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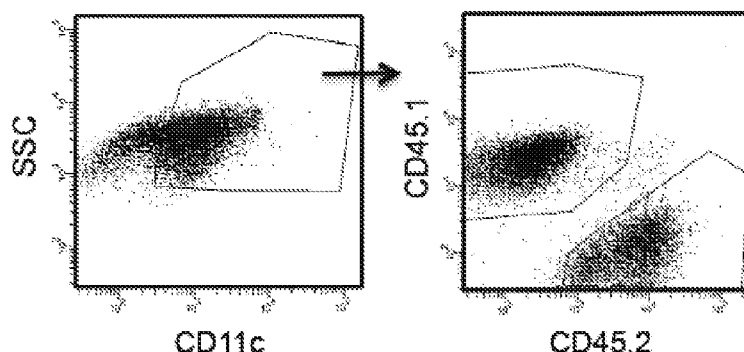


FIG. 1B

(57) **Abstract:** A method of repressing a target messenger RNA (mRNA) in a subject is provided. The method may include the step of loading exosomes with a microRNA (miRNA), wherein the miRNA is configured to repress the target mRNA. The method may further include the step of introducing the one or more miRNA-loaded exosomes into the subject.

EXOSOME DELIVERY OF MICRORNAS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Application No. 62/157,875, filed May 6, 2015, which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Immune cells utilize intercellular communication to coordinate inflammatory responses. Cytokines, chemokines, and cell surface receptors may be mediators of this process. In addition to these signaling molecules, emerging evidence may suggest that immune cells signal by secreting small lipid packages called exosomes, which carry a variety of different molecules that may be taken up by recipient cells (see Tian, T. *et al.*, J. Cell. Biochem. 111, 488–96 (2010); Raposo, G. and Stoorvogel, W., J. Cell Biol. 200, 373–83 (2013); Théry, C. *et al.*, Nat. Rev. Immunol. 2, 569–79 (2002); Valadi, H. *et al.*, Nat. Cell Biol. 9, 654–9 (2007); each of which is incorporated by reference herein). The functional relevance of exosomes in many different biological systems, including the immune system, may be beginning to be demonstrated (see Zhou, W. *et al.*, Cancer Cell 25, 501–15 (2014); Bang, C. *et al.*, J. Clin. Investig. 124, (2014); Aucher, A. *et al.*, J. Immunol. 191, 6250–60 (2013); Zhang, Y. *et al.*, Mol. Cell 288, 23586–96 (2010); Chairoungdua, A. *et al.*, J. Cell Biol. 190, 1079–91 (2010); Sheldon, H. *et al.*, Blood 116, 2385–94 (2010); Raposo, G. *et al.*, J. Exp. Med. 183, (1996); each of which is incorporated by reference herein).

[0003] MicroRNAs (miRNAs) may modulate gene expression by targeting mRNAs for degradation or preventing translation. Typically, miRNAs have been thought to function within the cells in which they are made; however, recently miRNAs have been observed in secreted exosomes (see Montecalvo, A. *et al.*, Blood 119, 756–66 (2012); Kosaka, N. *et al.*, J. Biol. Chem. 285, 17442–52 (2010); and Mittelbrunn, M. *et al.*, Nat. Commun. 2, 282 (2011); each of which is incorporated by reference herein). Immune cells, including antigen presenting dendritic cells and T lymphocytes, may both secrete and take up exosomal miRNAs, which may suggest that exosomal transfer of miRNAs may be a mechanism for intercellular communication (see Montecalvo, A. *et al.*, Blood 119, 756–66 (2012); Mittelbrunn, M. *et al.*, Nat. Commun. 2, 282 (2011); and Chen, X. *et al.*, Protein Cell 3, 28–37 (2012); each of which is incorporated by reference herein). Furthermore, recent studies may indicate that the loading of miRNAs into exosomes may be a selective process where specific motifs in miRNA sequences are recognized by the RNA binding protein, hnRNPA2B1 (see Villarroya-Beltri, C. *et al.*, Nat. Commun. 4, 2980 (2013); which is

incorporated by reference herein). Other reports may have found that miRNA loading into exosomes may be dependent on 3' end uridylated isoforms (see Koppers-Lalic, D. *et al.*, Cell Rep. 8, 1649–58 (2014); which is incorporated by reference herein) as well as the levels of miRNA targets in the producer cells (see Squadrito, M. L. *et al.*, Cell Rep. 1432–1446 (2014), doi:10.1016/j.celrep.2014.07.035; which is incorporated by reference herein). Consistent with this, exosomal miRNA signatures may not simply reflect the miRNA composition of the parent cell, but may be composed of a distinct set of miRNAs (see Villarroya-Beltri, C. *et al.*, Nat. Commun. 4, 2980 (2013); Squadrito, M. L. *et al.*, Cell Rep. 1432–1446 (2014), doi:10.1016/j.celrep.2014.07.035; Villarroya-Beltri, C. *et al.*, Semin. Cancer Biol. 28, 3–13 (2014); Gibbings, D. J. *et al.*, Nat. Cell Biol. 11, 1143–9 (2009); Guduric-Fuchs, J. *et al.*, BMC Genomics 13, 357 (2012); each of which is incorporated by reference herein). This may indicate that certain miRNAs have evolved to be packaged into exosomes in order to carry out their biological functions.

[0004] Exosomally transferred miRNAs may be regulators of cellular function. There is evidence in both immune cells and other cell types that transferred miRNAs may repress target mRNAs in recipient cells (see Montecalvo, A. *et al.*, Blood 119, 756–66 (2012); Kosaka, N. *et al.*, J. Biol. Chem. 285, 17442–52 (2010); Mittelbrunn, M. *et al.*, Nat. Commun. 2, 282 (2011); Katakowski, M., *et al.* Cancer Res. 70, 8259–63 (2010); Okoye, I. S. *et al.*, Immunity 41, 89–103 (2014); and Pegtel, D. M. *et al.*, Proc. Natl. Acad. Sci. U. S. A. 107, 6328–33 (2010); each of which is incorporated by reference herein). The transfer of miRNAs may also cause physiological changes in recipient cells (see Zhou, W. *et al.*, Cancer Cell 25, 501–15 (2014); Bang, C. *et al.*, J. Clin. Investig. 124, (2014); and Aucher, A., *et al.*, J. Immunol. 191, 6250–60 (2013); each of which is incorporated by reference herein), as demonstrated by miRNAs moving from cancer cells to endothelial cells, which may promote tumor metastasis (see Zhou, W. *et al.*, Cancer Cell 25, 501–15 (2014); which is incorporated by reference herein). Cancer cells may also receive miRNAs secreted from immune cells, which may have been shown to have an anti-proliferative effect on the tumor cells (see Aucher, A. *et al.* J. Immunol. 191, 6250–60 (2013); which is incorporated by reference herein). These data may suggest that different cell types secrete or receive miRNAs as a form of communication.

[0005] Within the immune system, several specific miRNAs may have recently emerged as regulators of immune cell function. Among these, miR-155 may be a promoter of inflammatory responses, while miR-146a may be a mediator of immune suppression (see Huffaker, T. B. *et al.*, Cell Rep. 2, 1697–709 (2012); Boldin, M. P. *et al.*, J. Exp. Med. 208,

1189–201 (2011); Turner, M. L., *et al.*, J. Immunol. 187, 3911–7 (2011); and O’Connell, R. M., *et al.*, Proc. Natl. Acad. Sci. U. S. A. 104, 1604–9 (2007); each of which is incorporated by reference herein). Despite progress in the understanding of how these miRNAs may influence immunity *in vivo*, there may be aspects of their regulation and function that remain unclear.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] The embodiments disclosed herein will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. These drawings depict only typical embodiments, which will be described with additional specificity and detail through use of the accompanying drawings in which:

[0007] FIGS. 1A-1K indicate that miR-155 may be transferred between BMDCs and may be present in exosomes. FIG. 1A is a schematic of a co-culture experiment according to an embodiment of the present disclosure. FIG. 1B are representative FACS plots where co-cultured *CD45.1⁺ Wt* and *CD45.2⁺ miR-155^{-/-} CD11c⁺* BMDCs were separated (n=4). FIG. 1C is a graph depicting that relative miR-155 levels were quantified via qRT-PCR from isolated *miR-155^{-/-}* BMDCs that had been cultured alone or with *Wt* BMDCs in the presence or absence of LPS for 24 hours (n=4). FIG. 1D is a graph depicting that relative miR-155 levels were measured via qRT-PCR in *miR-155^{-/-}* BMDCs either cultured alone or with *Wt* BMDCs separated by a .4 μ m filter for 24 hours with or without LPS (n=3). FIG. 1E is a micrograph depicting cryo electron microscopy (EM) of exosomes isolated from *Wt* BMDCs. Scale bar is 200nm. Boxed portion is enlarged in the upper left corner. FIG. 1F is an image depicting CD63 protein levels in the exosomal pellet from *Wt* and *miR-155^{-/-}* BMDCs treated with or without LPS. FIG. 1G is a graph depicting relative levels of miR-155 in exosomes derived from *Wt* or *miR-155^{-/-}* BMDCs treated with or without LPS (n=3). FIG. 1H is a graph depicting exosome quantification of *Wt* BMDCs treated with or without GW4869 (n=3). Limit of detection is 2×10^7 exosomes. FIG. 1I is a graph depicting that relative levels of miR-155 were measured in the exosomal pellet from *Wt* BMDCs treated with or without LPS and GW4869 as quantified by qRT-PCR (n=2). FIG. 1J is a graph depicting exosome quantification of *Wt* and *Rab27 DKO* BMDC derived exosomes (n=2). Limit of detection is 2×10^7 exosomes. FIG. 1K is a graph depicting miR-155 levels in exosome pellets from *Wt* and *Rab27 DKO* BMDC conditioned medium (n=2). Data presented in FIGS. 1A-1K represent two independent experiments and are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; **, $p < 0.01$, Student’s t-Test.

[0008] FIGS. 2A-2M depict the functional transfer of miR-155 via exosomes *in vitro*. FIG. 2A is a schematic of an exosome transfer experiment according to an embodiment of the present disclosure. FIG. 2B is a graph depicting qRT-PCR, which was used to measure relative miR-155 levels in *miR-155*^{-/-} BMDCs that received either *Wt* or *miR-155*^{-/-} exosomes derived from BMDCs treated with or without GW4869 (n=5). FIGS. 2C and 2D are graphs depicting mRNA levels of miR-155 targets, BACH1 and SHIP1, from the same experiment shown in FIG. 2B as measured by qRT-PCR (n=5). FIG. 2E depicts representative Western blots of SHIP1 and β -actin in *miR-155*^{-/-} BMDCs given either *Wt* or *miR-155*^{-/-} exosomes. FIG. 2F is a graph depicting protein levels of SHIP1, which were quantified using ImageJ software (n=2). FIG. 2G is a graph depicting relative miR-155 levels in *Wt* BMDCs given either *Wt* or *miR-155*^{-/-} exosomes as quantified by qRT-PCR (n=6). FIGS. 2H and 2I are graphs depicting BACH1 and SHIP1 mRNA levels, which were measured in the same experiment shown in FIG. 2G as quantified by qRT-PCR (n=6). FIG. 2J is a graph depicting qRT-PCR, which was used to quantify HO1 mRNA levels during the experiment in FIG. 2B (n=5). FIG. 2K are images of Western blotting for AGO2 and β -actin from *miR-155*^{-/-} BMDCs given *Wt* or *miR-155*^{-/-} exosomes. On the left is the input (whole cell lysate), the middle is from the pan-AGO pull-down where 1/3 of input was used, and the right is the IgG pull-down where 1/3 of the input was used. FIG. 2L is a graph depicting relative miR-155 levels, which were quantified via qRT-PCR in the same experiment shown in FIG. 2K. FIG. 2M is a graph depicting miR-146a levels, which were quantified using qRT-PCR during the experiment in FIG. 2K. Levels in FIGS. 2L and 2M are plotted as Ago:IgG. Dotted line separates input from pull-down groups. Data presented in FIGS. 2A-2M represent two independent experiments and are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; **, $p < 0.01$, Student's t-Test.

[0009] FIGS. 3A-3L depict functional transfer of miR-146a via exosomes *in vitro*. FIG. 3A is a graph depicting levels of miR-146a in the exosomal pellet derived from BMDCs that were treated with or without GW4869 and LPS (n=2). FIG. 3B is a graph depicting miR-146a levels in *Wt* and *Rab27 DKO* BMDC derived exosomal pellets (n=2). FIG. 3C is a schematic of an miR-146a exosome transfer experiment where *Wt* or *miR-146a*^{-/-} exosomes were isolated from BMDCs and transferred to recipient *miR-146a*^{-/-} BMDCs. RNA was isolated after 24 hours and the presence of miR-146a was assayed via qRT-PCR. FIG. 3D is a graph depicting the relative levels of miR-146a in *miR-146a*^{-/-} BMDCs given exosomes derived from *Wt* or *miR-146a*^{-/-} BMDCs (n=4). FIG. 3E is a graph depicting mRNA levels of miR-146a target, IRAK1, which were measured from the same cells as in

FIG. 3D via qRT-PCR (n=4). FIG. 3F depicts representative Western blots of IRAK1 and β -actin from *miR-146a*^{-/-} cells given either *Wt* or *miR-146a*^{-/-} exosomes. FIG. 3G is a graph depicting IRAK1 protein levels, which were quantified using ImageJ software (n=2). FIG. 3H is a graph depicting mRNA levels of *miR-146a* target, TRAF6, which were measured in the same cells as FIG. 3D via qRT-PCR. FIG. 3I depicts Western blots for TRAF6 and β -actin from *miR-146a*^{-/-} BMDCs given either *Wt* or *miR-146a*^{-/-} exosomes (n=2). FIG. 3J is a graph depicting Western results, which were quantified with ImageJ software. FIG. 3K is a graph depicting copy number of *miR-146a* in *Wt* and *miR-146a*^{-/-} exosomes (n=3). Copy number is calculated based on a standard curve where a known amount of synthetic *miR-146a* was spiked into *miR-146a*^{-/-} BMDC derived exosome pellet followed by RNA isolation and qRT-PCR. FIG. 3L is a graph depicting copy number of *miR-146a*, which was measured via qRT-PCR in *miR-146a*^{-/-} recipient BMDCs that received either *Wt* or *miR-146a*^{-/-} exosomes (*146a*^{-/-} BMDC + *Wt* exos and *146a*^{-/-} BMDC + *146a*^{-/-} exos) as well as in *Wt* and *miR-146a*^{-/-} donor BMDCs (n=3). Average copy number is displayed above. Copy number is calculated based on a standard curve where a known amount of synthetic *miR-146a* was spiked into *miR-146a*^{-/-} BMDC pellet followed by RNA isolation and qRT-PCR. Data presented in FIGS. 3A-3L represent two independent experiments and are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$, Student's t-Test.

[0010] FIGS. 4A-4I depict seed-dependent repression of miRNA targets by exosome-delivered *miR-155* and *miR-146a*. FIG. 4A is a schematic for a mimic experiment according to an embodiment of the present disclosure. FIGS. 4B and 4C are graphs depicting relative mRNA levels of the *miR-155* targets SHIP1 and BACH1, which were measured via qRT-PCR in recipient cells that received exosomes with no mimics (No) (n=7), *miR-155* seed mutant mimics (Seed) (n=4), with scrambled mimics (Scram) (n=3), or with *miR-155*-mimics (Mimic) (n=7). FIGS. 4D and 4E are graphs depicting qRT-PCR, which was performed to assay the mRNA levels of the *miR-146a* targets, IRAK1 and TRAF6, following treatment with exosomes containing *miR-146a* mimics and controls as in FIGS. 4B and 4C. Results are reported normalized to exosomes with no mimics added, which is set as 1. FIG. 4F is a series of images depicting the protein levels of TRAF6, IRAK1, and β -actin, which were determined via Western blotting using lysates from *miR-146a*^{-/-} BMDCs that received exosomes containing no mimics, seed mutant mimics, or *Wt* mimics. Numbers below the blot represent relative protein levels with no mimics set as 1 following normalization to β -actin. FIG. 4G is a schematic for the 3' UTR luciferase reporter assays as depicted in FIGS. 4H and 4I. FIG. 4H is a graph depicting results from 3' UTR luciferase reporter

assays where *miR-155*^{-/-} BMDCs were transfected with a pmiReport control vector, a BACH1 3' UTR vector (BACH1), a BACH1 miR-155 binding site (bs) mutant vector (BACH1 mutant), or a 2mer positive control vector. Transfected BMDCs were treated 6 hours later with or without *Wt* exosomes and percent change in luciferase activity of exosome treated BMDCs compared to no exosome treatment was calculated after 24 hours (n=4). FIG. 4I is a graph depicting results from a 3' UTR luciferase reporter assay where *miR-146a*^{-/-} BMDCs were transfected with a pmiReport control vector, a TRAF6 3' UTR vector (TRAF6), or TRAF6 miR-146a bs mutant vector (TRAF6 mutant). 6 hours later, the BMDCs were treated with or without *Wt* exosomes and percent repression of luciferase activity was calculated 24 hours after exosome transfer (n=4). Results presented in FIGS. 4A-4I represent two independent experiments, and all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, p < 0.05; **, p < 0.01, ****, p < .0001; Student's t-Test.

[0011] FIGS. 5A-5F depict exosomal transfer of miR-155 and miR-146a program response to LPS *in vitro*. FIG. 5A is a schematic of the experimental design for FIG. 5B. FIG. 5B is a graph depicting exosomes, which were isolated from *Wt* or *miR-155*^{-/-} BMDCs and given to *miR-155*^{-/-} BMDCs for 24 hours. Cells were then treated with or without LPS and media was taken after 6 hours for an IL-6 ELISA. Relative IL-6 protein levels are shown (n=4). FIG. 5C is a schematic for the experiments in FIGS. 5D-5F. FIGS. 5D, 5E, and 5F depict qRT-PCR, which was used to quantify mRNA levels of IL-10, IL-6, and IL-12 p40 in *miR-146a*^{-/-} BMDCs given exosomes from *Wt* or *miR-146a*^{-/-} BMDCs for 24 hours followed by stimulation with or without LPS for 6 hours (n=4). Data presented in FIGS. 5A-5F represent two independent experiments, and all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, p < 0.05; Student's t-Test.

[0012] FIGS. 6A-6K depict transfer of endogenous miR-155 between hematopoietic cells *in vivo*. FIG. 6A depicts a CD63 Western blot using exosomes isolated directly from the BM of *Wt* or *miR-155*^{-/-} mice. 1 and 2 stand for two biological replicates. FIGS. 6B and 6C depict levels of miR-155 and miR-146a in exosomes isolated from *Wt* and *miR-155*^{-/-} mouse BM as measured by qRT-PCR (n=2). FIG. 6D is a schematic of the *in vivo* experiment according to one embodiment as disclosed herein. FIG. 6E is a graph depicting qRT-PCR, which was used to quantify levels of miR-155 in *miR-155*^{-/-} CD45.2⁺ BM cells that were B220⁺, CD3⁺, or CD11b⁺ from *miR-155*^{-/-} mice that were either reconstituted with *Wt* (CD45.1⁺) and *miR-155*^{-/-} BM or *miR-155*^{-/-} BM alone as indicated (n=5). FIGS. 6F-6H depict representative FACS plots of the cell types in isolated BM shown in FIG. 6E (n=5). FIG. 6I-6K are graphs depicting where *miR-155*^{-/-} mice were i.p. injected multiple times

over a week with either *Wt* or *miR-155*^{-/-} exosomes. CD3⁺ T cells, B220⁺ B cells, and CD11b⁺ myeloid cells were sorted from mouse spleens and qRT-PCR was performed to analyze the delivery of miR-155 to each cell type (n=5). In FIGS. 6A-6K, all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; **, $p < 0.01$, *** $P < 0.001$, Student's t-Test.

[0013] FIGS. 7A-7I depict that miR-155-containing exosomes may promote a heightened response to LPS in *miR-155*^{-/-} mice. FIG. 7A is a schematic of the experimental design where *miR-155*^{-/-} mice were i.p. injected with either *Wt* or *miR-155*^{-/-} BMDC derived exosomes then challenged with LPS 24 hours later. Blood was taken 2 hours post LPS injection and the spleen, liver, and bone marrow (BM) were harvested 24 hours post injection. FIGS. 7B and 7C are graphs depicting serum TNF α and IL-6 concentrations, which were analyzed via ELISA 2 hours after injection of LPS in *miR-155*^{-/-} mice that had been pretreated with either *Wt* or *miR-155*^{-/-} exosomes (n=5). FIGS. 7D-7F are graphs depicting qRT-PCR, which was performed using RNA isolated from the spleen, liver, and BM to assay the relative levels of exosomally delivered miR-155 (n=5). FIGS. 7G-7I are graphs depicting mRNA levels of the miR-155 targets SHIP1 and BACH1, which were measured in the spleen, liver, and/or the BM using qRT-PCR (n=5). In FIGS. 7A-7I, all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; **, $p < 0.01$, Student's t-Test.

[0014] FIGS. 8A-8I indicate that miR-146a-containing exosomes reduce inflammatory responses to LPS in *miR-146a*^{-/-} mice. FIG. 8A is a schematic of the experimental design where *miR-146a*^{-/-} mice were i.p. injected with either *Wt* or *miR-146a*^{-/-} BMDC derived exosomes then challenged with LPS 24 hours later. Blood was taken 2 hours post LPS injection and the spleen, liver, and bone marrow (BM) were harvested 24 hours post injection. FIGS. 8B and 8C are graphs depicting serum TNF α and IL-6, which were analyzed via ELISA 2 hours after injection of LPS (n=5). FIGS 8D-8F depict graphs of qRT-PCR, which was performed using RNA isolated from the spleen, liver, and BM to assay the relative levels of exosomally delivered miR-146a (n=5). FIGS. 8G-8I are graphs depicting mRNA levels of the miR-146a targets, TRAF6 and IRAK1, which were measured in the spleen, liver, and/or the BM using qRT-PCR (n=5). In FIGS. 8A-8I, all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; **, $p < 0.01$, Student's t-Test.

