

(51) International Patent Classification:
C12N 15/11 (2006.01)(21) International Application Number:
PCT/US2016/012844(22) International Filing Date:
11 January 2016 (11.01.2016)

(25) Filing Language: English

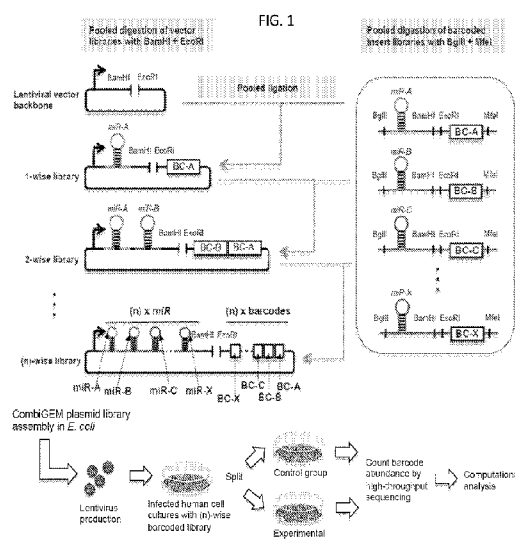
(26) Publication Language: English

(30) Priority Data:
62/102,255 12 January 2015 (12.01.2015) US(71) Applicant: MASSACHUSETTS INSTITUTE OF
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02210-2206 (US).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: MICRORNA COMBINATIONS FOR ANTI-CANCER THERAPEUTICS



(57) Abstract: Described herein are methods and compositions of combinations of microRNAs that enhance the sensitivity of cancer cells to chemotherapeutic agents or reduce proliferation of cancer cells. Also described herein are methods for the identification of combinations of microRNAs that result in desired effects.

MICRORNA COMBINATIONS FOR ANTI-CANCER THERAPEUTICS

RELATED APPLICATION

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional
5 application number 62/102,255, filed January 12, 2015, which is incorporated by reference
herein in its entirety.

GOVERNMENT FUNDING

This invention was made with government funding support under Grant No.
10 OD008435 awarded by the National Institutes of Health. The government has certain rights
in this invention.

FIELD OF INVENTION

This invention related to methods and compositions for reducing proliferation of
15 cancer cells or enhancing the susceptibility of cancer cells to a chemotherapeutic agent.

BACKGROUND

The concerted action of combinatorial gene sets play significant roles in regulating
complex biological traits (Dixon et al. *Annu. Rev. Genet.* (2009) 43, 601–625). For example,
20 multiple genetic factors are needed to reprogram somatic cells into induced pluripotent stem
cells or distinct lineages such as neurons and cardiomyocytes (Vierbuchen et al. *Mol. Cell.*
(2012) 47, 827-838). Combinatorial drug therapies can achieve enhanced efficacy over
conventional monotherapies since targeting multiple pathways can be synergistic (Al-
Lazikani et al. *Nat. Biotechnol.* (2012) 30, 679-692). Furthermore, although genome-wide
25 association studies have putatively implicated multiple individual loci in multifactorial
human diseases, these loci can only explain a minor fraction of disease heritability (Zuk et al.
Proc. Natl. Acad. Sci. (2012) 109, 1193-1198; Eichler et al. *Nat. Rev. Genet.* (2010) 11, 446-
450; Manolio et al. *Nature* (2009) 461, 747-753). Interactions between genes may be
important in accounting for this missing heritability but current technologies for
30 systematically characterizing the function of high-order gene combinations are limited.

SUMMARY OF INVENTION

Multiple genetic pathways may function independently to promote disease (*e.g.*, cancer) formation or progression. Thus, conventional monotherapies may have limited efficacy. The methods and compositions described herein provide combinations of microRNAs that may target multiple mRNAs, reducing or preventing their expression, resulting in reduced proliferation of the cell. The methods and compositions described herein also provide combinations of microRNAs that sensitize cells to chemotherapeutic agents. Also provided are screening methods for the identification of novel microRNA combinations that affect cell proliferation and/or sensitivity to agents.

Aspects of the present invention provide compositions comprising one or more recombinant expression vectors encoding a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10. Other aspects provide compositions comprising a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10. In some embodiments, the combination of three microRNAs are concatenated microRNAs, optionally with one or more linker and/or spacer sequence; conjugated to one or more nanoparticle, cell-permeating peptide, or polymer; or contained within a liposome. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-181a, and miR-132. In some embodiments, the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-211, and miR-132. In some embodiments, the combination of three microRNAs comprises miR-376a, miR-31, and miR-488. In some embodiments, the combination of three microRNAs comprises mir-128b, mir-212, and let-7i or miR-451a/451b/144/4732 cluster. In some embodiments, the combination of three microRNAs comprises mir128b, miR-451a/451b/144/4732 cluster, and miR-132 or miR-212. In some embodiments, the combination of three microRNAs comprises miR-128b, let-7i, and mir-212 or miR-196. In some embodiments, the combination of three microRNAs comprises miR-132, miR-15b/miR-16-2, and miR-31 or let-7i. In some embodiments, the combination of three microRNAs comprises miR-132, miR-451a/451b/144/4732 cluster, and miR-212 or miR-128b. In some embodiments, the combination of three microRNAs comprises miR-181c, let-7i, and miR-373 or miR-429. In some embodiments, the combination of three microRNAs comprises miR-181a, miR-429, and miR-29a or miR-31. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2, let-7i, and miR-132 or miR-181a. In some embodiments, the

combination of three microRNAs comprises miR-212, miR-451a/451b/144/4732 cluster, and miR-132 or miR-128b. In some embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, let-7e/miR-99b cluster, and miR-128b.

Other aspects provide compositions comprising one or more recombinant expression vectors encoding a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10. Yet other aspects provide compositions comprising a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10. In some embodiments, the combination of two microRNAs or the combination of three microRNAs are concatenated microRNAs, optionally with one or more linker and/or spacer sequence; conjugated to one or more nanoparticle, cell-permeating peptide, or polymer; or contained within a liposome. In some embodiments, the compositions further comprise a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is an anti-mitotic/anti-microtubule agent. In some embodiments, the anti-mitotic agent is docetaxel.

In some embodiments, the combination of three microRNA comprises miR-15b/miR-16-2 cluster, miR-181a, and miR-132. In some embodiments, the combination of three microRNA comprises miR-451a/451b/144/4732 cluster, miR-211, and miR-132. In some embodiments, the combination of three microRNA comprises miR-376a, miR-31, and miR-488. In some embodiments, the combination of two microRNAs comprises miR-376a and any one of the miRNAs selected from the group consisting of miR-16-1/15a cluster, miR-212, and miR-31. In some embodiments, the combination of two microRNAs comprises miR-216 and any one of the miRNAs selected from the group consisting of miR-181c, let-7a, miR-15b/miR-16-2 cluster, and miR-181a. In some embodiments, the combination of two microRNAs comprises miR-31 and miR-181a or miR-376a. In some embodiments, the combination of two microRNAs comprises miR-93/106b cluster and miR-16-1/15a cluster or miR-181a. In some embodiments, the combination of two microRNAs comprises miR-181a and any one of the miRNAs selected from the group consisting of miR-31, let-7i, miR-93/106b cluster, miR-373, miR-216, miR-15b/miR-16-2 cluster, and miR-16-1/15a cluster. In some embodiments, the combination of two microRNAs comprises miR-16-1/15a cluster and any one of the miRNAs selected from the group consisting of miR-376a, miR-93/10b cluster, let-7a, miR-10b, miR-181a, miR-9-1, and miR-99a. In some embodiments, the

combination of two microRNAs comprises miR-10b and any one of the miRNAs selected from the group consisting of miR-16-1/15a cluster, miR-212, miR-196, and miR-15b/miR-16-2 cluster. In some embodiments, the combination of two microRNAs comprises miR-15b/miR-161-2 cluster and any one of the miRNAs selected from the group consisting of miR-216, miR-181a, miR-9-1, and miR-10b. In some embodiments, the combination of two microRNAs comprises miR181c and mir-9-1 or miR-216. In some embodiments, the combination of two microRNAs comprises miR-212 and miR-376a or miR-10b. In some embodiments, the combination of two microRNAs comprises miR-9-1 and any one of the miRNAs selected from the group consisting of miR-15b/miR-16-2 cluster, miR-16-1/15a cluster, miR-324, and miR-181c. In some embodiments, the combination of two microRNAs comprises let-7a and miR-16-1/15a cluster or miR-216.

In some embodiments, the combination of three microRNAs comprises let-7c, miR-451a/451b/144/4732 cluster, and miR-324 or miR376a. In some embodiments, the combination of three microRNAs comprises let-7d, miR-181c, and miR-10b or miR-9-1. In some embodiments, the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-15b/miR-16-2 cluster, and miR-181a or miR-16-1/miR-15a cluster. In some embodiments, the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-16-1/15a cluster and miR-15b/miR-16-2 cluster or miR-181c. In some embodiments, the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-181a, and miR-324 or miR-15b/miR-16-2 cluster. In some embodiments, the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-181c, and miR-429 or miR-16-1/15a cluster. In some embodiments, the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-376a, and miR-199b/3154 cluster or miR-188. In some embodiments, the combination of three microRNAs comprises let-7i, miR-15b/miR-16-2 cluster, and miR-451a/451b/144/4732 cluster or let-7c. In some embodiments, the combination of three microRNAs comprises let-7i, miR-199b/3154 cluster, and miR-10b or miR-29a. In some embodiments, the combination of three microRNAs comprises miR-10b, miR-15b/miR-16-2 cluster, and any one of the miRNAs selected from the group consisting miR-373, miR-211, and miR-126. In some embodiments, the combination of three microRNAs comprises miR-10b, miR-373, and miR-15b/miR-16-2 cluster or miR-451a/451b/144/4732 cluster. In some embodiments, the combination of three microRNAs comprises miR-10b, miR-451a/451b/144/4732 cluster, and miR-373, miR-429, or miR-708. In some embodiments, the combination of three

microRNAs comprises miR-126, miR-15b/miR-16-2 cluster, and miR-10b or miR-181a. In some embodiments, the combination of three microRNAs comprises miR-126, miR-181a, and miR-451a/451b/144/4732 cluster or miR-15b/miR-16-2 cluster. In some embodiments, the combination of three microRNAs comprises miR-126, miR-181c, and miR-

5 451a/451b/144/4732 cluster or miR-29a. In some embodiments, the combination of three microRNAs comprises miR-126, miR-29a, and miR-211 or miR-181c. In some embodiments, the combination of three microRNAs comprises miR-126, miR-451a/451b/144/4732 cluster, and miR-181a or miR-181c. In some embodiments, the combination of three microRNAs comprises miR-128b, miR-16-1/15a cluster, and miR-181c

10 or miR-31. In some embodiments, the combination of three microRNAs comprises miR-128b, miR-31, and miR-24-2/27a/23a cluster or miR-16-1/15a cluster. In some embodiments, the combination of three microRNAs comprises miR-128b, miR-324, and miR-216 or miR-188. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-16-1/15a cluster, and any one of the microRNAs

15 selected from the group consisting of miR-216, miR-429, miR-451a/451b/144/4732 cluster, and let-7e/miR-99b cluster. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-181a, and any one of the microRNAs selected from the group consisting of miR-9-1, miR-126, miR-489, let-7e/miR-99b cluster, miR-216, and miR-488. In some embodiments, the combination of three microRNAs comprises miR-

20 15b/miR-16-2 cluster, miR-181c, and miR-328 or miR-488. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-216, and any one of the microRNAs selected from the group consisting of miR-373, miR-16-1/15a cluster, and miR-181a. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-373, and any one of the microRNAs selected from the group

25 consisting of miR-216, miR-9-1, and miR-10b. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-376a, and miR-24-2/27a/23a cluster or miR-324. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-451a/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of let-7a, miR-16-1/15a cluster, miR-708, and let-7i.

30 In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-488, and miR-181a or miR-181c. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-489, and miR-

128b or miR-181a. In some embodiments, the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-9-1, and miR-181a or miR-373. In some embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, miR-181c, and any one of the microRNAs selected from the group consisting of miR-489, miR-211, let-7e/miR-99b
5 cluster, miR-128b, and miR-29a. In some embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, miR-216, and miR-126 or miR-15b/miR-16-2 cluster. In some embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, miR-451/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of miR-489, miR-15b/miR-16-2 cluster, and miR-328. In some
10 embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, miR-489, and miR-181c or miR-451/451b/144/4732 cluster. In some embodiments, the combination of three microRNAs comprises miR-181a, miR-216, and any one of the microRNAs selected from the group consisting of miR-489, miR-15b/miR-16-2 cluster, and let-7i. In some embodiments, the combination of three microRNAs comprises miR-181a,
15 miR-324, and any one of the microRNAs selected from the group consisting of miR-708, miR-31, and let-7e/miR-99b cluster. In some embodiments, the combination of three microRNAs comprises miR-181a, miR-376a, and miR-24-2/27a/23a cluster or miR-29c. In some embodiments, the combination of three microRNAs comprises miR-181a, miR-451a/451b/144/4732 cluster, and miR-126 or miR-128b. In some embodiments, the
20 combination of three microRNAs comprises miR-181a, miR-488, and miR-15b/miR-16-2 cluster or miR-29a. In some embodiments, the combination of three microRNAs comprises miR-181a, miR-489, and miR-15b/miR-16-2 cluster or miR-216. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-29a, and miR-126, miR-16-1/15a cluster or miR-9-1. In some embodiments, the combination of three microRNAs
25 comprises miR-181c, miR-29c, and miR-31 or miR-324. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-31, and any one of the microRNAs selected from the group consisting of miR-328, miR-29c, and miR-99a. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-324, and miR-129-2 or miR-29c. In some embodiments, the combination of three microRNAs
30 comprises miR-181c, miR-328, and miR-15b/miR-16-2 cluster or miR-31. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-376a, and miR-708 or miR-212. In some embodiments, the combination of three microRNAs

comprises miR-181c, miR-451a/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of miR-126, miR-196, and miR-9-1. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-488, and miR-15b/miR-16-2 cluster or miR-132. In some embodiments, the combination of three microRNAs comprises miR-181c, miR-9-1, and any one of the microRNAs selected from the group consisting of miR-451a/451b/144/4732 cluster, let-7d, and miR-29a. In some embodiments, the combination of three microRNAs comprises miR-24-2/27a/23a cluster, miR-37a, and any one of the microRNAs selected from the group consisting of miR-328, miR-181a and miR-15b/miR-16-2 cluster. In some embodiments, the combination of three microRNAs comprises miR-29a, miR-199b/3154 cluster, and let-7i or let-7c. In some embodiments, the combination of three microRNAs comprises miR-29a, miR-9-1, and miR-181c or miR-451a/451b/144/4732 cluster. In some embodiments, the combination of three microRNAs comprises miR-31, miR-376a, and miR-16-1/15a cluster or miR-488. In some embodiments, the combination of three microRNAs comprises miR-328, miR-451a/451b/144/4732 cluster, and let-7e/miR-99b cluster or miR-16-1/15a cluster. In some embodiments, the combination of three microRNAs comprises miR-373, miR-451a/451b/144/4732 cluster, and miR-10b or miR-708. In some embodiments, the combination of three microRNAs comprises miR-376a, miR-451a/451b/144/4732 cluster, and let-7c or miR-9-1. In some embodiments, the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-708, and any one of the microRNAs selected from the group consisting of miR-10b, miR-15b/miR-16-2 cluster, and miR-373. In some embodiments, the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-9-1, and any one of the microRNAs selected from the group consisting of miR-181c, miR-29a, and miR-376a. In some embodiments, the combination of three microRNAs comprises miR-16-1/15a cluster, let-7e/miR-99b cluster, and miR-128b.

Aspects of the present invention provide methods for enhancing sensitivity of a cell to a chemotherapeutic agent, comprising contacting the cell with a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10. In some embodiments, the methods further comprise contacting the cell with the chemotherapeutic

agent. In some embodiments, the cell is a cancer cell. In some embodiments, the combination of microRNAs are expressed from one or more recombinant expression vectors.

Other aspects provide methods for treating cancer in a subject, comprising administering to the subject a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10 and a chemotherapeutic agent in an effective amount. In some embodiments, administering a combination of microRNAs comprises expressing the combination of microRNAs from one or more recombinant RNA expression vectors. In some embodiments, the effective amount of the chemotherapeutic agent administered with the combination of microRNAs is less than the effective amount of the chemotherapeutic agent when administered without the combination of microRNAs. In some embodiments, the combination of microRNAs comprises any of the combinations of microRNAs provided herein.

Other aspects provide methods for reducing cell proliferation, comprising contacting a cell with a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10. In some embodiments, the cell is a cancer cell. In some embodiments, the combination of microRNAs are expressed from one or more recombinant expression vectors.

Other aspects provide methods for treating cancer in a subject, comprising administering to the subject a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10. In some embodiments, administering a combination of microRNAs comprises expressing the combination of three microRNAs from one or more recombinant expression vectors. In some embodiments, the combination of microRNAs comprises any of the combinations of microRNAs provided herein.

Yet other aspects provide methods for identifying a combination of microRNAs that enhances sensitivity of a cell to an agent, comprising contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector; contacting the first population of cells with an agent, wherein the second population of cells is not contacted with the agent; identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells; comparing the abundance of each combination of two or more microRNAs in the first population of cells to

the abundance of each combination of two or more microRNAs in the second population of cells; identifying a combination of two or more microRNAs that is absent from or has reduced abundance in the first population of cells relative to the abundance of the same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances sensitivity a cell to the agent.

In some embodiments, the combinations of microRNAs that enhance sensitivity of a cell to the agent are compared to the combinations of microRNAs that reduce cell proliferation to identify the combinations of microRNAs that enhance sensitivity of a cell to the agent and reduce cell proliferation.

Other aspects provide methods for identifying a combination of microRNAs that enhances resistance of a cell to an agent, comprising contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector; contacting the first population of cells with an agent, wherein the second population of cells is not contacted with the agent; identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells; comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells; identifying a combination of two or more microRNAs that has increased abundance in the first population of cells relative to the abundance same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances resistance of a cell to the agent.

In some embodiments, the agent is a cytotoxic agent. In some embodiments, the cytotoxic agent is a chemotherapeutic agent. In some embodiments, the chemotherapeutic agent is an anti-mitotic/anti-microtubule agent. In some embodiments, the chemotherapeutic agent is docetaxel.

Other aspects provide methods for identifying a combination of microRNAs that reduces cell proliferation, comprising contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector; culturing the first population of cells and the second population of cells such that the second population of cells is cultured for a longer duration compared to the first population of cells; identifying the combinations of two or

more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells; comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells; identifying a combination of two or more microRNAs that is absent from or in reduced abundance in the second population of cells but present in or in increased abundance in the first population of cells as a combination of microRNAs that reduces cell proliferation.

In some embodiments, the combinations of microRNAs that reduce cell proliferation are compared to the combinations of microRNAs that enhance sensitivity of a cell to an agent to identify the combinations of microRNAs that reduce cell proliferation and enhance sensitivity of a cell to the agent.

Other aspects provide methods for identifying a combination of microRNAs that enhances cell proliferation, comprising contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector; culturing the first population of cells and the second population of cells such that the second population of cells is cultured for a longer duration compared to the first population of cells; identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells; comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells; identifying a combination of two or more microRNAs that is present in or in increased abundance in the second population of cells but absent from or in reduced abundance in the first population of cells as a combination of microRNAs that enhances cell proliferation.

In some embodiments, the microRNA expression vector is delivered to the first population of cells and/or the second population of cells by a virus. In some embodiments, the virus is a lentivirus.

