAAGA TGAAATGTGT CCTCTGTCTC CCGCTTAATA AAGAAATATC
 601 GGCCTTCAAA AGTTCGCCAT CAAACCTAAG GAAGCATATT GAGGTAAGTA CATTAAGTAT
 661 TTTGTTTTAC TGATAGTTTT TTTTTTTTTT TTTTTTTTTT TTTTTGGGTG TGCATGTTTT
 721 GACGTTGATG GCGCGCCTTT TATATGTGTA GTAGGCCTAT TTTCACTAAT GCATGCGATT
 781 GACAATATAA GGCTCACGTA ATAAAATGCT AAAATGCATT TGTAATTGGT AACGTTAGGT
 841 CCACGGGAAA TTTGGCGCCT ATTGCAGCTT TGAATAATCA TTATCATTCC GTGCTCTCAT
 901 TGTGTTTGAA TTCATGCAAA ACACAAGAAA ACCAAGCGAG AAATTTTTTT CCAAACATGT
 961 TGTATTGTCA AAACGGTAAC ACTTTACAAT GAGGTTGATT AGTTCATGTA TTAACTAACA
1021 TTAAATAACC ATGAGCAATA CATTTGTTAC TGTATCTGTT AATCTTTGTT AACGTTAGTT
1081 AATAGAAATA CAGATGTTCA TTGTTTGTTC ATGTTAGTTC ACAGTGCATT AACTAATGTT
1141 AACAAGATAT AAAGTATTAG TAAATGTTGA AATTAACATG TATACGTGCA GTTCATTATT
1201 AGTTCATGTT AACTAATGTA GTTAACTAAC GAACCTTATT GTAAAAGTGT TACCATCAAA
1261 ACTAATGTAA TGAAATCAAT TCACCCTGTC ATGTCAGCCT TACAGTCCTG TGTTTTTGTC
1321 AATATAATCA GAAATAAAAT TAATGTTTGA TTGTCACTAA ATGCTACTGT ATTTCTAAAA
1381 TCAACAAGTA TTTAACATTA TAAAGTGTGC AATTGGCTGC AAATGTCAGT TTTATTAAAG
1441 GGTTAGTTCA CCCAAAAATG AAAATAATGT CATTAATGAC TCGCCCTCAT GTCGTTCCAA
1501 GCCCGTAAGA CCTCCGTTCA TCTTCAGAAC ACAGTTTAAG ATATTTTAGA TTTAGTCCGA
1561 GAGCTTTCTG TGCCTCCATT GAGAATGTAT GTACGGTATA CTGTCCATGT CCAGAAAGGT
1621 AATAAAAAACA TCAAAGTAGT CCATGTGACA TCAGTGGGTT AGTTAGAATT TTTTGAAGCA
1681 TCGAATACAT TTTGGTCCAA AAATAACAAA ACCTACGACT TTATTCGGCA TTGTATTCTC
1741 TTCCGGGTCT GTTGTCAATC CGCGTTCACG ACTTCGCAGT GACGCTACAA TGCTGAATAA
1801 AGTCGTAGGT TTTGTTATTT TTGGACCAAA ATGTATTTTC GATGCTTCAA ATAATTCTAC
1861 CTAACCCACT GATGTCACAT GGACTACTTT GATGTTTTTA TTACCTTTCT GGACATGGAC
1921 AGTATACCGT ACATACATTT TCAGTGGAGG GACAGAAAGC TCTCGGACTA AATCTAAAAT
1981 ATCTTAAACT GTGTTCCGAA GATGAACGGA GGTGTTACGG GCTTGGAACG ACATGAGGGT
2041 GAGTCATTAA TGACATCTTT TCATTTTTGG GTGAACTAAC CCTTTAATGC TGTAATCAGA
2101 GAGTGTATGT GTAATTGTTA CATTTATTGC ATACAATATA AATATTTATT TGTTGTTTTT
2161 ACAGAGAATG CACCCAAATT ACCTCAAAAA CTACTCTAAA TTGACAGCAC AGAAGAGAAA
2221 GATCGGGACC TCCACCCATG CTTCCAGCAG TAAGCAACTG AAAGTTGACT CAGTTTTCCC
2281 AGTCAAACAT GTGTCTCCAG TCACTGTGAA CAAAGCTATA TTAAGGTACA TCATTCAAGG
2341 ACTTCATCCT TTCAGCACTG TTGATCTGCC ATCATTTAAA GAGCTGATTA GTACACTGCA
2401 GCCTGGCATT TCTGTCATTA CAAGGCCTAC TTTACGCTCC AAGATAGCTG AAGCTGCTCT
2461 GATCATGAAA CAGAAAGTGA CTGCTGCCAT GAGTGAAGTT GAATGGATTG CAACCACAAC
2521 GGATTGTTGG ACTGCACGTA GAAAGTCATT CATTGGTGTA ACTGCTCACT GGATCAACCC
2581 TGGAAGTCTT GAAAGACATT CCGCTGCACT TGCCTGCAAA AGATTAATGG GCTCTCATAC
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2641 TTTTGAGGTA CTGGCCAGTG CCATGAATGA TATCCACTCA GAGTATGAAA TACGTGACAA
2701 GGTTGTTTGC ACAACCACAG ACAGTGGTTC CAACTTTATG AAGGCTTTCA GAGTTTTTGG
2761 TGTGGAAAAC AATGATATCG AGACTGAGGC AAGAAGGTGT GAAAGTGATG ACACTGATTC
2821 TGAAGGCTGT GGTGAGGGAA GTGATGGTGT GGAATTCCAA GATGCCTCAC GAGTCCTGGA
2881 CCAAGACGAT GGCTTCGAAT TCCAGCTACC AAAACATCAA AAGTGTGCCT GTCACTTACT
2941 TAACCTAGTC TCAAGCGTTG ATGCCCAAAA AGCTCTCTCA AATGAACACT ACAAGAAACT
3001 CTACAGATCT GTCTTTGGCA AATGCCAAGC TTTATGGAAT AAAAGCAGCC GATCGGCTCT
3061 AGCAGCTGAA GCTGTTGAAT CAGAAAGCCG GCTTCAGCTT TTAAGGCCAA ACCAAACGCG
3121 GTGGAATTCA ACTTTTATGG CTGTTGACAG AATTCTTCAA ATTTGCAAAG AAGCAGGAGA
3181 AGGCGCACTT CGGAATATAT GCACCTCTCT TGAGGTTCCA ATGTAAGTGT TTTTCCCCTC
3241 TATCGATGTA AACAAATGTG GGTTGTTTTT GTTTAATACT CTTTGATTAT GCTGATTTCT
3301 CCTGTAGGTT TAATCCAGCA GAAATGCTGT TCTTGACAGA GTGGGCCAAC ACAATGCGTC
3361 CAGTTGCAAA AGTACTCGAC ATCTTGCAAG CGGAAACGAA TACACAGCTG GGGTGGCTGC
3421 TGCCTAGTGT CCATCAGTTA AGCTTGAAAC TTCAGCGACT CCACCATTCT CTCAGGTACT
3481 GTGACCCACT TGTGGATGCC CTACAACAAG GAATCCAAAC ACGATTCAAG CATATGTTTG
3541 AAGATCCTGA GATCATAGCA GCTGCCATCC TTCTCCCTAA ATTTCGGACC TCTTGGACAA
3601 ATGATGAAAC CATCATAAAA CGAGGTAAAT GAATGCAAGC AACATACACT TGACGAATTC
3661 TAATCTGGGC AACCTTTGAG CCATACCAAA ATTATTCTTT TATTTATTTA TTTTTGCACT
3721 TTTTAGGAAT GTTATATCCC ATCTTTGGCT GTGATCTCAA TATGAATATT GATGTAAAGT
3781 ATTCTTGCAG CAGGTTGTAG TTATCCCTCA GTGTTTCTTG AAACCAAACT CATATGTATC
3841 ATATGTGGTT TGGAAATGCA GTTAGATTTT ATGCTAAAAT AAGGGATTTG CATGATTTTA
3901 GATGTAGATG ACTGCACGTA AATGTAGTTA ATGACAAAAT CCATAAAATT TGTTCCCAGT
3961 CAGAAGCCCC TCAACCAAAC TTTTCTTTGT GTCTGCTCAC TGTGCTTGTA GGCATGGACT
4021 ACATCAGAGT GCATCTGGAG CCTTTGGACC ACAAGAAGGA ATTGGCCAAC AGTTCATCTG
4081 ATGATGAAGA TTTTTTCGCT TCTTTGAAAC CGACAACACA TGAAGCCAGC AAAGAGTTGG
4141 ATGGATATCT GGCCTGTGTT TCAGACACCA GGGAGTCTCT GCTCACGTTT CCTGCTATTT
4201 GCAGCCTCTC TATCAAGACT AATACACCTC TTCCCGCATC GGCTGCCTGT GAGAGGCTTT
4261 TCAGCACTGC AGGATTGCTT TTCAGCCCCA AAAGAGCTAG GCTTGACACT AACAATTTTG
4321 AGAATCAGCT TCTACTGAAG TTAAATCTGA GGTTTTACAA CTTTGAGTAG CGTGTACTGG
4381 CATTAGATTG TCTGTCTTAT AGTTTGATAA TTAAATACAA ACAGTTCTAA AGCAGGATAA
4441 AACCTTGTAT GCATTTCATT TAATGTTTTT TGAGATTAAA AGCTTAAACA AGAATCTCTA
4501 GTTTTCTTTC TTGCTTTTAC TTTTACTTCC TTAATACTCA AGTACAATTT TAATGGAGTA
4561 CTTTTTTACT TTTACTCAAG TAAGATTCTA GCCAGATACT TTTACTTTTA ATTGAGTAAA
4621 ATTTTCCCTA AGTACTTGTA CTTTCACTTG AGTAAAATTT TTGAGTACTT TTTACACCTC
4681 TG (SEQ ID NO: 25).

[046] The disclosure provides a vector comprising the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments, the vector is a viral vector.

[047] Viral vectors of the disclosure may comprise a sequence isolated or derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV) or any combination thereof. In certain embodiments, the viral vector comprises a sequence isolated or derived from a retrovirus. In certain embodiments, the retrovirus is a gammaretrovirus. In certain embodiments, the retrovirus is a lentivirus. In certain embodiments, viral vectors of the disclosure may be recombinant vectors.

[048] Viral vectors of the disclosure may comprise a sequence isolated or derived from an adeno-associated virus. In certain embodiments, the AAV comprises an AAV of a serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11. In certain embodiments, the AAV comprises a sequence from one or more of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11. In certain embodiments, the AAV comprises a sequence isolated, derived, or recombined from one or more of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11. In certain embodiments, the AAV comprises a sequence isolated, derived, or recombined from AAV2. In certain embodiments, including those in which the vector crosses the blood brain barrier (BBB), the AAV comprises a sequence isolated, derived, or recombined from AAV9. Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure include, but are not limited to, self-complementary AAV (scAAV) and AAV hybrids containing the genome of one serotype and the capsid of another serotype (e.g. AAV2/5, AAV-DJ and AAV-DJ8). Exemplary adeno-associated viruses and recombinant adeno-associated viruses of the disclosure include, but are not limited to, rAAV-LK03, rAAV-NP59 and rAAV-NP84. In certain embodiments, the AAV comprises a sequence isolated or derived from rAAV-LK03, rAAV-NP59 or rAAV-NP84. In certain embodiments, viral vectors of the disclosure may be recombinant vectors.

[049] The disclosure provides a vector comprising the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. In certain embodiments, the vector is a nanoparticle vector.

[050] Nanoparticle vectors of the disclosure may comprise a nucleic acid, an amino acid, a polymer, a micelle, lipid, an organic molecule, an inorganic molecule or any combination thereof. Nanoparticles may be comprised of polymers disclosed in, for example, International

Patent Publication No. WO 2012/094679, International Patent Publication No. WO 2016/022805, International Patent Publication No. WO/2011/133635, International Patent Publication No. WO/2016/090111, International Patent Publication No. WO/2017/004498, WO/2017/004509, International Patent Application No. PCT/US2017/030271, US Patent No. 6,835,394, US Patent No. 7,217,427, and US Patent No. 7,867,512.

[051] Nanoparticle vectors of the disclosure may further comprise at least one self-cleaving peptide. In certain embodiments, a nanoparticle vector of the disclosure may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure and another sequence linked to the nanoparticle. In certain embodiments, a nanoparticle vector of the disclosure may comprise at least one self-cleaving peptide and wherein a self-cleaving peptide is located upstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure and a second self-cleaving peptide is located downstream of the inducible proapoptotic polypeptide, inducible caspase polypeptide, inducible caspase 9 polypeptide, and/or inducible truncated caspase 9 polypeptide of the disclosure. The at least one self-cleaving peptide may comprise a T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide. In certain embodiments, the T2A peptide comprises an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 11). In certain embodiments, the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 12). In certain embodiments, the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 13). In certain embodiments, the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 14). In certain embodiments, the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 15). In certain embodiments, the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 16). In certain embodiments, the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP

(SEQ ID NO: 17). In certain embodiments, the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 18).

[052] The disclosure provides a composition comprising a vector of the disclosure.

[053] The disclosure provides a cell comprising an inducible proapoptotic polypeptide, an inducible caspase polypeptide, an inducible caspase 9 polypeptide, and/or an inducible truncated caspase 9 polypeptide of the disclosure. The disclosure provides a cell comprising a transposon of the disclosure. The disclosure provides a cell comprising a vector of the disclosure. In certain embodiments, the cell expresses the inducible caspase protein of the disclosure following contact with an induction agent.

[054] In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be human cells.

[055] In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be immune cells. Examples of immune cells include, but are not limited to, T-cells, Natural Killer (NK) cells, Natural Killer (NK)-like cells, hematopoietic progenitor cells, peripheral blood (PB) derived T cells and umbilical cord blood (UCB) derived T-cells. In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be T-cells. In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be activated T-cells. In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be activated T-cells that express an iC9 sequence of the disclosure. In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be artificial antigen presenting cells (APCs). Immune cells of the disclosure may further include any commercially-available cell line or modified cell line, including, but not limited to, cell lines of dendritic cells, B cells, macrophages and monocytes.

[056] In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be immune cells. Examples of immune cells include, but are not limited to, T-cells, Natural Killer (NK) cells, Natural Killer (NK)-like cells, hematopoietic progenitor cells, peripheral blood (PB) derived T cells and umbilical cord blood (UCB) derived T-cells. In certain embodiments, cells of the disclosure that

comprise a polypeptide, transposon or vector of the disclosure may be T-cells. In certain embodiments, the cell is an artificial antigen presenting cell.

[057] In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be stem cells. Stem cells of the disclosure may be human stem cells. Stem cells of the disclosure may be embryonic or adult stem cells. Stem cells of the disclosure may be totipotent, pluripotent, or multipotent. In certain embodiments, stem cells of the disclosure may be induced pluripotent stem cell (iPSC).

[058] In certain embodiments, cells of the disclosure that comprise a polypeptide, transposon or vector of the disclosure may be somatic cells. Somatic cells of the disclosure may be isolated or derived from any part of a body, and preferably a human body, including, but not limited to, a human heart; skeletal or smooth muscle; blood vessel, vein or capillary; spleen; thyroid; lymph node or lymph vessel; bone or bone marrow; skin or endothelium; adrenal gland; esophagus; larynx; brain or spinal cord; peripheral nervous system; eye; hypothalamus; liver; olfactory tissue; prostate; stomach; large or small intestine; lung or bronchi; kidney; pancreas; thymus gland; ureter or urethrae; bladder; auditory tissue; bladder; parathyroid gland; salivary gland; or trachea. Somatic cells of the disclosure may be isolated or derived from a precursor or stem cell that may differentiate into any part of a body, and preferably a human body including, but not limited to, a human heart; skeletal or smooth muscle; blood vessel, vein or capillary; spleen; thyroid; lymph node or lymph vessel; bone or bone marrow; skin or endothelium; adrenal gland; esophagus; larynx; brain or spinal cord; peripheral nervous system; eye; hypothalamus; liver; olfactory tissue; prostate; stomach; large or small intestine; lung or bronchi; kidney; pancreas; thymus gland; ureter or urethrae; bladder; auditory tissue; bladder; parathyroid gland; salivary gland; or trachea. Somatic cells of the disclosure may be isolated or derived from transdifferentiated cells.

[059] The disclosure provides a composition comprising a cell of the disclosure comprising a polypeptide, transposon, or vector of the disclosure.

[060] The disclosure provides a use of the compositions of the disclosure for an adoptive cell therapy. In certain embodiments, the cells of the composition may be autologous. In certain embodiments, the cells of the composition may be allogeneic.

[061] The disclosure provides a use of the compositions of the disclosure for an ex vivo gene therapy. In certain embodiments, the cells of the composition may be autologous. In certain embodiments, the cells of the composition may be allogeneic.

[062] The disclosure provides a use for the composition comprising a cell of the disclosure for an ex vivo gene therapy. In certain embodiments of the use of the composition comprising the cells of the disclosure, the cell is autologous. In certain embodiments, the cell is allogenic.

[063] The disclosure provides a method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent and an inducible caspase polypeptide of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

[064] The disclosure provides a method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent and a composition of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

[065] The disclosure provides a method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent, a transposon of the disclosure and a composition comprising a transposase of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

[066] The disclosure provides a method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent and a vector of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

[067] In certain embodiments of the methods of modifying a cell therapy of the disclosure, the cells of the composition may be autologous. In certain embodiments, the cells of the composition may be allogeneic. In certain embodiments of this method, the cell therapy is an adoptive cell therapy. In certain embodiments, the therapeutic agent has been introduced by ex vivo gene therapy. In certain embodiments, the therapeutic agent is a sequence encoding a modified endogenous gene, an exogenous gene, or a portion thereof. In certain embodiments of this method, the modifying of the cell therapy is a termination of the cell therapy. In

certain embodiments of this method, the modifying of the cell therapy is a depletion of a portion of the cells provided in the cell therapy. This depletion may be transient or may be maintained for a period of time, for example, during a period of remission of a disease or disorder. In certain embodiments, the method further comprises the step of administering an inhibitor of the induction agent to inhibit modification of the cell therapy, thereby restoring the function and/or efficacy of the cell therapy. For example, should a disease or disorder return following a remission or a subject's adverse reaction subside, the cell therapy may be resumed by administering to the subject an inhibitor of the induction agent.

[068] Methods of modifying a cell therapy of the disclosure may be used to terminate or dampen a therapy in response to, for example, a sign of recovery or a sign of decreasing disease severity/progression, a sign of disease remission/cessation, and/or the occurrence of an adverse event. Cell therapies of the disclosure may be resumed by inhibiting the induction agent should a sign or symptom of the disease reappear or increase in severity and/or an adverse event is resolved.

[069] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises between $2x10^5$ and $5x10^8$ cells per kg of body weight of the patient per administration, or any range, value or fraction thereof.

[070] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises between $0.2x10^6$ to $20x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $0.2x10^6$ cells per kg of body weight of the patient per administration, $2x10^6$ cells per kg of body weight of the patient per administration, $20x10^6$ cells per kg of body weight of the patient per administration, or any cells per kg of body weight of the patient per administration in between.

[071] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $1x10^6$ cells or about $1x10^6$ cells per kg of body weight of the patient per administration.

[072] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $3x10^6$ cells or about $3x10^6$ cells per kg of body weight of the patient per administration.

[073] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises between $0.7x10^6$ to $6.7x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $0.7x10^6$ cells per kg of body weight of the patient per administration, $6.7x10^6$ cells per kg of body weight of the patient per administration or any cells per kg of body weight of the patient per administration in between.

[074] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises between $0.7x10^6$ to $16x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $0.7x10^6$ cells per kg of body weight of the patient per administration, $2x10^6$ cells per kg of body weight of the patient per administration, $6x10^6$ cells per kg of body weight of the patient per administration, $10.7x10^6$ cells per kg of body weight of the patient per administration, $16x10^6$ cells per kg of

body weight of the patient per administration or any cells per kg of body weight of the patient per administration in between.

[075] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $1.2x10^6$ to $7.1x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $1.2x10^6$ cells per kg of body weight of the patient per administration, $7.1x10^6$ cells per kg of body weight of the patient per administration or any number of cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises between $2x10^6$ to $3x10^6$ cells per kg of body weight of the patient per administration.