[0015] FIGS. 9A-9I indicate that miR-146a-containing exosomes may reduce inflammatory response to LPS in *Wt* mice. FIG. 9A is a schematic of the experimental design where *Wt* mice were i.p. injected with either *Wt* or *miR-146a*^{-/-} BMDC derived

exosomes then challenged with LPS 24 hours later. Blood was taken 2 hours post LPS injection and the spleen, liver, and bone marrow (BM) were harvested 24 hours post injection. FIGS. 9B and 9C are graphs depicting serum TNF α and IL-6, which were analyzed via ELISA, 2 hours after injection of LPS (n=5). FIGS. 9D-9F are graphs depicting qRT-PCR using RNA isolated from the spleen, liver, and BM, which was performed to assay the relative levels of exosomally delivered miR-146a (n=5). FIGS. 9G-9I depict mRNA levels of the miR-146a targets, TRAF6 and IRAK1, which were measured in the spleen, liver, and/or the BM using qRT-PCR (n=5). Results presented in FIGS. 9A-9I represent two independent experiments, and all data are presented as the mean $\pm$ S.D. (error bars), as indicated. *, $p < 0.05$; ****, $p < 0.0001$, Student's t-Test.

[0016] FIGS. 10A and 10B depict exosome isolation from BMDCs treated with and without GW4869. FIG. 10A is a CD63 Western blot from the protein lysed exosome pellet isolated from BMDCs treated with GW4869 or DMSO vehicle control. FIG. 10B is an EM image of the exosome pellet from BMDCs treated with GW4869. Scale bar is 100 nm.

[0017] FIG. 11 is a graph depicting the quantification of exosomes produced by BMDCs. Exosomes were isolated from approximately 1 million miR-146a $^{-/-}$, miR-155 $^{-/-}$, or WT BMDCs that were cultured in media for 24 hours (n=3). Quantification was performed with the EXOCET kit. Limit of detection is 2×10^7 exosomes. All data are presented as the mean $\pm$ S.D. (error bars).

[0018] FIGS. 12A-12F are graphs depicting relative expression of miR-155 and miR-425 in donor cells, exosomes, and recipient cells. FIG. 12A is a graph depicting relative expression of miR-155 in Wt (Wt donor) and miR-155 $^{-/-}$ (155 $^{-/-}$ donor) donor BMDCs and miR-155 $^{-/-}$ recipient BMDC that received either Wt (155 $^{-/-}$ + Wt exos) or miR-155 $^{-/-}$ (155 $^{-/-}$ + 155 $^{-/-}$ exo) exosomes (n=3). FIG. 12B is a graph depicting relative expression of miR-155 in miR-155 $^{-/-}$ and Wt exosomes as determined by qRT-PCR (n=3). FIGS. 12C and 12D are graphs depicting relative miR-425 levels, which were measured via qRT-PCR in same cells as in FIG. 12A and same exosomes as in FIG. 12B (n=3). FIG. 12E is a graph depicting relative miR-425 levels, which were measured via qRT-PCR in Wt (Wt donor) or miR-146a $^{-/-}$ BMDCs (146a $^{-/-}$ donor) and miR-146a $^{-/-}$ recipient BMDCs that received either Wt (146a $^{-/-}$ + Wt exos) or miR-146a $^{-/-}$ exosomes (146a $^{-/-}$ + 146a $^{-/-}$ exos) (n=3). FIG. 12F is a graph depicting relative miR-425 levels, which were measured via qRT-PCR in Wt and miR-146a $^{-/-}$ exosomes (n=3). All data are presented as the mean $\pm$ S.D. (error bars).

[0019] FIGS. 13A-13D are charts depicting mature miRNA sequence differences between donor and exosome treated recipient BMDCs. FIGS. 13A and 13B are charts

depicting percentages of mature miR-155 without NTAs or with A, C, G, and U NTAs, which were determined by RNA-Seq using RNA from Wt donor BMDCs and DKO BMDCs that received Wt exosomes (n=3). FIGS. 13C and 13D are charts depicting percentages of mature miR-146a non-NTA sequences as well as A, C, G, and U NTAs, which were determined by RNA-Seq using RNA from Wt donor BMDCs and DKO BMDCs that received Wt exosomes (n=3).

[0020] FIGS. 14A-14C depict transfected miRNA mimics that are loaded into exosomes and transferred to recipient cells. FIG. 14A is a graph depicting that miR-155 mimics can be detected in miR-155^{-/-} BMDCs after transfection for 24 hours (n=2). FIG. 14B is a graph depicting that miR-155 mimics can be detected in exosomes from miR-155^{-/-} BMDCs transfected with mimics as assayed by qRT-PCR (n=2). FIG. 14C is a graph depicting qRT-PCR, which was used to determine levels of miR-155 mimics in recipient miR-155^{-/-} BMDCs given either miRNA loaded exosomes or exosomes lacking miR-155. All data are presented as the mean $\pm$ S.D. (error bars).

[0021] FIGS. 15A-15D depict that exosomally delivered miRNA mimics and seed mutant mimics can be detected in recipient cells following delivery by exosomes. FIG. 15A is a graph depicting levels of miR-155 mimics, which were measured via qRT-PCR in recipient miR-155^{-/-} BMDCs that had been given exosomes derived from donor miR-155^{-/-} BMDCs transfected with either no mimics, seed mutant mimics, scrambled mimics, or Wt miR-mimics (n=3). FIGS. 15B is a graph depicting levels of seed mutant miR-155 mimics in the same cells as in FIG. 15A. FIG. 15C is a graph depicting levels of miR-146a mimics, which were measured via qRT-PCR in miR-146a^{-/-} recipient BMDCs that had been given exosomes derived from donor miR-146a^{-/-} BMDCs transfected with either no mimics, seed mutant mimics, scrambled mimics, or Wt miR-mimics (n=3). FIG. 15D is a graph depicting levels of seed mutant miR-146a mimics in the same cells as in FIG. 15C. All data are presented as the mean $\pm$ S.D. (error bars). *, $p < 0.05$; **, $p < 0.01$, *** $P < 0.001$, Student's t-Test.

[0022] FIGS. 16A-16E are un-cropped Western blots from FIGS. 1F, 2E, and 2K. FIG. 16A is an un-cropped image of FIG. 1F (CD63). FIG. 16B is an un-cropped image of FIG. 2E (SHIP1). FIG. 16C is an un-cropped image of FIG. 2E (β -Actin). FIG. 16D is an un-cropped image of FIG. 2K (AGO2). FIG. 16E is an un-cropped image of FIG. 2K (β -Actin).

[0023] FIGS. 17A-17D are un-cropped Western blots for FIGS. 3F and 3I. FIG. 17A is an un-cropped image of FIG. 3F (IRAK1). FIG. 17B is an un-cropped image of FIG. 3F (β -

Actin). FIG. 17C is an uncropped image of FIG. 3I (TRAF6). FIG. 17D is an un-cropped image of FIG. 3I (β -Actin).

[0024] FIGS. 18A-18E are un-cropped Western blots for FIGS. 4F, 6A, and 10A. FIG. 18A is an un-cropped image of FIG. 4F (TRAF6). FIG. 18B is an un-cropped image of FIG. 4F (IRAK1). FIG. 18C is an un-cropped image of FIG. 4F (β -Actin). FIG. 18D is an un-cropped image of FIG. 6A (CD63). FIG. 18E is an un-cropped image of FIG. 10A (CD63).

[0025] FIGS. 19A and 19B are tables showing miR-155 nucleotide differences between Wt donor BMDCs and DKO BMDCs treated with Wt exosomes. FIG. 19A is a table showing the mature miR-155 nucleotide composition was analyzed in Wt BMDCs using RNA-Seq. Underline represents significant changes between Wt donors and DKO BMDCs that received Wt exosomes (n=3). FIG. 19B is a table showing the mature miR-155 nucleotide composition in DKO BMDCs that received Wt exosomes as determined by RNA-Seq. Underline represents significant changes between Wt donors and DKO BMDCs that received Wt exosomes (n=3).

[0026] FIGS. 20A and 20B are tables showing miR-146a nucleotide differences between Wt donor BMDCs and DKO BMDCs treated with Wt exosomes. FIG. 20A is a table showing the Mature miR-146a nucleotide composition in Wt BMDCs donors as determined by RNA-Seq. Underline represents significant changes between Wt donors and DKO BMDCs that received Wt exosomes (n=3). FIG. 20B is a table showing the mature miR-146a nucleotide composition in DKO BMDCs that received Wt exosomes as determined by RNA-Seq. Underline represents significant changes between Wt donors and DKO BMDCs that received Wt exosomes (n=3).

DETAILED DESCRIPTION

[0027] The following more detailed description of various embodiments is not intended to limit the scope of the present disclosure, but is merely representative of various embodiments. Moreover, the order of the steps or actions of the methods disclosed herein may be changed by those skilled in the art without departing from the scope of the present disclosure. In other words, unless a specific order of steps or actions is required for proper operation of the embodiment, the order or use of specific steps or actions may be modified. Each and every reference recited herein is incorporated by reference in its entirety.

[0028] It was investigated whether endogenous miR-155 and miR-146a may be functionally transferred between primary bone marrow-derived dendritic cells (BMDCs). It was found that both of these miRNAs may be released within exosomes and may be taken up by recipient BMDCs. Upon uptake, the miRNAs may be associated with Ago proteins,

knockdown their respective targets, and/or reprogram the response of BMDCs to endotoxin challenge. It was also shown that miR-155 may be transferred between immune cells *in vivo*. It was additionally demonstrated that injection of miR-146a-containing exosomes into mice may inhibit their inflammatory response to endotoxin while injection of miR-155-containing exosomes may promote inflammation following exposure to the same inflammatory stimulus. This study may support a model wherein exosomal miRNAs participate in the regulation of inflammatory responses.

miR-155 May Be Found in Exosomes and Transferred between BMDCs

[0029] miR-155 may be an immunomodulatory miRNA expressed by many types of immune cells including dendritic cells (DCs) (see Turner, M. L. *et al.*, J. Immunol. 187, 3911–7 (2011); which is incorporated by reference herein). It was sought to be determined if miR-155 could be passed between cultured bone marrow derived DCs (BMDCs). Co-cultures of primary mouse BMDCs derived from *CD45.1⁺ Wt* mice and *CD45.2⁺ miR-155^{-/-}* mice were set up at a 1:1 ratio with and without LPS treatment (see FIG. 1A). As a control, *miR-155^{-/-}* BMDCs were also cultured under substantially the same conditions without *Wt* cells. After 24 hours, the co-cultured *Wt* and *miR-155^{-/-}* *CD11c⁺* BMDCs were separated based upon their differential CD45 markers using fluorescence activated cell sorting (FACS) (see FIG. 1B). qRT-PCR was performed on RNA isolated from the *CD45.2⁺ miR-155^{-/-}* BMDCs. miR-155 was detected in *miR-155^{-/-}* BMDCs that were cultured with *Wt* cells, and the signal was above background levels established using *miR-155^{-/-}* BMDCs cultured alone (see FIG. 1C). When cells were treated with LPS, the transfer of miR-155 to *miR155^{-/-}* cells may have been increased, consistent with previous findings that cellular miR-155 concentrations may be elevated following LPS stimulation (see O'Connell, R. M. *et al.*, J. Exp. Med. 205, 585–94 (2008); which is incorporated herein by reference) (see FIG. 1C).

[0030] To determine if cell-cell contact may be substantially necessary for the transfer of miR-155, 0.4 μ m filters were used to separate *miR-155^{-/-}* and *Wt* BMDCs that were co-cultured in the presence or absence of LPS for 24 hours. The 0.4 μ m pore size may allow for small molecules and vesicles such as exosomes to pass through but may prevent cell-cell contact mediated exchange of material (see Okoye, I. S. *et al.*, Immunity 41, 89–103 (2014); which is incorporated by reference herein). miR-155 was detected in the *miR-155^{-/-}* BMDCs that were cultured with *Wt* BMDCs, which was above background (see FIG. 1D). This data may indicate that miR-155 may be passed between cells and that cell-cell contact may not be substantially necessary for transfer to occur between BMDCs.

[0031] As miRNAs have been shown to be transferred between immune cells within exosomes it was investigated whether miR-155 is contained within these secreted vesicles. To address this question, the exosomal pellet from *Wt* or *miR-155*^{-/-} BMDC conditioned-media was isolated using differential centrifugation. Both electron microscopy (EM) and a CD63 Western blotting of the isolated vesicles indicated that we may have isolated exosomes (see FIGS. 1E and 1F). Using qRT-PCR, it was found that miR-155 may be contained in exosomes derived from *Wt* BMDCs but not in exosomes derived from *miR-155*^{-/-} cells (see FIG. 1G). BMDCs treated with LPS have enhanced the levels of miR-155 found in the exosomal pellet consistent with higher levels of miR-155 being produced by the activated BMDCs. Additionally, exosome formation was blocked by treating donor BMDCs with GW4869, a drug that may hinder exosome biogenesis by blocking nSMase2 (see Kosaka, N. *et al.*, J. Biol. Chem. 285, 17442–52 (2010); and Chen, X. *et al.*, Protein Cell 3, 28–37 (2012); each of which is incorporated by reference herein). Following drug treatment, the pellet contained reduced exosomes as determined by EXOCET quantification (see FIG. 1H), CD63 Western blotting, and EM (see FIGS. 10A and 10B). Drug treatment also prevented the detection of miR-155 in the exosomal pellet (see FIG. 1I), suggesting that miR-155 may be contained within exosomes. Additionally, BMDCs were derived from *Rab27a* and *Rab27b* double knockout mice (*Rab27* DKO), which may have been shown to have decreased release of exosomes (see Okoye, I. S. *et al.*, Immunity 41, 89–103 (2014); which is incorporated by reference herein). It was found that *Rab27* DKO BMDCs may have both decreased exosome release (see FIG. 1J) and a corresponding decrease in miR-155 in the exosomal pellet (see FIG. 1K). Together, these data may show that miR-155 may be passed between BMDCs and that miR-155 may be contained in exosomes produced by BMDCs.

Exosomal Transfer of miR-155 May be Functionally Relevant

[0032] With the knowledge that miR-155 may be transferred between BMDCs, it was determined if exosomes may be sufficient for this transfer and whether transfer may result in knockdown of target mRNAs. To specifically investigate the impact of exosomally-transferred miRNA, without the effects of other factors that are released from BMDCs, exosomes were purified away from other components in the conditioned medium using differential centrifugation and washing. Next, the exosomes were re-suspended in fresh medium and administered to recipient cells. 1×10^6 *Wt* or *miR-155*^{-/-} BMDCs produced $\sim 5 \times 10^8$ exosomes in 24 hours (see FIG. 11). Exosomes isolated from the supernatant of both *Wt* and *miR-155*^{-/-} BMDCs treated with GW4869 or DMSO vehicle control were

transferred to *miR-155*^{-/-} recipient BMDCs. *miR-155*^{-/-} recipient BMDCs were incubated with donor exosomes for 24 hours to allow time for miRNA transfer and knockdown of miRNA targets (see FIG. 2A). Using qRT-PCR, increased miR-155 levels and decreased mRNA levels of miR-155 targets BACH1 and SHIP1 were detected when cells were treated with *Wt* exosomes (see FIGS. 2B-2D). These changes were substantially prevented if the exosomes were derived from *miR-155*^{-/-} BMDCs, or if the *Wt* donor cells were pretreated with GW4869. SHIP1 protein levels were also decreased in *miR-155*^{-/-} BMDCs that received *Wt* exosomes (see FIGS. 2E and 2F). Exosome delivery of miR-155 brought its levels in the *miR-155*^{-/-} recipient cells to about 20% of *Wt* miR-155 levels (see FIGS. 12A and 12B). Furthermore, the relative expression of a separate miRNA, miR-425, which may have been previously seen to be released in exosomes (see Taylor, D. D. and Gercel-Taylor, Gynecol. Oncol. 110, 13–21 (2008); which is incorporated by reference herein), was looked at as a control. The levels of miR-425 may have increased with exosome delivery and may have been about the same in *Wt* and knockout groups (see FIGS. 12C and 12D).

[0033] In addition to using *miR-155*^{-/-} recipient cells, which may provide a substantially clean background to detect the transferred miRNA, it was also examined if transferred miR-155 may be detected in *Wt* BMDC recipients. miR-155 levels may have been increased upon treatment of *Wt* BMDCs with *Wt* exosomes but may not have been increased when treated with *miR-155*^{-/-} exosomes (see FIG. 2G). Additionally, the mRNA levels of both BACH1 and SHIP1 may both have been decreased in *Wt* BMDCs receiving exosomal miR-155 (see FIGS. 2H and 2I). These data may indicate that miR-155 may be transferred between *Wt* BMDCs in exosomes, resulting in the knockdown of known miR-155 targets.

[0034] As it has been found that miR-155 may knockdown its targets in recipient cells, it was next investigated whether the transfer of miR-155 may alter downstream factors in recipient cells. It was found that exosomal miR-155 may increase expression of HO1 (see FIG. 2J), an oxidative stress response gene that may be repressed by BACH1, a gene that has been shown to be targeted by transferred miR-155 (see O'Connell, R. M. *et al.*, J. Exp. Med. 205, 585–94 (2008); which is incorporated by reference herein) (see FIG. 2C). These results may indicate that transferred miR-155 may not only repress its putative direct targets but may also affect factors that are downstream of these targets.

[0035] To further characterize the functional transfer of miR-155 between BMDCs it was examined whether transferred miR-155 may be associated with AGO proteins that may be involved in miRNA-mediated knockdown of targets. Following exosomal transfer of miR-155 into *miR-155*^{-/-} BMDCs, an AGO IP was performed using a pan-AGO antibody and

Western blotting for AGO2 was performed to verify if pull-down was occurring. Using qRT-PCR, it was found that miR-155 may be associated with AGO proteins in *miR-155*^{-/-} recipient cells (see FIGS. 2K and 2L). miR-155 associated AGO proteins were not detected when *miR-155*^{-/-} BMDCs were treated with *miR-155*^{-/-} exosomes. Further, AGO2 was not detected via Western blotting and miR-155 was not pulled down when an isotype control antibody was used. As an additional control it was found that another miRNA, miR-146a, may also be enriched in the AGO pull-down from both groups (see FIG. 2M). These data may demonstrate that exosomal miR-155 is associated with AGO proteins, components of the RISC complex, following its uptake by recipient BMDCs.

Exosomal Transfer of miR-146a May be Functionally Relevant

[0036] miR-146a may be an anti-inflammatory miRNA involved in DC function (see Turner, M. L. *et al.* J. Immunol. 187, 3911–7 (2011); which is incorporated by reference herein), and may play an opposing role to miR-155 during inflammatory responses (see Huffaker, T. B. *et al.*, Cell Rep. 2, 1697–709 (2012); and Hu, R. *et al.*, Immunity 41, 605–619 (2014); each of which is incorporated by reference herein). To determine if miR-146a may also be contained in BMDC derived exosomes, exosomes from *Wt* BMDCs were isolated that had been treated with or without GW4869 and/or LPS and it was found that miR-146a may be contained in exosomes from untreated BMDCs but may not be present in the exosomal pellet from BMDCs treated with GW4869 (see FIG. 3A). Additionally, reductions in miR-146a were observed in the extracellular exosomal fraction obtained from *Rab27 DKO* BMDCs compared to *Wt* controls (see FIG. 3B). These data may reveal that miR-146a may be contained within exosomes released from BMDCs.

[0037] To test if miR-146a may be functionally transferred between BMDCs exosomes from *Wt* or *miR-146a*^{-/-} BMDCs were isolated and administered to *miR-146a*^{-/-} BMDCs (see FIG. 3C). Similar to miR-155, it was observed that exosomal miR-146a may have been taken up by recipient BMDCs (see FIG. 3D), and that miR-146a targets, IRAK1 and TRAF6, may have been repressed in recipient BMDCs receiving *Wt* but not *miR-146a*^{-/-} exosomes looking at both the mRNA and protein levels (see FIGS. 3E-3J). Additionally, miR-146a copy number was calculated in *Wt* and *miR-146a*^{-/-} exosomes where there was found to be about 1 copy of miR-146a per exosome (see FIG. 3K). miR-146a copy number was also calculated in *Wt* and *miR-146a*^{-/-} donor BMDCs and BMDCs that received either *Wt* or *miR-146a*^{-/-} exosomes (see FIG. 3L). It was observed that an average of 370 copies were present in recipient BMDCs following exosomes treatment. It has been suggested that 100-1000 copies of miRNA per cell may be functionally relevant (see Mullokandov, G.

et al., Nat. Methods 9, 840–846 (2013); which is incorporated by reference herein). As a control, the relative expression of miR-425 was investigated, which was found to be similar between the genotypes (see FIGS. 12E and 12F). The copy number data disclosed herein along with the observations of target knockdown disclosed herein may be consistent with exosomally-transferred miR-146a having functional relevance.