Also provided are methods for determining a synergistic or antagonistic interaction of a combination of miRNAs on sensitivity of a cell to an agent and cell proliferation, comprising (1) contacting a first population of cells, a second population of cells, a third population of cells and a fourth population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector; (2) (a) contacting the first

population of cells with an agent, wherein the second population of cells is not contacted with the agent; (b) culturing the third population of cells and the fourth population of cells such that the fourth population of cells is cultured for a longer duration compared to the third population of cells; (3) identifying the combinations of two or more microRNAs in the first population of cells, the second population of cells, the third population of cells and the fourth population of cells; (4) (a) comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells; (b) comparing the abundance of each combination of two or more microRNAs in the third population of cells to the abundance of each combination of two or more microRNAs in the fourth population of cells; (5) (a) (1) identifying a combination of two or more microRNAs that is absent from or has reduced abundance in the first population of cells relative to the abundance of the same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances sensitivity a cell to the agent; and (2) identifying a combination of two or more microRNAs that has increased abundance in the first population of cells relative to the abundance same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances resistance of a cell to the agent (b) (1) identifying a combination of two or more microRNAs that is absent from or in reduced abundance in the fourth population of cells but present in or in increased abundance in the third population of cells as a combination of microRNAs that reduces cell proliferation, and (2) identifying a combination of two or more microRNAs that is present in or in increased abundance in the fourth population of cells but absent from or in reduced abundance in the third population of cells as a combination of microRNAs that enhances cell proliferation; (6) calculating a genetic interaction score for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation; (7) calculating an expected phenotype value for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation; and (8) comparing the genetic interaction score for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation with the expected phenotype value for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation, wherein a genetic interaction score greater than the expected phenotype value indicates a synergistic interaction between the microRNAs of the

combination, or wherein a genetic interaction score less than the expected phenotype value indicates an antagonistic interaction between the microRNAs of the combination.

In some embodiments, the expected phenotype value is calculated based on the additive model or the multiplicative model.

5 These and other aspects of the invention, as well as various embodiments thereof, will become more apparent in reference to the drawings and detailed description of the invention.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combination of elements can be included in each aspect of the invention.

10 This invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways.

15 BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

Figure 1 shows the strategy for assembling combinatorial genetic libraries and performing combinatorial miRNA screens. CombiGEM assembly uses iterative one-pot cloning of pooled single-genetic insert libraries into progressively more complex (n)-wise vector libraries. MicroRNA precursors were barcoded (BC) and four restriction sites (BglII, MfeI, BamHI, EcoRI) were positioned as shown in the right panel. The BglII/BamHI and EcoRI/MfeI pairs are unique restriction sites that generate compatible overhangs within the pair but are incompatible with the other pair. The pooled inserts and vectors were digested with BglII + MfeI and BamHI + EcoRI, respectively. A one-pot ligation created a pooled vector library, which was further iteratively digested and ligated with the same insert pool to produce higher-order combinations. All barcodes were localized into a contiguous stretch of DNA. The final combinatorial libraries were encoded in lentiviruses and delivered into targeted human cells. The integrated barcodes representing each genetic combination were amplified from the genomic DNA within the pooled cell populations in an unbiased fashion

and quantified using high-throughput sequencing to identify shifts in representation under different experimental conditions.

Figures 2A-2F show high-coverage combinatorial miRNA libraries can be efficiently generated and delivered to human cells. Figure 2A shows the cumulative distribution of sequencing reads for barcoded two-wise miRNA combinatorial libraries in the plasmid pools extracted from *E. coli* and the infected OVCAR8- ADR cell pools. Full coverage for all expected two-wise combinations within both the plasmid and infected cell pools was obtained, and less than 2% of two-wise combinations were covered by < 100 barcode reads. Figure 2B shows the cumulative distribution of sequencing reads for barcoded three-wise miRNA combinatorial libraries in the plasmid pools extracted from *E. coli* and the infected OVCAR8-ADR cell pools. High coverage of the three-wise library within the plasmid and infected cell pools (~89% and ~87%, respectively) was achieved, and ~10-15% of the combinations were covered by < 100 barcode reads. Figure 2C shows a high correlation between barcode representations ($\log_2$ values of normalized barcode counts) within the plasmid and infected OVCAR8-ADR cell pools indicating efficient lentiviral delivery of the two-wise libraries into human cells. Figure 2D shows a high correlation between barcode representations ($\log_2$ values of normalized barcode counts) within the plasmid and infected OVCAR8-ADR cell pools indicate efficient lentiviral delivery of the three-wise libraries into human cells. Combinations are considered underrepresented when the fold change of the barcode counts in cells relative to the plasmid libraries has a Z-score < -2, a cutoff set for the combinations that have two standard deviations below the population mean. The underrepresented combinations are highlighted in light gray. Figure 2E shows high reproducibility for barcode representations between two biological replicates in OVCAR8-ADR cells infected with the two-wise combinatorial miRNA libraries. Figure 2F shows high reproducibility for barcode representations between two biological replicates in OVCAR8-ADR cells infected with the three-wise combinatorial miRNA libraries. R is Pearson correlation coefficient.

Figures 3A-3E show a two-wise combinatorial screen revealing miRNA interactions that confer docetaxel resistance or sensitization in cancer cells. Figure 3A presents a schematic showing OVCAR8-ADR cells infected with the two-wise combinatorial miRNA

library were split into two groups and treated with 25 nM of docetaxel or vehicle control for four days. The barcodes of each combinatorial miRNA construct were amplified by PCR from the genomic DNA within the cell pools in an unbiased fashion, and counted using high throughput sequencing (Illumina HiSeq). Figure 3B presents two-wise miRNA combinations that modulated docetaxel sensitivity ranked by their mean $\log_2$ ratios of the normalized barcode count for docetaxel (25 nM)-treated cells to that for vehicle-treated cells from two biological replicates. The labeled miRNA combinations were further validated in the experiments described herein. Figure 3C shows validation of two-wise miRNA combinations conferring docetaxel sensitization. OVCAR8-ADR cells were infected with single miRNA, two-wise miRNAs, or vector control and subjected to 10 nM (light gray) or 25 nM (dark gray) of docetaxel for three days. Figure 3D shows viability of OVCAR8-ADR cells infected with two-wise miRNA combinations or vector control and treated with docetaxel (0-50 nM) or vehicle control (black line) for three days. Dose response analysis showed that OVCAR8-ADR cells infected with the combination of the *miR-16-1/15a* cluster with the *miR-93/106b* cluster (light gray line) or *miR-376a* (medium gray line) reduced the IC₅₀ of docetaxel by ~2-fold. Figure 3E shows validation of two-wise miRNA combinations conferring docetaxel resistance. OVCAR8-ADR cells were infected with single miRNA, two-wise miRNAs, or vector control and subjected to 10 nM (light gray) or 25 nM (dark gray) of docetaxel for three days. Cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Data represent the mean $\pm$ SD ($n \geq 10$), and data of Figures 3C and 3D was obtained from the same experiments. An asterisk indicates $P < 0.05$.

Figures 4A-4E show three-wise combinatorial screens identifying miRNA combinations modify docetaxel sensitivity or proliferation in cancer cells. Figure 4A presents a schematic showing OVCAR8-ADR cells infected with the three-wise combinatorial miRNA library were split into three groups, and treated with 25 nM of docetaxel or vehicle 25 for four days, or cultured with vehicle for one day. The barcodes of each combinatorial miRNA construct were amplified by PCR from the genomic DNA within the cell pools in an unbiased fashion, and counted using high throughput sequencing (Illumina HiSeq). Figure 4B presents three-wise miRNA combinations that modulated docetaxel sensitivity ranked by their mean $\log_2$ ratios of the normalized barcode count for docetaxel (25 nM)-treated cells versus four-day vehicle-treated cells. The labeled miRNA

combinations were further validated in the experiments described herein. Figure 4C shows validation of three-wise miRNA combinations that altered docetaxel sensitivity. OVCAR8-ADR cells were infected with the indicated three-wise miRNA combinations or vector control and subjected to 0-50 nM of docetaxel for three days. Figure 4D presents three-wise miRNA combinations that modulated cell proliferation ranked by their mean $\log_2$ ratios of the normalized barcode count for four-day versus one-day cultured cells. The labeled miRNA combinations were further validated in the experiments described herein. Figure 4E shows validation of the indicated three-wise miRNA combinations that altered cell proliferation. OVCAR8-ADR cells were infected with three-wise miRNA combinations or vector control and cultured for the indicated time periods. Cell viability was measured by the MTT assay and was compared to the no drug control ($n \geq 5$). Proliferation was characterized by absorbance measurements ($OD_{570} - OD_{650}$) ($n \geq 4$). Data represent the mean $\pm$ standard deviation.

Figures 5A-5F show high-throughput profiling of miRNA combinations revealing genetic interactions for modulating docetaxel sensitivity and/or cell proliferation phenotypes. Figure 5A shows a two-dimensional heat map (upper panel) and genetic interaction map (lower panel) depicting the docetaxel sensitivity of cells harboring two-wise miRNA combinations and the genetic interaction (GI) scores of the miRNA pairs respectively. Docetaxel sensitivity was measured by the $\log_2$ ratios of the normalized barcode counts for docetaxel-treated versus vehicle-treated OVCAR8-ADR cells. Drug resistance and sensitization phenotypes have the $\log_2$ ratios of > 0 and < 0 , respectively. The data for miRNA two-wise pairs with less than 100 absolute barcode reads in the control sample were filtered out and are denoted in light gray. MicroRNAs were clustered hierarchically based on the correlation of their $\log_2$ ratios. GI scores for all two-wise combinations were calculated and presented in the GI map (lower panel). Synergistic and buffering interactions are defined when an observed combinatorial phenotype deviates further from or less than the expected phenotype produced by the additive model. Synergistic and buffering interactions have GI scores of > 0 and < 0 , respectively. miRNA pairs for which no GIs were measured are indicated in light gray. The miRNAs were presented on the genetic interaction map in the same order as for the two-dimensional heatmap. Figure 5B shows three-dimensional plots illustrating the docetaxel-sensitizing effects of three-wise miRNA combinations. The $\log_2$

ratios of the normalized barcode counts for docetaxel-treated versus four-day vehicle-treated OVCAR8-ADR cells were determined for all three-wise miRNA combinations. Drug resistance ($\log_2$ ratio > 0) and sensitization ($\log_2$ ratio < 0) phenotypes are presented by the bubbles. Figure 5C shows three-dimensional plots illustrating the proliferation-modulating effects of three-wise miRNA combinations. The $\log_2$ ratios of the normalized barcode counts for four-day versus one-day cultured cells were determined for all three-wise miRNA combinations. Proproliferation ($\log_2$ ratio > 0) and anti-proliferation ($\log_2$ ratio < 0) phenotypes are represented by the bubbles. See Figure 14 for full panels of 39x39x39 miRNA combinations. Each two-dimensional plane was arranged in the same hierarchically clustered order as in Figure 5A, and the additional third miRNA element is labeled. Figure 5D presents the distribution of GI scores determined for the docetaxel-sensitivity screen using two-wise miRNA combinations. Figure 5E presents the distribution of GI scores determined for the docetaxel-sensitivity screen using three-wise miRNA combinations. Figure 5F presents the distribution of GI scores determined for the cell proliferation screen using three-wise miRNA combinations. In Figures 5D-5F, miRNA combinations were grouped based on their GI scores to evaluate the frequency of genetic interactions. GI scores of the validated miRNA combinations are indicated by arrows and labeled. GI scores of the three-wise miRNA combination represent the interaction between the additional third miRNA with the two-wise miRNA combination that modifies the biological phenotype. All $\log_2$ ratios and GI scores shown were determined from the mean of two biological replicates.

Figures 6A-6J shows microRNAs interact combinatorially to modulate docetaxel sensitivity and cancer cell proliferation. Figures 6A-6H present a scatter plot comparing the drug sensitization and proliferation-modulating effects of three-wise (triangles) miRNA combinations with their respective single (squares) and two-wise (diamonds) combinations for miRNAs. Relative cell viabilities plotted for three-day docetaxel (25 nM)-treated versus vehicle-treated OVCAR8-ADR cells and absorbances ($OD_{570} - OD_{650}$) plotted for seven-day versus one-day cultured cells were determined by MTT assays. Drug sensitivity (y-axis; $n \geq 5$) and cell proliferation (x-axis; $n \geq 3$) indexes were obtained by dividing the relative viability and absorbance determined for each miRNA combination by that for the empty vector control without miRNA. Data were obtained from the same sets of experiments. Figure 6I shows OVCAR8-ADR cells infected with the indicated miRNA combinations and

treated with 25 nM of docetaxel for three days. Viable cell numbers were determined by the trypan blue exclusion assay. Figure 6J shows OVCAR8-ADR cells infected with the indicated miRNA combinations, treated with 25 nM of docetaxel, and cultured for another eleven days, and stained with crystal violet. The colony area percentage for each sample was quantified. Data represent the mean $\pm$ standard deviation ($n = 3$). An asterisk indicates $P < 0.05$.

Figures 7A-7D show lentiviral delivery of combinatorial miRNA expression constructs provides efficient target gene repression. Figure 7A depicts design for lentiviral combinatorial miRNA expression and sensor constructs. Single or multiple miRNA precursor sequences are arranged in tandem downstream of a GFP gene to monitor expression driven by a CMV promoter in a lentiviral vector. Sensors harboring four repeats of the cognate miRNA target sequence(s) were cloned in the 3'UTR of a RFP gene expressed from an UBC promoter to report on miRNA activity. The constructs were delivered by lentiviruses to HEK293T cells and then analyzed for GFP and RFP expression using flow cytometry. Figure 7B shows repression of the RFP reporter activity by miRNA expression. Lentiviral constructs harboring the indicated miRNA, the cognate sensor, or both were introduced into HEK293T cells. Figure 7C shows the indicated combinatorial miRNA expression constructs effectively repressed RFP reporters containing the cognate miRNA sensors. Lentiviral constructs harboring two-wise or three-wise miRNA combinations, with or without the cognate sensors, were introduced into HEK293T cells, and RFP and GFP expression were assessed. Figure 7D shows there is limited cross-reactivity between miRNAs and non-cognate sensors. Lentiviral constructs harboring miRNAs paired with different (non-cognate) sensors were delivered into HEK293T cells. The percentages of RFP positive cells within GFP-positive cell populations were determined by flow cytometry. Data represent the mean $\pm$ standard deviation ($n = 3$).

Figures 8A-8C shows efficient lentiviral delivery of a dual-fluorescent protein reporter construct to human cells. Figure 8A depicts a strategy for testing lentiviral delivery of a dual-fluorescent protein reporter construct to human cells. Lentiviruses generated for delivering vectors containing a GFP gene expressed under control of a CMV or UBC promoter, or a single vector encoding RFP and GFP genes under control of the UBC and

CMV promoters, respectively, were delivered to HEK293T cells for analysis of GFP and RFP expression. Figure 8B presents fluorescence micrographs showing RFP and GFP expressed in UBCp-RFP-CMVp-GFP virus-infected cells, whereas only GFP was expressed in cells infected with UBCp-GFP and CMVp-GFP lentiviruses. The scale bar denotes 400 μm . Figure 8C shows results from flow cytometry analysis quantifying cell populations positive for RFP and GFP fluorescence, assessing delivery and expression of the dual-fluorescent protein reporter construct in human cells. Over 97 percent of UBCp-RFP-CMVp-GFP virus-infected HEK293T cells were positive for both RFP and GFP, and similar percentages of UBCp-GFP or CMVp-GFP virus-infected cells were GFP-positive.

Figures 9A-9D shows identification of the exponential phase during PCR for CombiGEM barcode amplification. Figure 9A shows a procedure for identifying the transition point from exponential to linear phase during PCR for CombiGEM barcode amplification from the one-wise miRNA vector library pooled-assembled in *E. coli* used as templates in replicate PCR reactions. Figure 9B shows a procedure for identifying the transition point from exponential to linear phase during PCR for CombiGEM barcode amplification from the genomic DNA isolated from human breast cancer cells (MCF7) infected with the two-wise library used as templates in replicate PCR reactions. In Figures 9A and 9B, the barcodes representing each miRNA combination were amplified using primers targeting the sequences located outside the barcode region. PCR products were collected from the reactions stopped at cycles between 10 to 20 (Figure 9A) or 19 to 28 (Figure 9B), and were then diluted as templates for quantitative PCR reactions. The mean difference of threshold cycle (Ct) between cycles was determined. Error bars indicate SD from triplicates. Primer efficiencies were estimated to be 102% and 100%, for Figure 9A and Figure 9B, respectively. PCR cycle numbers indicated with an arrow in Figure 9A and 9B were used in unbiased barcode amplification for subsequent high throughput (Illumina) sequencing. Figure 9C presents a stained agarose gel of the amplified PCR products with indicated cycle numbers from (Figure 9A). Figure 9D presents a stained agarose gel of the amplified PCR products with indicated cycle numbers from Figure 9B.

Figures 10A and 10B show the high reproducibility of barcode quantitation in biological replicates for combinatorial miRNA screens. Figure 10A presents scatter plots

showing high correlation between barcode representations ($\log_2$ number of normalized barcode counts) between two biological replicates of docetaxel (25 nM)-treated or vehicle-treated OVCAR8-ADR cells infected with the two-wise miRNA combinatorial libraries.

Figure 10B presents scatter plots showing high correlation between barcode representations ($\log_2$ number of normalized barcode counts) between two biological replicates of docetaxel (25 nM)-treated or vehicle-treated OVCAR8-ADR cells infected with the three-wise miRNA combinatorial libraries. R is Pearson correlation coefficient.

Figures 11A-11C show consistent fold changes of barcodes among same miRNA combinations arranged with different orders in the expression constructs. Figure 11A shows the coefficient of variation for two-wise combinations arranged in different orders for cells that received treatment with docetaxel (25 nM) versus vehicle control for four days. 92% of two-wise miRNA combinations had a CV of < 0.2 in the drug sensitivity screen. Figure 11B shows the coefficient of variation for three-wise combinations arranged in different orders for cells that received treatment with docetaxel (25 nM) versus vehicle control for four days. 95% of three-wise miRNA combinations had a CV of < 0.2 in the drug sensitivity screen. Figure 11C shows the coefficient of variation for three-wise combinations arranged in different orders for cells cultured four days versus one day. 98% of three-wise miRNA combinations had a CV of < 0.2 in the proliferation screen.

Figures 12A-12C show consistency between biological replicates for all individual hits in the pooled screens. Figure 12A shows consistency between biological replicates for hits identified docetaxel (25 nM)-treated versus vehicle treated OVCAR8-ADR cells for each two-wise miRNA combination. The top panel presents the $\log_2$ fold change of biological replicate 1 is plotted against replicate 2 for mean values of normalized barcode counts. The lower panel presents distributions of $\log_2$ fold change difference between two biological replicates at a bin size of 0.1. Figure 12B shows consistency between biological replicates for hits identified docetaxel (25 nM)-treated versus vehicle treated OVCAR8-ADR cells for each three-wise miRNA combination. The top panel presents the $\log_2$ fold change of biological replicate 1 is plotted against replicate 2 for mean values of normalized barcode counts. The lower panel presents distributions of $\log_2$ fold change difference between two biological replicates at a bin size of 0.1. Figure 12C shows consistency between biological

replicates for hit identified for relative cell viability at day 4 versus day 1 for each three-wise miRNA combination. The top panel presents the $\log_2$ fold change of biological replicate 1 is plotted against replicate 2 for mean values of normalized barcode counts. The lower panel presents distributions of $\log_2$ fold change difference between two biological replicates at a bin size of 0.1. Data points identified as hits (see Tables 3-7) are colored in dark gray. Screen hits show a higher Pearson correlation coefficient ($R = 0.636-0.788$). Each data point for the screening data represents the mean of two biological replicates. The majority of combinations (78-90%) have $< 0.3 \log_2$ fold change difference.