[076] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $1x10^6$ to $2x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $1x10^6$ cells per kg of body weight of the patient per administration, $2x10^6$ cells per kg of body weight of the patient per administration or any number of cells per kg of body weight of the patient per administration in between. In certain embodiments of the disclosure, modified cells of the disclosure are delivered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $0.7x10^6$ to $1.3x10^6$ cells per kg of body weight of the patient per administration. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises $0.7x10^6$ cells per kg of body weight of the patient per administration,

$1.3x10^6$ cells per kg of body weight of the patient per administration or any number of cells per kg of body weight of the patient per administration in between.

[077] In certain embodiments, a composition comprising a cell of the disclosure or a modified cell of the disclosure is administered to a patient via injection or intravenous infusion. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises a single or multiple doses. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises a split dose. In certain embodiments, a therapeutically effective dose of a composition of the disclosure or of compositions comprising modified cells of the disclosure comprises an initial dose and a maintenance dose.

[078] The disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject a composition comprising a cell comprising a kinetic agent and an inducible caspase polypeptide of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of toxicity in a non-target tissue of the subject, thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

[079] As used herein, a kinetic agent is meant to describe a therapeutic agent having specificity for a target tissue and either known/intentional or unknown/unintentional toxicity towards non-target tissue, which, in either case, may be controlled or limited such that the toxicity does not significantly affect non-target tissues. Kinetic agents specifically target a tissue, specifically and locally induce toxicity, but before a kinetic agent can induce significant toxicity or damage to a non-target tissue, an induction agent of the disclosure may be administered to reduce or prevent non-target tissue toxicity. The extent to which any toxicity affects off-target tissues (e.g. damage to non-target tissues) may be limited by the administration of the induction agent and may correspond to a duration of exposure of the non-target tissue to the kinetic agent. In certain embodiments of the disclosure, cells comprising a therapeutic agent may react to both on-target on-tumor cells and on-target off-

tumor cells at the same time and in multiple different tissues. The on-target off-tumor cells are preserved through the activation of the inducible caspase polypeptides of the disclosure in the cells comprising a therapeutic agent to eliminate the cells comprising the inducible caspase polypeptides of the disclosure, for example, after the tumor is eliminated. In certain embodiments, off-tumor off-target effects may be due to cross-reactivity of the kinetic agent. In certain embodiments, if the off-target effects are too great, the toxicity off the kinetic agent may be limited or eliminated by administration of an induction agent before the treatment of a disease of the subject is complete.

[080] The disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject a composition comprising a cell comprising a kinetic agent and a composition of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject, thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

[081] The disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject a composition comprising a cell comprising a kinetic agent, a transposon of the disclosure and a composition comprising a transposase of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject, thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

[082] The disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering to the subject a composition comprising a cell comprising a kinetic agent and a vector of the disclosure, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the

subject, and selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject, thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

[083] The term "significant toxicity in a non-target tissue" may described a level of toxicity in which the cells of the tissue are dead (e.g. have died as a result of necrosis or apoptosis), have otherwise become not viable or have ceased to perform one or more essential physiological function(s). In certain embodiments, significant toxicity denotes permanent damage to a cell or a tissue. In certain embodiments, significant toxicity denotes a transient loss of a function of a cell or a tissue. In certain embodiments, significant toxicity induces symptoms in a subject that are recognizable as such by one skilled in the art. In certain embodiments, significant toxicity leads to death, a reduced life span, or a reduced quality of life of a subject.

[084] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a proliferative disorder or cancer. In certain embodiments, the target tissue comprises a tumor. In certain embodiments, the tumor is benign. In certain embodiments, the tumor is malignant. In certain embodiments, the target tissue comprises an exposed tissue or margin of a resected tumor. In certain embodiments, the target tissue comprises a site of probable metastasis. In certain embodiments, the site of metastasis comprise one or more of a lymph node, lymph fluid, peripheral circulating blood, local circulating blood, a bone, a bone marrow and cerebral spinal fluid (CSF).

[085] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is an inflammatory disease or disorder. In certain embodiments, the target tissue comprises a site of inflammation.

[086] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is an immune or autoimmune disease or disorder. In certain embodiments, the target tissue comprises a site of exposed or infected tissue. In certain embodiments, the target tissue comprises a burned or a wounded tissue.

[087] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is an infectious disease or disorder. In certain embodiments, the target tissue comprises an infected tissue.

[088] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a genetic or epigenetic disease or disorder. In certain embodiments, the target tissue comprises one or more cells comprising the genetic or epigenetic modification when compared to a wild type cell.

[089] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a metabolic disorder. In certain embodiments, the target tissue comprises one or more cells with the metabolic disorder.

[090] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a vascular disorder. In certain embodiments, the target tissue comprises one or more cells of a vein, blood vessel, capillary or a component of circulating blood.

[091] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a respiratory disorder. In certain embodiments, the target tissue comprises one or more cells of a nasal passage, esophagus, or lung.

[092] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a fibrotic disorder. In certain embodiments, the target tissue comprises a fibroid mass or a cell in proximity to the fibroid mass.

[093] In certain embodiments of the methods of treating a disease or disorder of the disclosure, an adoptive cell therapy comprises the cell comprising the kinetic agent. In certain embodiments, the cell comprising the kinetic agent is autologous. In certain embodiments, the cell comprising the kinetic agent is allogeneic. In certain embodiments, the cell comprising the kinetic agent is a T-cell. In certain embodiments, the kinetic agent is a non-naturally occurring receptor. In certain embodiments, the non-naturally occurring receptor is a synthetic, modified, recombinant or chimeric receptor. In certain embodiments, the chimeric receptor is a chimeric antigen receptor (CAR).

[094] In certain embodiments of the methods of treating a disease or disorder of the disclosure, the disease or disorder is a proliferative disorder or a cancer. In certain embodiments, the target tissue comprises a tumor. In certain embodiments, treatment of the tumor comprises a composition comprising a cell comprising an inducible caspase polypeptide of the disclosure. In certain embodiments, the composition comprising a cell comprising an inducible caspase polypeptide of the disclosure further comprises a chimeric

antigen receptor (CAR). In certain embodiments, the chimeric antigen receptor (CAR) specifically binds a sequence expressed on a cell of a tumor, thereby conferring specificity of the CAR for the tumor cell. In certain embodiments, the cell expressing the CAR that specifically binds a tumor cell is a T-cell, thereby making a CAR-T that specifically binds a tumor cell. In certain embodiments, compositions comprising CAR-T cells specifically target tumor cells will selectively kill only those tumor cells expressing the antigen sequence. However, in certain embodiments, the tumor antigen may be expressed in other normal tissues leading to on-target off-tumor activity of the CAR-T cells in non-target tissues. For example, the antigen may be CD19 and CD19 is expressed almost exclusively in B cells. In the case of CD19, off target activity of anti-CD19 CAR-T cells is minimal. However, if, for example, the antigen is PSMA (folate hydrolase 1) and PSMA is expressed in several normal cell types, anti-PSMA CAR-T cells may target normal cells in addition to the pathological target cells in an activity called on-target off-tumor toxicity. In certain embodiments, once the pathological target cells of the disclosure (for example, the tumor cells) are eradicated by the anti-PSMA CAR-T cells, treating the subject with the induction agent of the disclosure to induce programmed cell death in the anti-PSMA CAR-T cells using the inducible caspase polypeptides of the disclosure eliminates on-target off-tumor effects.

[095] In certain methods of the disclosure, including those wherein an adoptive cell therapy comprises the cell comprising the kinetic agent, the kinetic agent comprises an anti-cancer agent. In certain embodiments, the anti-cancer agent comprises an anti-CD19 agent. In certain embodiments, the anti-cancer agent comprises an anti-BCMA agent. In certain embodiments, the anti-cancer agent comprises an anti-PSMA agent. In certain embodiments, the anti-cancer agent comprises an anti-Muc1 agent.In certain embodiments, the kinetic agent comprises a non-naturally occurring receptor. In certain embodiments, the non-naturally occurring receptor comprises a synthetic, modified, recombinant or chimeric receptor. In certain embodiments, the chimeric receptor is a chimeric antigen receptor (CAR). In certain embodiments, the CAR comprises one or more VHH sequence(s). In certain embodiments, the CAR is a VCAR. In certain embodiments, the kinetic agent induces an aplasia. In certain embodiments, the aplasia is not fatal. In certain embodiments, the induction agent eliminates the cell comprising the kinetic agent. In certain embodiments, the cell is a T-cell. In certain embodiments, the induction agent eliminates or reduces a sign or a symptom of the aplasia.

In certain embodiments, the CAR-T therapy eliminates the malignancy, but continues to target healthy B cells such that the subject must be treated with IVIG infusions. In certain embodiments, anti-BCMA CAR-T therapy eliminates the subject's own unmutated plasma cells such that the subject must also be treated with IVIG. In certain embodiments, the CAR-T causing the aplasia may be eliminated through the caspase 9 polypeptides of the disclosure and the induction agent. In certain embodiments, treatment of the subject with the induction agent of the disclosure alleviates a sign or a symptom of the aplasia.

BRIEF DESCRIPTION OF THE DRAWINGS

[096] Figure 1 is a schematic diagram depicting an exemplary inducible truncated caspase 9 polypeptide of the disclosure.

[097] Figure 2 is a series of flow cytometry plots depicting the abundance of cells moving from an area of live cells (the gated lower right quadrant) to an area populated by apoptotic cells (the upper left quadrant) as a function of increasing dosage of the induction agent (AP1903, also called Rimiducid) in cells modified to express a therapeutic agent (a CARTyrin) alone or in combination with an inducible caspase polypeptide of the disclosure (encoded by an iC9 construct (also known as a "safety switch") introduced into cells by a piggyBac (PB) transposase) at day 12 post nucleofection.

[098] Figure 3 is a series of flow cytometry plots depicting the abundance of cells moving from an area of live cells (the gated lower right quadrant) to an area populated by apoptotic cells (the upper left quadrant) as a function of increasing dosage of the induction agent (AP1903, also called Rimiducid) in cells modified to express a therapeutic agent (a CARTyrin) alone or in combination with an inducible caspase polypeptide of the disclosure (encoded by an iC9 construct (also known as a "safety switch") introduced into cells by a piggyBac (PB) transposase) at day 19 post nucleofection.

[099] Figure 4 is a pair of graphs depicting a quantification of the aggregated results shown either in Figure 2 (left graph) or Figure 3 (right graph). Specifically, these graphs show the impact of the iC9 safety switch on the percent cell viability as a function of the concentration of the induction agent (AP1903, also called Rimiducid) of the iC9 switch for each modified cell type at either day 12 (Figure 2 and left graph) or day 19 (Figure 3 and right graph).

[0100] Figure 5 is a diagram showing a study timeline and outline for an in vivo study. NSG mice were IV injected with MM.1S/luciferase$^+$ cells, staged at day 8, injected with T cells on day 9, and treated with AP1903 (Rimiducid) on day 12 at the indicated doses. 24 hours later, mice were euthanized and blood, spleen, and bone marrow cells were collected and stained for the presence of huCD45$^+$ cells.

[0101] Figure 6 is a graph demonstrating the highly efficient killing of cells comprising P-BCMA-101 using Rimiducid (AP1903) in vivo. Figure 6 shows the presence of CARTyrin$^+$ cells in blood, spleen, and bone marrow following AP1903 Treatment. Blood, spleen, and bone marrow cells were analyzed by flow cytometry for the presence of huCD45$^+$ cells. The relative viability was determined by: ((# of huCD45 cells/# of msCD45 cells)/(Average of huCD45/msCD45 in no treatment group))*100% per 1,500 bead events for each sample. Each data point represents a different mouse. On the x-axis is plotted percent Relative CAR-T Viability from 0 to 175 in increments of 25. On the y axis, from left to right for each panel, blood, spleen and bone marrow following AP1903 (Rimiducid) treatment. AP1903 (Rimiducid) increase by panel from left to right in the following order: 0 mg/kg, 0.005 mg/kg, 0.05 mg/kg, 0.5 mg. kg, 5 mg/kg and 5 mg/kg in the absence of the iC9 gene.

DETAILED DESCRIPTION

[0102] The disclosure provides inducible proapoptotic polypeptides as well as transposons, vectors, and cells comprising inducible proapoptotic polypeptides of the disclosure. Inducible proapoptotic polypeptides of the disclosure may be introduced into a cell simultaneously or sequentially with a therapeutic agent. For example, inducible proapoptotic polypeptides of the disclosure may be introduced into a cell simultaneously or sequentially with one or more sequences that encode a chimeric antigen receptor to produce a modified cell of the disclosure. Modified cells of the disclosure may be used in cell-based therapies. To control activity of modified cells of the disclosure comprising an inducible proapoptotic polypeptide, and, optionally, a therapeutic agent, the cell and/or the inducible proapoptotic polypeptide may contact an induction agent that specifically binds to the ligand binding region of the inducible proapoptotic polypeptide and ultimately causes initiation of apoptosis in the cell comprising the inducible proapoptotic polypeptide. When a modified cell of the disclosure is used as a cell therapy, the induction agent may be administered locally or systemically to the

subject who received the cell therapy. An inhibitor of the induction agent may be administered locally or systemically to the subject who received the cell therapy and the induction agent.

[0103] As used herein, the term "therapeutic agent" may refer to any molecule, organic or inorganic, that, when introduced into a cell intended for cell therapy, modified an activity, a signaling pathway, a signaling outcome, and/or an interaction of that cell with the cell's internal or external environment , including, but not limited to, neighboring cells, extracellular ligands and signaling molecules, immune system of the cell's host or a component thereof, infection of host, a diseased cell (e.g. a cancer cell) of the host, intracellular ligands and signaling molecules, epigenetic regulation, gene transcription, gene regulation, transcriptome, DNA/RNA translation, protein processing or secretion processes, and proteome. Therapeutic agents include, but are not limited to, recombinant and/or chimeric cell surface receptors, recombinant and/or chimeric transmembrane receptors, recombinant and/or chimeric ion channels, and recombinant and/or chimeric antigen receptors. In certain embodiments, the therapeutic agent is a chimeric antigen receptor (CAR), and, optionally, a chimeric antigen receptor in which the antigen recognition region comprises at least one Centyrin. As used throughout the disclosure, a CAR comprising a Centyrin is referred to as a CARTyrin.

[0104] The disclosure provides inducible proapoptotic polypeptides comprising a ligand binding region, a linker, and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. Inducible proapoptotic polypeptides of the disclosure dimerize through interaction with an induction agent. Dimerization of a first inducible proapoptotic polypeptide and a second inducible proapoptotic polypeptide facilitates or activates an interaction, a cross-linking, a cross-activation, or an activation of the caspase polypeptides. The interaction, cross-linking, cross-activation, or activation of the caspase polypeptides initiates apoptosis in a cell comprising the inducible proapoptotic polypeptides of the disclosure or a sequence encoding the inducible proapoptotic polypeptides of the disclosure. Inducible proapoptotic polypeptides of the disclosure do not initiate apoptosis unless and the inducible proapoptotic polypeptide of the disclosure contacts an induction agent. Contact between an induction agent and an inducible proapoptotic

polypeptides of the disclosure may occur in vivo, ex vivo, or in vitro. Contact between an induction agent and an inducible proapoptotic polypeptides of the disclosure may occur intracellularly.

[0105] With respect to cell therapies, the disclosure provides modified T cells for adoptive cell therapies comprising an inducible proapoptotic polypeptide or a sequence encoding an inducible proapoptotic polypeptide of the disclosure.

[0106] Modified T cells for adoptive cell therapies may be autologous or allogeneic. The term "allogeneic" as used herein, refers to HLA or MHC loci that are antigenically distinct. Cells or tissue transferred from the same species can be antigenically distinct. Syngeneic mice can differ at one or more loci (congenics) and allogeneic mice can have the same background.

[0107] Modified T cells for adoptive cell therapies may include activated T cells. T cells (also referred to as T lymphocytes) belong to a group of white blood cells referred to as lymphocytes. Lymphocytes generally are involved in cell-mediated immunity. The "T" in "T cells" refers to cells derived from or whose maturation is influence by the thymus. T cells can be distinguished from other lymphocytes types such as B cells and Natural Killer (NK) cells by the presence of cell surface proteins known as T cell receptors. The term "activated T cells" as used herein, refers to T cells that have been stimulated to produce an immune response (e.g., clonal expansion of activated T cells) by recognition of an antigenic determinant presented in the context of a Class II major histocompatibility (MHC) marker. T-cells are activated by the presence of an antigenic determinant, cytokines and/or lymphokines and cluster of differentiation cell surface proteins (e.g., CD3, CD4, CD8, the like and combinations thereof). Cells that express a cluster of differential protein often are said to be "positive" for expression of that protein on the surface of T-cells (e.g., cells positive for CD3 or CD4 expression are referred to as CD3+ or CD4+). CD3 and CD4 proteins are cell surface receptors or co-receptors that may be directly and/or indirectly involved in signal transduction in T cells.

[0108] Modified T cells for adoptive cell therapies may include "pan T cells". As used herein, pan T-cells include all T lymphocytes isolated from a biological sample, without sorting by subtype, activation status, maturation state, or cell-surface marker expression.

[0109] Modified T cells for adoptive cell therapies may be obtained and/or prepared from, for example, whole blood, peripheral blood, umbilical cord blood, lymph fluid, lymph node tissue, bone marrow, and cerebral spinal fluid (CSF). By "obtained or prepared" as, for example, in the case of cells, is meant that the cells or cell culture are isolated, purified, or partially purified from the source, where the source may be, for example, umbilical cord blood, bone marrow, or peripheral blood. The terms may also apply to the case where the original source, or a cell culture, has been cultured and the cells have replicated, and where the progeny cells are now derived from the original source. The term "peripheral blood" as used herein, refers to cellular components of blood (e.g., red blood cells, white blood cells and platelets), which are obtained or prepared from the circulating pool of blood and not sequestered within the lymphatic system, spleen, liver or bone marrow. Umbilical cord blood is distinct from peripheral blood and blood sequestered within the lymphatic system, spleen, liver or bone marrow. The terms "umbilical cord blood", "umbilical blood" or "cord blood", which can be used interchangeably, refers to blood that remains in the placenta and in the attached umbilical cord after child birth. Cord blood often contains stem cells including hematopoietic cells.

[0110] The disclosure provides inducible proapoptotic polypeptides comprising a ligand binding region, a linker, and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. In certain embodiments, the proapoptotic peptide is a caspase polypeptide. In certain embodiments, the caspase polypeptide is a caspase 9 polypeptide. In certain embodiments, the caspase 9 polypeptide is a truncated caspase 9 polypeptide. Inducible proapoptotic polypeptides of the disclosure may be non-naturally occurring.

[0111] Caspase polypeptides of the disclosure include, but are not limited to, caspase 1, caspase 2, caspase 3, caspase 4, caspase 5, caspase 6, caspase 7, caspase 8, caspase 9, caspase 10, caspase 11, caspase 12, and caspase 14. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides associated with apoptosis including caspase 2, caspase 3, caspase 6, caspase 7, caspase 8, caspase 9, and caspase 10. Caspase polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that initiate apoptosis, including caspase 2, caspase 8, caspase 9, and caspase 10. Caspase

polypeptides of the disclosure include, but are not limited to, those caspase polypeptides that execute apoptosis, including caspase 3, caspase 6, and caspase 7.

[0112] Caspase polypeptides of the disclosure may be encoded by an amino acid or a nucleic acid sequence having one or more modifications compared to a wild type amino acid or a nucleic acid sequence. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be codon optimized. The one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may increase an interaction, a cross-linking, a cross-activation, or an activation of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence. Alternatively, or in addition, the one or more modifications to an amino acid and/or nucleic acid sequence of a caspase polypeptide of the disclosure may decrease the immunogenicity of the caspase polypeptide of the disclosure compared to a wild type amino acid or a nucleic acid sequence.

[0113] Caspase polypeptides of the disclosure may be truncated compared to a wild type caspase polypeptide. For example, a caspase polypeptide may be truncated to eliminate a sequence encoding a Caspase Activation and Recruitment Domain (CARD) to eliminate or minimize the possibility of activating a local inflammatory response in addition to initiating apoptosis in the cell comprising an inducible caspase polypeptide of the disclosure. The nucleic acid sequence encoding a caspase polypeptide of the disclosure may be spliced to form a variant amino acid sequence of the caspase polypeptide of the disclosure compared to a wild type caspase polypeptide. Caspase polypeptides of the disclosure may be encoded by recombinant and/or chimeric sequences. Recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more different caspase polypeptides. Alternatively, or in addition, recombinant and/or chimeric caspase polypeptides of the disclosure may include sequences from one or more species (e.g. a human sequence and a non-human sequence). Caspase polypeptides of the disclosure may be non-naturally occurring.

