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Self-replicating cell selective gene delivery compositions, methods, and uses thereof

Hana Totary-Jain

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US010709759B1

(12) **United States Patent**
Totary-Jain(10) **Patent No.:** **US 10,709,759 B1**
(45) **Date of Patent:** **Jul. 14, 2020**(54) **SELF-REPLICATING CELL SELECTIVE
GENE DELIVERY COMPOSITIONS,
METHODS, AND USES THEREOF**(71) Applicant: **Hana Totary-Jain**, Wesley Chapel, FL
(US)(72) Inventor: **Hana Totary-Jain**, Wesley Chapel, FL
(US)(73) Assignee: **University of South Florida**, Tampa,
FL (US)(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.(21) Appl. No.: **16/247,022**(22) Filed: **Jan. 14, 2019****Related U.S. Application Data**(63) Continuation of application No. 15/333,074, filed on
Oct. 24, 2016, now Pat. No. 10,188,750.(60) Provisional application No. 62/264,609, filed on Dec.
8, 2015, provisional application No. 62/245,457, filed
on Oct. 23, 2015.(51) **Int. Cl.**
C07H 21/04 (2006.01)
A61K 38/17 (2006.01)
A61K 31/7088 (2006.01)(52) **U.S. Cl.**
CPC **A61K 38/1709** (2013.01); **A61K 31/7088**
(2013.01); **C12Y 207/07048** (2013.01); **C12N**
2310/113 (2013.01); **C12N 2710/10343**
(2013.01)(58) **Field of Classification Search**
None
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

4,921,475 A 5/1990 Sibalís
5,008,110 A 4/1991 Benecke et al.
5,087,240 A 2/1992 Sibalís
5,088,977 A 2/1992 Sibalís
5,139,941 A 8/1992 Muzyczka et al.
5,163,899 A 11/1992 Sibalís
5,164,189 A 11/1992 Farhadieh et al.
5,252,479 A 10/1993 Srivastava
5,254,346 A 10/1993 Tucker et al.
5,290,561 A 3/1994 Farhadieh et al.
5,328,470 A 7/1994 Nabel et al.
5,332,213 A 7/1994 Klose
5,336,168 A 8/1994 Sibalís
5,352,456 A 10/1994 Fallon et al.
5,407,713 A 4/1995 Wilfong et al.
5,641,670 A 6/1997 Treco et al.
5,747,469 A 5/1998 Roth et al.
6,017,524 A 1/2000 Roth et al.
6,143,290 A 11/2000 Zhang et al.
6,410,010 B1 6/2002 Zhang et al.
6,511,847 B1 1/2003 Zhang et al.
7,232,806 B2 6/2007 Tuschl et al.

8,398,968 B2 3/2013 Mayall
8,404,653 B2 3/2013 Zsebo
2004/0057937 A1 3/2004 Jahoda et al.
2006/0105360 A1 5/2006 Croce et al.
2010/0041737 A1 2/2010 Naldini et al.
2011/0218234 A1 9/2011 Annoni et al.
2012/0128643 A1 5/2012 Biffi et al.
2013/0202532 A1 8/2013 Benenson et al.
2015/0167003 A1 6/2015 Naldini et al.
2015/0190532 A1 7/2015 Totary-Jain et al.

FOREIGN PATENT DOCUMENTS

WO 1994013788 A1 6/1994
WO 2001032840 A2 5/2001
WO 2007100870 A2 9/2007
WO 2013152230 A1 10/2013

OTHER PUBLICATIONS

Malek et al., 2001. A mouse knock-in model exposes sequential
proteolytic pathways that regulate p27Kip1 in G1 and S phase,
Nature, 413, 323-327.
Fiers et al., 1973. Nature 273: 113-120.
Hager et al., 2002. Curr Opin Genet Dev, 12(2): 137-41.
Zeng et al., 2002. Molecular Cell 9:1327-1333.
Tuschl, 2002. Nat. Biotechnol, 20:446-448.
Brummelkamp et al., 2002. Science 296:550-553.
Miyagishi et al., 2002. Nat. Biotechnol. 20:497-500.
Paddison et al., 2002. Genes Dev. 16:948-958.
Lee et al., 2002. Nat. Biotechnol. 20:500-505.
Paul et al., 2002. Nat. Biotechnol. 20:505-508.
Rabinowitz et al., 2002. J Virol 76:791-801.
Eglitis, 1988. Biotechniques 6:608-614.
Miller, 1990. Hum. Gene Therap. 1:5-14.
Xia et al., 2002. Nat. Biotech. 20:1006-1010.
Samulski et al., 1987. J. Virol. 61:3096-3101.
Fisher et al., 1996. J. Virol. 70:520-532.
Samulski et al., 1989. J. Virol. 63:3822-3826.
Madzak et al., 1992. J Gen Virol. 73(Pt 6):1533-6.
Berkner, 1992. Curr Top Microbiol Immunol. 158:39-66.
Berkner, 1988. Biotechniques, 6(7):616-29.
Gorziglia & Kapikian, 1992. J Virol. 66(7):4407-12.
Quantin et al., 1992. Proc Natl Acad Sci USA. 89(7):2581-4.
Rosenfeld et al., 1992. Cell. 68(1):143-55.
Wilkinson et al., 1992. Nucleic Acids Res. 20(9):2233-9.
Stratford-Perricaudet et al., 1990. Hum Gene Ther. 1(3):241-56.
Moss, 1992. Curr Opin Biotechnol. 3(5):518-22.
Muzyczka, 1992. Curr Top Microbiol Immunol. 158:97-129.
Ohi et al., 1990. Gene. 89(2):279-82.
Margolskee, 1992. Curr Top Microbiol Immunol. 158:67-95.
Johnson et al., 1992. Brain Res Mol Brain Res. 12(1-3):95-102.

(Continued)

Primary Examiner — Kimberly Chong(74) *Attorney, Agent, or Firm* — Thomas|Horstemeyer,
LLP(57) **ABSTRACT**

Described herein are cell-selective mRNA constructs that
can contain a RNA of interest and one or more miRNA
targets. The cell-selective mRNA constructs described
herein can be used to express an RNA of interest to a cell in
a cell-selective manner.

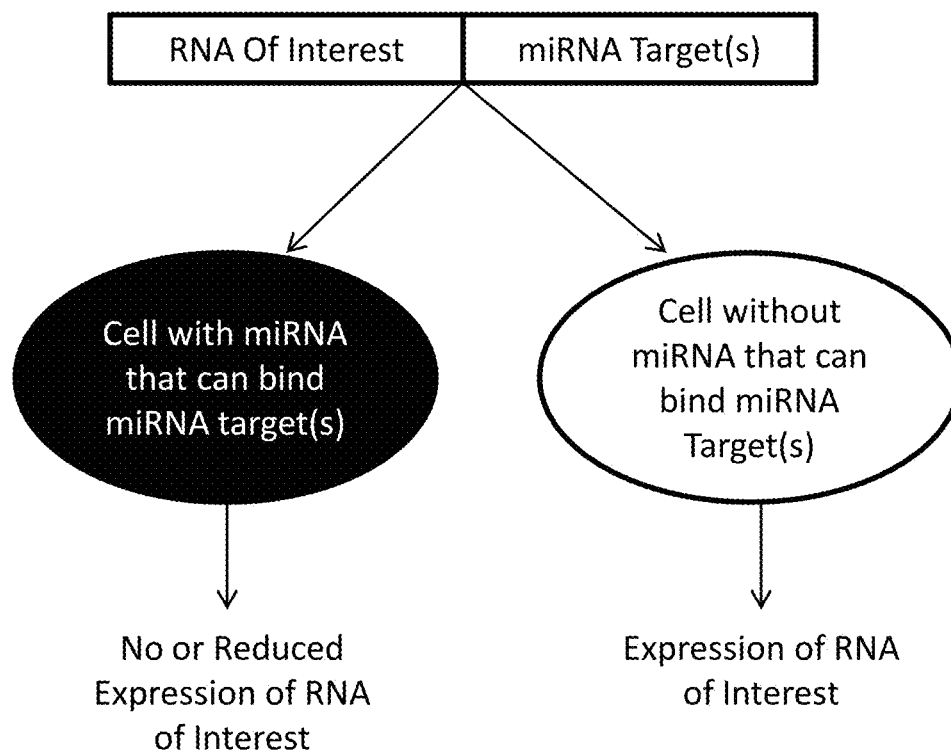
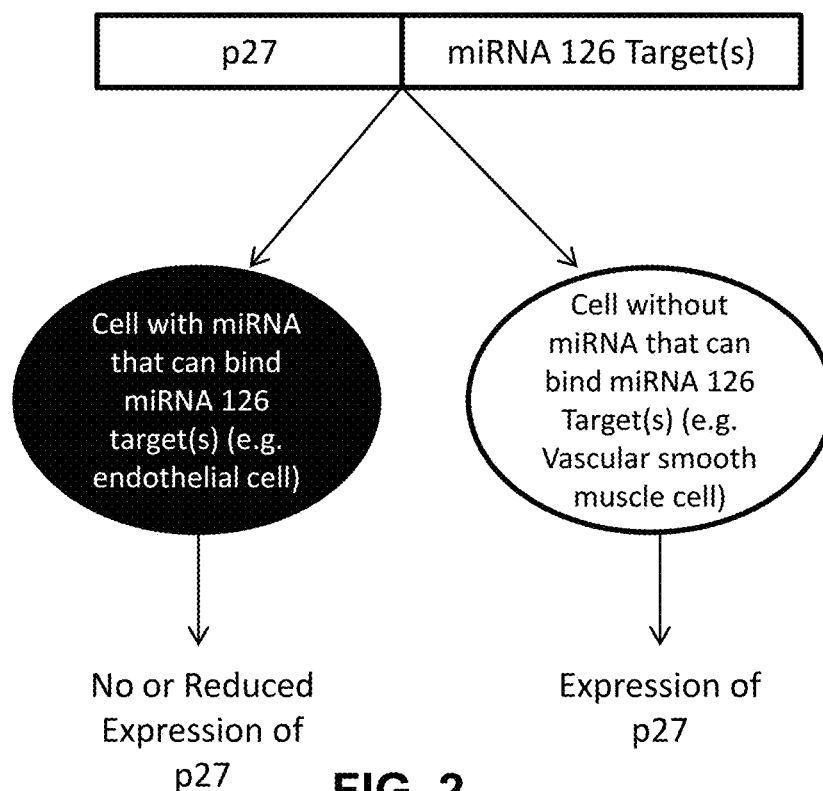
12 Claims, 34 Drawing Sheets**Specification includes a Sequence Listing.**

(56)

References Cited

OTHER PUBLICATIONS

- Fink et al., 1992. *Hum Gene Ther.* 3(1):11-9.
- Breakefield & Geller, 1987. *Mol Neurobiol.* 1(4):339-71.
- Freese et al., 1990. *Biochem Pharmacol.* 40(10):2189-99.
- Bandyopadhyay & Temin, 1984. *Mol Cell Biol.* 4(4):749-54.
- Petropoulos et al., 1992. *J Virol.* 66(6):3391-7.
- Miller et al., 1992. *Mol Cell Biol.* 12(7):3262-72.
- Miller et al., 1985. *J Virol.* 55(3):521-6.
- Sorge et al., 1984. *Mol Cell Biol.* 4(9):1730-7.
- Mann & Baltimore, 1985. *J Virol.* 54(2):401-7.
- Miller et al., 1988. *J Virol.* 62(11):4337-45.
- Shimada et al., 1991. *J Clin Invest.* 88(3):1043-7.
- Helseth et al., 1990. *J Virol.* 64(12):6314-8.
- Page et al., 1990. *J Virol.* 64(11):5270-6.
- Buchschacher & Panganiban, 1992. *J Virol.* 66(5):2731-9.
- Dzau et al., 1993. *Trends in Biotechnology* J 1:205-2W.
- Brower, 1998. *Nature Biotechnology*, 16:1304-1305.
- Chen et al., 1994. *Proc. Natl. Acad. Sci. USA* 91:3054-3057.
- Anderson et al., 1992. *Science* 256:808-813.
- Porter et al., 2011. *NEJM* 365:725-733.
- Kalos et al., 2011. *Sci. Transl. Med.* 201 3(95): 1-11.
- Friedmann, 1989. *Science*, 244:1275-1281.
- Verma, 1990. *Scientific American*: 68-84.
- Kikuchi et al., 2008. *J Dermatol Sci.* May; 50(2):87-98.
- Isaka et al., 2007. *Expert Opin Drug Deily. Sep.*; 4(5):561-71.
- Wachler et al., 2007. *Nat Rev Genet.* Aug.; 8(8):573-87.
- Jensen et al., 2007. *Ann Med.*;39(2): 108-15.
- Herweijer et al., 2007. *Gene Ther.* Jan.; 14(2):99-107.
- Eliyahu et al., 2005. *Molecules*, Jan. 31; 10(1):34-64.
- Altaras et al., 2005. *Adv Biochem Eng Biotechnol.*; 99:193-260.
- Cleveland et al., 1983. *J Immunol Methods*, 56(2): 221-234.
- Lee et al., 2004. *Biomaterials* 25:2461-2466.
- Peppas et al., 2006. *Adv Mater.* 18:1345-60.
- Hoffman, 2002. *Adv Drug Deliv Rev.* 43:3-12.
- Hoffman, 2001. *Ann Ny Acad Sci* 944:62-73.
- Zuker & Stiegler, 1981. *Nucleic Acids Res.* 9, 133-148.
- Carr & Church, 2009. *Nature Biotechnol.* 27: 1151-1162.
- Lieber, 2010. Author's Manuscript, 1-24. Published in: *Annu. Rev. Biochem.* 79: 181-211.
- Bleuyard et al., 2006. *DNA Repair* 5: 1-12.
- Kirik et al., 2000. *EMBO J.* 19: 5562-5566.
- Siebert & Puchta, 2002. *Plant Cell* 14:1121-1131.
- Pacher et al., 2007. *Genetics* 175: 21-29.
- Carillo & Lipman, 1988. *SIAM J. Applied Math.* 48: 1073.
- Needelman & Wunsch, 1970. *J. Mol. Biol.*, 48: 443-453.
- Konermann et al., 2015. Author Manuscript, 1-37. Published as: Genome-scale transcriptional activation by an engineered crisper-cas9 complex. *Nature*. 517:583-588.
- Geall, et al., Nonviral delivery of self-amplifying RNA vaccines, *PNAS U.S.A.* 2012, 109(36): 14604-14609.
- Yoshioka, et al., Efficient Generation of Human iPSCs by a Synthetic Self-Replicative RNA, *Cell Stem Cell* 2013, 13(2): 246-254.
- Bartel, 2009. MicroRNAs: Target Recognition and Regulatory Functions. *Cell*, 136(2), 215-233.
- Brown & Naldini, 2009. Exploiting and antagonizing microRNA regulation for therapeutic and experimental applications. *Nature Reviews. Genetics*, 10(8), 578-585.
- Janssen et al., 2013. Treatment of HCV infection by targeting microRNA. *The New England Journal of Medicine*, 368, 1685-94.
- Karikó et al., 2008. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Molecular Therapy : The Journal of the American Society of Gene Therapy*, 16(11), 1833-1840.
- Krek et al., 2005. Combinatorial microRNA target predictions. *Nature Genetics*, 37(5), 495-500.
- Laursen et al., 2010. Utilization of unlocked nucleic acid (UNA) to enhance siRNA performance in vitro and in vivo. *Molecular bioSystems*, 6(5), 862-870.
- Montgomery et al., 2011. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. *Circulation*, 124, 1-12 and S1-S19.
- Santulli et al., 2014. A selective microRNA-based strategy inhibits restenosis while preserving endothelial function. *Journal of Clinical Investigation*, 124(9), 4102-4114.
- Yeung et al., 2005. siRNA, miRNA and HIV: promises and challenges. *Cell Research*, 15(11-12), 935-946.
- Heron et al., 2009. Deaths: final data for 2006. *Natl Vital Stat Rep* 57(14), 1-134.
- Garg & Serruys, 2010. *Journal of the American College of Cardiology* 56, S1-42.
- Garg & Serruys, 2010. Coronary stents: looking forward. *J Am Coll of Cardiol* 56(10 Suppl), S43-78.
- Jukema et al., 2011. Restenosis after PCI. Part 1: pathophysiology and risk factors. *Nat. Rev. Cardiol.* Advance Online Publication, 1-10.
- Marx et al., 2011. Author Manuscript pp. 1-16. Published as: Vascular smooth muscle cell proliferation in restenosis. *Circ Cardiovasc Interv* 4(1), 104-111.
- Cassese & Kastrati, 2012. *JAMA* 308, 814-815.
- Kotani et al., 2006. *Journal of the American College of Cardiology* 47, 2108-2111.
- Liu et al., 2010. *Tex Heart Inst J* 37, 194-2010.
- Wenaweser et al., 2008. *Journal of the American College of Cardiology* 52, 1134-1140.
- Van Rooij & Olson, 2012. *Nat Rev Drug Discov* 11, 860-872.
- Brown et al., 2006. *Nat Med* 12, 585-591.
- Wang et al., 2008. *Dev Cell* 15, 261-271.
- Hayashi et al., 2009. *The American journal of pathology* 175, 2226--2234.
- Iakovou et al., 2005. *JAMA* 293, 2126-2130.
- Ciccarelli et al., 2008. Endothelial alpha 1-adrenoceptors regulate neoangiogenesis. *Br J Pharmacol* 153, 936-946.
- Santulli et al., 2011. Evaluation of the anti-angiogenic properties of the new selective alphaVbeta3 integrin antagonist RGDechiHCit J Transl Med 9, 7.
- Totary-Jain et al., 2012. Author's Manuscript, pp. 1-14. Published as: Rapamycin resistance is linked to defective regulation of Skp2. *Cancer Res* 72, 1836-1843.
- Iaccarino et al., 1999. Targeting Gbeta gamma signaling in arterial vascular smooth muscle proliferation: a novel strategy to limit restenosis. *Proc Natl Acad Sci USA* 96, 3945-3950.
- Iaccarino et al., 2004. AKT participates in endothelial dysfunction in hypertension. *Circulation* 109, 2587-2593.
- Yamaguchi et al., 2011. Local persistent hypercoagulability after sirolimus-eluting stent implantation in patients with stable angina. *Int J Cardio/153*, 272--276.
- Vestweber, 2008. VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arterioscler Thromb Vase Biol* 28, 223-232.
- Santulli et al., 2009. In vivo properties of the proangiogenic peptide QK. *J Transl Med* 7:41, 1-10.
- Santulli et al., 2012. CaMK4 Gene Deletion Induces Hypertension. *J Am Heart Assoc* 1, e001081.
- Marks, 2003. Sirolimus for the prevention of in-stent restenosis in a coronary artery. *N Engl J Med* 349(14), 1307-1309.
- Wijns, 2009. Late stent thrombosis after drug-eluting stent: seeing is understanding. *Circulation* 120(5), 364-365.
- Finn et al., 2007. Vascular responses to drug eluting stents: importance of delayed healing. *Arterioscler Thromb Vase Biol* 27(7), 1500-1510.
- Joner et al., 2006. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. *J Am Coll Cardiol* 48(1), 193-202.
- Calin et al., 2002. *Proc. Natl. Acad. Sci. USA* 99:15524-29.
- Landgraf et al., 2007. *Cell* 129:1401-1414.
- Mendell, 2005. *Cell Cycle* 4(9):1179-84.
- Shivdasani, 2006. *Blood* 108(12):3646-53.
- Hwang & Mendell, 2006. *Br J Cancer* 94(6):776-80.
- Hammond, 2006. *Curr Opin Genet Dev.* 16(1):4-9.
- Osada & Takahashi, 2007. *Carcinogenesis* 28(1):2-12.
- Zhang et al., 2007. *Dev Biol.* 302(1):1-12.
- Ishida et al., 2013. Author's Manuscript 1-14. Published as: miRNA-Based Therapeutic Strategies, *Curr Anesthesiol Rep.*, 1(1):63-70.

**FIG. 1****FIG. 2**

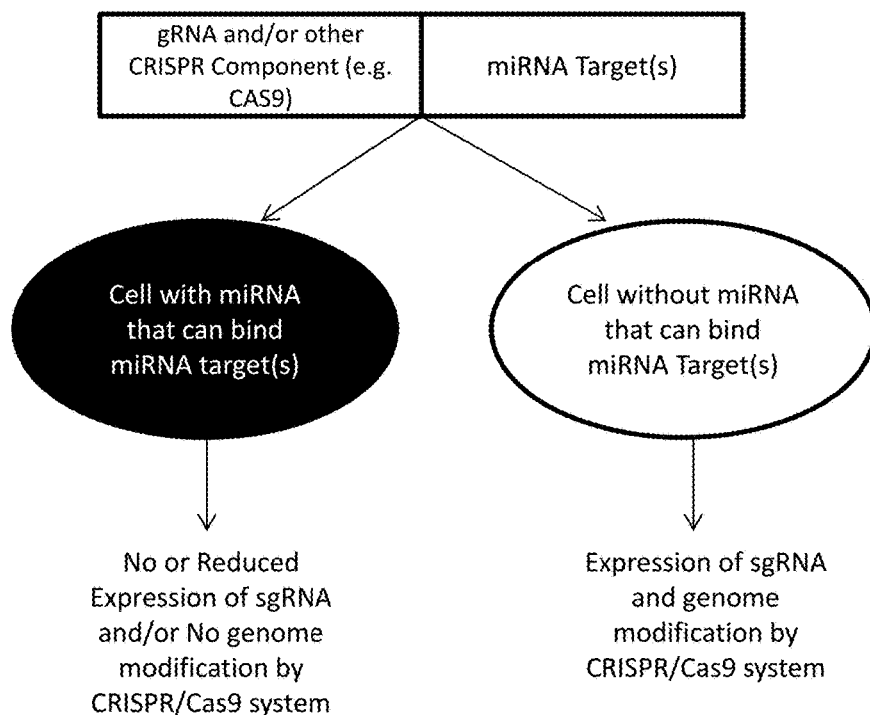


FIG. 3

Synthesizing mRNA

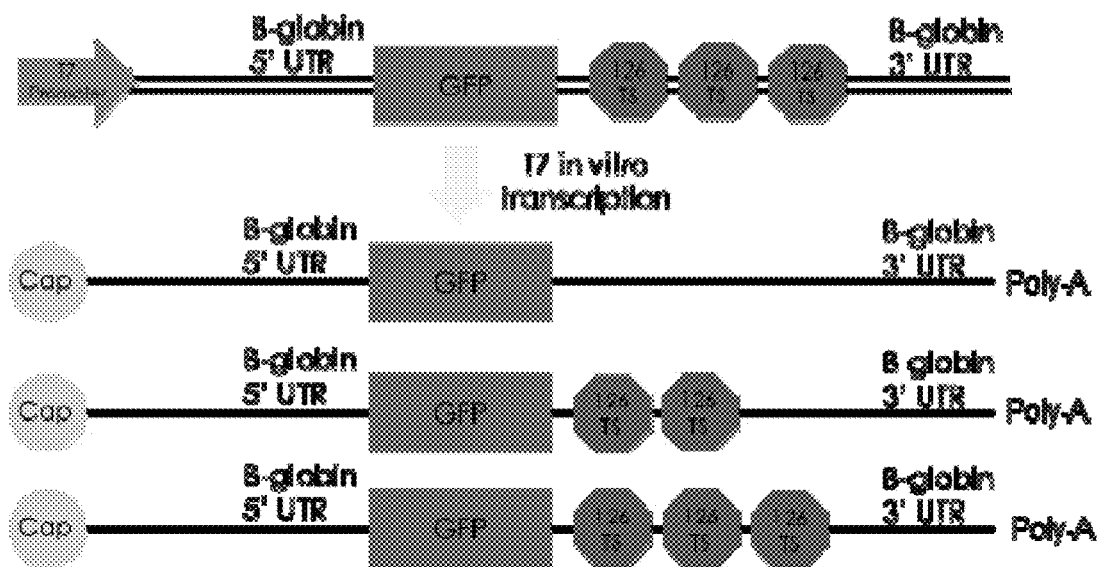
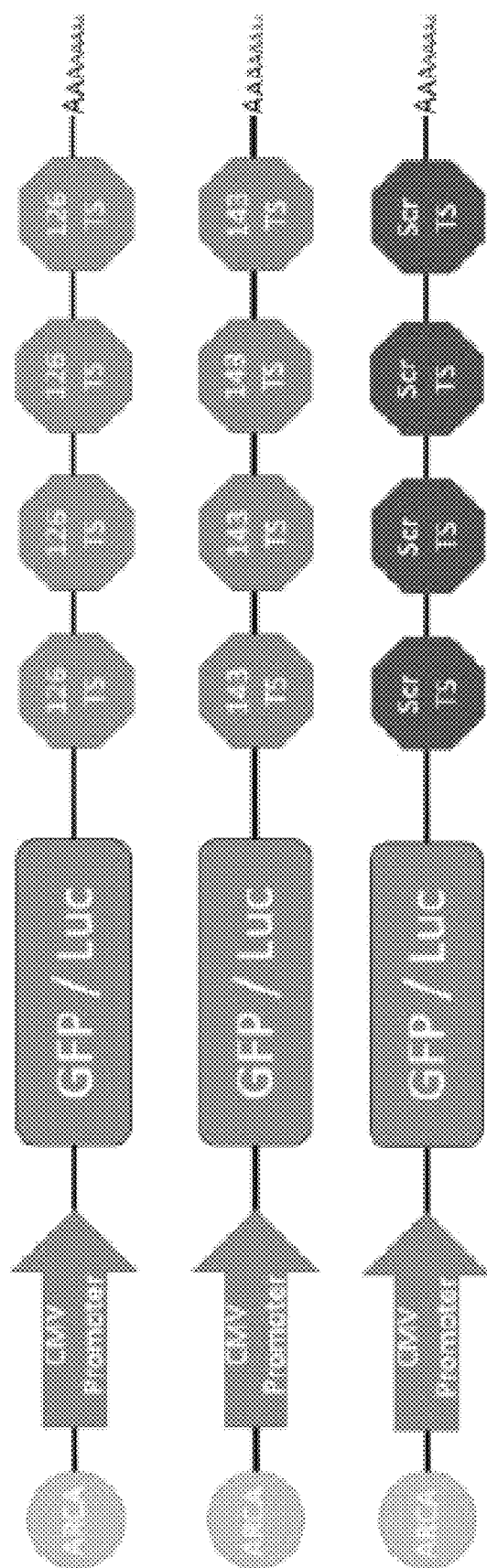


