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(54) Title: CARDIOMYOCYTE-DERIVED EXOSOMES INDUCING REGENERATION OF DAMAGED HEART TISSUE

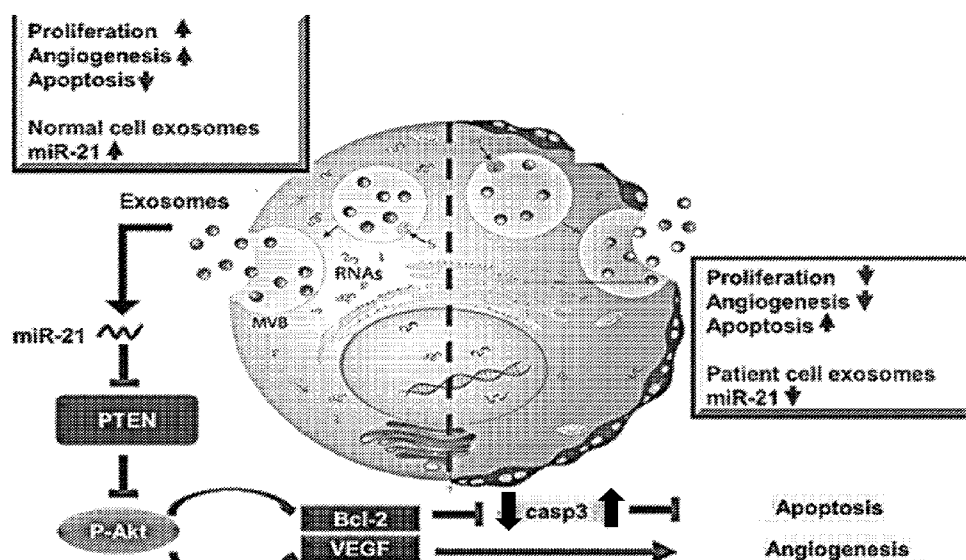


Fig. 8J

(57) Abstract: Exosome compositions of the disclosure comprise the miRNA species hsa-miR-21-5p but may also include a least one other miRNA species over-expressed in normal cardiac stromal cells compared to stromal cells from injured cardiac tissue. Further provided are methods of regenerating damaged cardiac tissue by administering to the site of injury a composition comprising exosomes that deliver at least one miRNA species that reduces the expression of phosphatase and tensin homolog (PTEN) thereby initiating new tissue formation.

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CARDIOMYOCYTE-DERIVED EXOSOMES INDUCING REGENERATION OF DAMAGED HEART TISSUE

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No.: 62/818,945, entitled "microRNA-21-5P DYSREGULATION IN EXOSOMES DERIVED FROM HEART-FAILURE PATIENTS IMPAIRS REGENERATIVE POTENTIAL" filed on March 15, 2019, the entirety of which is hereby incorporated by reference.

SEQUENCE LISTING

10 This application contains a sequence listing filed in electronic form as an ASCII.txt file entitled "2214042370_ST25" created on March 12, 2020. The content of the sequence listing is incorporated herein in its entirety.

TECHNICAL FIELD

15 The present disclosure is generally related to compositions comprising cardiomyocyte exosomes having an miRNA down-regulating PTEN. The present disclosure is also generally related to methods of using cardiomyocyte-derived exosomes to induce regeneration of damaged heart tissue.

BACKGROUND

20 Mounting lines of evidence (Luo *et al.*, (2017) *Circ. Res.* 120: 1768-1775; Tang. *et al.*, (2018) *Nat. Biomed. Engineer.* 2: 17-26; Tang *et al.*, (2017) *ACS Nano* 11: 9738-9749; Tang *et al.*, (2017) *Nat. Commun.* 8: 13724), have demonstrated that cell-based therapy holds great promise for the regeneration of injured heart muscle (Kreke *et al.*, (2012) *Expert Rev. Cardiovasc. Therapy* 10: 1185-1194; Makkar *et al.*, (2012) *Lancet* 379: 895-904; Chugh *et al.*, (2012) *Circulation* 126: S54-S64; Rhee *et al.*, (2018) *Am Heart Assoc.*; Qi *et al.*, (2008) *Chin. Med. J. Beijing* 121: 544; Bellamy *et al.*, (2015) *J. Heart Lung Transplant.* 34: 1198-1207; Kim *et al.*, (2014) *J. Am. Coll. Cardiol.* 64: 1681-1694; Joladarashi *et al.*, (2015) *J. Am. Coll. Cardiol.* 66: 2214-2226). However, cell-based products must be carefully preserved to maintain their viability and activity until transplantation and there are also some risks involved in cell transplantation. The modes of action for cell therapy products remain elusive, making it
30 difficult to standardize each cell lot. Recent meta-analyses indicate that cardiac cell therapies are overwhelmingly safe but only show none-to-marginal efficacy (Tang *et al.*, (2018) *Stem Cells Transl. Med.* 7: 354-359). The development of cell-free and non-living therapeutics (e.g. proteins, RNAs) has the potential to revolutionize cardiovascular regenerative medicine. These therapeutics have compounded the evidence showing that the benefits of stem cell
35 therapies mainly come from paracrine mechanisms instead of the direct differentiation of injected stem cells into cardiomyocytes (Chimenti *et al.*, (2010) *Circ. Res.* 106: 971-980; Malliaras *et al.*, (2011) *Circulation* 111.042598). The injected cells secrete proteins and

nucleic acids to promote endogenous repair (Raposo and Stoorvogel (2013) *J. Cell Biol.* 200: 373-383).

Exosomes are nanosized membrane vesicles secreted by most cell types, including stem cells and cancer cells (Kalra *et al.*, (2016) *Int. J. Mol. Sci* 17: 170; Melo *et al.*, (2018) *Frontiers Immunol.* 9: 730). They are extracellular nanoshuttles that facilitate cell-cell communications and are crucial for maintaining the normal physiological functions of cells. It has been reported that exosomes, as functional paracrine units of stem cells, can partially recapitulate the regenerative activities of their parent cells, suggesting that they may provide an alternative, cell-free therapeutic option (Gallet *et al.*, (2016) *Euro. Heart J.* 38: 201-211; Mathiyalagan *et al.*, (2017) *Circ. Res.* 116:310557; Lai *et al.*, (2011) *Regen. Med.* 6: 481-492; Sahoo and Losordo (2014) *Circ. Res.* 114: 333). The mechanisms that drive the exosome-mediated repair processes rely largely on the transferring of exosomal cargos, including microRNAs (miR), mRNA, and proteins, to the recipient cells (Prathipati *et al.*, (2017) *Stem Cell Revs. Reports* 13: 79-91). Accordingly, the constitution of exosomes, as well as their biological activity is largely dependent on the physiological state of their parent cells (Wang *et al.*, (2015) *Int. J. Cardiol.* 192: 61-69; Yue *et al.*, (2017) *Tissue Eng Part A* 23: 1241-1250).

SUMMARY

The present disclosure encompasses methods of regenerating damaged cardiac tissue by delivering to the site of injury a therapeutic composition comprising at least one miRNA that reduces the expression of phosphatase and tensin homolog (PTEN) and is over-expressed in normal cardiac stromal cells compared to stromal cells from injured cardiac tissue. The exosome compositions of the disclosure comprise the miRNA species hsa-miR-21-5p (SEQ ID NO: 1) but may also include a least one other miRNA species over-expressed in normal cardiac stromal cells compared to stromal cells from injured cardiac tissue.

One aspect of the disclosure, therefore, encompasses a composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes, wherein the exosomes comprise an miRNA species can have at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) or a heterologous nucleic acid that expresses the miRNA species when in recipient cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure, the composition can further comprise a pharmaceutically acceptable carrier.

In some embodiments of this aspect of the disclosure, the composition can be formulated for delivery to a site of cardiac injury.

In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart tissue.

5 In some embodiments of this aspect of the disclosure, at least one of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure, each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence
10 similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13),
15 hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

Another aspect of the disclosure encompasses embodiments of a method of modulating the activity of PTEN in the cardiac tissue of an animal or human subject, the method comprising administering to a cardiac tissue of an animal or human subject a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically
20 acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes can comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

25 In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species.

In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart
30 tissue.

In some embodiments of this aspect of the disclosure, at least one of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure, each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence
35 similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5),

hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

5 In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a heterologous nucleic acid that
10 expresses at least one miRNA species when in recipient cardiac tissue.

In some embodiments of this aspect of the disclosure the composition can be administered to the cardiac tissue of the animal or human subject by a percutaneous method.

In some embodiments of this aspect of the disclosure the percutaneous method is by injection into the cardiac tissue.

15 Yet another aspect of the disclosure encompasses embodiments of a method of repairing cardiac tissue damage, the method comprising administering to a cardiac tissue of an animal or human subject a therapeutic composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA
20 species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species.

25 In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue.

In some embodiments of this aspect of the disclosure at least some of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived
30 from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5),
35 hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a

(SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a heterologous nucleic acid that expresses at least one miRNA species when in recipient cardiac tissue.

In some embodiments of this aspect of the disclosure the therapeutic composition can be administered to the cardiac tissue of the animal or human subject by a percutaneous method.

In some embodiments of this aspect of the disclosure the percutaneous method is by injection into the cardiac tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

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

Figs. 1A-1J illustrate the effects of exosome treatment on cardiomyocytes, endothelial cells, and cardiac fibroblasts *in vitro*.

Fig. 1A shows representative fluorescent micrographs showing uptake of Dil-labeled exosomes derived from the cardiac cells of normal heart donors (NEXO) and exosomes derived from the cardiac cells of heart-failure patients (FEXO) by neonatal rat cardiomyocytes (NRCMs). Endocytosed exosomes can be seen within the cytoplasm of cardiomyocytes. Scale bar: 10 μ m.

Fig. 1B shows quantitation of exosomes uptake (n=10). Two-tailed t-test.

Fig. 1C shows NRCM proliferation in response to NEXO, FEXO, or phosphate-buffered saline (PBS) treatment. White arrows indicate Ki67⁺/α-SA⁺ cells. Scale bar: 20 μ m.

Fig. 1D shows quantitation of proliferating cardiomyocytes (n=6).

Fig. 1E shows apoptotic NRCMs in response to NEXO, FEXO, or PBS treatment. White arrows indicate TUNEL⁺/α-SA⁺ cells. Scale bar: 20 μ m.

Fig. 1F shows quantitation of apoptotic cardiomyocytes (n=6).

Fig. 1G shows measurement of tube formation in human umbilical vascular endothelial cells (HUVECs) co-cultured with NEXO, FEXO, or PBS. Scale bar: 100 μ m.

Fig. 1H shows quantitation of average HUVEC tube length (n=20).

Fig. 1I shows neonatal rat fibroblasts (NRCFs) underwent phenotypic transition to myofibroblasts in response to NEXO, FEXO, or PBS treatment. Scale bar: 20 μ m.

Fig. 1J shows quantitation of myofibroblasts (n=12). *, p<0.05. ***, p<0.001. N.S., no significance. (D, F, H, J) One-way ANOVA with Bonferroni post hoc correction. All values are mean $\pm$ S.D.

Figs. 2A-2G illustrate the effects of NEXO and FEXO treatment in a mouse model of acute MI.

Figs. 2A and 2B show left ventricular ejection fraction (LVEF) measured by echocardiography at baseline (one day before MI) (Fig. 2A) and endpoint (3 weeks after MI) (Fig. 2B).

Fig. 2C shows the treatment effects (changes in LVEF at 3 weeks relative to baseline) in each group (A-C, n=9 animals per treatment group, n=3 animals for the sham group).

Fig. 2D shows representative Masson's trichrome staining of myocardial section 3 weeks after treatment with NEXO, FEXO, or PBS. Scale bar: 0.5 mm.

Figs. 2E-2G show quantitative analyses of infarct size (Fig. 2E), infarct wall thickness (Fig. 2F), and viable tissue from Masson's trichrome-stained heart sections (Fig. 2G) (n = 8 animals per treatment group). *, p<0.05. ***, p<0.001. One-way ANOVA with Bonferroni post hoc correction.

Figs. 3A-3F show mechanisms of exosome-mediated cardiac repair.

Fig. 3A shows representative images of post-myocardial infarction (MI) heart sections stained with Ki67, α -SA, and DAPI. White boundaries show infarct area, and white arrows indicate Ki67⁺ cells in the peri-infarct zone. Scale bar: 10 μ m

Fig. 3B shows heart sections stained with vWF, α -SA in response to NEXO, FEXO or PBS treatment. White arrows indicate capillary structures in the peri-infarct zone. Scale bar: 50 μ m.

Fig. 3C shows heart sections stained with TUNEL, α -SA, and DAPI. White arrows indicate apoptotic cardiomyocytes in the peri-infarct zone. Scale bar: 50 μ m.

Fig. 3D shows quantification of cycling cardiomyocytes (Ki67⁺/ α -SA⁺)

Fig. 3E shows quantification of capillary density (vWF⁺).

Fig. 3F shows quantification of cardiomyocyte apoptosis (TUNEL⁺/ α -SA⁺). n=6 animals per group, 3 heart sections for each animal. *, p<0.05. **, p<0.01. ***, p<0.001. One-way ANOVA with Bonferroni post hoc correction.

Figs. 4A-4C illustrate dysregulation of hsa-miR-21-5p (SEQ ID NO: 1) in heart failure exosomes.

Fig. 4A shows an miRNA array showing fold changes of miRNA abundance in NEXO or FEXO (n = 3 biological replicates, 3 technical replicates for each biological replicate).

Fig. 4B shows a Venn diagram showing the variable miRNA profile between NEXO and FEXO.

Fig. 4C shows a qRT-PCR analysis validated that hsa-miR-21-5p (SEQ ID NO: 1) was

highly enriched in NEXO (n = 3 biological replicates, 3 technical replicates for each biological replicate). *, p<0.05. N.S., no significance. Two-tailed t-test.

Figs. 5A-5H illustrate that manipulation of hsa-miR-21-5p (SEQ ID NO: 1) in exosomes modulates their pro-angiogenic and anti-apoptosis activities *in vitro*. FEXO+miR-scr, exosomes derived from the cardiac cells of heart-failure patients transfected with scrambled miRNA oligo. FEXO+miR-21, exosomes derived from the cardiac cells of heart-failure patients transfected with hsa-miR-21-5p (SEQ ID NO: 1) oligo. NEXO+miR-scr, exosomes derived from the cardiac cells of the normal hearts transfected with scrambled miRNA oligo. NEXO+anti-miR-21, exosomes derived from the cardiac cells of the normal hearts transfected with anti-miR-21-5p (SEQ ID NO: 1) oligo. FEXO, exosomes derived from the cardiac cells of heart-failure patients. NEXO, exosomes derived from the cardiac cells of normal heart donors.

Fig. 5A shows anti-apoptotic effects were diminished after hsa-miR-21-5p (SEQ ID NO: 1) knockdown in NEXOs. White arrows indicate TUNEL⁺ cells. Scale bar: 50µm.

Fig. 5B shows quantitation of apoptotic cells (n = 6).

Fig. 5C shows the pro-angiogenic effects of NEXO were diminished by hsa-miR-21-5p (SEQ ID NO: 1) knockdown. Scale bar: 50µm.

Fig. 5D shows quantitation of average tube length (n = 10).

Fig. 5E shows enhancing hsa-miR-21-5p (SEQ ID NO: 1) expression in FEXO partly rescued its ability to promote cardiomyocyte proliferation. Scale bar: 50µm.

Fig. 5F shows quantitation of apoptotic cells (n = 6).

Fig. 5G shows tube formation assay showing enhanced pro-angiogenic activity of FEXO with restored hsa-miR-21-5p (SEQ ID NO: 1) expression. Scale bar: 50µm.

Fig. 5H shows quantitation of average tube length (n = 10). *, p<0.05. Two-tailed t-test.

All values are mean ± S.D.

Figs. 6A-6G illustrate the manipulation of miR21 in exosomes modulates their therapeutic potency. hsa-miR-21-5p (SEQ ID NO: 1)-deficient exosomes were produced by transfecting healthy cardiac cells with anti-miR-21-5p (SEQ ID NO: 1) oligo (NEXO+anti-miR-21). hsa-miR-21-5p (SEQ ID NO: 1)-restored exosomes were engineered by transfecting heart failure cardiac cells with hsa-miR-21-5p (SEQ ID NO: 1) oligo (FEXO+miR-21), followed by media conditioning and exosome isolation, as previously described. Scrambled miRNA oligo was used as a control (NEXO/FEXO+miR-scr).

Fig. 6A shows left ventricular ejection fraction (LVEF) was measured by echocardiography 3 weeks after treatment.

Fig. 6B shows treatment effects (changes in LVEF at 3 weeks relative to baseline) were calculated for each group.

Figs. 6C and 6D show representative Masson's trichrome staining of myocardial

section 3 weeks after treatments. Scale bar: 1 mm.

Figs. 6E-6H show quantitative analysis of infarct size, infarct wall thickness, and viable tissue from Masson's trichrome-stained images. $n = 6$ animals per group. *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$. One-way ANOVA with Bonferroni post hoc correction. All values are mean $\pm$ S.D.

Figs. 7A-7H illustrate manipulation of miR21 in exosomes modulates their pro-angiogenic and anti-apoptotic activities *in vivo*.

Fig. 7A shows representative images of post-MI heart sections stained with vWF, α -SA, and DAPI. White circles indicate capillaries in the peri-infarct zone. Scale bar: 100 μ m.

Fig. 7B shows quantitation of capillary density (vWF⁺).

Fig. 7C shows heart sections stained with TUNEL, α -SA, and DAPI. White squares indicate apoptotic cardiomyocytes in the peri-infarct zone. Scale bar: 50 μ m.

Fig. 7D shows quantitation of cardiomyocyte apoptosis (TUNEL⁺/ α -SA⁺).

Fig. 7E shows representative images of post-MI heart sections stained with vWF, α -SA, and DAPI. White circles indicate capillaries in the peri-infarct zone. Scale bar: 50 μ m.

Fig. 7F shows quantitation of capillary density (vWF⁺).

Fig. 7G shows heart sections stained with TUNEL, α -SA, and DAPI. White squares indicate apoptotic cardiomyocytes in the peri-infarct zone. Scale bar: 50 μ m.

Fig. 7H shows quantitation of cardiomyocyte apoptosis (TUNEL⁺/ α -SA⁺). (B, D, F, H) $n = 6$ animals for each group, and 3 heart sections for each animal. **, $p < 0.01$. Two-tailed t-test.

Figs. 8A-8J illustrate that hsa-miR-21-5p (SEQ ID NO: 1) targets the PTEN pathway in post-MI pathology.

Fig. 8A shows representative western blot images showing the expression of various PTEN/Akt pathway components.

Fig. 8B-8E show quantitation of the levels of PTEN (Fig. 8B), p-Akt (Fig. 8C), t-Akt (Fig. 8D), Bcl-2 (Fig. 8E), and caspase-3 (Fig. 8F) ($n = 3$).

Fig. 8F shows representative western blot images showing the expression of PCNA (proliferation marker), VEGF, and PDCD4 (miR-21 target).

Figs. 8G-8I show quantitation of the levels of PCNA (Fig. 8G), VEGF (Fig. 8H), and PDCD4 (Fig. 8I) ($n = 3$). *, $p < 0.05$. **, $p < 0.01$. ***, $p < 0.001$. N.S., no significance. Two-tailed t-test. All values are mean $\pm$ S.D.

Fig. 8J shows a schematic showing the working model of our study.

CM/H9C2/EC/CF+miR-scr, human cardiomyocytes/H9C2 cells/HUVECs/human cardiac fibroblasts transfected with scrambled miRNA. CM/H9C2/EC/CF+miR-21, human cardiomyocytes/H9C2 cells/HUVECs/human cardiac fibroblasts transfected with hsa-miR-21-5p (SEQ ID NO: 1) mimic.

Figs. 9A-9C illustrate the isolation of exosomes from cardiac explant-derived cells.

Fig. 9A shows the derivation of cardiac cells and exosomes.

Fig. 9B shows representative images of explant culture, outgrowth cells from explant tissues, and the cardiac cells before and after 14-day conditioning. Scale bar: 100µm.

Fig. 9C shows a Live/Dead assay showing cell viability during the conditioning period
5 (n=3).

Figs. 10A and 10B illustrate a phenotypic analysis of explant-derived cardiac cells.

Fig. 10A shows representative flow cytometry histogram of cardiac cells derived from heart-failure patients (FEDC) or healthy heart donors (NEDC). Black lines in the histograms are the unstained or isotype controls. Lighter lines show the particular markers.

Fig. 10B shows quantitative analyses of CD90, CD105, CD31, CD34, CD45, and c-kit
10 expressions (n=3-4). N.S., no significance. Two-tailed t-test. All values are mean ± S.D.

Figs. 11A-11E illustrate the characterization of exosomes from normal and failing hearts.

Fig. 11A shows exosome quantitation using NanoSight particle tracking images (n = 3
15 biological replicates, 5 technical replicates for each biological replicate).

Fig. 11B shows size distribution of NEXO and FEXO by NanoSight showing that the exosomes are within the expected size range.

Fig. 11C shows NEXO and FEXO express exosomal markers Alix, TSG 101, and CD81 as revealed by western blot analysis.

Fig. 11D shows transmission electron microscopy (TEM) and NanoSight showing
20 exosome morphology and motion. Scale bar: 100 µm.

Fig. 11E shows total RNA content measured in NEXO and FEXO by Nanodrop (n = 3 biological replicates). N.S., no significance. Two-tailed t-test. All values are mean ± S.D.

Figs. 12A-12H illustrate the effects of NEXO or FEXO treatment on cardiomyocytes *in vitro*.
25

Figs. 12A-12C show representative fluorescent micrographs showing adult human cardiomyocyte proliferation in response to NEXO, FEXO, or PBS treatment. Scale bar: 20 µm.

Figs. 12D-12F show the quantitation of cardiomyocyte proliferation, as detected by three proliferation markers: AURKB, Ki67, and PH3 (n=6).

Fig. 12G shows representative fluorescent micrographs showing adult human
30 cardiomyocyte apoptosis in response to NEXO, FEXO, or PBS treatment. Scale bar: 20 µm.

Fig. 12H shows quantitation of apoptotic cardiomyocytes detected by TUNEL (n=6). *, p<0.05. **, p<0.01. ***, p<0.001. One-way ANOVA with Bonferroni post correction.

Figs. 13A-13D illustrate the effects of NEXO or FEXO therapy on cardiac function and
35 morphometry.

Figs. 13A-13C show echocardiographic measurements of fractional shortening (FS) (Fig. 13), left ventricular end systolic volume (Fig. 13B), and left ventricular end diastolic

volume (Fig. 13C) of MI hearts treated with NEXO, FEXO, or PBS.

Fig. 13D shows quantitative analysis of circumference of scar tissue. n=6 animals per group. *, p<0.05. **, p<0.01. ***, p<0.001. N.S., no significance. One-way ANOVA with Bonferroni post correction. All values are mean $\pm$ S.D.

5 Figs. 14A-14C illustrate the effects of NEXO or FEXO treatment on cardiomyocyte proliferation *in vivo*.

Fig. 14A shows representative images of post-MI heart sections stained with mitosis marker PH3 or cytokinesis marker ARUKB. Scale bar: 20 μ m.

10 Figs. 14A and 14B shows the quantitation of proliferated cardiomyocytes (n=6). *, p<0.05. **, p<0.01. ***, p<0.001. One-way ANOVA with Bonferroni post correction. All values are mean $\pm$ S.D.

Figs. 15A-15C illustrate the heat map of microRNA PCR array identifies hsa-miR-21-5p (SEQ ID NO: 1) as the most differentially expressed microRNA between NEXO and FEXO.

15 Figs. 15A and 15B show a heat map showing miRNA fold changes between NEXO and FEXO.

Fig. 15C shows that infarcted mouse hearts treated with NEXO have elevated levels of hsa-miR-21 compared to FEXO-treated hearts. n = 3 animals per group. *, p<0.05. Two-tailed t-test. All values are mean $\pm$ S.D.

20 Figs. 16A-16C illustrate the direct manipulation of hsa-miR-21 in NEXO and FEXO. FEXO+miR-scr, exosomes derived from the cardiac cells of heart-failure patients transfected with scrambled miRNA oligo. FEXO+miR-21, exosomes derived from the cardiac cells of heart-failure patients transfected with hsa-miR-21-5p (SEQ ID NO: 1) oligo. NEXO+miR-scr, exosomes derived from the cardiac cells of the normal hearts transfected with scrambled miRNA oligo. NEXO+anti-miR-21, exosomes derived from the cardiac cells of the normal hearts transfected with anti-miR-21-5p (SEQ ID NO: 1) oligo.

Fig. 16A shows transfection of cardiac cells from heart-failure patients/healthy heart donors with hsa-miR-21/ anti-miR-21 oligo or scrambled miRNA oligo (as a negative control). Scale bar: 100 μ m.

30 Figs. 16B and 16C show the transfection efficiency of mi-R21/anti-miR-21 oligo was determined by qRT-PCR. n=3 biological replicates. ***, p<0.001. Two-tailed t-test. All values are mean $\pm$ S.D.

35 Figs. 17A and 17B show the effects of hsa-miR-21 on adult human cardiomyocytes *in vitro*. Adult human cardiomyocytes were transfected with hsa-miR-21-5p (SEQ ID NO: 1) mimic or scrambled control. CM+miR-scr: human cardiomyocytes transfected with scrambled miRNA. CM+miR-21: human cardiomyocytes transfected with hsa-miR-21-5p (SEQ ID NO: 1) mimic.

Fig. 17A shows representative images of transfected human cardiomyocytes stained with TUNEL. Scale bar: 20 μ m.

Fig. 17B shows quantitation of cardiomyocyte apoptosis (n=6). ***, p<0.001. Two-tailed t-test. All values are mean $\pm$ S.D.

5 Fig. 18 illustrates pro-angiogenic effects of hsa-miR-21 on post-MI heart. Functional vessels were confirmed by co-staining of the endothelial cell marker von Willebrand factor (vWF) and the red blood cell (RBC) marker. RBCs were detected in the blood vessels in the MI border zone of the heart treated with hsa-miR-21-over-expressed FEXO.

10 Figs. 19A-19G illustrate the effects of various microRNA treatments on cardiomyocytes, endothelial cells, and cardiac fibroblasts *in vitro*. Human cardiomyocytes (CM), human cardiac fibroblasts (CF), and human umbilical vein endothelial cells (HUVECs) were treated with let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR-146a-5p (SEQ ID NO: 4), hsa-miR-21-5p (SEQ ID NO: 1) mimics or scrambled control, followed by immunocytochemistry staining of proliferation or apoptotic markers.

15 Figs. 19A-19C show cardiomyocyte proliferation in response to let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR-146a-5p (SEQ ID NO: 4) or hsa-miR-21-5p (SEQ ID NO: 1), as calculated by three proliferation markers: Ki67, phospho-histone H3 (PH3, mitosis marker), and aurora kinase B (AURKB, cytokinesis marker) (n=6).

Fig. 19D shows apoptotic cardiomyocytes transfected with indicated miRNAs (n=6).

20 Fig. 19D shows measurement of tube length in HUVECs in the transfection of indicated miRNAs (n=22).

Figs. 19F and 19G show quantitation of apoptosis of HUVECs (Fig. 19F) and cardiac fibroblasts (Fig. 19G) after transfection with indicated miRNAs (n=6). *, p<0.05. **, p<0.01. ***, p<0.001. N.S., no significance. One-way ANOVA with Bonferroni post correction. All values are mean $\pm$ S.D.

25 Figs. 20A-20C illustrate that a signaling pathway phosphorylation array identifies PTEN/Akt as the hsa-miR-21-regulate genes in cardiomyocytes and endothelial cells. Three major cell types in the heart (cardiomyocytes (CM), endothelial cells (EC), and cardiac fibroblasts (CF)) were treated with hsa-miR-21 mimic or scrambled control, followed by the examination of 18 phosphorylated proteins with a signaling pathway phosphorylation array.

Fig. 20A shows a heat map showing expression level changes between cells transfected with hsa-miR-21 mimic or scrambled control.

Fig. 20A shows quantitation of expression changes in hsa-miR-21-upregulated cells relative to scrambled control. (n = 2 biological replicates, 2 technical replicates for each biological replicate). All values are mean $\pm$ S.D. Cardiomyocytes (using both human induced pluripotent stem cell-derived cardiomyocytes (iCM) and H9C2 cells), cardiac fibroblasts (using human cardiac fibroblasts, HCF) (Fig. 20B), and endothelial cells (using human umbilical vein

endothelial cells, HUVECs) (Fig. 20C).

DETAILED DESCRIPTION

This disclosure is not limited to particular embodiments described, and as such may, of course, vary. The terminology used herein serves the purpose of describing particular
5 embodiments only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

Where a range of values is provided, 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
10 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.

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

The following examples are put forth so as to provide those of ordinary skill in the art
20 with a complete disclosure and description of how to perform the methods and use the compositions and compounds disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (*e.g.*, amounts, temperature, *etc.*), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C, and pressure is at or near atmospheric. Standard temperature
25 and pressure are defined as 20 °C and 1 atmosphere.

Before the embodiments of the present disclosure are described in detail, it is to be understood that, unless otherwise indicated, the present disclosure is not limited to particular materials, reagents, reaction materials, manufacturing processes, dimensions, frequency ranges, applications, or the like, as such can vary. It is also to be understood that the
30 terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It is also possible in the present disclosure that steps can be executed in different sequence, where this is logically possible. It is also possible that the embodiments of the present disclosure can be applied to additional embodiments involving measurements beyond the examples described herein, which are not intended to be limiting. It is furthermore
35 possible that the embodiments of the present disclosure can be combined or integrated with other measurement techniques beyond the examples described herein, which are not intended to be limiting.

It should be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a support” includes a plurality of supports. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings unless a contrary intention is apparent.

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each issued patent; “application cited documents”), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference. Further, documents or references cited in this text, in a Reference List before the claims, or in the text itself; and each of these documents or references (“herein cited references”), as well as each document or reference cited in each of the herein-cited references (including any manufacturer’s specifications, instructions, etc.) are hereby expressly incorporated herein by reference.

Prior to describing the various embodiments, the following definitions are provided and should be used unless otherwise indicated.

Definitions

The term “cell” as used herein refers to an animal or human cell. The engineered cells of the disclosure can be removed (isolated) from a tissue and mixed with platelet-derived membrane vesicles without culturing of the cells, or after removal from a tissue or fluid of an animal or human may be cultured to increase the population size of the cells. If not immediately required for incorporation of the platelet-derived membrane vesicles, the animal or human cells may be maintained in a viable state either by culturing by serial passage in a culture medium or cryopreserved by methods well-known in the art. The cells may be obtained from the animal or human individual that has received an injury desired to be repaired by administration of the engineered cells of the disclosure or from a different individual.

Cells, and their extracellular vesicle such as an exosomes that are contemplated for use in the methods of the present disclosure may be derived from the same subject to be treated (autologous to the subject) or they may be derived from a different subject, preferably of the same species, (allogeneic to the subject).

Commercially available media may be used for the growth, culture and maintenance of mesenchymal stem cells. Such media include but are not limited to Dulbecco's modified Eagle's medium (DMEM). Components in such media that are useful for the growth, culture and maintenance of mesenchymal stem cells include but are not limited to amino acids, vitamins, a carbon source (natural and non-natural), salts, sugars, plant derived hydrolysates,

sodium pyruvate, surfactants, ammonia, lipids, hormones or growth factors, buffers, non-natural amino acids, sugar precursors, indicators, nucleosides and/or nucleotides, butyrate or organics, DMSO, animal derived products, gene inducers, non-natural sugars, regulators of intracellular pH, betaine or osmoprotectant, trace elements, minerals,

5 non-natural vitamins. Additional components that can be used to supplement a commercially available tissue culture medium include, for example, animal serum (e.g., fetal bovine serum (FBS), fetal calf serum (FCS), horse serum (HS)), antibiotics (e.g., including but not limited to, penicillin, streptomycin, neomycin sulfate, amphotericin B, blasticidin, chloramphenicol, amoxicillin, bacitracin, bleomycin, cephalosporin, chlortetracycline, zeocin, and puromycin),
 10 and glutamine (e.g., L-glutamine). Mesenchymal stem cell survival and growth also depends on the maintenance of an appropriate aerobic environment, pH, and temperature. Mesenchymal stem cells can be maintained using methods known in the art (see for example Pittenger *et al.*, (1999) *Science* 284:143-147).

The term "extracellular vesicle" as used herein can refer to a membrane vesicle
 15 secreted by cells that may have a larger diameter than that referred to as an "exosome". Extracellular vesicles (alternatively named "microvesicle" or "membrane vesicle") may have a diameter (or largest dimension where the particle is not spheroid) of between about 10 nm to about 5000 nm (e.g., between about 50 nm and 1500 nm, between about 75 nm and 1500 nm, between about 75 nm and 1250 nm, between about 50 nm and 1250 nm, between about 30
 20 nm and 1000 nm, between about 50 nm and 1000 nm, between about 100 nm and 1000 nm, between about 50 nm and 750 nm, etc.). Typically, at least part of the membrane of the extracellular vesicle is directly obtained from a cell (also known as a donor cell). Extracellular vesicles suitable for use in the compositions and methods of the present disclosure may originate from cells by membrane inversion, exocytosis, shedding, blebbing, and/or budding.
 25 Extracellular vesicles may originate from the same population of donor cells yet different subpopulations of extracellular vesicles may exhibit different surface/lipid characteristics. Alternative names for extracellular vesicles include, but are not limited to, exosomes, ectosomes, membrane particles, exosome-like particles, and apoptotic vesicles. Depending on the manner of generation (e.g., membrane inversion, exocytosis, shedding, or budding),
 30 the extracellular vesicles contemplated herein may exhibit different surface/lipid characteristics.

The term "exosomes" as used herein refers to small secreted vesicles (typically about 30 nm to about 150 nm (or largest dimension where the particle is not spheroid)) that may contain, or have present in their membrane, nucleic acid, protein, or other biomolecules and
 35 may serve as carriers of this cargo between diverse locations in a body or biological system. The term "exosomes" as used herein advantageously refers to extracellular vesicles that can

have therapeutic properties, including, but not limited to stem cell exosomes such as cardiac stem cell or mesenchymal stem cells.

Exosomes may be isolated from a variety of biological sources including mammals such as mice, rats, guinea pigs, rabbits, dogs, cats, bovine, horses, goats, sheep, primates or humans. Exosomes can be isolated from biological fluids such as serum, plasma, whole blood, urine, saliva, breast milk, tears, sweat, joint fluid, cerebrospinal fluid, semen, vaginal fluid, ascetic fluid and amniotic fluid. Exosomes may also be isolated from experimental samples such as media taken from cultured cells ("conditioned media", cell media, and cell culture media).

Exosomes may also be isolated from tissue samples such as surgical samples, biopsy samples, and cultured cells. When isolating exosomes from tissue sources it may be necessary to homogenize the tissue in order to obtain a single cell suspension followed by lysis of the cells to release the exosomes. When isolating exosomes from tissue samples it is important to select homogenization and lysis procedures that do not result in disruption of the exosomes.

Exosomes may be isolated from freshly collected samples or from samples that have been stored frozen or refrigerated. Although not necessary, higher purity exosomes may be obtained if fluid samples are clarified before precipitation with a volume-excluding polymer, to remove any debris from the sample. Methods of clarification include centrifugation, ultracentrifugation, filtration or ultrafiltration.

The genetic information within the extracellular vesicle such as an exosome may easily be transmitted by fusing to the membranes of recipient cells, and releasing the genetic information into the cell intracellularly. Though exosomes as a general class of compounds represent great therapeutic potential, the general population of exosomes are a combination of several class of nucleic acids and proteins which have a constellation of biologic effects both advantageous and deleterious. In fact, there are over 1000 different types of exosomes.

The terms "microRNA" and "miRNA" as used herein refer to a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses, and which function in RNA silencing and post-transcriptional regulation of gene expression.

miRNAs function via base-pairing with complementary sequences within mRNA molecules. As a result, these mRNA molecules are silenced, by one or more of cleavage of the mRNA strand into two pieces, destabilization of the mRNA through shortening of its poly(A) tail, and less efficient translation of the mRNA into proteins by ribosomes. miRNAs are derived from regions of RNA transcripts that fold back on themselves to form short hairpins.

The term "stem cell" as used herein refers to cells that have the capacity to self-renew and to generate differentiated progeny. The term "pluripotent stem cells" refers to stem cells that have complete differentiation versatility, i.e., the capacity to grow into any of the fetal or

adult mammalian body's approximately 260 cell types. For example, pluripotent stem cells have the potential to differentiate into three germ layers: endoderm (e.g., blood vessels), mesoderm (e.g., muscle, bone and blood) and ectoderm (e.g., epidermal tissues and nervous system), and therefore, can give rise to any fetal or adult cell type. The term "cardiac stem cells" refers to stem cells obtained from or derived from cardiac tissue. The term "cardiosphere-derived cells (CDCs)" as used herein refers to undifferentiated cells that grow as self-adherent clusters from subcultures of postnatal cardiac surgical biopsy specimens. CDCs can express stem cell as well as endothelial progenitor cell markers, and typically possess properties of adult cardiac stem cells. For example, human CDCs can be distinguished from human cardiac stem cells in that human CDCs typically do not express multidrug resistance protein 1 (MDR1; also known as ABCB1), CD45 and CD133 (also known as PROM1). CDCs are capable of long-term self-renewal, and can differentiate *in vitro* to yield cardiomyocytes or vascular cells after ectopic (dorsal subcutaneous connective tissue) or orthotopic (myocardial infarction) transplantation in SCID beige mouse.

The terms "cardiac progenitor cells" and "cardiac stem cells" as used herein can refer to a population of progenitor cells derived from human heart tissue. In some aspects the present disclosure, of the cardiac progenitor (stem) cells, at least 3%, 5%, 7%, 10%, 12%, or 15%, e.g., 3-50%, 3-20%, 3-10%, 5-30%, 5-10%, etc., of the cells express Isl1. In some aspects, CPCs comprise about 10%, 15%, 20%, 30%, 40%, or 50%, e.g., 10-50%, 10-40%, 10-30%, 15-40%, etc., GATA4 expressing cells. In some aspects, cardiac stem cells comprise about 5%, 8%, 10%, 13%, or 15%, e.g., 8-15%, 5-15%, 5-13%, etc., NKX2.5 expressing cells. Before fusion with platelet membrane vesicles by the methods of the present disclosure, cardiac stem cells are unmodified cells in that recombinant nucleic acids or proteins have not been introduced into them or the Sca-1⁺, CD45⁻ cell from which it is derived. As such, cardiac stem cells as isolated from cardiac tissue are non-transgenic, or in other words have not been genetically modified. For example, expression of genes such as Isl1, GATA4, and NKX2.5 in CPCs is from the endogenous gene. Cardiac stem cells may comprise Sca-1⁺, CD45⁻ cells, c-kit⁺ cells, CD90⁺ cells, CD133⁺ cells, CD31⁺ cells, Flk1⁺ cells, GATA4⁺ cells, or NKX2.5⁺ cells, or combinations thereof. In some aspects, cardiac stem cells comprise about 50% GATA4 expressing cells. In some aspects, cardiac stem cells comprise about 15% NKX2.5 expressing cells. Cardiac stem cells can replicate and are capable of differentiating into endothelial cells, cardiomyocytes, smooth muscle cells, and the like.