[0114] The ligand binding region of an inducible proapoptotic polypeptide of the disclosure may include any polypeptide sequence that facilitates or promotes the dimerization of a first inducible proapoptotic polypeptide of the disclosure with a second inducible proapoptotic polypeptide of the disclosure, the dimerization of which activates or induces cross-linking of the proapoptotic polypeptides and initiation of apoptosis in the cell.

[0115] The ligand-binding ("dimerization") region may comprise any polypeptide or functional domain thereof that will allow for induction using a natural or unnatural ligand (i.e. and induction agent), for example, an unnatural synthetic ligand. The ligand-binding region may be internal or external to the cellular membrane, depending upon the nature of the inducible proapoptotic polypeptide and the choice of ligand (i.e. induction agent). A wide variety of ligand-binding polypeptides and functional domains thereof, including receptors, are known. Ligand-binding regions of the disclosure may include one or more sequences from a receptor. Of particular interest are ligand-binding regions for which ligands (for example, small organic ligands) are known or may be readily produced. These ligand-binding regions or receptors may include, but are not limited to, the FKBPs and cyclophilin receptors, the steroid receptors, the tetracycline receptor, and the like, as well as "unnatural" receptors, which can be obtained from antibodies, particularly the heavy or light chain subunit, mutated sequences thereof, random amino acid sequences obtained by stochastic procedures, combinatorial syntheses, and the like. In certain embodiments, the ligand-binding region is selected from the group consisting of a FKBP ligand-binding region, a cyclophilin receptor ligand-binding region, a steroid receptor ligand-binding region, a cyclophilin receptors ligand-binding region, and a tetracycline receptor ligand-binding region.

[0116] The ligand-binding regions comprising one or more receptor domain(s) may be at least about 50 amino acids, and fewer than about 350 amino acids, usually fewer than 200 amino acids, either as the natural domain or truncated active portion thereof. The binding region may, for example, be small (< 25 kDa, to allow efficient transfection in viral vectors), monomeric, nonimmunogenic, have synthetically accessible, cell permeable, nontoxic ligands that can be configured for dimerization.

[0117] The ligand-binding regions comprising one or more receptor domain(s) may be intracellular or extracellular depending upon the design of the inducible proapoptotic polypeptide and the availability of an appropriate ligand (i.e. induction agent). For hydrophobic ligands, the binding region can be on either side of the membrane, but for hydrophilic ligands, particularly protein ligands, the binding region will usually be external to the cell membrane, unless there is a transport system for internalizing the ligand in a form in which it is available for binding. For an intracellular receptor, the inducible proapoptotic polypeptide or a transposon or vector comprising the inducible proapoptotic polypeptide may

encode a signal peptide and transmembrane domain 5′ or 3′ of the receptor domain sequence or may have a lipid attachment signal sequence 5′ of the receptor domain sequence. Where the receptor domain is between the signal peptide and the transmembrane domain, the receptor domain will be extracellular.

[0118] Antibodies and antibody subunits, e.g., heavy or light chain, particularly fragments, more particularly all or part of the variable region, or fusions of heavy and light chain to create high-affinity binding, can be used as a ligand binding region of the disclosure. Antibodies that are contemplated include ones that are an ectopically expressed human product, such as an extracellular domain that would not trigger an immune response and generally not expressed in the periphery (i.e., outside the CNS/brain area). Such examples, include, but are not limited to low affinity nerve growth factor receptor (LNGFR), and embryonic surface proteins (i.e., carcinoembryonic antigen). Yet further, antibodies can be prepared against haptenic molecules, which are physiologically acceptable, and the individual antibody subunits screened for binding affinity. The cDNA encoding the subunits can be isolated and modified by deletion of the constant region, portions of the variable region, mutagenesis of the variable region, or the like, to obtain a binding protein domain that has the appropriate affinity for the ligand. In this way, almost any physiologically acceptable haptenic compound can be employed as the ligand or to provide an epitope for the ligand. Instead of antibody units, natural receptors can be employed, where the binding region or domain is known and there is a useful or known ligand for binding.

[0119] For multimerizing the receptor, the ligand for the ligand-binding region/receptor domains of the inducible proapoptotic polypeptides may be multimeric in the sense that the ligand can have at least two binding sites, with each of the binding sites capable of binding to a ligand receptor region (i.e. a ligand having a first binding site capable of binding the ligand-binding region of a first inducible proapoptotic polypeptide and a second binding site capable of binding the ligand-binding region of a second inducible proapoptotic polypeptide, wherein the ligand-binding regions of the first and the second inducible proapoptotic polypeptides are either identical or distinct). Thus, as used herein, the term "multimeric ligand binding region" refers to a ligand-binding region of an inducible proapoptotic polypeptide of the disclosure that binds to a multimeric ligand. Multimeric ligands of the disclosure include dimeric ligands. A dimeric ligand of the disclosure may have two binding sites capable of binding to

the ligand receptor domain. In certain embodiments, multimeric ligands of the disclosure are a dimer or higher order oligomer, usually not greater than about tetrameric, of small synthetic organic molecules, the individual molecules typically being at least about 150 Da and less than about 5 kDa, usually less than about 3 kDa. A variety of pairs of synthetic ligands and receptors can be employed. For example, in embodiments involving natural receptors, dimeric FK506 can be used with an FKBP12 receptor, dimerized cyclosporin A can be used with the cyclophilin receptor, dimerized estrogen with an estrogen receptor, dimerized glucocorticoids with a glucocorticoid receptor, dimerized tetracycline with the tetracycline receptor, dimerized vitamin D with the vitamin D receptor, and the like. Alternatively higher orders of the ligands, e.g., trimeric can be used. For embodiments involving unnatural receptors, e.g., antibody subunits, modified antibody subunits, single chain antibodies comprised of heavy and light chain variable regions in tandem, separated by a flexible linker, or modified receptors, and mutated sequences thereof, and the like, any of a large variety of compounds can be used. A significant characteristic of the units comprising a multimeric ligand of the disclosure is that each binding site is able to bind the receptor with high affinity, and preferably, that they are able to be dimerized chemically. Also, methods are available to balance the hydrophobicity/hydrophilicity of the ligands so that they are able to dissolve in serum at functional levels, yet diffuse across plasma membranes for most applications.

[0120] Activation of inducible proapoptotic polypeptides of the disclosure may be accomplished through, for example, chemically induced dimerization (CID) mediated by an induction agent to produce a conditionally controlled protein or polypeptide. Proapoptotic polypeptides of the disclosure not only inducible, but the induction of these polypeptides is also reversible, due to the degradation of the labile dimerizing agent or administration of a monomeric competitive inhibitor.

[0121] In certain embodiments, the ligand binding region comprises a FK506 binding protein 12 (FKBP12) polypeptide. In certain embodiments, the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V), the induction agent may comprise AP1903 (Rimiducid), a synthetic drug (CAS Index Name: 2-Piperidinecarboxylic acid, 1-[(2S)-1-oxo-2-(3,4,5-trimethoxyphenyl)butyl]-,

1,2-ethanediylbis[imino(2-oxo-2,1-ethanediyl)oxy-3,1-phenylene[(1R)-3-(3,4-dimethoxyphenyl)propylidene]]ester, [2S-[1(R*),2R*[S*[S*[1(R*),2R*]]]]]-(9Cl) CAS Registry Number: 195514-63-7; Molecular Formula: C78H98N4O20; Molecular Weight: 1411.65)). In certain embodiments, in which the ligand binding region comprises a FKBP12 polypeptide having a substitution of valine (V) for phenylalanine (F) at position 36 (F36V), the induction agent may comprise AP20187 (CAS Registry Number: 195514-80-8 and Molecular Formula: C82H107N5O20). In certain embodiments, the induction agent is an AP20187 analog, such as, for example, AP1510. As used herein, the induction agents AP20187, AP1903 (Rimiducid) and AP1510 may be used interchangeably.

[0122] AP1903 (Rimiducid) API is manufactured by Alphora Research Inc. and AP1903 (Rimiducid)Drug Product for Injection is made by Formatech Inc. It is formulated as a 5 mg/mL solution of AP1903 (Rimiducid) in a 25% solution of the non-ionic solubilizer Solutol HS 15 (250 mg/mL, BASF). At room temperature, this formulation is a clear, slightly yellow solution. Upon refrigeration, this formulation undergoes a reversible phase transition, resulting in a milky solution. This phase transition is reversed upon re-warming to room temperature. The fill is 2.33 mL in a 3 mL glass vial (approximately 10 mg AP1903 (Rimiducid) for Injection total per vial). Upon determining a need to administer AP1903 (Rimiducid), patients may be, for example, administered a single fixed dose of AP1903 (Rimiducid) for Injection (0.4 mg/kg) via IV infusion over 2 hours, using a non-DEHP, non-ethylene oxide sterilized infusion set. The dose of AP1903 (Rimiducid) is calculated individually for all patients, and is not be recalculated unless body weight fluctuates by ≧10%. The calculated dose is diluted in 100 mL in 0.9% normal saline before infusion. In a previous Phase I study of AP1903 (Rimiducid), 24 healthy volunteers were treated with single doses of AP1903 (Rimiducid) for Injection at dose levels of 0.01, 0.05, 0.1, 0.5 and 1.0 mg/kg infused IV over 2 hours. AP1903 (Rimiducid) plasma levels were directly proportional to dose, with mean Cmax values ranging from approximately 10-1275 ng/mL over the 0.01-1.0 mg/kg dose range. Following the initial infusion period, blood concentrations demonstrated a rapid distribution phase, with plasma levels reduced to approximately 18, 7, and 1% of maximal concentration at 0.5, 2 and 10 hours post-dose, respectively. AP1903 (Rimiducid) for Injection was shown to be safe and well tolerated at all

dose levels and demonstrated a favorable pharmacokinetic profile. Iuliucci J D, et al., J Clin Pharmacol. 41: 870-9, 2001.

[0123] The fixed dose of AP1903 (Rimiducid) for injection used, for example, may be 0.4 mg/kg intravenously infused over 2 hours. The amount of AP1903 (Rimiducid) needed in vitro for effective signaling of cells is 10-100 nM (1600 Da MW). This equates to 16-160 µg/L or ~0.016-1.6 µg/kg (1.6-160 µg/kg). Doses up to 1 mg/kg were well-tolerated in the Phase I study of AP1903 (Rimiducid) described above. Therefore, 0.4 mg/kg may be a safe and effective dose of AP1903 (Rimiducid) for this Phase I study in combination with the therapeutic cells.

[0124] The amino acid and/or nucleic acid sequence encoding ligand binding of the disclosure may contain sequence one or more modifications compared to a wild type amino acid or nucleic acid sequence. For example, the amino acid and/or nucleic acid sequence encoding ligand binding region of the disclosure may be a codon-optimized sequence. The one or more modifications may increase the binding affinity of a ligand (e.g. an induction agent) for the ligand binding region of the disclosure compared to a wild type polypeptide. Alternatively, or in addition, the one or more modifications may decrease the immunogenicity of the ligand binding region of the disclosure compared to a wild type polypeptide. Ligand binding regions of the disclosure and/or induction agents of the disclosure may be non-naturally occurring.

[0125] Inducible proapoptotic polypeptides of the disclosure comprise a ligand binding region, a linker and a proapoptotic peptide, wherein the inducible proapoptotic polypeptide does not comprise a non-human sequence. In certain embodiments, the non-human sequence comprises a restriction site. The linker may comprise any organic or inorganic material that permits, upon dimerization of the ligand binding region, interaction, cross-linking, cross-activation, or activation of the proapoptotic polypeptides such that the interaction or activation of the proapoptotic polypeptides initiates apoptosis in the cell. In certain embodiments, the linker is a polypeptide. In certain embodiments, the linker is a polypeptide comprising a G/S rich amino acid sequence (a "GS" linker). In certain embodiments, the linker is a polypeptide comprising the amino acid sequence GGGGS (SEQ ID NO: 5). In preferred embodiments, the linker is a polypeptide and the nucleic acid encoding the

polypeptide does not contain a restriction site for a restriction endonuclease. Linkers of the disclosure may be non-naturally occurring.

[0126] Inducible proapoptotic polypeptides of the disclosure may be expressed in a cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in that cell. The term "promoter" as used herein refers to a promoter that acts as the initial binding site for RNA polymerase to transcribe a gene. For example, inducible proapoptotic polypeptides of the disclosure may be expressed in a mammalian cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a mammalian cell, including, but not limited to native, endogenous, exogenous, and heterologous promoters. Preferred mammalian cells include human cells. Thus, inducible proapoptotic polypeptides of the disclosure may be expressed in a human cell under the transcriptional regulation of any promoter capable of initiating and/or regulating the expression of an inducible proapoptotic polypeptide of the disclosure in a human cell, including, but not limited to, a human promoter or a viral promoter. Exemplary promoters for expression in human cells include, but are not limited to, a human cytomegalovirus (CMV) immediate early gene promoter, a SV40 early promoter, a Rous sarcoma virus long terminal repeat, β-actin promoter, a rat insulin promoter and a glyceraldehyde-3-phosphate dehydrogenase promoter, each of which may be used to obtain high-level expression of an inducible proapoptotic polypeptide of the disclosure. The use of other viral or mammalian cellular or bacterial phage promoters which are well known in the art to achieve expression of an inducible proapoptotic polypeptide of the disclosure is contemplated as well, provided that the levels of expression are sufficient for initiating apoptosis in a cell. By employing a promoter with well-known properties, the level and pattern of expression of the protein of interest following transfection or transformation can be optimized.

[0127] Selection of a promoter that is regulated in response to specific physiologic or synthetic signals can permit inducible expression of the inducible proapoptotic polypeptide of the disclosure. The ecdysone system (Invitrogen, Carlsbad, Calif.) is one such system. This system is designed to allow regulated expression of a gene of interest in mammalian cells. It consists of a tightly regulated expression mechanism that allows virtually no basal level

expression of a transgene, but over 200-fold inducibility. The system is based on the heterodimeric ecdysone receptor of *Drosophila*, and when ecdysone or an analog such as muristerone A binds to the receptor, the receptor activates a promoter to turn on expression of the downstream transgene high levels of mRNA transcripts are attained. In this system, both monomers of the heterodimeric receptor are constitutively expressed from one vector, whereas the ecdysone-responsive promoter, which drives expression of the gene of interest, is on another plasmid. Engineering of this type of system into a vector of interest may therefore be useful. Another inducible system that may be useful is the Tet-Off™ or Tet-On™ system (Clontech, Palo Alto, Calif.) originally developed by Gossen and Bujard (Gossen and Bujard, Proc. Natl. Acad. Sci. USA, 89:5547-5551, 1992; Gossen et al., Science, 268:1766-1769, 1995). This system also allows high levels of gene expression to be regulated in response to tetracycline or tetracycline derivatives such as doxycycline. In the Tet-On™ system, gene expression is turned on in the presence of doxycycline, whereas in the Tet-Off™ system, gene expression is turned on in the absence of doxycycline. These systems are based on two regulatory elements derived from the tetracycline resistance operon of *E. coli*: the tetracycline operator sequence (to which the tetracycline repressor binds) and the tetracycline repressor protein. The gene of interest is cloned into a plasmid behind a promoter that has tetracycline-responsive elements present in it. A second plasmid contains a regulatory element called the tetracycline-controlled transactivator, which is composed, in the Tet-Off™ system, of the VP16 domain from the herpes simplex virus and the wild-type tetracycline repressor. Thus in the absence of doxycycline, transcription is constitutively on. In the Tet-On™ system, the tetracycline repressor is not wild type and in the presence of doxycycline activates transcription. For gene therapy vector production, the Tet-Off™ system may be used so that the producer cells could be grown in the presence of tetracycline or doxycycline and prevent expression of a potentially toxic transgene, but when the vector is introduced to the patient, the gene expression would be constitutively on.

[0128] In some circumstances, it is desirable to regulate expression of a transgene in a gene therapy vector. For example, different viral promoters with varying strengths of activity are utilized depending on the level of expression desired. In mammalian cells, the CMV immediate early promoter is often used to provide strong transcriptional activation. The CMV promoter is reviewed in Donnelly, J. J., et al., 1997. Annu. Rev. Immunol. 15:617-48.

Modified versions of the CMV promoter that are less potent have also been used when reduced levels of expression of the transgene are desired. When expression of a transgene in hematopoietic cells is desired, retroviral promoters such as the LTRs from MLV or MMTV are often used. Other viral promoters that are used depending on the desired effect include SV40, RSV LTR, HIV-1 and HIV-2 LTR, adenovirus promoters such as from the E1A, E2A, or MLP region, AAV LTR, HSV-TK, and avian sarcoma virus.

[0129] In other examples, promoters may be selected that are developmentally regulated and are active in particular differentiated cells. Thus, for example, a promoter may not be active in a pluripotent stem cell, but, for example, where the pluripotent stem cell differentiates into a more mature cell, the promoter may then be activated.

[0130] Similarly tissue specific promoters are used to effect transcription in specific tissues or cells so as to reduce potential toxicity or undesirable effects to non-targeted tissues. These promoters may result in reduced expression compared to a stronger promoter such as the CMV promoter, but may also result in more limited expression, and immunogenicity (Bojak, A., et al., 2002. Vaccine. 20:1975-79; Cazeaux., N., et al., 2002. Vaccine 20:3322-31). For example, tissue specific promoters such as the PSA associated promoter or prostate-specific glandular kallikrein, or the muscle creatine kinase gene may be used where appropriate.

[0131] Examples of tissue specific or differentiation specific promoters include, but are not limited to, the following: B29 (B cells); CD14 (monocytic cells); CD43 (leukocytes and platelets); CD45 (hematopoietic cells); CD68 (macrophages); desmin (muscle); elastase-1 (pancreatic acinar cells); endoglin (endothelial cells); fibronectin (differentiating cells, healing tissues); and Flt-1 (endothelial cells); GFAP (astrocytes).

[0132] In certain indications, it is desirable to activate transcription at specific times after administration of the gene therapy vector. This is done with such promoters as those that are hormone or cytokine regulatable. Cytokine and inflammatory protein responsive promoters that can be used include K and T kininogen (Kageyama et al., (1987) J. Biol. Chem., 262, 2345-2351), c-fos, TNF-alpha, C-reactive protein (Arcone, et al., (1988) Nucl. Acids Res., 16(8), 3195-3207), haptoglobin (Oliviero et al., (1987) EMBO J., 6, 1905-1912), serum amyloid A2, C/EBP alpha, IL-1, IL-6 (Poli and Cortese, (1989) Proc. Nat'l Acad. Sci. USA, 86, 8202-8206), Complement C3 (Wilson et al., (1990) Mol. Cell. Biol., 6181-6191), IL-8, alpha-1 acid glycoprotein (Prowse and Baumann, (1988) Mol Cell Biol, 8, 42-51), alpha-1

antitrypsin, lipoprotein lipase (Zechner et al., Mol. Cell. Biol., 2394-2401, 1988), angiotensinogen (Ron, et al., (1991) Mol. Cell. Biol., 2887-2895), fibrinogen, c-jun (inducible by phorbol esters, TNF-alpha, UV radiation, retinoic acid, and hydrogen peroxide), collagenase (induced by phorbol esters and retinoic acid), metallothionein (heavy metal and glucocorticoid inducible), Stromelysin (inducible by phorbol ester, interleukin-1 and EGF), alpha-2 macroglobulin and alpha-1 anti-chymotrypsin. Other promoters include, for example, SV40, MMTV, Human Immunodeficiency Virus (MV), Moloney virus, ALV, Epstein Barr virus, Rous Sarcoma virus, human actin, myosin, hemoglobin, and creatine.

[0133] It is envisioned that any of the above promoters alone or in combination with another can be useful depending on the action desired. Promoters, and other regulatory elements, are selected such that they are functional in the desired cells or tissue. In addition, this list of promoters should not be construed to be exhaustive or limiting; other promoters that are used in conjunction with the promoters and methods disclosed herein.

Nucleic Acid Molecules

[0134] Nucleic acid molecules of the disclosure, including sequences encoding an inducible polypeptide of the disclosure, can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

[0135] Isolated nucleic acid molecules of the disclosure can include nucleic acid molecules comprising an open reading frame (ORF), optionally, with one or more introns, e.g., but not limited to, at least one specified portion of at least one sequence encoding a an inducible polypeptide of the disclosure; nucleic acid molecules comprising the coding sequence for a an inducible polypeptide of the disclosure; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode an inducible polypeptide of the disclosure as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic

acid variants that code for an inducible polypeptide of the disclosure. See, e.g., Ausubel, et al., supra, and such nucleic acid variants are included in the disclosure.