Figure 13 shows docetaxel dose-response curves for the OVCAR8 cell line and the docetaxel-resistant OVCAR8-ADR cell line. OVCAR8 cells (triangles) and OVCAR8-ADR (squares) cells (docetaxel-resistant derivative of OVCAR8) cells were treated with docetaxel at indicated doses for three days and subjected to the MTT assay. Cell viabilities were compared to the respective no drug controls. The OVCAR8-ADR cell line has a ~3-fold higher IC_{50} than the parental OVCAR8 cell line. Data represent the mean $\pm$ SD ($n = 3$).

Figures 14A and 14B presents three-dimensional plots depicting the effects of each of the three-wise miRNA combinations. Figure 14A shows the docetaxel-sensitizing effects of each of the three-wise miRNA combinations. The $\log_2$ ratios of the normalized barcode counts for docetaxel-treated versus four-day vehicle-treated OVCAR8-ADR cells were determined for all three-wise miRNA combinations, and were presented as the colored bubbles. MicroRNA combinations with drug resistance have the $\log_2$ ratios > 0 and < 0 , respectively. Figure 14B shows the proliferation-modulating effects of each of the three-wise miRNA combinations. The $\log_2$ ratios of the normalized barcode counts for four-day versus one-day cultured cells were determined for all three-wise miRNA combinations, and were presented as the colored bubbles. miRNA combinations with pro-proliferation and anti-proliferation effects have the $\log_2$ ratios > 0 and < 0 respectively. Each two-dimensional plane was arranged in the same hierarchically clustered order as in Figures 5A-5C, and the additional third miRNA element is labeled. All $\log_2$ ratios shown were determined from the mean of two biological replicates.

Figures 15 shows definitions of Genetic Interaction (GI) described herein. Synergistic or buffering interactions have positive and negative GI scores respectively, as described for Cases 1 to 7. Positive and negative phenotypes have fold changes of normalized barcode reads of > 1 and < 1 respectively, while no phenotype change results in a fold change = 1.

- 5 For miRNA [A] and [B] with individual phenotype “A” and “B”, the expected phenotype for the two-wise combination [A,B] is (“A” + “B” - 1) according to the additive model. Deviation was calculated by subtracting expected phenotype from observed phenotype (i.e. Observed phenotype – Expected phenotype).

- 10 Figures 16A-16D show synergistic interaction between *miR-16-1/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster to modulate the cell proliferation phenotype. Figure 16A shows the GI scores for a given three-wise miRNA combination [A,B,C] plotted and compared to the respective combinations harboring two same miRNAs and every other miRNA library members (denoted as X). GI score represents the interaction between the
- 15 additional third miRNA and the two-wise miRNA combination that modifies the biological phenotype. GI scores were determined for the three possible permutations (i.e. [A,B,C], [B,C,A], and [A,C,B]). miRNA combinations having GI scores beyond a |Z-score| cut-off value of 2 are considered statistically significant ($P < 0.05$). In this example, A, B, and C represent the *miR-16-1/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster, respectively,
- 20 and X represents all 39 library members. GI scores for the cell proliferation phenotype were determined for the three-wise combinations harboring the *miR-16-1/15a* cluster, *miR-128b*, and/or the *let-7e/miR-99b* cluster, and revealed their synergistic interactions that modify the phenotype. Figure 16B presents a GI map showing GI scores for the cell proliferation phenotype of all three-wise miRNA combinations that include the *miR-16-1/15a* cluster.
- 25 Figure 16C presents a GI map showing GI scores for the cell proliferation phenotype of all three-wise miRNA combinations that include *miR-128b*. Figure 16D presents a GI map showing GI scores for the cell proliferation phenotype of all three-wise miRNA combinations that include the *let-7e/miR-99b* cluster. The combinations for which no GIs were measured are indicated in light gray.

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Figure 17 presents a graph showing three-wise miRNA combinations have distinct docetaxel sensitivity and anti-proliferation phenotypes. The fold change of normalized

barcode counts for docetaxel (25 nM)-treated versus vehicle-treated OVCAR8-ADR cells (y-axis) and fold change for four-day versus one-day cultured cells (x-axis) were plotted for all three-wise miRNA combinations. Each data point represents the mean of two biological replicates.

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Figures 18A and 18B show combinatorial expression of the *miR-16-1/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster inhibits colony formation by viable OVCAR8-ADR cells. Figure 18A shows representative images of ~10,000 OVCAR8-ADR cells infected with each indicated miRNA combinations treated with 25 nM of docetaxel for three days, and were cultured for another eleven days. Cells were stained with crystal violet to visualize colony formation for quantification. Figure 18B presents the number of colonies for each sample from Figure 18A. The maximum number of discrete colonies that could be reliably counted was ~500 per well, and thus, samples with more than 500 colonies are presented as > 500 colonies. Data represent the mean $\pm$ SD (n = 3; *P < 0.05).

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Figure 19 presents a graph showing high consistency between pooled screening and validation data for individual hits. The fold change in the normalized barcode count for docetaxel (25 nM)-treated versus vehicle-treated OVCAR8-ADR cells, obtained from pooled screening data was plotted against its relative cell viability compared to vector control determined from individual drug sensitivity assays (R = 0.899) was plotted for each two-wise (diamonds) and three-wise (triangles) miRNA combination. The fold change in the normalized barcode count for four-day versus one-day cultured cells (circles), obtained from pooled screening data was plotted against its relative cell viability compared to vector control determined from individual drug sensitivity or proliferation assays respectively (R = 0.899) was plotted for each three-wise miRNA combination. Screening data are the mean of two biological replicates while the individual hit validation data represent the mean of three independent experiments. R is Pearson correlation coefficient.

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Figures 20A-20C show combinatorial expression of the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster reduced mRNA levels of targeted genes in OVCAR8-ADR cells. Figure 20A presents RT-qPCR quantification of relative mRNA levels in OVCAR8-ADR cells expressing the *miR-16/15a* cluster or coexpressing the *miR-16/15a*

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cluster, *miR-128b*, and the *let-7e/miR-99b* cluster. The targeted mRNA levels were normalized to GAPDH, and data represent mean $\pm$ SD (n = 3). mRNA sequences predicted or validated to contain conserved sites matching the seed region of the corresponding miRNAs using TargetScan and miRTarBase are shaded in medium gray as shown in the table below the graph. Significant difference of the mRNA levels of CCND1, CCND3, CCNE1 and CHEK1 in cells expressing the *miR-16/15a* cluster or co-expressing the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster was determined by comparing to vector control-infected cells (# P < 0.05). An asterisk represents a statistically significant difference (P < 0.05) of mRNA levels between cells expressing the *miR-16/15a* cluster or co-expressing the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster. Figure 20B shows relative mRNA levels of CDC14B among cells expressing different combinations of miRNAs composed of none, singles, doubles or triples of the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster. The mRNA level of CDC14B was significantly reduced in cells co-expressing the *let-7e/miR-99b* cluster and *miR-128b*, or the triples of the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster. Data represent the mean $\pm$ SD (n = 9; *P < 0.05). Figure 20C presents a summary diagram illustrating the potential roles of the *miR-16/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster in regulating multiple downstream targets responsible for the change in docetaxel resistance and/or proliferation phenotypes in OVCAR8-ADR cells.

Figure 21 presents graphs showing the anti-proliferative effects of miR-34a and the miR-15b/16-2 cluster vary across different cell lines. The proliferation rate of OVCAR8, OVCAR8-ADR, HOSE11-12, HOSE17-1, T1074 and MCF7 cells infected with *miR-34a* (light gray line), the *miR-15b/16-2* cluster (medium gray line), or vector control (black line) was characterized by absorbance measurements (OD570 - OD650) or MTT assay. Data represent the mean $\pm$ SD (n = 3; *P < 0.05).

DETAILED DESCRIPTION

Therapies that target multiple cellular pathways or multiple factors that may have independent roles that synergize for disease development and progression has proven to be a more effective approach to therapy compared to convention monotherapies. However, methods for the identification of multiple genetic targets can be very limited and laborious

due to the difficulty in generating high-order gene knock-out/silenced combinations, especially for high-throughput screening. The invention described herein is based on the surprising discovery of novel combinations of microRNAs that together have anti-cancer effects, such as enhancing sensitivity of cancer cells to chemotherapeutic agents and reducing proliferation of cancer. Also provided are methods of generating complex combinatorial microRNA expression libraries useful for a variety of high-throughput screening methods.

The methods and compositions described herein provide combinations of two or three microRNAs that enhance sensitivity of a cancer cell to a chemotherapeutic agent (see Tables 3 and 7). The methods and compositions also provide combinations of three microRNAs that reduce proliferation of cancer cells (see Table 7). As used herein, the terms “microRNA” and “miRNA” may be used interchangeably and refer to a small non-coding RNA molecule that plays a role in RNA interference (RNAi), particularly in a silencing an mRNA (“RNA silencing”) and regulation of gene expression. A microRNA that achieves RNA silencing or silences a mRNA means the target mRNA is not translated into protein. Without wishing to be bound by any particular theory, it is thought that RNA silencing with a microRNA may occur by any of several mechanisms, such as translational repression; mRNA cleavage, destabilization or decay; and deadenylation of the target mRNA. The terms “silence” or “RNA silencing” refers to complete silencing of a target mRNA, resulting in no detectable protein expression, or partial silencing, resulting in a reduction in protein expression as compared to protein expression in the absence of the microRNA.

A microRNA is complementary to at least one target mRNA or portion thereof. In some embodiments, the microRNA may be complementary to a portion of a mRNA in the 3'UTR of the mRNA. In other embodiments, the microRNA may be complementary to a portion of the protein coding region of the mRNA. In some embodiments, the miRNA is between 15-30 nucleotides, 18-28 nucleotides, or 21-25 nucleotides in length. In some embodiments, the miRNA is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

It will be appreciated that a microRNA is complementary to a target mRNA in a cell if the microRNA is capable of hybridizing to the target mRNA to an extent sufficient to silence the mRNA. In some embodiments, the microRNA is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or at least 100% complementary to a portion of the target mRNA. In some embodiments, a portion of the microRNA, referred to

as a seed region, is complementary to a target mRNA. In some embodiments, the seed region is between 2-7 nucleotides of the microRNA. In some embodiments, the seed region of the microRNA is at least 90%, 95%, 96%, 97%, 98%, 99%, or at least 100% complementary to a portion of the target mRNA.

5 In some embodiments, the combination of microRNAs is expressed in a cell (*e.g.*, a cancer cell) as a pri-microRNA or a pre-mRNA and is subsequently processed into a pre-microRNA in the nucleus of the cell. In some embodiments, the pre-microRNA is further processed in the cytoplasm to form a microRNA that is capable of hybridizing to its complementary target mRNA and silencing expression.

10 The methods and compositions described herein may be useful for reducing proliferation of a cell, such as a cancer cell or other cell for which reduced proliferation is desired. In some embodiments, contacting a cell with a combination of three microRNAs partially or completely reduces proliferation of the cell. In some embodiments, contacting a cell with a combination of three microRNAs partially or completely reduces proliferation of
15 the cell as compared to a cell that is not contacted with the combination of microRNAs. In some embodiments, contacting cells with a combination of three microRNAs reduces proliferation of the cells by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, or at least 65% as compared to cells that were not contacted with the combination of microRNAs. Cell proliferation may be assessed and quantified by any method known in the
20 art, for example using cell viability assays or BrdU cell proliferation assays.

The methods and compositions of combinations of microRNAs described herein may also be useful for enhancing the sensitivity of cells (*e.g.*, cancer cells) to a chemotherapeutic agent. In some embodiments, contacting a cell with a combination of two or three microRNAs leads to a reduction in the half minimal inhibitory concentration (IC₅₀) of the
25 chemotherapeutic agent. In some embodiments, contacting a cell with a combination of two or three microRNAs leads to a reduction in the IC₅₀ of the chemotherapeutic agent as compared to the IC₅₀ of the chemotherapeutic on a cell that is not contacted with the combination of microRNAs. In some embodiments, following contact with combination of microRNAs, the IC₅₀ of the chemotherapeutic agent is reduced by at least 1.1-, 1.2-, 1.3-, 1.4-
30 , 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 4.0-, or at least 5.0-fold. In some embodiments, following contact with combination of microRNAs, the IC₅₀ of the chemotherapeutic agent is reduced by at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-,

1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 4.0-, or at least 5.0-fold as compared to the IC₅₀ of the chemotherapeutic agent on cells that have not been contacts with the combination of microRNAs. Methods for determining chemotherapeutic sensitivity and IC₅₀ values will evident to one of skill in the art

5 The invention encompasses any cell type in which expression of a gene may be reduced or silenced using microRNAs. In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell, including a human cell (e.g., a human embryonic kidney cell (e.g., HEK293T cell), a human dermal fibroblast, a MC7 cell, OVCAR8 cell, OVCAR8-ADR cell, T1074 cell, HOSE 11-12 cell, or HOSE 17-1 cell) or a
10 rodent cell. In other embodiments, the cell is an algal cell, a plant cell, or an insect cell. In other embodiments, the cell is a fungal cell such as a yeast cell, e.g., *Saccharomyces* spp., *Schizosaccharomyces* spp., *Pichia* spp., *Phaffia* spp., *Kluyveromyces* spp., *Candida* spp., *Talaromyces* spp., *Brettanomyces* spp., *Pachysolen* spp., *Debaryomyces* spp., *Yarrowia* spp. and industrial polyploid yeast strains. Preferably the yeast strain is a *S. cerevisiae* strain.
15 Other examples of fungi include *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Rhizopus* spp., *Acremonium* spp., *Neurospora* spp., *Sordaria* spp., *Magnaporthe* spp., *Allomyces* spp., *Ustilago* spp., *Botrytis* spp., and *Trichoderma* spp. In some embodiments, the cell is in an multicellular organism, for example a plant or a mammal. In some embodiments, the mammal is a human.

20 Aspects of the invention relate to methods and compositions for enhancing the sensitivity of a cancer cell to a chemotherapeutic agent or to reducing proliferation of a cancer cell. Cancer is a disease characterized by uncontrolled or aberrantly controlled cell proliferation and other malignant cellular properties. As used herein, the term “cancer” refers to any type of cancer known in the art, including without limitation, breast cancer, biliary
25 tract cancer, bladder cancer, brain cancer, cervical cancer, choriocarcinoma, colon cancer, endometrial cancer, esophageal cancer, gastric cancer, hematological neoplasms, T-cell acute lymphoblastic leukemia/lymphoma, hairy cell leukemia, chronic myelogenous leukemia, multiple myeloma, AIDS-associated leukemias and adult T-cell leukemia/lymphoma, intraepithelial neoplasms, liver cancer, lung cancer, lymphomas, neuroblastomas, oral cancer,
30 ovarian cancer, pancreatic cancer, prostate cancer, rectal cancer, sarcomas, skin cancer, testicular cancer, thyroid cancer, and renal cancer. The cancer cell may be a cancer cell *in*

vivo (i.e., in an organism), *ex vivo* (i.e., removed from an organism and maintained *in vitro*), or *in vitro*.

Other aspects of the invention relate to methods and compositions for treating cancer in a subject. In some embodiments, the subject is a subject having, suspected of having, or at risk of developing cancer. In some embodiments, the subject is a mammalian subject, including but not limited to a dog, cat, horse, cow, pig, sheep, goat, chicken, rodent, or primate. In some embodiments, the subject is a human subject, such as a patient. The human subject may be a pediatric or adult subject. Whether a subject is deemed “at risk” of having a cancer may be determined by a skilled practitioner.

As used herein “treating” includes amelioration, cure, prevent it from becoming worse, slow the rate of progression, or to prevent the disorder from re-occurring (i.e., to prevent a relapse). An effective amount of a composition refers to an amount of the composition that results in a therapeutic effect. For example, in methods for treating cancer in a subject, an effective amount of a chemotherapeutic agent is any amount that provides an anti-cancer effect, such as reduces or prevents proliferation of a cancer cell or is cytotoxic towards a cancer cell. The effective amount of a chemotherapeutic agent may be presented as the half minimal inhibitory concentration (IC₅₀). In some embodiments, the effective amount of a chemotherapeutic agent is reduced when the chemotherapeutic agent is administered concomitantly with any of the combinations of microRNAs described herein as compared to the effective amount of the chemotherapeutic agent when administered in the absence of the combination of microRNAs. In some embodiments, the effective amount of a chemotherapeutic agent is reduced by at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 4.0-, 5.0-, 10.0-, 15.0-, 20.0-, 25.0-, 30.0-, 35.0-, 40.0-, 45.0-, 50.0-, 55.0-, 60.0-, 65.0-, 70.0-, 75.0-, 80.0-, 85.0-, 90.0-, 95.0-, 100-, 200-, 300-, 400-, or at least 500-fold or more when the chemotherapeutic agent is concomitantly administered with a combination of microRNAs (e.g., combinations of two microRNAs presented in Table 3 or combinations of three microRNAs presented in Table 5). In some embodiments, the IC₅₀ of the chemotherapeutic agent is reduced by at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 4.0-, 5.0-, 10.0-, 15.0-, 20.0-, 25.0-, 30.0-, 35.0-, 40.0-, 45.0-, 50.0-, 55.0-, 60.0-, 65.0-, 70.0-, 75.0-, 80.0-, 85.0-, 90.0-, 95.0-, 100-, 200-, 300-, 400-, or at least 500-fold or more when the

chemotherapeutic agent is concomitantly administered with any of the combinations of microRNAs described herein.

As used herein, the term “chemotherapeutic agent” refers to any agent that has an anti-cancer effect (e.g., kills or reduces proliferation of a cancer cell). Chemotherapeutic agents may include alkylating agents, such as mechlorethamine, chlorambucil, cyclophosphamide, ifosfamide, melphalan, streptozocin, carmustine (BCNU), lomustine, busulfan, dacarbazine (DTIC), temozolomide, thiotepa and altretamine (hexamethylmelamine); anti-mitotic agents (mitotic inhibitors), such as paclitaxel, docetaxel, izabepilone, vinblastine, vincristine, vinorelbine, and estramustine; antimetabolites, such as 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine, cladribine, clofarabine, cytarabine, floxuridine, fludarabine, gemcitabine, hydroxyurea, methotrexate, pemetrexed, pentostatin, and thioguanine; anti-tumor antibiotics, such as anthracyclines (daunorubicin, doxorubicin, epirubicin, idarubicin), actinomycin-D, bleomycin, and mitomycin-C; topoisomerase inhibitors, such as topoisomerase I inhibitors (topotecan and irinotecan (CPT-11)) and topoisomerase II inhibitors (etoposide (VP-16), teniposide, and mitoxantrone); and corticosteroids, such as prednisone, methylprednisolone, and dexamethasone. In some embodiments, the chemotherapeutic agent is an anti-mitotic agent. In some embodiments, the anti-mitotic agent is docetaxel.