The term "cardiac cells" as used herein refers to any cells present in the heart that provide a cardiac function, such as heart contraction or blood supply, or otherwise serve to maintain the structure of the heart. Cardiac cells as used herein encompass cells that exist in the epicardium, myocardium or endocardium of the heart. Cardiac cells also include, for example, cardiac muscle cells or cardiomyocytes; cells of the cardiac vasculatures, such as

cells of a coronary artery or vein. Other non-limiting examples of cardiac cells include epithelial cells, endothelial cells, fibroblasts, cardiac conducting cells and cardiac pacemaking cells that constitute the cardiac muscle, blood vessels and cardiac cell supporting structure.

The term "cardiac function" as used herein refers to the function of the heart, including global and regional functions of the heart. The term "global" cardiac function as used herein refers to function of the heart as a whole. Such function can be measured by, for example, stroke volume, ejection fraction, cardiac output, cardiac contractility, etc. The term "regional cardiac function" refers to the function of a portion or region of the heart. Such regional function can be measured, for example, by wall thickening, wall motion, myocardial mass, segmental shortening, ventricular remodeling, new muscle formation, the percentage of cardiac cell proliferation and programmed cell death, angiogenesis and the size of fibrous and infarct tissue. Techniques for assessing global and regional cardiac function are known in the art. For example, techniques that can be used to measure regional and global cardiac function include, but are not limited to, echocardiography (e.g., transthoracic echocardiogram, transesophageal echocardiogram or 3D echocardiography), cardiac angiography and hemodynamics, radionuclide imaging, magnetic resonance imaging (MRI), sonomicrometry and histological techniques.

The term "cardiac tissue" as used herein refers to tissue of the heart, for example, the epicardium, myocardium or endocardium, or portion thereof, of the heart. The term "injured" cardiac tissue as used herein refers to a cardiac tissue that is, for example, ischemic, infarcted, reperfused, or otherwise focally or diffusely injured or diseased. Injuries associated with a cardiac tissue include any areas of abnormal tissue in the heart, including any areas caused by a disease, disorder or injury and includes damage to the epicardium, endocardium and/or myocardium. Non-limiting examples of causes of cardiac tissue injuries include acute or chronic stress (e.g., systemic hypertension, pulmonary hypertension or valve dysfunction), atheromatous disorders of blood vessels (e.g., coronary artery disease), ischemia, infarction, inflammatory disease and cardiomyopathies or myocarditis.

The terms "generate", "generation", and "generating" as used herein shall be given their ordinary meaning and shall refer to the production of new cells in a subject and optionally the further differentiation into mature, functioning cells. Generation of cells may comprise regeneration of the cells. Generation of cells comprises improving survival, engraftment and/or proliferation of the cells.

The terms "regenerate," "regeneration" and "regenerating" as used herein refer to the process of growing and/or developing new cardiac tissue in a heart or cardiac tissue that has been injured, for example, injured due to ischemia, infarction, reperfusion, or other disease. Tissue regeneration may comprise activation and/or enhancement of cell proliferation. Cardiac tissue regeneration comprises activation and/or enhancement of cell migration.

The term "cell therapy" as used herein refers to the introduction of new cells into a tissue in order to treat a disease and represents a method for repairing or replacing diseased tissue with healthy tissue.

5 The term "derived from" as used herein refers to cells or a biological sample (e.g., blood, tissue, bodily fluids, etc.) and indicates that the cells or the biological sample were obtained from the stated source at some point in time. For example, a cell derived from an individual can represent a primary cell obtained directly from the individual (i.e., unmodified). In some instances, a cell derived from a given source undergoes one or more rounds of cell division and/or cell differentiation such that the original cell no longer exists, but the continuing
10 cell (e.g., daughter cells from all generations) will be understood to be derived from the same source. The term includes directly obtained from, isolated and cultured, or obtained, frozen, and thawed. The term "derived from" may also refer to a component or fragment of a cell obtained from a tissue or cell.

15 The term "isolating" or "isolated" when referring to a cell or a molecule (e.g., nucleic acids or protein) indicates that the cell or molecule is or has been separated from its natural, original or previous environment. For example, an isolated cell can be removed from a tissue derived from its host individual, but can exist in the presence of other cells (e.g., in culture), or be reintroduced into its host individual.

20 The term "culturing" as used herein refers to growing cells or tissue under controlled conditions suitable for survival, generally outside the body (e.g., *ex vivo* or *in vitro*). The term includes "expanding," "passaging," "maintaining," etc. when referring to cell culture of the process of culturing. Culturing cells can result in cell growth, differentiation, and/or division.

25 The term "disaggregating" includes separating, dislodging, or dissociating cells or tissue using mechanical or enzymatic disruption to isolate single cells or small clusters of cells. In some instances, enzymatic disruption can be replaced with one of more enzyme alternatives having substantially the same effect as the enzyme.

30 The term "tissue injury" as used herein refers to damage to a vascularized tissue of an animal or human, wherein the damage is adjacent to, or in close proximity to, a blood vessel that has also undergone injury, and in particular loss of endothelial cells lining the lumen of the blood vessel. For example, but not intended to be limiting, vascular ischemia can result in both loss of vascular endothelial cells to expose the underlying subendothelial matrix. The loss of adequate blood flow can result in loss of cell viability in such as cardiac tissue, brain or neurological tissue that is in contact with the occluded blood vessel.

35 The term "endothelial cell" refers to a cell necessary for the formation and development of new blood vessel from pre-existing vessels (e.g., angiogenesis). Typically, endothelial cells are the thin layer of cells that line the interior surface of blood vessels and lymphatic vessels. Endothelial cells are involved in various aspects of vascular biology,

including atherosclerosis, blood clotting, inflammation, angiogenesis, and control of blood pressure.

The term "smooth muscle cell" refers to a cell comprising non-striated muscle (e.g., smooth muscle). Smooth muscle is present within the walls of blood vessels, lymphatic vessels, cardiac muscle, urinary bladder, uterus, reproductive tracts, gastrointestinal tract, respiratory tract, and iris of the eye.

The term "cardiomyocyte cell" refers to a cell comprising striated muscle of the walls of the heart. Cardiomyocytes can contain one or more nuclei.

The term "cardiosphere" refers to a cluster of cells derived from heart tissue or heart cells. In some instances, a cardiosphere includes cells that express stem cell markers (e.g., c-Kit, Sca-1, and the like) and differentiating cells expressing myocyte proteins and the gap protein (connexin 43).

The term "autologous" refers to deriving from or originating in the same subject or patient. An "autologous transplant" refers to collection (e.g., isolation) and re-transplantation of a subject's own cells or organs. In some instances, an "autologous transplant" includes cells grown or cultured from a subject's own cells. For example, in the methods of the present disclosure, the cardiac stem cells may be derived from a cardiac tissue sample excised from the heart of the patient to be treated, cultured, engineered to be fused with platelet membrane vesicles according to the methods of the disclosure and then administered to the same patient for the treatment of a cardiovascular injury therein.

The term "allogeneic" refers to deriving from or originating in another subject or patient. An "allogeneic transplant" refers to collection (e.g., isolation) and transplantation of the cells or organs from one subject into the body of another. In some instances, an "allogeneic transplant" includes cells grown or cultured from another subject's cells.

The term "transplant" as used herein refers to cells, e.g., cardiac progenitor cells, introduced into a subject. The source of the transplanted material can include cultured cells, cells from another individual, or cells from the same individual (e.g., after the cells are cultured, enriched, or expanded *ex vivo* or *in vitro*).

The terms "treatment," "therapy," "amelioration" and the like refer to any reduction in the severity of symptoms. As used herein, the terms "treat" and "prevent" are not intended to be absolute terms. Treatment can refer to any delay in onset, amelioration of symptoms, improvement in patient survival, repair/regeneration of heart tissue or blood vessels, increase in survival time or rate, etc. The effect of treatment can be compared to an individual or pool of individuals not receiving the treatment. In some instances, the effect can be the same patient prior to treatment or at a different time during the course of therapy. In some aspects, the severity of disease, disorder or injury is reduced by at least 10%, as compared, e.g., to the individual before administration or to a control individual (e.g., healthy individual or an

individual no longer having the disease, disorder or injury) not undergoing treatment. In some instances, the severity of disease, disorder or injury is reduced by at least 20%, 25%, 50%, 75%, 80%, or 90%. In some cases, the symptoms or severity of disease are no longer detectable using standard diagnostic techniques.

The terms "subject," "patient," "individual" and the like are used interchangeably and refer to, except where indicated, mammals such as humans and non-human primates, as well as rabbits, rats, mice, dogs, cats, goats, pigs, cows, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease, but typically refers to an individual under medical supervision.

Abbreviations

PTEN, phosphatase and tensin homolog; NEXO, normal heart donor exosomes; FEXO, heart-failure patient exomes; NRCM, neonatal rat cardiomyocyte; PBS, phosphate-buffered saline; LVEF, left ventricular ejection fraction; MI, myocardial infarction; vWF, von Willebrand factor; CM, cardiomyocyte; CF, cardiac fibroblast (CF); EC, endothelial cell; DMEM, Dulbecco's modified Eagle's medium

Discussion

The present disclosure encompasses methods of regenerating damaged cardiac tissue by delivering to the site of injury a therapeutic composition comprising at least one miRNA or modified miRNA that reduces the expression of PTEN that is over-expressed in normal cardiac stromal cells compared to stromal cells from injured cardiac tissue. The
exosome compositions of the disclosure comprise the miRNA species hsa-miR-21-5p (SEQ ID NO: 1) but may also include a least one other miRNA species over-expressed in normal cardiac stromal cells compared to stromal cells from injured cardiac tissue and which may induce the expression of genes promoting the regeneration of cardiac cells, inhibit the
expression of such genes or induce directly or indirectly the differentiation of cardiac stem cells to provide new and healthy cardiac tissue and/or vascular formation. For example, but not intended to be limiting, the exosome compositions of the disclosure may induce angiogenesis to restore blood flow to damaged heart tissue.

It has been reported that stem cells derived from animals or patients with physiological stresses, such as systemic inflammation, have impaired regenerative activity (Pang *et al.*, (2013) *Eur. Rev. Med. Pharmacol. Sci.* 17:3169-3177; Desouza *et al.*, (2011) *Diabetes* 60: 1286-1294; Hong *et al.*, (2015) *Arthritis Res. Therapy* 17: 292). Since many patients with cardiac diseases, especially heart failure, suffer from these physiological stresses, it was hypothesized that cardiac-cell-secreted exosomes derived from these patients may have
impaired therapeutic activity, or possibly make the condition worse. The treatment effects of cardiac exosomes from healthy donor hearts (normal exosomes, or NEXO) were compared to those of cardiac exosomes from failing hearts (failure exosomes, or FEXO).

Exosomes, as functional paracrine units of therapeutic cells, can partially reproduce the reparative properties of their parental cells. The constitution of exosomes, as well as their biological activity, is largely dependent on the cells which secrete them. Exosomes from explant-derived cardiac stromal cells from heart-failure patients (FEXO), or from normal donor hearts (NEXO) were isolated and their regenerative activities compared *in vitro* and *in vivo*.

FEXO exhibited impaired ability to promote endothelial tube formation and cardiomyocyte proliferation *in vitro*. Intramyocardial injection of NEXO resulted in structural and functional improvements in a murine model of acute myocardial infarction. In contrast, FEXO therapy exacerbated cardiac dysfunction and left ventricular remodeling

MicroRNA array and PCR analysis has shown dysregulation of hsa-miR-21-5p (SEQ ID NO: 1) in exosomes from heart-failure patients. Restoring hsa-miR-21-5p (SEQ ID NO: 1) expression rescued FEXO's reparative function, while blunting hsa-miR-21-5p (SEQ ID NO: 1) expression in NEXO diminished its therapeutic benefits. Other mechanistic studies showed that hsa-miR-21-5p (SEQ ID NO: 1) augmented Akt kinase activity through the inhibition of PTEN. Taken together, the heart failure pathological condition altered the miRNA cargos of cardiac-derived exosomes and impaired their regenerative activities. hsa-miR-21-5p (SEQ ID NO: 1) contributes to exosome-mediated heart repair by enhancing angiogenesis and cardiomyocyte survival through the PTEN/Akt pathway.

Generation and characterization of NEXO and FEXO: Information regarding patients and healthy donors are presented in Table 1.

Table 1: Patient and Healthy Heart donors

Normal Healthy patients				
ID	Sex	Age (years)	Race	Cause of Death
#1	Male	43	Hispanic	Head trauma/motor vehicle accident
#2	Male	18	Hispanic	Head trauma/motor vehicle accident
#3	Male	41	Caucasian	Head trauma/motor vehicle accident
Heart-failure patients				
ID	#1	#2	#3	
Age	44	54	60	
Male/Female	Female	Female	Female	
Weight	78	62	116	
Body Mass index	32.5	26.6	35.6	
Smoker	No	No	Yes	
Diabetes	No	Yes	No	
Hyperlipidemia	Yes	Yes	Yes	
Renal dysfunction	No	No	No	
Hypertension	Yes	Yes	Yes	
Chronic lung disease	No	No	Yes	
Peripheral vascular disease	No	No	No	
Cerebrovascular disease	Yes	No	No	

Myocardial infarction	No	No	No
Coronary artery disease	No	No	Yes
Angina	Yes	No	No
Arrhythmia	Yes	No	Yes
Congestive heart failure	Yes	Yes	Yes
Classification NYHA ^A (I/II/III/IV)	IV	IV	IV
Cardiac surgical procedure	No	No	No

A: NYHA = New York Heart Association

There were no significant differences in age or body mass index between the two groups.

Explant-derived cardiac stromal cells were harvested from the donors' hearts as shown in Figs. 9A and 9B. Flow cytometry revealed that explant-derived cells contain a negligible subpopulation of c-kit⁺ cells and endothelial cells (CD31⁺, CD34⁺), along with a dominating subpopulation that phenotypically resembles mesenchymal cells or fibroblasts (CD90⁺, CD105⁺) (Fig. 10A). NEXO and FEXO were isolated from the 14-day conditioned media of cardiac cells from normal heart donors or heart-failure patients, respectively (Fig. 9A). Cell viability was confirmed at the end of the conditioning period (Figs. 9B and 9C). Cardiac cells from heart-failure patients, and those from healthy heart donors, had comparable exosome yields (Fig. 11A) and exosome sizes (Fig. 11B), as measured by NanoSight nanoparticle tracking analysis. Transmission electron microscopy (TEM) revealed the vesicular morphology of exosomes and confirmed their size did not exceed 200 nm (34) (Fig. 11D). In addition, western blot analysis indicated the presence of signature exosomal markers, such as protein CD81, Alix and TSG101 (Fig. 11C). Both types of exosomes were abundant in RNA, and the amount of RNA contents in NEXO and FEXO was indistinguishable (Fig. 11E).

Effects of NEXO or FEXO on cardiomyocytes, endothelial cells, and cardiac fibroblasts in vitro:

Cardiomyocytes, endothelial cells, and cardiac fibroblasts are the three major cell types in the heart. The uptake efficiency of NEXO and FEXO by those cells was first measured.

Dil-labeled exosomes were readily internalized by neonatal rat cardiomyocytes (NRCMs) (Fig. 1A). There was no significant difference in uptake efficiency between NEXO and FEXO by NRCMs (Fig. 1B).

NEXO significantly promoted cardiomyocyte proliferation, as evidenced by the higher percentage of Ki67-positive nuclei, while treatment with FEXO suppressed cardiomyocyte proliferation (Figs. 1C and 1D). In addition, cardiomyocytes treated with NEXO exhibited fewer TUNEL-positive nuclei, indicating less apoptosis. In contrast, FEXO did not show any anti-apoptotic effects (Figs. 1E and 1F).

To further validate the effects of NEXO and FEXO on adult cardiomyocytes, human cardiomyocytes were cultured on human cardiomyocyte cell culture extracellular matrix, then treated with NEXO or FEXO for 24 hours. NEXO significantly promoted cardiomyocyte proliferation, as indicated by the higher percentage of Ki67, while treatment with FEXO suppressed cardiomyocyte proliferation (Figs. 12A and 12D). This result was supported by

the assessment of two additional proliferation markers: phospho-histone H3 (PH3), a characteristic marker of mitosis, and aurora kinase B (AURKB), a cytokinesis marker, (Figs. 12B-12F). Furthermore, adult cardiomyocytes treated with NEXO exhibited fewer TUNEL-positive nuclei, suggesting less apoptosis. In contrast, FEXO did not show any anti-apoptotic effects (Figs. 12G-12H).

A tube formation assay using HUVECs, was used to evaluate the pro-angiogenic effects of exosomes. Treatment with FEXO inhibited tube formation of HUVECs, while NEXO promoted tube formation, compared to normal saline control (Figs. 1G and H). Alpha-smooth muscle actin (α -SMA) was employed as an indicator of transition from fibroblasts to myofibroblasts. The expression of α -SMA was up-regulated in FEXO-treated cardiac fibroblasts (Figs. 1I and 1J), suggesting a phenotype transition to myofibroblasts. Taken together, the data indicate that cardiac cells in the failing heart secreted exosomes with an impaired ability to promote cardiomyocyte proliferation, decrease programmed cell death, and stimulate angiogenesis *in vitro*. Moreover, such diseased exosomes drive the differentiation of fibroblasts to myofibroblasts.

Effects of NEXO and FEXO therapy on cardiac function and morphometry in a mouse model of acute MI: An acute myocardial infarction (MI) was induced in CD1 mice by coronary vessel ligation, then intramyocardially injected NEXO, FEXO, or PBS into the MI border zone. Left ventricular ejection fraction (LVEF) was used to assess the enhancement or preservation of heart function.

There was no significant difference in LVEF between the three treatment groups at baseline (Fig. 2A). LVEF progressively decreased in the control group over the next 3 weeks, whereas hearts injected with NEXO showed greater LVEFs, compared to those treated with FEXO or PBS control. FEXO-treated hearts exhibited a worsening of LVEFs when compared to PBS-treated control hearts (Fig. 2B).

To facilitate head-to-head comparisons, treatment effect was evaluated by calculating the changes in endpoint LVEF relative to baseline LVEF. Control treatment had a negative effect, as LVEF declined over time, while NEXO treatment robustly prevented the decline of LVEF post-MI (Fig. 2C). Likewise, the animals that received NEXO treatment showed a recovery in fractional shortening (FS), whereas the ones injected with FEXO, suffered from FS deterioration (Fig. 13A). These results demonstrate that exosomes from heart-failure patients have reduced reparative activity, and may exacerbate LV dysfunction post-MI. At the histological level, Masson's trichrome staining was performed to simultaneously explore infarcted and viable cardiac tissues (Fig. 2D).

NEXO-treated hearts exhibited decreased infarct size (Fig. 2E), increased infarcted wall thickness (Fig. 2F), and increased viable tissue (Fig. 2G) compared to FEXO and PBS controls. Likewise, injured hearts treated with NEXO displayed less chamber dilation (Figs.

13B and 13C) and smaller infarct circumference (Fig. 13D) when compared to control or FEXO treated hearts. These data indicated that exosomes from healthy hearts attenuated LV remodeling after MI, while exosomes from failing hearts exacerbate it.

Effects of NEXO and FEXO treatment on post-MI heart angiomyogenesis and apoptosis: It

has been well established that adult stem cells exert their therapeutic benefits through indirect regenerative mechanisms. The secretome from the injected cells enhances cardiomyocyte proliferation, promotes neovascularization, and inhibits cardiomyocyte apoptosis (Chimenti *et al.*, (2010) *Circ. Res.* 106: 971-980; Gnecci *et al.*, (2005) *Nat. Med* 11: 367). Three weeks after MI and exosome injections, the percentage of cycling cardiomyocytes (Ki67⁺/a-SA⁺, Figs. 3A and 3D) in the peri-infarct zone of NEXO-treated hearts was significantly higher than in those that received FEXO or PBS control injections. This was further confirmed by mitosis marker PH3 and cytokinesis marker AURKB (Figs. 14A-14C). A similar trend was detected in capillary density (vWF⁺ capillaries, Figs. 3B and 3E), indicating the proangiogenic role of NEXO but not of FEXO. Hearts injected with NEXO displayed significantly fewer apoptotic cardiomyocytes (TUNEL⁺/a-SA⁺, Figs. 3C and F), suggesting NEXO therapy led to tissue preservation. Overall, FEXO treatment is detrimental to post-MI healing, as it inhibits angiogenesis and cardiomyocyte proliferation.

Dysregulation of hsa-miR-21-5p (SEQ ID NO: 1) in heart failure exosomes: Several studies imply that the mechanism of exosome-mediated repair involves exosomal microRNAs, which target specific signaling pathways in the recipient cells (e.g. injured cardiomyocytes) (Ibrahim *et al.*, (2014) *Stem Cell Report.* 2: 606-619; Barile *et al.*, (2014) *Cardiovascular Res.* 103: 530-541). It was possible, therefore, that the functional impairment of FEXO might result from altered miRNA cargos.

The miRNA components of NEXO to that of FEXO were compared using PCR microarrays for 84 well-reported miRNAs (Figs. 15A and 15B). Twenty-three miRNAs were differentially expressed in the two groups of exosomes. Among those, hsa-miR-21-5p (SEQ ID NO: 1) was the most dysregulated in FEXO, as compared to the expression in NEXO (Figs. 4A and 4B). qRT-PCR was then performed to verify the results. hsa-miR-21-5p (SEQ ID NO: 1), but not hsa-miR-21-3p (SEQ ID NO: 9), was dysregulated in FEXO (Figs. 4C). The myocardial tissue levels of hsa-miR-21-5p (SEQ ID NO: 1) were decreased in the post-MI hearts injected with FEXO (Fig. 15C), as compared to the ones injected with NEXO.

The role of hsa-miR-21-5p (SEQ ID NO: 1) in exosome-mediated heart repair: Various gain- and loss-of-function studies were designed to verify the role of hsa-miR-21-5p (SEQ ID NO: 1) in exosome-mediated, post-MI repair. The silencing of exosomal hsa-miR-21-5p (SEQ ID NO: 1) by transfecting healthy cardiac cells with anti-miR-21-5p (SEQ ID NO: 1) oligo (NEXO+anti-miR-21) was confirmed by qRT-PCR on the exosome products (Figs. 16A and 16C). Knocking down hsa-miR-21-5p (SEQ ID NO: 1) in NEXO led to impaired anti-apoptotic

effects of NEXO on cultured H9C2 cells (Figs. 5A and B). In contrast to the control NEXO, NEXO+anti-miR-21 lost the ability to promote tube formation (Figs. 5C and D).

miR-21-rescued exosomes were also engineered by transfecting heart failure cardiac cells with hsa-miR-21 oligo. These cells were engineered to produce exosomes with hsa-miR-21-5p (SEQ ID NO: 1) over-expression (FEXO+miR-21) (Figs. 16A and 16B). As expected, upregulation of hsa-miR-21-5p (SEQ ID NO: 1) in FEXO rescued their ability to inhibit apoptosis (Figs. 5E and 5F) and promote tube formation (Figs. 5G and H). To further validate the functions of hsa-miR-21 in adult cardiomyocytes, adult human cardiomyocytes were transfected with hsa-miR-21-mimic or scrambled control. Over-expression of hsa-miR-21 led to improved survival of adult cardiomyocytes, as indicated by less apoptotic cells (TUNEL-positive nuclei) (Figs. 17A and 17B).

To confirm those results *in vivo*, the same mouse MI model was used to test the therapeutic effects of FEXO+miR-21 and NEXO+anti-miR-21. Mice injected with NEXO+anti-miR-21 exhibited decreased pump function (Figs. 6A and 6B), increased scar mass, and decreased viable heart tissue, compared to the hearts injected with NEXO+miR-scr (Figs. 6C-6G). In contrast, hsa-miR-21-5p (SEQ ID NO: 1) enhancement rescued the therapeutic functions of heart failure exosomes. FEXO+miR-21 treatment led to improvement in cardiac function (Figs. 6A and 6B) and attenuation of LV remodeling (Figs. 6D-6G).

Histological analysis showed that hsa-miR-21-5p (SEQ ID NO: 1) inhibition abolished the ability of NEXO therapy to promote angiogenesis (Figs. 7A and 7B) and inhibit apoptosis (Figs. 7C and 7D). Vice versa, restoring hsa-miR-21-5p (SEQ ID NO: 1) expression in FEXO rescued those paracrine effects (Figs. 7E-7H). Moreover, the pro-angiogenic effects of hsa-miR-21-5p (SEQ ID NO: 1) was confirmed by the increased number of functional vessels.

The functionality of a blood vessel was confirmed by co-staining the endothelial cell marker, Von Willebrand factor (vWF), and the red blood cell (RBC) marker. As shown in Fig. 18, RBCs were detected in the blood vessels in the MI border zone of the hearts treated with hsa-miR-21-over-expressed FEXO. Through those gain- and loss-of-function studies, the pivotal role of hsa-miR-21-5p (SEQ ID NO: 1) in exosome-mediated heart repair was shown, possibly through pro-angiogenic and anti-apoptotic mechanisms.

Alongside with hsa-miR-21 such as, but not limited to, let-7b-5p (SEQ ID NO: 2), miR125a-5p (SEQ ID NO: 3), and hsa-miR-146a-5p (SEQ ID NO: 4) were examined for their effects on cardiomyocytes, cardiac fibroblasts, and endothelial cells. The results indicated that hsa-miR-146a inhibited apoptosis in cardiomyocyte but promoted tube formation in endothelial cells (Fig. 19). This was consistent with previous studies (Ibrahim *et al.*, (2014) *Stem Cell Report.* 2: 606-619). In contrast, neither let-7b-5p (SEQ ID NO: 2) nor hsa-miR-125a-5p (SEQ ID NO: 3) showed beneficial effects on cardiomyocyte survival or

endothelial tube formation (Figs. 19A-19G).

Effects of hsa-miR-21-5p (SEQ ID NO: 1) on PTEN/Akt signaling: It has been established that hsa-miR-21-5p (SEQ ID NO: 1) promotes cancer cell proliferation, angiogenesis, migration, and invasion by targeting pathways involving phosphatase and tensin homolog (PTEN),
 5 Programmed Cell Death 4 (PDCD4), Forkhead Box O1 (FOXO1), SMAD Family Member 7 (SMAD7), Tumor necrosis factor- α induced protein-8-like 2 (TIPE2), Sprouty2 (SPRY2) (Melnik, B.C. (2015) *J. Translational Med.* 13: 202). Recent studies have also shown that PTEN and SPRY1 are targeted by hsa-miR-21-5p (SEQ ID NO: 1) in cardiovascular diseases (Thum *et al.* (2008) *Nature* 456: 980-984; Wang *et al.*, (2017) *Stem Cells Translational Med.* 6:
 10 209-222). To identify the potential target genes of hsa-miR-21 on three major cell types in the heart, cardiomyocytes (using human induced-pluripotent stem cell-derived cardiomyocytes (iCM) and H9C2 cells), cardiac fibroblasts (using human cardiac fibroblasts, CF), and endothelial cells (using human umbilical vein endothelial cells, HUVECs) were incubated with hsa-miR-21 mimic or scrambled control. Eighteen phosphorylated proteins were then
 15 examined with a signaling pathway phosphorylation array. The signaling pathway array pointed at PTEN, AKT, and BAD as the hsa-miR-21-regulated genes in cardiomyocytes and endothelial cells, but not in cardiac fibroblasts (Figs. 20A-20C).

Western blot studies further confirmed that PTEN expression was significantly attenuated in human cardiomyocytes (CMs) and HUVECs treated with hsa-miR-21 mimics
 20 (Figs. 8A and 8B). This coincides with enhanced Akt phosphorylation, as well as the alteration of other downstream targets such as caspase-3 and Bcl-2 (Figs. 8A and 8C-8E). Upregulation of hsa-miR-21 in cardiomyocytes and HUVECs also promoted PCNA (a proliferation marker) and vascular endothelial growth factor (VEGF) expressions (Figs. 8F-8H), which are consistent with the data in Figs. 5A-5H showing the pro-angiogenic effects of hsa-miR-21.

25 Previous studies indicated that programmed cell death 4 (PDCD4), a pro-apoptotic protein, is a known hsa-miR-21 target (Khan *et al.*, (2015) *Circulation Res.* 115: 305990), so PDCD4 expressions was checked in CMs, HCFs, and HUVECs treated with hsa-miR-21 mimics. Western blot analysis showed reduced PDCD4 expression in CMs only, but not in HCFs or HUVECs (Figs. 8F and 8I).

30 Taken together, the data indicate that hsa-miR-21 inhibits cardiomyocyte apoptosis by targeting PDCD4, promotes angiogenesis by activating PTEN/Akt signaling, and promotes the expression of VEGF in endothelial cells. Since the flow cytometry showed a significant proportion of mesenchymal cells or fibroblasts (CD90+, CD105+) in the cardiac cells (Figs. 10A and 10B), it is possible that those cardiac cells secreted exosomes containing hsa-miR-21
 35 to communicate with nearby cardiomyocytes and endothelial cells under physiological and heart failure conditions.

The results show that exosomes derived from heart-failure patients have impaired

therapeutic potency in an animal model of myocardial infarction and are incapable of promoting angiomyogenesis *in vitro* and *in vivo*. MicroRNA array and a series of gain- and loss-of-function experiments revealed that the impairment of regenerative activities of heart failure exosomes is related to dysregulation of hsa-miR-21-5p (SEQ ID NO: 1) (Fig. 8J).

5 Cell therapy has emerged as a promising therapeutic option for cardiac regeneration and protection. As a major paracrine component, exosomes have been revealed to play a vital role in tissue repair by packaging and delivering RNAs and proteins. It has been reported that injured mouse hearts treated with cardiac stem cell-secreted exosomes exhibited regenerative and functional improvements produced by the stem cells themselves (Gallet *et al.*, (2016) *Euro. Heart J.* 38: 201-211; Ibrahim *et al.*, (2014) *Stem Cell Report.* 2: 606-619).
10 Exosomes derived from mouse embryonic stem cells promote neovascularization and cardiomyocyte survival, and reduce fibrosis after myocardial infarction, consistent with the resurgence of the cardiac proliferative response produced by embryonic stem cells (Kervadec *et al.*, (2016) *J. Heart Lung Transplantation* 35: 795-807). However, most of these exosome
15 products are derived from young and healthy donors. Since most patients with cardiac diseases, especially heart failure, suffer from systemic inflammation and are of old age, it is possible that heart failure modifies exosome contents and further compromises their reparative properties. However, now it is found that exosomes derived from the cardiac cells of heart-failure patients lose their pro-angiogenic drive of HUVEC tube formations and fail to
20 promote cardiomyocyte proliferation in injured mouse hearts, suggesting a dysfunction of heart failure exosomes in ischemic tissue repair. The finding can partly explain the compromised therapeutic effects in clinical trials using autologous stem cells. The advanced cardiomyopathy condition modifies exosome contents and renders the stem cells ineffective in therapeutic repair.

25 The mechanisms that drive exosome-mediated repair rely on the transfer of specific donor cell microRNAs and proteins to recipient cells (Dhanabal *et al.*, (2005) *Cancer Biol. Therapy* 4: 659-668). MicroRNA array analysis was performed to compare exosomes secreted from heart-failure patients with those from healthy donors. Exosomal hsa-miR-21-5p (SEQ ID NO: 1), secreted from healthy cardiac cells, can be delivered to recipient cardiac cells
30 to regulate apoptosis and angiogenesis, and improve heart function in a mouse MI model. The silencing of hsa-miR-21-5p (SEQ ID NO: 1) in normal exosomes (NEXO) eliminated these benefits, and the restoration of hsa-miR-21-5p (SEQ ID NO: 1) in heart failure exosomes (FEXO) rescued their therapeutic potency, further supporting the essential role of hsa-miR-21-5p (SEQ ID NO: 1) in exosome-mediated heart repair.

35 Although the studies focused on hsa-miR-21-5p (SEQ ID NO: 1), there are several other miRNAs dysregulated in FEXO, such as hsa-miR-146a, let-7b, hsa-miR-125a, and hsa-miR-23. Exosomal hsa-miR-146a leads to beneficial effects in post MI hearts by targeting

Irak-1 and Traf6, both associated with the toll-like receptor signaling pathway (Ibrahim *et al.*, (2014) *Stem Cell Report.* 2: 606-619). It has also been shown that hsa-miR-23 can promote angiogenesis in cardiac endothelial cells by activating proangiogenic signaling through the inhibition of Sprouty2 and Sema6A (Impagnatiello *et al.*, (2001) *J. Cell Biol.* 152: 1087-1098).

5 The results in the present disclosure indicate that hsa-miR-146a inhibited apoptosis in cardiomyocytes but promoted tube formation in endothelial cells, which is consistent with previous studies, while neither let-7b-5p (SEQ ID NO: 2) nor hsa-miR-125a-5p (SEQ ID NO: 3) showed beneficial effects on cardiomyocyte survival or endothelial tube formation.

miR-21 expression has been associated with various disorders (Thum *et al.* (2008) *Nature* 456: 980-984, Thum *et al.*, (2008) *Nature* 456: 980; Bang *et al.*, (2014) *J. Clinical Invest.* 124: 2136-2146; Patrick *et al.*, (2010) *J. Clinical Invest.* 120: 3912-3916.). However, the precise functional role of hsa-miR-21 in cardiac diseases is still controversial. Thomas *et al.* reported that hsa-miR-21 expression is highly upregulated by cardiac stress, leading to activation of ERK/MAP kinase signaling through inhibition of Spry1, which in turn promoted cardiac fibrosis and heart dysfunction (Thum *et al.* (2008) *Nature* 456: 980-984). hsa-miR-21 also reportedly induced cardiac fibrosis by inhibiting PTEN expression in cardiac fibroblasts at the infarct zone after myocardial ischemia-reperfusion (Roy *et al.*, (2009) *Cardiovascular Res.* 82: 21-29). However, Patrick *et al.* reported that miR21 was not essential for cardiac fibrosis, as hsa-miR-21-null mice still display cardiac hypertrophy, fibrosis, and the loss of cardiac contractility (Patrick *et al.*, (2010) *J. Clinical Invest.* 120: 3912-3916).

In the present study, hsa-miR-21 did not affect the survival of cardiac fibroblasts (Fig. 19G). Moreover, hsa-miR-21 regulated the PTEN/Akt signaling pathway in cardiomyocytes and endothelial cells instead of fibroblasts (Figs. 8A-8J), indicating that hsa-miR-21 does not promote cardiac fibrosis, as reported in previous studies.

25 Exosomal hsa-miR-21, secreted by induced pluripotent stem cells (iPSCs), promoted angiogenesis in transfected bronchial epithelial cells (Xu *et al.*, (2015) *Arch. Toxicol.* 89: 1071-1082). Kristin *et al.* recently reported that hsa-miR-21a-5p is the most abundant miRNA in murine mesenchymal stem cell (MSC) exosomes, and that it contributes to myocardial salvage via synergistic anti-apoptotic activity. hsa-miR-21-5p (SEQ ID NO: 1) plays a crucial role in the MSC-exosome-mediated effects on cardiac contractility through PI3K signaling (Mayourian *et al.*, (2018) *Circulation Res.* 122: 933-944).

A unique aspect of the methods and compositions of the present disclosure is the cell source. While therapeutic MSCs can be readily derived from bone marrow and adipose tissues, these cells do not naturally reside in the heart. Focusing on intrinsic cardiac cells in failing or normal hearts demonstrated that heart failure pathology leads to a severe dysregulation of hsa-miR-21-5p (SEQ ID NO: 1) in intrinsic cardiac cells, which further leads to the impaired regenerative function of the intrinsic heart exosomes. Therefore, the present

findings demonstrate that hsa-miR-21 not only plays a key role in therapeutic interventions (e.g. in the case of MSCs), but also represents a crucial component in the naturally-occurring repair process post heart injury.

The regulation of PTEN by hsa-miR-21 was reported in carcinoma (Meng *et al.*, (2007) *Gastroenterology* 133: 647-658) and cardiovascular cells (Roy *et al.*, (2009) *Cardiovascular Res.* 82: 21-29). Consistent with this, upregulation of hsa-miR-21-5p (SEQ ID NO: 1) in exosomes augmented phosphorylation of Akt via the inhibition of PTEN in the recipient cells. These effects were eliminated by the knockdown of hsa-miR-21-5p (SEQ ID NO: 1), indicating a possible molecular mechanism for the positive role of hsa-miR-21-5p (SEQ ID NO: 1) in post-MI repair. Based on the flow cytometry data, cardiac stromal cells/fibroblasts might be the main source of exosomes with altered hsa-miR-21.

It has been shown that strong hsa-miR-21 expression occurs primarily in cardiac fibroblasts but was low in cardiomyocytes. This is consistent with the present findings. There is every probability that cardiac fibroblasts released exosomal hsa-miR-21 to support survival of neighboring cardiomyocytes and endothelial cells under physiological conditions.

These findings have important clinical implications. Unlike cell-based therapy products, exosomes can offer an off-the-shelf and universal therapeutic option. The cryo-preservation of exosomes normally does not require the sophisticated steps associated with cellular products. In addition, injection of allogeneic or even xenogeneic exosomes is well tolerated by the host immune system (Vandergriff *et al.*, (2018) *Theranostics* 8: 1869-1878; Vandergriff *et al.*, (2015) *Stem Cells Int.* 2015: 960926). The present findings indicate that exosomes from heart-failure patients carry significantly altered miRNA signatures compared to those from healthy heart cells and are functionally deficient in their ischemic myocardial repair capabilities. This may explain why patients' autologous cell therapies only show modest therapeutic effects in clinical trials. More importantly, the present data showed that the dysfunction of heart failure exosomes can be rescued by modulation of a specific miRNA in the exosomes. The study provides new mechanistic insights into the therapeutic potential of exosomes and new strategies to rescue patients' defective exosomes through modulation of specific miRNA cargos.

One aspect of the disclosure, therefore, encompasses a composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes, wherein the exosomes comprise an miRNA species can have at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) or a heterologous nucleic acid that expresses the miRNA species when in recipient cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure, the composition can further comprise a pharmaceutically acceptable carrier.

In some embodiments of this aspect of the disclosure, the composition can be formulated for delivery to a site of cardiac injury.