[0038] It has been shown in human B cell lines that miRNAs may be selectively packaged into exosomes based on 3' non-templated nucleotide additions (NTAs) (see Koppers-Lalic, D. *et al.*, Cell Rep. 8, 1649–58 (2014); which is incorporated by reference herein), where 3' uridylation was enriched in miRNAs contained in exosomes and 3' adenylation was enriched in miRNAs retained in cells. To address whether a similar phenomenon can be observed, RNA-Seq was performed using RNA from *Wt* donor BMDCs and *miR-155* and *miR-146a* double knockout (*DKO*) BMDCs that had received *Wt* exosomes. Next, a previously reported approach was used to identify NTAs in the data set of the present disclosure (see Koppers-Lalic, D. *et al.*, Cell Rep. 8, 1649–58 (2014); which is incorporated by reference herein). However, an enrichment of 3' uridylation was not observed in transferred miR-155 or miR-146a (see FIGS. 13A-13D). This difference from previous findings may be due to species and cellular differences (mouse primary BMDCs versus human B cell lines) or further processing of the transferred miRNAs in recipient cells. However, some differences were observed at certain nucleotide positions in each respective mature miRNA sequence when comparing *Wt* donor BMDCs to *DKO* BMDCs that received *Wt* exosomes (see FIGS. 19A-20B). These results may be consistent with the idea that alterations to the mature miRNA sequence may influence miRNA loading into exosomes versus cellular retention.

Seed-Dependent Repression of miRNA Targets

[0039] It was next determined whether miR-155 and miR-146a mimic loaded exosomes were sufficient to mediate direct target knockdown in recipient cells. *miR-155*^{-/-} or *miR-146a*^{-/-} BMDCs were transfected with either a corresponding miRNA mimics, scrambled miRNA mimics, or seed mutant miRNA mimics for 24 hours then washed 3 times with PBS to remove any mimics that did not make it into the cells (see FIG. 4A). Exosomes were isolated from the cells after 24 hours and the isolated exosomes were transferred to recipient knockout BMDCs. After 24 hours, RNA was isolated from the cells and qRT-PCR was performed to assay the delivery of mimics and the knockdown of target mRNAs. It was found that miRNA mimics may be loaded into exosomes and delivered to recipient cells (see FIGS. 14A-14C and 15A-15D). The transfer of miRNA mimics containing exosomes

may result in knockdown of respective target mRNAs in recipient BMDCs (see FIGS. 4B-4F). However, exosomes that did not carry mimics, or that carried scrambled, or seed mutant mimics showed substantially no change in the mRNA targets. These results may indicate that exosomal miRNAs may be responsible for direct target repression and may be able to complement the target knockdown phenotype.

[0040] To further assess whether exosomal miRNA target repression was direct, 3' UTR luciferase reporter assays were utilized. Knockout BMDCs were transfected with 3' UTR luciferase reporters for 6 hours followed by treatment with or without *Wt* exosomes (see FIG. 4G). *miR-155*^{-/-} BMDCs were transfected with either a pmiReport empty vector control, BACH1 3' UTR, BACH1 miR-155 binding site (bs) mutant 3' UTR, or a miR-155 positive control (2mer). Luciferase activity in cells receiving the BACH1 3' UTR or 2mer reporter constructs may have been reduced in response to miRNAs delivered by exosomes, while the exosomal miRNAs may have had little impact on luciferase activity in cells receiving the pmiReport empty vector or the BACH1 miR-155 bs mutant 3' UTR reporter (see FIG. 4H). In a separate experiment, *miR-146a*^{-/-} BMDCs were transfected with either a pmiReport empty vector control, TRAF6 3' UTR, or a TRAF6 miR-146a bs mutant 3' UTR. The BMDCs transfected with the TRAF6 3' UTR may have had decreased luciferase activity compared to the pmiReport empty vector and the TRAF6 miR-146a bs mutant 3' UTR following exosome delivery of miRNAs (see FIG. 4I). These results indicate that exosomally transferred miRNAs may repress, or directly repress, their targets via direct 3' UTR interactions.

Exosomal miR-155 and miR-146a May Modulate the Response to LPS

[0041] To determine whether the transfer of miR-155 via exosomes may affect the BMDC response to LPS, exosomes were isolated from *Wt* or *miR-155*^{-/-} BMDCs and transferred to *miR-155*^{-/-} BMDCs. 24 hours later, cells were treated with LPS for 6 hours (see FIG. 5A). Substantially consistent with a previously reported role for miR-155 in promoting IL-6 expression (see Kurowska-Stolarska, M. *et al.*, Proc. Natl. Acad. Sci. U. S. A. 108, 11193–8 (2011); which is incorporated by reference herein) and previously reported roles of miR-155 regulated responses to endotoxin (see Tili, E. *et al.*, J. Immunol. 179(8), 5082-5089 (2007); which is incorporated by reference herein), cells that were treated with miR-155-containing exosomes produced more IL-6 upon treatment with LPS for 6 hours than cells having received *miR-155*^{-/-} exosomes (see FIG. 5B). These findings may indicate that exosomes containing miR-155 may reprogram recipient BMDCs in a manner that enhances their response to LPS.

[0042] miR-146a may induce an anti-inflammatory responses to LPS (see Boldin, M. P. *et al.*, J. Exp. Med. 208, 1189–201 (2011); which is incorporated by reference herein). Therefore, it was investigated whether exosomally transferred miR-146a may program BMDCs to respond in an anti-inflammatory manner using a similar experimental setup as was done for miR-155 (see FIG. 5C). BMDCs pre-treated with *Wt* exosomes produced more IL-10, but less IL-6 and IL-12 p40, following LPS stimulation than BMDCs that received *miR-146a*^{-/-} exosomes (see FIGS. 5D-5F). This gene expression profile may demonstrate that miR-146a-containing exosomes reduce the pro-inflammatory response by BMDCs following LPS treatment. Without LPS treatment there was no substantial difference in IL-10, IL-6, or IL-12 p40 expression by cells receiving *Wt* vs. *miR-146a*^{-/-} exosomes (see FIGS. 5D-5F), indicating that exosomal miR-146a may specifically alter how these cells respond to LPS. Like miR-155, the instant data may indicate that miR-146a is functionally transferred in exosomes and able to cause physiological changes in recipient cells. However, unlike miR-155, exosomal miR-146a may act to dampen the inflammatory response to LPS. These results may be substantially consistent with previous observations that miR-155 and miR-146a may play substantially opposing roles during inflammation (see Huffaker, T. B. *et al.*, Cell Rep. 2, 1697–709 (2012); and Hu, R. *et al.*, Immunity 41, 605–619 (2014); each of which is incorporated by reference herein).

miR-155 May be Transferred between Immune Cells *In Vivo*

[0043] It was determined if miRNAs may be transferred between immune cells *in vivo*. It was first investigated whether exosomes were present in mouse BM by isolating exosomes directly from the BM of *Wt* and *miR-155*^{-/-} mice using differential centrifugation. It was found that both genotypes had exosomes present in the BM (see FIG. 6A) and that miR-155 was expressed in *Wt* BM exosomes (see FIG. 6B) while miR-146a was present in exosomes from both genotypes (see FIG. 6C). It was next determined whether miR-155 was passed between immune cells *in vivo*. To investigate this, *miR-155*^{-/-} mice were lethally irradiated and reconstituted with either an equal mix of *CD45.1*⁺ *Wt* and *CD45.2*⁺ *miR-155*^{-/-} BM or just *miR-155*^{-/-} BM. After allowing 3 months for reconstitution, mice were injected with LPS to stimulate production of miR-155 by BM cells (see FIG. 6D). BM cells were isolated 24 hours after LPS stimulation, and *miR-155*^{-/-} hematopoietic cells were sorted via FACS according to their different CD45 alleles (see Fig. 6F). Further, the *miR-155*^{-/-} BM was fractionated into B cell, myeloid cell, and T cell fractions using the surface markers B220, CD11b, and CD3, respectively (see FIGS. 6G and 6H). Using qRT-PCR, miR-155 expression was detected in *miR-155*^{-/-} B cells, T cells, and myeloid cells taken

from *miR-155*^{-/-} mice that had been reconstituted with both *Wt* and *miR-155*^{-/-} BM (see FIG. 6E). As a control, no, or substantially no, miR-155 expression was observed in cells from mice reconstituted with only *miR-155*^{-/-} BM. These data may provide evidence that miR-155 may be located within exosomes within the BM and may be transferred between immune cells *in vivo*.

[0044] To determine whether miRNA-containing exosomes could deliver miRNAs to various cell types *in vivo*, approximately 10⁹ exosomes derived from *miR-155*^{-/-} or *Wt* BMDCs were intraperitoneal (i.p.) injected into *miR-155*^{-/-} mice. After multiple injections, over a week, the spleens of these mice were harvested and CD3⁺ T cells, B220⁺ B cells, and CD11b⁺ myeloid cells were sorted via FACS. It was found that miR-155 may be delivered to all three of these cell types in the spleen (see FIGS. 6I-6K). This may indicate that exosomes are able to deliver miRNAs to various immune cell types.

Exosomal miR-155 May Enhance Inflammatory Responses *In Vivo*

[0045] The present *in vitro* data may suggest that exosomally delivered miR-155 may increase the BMDC response to LPS (see FIG. 5B). It was investigated whether the same effect, or substantially the same effect, may be seen *in vivo*. About 10⁹ *Wt* or *miR-155*^{-/-} BMDC derived exosomes were i.p. injected into *miR-155*^{-/-} mice, followed by administration of LPS 24 hours later, and collection of serum 2 hours after that (see FIG. 7A). The injection of *Wt* exosomes before LPS administration resulted in increased TNF α , and trending elevations in IL-6 serum concentrations compared to mice pretreated with *miR-155*^{-/-} exosomes (see FIGS. 7B and 7C). Additionally, it was observed that miR-155 was delivered to the spleen, liver, and bone marrow, where reduced target mRNA levels were found, consistent with miR-155 activity in these tissues (see FIGS. 7D-7I). These data may demonstrate that miR-155 may be functionally delivered to a variety of tissues and cell types via exosome injection, and that this may increase the response to LPS *in vivo*.

Exosomal miR-146a May Reduce Inflammatory Responses *In Vivo*

[0046] It was next investigated whether exosomes containing miR-146a would have an anti-inflammatory impact following LPS administration to mice. About 10⁹ exosomes were isolated from *Wt* or *miR-146a*^{-/-} BMDCs and injected i.p. into *miR-146a*^{-/-} mice. 24 hours later, the mice were given LPS and serum was collected after 2 hours to assay inflammatory cytokine levels (see FIG. 8A). Mice having received *Wt*, miR-146a-containing exosomes had reduced TNF α and IL-6 serum concentrations after LPS administration compared to mice having received miR-146a-deficient exosomes (see FIGS. 8B and 8C). Exosomes alone had a negligible effect, or substantially negligible effect, on cytokine levels

in vivo. Further, 24 hours after LPS injection the spleen, liver, and bone marrow were isolated and it was found that miR-146a was present in these tissues from mice receiving *Wt* exosomes but was not present in tissues from mice that received *miR-146a*^{-/-} exosomes (see FIGS. 8D-8F). Additionally, miR-146a targets involved in TLR signaling were repressed in tissues in mice that received *Wt* exosomes (see FIGS. 8G-8I). Substantially similar results were obtained when miR-146a-containing exosomes were administered to *Wt* recipients (see FIGS. 9A-9I). Together, these data may demonstrate that exosomal miR-146a may reduce the inflammatory response to LPS in mice.

[0047] The present data may demonstrate that miRNAs 155 and 146a are released from BMDCs in exosomes, are taken up by recipient BMDCs, and subsequently mediate target gene repression. Additionally, it has been found that the transfer of miR-155 or miR-146a may alter the ability of recipient cells to respond to inflammatory cues both *in vitro* and *in vivo*. The capacity of these transferred miRNAs to influence the response of BMDCs to a pro-inflammatory stimulus may suggest that the transfer of miRNAs is a mechanism by which immune cells are primed to respond to an encounter (*i.e.*, and imminent encounter) with a microbe. Because miR-155 and miR-146a have been shown to regulate inflammation in a variety of contexts, the present findings may provide insights into how and where miR-155 and miR-146a function, and may provide a greater understanding of how they regulate mammalian immunity. Furthermore, the present disclosure may add to the evidence that miRNA transfer within exosomes is part of the intercellular communication networks that may coordinate complex immune responses (see Zhang, Y. *et al.*, Mol. Cell 288, 23586–96 (2010); Montecalvo, A. *et al.*, Blood 119, 756–66 (2012); and Mittelbrunn, M. *et al.*, Nat. Commun. 2, 282 (2011); each of which is incorporated by reference herein).

[0048] In the present disclosure, miRNAs were produced at substantially endogenous levels by primary cells, and established endogenous miRNA target genes were used as readouts for miRNA activity in recipient cells. Furthermore, exosomes were purified away from other BMDC factors, such as cytokines, and miR-155 and miR-146a deficient recipient cells were used to track the delivery and specific effects of the exosomally delivered miRNA both *in vitro* and *in vivo*.

[0049] As discussed above, copy number analysis found there to be about 1 copy of miR-146a per exosome, consistent with exosomes having low content of individual miRNAs. However, it was found that BMDCs produce ~500 exosomes after 24 hours of culture, indicating that each cell may be able to release at least hundreds of copies of miR-146a in exosomes to be delivered to recipient BMDCs and mediate target knockdown.

Thus, the substantially large numbers of exosomes produced per cell may allow for the loading of low miRNA numbers per exosome to achieve functional relevance.

[0050] Exosome populations produced by *Wt* cells may also contain both miR-155 and miR-146a, which, as shown herein, may have either pro- or anti-inflammatory effects, respectively. There may be several possible reasons why exosome populations may contain both miR-155 and miR-146a. Without being bound by theory, first, exosomes may be transferring both pro- and anti-inflammatory miRNAs together to buffer inflammatory responses by recipient cells to achieve the optimal magnitude of response. Second, miR-155 and miR-146a may be disposed or located in separate exosomes that are delivered to different target cell types. A third possibility may be that miR-155 and miR-146a release in exosomes is a dynamically regulated process where the ratio of miR-155 to miR-146a may change over time. For example, immune cells that have sensed a pathogen may initially release exosomes with high levels, or substantially high levels, of pro-inflammatory miRNAs like miR-155 followed by a shift to anti-inflammatory miRNAs like miR-146a during the resolution phase of the response.

[0051] Exosomes may be complex vesicles that comprise an assortment of different membrane and soluble proteins as well as different types of RNAs, including miRNAs (see Théry, C. *et al.*, Nat. Rev. Immunol. 2, 569–79 (2002); which is incorporated by reference herein). Thus, it may not be ruled out that exosomes produced by *Wt* vs. *miR-155*^{-/-} or *miR-146a*^{-/-} BMDCs may differ in some aspect other than the presence or absence of the corresponding miRNA that has been genetically deleted, and that this may also have some influence on the inflammatory response by recipient cells. However, this possibility may have been addressed, at least partially, herein by complementing the exosomal miRNA target gene phenotypes by loading miRNA mimics into miRNA knockout exosomes. Further, it was also demonstrated that target repression may be direct through the use of seed mutant mimics that failed, or generally failed, to repress target gene expression in recipient cells as well as 3' UTR luciferase reporter assays where binding site mutant 3' UTRs were not repressed, or not substantially repressed, by exosomally transferred miRNAs. These data may suggest that individual miRNAs in exosomes are transferred between cells in a functionally relevant manner.

[0052] The transfer of miRNAs between immune cells *in vivo* was also observed, indicating that this mechanism of cellular communication may also occur in a physiologically relevant setting. Without being bound by theory, functional miRNA transfer via exosomes

may be relevant in defined microenvironments such as stem cell niches, or within tumors, where exosome concentrations may be at their highest.

[0053] Producing exosomes from patients' own cells may serve as a vehicle for autologous therapies involving miRNA delivery, and the capacity to load miRNA mimics, as disclosed herein, may suggest that the miRNA content of exosomes may be manipulated. Further, as disclosed herein, injection of miR-146a and miR-155 containing exosomes may result in delivery of these miRNAs to a variety of mouse tissues, repression of target genes, and an altered inflammatory response *in vivo*, where miR-155 may promote and miR-146a may repress inflammation in response to endotoxin. This may suggest that exosomal miR-146a may be used as a prophylaxis or therapy to treat inflammatory diseases, such as bacterial sepsis. Conversely, exosomal miR-155 may be used as an adjuvant to improve vaccine efficacy.

Methods

[0054] Mice: *miR-155*^{-/-} (Allan Bradley Lab, Sanger Institute), *miR-146a*^{-/-} (David Baltimore Lab, California Institute of Technology), *miR-155* and *miR-146a* double knockout mice (DKO) (Ryan O'Connell, University of Utah), *Wt* (Jackson Labs), and *CD45.1 Wt* (Jackson Labs) are on a C57BL6 genetic background and housed in the animal facility at the University of Utah. *Rab27 DKO* (*Rab27a*^{ash/ash} *Rab27b*^{-/-}) mice (Tanya Tolmachova and Miguel C. Seabra, Imperial College London) were housed at the Imperial College London under the UK Home Office animal project license 70/7078 and BM was sent to Utah for experiments together with BM from the *Wt* animals of similar age, sex, and background (C57BL6). Experiments were approved by the Institutional Animal Care and Use Committee at the University of Utah. Mice were age matched and sex matched and were in the age range of 8-16 weeks old. For bone marrow reconstitutions lethal irradiation (1000 rads) was delivered using an X-ray source. Following irradiation, mice were injected with about 3 million bone marrow cells via retro-orbital injection. *E. Coli* LPS (Sigma) was administered through i.p. injections at a sub-lethal concentration of 50 µg (see O'Connell, R. M. et al., J. Exp. Med. 205, 585–94 (2008); which is incorporated by reference herein). In other experiments, exosomes were i.p. injected 24 hours before LPS injection of the same concentration.

[0055] Cells culture: BMDCs were derived from mouse bone marrow by culturing red blood cell (RBC) depleted BM in complete RPMI (10% fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin, β-mercaptoethanol, glutamate, sodium pyruvate, HEPES, and non-essential amino acids) with 20 ng/ml GM-CSF for 3-4 days at 37 °C with

5% CO². The cells were then cultured in 5 ml complete RPMI with 20 ng/ml GM-CSF for an additional 3-4 days for a total of 7 days in culture. LPS stimulation was performed at a concentration of 500 ng/ml. Cells were separated using a Transwell Permeable Support 0.4 µm Polycarbonate Membrane 24 mm insert 6 well plates (Costar).

[0056] RNA-Seq: *Wt* exosomes were transferred to recipient *miR-155 miR-146a double knockout BMDCs (DKO)*. 3 biological replicates from *Wt* donor and exosome recipient *DKO* BMDCs were submitted to the University of Utah's High Throughput Genomic Core for Illumina TrueSeq Small RNA Sample Prep. Non-templated nucleotide additions (NTAs) were identified and frequencies of A, G, C, and U additions were calculated as described previously (see Koppers-Lalic, D. *et al.* Cell Rep. 8, 1649–58 (2014); which is incorporated by reference herein) by the University of Utah's bioinformatics core facility. Additionally, each position was analyzed in the mature miRNA sequences of miR-155 and miR-146a and the percentage of observed bases at each position was calculated to determine changes in nucleotide composition between miRNAs in donor versus exosome recipient BMDCs. RNA-seq data is deposited in GEO with the accession number GSE67946.

[0057] Copy number analysis: miRNA copy number was calculated in *Wt* and *miR-146a-/-* donor cells, exosomes, and *miR-146a-/-* BMDCs that received *Wt* exosomes. Total RNA was isolated (using the miRNeasy kit) from about 1 million donor BMDCs and about 1 million recipient BMDCs that were cultured with exosomes from about 1 million donor BMDCs collected after 24 hours, or exosomes isolated from about 1 million BMDCs after 24 hours. 30 ng of RNA isolated from these samples was then used for qRT-PCR. To make a standard curve, 1 ng of synthetic single stranded miR-146a (IDT custom RNA oligo – sequence: UGAGAACUGAAUCCAUUGGGUU (SEQ ID NO:1)) was spiked into either about 1 million *miR-146a-/-* BMDCs or exosomes isolated from about 1 million *miR-146a-/-* BMDCs and total RNA was isolated in the same manner as the experimental samples (miRNeasy). 30 ng of this isolated RNA was used to perform a cDNA reaction to use for standard curves. Standard curves for cells and exosomes were made with these cDNA samples via serial dilutions and cp values were determined via qPCR with miR-146a primers. The BMDC standard curve was then used to determine copy number in our cellular samples and the BMDC exosome standard curve was used to determine the copy number of miR-146a in the exosome samples.

[0058] Mimic: miRNA mimics were purchased from Qiagen. Scrambled, seed mutant, and miR-mimic sequences are as follows:
miR-146a scramble (5'-ACGAGUUACGUGGUACGUUAAU-3' (SEQ ID NO:2)),

miR-146a seed mutant (5'-UGUCAAGAGAAUCCAUGGGUU-3' (SEQ ID NO:3)),
miR-146a mimic (5'-UGAGAACUGAAUCCAUGGGUU-3' (SEQ ID NO:4)),
miR-155 scramble (5'-GGAUGUUAUUGCGUAUAUUAGGA-3' (SEQ ID NO:5)),
miR-155 seed mutant (5'-UUUGCUAAAAUUGUGAUAGGGGU-3' (SEQ ID NO:6)),
miR-155 mimic (5'-UUA AUGCUAAUUGUGAUAGGGGU-3' (SEQ ID NO:7)).

[0059] Donor cells were transfected with 30 μ l of the hi-perfect transfection reagent (Qiagen) in 2 ml of serum free media with 60 ng of each mimic. After 24 hours, cells were washed 3 times with PBS and given fresh medium. Exosomes were isolated 24 hours after washing and transferred to recipient cells for 24 hours.