Also within the scope of the present invention are methods for screening cell populations for combinations of microRNAs that, when administered to cells, result in an increase or decrease in sensitivity of the cells to an agent. In some embodiments, the agent is a chemotherapeutic agent. As depicted in Figure 3A, the methods involve contacting two populations of cells with a combinatorial library of microRNAs (e.g., a barcoded microRNA library generated with the CombiGEM method). One of the populations of cells is also contacted with an agent, such as a cytotoxic agent (e.g., a toxin, chemotherapeutic agent). Following a duration of time, the identification of the combinations of microRNAs are determined, for example by sequencing methods. The abundance of each combination of microRNAs in the first population of cells that was also contacted with the cytotoxic agent is compared to the abundance of each combination of microRNAs in the population of cells that was not contacted with the agent. Combinations of microRNAs that enhanced sensitivity of the cells to the agent will be less abundant or absent from the population of cells that was exposed to the agent. Combinations of microRNAs that reduced sensitivity of the cells to the

agent will be more abundant in the population of cells that was exposed to the agent. The combinations of microRNAs that enhance sensitivity of cells to an agent (*e.g.*, a chemotherapeutic agent) may be compared to combinations of microRNAs that reduce proliferation of cells to identify combinations of microRNAs that both enhance sensitivity of
5 cells to the agent and reduce proliferation of cells.

Other methods are provided for screening cell populations for combinations of microRNAs that, when administered to the cell populations, result in an enhancement or reduction of cell proliferation. As depicted in Figure 4A, the methods involve contacting two populations of cells a combinatorial library of microRNAs (*e.g.*, a barcoded microRNA
10 library). The two populations of cells are cultured for different durations of time. For example, one population of cells may be cultured for one day and the other population of cells is cultured for four days. The identification of the combinations of microRNAs are determined for each population of cells, for example by sequencing methods. The abundance of each combination of microRNAs in the population of cells that was for a longer duration
15 of time is compared to the abundance of each combination of microRNAs in the population of cells that was for the shorter duration of time. Combinations of microRNAs that enhanced proliferation of the cells will be more abundant in the population of cells that was cultured for the longer duration of time. Combinations of microRNAs that reduced proliferation of the cells will be less abundant in the population of cells that was cultured for the longer duration
20 of time. The combinations of microRNAs that reduced proliferation of cells may be compared to combinations of microRNAs that enhance sensitivity of cells to an agent (*e.g.*, a chemotherapeutic agent) to identify combinations of microRNAs that both reduce proliferation of cells and enhance sensitivity of cells to the agent.

The combinations of microRNAs described herein may be administered to a subject,
25 or delivered to or contacted with a cell in any form known in the art. In some embodiments, the combination of microRNAs are concatenated microRNAs. In some embodiments, the concatenated microRNAs also contain one or more linker and/or spacer sequence. In other embodiments, the combination of microRNAs are conjugated to one or more nanoparticle, cell-permeating peptide, and/or polymer. In other embodiments, the combination of
30 microRNAs are contained within a liposome.

The combinations of microRNAs described herein may be administered to a subject, or delivered to or contacted with a cell by any methods known in the art. In some

embodiments, the combination of microRNAs are delivered to the cell by a nanoparticle, cell-permeating peptide, polymer, liposome, or recombinant expression vector.

In some embodiments, one or more genes encoding the microRNAs associated with the invention is expressed in a recombinant expression vector. As used herein, a “vector” may be any of a number of nucleic acids into which a desired sequence or sequences may be inserted by restriction digestion and ligation (*e.g.*, using the CombiGEM method) or by recombination for transport between different genetic environments or for expression in a host cell (*e.g.*, a cancer cell). Vectors are typically composed of DNA, although RNA vectors are also available. Vectors include, but are not limited to: plasmids, fosmids, plagemids, virus genomes, and artificial chromosomes. In some embodiments, the vector is a lentiviral vector. In some embodiments, each of the genes encoding the combination of two or three microRNAs are expressed on the same recombinant expression vector. In some embodiments, the genes encoding the combination of two or three microRNAs are expressed on two recombinant expression vectors. In some embodiments, the genes encoding the combination of three microRNAs are expressed on three recombinant expression vectors.

A recombinant expression vector is one into which a desired DNA sequence may be inserted by restriction digestion and ligation or recombination such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (*e.g.*, galactosidase, fluorescence, luciferase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (*e.g.*, green fluorescent protein, red fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

As used herein, a coding sequence and regulatory sequences are said to be “operably” joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5’

regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript can be translated into the desired protein or polypeptide.

When the nucleic acid molecule is expressed in a cell, a variety of transcription control sequences (e.g., promoter/enhancer sequences) can be used to direct its expression. The promoter can be a native promoter, *i.e.*, the promoter of the gene in its endogenous context, which provides normal regulation of expression of the gene. In some embodiments the promoter can be constitutive, *i.e.*, the promoter is unregulated allowing for continual transcription of its associated gene. A variety of conditional promoters also can be used, such as promoters controlled by the presence or absence of a molecule. In some embodiments, the promoter is a human cytomegalovirus promoter (CMVp).

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. In particular, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene. Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

Recombinant expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, *e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Fourth Edition, Cold Spring Harbor Laboratory Press, 2012. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA). That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

A nucleic acid molecule associated with the invention can be introduced into a cell or cells using methods and techniques that are standard in the art. For example, nucleic acid molecules can be introduced by standard protocols such as transformation including chemical transformation and electroporation, viral transduction, particle bombardment, etc. In some
5 embodiments, the recombinant expression vector is introduced by viral transduction. In some
embodiments, the viral transduction is achieved using a lentivirus. Expressing the nucleic acid molecule may also be accomplished by integrating the nucleic acid molecule into the genome.

Also disclosed herein are methods for determining a synergistic or antagonistic
10 interaction by calculating a genetic interaction score for each combination of microRNAs
(see Example and Figures 5D-5F, 15 and 16). An expected phenotype value can also be calculated for each combination of microRNAs, for example using the additive model or the multiplicative model. The genetic interaction score for the combination of microRNAs may be compared with the expected phenotype value for the combination of microRNAs. A
15 genetic interaction score greater than the expected phenotype value indicates a synergistic
interaction between the microRNAs of the combination. A genetic interaction score less than the expected phenotype value indicates an antagonistic interaction between the microRNAs of the combination. Methods for calculating the genetic interaction score will be evident to one of skill in the art (see, for example, Bassik et al. *Cell* (2013) 152(4): 909-22 and
20 Kampmann et al *PNAS* (2013) 110(25) E2317-26.

EXAMPLE

Massively Parallel High-Order Combinatorial Genetics in Human Cells

25 *Combinatorial Genetics En Masse (CombiGEM) for Human Systems*

To address the limitations of conventional methods for generating high-order combinatorial libraries for high-throughput screening, a technology was developed for the scalable pooled assembly of barcoded high-order combinatorial genetic libraries for human cells. This approach, referred to as Combinatorial Genetics *En Masse* (CombiGEM), enables
30 high-throughput tracking of the barcoded combinatorial populations with next-generation sequencing (Figure 1). CombiGEM uses an iterative cloning strategy starting with an insert library of barcoded DNA elements. Restriction digestion of pooled insert libraries and the

destination vector, followed by a one-pot ligation reaction, create a library of genetic combinations. The newly produced combinatorial library and the same insert pool can be combined to generate higher-order combinations with concatenated barcodes that are unique for each combination, thus enabling tracking using high-throughput sequencing.

5 The final barcoded combinatorial genetic libraries were encoded in lentiviruses to enable efficient delivery and stable genomic integration in a wide range of human cell types. Lentiviral

vectors have been widely used to deliver pooled libraries for large-scale genetic screening (Johannessen et al. *Nature* (2013) 504, 138-142; Koike-Yusa et al. *Nat. Biotechnol.* (2014)

10 32,267-273; Shalem et al. *Science* (2014) 343, 84-87; Wang et al. *Science* (2014) 343, 80-84; Bassik et al. *Cell* (2013) 152, 909-922). After delivering combinatorial libraries into human

cells, pooled assays were performed and genomic DNA was extracted for unbiased amplification of the integrated barcodes. Illumina HiSeq sequencing was used to quantify the abundances of the contiguous DNA barcode sequences, which represent each genetic

15 combination within the pooled populations, and to identify shifts in representation of each combination under the different experimental conditions. The CombiGEM strategy was applied to identify genetic combinations (miRNAs in this study) that sensitize cancer cells to drugs and/or inhibit cancer cell proliferation, with the ultimate goal to validate novel and promising combinatorial effectors for anti-cancer treatment.

20 *Combinatorial miRNA Expression System*

Previous work showed that multiple miRNAs can be expressed by arranging their precursor sequences in tandem (Yoo et al. *Nature* (2011) 476, 228-231). The lentiviral vector was confirmed to express combinatorial sets of functional miRNAs. Lentiviral vectors were

25 generated to encode miRNA precursors cloned downstream of a green fluorescent protein (GFP) gene to monitor expression from the cytomegalovirus (CMVp) promoter (Figure 7A). In addition, miRNA sensor sequences, which are targeted by their cognate miRNAs (Brown et al. *Nat. Rev. Genet.* (2009) 10, 578-585), were added to the 3' untranslated region of a red fluorescent protein (RFP) gene driven by the ubiquitin C (UBCp) promoter in order to report

30 on miRNA activity (Figure 7A). The miRNA expression and sensor cassettes were placed in a single vector to ensure constant ratios between the two components in infected cells.

Efficient delivery of lentiviral vectors into human embryonic kidney cells (HEK293T; Figure 8) and human dermal fibroblasts was confirmed (data not shown).

It was anticipated that active miRNAs would target their sensor sequences, thus reducing RFP fluorescence levels. Flow cytometry analysis showed that cells expressing miRNAs but without sensors produced both GFP and RFP, whereas those cells expressing miRNAs and harboring cognate sensors lost RFP fluorescence, indicating repression by miRNAs (Figure 7B). In addition, distinct two-wise and three-wise miRNA combinations exhibited repression activities (Figure 7C) comparable to their respective one-wise individual miRNA constructs (Figure 7B). This effect did not result from cross-reactivity between the miRNAs and non-cognate sensors (Figure 7D). These results demonstrate the ability of lentiviral vectors to encode combinatorial miRNA expression in human cells.

Generation of High-Coverage Combinatorial miRNA Libraries

Given the high efficiency of gene repression achieved by the lentiviral combinatorial miRNA expression system, high-coverage barcoded combinatorial miRNA libraries were generated. The goal of these studies was to systematically evaluate the combinatorial effects of miRNA overexpression on anti-cancer phenotypes since rational combination therapy can enhance therapeutic efficacy (Al-Lazikani et al. *Nat. Biotechnol.* (2012) 30, 679-692) and miRNA-based therapeutics have been shown to be effective in various animal models and are in preclinical and clinical development (Li et al. *Nat. Rev. Drug Discov.* (2014) 13, 622-638). To build the libraries, 39 miRNAs were selected that were previously reported to be down-regulated in drug-resistant cancer cells or exhibited altered expression in ovarian cancer cells (Tables 1 and 2). The expression of these 39 miRNAs in human ovarian cancer (OVCAR8) cells and its drug-resistant derivative OVCAR8-ADR cells (Patnaik et al. *PLoS One* (2012) 7) was previously shown in miRNA profiling studies (Creighton et al. *Breast Cancer Res.* (2010) 12, R40; Gholami et al. *Cell Rep.* (2008) 4, 609-620; Hsu et al. *Nucleic Acid Res.* (2014) 42, D78-85). Using ProteomicsDB (Honma et al. *Nat. Med.* (2008) 14, 939-948), it was found that at least ~60% (2716 out of 4532) of the experimentally validated targets of these 39 miRNAs, which were retrieved from miRTarBase (Strezoska et al. *PLoS One* (2012) 7, e42341) are expressed in OVCAR8-ADR cells. A barcoded library comprising the 39 miRNA precursor sequences was first cloned into storage vectors. Using CombiGEM, two-wise (39 x 39 miRNAs = 1,521 total combinations) and three-wise (39 x 39 x 39 miRNAs =

59,319 total combinations) pooled miRNA libraries were generated in just two subsequent steps (Figure 1).

Specifically, a library of barcoded miRNAs was first cloned into storage vectors with BamHI and EcoRI sites in between the miRNA sequences and the barcode sequences and BglII and MfeI sites at the ends (Figure 1). To construct the one-wise library, pooled inserts were generated by digestion of the pooled storage vectors with BglII and MfeI. The lentiviral destination vector was digested with BamHI and EcoRI. The digested sites on the inserts and lentiviral vectors had compatible sticky ends (i.e., BamHI + BglII & EcoRI + MfeI) and were ligated in a single-pot reaction to generate a pooled one-wise library. The vector library was then digested again in pooled format at the BamHI and EcoRI sites located in between the miRNAs and their barcodes, and then ligated with the same pooled inserts to generate two-wise and three-wise libraries. This process can be iteratively repeated to generate progressively more complex libraries with all the respective barcodes localized at one end.

Lentiviral pools were then produced to deliver the combinatorial libraries into human cells. To facilitate single-copy lentiviral integration in most infected cells, lentiviruses were titrated to a multiplicity of infection (MOI) of about 0.3 to 0.5. To ensure high-quality screens with high-coverage libraries containing a significant representation for most combinations (Bhattacharya et al. *Cancer Res.* (2009) 69, 9090-9095), ~300-fold more cells for lentiviral infection were used than the size of the combinatorial library being tested.

Thus, any spurious phenotype resulting from any given random integrant should be diminished by averaging over the population.

Genomic DNA from pooled populations was isolated for barcode amplification by polymerase chain reaction (PCR). The PCR conditions were optimized to achieve unbiased amplification in order to ensure accurate quantification of the barcodes (Figure 9). Illumina HiSeq sequencing was then used to quantify the representation of individual barcoded combinations in the plasmid pools stored in *Escherichia coli* and also the infected human cell pools (Figure 2A and 2B). Full coverage for the two-wise library within both the plasmid and infected cell pools was achieved from ~5-10 million reads per sample (Figure 2A). Ten two-wise combinations composed of the miR-16-1/15a cluster paired with 10 different miRNAs were found to be under-represented in infected cells relative to the plasmid pool (highlighted in light gray in Figure 2C). This observation may be consistent with reports indicating that miR-16-1 or miR-15a overexpression can inhibit clonal growth and

proliferation in ovarian cancer cells (Cheng et al. *Proc. Natl. Acad. Sci.* (2014) 111, 12462-12467).

In addition, high coverage of the three-wise library within the plasmid and infected cell pools (~89% and ~87%, respectively) was achieved with ~30 million reads per sample (Figure 2B). It was previously demonstrated that greater library coverage could be achieved by scaling up library transformations and increasing the number of sequencing reads per sample (Xia et al. *Int. J. Cancer* (2008) 123, 372-379). Such efforts could help to increase coverage of the missing three-wise combinations (~11% of the total expected combinations) in the plasmid library. A small set (~2%) of three-wise combinations were detected that were present in the plasmid library, but were absent in the infected cell pools. These combinations could have been lost due to low representation in the plasmid library or inhibitory effects on cell survival/proliferation. Furthermore, high correlations were observed between barcodes represented in the plasmid and infected cell pools (Figures 2C and 2D), as well as high reproducibility in barcode representation between biological replicates within infected cell pools (Figures 2E and 2F). Thus, CombiGEM can be used to efficiently assemble and deliver high-order combinatorial genetic libraries into human cells.

High-Throughput Two-Wise Combinatorial Screen

To identify combinatorial miRNAs that modify chemotherapy drug sensitivity, OVCAR8-ADR cells were infected with the two-wise barcoded combinatorial miRNA library (Figure 3A). One half of the pooled population was treated with the chemotherapeutic drug docetaxel while the other half was exposed to vehicle control. After four days, genomic DNA was isolated from both cell populations for unbiased amplification and quantification of the integrated barcodes. Comparison of the barcode abundances (normalized per million reads) between the drug-treated and control groups yielded $\log_2$ (barcode count ratios) values, which were used as a measure of drug sensitivity. Cells with miRNA combinations conferring enhanced drug resistance or sensitivity were expected to have positive or negative $\log_2$ ratios, respectively. This screen was performed in duplicate, and high reproducibility of barcode representation was observed between biological replicates (Pearson correlation coefficient > 0.95) (Figure 10A). To reduce variability, combinations with less than ~100 absolute reads in the control group were filtered out, and the $\log_2$ ratios of the two potential arrangements for each miRNA pair were averaged (i.e., for two-wise miRNA combinations:

miR-A + miR-B and miR-B + miR-A) (Figure 11). The miRNA combinations were then ranked based on their mean log₂ ratios from two biological replicates (Figure 3B and Figure 12). Twenty-four two-wise miRNA combinations were defined as hits for drug sensitizers (log₂ ratio < -0.42; i.e. with >25% fewer barcode counts in docetaxel-treated cells compared to control) (Table 3), while 36 combinations were considered as hits for enhancing docetaxel resistance (log₂ ratio > 0.32; i.e. with > 25% more barcode counts in docetaxel-treated cells relative to control) (Table 4).

The drug-sensitizing or resistance-enhancing effects of selected miRNA pairs from these hits were confirmed with individual drug sensitivity assays. It was further revealed that miRNA combinations could enhance drug sensitivity over their individual components. Previous work has shown that expression of the *miR-16/15* precursor family sensitized drug-resistant gastric cancer cells to chemotherapeutic drugs (Kastl et al. *Breast Cancer Res. Treat.* (2012) 131, 445-454). In line with this finding, it was found that expression of the *miR-16-1/15a* cluster increased docetaxel sensitivity in OVCAR8-ADR cells, resulting in a ~10-20% decrease in cell viability when co-applied with docetaxel compared to the vector control (Figure 3C). Intriguingly, the drug sensitization effect of the *miR-16-1/15a* cluster approximately doubled when it was expressed in combination with the *miR-93/106b* cluster or *miR-376a*. The *miR-93/106b* cluster or *miR-376a* on their own only slightly altered docetaxel sensitivity and resulted in less than ~5-10% reductions in cell viability when co-administered with docetaxel (Figure 3C). When the *miR-16-1/15a* cluster was combined with the *miR-93/106b* cluster or *miR-376a*, the half maximal inhibitory concentration (IC₅₀) of docetaxel was reduced by ~2-fold (Figure 3D), resulting in nearly comparable killing to the parental OVCAR8 cells treated with the same drug dose (Figure 13). These results demonstrate the ability of CombiGEM to identify effective combinatorial miRNAs for sensitizing drug-resistant cancer cells to chemotherapy.

MicroRNA combinations that enhanced docetaxel resistance in OVCAR8-ADR cells were also evaluated. It has been demonstrated that overexpression of *miR-34a* conferred docetaxel resistance in breast cancer cells (Krek et al. *Nat. Genet.* (2005) 37, 495-500). Consistent with this observation, *miR-34a* was frequently represented in combinations that showed increased docetaxel resistance in OVCAR-ADR cells (23 out of 36 combinations) (Table 4). It was confirmed that cells expressing *miR-34a* in combination with the *miR-199b/3154* cluster, *miR-328*, or *miR-429* developed profound resistance towards 25 nM of

docetaxel treatment, resulting in increased cell viability by ~1.6 to 1.9-fold in the presence of drug when compared to the vector control (Figure 3E). Elevated resistance resulted from interactions between *miR-34a* and each of these three additional miRNAs, since *miR-34a* expression only slightly enhanced docetaxel resistance by ~1.3-fold while expression of the *miR-199b/3154* cluster, *miR-328*, or *miR-429* on their own did not significantly affect docetaxel sensitivity (Figure 3E). These results thus support a central role for *miR-34a* overexpression in increasing docetaxel resistance in OVCAR8-ADR cells and demonstrate that *miR-34a* can act in concert with other miRNAs to regulate important cellular phenotypes. In summary, an experimental pipeline was established and validated for the systematic screening of barcoded two-wise miRNA combinations that modulate biological phenotypes.