FIG. 4



5
6
7

miRNA-mediated silencing corresponds
to the number of target sites

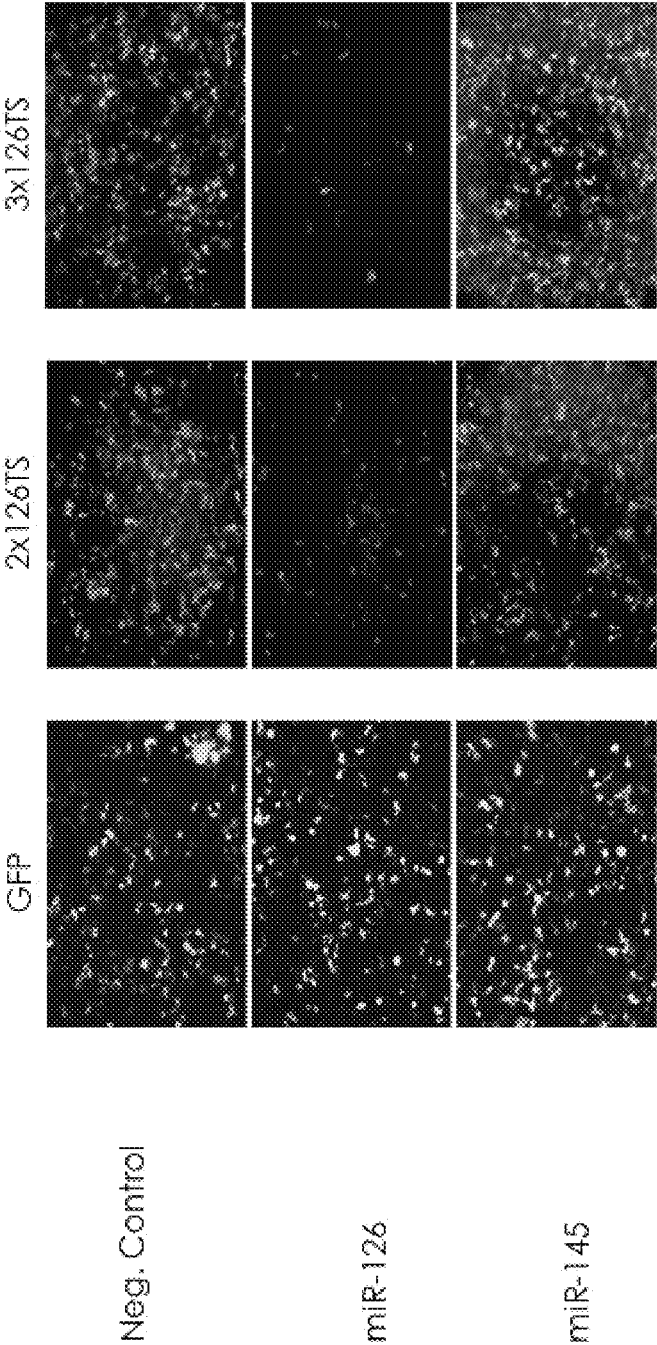


FIG. 6

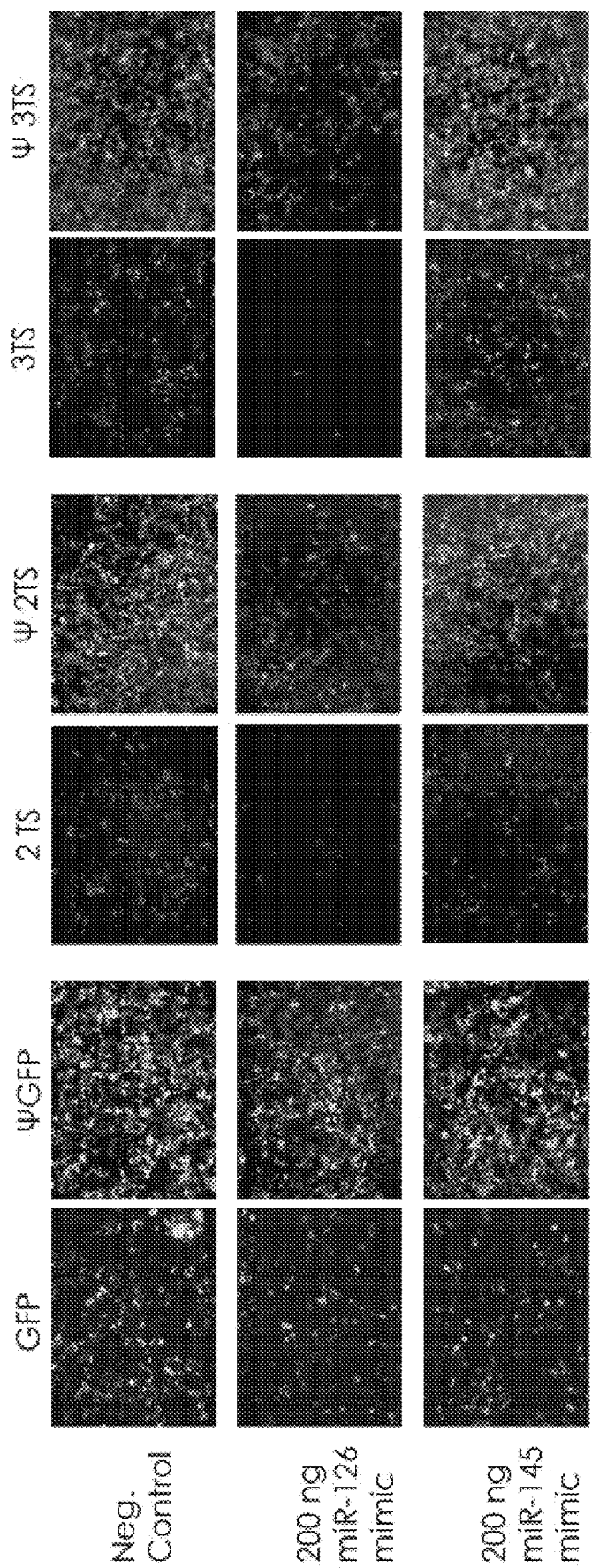


FIG. 7

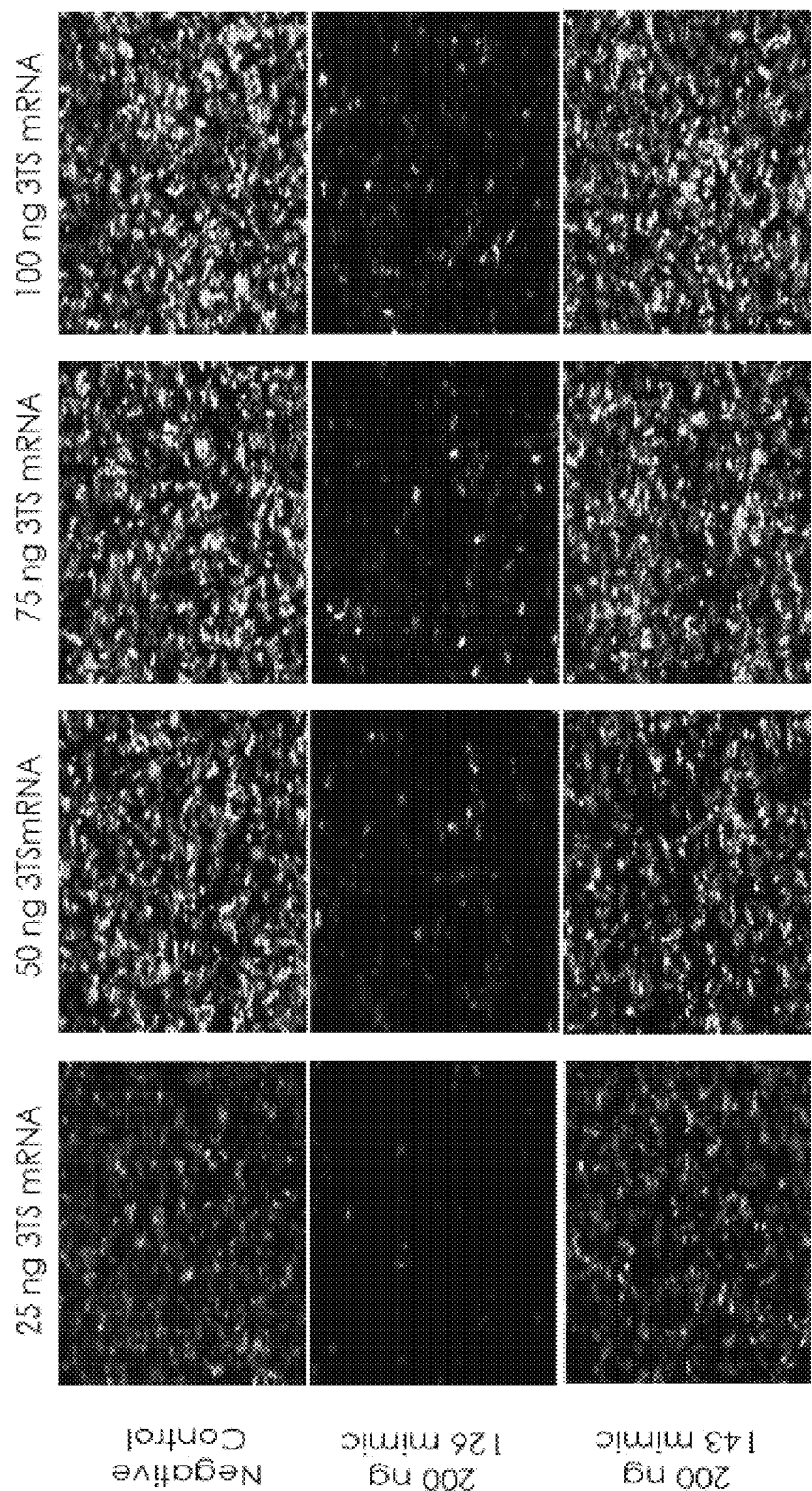
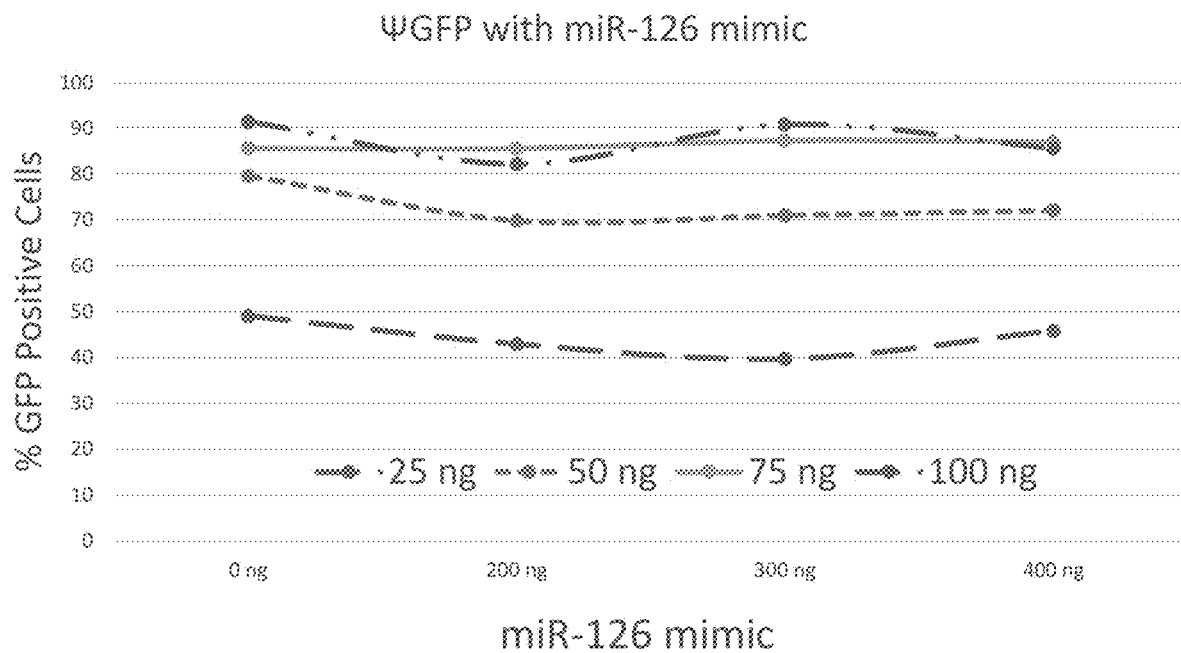
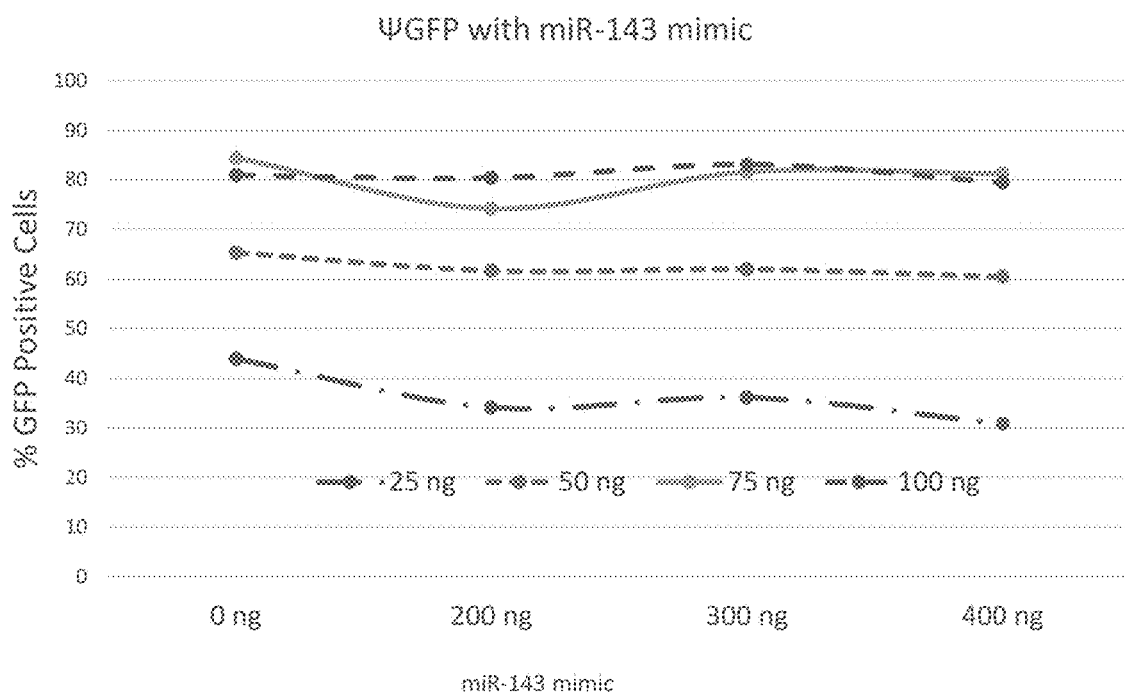
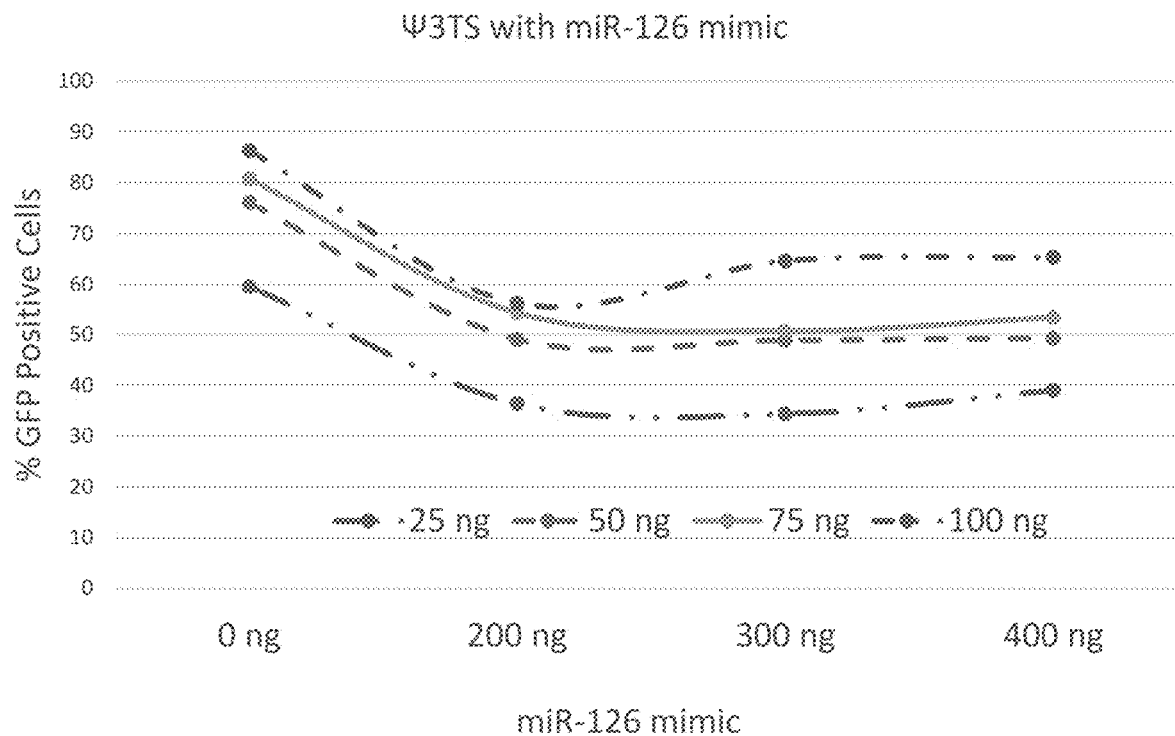
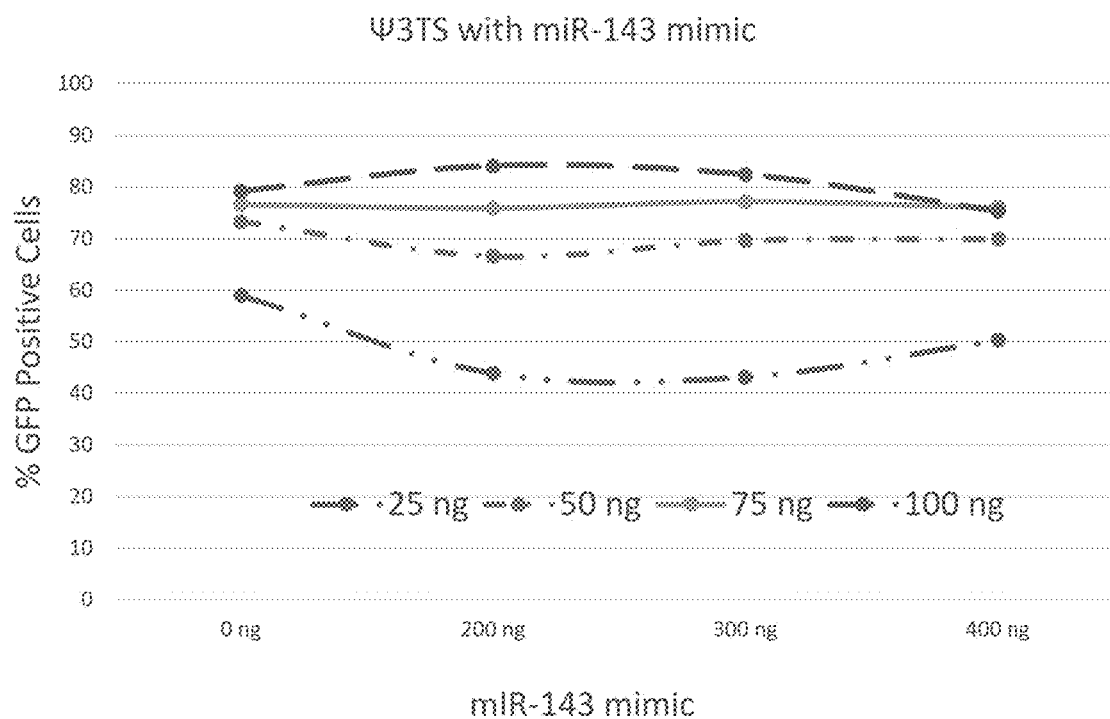
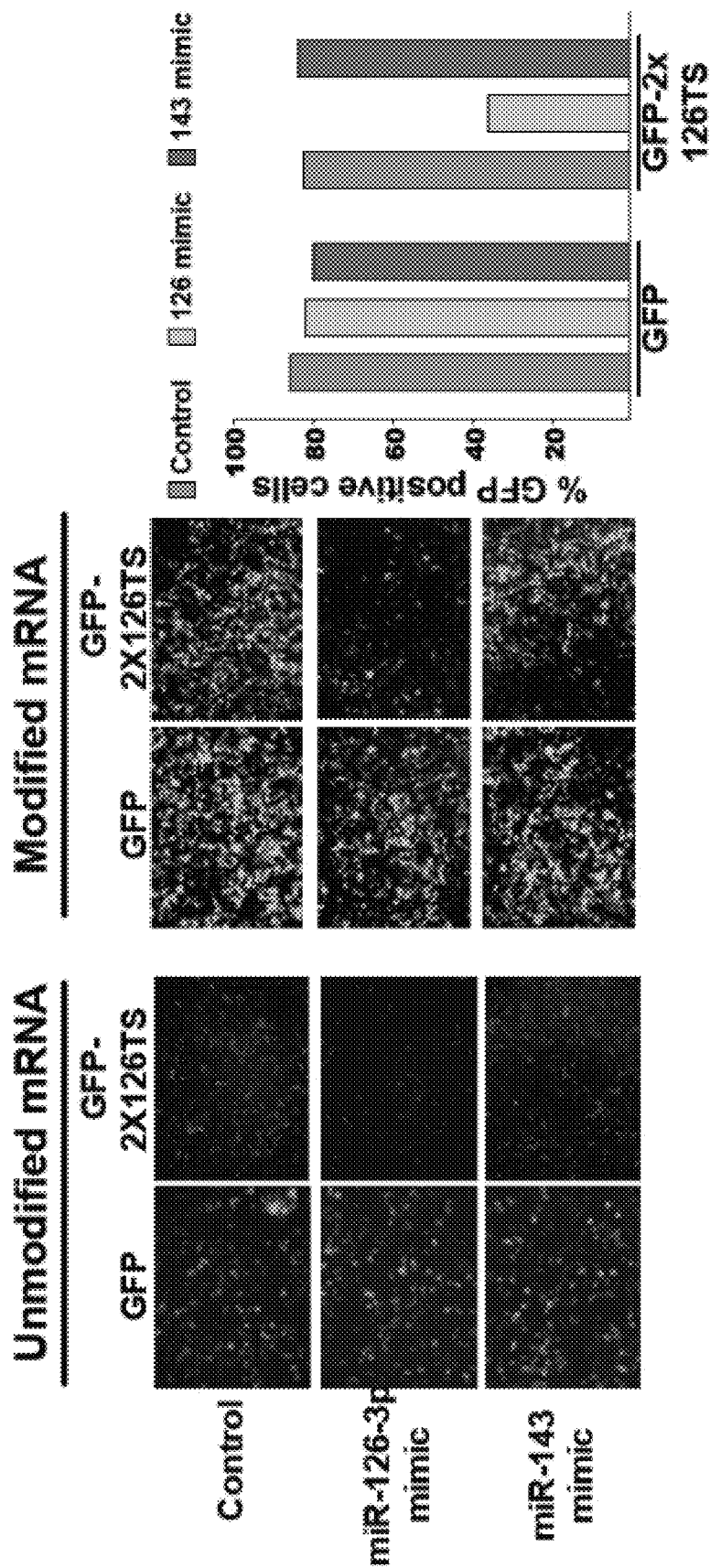