[0060] Luciferase reporter assay: 2.5×10^5 knockout BMDCs were transfected with 3' UTR luciferase reporter constructs (for *mir-155*^{-/-}: pmiReport, Bach1, Bach1 155 mutant, 2mer (see O'Connell, R. M. *et al.*, J. Exp. Med. 205, 585–94 (2008); which is incorporated by reference herein)) (for miR-146^{-/-}: pmiReport, Traf6, Traf6 146a mutant (see Taganov, K. D. *et al.*, Proc. Natl. Acad. Sci. U. S. A 103(33), 12481–12486 (2006); which is incorporated by reference herein)) using Lonza's Amaxa Mouse Dendritic Cell Nucleofector Kit according to manufacturer's instructions. After 6 hours of nucleofection, BMDCs were treated with or without *Wt* BMDC derived exosomes and luciferase activity was measured 24 hours later using a Dual Luciferase Kit (Promega). Luciferase repression of exosome treated BMDCs compared to no exosome treatment was calculated and graphed as percent change in luciferase activity.

[0061] Exosome isolation and procedures: For *in vitro* experiments exosomes were isolated from about 1 million BMDCs cultured in media for 24 hours and they were transferred to the same number of recipient BMDCs. Differential centrifugation was performed to isolate exosomes from conditioned medium. Initial spins consisted of a 10 minute spin at 1000xg, a 2000xg spin for 10 minutes, and a 10,000xg spin for 30 minutes. The supernatant was retained each time. The supernatant was then spun at 100,000xg for 70 minutes and the pellet was re-suspended in 1x PBS to dilute remaining soluble factors, followed by another centrifugation at 100,000xg for 70 minutes. The final pellet comprised the exosomes, which were re-suspended in tissue culture media. This protocol is based on previous exosome isolation methods (see Théry, C., *et al.* Curr. Protoc. Cell Biol. 1–29 (2006); which is incorporated by reference herein). Either a Beckman ultracentrifuge with a TI75 fixed angle rotor or a Thermo Scientific Sorvall Lynx 6000 with a T26-8X50 rotor was utilized. GW4869 is a neutral sphingomyelinase inhibitor that has been previously used to prevent exosome release (see Kosaka, N. *et al.*, J. Biol. Chem. 285, 17442–52 (2010); and

Chen, X. *et al.*, Protein Cell 3, 28–37 (2012); each of which is incorporated by reference herein). In some experiments BMDCs were treated with 10 μ M GW4869 (Sigma-Aldrich) or vehicle for 24 hours.

[0062] Exosome numbers for the miR-146a and miR-155 *in vivo* experiments were determined using the EXOCET Exosome Quantification Assay Kit from System Biosciences according to kit instructions. Three plates of approximately 3 million BMDCs each were cultured in media for 3 days. The supernatant from these plates was collected and exosomes were isolated as described above. For *in vitro* experiments, the supernatant was taken from about 1 million BMDCs that had been cultured for 24 hours.

[0063] Western blotting and ELISA: Protein was isolated with RIPA lysis buffer (RIPA buffer, PMSF, NaF, NaVO₄, and protease inhibitor). Total protein levels were quantified using a Bio-Rad protein assay and equal amounts of protein were loaded and separated using 12% (TRAF6, Ago2, and CD63) or 8% (SHIP1 and IRAK1) SDS-PAGE followed by immunoblotting with appropriate antibodies. Antibodies include the following: α -TRAF6 at 1:500 dilution (EP591Y Abcam, ab33915), α - β -Actin antibody at 1:1000 dilution (mAbcam 8226, ab8226), α -Ago2/eIF2C2 antibody at 1:200 dilution (Abcam, ab32381), α -CD63 (H-193) at 1:200 dilution (Santa Cruz Biotechnology, sc-15363), α -SHIP1 (V-19) at 1:250 dilution (Santa Cruz Biotechnology, sc-1963), α -IRAK1 D5167 at 1:500 Dilution (Cell Signaling, #4504). Western blots were quantified using ImageJ software. The ELISA assay used to quantify mouse IL-6 and TNF α concentrations were obtained from eBioscience and performed using the manufacturer's suggested protocol. Some images have been cropped for presentation. Full size images are presented in FIGS. 16A-18E.

[0064] RNA isolation and qRT-PCR: RNA isolation was performed using Qiagen's miRNeasy kit according to manufacturer's instructions. Mature miRNA cDNA was made with a miRCURY LNA universal RT microRNA PCR kit using 10 ng of RNA from each sample (Exiqon). qPCR of mature miRNA was performed with the miRCURY LNA universal RT microRNA PCR kit SYBR green master mix (Exiqon) with LNA primers for miR-146a-5p (Exiqon), mmu-miR155-5p (Exiqon), mmu-miR-425-5p (Exiqon), and 5s rRNA (Exiqon). Custom LNA primers were also made and designed by Exiqon to detect the miR-155 and miR-146a seed mutant mimics (miR-146a design ID 410833-1) (miR-155 design ID 410829-1). 5s was used to normalize expression. cDNA from total RNA was made with qScript using 10 ng of RNA from each sample (Quanta). qPCR was performed with Promega GoTaq pPCR master mix. Primer sequences are as follows: SHIP1-F (5'-GAGCGGGATGAATCCAGTGG-3' (SEQ ID NO:8)),

SHIP1-R (5'-GGACCTCGGTTGGCAATGGTA-3' (SEQ ID NO:9)),
 BACH1-F (5'-TGAGTGAGAGTGCGGTATTTGC-3' (SEQ ID NO:10)),
 BACH1-R (5'-GTCAGTCTGGCCTACGATTCT-3' (SEQ ID NO:11)),
 HO1-F (5'-TGACACCTGAGGTCAAGCAC-3' (SEQ ID NO:12)),
 HO1-R (5'-TCCTCTGTCAGCATCACCTG-3' (SEQ ID NO:13)),
 IRAK-F (5'-TGTGCCGCTTCTACAAAGTG-3' (SEQ ID NO:14)),
 IRAK-R (5'-TGTGAACGAGGTCAGCTACG-3' (SEQ ID NO:15)),
 TRAF6-F (5'-AAGCCTGCATCATCAAATCC-3' (SEQ ID NO:16)),
 TRAF6-R (5'-CTGGCACTTCTGGAAAGGAC-3' (SEQ ID NO:17)).
 L32-F (5'-AGCTCCCAAAAATAGACGCAC-3' (SEQ ID NO:18)),
 L32-R (5'-TTCATAGCAGTAGGCACAAAGG-3' (SEQ ID NO:19)). L32 levels were used to normalize mRNA expression levels.

[0065] Electron microscopy: EM samples were prepared using differential centrifugation from BMDC conditioned media. Exosomal pellets were re-suspended in PBS and processed by the University of Utah's EM core facility for cryo EM analysis.

[0066] Immunoprecipitations: An anti-pan Ago antibody (clone 2A8, Millipore) was used to IP Ago proteins. α -AGO and IgG control coated beads were prepared by incubating magnetic protein G beads (Active motif) with each respective antibody in IP lysis buffer (0.5% NP40, 150mM KCl, 1mM NaF, 25mM Tris, 2mM EDTA, protease inhibitor, and 0.5mM DTT) with rotation overnight at 4 °C. One third of the protein lysate prepared from BMDCs that had received either *Wt* or knockout exosomes using IP lysis buffer was used for the IP. Bead-antibody mixes were washed 3 times with lysis buffer with rotation at 4 °C, re-suspended in lysis buffer and added to the lysates. Lysates were incubated with bead-Ab mix at 4 °C with rotation overnight and then washed 6 times with IP wash buffer (300 mM NaCl, 50 mM Tris, .01% NP40, 5 mM MgCl₂, 129 ml dH₂O) with the last wash using PBS. Protein was isolated from a fraction of the sample with 1X Laemmli diluted in lysis buffer and RNA was isolated from another fraction using miRNeasy extraction. A Western blot for AGO2 (Abcam) and qRT-PCR for miR-155 and miR-146a were performed to confirm AGO pulldown and association with these miRNAs.

[0067] Flow cytometry: Fluorophore-conjugated monoclonal antibodies specific to CD45.1, CD45.2, B220, CD3, CD11b, or CD11c (Biolegend) were used to stain RBC-depleted bone marrow and spleen cells. These populations were sorted using a FACS Aria II in the Flow Cytometry Core Facility at the University of Utah.

[0068] Statistics: Data were analyzed using Student's t-Tests to determine statistically significant differences between relevant samples. P values were either listed or represented by the following number of stars: *, $p < 0.05$; **, $p < 0.01$; ***, $p < .001$; ****, $p < .0001$.

[0069] In some embodiments, the human miR-146a sequence is UGAGAACUGAAUCCAUGGGUU (SEQ ID NO:20), the mouse miR-146a sequence is UGAGAACUGAAUCCAUGGGUU (SEQ ID NO:21), the human miR-155 sequence is UUA AUGCUAAUCGUGAUAGGGGU (SEQ ID NO:22), and the mouse miR-155 sequence is UUA AUGCUAAUUGUGAUAGGGGU (SEQ ID NO:23).

[0070] References to approximations are made throughout this specification, such as by use of the term "about." For each such reference, it is to be understood that, in some embodiments, the value, feature, or characteristic may be specified without approximation. For example, where qualifiers such as "about" and "generally" are used, these terms include within their scope the qualified words in the absence of their qualifiers. For example, where the term "about 200 μm " is recited with respect to a feature, it is understood that in further embodiments, the feature can be precisely 200 μm .

[0071] Reference throughout this specification to "an embodiment" or "the embodiment" means that a particular feature, structure, or characteristic described in connection with that embodiment is included in at least one embodiment. Thus, the quoted phrases, or variations thereof, as recited throughout this specification are not necessarily all referring to the same embodiment.

[0072] Similarly, it should be appreciated that in the above description of embodiments, various features are sometimes grouped together in a single embodiment, figure, or description thereof for the purpose of streamlining the disclosure. This method of disclosure, however, is not to be interpreted as reflecting an intention that any claim require more features than those expressly recited in that claim. Rather, as the following claims reflect, inventive aspects lie in a combination of fewer than all features of any single foregoing disclosed embodiment.

[0073] The claims following this written disclosure are hereby expressly incorporated into the present written disclosure, with each claim standing on its own as a separate embodiment. This disclosure includes all permutations of the independent claims with their dependent claims. Moreover, additional embodiments capable of derivation from the independent and dependent claims that follow are also expressly incorporated into the present written description.

[0074] Without further elaboration, it is believed that one skilled in the art can use the preceding description to utilize the invention to its fullest extent. The claims and embodiments disclosed herein are to be construed as merely illustrative and exemplary, and not a limitation of the scope of the present disclosure in any way. It will be apparent to those having ordinary skill in the art, with the aid of the present disclosure, that changes may be made to the details of the above-described embodiments without departing from the underlying principles of the disclosure herein. In other words, various modifications and improvements of the embodiments specifically disclosed in the description above are within the scope of the appended claims. Moreover, the order of the steps or actions of the methods disclosed herein may be changed by those skilled in the art without departing from the scope of the present disclosure. In other words, unless a specific order of steps or actions is required for proper operation of the embodiment, the order or use of specific steps or actions may be modified. The scope of the invention is therefore defined by the following claims and their equivalents.

[0075] It will be apparent to those having skill in the art that many changes may be made to the details of the above-described embodiments without departing from the underlying principles of the invention. The scope of the present invention should, therefore, be determined only by the following claims.

Claims:

1. A method of repressing a target messenger RNA (mRNA) in a subject, comprising the steps of:
loading one or more exosomes with a microRNA (miRNA), wherein the miRNA is configured to repress the target mRNA; and
introducing the one or more miRNA-loaded exosomes into the subject.
2. The method of claim 1, further comprising the step of:
monitoring the level of the target mRNA in the subject.
3. The method of claim 1 or 2, wherein the miRNA is a synthetic miRNA.
4. The method of any one of claims 1–3, wherein the miRNA is further configured to mediate repression of a target gene.
5. The method of any one of claims 1–4, further comprising the steps of:
isolating one or more cells from the subject; and
harvesting the one or more exosomes from the one or more isolated cells.
6. The method of claim 5, wherein the step of harvesting of the one or more exosomes comprises differential centrifugation and washing.
7. The method of any one of claims 1–6, further comprising the steps of:
obtaining one or more genetically-modified cells, wherein the one or more genetically-modified cells are configured to not produce miRNAs; and
harvesting the one or more exosomes from the one or more genetically-modified cells.
8. The method of claim 7, wherein the step of harvesting of the one or more exosomes comprises differential centrifugation and washing.

9. The method of any one of claims 1–8, wherein the one or more miRNA-loaded exosomes are introduced into the subject via at least one intraperitoneal injection (IP injection).

10. The method of claim 9, wherein the one or more miRNA-loaded exosomes are introduced at or adjacent at least one of a predetermined tissue type or a predetermined cell type via the IP injection.

11. The method of claim 10, wherein the predetermined cell type comprises dendritic cells.

12. The method of claim 10, wherein the predetermined cell type comprises tumor cells.

13. The method of any one of claims 1–12, wherein the one or more miRNA-loaded exosomes are introduced into the subject via multiple intraperitoneal injections (IP injections).

14. The method of any one of claims 1–13, wherein the subject is a mammal.

15. The method of any one of claims 1–14, wherein the subject is a human.

16. A method of repressing a target messenger RNA (mRNA) in a subject, comprising the steps of:

loading one or more vesicles with a microRNA (miRNA), wherein the miRNA is configured to repress the target mRNA; and

introducing the one or more miRNA-loaded exosomes into the subject.

17. The method of claim 16, wherein the one or more vesicles are exosomes.

18. The method of claim 17, further comprising the steps of:

obtaining one or more genetically-modified cells, wherein the one or more genetically-modified cells are configured to not produce miRNAs; and

harvesting the one or more exosomes from the one or more genetically-modified cells.

19. A method for treating a subject having, or at risk of developing, an inflammatory disorder, comprising the steps of:

loading one or more exosomes with a microRNA (miRNA), wherein the miRNA is configured to reduce a pathological effect or symptom of the inflammatory disorder, or to reduce the risk of developing the inflammatory disorder; and

administering the one or more miRNA-loaded exosomes to the subject.

20. The method of claim 19, wherein the miRNA is miR-146a (SEQ ID NO:20).

21. The method of claim 19, wherein the sequence of the miRNA is at least eighty percent identical to the sequence of miR-146a (SEQ ID NO:20).

22. The method of claim 19, wherein the sequence of the miRNA is at least ninety percent identical to the sequence of miR-146a (SEQ ID NO:20).

23. The method of claim 19, wherein the sequence of the miRNA differs from the sequence of miR-146a (SEQ ID NO:20) at one nucleobase position.

24. The method of any one of claims 19–23, wherein the inflammatory disorder comprises bacterial sepsis.

25. A method for improving vaccine efficacy in a subject, comprising the steps of:
loading one or more exosomes with a microRNA (miRNA), wherein the miRNA is configured to act as an adjuvant to a vaccine; and
administering the one or more miRNA-loaded exosomes to the subject.

26. The method of claim 25, wherein the miRNA is miR-155 (SEQ ID NO:22).

27. The method of claim 25, wherein the sequence of the miRNA is at least eighty percent identical to the sequence of miR-155 (SEQ ID NO:22).

28. The method of claim 25, wherein the sequence of the miRNA is at least ninety percent identical to the sequence of miR-155 (SEQ ID NO:22).

29. The method of claim 25, wherein the sequence of the miRNA differs from the sequence of miR-155 (SEQ ID NO:22) at one nucleobase position.

30. A method of delivering a therapeutic microRNA (miRNA) to a subject, comprising the steps of:

isolating one or more cells from the subject;

harvesting one or more exosomes from the one or more isolated cells;

loading the one or more exosomes with the therapeutic miRNA, wherein the therapeutic miRNA is configured to repress a target mRNA; and

introducing the one or more therapeutic miRNA-loaded exosomes into the subject.

31. The method of claim 30, wherein the therapeutic miRNA is miR-146a (SEQ ID NO:20).

32. The method of claim 30, wherein the therapeutic miRNA is miR-155 (SEQ ID NO:22).

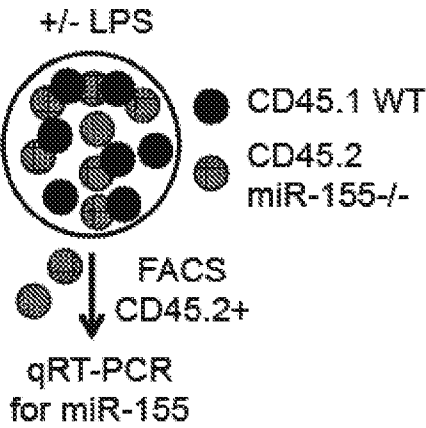


FIG. 1A

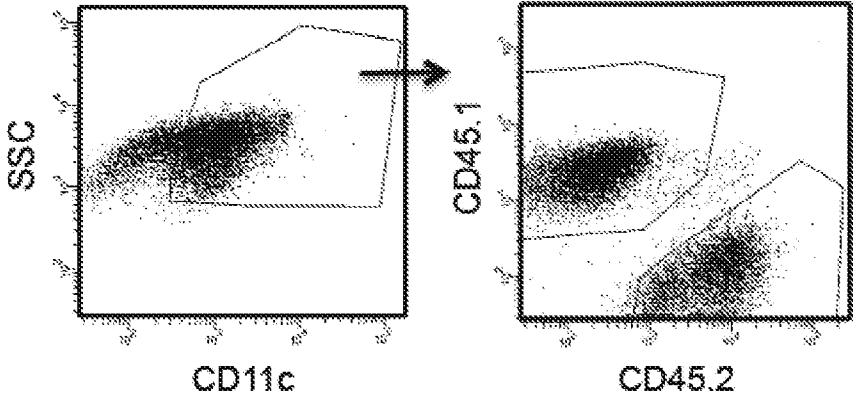
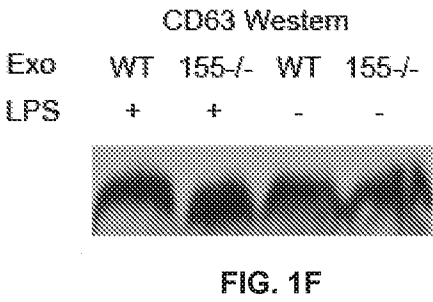
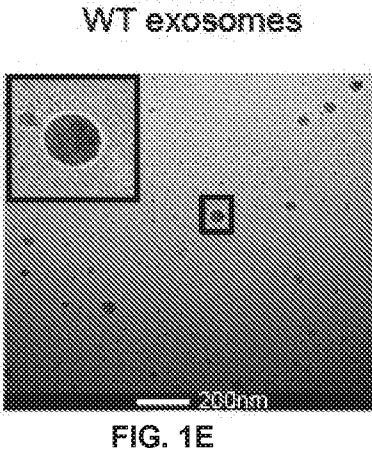
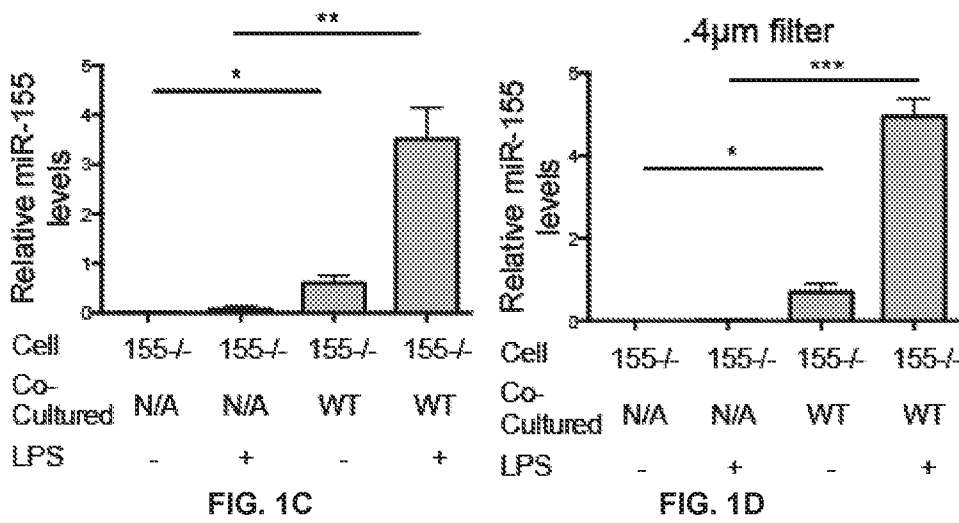