Massively Parallel Three-Wise Combinatorial Screens

High-throughput genetic screens with higher-order combinatorial libraries were performed to demonstrate the scalability of the CombiGEM approach (Figure 4A). OVCAR-ADR cells were infected with the three-wise barcoded combinatorial miRNA library. Using the same experimental procedures as for the two-wise screen described in Example 2, a massively parallel pooled screen was conducted to isolate three-wise miRNA combinations that modulated docetaxel sensitivity. The infected cell pool was split in half, and treated with docetaxel or vehicle control for four days. Integrated barcodes were then PCR amplified and quantified. Highly reproducible barcode representation was observed between two biological replicates in both experimental conditions (Pearson correlation coefficient > 0.97) (Figure 10B). To measure drug sensitivity, the log₂ ratio was determined between the normalized barcode reads in the treatment versus control groups (Figures 11 and 12). MicroRNA combinations were ranked based on their mean log₂ ratios from the two replicates (Figure 4B). Ninety-one and 36 three-wise miRNA combinations were identified as drug-sensitizing (Table 5) and resistance-enhancing (Table 6) hits with docetaxel treatment, respectively (log₂ ratio < -0.42 or > 0.32). The effects of select three-wise miRNA combinations were confirmed with individual drug sensitivity assays (Figure 4C). For example, expression of the *miR-16-1/15a* cluster, *miR-181c*, and the *let-7e/miR-99b* cluster together led to a ~2-fold reduction in the IC₅₀ of docetaxel compared with untreated cells.

Using the same three-wise combinatorial miRNA library and experimental pipeline, the effect of combinatorial miRNAs on cancer cell proliferation was systematically evaluated

(Figure 4A). OVCAR8-ADR cells infected with the three-wise combinatorial miRNA library were cultured for one or four days, and each integrated barcode was quantified to obtain a $\log_2$ ratio between its abundance at day four versus day one. MicroRNA combinations conferring a growth advantage were expected to have positive $\log_2$ ratios, while miRNA combinations inhibiting cell proliferation were expected to yield negative $\log_2$ ratios. $\log_2$ ratios for each miRNA combination were averaged from two biological replicates (Figures 11 and 12) and ranked (Figure 4D). Twenty-seven miRNA combinations were shown to exert considerable anti-proliferative effects ($\log_2$ ratio < -0.42) (Table 7). These three-wise miRNA hits were validated by demonstrating the ability of each combination to inhibit the growth of OVCAR8-ADR cells in individual cell proliferation assays (Figure 4E). For example, the three-wise expression of the *miR-16-1/15a* cluster, *miR-128b*, and the *let-7e/miR-99b* cluster led to a large reduction in cell growth (i.e. $> 55\%$ decrease in viable cell numbers on Day 7). Collectively, these results demonstrate that CombiGEM enables the identification of high-order miRNA combinations that are capable of achieving drug sensitization and anti-proliferative effects.

MicroRNA Interactions Control Anti-Cancer Phenotypes

Combining the high-throughput screening data for drug sensitization and inhibition of cell proliferation, miRNA combinations were profiled based on their ability to modulate drug resistance as well as cancer cell growth (Figure 14). Using the $\log_2$ ratios from the screens as indices for drug sensitivity and proliferation, a two-dimensional heat map (Figure 5A) and three-dimensional plots were constructed (Figures 5B, 5C and 14) presenting docetaxel sensitivity and cell proliferation phenotypes conferred by the two-wise and three-wise miRNA combinations, respectively. Hierarchical clustering was carried out to group miRNA combinations that shared similar drug sensitization profiles together in order to enhance visualization.

These plots revealed insights into previously unexamined roles that combinatorial miRNAs play in modulating drug resistance and cell growth phenotypes. For instance, most two wise and three-wise combinations that contained *miR-34a* conferred cellular resistance against docetaxel and anti-proliferative effects (Figures 5A-C). In addition, many combinations encoding miRNAs such as the *miR-16-1/15a* cluster or the *miR-15b/16-2*

cluster sensitized cells to docetaxel (Figures 5A and 5B), while exerting differential effects on proliferation (Figure 5C).

Genetic interaction (GI) scores were defined for each two-wise and three-wise combinations using a previously described scoring system (Bassik et al. *Cell* (2013) 152, 909-922). Generally, combinations that exhibited stronger phenotypes than predicted via the additive effect of individual phenotypes were defined as synergistic, whereas combinations with weaker than expected phenotypes based on an additive model were defined as buffering (see *Materials and Methods* and Figure 15). GI maps encompassing GI scores of all two-wise miRNA interactions were constructed (Figure 5A) and revealed that our validated two-wise miRNA combinations showed synergy based on their GI scores. For example, synergistic phenotype-modifying effects on *miR-34a* by the *miR-199b/3154* cluster to enhance drug resistance were observed (Figure 5D). Furthermore, a synergistic effect on the *miR-16-1/15a* cluster by the *miR-93/106b* cluster to increase drug sensitization was detected (Figure 5D). GI scores were further computed for the three-wise miRNA combinations and found that the addition of a third miRNA element could interact with two-wise miRNA combinations to modify biological phenotypes (Figures 5E, 5F and 16).

MicroRNA Combinations with Both Drug-Sensitizing and Anti-Proliferation Phenotypes

Combining the high-throughput screening data for drug sensitization and inhibition of cell proliferation, miRNA combinations were profiled based on their ability to modulate both drug resistance and cancer cell growth (Figure 17). The drug sensitization and anti-proliferation effects of three-wise miRNA combinations were compared with their respective single and two-wise combinations in individual drug sensitivity and cell proliferation assays (Figures 6A-6J). It was found that the expression of the *miR-16-1/15a* cluster alone or together with the *let-7e/miR-99b* cluster resulted in slight sensitization of cells to docetaxel and reduced cell viability by <10% when co-administrated with drug (Figures 6A-6C). This docetaxel sensitization was increased by ~2-fold in cells co-expressing the *miR-16-1/15a* cluster, the *let-7e/miR-99b* cluster, and the *miR-15b/16-2* (Figure 6C). In the absence of the *miR-16-1/15a* precursor family, miRNAs such as the *let-7e/miR-99b* cluster, *miR-128b*, *miR-181c*, and *miR-132* by themselves and many of their respective paired combination did not exhibit docetaxel-sensitizing phenotypes (Figures 6A-6F). These results therefore demonstrate that the *miR-16/15* precursor family plays a critical role within miRNA

combinations in promoting docetaxel sensitization, and that its sensitizing capacity can be modulated by the co-expression of specific miRNA partners.

The results also identified interacting miRNAs that regulate cancer cell growth. It was found that *miR-181c* expression inhibited cancer cell growth by ~30% and that this anti-proliferative effect was potentiated to ~50-60% when *miR-181c* was expressed in combination with the *let-7e/miR-99b* cluster (Figure 6B), *let-7i*, or *miR-373* (Figure 6H), even though these miRNAs did not inhibit cell proliferation on their own. Furthermore, the three-wise expression of the *miR-16-1/15a* cluster, the *let-7e/miR-99b* cluster, and *miR-128b* resulted in >2.5-fold increase in anti-proliferative effects compared to when they were expressed individually and in pairs (Figure 6A). Table 10 presents additional three-wise miRNA combinations that both inhibit cell proliferation and increase docetaxel sensitivity in OVCAR8-ADR cells based on pooled screening.

Via these analyses, miRNA combination that could modulate both drug-sensitization and cell-growth phenotypes were identified and validated. These miRNA combinations may serve as candidates for novel anti-cancer therapeutics. For example, the integrated docetaxel-sensitizing and anti-proliferative functions of the *miR-16-1/15a* cluster, the *let-7e/miR-99b* cluster, and *miR-128b* together (Figure 6A) led to significantly enhanced killed of drug-resistant OVCAR8-ADR cells with docetaxel, and resulted in a reduction of >90% in viable cells compared to the vector control group (Figure 6I). This three-wise combination greatly reduced the ability of treated OVCAR8-ADR cells to form viable colonies after drug treatment by ~99.5% (Figures 6J and 18), thus highlighting the potential for using combinatorial miRNAs as a novel form of effective therapeutic agents.

Methods

Construction of Combinatorial miRNA Expression and Sensor Vectors

The vectors used (Table 8) were constructed using standard molecular cloning techniques, including PCR, restriction enzyme digestion, ligation, and Gibson assembly. Custom oligonucleotides and gene fragments were purchased from Integrated DNA Technologies and GenScript. The vector constructs were transformed into *E. coli* strain DH5 α , and 50 μ g/ml of carbenicillin (Teknova) was used to isolate colonies harboring the constructs. DNA was extracted and purified using Qiagen Plasmid Mini or Midi Kit

(Qiagen). Sequences of the vector constructs were verified with Genewiz's DNA sequencing service.

To create a lentiviral vector for expression of dual fluorescent protein reporters (pAWp7; pFUGW-UBCp-RFP-CMVp-GFP), turboRFP (Addgene #31779) (Yoo et al. *Nature* (2011) 476, 228-231), and CMV promoter sequences were amplified by PCR using Phusion DNA polymerase (New England Biolabs) and cloned into the pAWp6 vector backbone (pFUGW-UBCp-GFP) using Gibson Assembly Master Mix (New England Biolabs). To express miRNAs, miRNA precursor sequences of miR-124 (Addgene #31779) (Yoo et al. *Nature* (2011) 476, 228-231), *miR-128* (Bruno et al. *Mol. Cell* (2011) 42, 500-510), and *miR-132* (Klein et al. *Nat. Neurosci.* (2007) 10, 1513-1514) were amplified by PCR and cloned downstream of the GFP sequence in pAWp7 vector using Gibson assembly. During PCR, four restriction digestion sites (BglII, BamHI, EcoRI and MfeI) were added to flank the miRNA precursor sequences, resulting in a BglII-BamHI-EcoRI-miRNA precursor-MfeI configuration that facilitated cloning of additional miRNA precursors for generating combinatorial miRNA expression cassettes. To construct two-wise miRNA precursor expression cassettes, the single miRNA precursor expression vectors were digested with BamHI and EcoRI (Thermo Scientific), and ligated using T4 DNA ligase (New England Biolabs) with the compatible sticky ends of the miRNA precursor inserts prepared from digestion of the respective PCR product with BglII and MfeI (Thermo Scientific). Likewise, three-wise miRNA precursor expression cassettes were built by ligating the BglII- and MfeI-digested two-wise miRNA precursor expression vectors with BamHI- and EcoRI- digested miRNA precursor inserts. To report on miRNA activities, miRNA sensors harboring four tandem repeats of the reverse-complemented sequences of the mature miRNAs were amplified by PCR from synthesized gene fragments, and inserted via a SbfI cleavage site into the 3' UTR of RFP of pAWp7 or the combinatorial miRNA precursor expression vectors using Gibson assembly.

Creation of the Barcoded Single miRNA Precursor Library

Each of the 39 miRNA precursor sequences (with lengths of ~261-641 base pairs) was amplified from human genomic DNA (Promega) as described previously (Voorhoeve et al. *Cell* (2007) 131, 102-114) by PCR using Phusion (New England Biolabs) or Kapa HiFi (Kapa Biosystems) DNA polymerases and primers listed in Table 1. Eight-base pair barcodes

unique to each miRNA precursor were added during PCR. The barcode sequences differed from each other by at least two bases. In addition, restriction enzyme sites BglII and MfeI were added to flank the ends, and cleavage sites BamHI and EcoRI were introduced in between the miRNA precursor and the barcode sequences. Each PCR product herein was thus configured as BglII-miRNA precursor-BamHI-EcoRI-Barcode-MfeI. The PCR product of each barcoded miRNA precursor was then ligated into the pBT264 storage vector (Addgene #27428)⁵⁷ using sites BglII and MfeI.

Pooled Combinatorial miRNA Library Assembly for High-Throughput Screening

Storage vectors harboring the 39 barcoded miRNA precursors were mixed at equal molar ratios. Pooled inserts were generated by single-pot digestion of the pooled storage vectors with BglII and MfeI. The destination lentiviral vector (pAWp11; modified from the pAWp7 vector) was digested with BamHI and EcoRI. The digested inserts and vectors were ligated via their compatible sticky ends (i.e., BamHI + BglII & EcoRI + MfeI) to create a pooled one-wise miRNA precursor library in lentiviral vector. The one-wise miRNA precursor vector library was digested again with BamHI and EcoRI, and ligated with the same 39 miRNA precursor insert pool to assemble the two-wise miRNA precursor library (39×39 miRNAs = 1,521 total combinations). Ligation was performed with the BamHI- and EcoRI- digested two-wise miRNA precursor vector library and the same pooled inserts to generate the three-wise miRNA precursor library ($39 \times 39 \times 39$ miRNAs = 59,319 total combinations). After each pooled assembly step, the miRNA precursors were localized to one end of the vector construct and their respective barcodes were concatenated at the other end.

Generation of Combinatorial miRNA vectors for Individual Validation Assays

Lentiviral vectors harboring single, two-wise, or three-wise miRNA precursors were constructed with same strategy as for the generation of combinatorial miRNA libraries described above, except that the assembly was performed with individual inserts and vectors, instead of pooled ones.

Human Cell Culture

HEK293T and MCF7 cells were obtained from ATCC. T1074 cells were obtained from Applied Biological Materials. HOSE 11-12 and HOSE 17-1 cells were obtained from G. S. W. Tsao (University of Hong Kong, Hong Kong). OVCAR8 and OVCAR8-ADR cells were previously described (Gaj et al. *Trends Biotechnol.* (2013) 31, 397-405; Patnaik et al. *PLoS One* (2007) 7). The identity of the OVCAR8-ADR cells was confirmed by a cell line authentication test (Genetica DNA Laboratories). HEK293T cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum and 1X antibiotic-antimycotic (Life Technologies) at 37°C with 5% CO₂. MCF7, T1074, HOSE 11-12, HOSE 17-1, OVCAR8, and OVCAR8-ADR cells were cultured in RPMI supplemented with 10% heat-inactivated fetal bovine serum and 1X antibiotic-antimycotic (Life Technologies) at 37°C with 5% CO₂. For drug sensitivity assays, docetaxel (LC Laboratories) or vehicle control was added to the cell cultures at indicated doses and time periods.

Lentivirus Production and Transduction

Lentiviruses were produced in 6-well plates with 250,000 HEK293T cells per well. Cells were transfected using FuGENE HD transfection reagents (Promega) with 0.5 µg of lentiviral vector, 1 µg of pCMV-dR8.2-dvpr vector, and 0.5 µg of pCMV-VSV-G vector mixed in 100 µl of OptiMEM medium (Life Technologies) for 10 minutes. The medium was replaced with fresh culture medium one day after transfection. Viral supernatants were then collected every 24 hours between 48 to 96 hours after transfection, pooled together, and filtered through a 0.45 µm polyethersulfone membrane (Pall). For transduction with individual vector constructs, 500 µl filtered viral supernatant was used to infect 250,000 cells in the presence of 8 µg/ml polybrene (Sigma) overnight. For transduction with pooled libraries, lentivirus production was scaled up using the same experimental conditions. Filtered viral supernatant was concentrated at 50-fold using an Amicon Ultra Centrifugal Filter Unit (Millipore) and used to infect a starting cell population containing ~300-fold more cells than the library size to be tested. MOIs of 0.3 to 0.5 were used to give an infection efficiency of about 30 to 40% in the presence of 8 µg/ml polybrene. Cells were washed with fresh culture medium one day after infection, and cultured for three more days prior to experiments.

Sample Preparation for Barcode Sequencing

For the combinatorial miRNA vector libraries, plasmid DNA was extracted from *E. coli* transformed with the vector library using the Qiagen Plasmid Mini kit (Qiagen). For the human cell pools infected with the combinatorial miRNA libraries, genomic DNA of cells collected from various experimental conditions was extracted using DNeasy Blood & Tissue Kit (Qiagen). DNA concentrations were measured by Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies).

PCR amplification of a 359 base-pair fragment containing unique CombiGEM barcodes representing each combination within the pooled vector and infected cell libraries was performed using Kapa HiFi Hotstart Ready-mix (Kapa Biosystems). During the PCR, each sample had Illumina anchor sequences and an 8 base-pair indexing barcode for multiplexed sequencing added. The forward and reverse primers used were 5'-

AATGATACGGCGACCACCGAGATC

TACACGGATCCGCAACGGAATTC-3' (SEQ ID NO:1) and 5'-

CAAGCAGAAGACGGCAT

ACGAGATNNNNNNNNGGTTGCGTCAGCAAACACAG-3' (SEQ ID NO:2), where NNNNNNNN denotes a specific indexing barcode assigned for each experimental sample.

0.5 ng of plasmid DNA was added as template in a 12.5 µl PCR reaction, while 800 ng of genomic DNA was used per 50-µl PCR reaction. Eight and 80 PCR reactions were performed for human cell pools infected with two-wise and three-wise miRNA library respectively to reach at least 50-fold representation for each combination. To prevent PCR bias that would skew the population distribution, PCR conditions were optimized to ensure the amplification occurred during the exponential phase. PCR products were run on a 1.5% agarose gel, and the 359 basepair fragment was isolated using QIAquick Gel Extraction Kit (Qiagen). Concentrations of the PCR products were determined by quantitative PCR using

Kapa SYBR Fast qPCR Master Mix (Kapa Biosystems) with a Mastercycler Ep Realplex machine (Eppendorf). Forward and reverse primers used for quantitative PCR were 5'-

AATGATACGGCGACCACCGA-3' (SEQ ID NO:3) and 5'-

CAAGCAGAAGACGGCATACGA-3' (SEQ ID NO:4), respectively. The quantified PCR products were then pooled at desired ratio for multiplexing samples and run for Illumina

HiSeq using CombiGEM barcode primer (5'-CCACCGAGATCTACACGGATCCGC AACGGAATTC-3' (SEQ ID NO:5)) and indexing barcode primer (5'-

GTGGCGTGGTGTGCA

CTGTGTTTGCTGACGCAACC-3' (SEQ ID NO:6)).

Data Analysis

Screens were performed in two biological replicates with independent infections of the same lentiviral libraries, and the mean $\log_2$ ratio was used as a measure of drug sensitivity or cell proliferation. A majority (78-90%) of combinations showed a small difference (<0.3) of $\log_2$ ratios between biological replicates (Figure 12). Combinations were ranked by the mean $\log_2$ ratio across all experimental conditions. The set of top hits was defined as a $\log_2$ ratio that was greater than 0.32 or less than -0.42 (with $>25\%$ more or fewer barcode counts in experimental versus control group). Consistent phenotype-modifying effects were observed for the screen hits between biological replicates (Pearson correlation coefficients = 0.636-0.788) (Figure 12). Differences in phenotype-modifying effects measured between independent experiments can result from modest differences in the levels of the toxic selection pressure applied (Kampmann et al. *Proc. Natl. Acad. Sci. U.S.A.* (2013) 110, E2317-2326), as well as the Poisson sampling error from repeated passaging of cells through a population bottleneck (Pierce et al. *Nat. Protoc.* (2007) 2, 2958, 2974). The reproducibility between biological replicates can be improved by increasing the fold representation of cells per combination in the pooled screens (Bassik et al. *Cell* (2013) 152, 909-922). To enhance visualization in the two-dimensional heatmap and three-dimensional plots, hierarchical clustering was performed to group combinations that shared similar $\log_2$ ratio profiles based on Euclidean correlation.

To determine miRNA interactions, a scoring system similar to one previously described for measuring genetic interactions was applied (Bassik et al. *Cell* (2013) 152, 909-922), and genetic interaction (GI) scores for each two- and three- wise combination were calculated. Combinations were grouped based on their GI scores to evaluate the frequency of genetic interactions as shown in the histograms in Figures 5D-5F. In general, combinations that exhibited stronger phenotypes than predicted via the additive effect of individual phenotypes were defined as synergistic, whereas combinations with weaker than expected phenotypes based on an additive model were defined as buffering. The detailed definitions are illustrated below and in Figure 15.