[0136] As indicated herein, nucleic acid molecules of the disclosure which comprise a nucleic acid encoding an inducible polypeptide of the disclosure can include, but are not limited to, those encoding the amino acid sequence of an inducible polypeptide or fragment, by itself; the coding sequence for the entire an inducible polypeptide or a portion thereof; the coding sequence for an inducible polypeptide of the disclosure, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5′ and 3′ sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example, ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an inducible polypeptide of the disclosure can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused protein scaffold comprising a protein scaffold fragment or portion.

Construction of Nucleic Acids

[0137] The isolated nucleic acids of the disclosure can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, and/or (d) combinations thereof, as well-known in the art.

[0138] The nucleic acids can conveniently comprise sequences in addition to a sequence encoding an inducible polypeptide of the disclosure. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the sequence encoding an inducible polypeptide of the disclosure. Also, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide of the disclosure. For example, a hexa-histidine marker sequence provides a convenient means to purify the caspase proteins of the disclosure. The nucleic acid of the disclosure, excluding the coding sequence, is optionally a vector, adapter, or linker for cloning and/or expression of a sequence encoding an inducible polypeptide of the disclosure or an inducible polypeptide of the disclosure.

[0139] Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide sequence encoding an inducible polypeptide of the disclosure, or to improve the introduction of the polynucleotide sequence encoding an inducible polypeptide of the disclosure into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Recombinant Methods for Constructing Nucleic Acids

[0140] The isolated nucleic acid compositions of this disclosure, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, construction of cDNA and genomic libraries are well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra).

Nucleic Acid Screening and Isolation Methods

[0141] A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the disclosure or fragment thereof. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent, such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70-100%, or any range or value therein. However, it should be understood that minor

sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

[0142] Methods of amplification of RNA or DNA are well known in the art and can be used according to the disclosure without undue experimentation, based on the teaching and guidance presented herein.

[0143] Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; 4,795,699 and 4,921,794 to Tabor, et al; 5,142,033 to Innis; 5,122,464 to Wilson, et al.; 5,091,310 to Innis; 5,066,584 to Gyllensten, et al; 4,889,818 to Gelfand, et al; 4,994,370 to Silver, et al; 4,766,067 to Biswas; 4,656,134 to Ringold) and RNA mediated amplification that uses anti-sense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

[0144] For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the disclosure and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids

[0145] The isolated nucleic acids of the disclosure can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded

DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA may be limited to sequences of about 100, 500, and 1000 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

[0146] The disclosure further provides recombinant expression cassettes comprising a nucleic acid of the disclosure. A nucleic acid sequence of the disclosure, for example, a cDNA or a genomic sequence encoding a portion of an inducible polypeptide of the disclosure, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the disclosure operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the disclosure.

[0147] In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in the intron) of a non-heterologous form of a polynucleotide of the disclosure so as to up or down regulate expression of a polynucleotide of the disclosure. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors and Host Cells

[0148] The disclosure also relates to vectors that include a sequence encoding an inducible polypeptide of the disclosure, host cells that are genetically engineered with the recombinant vectors, and the production of at least one inducible polypeptide of the disclosure by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

[0149] Polynucleotides, including a sequence encoding an inducible polypeptide of the disclosure, can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

[0150] The disclosure provides a composition comprising the transposon the disclosure. In certain embodiments, the composition may further comprise a plasmid comprising a sequence encoding a transposase enzyme. The sequence encoding a transposase enzyme may be an mRNA sequence.

[0151] Transposons of the disclosure be episomally maintained or integrated into the genome of the recombinant/modified cell. The transposon may be part of a two component piggyBac system that utilizes a transposon and transposase for enhanced non-viral gene transfer. In certain embodiments of this method, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBac™ or a Super piggyBac™ (SPB) transposase.

[0152] Transposons of the disclosure may comprise piggyBac transposons. In certain embodiments of the methods of the disclosure, the transposon is a plasmid DNA transposon with a sequence encoding the inducible caspase polypeptide of the disclosure flanked by two cis-regulatory insulator elements. In certain embodiments, the transposon is a piggyBac transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a piggyBac transposon, the transposase is a piggyBac™ or a Super piggyBac™ (SPB) transposase. In certain embodiments, and, in particular, those embodiments wherein the transposase is a Super piggyBac™ (SPB) transposase, the sequence encoding the transposase is an mRNA sequence.

[0153] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme. The piggyBac (PB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEI SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG
 61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG
121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTGATFRD TNEDEIYAFF
181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV
241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RMYIPNKPSK YGIKILMMCD
301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ
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361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC

421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN

481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPNEV

541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO: 1).

[0154] In certain embodiments of the methods of the disclosure, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at one or more of positions 30, 165, 282, or 538 of the sequence:

1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEI SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG

61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG

121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTGATFRD TNEDEIYAFF

181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV

241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RMYIPNKPSK YGIKILMMCD

301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ

361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC

421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN

481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPNEV

541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO: 1).

[0155] In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at two or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at three or more of positions 30, 165, 282, or 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the transposase enzyme is a piggyBac™ (PB) transposase enzyme that comprises or consists of an amino acid sequence having an amino acid substitution at each of the following positions 30, 165, 282, and 538 of the sequence of SEQ ID NO: 1. In certain embodiments, the amino acid substitution at position 30 of the sequence of SEQ ID NO: 1 is a substitution of a valine (V) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 165 of the sequence of SEQ ID NO: 1 is

a substitution of a serine (S) for a glycine (G). In certain embodiments, the amino acid substitution at position 282 of the sequence of SEQ ID NO: 1 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 538 of the sequence of SEQ ID NO: 1 is a substitution of a lysine (K) for an asparagine (N).

[0156] In certain embodiments of the methods of the disclosure, the transposase enzyme is a Super piggyBac™ (SPB) transposase enzyme. In certain embodiments, the Super piggyBac™ (SPB) transposase enzymes of the disclosure may comprise or consist of the amino acid sequence of the sequence of SEQ ID NO: 1 wherein the amino acid substitution at position 30 is a substitution of a valine (V) for an isoleucine (I), the amino acid substitution at position 165 is a substitution of a serine (S) for a glycine (G), the amino acid substitution at position 282 is a substitution of a valine (V) for a methionine (M), and the amino acid substitution at position 538 is a substitution of a lysine (K) for an asparagine (N). In certain embodiments, the Super piggyBac™ (SPB) transposase enzyme may comprise or consist of an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGSSLDDEHI LSALLQSDDE LVGEDSDSEV SDHVSEDDVQ SDTEEAFIDE VHEVQPTSSG
 61 SEILDEQNVI EQPGSSLASN RILTLPQRTI RGKNKHCWST SKSTRRSRVS ALNIVRSQRG
121 PTRMCRNIYD PLLCFKLFFT DEIISEIVKW TNAEISLKRR ESMTSATFRD TNEDEIYAFF
181 GILVMTAVRK DNHMSTDDLF DRSLSMVYVS VMSRDRFDFL IRCLRMDDKS IRPTLRENDV
241 FTPVRKIWDL FIHQCIQNYT PGAHLTIDEQ LLGFRGRCPF RVYIPNKPSK YGIKILMMCD
301 SGTKYMINGM PYLGRGTQTN GVPLGEYYVK ELSKPVHGSC RNITCDNWFT SIPLAKNLLQ
361 EPYKLTIVGT VRSNKREIPE VLKNSRSRPV GTSMFCFDGP LTLVSYKPKP AKMVYLLSSC
421 DEDASINEST GKPQMVMYYN QTKGGVDTLD QMCSVMTCSR KTNRWPMALL YGMINIACIN
481 SFIIYSHNVS SKGEKVQSRK KFMRNLYMSL TSSFMRKRLE APTLKRYLRD NISNILPKEV
541 PGTSDDSTEE PVMKKRTYCT YCPSKIRRKA NASCKKCKKV ICREHNIDMC QSCF (SEQ ID NO: 2).
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[0157] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 3, 46, 82, 103, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 258, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 486, 503, 552, 570 and 591 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein

the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ or Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of positions 46, 119, 125, 177, 180, 185, 187, 200, 207, 209, 226, 235, 240, 241, 243, 296, 298, 311, 315, 319, 327, 328, 340, 421, 436, 456, 470, 485, 503, 552 and 570. In certain embodiments, the amino acid substitution at position 3 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an asparagine (N) for a serine (S). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an alanine (A). In certain embodiments, the amino acid substitution at position 46 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 82 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for an isoleucine (I). In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 119 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for an arginine (R). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) a cysteine (C). In certain embodiments, the amino acid substitution at position 125 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 177 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a histidine (H) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 180 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 185 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 187 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for an alanine (A). In certain embodiments, the amino acid substitution at position 200 of SEQ ID

NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a phenylalanine (F).In certain embodiments, the amino acid substitution at position 207 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a valine (V). In certain embodiments, the amino acid substitution at position 209 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a valine (V). In certain embodiments, the amino acid substitution at position 226 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a methionine (M). In certain embodiments, the amino acid substitution at position 235 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a leucine (L). In certain embodiments, the amino acid substitution at position 240 of SEQ ID NO: 1 or SEQ ID NO: 1 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 241 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a phenylalanine (F). In certain embodiments, the amino acid substitution at position 243 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a proline (P). In certain embodiments, the amino acid substitution at position 258 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tryptophan (W) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a leucine (L). In certain embodiments, the amino acid substitution at position 296 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for a methionine (M). In certain embodiments, the amino acid substitution at position 298 of SEQ ID NO: 1or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a proline (P). In certain embodiments, the amino acid substitution at position 311 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine for a proline (P). In certain embodiments, the amino acid substitution at position 315 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for an arginine (R).In certain embodiments, the amino acid substitution at position 319 of

SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for a threonine (T). In certain embodiments, the amino acid substitution at position 327 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 328 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a tyrosine (Y). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for a cysteine (C). In certain embodiments, the amino acid substitution at position 340 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a cysteine (C). In certain embodiments, the amino acid substitution at position 421 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a histidine (H) for the aspartic acid (D). In certain embodiments, the amino acid substitution at position 436 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a valine (V). In certain embodiments, the amino acid substitution at position 456 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a tyrosine (Y) for a methionine (M). In certain embodiments, the amino acid substitution at position 470 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a phenylalanine (F) for a leucine (L). In certain embodiments, the amino acid substitution at position 485 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a serine (S). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a leucine (L) for a methionine (M). In certain embodiments, the amino acid substitution at position 503 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an isoleucine (I) for a methionine (M). In certain embodiments, the amino acid substitution at position 552 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a lysine (K) for a valine (V). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a threonine (T) for an alanine (A). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a glutamine (Q). In certain embodiments, the amino acid substitution at position 591 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an arginine (R) for a glutamine (Q).

[093] In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at one or more of

positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments of the methods of the disclosure, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at two, three, four, five, six or more of positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, including those embodiments wherein the transposase comprises the above-described mutations at positions 30, 165, 282 and/or 538, the piggyBac™ transposase enzyme may comprise or the Super piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 103, 194, 372, 375, 450, 509 and 570 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, the amino acid substitution at position 103 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a proline (P) for a serine (S). In certain embodiments, the amino acid substitution at position 194 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a valine (V) for a methionine (M). In certain embodiments, the amino acid substitution at position 372 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for an arginine (R). In certain embodiments, the amino acid substitution at position 375 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an alanine (A) for a lysine (K). In certain embodiments, the amino acid substitution at position 450 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of an asparagine (N) for an aspartic acid (D). In certain embodiments, the amino acid substitution at position 509 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a glycine (G) for a serine (S). In certain embodiments, the amino acid substitution at position 570 of SEQ ID NO: 1 or SEQ ID NO: 2 is a substitution of a serine (S) for an asparagine (N). In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1. In certain embodiments, including those embodiments wherein the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, the piggyBac™ transposase enzyme may further comprise an amino acid substitution at positions 372, 375 and 450 of the sequence of SEQ ID NO: 1 or SEQ ID NO: 2. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, a substitution of an alanine (A)

for an arginine (R) at position 372 of SEQ ID NO: 1, and a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 1. In certain embodiments, the piggyBac™ transposase enzyme may comprise a substitution of a valine (V) for a methionine (M) at position 194 of SEQ ID NO: 1, a substitution of an alanine (A) for an arginine (R) at position 372 of SEQ ID NO: 1, a substitution of an alanine (A) for a lysine (K) at position 375 of SEQ ID NO: 1 and a substitution of an asparagine (N) for an aspartic acid (D) at position 450 of SEQ ID NO: 1.

[0158] In certain embodiments of the methods of the disclosure, the transposon is a Sleeping Beauty transposon. In certain embodiments, and, in particular, those embodiments wherein the transposon is a Sleeping Beauty transposon, the transposase is a Sleeping Beauty transposase or a hyperactive Sleeping Beauty transposase (SB100X).

[0159] In certain embodiments of the methods of the disclosure, the Sleeping Beauty transposase enzyme comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGKSKEISQD LRKKIVDLHK SGSSLGAISK RLKVPRSSVQ TIVRKYKHHG TTQPSYRSGR
 61 RRVLSPRDER TLVRKVQINP RTTAKDLVKM LEETGTKVSI STVKRVLYRH NLKGRSARKK
121 PLLQNRHKKA RLRFATAHGD KDRTFWRNVL WSDETKIELF GHNDHRYVWR KKGEACKPKN
181 TIPTVKHGGG SIMLWGCFAA GGTGALHKID GIMRKENYVD ILKQHLKTSV RKLKLGRKWV
241 FQMDNDPKHT SKVVAKWLKD NKVKVLEWPS QSPDLNPIEN LWAELKKRVR ARRPTNLTQL
301 HQLCQEEWAK IHPTYCGKLV EGYPKRLTQV KQFKGNATKY (SEQ ID NO: 19).
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[0160] In certain embodiments of the methods of the disclosure, the hyperactive Sleeping Beauty (SB100X) transposase enzyme comprises an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 99% or any percentage in between identical to:

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  1 MGKSKEISQD LRKRIVDLHK SGSSLGAISK RLAVPRSSVQ TIVRKYKHHG TTQPSYRSGR
 61 RRVLSPRDER TLVRKVQINP RTTAKDLVKM LEETGTKVSI STVKRVLYRH NLKGHSARKK
121 PLLQNRHKKA RLRFATAHGD KDRTFWRNVL WSDETKIELF GHNDHRYVWR KKGEACKPKN
181 TIPTVKHGGG SIMLWGCFAA GGTGALHKID GIMDAVQYVD ILKQHLKTSV RKLKLGRKWV
241 FQHDNDPKHT SKVVAKWLKD NKVKVLEWPS QSPDLNPIEN LWAELKKRVR ARRPTNLTQL
301 HQLCQEEWAK IHPNYCGKLV EGYPKRLTQV KQFKGNATKY (SEQ ID NO: 20).
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[0161] In certain embodiments of the methods of the disclosure, the transposase is a Helitron transposase. Helitron transposases mobilize the Helraiser transposon, an ancient element from the bat genome that was active about 30 to 36 million years ago. An exemplary Helraiser transposon of the disclosure includes Helibat1, which comprises a nucleic acid sequence comprising:

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   1 TCCTATATAA TAAAAGAGAA ACATGCAAAT TGACCATCCC TCCGCTACGC TCAAGCCACG
  61 CCCACCAGCC AATCAGAAGT GACTATGCAA ATTAACCCAA CAAAGATGGC AGTTAAATTT
 121 GCATACGCAG GTGTCAAGCG CCCCAGGAGG CAACGGCGGC CGCGGGCTCC CAGGACCTTC
 181 GCTGGCCCCG GGAGGCGAGG CCGGCCGCGC CTAGCCACAC CCGCGGGCTC CCGGGACCTT
 241 CGCCAGCAGA GAGCAGAGCG GGAGAGCGGG CGGAGAGCGG GAGGTTTGGA GGACTTGGCA
 301 GAGCAGGAGG CCGCTGGACA TAGAGCAGAG CGAGAGAGAG GGTGGCTTGG AGGGCGTGGC
 361 TCCCTCTGTC ACCCCAGCTT CCTCATCACA GCTGTGGAAA CTGACAGCAG GGAGGAGGAA
 421 GTCCCACCCC CACAGAATCA GCCAGAATCA GCCGTTGGTC AGACAGCTCT CAGCGGCCTG
 481 ACAGCCAGGA CTCTCATTCA CCTGCATCTC AGACCGTGAC AGTAGAGAGG TGGGACTATG
 541 TCTAAAGAAC AACTGTTGAT ACAACGTAGC TCTGCAGCCG AAAGATGCCG GCGTTATCGA
 601 CAGAAAATGT CTGCAGAGCA ACGTGCGTCT GATCTTGAAA GAAGGCGGCG CCTGCAACAG
 661 AATGTATCTG AAGAGCAGCT ACTGGAAAAA CGTCGCTCTG AAGCCGAAAA ACAGCGGCGT
 721 CATCGACAGA AAATGTCTAA AGACCAACGT GCCTTTGAAG TTGAAAGAAG GCGGTGGCGA
 781 CGACAGAATA TGTCTAGAGA ACAGTCATCA ACAAGTACTA CCAATACCGG TAGGAACTGC
 841 CTTCTCAGCA AAAATGGAGT ACATGAGGAT GCAATTCTCG AACATAGTTG TGGTGGAATG
 901 ACTGTTCGAT GTGAATTTTG CCTATCACTA AATTTCTCTG ATGAAAAACC ATCCGATGGG
 961 AAATTTACTC GATGTTGTAG CAAAGGGAAA GTCTGTCCAA ATGATATACA TTTTCCAGAT
1021 TACCCGGCAT ATTTAAAAAG ATTAATGACA AACGAAGATT CTGACAGTAA AAATTTCATG
1081 GAAAATATTC GTTCCATAAA TAGTTCTTTT GCTTTTGCTT CCATGGGTGC AAATATTGCA
1141 TCGCCATCAG GATATGGGCC ATACTGTTTT AGAATACACG GACAAGTTTA TCACCGTACT
1201 GGAACTTTAC ATCCTTCGGA TGGTGTTTCT CGGAAGTTTG CTCAACTCTA TATTTTGGAT
1261 ACAGCCGAAG CTACAAGTAA AAGATTAGCA ATGCCAGAAA ACCAGGGCTG CTCAGAAAGA
1321 CTCATGATCA ACATCAACAA CCTCATGCAT GAAATAAATG AATTAACAAA ATCGTACAAG
1381 ATGCTACATG AGGTAGAAAA GGAAGCCCAA TCTGAAGCAG CAGCAAAAGG TATTGCTCCC
1441 ACAGAAGTAA CAATGGCGAT TAAATACGAT CGTAACAGTG ACCCAGGTAG ATATAATTCT
1501 CCCCGTGTAA CCGAGGTTGC TGTCATATTC AGAAACGAAG ATGGAGAACC TCCTTTTGAA
1561 AGGGACTTGC TCATTCATTG TAAACCAGAT CCCAATAATC CAAATGCCAC TAAAATGAAA
1621 CAAATCAGTA TCCTGTTTCC TACATTAGAT GCAATGACAT ATCCTATTCT TTTTCCACAT
1681 GGTGAAAAAG GCTGGGGAAC AGATATTGCA TTAAGACTCA GAGACAACAG TGTAATCGAC
1741 AATAATACTA GACAAAATGT AAGGACACGA GTCACACAAA TGCAGTATTA TGGATTTCAT
1801 CTCTCTGTGC GGGACACGTT CAATCCTATT TTAAATGCAG GAAAATTAAC TCAACAGTTT
1861 ATTGTGGATT CATATTCAAA AATGGAGGCC AATCGGATAA ATTTCATCAA AGCAAACCAA
1921 TCTAAGTTGA GAGTTGAAAA ATATAGTGGT TTGATGGATT ATCTCAAATC TAGATCTGAA
1981 AATGACAATG TGCCGATTGG TAAAATGATA ATACTTCCAT CATCTTTTGA GGGTAGTCCC
2041 AGAAATATGC AGCAGCGATA TCAGGATGCT ATGGCAATTG TAACGAAGTA TGGCAAGCCC
2101 GATTTATTCA TAACCATGAC ATGCAACCCC AAATGGGCAG ATATTACAAA CAATTTACAA
2161 CGCTGGCAAA AAGTTGAAAA CAGACCTGAC TTGGTAGCCA GAGTTTTTAA TATTAAGCTG
2221 AATGCTCTTT TAAATGATAT ATGTAAATTC CATTTATTTG GCAAAGTAAT AGCTAAAATT
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2281 CATGTCATTG AATTTCAGAA ACGCGGACTG CCTCACGCTC ACATATTATT GATATTAGAT
2341 AGTGAGTCCA AATTACGTTC AGAAGATGAC ATTGACCGTA TAGTTAAGGC AGAAATTCCA
2401 GATGAAGACC AGTGTCCTCG ACTTTTTCAA ATTGTAAAAT CAAATATGGT ACATGGACCA
2461 TGTGGAATAC AAAATCCAAA TAGTCCATGT ATGGAAAATG GAAAATGTTC AAAGGGATAT
2521 CCAAAAGAAT TTCAAAATGC GACCATTGGA AATATTGATG GATATCCCAA ATACAAACGA
2581 AGATCTGGTA GCACCATGTC TATTGGAAAT AAAGTTGTCG ATAACACTTG GATTGTCCCT
2641 TATAACCCGT ATTTGTGCCT TAAATATAAC TGTCATATAA ATGTTGAAGT CTGTGCATCA
2701 ATTAAAAGTG TCAAATATTT ATTTAAATAC ATCTATAAAG GGCACGATTG TGCAAATATT
2761 CAAATTTCTG AAAAAAATAT TATCAATCAT GACGAAGTAC AGGACTTCAT TGACTCCAGG
2821 TATGTGAGCG CTCCTGAGGC TGTTTGGAGA CTTTTTGCAA TGCGAATGCA TGACCAATCT
2881 CATGCAATCA CAAGATTAGC TATTCATTTG CCAAATGATC AGAATTTGTA TTTTCATACC
2941 GATGATTTTG CTGAAGTTTT AGATAGGGCT AAAAGGCATA ACTCGACTTT GATGGCTTGG
3001 TTCTTATTGA ATAGAGAAGA TTCTGATGCA CGTAATTATT ATTATTGGGA GATTCCACAG
3061 CATTATGTGT TTAATAATTC TTTGTGGACA AAACGCCGAA AGGGTGGGAA TAAAGTATTA
3121 GGTAGACTGT TCACTGTGAG CTTTAGAGAA CCAGAACGAT ATTACCTTAG ACTTTTGCTT
3181 CTGCATGTAA AAGGTGCGAT AAGTTTTGAG GATCTGCGAA CTGTAGGAGG TGTAACTTAT
3241 GATACATTTC ATGAAGCTGC TAAACACCGA GGATTATTAC TTGATGACAC TATCTGGAAA
3301 GATACGATTG ACGATGCAAT CATCCTTAAT ATGCCCAAAC AACTACGGCA ACTTTTTGCA
3361 TATATATGTG TGTTTGGATG TCCTTCTGCT GCAGACAAAT TATGGGATGA GAATAAATCT
3421 CATTTTATTG AAGATTTCTG TTGGAAATTA CACCGAAGAG AAGGTGCCTG TGTGAACTGT
3481 GAAATGCATG CCCTTAACGA AATTCAGGAG GTATTCACAT TGCATGGAAT GAAATGTTCA
3541 CATTTCAAAC TTCCGGACTA TCCTTTATTA ATGAATGCAA ATACATGTGA TCAATTGTAC
3601 GAGCAACAAC AGGCAGAGGT TTTGATAAAT TCTCTGAATG ATGAACAGTT GGCAGCCTTT
3661 CAGACTATAA CTTCAGCCAT CGAAGATCAA ACTGTACACC CCAAATGCTT TTTCTTGGAT
3721 GGTCCAGGTG GTAGTGGAAA AACATATCTG TATAAAGTTT TAACACATTA TATTAGAGGT
3781 CGTGGTGGTA CTGTTTTACC CACAGCATCT ACAGGAATTG CTGCAAATTT ACTTCTTGGT
3841 GGAAGAACCT TTCATTCCCA ATATAAATTA CCAATTCCAT TAAATGAAAC TTCAATTTCT
3901 AGACTCGATA TAAAGAGTGA AGTTGCTAAA ACCATTAAAA AGGCCCAACT TCTCATTATT
3961 GATGAATGCA CCATGGCATC CAGTCATGCT ATAAACGCCA TAGATAGATT ACTAAGAGAA
4021 ATTATGAATT TGAATGTTGC ATTTGGTGGG AAAGTTCTCC TTCTCGGAGG GGATTTTCGA
4081 CAATGTCTCA GTATTGTACC ACATGCTATG CGATCGGCCA TAGTACAAAC GAGTTTAAAG
4141 TACTGTAATG TTTGGGGATG TTTCAGAAAG TTGTCTCTTA AAACAAATAT GAGATCAGAG
4201 GATTCTGCTT ATAGTGAATG GTTAGTAAAA CTTGGAGATG GCAAACTTGA TAGCAGTTTT
4261 CATTTAGGAA TGGATATTAT TGAAATCCCC CATGAAATGA TTTGTAACGG ATCTATTATT
4321 GAAGCTACCT TTGGAAATAG TATATCTATA GATAATATTA AAAATATATC TAAACGTGCA
4381 ATTCTTTGTC CAAAAAATGA GCATGTTCAA AAATTAAATG AAGAAATTTT GGATATACTT
4441 GATGGAGATT TTCACACATA TTTGAGTGAT GATTCCATTG ATTCAACAGA TGATGCTGAA
4501 AAGGAAAATT TTCCCATCGA ATTTCTTAAT AGTATTACTC CTTCGGGAAT GCCGTGTCAT

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4561 AAATTAAAAT TGAAAGTGGG TGCAATCATC ATGCTATTGA GAAATCTTAA TAGTAAATGG
4621 GGTCTTTGTA ATGGTACTAG ATTTATTATC AAAAGATTAC GACCTAACAT TATCGAAGCT
4681 GAAGTATTAA CAGGATCTGC AGAGGGAGAG GTTGTTCTGA TTCCAAGAAT TGATTTGTCC
4741 CCATCTGACA CTGGCCTCCC ATTTAAATTA ATTCGAAGAC AGTTTCCCGT GATGCCAGCA
4801 TTTGCGATGA CTATTAATAA ATCACAAGGA CAAACTCTAG ACAGAGTAGG AATATTCCTA
4861 CCTGAACCCG TTTTCGCACA TGGTCAGTTA TATGTTGCTT TCTCTCGAGT TCGAAGAGCA
4921 TGTGACGTTA AAGTTAAAGT TGTAAATACT TCATCACAAG GGAAATTAGT CAAGCACTCT
4981 GAAAGTGTTT TTACTCTTAA TGTGGTATAC AGGGAGATAT TAGAATAAGT TTAATCACTT
5041 TATCAGTCAT TGTTTGCATC AATGTTGTTT TTATATCATG TTTTTGTTGT TTTTATATCA
5101 TGTCTTTGTT GTTGTTATAT CATGTTGTTA TTGTTTATTT ATTAATAAAT TTATGTATTA
5161 TTTTCATATA CATTTTACTC ATTTCCTTTC ATCTCTCACA CTTCTATTAT AGAGAAAGGG
5221 CAAATAGCAA TATTAAAATA TTTCCTCTAA TTAATTCCCT TTCAATGTGC ACGAATTTCG
5281 TGCACCGGGC CACTAG (SEQ ID NO: 21).
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[0162] Unlike other transposases, the Helitron transposase does not contain an RNase-H like catalytic domain, but instead comprises a RepHel motif made up of a replication initiator domain (Rep) and a DNA helicase domain. The Rep domain is a nuclease domain of the HUH superfamily of nucleases.

[0163] An exemplary Helitron transposase of the disclosure comprises an amino acid sequence comprising:

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   1 MSKEQLLIQR SSAAERCRRY RQKMSAEQRA SDLERRRRLQ QNVSEEQLLE KRRSEAEKQR
  61 RHRQKMSKDQ RAFEVERRRW RRQNMSREQS STSTTNTGRN CLLSKNGVHE DAILEHSCGG
 121 MTVRCEFCLS LNFSDEKPSD GKFTRCCSKG KVCPNDIHFP DYPAYLKRLM TNEDSDSKNF
 181 MENIRSINSS FAFASMGANI ASPSGYGPYC FRIHGQVYHR TGTLHPSDGV SRKFAQLYIL
 241 DTAEATSKRL AMPENQGCSE RLMININNLM HEINELTKSY KMLHEVEKEA QSEAAAKGIA
 301 PTEVTMAIKY DRNSDPGRYN SPRVTEVAVI FRNEDGEPPF ERDLLIHCKP DPNNPNATKM
 361 KQISILFPTL DAMTYPILFP HGEKGWGTDI ALRLRDNSVI DNNTRQNVRT RVTQMQYYGF
 421 HLSVRDTFNP ILNAGKLTQQ FIVDSYSKME ANRINFIKAN QSKLRVEKYS GLMDYLKSRS
 481 ENDNVPIGKM IILPSSFEGS PRNMQQRYQD AMAIVTKYGK PDLFITMTCN PKWADITNNL
 541 QRWQKVENRP DLVARVFNIK LNALLNDICK FHLFGKVIAK IHVIEFQKRG LPHAHILLIL
 601 DSESKLRSED DIDRIVKAEI PDEDQCPRLF QIVKSNMVHG PCGIQNPNSP CMENGKCSKG
 661 YPKEFQNATI GNIDGYPKYK RRSGSTMSIG NKVVDNTWIV PYNPYLCLKY NCHINVEVCA
 721 SIKSVKYLFK YIYKGHDCAN IQISEKNIIN HDEVQDFIDS RYVSAPEAVW RLFAMRMHDQ
 781 SHAITRLAIH LPNDQNLYFH TDDFAEVLDR AKRHNSTLMA WFLLNREDSD ARNYYYWEIP
 841 QHYVFNNSLW TKRRKGGNKV LGRLFTVSFR EPERYYLRLL LLHVKGAISF EDLRTVGGVT
 901 YDTFHEAAKH RGLLLDDTIW KDTIDDAIIL NMPKQLRQLF AYICVFGCPS AADKLWDENK
 961 SHFIEDFCWK LHRREGACVN CEMHALNEIQ EVFTLHGMKC SHFKLPDYPL LMNANTCDQL
1021 YEQQQAEVLI NSLNDEQLAA FQTITSAIED QTVHPKCFFL DGPGGSGKTY LYKVLTHYIR
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1081 GRGGTVLPTA STGIAANLLL GGRTFHSQYK LPIPLNETSI SRLDIKSEVA KTIKKAQLLI
1141 IDECTMASSH AINAIDRLLR EIMNLNVAFG GKVLLLGGDF RQCLSIVPHA MRSAIVQTSL
1201 KYCNVWGCFR KLSLKTNMRS EDSAYSEWLV KLGDGKLDSS FHLGMDIIEI PHEMICNGSI
1261 IEATFGNSIS IDNIKNISKR AILCPKNEHV QKLNEEILDI LDGDFHTYLS DDSIDSTDDA
1321 EKENFPIEFL NSITPSGMPC HKLKLKVGAI IMLLRNLNSK WGLCNGTRFI IKRLRPNIIE
1381 AEVLTGSAEG EVVLIPRIDL SPSDTGLPFK LIRRQFPVMP AFAMTINKSQ GQTLDRVGIF
1441 LPEPVFAHGQ LYVAFSRVRR ACDVKVKVVN TSSQGKLVKH SESVFTLNVV YREILE (SEQ ID NO: 22).

[0164] In Helitron transpositions, a hairpin close to the 3' end of the transposon functions as a terminator. However, this hairpin can be bypassed by the transposase, resulting in the transduction of flanking sequences. In addition, Helraiser transposition generates covalently closed circular intermediates. Furthermore, Helitron transpositions can lack target site duplications. In the Helraiser sequence, the transposase is flanked by left and right terminal sequences termed LTS and RTS. These sequences terminate with a conserved 5'-TC/CTAG-3' motif. A 19 bp palindromic sequence with the potential to form the hairpin termination structure is located 11 nucleotides upstream of the RTS and consists of the sequence GTGCACGAATTTCGTGCACCGGGCCACTAG (SEQ ID NO: 23).

[0165] In certain embodiments of the methods of the disclosure, the transposase is a Tol2 transposase. Tol2 transposons may be isolated or derived from the genome of the medaka fish, and may be similar to transposons of the hAT family. Exemplary Tol2 transposons of the disclosure are encoded by a sequence comprising about 4.7 kilobases and contain a gene encoding the Tol2 transposase, which contains four exons. An exemplary Tol2 transposase of the disclosure comprises an amino acid sequence comprising the following:

1 MEEVCDSSAA ASSTVQNQPQ DQEHPWPYLR EFFSLSGVNK DSFKMKCVLC LPLNKEISAF
61 KSSPSNLRKH IERMHPNYLK NYSKLTAQKR KIGTSTHASS SKQLKVDSVF PVKHVSPVTV
121 NKAILRYIIQ GLHPFSTVDL PSFKELISTL QPGISVITRP TLRSKIAEAA LIMKQKVTAA
181 MSEVEWIATT TDCWTARRKS FIGVTAHWIN PGSLERHSAA LACKRLMGSH TFEVLASAMN
241 DIHSEYEIRD KVVCTTTDSG SNFMKAFRVF GVENNDIETE ARRCESDDTD SEGCGEGSDG
301 VEFQDASRVL DQDDGFEFQL PKHQKCACHL LNLVSSVDAQ KALSNEHYKK LYRSVFGKCQ
361 ALWNKSSRSA LAAEAVESES RLQLLRPNQT RWNSTFMAVD RILQICKEAG EGALRNICTS
421 LEVPMFNPAE MLFLTEWANT MRPVAKVLDI LQAETNTQLG WLLPSVHQLS LKLQRLHHSL
481 RYCDPLVDAL QQGIQTRFKH MFEDPEIIAA AILLPKFRTS WTNDETIIKR GMDYIRVHLE
541 PLDHKKELAN SSSDDEDFFA SLKPTTHEAS KELDGYLACV SDTRESLLTF PAICSLSIKT
601 NTPLPASAAC ERLFSTAGLL FSPKRARLDT NNFENQLLLK LNLRFYNFE (SEQ ID NO: 24).

[0166] An exemplary Tol2 transposon of the disclosure, including inverted repeats, subterminal sequences and the Tol2 transposase, is encoded by a nucleic acid sequence comprising the following:

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   1 CAGAGGTGTA AAGTACTTGA GTAATTTTAC TTGATTACTG TACTTAAGTA TTATTTTTGG
  61 GGATTTTTAC TTTACTTGAG TACAATTAAA AATCAATACT TTTACTTTTA CTTAATTACA
 121 TTTTTTTAGA AAAAAAAGTA CTTTTTACTC CTTACAATTT TATTTACAGT CAAAAAGTAC
 181 TTATTTTTTG GAGATCACTT CATTCTATTT TCCCTTGCTA TTACCAAACC AATTGAATTG
 241 CGCTGATGCC CAGTTTAATT TAAATGTTAT TTATTCTGCC TATGAAAATC GTTTTCACAT
 301 TATATGAAAT TGGTCAGACA TGTTCATTGG TCCTTTGGAA GTGACGTCAT GTCACATCTA
 361 TTACCACAAT GCACAGCACC TTGACCTGGA AATTAGGGAA ATTATAACAG TCAATCAGTG
 421 GAAGAAAATG GAGGAAGTAT GTGATTCATC AGCAGCTGCG AGCAGCACAG TCCAAAATCA
 481 GCCACAGGAT CAAGAGCACC CGTGGCCGTA TCTTCGCGAA TTCTTTTCTT TAAGTGGTGT
 541 AAATAAAGAT TCATTCAAGA TGAAATGTGT CCTCTGTCTC CCGCTTAATA AAGAAATATC
 601 GGCCTTCAAA AGTTCGCCAT CAAACCTAAG GAAGCATATT GAGGTAAGTA CATTAAGTAT
 661 TTTGTTTTAC TGATAGTTTT TTTTTTTTTT TTTTTTTTTT TTTTTGGGTG TGCATGTTTT
 721 GACGTTGATG GCGCGCCTTT TATATGTGTA GTAGGCCTAT TTTCACTAAT GCATGCGATT
 781 GACAATATAA GGCTCACGTA ATAAAATGCT AAAATGCATT TGTAATTGGT AACGTTAGGT
 841 CCACGGGAAA TTTGGCGCCT ATTGCAGCTT TGAATAATCA TTATCATTCC GTGCTCTCAT
 901 TGTGTTTGAA TTCATGCAAA ACACAAGAAA ACCAAGCGAG AAATTTTTTT CCAAACATGT
 961 TGTATTGTCA AAACGGTAAC ACTTTACAAT GAGGTTGATT AGTTCATGTA TTAACTAACA
1021 TTAAATAACC ATGAGCAATA CATTTGTTAC TGTATCTGTT AATCTTTGTT AACGTTAGTT
1081 AATAGAAATA CAGATGTTCA TTGTTTGTTC ATGTTAGTTC ACAGTGCATT AACTAATGTT
1141 AACAAGATAT AAAGTATTAG TAAATGTTGA AATTAACATG TATACGTGCA GTTCATTATT
1201 AGTTCATGTT AACTAATGTA GTTAACTAAC GAACCTTATT GTAAAAGTGT TACCATCAAA
1261 ACTAATGTAA TGAAATCAAT TCACCCTGTC ATGTCAGCCT TACAGTCCTG TGTTTTTGTC
1321 AATATAATCA GAAATAAAAT TAATGTTTGA TTGTCACTAA ATGCTACTGT ATTTCTAAAA
1381 TCAACAAGTA TTTAACATTA TAAAGTGTGC AATTGGCTGC AAATGTCAGT TTTATTAAAG
1441 GGTTAGTTCA CCCAAAAATG AAAATAATGT CATTAATGAC TCGCCCTCAT GTCGTTCCAA
1501 GCCCGTAAGA CCTCCGTTCA TCTTCAGAAC ACAGTTTAAG ATATTTTAGA TTTAGTCCGA
1561 GAGCTTTCTG TGCCTCCATT GAGAATGTAT GTACGGTATA CTGTCCATGT CCAGAAAGGT
1621 AATAAAAACA TCAAAGTAGT CCATGTGACA TCAGTGGGTT AGTTAGAATT TTTTGAAGCA
1681 TCGAATACAT TTTGGTCCAA AAATAACAAA ACCTACGACT TTATTCGGCA TTGTATTCTC
1741 TTCCGGGTCT GTTGTCAATC CGCGTTCACG ACTTCGCAGT GACGCTACAA TGCTGAATAA
1801 AGTCGTAGGT TTTGTTATTT TTGGACCAAA ATGTATTTTC GATGCTTCAA ATAATTCTAC
1861 CTAACCCACT GATGTCACAT GGACTACTTT GATGTTTTTA TTACCTTTCT GGACATGGAC
1921 AGTATACCGT ACATACATTT TCAGTGGAGG GACAGAAAGC TCTCGGACTA AATCTAAAAT
1981 ATCTTAAACT GTGTTCCGAA GATGAACGGA GGTGTTACGG GCTTGGAACG ACATGAGGGT
2041 GAGTCATTAA TGACATCTTT TCATTTTTGG GTGAACTAAC CCTTTAATGC TGTAATCAGA
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2101 GAGTGTATGT GTAATTGTTA CATTTATTGC ATACAATATA AATATTTATT TGTTGTTTTT
2161 ACAGAGAATG CACCCAAATT ACCTCAAAAA CTACTCTAAA TTGACAGCAC AGAAGAGAAA
2221 GATCGGGACC TCCACCCATG CTTCCAGCAG TAAGCAACTG AAAGTTGACT CAGTTTTCCC
2281 AGTCAAACAT GTGTCTCCAG TCACTGTGAA CAAAGCTATA TTAAGGTACA TCATTCAAGG
2341 ACTTCATCCT TTCAGCACTG TTGATCTGCC ATCATTTAAA GAGCTGATTA GTACACTGCA
2401 GCCTGGCATT TCTGTCATTA CAAGGCCTAC TTTACGCTCC AAGATAGCTG AAGCTGCTCT
2461 GATCATGAAA CAGAAAGTGA CTGCTGCCAT GAGTGAAGTT GAATGGATTG CAACCACAAC
2521 GGATTGTTGG ACTGCACGTA GAAAGTCATT CATTGGTGTA ACTGCTCACT GGATCAACCC
2581 TGGAAGTCTT GAAAGACATT CCGCTGCACT TGCCTGCAAA AGATTAATGG GCTCTCATAC
2641 TTTTGAGGTA CTGGCCAGTG CCATGAATGA TATCCACTCA GAGTATGAAA TACGTGACAA
2701 GGTTGTTTGC ACAACCACAG ACAGTGGTTC CAACTTTATG AAGGCTTTCA GAGTTTTTGG
2761 TGTGGAAAAC AATGATATCG AGACTGAGGC AAGAAGGTGT GAAAGTGATG ACACTGATTC
2821 TGAAGGCTGT GGTGAGGGAA GTGATGGTGT GGAATTCCAA GATGCCTCAC GAGTCCTGGA
2881 CCAAGACGAT GGCTTCGAAT TCCAGCTACC AAAACATCAA AAGTGTGCCT GTCACTTACT
2941 TAACCTAGTC TCAAGCGTTG ATGCCCAAAA AGCTCTCTCA AATGAACACT ACAAGAAACT
3001 CTACAGATCT GTCTTTGGCA AATGCCAAGC TTTATGGAAT AAAAGCAGCC GATCGGCTCT
3061 AGCAGCTGAA GCTGTTGAAT CAGAAAGCCG GCTTCAGCTT TTAAGGCCAA ACCAAACGCG
3121 GTGGAATTCA ACTTTTATGG CTGTTGACAG AATTCTTCAA ATTTGCAAAG AAGCAGGAGA
3181 AGGCGCACTT CGGAATATAT GCACCTCTCT TGAGGTTCCA ATGTAAGTGT TTTTCCCCTC
3241 TATCGATGTA AACAAATGTG GGTTGTTTTT GTTTAATACT CTTTGATTAT GCTGATTTCT
3301 CCTGTAGGTT TAATCCAGCA GAAATGCTGT TCTTGACAGA GTGGGCCAAC ACAATGCGTC
3361 CAGTTGCAAA AGTACTCGAC ATCTTGCAAG CGGAAACGAA TACACAGCTG GGGTGGCTGC
3421 TGCCTAGTGT CCATCAGTTA AGCTTGAAAC TTCAGCGACT CCACCATTCT CTCAGGTACT
3481 GTGACCCACT TGTGGATGCC CTACAACAAG GAATCCAAAC ACGATTCAAG CATATGTTTG
3541 AAGATCCTGA GATCATAGCA GCTGCCATCC TTCTCCCTAA ATTTCGGACC TCTTGGACAA
3601 ATGATGAAAC CATCATAAAA CGAGGTAAAT GAATGCAAGC AACATACACT TGACGAATTC
3661 TAATCTGGGC AACCTTTGAG CCATACCAAA ATTATTCTTT TATTTATTTA TTTTTGCACT
3721 TTTTAGGAAT GTTATATCCC ATCTTTGGCT GTGATCTCAA TATGAATATT GATGTAAAGT
3781 ATTCTTGCAG CAGGTTGTAG TTATCCCTCA GTGTTTCTTG AAACCAAACT CATATGTATC
3841 ATATGTGGTT TGGAAATGCA GTTAGATTTT ATGCTAAAAT AAGGGATTTG CATGATTTTA
3901 GATGTAGATG ACTGCACGTA AATGTAGTTA ATGACAAAAT CCATAAAATT TGTTCCCAGT
3961 CAGAAGCCCC TCAACCAAAC TTTTCTTTGT GTCTGCTCAC TGTGCTTGTA GGCATGGACT
4021 ACATCAGAGT GCATCTGGAG CCTTTGGACC ACAAGAAGGA ATTGGCCAAC AGTTCATCTG
4081 ATGATGAAGA TTTTTTCGCT TCTTTGAAAC CGACAACACA TGAAGCCAGC AAAGAGTTGG
4141 ATGGATATCT GGCCTGTGTT TCAGACACCA GGGAGTCTCT GCTCACGTTT CCTGCTATTT
4201 GCAGCCTCTC TATCAAGACT AATACACCTC TTCCCGCATC GGCTGCCTGT GAGAGGCTTT
4261 TCAGCACTGC AGGATTGCTT TTCAGCCCCA AAAGAGCTAG GCTTGACACT AACAATTTTG
4321 AGAATCAGCT TCTACTGAAG TTAAATCTGA GGTTTTACAA CTTTGAGTAG CGTGTACTGG