In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart tissue.

In some embodiments of this aspect of the disclosure, at least one of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure, each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

Another aspect of the disclosure encompasses embodiments of a method of modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject, the method comprising administering to a cardiac tissue of an animal or human subject a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes can comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species.

In some embodiments of this aspect of the disclosure, the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart tissue.

In some embodiments of this aspect of the disclosure, at least one of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure, each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence

similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a heterologous nucleic acid that expresses at least one miRNA species when in recipient cardiac tissue.

In some embodiments of this aspect of the disclosure the composition can be administered to the cardiac tissue of the animal or human subject by a percutaneous method.

In some embodiments of this aspect of the disclosure the percutaneous method is by injection into the cardiac tissue.

Yet another aspect of the disclosure encompasses embodiments of a method of repairing cardiac tissue damage, the method comprising administering to a cardiac tissue of an animal or human subject a therapeutic composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a plurality of miRNA species expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue.

In some embodiments of this aspect of the disclosure at least some of the plurality of miRNA species can be over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

In some embodiments of this aspect of the disclosure each miRNA species of the plurality of miRNA species can be selected from and can have at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID

NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

In some embodiments of this aspect of the disclosure the population of cardiac stromal cell/fibroblast-derived exosomes can further comprise a heterologous nucleic acid that expresses at least one miRNA species when in recipient cardiac tissue.

In some embodiments of this aspect of the disclosure the therapeutic composition can be administered to the cardiac tissue of the animal or human subject by a percutaneous method.

In some embodiments of this aspect of the disclosure the percutaneous method is by injection into the cardiac tissue.

As mentioned above, compounds of the present disclosure and pharmaceutical compositions can be used in combination of one or more other therapeutic agents for treating viral infection and other diseases. For example, compounds of the present disclosure and pharmaceutical compositions provided herein can be employed in combination with other anti-viral agents to treat viral infection.

While embodiments of the present disclosure are described in connection with the Examples and the corresponding text and figures, there is no intent to limit the disclosure to the embodiments in these descriptions. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

EXAMPLES

Example 1

Cell Culture and Exosome Isolation: Diseased heart tissues were received from patients who underwent heart transplantation at UNC Hospital after heart failure. Healthy heart tissues were received from normal, non-damaged, human donor hearts. Cardiac cells were separated and cultured as previously described (Makkar *et al.*, (2012) *Lancet* 379: 895-904). In brief, myocardial tissues harvested from donors' hearts were separated and washed with PBS, then cut into smaller 2 mm³ fragments, and partially enzymatically digested at 37°C with collagenase type IV (C1889, Sigma-Aldrich). The tissue samples were plated onto fibronectin-coated petri dishes in Iscove's modified Dulbecco's medium (IMDM, Gibco) infused with 20% fetal bovine serum (FBS; Corning). After 3-5 days, cardiac cells began to grow from the explants. Exosomes were isolated from the conditioned medium of these cardiac cells. Passage 1-3 cardiac cells were cultured to 80% confluency. The medium was then switched

to serum-free IMDM and conditioned for 14 days. Then, exosomes were isolated from the conditioned medium by ultrafiltration, as previously described (Vandergriff *et al.*, (2018) *Theranostics* 8: 1869-1878; Vandergriff *et al.*, (2015) *Stem Cells Int.* 2015: 960926). In brief, conditioned medium was filtered through 0.22 μ m Steriflip filters to remove cellular debris and large vesicles. The filtrate was then added to Amicon Ultra-15 100 kDa filters (SCGP00525, Millipore) to centrifuge at 5,000 $\times$ g for 5min. The flow-through was discarded. The concentrated exosomes were collected and washed with PBS three times, and stored at – 80 °C. Labeling was performed using 10 μ M Dil (V22889, Thermo Fisher Scientific)

Example 2

Flow Cytometry: Flow cytometry was performed to examine the antigenic phenotypes of cardiac cells. Cells were incubated with antibodies against CD90 (555595, BD), CD105 (ab11414, Abcam), CD31 (555445, BD), CD 34 (ab81289, Abcam), CD 45 (555482, BD), and c-kit (550412, BD) for 60 mins at 4 °C. Both unstained and isotype controls (555748, 559320, BD) were used as negative controls. Flow cytometry was conducted with a CytoFlex Flow Cytometer (Beckman Coulter) and data were analyzed with FCS Express software (De Novo).

Example 3

Exosome Characterization: Nanoparticle tracking analysis (NanoSight NS300, Malvern) was used to measure the concentration and size of exosomes. Each sample was imaged five times for 60 secs and analyzed. Transmission electron microscopy was used to assess the morphology of exosomes. In preparation for TEM, exosomes were fixed with 4% PFA and 1% glutaraldehyde. They were stored at room temperature. Microscopy was conducted with a transmission electron microscope (JEM-2000FX, JEOL).

Example 4

Immunocytochemistry: Neonatal rat cardiomyocytes (NRCMs) and cardiac fibroblasts were isolated and cultured as previously described (Vandergriff *et al.*, (2015) *J. Visualized Expts* 98: 52726). The H9C2 cell line was purchased from Sigma-Aldrich (88092904, Sigma-Aldrich). Adult human cardiomyocytes were purchased from Celprogen and cultured on human cardiomyocyte cell culture extracellular matrix (36044-15, Celprogen). Human induced pluripotent stem cell-derived cardiomyocytes (iCM) were kindly provided by Dr. Freytes (UNC/NC State Joint Department of Biomedical Engineering, Chapel Hill/Raleigh). After 3-days of cultivation, the culture medium was supplemented with 7×10^8 exosomes or PBS for an additional 24 hours. They were fixed with 4% paraformaldehyde (PFA) and blocked with Protein Block Solution (DAKO) with 1% saponin (Sigma-Aldrich). Subsequently, the cells were stained with anti- α -sarcomeric actin (α -SA, a7811, Sigma-Aldrich), anti-ki67 (ab15580, Abcam), anti- α -smooth muscle actin (ab5694, Abcam), or anti-von Willebrand factor (vWF, ab6994, Abcam) antibodies. Flour 488 or Texas-Red conjugated secondary antibodies (ab150117, ab6787, ab6719, ab150077, Abcam) were used for detection. Sections treated

with terminal deoxynucleotidyl transferase nick end labeling (TUNEL, 12156792910, Roche) were incubated for 30 minutes after the secondary antibody incubation. Cell nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI). Images were taken by a fluorescent microscope (Olympus IX81; Olympus).

5 **Example 5**

Angiogenesis Assay: Human umbilical vein endothelial cells (HUVECs) were co-incubated with 1.5×10^8 exosomes for 24 hours, then plated on growth factor-deprived Matrigel (356230, Corning) to evaluate angiogenesis (Manoussaki *et al.*, (1996) *Acta Biotheoretica* 44: 271-282). Eight hours later, tube formation was examined with a white light microscope and analyzed with NIH Image J software.

10 **Example 6**

miR-21-5p (SEQ ID NO: 1) transfection: hsa-miR-21-5p (SEQ ID NO: 1) oligo, anti-miR-21-5p (SEQ ID NO: 1) or scrambled miRNA oligo transfection was performed according to the XMIR exosome RNA packaging protocol (XMIR-21, AXMIR-21, XMIR-POS, SBI System Biosciences) with slight modifications. Briefly, cardiac cells were cultured to 70% confluency. hsa-miR-21-5p (SEQ ID NO: 1), anti-miR-21-5p (SEQ ID NO: 1), or scrambled miRNA oligo was mixed with a transfection reagent, then added to the cell culture at a final concentration of 20 nM. Cells were returned to the incubator for exosome production for 24 hours. Culture media was collected, and exosomes were isolated as previously described. Transfection efficiency was determined by performing qRT-PCR on exosomes derived from the transfected cells. Hs_miR-21_2 miScript Primer Assay (MS00009079, Qiagen) was performed according to the manufacturer's protocol. qRT-PCR was performed using Roche Light Cycler 480 Instrument II (Roche) and relative exosome abundance levels were calculated using the delta-delta Ct method. hsa-miR-16 (MS00031493, Qiagen) was used as a reference control.

25 **Example 7**

Animal Studies: The acute myocardial infarction (MI) model was created as previously described (Luo *et al.*, (2017) *Circ. Res.* 120: 1768-1775; Tang *et al.*, (2018) *Nat. Biomed. Engineer.* 2: 17-26; Tang *et al.*, (2017) *ACS Nano* 11: 9738-9749; Tang *et al.*, (2017) *Nat. Commun.* 8: 13724). Sixty 6-week-old female CD-1 mice (Charles River Labs, Wilmington, MA) underwent left thoracotomy under general anesthesia. The left anterior descending (LAD) coronary artery was permanently ligated. Subsequently, intramyocardial injections were performed at four sites in the peri-infarct zone. All mice were randomly assigned to the following groups. (1) NEXO: intramyocardial injection of 50 μ l PBS containing 30×10^9 NEXO; (2) FEXO: injection of 50 μ l PBS containing 30×10^9 FEXO; (3) control: injection of 50 μ l PBS; and (4) Sham: mice with sham surgery underwent the same procedures except for the permanent ligation.

To validate the role of hsa-miR-21-5p (SEQ ID NO: 1) in exosome-mediated heart repair, we created the same MI models in mice. hsa-miR-21-5p (SEQ ID NO: 1)-deficient exosomes were engineered by transfecting healthy cardiac cells with anti-miR-21-5p (SEQ ID NO: 1) oligo (NEXO+anti-miR-21). We also produced hsa-miR-21-rescued exosomes by transfecting heart failure cardiac cells with hsa-miR-21 RNA oligo (FEXO+miR-21) to harvest exosomes with hsa-miR-21-5p (SEQ ID NO: 1) over-expression. Scrambled miRNA oligo was used as a control (NEXO/FEXO+miR-scr): (1) NEXO+anti-miR-21: injection of 50µl PBS containing 30 x 10⁹ NEXO+anti-miR-21; (2) NEXO+miR-scr: injection of 50µl PBS containing 30 x 10⁹ NEXO+miR-scr; (3) FEXO+miR-21: injection of 50µl PBS containing 30 x 10⁹ FEXO+miR-21; (4) FEXO+miR-scr: injection of 50µl PBS containing 30 x 10⁹ FEXO+miR-scr.

Example 8

Heart Function Assessment: Cardiac function was measured by blinded echocardiography analysis using a Philips Cx-70 Ultrasound System with an L15-7io high-frequency probe. Each measurement was performed 3 times. Long-axis views were measured at the greatest left ventricular diameter. Left ventricular ejection fraction was measured from views taken through the infarcted area.

Example 9

Heart Morphometry: Animals were sacrificed 3 weeks after treatments. Six cryo-sections from each heart, collected at 400-µm intervals, were stained with Masson's trichrome. Infarct size, infarct circumference, infarct wall thickness, and viable tissue in the risk area were measured with NIH Image J software, as previously described (Luo *et al.*, (2017) *Circ. Res.* 120: 1768-1775; Tang. *et al.*, (2018) *Nat. Biomed. Engineer.* 2: 17-26; Tang *et al.*, (2017) *ACS Nano* 11: 9738-9749; Tang *et al.*, (2017) *Nat. Commun.* 8: 13724).

Example 10

Antibody Array and Western Blot Analysis: Adult human cardiomyocytes (CM, 36044-15, Celprogen), human induced pluripotent stem cell-derived cardiomyocytes (iCM), H9C2 cells (88092904, Sigma), human cardiac fibroblasts (HCF, 306-05A, Sigma), and human umbilical vein endothelial cells (HUVECs, PCS-100-010, ATCC) were transfected with hsa-miR-21-5p (SEQ ID NO: 1) mimic or scrambled control (miScript miRNA Mimic, 219600, Qiagen), according to the manufacturer's protocol with slight modifications. Briefly, cells were cultured to 70% confluency. hsa-miR-21-5p (SEQ ID NO: 1) mimic or scrambled control was mixed with HiPerFect Transfection Reagent and culture medium without serum, at a final concentration of 5 nM. Cells were returned to the incubator for 48 hours. Cells were collected, lysed by RIPA Lysis Buffer (89900, Thermo Fisher Scientific), and centrifuged at 14,000 × g for 15 minutes to pellet the cell debris. The protein concentration was quantified with a BCA Protein Assay (23227, Thermo Fisher Scientific). Protein from transfected iCM, H9C2, HCF, and HUVEC cells was used on the signaling

pathway phosphorylation array (AAH-AKT-1-2, RayBiotech). The protein array assay was performed according to the manufacturer's protocol with slight modification. Briefly, the antibody membranes were blocked for 30 minutes at RT. The samples were then added and incubated overnight at 4°C. The detection antibody cocktail was prepared, pipetted onto the membranes, and incubated overnight at 4°C. Then, the samples were incubated with HRP-anti-rabbit IgG and visualized with the ChemiDoc Touch Imaging System (ChemiDoc, Bio-Rad). Quantitative analysis was performed with ImageJ or Image Lab software. Protein from transfected CM, H9C2, HCF and HUVEC cells was used on western blot analysis. The equivalent of 25 mg of total protein per lane was loaded onto Mini-PROTEAN TGX Stain-Free Protein Gels (4568083, Bio-RAD) and transferred to Immun-Blot® PVDF Membranes (1620177, Bio-RAD). Membranes were blocked with 5% blocking buffer for 1 hour and incubated with primary antibodies against PTEN (ab31392, Abcam), p-akt (phospho T308) (ab38449, Abcam), t-akt (ab8805, Abcam), BCL-2 (ab59348, Abcam), caspase-3 (ab13847, Abcam), PDCD4 (ab51495, Abcam), PCNA (ab29, Abcam), or VEGF (PA5-16754, Thermo Fisher Scientific) overnight, at 4°C on a rocker. Subsequently, the samples were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 hour on a shaker. The blots were exposed with Clarity Western ECL Blotting Substrates (1705061, Bio-Rad) and visualized with the ChemiDoc Touch Imaging System (ChemiDoc, Bio-Rad). Quantitative analysis was performed with ImageJ or Image Lab software, and expressions were normalized to GAPDH.

Example 11

Statistics: GraphPad Prism (GraphPad Software, La Jolla, CA) was used for statistical analysis. Results were presented as mean $\pm$ SD. All comparisons between two groups were performed with two-tailed unpaired Student's t-test. One-way ANOVA analysis, with post hoc Bonferroni correction, was used to compare means among more than two groups. Differences were considered statistically significant when $p < 0.05$.

Example 12

Table 2

miRNA	Accession Number ^a	Sequence
hsa-miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA (SEQ ID NO: 1)
hsa-let-7b-5p	MIMAT0000063	UGAGGUAGUAGGUUGUGUGGUU (SEQ ID NO: 2)
hsa-miR-125a-5p	MIMAT0000443	UCCCUGAGACCCUUAACCUUGUGA (SEQ ID NO: 3)
hsa-miR-146a-5p	MIMAT0000449	UGAGAACUGAAUCCAUGGGUU (SEQ ID NO: 4)
hsa-miR-125b-5p	MIMAT0000423	UCCCUGAGACCCUUAACCUUGUGA (SEQ ID NO: 5)
hsa-miR-126-3p	MIMAT0000445	UCGUACCGUGAGUAAUAAUGCG

		(SEQ ID NO: 6)
hsa-miR-16-1-3p	MIMAT0004489	CCAGUAUUAACUGUGCUGCUGA (SEQ ID NO: 7)
hsa-miR-23a-5p	MIMAT0004496	GGGUUCCUGGGGAUGGGAUUU (SEQ ID NO: 8)
hsa-miR-21-3p	MIMAT0004494	CAACACCAGUCGAUGGGCUGU (SEQ ID NO: 9)
hsa-miR-26a-5p	MIMAT0000082	UUCAAGUAAUCCAGGAUAGGCU (SEQ ID NO: 10)
hsa-mir-320a	MI0000542	CUCCCCUCCGCCUUCUCUCCCGGU UCUCCCCGGAGUCGGGAAAAGCUG GGUUGAGAGGGCGAAAAAGGAUG (SEQ ID NO: 11)
hsa-miR-29a-3p	MIMAT0000086	UAGCACCAUCUGAAAUCGGUUA (SEQ ID NO: 12)
hsa-miR-23a-3p	MIMAT0000069	UAGCAGCACGUAAAUAUUGGCG (SEQ ID NO: 13)
hsa-miR-23a-3p	MIMAT0000078	AUCACAUUGCCAGGGAUUUCC (SEQ ID NO: 14)
hsa-miR-16-5p	MIMAT0000069	UAGCAGCACGUAAAUAUUGGCG (SEQ ID NO: 15)

^amiRBase accession number

CLAIMS

What is claimed:

1. A composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes, wherein the exosomes comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) or a heterologous nucleic acid that expresses the miRNA species when in recipient cardiac stromal cells or fibroblasts cells.
2. The composition of claim 1, wherein the composition further comprises a pharmaceutically acceptable carrier.
3. The composition of claim 2, wherein the composition is formulated for delivery to a site of cardiac injury.
4. The composition of claim 1, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart tissue.
5. The composition of claim 4, wherein at least one of the plurality of miRNA species is over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.
6. The composition of claim 4, wherein each miRNA species of the plurality of miRNA species is selected from and has at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).
7. A method of modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject, the method comprising administering to a cardiac tissue of an animal or human subject a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA

species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

5 8. The method of claim 7, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species.

9. The method of claim 7, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species expressed in at least one of cardiac stromal cells or fibroblasts derived from a normal heart tissue.

10

10. The method of claim 9, wherein at least one of the plurality of miRNA species is over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

15 11. The method of claim 9, wherein each miRNA species of the plurality of miRNA species is selected from and has at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR-146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9),
20 hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

25 12. The method of claim 7, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

30 13. The method of claim 8, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a heterologous nucleic acid that expresses at least one miRNA species when in recipient cardiac tissue.

14. The method of claim 7, wherein the composition is administered to the cardiac tissue of the animal or human subject by a percutaneous method.

35 15. The method of claim 14, wherein the percutaneous method is by injection into the cardiac tissue.

16. A method of repairing cardiac tissue damage, the method comprising administering to a cardiac tissue of an animal or human subject a therapeutic composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, , wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) that when delivered to recipient cardiac tissue of the animal or human subject reduces the activity of PTEN in the recipient cells thereof.

17. The method of claim 16, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species.

18. The method of claim 16, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a plurality of miRNA species expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue.

19. The method of claim 17, wherein at least some of the plurality of miRNA species are over-expressed in cardiac stromal cells or fibroblasts cells derived from a normal heart tissue compared to a heart tissue having a pathological injury.

20. The method of claim 17, wherein each miRNA species of the plurality of miRNA species is selected from and has at least 90% sequence similarity to one of the group consisting of hsa-let-7b-5p (SEQ ID NO: 2), hsa-miR-125a-5p (SEQ ID NO: 3), hsa-miR146a-5p (SEQ ID NO: 4), hsa-miR-125b-5p (SEQ ID NO: 5), hsa-miR-126-3p (SEQ ID NO: 6), hsa-miR-16-1-3p (SEQ ID NO: 7), hsa-miR-23a-5p (SEQ ID NO: 8), hsa-miR-21-3p (SEQ ID NO: 9), hsa-miR-26a-5p (SEQ ID NO: 10), hsa-miR-320a (SEQ ID NO: 11), hsa-miR-29a-3p (SEQ ID NO: 12), hsa-miR-16-5p (SEQ ID NO: 13), hsa-miR-23a-3p (SEQ ID NO: 14), and hsa-miR-16-5p (SEQ ID NO: 15).

21. The method of claim 17, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises at least one nucleic acid species not derived from cardiac stromal cells or fibroblasts cells.

22. The method of claim 17, wherein the population of cardiac stromal cell/fibroblast-derived exosomes further comprises a heterologous nucleic acid that expresses at least one miRNA species when in recipient cardiac tissue.

23. The method of claim 17, wherein the therapeutic composition is administered to the cardiac tissue of the animal or human subject by a percutaneous method.

24. The method of claim 17, wherein the percutaneous method is by injection into the cardiac
5 tissue.

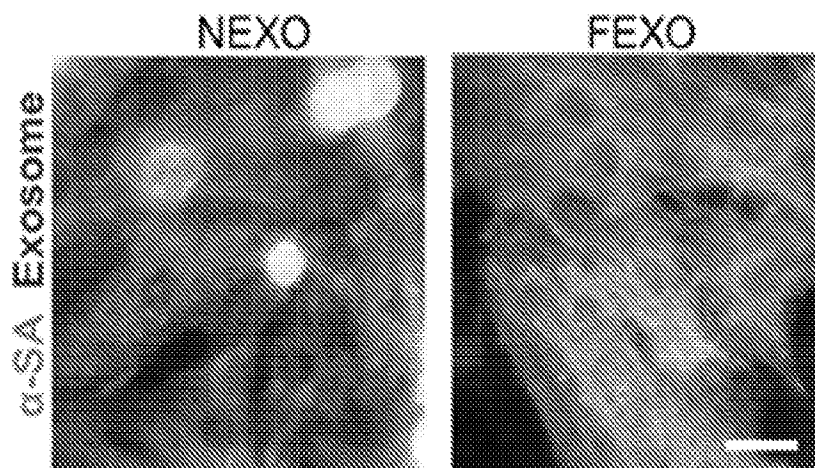


Fig. 1A

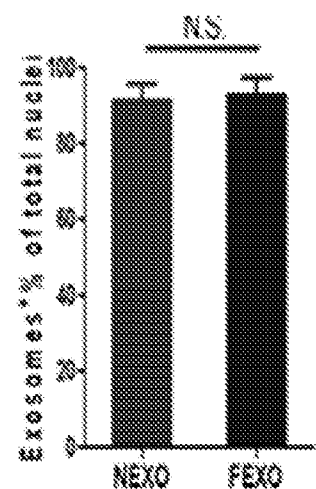


Fig. 1B

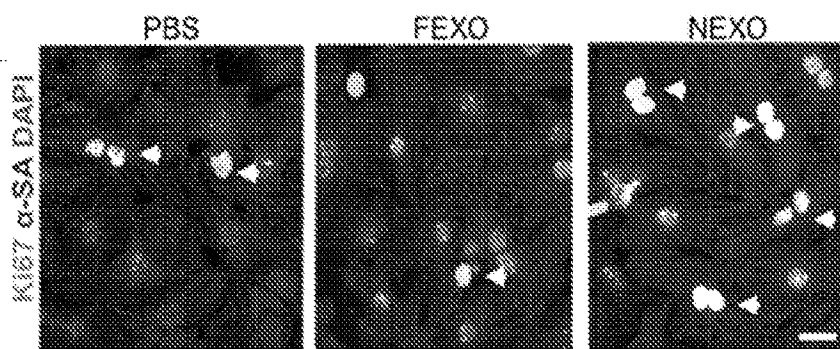


Fig. 1C

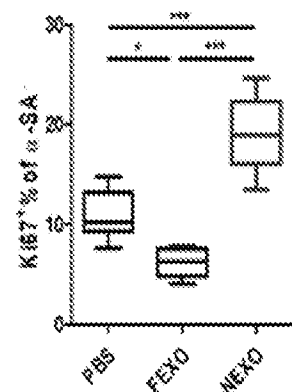


Fig. 1D

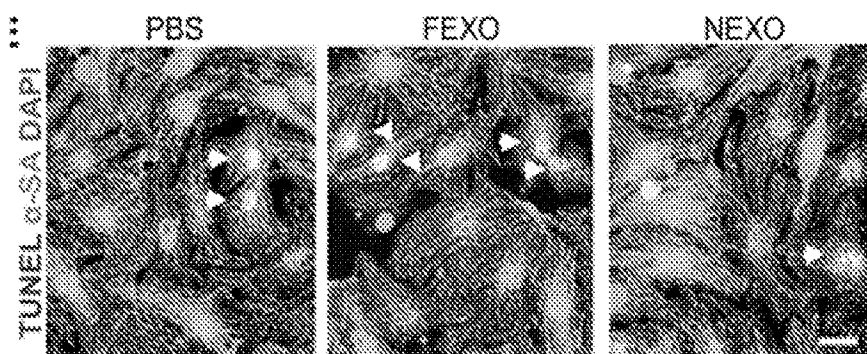


Fig. 1E

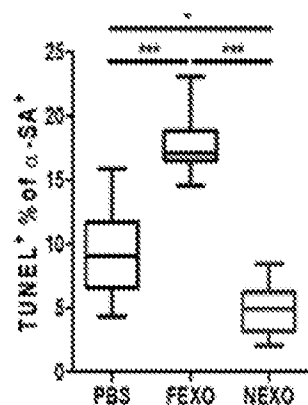


Fig. 1F

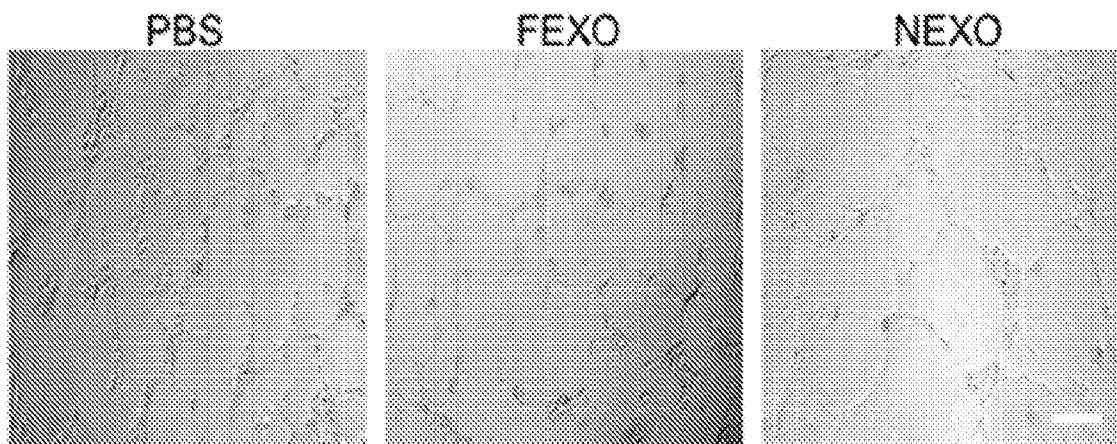


Fig. 1G

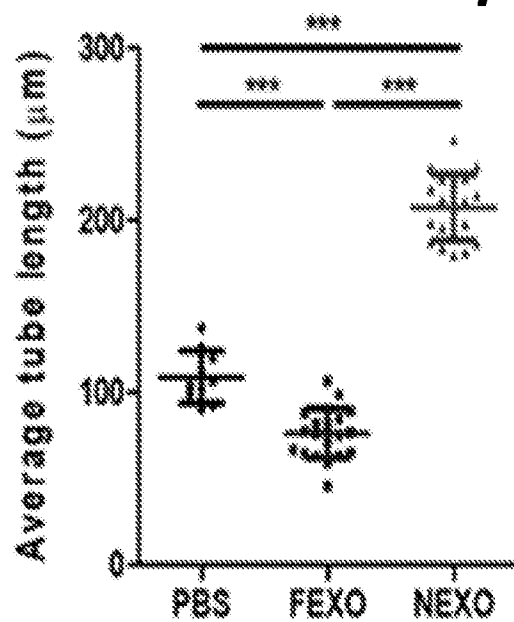


Fig. 1H

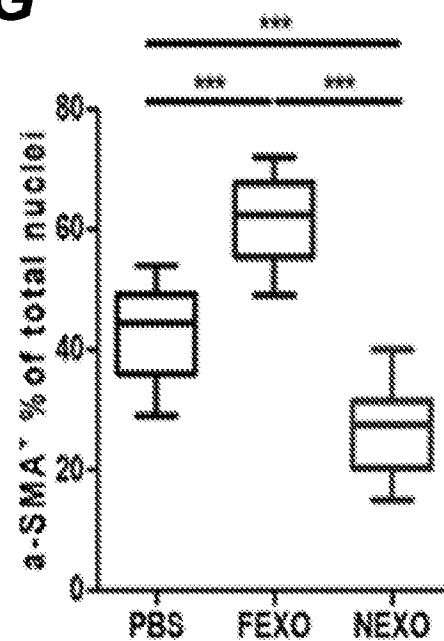


Fig. 1J

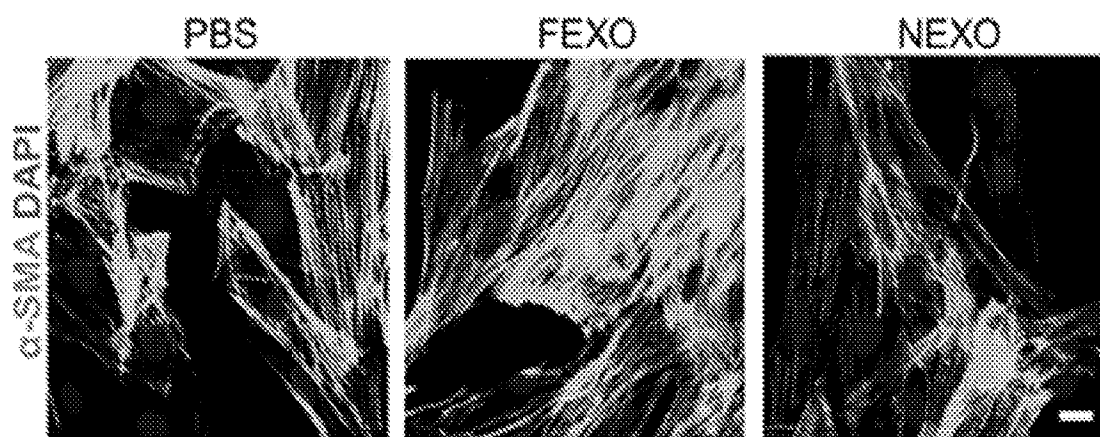
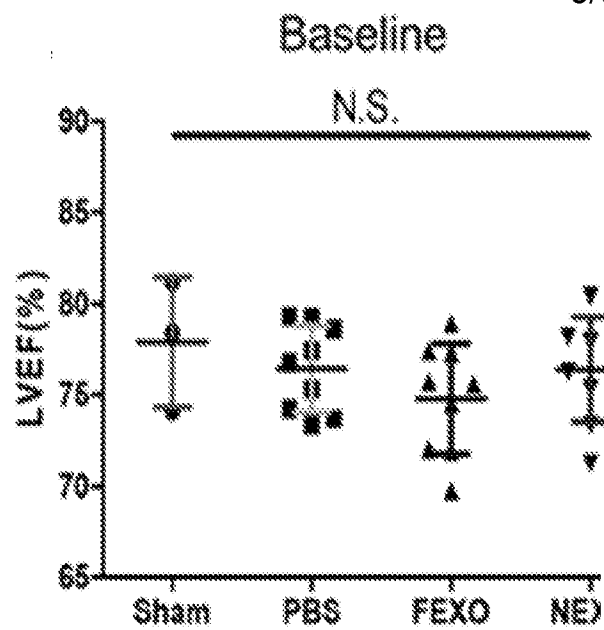
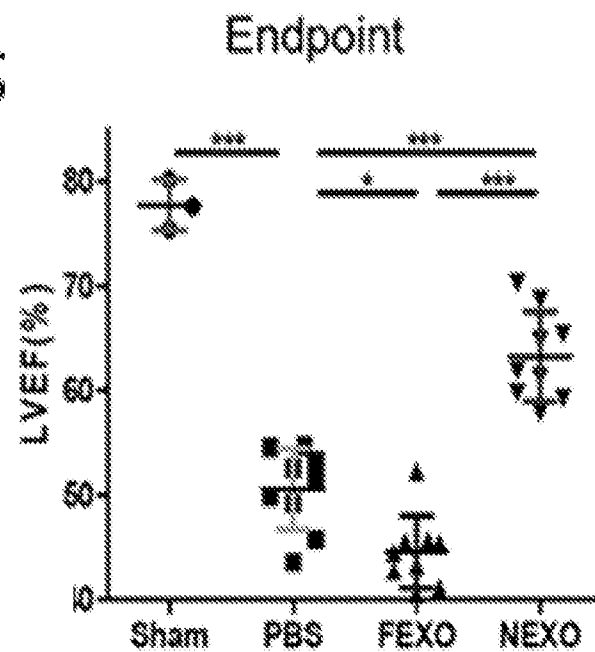
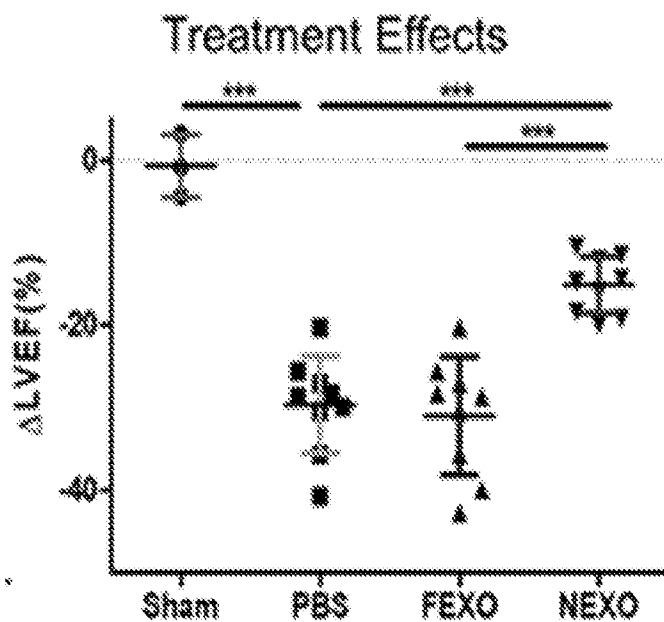


Fig. 1I

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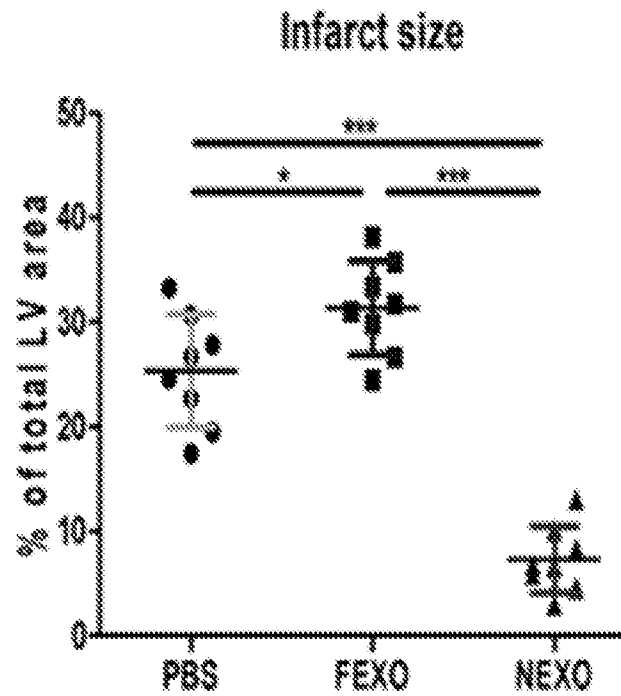
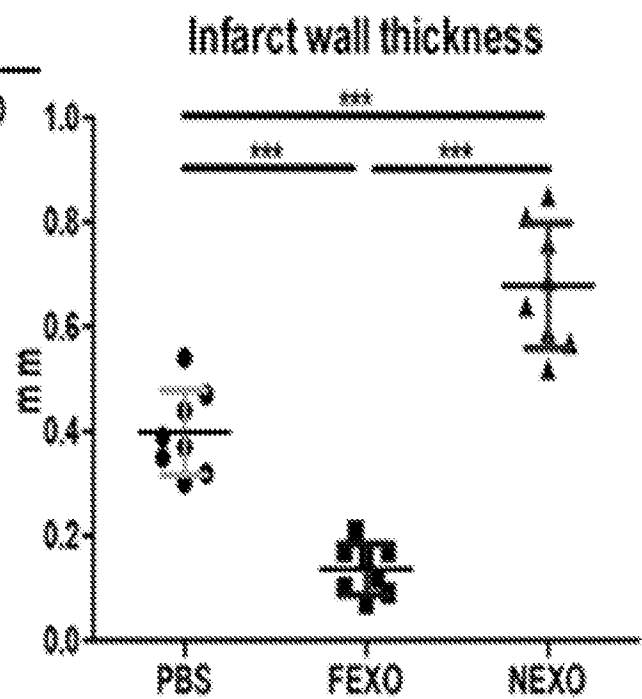
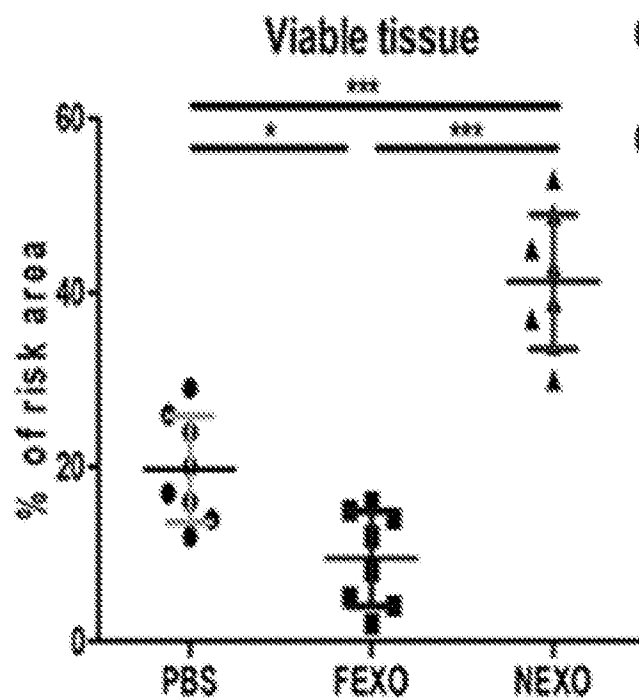
**Fig. 2A****Fig. 2B****Fig. 2C**