As described above, positive and negative phenotypes had averaged fold changes of normalized barcode reads of > 1 and < 1 respectively, while no phenotypic change resulted in

a fold change = 1. For miRNA [A] and [B] with individual phenotypes “A” and “B”, the expected phenotype for the two-wise combination [A,B] is (“A” + “B” - 1), according to the additive model, where “A” and “B” are calculated based on the median fold changes of normalized barcode reads determined for combinations [A,X] and [B,X] respectively and [X] represents all 39 library members. Similarly, the expected phenotype for three-wise combination [A,B,C] is (“A,B” + “C” - 1), where “A,B” and “C” are the median fold changes of normalized barcode reads determined for combinations [A,B,X] and [C,X,X] respectively and [X] represents all 39 library members.

The GI score of a given two-wise combination was determined as follows (Figure 15):

Definition of Deviation = Observed phenotype – Expected phenotype,

1) If phenotype “A” and “B” are both > 1 and Deviation > 0, the interaction is defined as synergistic. GI score = | Deviation |

2) If phenotype “A” and “B” are both > 1 and Deviation < 0, the interaction is defined as buffering. GI score = - | Deviation |

3) If phenotype “A” and “B” are both < 1 and Deviation > 0, the interaction is defined as buffering. GI score = - | Deviation |

4) If phenotype “A” and “B” are both < 1 and Deviation < 0, the interaction is defined as synergistic. GI score = | Deviation |

5) If phenotype “A” > 1 and “B” < 1, or vice versa, and Observed Phenotype > both “A” and “B”, the interaction is defined as synergistic. GI score = | Deviation |

6) If phenotype “A” > 1 and “B” < 1, or vice versa, and Observed Phenotype < both “A” and “B”, the interaction is defined as synergistic. GI score = | Deviation |

7) If phenotype “A” > 1 and “B” < 1, or vice versa, and Observed Phenotype is neither > both “A” and “B” nor < both “A” and “B”, the interaction is defined as buffering.

GI score = - | Deviation |

The GI score for a given three-wise combination was calculated using the same method. For each three-wise combination, three GI scores were determined for the three possible permutations (i.e. “A,B” + “C”, “A,C” + “B”, “B,C” + “A”). The GI score of “B,A” + “C” was the same as of “A,B” + “C” since the fold changes for different orders of the same pair of miRNAs were averaged as described above. In Figure 5F, the GI scores for all three permutations of the combinations labeled (iii) were 0.296/0.297/0.330.

To determine the significance of GI, the GI scores were Z-score-normalized as previously described⁶¹, and a |Z-score| cut-off value of 2 was considered statistically significant ($P < 0.05$). The GI scores for significant synergistic and buffering interactions were determined to be $> .198$ and < -0.186 respectively for the drug-sensitivity screen with the two-wise miRNA combinations (Figure 5D), > 0.199 and < -0.191 respectively for the drug-sensitivity screen with the three-wise miRNA combinations (Figure 5E), and > 0.146 and < -0.110 respectively for the cell-proliferation screen with the three-wise miRNA combinations (Figure 5F). To generate the GI heatmaps in Figures 5A and 17A-17D, the calculated GI scores for two- and three- wise combinations were displayed in the same order as for the two-dimensional heatmap for easy comparison. Sign epistasis is more difficult to present using current scoring methods⁵⁹. Within the definitions, sign epistasis is referred to as synergistic while reciprocal sign epistasis is classified as buffering. GIs were also formulated for each two- and three- wise combination based on the expected phenotype produced by the multiplicative model^{1,16}, and similar GIs were observed as with the additive model (data not shown). Enhanced utility of GI maps could be achieved by including a one-wise library in the pooled screens to enable comparisons of genetic combinations with their single-gene constituents and by increasing the representation of each genetic combination to minimize potential errors due to limited sample sizes.

Flow Cytometry

Four days post-infection, cells were washed and resuspended with 1X PBS supplemented with 2% heat-inactivated fetal bovine serum, and assayed with a LSRII Fortessa flow cytometer (Becton Dickinson). Cells were gated on forward and side scatter. At least 20,000 cells were recorded per sample in each data set.

Fluorescence Microscopy

To visualize GFP and RFP, cells were directly observed under an inverted fluorescence microscope (Zeiss) after four days post-infection.

Cell Viability Assays

For the MTT assay, 100 μ l of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (Sigma) was added to the cell cultures in 96-well

plates and incubated at 37°C with 5% CO₂ for 2 hours. Viable cells convert the soluble MTT salt to insoluble blue formazan crystals. Formazan crystals formed were dissolved with 100 µl of solubilization buffer at 37°C. The absorbance of the solubilized formazan was measured at an optical density (OD) of 570 nm (along with the reference OD at 650 nm) using a Synergy H1 Microplate Reader (BioTek). For the trypan blue exclusion assay, cells were trypsinized and stained with 0.4% trypan blue dye solution (Sigma). Viable cells were counted in four different fields of a hemacytometer under microscopy.

Colony Formation Assay

10,000 cells were plated in 96-well plates and treated with 25 nM of docetaxel. Cells were trypsinized and transferred to 6-well plates. After eleven days, cells were fixed in ice-cold 100% methanol for 10 minutes, and stained with crystal violet solution for 20 minutes. The colony area percentage and number of colonies in each sample were determined using ImageJ software.

RNA extraction and quantitative RT-PCR (qRT-PCR)

RNA was extracted from cells using TRIzol Plus RNA Purification Kit (Invitrogen) and treated with DNase using PureLink DNase Set (Invitrogen), according to the manufacturer's protocols and quantified using a Nanodrop Spectrophotometer. RNA samples were reverse-transcribed using GoScript Reverse Transcriptase (Promega), Random Primer Mix (New England Biolabs) and RNase OUT (Invitrogen). qRT-PCR was performed on the LightCycler480 system (Roche) using SYBR FAST qPCR Master Mix (KAPA). LightCycler 480 SW 1.1 was used for TM curves evaluation and quantification. PCR primers are listed in Table 9.

Table 1: List of Candidate miRNAs and Primers Used for Barcoded Library Cloning

miRNA precursor	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>let-7a</i>	CTCTAGAGATCTGGAGCGGAT TCAGATAACCAAGC (SEQ ID NO:7)	ATCGCAATTGTCGCGTTTGAATTCCGTTGCG GATCCGGTTTCCCCACCCCATCCAGTG (SEQ ID NO:8)
<i>let-7b/miR-4763</i> cluster	CTCTAGAGATCTGGCAGACAG TGGCTCCTCTGTACC (SEQ ID NO:9)	ATCGCAATTGATGACCTTGAATTCCGTTGCG GATCCCACTGTCCCGCAGCAGACGCGC (SEQ ID NO:10)
<i>let-7c</i>	CTCTAGAGATCTGGCAGGTTA GATGGTCAGAAGAC (SEQ ID NO:11)	ATCGCAATTGCGTTCTGTGAATTCCGTTGCG GATCCCTCGACGGCTCAAGTGTGCTCCA (SEQ ID NO:12)
<i>let-7d</i>	CTCTAGAGATCTCAGGTAAAT	ATCGCAATTGCGAAAGGTGAATTCCGTTGC

	TTGAAGTGCATCTGCC (SEQ ID NO:13)	GGATCCGAGGAGGAACAGCTGAGAGTCTC (SEQ ID NO:14)
<i>let-7e/miR-99b</i> cluster	CTCTAGAGATCTGGCTGAGTC CTTGGATTCCAGGAAC (SEQ ID NO:15)	ATCGCAATTGCTCCTTCGGAATTCCGTTGCG GATCCGAAAGCTAGGAGGCCGTATAGTG (SEQ ID NO:16)
<i>let-7i</i>	CTCTAGAGATCTCACCCGGGC GCCGGCGCCGCCTC (SEQ ID NO:17)	ATCGCAATTGTTGGCAGTGAATTCCGTTGC GGATCCGCATCGCGGTCCACCCCACTC (SEQ ID NO:18)
<i>miR-10b</i>	CTCTAGAGATCTAGAAGAATA TTCTGGTTGTTTCGCCTG (SEQ ID NO:19)	ATCGCAATTGTATGACGTGAATTCCGTTGC GGATCCAGGATACTCTGTTTAAAGGTGAGG (SEQ ID NO:20)
<i>miR-126</i>	CTCTAGAGATCTGGAAGGCGG TGGGGACTCCCTCTCC (SEQ ID NO:21)	ATCGCAATTGTGGTAACGGAATTCCGTTGC GGATCCGCCTGCCTGGCGCTGGCCAGAGG (SEQ ID NO:22)
<i>miR-128b</i>	CTCTAGAGATCTGAAGAGAGT GCTTCTCTGTCTTAAG (SEQ ID NO:23)	ATCGCAATTGTTGCGTGCGAATTCCGTTGCG GATCCCAGTGCAGAAATCAGATCACGGAG (SEQ ID NO:24)
<i>miR-129-2</i>	CTCTAGAGATCTCCTTCTCGC CCTCCACACACTTCTC (SEQ ID NO:25)	ATCGCAATTGGTCTATGCGAATTCCGTTGCG GATCCGAAGACAGGTGACCAAAGCCTCG (SEQ ID NO:26)
<i>miR-132</i>	CTCTAGAGATCTTGATCAACG CAGGCGCCGCCATC (SEQ ID NO:27)	ATCGCAATTGTAGGACGCGAATTCCGTTGC GGATCCGAGCCCTGGCTGGGATACCTTGG (SEQ ID NO:28)
<i>miR-15b/16-2</i> cluster	CTCTAGAGATCTCGGCCTGCA GAGATAATACTTCTGTC (SEQ ID NO:29)	ATCGCAATTGCTATGACTGAATTCCGTTGCG GATCCTAGTTGCTGTATCCCTGTACAC (SEQ ID NO:30)
<i>miR-16-1/15a</i> cluster	CTCTAGAGATCTAGTTGTATT GCCCTGTAAAGTTGGC (SEQ ID NO:31)	ATCGCAATTGGGGATACTGAATTCCGTTGC GGATCCACAGAATCATACTAAAAATAACA (SEQ ID NO:32)
<i>miR-181a</i>	CTCTAGAGATCTCTGCACAGT CTATCCACAGTTC (SEQ ID NO:33)	ATCGCAATTGTATAAGCCGAATTCCGTTGC GGATCCATCATGGACTGCTCCTTACCTTG (SEQ ID NO:34)
<i>miR-181c</i>	CTCTAGAGATCTAGGTGCAAA CAGCACCTGAAAAGCG (SEQ ID NO:35)	ATCGCAATTGATTTGGACGAATTCCGTTGC GGATCCGGAAGGTCAGAGTCACCGGCAGG (SEQ ID NO:36)
<i>miR-188</i>	CTCTAGAGATCTGCATGAGCA CATGGACAGGTACACC (SEQ ID NO:37)	ATCGCAATTGTACGCCACGAATTCCGTTGC GGATCCCTGAGATGGGAGAAAGGACATGG (SEQ ID NO:38)
<i>miR-196</i>	CTCTAGAGATCTGTCTCTGGA ATCTGAGCTATCAGG (SEQ ID NO:39)	ATCGCAATTGGATTACAGGAATTCCGTTGC GGATCCGTTGCTCCTGGATGAAGGACCTC (SEQ ID NO:40)
<i>miR-199b/3154</i> cluster	CTCTAGAGATCTCTGGAACCG TTCGAGAGAGGCC (SEQ ID NO:41)	ATCGCAATTGTCATCTTCGAATTCCGTTGCG GATCCCCACATCCCCAGTCCCCATTGA (SEQ ID NO:42)
<i>miR-211</i>	CTCTAGAGATCTCGAAGAATA CATTGGTCGATGACTG (SEQ ID NO:43)	ATCGCAATTGTCCATTCCGAATTCCGTTGCG GATCCGCTGTGTCCACCAGAGTAATTA (SEQ ID NO:44)
<i>miR-212</i>	CTCTAGAGATCTTCTGCGAGC GGAGCTGTCCTCTCA (SEQ ID NO:45)	ATCGCAATTGCTCTAAACGAATTCCGTTGC GGATCCTGCGTTGATCAGCACCGCGGACA (SEQ ID NO:46)
<i>miR-216</i>	CTCTAGAGATCTCAGCAAGAG GAGTAGCTTGATAATGTCGC (SEQ ID NO:47)	ATCGCAATTGCAGTCACCGAATTCCGTTGC GGATCCGTTTCTTTCCAGCACAAACAG (SEQ ID NO:48)
<i>miR-24-2/27a/23a</i> cluster	CTCTAGAGATCTCCTGTGATC AAAGGAAGCATCTGGG (SEQ ID NO:49)	ATCGCAATTGAACTCCGGAATTCCGTTGC GGATCCCATCTCTGCTCCAAGCATCAGCC (SEQ ID NO:50)
<i>miR-29a</i>	CTCTAGAGATCTGGGGCTTTC	ATCGCAATTGTGGTGGATGAATTCCGTTGC

	TGGAACCAATCCCTCA (SEQ ID NO:51)	GGATCCTCATGATATGCTAATAGTGAACC (SEQ ID NO:52)
<i>miR-29b</i>	CTCTAGAGATCTCCATCAATA ACAAATTCAGTGAC (SEQ ID NO:53)	ATCGCAATTGTACAGATGAATTCCGTTGC GGATCCGCCAGTGCAGAGACCTGACTGCC (SEQ ID NO:54)
<i>miR-29c</i>	CTCTAGAGATCTGATTGTCAT GGGGCAGGGGAGAG (SEQ ID NO:55)	ATCGCAATTGGGTCTTTGGAATTCCGTTGCG GATCCAGAATTTAGAACAGCACTAC (SEQ ID NO:56)
<i>miR-31</i>	CTCTAGAGATCTTAGTCCATA AACATTCTCGAGGTTC (SEQ ID NO:57)	ATCGCAATTGATCGTCCGGAATTCCGTTGC GGATCCGGACACAATACATAGCAGGACAG (SEQ ID NO:58)
<i>miR-324</i>	CTCTAGAGATCTCCAAGATAA GGGCTGACCTAGCTTG (SEQ ID NO:59)	ATCGCAATTGCGAACTGAGAATTCCGTTGC GGATCCCATCCAGCGTAGACTGAACTCTG (SEQ ID NO:60)
<i>miR-328</i>	CTCTAGAGATCTCCAAGCTCA GCTCAGGGCCTAAGC (SEQ ID NO:61)	ATCGCAATTGCTGTGGACGAATTCCGTTGC GGATCCCCAGCGCGTACAGCCGGGTCCG (SEQ ID NO:62)
<i>miR-34a</i>	CTCTAGAGATCTCTGGCCTCT CCAGTAGCTAGGACTAC (SEQ ID NO:63)	ATCGCAATTGAGGATGTGGAATTCCGTTGC GGATCCCTGGCTACTATTCTCCCTACGTG (SEQ ID NO:64)
<i>miR-373</i>	CTCTAGAGATCTGCAGCTGTG ACCAAGGGGCTGTA (SEQ ID NO:65)	ATCGCAATTGTGGAGATAGAATTCCGTTGC GGATCCCTGCAGGTGAACCCCGTATCCT (SEQ ID NO:66)
<i>miR-376a</i>	CTCTAGAGATCTCCGTAGGGC CTGAGTAGGTGC (SEQ ID NO:67)	ATCGCAATTGGGTAGTCAGAATTCCGTTGC GGATCCCAGCCCACTCAGGTATTCTC (SEQ ID NO:68)
<i>miR-429</i>	CTCTAGAGATCTTAACCCGAC CGAGCTTCAGGAAGC (SEQ ID NO:69)	ATCGCAATTGCTGAATGAGAATTCCGTTGC GGATCCCTCCGGGTATCTGTGACTGTGAC (SEQ ID NO:70)
<i>miR-451/144/4732 cluster</i>	CTCTAGAGATCTCAGCCCTGA CCTGTCTGTCTG (SEQ ID NO:71)	ATCGCAATTGGGCAACGAGAATTCCGTTGC GGATCCCCTGCCTTGTTTGAGCTGGAGTC (SEQ ID NO:72)
<i>miR-488</i>	CTCTAGAGATCTGGCGTAGTA GAGGTAGGAATGATAG (SEQ ID NO:73)	ATCGCAATTGAACGCGAAGAATTCCGTTGC GGATCCCCTCAACTACACTGCCCCCAATC (SEQ ID NO:74)
<i>miR-489</i>	CTCTAGAGATCTATCAGATTC CTTCTCTGTGGAT (SEQ ID NO:75)	ATCGCAATTGATGACTCAGAATTCCGTTGC GGATCCAAGTGTTATGTCTATACTACTT (SEQ ID NO:76)
<i>miR-708</i>	CTCTAGAGATCTGTGAAGGGG CAAGCTCTACTAAAGG (SEQ ID NO:77)	ATCGCAATTGCTGACCAAGAATTCCGTTGC GGATCCCTCAGGAGACAATTACAGGCCTAG (SEQ ID NO:78)
<i>miR-9-1</i>	CTCTAGAGATCTTTTCGGTCTC TGTCGTGTCTGTATCTC (SEQ ID NO:79)	ATCGCAATTGCGTGAGCTGAATTCCGTTGC GGATCCTGTGGGGGAAAAATATGGCACC (SEQ ID NO:80)
<i>miR-93/106b cluster</i>	CTCTAGAGATCTCATGTGCCG CGAGAAGCAGCCCATG (SEQ ID NO:81)	ATCGCAATTGATCTTGAGGAATTCCGTTGC GGATCCAGTGCTAGCTCAGCAGTAGGTTG (SEQ ID NO:82)
<i>miR-99a</i>	CTCTAGAGATCTTTTAGTTTTG AATATTTATGAAGGCC (SEQ ID NO:83)	ATCGCAATTGGGATTCCGGAATTCCGTTGC GGATCCGTACATGGAATCGAACTTGAATG (SEQ ID NO:84)

Table 2: Reported Expression of Candidate miRNAs in Cancer Cells

miRNA expression in cancer cells	miRNAs	References
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miRNAs reported to be down-regulated in cancer cells resistant to taxanes	<i>miR-15b/16-2 cluster, miR-24-2/27a-23a cluster, miR-29a, miR-29b, miR-126, miR-128b, and miR-324</i>	62-64
miRNAs reported to be down-regulated in cancer cells resistant to other chemotherapy drugs	<i>let-7a, let-7b, let-7c, let-7d, let-7i, miR-9-1, miR-10b, miR-29a, miR-29c, miR-31, miR-34a, miR-126, miR-129-2, miR-132, miR-181a, miR-181c, miR-196, miR-199b, miR-211, miR-212, miR-216, miR-328, miR-373, miR-429, miR-451/144/4732 cluster, and miR-489</i>	64-79
miRNAs reported to have altered expression in ovarian cancer cell lines	<i>miR-16-1/15a cluster, miR-93/106b cluster, miR-99, miR-376a, and miR-488</i>	30, 80, 81