FIG. 1B



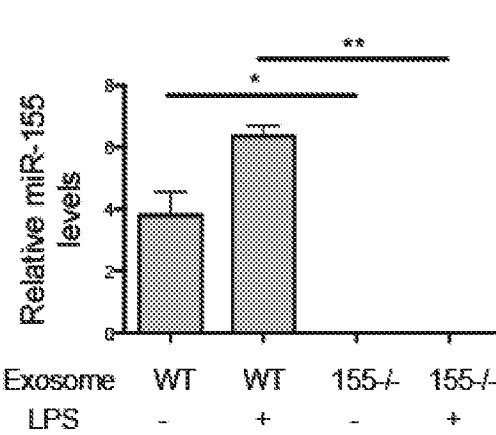


FIG. 1G

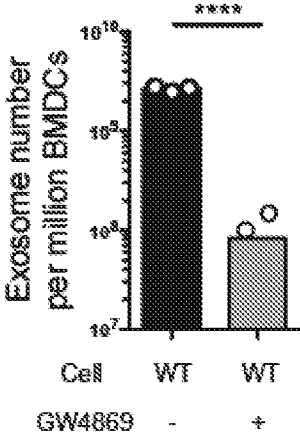


FIG. 1H

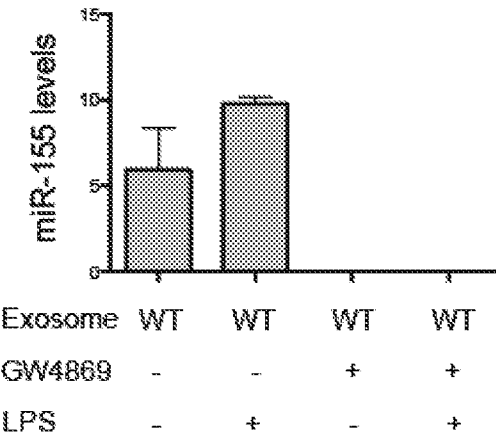


FIG. 1I

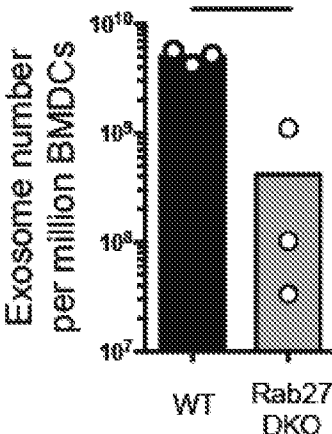


FIG. 1J

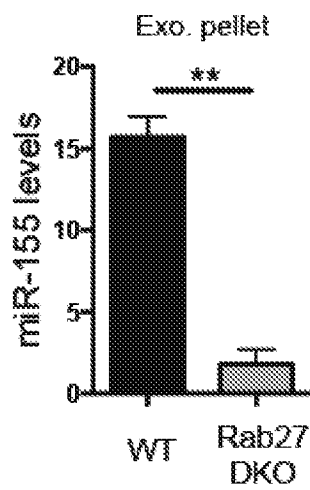


FIG. 1K

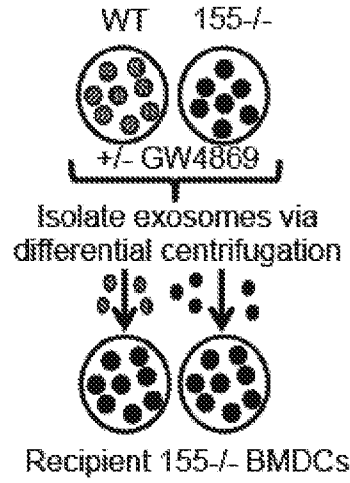


FIG. 2A

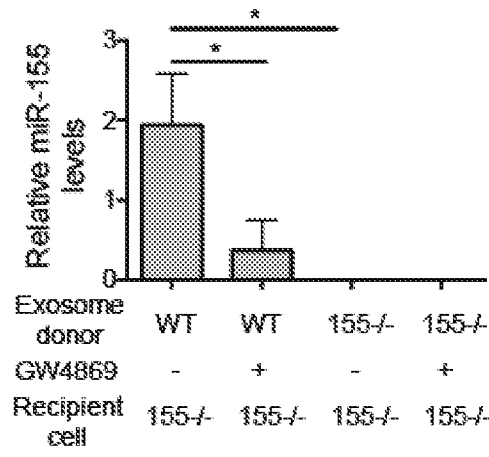


FIG. 2B

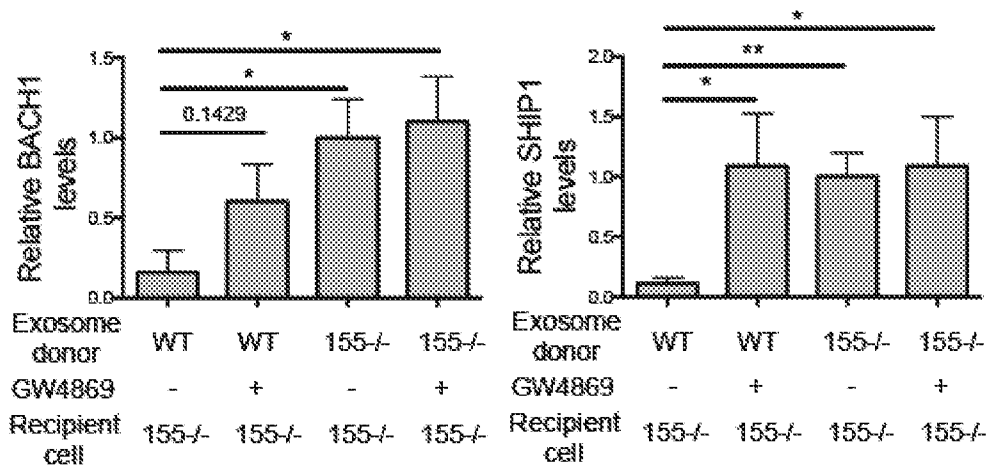


FIG. 2C

FIG. 2D

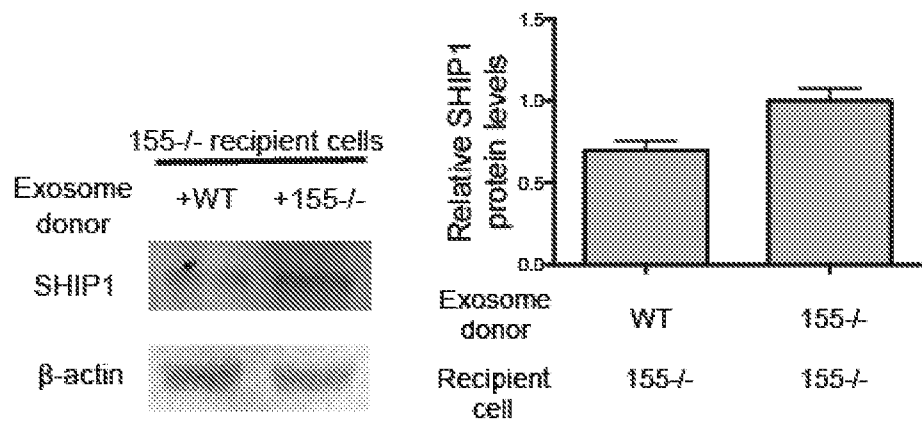


FIG. 2E

FIG. 2F

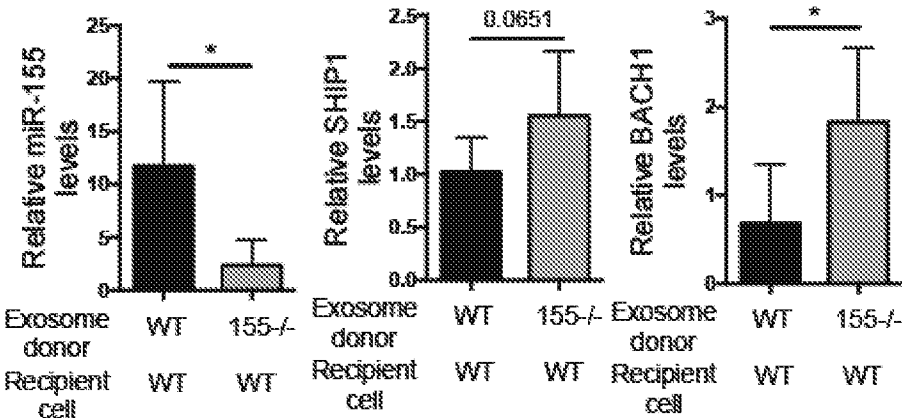


FIG. 2G

FIG. 2H

FIG. 2I

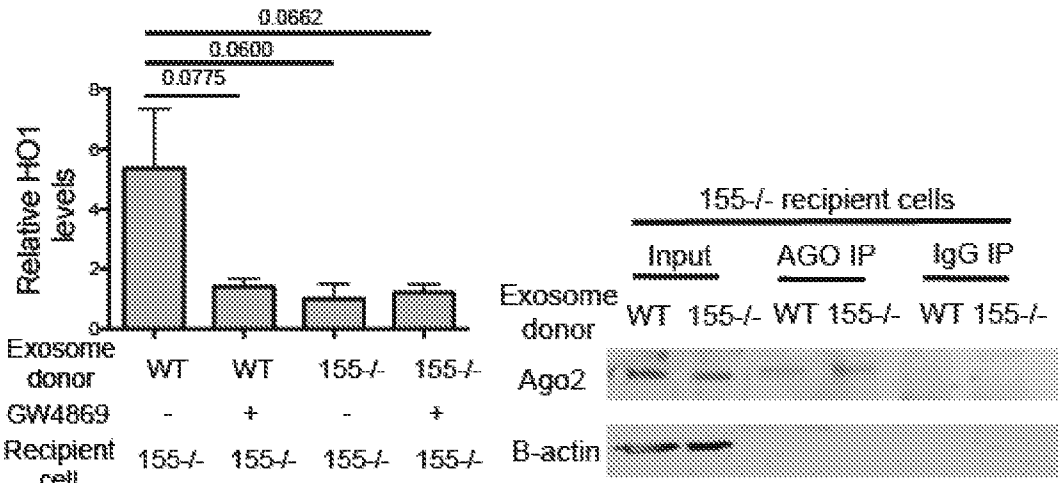


FIG. 2J

FIG. 2K

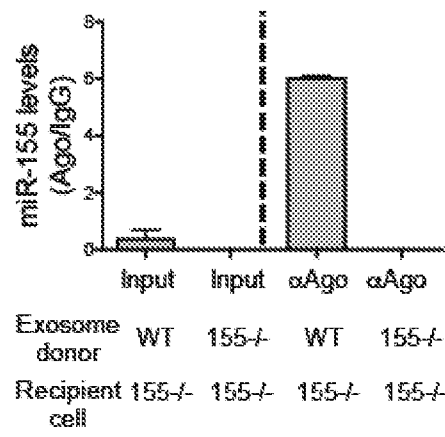


FIG. 2L

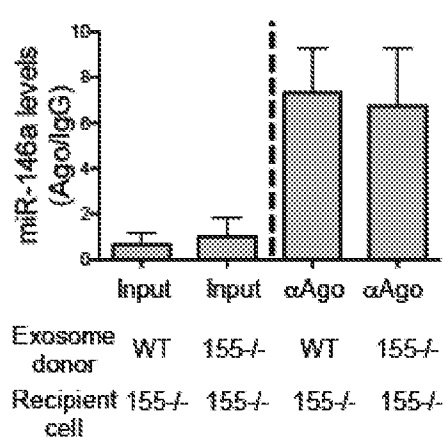


FIG. 2M

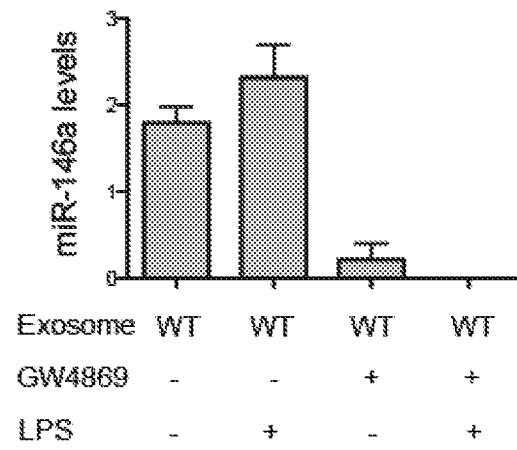


FIG. 3A

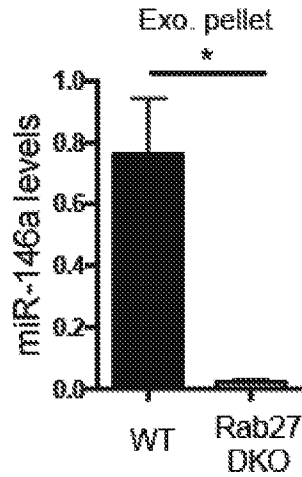


FIG. 3B

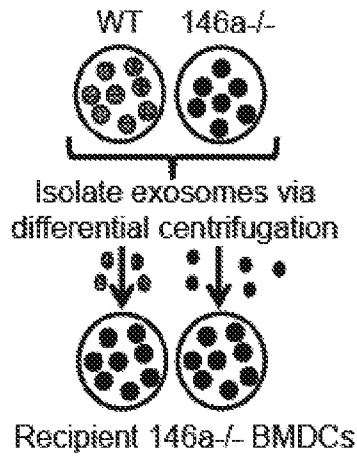


FIG. 3C

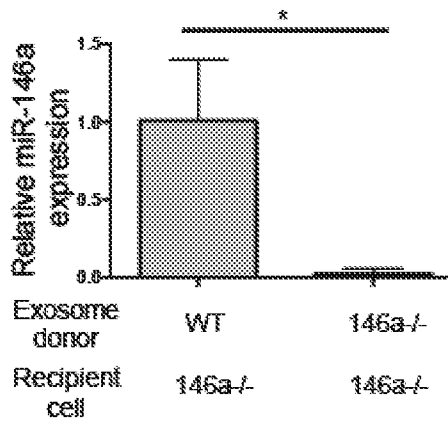


FIG. 3D

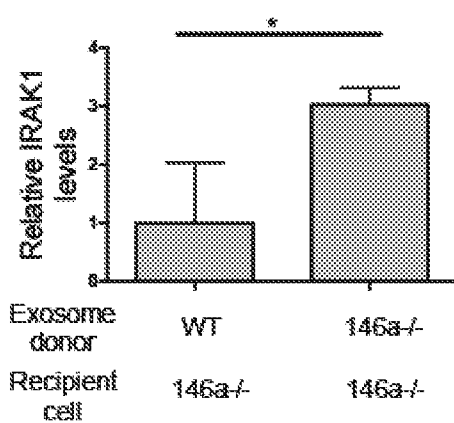


FIG. 3E

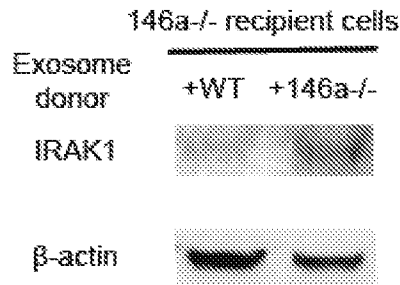


FIG. 3F

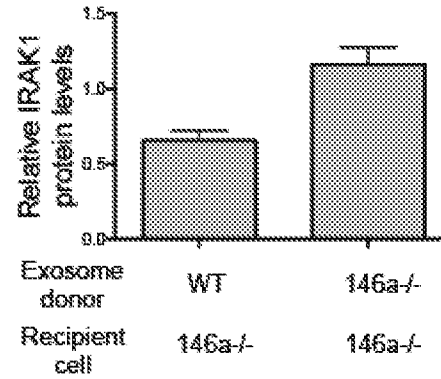


FIG. 3G

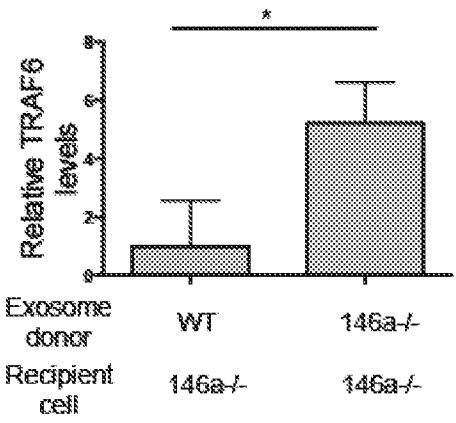


FIG. 3H

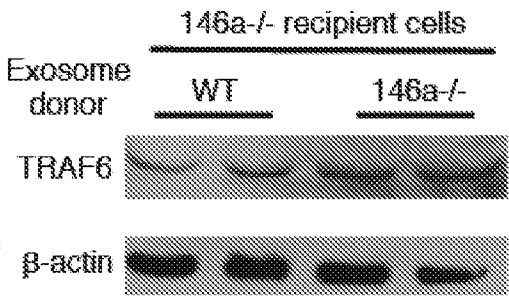


FIG. 3I

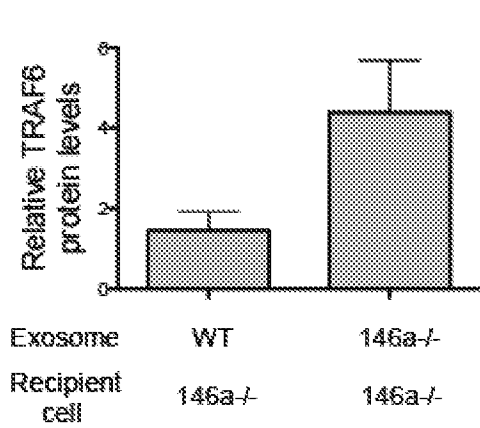


FIG. 3J

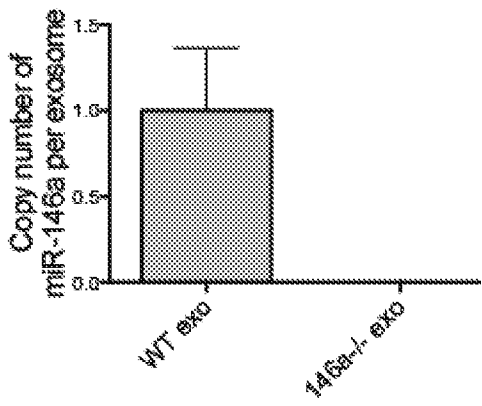


FIG. 3K

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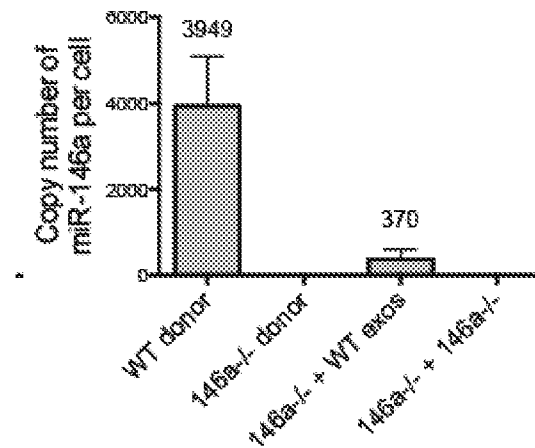