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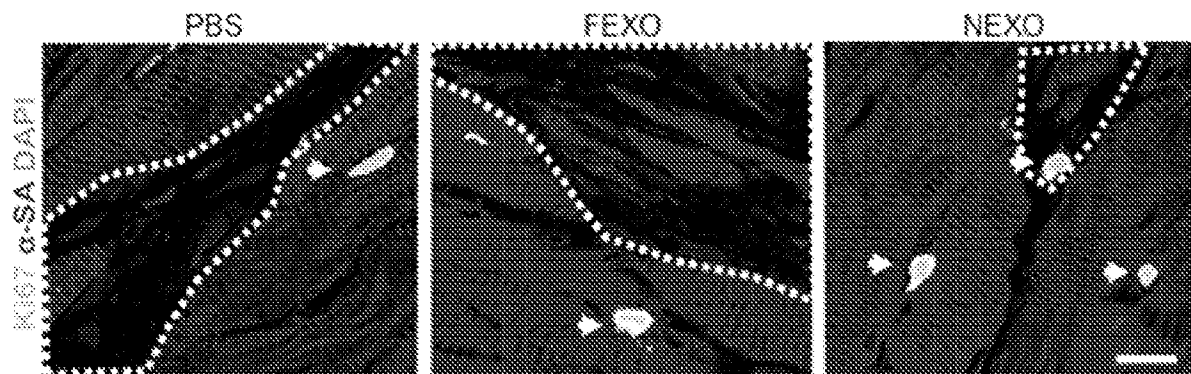
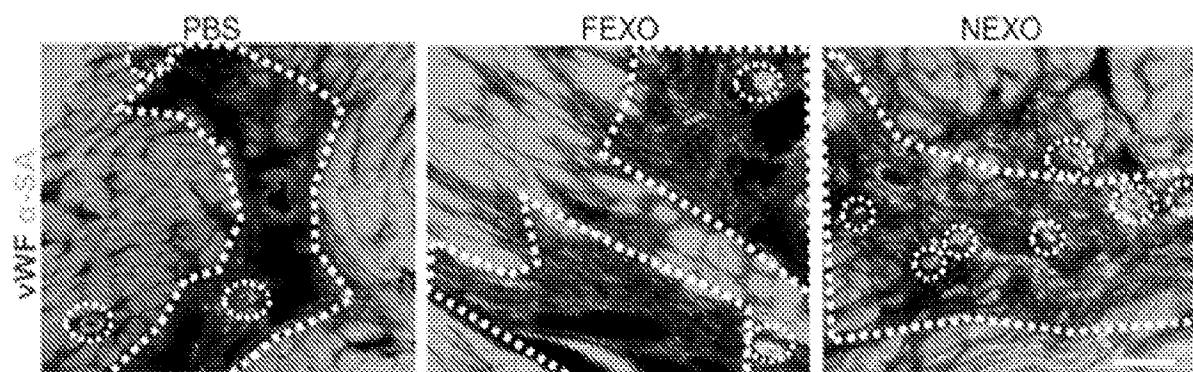
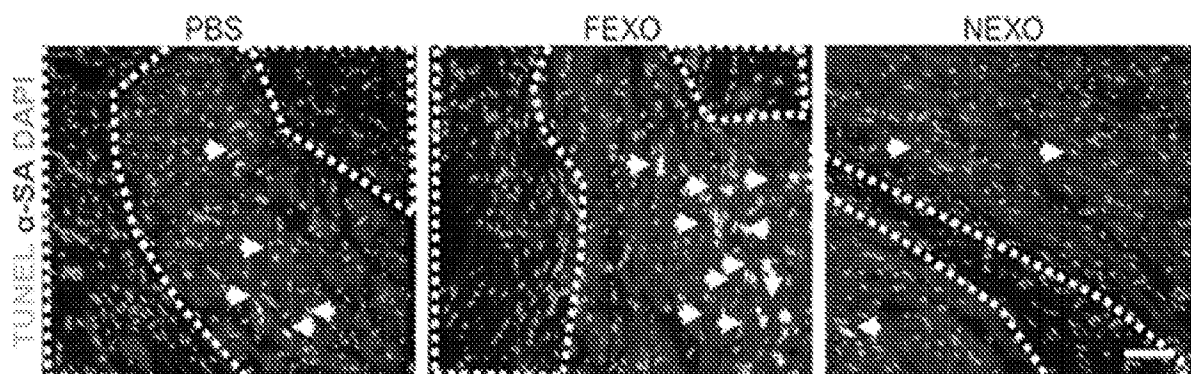


Fig. 2D

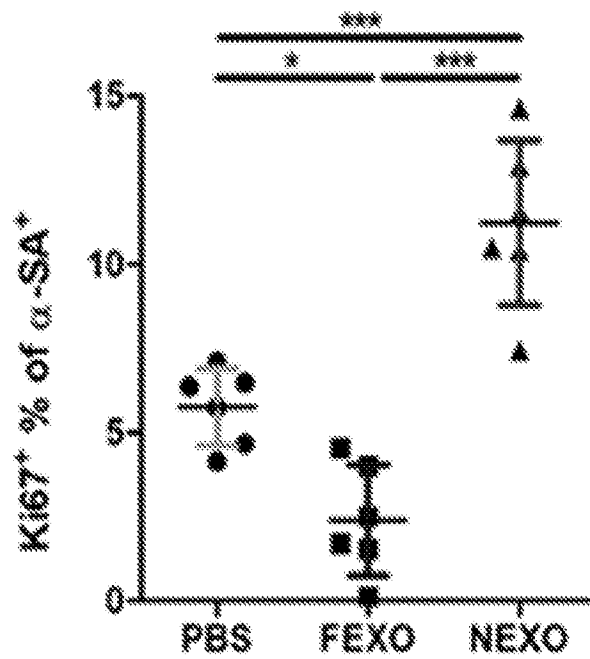
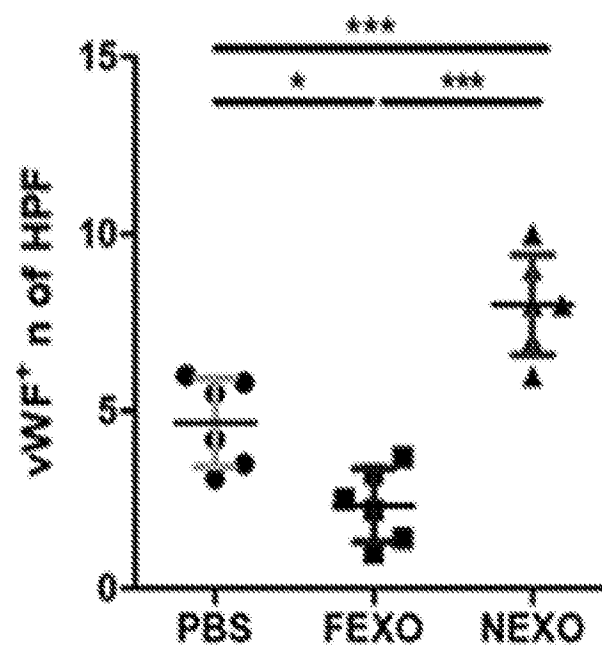
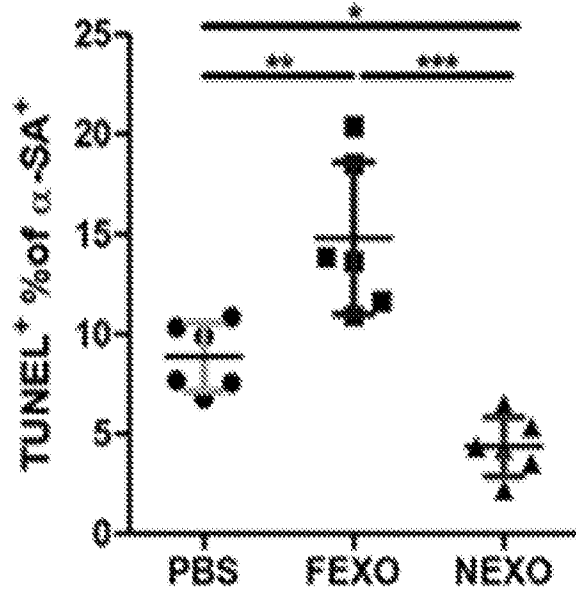
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**Fig. 2E****Fig. 2F****Fig. 2G**

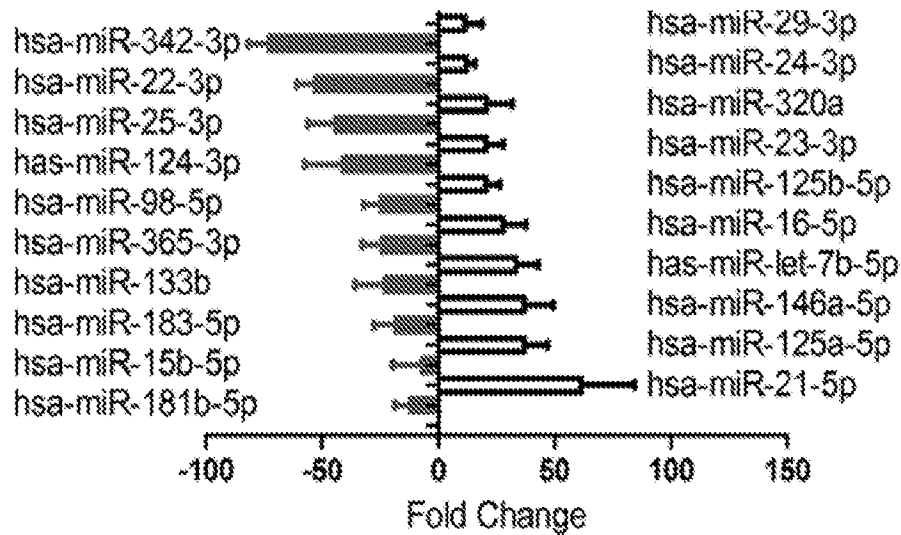
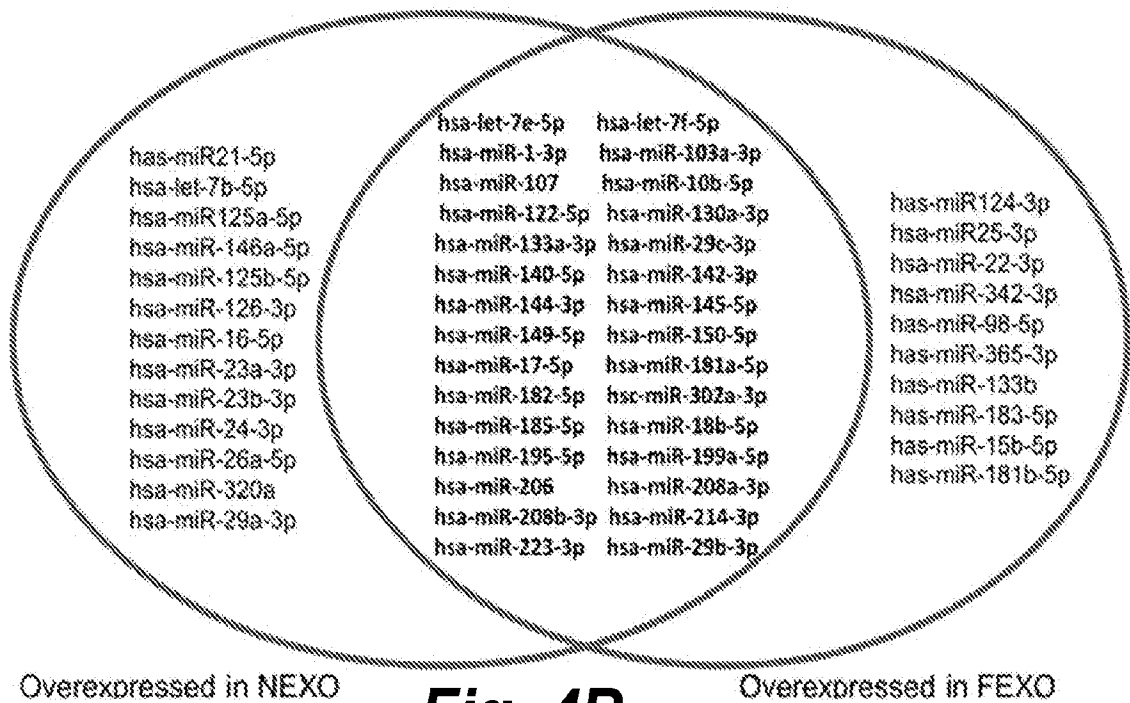
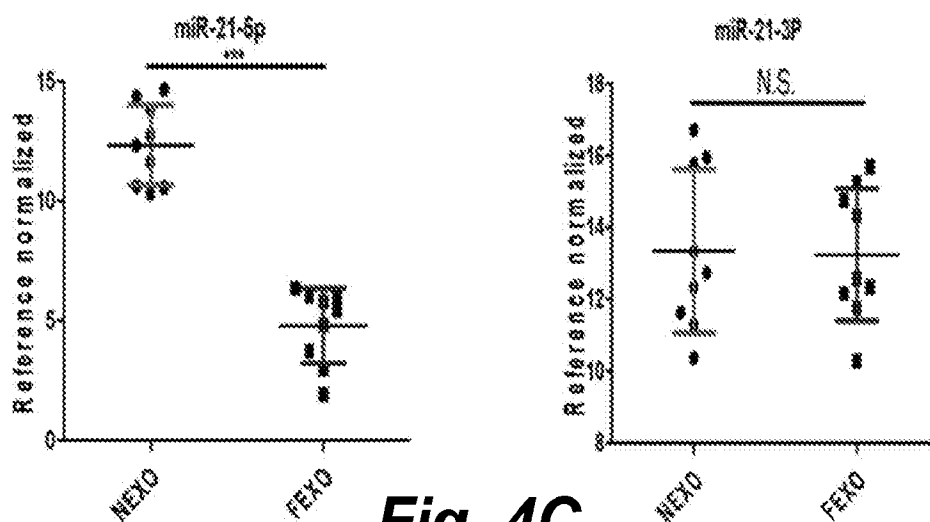
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**Fig. 3A****Fig. 3B****Fig. 3C**

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**Fig. 3D****Fig. 3E****Fig. 3F**

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**Fig. 4A****Fig. 4B****Fig. 4C**

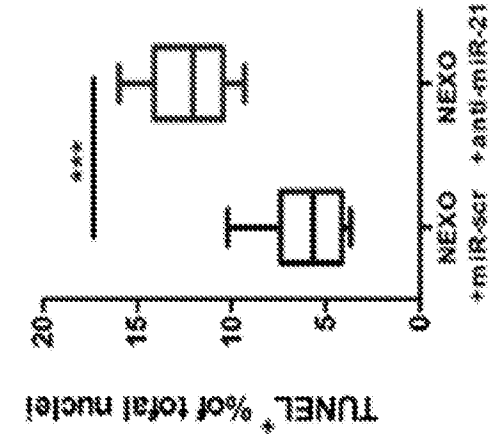


Fig. 5B

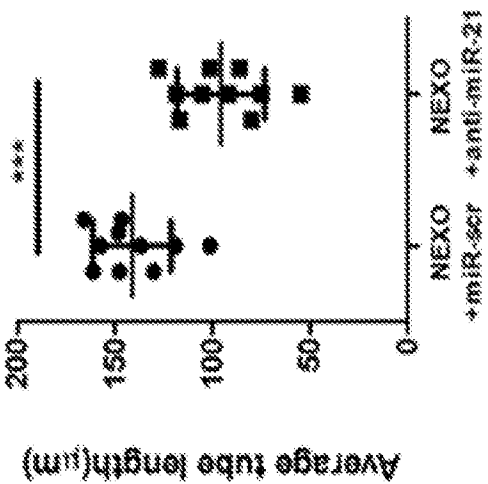


Fig. 5D

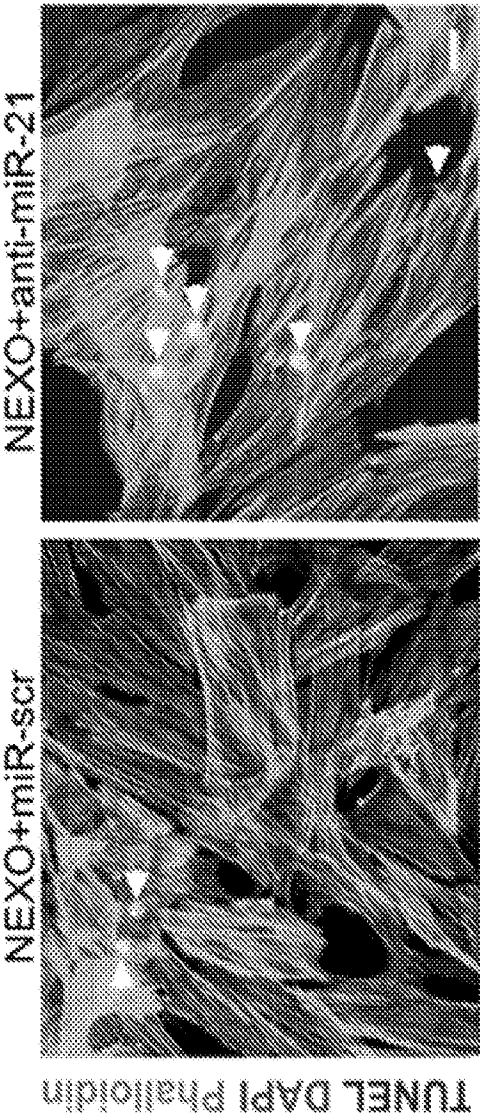


Fig. 5A

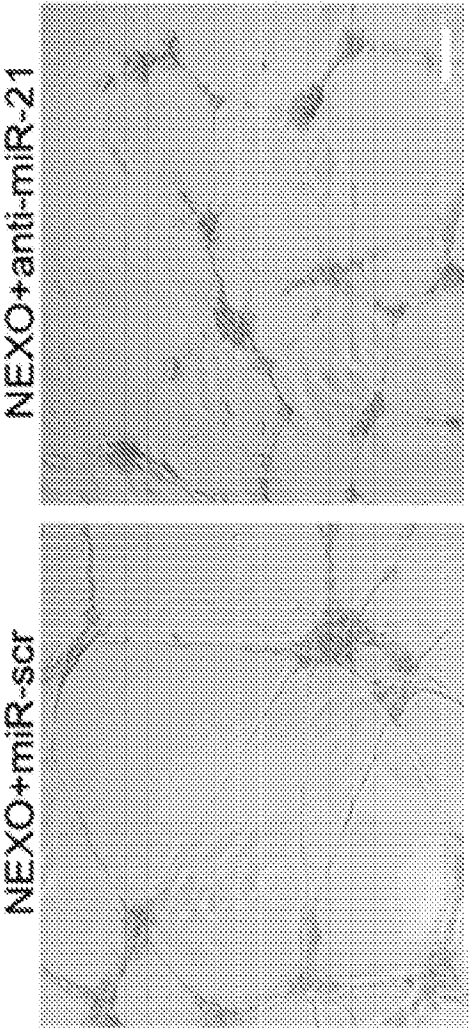


Fig. 5C

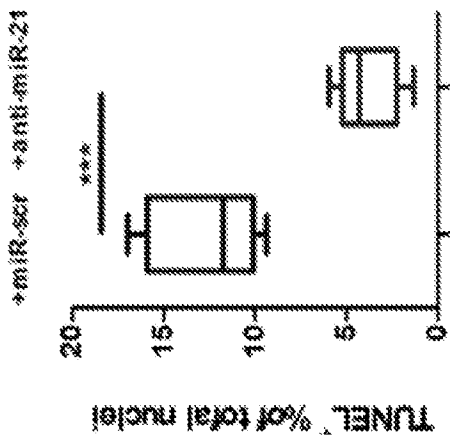


Fig. 5F

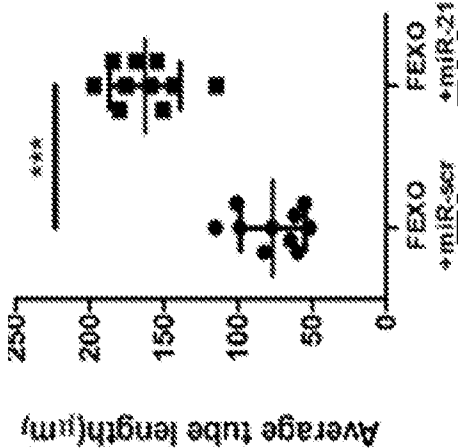


Fig. 5H

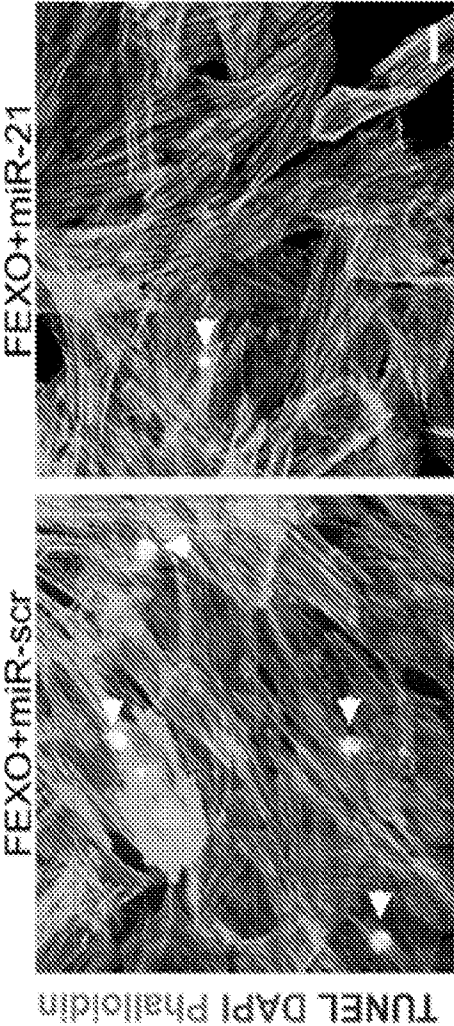


Fig. 5E

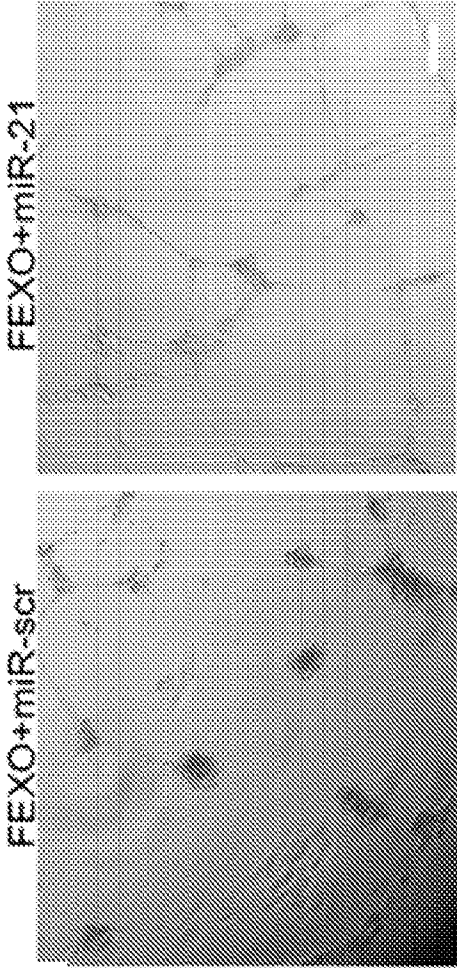
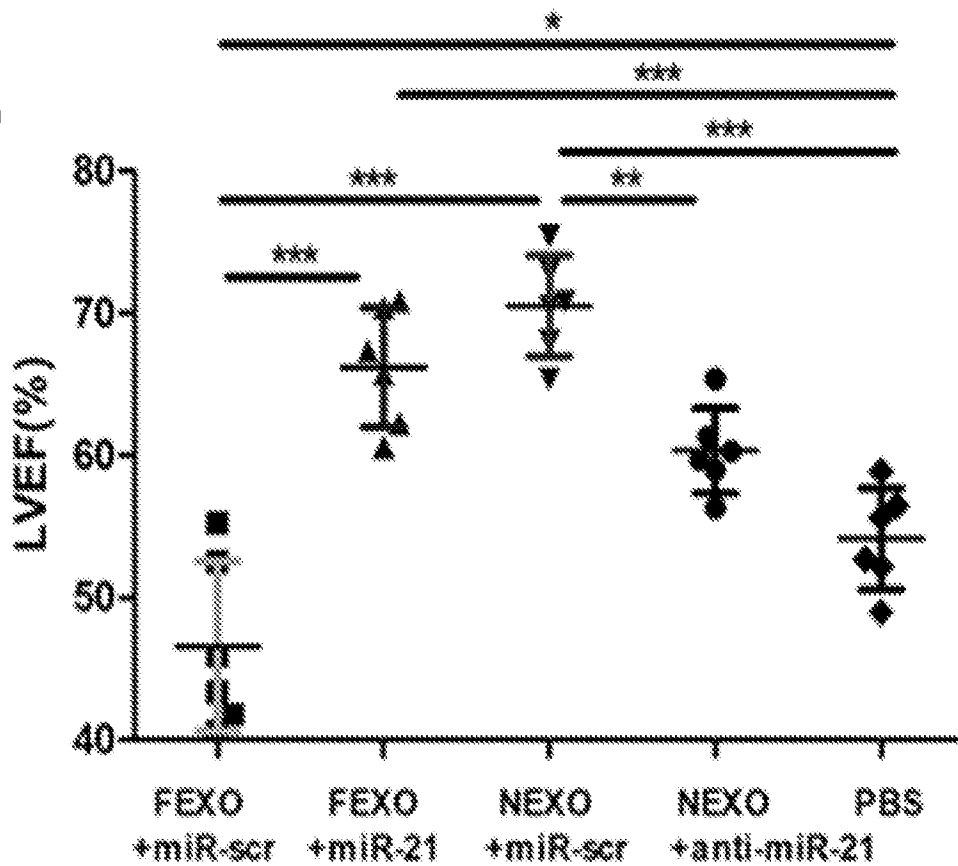
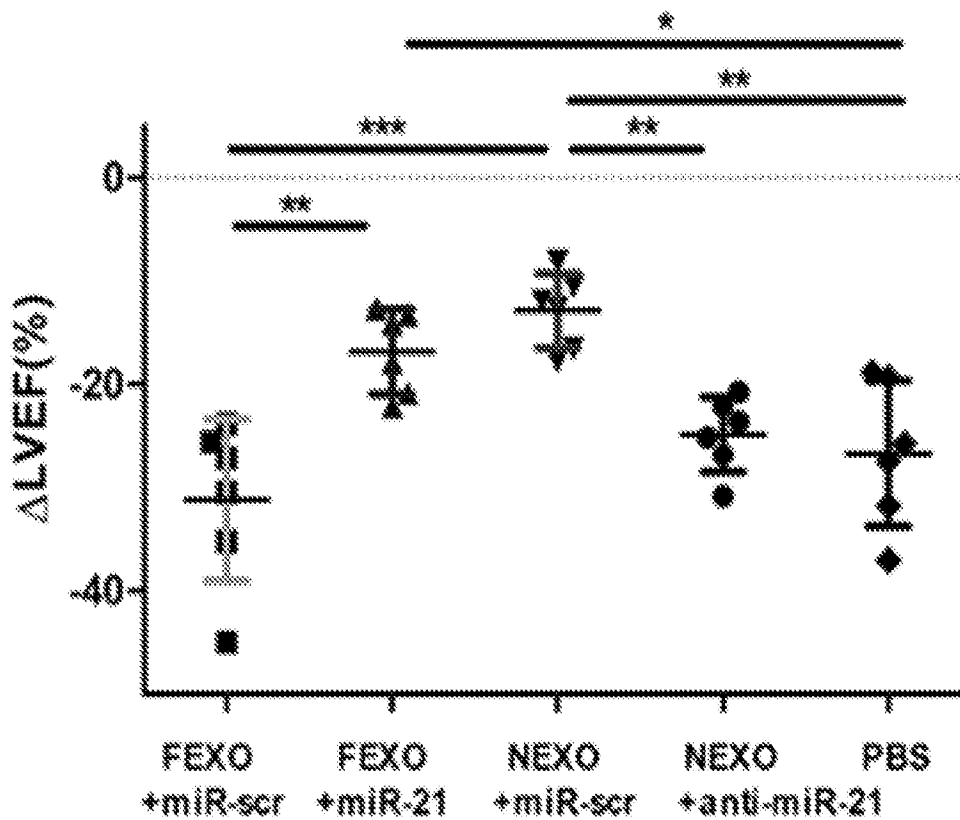


Fig. 5G

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**Fig. 6A****Fig. 6B**

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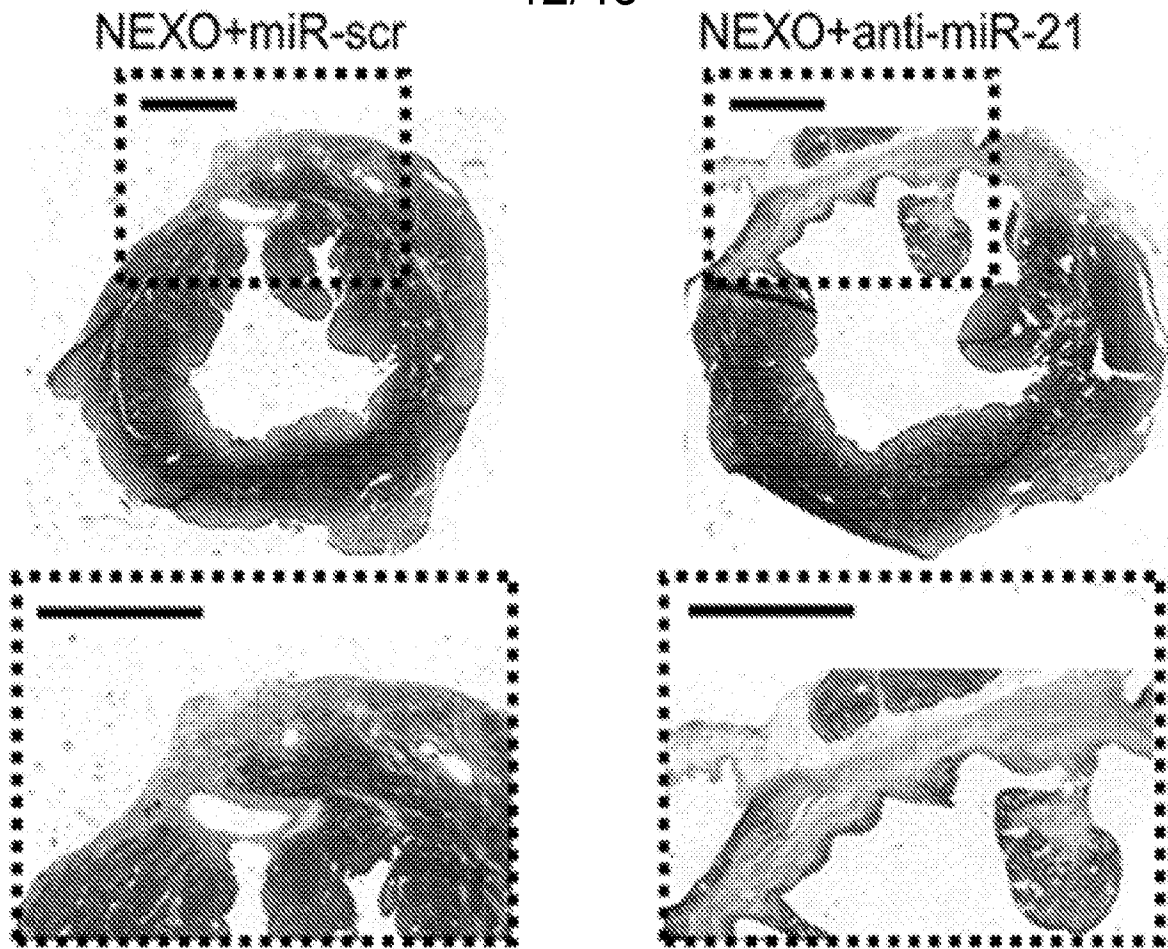


Fig. 6C

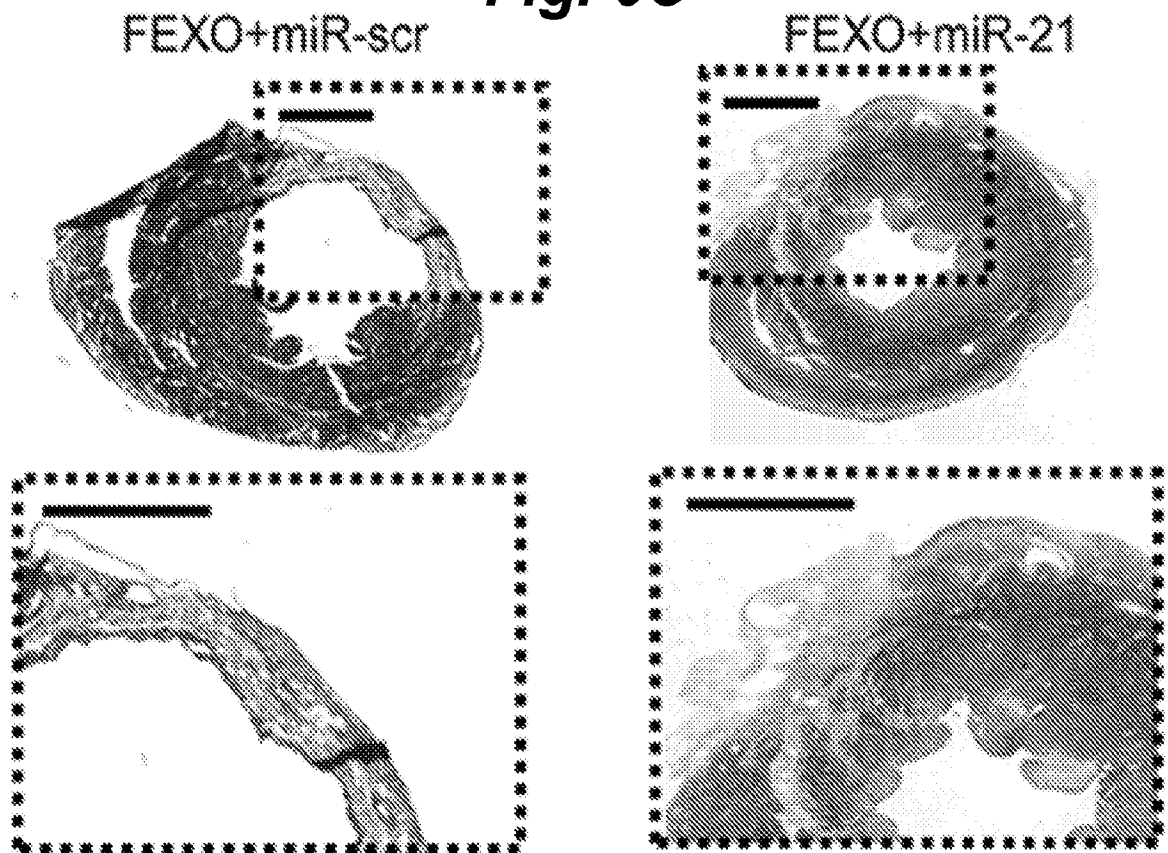
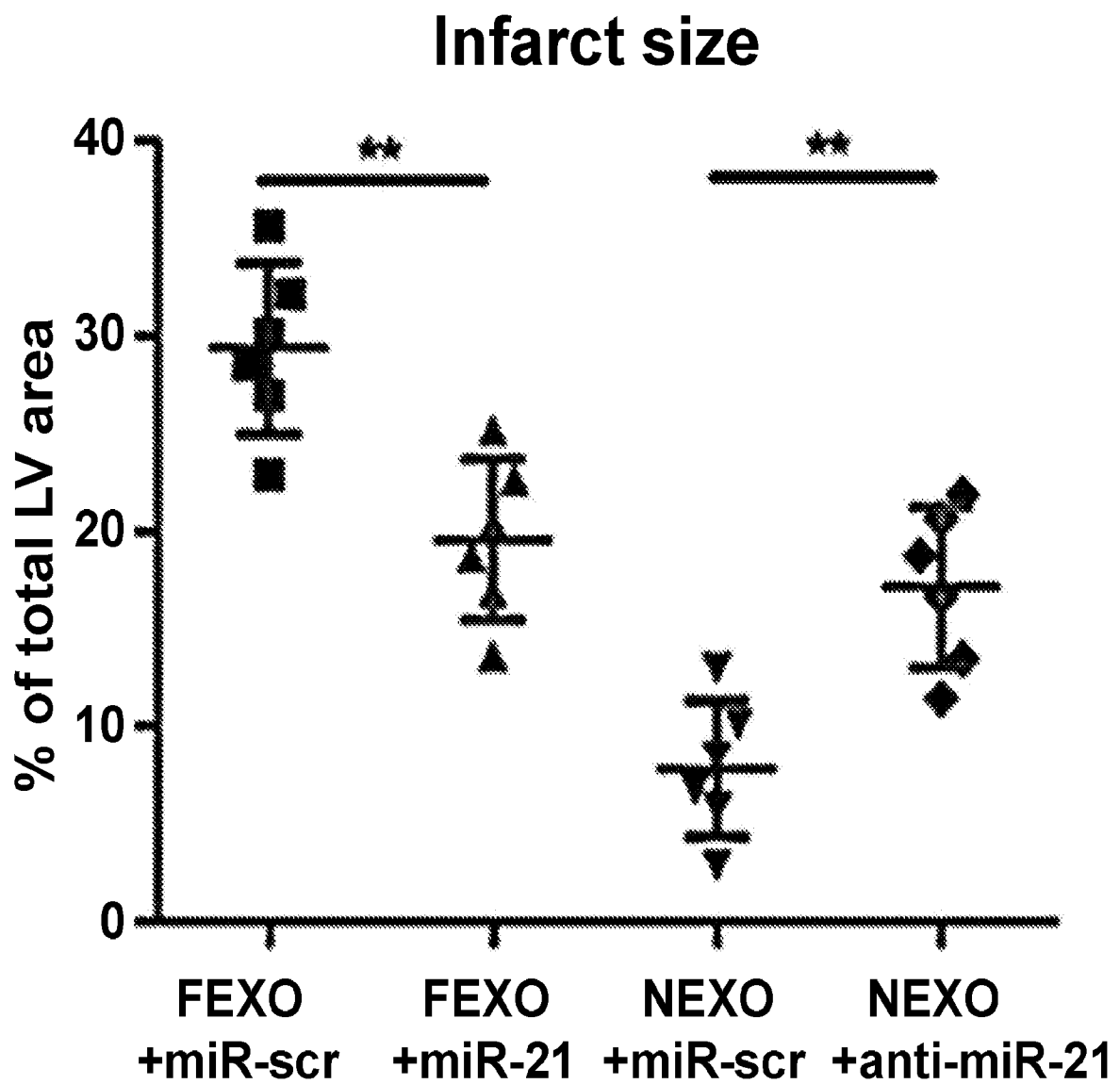
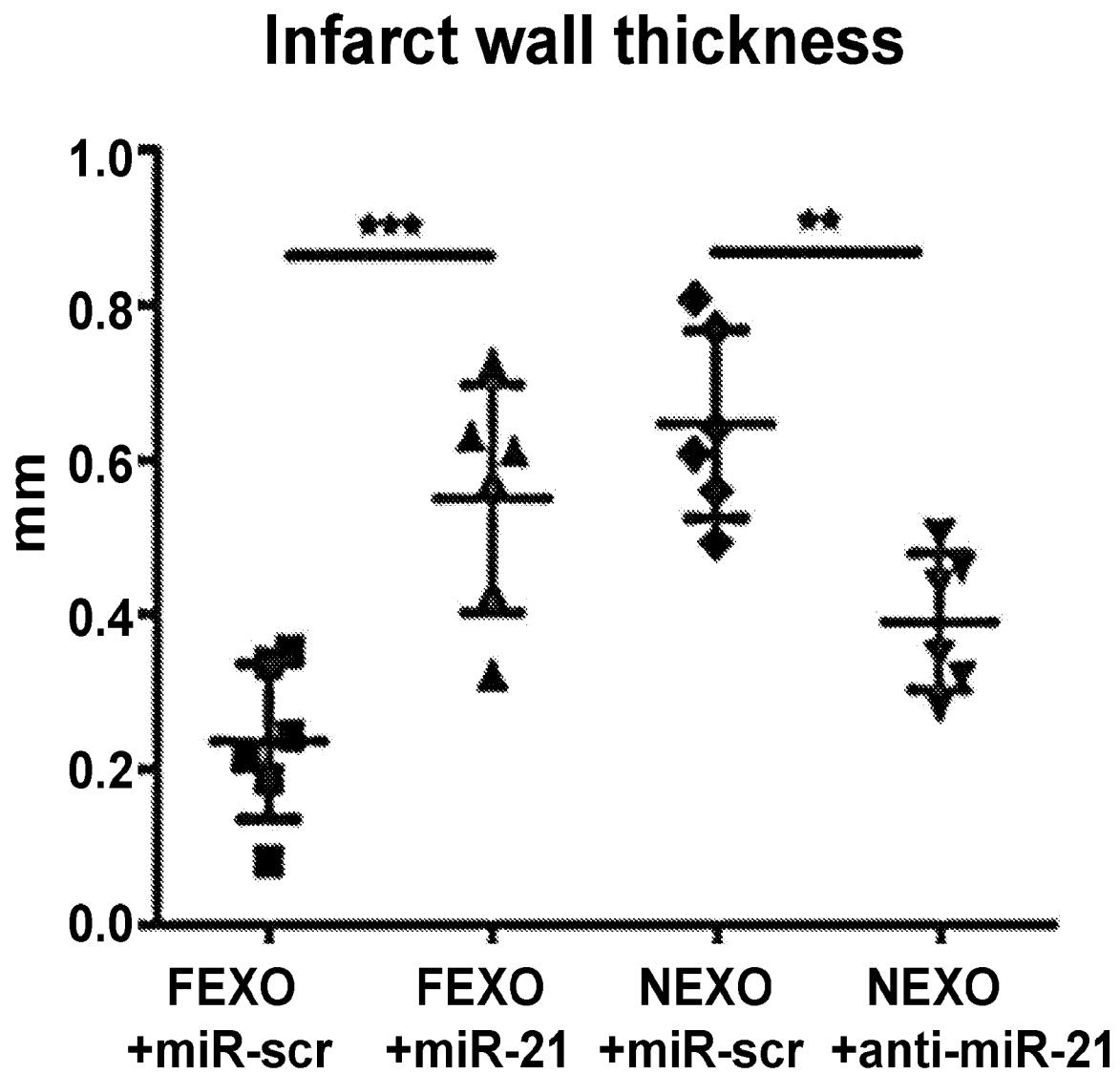