FIG. 8

**FIG. 9****FIG. 10**

**FIG. 11****FIG. 12**



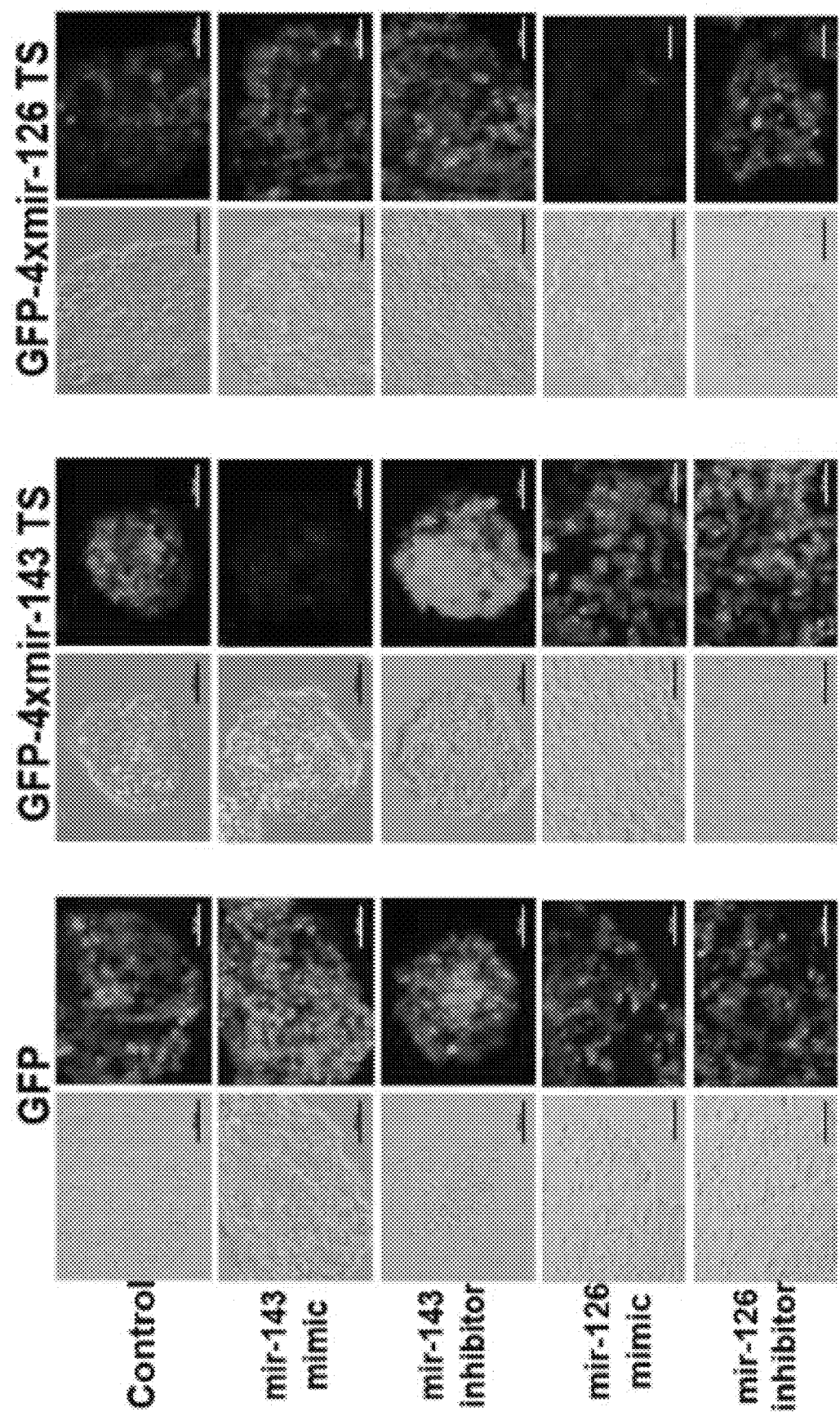


FIG. 15

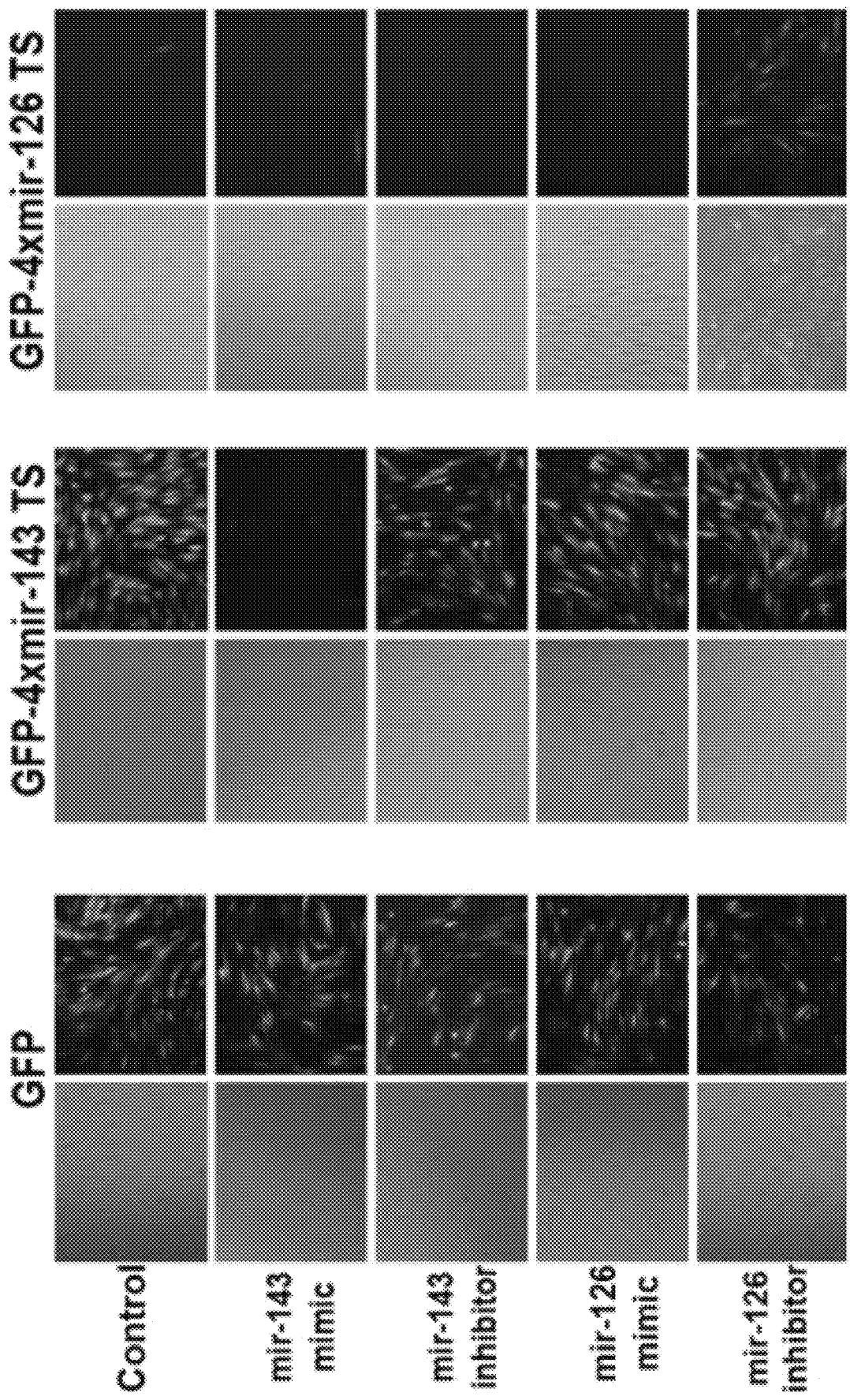


FIG. 16

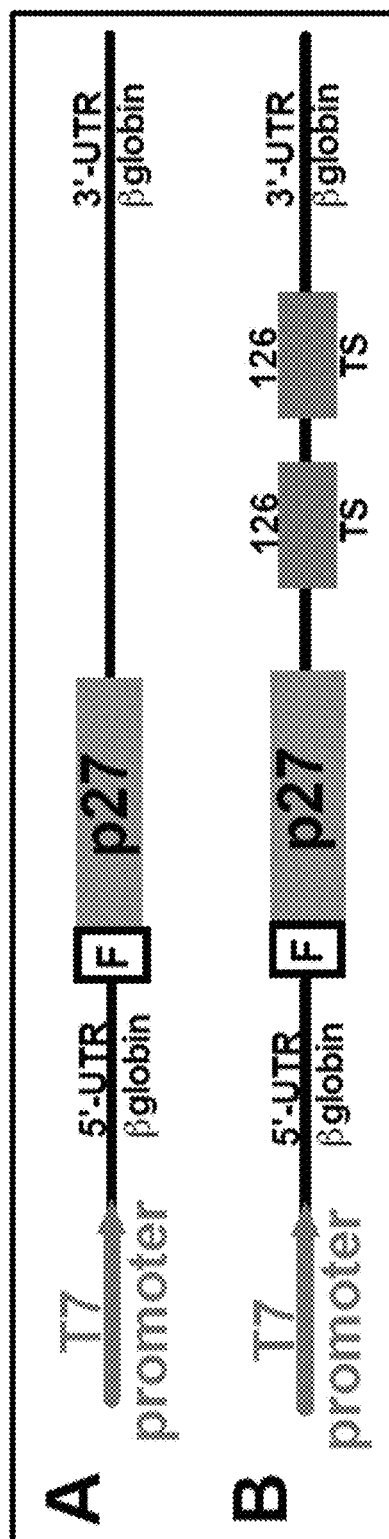


FIG. 17

Self-replicating mRNA synthesis

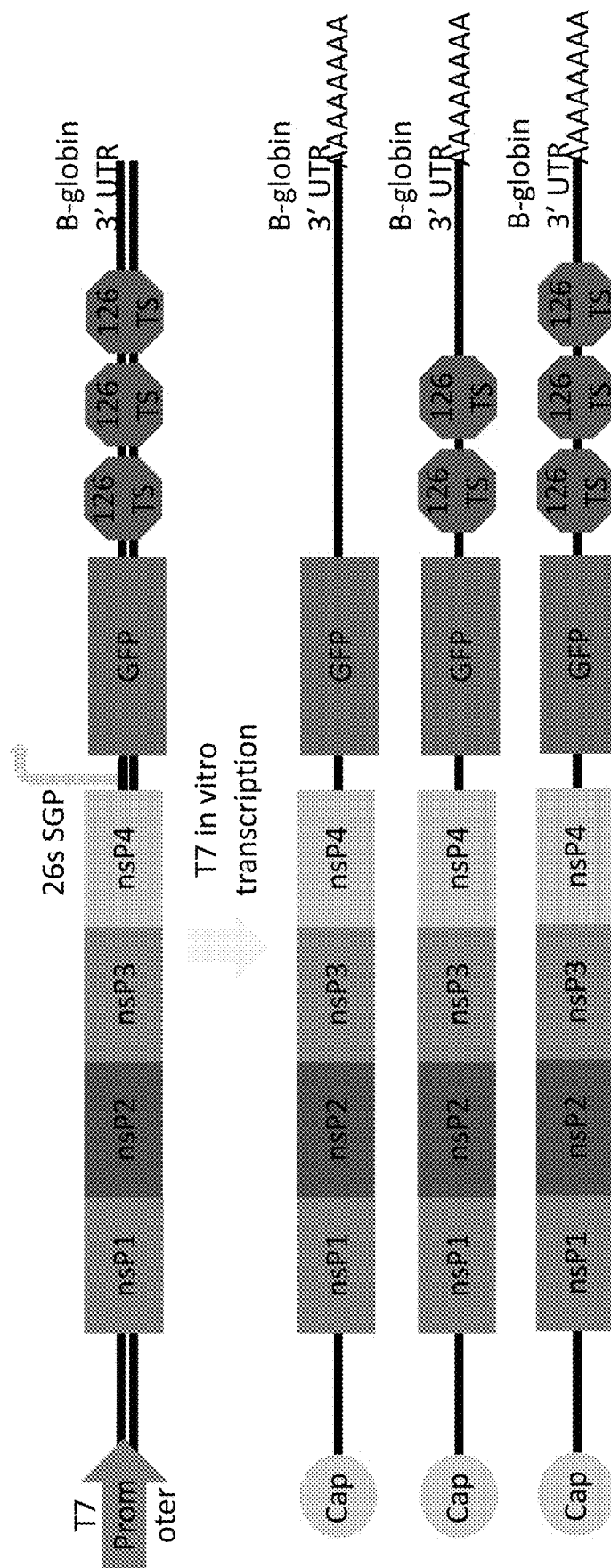


FIG. 18

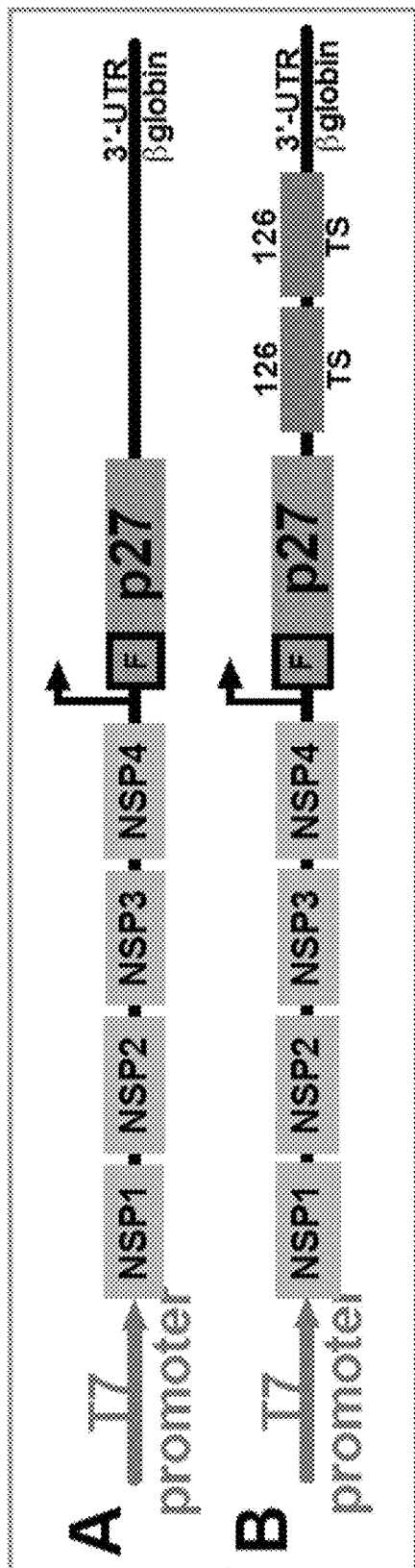


FIG. 19

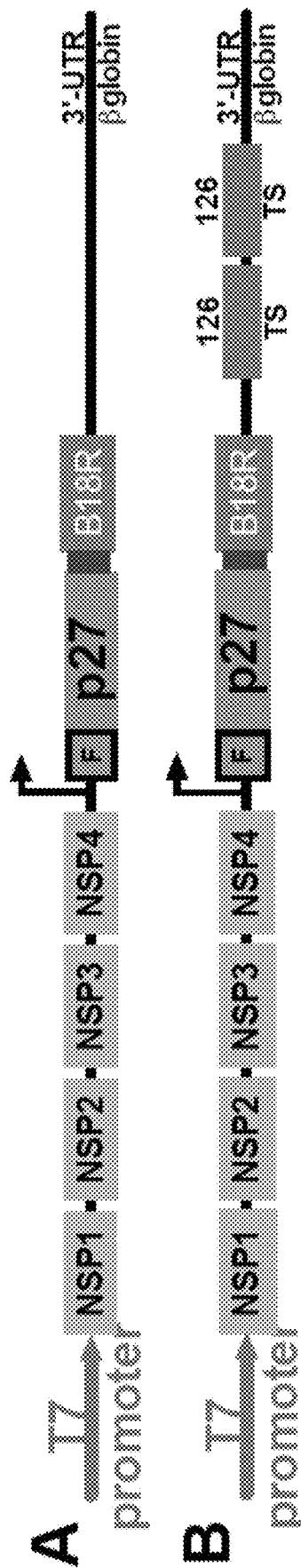


FIG. 20

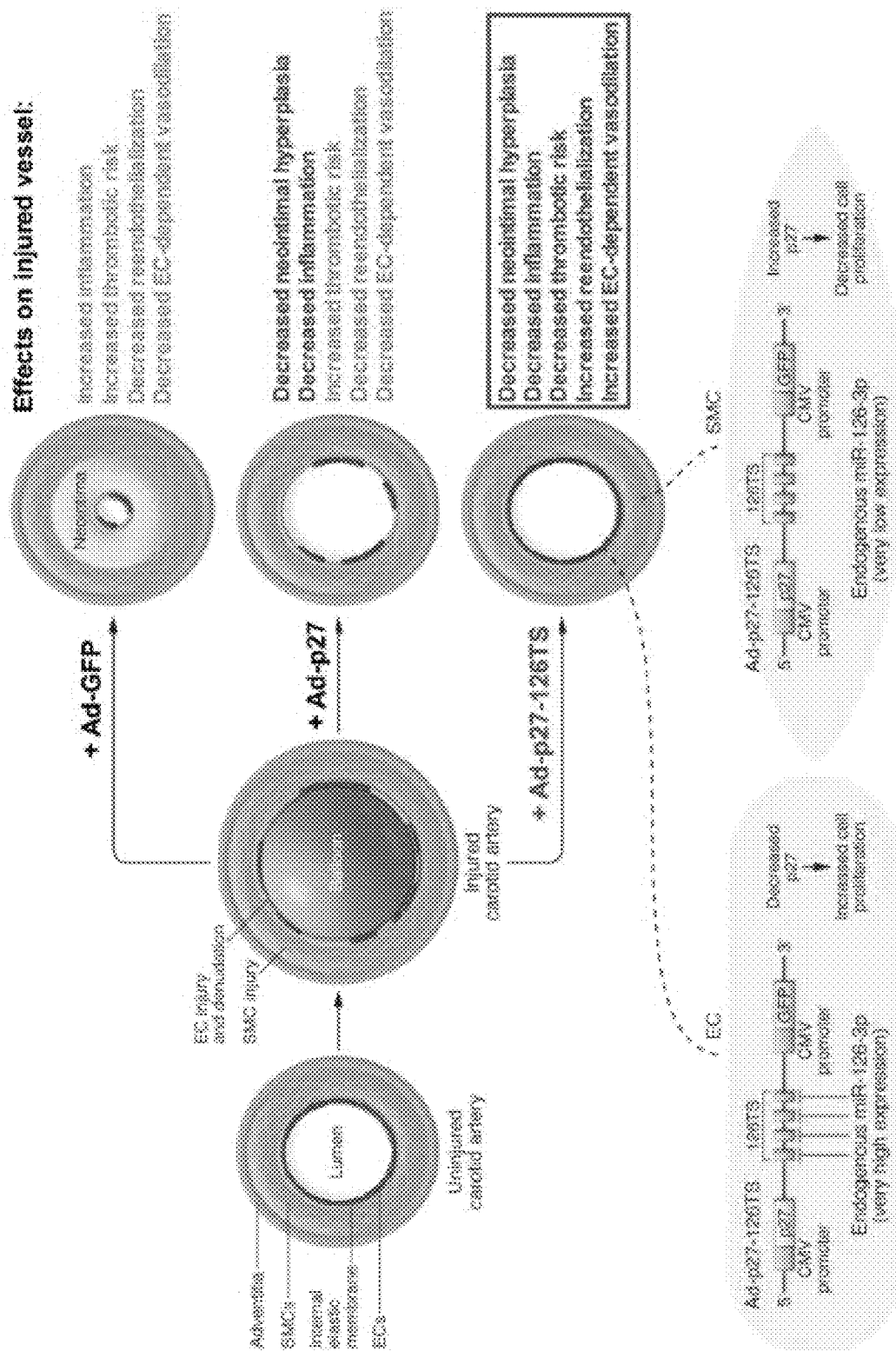


FIG. 21

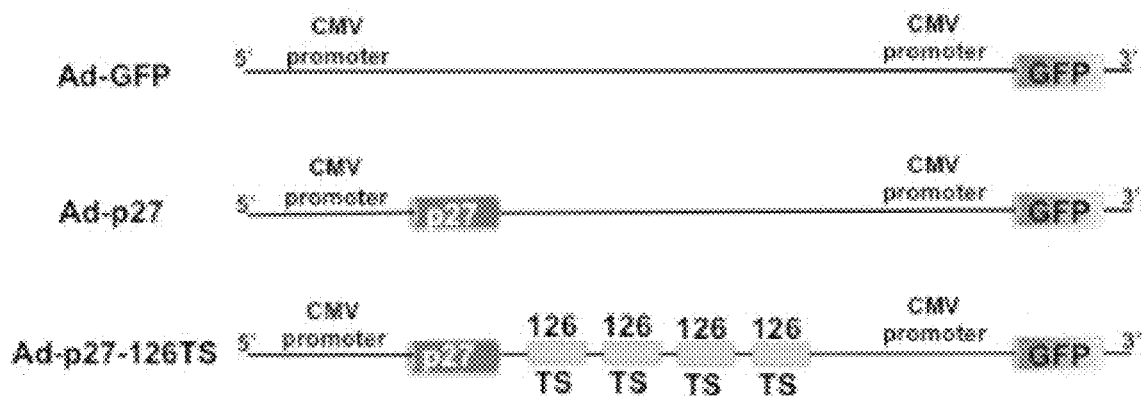


FIG. 22

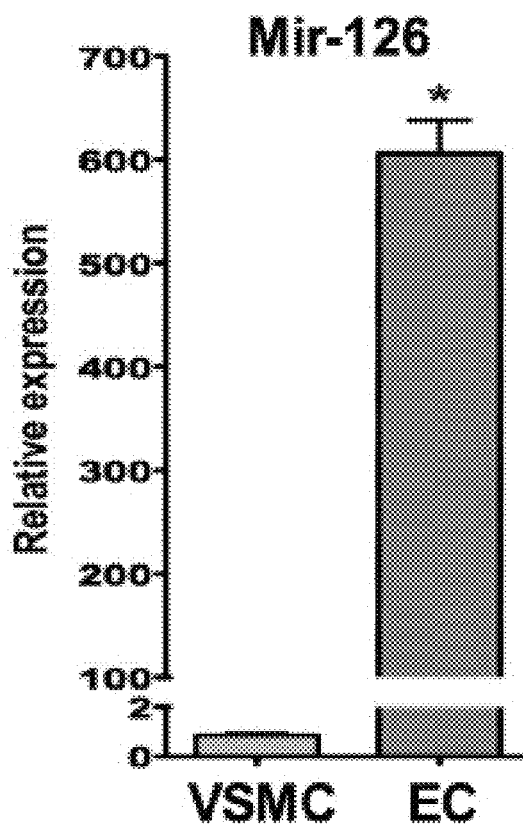


FIG. 23

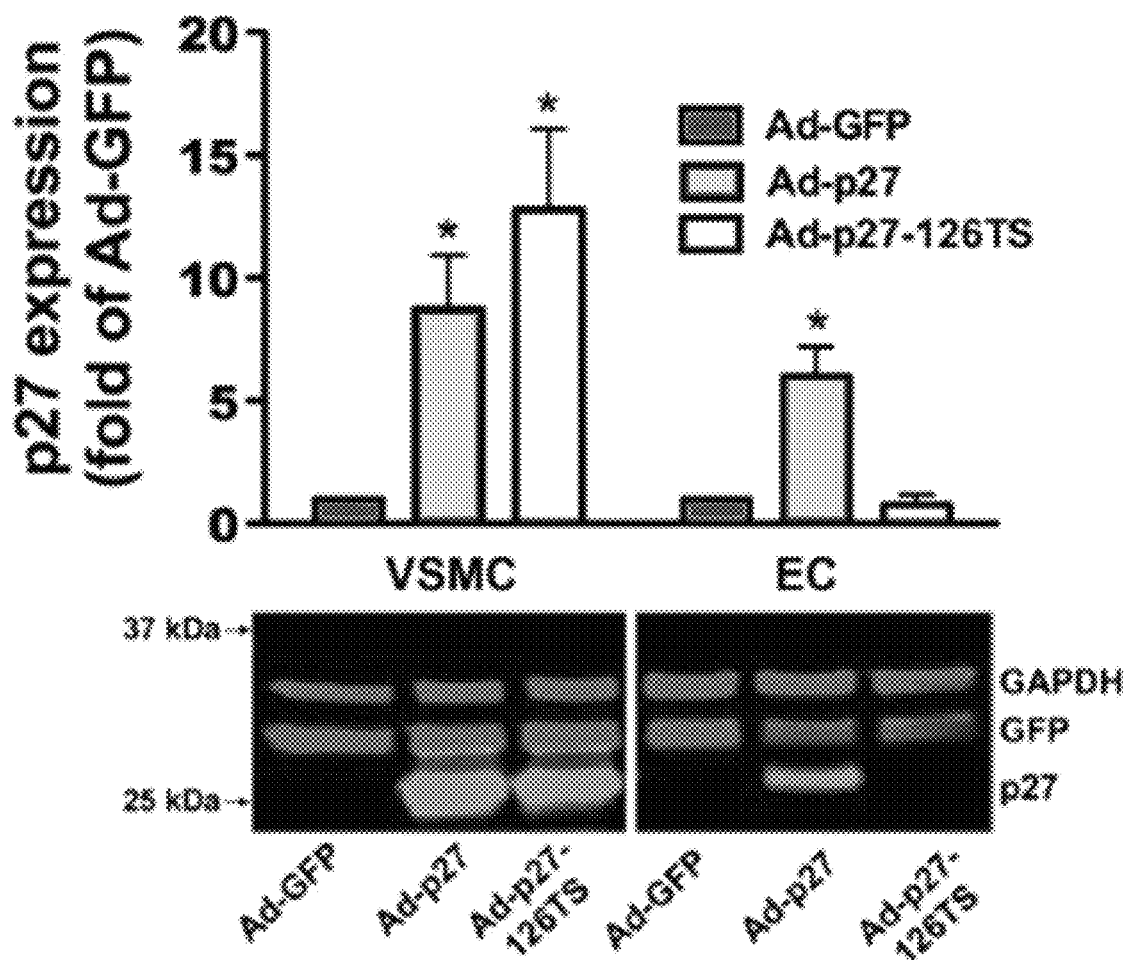


FIG. 24

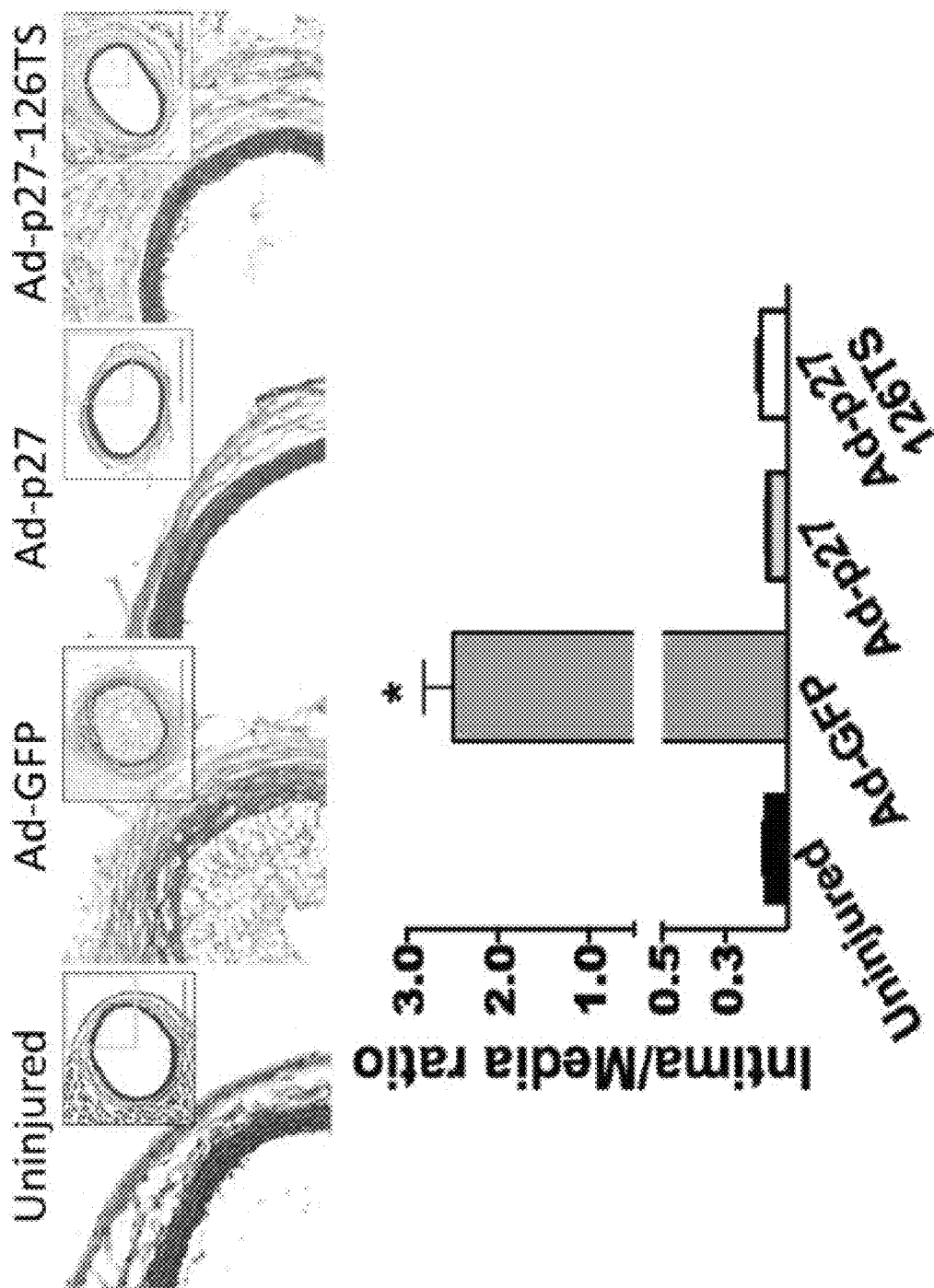


FIG. 25

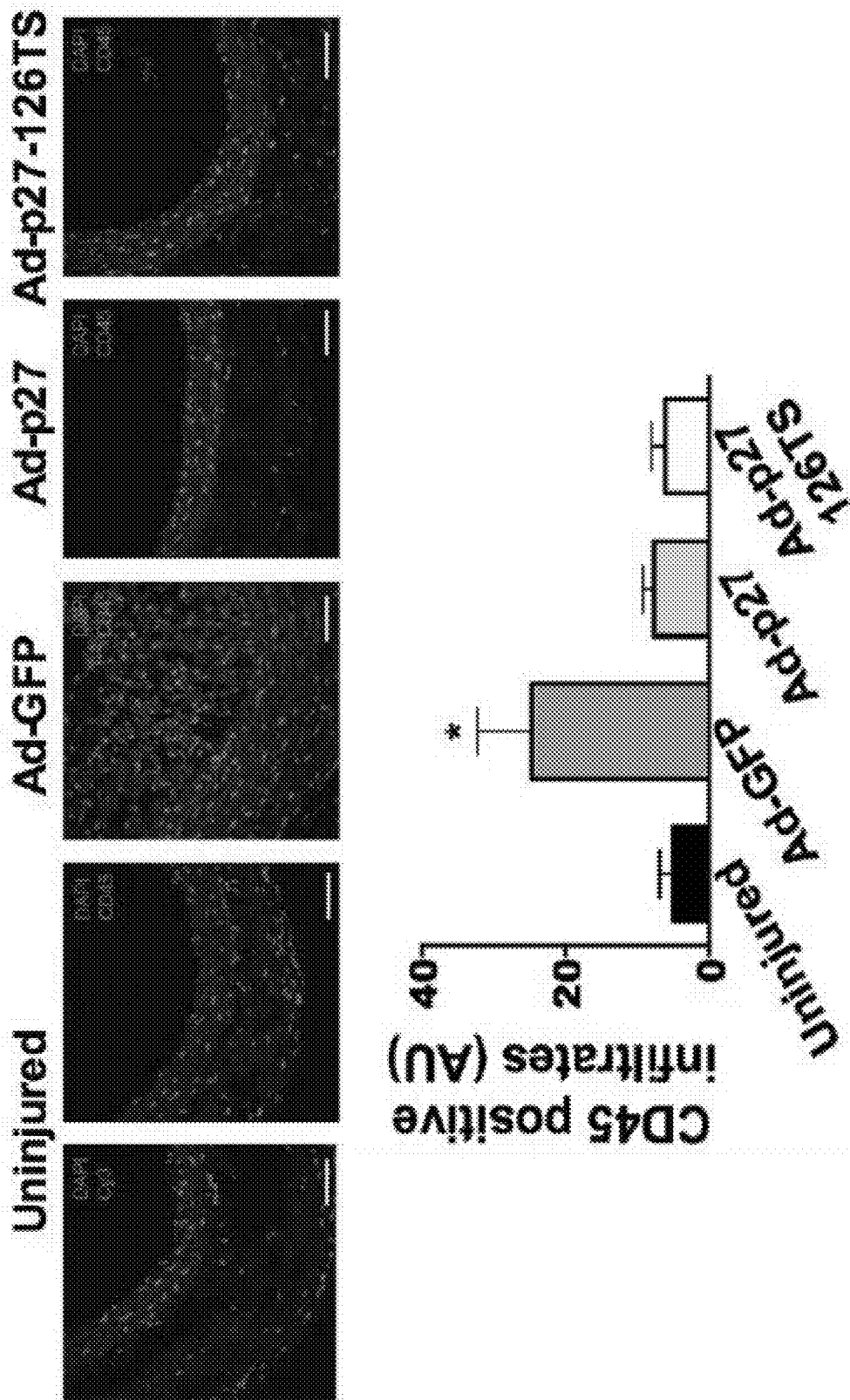


FIG. 26

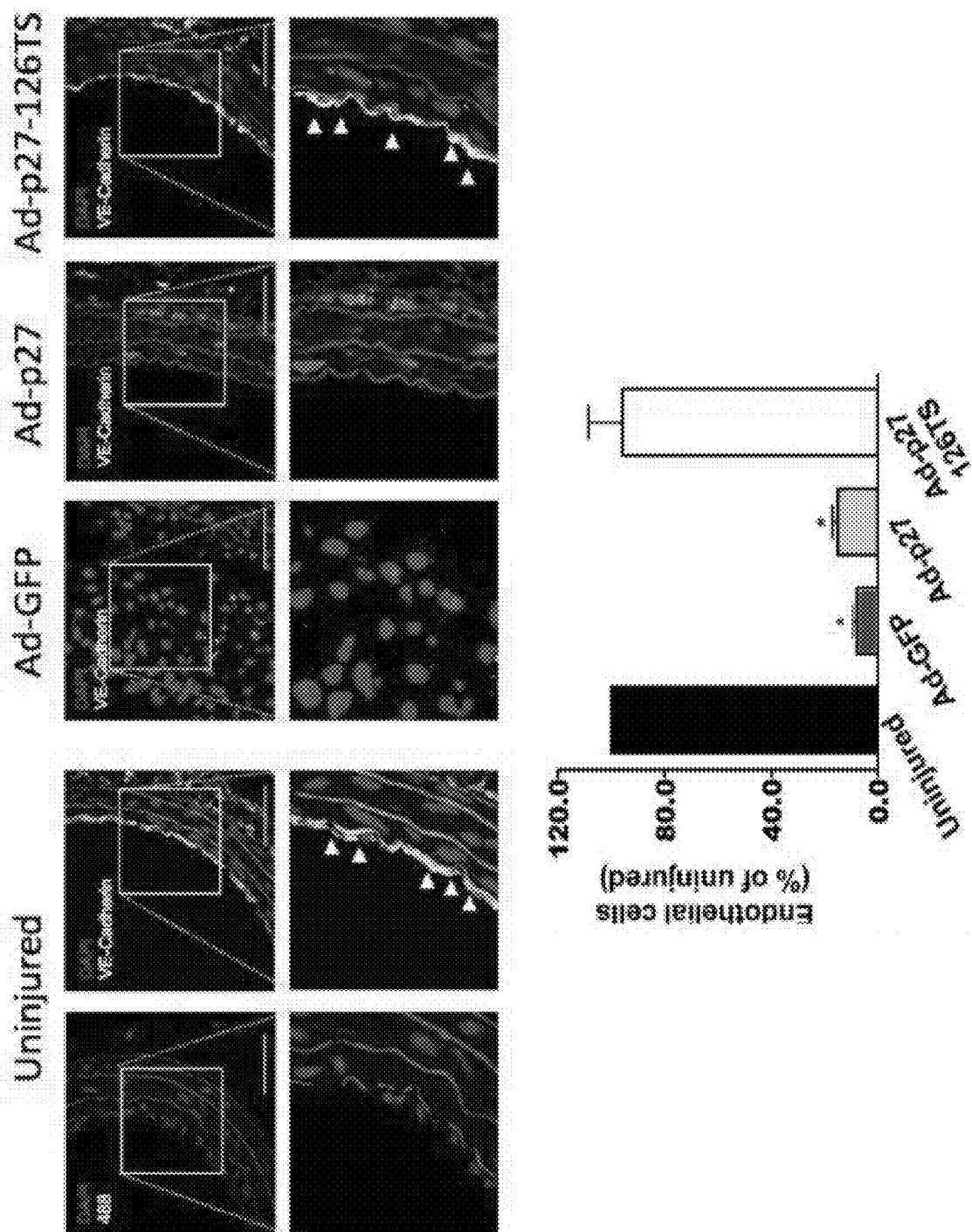


FIG. 27A

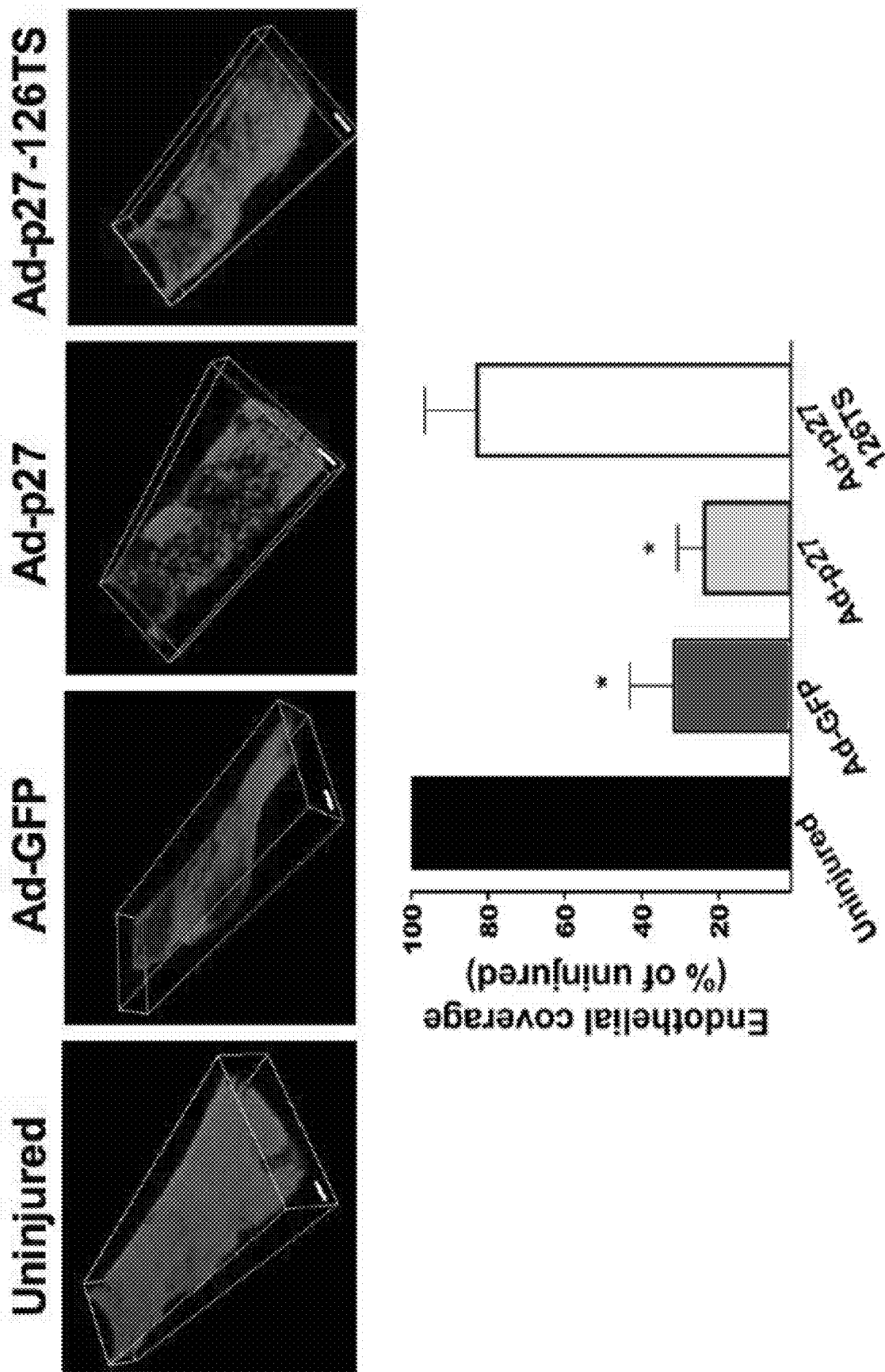


FIG. 27B

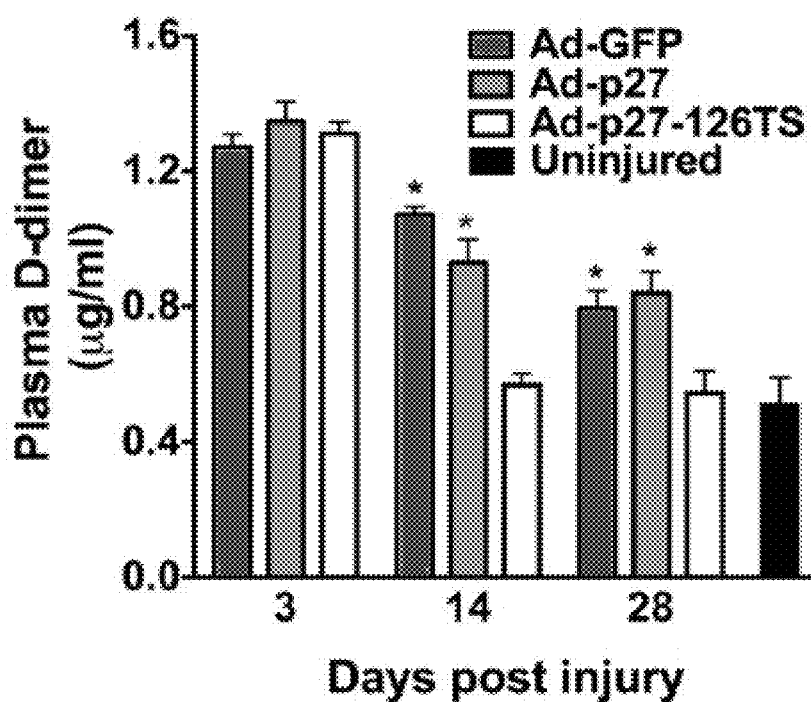


FIG. 28

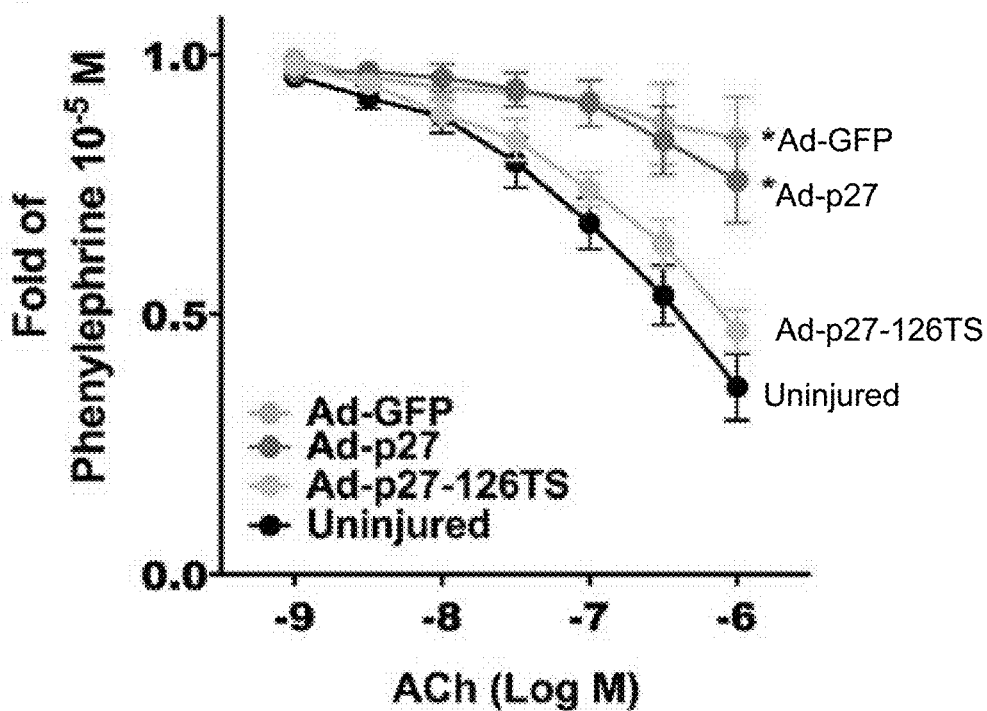


FIG. 29

Nucleotide	Catalog Number
Pseudouridine	N-1019
N-1-methylpseudouridine	N-1081
5-methoxyuridine	N-1093
5-hydroxymethylcytidine	N-1087
Immune Stimulation Reduction Transcription Nucleotide Set	K-1018
ARCA Cap	N-7003
Unmodified dNTP Set	N-2505