Table 3: List of Two-Wise miRNA Hits that Increase Docetaxel Sensitivity in OVCAR8-ADR Cells Based on Pooled Screening with $\log_2$ ratio <-0.42 and $>25\%$ fewer barcode counts in experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	<i>Log₂ ratio – Drug/Vehicle</i>
<i>miR-376a</i>	<i>miR-16-1/15a</i> cluster	-0.92
<i>miR-216</i>	<i>miR-181c</i>	-0.89
<i>miR-31</i>	<i>miR-181a</i>	-0.88
<i>miR-376a</i>	<i>miR-212</i>	-0.73
<i>let-7i</i>	<i>miR-181a</i>	-0.70
<i>miR-93/106b</i> cluster	<i>miR-16-1/15a</i> cluster	-0.66
<i>miR-181a</i>	<i>miR-93/106b</i> cluster	-0.64
<i>miR-15b/ miR-16-2</i> cluster	<i>miR-9-1</i>	-0.62
<i>miR-373</i>	<i>miR-181a</i>	-0.62
<i>miR-16-1/15a</i> cluster	<i>let-7a</i>	-0.58
<i>miR-10b</i>	<i>miR-16-1/15a</i> cluster	-0.57
<i>miR-216</i>	<i>miR-15b/ miR-16-2</i> cluster	-0.56
<i>miR-10b</i>	<i>miR-212</i>	-0.49
<i>miR-216</i>	<i>miR-181a</i>	-0.48
<i>miR-15b/ miR-16-2</i> cluster	<i>miR-181a</i>	-0.47
<i>miR-181a</i>	<i>miR-16-1/15a</i> cluster	-0.47
<i>miR-16-1/15a</i> cluster	<i>miR-9-1</i>	-0.46
<i>miR-324</i>	<i>miR-9-1</i>	-0.46
<i>miR-181c</i>	<i>miR-9-1</i>	-0.46
<i>miR-10b</i>	<i>miR-196</i>	-0.44
<i>miR-99a</i>	<i>miR-16-1/15a</i> cluster	-0.44
<i>miR-31</i>	<i>miR-376a</i>	-0.44
<i>miR-216</i>	<i>let-7a</i>	-0.44
<i>miR-10b</i>	<i>miR-15b/mir-16-2</i> cluster	-0.44

Table 4: List of Two-Wise miRNA Hits that Increase Docetaxel Resistance in OVCAR8-ADR Cells Based on Pooled Screening with log₂ ratio <-0.42 and >25% fewer barcode counts in experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	Log ₂ ratio – Drug/Vehicle
<i>miR-199b/3154</i> cluster	<i>miR-34a</i>	0.68
<i>miR-24-2/27a/23a</i> cluster	<i>miR-93/106b</i> cluster	0.64
<i>miR-93/106b</i> cluster	<i>miR-34a</i>	0.62
<i>miR-24-2/27a/23a</i> cluster	<i>miR-34a</i>	0.62
<i>miR-376a</i>	<i>miR-29b</i>	0.61
<i>miR-126</i>	<i>miR-34a</i>	0.59
<i>miR-196</i>	<i>miR-34a</i>	0.55
<i>miR-328</i>	<i>miR-34a</i>	0.54
<i>miR-34a</i>	<i>miR-29a</i>	0.52
<i>miR-34a</i>	<i>miR-29c</i>	0.51
<i>miR-128b</i>	<i>miR-34a</i>	0.51
<i>miR-129-2</i>	<i>miR-34a</i>	0.50
<i>miR-29b</i>	<i>miR-16-1/15a</i> cluster	0.48
<i>miR-429</i>	<i>miR-93/106b</i> cluster	0.47
<i>miR-34a</i>	<i>let-7b/miR-4763</i> cluster	0.46
<i>miR-199b/3154</i> cluster	<i>miR-93/106b</i> cluster	0.46
<i>miR-429</i>	<i>miR-34a</i>	0.44
<i>miR-489</i>	<i>miR-34a</i>	0.43
<i>miR-376a</i>	<i>miR-34a</i>	0.42
<i>miR-429</i>	<i>miR-199b/3154</i> cluster	0.40
<i>miR-324</i>	<i>miR-34a</i>	0.40
<i>miR-29b</i>	<i>let-7b/miR-4763</i> cluster	0.40
<i>miR-132</i>	<i>miR-34a</i>	0.39
<i>miR-24-2/27a/23a</i> cluster	<i>miR-129-2</i>	0.38
<i>miR-34a</i>	<i>let-7c</i>	0.38
<i>miR-211</i>	<i>miR-34a</i>	0.38
<i>let-7e/miR-99b</i> cluster	<i>miR-34a</i>	0.38
<i>let-7i</i>	<i>miR-34a</i>	0.37
<i>miR-24-2/27a/23a</i> cluster	<i>miR-29c</i>	0.37
<i>miR-212</i>	<i>miR-188</i>	0.37
<i>miR-24-2/27a/23a</i> cluster	<i>let-7d</i>	0.36
<i>miR-31</i>	<i>miR-34a</i>	0.36
<i>miR-451a/451b/144/4732</i> cluster	<i>miR-34a</i>	0.35
<i>miR-451a/451b/144/4732</i> cluster	<i>miR-24-2/27a/23a</i> cluster	0.34
<i>miR-488</i>	<i>miR-34a</i>	0.33
<i>miR-489</i>	<i>miR-93/106b</i> cluster	0.33

Table 5: List of Three-Wise miRNA Hits that Increase Docetaxel Sensitivity in OVCAR8-ADR Cells Based on Pooled Screening with log₂ ratio <-0.42 and >25% fewer barcode counts in experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	<i>miR-C</i>	Log ₂ ratio – Drug/Vehicle
<i>miR-16-1/15a</i> cluster	<i>miR-29a</i>	<i>miR-181c</i>	-0.95
<i>miR-129-2</i>	<i>miR-181c</i>	<i>miR-324</i>	-0.94
<i>let-7c</i>	<i>miR-29a</i>	<i>miR-199b/3154</i> cluster	-0.83
<i>miR-181a</i>	<i>miR-126</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.80
<i>miR-181a</i>	<i>miR-181c</i>	<i>miR-199b/3154</i> cluster	-0.77
<i>let-7e/miR-99b</i> cluster	<i>miR-328</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.76
<i>miR-9-1</i>	<i>miR-376a</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.70
<i>miR-16-1/15a</i> cluster	<i>miR-15b/miR-16-2</i> cluster	<i>miR-429</i>	-0.66
<i>miR-324</i>	<i>miR-24-2/27a/23a</i> cluster	<i>miR-373</i>	-0.66
<i>let-7c</i>	<i>miR-128b</i>	<i>miR-10b</i>	-0.65
<i>miR-29a</i>	<i>let-7i</i>	<i>miR-199b/3154</i> cluster	-0.63
<i>miR-16-1/15a</i> cluster	<i>miR-126</i>	<i>miR-216</i>	-0.62
<i>miR-16-1/15a</i> cluster	<i>miR-15b/miR-16-2</i> cluster	<i>miR-216</i>	-0.61
<i>miR-708</i>	<i>miR-451a/451b/144/4732</i> cluster	<i>miR-10b</i>	-0.61
<i>miR-29a</i>	<i>miR-181a</i>	<i>miR-488</i>	-0.59
<i>miR-181a</i>	<i>let-7i</i>	<i>miR-216</i>	-0.59
<i>miR-16-1/15a</i> cluster	<i>miR-128b</i>	<i>miR-181c</i>	-0.58
<i>let-7d</i>	<i>miR-181c</i>	<i>miR-10b</i>	-0.58
<i>miR-181a</i>	<i>miR-216</i>	<i>miR-489</i>	-0.57
<i>miR-324</i>	<i>miR-376a</i>	<i>miR-15b/miR-16-2</i> cluster	-0.55
<i>miR-708</i>	<i>miR-373</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.55
<i>miR-181c</i>	<i>miR-196</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.55
<i>miR-181a</i>	<i>miR-324</i>	<i>miR-708</i>	-0.55
<i>miR-16-1/15a</i> cluster	<i>miR-451a/451b/144/4732</i> cluster	<i>miR-489</i>	-0.54
<i>let-7a</i>	<i>miR-16-1/15a</i> cluster	<i>miR-199b/3154</i> cluster	-0.54
<i>miR-16-1/15a</i> cluster	<i>let-7e/miR-99b</i> cluster	<i>miR-15b/miR-16-2</i> cluster	-0.54
<i>let-7c</i>	<i>miR-376a</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.54
<i>miR-181c</i>	<i>miR-212</i>	<i>miR-376a</i>	-0.54
<i>miR-9-1</i>	<i>miR-29a</i>	<i>miR-181c</i>	-0.54
<i>miR-15b/miR-16-2</i> cluster	<i>miR-31</i>	<i>miR-196</i>	-0.54
<i>miR-128b</i>	<i>miR-24-2/27a/23a</i> cluster	<i>miR-31</i>	-0.53
<i>let-7c</i>	<i>miR-324</i>	<i>miR-451a/451b/144/4732</i> cluster	-0.53
<i>miR-128b</i>	<i>miR-188</i>	<i>miR-324</i>	-0.53
<i>miR-181a</i>	<i>miR-15b/miR-16-2</i> cluster	<i>miR-489</i>	-0.53
<i>miR-429</i>	<i>miR-451a/451b/144/4732</i> cluster	<i>miR-10b</i>	-0.52
<i>miR-16-1/15a</i> cluster	<i>miR-376a</i>	<i>miR-31</i>	-0.51
<i>let-7e/miR-99b</i> cluster	<i>miR-181a</i>	<i>miR-324</i>	-0.51
<i>let-7e/miR-99b</i> cluster	<i>miR-188</i>	<i>miR-376a</i>	-0.51
<i>miR-128b</i>	<i>miR-324</i>	<i>miR-216</i>	-0.51
<i>miR-93/106b</i> cluster	<i>miR-181c</i>	<i>miR-373</i>	-0.51
<i>miR-181a</i>	<i>miR-15b/miR-16-2</i> cluster	<i>miR-126</i>	-0.50
<i>miR-181c</i>	<i>miR-15b/miR-16-2</i> cluster	<i>miR-328</i>	-0.50
<i>miR-181a</i>	<i>miR-488</i>	<i>miR-15b/miR-16-2</i> cluster	-0.50
<i>miR-29c</i>	<i>miR-181a</i>	<i>miR-376a</i>	-0.50
<i>let-7c</i>	<i>let-7i</i>	<i>miR-15b/miR-16-2</i> cluster	-0.50
<i>miR-708</i>	<i>miR-15b/miR-16-2</i> cluster	<i>miR-451a/451b/144/4732</i> cluster	-0.50

<i>miR-181c</i>	<i>miR-488</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.50
<i>let-7e/miR-99b cluster</i>	<i>miR-181a</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.49
<i>miR-181a</i>	<i>miR-376a</i>	<i>miR-24-2/27a/23a cluster</i>	-0.49
<i>miR-376a</i>	<i>miR-488</i>	<i>miR-31</i>	-0.49
<i>miR-181c</i>	<i>miR-376a</i>	<i>miR-708</i>	-0.48
<i>miR-128b</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-489</i>	-0.48
<i>miR-16-1/15a cluster</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.48
<i>miR-9-1</i>	<i>miR-181c</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.48
<i>miR-29c</i>	<i>miR-181c</i>	<i>miR-31</i>	-0.47
<i>miR-29a</i>	<i>miR-181c</i>	<i>miR-126</i>	-0.47
<i>miR-708</i>	<i>let-7i</i>	<i>miR-429</i>	-0.47
<i>miR-29a</i>	<i>miR-126</i>	<i>miR-211</i>	-0.47
<i>miR-16-1/15a cluster</i>	<i>miR-128b</i>	<i>miR-31</i>	-0.47
<i>miR-132</i>	<i>miR-181c</i>	<i>miR-488</i>	-0.47
<i>miR-376a</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-24-2/27a/23a cluster</i>	-0.47
<i>let-7a</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.47
<i>miR-16-1/15a cluster</i>	<i>miR-328</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.47
<i>miR-99a</i>	<i>miR-181c</i>	<i>miR-31</i>	-0.46
<i>miR-16-1/15a cluster</i>	<i>miR-181c</i>	<i>miR-489</i>	-0.46
<i>let-7e/miR-99b cluster</i>	<i>miR-376a</i>	<i>miR-199b/3154 cluster</i>	-0.46
<i>miR-128b</i>	<i>miR-376a</i>	<i>miR-373</i>	-0.46
<i>miR-9-1</i>	<i>miR-29a</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.46
<i>miR-376a</i>	<i>miR-24-2/27a/23a cluster</i>	<i>miR-328</i>	-0.46
<i>miR-373</i>	<i>miR-10b</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.46
<i>miR-181a</i>	<i>miR-324</i>	<i>miR-31</i>	-0.46
<i>miR-16-1/15a cluster</i>	<i>miR-181c</i>	<i>miR-211</i>	-0.45
<i>miR-16-1/15a cluster</i>	<i>let-7i</i>	<i>miR-196</i>	-0.45
<i>miR-15b/miR-16-2 cluster</i>	<i>miR-126</i>	<i>miR-10b</i>	-0.45
<i>miR-29c</i>	<i>miR-181c</i>	<i>miR-324</i>	-0.45
<i>let-7e/miR-99b cluster</i>	<i>miR-181c</i>	<i>miR-429</i>	-0.44
<i>miR-181c</i>	<i>miR-126</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.44
<i>miR-181c</i>	<i>miR-31</i>	<i>miR-328</i>	-0.44
<i>miR-9-1</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-373</i>	-0.44
<i>let-7d</i>	<i>miR-29a</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.43
<i>miR-9-1</i>	<i>let-7d</i>	<i>miR-181c</i>	-0.43
<i>miR-16-1/15a cluster</i>	<i>let-7e/miR-99b cluster</i>	<i>miR-181c</i>	-0.43
<i>miR-15b/miR-16-2 cluster</i>	<i>miR-373</i>	<i>miR-216</i>	-0.43
<i>miR-93/106b cluster</i>	<i>let-7i</i>	<i>miR-324</i>	-0.43
<i>let-7i</i>	<i>miR-199b/3154 cluster</i>	<i>miR-10b</i>	-0.43
<i>miR-373</i>	<i>miR-10b</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.43
<i>miR-15b/miR-16-2 cluster</i>	<i>miR-10b</i>	<i>miR-211</i>	-0.43
<i>miR-128b</i>	<i>miR-451a/451b/144/4732 cluster</i>	<i>miR-181a</i>	-0.43
<i>let-7i</i>	<i>miR-451a/451b/144/4732 cluster</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.43
<i>miR-9-1</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-181a</i>	-0.43
<i>miR-181a</i>	<i>miR-216</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.43

Table 6: List of Three-Wise miRNA Hits that Increase Docetaxel Resistance in OVCAR8-ADR Cells Based on Pooled Screening with log₂ ratio <-0.42 and >25% fewer barcode counts in experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	<i>miR-C</i>	Log ₂ ratio – Drug/Vehicle
<i>miR-34a</i>	<i>miR-376a</i>	<i>miR-31</i>	0.67
<i>miR-29a</i>	<i>miR-34a</i>	<i>miR-31</i>	0.52
<i>miR-132</i>	<i>miR-429</i>	<i>miR-451a/451b/144/4732</i> cluster	0.46
<i>miR-34a</i>	<i>miR-376a</i>	<i>let-7i</i>	0.46
<i>miR-128b</i>	<i>miR-181c</i>	<i>miR-188</i>	0.45
<i>miR-181c</i>	<i>miR-212</i>	<i>miR-31</i>	0.43
<i>miR-34a</i>	<i>miR-93/106b</i> cluster	<i>miR-181c</i>	0.41
<i>miR-34a</i>	<i>miR-128b</i>	<i>miR-15b/miR-16-2</i> cluster	0.41
<i>miR-129-2</i>	<i>miR-181c</i>	<i>miR-376a</i>	0.40
<i>miR-132</i>	<i>miR-376a</i>	<i>miR-211</i>	0.40
<i>miR-34a</i>	<i>miR-376a</i>	<i>miR-429</i>	0.39
<i>miR-34a</i>	<i>miR-24-2/27a/23a</i> cluster	<i>miR-196</i>	0.38
<i>miR-132</i>	<i>miR-181c</i>	<i>miR-376a</i>	0.38
<i>miR-29b</i>	<i>miR-34a</i>	<i>miR-93/106b</i> cluster	0.38
<i>miR-34a</i>	<i>miR-128b</i>	<i>miR-132</i>	0.38
<i>miR-29b</i>	<i>miR-376a</i>	<i>miR-328</i>	0.38
<i>miR-34a</i>	<i>miR-93/106b</i> cluster	<i>miR-132</i>	0.37
<i>miR-34a</i>	<i>miR-93/106b</i> cluster	<i>miR-199b/3154</i> cluster	0.37
<i>miR-29c</i>	<i>miR-34a</i>	<i>miR-429</i>	0.37
<i>miR-181c</i>	<i>miR-211</i>	<i>miR-10b</i>	0.37
<i>let-7a</i>	<i>miR-212</i>	<i>miR-376a</i>	0.36
<i>miR-29a</i>	<i>let-7e/miR-99b</i> cluster	<i>miR-31</i>	0.36
<i>miR-34a</i>	<i>let-7e/miR-99b</i> cluster	<i>miR-196</i>	0.36
<i>miR-128b</i>	<i>miR-132</i>	<i>miR-31</i>	0.36
<i>miR-34a</i>	<i>miR-132</i>	<i>miR-181c</i>	0.36
<i>miR-29c</i>	<i>miR-34a</i>	<i>miR-132</i>	0.35
<i>miR-29c</i>	<i>miR-34a</i>	<i>miR-93/106b</i> cluster	0.34
<i>miR-29c</i>	<i>miR-34a</i>	<i>miR-24-2/27a/23a</i> cluster	0.34
<i>miR-34a</i>	<i>miR-93/106b</i> cluster	<i>miR-429</i>	0.34
<i>miR-34a</i>	<i>miR-211</i>	<i>miR-429</i>	0.34
<i>miR-34a</i>	<i>miR-132</i>	<i>miR-196</i>	0.34
<i>miR-34a</i>	<i>let-7e/miR-99b</i> cluster	<i>miR-429</i>	0.34
<i>miR-29a</i>	<i>miR-34a</i>	<i>miR-429</i>	0.33
<i>miR-29a</i>	<i>miR-34a</i>	<i>miR-93/106b</i> cluster	0.33
<i>miR-16-1/15a</i> cluster	<i>let-7e/miR-99b</i> cluster	<i>miR-429</i>	0.33
<i>miR-29b</i>	<i>miR-181c</i>	<i>miR-489</i>	0.33

Table 7: List of Three-Wise miRNA Hits that Inhibit OVCAR8-ADR Cell Proliferation
Based on Pooled Screening with log₂ ratio <-0.42 and >25% fewer barcode counts in
experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	<i>miR-C</i>	Log ₂ ratio – Day 4/Day1
<i>let-7d</i>	<i>miR-488</i>	<i>let-7i</i>	-0.87
<i>miR-128b</i>	<i>miR-212</i>	<i>let-7i</i>	-0.85
<i>miR-132</i>	<i>miR-15b/miR-16-2 cluster</i>	<i>miR-31</i>	-0.82
<i>miR-708</i>	<i>let-7i</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.72
<i>miR-181c</i>	<i>let-7i</i>	<i>miR-373</i>	-0.70
<i>miR-132</i>	<i>miR-212</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.64
<i>let-7d</i>	<i>miR-132</i>	<i>miR-181c</i>	-0.62
<i>miR-9-1</i>	<i>miR-181c</i>	<i>miR-324</i>	-0.56
<i>miR-29a</i>	<i>miR-99a</i>	<i>miR-376a</i>	-0.56
<i>miR-128b</i>	<i>miR-132</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.55
<i>miR-16-1/15a cluster</i>	<i>let-7e/miR-99b cluster</i>	<i>miR-128b</i>	-0.51
<i>miR-132</i>	<i>let-7i</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.51
<i>miR-181a</i>	<i>let-7i</i>	<i>miR-15b/miR-16-2 cluster</i>	-0.51
<i>miR-16-1/15a cluster</i>	<i>miR-29a</i>	<i>miR-488</i>	-0.50
<i>miR-34a</i>	<i>miR-328</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.49
<i>miR-128b</i>	<i>miR-212</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.48
<i>let-7c</i>	<i>miR-128b</i>	<i>miR-376a</i>	-0.48
<i>miR-29a</i>	<i>let-7i</i>	<i>miR-126</i>	-0.47
<i>miR-181c</i>	<i>miR-212</i>	<i>miR-199b/3154 cluster</i>	-0.47
<i>miR-29a</i>	<i>miR-29b</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.47
<i>miR-181a</i>	<i>miR-31</i>	<i>miR-429</i>	-0.45
<i>miR-34a</i>	<i>miR-181a</i>	<i>miR-181c</i>	-0.45
<i>miR-181c</i>	<i>let-7i</i>	<i>miR-429</i>	-0.45
<i>miR-29a</i>	<i>miR-181a</i>	<i>miR-429</i>	-0.44
<i>miR-181a</i>	<i>miR-24-2/27a/23a cluster</i>	<i>miR-451a/451b/144/4732 cluster</i>	-0.44
<i>miR-34a</i>	<i>miR-324</i>	<i>miR-376a</i>	-0.44
<i>miR-128b</i>	<i>let-7i</i>	<i>miR-196</i>	-0.44