Fig. 6D

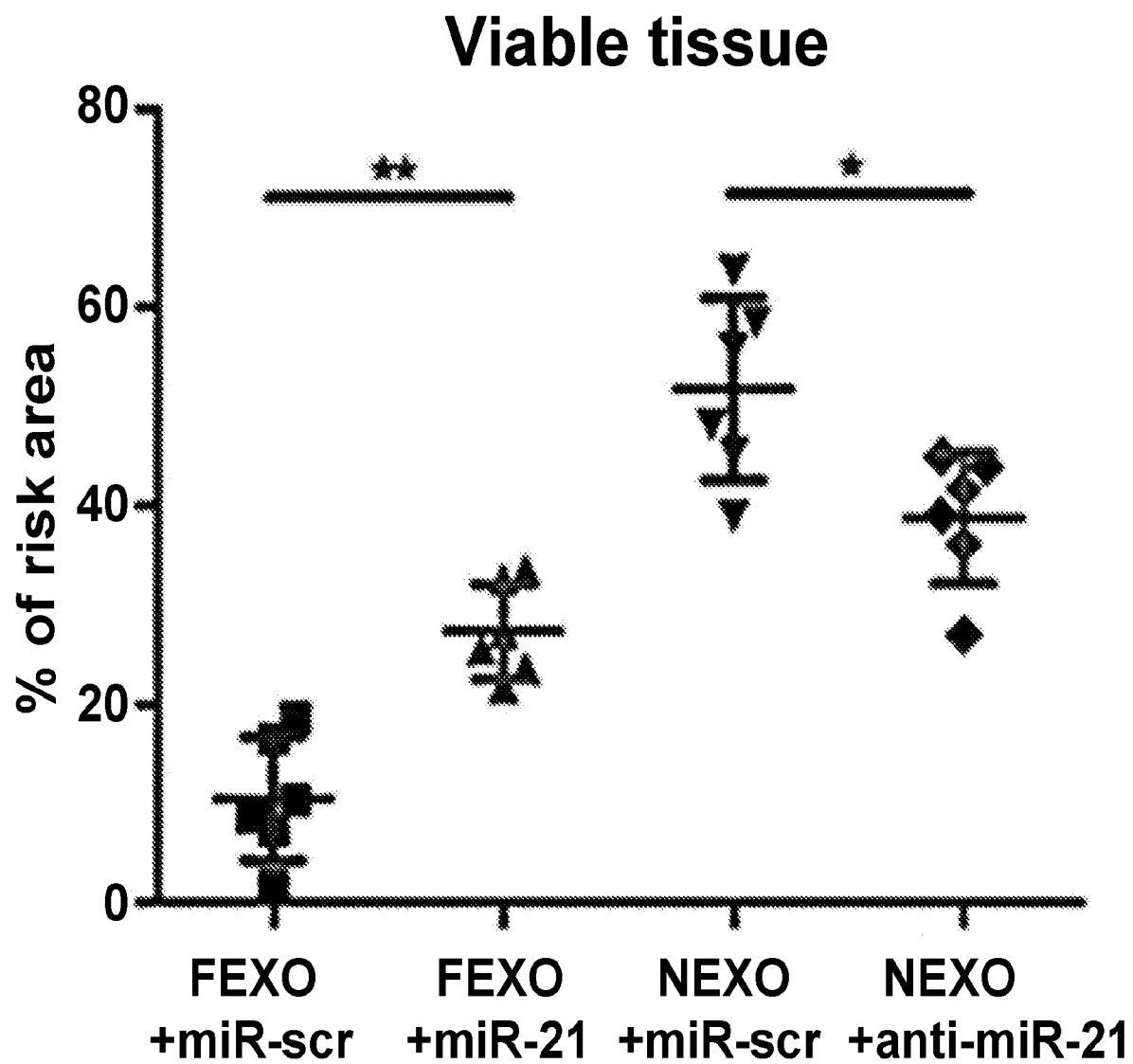
13/46

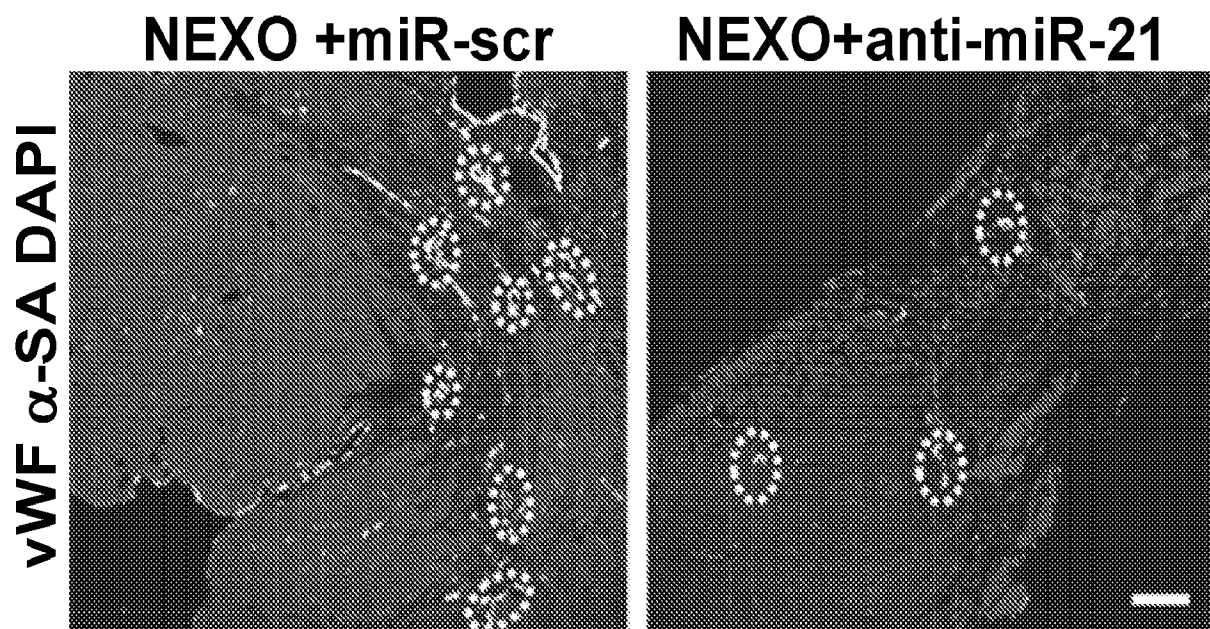
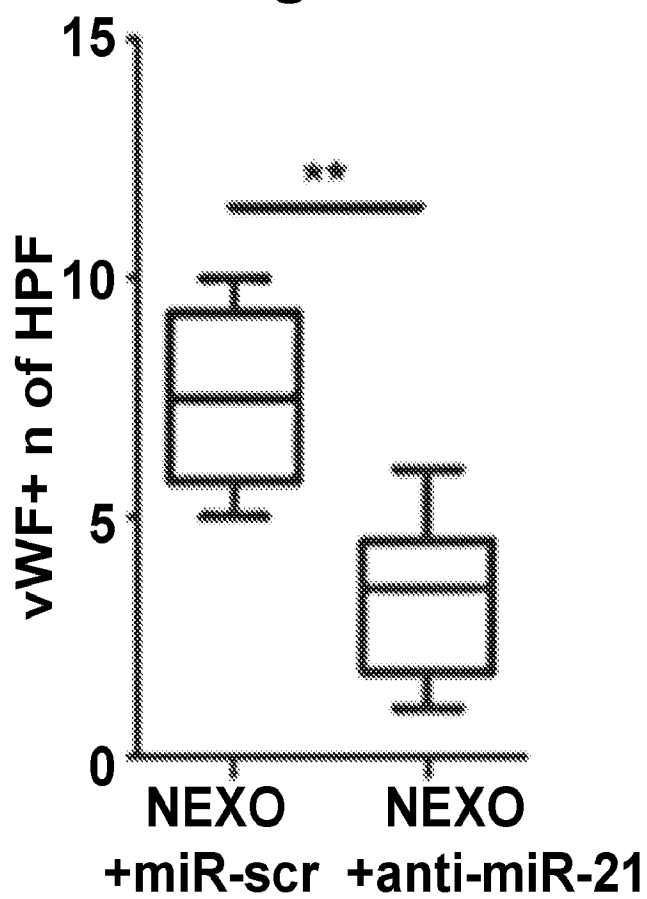
***Fig. 6E***

14/46

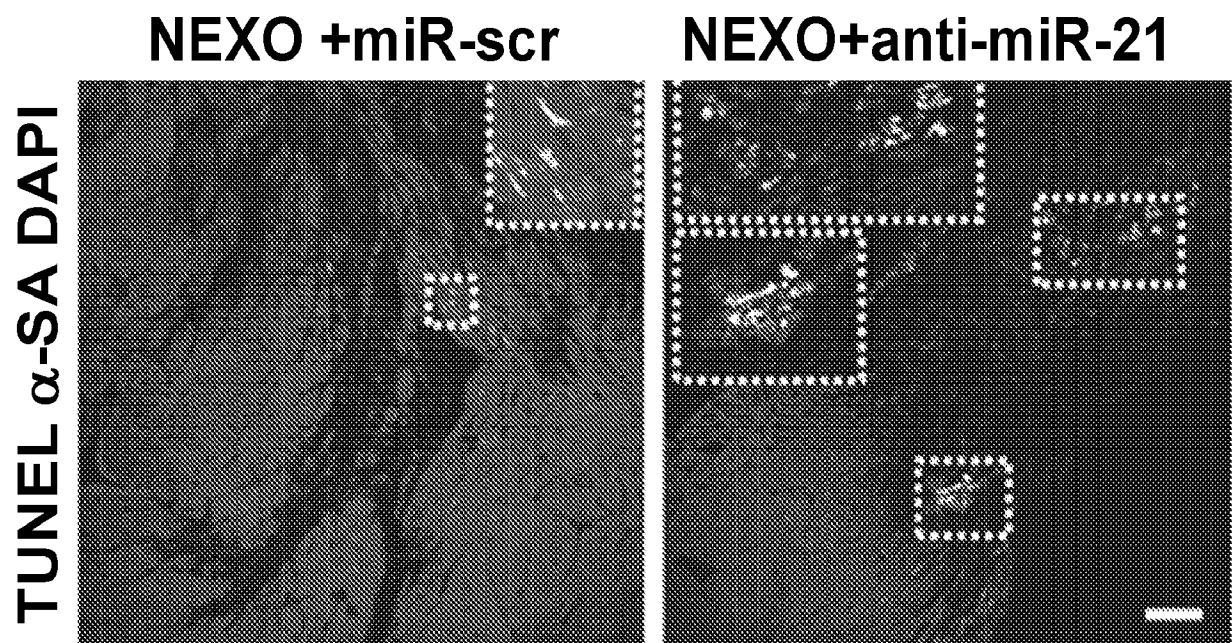
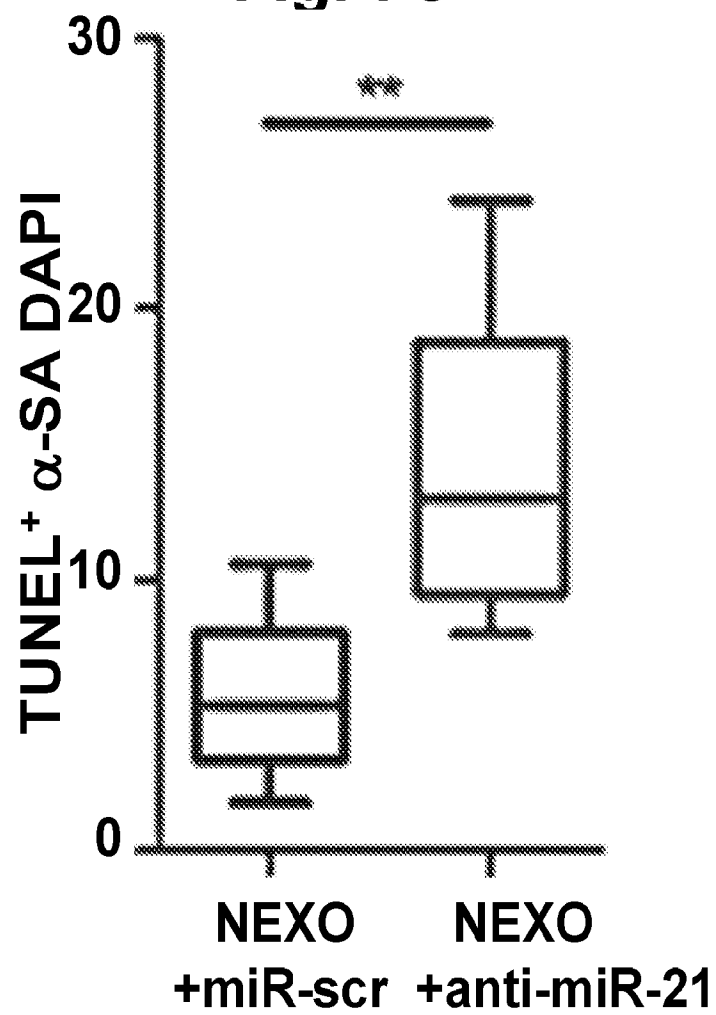
***Fig. 6F***

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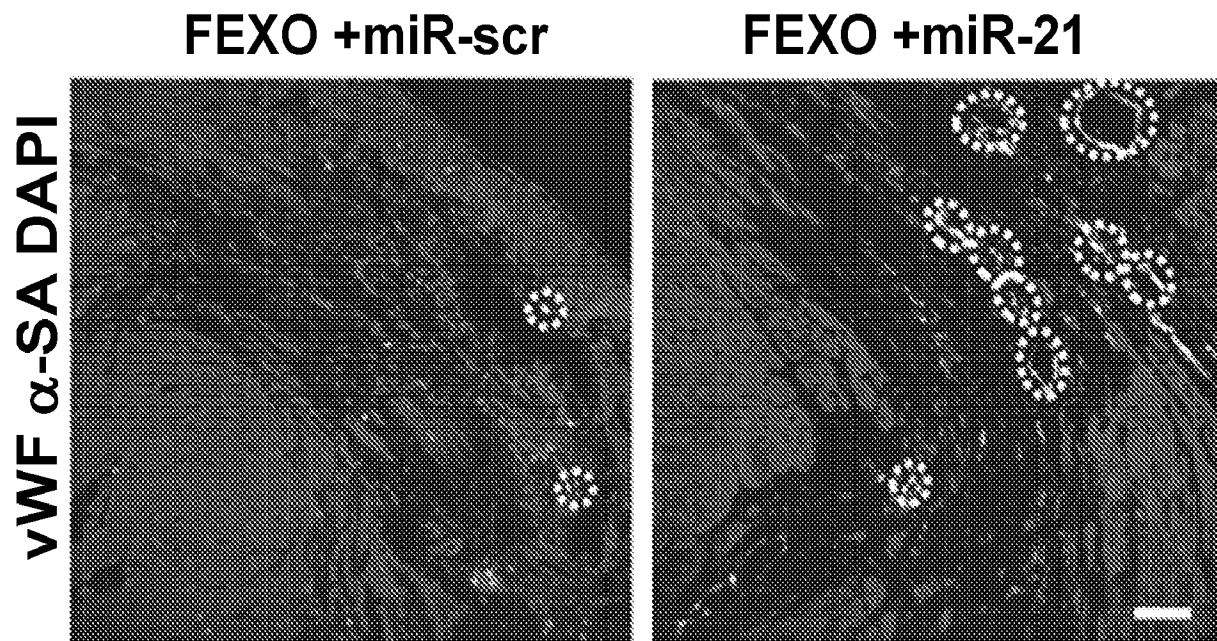
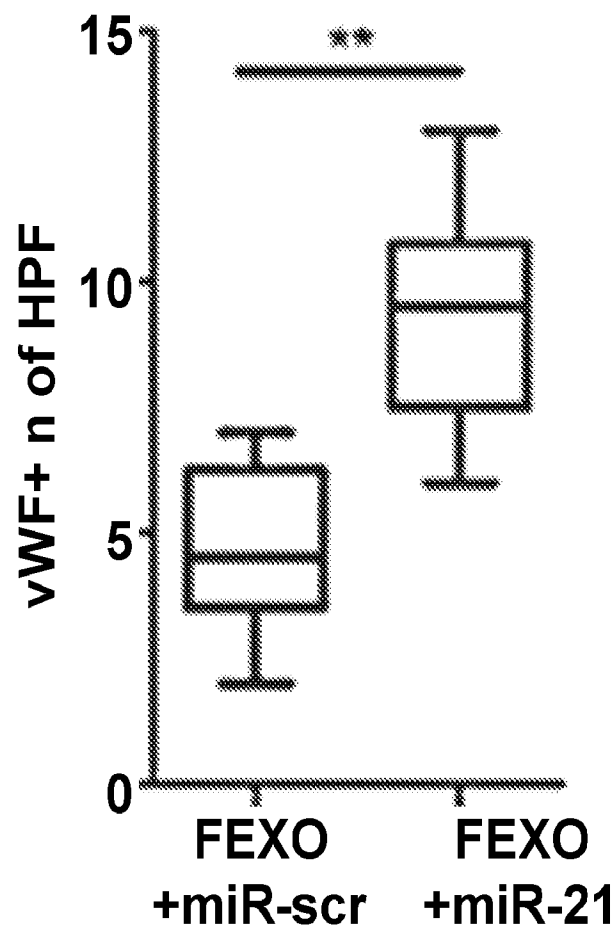
**Fig. 6G**

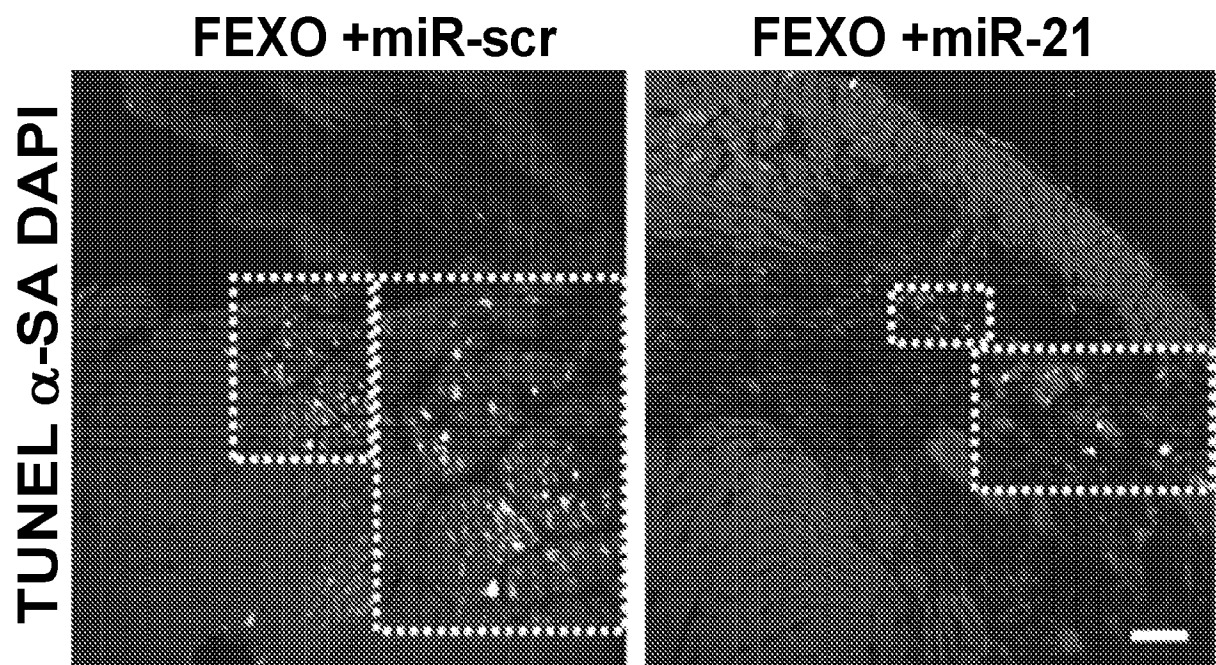
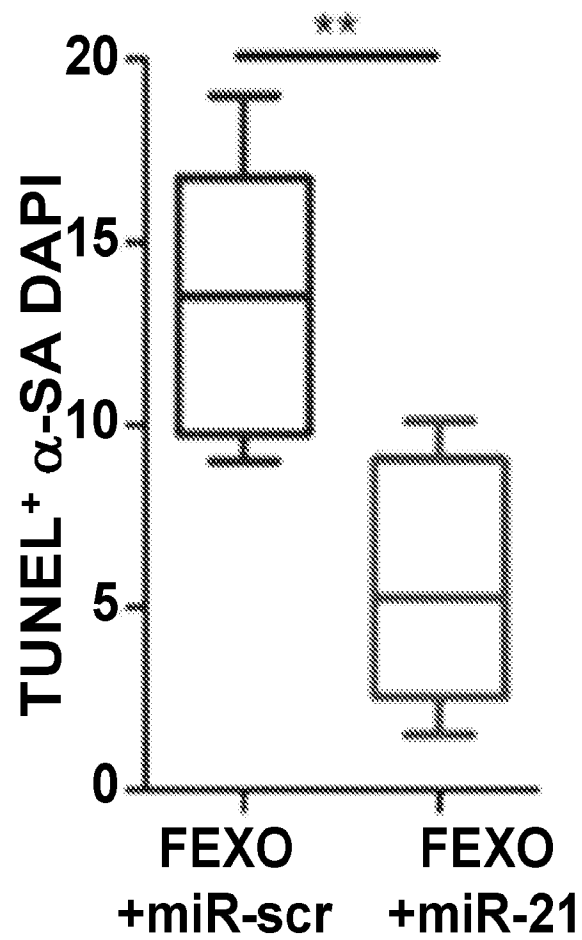
**Fig. 7A****Fig. 7B**

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**Fig. 7C****Fig. 7D**

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**Fig. 7E****Fig. 7F**

**Fig. 7G****Fig. 7H**

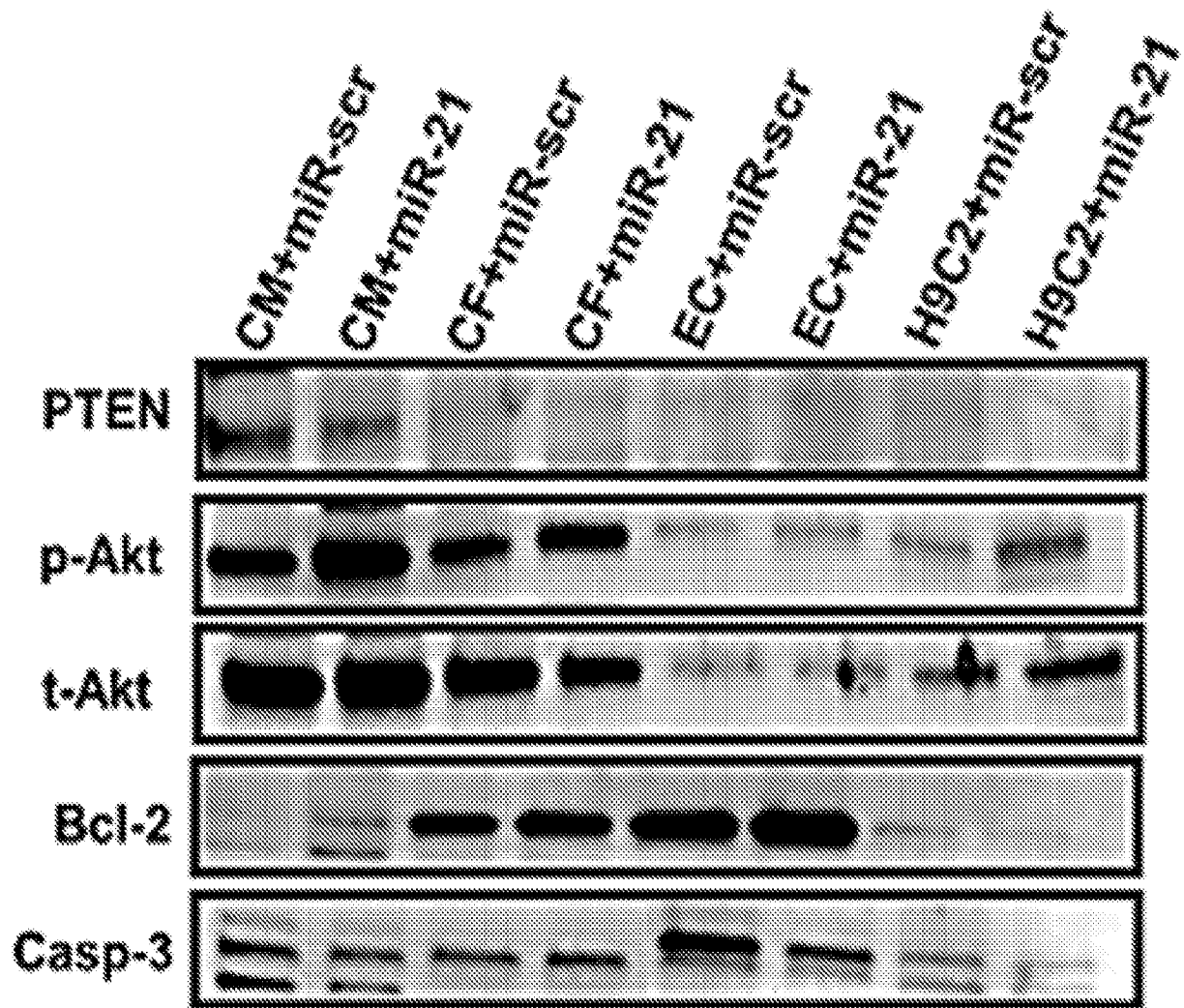
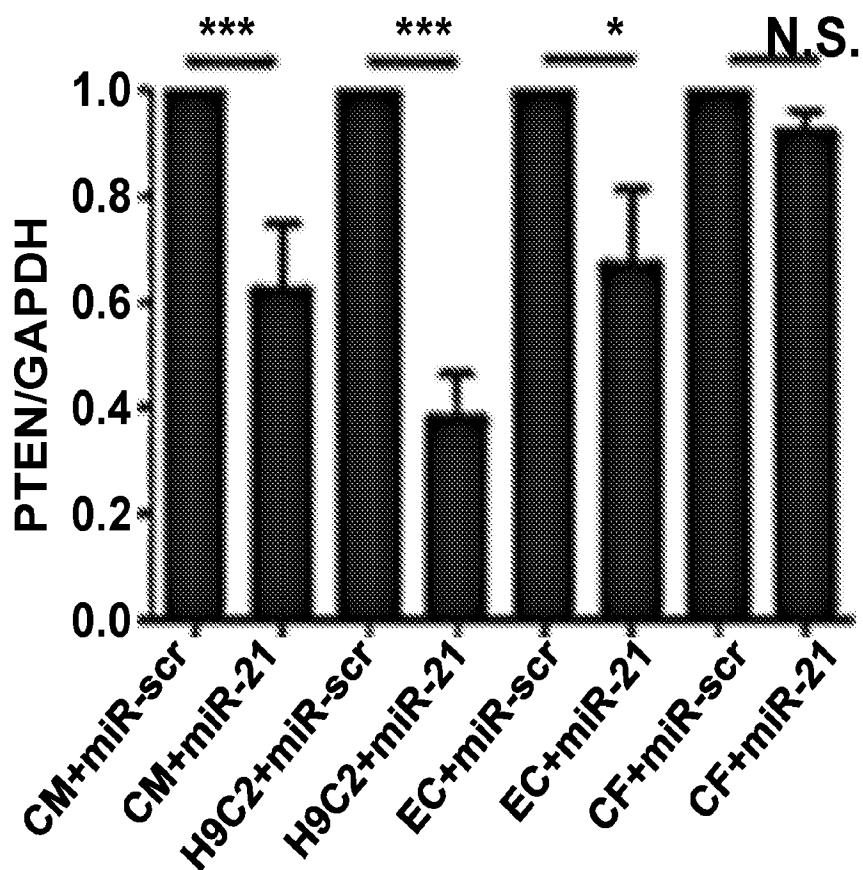
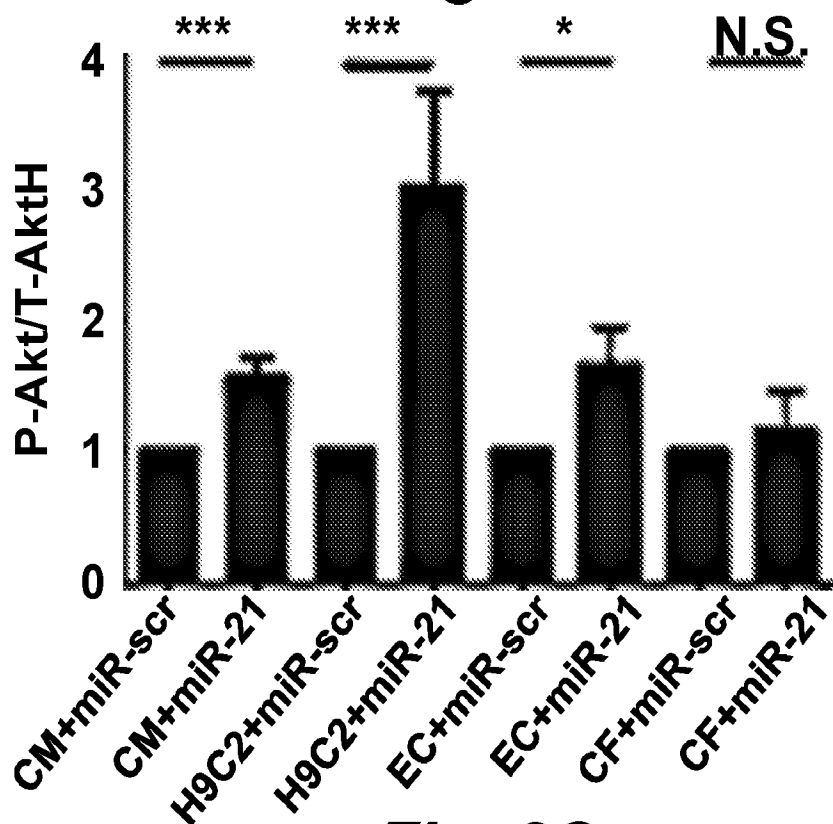
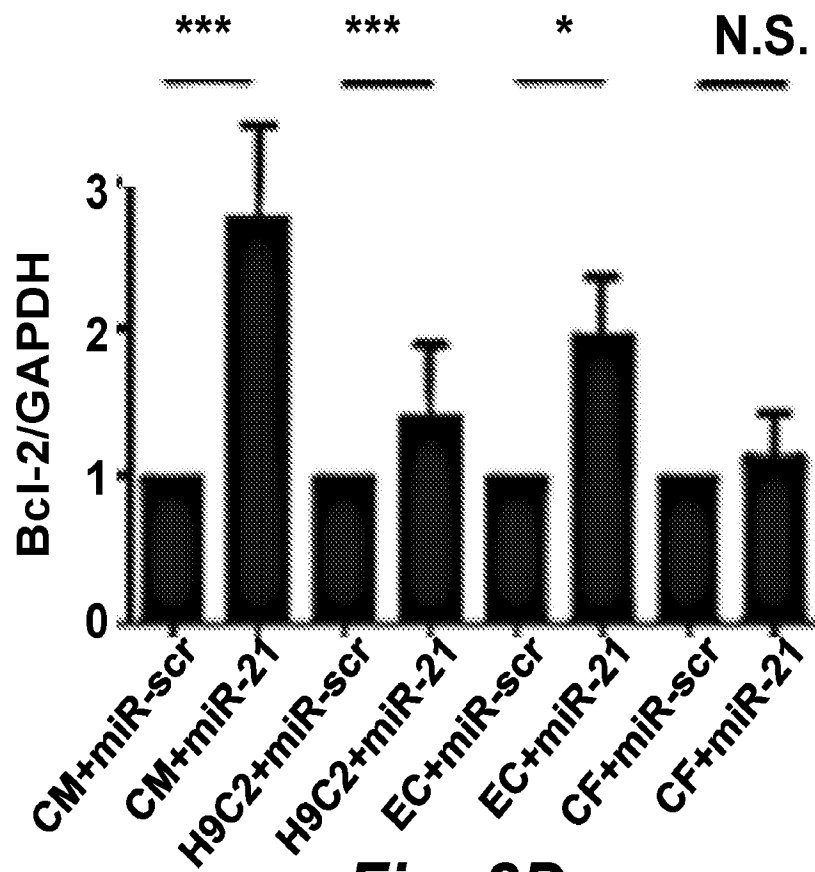
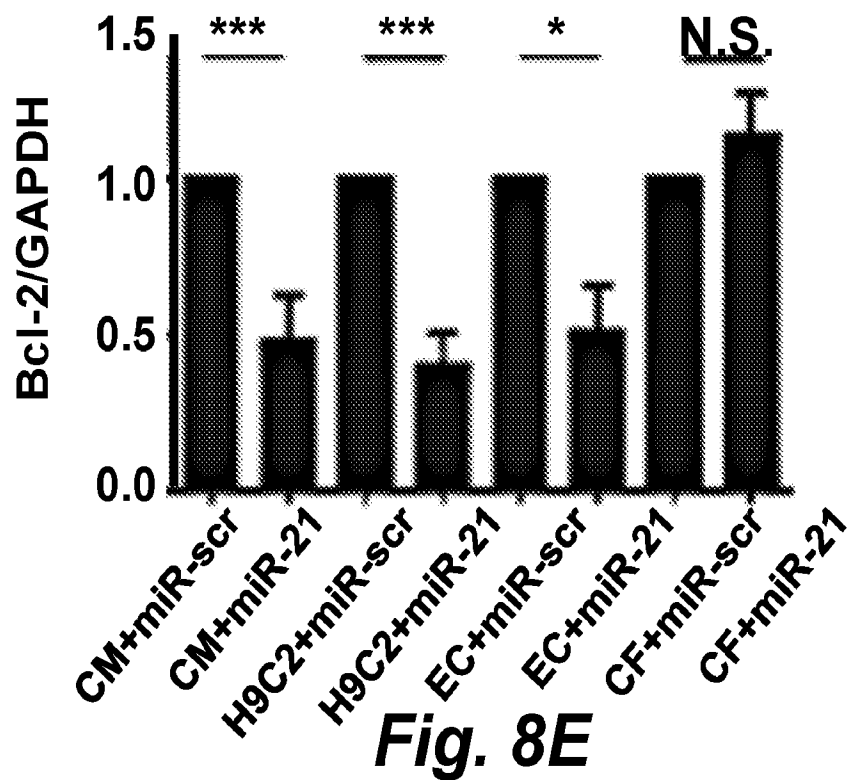