FIG. 30

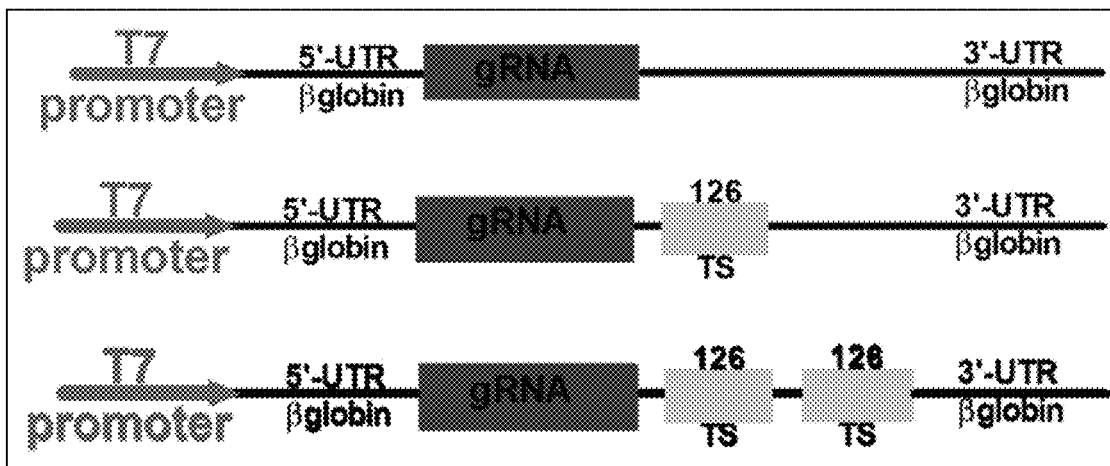


FIG. 31

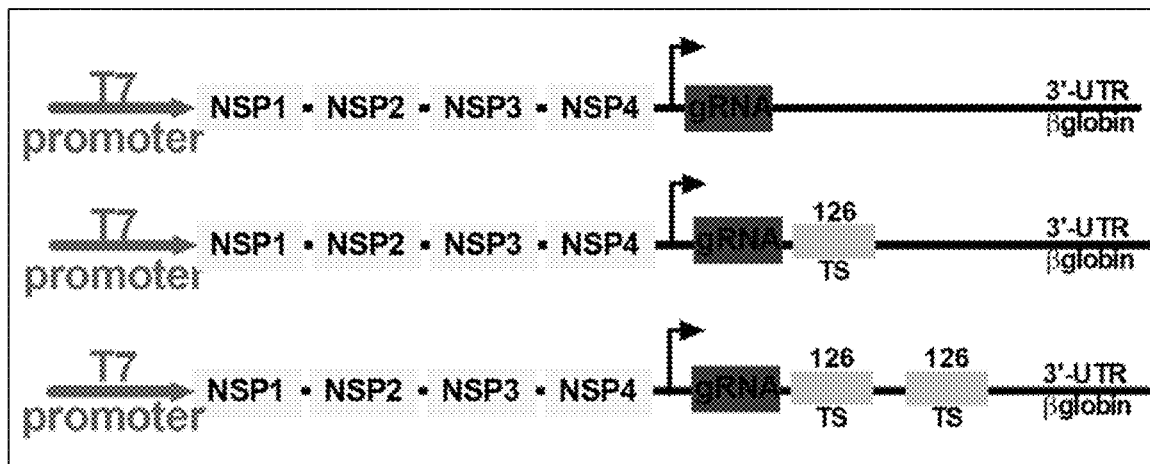


FIG. 32

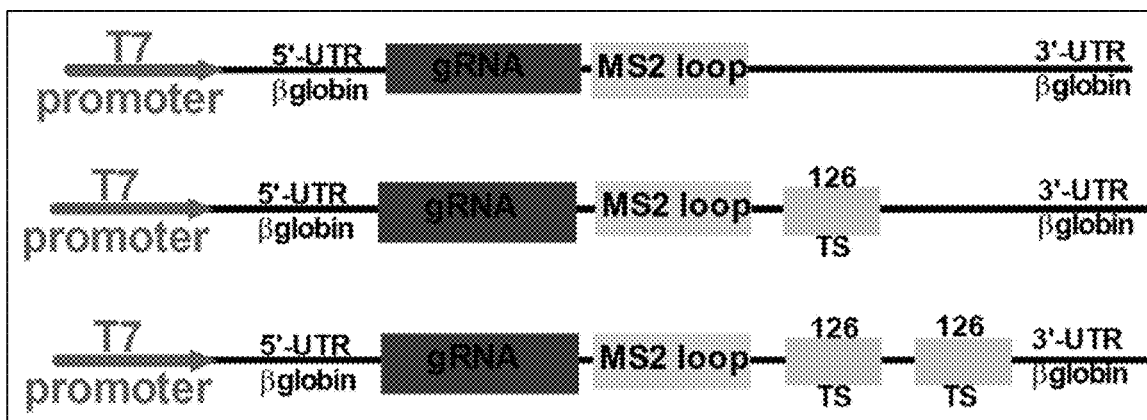


FIG. 33

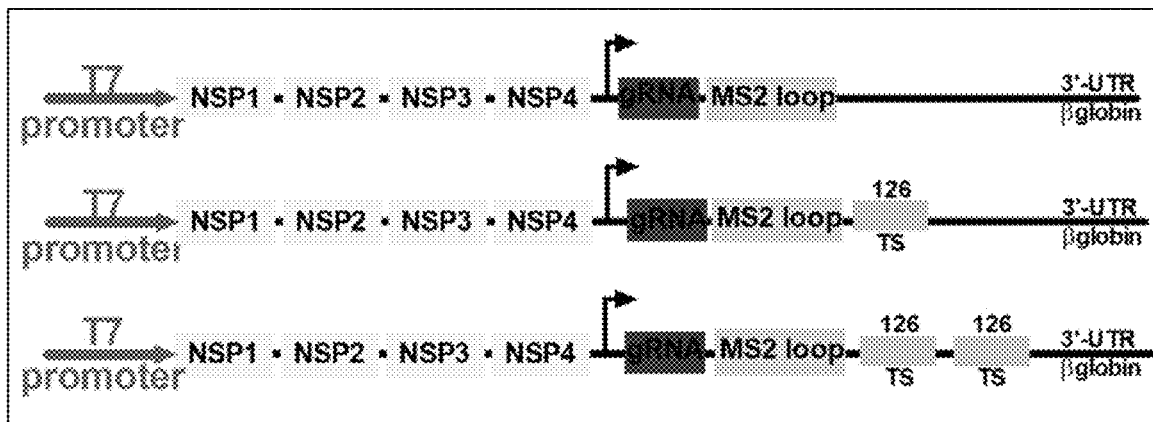


FIG. 34

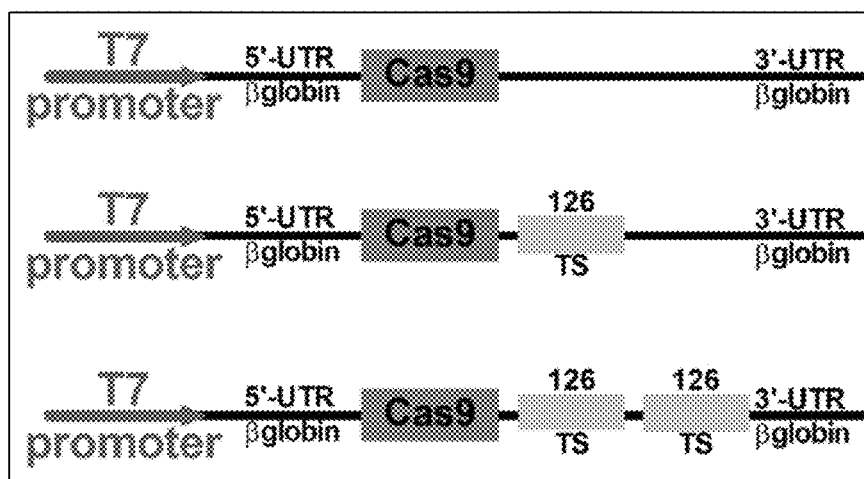


FIG. 35

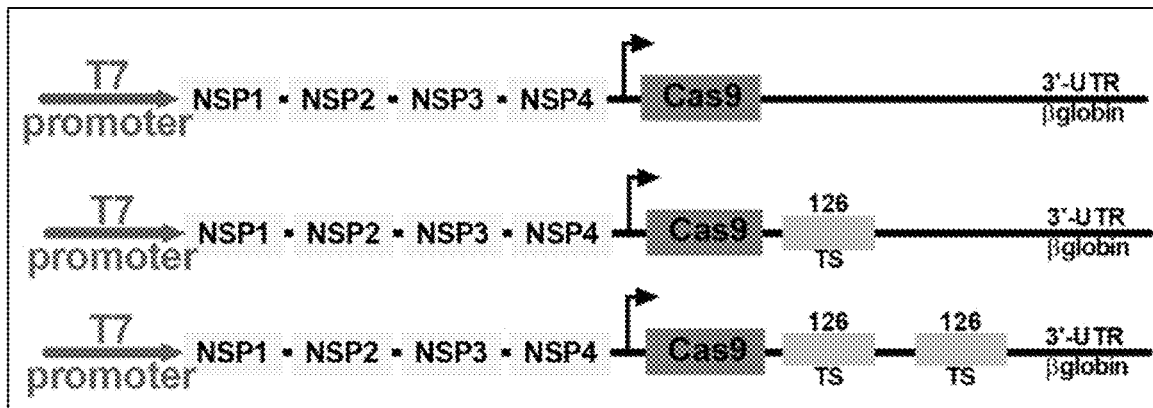


FIG. 36

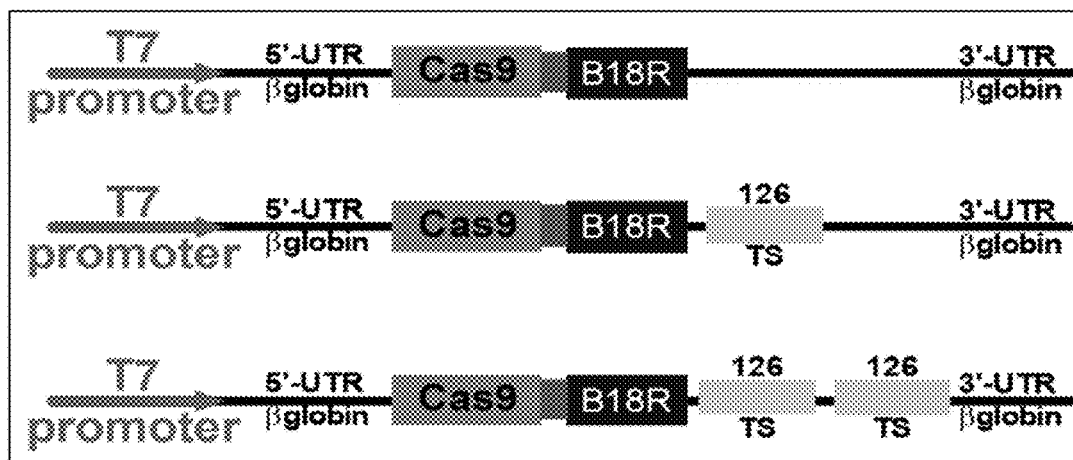


FIG. 37

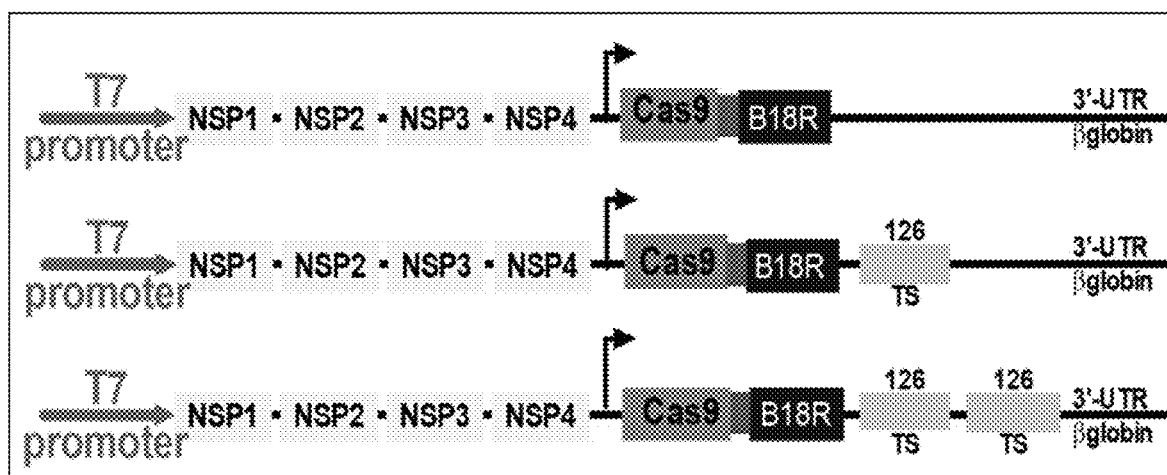


FIG. 38

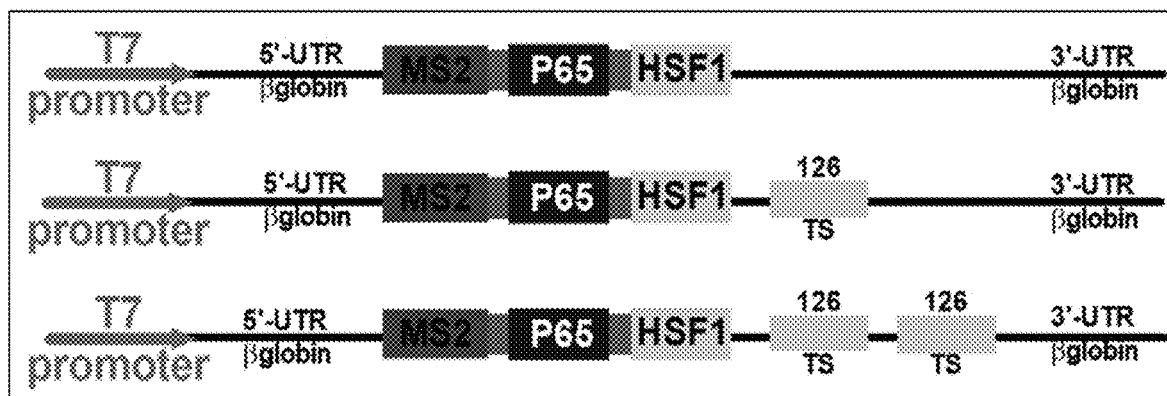


FIG. 39

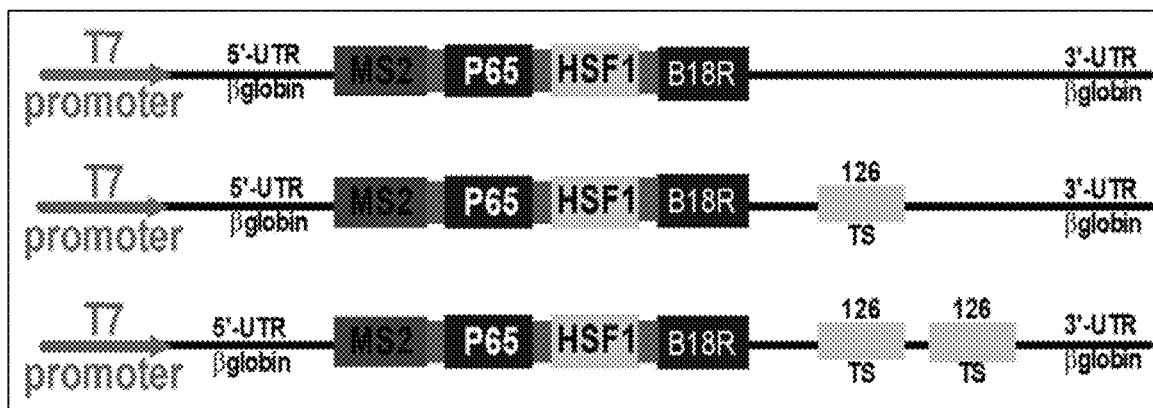


FIG. 40

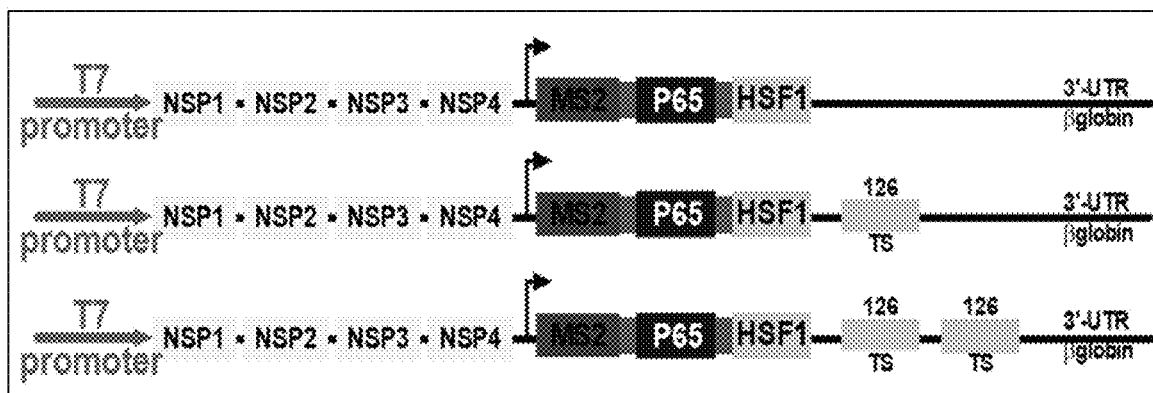


FIG. 41

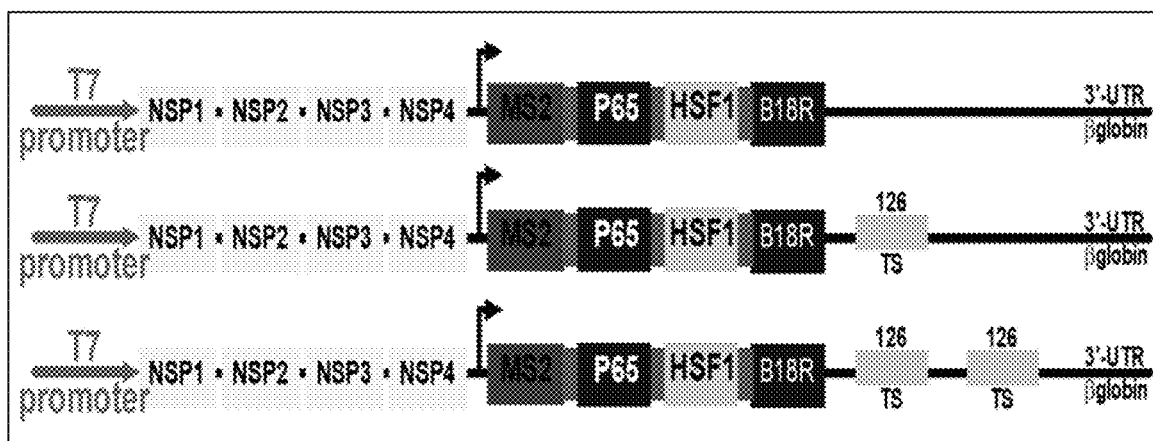


FIG. 42

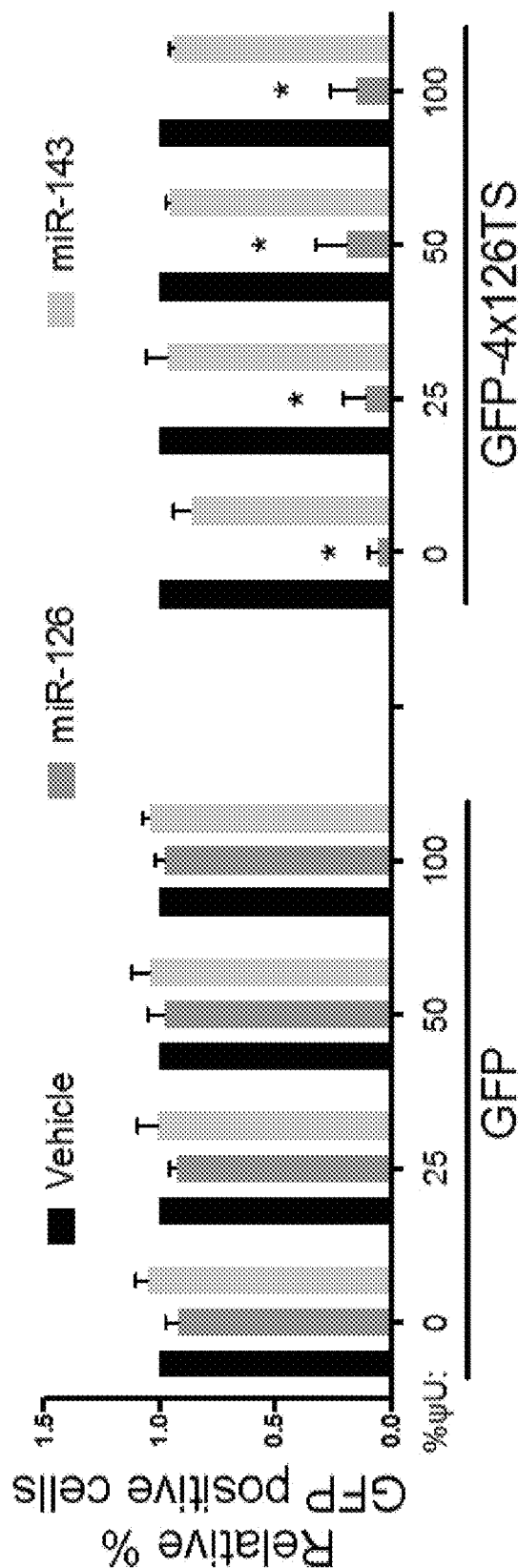


FIG. 43A

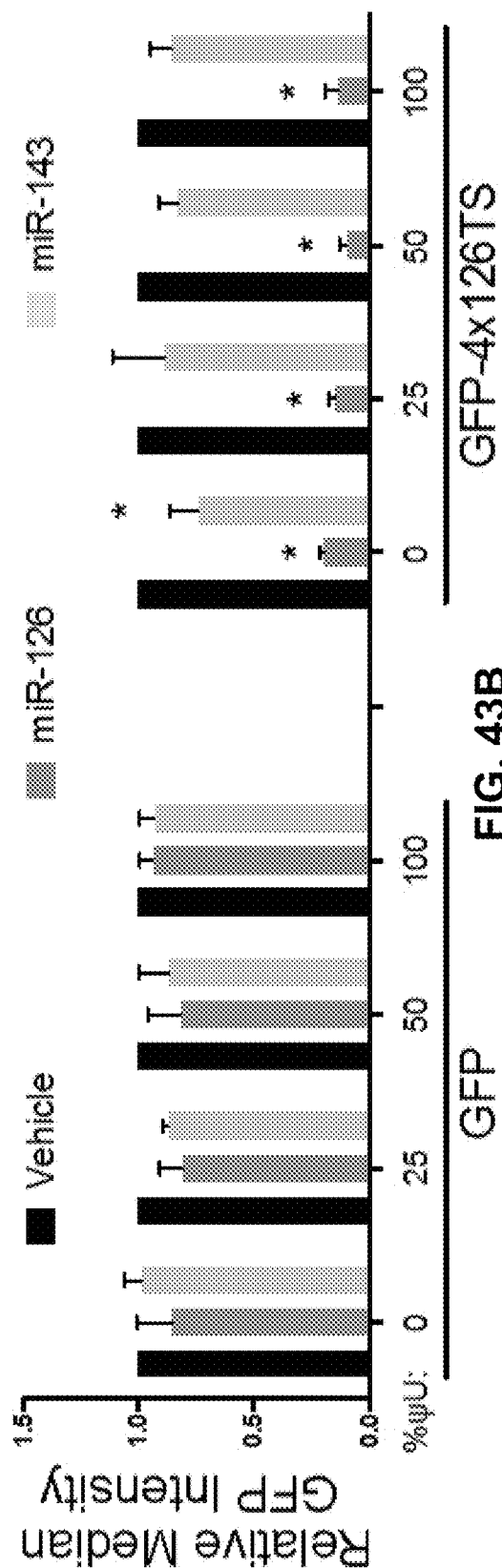


FIG. 43B

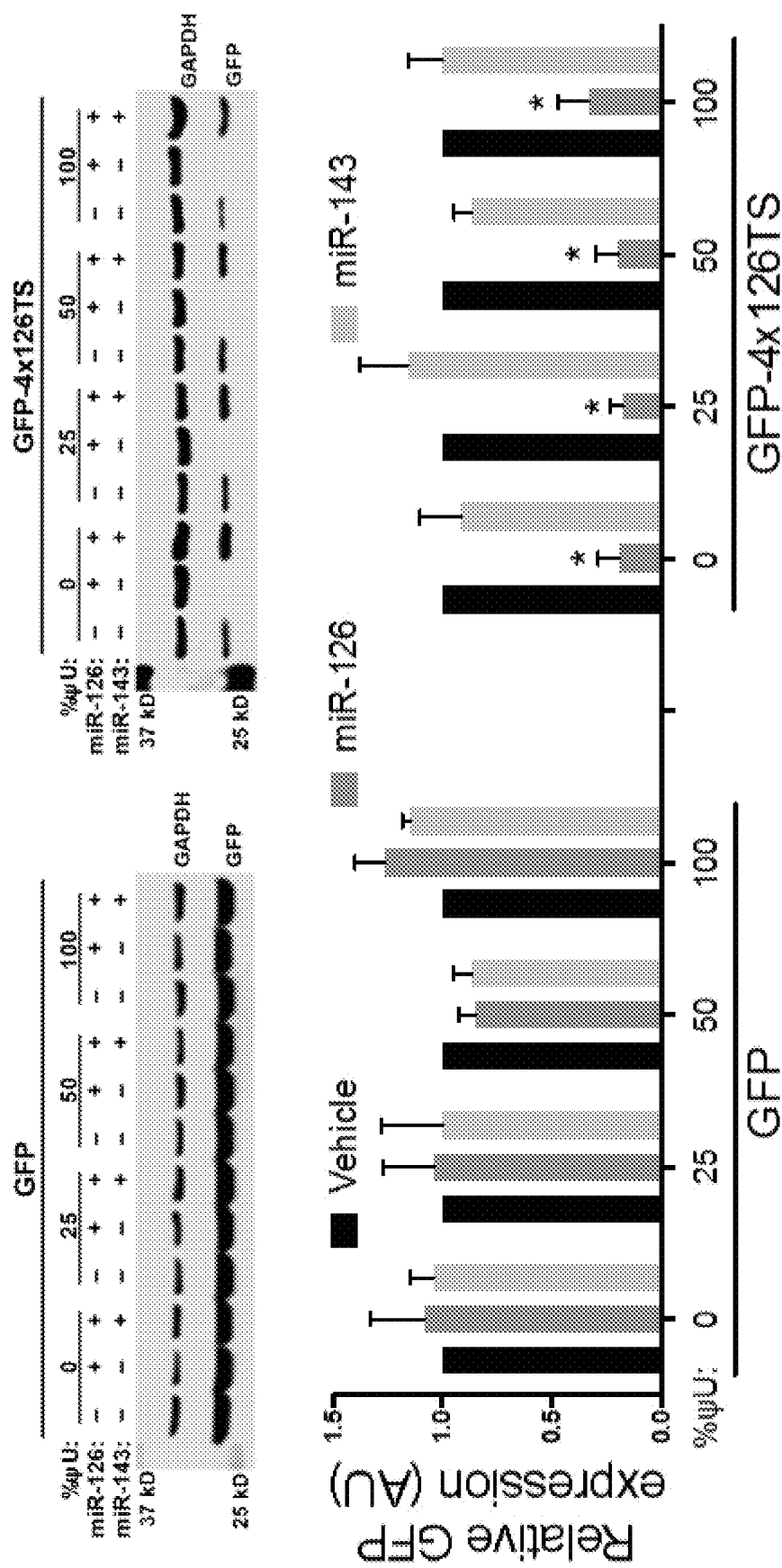


FIG. 43C

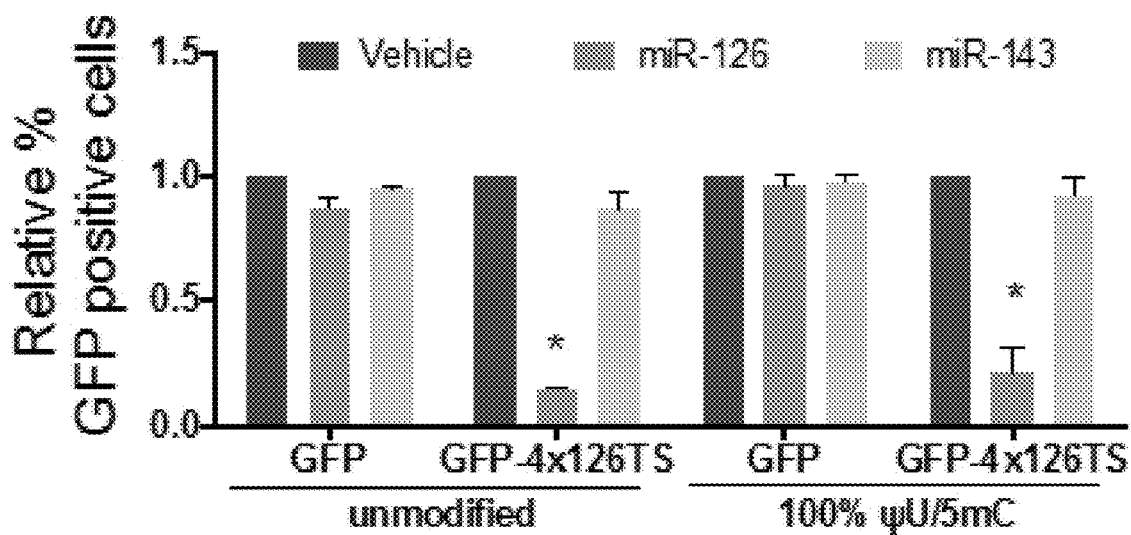


FIG. 44A

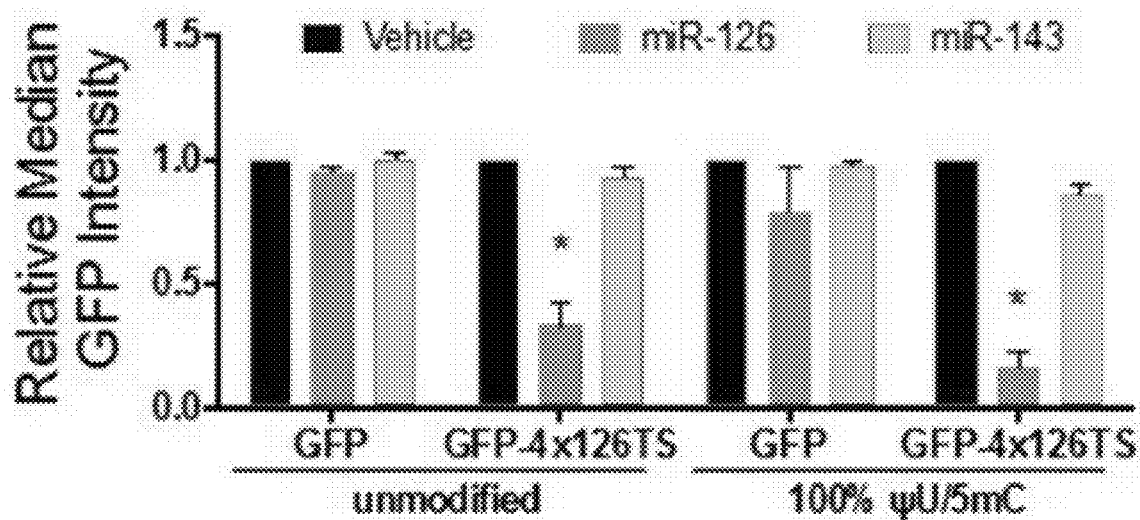


FIG. 44B

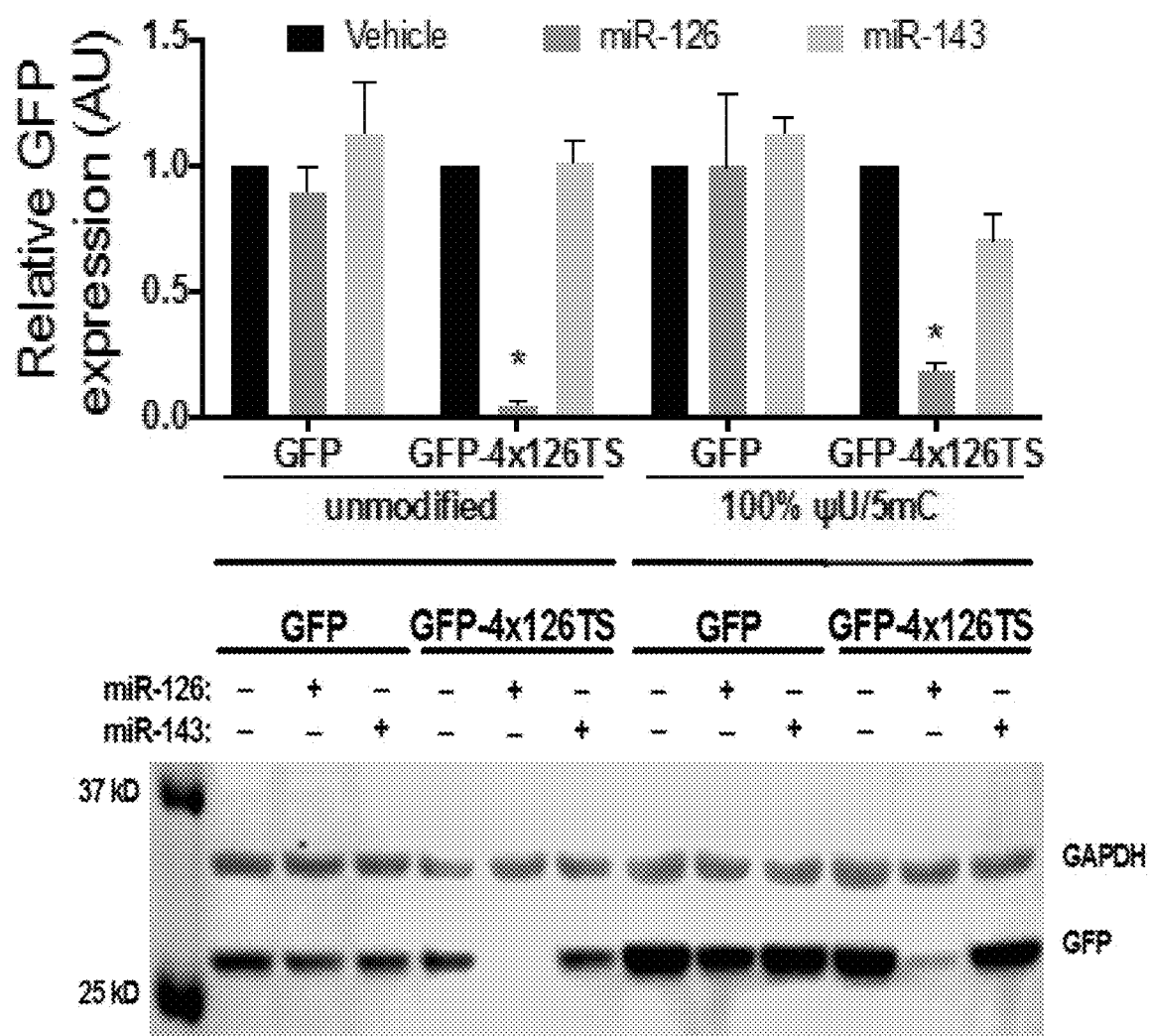
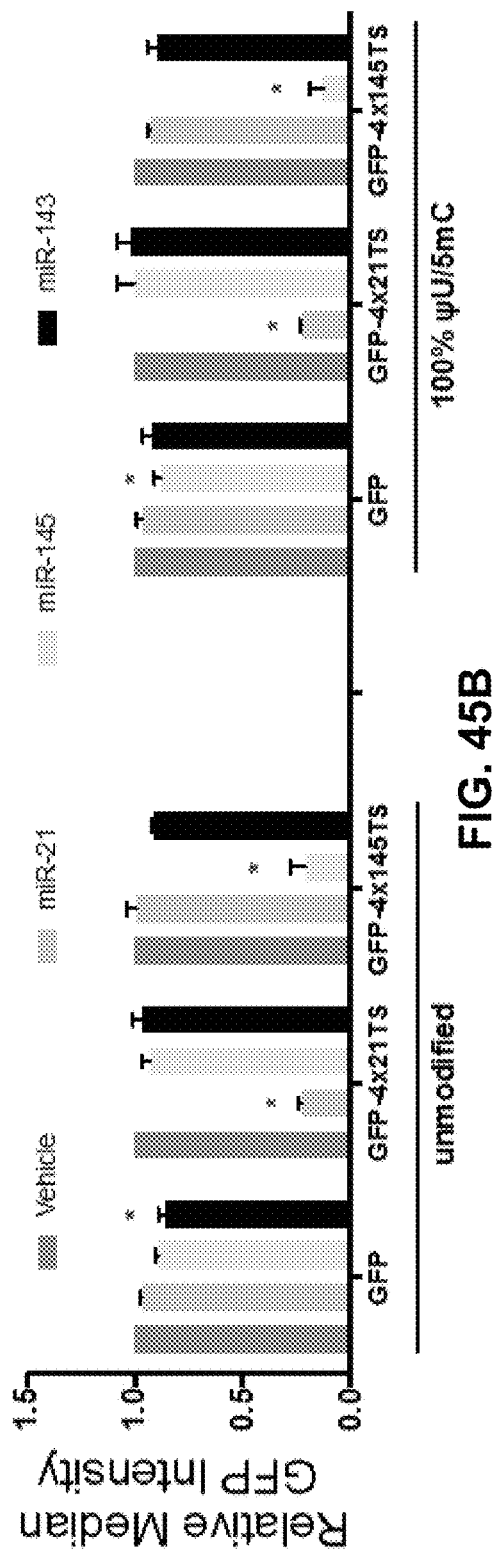
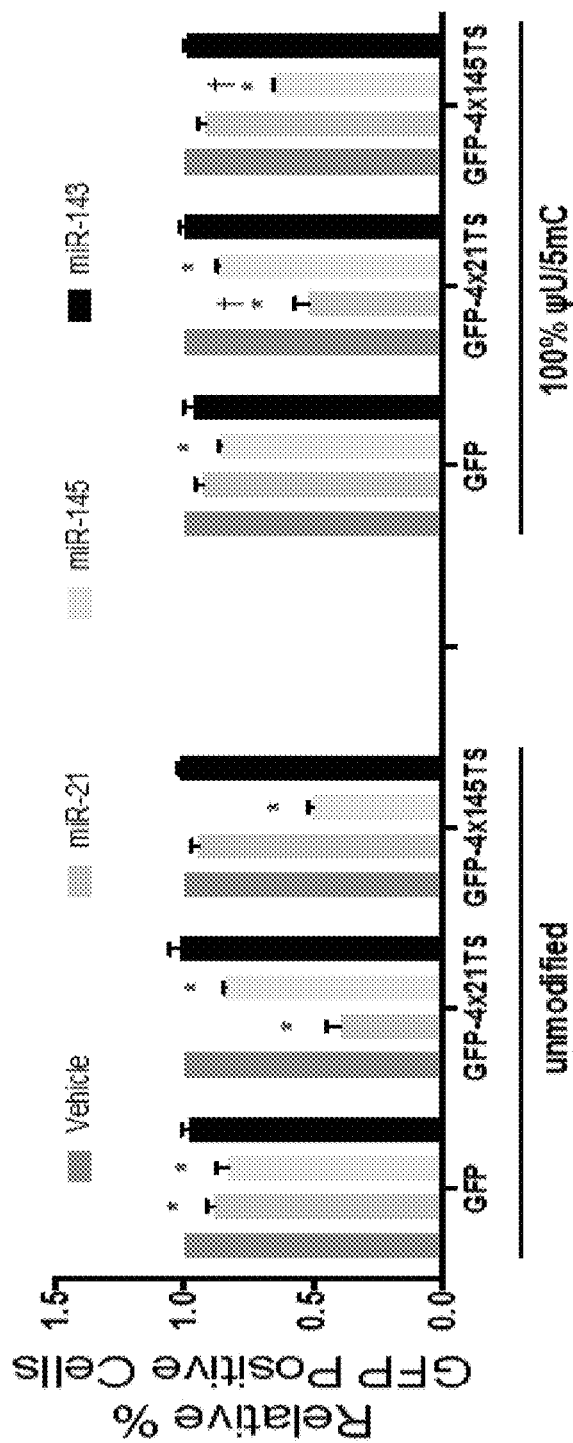


FIG. 44C



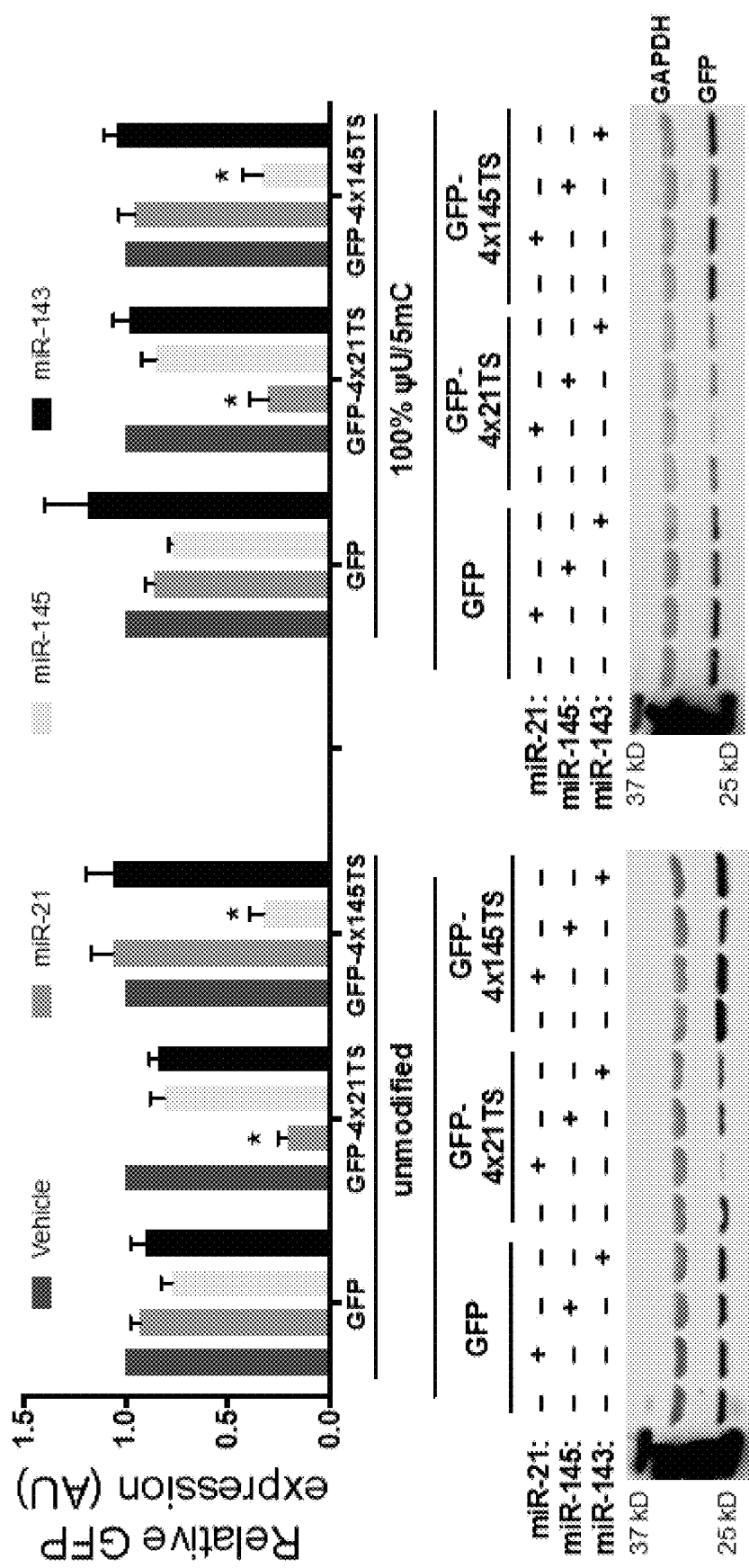


FIG. 45C

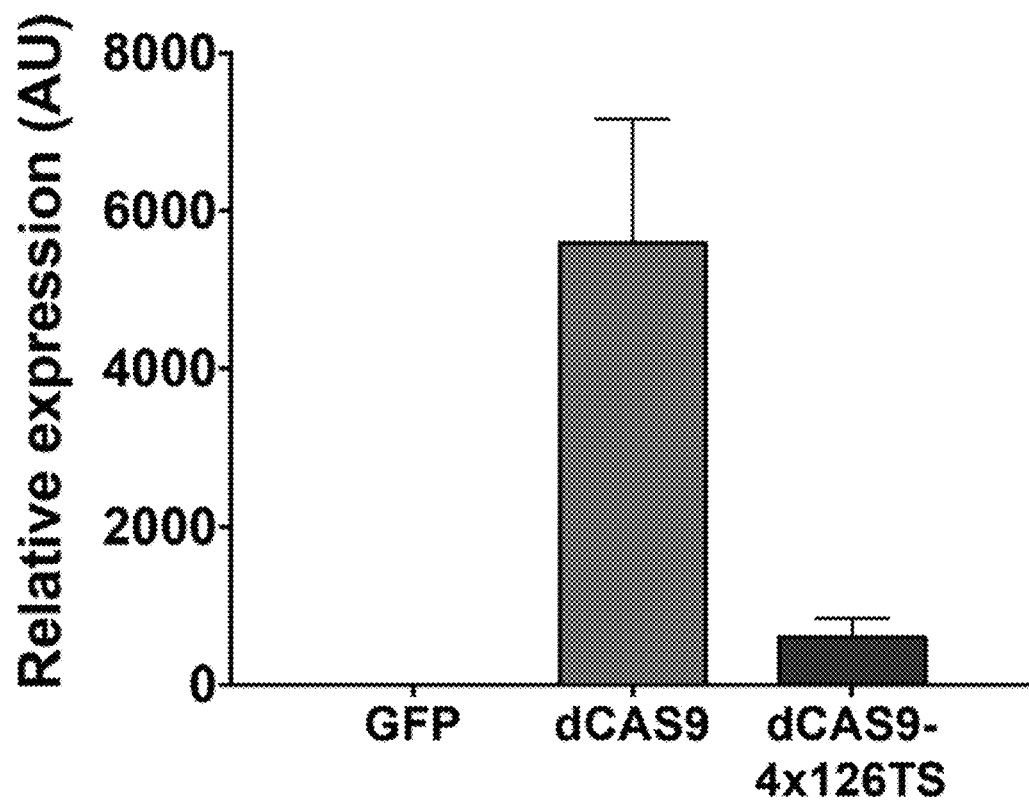


FIG. 46

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SELF-REPLICATING CELL SELECTIVE GENE DELIVERY COMPOSITIONS, METHODS, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/333,074, filed on Oct. 24, 2016, entitled "SELF-REPLICATING CELL SELECTIVE GENE DELIVERY COMPOSITIONS, METHODS, AND USES THEREOF," the contents of which is incorporated by reference herein in its entirety.