4381 CATTAGATTG TCTGTCTTAT AGTTTGATAA TTAAATACAA ACAGTTCTAA AGCAGGATAA
4441 AACCTTGTAT GCATTTCATT TAATGTTTTT TGAGATTAAA AGCTTAAACA AGAATCTCTA
4501 GTTTTCTTTC TTGCTTTTAC TTTTACTTCC TTAATACTCA AGTACAATTT TAATGGAGTA
4561 CTTTTTTACT TTTACTCAAG TAAGATTCTA GCCAGATACT TTTACTTTTA ATTGAGTAAA
4621 ATTTTCCCTA AGTACTTGTA CTTTCACTTG AGTAAAATTT TTGAGTACTT TTTACACCTC
4681 TG (SEQ ID NO: 25).

[0167] The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

[0168] Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but are not limited to, ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene), mycophenolic acid, or glutamine synthetase (GS, U.S. Pat. Nos. 5,122,464; 5,770,359; 5,827,739), blasticidin (*bsd* gene), resistance genes for eukaryotic cell culture as well as ampicillin, zeocin (*Sh bla* gene), puromycin (*pac* gene), hygromycin B (*hygB* gene), G418/Geneticin (*neo* gene), kanamycin, spectinomycin, streptomycin, carbenicillin, bleomycin, erythromycin, polymyxin B, or tetracycline resistance genes for culturing in E. coli and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1-4 and 16-18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

[0169] Expression vectors will preferably but optionally include at least one selectable cell surface marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable cell surface markers of the disclosure comprise surface proteins,

glycoproteins, or group of proteins that distinguish a cell or subset of cells from another defined subset of cells. Preferably the selectable cell surface marker distinguishes those cells modified by a composition or method of the disclosure from those cells that are not modified by a composition or method of the disclosure. Such cell surface markers include, e.g., but are not limited to, "cluster of designation" or "classification determinant" proteins (often abbreviated as "CD") such as a truncated or full length form of CD19, CD271, CD34, CD22, CD20, CD33, CD52, or any combination thereof. Cell surface markers further include the suicide gene marker RQR8 (Philip B et al. Blood. 2014 Aug 21; 124(8):1277-87).

[0170] Expression vectors will preferably but optionally include at least one selectable drug resistance marker for isolation of cells modified by the compositions and methods of the disclosure. Selectable drug resistance markers of the disclosure may comprise wild-type or mutant Neo, DHFR, TYMS, FRANCF, RAD51C, GCS, MDR1, ALDH1, NKX2.2, or any combination thereof.

[0171] At least one inducible polypeptide of the disclosure can be expressed in a modified form, such as a fusion protein, and can include additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of a protein scaffold to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to a protein scaffold of the disclosure to facilitate purification. Such regions can be removed prior to final preparation of an inducible polypeptide or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29-17.42 and 18.1-18.74; Ausubel, supra, Chapters 16, 17 and 18.

[0172] Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the disclosure. Alternatively, nucleic acids of the disclosure can be expressed in a host cell by turning on (by manipulation) in a host cell that contains a sequence encoding an inducible polypeptide of the disclosure. Such methods are well known in the art, e.g., as described in U.S. Pat. Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

[0173] Expression vectors for modified cells can include one or more of the following expression control sequences, such as, but not limited to, an origin of replication; a promoter

(e.g., late or early SV40 promoters, the CMV promoter (U.S. Pat. Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (U.S. Pat. No. 5,266,491), at least one human promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

[0174] When eukaryotic host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773-781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

Amino Acid Codes

[0175] The amino acids that make up protein scaffolds of the disclosure are often abbreviated. The amino acid designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994). An inducible polypeptide of the disclosure can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein. Amino acids in an inducible polypeptide of the disclosure that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to, at least one neutralizing activity. Sites that are critical for function (i.e., inducing apoptosis) can also be identified by structural analysis, such as crystallization, nuclear magnetic

resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899-904 (1992) and de Vos, et al., Science 255:306-312 (1992)).

[0176] Biologically active an inducible polypeptide of the disclosure include one or more proteins or enzymes (e.g. a caspase such as caspase 9) capable of inducing apoptosis in a cell with an efficacy that is at least 20%, 30%, or 40%, and, preferably, at least 50%, 60%, or 70%, and, most preferably, at least 80%, 90%, or 95%-99% or more efficacious as expression or induction of the cell's native (non-synthetic), endogenous or related and known protein or enzyme. Methods of assaying and quantifying measures of protein binding and enzymatic activity are well known to those of skill in the art.

Infusion of Modified Cells as Adoptive Cell Therapy

[0177] The disclosure provides modified cells that express one or more inducible polypeptide of the disclosure that have been selected for administration to a subject in need thereof. Modified cells of the disclosure may be formulated for storage at any temperature including room temperature and body temperature. Modified cells of the disclosure may be formulated for cryopreservation and subsequent thawing. Modified cells of the disclosure may be formulated in a pharmaceutically acceptable carrier for direct administration to a subject from sterile packaging.

EXAMPLES

Example 1: Expression and Function of piggyBac integrated iC9 safety switch into human pan T-cells

[0178] Human pan T-cells were nucleofected using an Amaxa 4D nucleofector with one of four piggyBac transposons. Modified T cells receiving the "mock" condition were nucleofected with an empty piggyBac transposon. Modified T cells received either a piggyBac transposase containing a therapeutic agent alone (a sequence encoding a CARTyrin) or a piggyBac transposase containing an integrated iC9 sequence and a therapeutic agent (a sequence encoding a CARTyrin).

[0179] Figure 1 provides a schematic diagram of the iC9 safety switch, which contains a ligand binding region, a linker, and a truncated caspase 9 polypeptide. Specifically, the iC9 polypeptide contains a ligand binding region comprising a FK506 binding protein 12 (FKBP12) polypeptide including a substitution of valine (V) for phenylalanine (F) at position 36 (F36V). The FKBP12 polypeptide of the iC9 polypeptide is encoded by an amino acid

sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID
NO: 3). The FKBP12 polypeptide of the iC9 polypeptide is encoded by a nucleic acid
sequence comprising
GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA
GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC
AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC
ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAACTGCTGAAGCTGGAG (SEQ
ID NO: 4). The linker region of the iC9 polypeptide is encoded by an amino acid comprising
GGGGS (SEQ ID NO: 5) and a nucleic acid sequence comprising GGAGGAGGAGGATCC
(SEQ ID NO: 6). The amino acid sequence encoding the iC9 polypeptide is encoded by an
amino acid comprising GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRES
GLRTRTGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCV
VVILSHGCQASHLQFPGAVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGG
EQKDHGFEVASTSPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFP
GFVSWRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFN
FLRKKLFFKTS (SEQ ID NO: 7). The nucleic acid sequence encoding the iC9 polypeptide
is encoded by a nucleic acid sequence comprising
TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTAC
ATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAACAATGTGAACTTCT
GCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGC
TGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGAC
CGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGC
TCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTG
CAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAG
ATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGGAAAGCCAAAAC
TGTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGG
CCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAA

CTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCT
GCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCAT
GGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTG
AACAGTGGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACG
CTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAG
AAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 8).

[0180] To test the iC9 safety switch, each of the four modified T cells were incubated for 24 hours with 0, 0.1 nM, 1 nM, 10 nM, 100 nM or 1000 nM AP1903 (an induction agent for AP1903, also known as Rimiducid). Viability was assessed by flow cytometry using 7-aminoactinomycin D (7-AAD), a fluorescent intercalator, as a marker for cells undergoing apoptosis.

[0181] Cell viability was assessed at day 12 (see Figure 2). The data demonstrate a shift of cell populations from the lower right to the upper left quadrants with increasing concentration of the induction agent in cells containing the iC9 construct; however, this effect is not observed in cells lacking the iC9 construct (those receiving only the CARTyrin), in which cells are evenly distributed among these two areas regardless of the concentration of the induction agent. Moreover, cell viability was assessed at day 19 (see Figure 3). The data reveal the same trend as shown in Figure 2 (day 12 post-nucleofection); however, the population shift to the upper left quadrant is more pronounced at this later time point (day 19 post-nucleofection).

[0182] A quantification of the aggregated results was performed and is provided in Figure 4, showing the significant impact of the iC9 safety switch on the percent cell viability as a function of the concentration of the induction agent (AP1903, also known as Rimiducid) of the iC9 switch for each modified cell type at either day 12 (Figure 2 and left graph) or day 19 (Figure 3 and right graph). The presence of the iC9 safety switch induces apoptosis in a significant majority of cells by day 12 and the effect is even more dramatic by day 19.

[0183] The results of this study show that the iC9 safety switch is extremely effective at eliminating active cells upon contact with an induction agent (e.g. AP1903, also known as Rimiducid) because AP1903 (Rimiducid) induces apoptosis at even the lowest concentrations of the study (0.1 nM). Furthermore, the iC9 safety switch may be functionally expressed as part of a tricistronic vector.

Example 2: highly efficient killing of cells comprising P-BCMA-101 using the iC9 safety switch in NGS mice in vivo

[0184] NSG mice were IV injected with MM.1S/luciferase$^+$ cells, staged at day 8, injected with T cells on day 9, and treated with AP1903 (Rimiducid) on day 12 at the indicated doses. 24 hours later, mice were euthanized and blood, spleen, and bone marrow cells were collected and stained for the presence of huCD45$^+$ cells (Figure 5). Blood, spleen, and bone marrow cells were analyzed by flow cytometry for the presence of huCD45$^+$ cells. The relative viability was determined by dividing the number of huCD45 cells by the number of msCD45 cells and normalizing to the average of huCD45/msCD45 in the no treatment group times, 100% per 1,500 bead events for each sample. Each data point represents a different mouse (Figure 6).

INCORPORATION BY REFERENCE

[0185] Every document cited herein, including any cross referenced or related patent or application is hereby incorporated herein by reference in its entirety unless expressly excluded or otherwise limited. The citation of any document is not an admission that it is prior art with respect to any invention disclosed or claimed herein or that it alone, or in any combination with any other reference or references, teaches, suggests or discloses any such invention. Further, to the extent that any meaning or definition of a term in this document conflicts with any meaning or definition of the same term in a document incorporated by reference, the meaning or definition assigned to that term in this document shall govern.

OTHER EMBODIMENTS

[0186] While particular embodiments of the disclosure have been illustrated and described, various other changes and modifications can be made without departing from the spirit and scope of the disclosure. The scope of the appended claims includes all such changes and modifications that are within the scope of this disclosure.

CLAIMS

What is claimed is:

1. An inducible caspase polypeptide comprising
(a) a ligand binding region,
(b) a linker, and
(c) a truncated caspase 9 polypeptide,
wherein the inducible caspase polypeptide does not comprise a non-human sequence.

2. The polypeptide of claim 1, wherein the non-human sequence is a restriction site.

3. The polypeptide of claim 1 or 2, wherein the ligand binding region comprises a FK506 binding protein 12 (FKBP12) polypeptide.

4. The polypeptide of any one of claims 1-3, wherein the amino acid sequence comprises a modification at position 36 of the sequence.

5. The polypeptide of claim 4, wherein the modification is a substitution of valine (V) for phenylalanine (F) at position 36 (F36V).

6. The polypeptide of any one of claims 3 to 5, wherein the FKBP12 polypeptide is encoded by an amino acid sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 3).

7. The polypeptide of claim 6, wherein the FKBP12 polypeptide is encoded by a nucleic acid sequence comprising
GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGG
GGCCAGACTTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTG
GACAGCTCCAGGGATCGCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAA

GTGATCCGAGGATGGGAGGAAGGCGTGGCACAGATGTCAGTCGGCCAGCGGGCC AAACTGACCATTAGCCCTGACTACGCTTATGGAGCAACAGGCCACCCAGGGATC ATTCCCCCTCATGCCACCCTGGTCTTCGAT GTGGAACTGCTGAAGCTGGAG (SEQ ID NO: 4).

8. The polypeptide of any one of claims 1 to 7, wherein the linker region is encoded by an amino acid comprising GGGGS (SEQ ID NO: 5).

9. The polypeptide of claim 8, wherein the linker region is encoded by a nucleic acid sequence comprising GGAGGAGGAGGATCC (SEQ ID NO: 6).

10. The polypeptide of any one of claims 1 to 9, wherein the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an arginine (R) at position 87 of the sequence.

11. The polypeptide of any one of claims 1 to 10, wherein the truncated caspase 9 polypeptide is encoded by an amino acid sequence that does not comprise an alanine (A) at position 282 the sequence.

12. The polypeptide of any one of claims 1 to 11, wherein the truncated caspase 9 polypeptide is encoded by an amino acid comprising GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRR RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG AVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID NO: 7).

13. The polypeptide of claim 12, wherein the truncated caspase 9 polypeptide is encoded by a nucleic acid sequence comprising

TTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGAGGAAATGCCGATCTGGCTTAC
ATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAACAATGTGAACTTCT
GCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTGAGAAGC
TGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGAC
CGCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGC
TCTGGATTGCTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTG
CAGTTCCCCGGAGCAGTGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAG
ATCGTCAACATCTTCAACGGCACTTCTTGCCCTAGTCTGGGGGGAAAGCCAAAAC
TGTTCTTTATCCAGGCCTGTGGCGGGGAACAGAAAGATCACGGCTTCGAGGTGG
CCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACCCTGAACCAGATGCAA
CTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATCTCAAGCCT
GCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTCAT
GGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTG
AACAGTGGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACG
CTGTCTCTGTGAAGGGCATCTACAAACAGATGCCCGGGTGCTTCAATTTTCTGAG
AAAGAAACTGTTCTTTAAGACTTCC (SEQ ID NO: 8).

14. The polypeptide of any one of claims 1 to 13, wherein the polypeptide is encoded by an amino acid sequence comprising
GVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGKQEVI
RGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLEGGGGS
GFGDVGALESLRGNADLAYILSMEPCGHCLIINNVNFCRESGLRTRTGSNIDCEKLRR
RFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHLQFPG
AVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTSPEDE
SPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGSWYVE
TLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS (SEQ ID NO: 9).

15. The polypeptide of claim 14, wherein the polypeptide is encoded by a nucleic acid sequence comprising
GGGGTCCAGGTCGAGACTATTTCACCAGGGGATGGGCGAACATTTCCAAAAAGGGGCCAGAC

TTGCGTCGTGCATTACACCGGGATGCTGGAGGACGGGAAGAAAGTGGACAGCTCCAGGGATC
GCAACAAGCCCTTCAAGTTCATGCTGGGAAAGCAGGAAGTGATCCGAGGATGGGAGGAAGGC
GTGGCACAGATGTCAGTCGGCCAGCGGGCCAAACTGACCATTAGCCCTGACTACGCTTATGG
AGCAACAGGCCACCCAGGGATCATTCCCCCTCATGCCACCCTGGTCTTCGATGTGGAACTGC
TGAAGCTGGAGGGAGGAGGAGGATCCGGATTTGGGGACGTGGGGGCCCTGGAGTCTCTGCGA
GGAAATGCCGATCTGGCTTACATCCTGAGCATGGAACCCTGCGGCCACTGTCTGATCATTAA
CAATGTGAACTTCTGCAGAGAAAGCGGACTGCGAACACGGACTGGCTCCAATATTGACTGTG
AGAAGCTGCGGAGAAGGTTCTCTAGTCTGCACTTTATGGTCGAAGTGAAAGGGGATCTGACC
GCCAAGAAAATGGTGCTGGCCCTGCTGGAGCTGGCTCAGCAGGACCATGGAGCTCTGGATTG
CTGCGTGGTCGTGATCCTGTCCCACGGGTGCCAGGCTTCTCATCTGCAGTTCCCCGGAGCAG
TGTACGGAACAGACGGCTGTCCTGTCAGCGTGGAGAAGATCGTCAACATCTTCAACGGCACT
TCTTGCCCTAGTCTGGGGGGAAAGCCAAAACTGTTCTTTATCCAGGCCTGTGGCGGGGAACA
GAAAGATCACGGCTTCGAGGTGGCCAGCACCAGCCCTGAGGACGAATCACCAGGGAGCAACC
CTGAACCAGATGCAACTCCATTCCAGGAGGGACTGAGGACCTTTGACCAGCTGGATGCTATC
TCAAGCCTGCCCACTCCTAGTGACATTTTCGTGTCTTACAGTACCTTCCCAGGCTTTGTCTC
ATGGCGCGATCCCAAGTCAGGGAGCTGGTACGTGGAGACACTGGACGACATCTTTGAACAGT
GGGCCCATTCAGAGGACCTGCAGAGCCTGCTGCTGCGAGTGGCAAACGCTGTCTCTGTGAAG
GGCATCTACAAACAGATGCCCCGGGTGCTTCAATTTTCTGAGAAAGAAACTGTTCTTTAAGAC
TTCC (SEQ ID NO: 10).