FIG. 3L

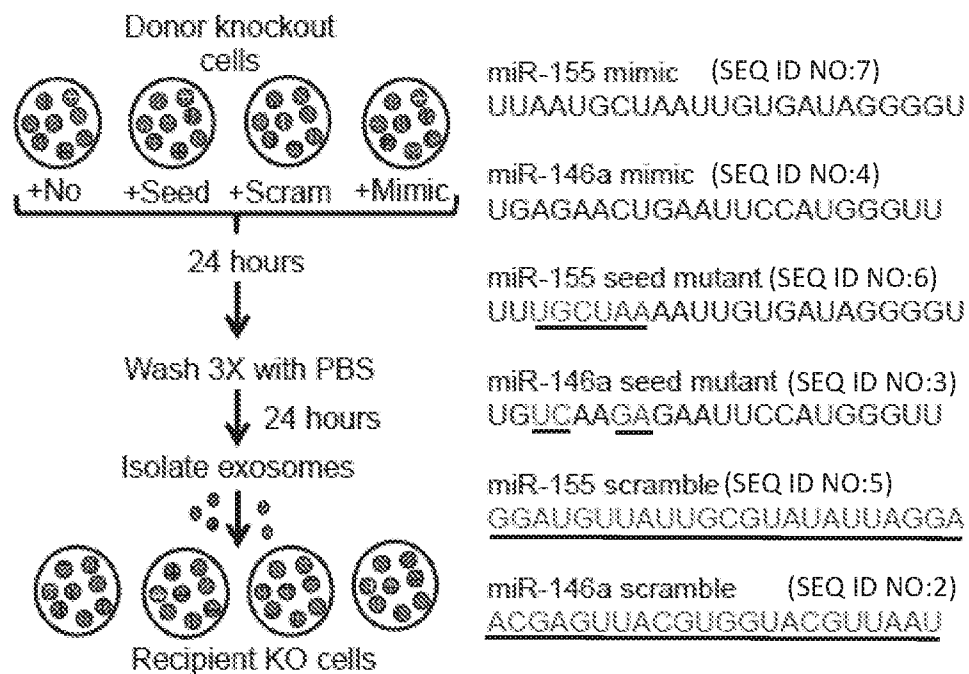


FIG. 4A

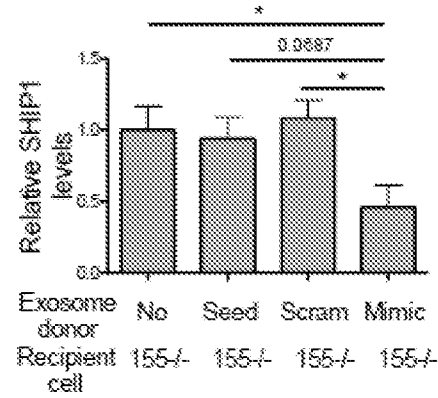


FIG. 4B

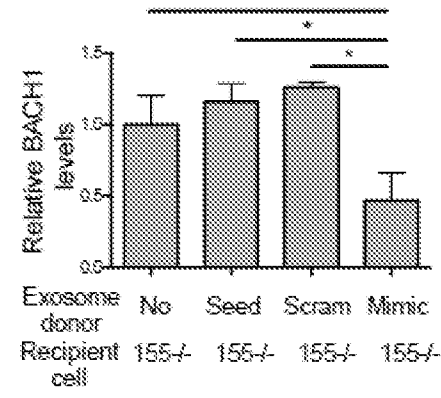


FIG. 4C

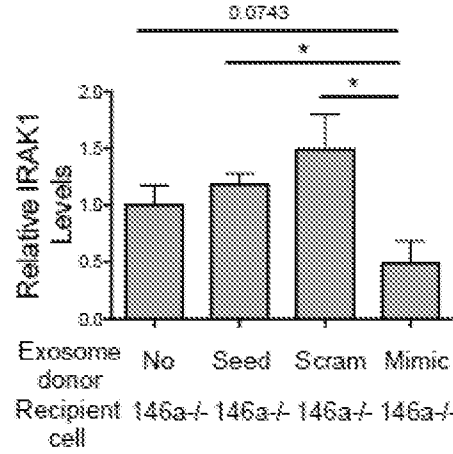


FIG. 4D

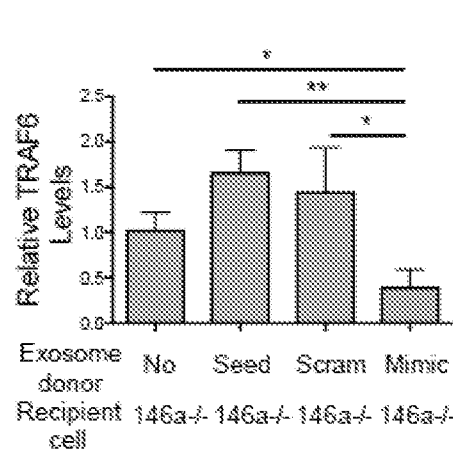


FIG. 4E

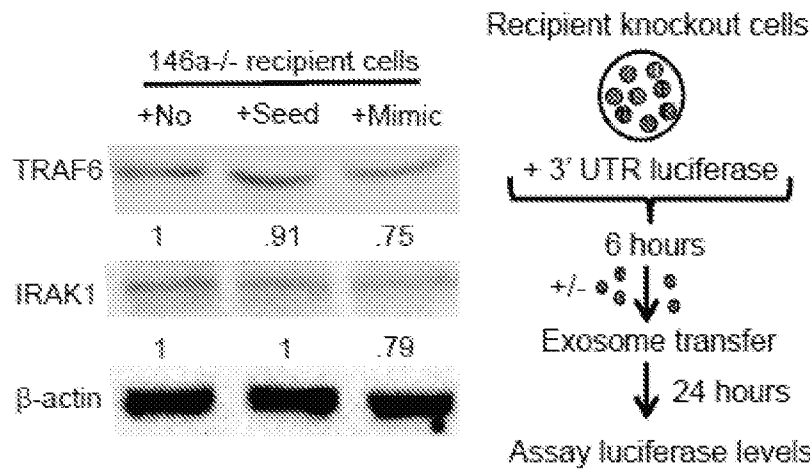


FIG. 4F

FIG. 4G

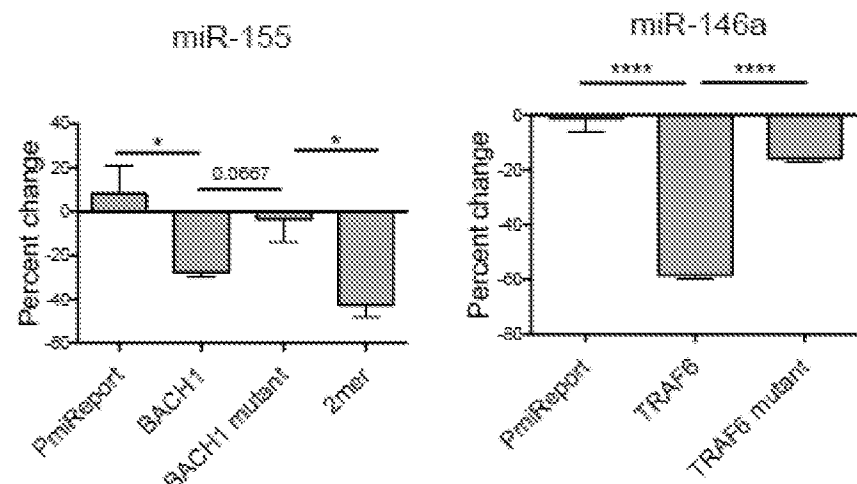


FIG. 4H

FIG. 4I

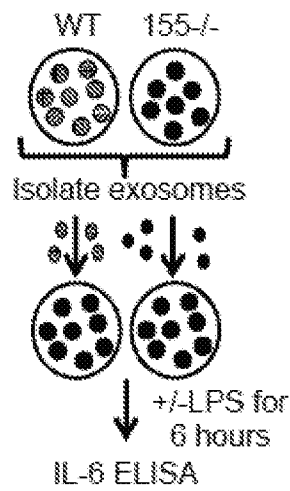


FIG. 5A

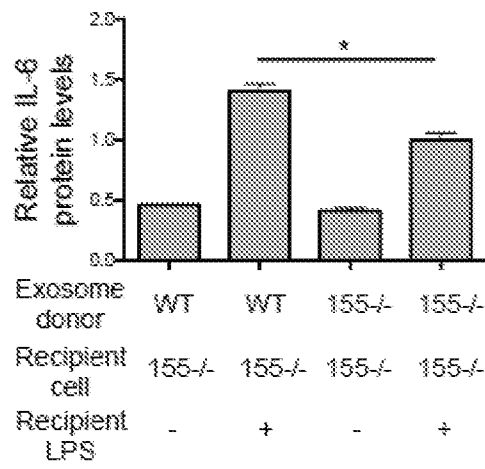


FIG. 5B

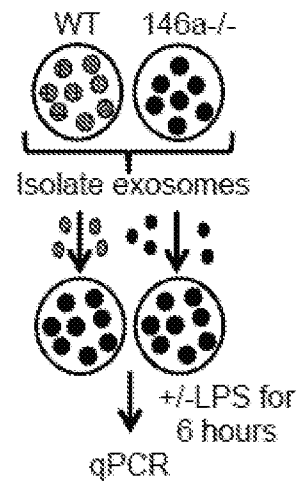


FIG. 5C

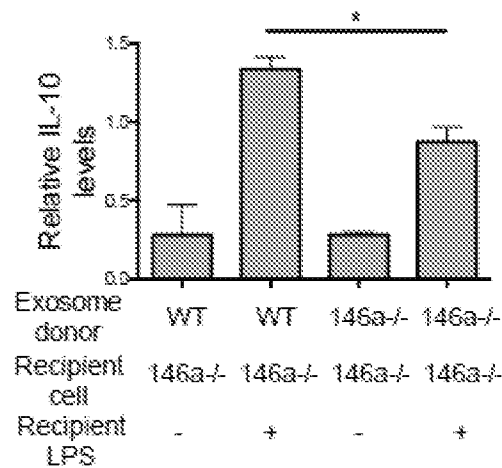


FIG. 5D

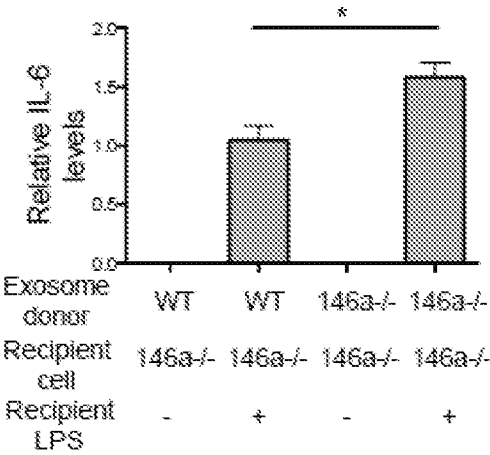


FIG. 5E

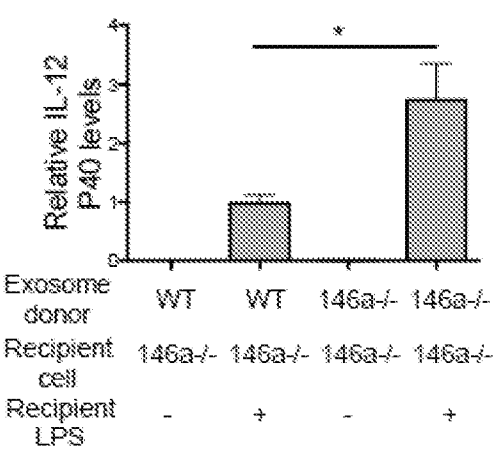


FIG. 5F

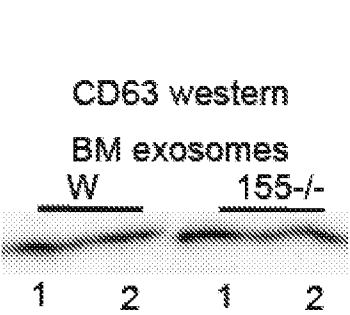


FIG. 6A

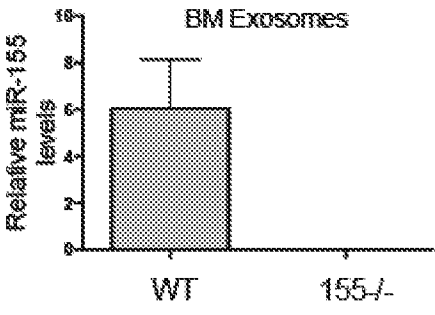


FIG. 6B

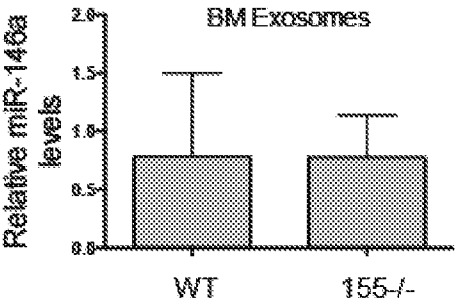
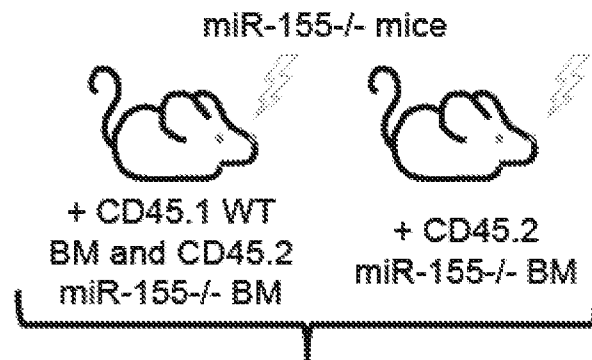


FIG. 6C

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After 3 months i.p. inject LPS and isolate
B (B220+), T (CD3+) and myeloid cells
(CD11b+) via FACS 24 hours later

FIG. 6D

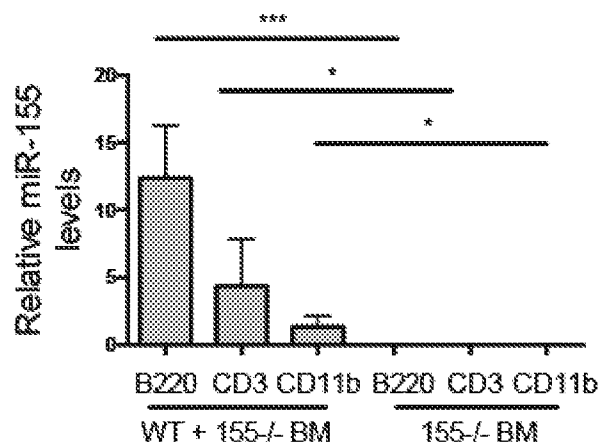


FIG. 6E

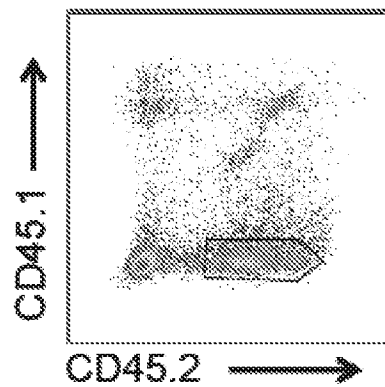
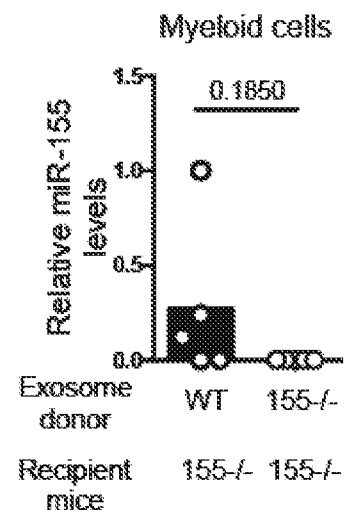
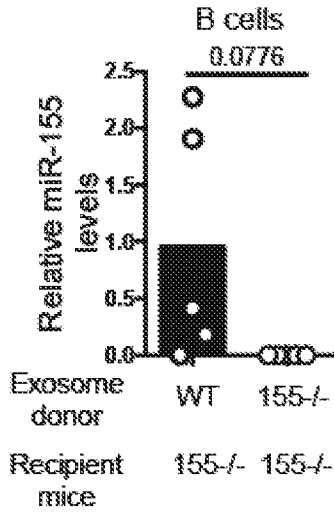
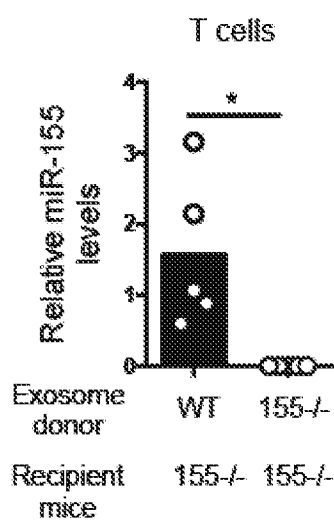
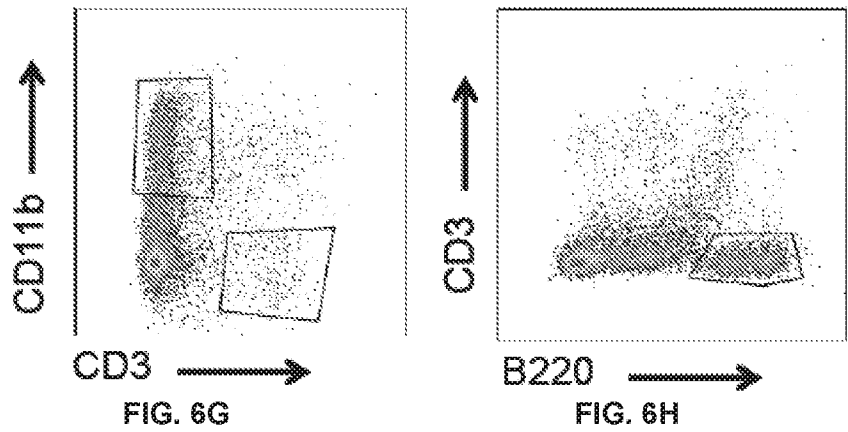