This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/245,457, filed on Oct. 23, 2015, entitled "CELL-SELECTIVE GENE EDITING," the contents of which is incorporated by reference herein in its entirety.

This application also claims the benefit of and priority to U.S. Provisional Patent Application No. 62/264,609, filed on Dec. 8, 2015, entitled "SELF-REPLICATING CELL SELECTIVE GENE DELIVERY COMPOSITIONS, METHODS, AND USES THEREOF" the contents of which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant Number HL109133 awarded by National Institutes of Health. The government has certain rights to the invention.

SEQUENCE LISTING

This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled 292105-1041_ST25.txt, created on Mar. 29, 2019. The content of the sequence listing is incorporated herein in its entirety.

BACKGROUND

Despite the remarkable advance in therapeutic strategies over the past decades, the lack of cell selective therapies remains a major hurdle in clinical medicine. As such, there exists a need for improved cell selective therapies.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

FIG. 1 shows embodiments of a cell-selective RNA molecule and its operation.

FIG. 2 shows an embodiment of a cell-selective RNA molecule and its operation.

FIG. 3 shows an embodiment of a cell-selective RNA molecule and its operation.

FIG. 4 shows several embodiments of constructs for synthesizing cell-selective mRNA molecules with 0, 2 or 3 microRNA target sites.

FIG. 5 shows embodiments of in vitro transcription of GFP, Luc or any gene of interest mRNA constructs. All mRNA constructs can be placed under the CMV promoter, capped and polyadenylated. The 3' UTR of each construct can have 4 target sites for miR-126, miR-143 or a scrambled

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control. The constructs can be used for in vitro transcription of mRNAs that incorporate the selected modified nucleotides.

FIG. 6 shows images demonstrating the effect of the number of miR-126 target sites of unmodified mRNA molecule on GFP silencing in HEK cells transfected with miR-126 mimics but not in HEK cells transfected with miR-145 or control mimic.

FIG. 7 shows images demonstrating GFP silencing by miR-126 mimics in HEK cells transfected with unmodified or pseudouridine modified mRNA molecule that includes 2 or 3 miR-126 target sites.

FIG. 8 demonstrates the dose dependency of GFP mRNA silencing of pseudouridine modified cell-selective mRNA by miR-126 mimics but not by miR-143 mimics.

FIG. 9 shows a graph demonstrating flow cytometry analysis of pseudouridine modified GFP expressing mRNA construct with no miR-126 target sites exposed to a miR-126 mimic at varying amounts.

FIG. 10 shows a graph demonstrating flow cytometry analysis of pseudouridine modified GFP expressing mRNA construct with no miR-126 target sites exposed to a miR-143 mimic at varying amounts.

FIG. 11 shows a graph demonstrating flow cytometry analysis of pseudouridine modified GFP expressing mRNA containing 3 miR-126 target sites at its 3'UTR silencing when exposed to miR-126 mimic at varying amounts.

FIG. 12 shows a graph demonstrating flow cytometry analysis of pseudouridine modified GFP expressing mRNA construct containing 3 miR-126 target sites at its 3'UTR exposed to miR-143 mimic at varying amounts.

FIG. 13 shows fluorescent images demonstrating GFP expression in HEK cells transfected with 200 nM of the indicated miR-mimic or control. After 24 hr cells were transfected with 100 nM unmodified or pseudouridine modified GFP or GFP-2×miR-126TS and the fluorescent images were taken 24 hr after the second transfection.

FIG. 14 shows a graph demonstrating the percent GFP positive cells transfected with pseudouridine modified mRNAs of FIG. 13 as determined by Flow Cytometry. The total number of cells analyzed was 10,000.

FIG. 15 shows images of HEK cells infected with lentivirus vectors encoding GFP, GFP-4×miR-143TS or GFP-4×miR-126TS (10⁹ transducing units TU/ml). After 24 h incubation, cells were transfected with 200 nM of the indicated miR-mimic, inhibitor or control and fluorescent images were taken after 72 hrs.

FIG. 16 shows images of VECs infected with lentivirus vectors encoding GFP, GFP-4×miR-143TS or GFP-4×miR-126TS (10⁹ transducing units TU/ml). After 24 h incubation, cells were transfected with 200 nM of the indicated miR-mimic, inhibitor or control and photographed after 72 hours.

FIG. 17 shows schematics of (A) p27 coding sequence flanked with the 5'- and 3'-UTR of β -globin and placed under the control of the bacteriophage T7 RNA polymerase promoter (p27) and (B) p27 coding sequence flanked with the 5'- and 3'-UTR of β -globin and placed under the control of T7 RNA polymerase promoter, containing 2 fully complementary target sequences for the mature miR-126-3p strand at its 3'-UTR (p27-2×126TS). F, Flag-tag.

FIG. 18 shows schematics of embodiments of self-replicating VEE based cell-selective mRNA constructs.

FIG. 19 shows embodiments of a self-replicating mRNA based, cell-selective construct for in vitro transcription. (A) Shows a schematic illustration of a self-replicating RNA derived from an alphavirus contains a 5' T7 RNA polymerase, nonstructural genes (NSP1-4), 26S subgenomic

promoter (black arrow), p27 coding sequence followed by β -globin 3'-UTR (VEE-p27); (B) same as in A, p27 coding sequence with 2 fully complementary target sequences for the mature miR-126-3p strand at its 3'-UTR (p27-2 \times 126TS). F, Flag-tag.

FIG. 20 shows embodiments of a VEE polyprotein constructs. (A) and (B) show constructs that contain a 5' T7 RNA polymerase, nonstructural genes (NSP1-4), 26S subgenomic promoter (black arrow), p27 and B18R polyprotein separated by 2A peptide (in green); 26S subgenomic promoter (black arrow) without (A) or with 2 fully complementary target sequences for the mature miR-126-3p strand at its 3'-UTR (p27-2 \times 126TS) (B).

FIG. 21 shows an adenoviral based cell-selective construct for overexpression of exogenous p27 in VSMCs to reduce neointimal hyperplasia while sparing ECs to improve reendothelialization. This figure is a modification from J Clin Invest. 2014 September; 124(9):3694-7. PMID: 25133421, PMCID: PMC4151224.

FIG. 22 shows embodiments of adenoviral vector constructs for cell-selective gene delivery.

FIG. 23 shows a graph demonstrating high levels of expression of endogenous miR-126-3p in human ECs as determined by RT-qPCR.

FIG. 24 shows representative immunoblots of p27 expression and corresponding graphs demonstrating densitometric quantification (n=3). Data represent the mean \pm SEM. Data comparisons were made using 1-way ANOVA with Tukey-Kramer's post hoc test. *P<0.01 versus Ad-GFP.

FIG. 25 shows images and corresponding graph demonstrating that treatment with Ad-p27-126TS inhibits neointimal hyperplasia. Representative H&E-stained sections 2 weeks after balloon injury. Scale bars: 500 μ m; original magnification, \times 10 (insets show the whole arterial section at \times 5 original magnification). Intima/media ratios were calculated from at least 6 rats/group. Data represent the mean \pm SEM and were compared using 1-way repeated measures ANOVA followed by Tukey-Kramer's post hoc test. *P<0.01 versus uninjured arteries.

FIG. 26 shows images and corresponding graph demonstrating that treatment with Ad-p27 or Ad-p27-126TS inhibited infiltration of inflammatory cells to the balloon injured vessel. Representative cross-sections of rat carotid arteries immunostained for CD45, 2 weeks after balloon injury. Nuclei were counterstained with DAPI. No positive staining was observed in the negative control sections (Cy3 alone). White scale bars: 100 μ m (magnification \times 40). CD45 positive cells were quantified by counting the number of CD45 positive cells in the intimal and medial areas from at least 3 sections/group. Data represent the mean \pm SEM and were compared using 1-way repeated measures ANOVA followed by Tukey-Kramer's post hoc test. *P<0.01 versus uninjured arteries.

FIGS. 27A-27B show images and graphs demonstrating that treatment with Ad-p27-126TS can allow for rapid and extensive reendothelialization of injured arteries within 2 weeks. Representative confocal images of cross-sections (FIG. 27A) and tridimensional en-face longitudinal arterial preparations (FIG. 27B) of rat carotid arteries immunostained for VE cadherin 2 weeks after injury. Nuclei were counterstained with DAPI. Scale bars: 100 μ m; original magnification, \times 60 and \times 120 (insets). Arrowheads indicate ECs beyond the inner autofluorescent elastic laminae. Endothelial coverage was quantified by counting the number of VEcadherin-positive cells in the circumference of the lumens from at least 6 rats/group. Data represent the mean \pm SEM and were compared using 1-way repeated mea-

sures ANOVA followed by Tukey-Kramer's post hoc test. *P<0.01 versus uninjured arteries.

FIG. 28 shows a graph demonstrating that treatment with Ad-p27-126TS can reduce plasma D-Dimer levels of injured arteries. Immunoassay of plasma D-dimer levels before (uninjured) and 3, 14, and 28 days after balloon injury. n=5 rats/group. Data represent the mean \pm SEM and were compared using 1-way repeated measures ANOVA followed by Tukey-Kramer's post hoc test. *P<0.01 versus uninjured arteries.

FIG. 29 shows a graph demonstrating that treatment with Ad-p27-126TS can restore the endothelium-dependent vasodilatory response of injured arteries to levels of uninjured controls. Vascular reactivity analysis on carotid rings 2 weeks after balloon injury Ad-p27-126TS treated vessels show vasodilatory in response to acetylcholine (ACh). n=5-6 rats/group. Data represent the mean \pm SEM and were compared using 2-way repeated measures ANOVA followed by Tukey-Kramer's post hoc test. *P<0.01 versus uninjured arteries.

FIG. 30 shows a table listing modified nucleotides that can be used in the cell-selective RNA molecule.

FIG. 31 shows embodiments of a cell-selective gRNA construct.

FIG. 32 shows embodiments of a self-replicating, cell-selective gRNA construct.

FIG. 33 shows embodiments of a cell-selective gRNA 2.0 construct for CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. *Nature* 517, 583-588 (29 Jan. 2015).

FIG. 34 shows embodiments of a self-replicating, cell-selective gRNA 2.0 construct for CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing (*Nature* 517, 583-588 (29 Jan. 2015)).

FIG. 35 shows embodiments of a cell-selective Cas9 or dCas9 construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing *Nature* 517, 583-588 (29 Jan. 2015).

FIG. 36 shows embodiments of a self-replicating, cell-selective Cas9 or dCas9 construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing *Nature* 517, 583-588 (29 Jan. 2015).

FIG. 37 shows embodiments of a cell selective Cas9 or dCas9 construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. Embodiments can also include a B18R, separated from the Cas9 or dCas9 by a 2A polypeptide.

FIG. 38 shows embodiments of a self-replicating, cell-selective Cas9 or dCas9 construct that can be used in CRISPR/Cas9 Synergistic ActivationM (SAM) genome editing. Embodiments can also include a B18R, separated from the Cas9 or dCas9 by a 2A polypeptide.

FIG. 39 shows embodiments of a cell-selective construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. The construct can include a MS2-p65-HSF1 fusion polypeptide.

FIG. 40 shows embodiments of a cell-selective construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. The construct can include a MS2-p65-HSF1 and B18R polypeptide separated by a 2A polypeptide.

FIG. 41 shows embodiments of a self-replicating, cell-selective construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. The construct can include a MS2-p65-HSF1 fusion polypeptide.

FIG. 42 shows embodiments of a self-replicating, cell-selective construct that can be used in CRISPR/Cas9 Synergistic Activation Mediator (SAM) genome editing. The

construct can include a MS2-p65-HSF1 and B18R polypeptide separated by a 2A polypeptide.

FIGS. 43A-43C show graphs demonstrating and an image of an immunoblot for GFP demonstrating the relative percent of GFP positive cells (FIG. 43A), relative median GFP intensity (FIG. 43B), and relative GFP expression (FIG. 43C) post transfection of HEK cells with GFP or GFP 4×126TS unmodified or with substitutions of uridine with pseudouridine (0%, 25%, 50% or 100%) when exposed to miR-126 or miR-143 mimics.

FIGS. 44A-44C show graphs and an image of an immunoblot for GFP demonstrating the relative percent of GFP positive cells (FIG. 44A), relative median GFP intensity (FIG. 44B), and relative GFP expression (FIG. 44C) post transfection with unmodified GFP or GFP 4×126TS or with 100% of uridines and cytosines substituted with pseudouridine and 5-methylcytosine when exposed to miR-126 or miR-143 mimics.

FIGS. 45A-45C show graphs and an image of an immunoblot for GFP demonstrating the relative percent of GFP positive cells (FIG. 45A), relative median GFP intensity (FIG. 45B), and relative GFP expression (FIG. 45C) post transfection with unmodified GFP, GFP 4×21TS, or GFP 4×145TS or with 100% of uridines and cytosines substituted with pseudouridine and 5-methylcytosine when exposed to miR-21 or miR-145, miR143 mimics

FIG. 46 shows a graph demonstrating the relative expression levels of LIN28A in cells transfected with miR-126 mimic about 24 hours prior to transfection with CRISPR-SAM components containing dCas9 or dCas9-4×126TS.

DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to

antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, nanotechnology, organic chemistry, biochemistry, botany and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Definitions

As used herein, “control” is an alternative subject or sample used in an experiment for comparison purpose and included to minimize or distinguish the effect of variables other than an independent variable.

As used herein, “biocompatible” or “biocompatibility” refers to the ability of a material to be used by a patient without eliciting an adverse or otherwise inappropriate host response in the patient to the material or a derivative thereof, such as a metabolite, as compared to the host response in a normal or control patient.

As used herein, “biodegradable” refers to the ability of a material or compound to be decomposed by bacteria or other living organisms or organic processes.

As used herein, “about,” “approximately,” and the like, when used in connection with a numerical variable, generally refers to the value of the variable and to all values of the variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within $\pm 10\%$ of the indicated value, whichever is greater.

As used herein, “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications, or dosages.

As used herein, “administering” can refer to an administration that is oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation, by catheters, stents or via an implanted reservoir or other device that administers, either actively or passively (e.g. by diffusion) a composition the perivascular space and adventitia. For example a medical device such as a stent can contain a composition or formulation disposed on its surface, which can then dissolve or be otherwise distributed to the surrounding tissue and cells. The term “parenteral” can include subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques.

As used herein, “preventative” refers to hindering or stopping a disease or condition before it occurs or while the disease or condition is still in the sub-clinical phase.

As used herein, “therapeutic” can refer to treating or curing a disease or condition.

As used interchangeably herein, “subject,” “individual,” or “patient,” refers to a vertebrate, preferably a mammal,

more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. The term “pet” includes a dog, cat, guinea pig, mouse, rat, rabbit, ferret, and the like. The term farm animal includes a horse, sheep, goat, chicken, pig, cow, donkey, llama, alpaca, turkey, and the like.

As used herein, the terms “cancer,” “cancer cells,” “neoplastic cells,” “neoplasia,” “tumor,” and “tumor cells” (used interchangeably) refer to cells which exhibit relatively autonomous growth so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation (i.e., de-regulated cell division). Neoplastic cells can be malignant or benign. A metastatic cell or tissue means that the cell can invade and destroy neighboring body structures. The cancer can be selected from astrocytoma, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brain cancer, brain stem glioma, breast cancer, cervical cancer, colon cancer, colorectal cancer, cutaneous T-cell lymphoma, ductal cancer, endometrial cancer, ependymoma, Ewing sarcoma, esophageal cancer, eye cancer, gallbladder cancer, gastric cancer, gastrointestinal cancer, germ cell tumor, glioma, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, macroglobulinemia, melanoma, mesothelioma, mouth cancer, multiple myeloma, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pituitary cancer, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, sarcoma, skin cancer, small cell lung cancer, small intestine cancer, squamous cell carcinoma, stomach cancer, T-cell lymphoma, testicular cancer, throat cancer, thymoma, thyroid cancer, trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer and Wilms tumor. In some embodiments, the cancer is prostate cancer.

The terms “guide polynucleotide,” “guide sequence,” or “guide RNA” as used herein refers to any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. The degree of complementarity between a guide polynucleotide and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting examples of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). A guide polynucleotide (also referred to herein as a guide sequence and includes single guide sequences (sgRNA)) can be about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, 90, 100, 110, 112, 115, 120, 130, 140, or more nucleotides in length. The guide polynucleotide can include a nucleotide sequence that is complementary to a target DNA sequence. This portion of the guide sequence can be referred to as the complementary region of the guide RNA. In some contexts, the two are distinguished from one another by calling one the complementary region

or target region and the rest of the polynucleotide the guide sequence or trans-activating crRNA (tracrRNA). The guide sequence can also include one or more miRNA target sequences coupled to the 3' end of the guide sequence. The guide sequence can include one or more MS2 RNA aptamers incorporated within the portion of the guide strand that is not the complementary portion. As used herein the term guide sequence can include any specially modified guide sequences, including but not limited to those configured for use in synergistic activation mediator (SAM) implemented CRISPR (*Nature* 517, 583-588 (29 Jan. 2015)). A guide polynucleotide can be less than about 150, 125, 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. In some embodiments, a guide polynucleotide can be 150 nucleotides or more, including, but not limited to 175, 200, 250, 300, 450, 500 or more. It will also be appreciated that the exact number of nucleotides may be any integer between any of the specific numbers given, for example 1, 4, 123, 36, etc. and are all within the spirit and scope of this disclosure. The ability of a guide polynucleotide to direct sequence-specific binding of a CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide polynucleotide to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide polynucleotide to be tested and a control guide polynucleotide different from the test guide polynucleotide, and comparing binding or rate of cleavage at the target sequence between the test and control guide polynucleotide reactions. Other assays are possible, and will occur to those skilled in the art.

A complementary region of the gRNA can be configured to target any DNA region of interest. The complementary region of the gRNA and the gRNA can be designed using a suitable gRNA design tool. Suitable tools are known in the art and are available to the skilled artisan. As such, the constructs described herein are enabled for any desired target DNA so long as it is CRISPR compatible according to the known requirements for CRISPR activation.

A guide polynucleotide can be selected to reduce the degree of secondary structure within the guide polynucleotide. Secondary structure may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is mFold, as described by Zuker & Stiegler ((1981) *Nucleic Acids Res.* 9, 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g. Gruber et al., (2008) *Cell* 106: 23-24; and Carr & Church (2009) *Nature Biotechnol.* 27: 1151-1162).

Homology-directed repair (HDR) refers to a mechanism in cells to repair double-stranded and single stranded DNA breaks. Homology-directed repair includes homologous recombination (HR) and single-strand annealing (SSA) (Lieber. (2010) *Annu. Rev. Biochem.* 79: 181-211). The most common form of HDR is called homologous recombination (HR), which has the longest sequence homology requirements between the donor and acceptor DNA. Other forms of HDR include single-stranded annealing (SSA) and break-

age-induced replication, and these require shorter sequence homology relative to HR. Homology-directed repair at nicks (single-stranded breaks) can occur via a mechanism distinct from HDR at double-strand breaks.

Error-prone DNA repair refers to mechanisms that can produce mutations at double-strand break sites. The Non-Homologous-End-Joining (NHEJ) pathways are the most common repair mechanism to bring the broken ends together (Bleuyard et al., (2006) *DNA Repair* 5: 1-12). The structural integrity of chromosomes is typically preserved by the repair, but deletions, insertions, or other rearrangements are possible. The two ends of one double-strand break are the most prevalent substrates of NHEJ (Kirik et al., (2000) *EMBO J.* 19: 5562-5566), however if two different double-strand breaks occur, the free ends from different breaks can be ligated and result in chromosomal deletions (Siebert & Puchta, (2002) *Plant Cell* 14:1121-1131), or chromosomal translocations between different chromosomes (Pacher et al., (2007) *Genetics* 175: 21-29).

It will also be appreciated that CRISPR can also be used to activate specific genes through CRISPR/synergistic activation mediator procedures. These procedures can utilize a guide polynucleotide that incorporates 2 MS2 RNA aptamers at the tetraloop and the stem-loop of the guide RNA such as that described in, but not limited to (*Nature* 517, 583-588 (29 Jan. 2015)).

The term “operatively linked” as used herein can refer to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operatively linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operatively linked to regulatory sequences in a sense or antisense orientation. In one example, the complementary RNA regions can be operatively linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA. The term “operatively linked” as used herein can also refer to the direct or indirect linkage of any two nucleic acid sequences on a singly nucleic acid fragment such that they are indirectly or directly physically connected on the same nucleic acid fragment. The term “operatively linked” as used herein can also refer to the insertion of a nucleic acid within the 5' and 3' end of another nucleic or the direct coupling of a nucleic acid to the 5' or 3' end of another nucleic acid.

As used herein, “specific binding” can refer to binding which occurs between such paired species as enzyme/substrate, receptor/agonist, antibody/antigen, and lectin/carbohydrate which may be mediated by covalent or non-covalent interactions or a combination of covalent and non-covalent interactions. When the interaction of the two species produces a non-covalently bound complex, the binding which occurs is typically electrostatic, hydrogen-bonding, or the result of lipophilic interactions. Accordingly, “specific binding” occurs between a paired species where there is interaction between the two which produces a bound complex having the characteristics of an antibody/antigen or enzyme/substrate interaction. In particular, the specific binding is characterized by the binding of one member of a pair to a particular species and to no other species within the family of compounds to which the corresponding member of the binding member belongs. Thus, for example, an antibody preferably binds to a single epitope and to no other epitope within the family of proteins. As another non-limiting

example, a miRNA can specifically bind preferably to a miRNA target and not to a non-specific nucleic acid sequence or if binding to a non-specific nucleic acid sequence occurs that no change in the expression or function of the non-specific nucleic acid can be observed or detected.

As used herein, “differentially expressed,” refers to the differential production of RNA, including but not limited to mRNA, tRNA, miRNA, siRNA, snRNA, and piRNA transcribed from a gene or regulatory region of a genome or the protein product encoded by a gene as compared to the level of production of RNA or protein by the same gene or regulator region in a normal or a control cell. In another context, “differentially expressed,” also refers to nucleotide sequences or proteins in a cell or tissue which have different temporal and/or spatial expression profiles as compared to a normal or control cell.

As used herein, “polypeptides” or “proteins” are amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus. In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

As used herein, “gene” can refer to a hereditary unit corresponding to a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a characteristic(s) or trait(s) in an organism. “Gene” also refers to the specific sequence of DNA that is transcribed into an RNA transcript that can be translated into a polypeptide or be a catalytic RNA molecule including but not limited to tRNA, siRNA, piRNA, miRNA, long-non-coding RNA and shRNA.

As used herein, “deoxyribonucleic acid (DNA)” and “ribonucleic acid (RNA)” generally refer to any polynucleotide or polydeoxynucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. RNA can be in the form of non-coding RNA such as tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), anti-sense RNA, RNAi (RNA interference construct), siRNA (short interfering RNA), microRNA (miRNA), or ribozymes, aptamers or coding mRNA (messenger RNA).

As used herein, “nucleic acid sequence” and “oligonucleotide” also encompasses a nucleic acid and polynucleotide as defined elsewhere herein.

As used herein, “DNA molecule” includes nucleic acids/polynucleotides that are made of DNA.

As used herein, “nucleic acid” and “polynucleotide” generally refer to a string of at least two base-sugar-phosphate combinations and refers to, among others, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The strands in such regions can be from the same molecule or from different molecules. The regions can include all of one or more of the molecules, but more typically involve

only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. "Polynucleotide" and "nucleic acids" also encompasses such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia. For instance, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. "Polynucleotide" and "nucleic acids" also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids. Natural nucleic acids have a phosphate backbone, artificial nucleic acids can contain other types of backbones, but contain the same bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "nucleic acids" or "polynucleotide" as that term is intended herein.

As used herein, "microRNA" can refer to a small non-coding RNA molecule containing about 21 to about 23 nucleotides found in organisms, which functions in transcriptional and post-transcriptional regulation of transcription and translation of RNA. "MicroRNA" can exist as part of a larger nucleic acid molecule such as a stem-loop structure that can be processed by a cell and yield a microRNA of about 21-23 nucleotides.

As used herein, "pharmaceutically acceptable carrier, diluent, binders, lubricants, glidant, preservative, flavoring agent, coloring agent, and excipient" refers to a carrier, diluent, binder, lubricant, glidant, preservative, flavoring agent, coloring agent, or excipient that is useful in preparing a pharmaceutical formulation that is generally safe, non-toxic, and is neither biologically or otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use.

The term "treating", as used herein, can include inhibiting the disease, disorder or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

As used herein, "overexpressed" or "overexpression" refers to an increased expression level of an RNA (coding or non-coding RNA) or protein product encoded by a gene as compared to the level of expression of the RNA or protein product in a normal or control cell.

As used herein, "underexpressed" or "underexpression" refers to decreased expression level of an RNA (coding or non-coding RNA) or protein product encoded by a gene as compared to the level of expression of the RNA or protein product in a normal or control cell.

As used herein, "expression" refers to the process by which polynucleotides are transcribed into RNA transcripts. In the context of mRNA and other translated RNA species, "expression" also refers to the process or processes by which the transcribed RNA is subsequently translated into peptides, polypeptides, or proteins.

As used herein "gene editing", "genome editing," "genome modification" can refer to the non-natural manipulation of genomic DNA such that the genomic DNA contains one or more additional nucleotides or has one or more

nucleotides removed from the genomic sequence. Such genome editing can be achieved by techniques such as viral integration of a transgene, homologous recombination insertion of a transgene, and CRISPR related methods and techniques, including but not limited to, the self-replicating RNA and self-replicating cell-selective CRISPR methods described herein. "Gene editing" and "genome editing" or "genome modification" can refer to the deletion and/or addition of nucleotides into the genomic DNA.

As used herein with reference to the relationship between DNA, cDNA, mRNA, RNA, and protein/peptides, "corresponding to" can refer to the underlying biological relationship between these different molecules. As such, one of skill in the art would understand that operatively "corresponding to" can direct them to determine the possible underlying and/or resulting sequences of other molecules given the sequence of any other molecule which has a similar biological relationship with these molecules. For example, from a DNA sequence an RNA sequence can be determined and from an RNA sequence a cDNA sequence can be determined.

As used herein, "promoter" can refer to all nucleotide sequences capable of driving or initiating transcription of a coding or a non-coding DNA sequence. The term "promoter" as used herein can refer to a DNA sequence generally described as the 5' regulator region of a gene, located proximal to the start codon. The transcription of an adjacent coding sequence(s) is initiated at the promoter region. The term "promoter" also includes fragments of a promoter that are functional in initiating transcription of the gene.

As used herein, "selectable marker" can refer to a gene whose expression allows one to identify cells that have been transformed or transfected with a vector containing the marker gene. For instance, a recombinant nucleic acid may include a selectable marker operatively linked to a gene of interest and a promoter, such that expression of the selectable marker indicates the successful transformation of the cell with the gene of interest.

As used herein, "constitutive promoter" can refer to a promoter that allows for continual or ubiquitous transcription of its associated gene or polynucleotide. Constitutive promoters are generally are unregulated by cell or tissue type, time, or environment.

As used herein, "inducible promoter" can refer to a promoter that allows transcription of its associated gene or polynucleotide in response to a substance or compound (e.g. an antibiotic, or metal), an environmental condition (e.g. temperature), developmental stage, or tissue type.

As used herein, "electroporation" is a transformation method in which a high concentration of plasmid DNA (containing exogenous DNA) or RNA is added to a suspension of host cell protoplasts, and the mixture shocked with an electrical field of about 200 to 600 V/cm.

As used herein, "plasmid" can refer to a non-chromosomal double-stranded DNA sequence including an intact "replicon" such that the plasmid is replicated in a host cell.

As used herein, the term "vector" can refer to a vehicle used to introduce an exogenous nucleic acid sequence into a cell. A vector can include a DNA molecule, linear or circular (e.g. plasmids), which includes a segment encoding a polypeptide of interest operatively linked to additional segments that provide for its transcription and translation upon introduction into a host cell or host cell organelles. Such additional segments can include promoter and terminator sequences, internal ribosome entry site, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal,

microRNA target sequences etc. Expression vectors are generally derived from yeast or bacterial genomic or plasmid DNA, or viral DNA, or can contain elements of both. The term “vector” can also include RNA or circular RNA vectors linked to additional segments that provide for its translation upon introduction into a host cell or host cell organelles. Such additional segments can include 5'Cap, one or more selectable markers, an enhancer, a polyadenylation signal, polyA tail, microRNA target sequences etc.

As used herein, “identity,” can refer to a relationship between two or more polypeptide or polynucleotide sequences, as determined by comparing the sequences. In the art, “identity” also refers to the degree of sequence relatedness between polypeptides or polynucleotides as determined by the match between strings of such sequences. “Identity” can be readily calculated by known methods, including, but not limited to, those described in (Computational Molecular Biology, Lesk, A. M., Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., Eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math. 1988, 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the Needleman and Wunsch, (J. Mol. Biol., 1970, 48: 443-453,) algorithm (e.g., NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides of the present disclosure.

As used herein, the term “transfection” can refer to the introduction of an exogenous and/or recombinant nucleic acid sequence into the interior of a membrane enclosed space of a living cell, including introduction of the nucleic acid sequence into the cytosol of a cell as well as the interior space of a mitochondria, nucleus, or chloroplast. The nucleic acid may be in the form of naked DNA or RNA (unmodified or modified), it may be associated with various proteins or regulatory elements (e.g., a promoter and/or signal element, miRNA target sequences as described herein), or the nucleic acid may be incorporated into a vector or a chromosome.

As used herein, “transformation” or “transformed” can refer to the introduction of a nucleic acid (e.g., DNA or RNA) into cells in such a way as to allow expression of the coding or non-coding portions of the introduced nucleic acid.

As used herein a “transformed cell” can refer to a cell transfected with a nucleic acid sequence.

As used herein, a “transgene” can refer to an artificial gene which is used to transform a cell of an organism, such as a bacterium or a plant.

As used herein, “transgenic” can refer to a cell, tissue, or organism that contains a transgene.

As used herein, the term “recombinant” generally refers to a non-naturally occurring nucleic acid, nucleic acid construct, or polypeptide. Such non-naturally occurring nucleic acids can include natural nucleic acids that have been modified, for example that have deletions, substitutions, inversions, insertions, etc., and/or combinations of nucleic acid sequences of different origin that are joined using

molecular biology technologies (e.g., a nucleic acid sequences encoding a “fusion protein” (e.g., a protein or polypeptide formed from the combination of two different proteins or protein fragments), the combination of a nucleic acid encoding a polypeptide to a promoter sequence, where the coding sequence and promoter sequence are from different sources or otherwise do not typically occur together naturally (e.g., a nucleic acid and a constitutive promoter etc.). Recombinant also refers to the polypeptide encoded by the recombinant nucleic acid. Non-naturally occurring nucleic acids or polypeptides include nucleic acids and polypeptides modified by man, including but not limited to miRNA target sequences described herein.

As used herein, the term “exogenous DNA” or “exogenous RNA” or exogenous nucleic acid sequence” or “exogenous polynucleotide” can refer to a nucleic acid sequence that was introduced into a cell, organism, or organelle via transfection. Exogenous nucleic acids originate from an external source, for instance, the exogenous nucleic acid may be from another cell or organism and/or it may be synthetic and/or recombinant. While an exogenous nucleic acid sometimes originates from a different organism or species, it may also originate from the same species (e.g., an extra copy or recombinant form of a nucleic acid that is introduced into a cell or organism in addition to or as a replacement for the naturally occurring nucleic acid). Typically, the introduced exogenous sequence is a recombinant sequence.

As used herein “miRNA target” or “miRNA target sequence” can refer to the nucleic acid sequence, typically RNA, that a miRNA specifically binds to. The miRNA target can be or include a sequence that is complementary to the miRNA. As an example, microRNA 126 (miR-126) can specifically bind a miR-126 target. Binding of a miRNA to a miRNA target can result in transcription and/or translation inhibition of the nucleic acid sequence, such as through degradation of the nucleic acid sequence (typically mRNA or other type of RNA), that the miRNA target is part of). A microRNA does not have to have perfect complementarity to a miRNA target for specific binding or transcription inhibition to occur.

As used herein “seed sequence” or “seed region” can refer to the conserved heptametrical sequence of a microRNA that has perfect complementarity to the miRNA target. The seed sequence can be at about positions 2-7 from the miRNA 5'-end.

As used herein, “nonstructural viral protein” and similar phrases can refer to proteins encoded by a virus, but are not incorporated into the viron particle.

As used herein “differentially expressed”, “differential expression,” and the like can refer to the difference in spatial, temporal, and/or amount of expression of a gene, transcript, and/or protein that can be observed between the same or different genes, transcripts, and/or proteins.

Discussion

Despite the remarkable advance in therapeutic strategies over the past decades, the lack of cell selective therapies remains a major hurdle in clinical medicine. Side effects and reduced efficacy due to poor or no selectivity remains the Achilles' heel in the treatment of many diseases and contributes to the significant morbidity and mortality rates associated with many diseases.

Gene therapy holds significant promise to treat, if not cure, many diseases for which there currently is no effective treatment or cure. However, the benefits of gene therapy still

remain illusive. Traditional gene therapy approaches rely on pseudo-viral packaging and delivery of the gene to cells. Off-target effects due to ubiquitous overexpression and oncogenesis due to insertional mutagenesis as a result of poor cell selectivity and insertion control remain issues with virus-based gene therapy. Further, size limitations of currently viral-based systems prevent the use of many viral-based systems for the delivery of many genes of interest.