16. A composition comprising the inducible caspase polypeptide of any one of the preceding claims.

17. A transposon comprising the inducible caspase polypeptide of any one of the preceding claims.

18. The transposon of claim 17, further comprising a sequence encoding a therapeutic protein.

19. The transposon of claim 18, wherein the therapeutic protein is naturally-occurring.

20. The transposon of claim 18, wherein the therapeutic protein is not naturally-occurring.

21. The transposon of any one of claims 18-20, wherein the therapeutic protein comprises a cell surface protein, a membrane-bound protein, an extracellular membrane-bound protein, an intracellular membrane-bound protein, an intracellular protein, a nuclear localized protein, a nuclear protein, a cytoplasmic protein, a cytosolic protein, a secreted protein, a lysosomal protein, an endosomal protein, a vesicle-associated protein, a mitochondrial protein, an endoplasmic reticulum protein, a cytoskeletal protein, a protein involved in intracellular signaling and/or a protein involved in extracellular signaling.

22. The transposon any one of claims 18-20, wherein the therapeutic protein comprises an antigen receptor.

23. The transposon of claim 22, wherein the antigen receptor comprises a T-Cell Receptor (TCR).

24. The transposon of claim 23, wherein the antigen receptor comprises a variant or recombinant T-Cell Receptor (TCR).

25. The transposon of any one of claims 23-25, wherein the antigen receptor is a Chimeric Antigen Receptor (CAR).

26. The transposon of claim 26, wherein the CAR comprises one or more Centyrin sequence(s).

27. The transposon of claim 26, wherein the CAR is a CARTyrin.

28. The transposon of claim 25, wherein the CAR comprises one or more VHH sequence(s).

29. The transposon of claim 28, wherein the CAR is a VCAR.

30. The transposon of any one of claims 17-29, wherein the transposon comprises at least one self-cleaving peptide.

31. The transposon of claim 30, wherein the transposon comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the inducible caspase polypeptide and another sequence in the transposon.

32. The transposon of claim 30, wherein the transposon comprises at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of the inducible caspase polypeptide and a second self-cleaving peptide is located downstream of the inducible caspase polypeptide.

33. The transposon of any one of claims 30-32, wherein the at least one self-cleaving peptide comprises T2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide.

34. The transposon of claim 33, wherein the T2A peptide comprises an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 11).

35. The transposon of claim 33, wherein the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 12).

36. The transposon of claim 33, wherein the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 13).

37. The transposon of claim 33, wherein the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 14).

38. The transposon of claim 33, wherein the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 15).

39. The transposon of claim 33, wherein the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 16).

40. The transposon of claim 33, wherein the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 17).

41. The transposon of claim 44, wherein the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 18).

42. The transposon of any one of claims 17-41, wherein the transposon is a piggyBac transposon.

43. A composition comprising the transposon of any one of claims 17-42.

44. The composition of claim 43, wherein the transposon is a piggyBac transposon and wherein the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme.

45. The composition of claim 44, wherein the sequence encoding a transposase enzyme is an mRNA sequence.

46. The composition of claim 44 or 45, wherein the transposase is a piggyBac transposase.

47. The composition of claim 46, wherein the piggyBac transposase comprises an amino acid sequence comprising SEQ ID NO: 1.

48. The composition of claim 46 or 47, wherein the piggyBac transposase is a hyperactive variant and wherein the hyperactive variant comprises an amino acid substitution at one or more of positions 30, 165, 282 and 538 of SEQ ID NO: 1.

49. The composition of claim 48, wherein the amino acid substitution at position 30 of SEQ ID NO: 1 is a substitution of a valine (V) for an isoleucine (I) (I30V).

50. The composition of claim 48, wherein the amino acid substitution at position 165 of SEQ ID NO: 1 is a substitution of a serine (S) for a glycine (G) (G165S).

51. The composition of claim 48, wherein the amino acid substitution at position 282 of SEQ ID NO: 1 is a substitution of a valine (V) for a methionine (M) (M282V).

52. The composition of claim 48, wherein the amino acid substitution at position 538 of SEQ ID NO: 1 is a substitution of a lysine (K) for an asparagine (N) (N538K).

53. The composition of any one of claims 48-52, wherein the transposase is a Super piggyBac (SPB) transposase.

54. The composition of claim 53, wherein the Super piggyBac (sPBo) transposase comprises an amino acid sequence comprising SEQ ID NO: 2.

55. The composition of claim 43, wherein the transposon is a Sleeping Beauty transposon and wherein the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme.

56. The composition of claim 55, wherein the sequence encoding the transposase enzyme comprises a sequence encoding a Sleeping Beauty transposase or hyperactive Sleeping Beauty transposase (SB100X).

57. The composition of claim 43, wherein the transposon is a Helraiser transposon and wherein the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme.

58. The composition of claim 57, wherein the sequence encoding the transposase enzyme comprises a sequence encoding a Helitron transposase.

59. The composition of claim 43, wherein the transposon is a Tol2 transposon and wherein the composition further comprises a plasmid comprising a sequence encoding a transposase enzyme.

60. The composition of claim 59, wherein the sequence encoding the transposase enzyme comprises a sequence encoding a Tol2 transposase.

61. The composition of any one of claims 56, 58 or 60, wherein the sequence encoding the transposase enzyme is an mRNA sequence.

62. A vector comprising the inducible caspase polypeptide of any one of claims 1-15.

63. The vector of claim 62, wherein the vector is a viral vector.

64. The vector of claim 63, wherein the viral vector comprises a sequence isolated or derived from a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV) or any combination thereof.

65. The vector of claim 63 or 64, wherein the viral vector comprises a sequence isolated or derived from a retrovirus.

66. The vector of claim 65, wherein the retrovirus is a gammaretrovirus.

67. The vector of claim 65, wherein the retrovirus is a lentivirus.

68. The vector of claim 63 or 64, wherein the viral vector comprises a sequence isolated or derived from an adeno-associated virus (AAV).

69. The vector of claim 68, wherein the AAV comprises an AAV of serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11.

70. The vector of claim 68, wherein the AAV comprises a sequence isolated or derived from rAAV-LK03, rAAV-NP59 or rAAV-NP84.

71. The vector of any one of claims 63-70, wherein the viral vector is a recombinant vector.

72. The vector of claim 62, wherein the vector is a nanoparticle vector.

73. The vector of claim 72, wherein the nanoparticle vector comprises a nucleic acid, an amino acid, a polymers, a micelle, lipid, an organic molecule, an inorganic molecule or any combination thereof.

74. The vector of any one of claims 62-73, wherein the vector comprises at least one self-cleaving peptide.

75. The vector of any one of claims 62-74, wherein the vector comprises at least one self-cleaving peptide and wherein a self-cleaving peptide is located between the inducible caspase polypeptide and another sequence in the vector.

76. The vector of any one of claims 62-74, wherein the vector comprises at least one self-cleaving peptide and wherein a first self-cleaving peptide is located upstream of the inducible caspase polypeptide and a second self-cleaving peptide is located downstream of the inducible caspase polypeptide.

77. The vector of any one of claims 74-76, wherein the at least one self-cleaving peptide comprises aT2A peptide, GSG-T2A peptide, an E2A peptide, a GSG-E2A peptide, an F2A peptide, a GSG-F2A peptide, a P2A peptide, or a GSG-P2A peptide.

78. The vector of claim 77, wherein the T2A peptide comprises an amino acid sequence comprising EGRGSLLTCGDVEENPGP (SEQ ID NO: 11).

79. The vector of claim 77, wherein the GSG-T2A peptide comprises an amino acid sequence comprising GSGEGRGSLLTCGDVEENPGP (SEQ ID NO: 12).

80. The vector of claim 77, wherein the E2A peptide comprises an amino acid sequence comprising QCTNYALLKLAGDVESNPGP (SEQ ID NO: 13).

81. The vector of claim 77, wherein the GSG-E2A peptide comprises an amino acid sequence comprising GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 14).

82. The vector of claim 77, wherein the F2A peptide comprises an amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 15).

83. The vector of claim 77, wherein the GSG-F2A peptide comprises an amino acid sequence comprising GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 16).

84. The vector of claim 77, wherein the P2A peptide comprises an amino acid sequence comprising ATNFSLLKQAGDVEENPGP (SEQ ID NO: 17).

85. The vector of claim 77, wherein the GSG-P2A peptide comprises an amino acid sequence comprising GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 18).

86. A composition comprising the vector of any one of claims 62-85.

87. A cell comprising the inducible caspase polypeptide of any one of claims 1-15.

88. A cell comprising the transposon of any one of claims 17-42.

89. A cell comprising the composition of any one of claims 16 or 43-61.

90. A cell comprising the vector of any one of claims 62-85.

91. The cell of any one of claims 87-90, wherein the cell expresses the inducible caspase protein following contact with an induction agent.

92. The cell of any one of claims 87-91, wherein the cell is a human cell.

93. The cell of any one of claims 87-92, wherein the cell is an immune cell.

94. The cell of claim 93, wherein the immune cell is a T-cell, a Natural Killer (NK) cell, a Natural Killer (NK)-like cell, a hematopoietic progenitor cell, a peripheral blood (PB) derived T cell or an umbilical cord blood (UCB) derived T-cell.

95. The cell of claim 94, wherein the immune cell is a T-cell.

96. The cell of any one of claims 87-91, wherein the cell is an artificial antigen presenting cell.

97. The cell of any one of claims 87-91, wherein the cell is a stem cell.

98. The cell of claim 97, wherein the stem cell is an embryonic stem cell.

99. The cell of claim 97, wherein the stem cell is an adult stem cell.

100. The cell of any one of claims 97-99, wherein the stem cell is totipotent, pluripotent, or multipotent.

101. The cell of claim 97 or 99, wherein the stem cell is an induced pluripotent stem cell (iPSC).

102. The cell of any one of claims 87-91, wherein the cell is a somatic cell.

103. The cell of claim 102, wherein the cell is isolated or derived from a human heart; skeletal or smooth muscle; blood vessel, vein or capillary; spleen; thyroid; lymph node or lymph vessel; bone or bone marrow; skin or endothelium; adrenal gland; esophagus; larynx; brain or spinal cord; peripheral nervous system; eye; hypothalamus; liver; olfactory tissue; prostate; stomach; large or small intestine; lung or bronchi; kidney; pancreas; thymus gland; ureter or urethrae; bladder; auditory tissue; bladder; parathyroid gland; salivary gland; or trachea.

104. A composition comprising the cell of any one of claims 87-103.

105. A use of the composition of claim 104 for an adoptive cell therapy.

106. The use of claim 105, wherein the cell is autologous.

107. The use of claim 105, wherein the cell is allogeneic.

108. A use of the composition of claim 104 for an ex vivo gene therapy.

109. The use of claim 108, wherein the cell is autologous.

110. The use of claim 108, wherein the cell is allogeneic.

111. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent

and an inducible caspase polypeptide of any one of claims 1-15, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

112. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent and a composition of any one of claims 16, 43-61, or 86, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

113. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent, a transposon of any one of claims 17-42 and a composition comprising a transposase of any one of claims 43-61, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

114. A method of modifying a cell therapy in a subject in need thereof, comprising administering to the subject a composition comprising a cell comprising a therapeutic agent and a vector of any one of claims 62-85, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent.

115. The method of any one of claims 111-114, wherein the cell is autologous.

116. The method of any one of claims 111-114, wherein the cell is allogeneic.

117. The method of any one of claims 111-116, wherein the cell therapy is an adoptive cell therapy.

118. The method of any one of claims 111-117, wherein the therapeutic agent has been introduced by ex vivo gene therapy.

119. The method of claim 118, wherein the therapeutic agent is a sequence encoding a modified endogenous gene, an exogenous gene, or a portion thereof.

120. The method of any one of claims 111-119, wherein the modifying is a termination of the cell therapy.

121. The method of any one of claims 111-119, wherein the modifying is a depletion of a portion of the cells provided in the cell therapy.

122. The method of any one of claims 111-121, further comprising the step of administering an inhibitor of the induction agent to inhibit modification of the cell therapy, thereby restoring the function and/or efficacy of the cell therapy.

123. A method of treating a disease or disorder in a subject in need thereof comprising

administering to the subject a composition comprising a cell comprising a kinetic agent and an inducible caspase polypeptide of any one of claims 1-15, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and

selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject,

thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

124. A method of treating a disease or disorder in a subject in need thereof comprising

administering to the subject a composition comprising a cell comprising a kinetic agent and a composition of any one of claims 16, 43-61, or 86, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and

selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject,

thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

125. A method of treating a disease or disorder in a subject in need thereof comprising

administering to the subject a composition comprising a cell comprising a kinetic agent, a transposon of any one of claims 17-42 and a composition comprising a transposase of any one of claims 43-61, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and

selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject,

thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

126. A method of treating a disease or disorder in a subject in need thereof comprising

administering to the subject a composition comprising a cell comprising a kinetic agent and a vector of any one of claims 62-85, wherein apoptosis may be selectively induced in the cell by contacting the cell with an induction agent, and wherein the cell comprising the kinetic agent induces local tissue toxicity within a target tissue of the subject, and

selectively inducing apoptosis in the cell comprising the kinetic agent prior to induction of significant toxicity in a non-target tissue of the subject,

thereby treating eliminating the target tissue, preserving non-target tissue and treating the disease or disorder in the subject.

127. The method of any one of claims 123-126, wherein the disease or disorder is a proliferative disorder or cancer.

128. The method of claim 127, wherein the target tissue comprises a tumor.

129. The method of claim 128, wherein the tumor is benign.

130. The method of claim 128, wherein the tumor is malignant.

131. The method of any one of claims 127-130, wherein the target tissue comprises an exposed tissue or margin of a resected tumor.

132. The method of any one of claims 127-130, wherein the target tissue comprises a site of probable metastasis.

133. The method of claim 132, wherein the site of metastasis comprise one or more of a lymph node, lymph fluid, peripheral circulating blood, local circulating blood, a bone, a bone marrow and cerebral spinal fluid (CSF).

134. The method of any one of claims 123-126, wherein the disease or disorder is an inflammatory disease or disorder.

135. The method of claim 134, wherein the target tissue comprises a site of inflammation.

136. The method of any one of claims 123-126, wherein the disease or disorder is an immune or autoimmune disease or disorder.

137. The method of claim 136, wherein the target tissue comprises a site of exposed or infected tissue.

138. The method of claim 136, wherein the target tissue comprises a burned or a wounded tissue.

139. The method of any one of claims 123-126, wherein the disease or disorder is an infectious disease or disorder.

140. The method of claim 139, wherein the target tissue comprises an infected tissue.

141. The method of any one of claims 123-126, wherein the disease or disorder is a genetic or epigenetic disease or disorder.

142. The method of claim 141, wherein the target tissue comprises one or more cells comprising the genetic or epigenetic modification when compared to a wild type cell.

143. The method of any one of claims 123-126, wherein the disease or disorder is a metabolic disorder.

144. The method of claim 141, wherein the target tissue comprises one or more cells with the metabolic disorder.

145. The method of any one of claims 123-126, wherein the disease or disorder is a vascular disorder.

146. The method of claim 145, wherein the target tissue comprises one or more cells of a vein, blood vessel, capillary or a component of circulating blood.

147. The method of any one of claims 123-126, wherein the disease or disorder is a respiratory disorder.

148. The method of claim 147, wherein the target tissue comprises one or more cells of a nasal passage, esophagus, or lung.

149. The method of any one of claims 123-126, wherein the disease or disorder is a fibrotic disorder.

150. The method of claim 149, wherein the target tissue comprises a fibroid mass or a cell in proximity to the fibroid mass.

151. The method of any one of claims 123-150, wherein an adoptive cell therapy comprises the cell comprising the kinetic agent.

152. The method of any one of claims 123-151, wherein the cell comprising the kinetic agent is autologous.

153. The method of any one of claims 123-151, wherein the cell comprising the kinetic agent is allogeneic.

154. The method of any one of claims 123-153, wherein the cell comprising the kinetic agent is a T-cell.

155. The method of any one of claims 123-154, wherein the kinetic agent is a non-naturally occurring receptor.

156. The method of claim 155, wherein the non-naturally occurring receptor is a synthetic, modified, recombinant or chimeric receptor.

157. The method of claim 156, wherein the chimeric receptor is a chimeric antigen receptor (CAR).

158. The method of any one of claims 151-154, wherein the kinetic agent comprises an anti-cancer agent.

159. The method of claim 158, wherein the anti-cancer agent comprises an anti-CD19 agent.

160. The method of claim 158, wherein the anti-cancer agent comprises an anti-BCMA agent.

161. The method of claim 158, wherein the anti-cancer agent comprises an anti-PSMA agent.

162. The method of claim 158, wherein the anti-cancer agent comprises an anti-Muc1 agent.

163. The method of any one of claims 158-162, wherein the kinetic agent comprises a non-naturally occurring receptor.

164. The method of claim 163, wherein the non-naturally occurring receptor comprises a synthetic, modified, recombinant or chimeric receptor.

165. The method of claim 164, wherein the chimeric receptor is a chimeric antigen receptor (CAR).

166. The method of claim 165, wherein the CAR comprises one or more VHH sequence(s).

167. The method of claim 166, wherein the CAR is a VCAR.

168. The method of any one of claims 158-167, wherein the kinetic agent induces an aplasia.

169. The method of claim 168, wherein the aplasia is not fatal.

170. The method of any one of claims 158-169, wherein the induction agent eliminates the cell comprising the kinetic agent.

171. The method of any one of claims 158-170, wherein the induction agent eliminates or reduces a sign or a symptom of the aplasia.