FIG. 6F



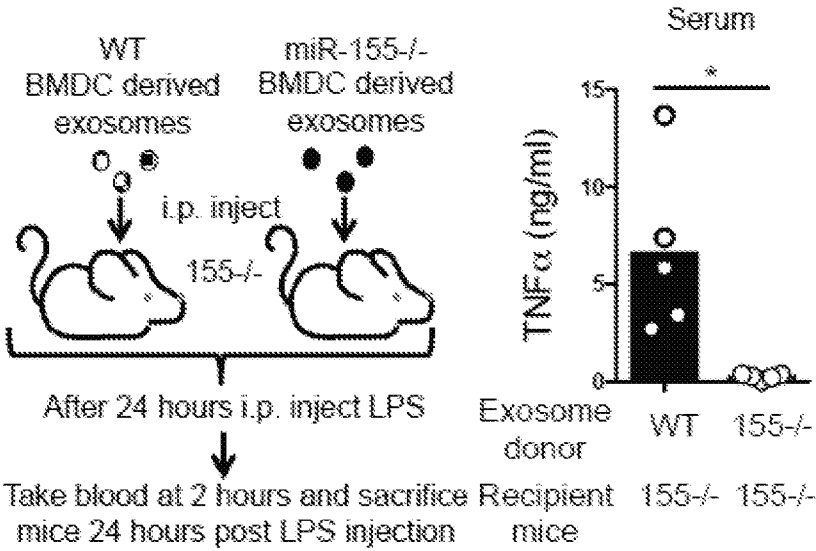


FIG. 7A

FIG. 7B

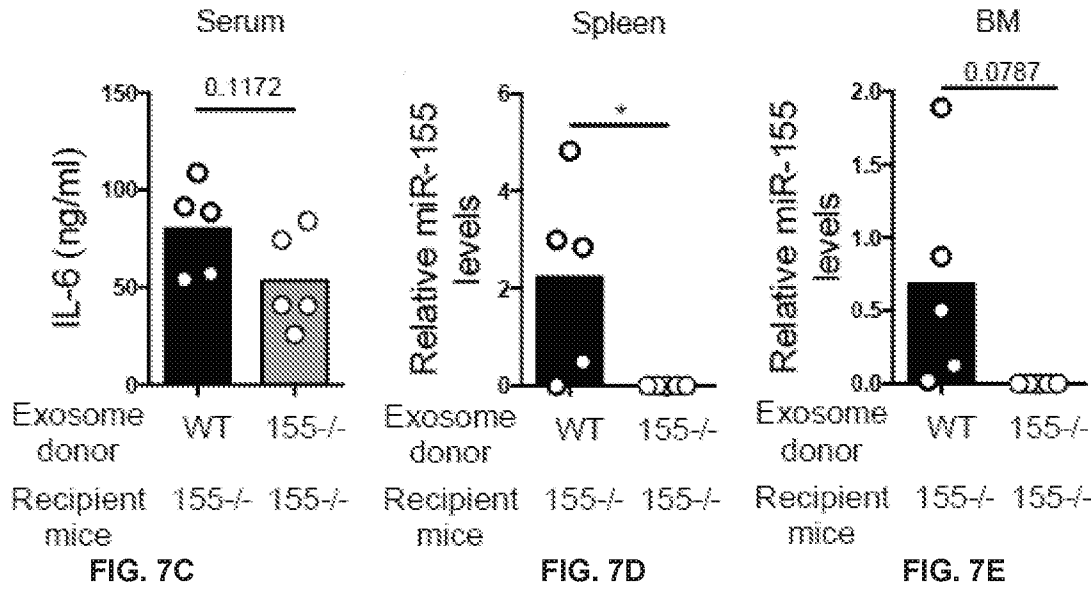


FIG. 7C

FIG. 7D

FIG. 7E

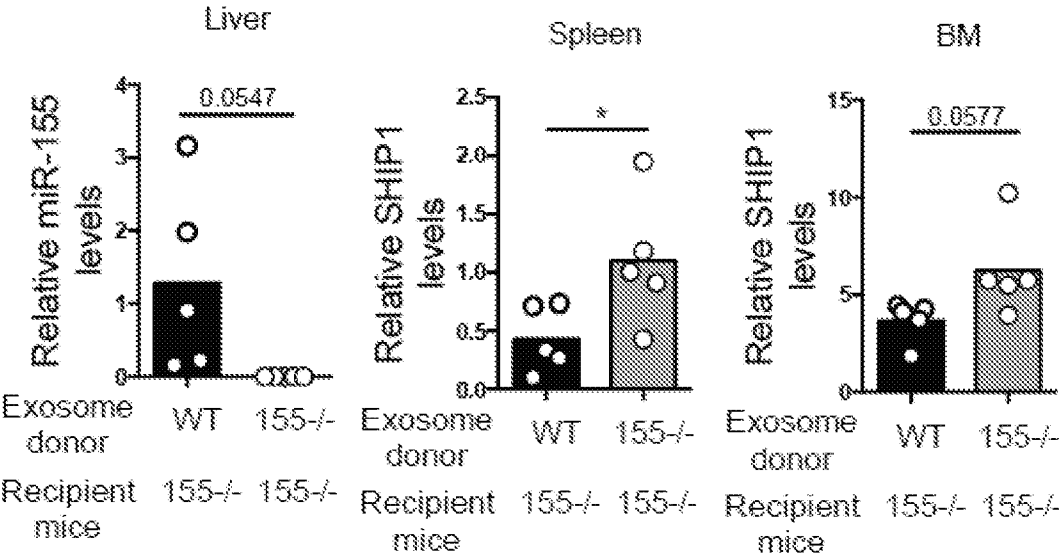


FIG. 7F

FIG. 7G

FIG. 7H

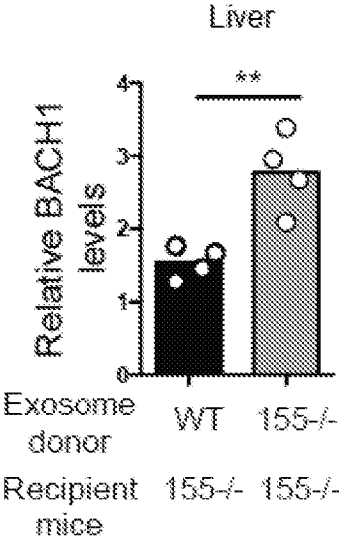


FIG. 7I

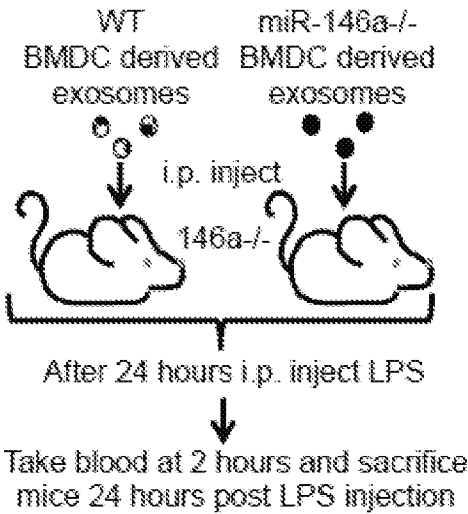


FIG. 8A

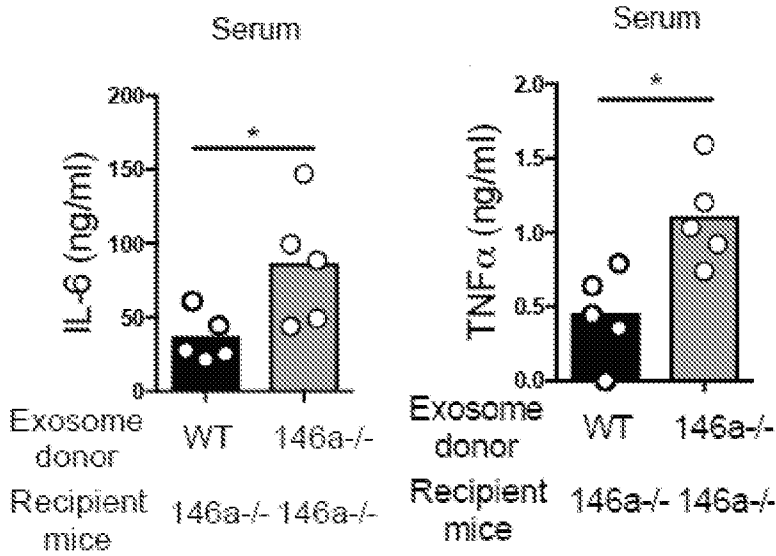


FIG. 8B

FIG. 8C

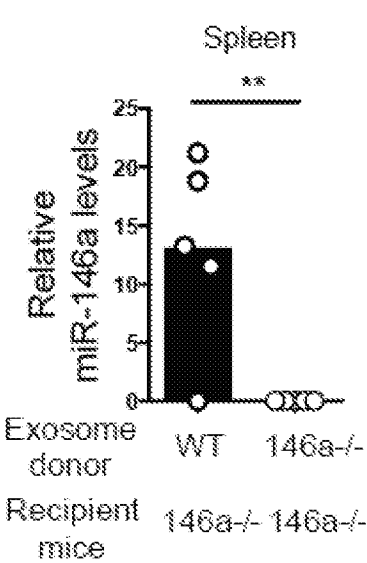


FIG. 8D

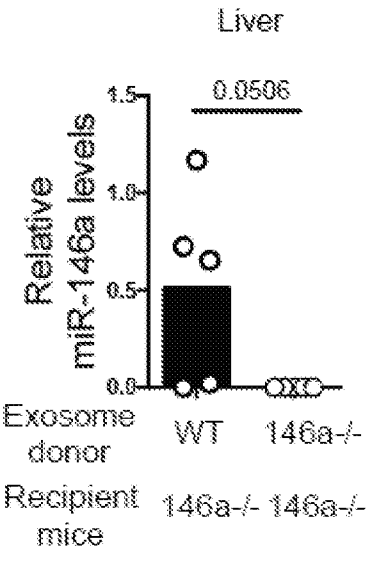


FIG. 8E

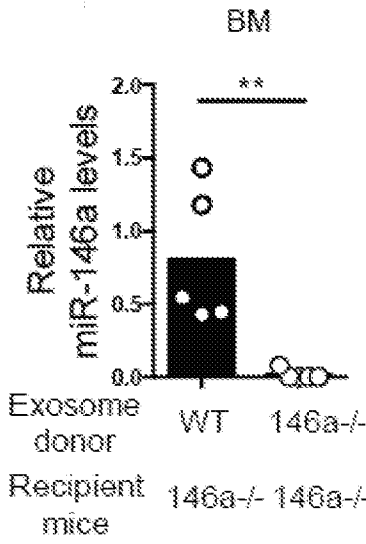


FIG. 8F

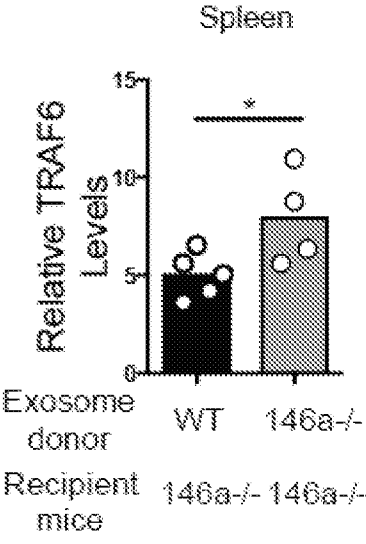


FIG. 8G

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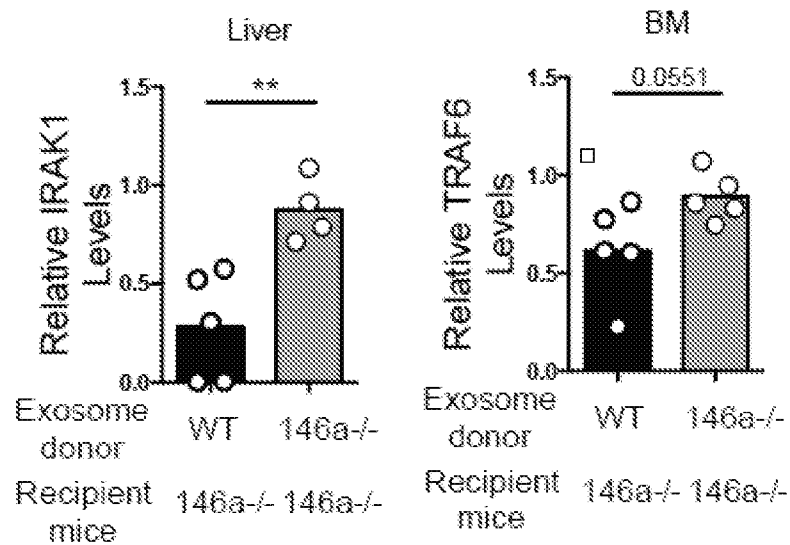


FIG. 8H

FIG. 8I

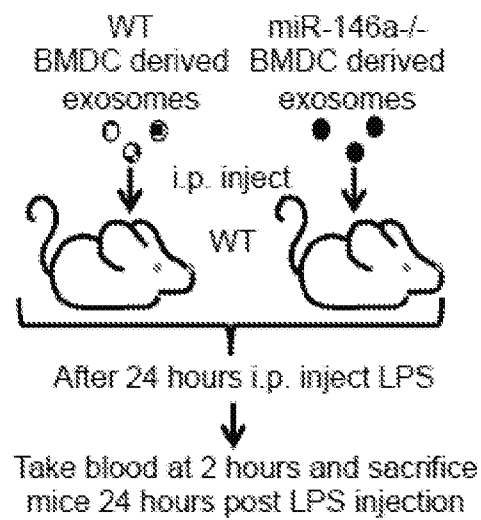


FIG. 9A

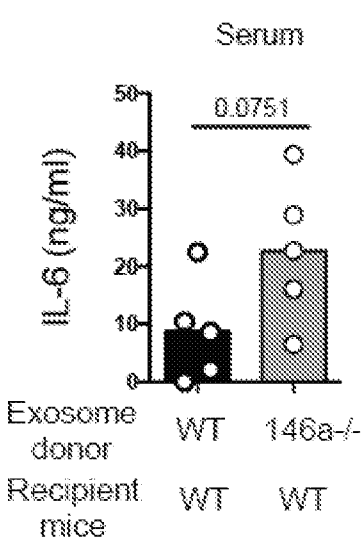


FIG. 9B

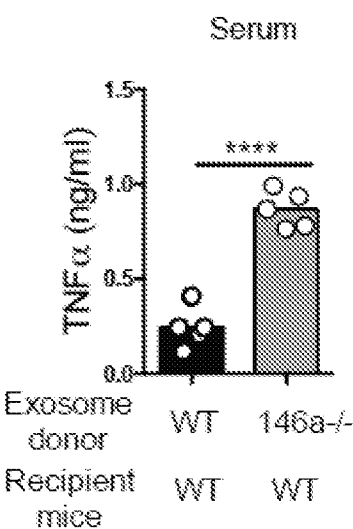


FIG. 9C

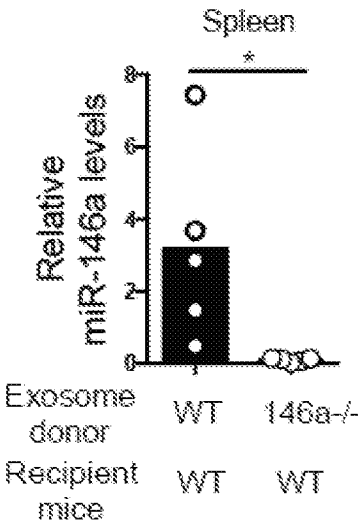


FIG. 9D

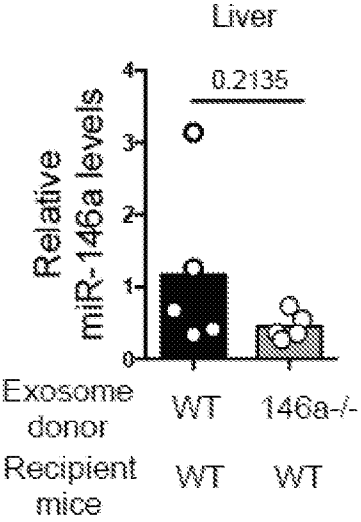


FIG. 9E

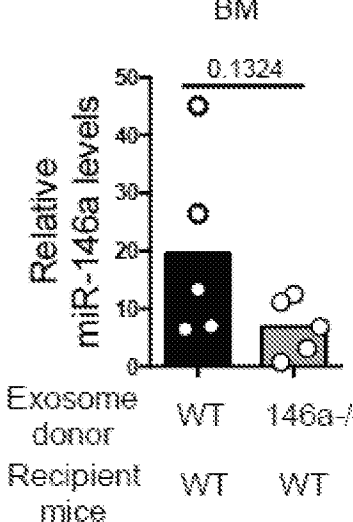


FIG. 9F

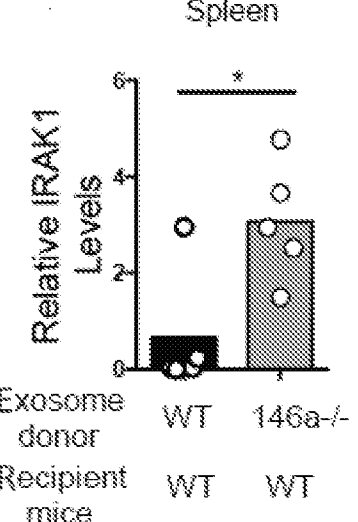
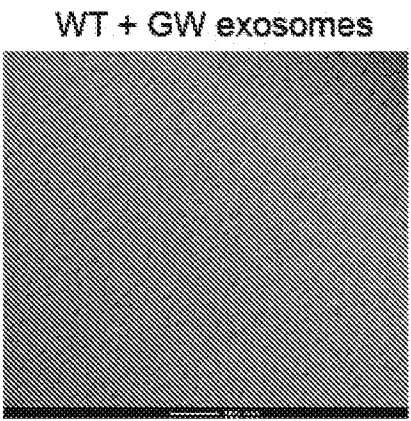
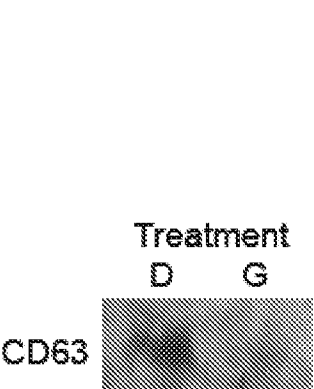
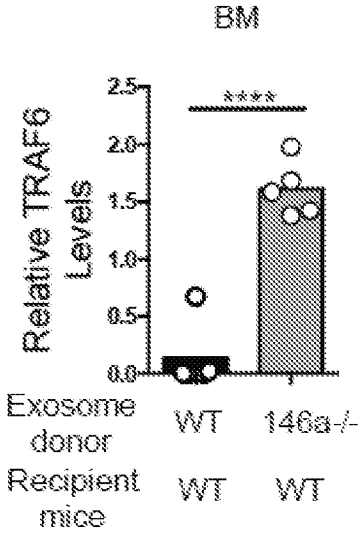
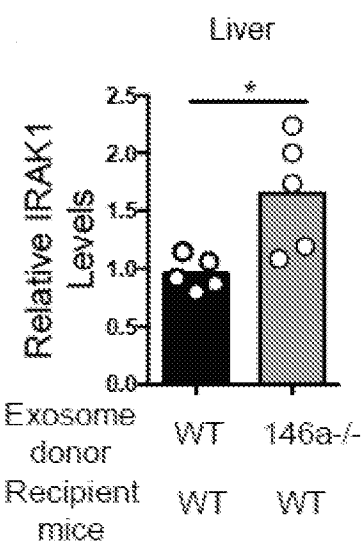


FIG. 9G



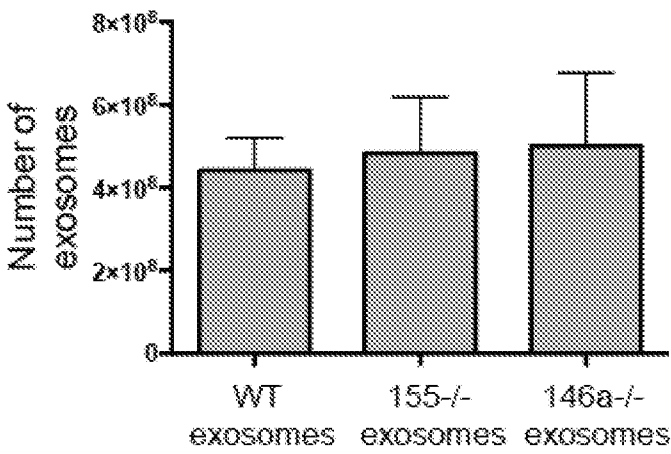


FIG. 11

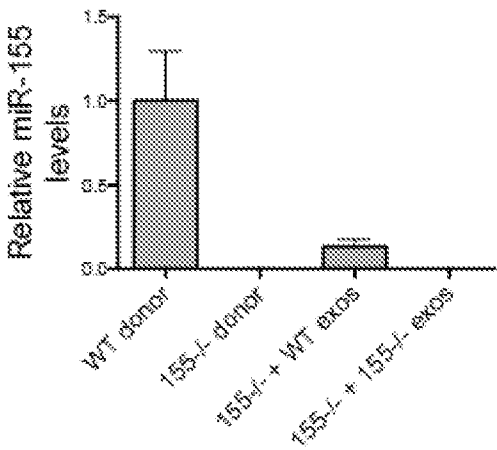


FIG. 12A

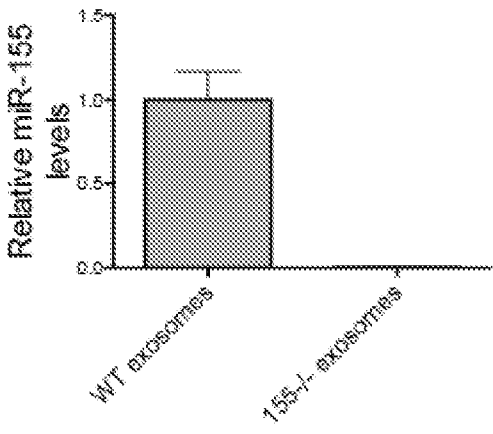


FIG. 12B

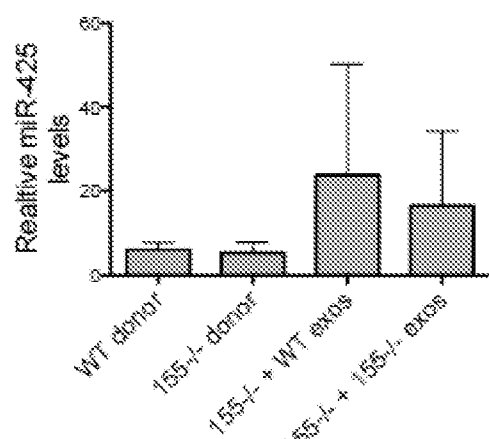


FIG. 12C

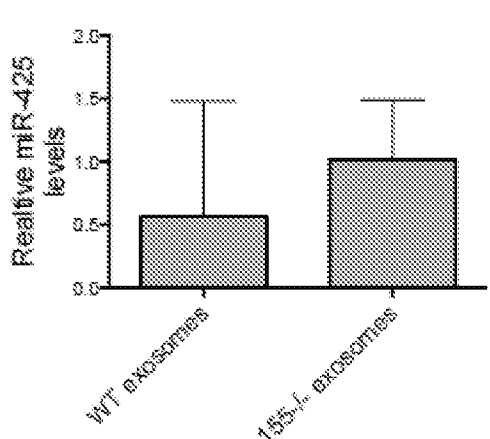


FIG. 12D

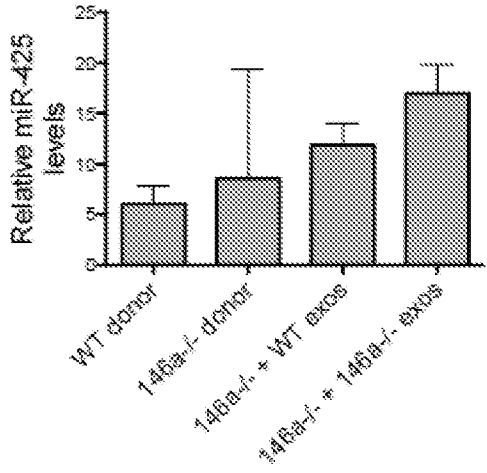


FIG. 12E

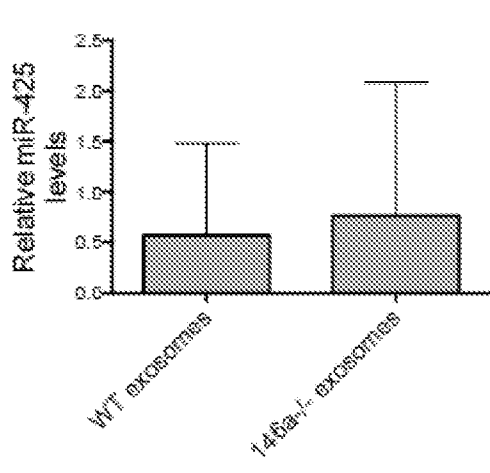
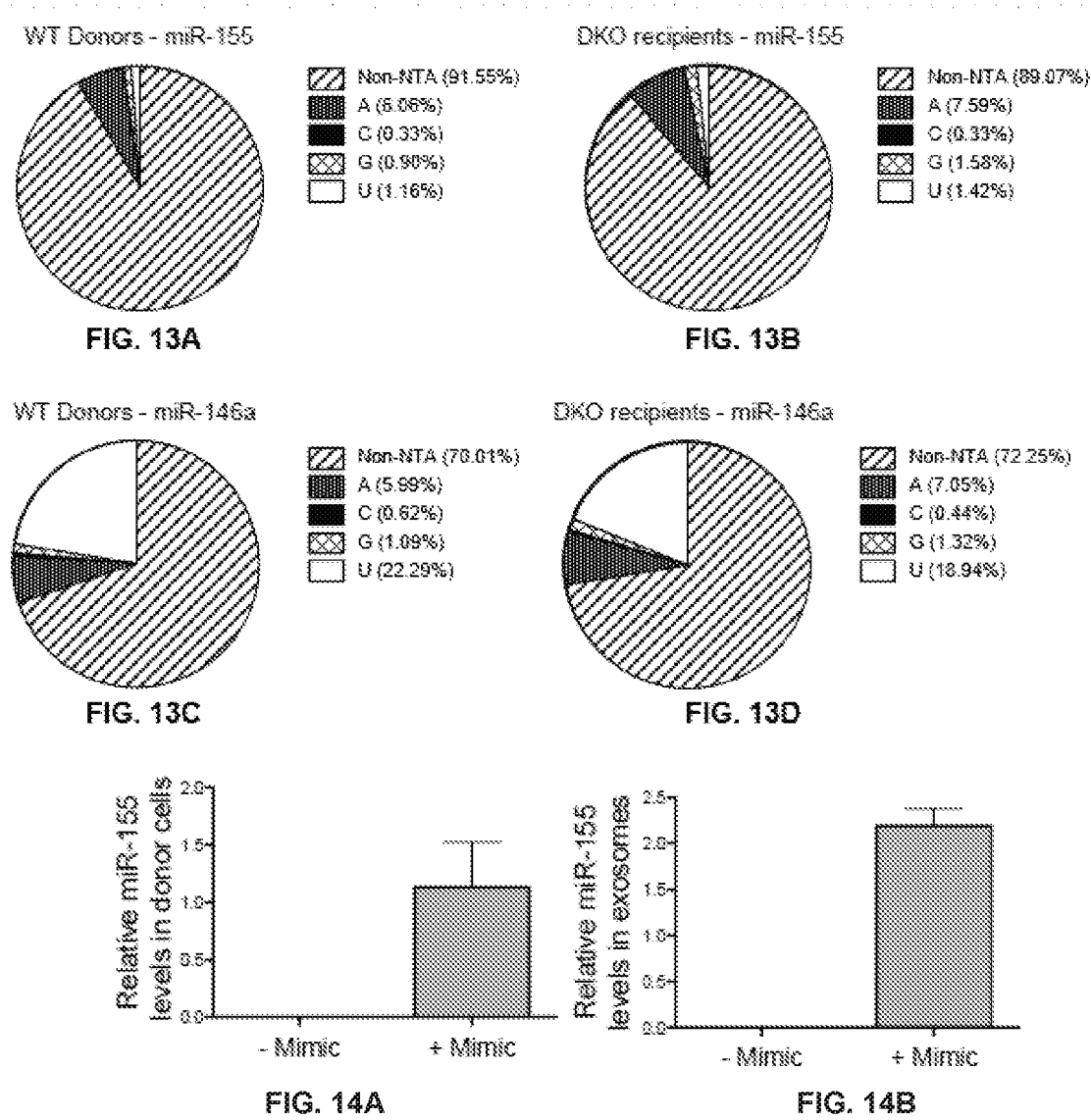