With that said, described herein are cell-selective RNA molecules that can include a gene of interest or guide sequence RNA (gRNA) operatively coupled to one or more miRNA target sites. The cell-selective RNA molecules can be linear or circular, self-replicating, and/or contain one or more chemical modifications that can reduce immunogenicity of the cell-selective RNA molecules. The compositions described herein can be used for the treatment of diseases or symptoms thereof and/or cell selective gene editing. Other compositions, compounds, methods, features, and advantages of the present disclosure will be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Cell Selective RNA Molecules

As shown in FIG. 1, the cell-selective RNA molecules can contain one or more RNA molecules of interest. The RNA molecule(s) can be operatively coupled to one or more miRNA target sequences. When introduced to cells that have miRNA that can specifically bind to the miRNA target(s), the miRNA can specifically bind the miRNA target(s) of the cell-selective RNA molecules. This can result in degradation and/or prevent translation of the cell-selective RNA molecules through endogenous pathways (e.g. RISC complex-mediated degradation). Thus, there is no observable expression of the RNA of interest. When introduced into cells that do not express miRNA that can specifically bind to the miRNA target(s) of the cell-selective RNA molecule, the cell-selective RNA molecule can be translated by the cell. It will be appreciated that the same outcome can be realized if a cell expresses the miRNA that can specifically bind the miRNA target(s) at such a low level that binding to the cell-selective RNA does not reduce translation (through degradation or otherwise) of enough cell-selective RNA molecules to inhibit or ablate effective expression of the RNA of interest. In this way expression and/or translation of the RNA of interest can be selective to only cells that do not express miRNA that can bind to the miRNA target(s) in the cell-selective RNA molecule. By way of non-limiting example, FIGS. 2-3 show cell-selective expression of p27, Cas9, and/or gRNA that can be used as part of a CRISPR/Cas9 genome modification system.

In some embodiments, the cell-selective RNA molecule can be a RNA molecule. In other embodiments, the cell-selective molecule can be a DNA molecule that corresponds to (or encodes) cell-selective RNA molecule. The cell-selective RNA molecule or its corresponding DNA molecule can exist as naked RNA or DNA molecule (i.e. not contained in a vector) or contained within a vector.

RNA Molecules of Interest

The cell-selective RNA molecules can contain one or more RNA molecules of interest (ROI). The RNA molecule can correspond to a gene of interest. The ROI can correspond to an untranslated RNA molecule. The ROI can be any RNA molecule, linear or circular. The ROI can contain one or more ROI separated by a self-cleaving 2a peptide

sequence. The ROI can contain a sequence corresponding to B18R to mitigate the innate immune response. The B18R can have a sequence that can be 90-100% identical to a sequence that corresponds to SEQ ID NO. 1. ROI can correspond to p27 that can be 90-100% identical to a sequence that corresponds to SEQ ID NO. 2, or any other tumor suppressor gene or suicide gene.

The ROI can be a RNA corresponding to a Cas9 protein. The Casp can have a polypeptide sequence that is identical to or that corresponds to SEQ NO. 3, Cas9n (D10A nickase version of the Cas9 enzyme generates a single-strand DNA break) that correspond to SEQ NO. 4, dCas9 (A catalytically inactive Cas9 or dCas9-repressor peptide fusion can be used to knock-down gene expression by interfering with transcription of the gene) that correspond to SEQ NO. 5 or dCAS9-VP64 activator that corresponds to SEQ NO. 6. The ROI can have or include a sequence 100% identical to any one of SEQ ID NOs: 2-6. The ROI can have or include a sequence that is 90-100% identical to a sequence corresponding to any one of SEQ ID NOs: 2-6. The ROI can be a guide RNA (gRNA).

The ROI can have or include a sequence that is 90-100% identical to SEQ ID NOs: 7. The ROI can be gRNA incorporating two MS2 RNA aptamers gRNA(MS2) cloning backbone include a sequence that is 90-100% identical to SEQ ID NO: 8. The ROI can encode MS2-P65-HSF1 activation helper proteins separated by 2a peptide sequence that have or include a sequence that is 90-100% identical to SEQ ID NO: 9 that can be used as part of a CRISPR/Cas9 genome modification system. The ROI can be self-replicating cell-selective RNA molecules (described below).

miRNA Targets

The cell-selective RNA molecules can contain one or more miRNA targets. In some embodiments, the number of miRNA targets can range from 1 to 20 or more. For example, in some embodiments, the cell-selective RNA molecules can contain 1, 2, 3, 4, or 5 miRNA targets. The miRNA target(s) can be operatively linked to the 5' and/or 3' end of the ROI. The miRNA target(s) can be operatively linked to a 5' untranslated region (UTR) of the ROI and/or a 3' UTR of the ROI. In embodiments having one or more miRNA targets, the miRNA targets can be the same miRNA target, they can each be a different miRNA target. In some embodiments at least two of the miRNA targets are the same. In some embodiments, at least two of the miRNA targets are different. The miRNA targets can be operatively linked to each other and/or the ROI of interest. The miRNA target(s) are nucleotide sequences that can specifically bind one or more miRNAs. The miRNA(s) can have differential spatial and temporal expression. As such, effective expression of the ROI can be controlled both spatially and temporally depending on the miRNA target(s) included in the cell-selective RNA molecule as previously described. Some exemplary constructs incorporating various ROIs are demonstrated in FIGS. 4, 5, 17, 18, 19, 20, 22, and 31-42.

Suitable miRNA targets include, but are not limited to, targets for miR-126, miR-145, miR-296, miR-21, miR-22, miR-15a, miR-16, miR-19b, miR-92, miR-93, miR-96, miR-130, miR-130b, miR-128, miR-9, miR-125b, miR-131, miR-178, miR-124a, miR-266, miR-103, miR-9*, miR-125a, miR-132, miR-137, miR-139, miR-7, miR-124b, miR-135, miR-153, miR-149, miR-183, miR-190, miR-219, miR-18, miR-19a, miR-24, miR-32, miR-213, miR-20, miR-141, miR-193, miR-200b, miR99a, miR127, miR142-a, miR-142-s, miR-151, miR-189b, miR-223, miR-142, miR-122a, miR-152, miR-194, miR-199, miR-215, miR-1b,

miR-1d, miR-133, miR-206, miR-208, miR-143, miR-30b, miR-30c, miR-26a, miR-27a, let-7a, and miR-7b.

In some embodiments, an miRNA target can have a sequence or include a sequence that is about 20-100% identical to the complement of any one of SEQ ID NOs: 10-135. In some embodiments, an miRNA target can have a sequence or include a sequence that is about 30-100% identical to the complement of a portion of any one of SEQ ID NOs: 10-135, where the portion is at least 5 consecutive nucleotides that corresponds to the seed sequence. The miRNAs that can specifically bind to the miRNA target(s) included in the cell-selective RNA molecule can have or include a sequence or portion thereof that is about 20-100% identical to any of SEQ ID NOs: 10-135, where a portion is at least 6 consecutive nucleotides that corresponds to the seed sequence. Where a stem-loop sequence is provided, those of skill in the art will appreciate, which portions correspond to the mature miRNA sequences that can be produced from the stem-loop sequences.

Self-Replicating Cell-Selective RNA Molecules

The cell-selective RNA molecules can be configured such that they are self-replicating. In other words, the cell-selective RNA molecules can be RNA replicons. The cell-selective RNA molecules can also include one or more viral RNA sequences that confer self-replication functionality once the cell-selective RNA molecule is introduced to a cell. The viral RNA sequence(s) can be alphavirus RNA sequences. The viral RNA sequence(s) can be Venezuelan Equine Encephalitis, Sindbis, and/or Semliki Forest virus sequence(s). Such sequences will be appreciated and determined by those of skill in the art. The sequences can encode one or more nonstructural proteins and/or an RNA replicase. The viral sequences can be operatively linked to the ROI and/or miRNA target(s). Noninfectious self-replicating viral RNA that lacks the genes encoding the viral structural proteins that encodes four nonstructural replication complex proteins (NSPs) as a single open reading frame (ORF) in addition to the ROI. In some embodiments, the cell-selective RNA molecules do not include an RNA replicase. Replicases are generally known in the art. Non-limiting examples include, but are not limited to, those set forth in Geall et al., (2012) PNAS 109(36) 14604-14609 and Yoshioka et al. (2013) Stem Cell. 13(2):246-254, which are incorporated by reference as if expressed in their entirety.

Promoter Sequences and Other Transcription Elements

The cell-selective RNA molecules can contain one or more promoters. The promoter(s) can be operatively linked to the 5' end of the cell selective RNA molecule. The promoter(s) can be operatively linked at any position between the 5' and 3' end of the cell-selective RNA molecules. The promoter(s) can be operatively linked to the 5' end of an ROI. The promoter(s) can be operatively linked to the 5' end of a miRNA target. The promoter(s) can drive in vitro and/or in vivo transcription of the cell-selective RNA molecule or corresponding DNA molecule.

The promoter can be a eukaryotic promoter. The promoter can be a prokaryotic promoter. The promoter can be a Pol I promoter. The promoter can be a Pol II promoter. The promoter can be a Pol III promoter. The promoter can be a T7, Sp6 or T3 polymerase promoter. Example promoters include, but are not limited to 26S subgenomic promoter, CMV, CAG, SV40, EF1a, PGK1, Unc, beta actin promoter, TRE, UAS, Ac5, Polyhedrin, CaMKIIa, GAL1, GAL10, TEF1, GDS, GAPDH promoter, ADH1, cAMV35S, Ubi, H1, 7SK, U6, T7, SP6, T7lac, araBAD, trp, lac, Ptac, and pL. The sequences and variants thereof of these promoters as well as other promoters that would be suitable to one of

ordinary skill in the art in view of this description are generally known in the art. See also Yoshioka et al. (2013) Stem Cell. 13(2):246-254 and Addgene Plasmid No.: 58976, which are incorporated by reference as if expressed in their entirety.

Markers

The cell-selective RNA molecule can include one or more markers or reporter molecules. Example markers and reporter molecules include, but are not limited to, Examples of selectable markers include, but are not limited to, DNA and/or RNA segments that contain restriction enzyme sites; DNA segments that encode products that provide resistance against otherwise toxic compounds including antibiotics, such as, spectinomycin, ampicillin, kanamycin, tetracycline, Basta, neomycin phosphotransferase II (NEO), hygromycin phosphotransferase (HPT) and the like; DNA and/or RNA segments that encode products that are otherwise lacking in the recipient cell (e.g., tRNA genes, auxotrophic markers); DNA and/or RNA segments that encode products which can be readily identified (e.g., phenotypic markers such as β -galactosidase, GUS; fluorescent proteins such as green fluorescent protein (GFP), cyan (CFP), yellow (YFP), red (RFP), luciferase, and cell surface proteins); the generation of new primer sites for PCR (e.g., the juxtaposition of two DNA sequence not previously juxtaposed), the inclusion of DNA sequences not acted upon or acted upon by a restriction endonuclease or other DNA modifying enzyme, chemical, etc.; epitope tags (e.g. FLAG- and His-tags), and, the inclusion of a DNA sequences required for a specific modification (e.g., methylation) that allows its identification. Other suitable markers will be appreciated by those of skill in the art.

The terms "plasmid", "vector" and "cassette" as used herein can refer to an extra chromosomal element often carrying genes that are not part of the central metabolism of the cell, and usually in the form of double-stranded DNA. Such elements may be autonomously replicating sequences, genome integrating sequences, phage, or nucleotide sequences, in linear or circular form, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a polynucleotide of interest into a cell.

Vectors

Also provided herein are nucleic acid vectors containing a nucleic acid molecule corresponding to a cell-selective RNA molecule described herein. All or part of the vectors can be capable of being transcribed in vitro without a host cell or in a host cell. The vectors can be capable of being replicated by a host cell. All or part of the vector or a RNA molecule produced from the vector template can be capable of being integrated directly or indirectly into a host cell genome. The vectors can be viral vectors, i.e. vectors that are virus based or incorporate viral proteins or nucleic acids corresponding to a viral protein. Suitable viral vectors can include adenoviral, lentiviral, retroviral, and alpha viral vectors.

Synthesis of the Cell-Selective RNA Molecules

The cell-selective RNA molecules can be synthesized using de novo chemical synthesis. The cell-selective RNA molecules can be synthesized using in vitro transcription from a DNA molecule template. In vitro transcription can occur within a host cell or without a host cell. The cell-selective RNA molecules can also be transcribed in vivo after delivery to a subject. The DNA molecule template can be a DNA molecule or DNA vector containing a DNA sequence corresponding to the cell-selective RNA mol-

ecules. These DNA molecules and vectors are described elsewhere herein. The cell-selective RNA molecules can be polyadenylated at the 3' end. The cell-selective RNA molecules can be 5' capped in vitro. The cell-selective RNA molecules can be synthesized with an ARCA Cap at the 5' end. The RNA molecule can be expressed in a bacterial, viral, yeast, plant, insect, or mammalian expression system. Suitable systems will be appreciated by those skilled in the art. The RNA molecule produced from transcription can be purified from a solution and/or other cellular components. Methods of RNA purification will be appreciated by those of skill in the art.

In some embodiments, the cell-selective RNA molecules are modified. The modification can occur during or after synthesis (transcription of the RNA molecule). The modification can be a nucleotide modification. As such, the cell-selective RNA molecules can be synthesized with one or more modified nucleotides. Suitable nucleotide modifications include, but are not limited to, pseudouridine (ψ U), N-1-methylpseudouridine, 5-methoxyuridine, and 5-hydroxymethylcytidine. In some embodiments 0-100% of the nucleotides are substituted. The modifications can reduce immunogenicity of the cell-selective RNA molecules. The modifications can increase transcription and/or translation of the cell-selective RNA molecules.

Delivery of the Cell-Selective RNA Molecules

The cell-selective RNA molecules, corresponding DNA molecule, vectors, virions, or pseudoviral particles described herein can be delivered to a subject as part of a pharmaceutical formulation as described herein. As described elsewhere herein, the cell-selective RNA molecules can be delivered to a cell by transfection, transduction, or by other suitable method. In other embodiments, a DNA based vector or polynucleotide that encodes a cell-selective RNA molecule as described herein can be configured to be delivered to a cell as being incorporated in a virus, pseudovirus, or virus particle. In these embodiments, the cells can be transduced or infected with a virus, pseudovirus, or other virus particle that can deliver the corresponding DNA molecule to the cell. In other embodiments, the cell-selective RNA molecules and/or corresponding DNA molecule can be delivered to a cell via chemical transfection of a cell. Chemical transfection methods include encapsulating the cell-selective RNA molecules and/or corresponding DNA molecules in a liposome or micelle (e.g. cationic liposome), which can then be taken in by the cell via endocytosis. Suitable transfection reagents will be appreciated by those of skill in the art. In further embodiments, the cell-selective RNA molecules and/or corresponding DNA molecules can be incorporated with mesoporous silica nanoparticles that can include a polycation adjunct or large pore mesoporous silica nanoparticles. The mesoporous silica nanoparticles that include the cell-selective RNA molecules and/or corresponding DNA molecules can be taken up by the cell via endocytosis. In other embodiments, the cell-selective RNA molecules and/or corresponding DNA molecules can couple to organic/inorganic silica hybrid nanoparticles which can be taken up by a cell via endocytosis. Other delivery methods include electroporation or chitosan polymers. Other delivery methods will be appreciated by those of ordinary skill in the art.

Pharmaceutical Formulations

Also provided herein are pharmaceutical formulations containing an amount of a cell-selective RNA molecule, corresponding DNA molecule (including vectors), and/or viron particle as described herein. The amount can be an effective amount. Pharmaceutical formulations can be for-

mulated for delivery via a variety of routes and can contain a pharmaceutically acceptable carrier. Techniques and formulations generally can be found in *Remington's Pharmaceutical Sciences*, Meade Publishing Co., Easton, Pa. (20th Ed., 2000), the entire disclosure of which is herein incorporated by reference. For systemic administration, an injection is useful, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the therapeutic compositions of the invention can be formulated in liquid solutions, for example in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the therapeutic compositions can be formulated in solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included. Pharmaceutical compositions of the present invention are characterized as being at least sterile and pyrogen-free. These pharmaceutical formulations include formulations for human and veterinary use.

Suitable pharmaceutically acceptable carriers include, but are not limited to water, salt solutions, alcohols, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxyl methylcellulose, and polyvinyl pyrrolidone, which do not deleteriously react with the active composition.

The pharmaceutical formulations can be sterilized, and if desired, mixed with auxiliary agents, such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances, and the like which do not deleteriously react with the active composition.

The pharmaceutical formulations can be administered to a subject in need thereof. The subject in need thereof can have a disease, disorder, or a symptom thereof. Example disease or disorder can include, but are not limited to, a cardiovascular disease, a pulmonary disease, a brain disease, a renal disease, a liver disease, a blood disease, a nervous system disease, an intestinal disease, an ocular disease, and cancer. The pharmaceutical formulations can be disposed on or otherwise coupled to or integrated with a medical device, such as, but not limited to, catheters or stents, such that the pharmaceutical formulation is eluted from the medical device over a time period. The pharmaceutical formulation can therefore be delivered to a subject in need thereof during and/or after a procedure such as an angioplasty, vein graft or organ transplant. Other procedures where such a medical device would be useful will be appreciated by those of skill in the art.

A pharmaceutical formulation can be formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable

syringes or multiple dose vials made of glass or plastic. The construct, biologic molecules and pharmaceutical formulations thereof described herein can be disposed on or otherwise integrated with or coupled to a medical device such as, but not limited to, a catheter or stent, such that the construct, biological molecule can be released to the surrounding local area or systemically over a period of time after insertion or implantation into a subject in need thereof. These can also be referred to as drug eluting medical devices.

Pharmaceutical formulations suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers can include physiological saline, bacteriostatic water, Cremophor EM™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). Injectable pharmaceutical formulations can be sterile and can be fluid to the extent that easy syringability exists. Injectable pharmaceutical formulations can be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, a pharmaceutically acceptable polyol like glycerol, propylene glycol, liquid polyethylene glycol, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it can be useful to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of injectable compositions can be brought about by incorporating an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating any of the cell-selective RNA molecules, corresponding DNA molecules, or viron particles as described herein in an amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions can be prepared by incorporating the nucleic acid vectors into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, examples of useful preparation methods are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fluidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the cell-selective RNA molecules, corresponding DNA molecules, and/or viron particles can be formulated into ointments, salves, gels, or creams as generally known in the art. In some embodiments, the cell-selective RNA molecules, corresponding DNA molecules, and/or viron particles can be applied via transdermal delivery systems, which can slowly release the cell-selective

RNA, corresponding DNA molecule, and/or viron particles for percutaneous absorption. Permeation enhancers can be used to facilitate transdermal penetration of the active factors in the conditioned media. Transdermal patches are described in for example, U.S. Pat. Nos. 5,407,713; 5,352,456; 5,332,213; 5,336,168; 5,290,561; 5,254,346; 5,164,189; 5,163,899; 5,088,977; 5,087,240; 5,008,110; and 4,921,475.

Administration of the cell-selective RNA molecules, corresponding DNA molecules, and/or viron particles is not restricted to a single route, but may encompass administration by multiple routes. For instance, exemplary administrations by multiple routes include, among others, a combination of intradermal and intramuscular administration, or intradermal and subcutaneous administration. Multiple administrations may be sequential or concurrent. Other modes of application by multiple routes will be apparent to the skilled artisan.

The pharmaceutical formulations can be administered to a subject by any suitable method that allows the agent to exert its effect on the subject in vivo. For example, the formulations or other compositions described herein can be administered to the subject by known procedures including, but not limited to, by oral administration, sublingual or buccal administration, parenteral administration, transdermal administration, via inhalation, via nasal delivery, vaginally, rectally, and intramuscularly. The formulations or other compositions described herein can be administered parenterally, by epifascial, intracapsular, intracutaneous, subcutaneous, intradermal, intrathecal, intramuscular, intraperitoneal, intrasternal, intravascular, intravenous, parenchymatous, and/or sublingual delivery. Delivery can be by injection, infusion, catheter delivery, or some other means, such as by tablet or spray. In some embodiments, the nucleic acid vectors of the invention are administered to the subject by way of delivery directly to the heart tissue, such as by way of a catheter inserted into, or in the proximity of the subject's heart, or by using delivery vehicles capable of targeting the drug to the heart. For example, the cell-selective RNA molecules, corresponding DNA molecules, and/or viron particles described herein can be conjugated to or administered in conjunction with an agent that is targeted to the heart, such as an aptamer, antibody or antibody fragment. The cell-selective RNA molecules, corresponding DNA molecules, and/or viron particles can be administered to the subject by way of delivery directly to the tissue of interest, such as by way of a catheter inserted into, or in the proximity of the subject's tissue of interest, or by using delivery vehicles capable of targeting the nucleic acid vectors to the muscle, such as an antibody or antibody fragment.

For oral administration, a formulation as described herein can be presented as capsules, tablets, powders, granules, or as a suspension or solution. The formulation can contain conventional additives, such as lactose, mannitol, cornstarch or potato starch, binders, crystalline cellulose, cellulose derivatives, acacia, cornstarch, gelatins, disintegrators, potato starch, sodium carboxymethylcellulose, dibasic calcium phosphate, anhydrous or sodium starch glycolate, lubricants, and/or or magnesium stearate.

For parenteral administration (i.e., administration by through a route other than the alimentary canal), the formulations described herein can be combined with a sterile aqueous solution that is isotonic with the blood of the subject. Such a formulation can be prepared by dissolving the active ingredient in water containing physiologically-compatible substances, such as sodium chloride, glycine and the like, and having a buffered pH compatible with physi-

ological conditions, so as to produce an aqueous solution, then rendering the solution sterile. The formulation can be presented in unit or multi-dose containers, such as sealed ampoules or vials. The formulation can be delivered by injection, infusion, or other means known in the art.

For transdermal administration, the formulation described herein can be combined with skin penetration enhancers, such as propylene glycol, polyethylene glycol, isopropanol, ethanol, oleic acid, N-methylpyrrolidone and the like, which increase the permeability of the skin to the nucleic acid vectors of the invention and permit the nucleic acid vectors to penetrate through the skin and into the bloodstream. The formulations and/or compositions described herein can be further combined with a polymeric substance, such as ethylcellulose, hydroxypropyl cellulose, ethylene/vinyl acetate, polyvinyl pyrrolidone, and the like, to provide the composition in gel form, which can be dissolved in a solvent, such as methylene chloride, evaporated to the desired viscosity and then applied to backing material to provide a patch.

Dosage Forms

The pharmaceutical formulations or compositions described herein can be provided in unit dose form such as a tablet, capsule or single-dose injection or infusion vial. Where appropriate, the dosage forms described herein can be microencapsulated. The dosage form can also be prepared to prolong or sustain the release of any ingredient. In some embodiments, the complexed active agent can be the ingredient whose release is delayed. In other embodiments, the release of an auxiliary ingredient is delayed. Suitable methods for delaying the release of an ingredient include, but are not limited to, coating or embedding the ingredients in material in polymers, wax, gels, and the like. Delayed release dosage formulations can be prepared as described in standard references such as "Pharmaceutical dosage form tablets," eds. Liberman et. al. (New York, Marcel Dekker, Inc., 1989), "Remington—The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, Md., 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6th Edition, Ansel et al., (Media, Pa.: Williams and Wilkins, 1995). These references provide information on excipients, materials, equipment, and processes for preparing tablets and capsules and delayed release dosage forms of tablets and pellets, capsules, and granules. The delayed release can be anywhere from about an hour to about 3 months or more.

Coatings may be formed with a different ratio of water soluble polymer, water insoluble polymers, and/or pH dependent polymers, with or without water insoluble/water soluble non polymeric excipient, to produce the desired release profile. The coating is either performed on the dosage form (matrix or simple) which includes, but is not limited to, tablets (compressed with or without coated beads), capsules (with or without coated beads), beads, particle compositions, "ingredient as is" formulated as, but not limited to, suspension form or as a sprinkle dosage form.

Examples of suitable coating materials include, but are not limited to, cellulose polymers such as cellulose acetate phthalate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and hydroxypropyl methylcellulose acetate succinate; polyvinyl acetate phthalate, acrylic acid polymers and copolymers, and methacrylic resins that are commercially available under the trade name EUDRAGIT® (Roth Pharma, Westerstadt, Germany), zein, shellac, and polysaccharides.

In some embodiments, such as for treatments of plants, the topical formulation of a composition or pharmaceutical formulation described herein can be further formulated as a

spray and can include a suitable surfactant, wetting agent, adjuvants/surfactant (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents), or any combination thereof so as to formulated as a spray. The compounds, any optional auxiliary active ingredient, suitable surfactant, wetting agent, adjuvants, or any combination thereof can be formulated as a solution, suspension, or emulsion. The spray dosage form can be administered through a spraying device. In some embodiments, the spraying device can be configured to generate the sprayable formulation as a liquid solution is contacted with the complexed active agent compound or formulation thereof. In other embodiments, the sprayable dosage form is pre-made prior to spraying. As such, the spraying device can act solely as an applicator for these embodiments.

In further embodiments, such as for treatments of plants (e.g. such as a herbicide), the dosage form of composition or pharmaceutical formulation described herein thereof can be further formulated as a dust and can include a suitable dry inert carrier (e.g. talc chalk, clay, nut hull, volcanic ash, or any combination thereof so as to be formulated as a dust. The dust can contain dust particles of varying sizes. In some embodiments, the particle size can be substantially homogeneous. In other embodiments, the particle size can be heterogeneous. Dosage forms adapted as a dust can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

In some embodiments, the dosage form can be formulated as a bait. In these embodiments, the complexed active agent compound or other formulation thereof can be further formulated to include a food or other attractive substance that can attract one or more insect or other pest. The bait dosage form can be formulated as a dust, paste, gel, or granule. Dosage forms adapted as baits can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

In additional embodiments, the dosage form can be formulated as granules or pellets that can be applied to the environment. These dosage formulations are similar to dust formulations, but the particles are larger and heavier. The granules can be applied to soil or other environmental area. Dosage forms adapted as granules or pellets can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

The dusts, granules, and pellets described herein can be formulated as wettable dusts, granules, and pellets, soluble dusts granules, and pellets, and/or water-dispersible granules, and/or dry flowables.

The dosage form can be adapted for impregnating (saturating) an object or device, which then can be carried by, worn, or otherwise coupled to an organism in need thereof. In some embodiments, the dosage form can be impregnated onto a collar, bracelet, patch, adhesive tape, livestock ear tags, clothing, blankets, plastics, nets, and paints. The composition or pharmaceutical formulation thereof can be formulated and impregnated in the object or device such that the composition or pharmaceutical formulation evaporates over time, which releases the composition and/or pharmaceutical formulation into the air and/or environment surrounding the organism and/or onto the organism.

The dosage form can be adapted as a fumigant, which is a formulation that forms a gas when utilized or applied. In some embodiments, the composition and/or pharmaceutical

formulation thereof can be supplied as a liquid when packaged under pressure and change to a gas when they are released. In other embodiments, the composition and/or pharmaceutical formulation thereof can be supplied as a volatile liquid when enclosed in a container (not under pressure). Others can be formulated as solids that release gases when applied under conditions of high humidity or in the presence of high water vapor. Dosage forms adapted as fumigants can contain one or more adjuvants/surfactants (stickers, extender, plant penetrant, compatibility agents, buffers, drift control additives, and defoaming agents).

Effective Amounts

The pharmaceutical formulations can contain an effective amount of a composition described herein and/or an effective amount of an auxiliary agent. In some embodiments, the effective amount ranges from about 0.001 pg to about 1,000 g or more of the composition described herein. In some embodiments, the effective amount of the composition described herein can range from about 0.001 mg/kg body weight to about 1,000 mg/kg body weight. In yet other embodiments, the effective amount of the composition can range from about 1% w/w to about 99% or more w/w, w/v, or v/v of the total pharmaceutical formulation.

Combination Therapy

The pharmaceutical formulations or other compositions described herein can be administered to a subject either as a single agent, or in combination with one or more other agents. Additional agents include but are not limited to DNA, RNA, amino acids, peptides, polypeptides, antibodies, aptamers, ribozymes, guide sequences for ribozymes that inhibit translation or transcription of essential tumor proteins and genes, hormones, immunomodulators, antipyretics, anxiolytics, antipsychotics, analgesics, antispasmodics, anti-inflammatories, anti-histamines, anti-infectives, and chemotherapeutics. Multiple cell-selective ROI can be administered simultaneously in a combination treatment.

Suitable antipyretics include, but are not limited to, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), aspirin and related salicylates (e.g. choline salicylate, magnesium salicylate, and sodium salicylate), paracetamol/acetaminophen, metamizole, nabumetone, phenazone, and quinine.

Suitable anxiolytics include, but are not limited to, benzodiazepines (e.g. alprazolam, bromazepam, chlordiazepoxide, clonazepam, clorazepate, diazepam, flurazepam, lorazepam, oxazepam, temazepam, triazolam, and tofisopam), serotonergic antidepressants (e.g. selective serotonin reuptake inhibitors, tricyclic antidepressants, and monoamine oxidase inhibitors), mebicar, afobazole, selank, bromantane, emoxypine, azapirone, barbiturates, hydroxyzine, pregabalin, validol, and beta blockers.

Suitable antipsychotics include, but are not limited to, benperidol, bromoperidol, droperidol, haloperidol, moperone, pipaperone, timiperone, fluspirilene, penfluridol, pimozide, acepromazine, chlorpromazine, cyamemazine, dizyrazine, fluphenazine, levomepromazine, mesoridazine, perazine, pericyazine, perphenazine, pipotiazine, prochlorperazine, promazine, promethazine, prothipendyl, thiochlorpromazine, thioridazine, trifluoperazine, trifluorpromazine, chlorprothixene, clopenthixol, flupentixol, tiotixene, zuclopenthixol, clotiapine, loxapine, prothipendyl, caripramine, clocapramine, molindone, mosapramine, sulpiride, veralipride, amisulpride, amoxapine, aripiprazole, asenapine, clozapine, blonanserin, iloperidone, lurasidone, melperone, nemonapride, olanzapine, paliperidone, perospirone, quetiapine, remoxipride, risperidone, sertindole, trimipramine,

ziprasidone, zotepine, alstonie, befeprunox, bitopertin, brexpiprazole, cannabidiol, cariprazine, pimavanserin, pomaglutmetad methionil, vabicaserin, xanomeline, and zicronapine.

Suitable analgesics include, but are not limited to, paracetamol/acetaminophen, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), COX-2 inhibitors (e.g. rofecoxib, celecoxib, and etoricoxib), opioids (e.g. morphine, codeine, oxycodone, hydrocodone, dihydromorphine, pethidine, buprenorphine), tramadol, norepinephrine, flupiretine, nefopam, orphenadrine, pregabalin, gabapentin, cyclobenzaprine, scopolamine, methadone, ketobemidone, piritramide, and aspirin and related salicylates (e.g. choline salicylate, magnesium salicylate, and sodium salicylate).

Suitable antispasmodics include, but are not limited to, mebeverine, papaverine, cyclobenzaprine, carisoprodol, orphenadrine, tizanidine, metaxalone, methocarbamol, chlorzoxazone, baclofen, dantrolene, baclofen, tizanidine, and dantrolene.

Suitable anti-inflammatories include, but are not limited to, prednisone, non-steroidal anti-inflammants (e.g. ibuprofen, naproxen, ketoprofen, and nimesulide), COX-2 inhibitors (e.g. rofecoxib, celecoxib, and etoricoxib), and immune selective anti-inflammatory derivatives (e.g. submandibular gland peptide-T and its derivatives).

Suitable anti-histamines include, but are not limited to, H₁-receptor antagonists (e.g. acrivastine, azelastine, bilastine, brompheniramine, buclizine, bromodiphenhydramine, carbinoxamine, cetirizine, chlorpromazine, cyclizine, chlorpheniramine, clemastine, cyproheptadine, desloratadine, dexbrompheniramine, dexchlorpheniramine, dimenhhydrinate, dimetindene, diphenhydramine, doxylamine, ebastine, embramine, fexofenadine, hydroxyzine, levocetirizine, loratadine, meclozine, mirtazapine, olopatadine, orphenadrine, phenindamine, pheniramine, phenyltoloxamine, promethazine, pyrilamine, quetiapine, rupatadine, tripeleminamine, and triprolidine), H₂-receptor antagonists (e.g. cimetidine, famotidine, lafutidine, nizatidine, ranitidine, and roxatidine), triptolamine, catechin, cromoglicate, nedocromil, and β_2 -adrenergic agonists.

Suitable anti-infectives include, but are not limited to, amebicides (e.g. nitazoxanide, paromomycin, metronidazole, tinidazole, chloroquine, and iodoquinol), aminoglycosides (e.g. paromomycin, tobramycin, gentamicin, amikacin, kanamycin, and neomycin), anthelmintics (e.g. pyrantel, mebendazole, ivermectin, praziquantel, albendazole, miltefosine, thiabendazole, oxamniquine), antifungals (e.g. azole antifungals (e.g. itraconazole, fluconazole, posaconazole, ketoconazole, clotrimazole, miconazole, and voriconazole), echinocandins (e.g. caspofungin, anidulafungin, and micafungin), griseofulvin, terbinafine, flucytosine, and polyenes (e.g. nystatin, and amphotericin b), antimalarial agents (e.g. pyrimethamine/sulfadoxine, artemether/lumefantrine, atovaquone/proquanil, quinine, hydroxychloroquine, mefloquine, chloroquine, doxycycline, pyrimethamine, and halofantrine), antituberculosis agents (e.g. aminosaliclates (e.g. aminosaliclic acid), isoniazid/rifampin, isoniazid/pyrazinamide/rifampin, bedaquiline, isoniazid, ethambutol, rifampin, rifabutin, rifapentine, capreomycin, and cycloserine), antivirals (e.g. amantadine, rimantadine, abacavir/lamivudine, emtricitabine/tenofovir, cobicistat/elvitegravir/emtricitabine/tenofovir, efavirenz/emtricitabine/tenofovir, avacavir/lamivudine/zidovudine, lamivudine/zidovudine, emtricitabine/tenofovir, emtricitabine/opinavir/ritonavir/tenofovir, interferon alfa-2v/ribavirin, peginterferon alfa-2b, maraviroc, raltegravir, dolutegravir, enfuvirtide, foscarnet, fomivirsen, oseltamivir, zanamivir, nevirapine, efavirenz,

Despite the remarkable advances in therapeutic strategies over the past decades the lack of cell selective therapies

remain a major overarching problem in clinical medicine. One clear example for this challenge is the deadly consequences of the non-selective agent used on the drug-eluting stents (DES) that were developed to inhibit neointimal overgrowth of vascular smooth muscle cells (VSMCs) following percutaneous intervention.

While DES significantly reduced the occurrence of restenosis compared with a bare-metal stent (BMS), they do not completely eliminate this challenging problem. Moreover, stent thrombosis (ST), reinfarction, and neoatherosclerosis within stent segments have emerged as major safety concerns with DES, all predominantly attributed to the lack of reendothelialization of diseased vessel walls with competent endothelial cells (ECs). DES deployment inevitably traumatizes the normal competent endothelium structure. Compounding this insult, the drugs eluted from the stents, while not delivered systemically, are still universally deleterious to all cell types exposed to the eluted drug, proving toxic to ECs and drastically reducing the quality of regenerating endothelium. This incompetent endothelium, with poorly formed cell-to-cell junctions reduced expression of anti-thrombotic molecules and endothelial nitric oxide synthase (eNOS), can no longer function normally to maintain vascular tone and fluid-tissue homeostasis. Thus, this requires patients to comply with at least 12 months of dual anti-platelet therapy.