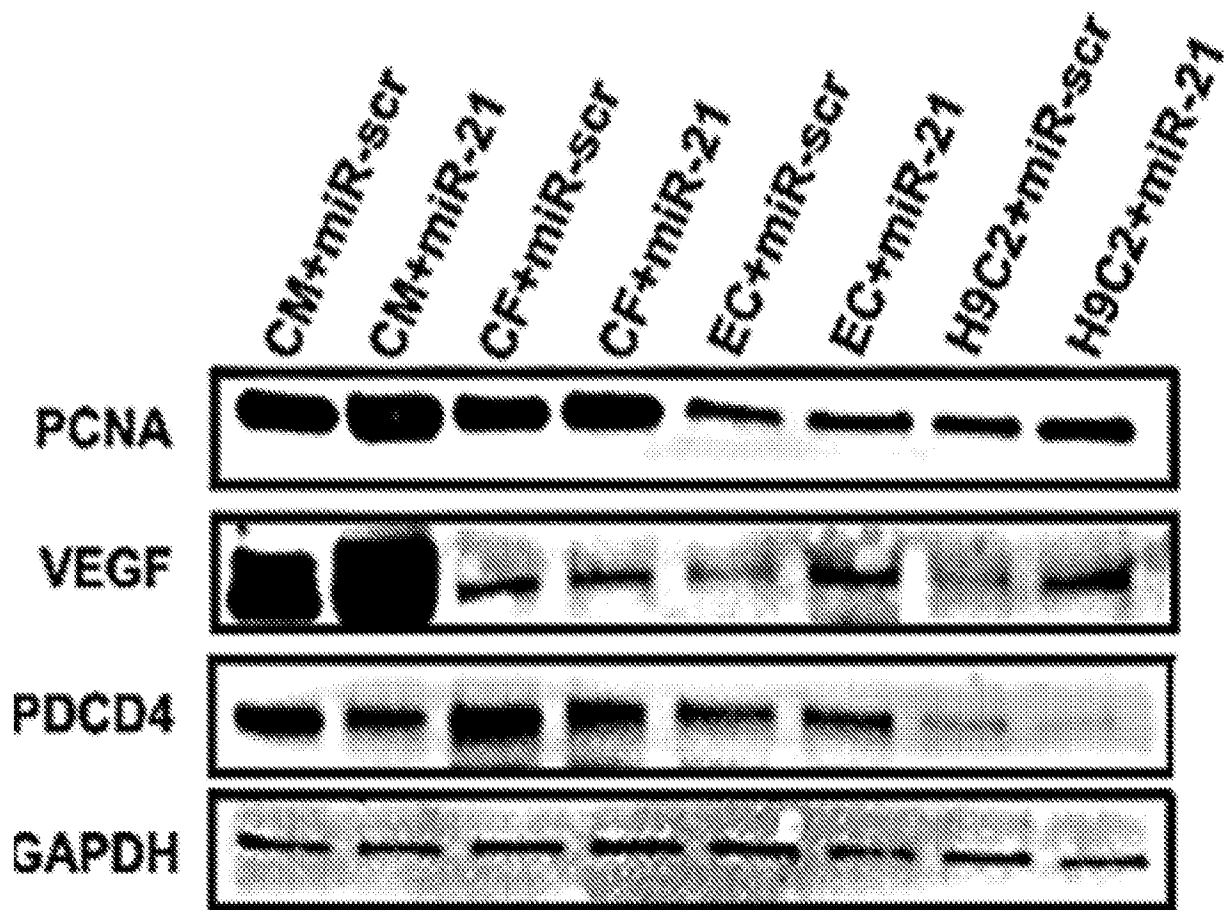
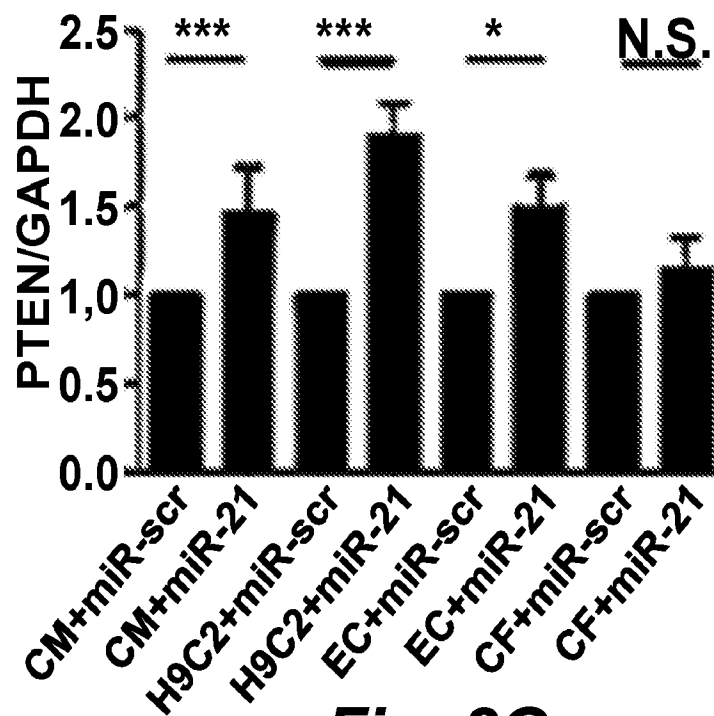
Fig. 8A

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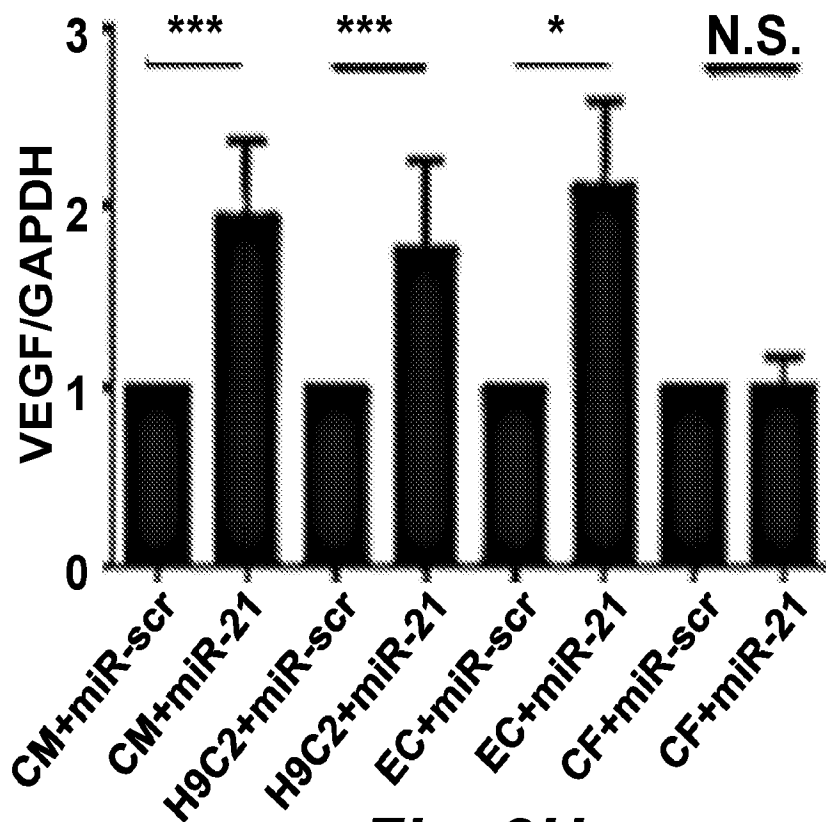
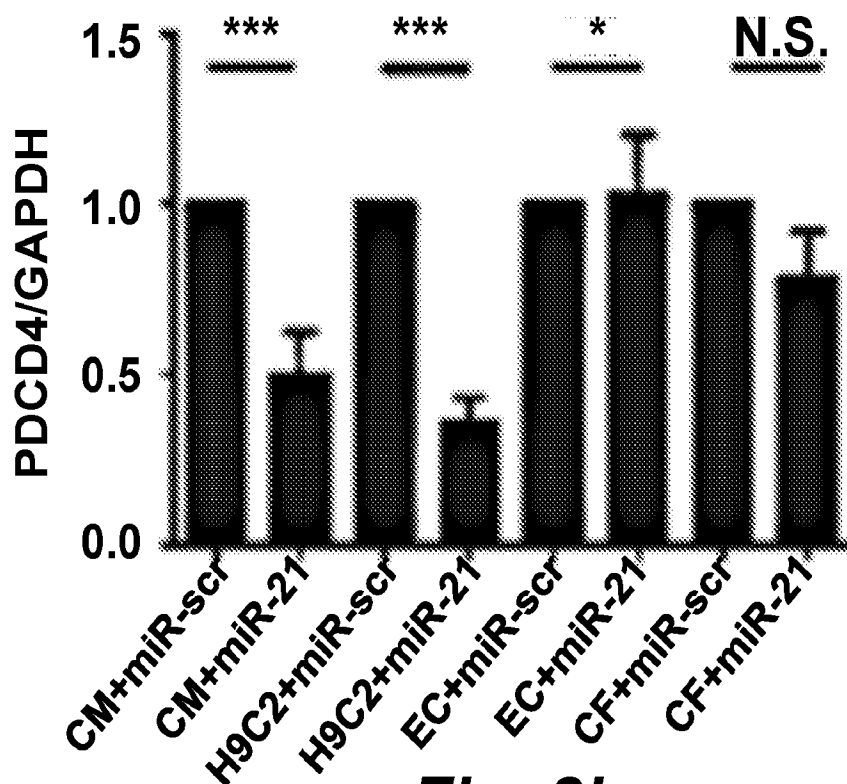
**Fig. 8B****Fig. 8C**

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**Fig. 8D****Fig. 8E**

**Fig. 8F****Fig. 8G**

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*Fig. 8H**Fig. 8I*

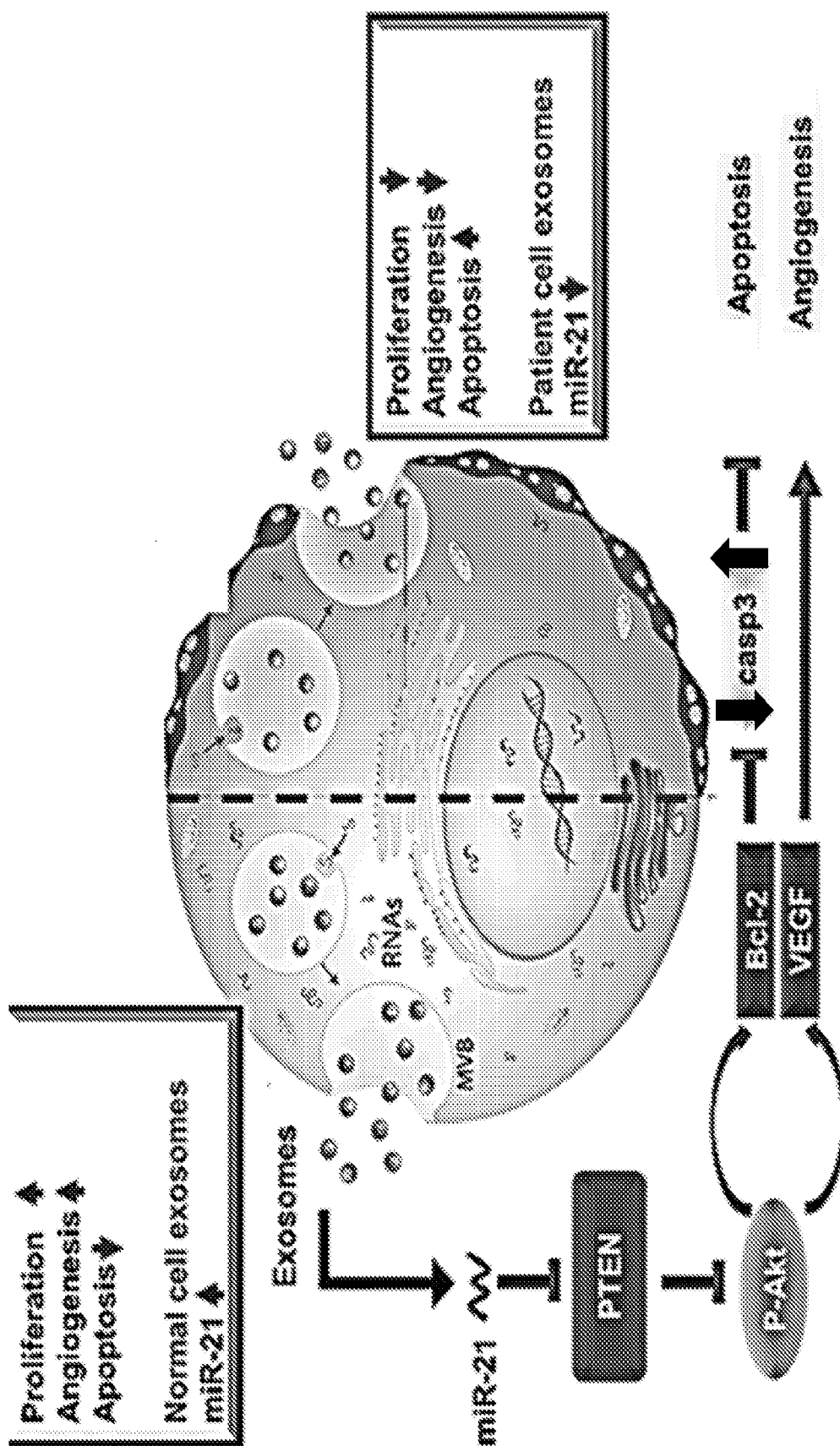
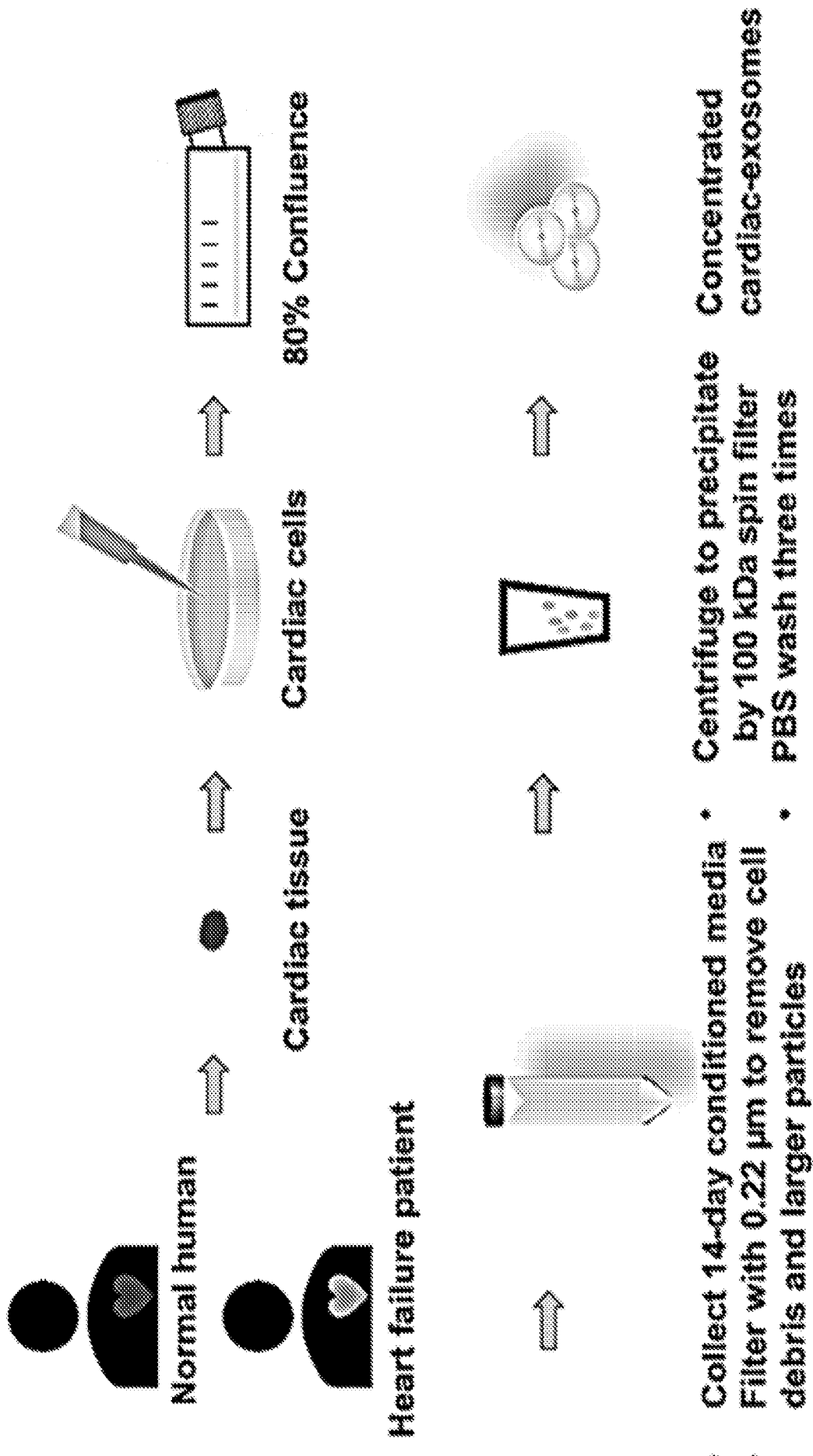
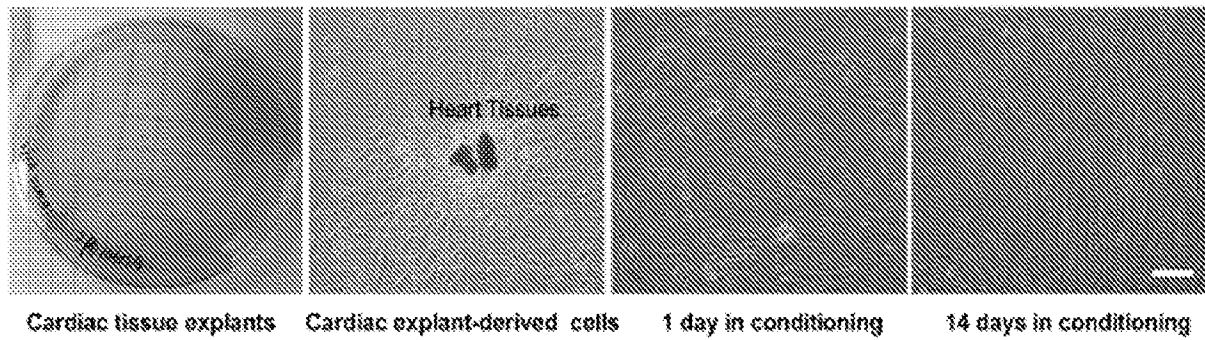
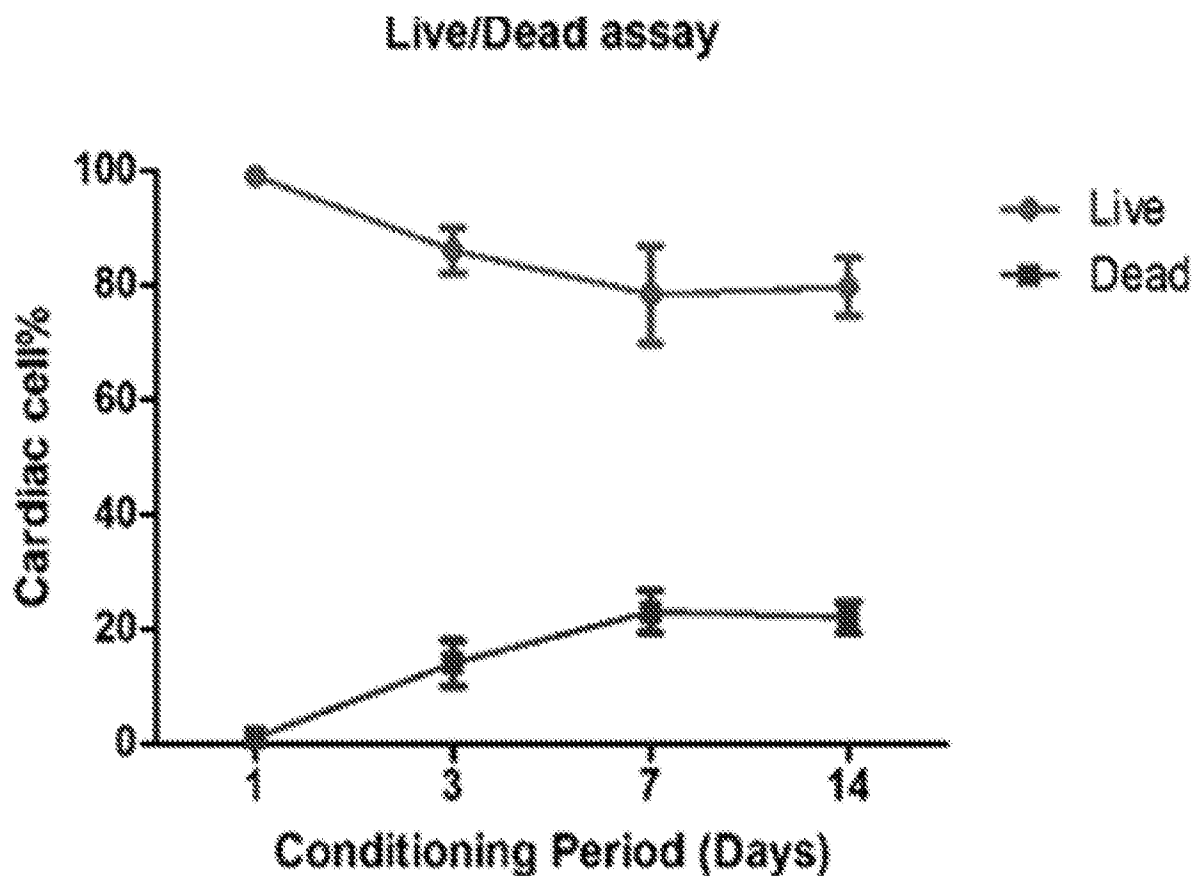


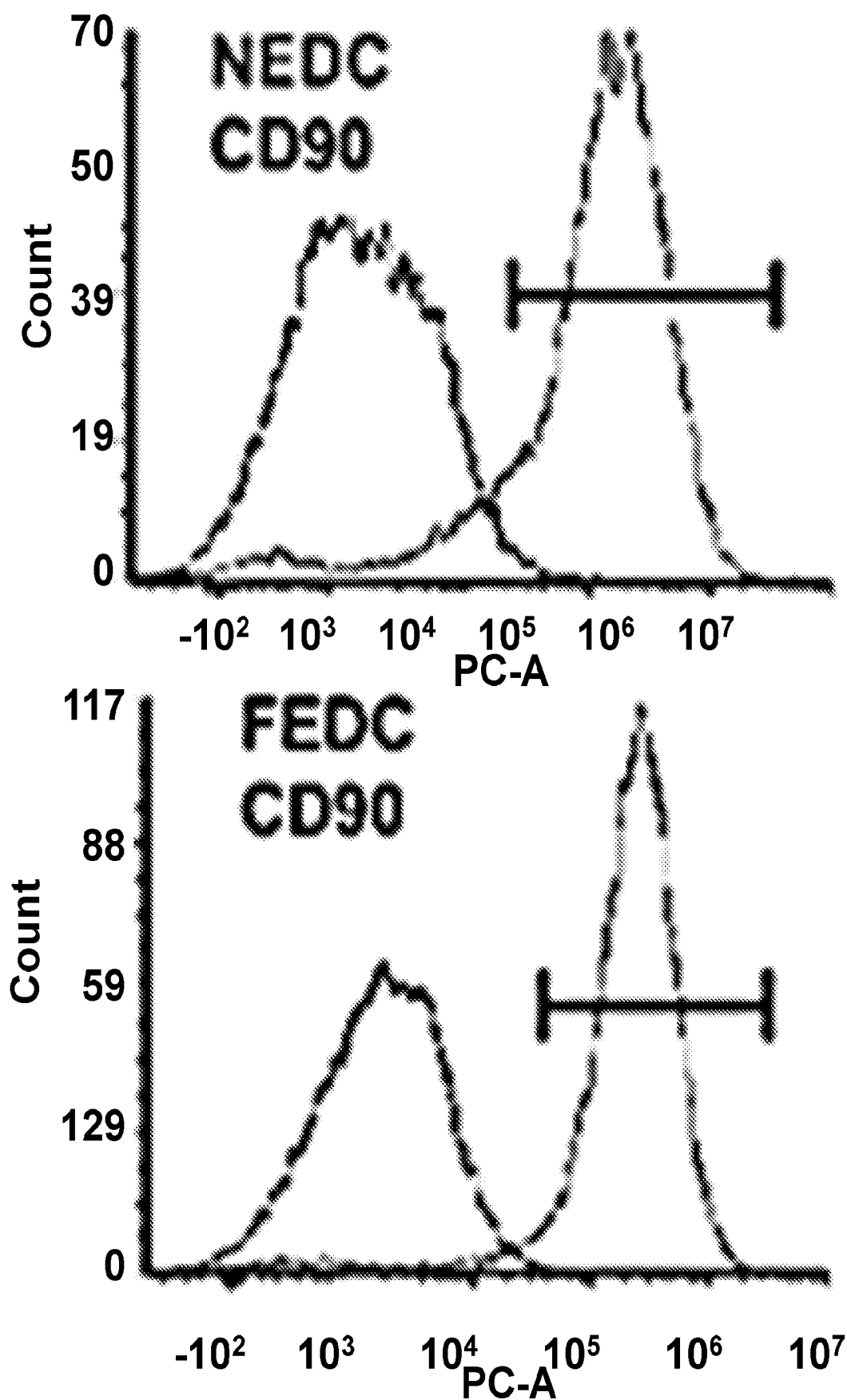
Fig. 8J



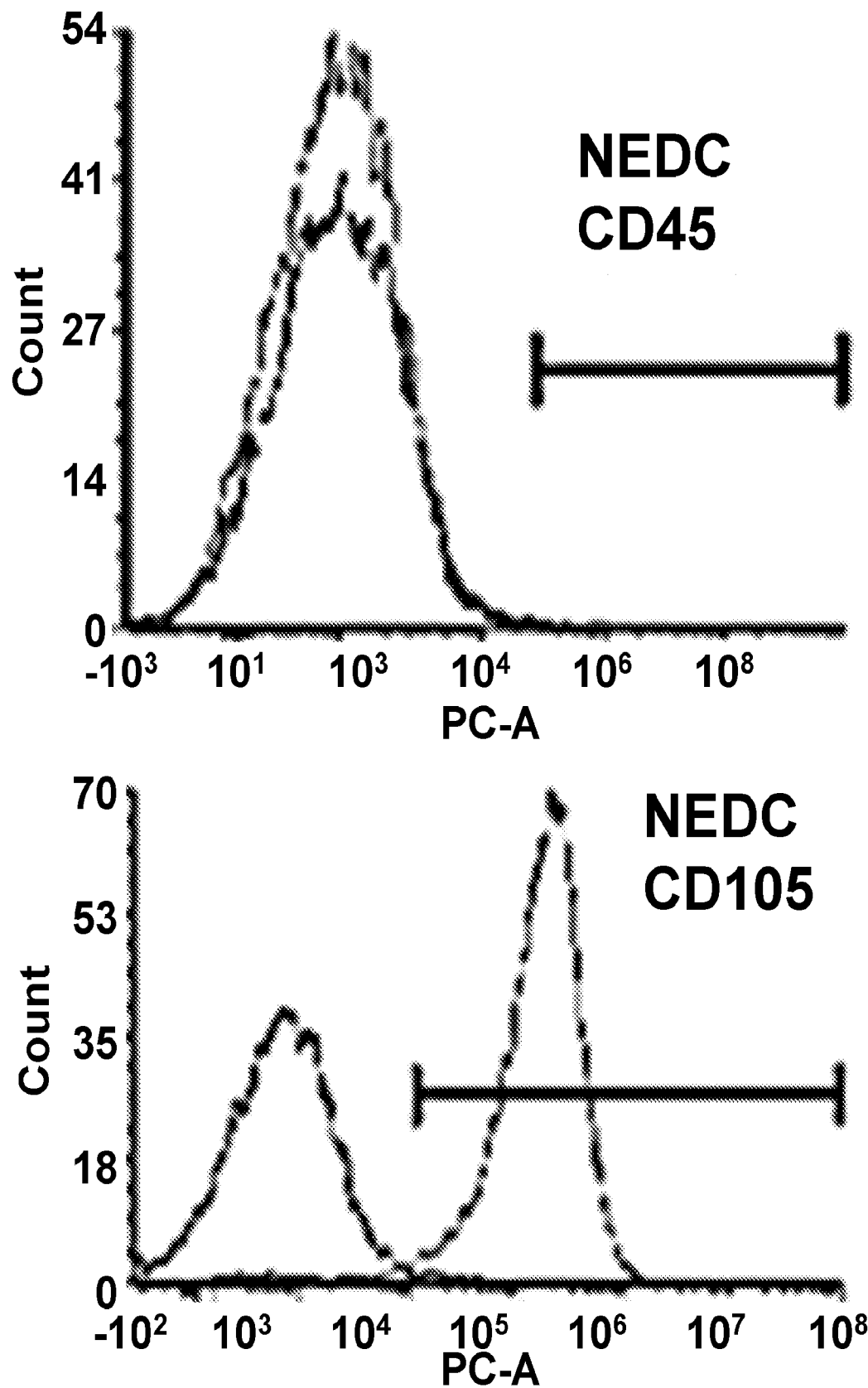
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**Fig. 9B****Fig. 9C**

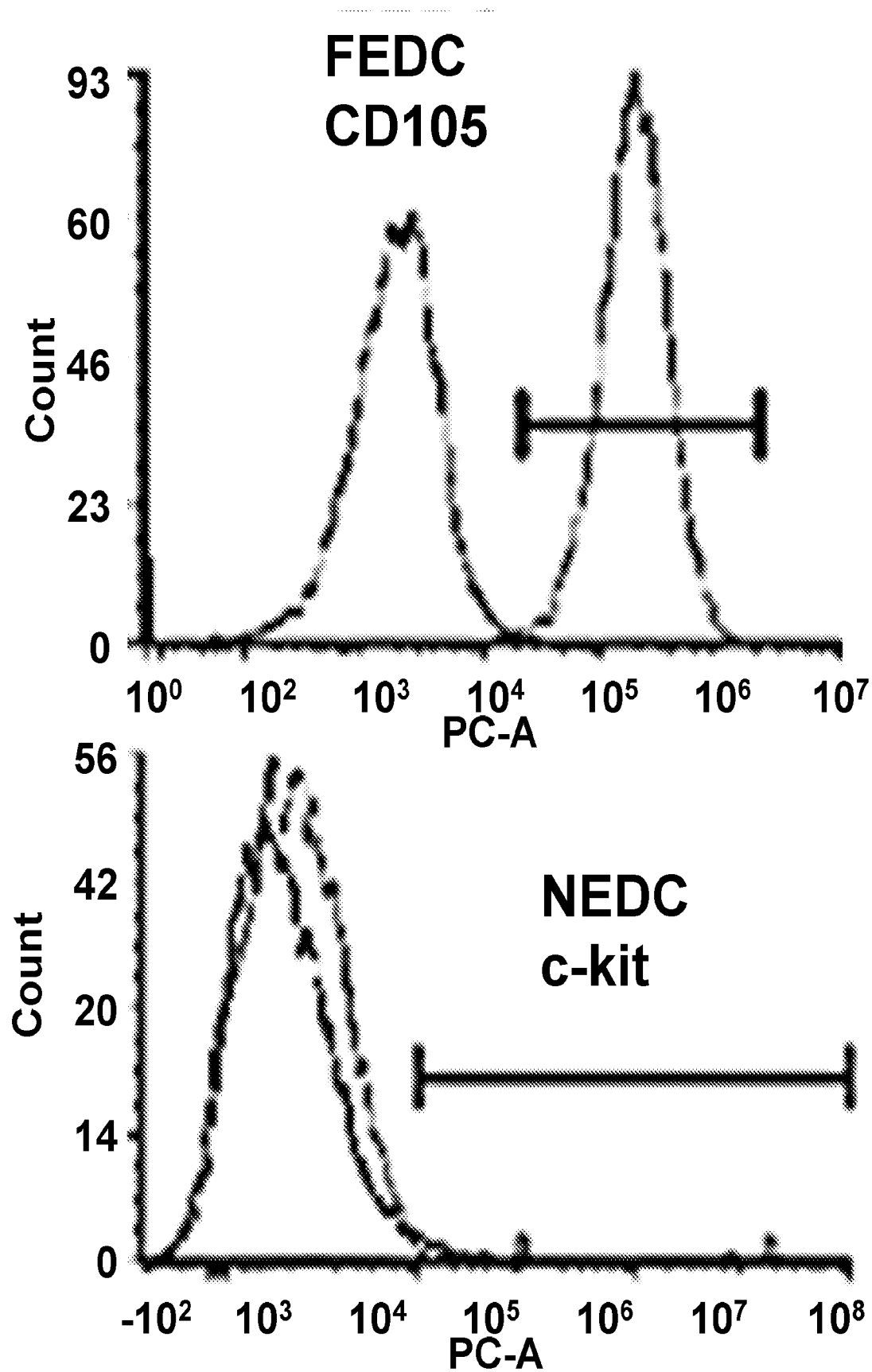
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**Fig. 10**

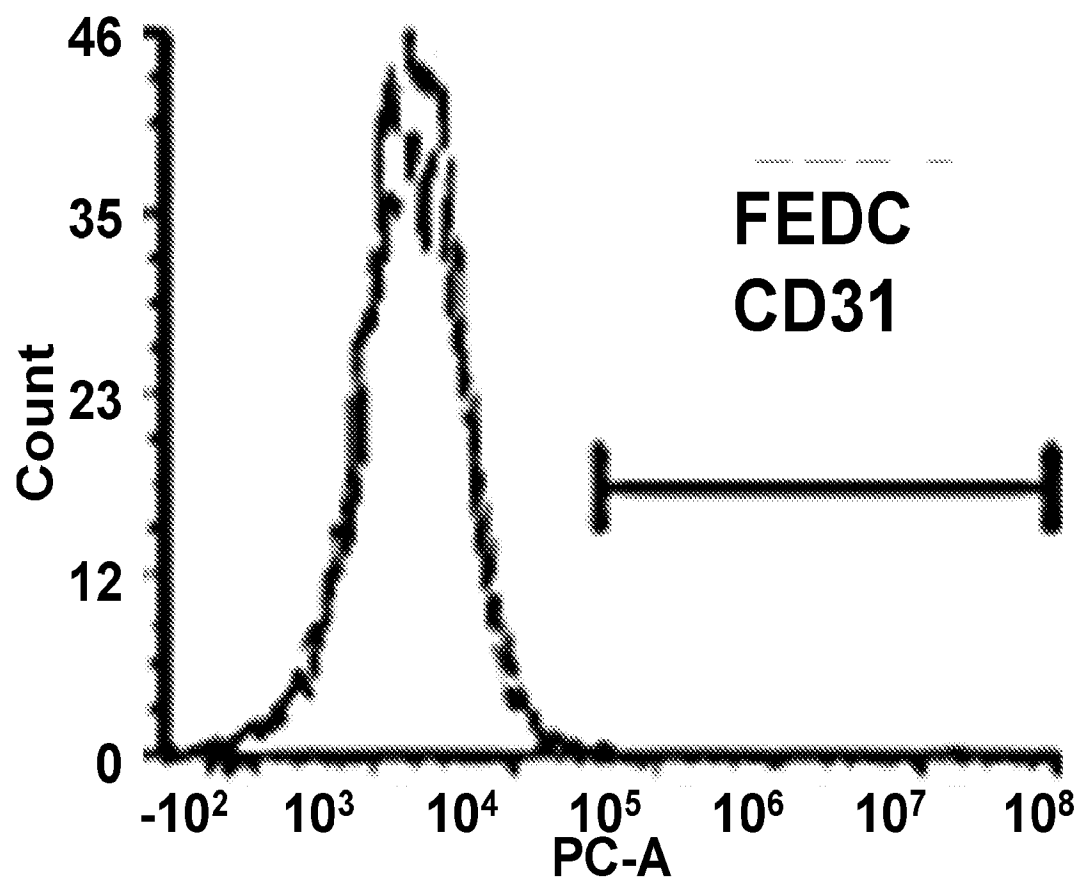
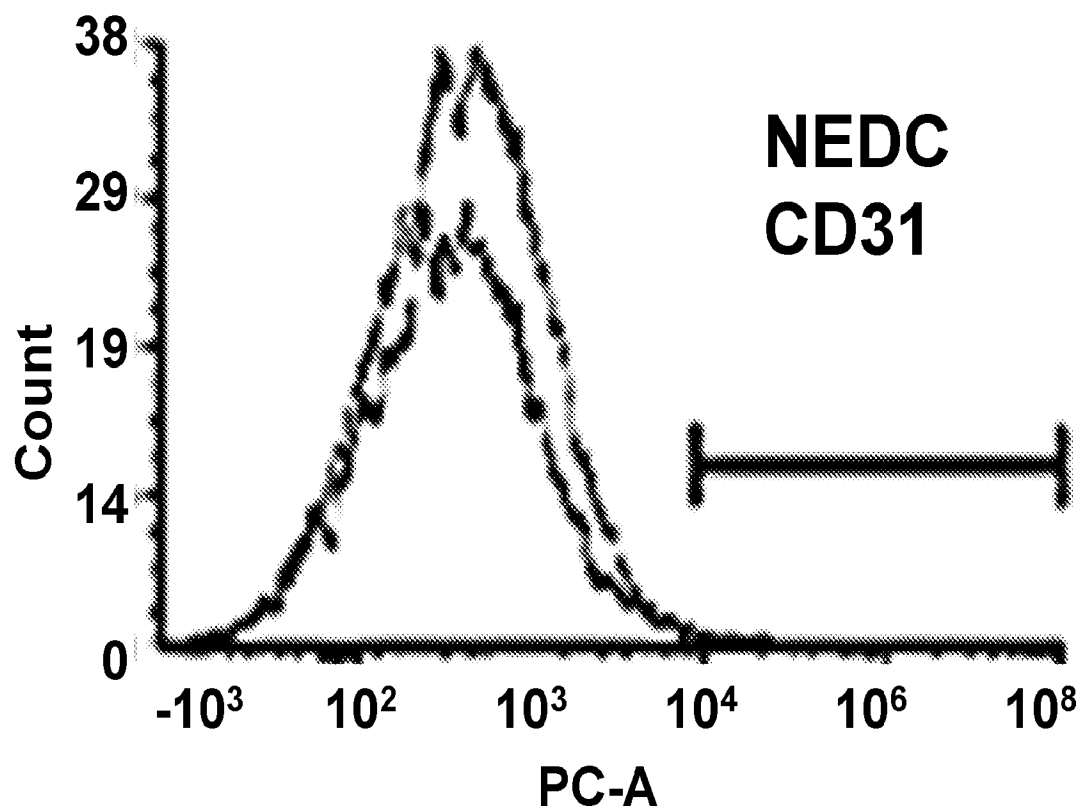
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**Fig. 10-Cont'd**