FIGURE 1

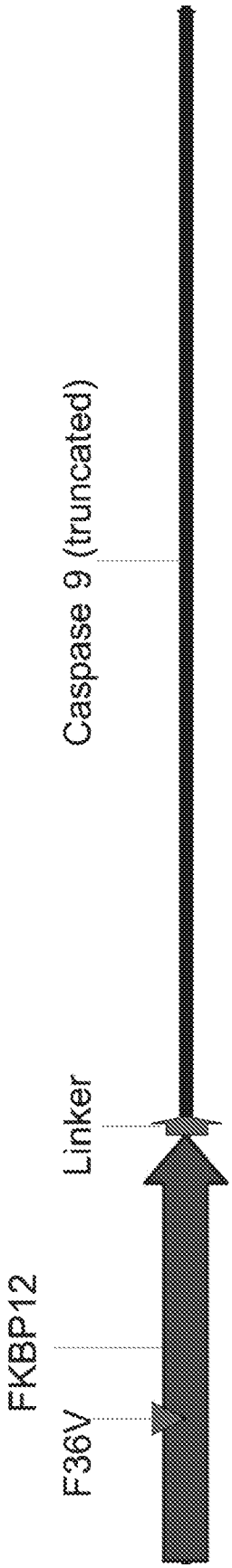


FIGURE 2

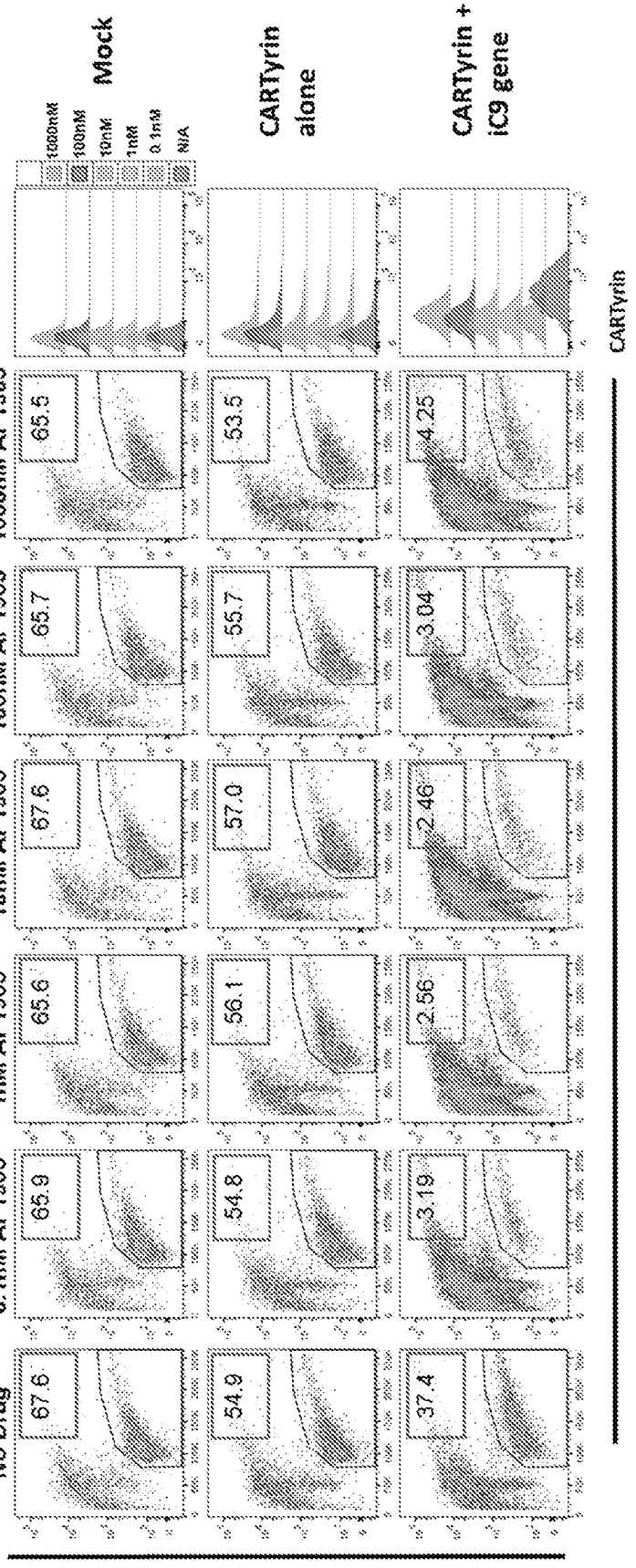

iC9/AP1903 Safety Switch Day 12
No Drug
0.1nM AP1903
1nM AP1903
10nM AP1903
100nM AP1903
1000nM AP1903
Mock
CARTyrin alone
CARTyrin + iC9 gene
1000nM
100nM
10nM
1nM
0.1nM
N/A
67.6
65.9
65.6
67.6
65.7
65.5
54.9
54.8
56.1
57.0
55.7
53.5
37.4
3.19
2.56
2.46
3.04
4.25
CARTyrin

FIGURE 3

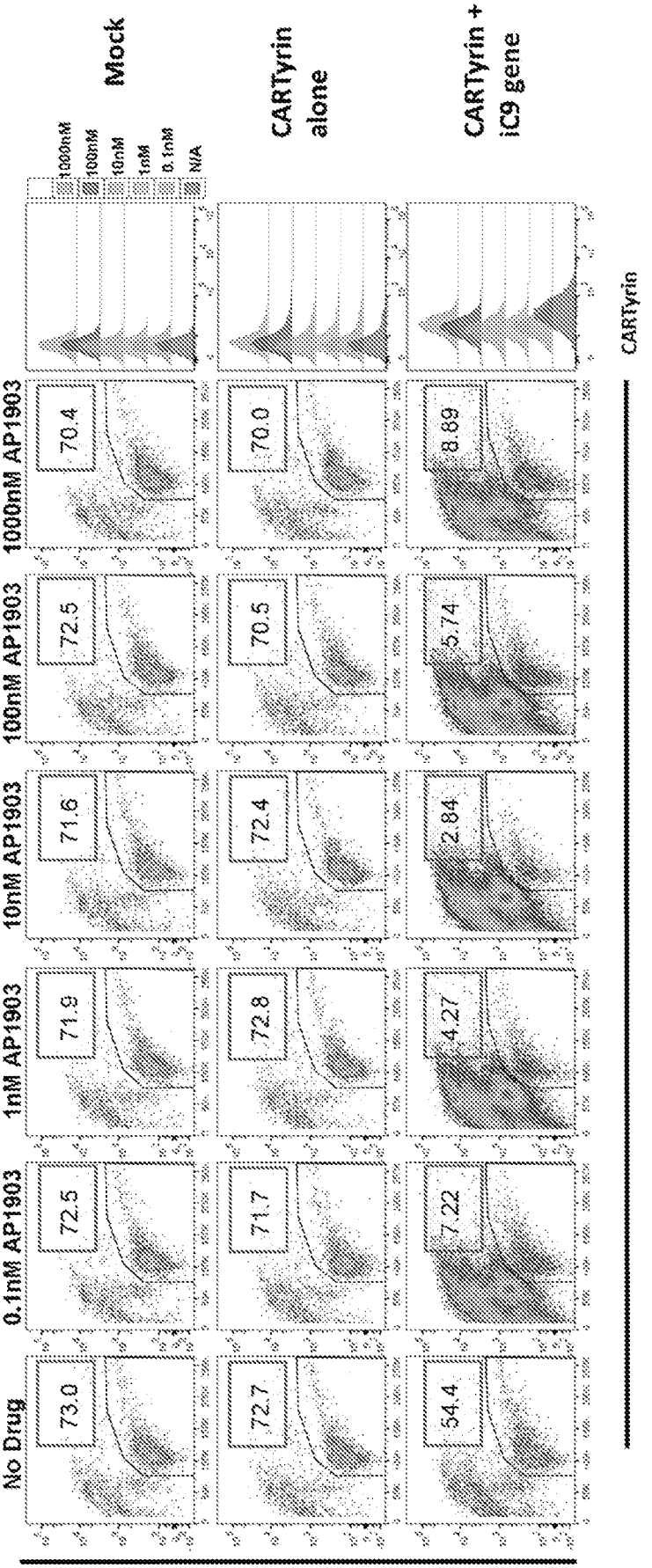
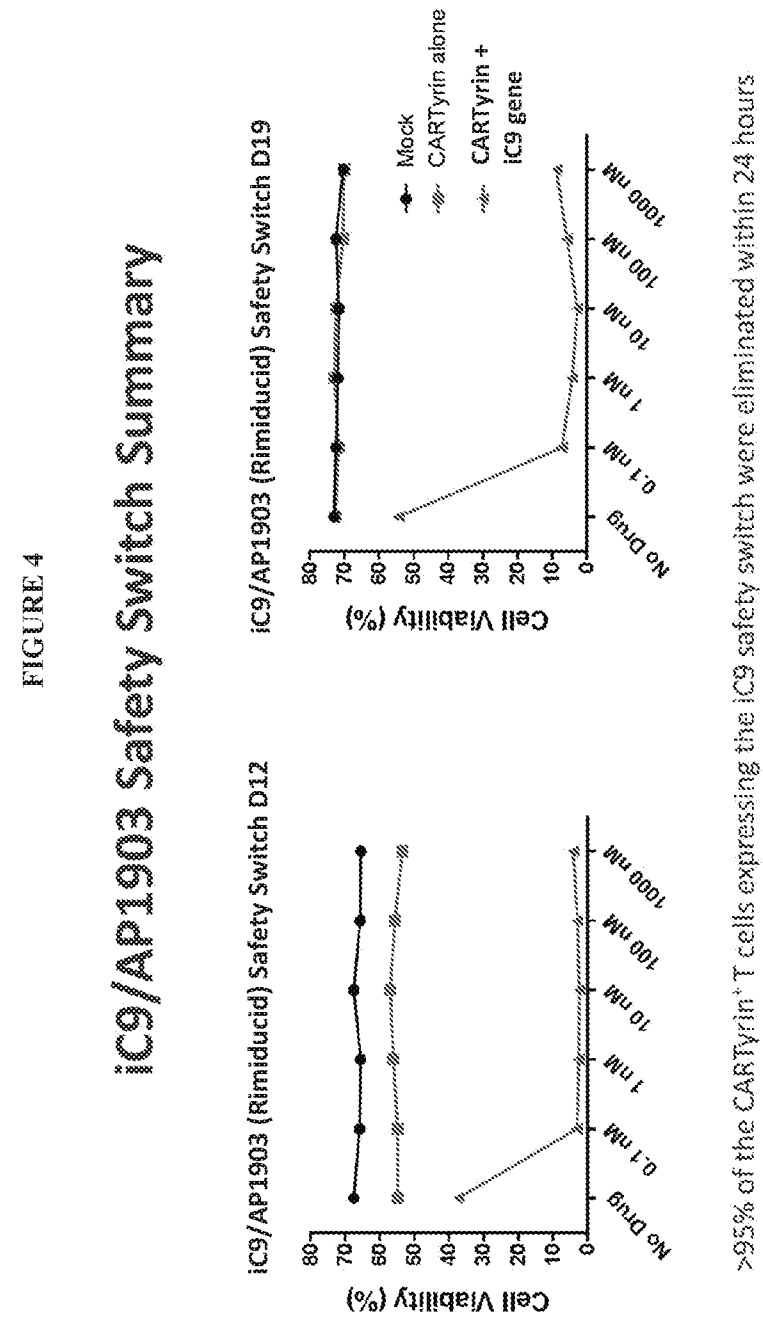

iC9/AP1903 Safety Switch Day 19
No Drug
0.1nM AP1903
1nM AP1903
10nM AP1903
100nM AP1903
1000nM AP1903
Mock
CARTyrin alone
CARTyrin + iC9 gene
1000nM
100nM
10nM
1nM
0.1nM
N/A
73.0
72.5
71.9
71.6
72.5
70.4
72.7
71.7
72.8
72.4
70.5
70.0
54.4
7.22
4.27
2.84
5.74
8.89
CARTyrin

FIGURE 4



>95% of the CARTyrin+ T cells expressing the iC9 safety switch were eliminated within 24 hours

FIGURE 5

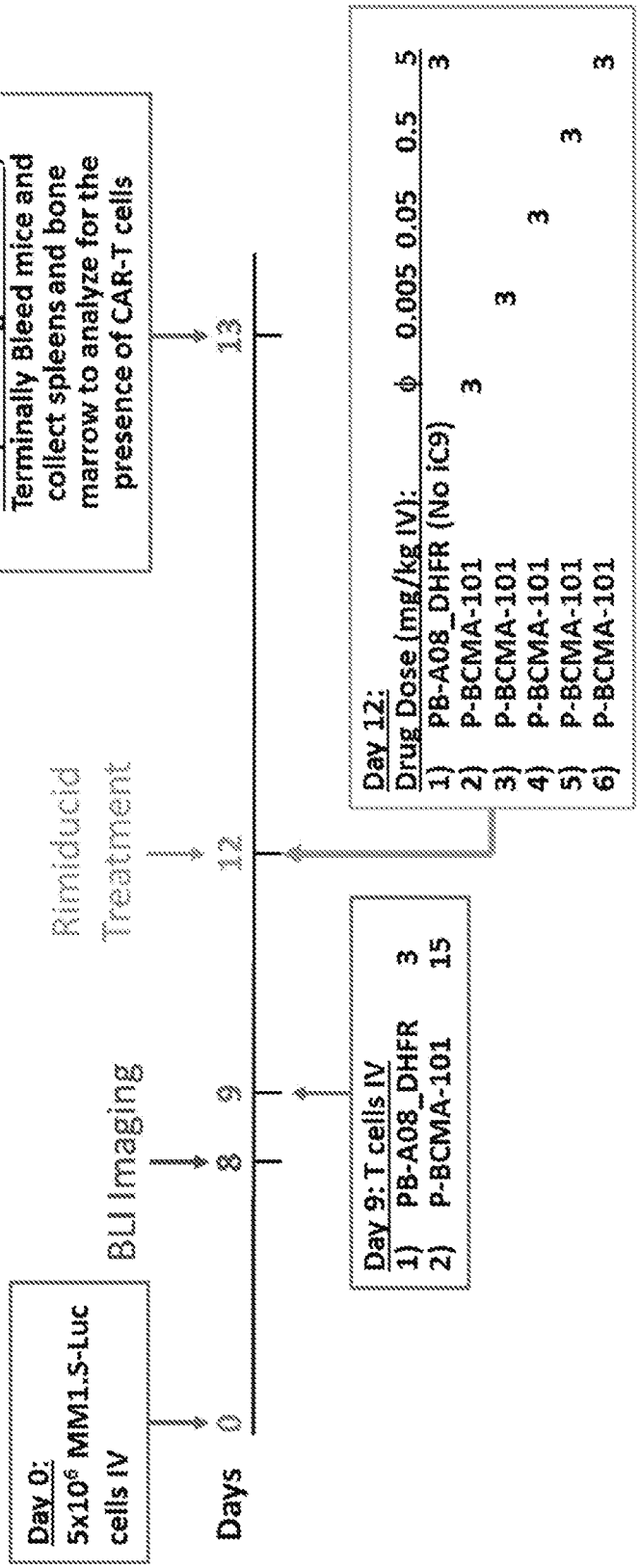
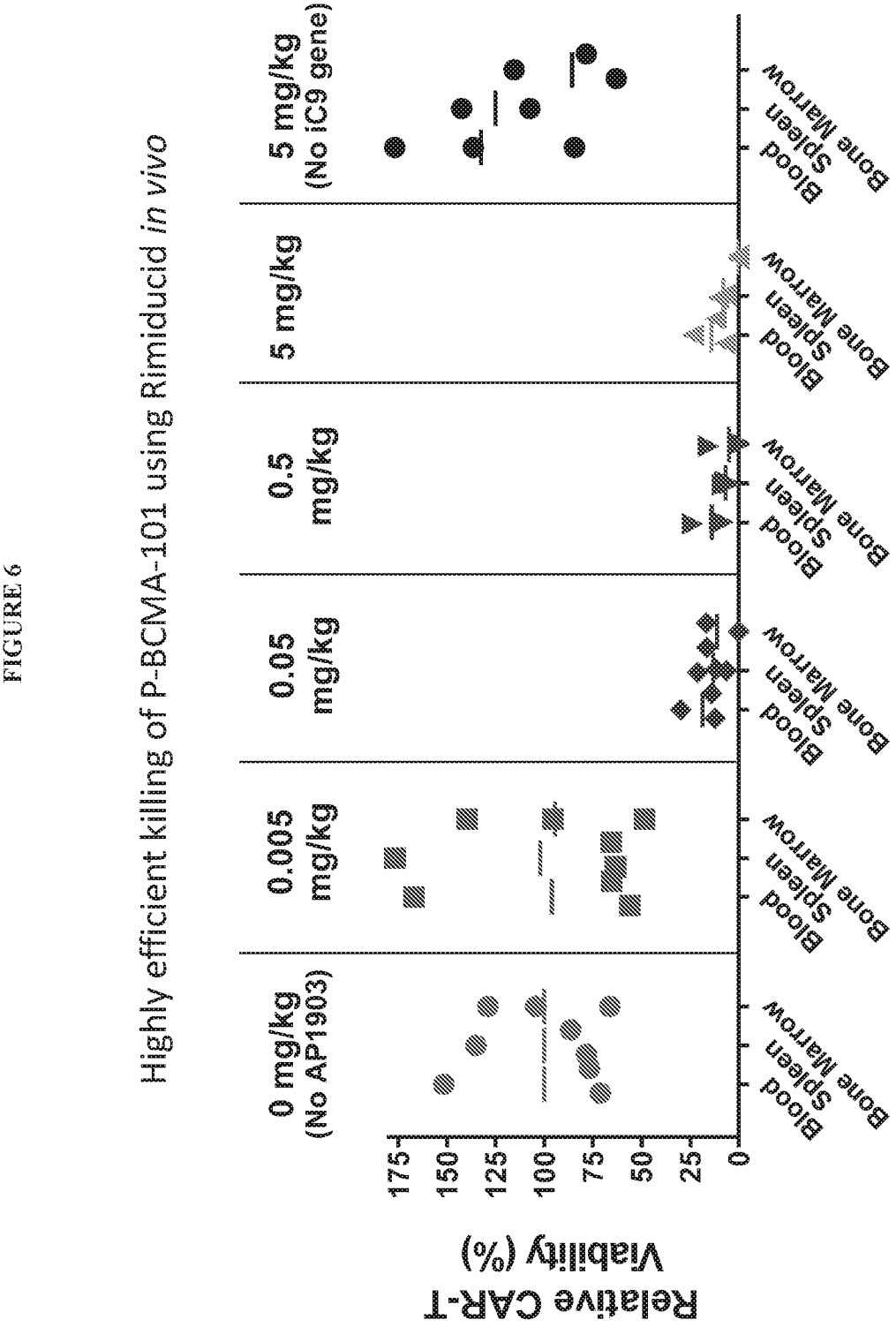

Day 0:
5x10⁶ MM1.S-Luc cells IV
Days 0 8 9 12 13
BLI Imaging
Rimiducid Treatment
Day 9: T cells IV
1) PB-A08_DHFR 3
2) P-BCMA-101 15
Day 12:
Drug Dose (mg/kg IV): ϕ 0.005 0.05 0.5 5
1) PB-A08_DHFR (No iC9) 3
2) P-BCMA-101 3
3) P-BCMA-101 3
4) P-BCMA-101 3
5) P-BCMA-101 3
6) P-BCMA-101 3
24h post-Drug Treatment; Terminally Bleed mice and collect spleens and bone marrow to analyze for the presence of CAR-T cells

FIGURE 6


Highly efficient killing of P-BCMA-101 using Rimiducid *in vivo*
Relative CAR-T Viability (%)
0
25
50
75
100
125
150
175
0 mg/kg (No AP1903)
0.005 mg/kg
0.05 mg/kg
0.5 mg/kg
5 mg/kg
5 mg/kg (No iC9 gene)
Blood
Spleen
Bone Marrow

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/055661

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/705 C07K14/47
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KARIN C STRAATHOF ET AL: "A inducible caspase 9 safety switch for T-cell therapy", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 105, no. 11, 1 June 2005 (2005-06-01) , pages 4247-4254, XP008126232, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2004-11-4564 the whole document pages 4248,4252-4253 and Figures 2, 4, 6 ----- -/--	1-16, 62-121, 123-171

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

[X] See patent family annex.

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 November 2017	13/12/2017

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TEY ET AL: "Inducible Caspase 9 Suicide Gene to Improve the Safety of Allodepleted T Cells after Haploidentical Stem Cell Transplantation", BIOLOGY OF BLOOD AND MARROW TRANSPLANTATION, KLUGE CARDEN JENNINGS PUBLISHING, CHARLOTTESVILLE, VA, US, vol. 13, no. 8, 18 July 2007 (2007-07-18), pages 913-924, XP022157037, ISSN: 1083-8791, DOI: 10.1016/J.BBMT.2007.04.005 abstract pages 914-915; figure 1	1-171
X	WO 2016/135470 A1 (UCL BUSINESS PLC [GB]; SYNCONA PARTNERS LLP [GB]) 1 September 2016 (2016-09-01) pages 1, 2, 27, 28, 30, 31; claims 1-33; sequences 2, 16, 17 & DATABASE Geneseq [Online] 20 October 2016 (2016-10-20), "Human FKBP12-dCasp9 chimeric protein SEQ ID NO: 2.", retrieved from EBI accession no. GSP:BDE42393 Database accession no. BDE42393 sequence	1-171
X	WO 2015/134877 A1 (BELLICUM PHARMACEUTICALS INC [US]; SPENCER DAVID [US]; CHANG WEI-CHUN) 11 September 2015 (2015-09-11) pages 2-14, 37-42, 47-54, 75-81; claims 1-65; figure 1A	1-171
A	NAKAZAWA YOZO ET AL: "Evaluation of Long-term Transgene Expression in piggyBac-Modified Human T Lymphocytes", JOURNAL OF IMMUNOTHERAPY,, vol. 36, no. 1, 1 January 2013 (2013-01-01), pages 3-10, XP002769988, abstract	1-171
A	Z JIN ET AL: "The hyperactive Sleeping Beauty transposase SB100X improves the genetic modification of T cells to express a chimeric antigen receptor", GENE THERAPY, vol. 18, no. 9, 31 March 2011 (2011-03-31) , pages 849-856, XP55399030, GB ISSN: 0969-7128, DOI: 10.1038/gt.2011.40 abstract	1-171

2 -/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M. V. MAUS ET AL: "Antibody-modified T cells: CARs take the front seat for hematologic malignancies", BLOOD, vol. 123, no. 17, 24 April 2014 (2014-04-24), pages 2625-2635, XP55185132, ISSN: 0006-4971, DOI: 10.1182/blood-2013-11-492231 abstract -----	1-171

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/055661

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		CN 107207621 A	26-09-2017
		EP 3197453 A1	02-08-2017
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		WO 2016135470 A1	01-09-2016
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		EP 3114217 A1	11-01-2017
		US 2015328292 A1	19-11-2015
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