Newer generations of DES, with more sophisticated platforms, thinner struts, and biocompatible polymers have been developed to combat DES-associated risks. Yet, they still deploy the same non-selective drugs (paclitaxel, sirolimus or its analogs, everolimus, zotarolimus and biolimus, with improved pharmacokinetics). Current DES devices with low rates of restenosis and ST translate into a significant number of patients that will suffer from myocardial infarction and death due to the large number of percutaneous coronary intervention (PCI) procedures performed worldwide every year. In fact, percutaneous interventions are among the most performed procedures in Medicine. In the US alone, nearly 1 million patients undergo PCI for symptomatic coronary artery disease (CAD) every year, and non-selective DES are deployed in at least 75% of these cases. In 2010, the DES segment contributed 55%-60% of the global coronary stent market and is expected to reach USD 5.3 billion in 2016 due to the growing aging population and lifestyle changes leading to obesity. The market for DES is growing at 9.0% in the United States, 3.1% in Europe, 10% in Asia-Pacific and 3.1% in the rest of the world. These numbers will potentially expand as DES deployment is used in percutaneous interventions to treat peripheral artery disease (PAD) that affects more than 10 million people in the US, with symptomatic lower-extremity PAD, renal artery stenosis and carotid artery disease.

Although percutaneous transluminal angioplasty (PTA) and stenting achieve a greater lumen diameter, vessel remodeling and restenosis remain its Achilles' heel contributing to significant morbidity and mortality rates in these patients. To date, cell-selective drugs that can discriminate between proliferating VSMCs, inflammatory cells and ECs are not available. Since vascular ECs provide crucial protection against thrombosis, lipid uptake and inflammation, there is a need to develop a cell-selective therapy that can inhibit VSMC proliferation and inhibit infiltration of inflammatory cells, yet spare ECs to carry on their vital functions.

A viral vector approach has been previously developed that contained an EC specific miRNA target sequence (target for miR-126). In that work an adenoviral vector (Ad-p27-126TS) containing target sequences complementary to the

mature miR-126-3p strand at the 3'-end of the cyclin-dependent kinase inhibitor p27Kip1 (p27). This approach allowed for exogenous p27 to be selectively overexpressed in VSMCs and infiltrate inflammatory cells, yet preserving the ECs. This therapy achieved results in an established rat model of balloon angioplasty, selectively inhibiting neointimal hyperplasia and inflammation while simultaneously promoting vessel reendothelialization, reducing hypercoagulability and restoring the endothelium-dependent vasodilatory response to levels indistinguishable from uninjured controls. (FIGS. 21-29).

Due to complications with viral gene delivery, this Example describes a messenger RNA based, cell-selective nanotherapy utilizing the mRNA or self-replicating RNA that can be based on an alphavirus genome. This strategy can match the potency of viral vectors yet avoid the serious safety concerns associated with recombinant virus-based therapeutics. This approach can reduce the need for repeated revascularization, reduce the need for prolonged dual anti-platelet drug regimens, and ultimately reduce the morbidity and mortality of CAD and PAD patients. This mRNA based cell-selective nanotherapy could not only replace the use of stents, but potentially treat lesions where DES cannot be deployed or DES is contraindicated, such as in stent restenosis, bifurcations, torturous vessels, small vessels or long calcifications. In addition to its potential benefit in CAD and PAD, this approach can be applied to benefit multiple other stenotic conditions, including transplant vasculopathy, arteriovenous fistulae, and vein graft failure, which all result from VSMC hyperplasia and EC dysfunction. Over 200,000 cardiovascular surgical procedures utilizing venous grafts fail each year in the US, primarily due to restenosis caused by VSMC hyperplasia. This mRNA based cell-selective approach may also inhibit vascular remodeling in deadly diseases such as pulmonary hypertension and pulmonary fibrosis, which still has no cure. The versatile concept of mRNA based, cell-selective nanotherapy can be broadly applied across disciplines and for treating any disease, including but not limited to, liver cirrhosis and cancer. This tailored mRNA-based, cell-selective nanotherapy represents an exciting translational and potentially transformative approach in modern clinical medicine.

mRNA-based therapeutics represent a game-changing technology and hold great promise for the treatment of human diseases including genetic disorders, infections, degenerative diseases and cancer. Many are the advantages of these versatile mRNA-based therapies. They can be produced very quickly, cost effectively and in a cell-free system at good manufacturing practice quality. Furthermore, any nucleotide sequence needed can be synthetically produced and stored at room temperature. Importantly, chemically modified mRNA that does not change the amino acid sequence of the corresponding protein not only decreases activation of the innate immune pathway but also improves the stability and enhances the translation levels. mRNA is non-replicative and therefore considered a very safe biomolecule that allows transient protein expression of every protein in virtually all cell types including non-dividing cells. Moreover, no nuclear localization, promoter elements or transcription is required and unlike recombinant virus-vectors the probability of genomic integration is nearly nonexistent. Lastly, chemically modified mRNAs that elude the body's innate immune response make therapeutic gene products and protein replacement therapies possible. However, there still exists the challenge of cell- or tissue-specific delivery that hinders virtually any type of therapeutic agent.

To overcome the deficiencies stated above the miRNA-based cell selective approach can be utilized in a chemically modified self-replicating RNA platform to achieve long lasting cell-selective targeting that can inhibit proliferating VSMCs and infiltration of inflammatory cells while allowing ECs to reendothelialize vessel walls and maintain their crucial function. miR-126 is enriched in ECs and is a pivotal regulator of vascular integrity and angiogenesis. Moreover, miR-126 was observed to be up-regulated following arterial injury and in atherosclerotic plaques. The approach described herein utilizes EC-specific expression of miR-126 to drive cell-specific expression of therapeutic genes of interest.

As proof of principle, an adenoviral vector (Ad-p27-126TS) containing target sequences complementary to the mature miR-126-3p strand at the 3'-end of the p27 to selectively avoid p27 overexpression in ECs, but not in VSMCs was designed FIG. 22-23. Employing this single comprehensive nanotherapy in a rat carotid balloon injury model we were able to inhibit neointimal hyperplasia, inhibit infiltration of inflammatory cells to the injury site, and at the same time complete reendothelialization of the vessels was achieved as soon as 2 weeks post injury restoring the endothelium-dependent vasodilatory response to levels indistinguishable from uninjured controls and reducing the plasma D-Dimer levels of injured vessels to levels observed in uninjured controls FIG. 25-29.

Incomplete and incompetent endothelial coverage in the vessel when deployed with current DES in PCI procedures greatly increased the risk of potentially catastrophic events such as late ST, primarily caused by the lack of drug specificity. Therefore, a therapy that would selectively inhibit VSMC proliferation, migration and inflammatory cell infiltration without affecting reendothelialization and EC function in the treated vessels would be advantageous. To establish proof of principle for cell-selective inhibition we chose to overexpress p27 in a cells selective manner due to the proven role it plays in the pathophysiology of vascular remodeling. As one of the most potent members of the Cip/Kip family of cyclin-dependent kinase (CDK) inhibitors, p27 binds and modulates cyclin D-, E- and A-dependent kinases, resulting in G1/S transition failure and cell cycle arrest. In healthy arteries, p27 is constitutively expressed in quiescent VSMCs. Upon vascular injury, however, multiple response mechanisms are initiated to conclude with a rapid downregulation of p27, activating and enabling VSMCs to resume cell division. This reentry into the cell cycle triggers intimal hyperplasia, leading to vascular restenosis. p27 knockout mice display a significant increase in VSMC proliferation and develop extensive arterial lesions. In contrast, overexpression of exogenous p27 in VSMCs instigates G1 phase arrest, resulting in VSMC growth inhibition and a significant reduction of neointimal lesion formation in both a porcine femoral arterial injury model as well as a rat carotid model of balloon angioplasty. Moreover, p27 has been shown to play a key role in atherosclerosis. Indeed, p27 deficiency leads to increased atherosclerotic plaque formation in Apoe^{-/-} mice. Lastly, previous reports show that p27 directly regulates the proliferation and migration of bone marrow-derived cells (hematopoietic and nonhematopoietic) to the damaged vessels to reconstitute vascular lesions. Therefore, p27 is a particularly ideal candidate for cell-selective regulation. This approach also takes advantage of the established fact that miR-126 is robustly enriched in EC and is a pivotal regulator of vascular integrity

and angiogenesis. Moreover, miR-126 was shown to be up-regulated following arterial injury and in atherosclerotic plaques.

An adenoviral (Ad) vector encoding p27 a known cell-cycle inhibitor, and incorporating 4 complementary target sequences for the mature miR-126-3p strand at its 3' end (Ad-p27-126TS) FIGS. 22-23. Our aim was to overexpress exogenous p27, yet effectively regulate its overexpression in a cell-specific manner with the incorporated EC-specific miR-126-3p target sequences FIG. 24. Employing this single comprehensive nanotherapy (Ad-p27-126TS) in a rat carotid balloon injury model the following was achieved: 1) inhibition of neointimal hyperplasia (FIG. 25); 2) inhibition of infiltration of inflammatory cells to the injury site (FIG. 26); 3) reendothelialize vessels rapidly and extensively (FIGS. 27A-B) 4) reduce the plasma D-Dimer levels of injured vessels to levels observed in uninjured controls (FIG. 28); and 5) restore the endothelium-dependent vasodilatory response to levels indistinguishable from uninjured controls (FIG. 29).

These data demonstrate that the simple incorporation of miR-126 target sequences within the Ad-p27-126TS vector provided robust EC protection and at the same time achieved significant inhibition of the neointimal hyperplasia and infiltration of inflammatory cells to the injury site. Although viral delivery systems are very efficient for in vivo transduction and delivery of nucleic acids, they suffer major drawbacks including their possible toxicity, immunogenicity, insertional mutagenicity and oncogenicity. Additionally, viral vectors are difficult and expensive to produce in large quantities. As such the design and engineering of alternative nonviral systems for delivery of therapeutic agents is needed. Presented here are mRNA-based, cell-selective nanotherapies that can self-replicate and can consistently express high levels of exogenous p27 and at the same time retain its cell-selective degradation in a miRNA-controlled fashion, in some cases over multiple cellular divisions.

To provide proof of concept and to show the feasibility of our cell-selective mRNA approach we constructed the following GFP-encoding plasmids: A) GFP coding sequence placed under the control of the bacteriophage T7 RNA polymerase promoter. To increase the stability of the mRNA we flanked GFP coding sequence with β -globin 5'- and 3'-UTR (FIG. 4); B) GFP-2 \times 126TS, a GFP-coding sequence placed under the control of the bacteriophage T7 RNA polymerase promoter, flanked with β -globin 5'- and 3'-UTR containing 2 or 3 tandem copies of a 22-bp target sequence perfectly complementary to the mature miR-126-3p strand at its 3' end (FIG. 4).

In vitro transcription reaction from the linear plasmid templates described in FIG. 4 was performed using T7 RNA polymerase to produce unmodified (regular nucleotides, NTPs) or modified RNA in which we substituted 100% of the uridine with pseudouridine. After removal of free NTPs, 5' capping and poly(A) tail addition was performed resulting in a high-yield RNA transcript. HEK cells were transfected with miR-126, miR-143 or control mimic and after 24 hr cells were transfected with either unmodified or modified GFP, GFP-2 \times 126TS or GFP-3 \times 126TS mRNAs. The effect of over-expression miR-126, miR-143 or control on GFP protein levels was assessed after 24 hr (FIGS. 6-14). First, a significant increase in GFP expression was observed when uridine was 100% substituted with pseudouridine (FIGS. 7 and 13. In cells transfected with the control unmodified or modified GFP mRNA neither over-expression of miR-126-3p or miR-143 had an effect on GFP expression (FIGS. 6-10 and 13-14). HEK cells transfected with GFP-2 \times miR-126TS

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or GFP-3×126TS mRNA, over-expression of miR-126 dramatically inhibited GFP expression of the unmodified or the modified mRNA (FIGS. 6-8 and 11-14). These data demonstrate that pseudouridine modified GFP-2×126TS mRNA is susceptible to argonaute-dependent gene silencing by miR-126. This effect was miR-126 specific since over-expression of miR-143 had no effect on GFP expression in cells transfected with either unmodified or modified GFP-2×miR-126TS or GFP-3×126TS (FIGS. 6-8 and 11-14). The data show that pseudouridine modified mRNA is susceptible to the endogenous miRNA machinery. Adding target sites in the 3'UTR of chemically modified mRNA can affect its expression only when this specific miRNA is expressed.

Example 2

A Flag tagged p27 encoding plasmids can be engineered to facilitate in vitro transcription of p27 encoding mRNA (FIG. 17): A) Flag-tagged p27; B) Flag-tagged p27 followed by two 2 fully complementary target sequences for the mature miR-126-3p strand at its 3'-UTR (p27-2×126TS). A Flag-tag can be incorporated to distinguish between endogenous and exogenous p27 expression.

To reduce innate immune responses and toxicity and at the same time maximize the efficiency and duration of expression of the mRNA encoding p27 described in FIG. 17, the following modified nucleotide substitutions or combinations thereof can be used: 1) Pseudouridine; 2) N-1-methylpseudouridine; 3) 5-methoxy-U; 4) 5-hydroxymethyl-C; 5) 5-methyl-C and 6) combination of Pseudouridine and 5-methyl-C. mRNAs can be in vitro transcribed using T7 RNA polymerase followed by 5' capping and poly(A) tail addition using a Vaccinia Capping Enzyme and *E. coli* Poly(A) Polymerase (New England BioLabs Inc.), respectively.

Example 3

Example 1 demonstrates that substitution of uridine with pseudouridine and addition of miR-126 target sequences at the 3'-UTR of GFP mRNA increased the translational efficiency in a cell-selective manner. However, to increase its therapeutic potential, it is desirable that sustained cell selective inhibition matches the potency and the durability of the drugs eluted from the DES. To develop a long-lasting cell-selective mRNA-based therapy, we focused our efforts on an approach that (1) utilizes a single RNA species capable of self-replicating for a limited number of cell divisions; (2) is capable of encoding our gene of interest; (3) consistently expresses the protein at high threshold levels over multiple cellular divisions; and/or (4) can be susceptible to endogenous miRNA degradation in a cell-selective fashion. In this Example, an approach utilizing a noninfectious, self-replicating Venezuelan equine encephalitis (VEE) virus (lacks the genes encoding the viral structural proteins) and mimics cellular mRNA with a 5'-cap and poly(A) tail and does not utilize a DNA intermediate is described. This approach lacks the potential for genomic integration. VEE virus is a positive-stranded RNA that encodes four nonstructural replication complex proteins (NSPs) as a single open reading frame (ORF).

Design of self-replicating, cell-selective mRNA vectors: To ectopically express p27 in a cell-selective manner, a p27-coding sequence can be placed at the 5' end of VEE-structural protein genes. The following VEE10 GFP or Flag tagged p27 encoding plasmids will be engineered: A) VEE-p27, 3'-ORF replaced with flag-tag p27 coding sequence followed by β -globin 3'-UTR (FIG. 19); (B) VEE-p27-2×126TS, 3'-ORF replaced with flag-tag p27-coding sequence followed by two tandem copies of a 22-bp target sequence

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perfectly complementary to the mature miR-126-3p at its 3' end (FIG. 19). A Flag-tag can be included to distinguish between endogenous and exogenous p27 expression. More than two miR-126 targets can be incorporated (FIG. 18).

Self-replicating mRNA depicted in FIGS. 18-19 can be in vitro transcribed using T7 RNA polymerase in the presence of the unmodified and modified nucleotides (Pseudouridine, N-1-methylpseudouridine, 5-methoxy-U, 5-hydroxymethyl-C, 5-methyl-C or combination of Pseudouridine and 5-methyl-C). mRNAs can be in vitro transcribed using T7 RNA polymerase followed by 5' capping and poly(A) tailing.

The self-replicating mRNA can also be designed to include RNA encoding the B18R protein. This can reduce immunogenic reactions (FIG. 20).

The self-replicating mRNA can also incorporate RNA encoding a marker protein such as GFP. FIG. 18.

Example 4

Cell-selective alternatives to the current DES used in percutaneous interventions are needed to inhibit restenosis while promoting reendothelialization. The advantage of such a treatment is the local non-invasive administration of drug in conjunction with balloon angioplasty limiting systemic toxicity. In this Example, an approach using FDA approved polymers to encapsulate the cell-selective mRNA or cell-selective self-replicating mRNA transcripts to facilitate efficient gene delivery in vivo is presented. The cell-selective RNA molecules can be encapsulated with a polymer to form RNA polymer nanoparticles. The RNA polymer nanoparticles can be attached to the stent or other medical device using a suitable method such as surface by dip- or by spray-coating. The following FDA approved polymers can be used: Phosphorylcholine-based polymers, Poly lactic-co-glycolic acid (PLGA), chitosan, cationic nanoemulsion, cationic electrodeposition coating or lipid nanoparticles.

Example 5

Manipulation of gene expression via the RNAi system is a powerful new tool for the treatment of many diseases, including Hepatitis C and heart failure. The current trend in miRNA therapies focuses on the use of antagomirs, short RNA fragments that block the activity of a specific miRNA. RNAs with numerous target sites for miRNAs, referred to as miRNA sponges, are also able to effectively restore the repressed targeted mRNAs by sequestering the endogenous miRNA. Cell-specific miRNAs can be used to confer cell-protectivity to a gene therapy by incorporating miRNA target sites into the 3' UTR. This strategy prevents expression of the inserted gene in a specific cell type based on the miRNA target sequences.

To demonstrate that miRNA can be used to specifically regulate gene of interest a GFP-expressing lentiviral vector was used in which four target sequences for miR-126 were inserted, a vascular endothelial cell (VEC)-specific miRNA, in the GFP 3'UTR (GFP-4×miR-126TS). To control for miRNA specificity, four target sequences for miR-143 (GFP-4×miR-143TS) were inserted, which is vascular smooth muscle cell (VSMC)-specific, or four scramble sequences (GFP) in the GFP-3'UTR region.

HEK cells were transduced with the different GFP expressing lentiviruses, and the effect of over-expression or inhibition of miR-143, miR-126 or control on GFP expression was assessed. In cells transduced with the control GFP virus, neither over-expression nor inhibition of miR-143 or miR-126 had an effect on GFP expression (FIG. 15, left). However, in HEK cells transduced with GFP-4×miR-143TS virus, only over-expression of miR-143 dramatically inhibited GFP expression (FIG. 15, middle). Similarly, over-

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expression of miR-143 had no effect on GFP expression in cells transduced with GFP-4×miR-126TS, while over-expression of miR-126 did inhibit GFP expression in these cells (FIG. 15, right).

This strategy was used in VEC to ensure that the endogenous miR-126 targets the viral constructs as expected. VEC were transduced with the same lentiviruses. In control GFP virus transduced cells, neither over-expression nor inhibition of miR-143 or miR-126 had an effect on GFP expression (FIG. 16, left). Similarly, over-expression of miR-143 inhibited GFP expression only in GFP-4×miR-143TS transduced cells, while over-expression of miR-126 had no effect (FIG. 16, middle). Interestingly, because miR-126 is highly expressed in VEC, it abolished the expression of GFP in the GFP-4×miR-126TS infected cells. Only inhibition of the endogenous miR-126 with a miR-126 antagomir rescued the expression of GFP (FIG. 16, right).

These results demonstrate the sensitivity and specificity of endogenous miRNA for target sites in the 3' UTR of unmodified mRNAs. Predictable patterns of expression can be observed when using cell-specific miRNA target to regulate expression of the inserted gene.

Example 6

Modified nucleotides can increase the translational efficiency and reduce cellular toxicity caused by the immunogenic response to exogenous mRNA. Here, 4 different representative modified nucleotide compositions (FIG. 30) can be included in the cell-selective mRNA. Each of the modified nucleotides can be used as a complete substitute for the unmodified nucleotides to achieve the maximum effect. Modified and unmodified mRNA can be synthesized with a 5' cap using an ARCA cap analog (TriLink) and PolyA tail using reagents to increase the stability of the mRNAs (TriLink).

To determine if modified nucleic acids affect target site recognition, modified and unmodified mRNA encoding green fluorescent protein (GFP) or luciferase (Luc) followed by 4 target sequences (TS) for miR-126, miR-143 or control scrambled sequences will be transcribed in vitro by T7 RNA polymerase (FIG. 5).

Example 7

The number of miRNA silencing can be dependent on the number of miRNA target sites present in the construct. As demonstrated in FIGS. 6 and 7, an increase from 0 (GFP) to 2 (2×126TS) to 3 (3×126TS) miRNA target sites within the construct can result in a positively correlated increase in silencing. The data of FIGS. 6 and 7 further demonstrates the specificity of the miRNA target sequences.

Example 8

To test whether modified nucleic acids affect the efficiency of miRNA-target site recognition and miRNA-dependent gene silencing, GFP or GFP4×126TS mRNA was in vitro transcribed with substitutions of uridine with pseudouridine (0%, 25%, 50% or 100%). Since ad-HEK293 cells do not express miR-126 or miR-143, we transfected with miR-126 mimic or miR-143 mimic 24 hr prior to transfection with GFP or GFP4×126TS mRNAs to ensure for miRNA/RISC assembly and GFP expression levels were assessed after 24 hr. In the control, unmodified (0% Pseudouridine) GFP transfected cells, the over-expression of miR-126-3p or miR-143 did not have any effect on GFP expression (FIGS. 43A-43C). This was also observed in cells transfected with GFP-mRNA containing increasing percentage of Pseudouridine (FIGS. 43A-43C). However, in cells transfected with GFP4×126TS mRNA, the over-expression of miR-126 and

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not miR-143 dramatically reduced the percentage of GFP positive cells and inhibited GFP expression. The miR-126-specific inhibition was not affected by increasing the percentage of pseudouridine substitution (FIGS. 43A-43C).

Example 9

It was also tested whether 100% substitution of both uridine and cytosine with pseudouridine and 5-methylcytosine nucleotides would affect miRNA-dependent gene silencing. To that end, GFP or GFP-4×126TS mRNA were in vitro transcribed with 100% pseudouridine and 100% 5-methylcytosine and transfected into Ad-HEK293 cells that were priorly (24 hr) transfected with miR-126 or miR-143 mimics. The inhibitory effect of miR-126 on the expression of the double-modified GFP-4×126TS mRNA were compared to the unmodified mRNA after 24 hr. Cells transfected with pseudouridine and 5-methylcytosine modified mRNA, miR-126 and not miR-143 reduced the percentage of GFP positive cells and inhibited GFP expression to the same extent as the unmodified mRNA (FIGS. 44A-44C). Thus, our data show that complete substitution of pseudouridine, or combination of pseudouridine and 5-methylcytosine modified mRNA, can still be targeted by microRNA-dependent silencing.

Example 10

To test whether this miRNA dependent silencing of modified mRNAs is not limited to miR-126, GFP coding mRNA containing target sites for miR-21 or miR-145 was designed. Ad-HEK293 cells were transfected with miR-21, miR-145 or miR-143 mimics 24 hr prior to transfection with unmodified or pseudouridine and 5-methylcytosine modified GFP, GFP4×21TS or GFP4×145TS mRNAs to ensure for RISC assembly and the GFP expression levels were assessed after 24 hr. Cells transfected with 100% Pseudouridine and 5-methylcytosine substituted GFP4×21TS mRNA, the over-expression of miR-21 and not miR-145, miR-21 or miR-143 reduced the expression GFP to the same extent as cells transfected with unmodified GFP4×21TS mRNA (FIGS. 45A-45C). Cells transfected with 100% Pseudouridine and 5-methylcytosine substituted GFP4×154TS mRNA, showed the same reduction in GFP expression as unmodified mRNA when miR-145 mimic was added and not when miR-21, miR-126 or miR-143 mimics were added (FIGS. 45A-45C). In the control transfected cells with unmodified or 100% substitution with Pseudouridine and 5-methylcytosine GFP mRNA (with no miRNA target sites), the over-expression of miR-21, miR-145 or miR-143 did not have an effect on GFP expression (FIGS. 45A-45C).

Example 11

Cell-Selective Gene Activation Using CRISPR/CAS9 Synergistic Activation Mediator (SAM) System

As proof of principle the modified CRISPR/dCas 9 Synergistic Activation Mediator (SAM) system (Konermann et al. 2015. Nature, 517:583-588) was employed that included the sgRNA2.0, MS2-p65-HSF1 and NLS-dCas9-VP64. To activate LIN28A in a cell selective manner, we added four tandem copies of a 22-bp target sequence perfectly complementary to the mature miR-126-3p strand at dCas9 3'UTR (dCas9-4×126TS). Ad-HEK293 cells were transfected with miR-126 mimic 24 hours prior to transfection with CRISPR-SAM components containing dCas9 or dCas9-4×126TS and the expression levels of LIN28A was assessed after 72 hours after. Transient transfected adHEK293 cells transfected with CRISPR-SAM components with dCas9 activated the transcription of LIN28A by >5000 fold while adHEK293 cells transfected with CRISPR-SAM and dCas9-4×126TS showed only 600-fold in LIN28A (FIG. 46).

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#44246

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tacggctaca	ctagaagaac	agtatttggg	atctgcgctc	tgctgaagcc	agttaccttc	10140
ggaaaaagag	ttggtagctc	ttgatccggc	aaacaaacca	ccgctggtag	cgggtggttt	10200
tttgtttgca	agcagcagat	tacgcgcaga	aaaaaaggat	ctcaagaaga	tcctttgatc	10260
ttttctacgg	ggtctgacgc	tcagtgggaa	gaaaactcac	gttaagggat	tttggtcatg	10320
agattatcaa	aaaggatctt	cacctagatc	cttttaaat	aaaaatgaag	ttttaaatca	10380
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cctatctcag	cgatctgtct	atttcgttca	tccatagttg	cctgactccc	cgtcgtgtag	10500
ataactacga	tacgggaggg	cttaccatct	ggccccagtg	ctgcaatgat	accgcgagac	10560
ccacgctcac	cggctccaga	tttatcagca	ataaaccagc	cagccggaag	ggccgagcgc	10620
agaagtggtc	ctgcaacttt	atccgcctcc	atccagtcta	ttaattgttg	ccgggaagct	10680
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gtggtgtcac	gctcgtcggt	tggtatggct	tcattcagct	ccggttccca	acgatcaagg	10800
caggttacat	gateccccat	gttgtgcaaa	aaagcgggta	gctccttcgg	tcctccgatc	10860
gttgtcagaa	gtaagttggc	cgcagtggtta	tcactcatgg	ttatggcagc	actgcataat	10920

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tctcttactg tcatgccatc cgtaagatgc ttttctgtga ctggtgagta ctcaaccaag 10980
tcattctgag aatagtgtat gcggcgaccg agttgctctt gcccggcgtc aatacgggat 11040
aataccgcgc cacatagcag aactttaaaa gtgctcatca ttggaaaacg ttcttcgggg 11100
cgaaaactct caaggatctt accgctgttg agatccagtt cgatgtaacc cactcgtgca 11160
cccaactgat cttcagcatc ttttactttc accagcgttt ctgggtgagc aaaaacagga 11220
aggcaaaatg cgcacaaaaa gggaataagg gcgacacgga aatgttgaat actcactatc 11280
ttcctttttc aatattattg aagcatttat cagggttatt gtctcatgag cggatacata 11340
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ccacctgac 11409

```

```

<210> SEQ ID NO 10
<211> LENGTH: 85
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000471: human miR126
(stem-loop)

```

```

<400> SEQUENCE: 10

```

```

cgcugggcgc gggacauuau uacuuuuggu acgcgcugug acacuucaaa cucguaccgu 60
gaguaauuuu gcgccgucca cggca 85

```

```

<210> SEQ ID NO 11
<211> LENGTH: 88
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000461: human miR145
(stem-loop)

```

```

<400> SEQUENCE: 11

```

```

caccuugucc ucacggucca guuuuuccag gaaucuuua gaugcuaaga ugaggauucc 60
uggaaaaacu guucugagg ucaugguu 88

```

```

<210> SEQ ID NO 12
<211> LENGTH: 106
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000459: human miR143
(stem-loop)

```

```

<400> SEQUENCE: 12

```

```

gcgcagcgcc cugucucca gccugaggug cagugcugca ucucugguca guugggaguc 60
ugagaugaag cacuguagcu caggaagaga gaaguuguuc ugcagc 106

```

```

<210> SEQ ID NO 13
<211> LENGTH: 85
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000442 Human miR-122
(stem-loop)

```

```

<400> SEQUENCE: 13

```

```

ccuuagcaga gcuguggagu gugacaaugg uguuuguguc uaaacuauc aacgccauua 60
ucacacuaaa uagcuacugc uaggc 85

```

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<210> SEQ ID NO 14
<211> LENGTH: 73
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0017383 Human miR-3591
      (stem-loop)

```

```

<400> SEQUENCE: 14

caguagcuau uuagugugau aaggcguuu gauaguuuag acacaaacac cauugucaca      60
cuccacagcu cug                                                         73

```

```

<210> SEQ ID NO 15
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000251 Human miR-208a
      (stem-loop)

```

```

<400> SEQUENCE: 15

ugacgggcga gcuuuuggcc cgguuauuac cugaugcuca cguauaagac gagcaaaaag      60
cuuguugguc a                                                         71

```

```

<210> SEQ ID NO 16
<211> LENGTH: 77
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0005570 Human miR-208b
      (stem-loop)

```

```

<400> SEQUENCE: 16

ccucucaggg aagcuuuuug cucgaauuau guuucugauc cgaauauaag acgaacaaaa      60
gguuugucug agggcag                                                         77

```

```

<210> SEQ ID NO 17
<211> LENGTH: 88
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000450 Human
      miR-133a-1 (stem-loop)

```

```

<400> SEQUENCE: 17

acaaugcuuu gcuagagcug guaaaaugga accaaaucgc cucuucaaug gauuuggucc      60
ccuucacca gcuguagcua ugcauuga                                                         88

```

```

<210> SEQ ID NO 18
<211> LENGTH: 102
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000451 Human
      miR-133a-2 (stem-loop)

```

```

<400> SEQUENCE: 18

gggagccaaa ugcuuugcua gacugguuaa aauggaacca aaucgacugu ccaauggauu      60
ugguccccuu caaccagcug uagcugugca ugauggcgcg cg                         102