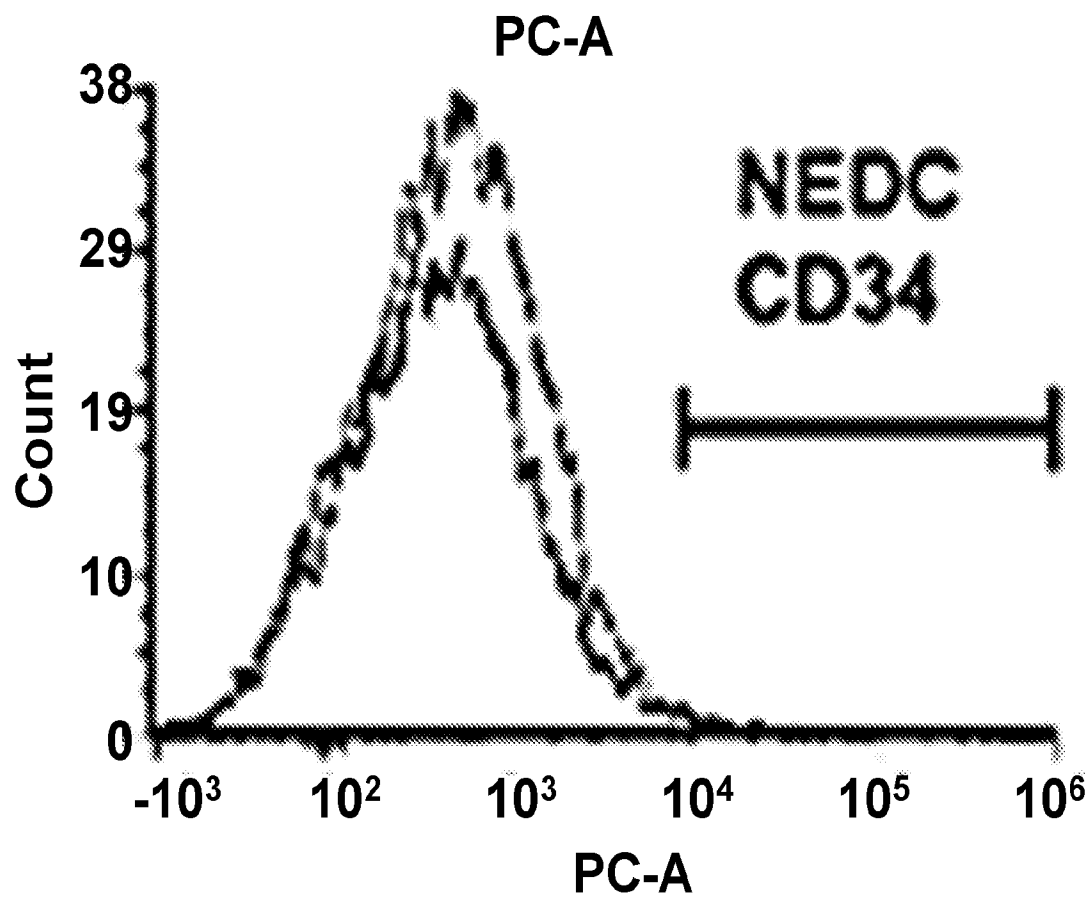
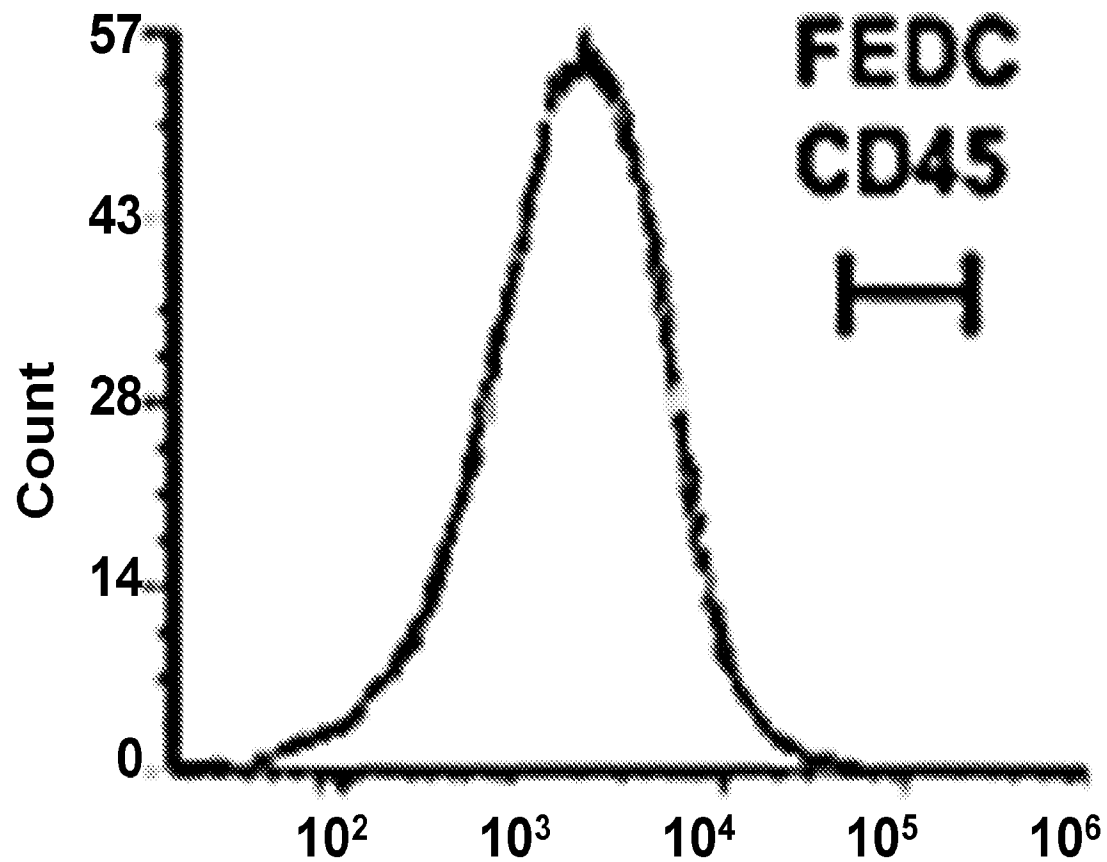
30/46

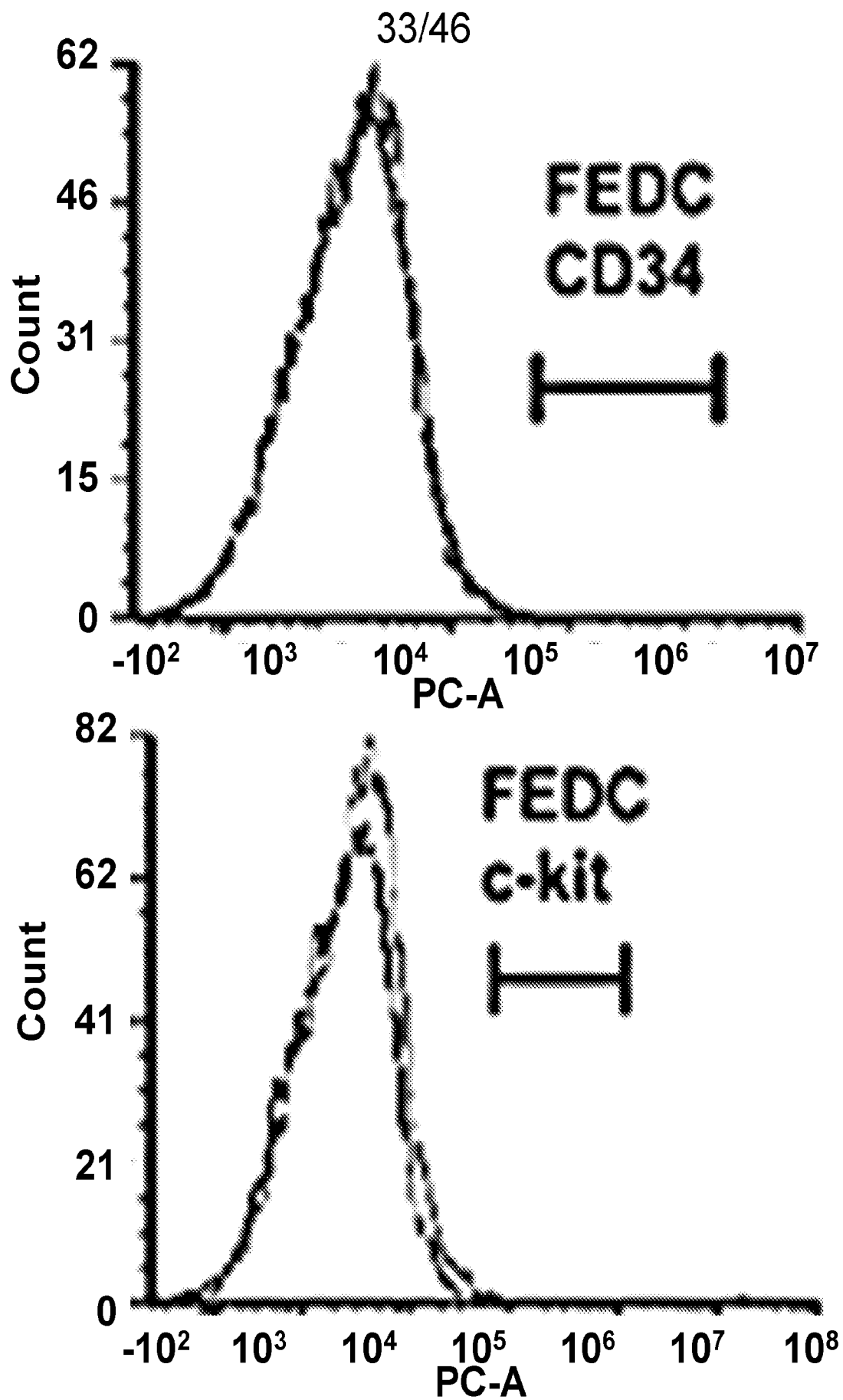
***Fig. 10-Cont'd***

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**Fig. 10-Cont'd**

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***Fig. 10-Cont'd***

**Fig. 10-Cont'd**

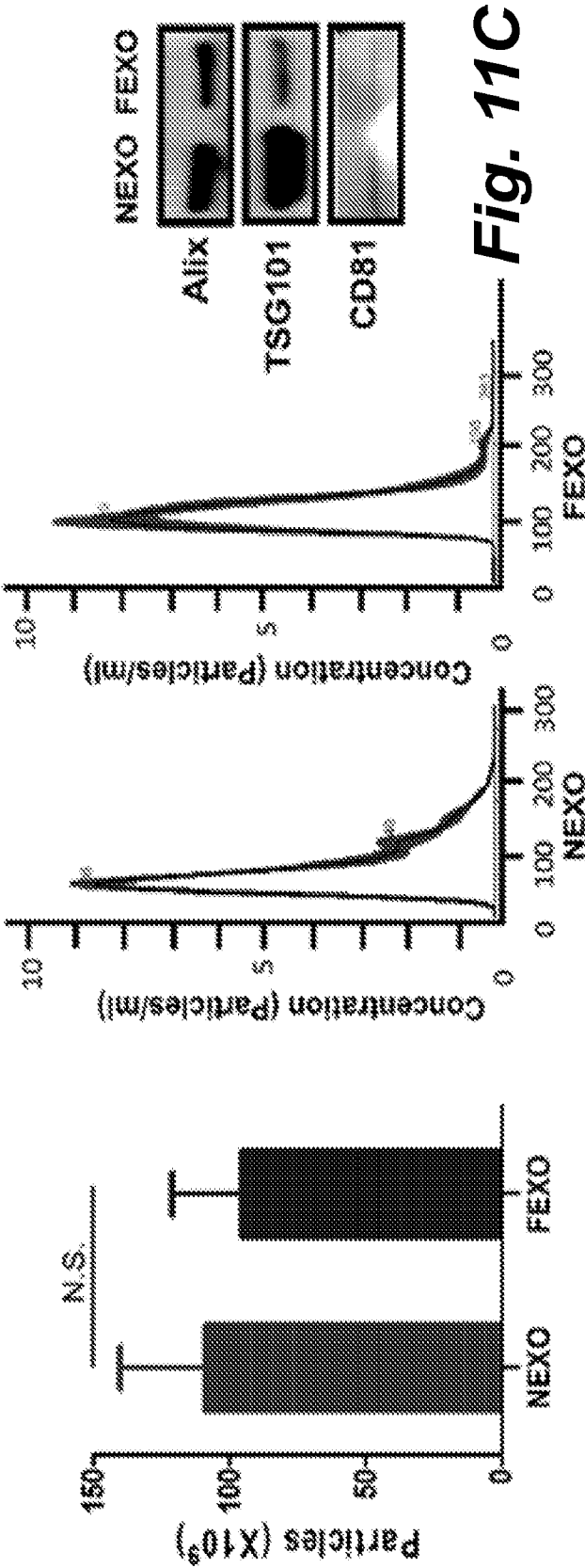


Fig. 11A

Fig. 11B

Fig. 11C

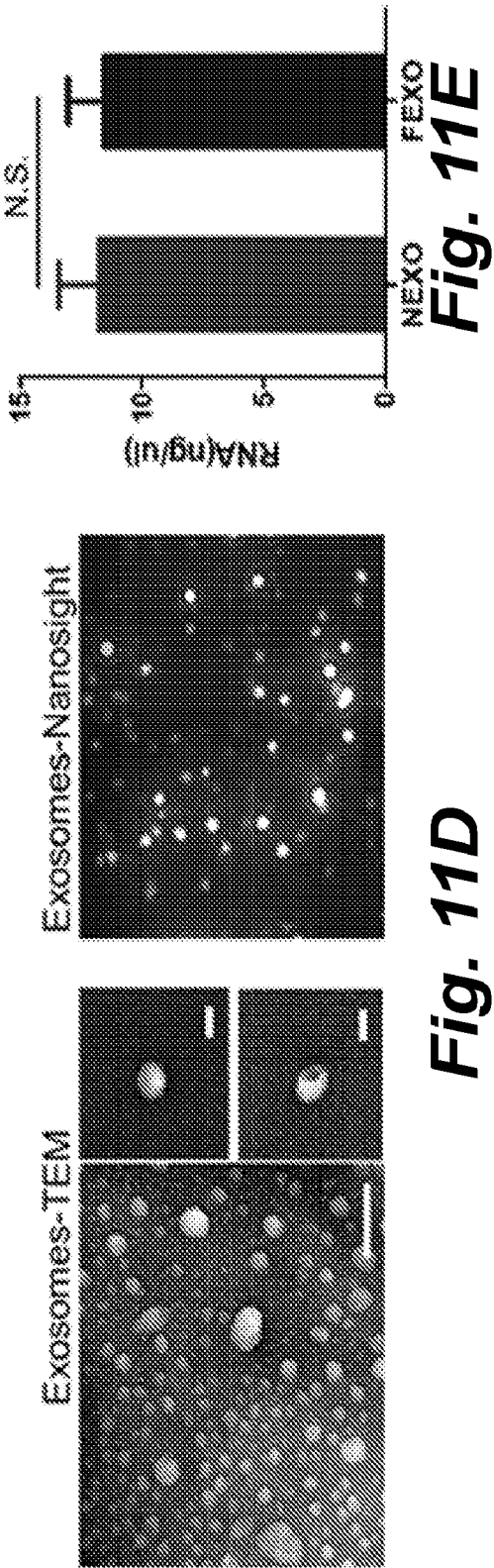


Fig. 11D

Fig. 11E

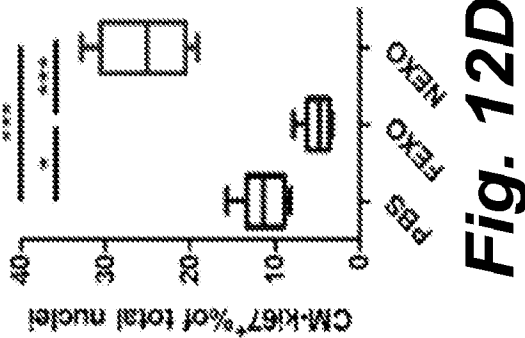


Fig. 12D

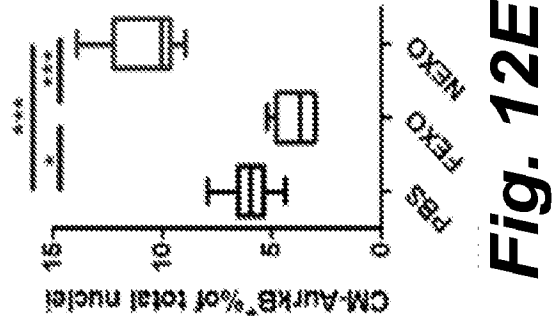


Fig. 12E

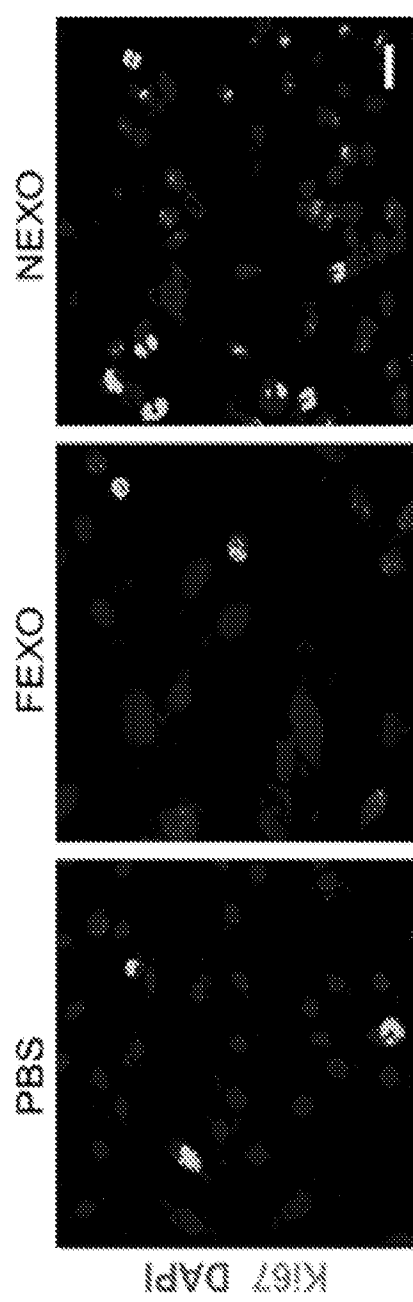


Fig. 12A

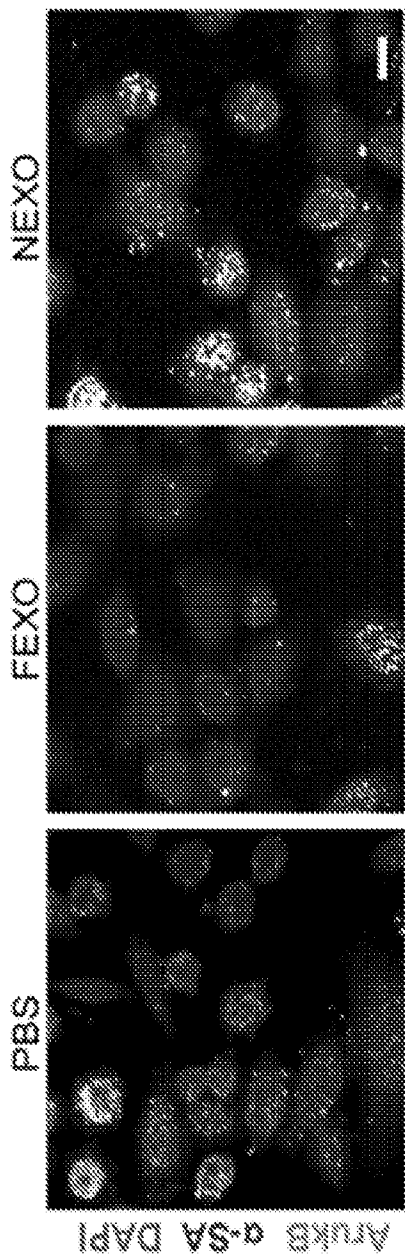


Fig. 12B

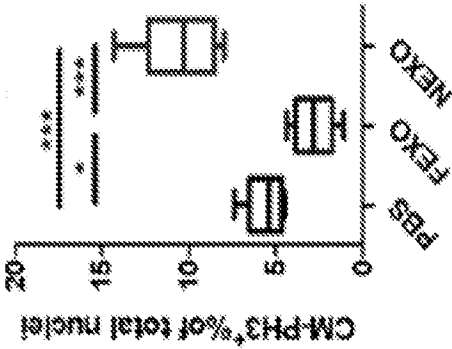


Fig. 12F

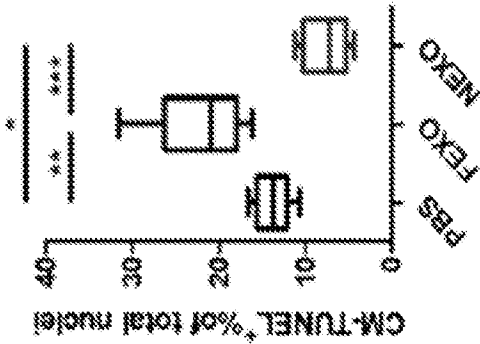


Fig. 12H

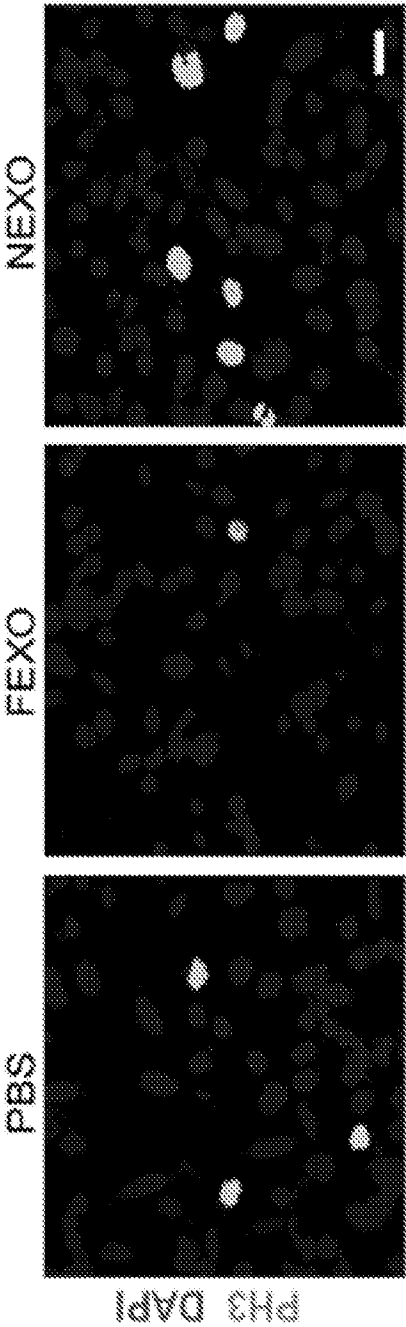


Fig. 12C

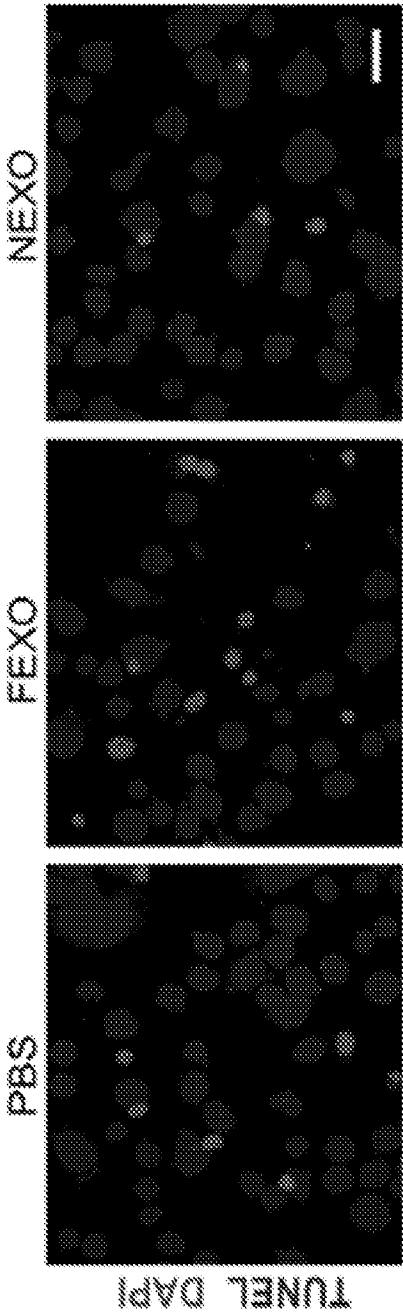
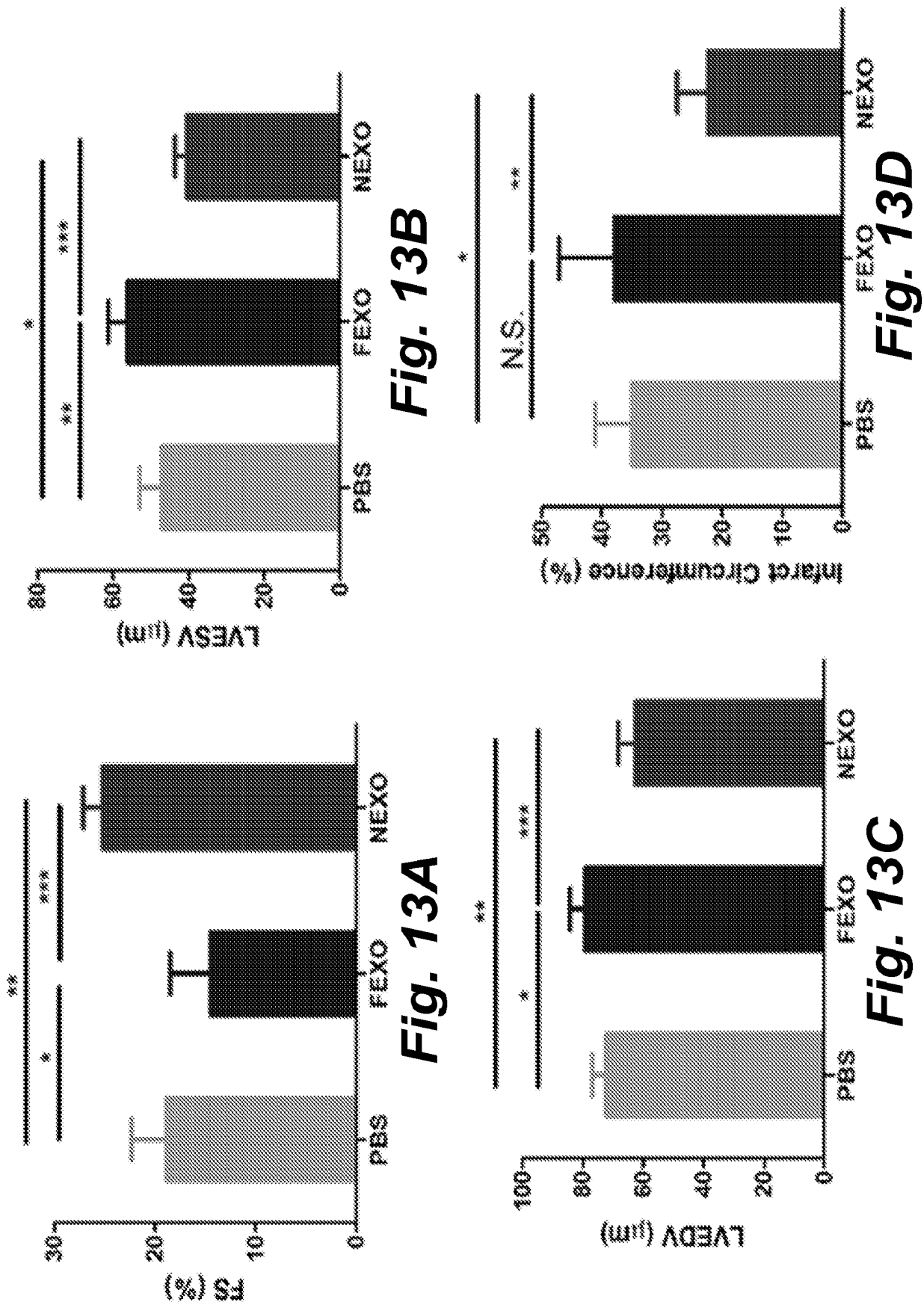
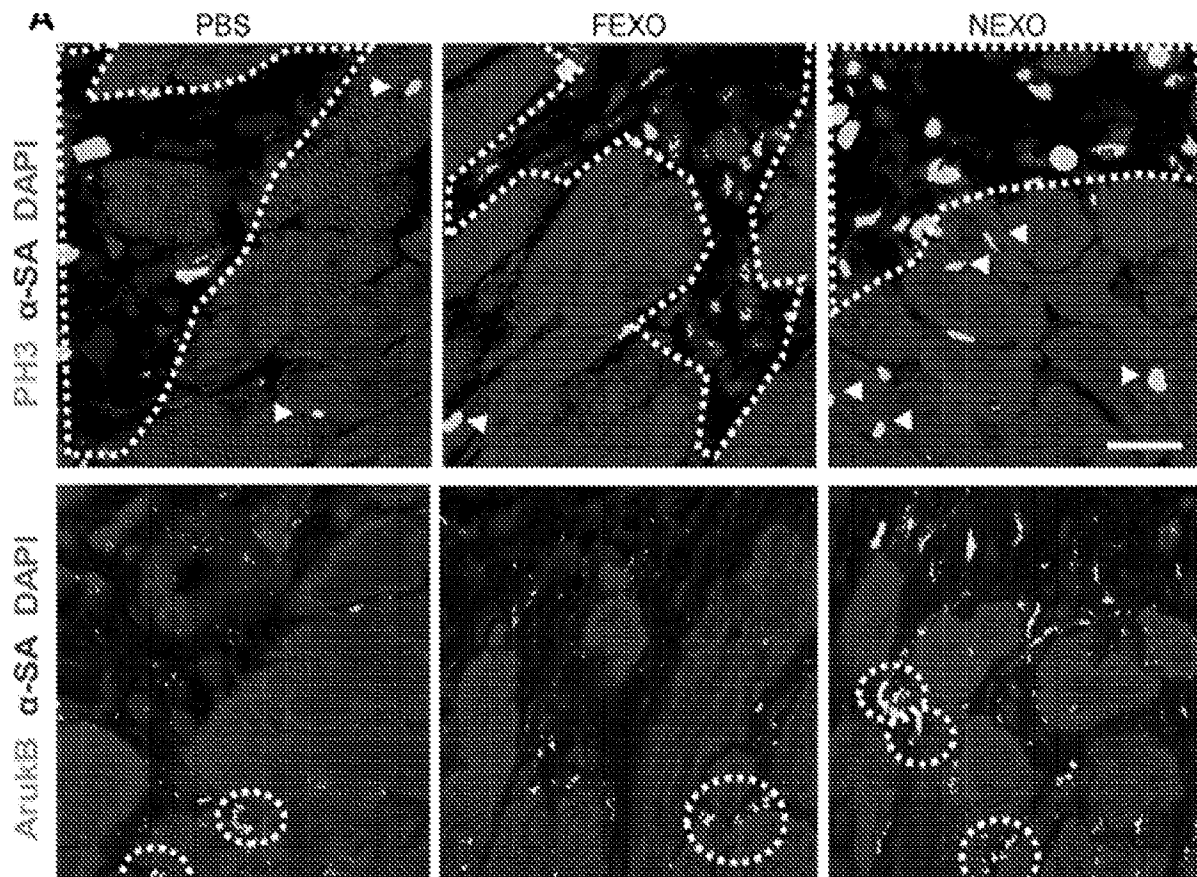
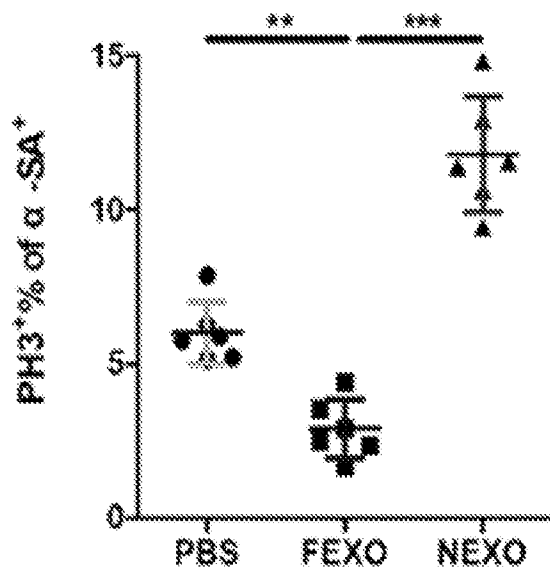
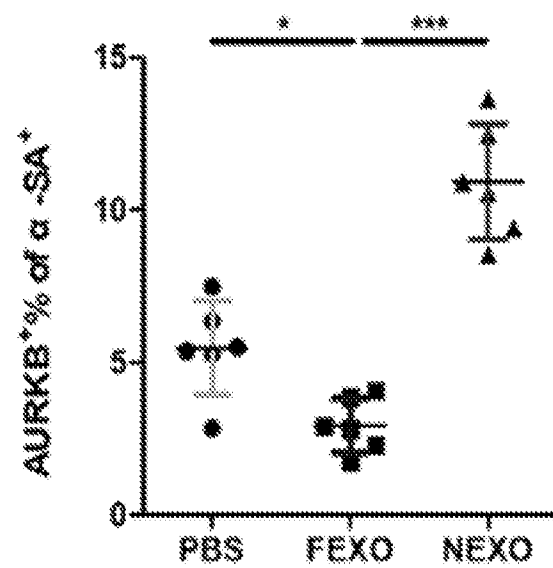


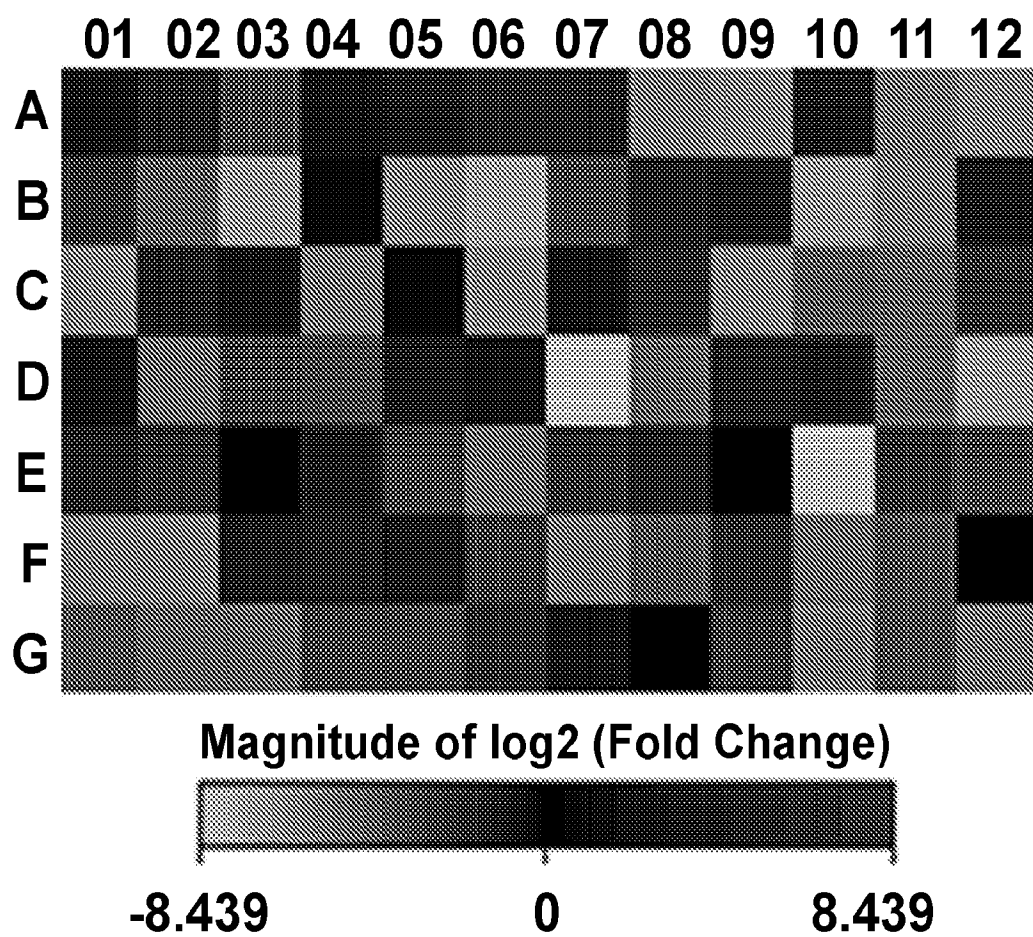
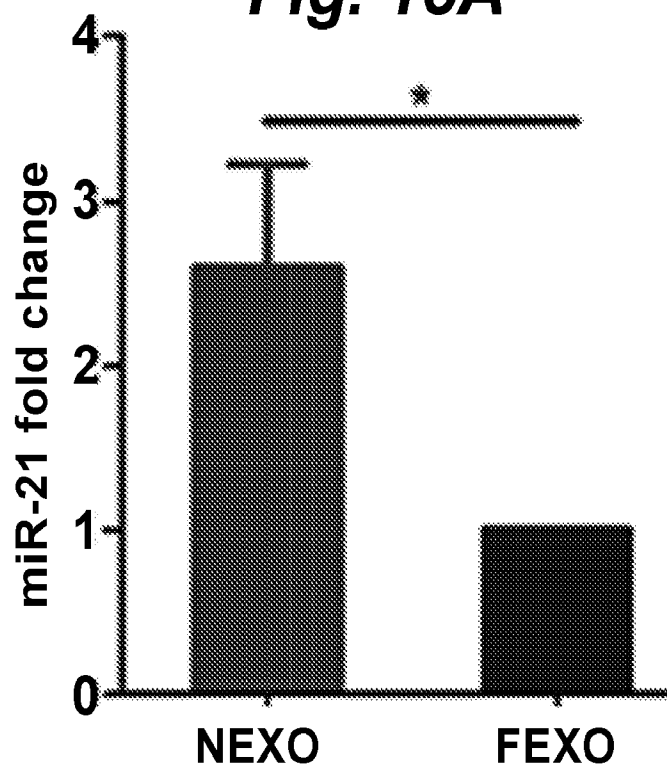
Fig. 12G



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**Fig. 14A****Fig. 14B****Fig. 14C**