FIG. 12F



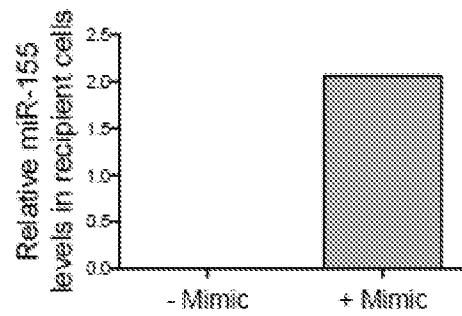


FIG. 14C

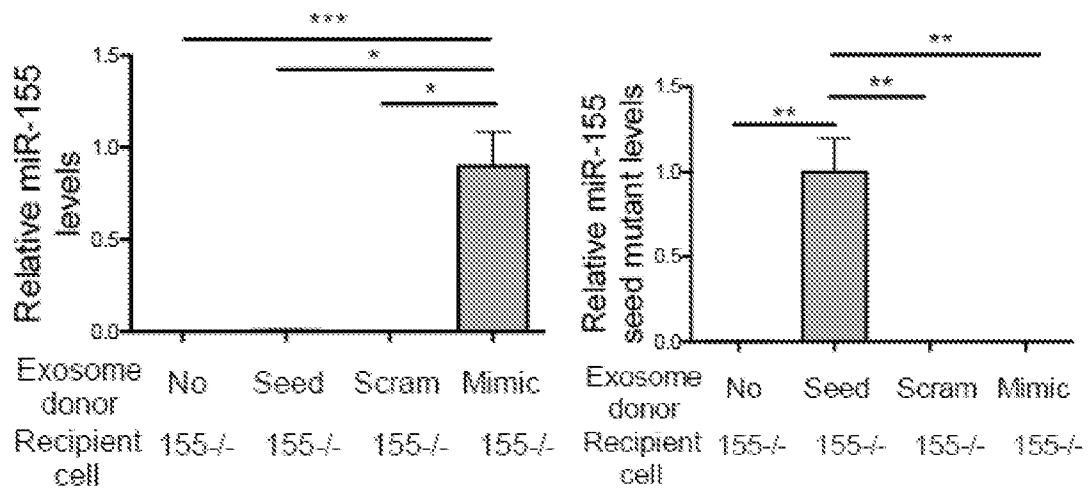


FIG. 15A

FIG. 15B

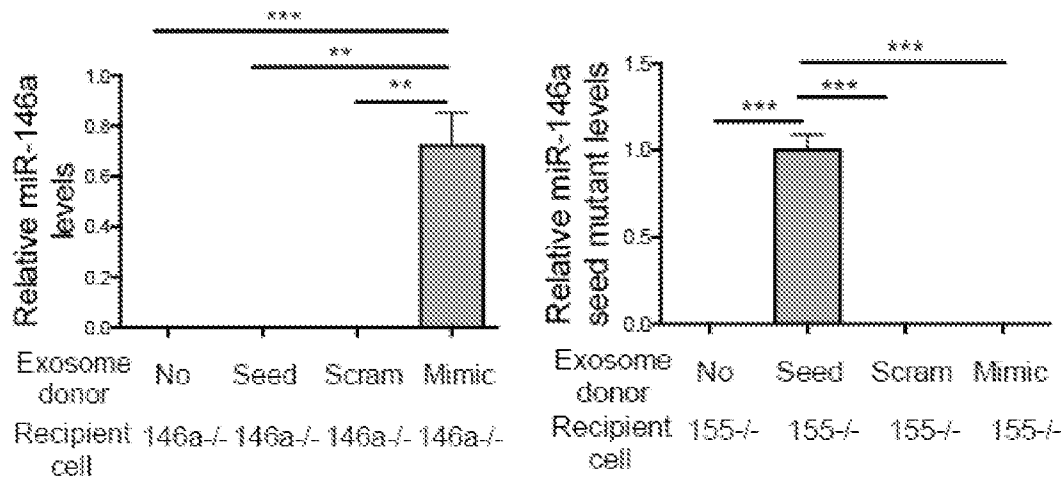


FIG. 15C

FIG. 15D

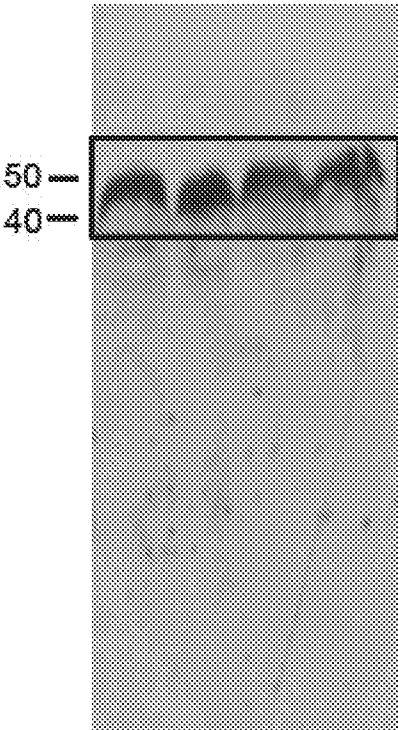


FIG. 16A

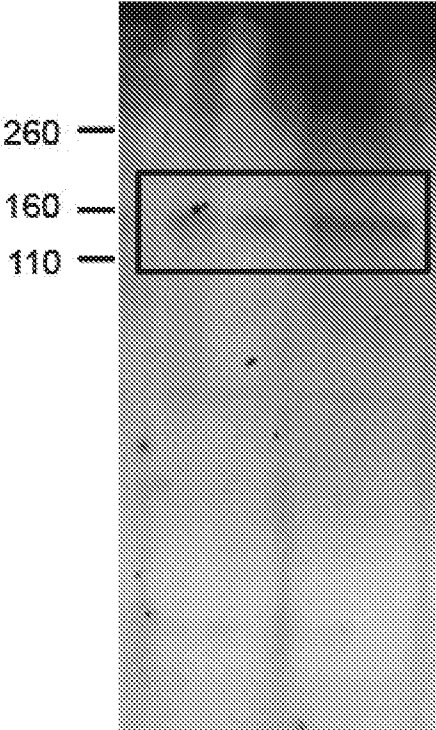


FIG. 16B

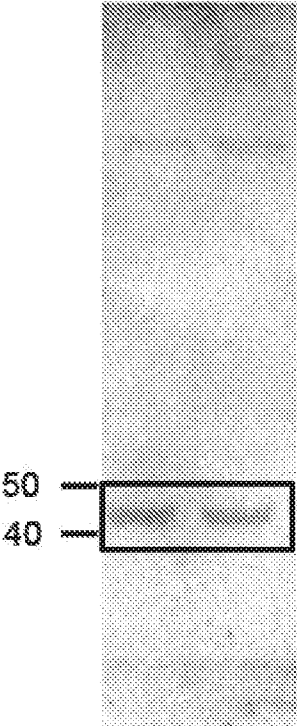


FIG. 16C

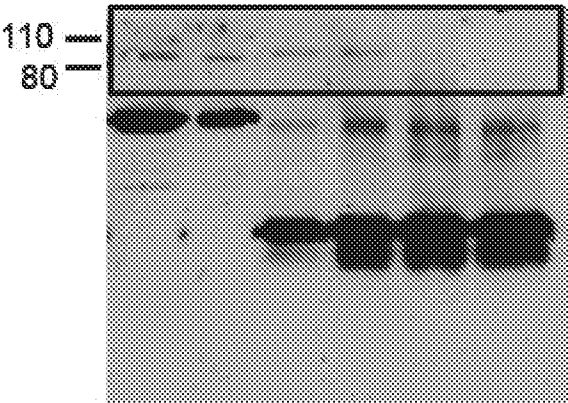


FIG. 16D

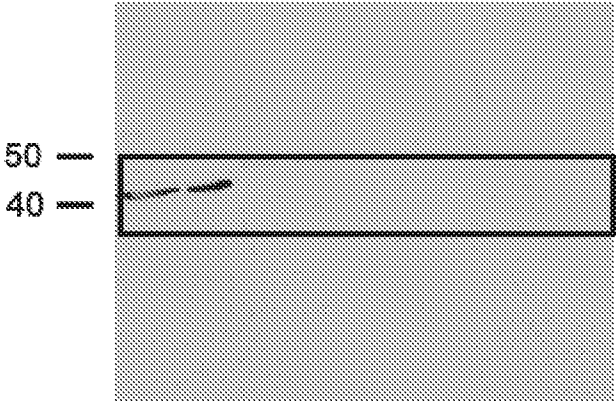


FIG. 16E

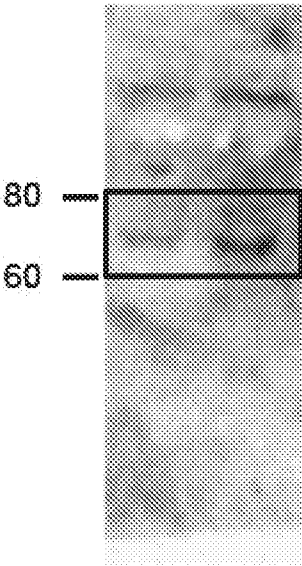


FIG. 17A

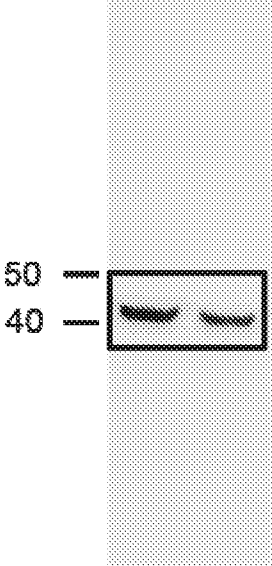


FIG. 17B

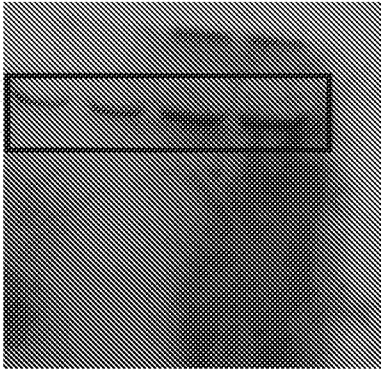


FIG. 17C

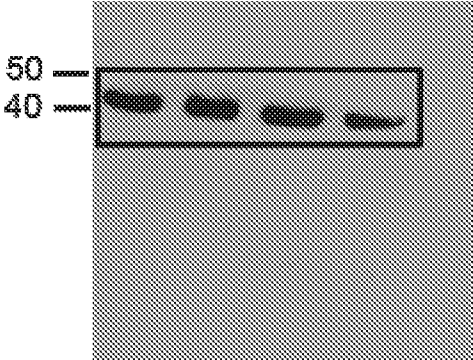


FIG. 17D

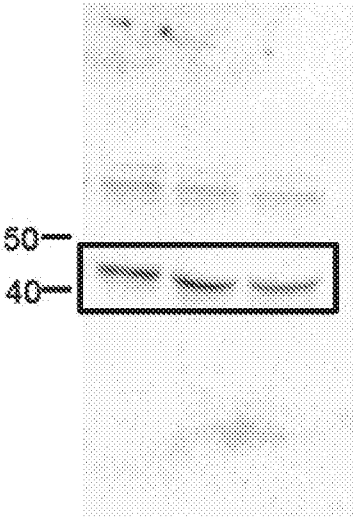


FIG. 18A

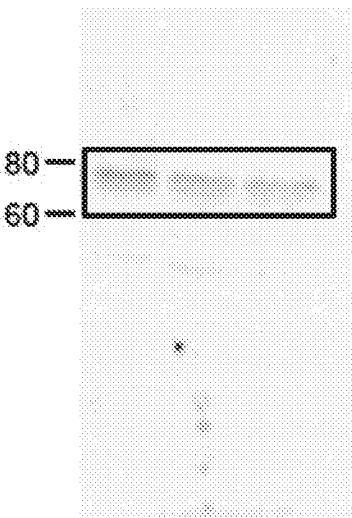


FIG. 18B

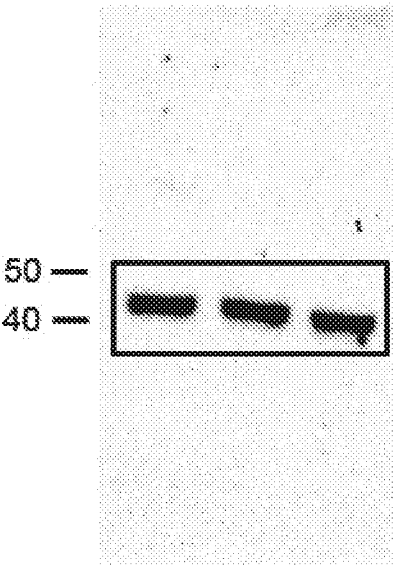


FIG. 18C

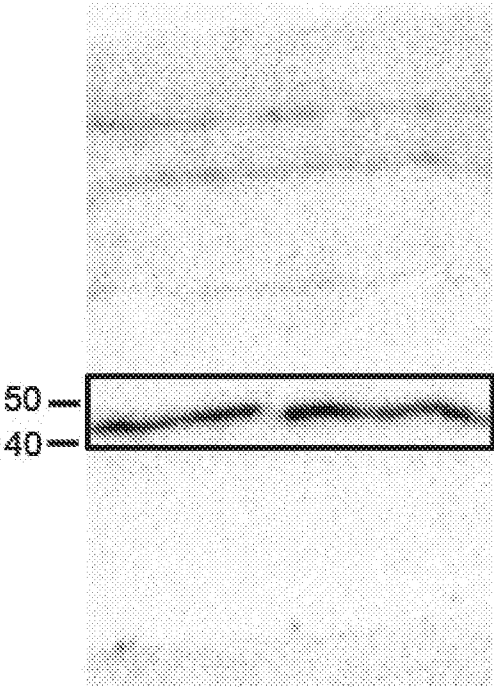


FIG. 18D

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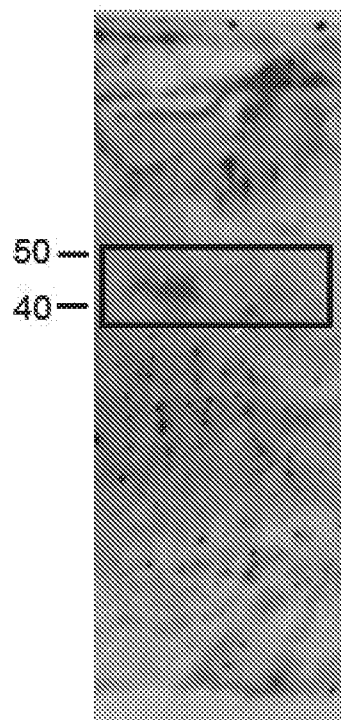


FIG. 18E

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miRbase mature miR-146a

WT BMDC donor miR-155					
Ref	%A	%C	%G	%T	Percent coverage
U	0.02%	0.03%	0.06%	99.89%	99.22%
U	0.02%	0.02%	0.05%	99.91%	99.92%
A	99.88%	0.03%	0.06%	0.02%	99.93%
A	99.85%	0.13%	0.01%	0.01%	99.96%
U	0.06%	0.03%	0.00%	99.88%	99.96%
G	0.14%	0.07%	99.49%	0.31%	99.98%
C	0.04%	99.91%	0.02%	0.03%	100.00%
U	0.01%	0.01%	0.01%	99.97%	100.00%
A	99.92%	0.05%	0.02%	0.02%	100.00%
A	99.97%	0.01%	0.01%	0.01%	100.00%
U	0.01%	0.01%	0.00%	99.98%	100.00%
U	0.01%	0.02%	0.01%	99.97%	100.00%
G	0.03%	0.02%	99.90%	0.05%	100.00%
U	0.02%	0.03%	0.01%	99.94%	100.00%
G	0.16%	0.08%	99.65%	0.11%	100.00%
A	99.96%	0.02%	0.01%	0.02%	100.00%
U	0.01%	0.01%	0.01%	99.97%	100.00%
A	99.91%	0.04%	0.03%	0.02%	100.00%
G	0.03%	0.00%	99.95%	0.02%	99.99%
G	0.06%	0.01%	99.88%	0.05%	99.63%
G	0.25%	0.01%	99.61%	0.12%	98.52%
G	1.85%	0.24%	97.26%	0.65%	95.16%
U	3.10%	0.11%	0.66%	96.14%	88.04%
U	5.10%	0.24%	0.38%	94.28%	44.99%
U	<u>20.08%</u>	1.39%	1.65%	<u>76.87%</u>	13.29%
U	21.42%	1.66%	4.83%	72.09%	2.90%
G	20.17%	1.97%	6.34%	71.52%	0.64%

FIG. 19A

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DKO BMDC +WT exosomes miR-155					
Ref	%A	%C	%G	%T	Percent coverage
U	0.33%	0.89%	0.74%	98.04%	99.09%
U	0.17%	0.25%	0.08%	99.49%	99.83%
A	99.83%	0.07%	0.10%	0.00%	99.83%
A	99.92%	0.08%	0.00%	0.00%	99.83%
U	0.62%	0.15%	0.00%	99.23%	99.83%
G	0.50%	0.15%	99.12%	0.23%	99.92%
C	0.50%	99.07%	0.18%	0.25%	100.00%
U	0.15%	0.33%	0.17%	99.34%	100.00%
A	99.61%	0.25%	0.07%	0.07%	100.00%
A	99.49%	0.44%	0.00%	0.07%	100.00%
U	0.00%	0.07%	0.15%	99.77%	100.00%
U	0.25%	0.07%	0.25%	99.42%	100.00%
G	0.40%	0.35%	98.25%	1.00%	100.00%
U	0.40%	0.25%	0.23%	99.12%	100.00%
G	0.68%	0.55%	98.04%	0.74%	100.00%
A	99.14%	0.47%	0.15%	0.23%	100.00%
U	0.00%	0.00%	0.08%	99.92%	100.00%
A	98.68%	0.88%	0.26%	0.17%	100.00%
G	0.07%	0.00%	99.93%	0.00%	100.00%
G	0.29%	0.22%	99.04%	0.45%	99.67%
G	0.27%	0.33%	98.21%	1.18%	98.51%
G	2.44%	0.39%	96.45%	0.72%	95.87%
U	3.94%	0.64%	1.37%	94.05%	89.35%
U	5.84%	0.17%	0.16%	93.83%	46.08%
U	<u>24.67%</u>	0.00%	2.84%	<u>72.49%</u>	13.63%
U	19.21%	5.29%	6.88%	68.62%	3.30%
G	12.50%	0.00%	0.00%	87.50%	1.36%

FIG. 19B

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miRbase mature miR-146a

WT BMDC donor miR-146a					
Ref	%A	%C	%G	%T	Percent coverage
U	0.01%	0.02%	33.37%	66.60%	99.96%
G	0.07%	0.02%	99.87%	0.04%	100.00%
A	66.62%	0.02%	0.03%	33.33%	100.00%
G	33.34%	0.02%	66.62%	0.02%	100.00%
A	66.60%	33.26%	0.05%	0.07%	100.00%
A	66.53%	33.35%	0.06%	0.07%	100.00%
C	0.02%	66.63%	0.02%	33.33%	100.00%
U	0.01%	0.01%	0.01%	99.98%	100.00%
G	33.34%	0.01%	66.62%	0.03%	100.00%
A	99.97%	0.01%	0.01%	0.01%	100.00%
A	66.66%	0.01%	33.33%	0.01%	100.00%
U	0.01%	0.01%	0.00%	99.97%	100.00%
U	0.01%	<u>33.33%</u>	0.01%	66.66%	100.00%
C	33.26%	66.64%	0.05%	0.05%	100.00%
C	33.36%	66.44%	0.09%	0.11%	100.00%
A	66.66%	0.01%	33.31%	0.02%	100.00%
U	33.31%	0.02%	0.02%	66.65%	99.99%
G	<u>0.13%</u>	<u>0.02%</u>	99.80%	<u>0.06%</u>	99.99%
G	0.01%	0.01%	99.93%	0.04%	99.98%
G	0.03%	0.04%	66.60%	33.33%	99.94%
U	0.32%	0.14%	0.04%	99.49%	99.69%
U	2.02%	0.20%	0.39%	97.39%	94.42%
A	<u>40.89%</u>	1.25%	2.05%	55.81%	41.04%
U	39.65%	0.77%	3.71%	<u>55.87%</u>	11.63%
A	<u>63.63%</u>	1.84%	3.21%	<u>31.32%</u>	1.39%
U	15.77%	0.98%	4.90%	78.35%	0.22%

FIG. 20A

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DKO BMDCs + WT exosomes miR-146a					
Ref	%A	%C	%G	%T	Percent coverage
U	0.00%	0.87%	0.43%	98.70%	100.00%
G	0.00%	0.43%	99.25%	0.32%	100.00%
A	100.00%	0.00%	0.00%	0.00%	100.00%
G	0.00%	0.00%	100.00%	0.00%	100.00%
A	100.00%	0.00%	0.00%	0.00%	100.00%
A	99.57%	0.43%	0.00%	0.00%	100.00%
C	0.00%	99.68%	0.32%	0.00%	100.00%
U	0.00%	0.74%	0.00%	99.26%	100.00%
G	0.00%	0.00%	100.00%	0.00%	100.00%
A	99.68%	0.00%	0.32%	0.00%	100.00%
A	100.00%	0.00%	0.00%	0.00%	100.00%
U	0.74%	0.00%	0.00%	99.26%	100.00%
U	0.00%	0.00%	0.00%	100.00%	100.00%
C	0.00%	100.00%	0.00%	0.00%	100.00%
C	0.00%	99.68%	0.00%	0.32%	100.00%
A	99.25%	0.32%	0.43%	0.00%	100.00%
U	0.00%	0.74%	0.00%	99.26%	100.00%
G	<u>21.65%</u>	<u>4.27%</u>	73.44%	<u>0.64%</u>	100.00%
G	0.00%	0.00%	100.00%	0.00%	100.00%
G	0.32%	1.07%	97.87%	0.74%	100.00%
U	1.73%	21.64%	0.00%	<u>76.63%</u>	99.56%
U	3.73%	0.70%	0.35%	95.22%	91.15%
A	<u>60.41%</u>	0.51%	8.50%	30.58%	58.85%
U	15.67%	0.00%	2.75%	<u>81.58%</u>	32.30%
A	<u>95.45%</u>	0.00%	0.00%	<u>4.55%</u>	19.03%
U	5.56%	0.00%	0.00%	94.44%	11.06%

FIG. 20B