```

```

<210> SEQ ID NO 19
<211> LENGTH: 119
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000822 Human miR-133b
(stem-loop)

<400> SEQUENCE: 19

ccucagaaga aagaugcccc cugcucuggc uggucaaaacg gaaccaaguc cgucuuccug      60
agagguuugg ucccuucaa ccagcuacag cagggcuggc aaugcccagu ccuuggaga      119

<210> SEQ ID NO 20
<211> LENGTH: 69
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000738 Human miR-302a
(stem-loop)

<400> SEQUENCE: 20

ccaccacuua aacguggaug uacuugcuuu gaaacuaaag aaguaaguc uccauguuu      60
uggugaugg                                         69

<210> SEQ ID NO 21
<211> LENGTH: 73
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000772 Human miR-302b
(stem-loop)

<400> SEQUENCE: 21

gcucccuuca acuuuaacau ggaagugcuu ucugugacuu uaaaaguaag ugcuuccaug      60
uuuuaguagg agu                                         73

<210> SEQ ID NO 22
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000773 Human miR-302c
(stem-loop)

<400> SEQUENCE: 22

ccuuugcuuu aacauggggg uaccugcugu gugaaacaaa aguaagugcu uccauguuuc      60
aguggagg                                         68

<210> SEQ ID NO 23
<211> LENGTH: 68
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: . miRBase Accession No.: MI0000774 Human
miR-302d (stem-loop)

<400> SEQUENCE: 23

ccucuacuuu aacauggagg cacuugcugu gacaugacaa aaauaaguc uccauguuu      60
gagugugg                                         68

<210> SEQ ID NO 24
<211> LENGTH: 51
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0006418 Human miR-302f
(stem-loop)

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<400> SEQUENCE: 24

ucuguguaaa ccuggcaauu uucacuaau ugcuccaug uuuaaaaaag a	51
--	----

<210> SEQ ID NO 25

<211> LENGTH: 71

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0006418 Human miR-1-1
(stem-loop)

<400> SEQUENCE: 25

ugggaaacau acuucuuuau augcccauau ggaccugcua agcuauaggaa uguaaagaag	60
--	----

uauauaucuc a	71
--------------	----

<210> SEQ ID NO 26

<211> LENGTH: 85

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000437 Human miR-1-2
(stem-loop)

<400> SEQUENCE: 26

accuacucag aguacauacu ucuuuuauua ccccauaggaa cauacaauugc uauaggaaugu	60
--	----

aaagaaguau guuuuuuugg uaggc	85
-----------------------------	----

<210> SEQ ID NO 27

<211> LENGTH: 86

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000490 Human miR-206
(stem-loop)

<400> SEQUENCE: 27

ugcuucccga ggcccauugc ucuuuuauau ccccauagg auuacuuugc uauaggaaugu	60
---	----

aagggaugug gugguuucgg caagug	86
------------------------------	----

<210> SEQ ID NO 28

<211> LENGTH: 73

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000474 Human miR-134
(stem-loop)

<400> SEQUENCE: 28

cagggugugug gacugguuga ccagaggggc augcacugug uucacccugug ggccaccua	60
--	----

gucaccaacc cuc	73
----------------	----

<210> SEQ ID NO 29

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000487 Human miR-193a
(stem-loop)

<400> SEQUENCE: 29

cgaggauagg agcugagggc ugguuuuug cgggcgagau gagggugugc gaucaacugg	60
--	----

ccuacaaagu cccaguucuc ggcccccg	88
--------------------------------	----

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<210> SEQ ID NO 30
 <211> LENGTH: 83
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0003137 Human miR-193b
 (stem-loop)

<400> SEQUENCE: 30

guggucucag aaucgggggu uugagggcga gaugaguuaa uguuuuaucc aacuggcccu 60
 caaagucccg cuuuuggggu cau 83

<210> SEQ ID NO 31
 <211> LENGTH: 82
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000447 Human
 miR-128-1 (stem-loop)

<400> SEQUENCE: 31

ugagcuguug gauucggggc cguagcacug ucugagaggu uuacauuuu cacagugaac 60
 cggucucuuu uucagcugcu uc 82

<210> SEQ ID NO 32
 <211> LENGTH: 84
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000727 Human
 miR-128-2 (stem-loop)

<400> SEQUENCE: 32

ugugcagugg gaaggggggc cgauacacug uacgagagug aguagcaggu cucacaguga 60
 accggucucu uucccuacug uguc 84

<210> SEQ ID NO 33
 <211> LENGTH: 81
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000097 Human miR-95
 (stem-loop)

<400> SEQUENCE: 33

aacacagugg gcacucauaa aaugucuguu gaauugaaa gcguuacau caacggguau 60
 uuauugagca cccacucugu g 81

<210> SEQ ID NO 34
 <211> LENGTH: 84
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000082 Human miR-25
 (stem-loop)

<400> SEQUENCE: 34

ggccaguguu gagaggcgga gacuugggca auugcuggac gcugcccugg gcauugcacu 60
 ugucucgguc ugacagugcc ggcc 84

<210> SEQ ID NO 35
 <211> LENGTH: 78
 <212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000093 Human
        miR-92a-1 (stem-loop)

<400> SEQUENCE: 35

cuuucacac agguugggau cgguugcaau gcuguguuuc uguaugguau ugcacuuguc      60
ccggccuguu gaguuugg                                         78

<210> SEQ ID NO 36
<211> LENGTH: 75
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000094 Human
        miR-92a-2 (stem-loop)

<400> SEQUENCE: 36

ucauuccugg guggggauuu guugcauuac uuguguucua uauaaaguau ugcacuuguc      60
ccggccugug gaaga                                           75

<210> SEQ ID NO 37
<211> LENGTH: 96
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003560 Human miR-92b
        (stem-loop)

<400> SEQUENCE: 37

cgggccccgg gcgggcgga gggacgggac gcgugcagu guuguuuuu cccccccaa      60
uauugcacuc gucccggccu ccggcccccc cgcccc                      96

<210> SEQ ID NO 38
<211> LENGTH: 95
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000457 Human miR-141
        (stem-loop)

<400> SEQUENCE: 38

cggcggccc ugguccauc uuccaguaca guguggaug gucuaauugu gaaguccua      60
acacugucug guaaagaugg cuccgggug gguuc                      95

<210> SEQ ID NO 39
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000737 Human miR-200a
        (stem-loop)

<400> SEQUENCE: 39

cgggccccc gugagcauc uaccggacag ugcuggauu cccagcuuga cucuaacacu      60
gucugguaac gauguucaa ggugaccgc                               90

<210> SEQ ID NO 40
<211> LENGTH: 95
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000342 Human miR-200b
        (stem-loop)

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<400> SEQUENCE: 40

ccagcucggg cagccguggc caucuacug ggcagcauug gauggaguca ggucucuaau 60

acugccuggu aaugaugacg gcggagcccu gcacg 95

<210> SEQ ID NO 41

<211> LENGTH: 68

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000650 Human miR-200c (stem-loop)

<400> SEQUENCE: 41

cccucgucuu acccagcagu guuugggugc gguugggagu cucuaauacu gccggguaau 60

gauggagg 68

<210> SEQ ID NO 42

<211> LENGTH: 70

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000238 Human miR-196a-1 (stem-loop)

<400> SEQUENCE: 42

gugaauuagg uaguuucaug uuguugggcc ugguuuucug aacacaacaa cauuuaacca 60

cccgauucac 70

<210> SEQ ID NO 43

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000279 Human miR-196a-2 (stem-loop)

<400> SEQUENCE: 43

ugcucgcuca gcugaucugu ggcuuaggua guuucauguu guugggauug aguuuugaac 60

ucggcaacaa gaaacugccu gaguuacac agucgguuuu cgucgagggc 110

<210> SEQ ID NO 44

<211> LENGTH: 84

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0001150 Human miR-196b (stem-loop)

<400> SEQUENCE: 44

acuggucggu gauuuaggua guuuccuguu guugggaucc accuuucucu cgacagcacg 60

acacugccuu cauucacuca guug 84

<210> SEQ ID NO 45

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000266 Human miR-10a (stem-loop)

<400> SEQUENCE: 45

gaucugucug ucuucuguau auaccugua gaucggaau uguguaagga auuuuguggu 60

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cacaaaaucg uaucuagggg aaauaguagu ugacauaaac acuccgcucu 110

<210> SEQ ID NO 46
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000267 Human miR-10b
 (stem-loop)

<400> SEQUENCE: 46

ccagagguug uaacguuguc uauauauacc cuguagaacc gaauuugugu gguaucggu 60

uagucacaga uucgauucua ggggaauua uggucgaugc aaaaacuca 110

<210> SEQ ID NO 47
 <211> LENGTH: 81
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000101 Human miR-99a
 (stem-loop)

<400> SEQUENCE: 47

cccauuggca uaaacccgua gaucggauc uugugugaag uggaccgcac aagcucgcu 60

cuaugggucu gugucagugu g 81

<210> SEQ ID NO 48
 <211> LENGTH: 70
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000746 Human miR-99b
 (stem-loop)

<400> SEQUENCE: 48

ggcaccacc cguagaaccg accuugcggg gccuucgccc cacacaagcu cgugucugug 60

gguccguguc 70

<210> SEQ ID NO 49
 <211> LENGTH: 80
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000102 Human miR-100
 (stem-loop)

<400> SEQUENCE: 49

ccguugcca caaacccgua gaucggaacu uguguaaua guccgcacaa gcuugauuc 60

auagguaugu gucuguuagg 80

<210> SEQ ID NO 50
 <211> LENGTH: 86
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000469 Human miR-125a
 (stem-loop)

<400> SEQUENCE: 50

ugccagucuc uagguccug agaccuuua accugugagg acauccaggg ucacagguga 60

gguuuuggg agccuggcgu cuggcc 86

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<210> SEQ ID NO 51
<211> LENGTH: 88
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000446 Human
      miR-125b-1 (stem-loop)

<400> SEQUENCE: 51

ugcguccuc ucaguccug agaccuaac uugugaugu uaccguuuuauuccacgggu      60
uaggcucuug ggagcugcga gucgugcu                                     88

<210> SEQ ID NO 52
<211> LENGTH: 89
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000470 Human
      miR-125b-2 (stem-loop)

<400> SEQUENCE: 52

accagacuuu uccuagucc ucagaccua acuugugagg uauuuuagua acaucacaag      60
ucaggcucu gggaccuagg cggagggga                                     89

<210> SEQ ID NO 53
<211> LENGTH: 99
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000477 Human miR-146a
      (stem-loop)

<400> SEQUENCE: 53

ccgaugugua uccucagcu ucagaacuga auuccauggg uugugucagu gucagaccuc      60
ugaaaauucag uucuucagcu gggauaucuc ugucaucgu                                     99

<210> SEQ ID NO 54
<211> LENGTH: 73
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003129 Human miR-146b
      (stem-loop)

<400> SEQUENCE: 54

ccuggcacug agaacugaau uccauaggcu gugagcucua gcaaugcccu guggacucag      60
uucuggugcc cgg                                                     73

<210> SEQ ID NO 55
<211> LENGTH: 71
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000088 Human miR-30a
      (stem-loop)

<400> SEQUENCE: 55

gcgacuguaa acauccucga cuggaagcug ugaagccaca gaugggcuuu cagucggau      60
uuugcagcug c                                                       71

<210> SEQ ID NO 56
<211> LENGTH: 88
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000441 Human miR-30b
(stem-loop)

<400> SEQUENCE: 56

accaaguuuu aguuaugua aacaucuuac acucagcugu aaaucaugga uuggcuggga      60
gguggauguu uacuucagcu gacuugga                                          88

<210> SEQ ID NO 57
<211> LENGTH: 89
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000736 Human
miR-30c-1 (stem-loop)

<400> SEQUENCE: 57

accaugcugu agugugugua aacaucuuac acucucagcu gugagcucaa gguggcuggg      60
agagggguugu uuacuccuuc ugccaugga                                          89

<210> SEQ ID NO 58
<211> LENGTH: 72
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000254 Human
miR-30c-2 (stem-loop)

<400> SEQUENCE: 58

agauacugua aacaucuuac acucucagcu guggaaagua agaaagcugg gagaaggcug      60
uuuacuuuuu cu                                                              72

<210> SEQ ID NO 59
<211> LENGTH: 70
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000255 Human miR-30d
(stem-loop)

<400> SEQUENCE: 59

guuguuguua acauucccgga cuggaagcug uaagacacag cuaagcuuuc agucagaugu      60
uugcugcuac                                                                70

<210> SEQ ID NO 60
<211> LENGTH: 92
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000749 Human miR-30e
(stem-loop)

<400> SEQUENCE: 60

gggcagucuu ugcuaucugua aacaucuuug acuggaagcu gaaagguguu cagaggagcu      60
uucagucgga uguuuacagc gccaggcugc ca                                      92

<210> SEQ ID NO 61
<211> LENGTH: 76
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0002467 Human miR-483
(stem-loop)

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<400> SEQUENCE: 61

gagggggaag acgggaggaa agaaggaggu gguuccauca cgccuccuca cuccucuccu 60

cccgucuucu ccucuc 76

<210> SEQ ID NO 62

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000234 Human miR-192
(stem-loop)

<400> SEQUENCE: 62

gccgagaccg agugcacagg gcucugaccu augaaugac agccagugcu cucgucuccc 60

cucuggcugc caauuccaua ggucacaggu auguucgccu caaugccagc 110

<210> SEQ ID NO 63

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000291 Human miR-215
(stem-loop)

<400> SEQUENCE: 63

aucuucaga aaugguauac agggaaauga ccuaugaauu gacagacaau auagcugagu 60

uugucuguca uuucuuuagg ccaauauucu guaugacugu gcuacuucaa 110

<210> SEQ ID NO 64

<211> LENGTH: 71

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000242 Human
miR-199a-1 (stem-loop)

<400> SEQUENCE: 64

gccaacccag uguucagacu accuguucag gaggcucuca auguguacag uagucugcac 60

auugguuagg c 71

<210> SEQ ID NO 65

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000281 Human
miR-199a-2 (stem-loop)

<400> SEQUENCE: 65

aggaagcuuc uggagauccu gcuccgucgc ccaguguuuc agacuaccug uucaggacaa 60

ugccguugua caguagucug cacauugguu agacugggca agggagagca 110

<210> SEQ ID NO 66

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000282 Human miR-199b
(stem-loop)

<400> SEQUENCE: 66

ccagaggaca ccuccacucc gucuaccag uguuuagacu aucuguucag gacucccaaa 60

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uuguacagua gucugcacau ugguuaggcu gggcuggguu agaccucgg 110

<210> SEQ ID NO 67
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000290 Human miR-214
 (stem-loop)

<400> SEQUENCE: 67

ggccuggcug gacagaguug ucaugugucu gccugucuac acuugcugug cagaacaacc 60

gcucaccugu acagcaggca cagacaggca gucacaugac aaccagccu 110

<210> SEQ ID NO 68
 <211> LENGTH: 81
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0014136 Human miR-3120
 (stem-loop)

<400> SEQUENCE: 68

gucaugugac ugccugucug ugccugcugu acaggugagc ggauguucug cacagcaagu 60

guagacaggc agacacauga c 81

<210> SEQ ID NO 69
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000266 Human miR-10a
 (stem-loop)

<400> SEQUENCE: 69

gaucugucug ucuucuguau auaccugua gaucggaau uguguaagga auuuuguggu 60

cacaaaucg uaucuagggg aauauguagu ugacauaac acuccgcucu 110

<210> SEQ ID NO 70
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000267 Human miR-10b
 (stem-loop)

<400> SEQUENCE: 70

ccagaggguug uaacguuguc uauauauacc cuguagaacc gaauuugugu gguauccgua 60

uagucacaga uucgauucua ggggaauuaa ugguagaucg aaaaacuua 110

<210> SEQ ID NO 71
 <211> LENGTH: 81
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000101 Human miR-99a
 (stem-loop)

<400> SEQUENCE: 71

cccauuggca uaaaccgua gaucggaucu uguggugaag uggaccgcac aagcucgcuu 60

cuaugggucu gugucagugu g 81

<210> SEQ ID NO 72

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<211> LENGTH: 70
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000746 Human miR-99b
(stem-loop)

<400> SEQUENCE: 72

ggcaccacc cguagaaccg accuugcggg gccuucgccg cacacaagcu cgugucugug      60
gguccguguc                                                                    70

<210> SEQ ID NO 73
<211> LENGTH: 80
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000102 Human miR-100
(stem-loop)

<400> SEQUENCE: 73

ccuguugcca caaaccgua gauccgaacu uguguaaua guccgcacaa gcuuguaucu      60
auagguaugu gucuguuagg                                                            80

<210> SEQ ID NO 74
<211> LENGTH: 86
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000469 Human miR-125a
(stem-loop)

<400> SEQUENCE: 74

ugccagucuc uaggucccug agaccuuua accugugagg acauccaggg ucacagguga      60
gguucuuuggg agccuggcgu cuggcc                                                    86

<210> SEQ ID NO 75
<211> LENGTH: 88
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000446 Human
miR-125b-1(stem-loop)

<400> SEQUENCE: 75

ugcguccuc ucagucccug agaccuaac uugugauguu uaccguuuua auccacgggu      60
uaggcucuug ggagcugcga gucgugcu                                                  88

<210> SEQ ID NO 76
<211> LENGTH: 89
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000470 Human
miR-125b-2 (stem-loop)

<400> SEQUENCE: 76

accagacuuu uccuaguccc ugagaccua acuugugagg uauuuuagua acaucacaag      60
ucaggcucuu gggaccuagg cggagggga                                                89

<210> SEQ ID NO 77
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: 78

<400> SEQUENCE: 77

uuggauguug gccuaguucu guguggaaga cuagugauuu uguuguuuuu agauaacuaa 60

aucgacaaca aaucacaguc ugccauaugg cacaggccau gccucuacag 110

<210> SEQ ID NO 78

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000264 Human miR-7-2
(stem-loop)

<400> SEQUENCE: 78

cuggauacag aguggaccgg cuggccccc au cuggaagacu agugauuuug uguugucuu 60

acugcgcuca acaacaaauc ccagucuaacc uaauggugcc agccaucgca 110

<210> SEQ ID NO 79

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000265 Human miR-7-3
(stem-loop)

<400> SEQUENCE: 79

agauuagagu ggcugugguc uagugcugug uggaagacua gugauuuugu uguucugaug 60

uacuacgaca acaagucaca gccggccuca uagcgcagac ucccuucgac 110

<210> SEQ ID NO 80

<211> LENGTH: 71

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000089 Human miR-31
(stem-loop)

<400> SEQUENCE: 80

ggagaggagg caaugcug gcauagcugu ugaacuggga accugcuaug ccaacauuu 60

gccaucuuuc c 71

<210> SEQ ID NO 81

<211> LENGTH: 72

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000252 Human
miR-129-1 (stem-loop)

<400> SEQUENCE: 81

ggaucuuuuu gcgucuggg cuugcuguuc cucucaacag uagucaggaa gccuuaccc 60

caaaaaguau cu 72

<210> SEQ ID NO 82

<211> LENGTH: 90

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000473 Human
miR-129-2 (stem-loop)

<400> SEQUENCE: 82

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ugcccuucgc gaaucuuuuu gcggucuggg cuugcuguac auaacucaau agccggaagc    60
ccuuacccca aaaagcauuu gcggagggcg                                     90

```

```

<210> SEQ ID NO 83
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000294 Human
        miR-218-1 (stem-loop)

```

```

<400> SEQUENCE: 83

```

```

gugauaaugu agcgagauuu ucuguugugc uugaucuaac caugugguug cgagguauga    60
guaaaacaug guuccgucaa gcaccaugga acgucacgca gcuuucuaca               110

```

```

<210> SEQ ID NO 84
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000295 Human
        miR-218-2 (stem-loop)

```

```

<400> SEQUENCE: 84

```

```

gaccagucgc ugcggggguu uccuuugugc uugaucuaac cauguggugg aacgauggaa    60
acggaacaug guucugucua gcacccgcca aagcaccgug cucuccugca               110

```

```

<210> SEQ ID NO 85
<211> LENGTH: 89
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000466 Human miR-9-1
        (stem-loop)

```

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<400> SEQUENCE: 85

```

```

cggggguuggu uguuauuuu gguuauuag cuguaugagu gguguggagu cuucauaaag    60
cuagauaacc gaaaguaaaa auaacccca                                     89

```

```

<210> SEQ ID NO 86
<211> LENGTH: 87
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000467 Human miR-9-2
        (stem-loop)

```

```

<400> SEQUENCE: 86

```

```

ggaagcgagu uguuauuuu gguuauuag cuguaugagu guauuggucu ucauaaagcu    60
agauaaccga aaguaaaaac uccuucua                                     87

```

```

<210> SEQ ID NO 87
<211> LENGTH: 90
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000468 Human miR-9-3
        (stem-loop)

```

```

<400> SEQUENCE: 87

```

```

ggaggcccgu uucucuuuu gguuauuag cuguaugagu gccacagagc cgucuaaag    60
cuagauaacc gaaaguagaa augauucua                                     90

```

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<210> SEQ ID NO 88
<211> LENGTH: 82
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000447 Human
      miR-128-1 (stem-loop)

<400> SEQUENCE: 88
ugagcuguug gauucggggc cguagcacug ucugagaggu uuacauuucu cacagugaac      60
cggucucuuu uucagcugcu uc                                             82

<210> SEQ ID NO 89
<211> LENGTH: 84
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000727 Human
      miR-128-2 (stem-loop)

<400> SEQUENCE: 89
ugugcagugg gaaggggggc cgauacacug uacgagagug aguagcaggu cucacaguga      60
accggucucu uucccuacug uguc                                           84

<210> SEQ ID NO 90
<211> LENGTH: 86
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000483 Human miR-186
      (stem-loop)

<400> SEQUENCE: 90
ugcuuguaac uuuccaaaga auucuccuuu ugggcuuucu gguuuuuuuu uaagcccaaa      60
ggugaauuuu uugggaaguu ugagcu                                           86

<210> SEQ ID NO 91
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000272 Human miR-182
      (stem-loop)

<400> SEQUENCE: 91
gagcugcuug ccucccccg uuuuuggcaa ugguagaacu cacacuggug agguaacagg      60
auccgguggu ucuagacuug ccaacuaugg ggcgaggacu cagccggcac              110

<210> SEQ ID NO 92
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000285 Human miR-205
      (stem-loop)

<400> SEQUENCE: 92
aaagauccuc agacaaucca ugugcuucuc uuguccuua uuccaccgga gucugucuca      60
uacccaacca gauuucagug gagugaaguu caggaggcau ggagcugaca              110

<210> SEQ ID NO 93
<211> LENGTH: 85
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003167 Human
        miR-516b-2 (stem-loop)

<400> SEQUENCE: 93

ucucaugaug ugaccaucug gagguaagaa gcacuuugug uuuugugaaa gaaagugcuu      60
ccuuucagag gguuacucuu ugaga                                              85

<210> SEQ ID NO 94
<211> LENGTH: 84
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003145 Human miR-519e
        (stem-loop)

<400> SEQUENCE: 94

ucucaugcag ucauucucca aaagggagca cuuucuguuu gaaagaaaac aaagugccuc      60
uuuuuagagu guuacuguuu gaga                                              84

<210> SEQ ID NO 95
<211> LENGTH: 85
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003178 Human
        miR-519a-1 (stem-loop)

<400> SEQUENCE: 95

cucaggcgug gacacucuag aggggaagcg uuucuguugu cugaaagaaa ggaaagugca      60
uccuuuuaga guguuacugu uugag                                              85

<210> SEQ ID NO 96
<211> LENGTH: 124
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003193 Human miR-506
        (stem-loop)

<400> SEQUENCE: 96

gccaccacca ucagccauac uauguguagu gccuuauuca ggaagguguu acuuauuaga      60
uuauuuuuug uaaggcaccc uucugaguag aguaaugugc acauggaca acauuugugg      120
uggc                                              124

<210> SEQ ID NO 97
<211> LENGTH: 115
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0003195 Human miR-508
        (stem-loop)

<400> SEQUENCE: 97

ccaccuucag cugaguguag ugcccuacuc cagagggcgu cacucaugua aacuaaaaca      60
ugauuguagc cuuuuggagu agaguaauac acaucacgua acgcuuuuu ggugg          115

<210> SEQ ID NO 98
<211> LENGTH: 94
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: miRBase Accession No.: MI0003196 Human
miR-509-1 (stem-loop)

<400> SEQUENCE: 98

caugcugugu gugguacccu acugcagaca guggcaauca uguauaaaua aaaaugauug 60

guacgucugu gguuagagua cugcaugaca caug 94

<210> SEQ ID NO 99

<211> LENGTH: 91

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0005530 Human
miR-509-2 (stem-loop)

<400> SEQUENCE: 99

caugcugugu gugguacccu acugcagaca guggcaauca uguauaaaua aaaaugauug 60

guacgucugu gguuagagua cugcaugaca c 91

<210> SEQ ID NO 100

<211> LENGTH: 75

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0005717
Human miR-509-3 (stem-loop)

<400> SEQUENCE: 100

gugguacccu acugcagacg uggcaaucau guauaaaua aaaugauugg uacgucugug 60

gguagaguac ugcau 75

<210> SEQ ID NO 101

<211> LENGTH: 74

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0003197 Human miR-510
(stem-loop)

<400> SEQUENCE: 101

gugguguccu acucaggaga guggcaauca caugaaaua ggugugauug aaaccucuaa 60

gaguggagua acac 74

<210> SEQ ID NO 102

<211> LENGTH: 84

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0006649 Human miR-513c
(stem-loop)

<400> SEQUENCE: 102

gcguacagug ccuucucuaa ggaggugucg uuuaugugaa cuaaaaaua aauuucaccu 60

uucugagaag aguaauguac agca 84

<210> SEQ ID NO 103

<211> LENGTH: 98

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0003198 Human
miR-514a-1 (stem-loop)

<400> SEQUENCE: 103

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aacauguugu cugugguacc cuacucugga gagugacaau cauguauau uaaaauugau 60

ugacacuucu gugaguagag uacgcgauga cacguacg 98

<210> SEQ ID NO 104

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0003199 Human
miR-514a-2 (stem-loop)

<400> SEQUENCE: 104

guugucugug guaccuacu cuggagagug acaaucaugu auaacuaau uugauugaca 60

cuucugugag uagaguaacg caugacac 88

<210> SEQ ID NO 105

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0003200
Human miR-514a-3 (stem-loop)

<400> SEQUENCE: 105

guugucugug guaccuacu cuggagagug acaaucaugu auaacuaau uugauugaca 60

cuucugugag uagaguaacg caugacac 88

<210> SEQ ID NO 106

<211> LENGTH: 80

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0014251 Human miR-514b
(stem-loop)

<400> SEQUENCE: 106

caugugguac ucuucucaag agggaggcaa ucauguguaa uuagauauga uugacaccuc 60

ugugagugga guaacacaug 80

<210> SEQ ID NO 107

<211> LENGTH: 83

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000069 Human miR-15a
(stem-loop)

<400> SEQUENCE: 107

ccuuggagua aaguagcagc acauauggu uuguggauuu ugaaaaggug caggccauu 60

ugugcugccu caaaaauaca agg 83

<210> SEQ ID NO 108

<211> LENGTH: 98

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: miRBase Accession No.: MI0000438 Human miR-15b
(stem-loop)

<400> SEQUENCE: 108

uugaggccuu aaaguacugu agcagcacau caugguuuac augcuacagu caagaugcga 60

aucuuuuuu gcugcucuag aaaaauaagg aaauucau 98

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<210> SEQ ID NO 109
 <211> LENGTH: 89
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000070 Human miR-16-1
 (stem-loop)

<400> SEQUENCE: 109

gucagcagug ccuagcagc acguaaaauu uggcguuaag auucuaaaa uaucuccagu 60
 auuaacugug cugcugaagu aagguugac 89

<210> SEQ ID NO 110
 <211> LENGTH: 81
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000115 Human miR-16-2
 (stem-loop)

<400> SEQUENCE: 110

guuccacucu agcagcacgu aaauauuggc guagugaaa auauauuaaa caccauuuu 60
 acugugcugc uuuguguga c 81

<210> SEQ ID NO 111
 <211> LENGTH: 87
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000489 Human miR-195
 (stem-loop)

<400> SEQUENCE: 111

agcuucccug gcucuagcag cacagaaaau uggcacagg gaagcgaguc ugccaauuu 60
 ggcuugcugc cuccaggcag gguggug 87

<210> SEQ ID NO 112
 <211> LENGTH: 119
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000100 Human miR-98
 (stem-loop)

<400> SEQUENCE: 112

aggauucugc ucaugccagg gugagguagu aaguuguauu guuguggggu agggauauua 60
 ggccccauu agaagauaac uauacaacuu acuaauuucc cuggugugug gcuaauuca 119

<210> SEQ ID NO 113
 <211> LENGTH: 80
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000060 Human let-7a-1
 (stem-loop)

<400> SEQUENCE: 113

ugggaugagg uaguagguug uauaguuuua gggucacacc caccacuggg agauaacuau 60
 acaaucuacu gucuuuccua 80

<210> SEQ ID NO 114
 <211> LENGTH: 72

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000061 Human let-7a-2
(stem-loop)

<400> SEQUENCE: 114
agguugaggu aguagguugu auaguuuaga auuacaucaa gggagauaac uguacagccu      60
ccuagcuuuc cu                                                                72

<210> SEQ ID NO 115
<211> LENGTH: 74
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000062 Human let-7a-3
(stem-loop)

<400> SEQUENCE: 115
gggugaggua guagguugua uaguuuagggg cucugcccug cuaugggaua acuaauacaau      60
cuacugucuu uccu                                                                74

<210> SEQ ID NO 116
<211> LENGTH: 83
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000063 Human let-7b
(stem-loop)

<400> SEQUENCE: 116
cggggugagg uaguagguug ugugguuuga gggcagugau guugcccuc ggaagauaac      60
uauacaaccu acugccuucc cug                                                    83

<210> SEQ ID NO 117
<211> LENGTH: 84
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000064 Human let-7c
(stem-loop)

<400> SEQUENCE: 117
gcauccgggu ugagguagua gguuguauug uuagaguua caccuggga guuaacugua      60
caaccuucua gcuuuccuug gagc                                                  84

<210> SEQ ID NO 118
<211> LENGTH: 87
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000065 Human let-7d
(stem-loop)

<400> SEQUENCE: 118
ccuaggaaga gguaguaggu ugcuauguuu uagggcaggg auuuugccca caaggaggua      60
acuauacgac cugcugccuu ucuuagg                                              87

<210> SEQ ID NO 119
<211> LENGTH: 79
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000066 Human let-7e

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      (stem-loop)

<400> SEQUENCE: 119

cccgggcuga gguaggaggu uguauaguug aggaggacac ccaaggagau cacuauacgg      60
ccuccuagcu uuccccagg      79

<210> SEQ ID NO 120
<211> LENGTH: 87
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000067 Human let-7f-1
      (stem-loop)

<400> SEQUENCE: 120

ucagagugag guaguagauu guauaguugu gggguaguga uuuuaccug uucaggagau      60
aacuauacaa ucuauugccu ucccuga      87

<210> SEQ ID NO 121
<211> LENGTH: 83
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000068 Human let-7f-2
      (stem-loop)

<400> SEQUENCE: 121

ugugggauga gguaguagau uguauaguuu uagggucaua cccaucuuug gagauaacua      60
uacagucuac ugucuuucc acg      83

<210> SEQ ID NO 122
<211> LENGTH: 84
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000433 Human let-7g
      (stem-loop)

<400> SEQUENCE: 122

aggcugaggu aguaguuuugu acaguuuugag ggucuaugau accaccggu acaggagaua      60
acuguacagg ccacugccuu gcca      84

<210> SEQ ID NO 123
<211> LENGTH: 84
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000434 Human let-7i
      (stem-loop)

<400> SEQUENCE: 123

cuggcugagg uaguaguuuug ugcuguuggu cggguuguga cauugccgc uguggagaua      60
acugcgcaag cuacugccuu gcua      84

<210> SEQ ID NO 124
<211> LENGTH: 110
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miRBase Accession No.: MI0000289 Human
      miR-181a-1 (stem-loop)

<400> SEQUENCE: 124

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 ugaguuuuga gguugcuuca gugaacauuc aacgcugucg gugaguuugg aauiuuuuuuc 60

aaaaccaucg accguugauu guaccuauug gcuaaccauc aucuacucca 110

<210> SEQ ID NO 125

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: miRBase Accession No.: MI0000269 Human
 miR-181a-2 (stem-loop)

<400> SEQUENCE: 125

agaagggcua ucagggcagc cuucagagga cuccaaggaa cauucaacgc ugucggugag 60

uuuggggaauu gaaaaaacca cugaccguug acuguaccuu gggguccuua 110

<210> SEQ ID NO 126

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: miRBase Accession No.: MI0000270 Human
 miR-181b-1 (stem-loop)

<400> SEQUENCE: 126

ccugugcaga gauuuuuuuu uaaaaggua caaucaacau ucauugcugu cgugggguug 60

aacugugugg acaagcucac ugaacauga augcaacugu ggccccguu 110

<210> SEQ ID NO 127

<211> LENGTH: 89

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: miRBase Accession No.: MI0000683 Human
 miR-181b-2 (stem-loop)

<400> SEQUENCE: 127

cugauggcug cacucaacau ucauugcugu cgugggguu gagucugaau caacucacug 60

aucaaugaau gcaaacugcg gaccaaaca 89

<210> SEQ ID NO 128

<211> LENGTH: 110

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: miRBase Accession No.: MI0000271 Human miR-181c
 (stem-loop)

<400> SEQUENCE: 128

cggaaaauuu gccaggguu ugagggaaca uucaaccugu cgugaguuu gggcagcuca 60

ggcaaaccau cgaccguuga guggaccug aggcuggaa uugccauccu 110

<210> SEQ ID NO 129

<211> LENGTH: 137

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

 <223> OTHER INFORMATION: miRBase Accession No.: MI0003139 Human miR-181d
 (stem-loop)

<400> SEQUENCE: 129

guccccucc cuaggccaca gccaggguca caaucaacau ucauuguugu cgugggguug 60

ugaggacuga ggccagacc accgggggau gaaugucacu guggcugggc cagacacggc 120

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uuaaggggaa ugaggac 137

 <210> SEQ ID NO 130
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000284 Human miR-204
 (stem-loop)

 <400> SEQUENCE: 130

 ggcuacaguc uuucucaug ugacucgugg acuuccuuu gucauccuau gccugagaau 60
 auaugaagga ggcugggaag gcaaaggac guucaauugu caucacuggc 110

 <210> SEQ ID NO 131
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000287 Human miR-211
 (stem-loop)

 <400> SEQUENCE: 131

 ucaccuggcc augugacuug ugggcuucc uuugucaucc uucgccuagg gcucugagca 60
 gggcaggac agcaaagggg ugcucaguug ucacuucca cagcacggag 110

 <210> SEQ ID NO 132
 <211> LENGTH: 85
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000078 Human miR-22
 (stem-loop)

 <400> SEQUENCE: 132

 ggcugagccg caguaguucu ucaguggcaa gcuuuaugc cugaccagc uaaagcugcc 60
 aguugaagaa cuguugcccu cugcc 85

 <210> SEQ ID NO 133
 <211> LENGTH: 110
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000268 Human miR-34a
 (stem-loop)

 <400> SEQUENCE: 133

 ggccagcugu gaguguuucu uuggcagugu cuuagcuggu uguugugagc aauguaagg 60
 aagcaaucag caaguauacu gccuagaag ugcugcacgu uguggggccc 110

 <210> SEQ ID NO 134
 <211> LENGTH: 84
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000742 Human miR-34b
 (stem-loop)

 <400> SEQUENCE: 134

 gugcucgguu uguaggcagu gucauuagcu gauuguacug uggugguuac aaucacuaac 60
 uccacugcca ucaaaacaag gcac 84

 <210> SEQ ID NO 135

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<211> LENGTH: 77
 <212> TYPE: RNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: miRBase Accession No.: MI0000743 Human miR-34c
 (stem-loop)

<400> SEQUENCE: 135

agucuaguua cuaggcagug uaguuagcug auugcuaaua guaccaauca cuaaccacac 60
 ggccagguaa aaagauu 77

I claim:

1. A polyribonucleotide comprising:
 an RNA molecule of interest (ROI), wherein the ROI comprises a nucleic acid sequence encoding a protein, wherein the protein comprises BAX, PTEN, p16, P15, P18, P19, P21, P57 or CDKN3;
 a microRNA (miRNA) target sequence operatively linked to the ROI, wherein the miRNA target sequence is a target for miR-126, miR-122, miR-208, miR-145, or miR-143; and
 an RNA molecule comprising a nucleic acid sequence encoding a viral RNA replicase operatively linked to the ROI, the miRNA target sequence, or both the ROI and miRNA target sequence.
2. The polyribonucleotide of claim 1, wherein the viral replicase is a Venezuelan Equine Encephalitis viral replicase, a Sindbis viral replicase, or a Semliki Forest virus replicase.
3. The polyribonucleotide of claim 1, further comprising a RNA molecule capable of being translated into one or more additional nonstructural viral proteins, wherein the RNA molecule capable of being translated into one or more additional nonstructural viral proteins is operatively linked to the ROI, the miRNA target sequence, or both the ROI and miRNA target sequence.
4. The polyribonucleotide of claim 1, wherein the polyribonucleotide is a linear polyribonucleotide or a circular polyribonucleotide.
5. The polyribonucleotide of claim 1, wherein one or more ribonucleotides of the polyribonucleotide is modified.
6. The polyribonucleotide of claim 5, wherein the modification is a Pseudouridine, N-1-methylpseudouridineridine, 5-methoxy-Uridine, a 5-hydroxymethyl-C, a 5-methyl-C, or a combination thereof.
7. The polyribonucleotide of claim 1, wherein the ROI is differentially expressed.
8. The polyribonucleotide of claim 1, wherein the miRNA-126 target has a sequence that is complementary to a sequence that is 20-100% identical to SEQ ID NO: 10, wherein the miRNA-145 target has a sequence that is complementary to a sequence that is 20-100% identical to SEQ ID NO: 11,
- 15 wherein the miRNA-143 target has a sequence that is complementary to a sequence that is 20-100% identical to SEQ ID NO:12, wherein the miRNA-122 target has a sequence that is complementary to a sequence that is 20-100% identical to SEQ ID NO: 13, and
 20 wherein the miRNA-208 target has a sequence that is complementary to a sequence that is 20-100% identical to SEQ ID NO: 15.
9. The polyribonucleotide of claim 1, wherein the miRNA-126 target has a sequence that is complementary to a sequence that is 90-100% identical to a portion of SEQ ID NO: 10 and the portion is 5 or more consecutive nucleotides, wherein the miRNA-145 target has a sequence that is complementary to a sequence that is 90-100% identical to a portion of SEQ ID NO: 11 and the portion is 5 or more consecutive nucleotides,
 25 wherein the miRNA-143 target has a sequence that is complementary to a sequence that is 90-100% identical to a portion of SEQ ID NO: 12 and the portion is 5 or more consecutive nucleotides,
 30 wherein the miRNA-122 target has a sequence that is complementary to a sequence that is 90-100% identical to a portion of SEQ ID NO: 13 and the portion is 5 or more consecutive nucleotides, and
 35 wherein the miRNA-208 target has a sequence that is complementary to a sequence that is 90-100% identical to a portion of SEQ ID NO: 15 and the portion is 5 or more consecutive nucleotides.
10. A pharmaceutical formulation comprising the polyribonucleotide of claim 1 in a pharmaceutically acceptable carrier.
11. The pharmaceutical formulation of claim 10, wherein the polynucleotide is present in the pharmaceutical formulation at an amount effective to treat cardiovascular disease, coronary artery disease, pulmonary hypertension, pulmonary fibrosis or a symptom thereof in a subject.
12. The pharmaceutical formulation of claim 10, wherein the polynucleotide is present in the pharmaceutical formulation at an amount effective to overexpress BAX in a vascular smooth muscle cell but not overexpress BAX in a vascular endothelial cell.

* * * * *