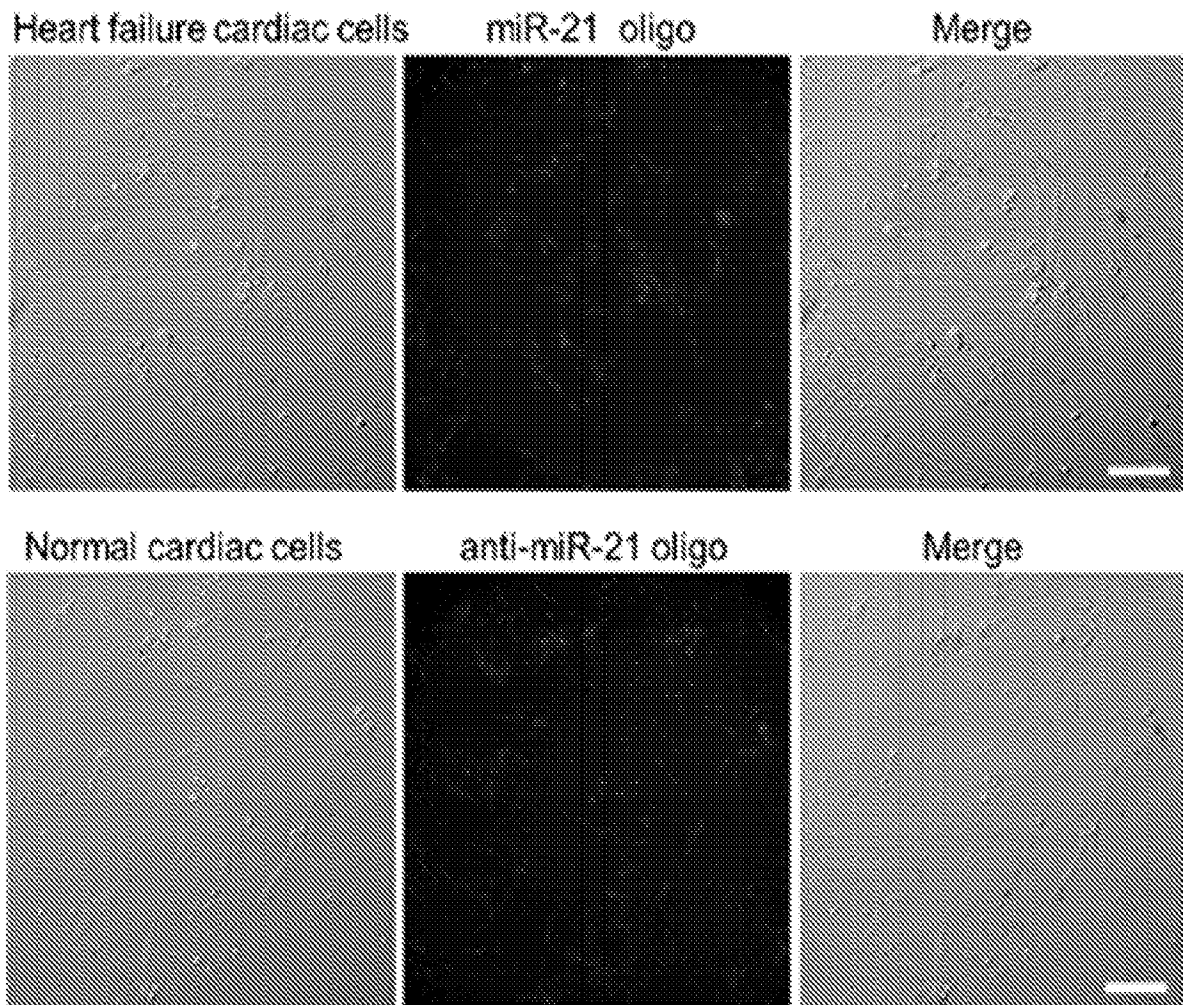
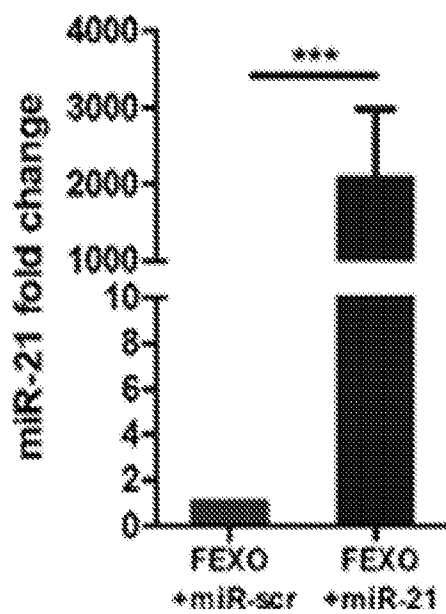
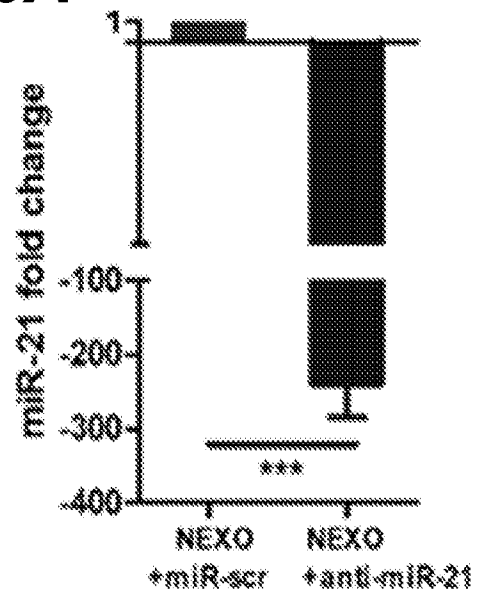
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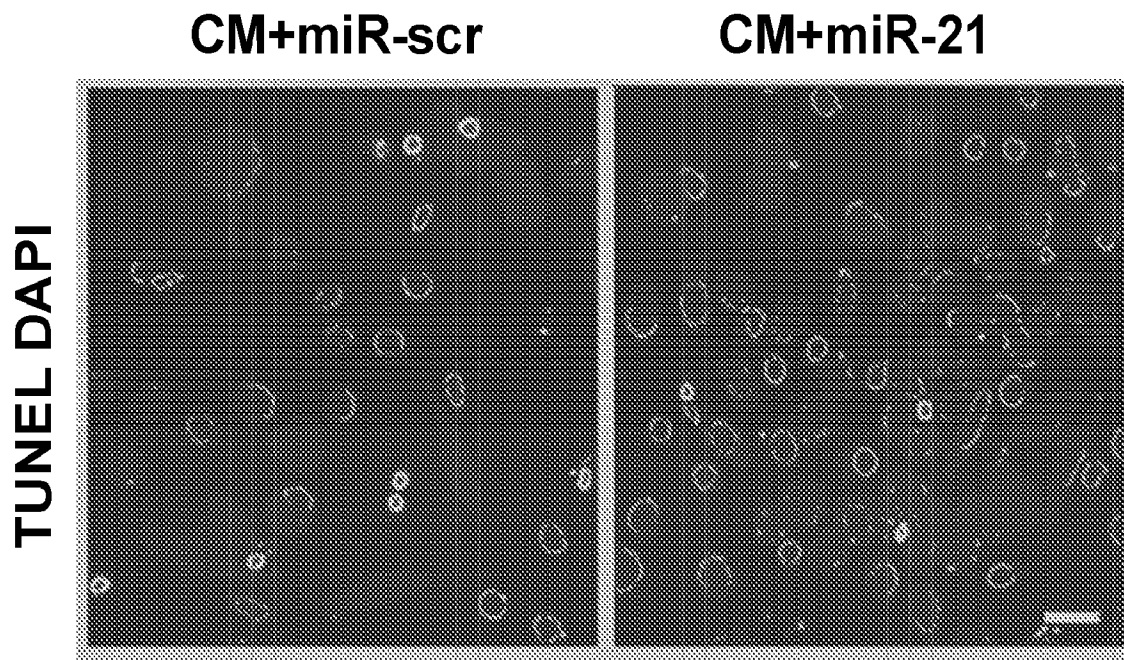
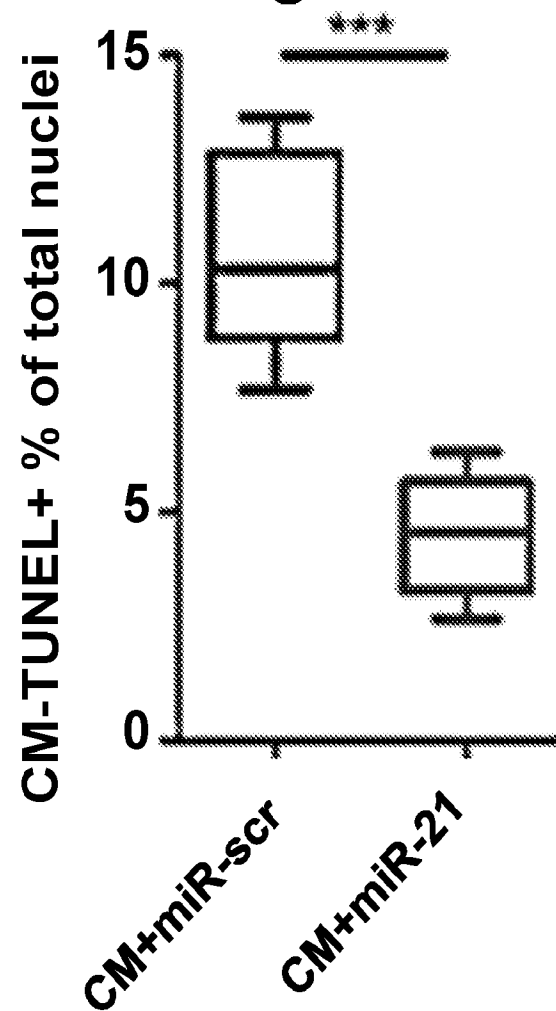
**Fig. 15A****Fig. 15C**

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hsa-let-7a- 5p	hsa-let-7b- 6p	hsa-let-7c- 6p	hsa-let-7d- 7p	hsa-let-7e- 7p	hsa-let-7f- 8p	hsa-miR-1- 3p	hsa-miR-1- 100-5p	hsa-miR- 103a-3p	hsa-miR- 107	hsa-miR- 10b-5p	hsa-miR- 122-5p
B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12
hsa-miR-124- 3p	hsa-miR- 125b-5p	hsa-miR- 125b-5p	hsa-miR- 126-3p	hsa-miR- 130a-3p	hsa-miR- 133a-3p	hsa-miR- 133b	hsa-miR- 140-5p	hsa-miR- 142-3p	hsa-miR- 143-3p	hsa-miR- 144-3p	hsa-miR- 145-5p
C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
hsa-miR-146- 5p	hsa-miR- 149-5p	hsa-miR- 150-5p	hsa-miR- 155-5p	hsa-miR- 15b-5p	hsa-miR- 16-5p	hsa-miR- 17-5p	hsa-miR- 181a-5p	hsa-miR- 181b-5p	hsa-miR- 182-5p	hsa-miR- 183-5p	hsa-miR- 185-5p
D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12
hsa-miR-18b- 5p	hsa-miR- 195-5p	hsa-miR- 199-5p	hsa-miR- 206	hsa-miR- 208a-3p	hsa-miR- 208b-3p	hsa-miR- 21-5p	hsa-miR- 26b-4p	hsa-miR- 214-3p	hsa-miR- 22-3p	hsa-miR- 221-3p	hsa-miR- 222-3p
E01	E02	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12
hsa-miR-223- 3p	hsa-miR- 224-5p	hsa-miR- 23a-3p	hsa-miR- 23b-3p	hsa-miR- 24-3p	hsa-miR- 25-3p	hsa-miR- 26a-5p	hsa-miR- 210-3p	hsa-miR- 27a-3p	hsa-miR- 27b-3p	hsa-miR- 29a-3p	hsa-miR- 29b-3p
F01	F02	F03	F04	F05	F06	F07	F08	F09	F10	F11	F12
hsa-miR-29c- 3p	hsa-miR- 302a-3p	hsa-miR- 302b-3p	hsa-miR- 30a-5p	hsa-miR- 30c-5p	hsa-miR- 30d-5p	hsa-miR- 30a-5p	hsa-miR- 31-5p	hsa-miR- 320a	hsa-miR- 328-3p	hsa-miR- 342-3p	hsa-miR- 365a-3p
G01	G02	G03	G04	G05	G06	G07	G08	G09	G10	G11	G12
hsa-miR- 378a-3p	hsa-miR- 423-3p	hsa-miR- 424-5p	hsa-miR- 451a	hsa-miR- 3486-5p	hsa-miR- 494-3p	hsa-miR- 499a-5p	hsa-miR- 5p	hsa-miR-7- 92a-3p	hsa-miR- 93-5p	hsa-miR- 98-5p	hsa-miR- 99a-5p

Fig. 15B

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**Fig. 16A****Fig. 16B****Fig. 16C**

**Fig. 17A****Fig. 17B**

vWF RBC DAPI

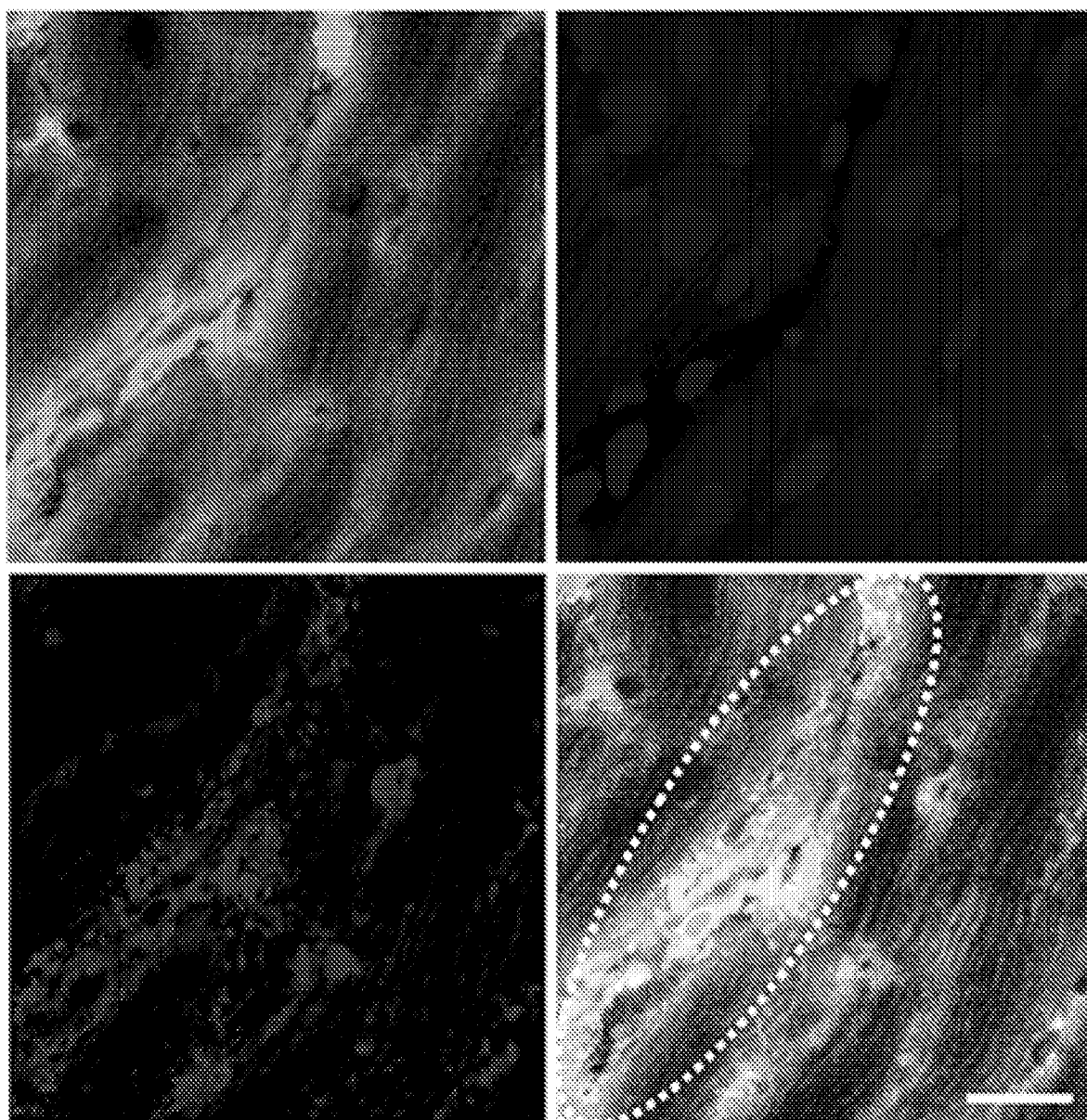
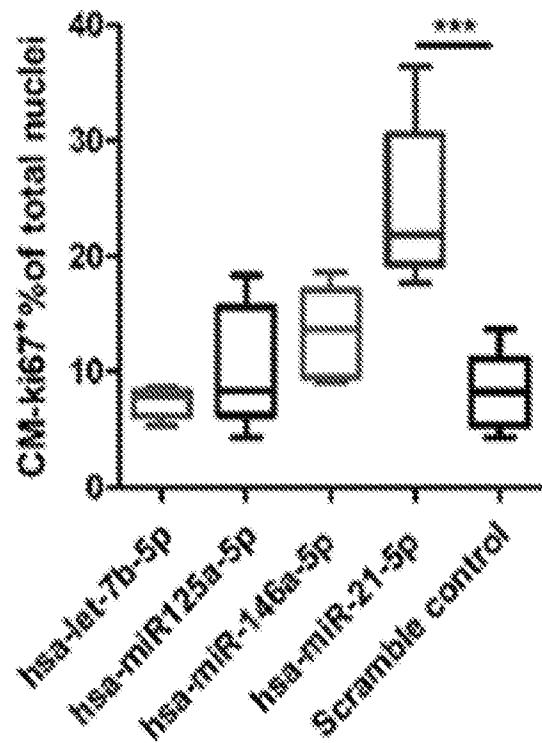
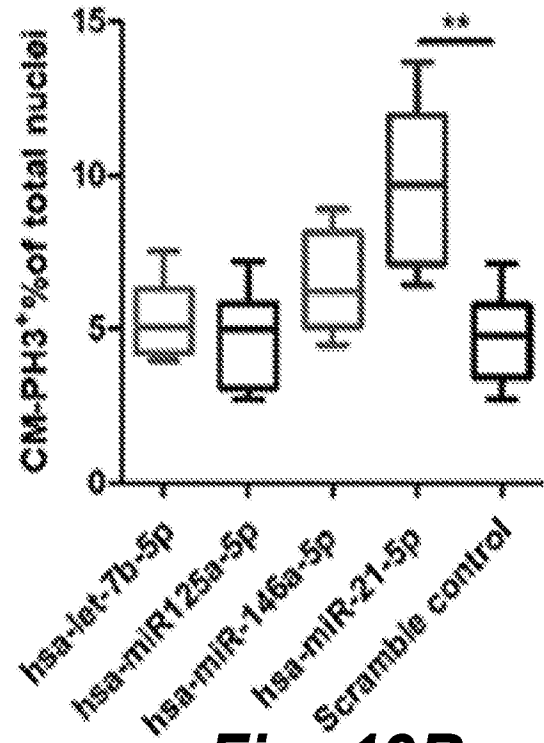
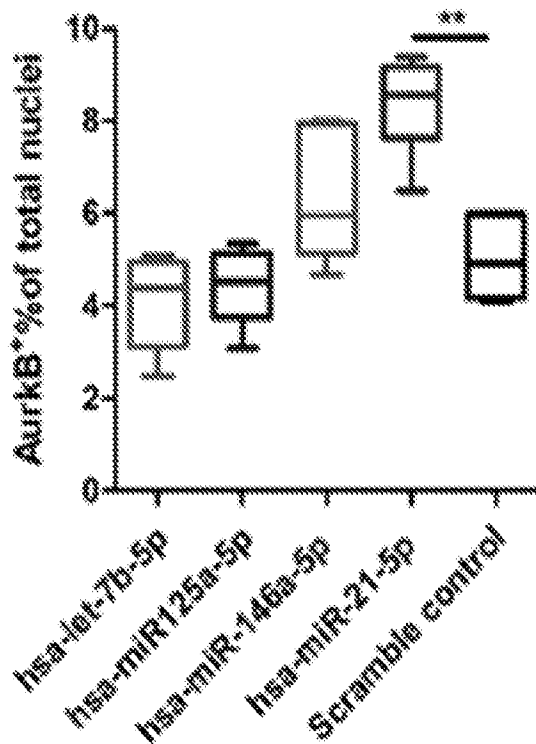
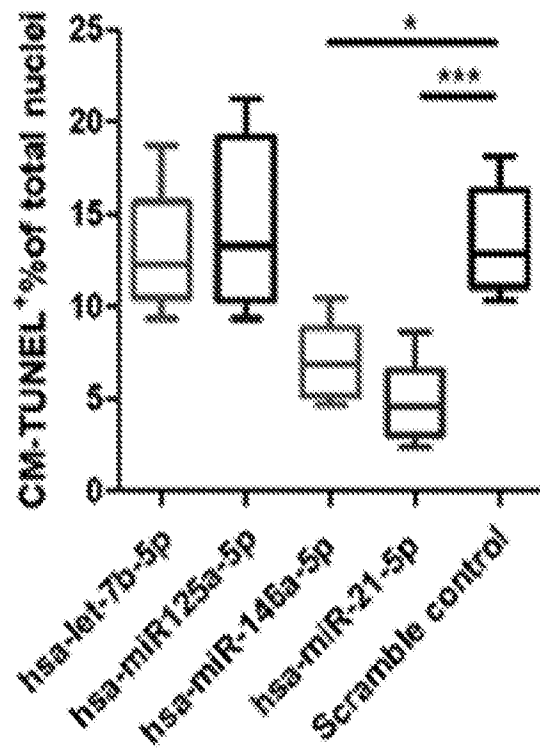
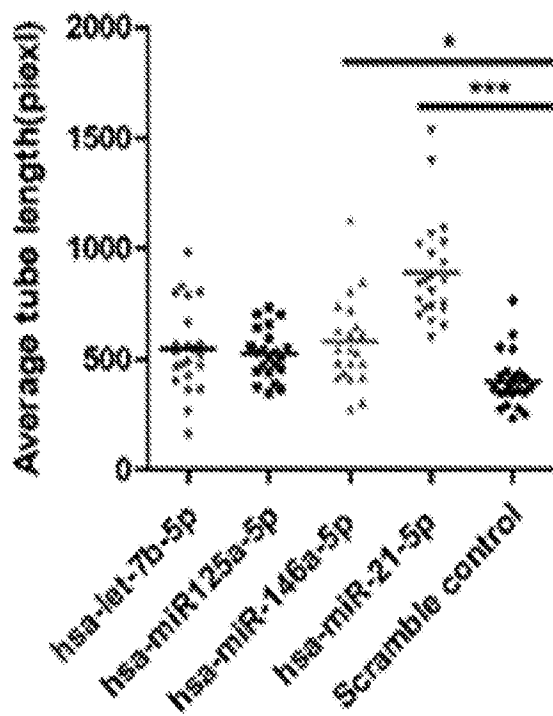
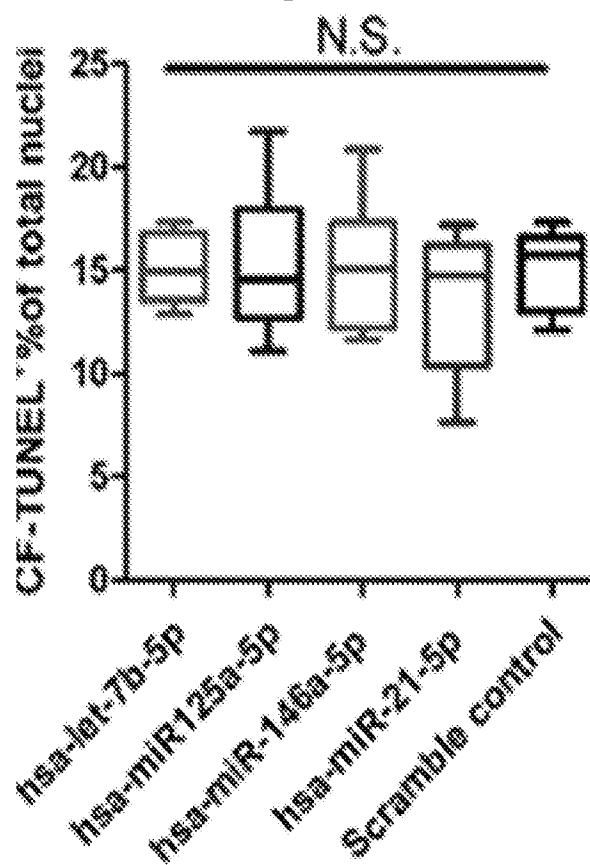
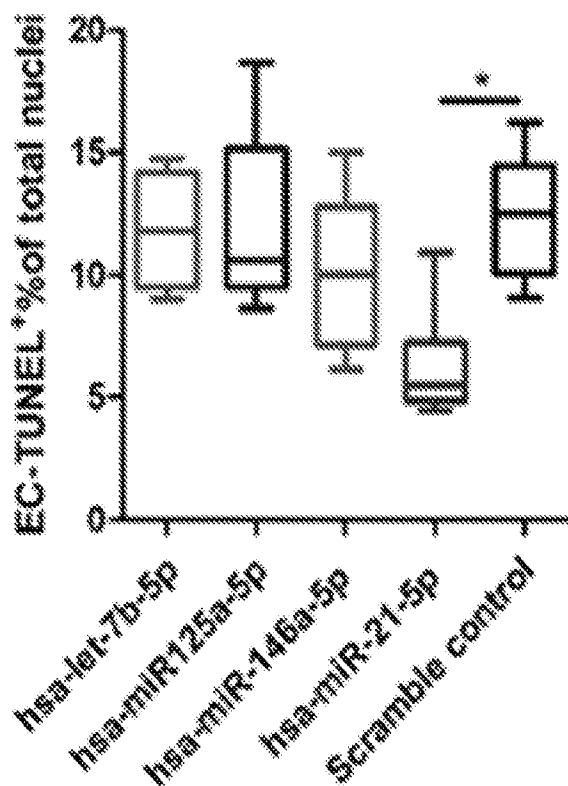


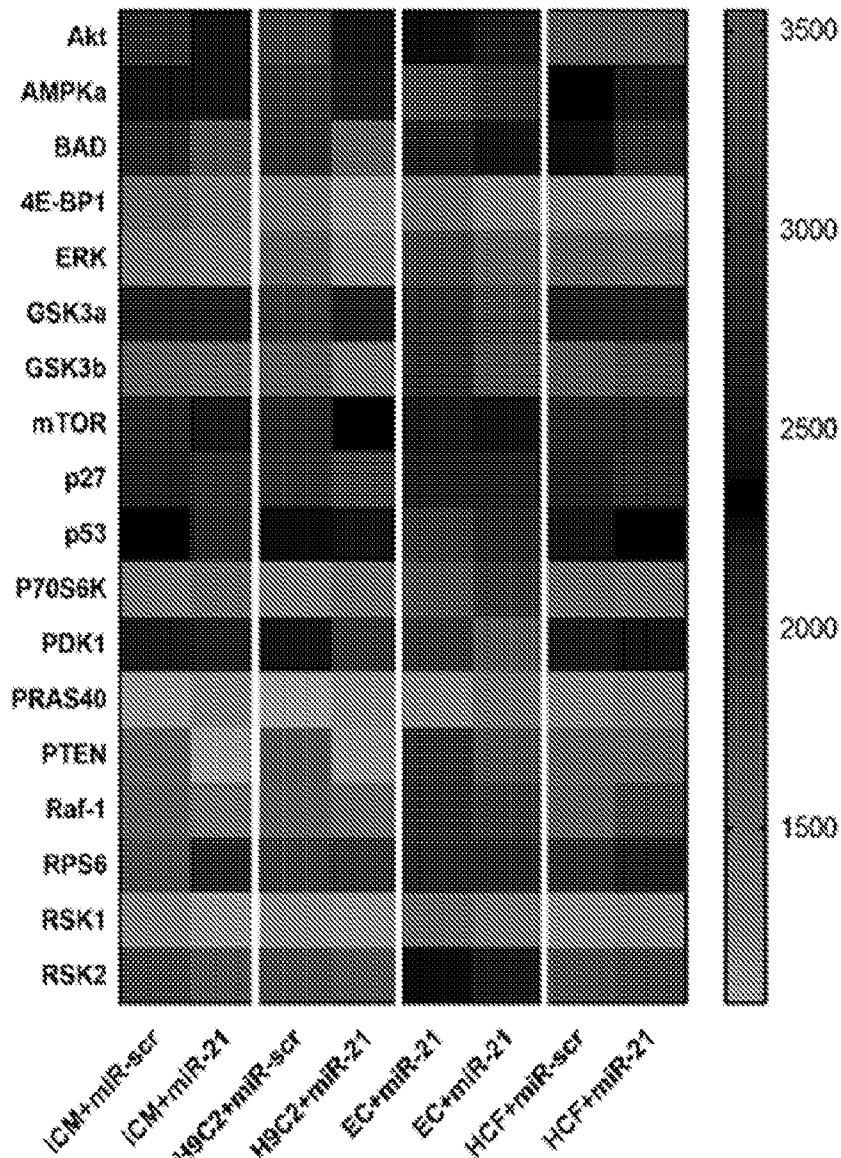
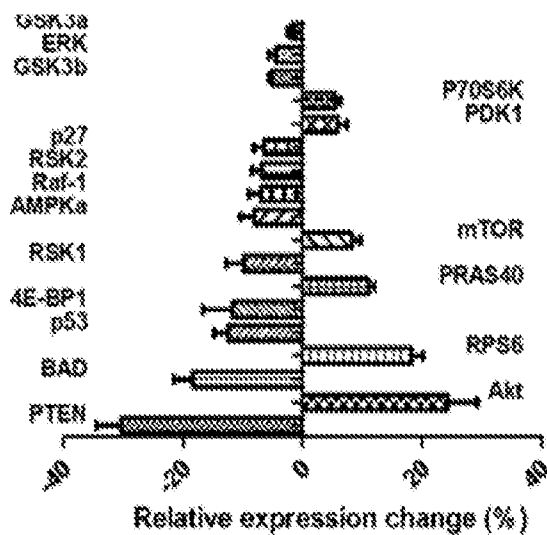
Fig. 18

**Fig. 19A****Fig. 19B****Fig. 19C****Fig. 19D**

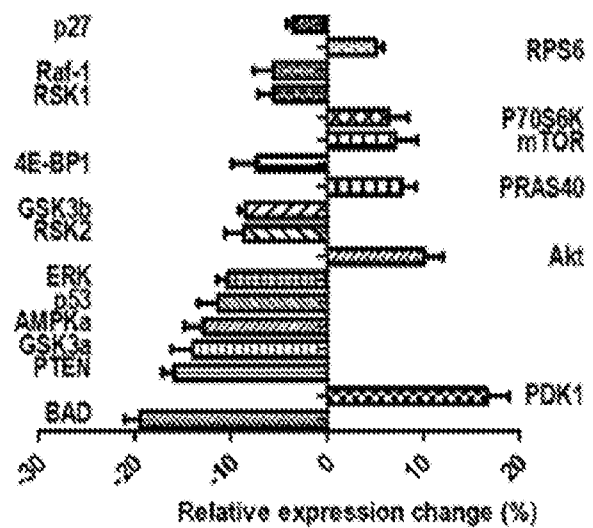
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**Fig. 19E****Fig. 19G****Fig. 19F**

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**Fig. 20A**

Cardiomyocyte

Fig. 20B

Endothelial cell

Fig. 20C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/22524

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 35/34, A61K 47/46, A61P 9/00, A61P 9/10, A61P 9/14, A61P 35/00 (2020.01)

CPC - C12N 2310/141, C12N 15/113, A61K 31/7105, C12N 5/0657, A61K 9/0019, C12N 2502/1329, A61K 47/42, A61P 43/00, A61P 29/00, C12N 2502/28

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018/0100149 A1 (CEDARS-SINAI MEDICAL CENTER) 12 April 2018 (12.04.2018) Abstract; Claim 1; Claim 5; Claim 18; para [0006]; para [0008]; para [0015]; para [0017]; para [0045]; para [0069]; para [0078]; para [0086]	1-6
Y	GenBank Accession No. AJ421741.1, Homo sapiens microRNA miR-21, 11 June 2003 [online]. [Retrieved on 27 June 2020]. Retrieved from the internet: <URL: https://www.ncbi.nlm.nih.gov/nuccore/AJ421741 , Entire document	1-6
Y	ROOIJ et al., A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. PNAS, 28 November 2006, Vol. 103, No. 48, p 18255-18260. Abstract; p18256, Fig. 1 legend	5
Y	GenBank Accession No. AJ421727.1, Homo sapiens microRNA let-7b, 11 June 2003 [online]. [Retrieved on 27 June 2020]. Retrieved from the internet: URL: https://www.ncbi.nlm.nih.gov/nuccore/AJ421727.1 , Entire document	6

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

22 July 2020

Date of mailing of the international search report

06 AUG 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/22524

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

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

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

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

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

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

This International Searching Authority found multiple inventions in this international application, as follows:
 ----Please see continuation in first extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, limited to SEQ ID NOS: 1 and 2

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 20/22524

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+, Claims 1-6, directed to a composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes containing miRNA species. The composition will be searched to the extent that the exosomes encompasses a miRNA having at least 90% sequence similarity to cardiac stromal cell/fibroblast derived miRNA species miRNA-21-5p (SEQ ID NO: 1) and hsa-let-7b-5p (SEQ ID NO: 2), (note, these are the first claimed sequences for miRNA). It is believed that claims 1-6 encompass this first named invention (because these claims are drawn to compositions comprising a single miRNA species), and thus these claims will be searched without fee to the extent that the miRNA species comprises SEQ ID NOs: 1 and 2. Additional composition(s) comprising additional miRNA(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected composition(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a composition comprising miRNAs having at least 90% sequence similarity to miRNA-21-5p (SEQ ID NO: 1) and hsa-miR-125a-5p (SEQ ID NO: 3) (claims 1-6, limited to SEQ ID NOs: 1 and 3).

Group II+, claims 7-24, directed to a method of modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to encompass exosomes comprising an miRNA species having at least 90% sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species miRNA-21-5p (SEQ ID NO: 1) and hsa-let-7b-5p (SEQ ID NO: 2) for an additional fee and election as such. It is believed that claims 7-24 read on this exemplary invention. Additional methods comprising additional miRNAs will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected methods and miRNAs. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be miRNA species having at least 90% sequence similarity to miRNA-21-5p (SEQ ID NO: 1) and hsa-miR-125a-5p (SEQ ID NO: 3). (claims 7-24).

The inventions listed as Groups I+ and II+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

The inventions of Groups I+ and II+ each include the special technical feature of a unique miRNA sequence. Each miRNA sequence comprises a unique nucleic acid, and is considered a distinct technical feature.

Additionally, Group I+ has the special technical feature of a composition comprising a population of cardiac stromal cell/fibroblast-derived exosomes containing miRNA species, that is not required by Group II+.

Group II+ has the special technical feature of a method of repairing cardiac tissue damage by modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject, that is not required by Group I+.

Common technical features

No technical features are shared between the miRNA nucleic acid sequences in each of Groups I+ and II+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of including: a population of cardiac stromal cell/fibroblast-derived exosomes, wherein the exosomes comprise a miRNA species having sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species, these shared technical features are previously disclosed by US 2018/0100149 A1 to Cedars-Sinai Medical Center, (hereinafter 'Cedars-Sinai').

--please see continuation on next extra sheet-----

Continuation of Box No. III. Observations where unity of invention is lacking.

-----continued from previous sheet-----

Cedars-Sinai teaches a composition of cardiosphere derived exosomes comprising miRNA species with sequence similarity to (Claim 1 - 'A method for producing a therapeutic composition comprising: harvesting a population of exosomes from cardiospheres or cardiosphere-derived cells (CDCs) wherein said exosomes comprise microRNAs mir-210 and miR-146a.'; para [0006] - 'In some such embodiments, the synthetic exosomes are generated in order to replicate, substantially, or closely mimic exosomes that are secreted from regenerative cells.'; para [0008] - 'In one embodiment, the microRNA fragments, or derivatives thereof are synthesized with a sequence that mimics one or more endogenous microRNA molecules. Alternatively, in several embodiments, miRNAs are complementary to certain genes in the target cell and can reduce the expression of target genes').

Additionally, Group II+ inventions further share the technical feature of:

a method of modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject, the method comprising administering to a cardiac tissue of an animal or human subject a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA species having sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species. However, these shared technical features are made obvious by Cedars-Sinai in view of the article entitled "Ischemic Postconditioning-Mediated miRNA-21 Protects against Cardiac ischemia/reperfusion Injury via PTEN/Akt Pathway" by Tu et al., (PLoS One. 3 October 2013, Vol. 8, No.10, e75872, p1-14) (hereinafter 'Tu').

Cedars-Sinai teaches a method of inducing regeneration in the cardiac tissue of an animal or human subject, the method comprising administering to a cardiac tissue of an animal or human subject a population of cardiac stromal cell/fibroblast-derived exosomes and a pharmaceutically acceptable carrier, wherein the population of cardiac stromal cell/fibroblast-derived exosomes comprise an miRNA species having sequence similarity to cardiac stromal cell/fibroblast-derived miRNA species (para [0006] - 'As such, there are provided herein methods for regenerating tissue in an individual having damaged tissue, comprising, identifying an individual having damaged tissue and administering a plurality of exosomes to the individual, wherein the exosomes are secreted from regenerative cells, wherein the exosomes comprise one or more microRNA fragments, and wherein after administration of the plurality of exosomes, the one or more microRNA fragments alter gene expression in the damaged tissue, improve the viability of the damaged tissue, and/or facilitate the formation of new tissue in the individual. In several embodiments, administration of the exosomes results in functional improvement in the tissue, in combination with one or more of the above-mentioned positive results.'; para [0009] - 'In several embodiments, the damaged tissue comprises cardiac tissue. In several embodiments, the regenerative cells comprise cardiospheres. In several embodiments, the regenerative cells comprise cardiosphere-derived cells (CDCs). In several embodiments, the use of cardiospheres and/or CDCs as a source of exosomes is particularly advantageous, as the resultant exosomes provide unexpectedly superior therapeutic benefits (as compared to exosomes from other cell types).'; para [0010] - 'In some embodiments, administration of the exosomes results in an increase in cardiac wall thickness in the area subjected to the infarction.'; para [0008] - 'In one embodiment, the microRNA fragments, or derivatives thereof are synthesized with a sequence that mimics one or more endogenous microRNA molecules. Alternatively, in several embodiments, miRNAs are complementary to certain genes in the target cell and can reduce the expression of target genes'). Cedars-Sinai does not expressly teach miRNA modulation of PTEN.

Tu teaches miR-21 is cardioprotective via modulation of PTEN/Akt signaling pathway (Abstract - 'Our data clearly demonstrate that miR-21 is involved in IPost-mediated cardiac protection against I/R injury and dysfunction through the PTEN/Akt signaling pathway in vivo.'). Since Cedars-Sinai teaches use of miR-21 for treatment of cardiac disease (para [0017] - 'In additional embodiments, the miRNA comprises one or more of miR-17, miR-21'), it would have been obvious to one of ordinary skill in the art that administration of miR-21 according to the method of Cedars-Sinai provides a method of modulating the activity of phosphatase and tensin homolog (PTEN) in the cardiac tissue of an animal or human subject according to Tu, for cardiac regeneration.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.