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Table 8: Constructs Used in this Work

Construct ID	Design
pAWp6	pFUGW-UBCp- <i>GFP</i>
pAWp12	pFUGW-CMVp- <i>GFP</i>
pAWp7	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i>
pAWp7-1	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-124</i>]
pAWp7-2	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-128</i>]
pAWp7-3	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-132</i>]
pAWp7-4	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-124</i> + <i>miR-128</i>]
pAWp7-5	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-128</i> + <i>miR-132</i>]
pAWp7-6	pFUGW-UBCp- <i>RFP</i> -CMVp- <i>GFP</i> -[<i>miR-124</i> + <i>miR-128</i> + <i>miR-132</i>]
pAWp7-7	pFUGW-UBCp- <i>RFP</i> -[<i>miR-124</i> sensor]-CMVp- <i>GFP</i>
pAWp7-8	pFUGW-UBCp- <i>RFP</i> -[<i>miR-128</i> sensor]-CMVp- <i>GFP</i>
pAWp7-9	pFUGW-UBCp- <i>RFP</i> -[<i>miR-132</i> sensor]-CMVp- <i>GFP</i>
pAWp7-10	pFUGW-UBCp- <i>RFP</i> -[<i>miR-124</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-124</i>]
pAWp7-11	pFUGW-UBCp- <i>RFP</i> -[<i>miR-128</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-124</i>]
pAWp7-12	pFUGW-UBCp- <i>RFP</i> -[<i>miR-132</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-124</i>]

pAWp7-13	pFUGW-UBCp- <i>RFP</i> -[<i>miR-124</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-128</i>]
pAWp7-14	pFUGW-UBCp- <i>RFP</i> -[<i>miR-128</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-128</i>]
pAWp7-15	pFUGW-UBCp- <i>RFP</i> -[<i>miR-132</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-128</i>]
pAWp7-16	pFUGW-UBCp- <i>RFP</i> -[<i>miR-124</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-132</i>]
pAWp7-17	pFUGW-UBCp- <i>RFP</i> -[<i>miR-128</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-132</i>]
pAWp7-18	pFUGW-UBCp- <i>RFP</i> -[<i>miR-132</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-132</i>]
pAWp7-19	pFUGW-UBCp- <i>RFP</i> -[<i>miR-124</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-124</i> + <i>miR-128</i>]
pAWp7-20	pFUGW-UBCp- <i>RFP</i> -[<i>miR-128</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-128</i> + <i>miR-132</i>]
pAWp7-21	pFUGW-UBCp- <i>RFP</i> -[<i>miR-132</i> sensor]-CMVp- <i>GFP</i> -[<i>miR-124</i> + <i>miR-128</i> + <i>miR-132</i>]
pAWSV-1	pBT264-[<i>let-7a</i>]
pAWSV-2	pBT264-[<i>let-7b/miR-4763</i> cluster]
pAWSV-3	pBT264-[<i>let-7c</i>]
pAWSV-4	pBT264-[<i>let-7d</i>]
pAWSV-5	pBT264-[<i>let-7e/miR-99b</i> cluster]
pAWSV-6	pBT264-[<i>let-7i</i>]
pAWSV-7	pBT264-[<i>miR-10b</i>]
pAWSV-8	pBT264-[<i>miR-126</i>]
pAWSV-9	pBT264-[<i>miR-128b</i>]
pAWSV-10	pBT264-[<i>miR-129-2</i>]
pAWSV-11	pBT264-[<i>miR-132</i>]
pAWSV-12	pBT264-[<i>miR-15b/16-2</i> cluster]
pAWSV-13	pBT264-[<i>miR-16-1/15a</i> cluster]
pAWSV-14	pBT264-[<i>miR-181a</i>]
pAWSV-15	pBT264-[<i>miR-181c</i>]
pAWSV-16	pBT264-[<i>miR-188</i>]
pAWSV-17	pBT264-[<i>miR-196</i>]
pAWSV-18	pBT264-[<i>miR-199b/3154</i> cluster]
pAWSV-19	pBT264-[<i>miR-211</i>]
pAWSV-20	pBT264-[<i>miR-212</i>]
pAWSV-21	pBT264-[<i>miR-216</i>]
pAWSV-22	pBT264-[<i>miR-24-2/27a/23a</i> cluster]
pAWSV-23	pBT264-[<i>miR-29a</i>]
pAWSV-24	pBT264-[<i>miR-29b</i>]
pAWSV-25	pBT264-[<i>miR-29c</i>]
pAWSV-26	pBT264-[<i>miR-31</i>]
pAWSV-27	pBT264-[<i>miR-324</i>]
pAWSV-28	pBT264-[<i>miR-328</i>]
pAWSV-29	pBT264-[<i>miR-34a</i>]
pAWSV-30	pBT264-[<i>miR-373</i>]
pAWSV-31	pBT264-[<i>miR-376a</i>]
pAWSV-32	pBT264-[<i>miR-429</i>]
pAWSV-33	pBT264-[<i>miR-451/144/4732</i> cluster]
pAWSV-34	pBT264-[<i>miR-488</i>]
pAWSV-35	pBT264-[<i>miR-489</i>]
pAWSV-36	pBT264-[<i>miR-708</i>]
pAWSV-37	pBT264-[<i>miR-9-1</i>]
pAWSV-38	pBT264-[<i>miR-93/106b</i> cluster]
pAWSV-39	pBT264-[<i>miR-99a</i>]
pAWp11	pFUGW-CMVp
pAWp11-1	pFUGW-CMVp-[<i>let-7e/miR-99b</i> cluster + <i>miR-15b/16-2</i> cluster]
pAWp11-2	pFUGW-CMVp-[<i>let-7e/miR-99b</i> cluster]
pAWp11-3	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-128b</i> + <i>miR-212</i>]
pAWp11-4	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-128b</i>]
pAWp11-5	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-132</i> + <i>miR-15b/16-2</i> cluster]
pAWp11-6	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-132</i>]
pAWp11-7	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-15b/16-2</i> cluster]

pAWp11-8	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-181c</i>]
pAWp11-9	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-212</i>]
pAWp11-10	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-373</i> + <i>miR-181c</i>]
pAWp11-11	pFUGW-CMVp-[<i>let-7i</i> + <i>miR-373</i>]
pAWp11-12	pFUGW-CMVp-[<i>let-7i</i>]
pAWp11-13	pFUGW-CMVp-[<i>miR-126</i> + <i>miR-181a</i> + <i>miR-451/144/4732</i> cluster]
pAWp11-14	pFUGW-CMVp-[<i>miR-128b</i> + <i>let-7e/miR-99b</i> cluster]
pAWp11-15	pFUGW-CMVp-[<i>miR-128b</i> + <i>miR-212</i>]
pAWp11-16	pFUGW-CMVp-[<i>miR-128b</i>]
pAWp11-17	pFUGW-CMVp-[<i>miR-132</i> + <i>miR-15b/16-2</i> cluster]
pAWp11-18	pFUGW-CMVp-[<i>miR-132</i>]
pAWp11-19	pFUGW-CMVp-[<i>miR-15b/16-2</i> cluster]
pAWp11-20	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>let-7e/miR-99b</i> cluster + <i>miR-15b/16-2</i> cluster]
pAWp11-21	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>let-7e/miR-99b</i> cluster]
pAWp11-22	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>miR-128b</i> + <i>let-7e/miR-99b</i> cluster]
pAWp11-23	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>miR-128b</i>]
pAWp11-24	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>miR-15b/16-2</i> cluster]
pAWp11-25	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>miR-181c</i> + <i>let-7e/miR-99b</i> cluster]
pAWp11-26	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster + <i>miR-181c</i>]
pAWp11-27	pFUGW-CMVp-[<i>miR-16-1/15a</i> cluster]
pAWp11-28	pFUGW-CMVp-[<i>miR-181c</i> + <i>let-7e/miR-99b</i> cluster]
pAWp11-29	pFUGW-CMVp-[<i>miR-181c</i>]
pAWp11-30	pFUGW-CMVp-[<i>miR-199b/3154</i> cluster]
pAWp11-31	pFUGW-CMVp-[<i>miR-212</i>]
pAWp11-32	pFUGW-CMVp-[<i>miR-29a</i> + <i>miR-34a</i>]
pAWp11-33	pFUGW-CMVp-[<i>miR-29a</i>]
pAWp11-34	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-132</i> + <i>miR-15b/16-2</i> cluster]
pAWp11-35	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-132</i>]
pAWp11-36	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-15b/16-2</i> cluster]
pAWp11-37	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-29a</i> + <i>miR-34a</i>]
pAWp11-38	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-29a</i>]
pAWp11-39	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-34a</i>]
pAWp11-40	pFUGW-CMVp-[<i>miR-31</i> + <i>miR-376a</i> + <i>miR-34a</i>]
pAWp11-41	pFUGW-CMVp-[<i>miR-31</i>]
pAWp11-42	pFUGW-CMVp-[<i>miR-328</i>]
pAWp11-43	pFUGW-CMVp-[<i>miR-34a</i> + <i>miR-199b/3154</i> cluster]
pAWp11-44	pFUGW-CMVp-[<i>miR-34a</i> + <i>miR-328</i>]
pAWp11-45	pFUGW-CMVp-[<i>miR-34a</i> + <i>miR-429</i>]
pAWp11-46	pFUGW-CMVp-[<i>miR-34a</i>]
pAWp11-47	pFUGW-CMVp-[<i>miR-373</i> + <i>miR-181c</i>]
pAWp11-48	pFUGW-CMVp-[<i>miR-373</i>]
pAWp11-49	pFUGW-CMVp-[<i>miR-376a</i> + <i>miR-16-1/15a</i> cluster]
pAWp11-50	pFUGW-CMVp-[<i>miR-376a</i>]
pAWp11-51	pFUGW-CMVp-[<i>miR-429</i>]
pAWp11-52	pFUGW-CMVp-[<i>miR-93/106b</i> cluster + <i>miR-16-1/15a</i> cluster]
pAWp11-53	pFUGW-CMVp-[<i>miR-93/106b</i> cluster]

Table 9: List of Primers used for qRT-PCR

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
<i>AKT3</i>	TGCAGCCACCATGAAGACAT (SEQ ID NO:85)	GTCCTCCACCAAGGCGTTTA (SEQ ID NO:86)
<i>BCL2</i>	GGAGGCTGGGATGCCTTTGT (SEQ ID NO:87)	GACTTCACTTGTGGCCAGAT (SEQ ID NO:88)
<i>BMI1</i>	GCTGGTTGCCATTGACAG (SEQ ID NO:89)	AAAAATCCCGGAAAGAGCAGC (SEQ ID NO:90)
<i>CAPRIN1</i>	CTGGCTATCAACGGGATGGA (SEQ ID NO:91)	GCCAGAACAGAAGCTCCACT (SEQ ID NO:92)
<i>CCND1</i>	GGC AGC AGA AGC GAG AGC (SEQ ID NO:93)	CTC GCA GAC CTC CAG CAT (SEQ ID NO:94)
<i>CCND3</i>	GAGCTGCTGTGTTGCGAAGG (SEQ ID NO:95)	CGCTGCTCCTCACATACCTCC (SEQ ID NO:96)
<i>CCNE1</i>	CCCATCATGCCGAGGGAG (SEQ ID NO:97)	TATTGTCCCAAGGCTGGCTC (SEQ ID NO:98)
<i>CCNT2</i>	CGGAGGAGGAAGTGTCATGG (SEQ ID NO:99)	GCTGAGAGACATTGAGACGCT (SEQ ID NO:100)
<i>CDC14B</i>	TAAACTTCGGGGGTGTGGTC (SEQ ID NO:101)	CAAAATCTGCGTAGAAGTTCTCAT (SEQ ID NO:102)
<i>CDK1</i>	AAGCCGGGATCTACCATACC (SEQ ID NO:103)	CATGGCTACCACTTGACCTGT (SEQ ID NO:104)
<i>CDK6</i>	GCTGGTAACTCCTTCCCCAG (SEQ ID NO:105)	GTCCAGAATCATTGCACCTGAG (SEQ ID NO:106)
<i>CHEK1</i>	AGGGATCAGCTTTTCCCAGC (SEQ ID NO:107)	CTCCAATCCATCACCTGATTC (SEQ ID NO:108)
<i>DMTF1</i>	TGGTGGACCATCAAAAGGCA (SEQ ID NO:109)	AGGAGAGTCTGCTGAAGAAACA (SEQ ID NO:110)
<i>E2F3</i>	TCTACACCACGCCGCAC (SEQ ID NO:111)	CCTTCGCTTTGCCGGAGG (SEQ ID NO:112)
<i>FBXW7</i>	AGTTTGTGTTGCCGGTTCTGC (SEQ ID NO:113)	TGGTCCAACCTTTCTTTTCATTTTGT (SEQ ID NO:114)
<i>HMGA1</i>	TGCTGCGCTCCTCTAATGG (SEQ ID NO:115)	GCAGGTGGAAGAGTGATGG (SEQ ID NO:116)
<i>KLF4</i>	CTGGGTCTTGAGGAAGTGCT (SEQ ID NO:117)	GGCATGAGCTCTTGGTAATGG (SEQ ID NO:118)
<i>KMT2A</i>	AAGGCGAAGTGGTTCCTGAG (SEQ ID NO:119)	AGGACGGCACTCCACTATCT (SEQ ID NO:120)
<i>PIM1</i>	CTGGGGAGAGCTGCCTAATG (SEQ ID NO:121)	GCTCCCTTTCCGTGATGAA (SEQ ID NO:122)
<i>PURA</i>	GGCGCTCAAAAGCGAGTTC (SEQ ID NO:123)	CTCCTCCACTCCGTAGTCGT (SEQ ID NO:124)
<i>RUNX1</i>	AATCGGCTTGTTGTGATGCG (SEQ ID NO:125)	GCCACCACCTTGAAAGCGAT (SEQ ID NO:126)
<i>ZYX</i>	CTGCTTCACCTGTGTGGTCT (SEQ ID NO:127)	GGCGTACTGCTTGTGGTAGT (SEQ ID NO:128)

Table 10: List of Three-Wise miRNA Hits that Both Inhibit Cell Proliferation and Increase Docetaxel Sensitivity in OVCAR8-ADR Cells Based on Pooled Screening with $\log_2$ ratio <-0.32 and $>20\%$ fewer barcode counts in experimental vs. control group

<i>miR-A</i>	<i>miR-B</i>	<i>miR-C</i>	Log₂ ratio – Day 4/Day1	Log₂ ratio – Drug/Vehicle
<i>miR-15b/miR-16-2</i> cluster	<i>miR-181a</i>	<i>miR-132</i>	-0.33	-0.32
<i>miR-451a/451b/144/4732</i> cluster	<i>miR-211</i>	<i>miR-132</i>	-0.35	-0.42
<i>miR-376a</i>	<i>miR-31</i>	<i>miR-488</i>	-0.34	-0.49

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30 human epithelial ovarian cancer. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7004–7009 (2008).

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention.

5 Accordingly, the foregoing description and drawings are by way of example only.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein,
10 those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. In addition, any combination of two or more of such features, systems, articles, materials, kits, and/or
15 methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

20 The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple
25 elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one
30 embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or,” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally,
5 additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e., “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or
10 “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily
15 including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least
20 one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one,
25 optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method
30 are recited. All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

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What is claimed is:

CLAIMS

1. A composition comprising one or more recombinant expression vectors encoding a combination of three microRNAs selected from the combinations set forth in Table 7 or
5 Table 10.
2. A composition comprising a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10.
- 10 3. The composition of claim 2, wherein the combination of three microRNAs are concatenated microRNAs, optionally with one or more linker and/or spacer sequence; conjugated to one or more nanoparticle, cell-permeating peptide, or polymer; or contained within a liposome.
- 15 4. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-16-1/15a cluster, let-7e/miR-99b cluster, and miR-128b.
5. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-181a, and miR-132.
20
6. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-211, and miR-132.
7. The composition of any one of claims 1-3, wherein the combination of three
25 microRNAs comprises miR-376a, miR-31, and miR-488.
8. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises mir-128b, mir-212, and let-7i or miR-451a/451b/144/4732 cluster.
- 30 9. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises mir128b, miR-451a/451b/144/4732 cluster, and miR-132 or miR-212.

10. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-128b, let-7i, and mir-212 or miR-196.

11. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-132, miR-15b/miR-16-2, and miR-31 or let-7i.

12. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-132, miR-451a/451b/144/4732 cluster, and miR-212 or miR-128b.

13. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-181c, let-7i, and miR-373 or miR-429.

14. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-181a, miR-429, and miR-29a or miR-31.

15. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-15b/miR-16-2, let-7i, and miR-132 or miR-181a.

16. The composition of any one of claims 1-3, wherein the combination of three microRNAs comprises miR-212, miR-451a/451b/144/4732 cluster, and miR-132 or miR-128b.

17. A composition comprising one or more recombinant expression vectors encoding a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10.

18. A composition comprising a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10.

19. The composition of claim 18, wherein the combination of two microRNAs or the combination of three microRNAs are concatenated microRNAs, optionally with one or more linker and/or spacer sequence; conjugated to one or more nanoparticle, cell-permeating peptide, or polymer; or contained within a liposome.

5

20 The composition of any one of claims 17-19, further comprising a chemotherapeutic agent.

21. The composition of claim 20, wherein the chemotherapeutic agent is an anti-
10 mitotic/anti-microtubule agent.

22. The composition of claim 21, wherein the anti-mitotic agent is docetaxel.

23. The composition of any one of claims 17-22, wherein the combination of three
15 microRNAs comprises miR-16-1/15a cluster, let-7e/miR-99b cluster, and miR-128b.

24. The composition of any one of claims 17-22, wherein the combination of three microRNA comprises miR-15b/miR-16-2 cluster, miR-181a, and miR-132.

20 25. The composition of any one of claims 17-22, wherein the combination of three microRNA comprises miR-451a/451b/144/4732 cluster, miR-211, and miR-132.

26. The composition of any one of claims 17-22, wherein the combination of three microRNA comprises miR-376a, miR-31, and miR-488.

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27. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-376a and any one of the miRNAs selected from the group consisting of miR-16-1/15a cluster, miR-212, and miR-31.

30 28. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-216 and any one of the miRNAs selected from the group consisting of miR-181c, let-7a, miR-15b/miR-16-2 cluster, and miR-181a.

29. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-31 and miR-181a or miR-376a.

5 30. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-93/106b cluster and miR-16-1/15a cluster or miR-181a.

31. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-181a and any one of the miRNAs selected from the group
10 consisting of miR-31, let-7i, miR-93/106b cluster, miR-373, miR-216, miR-15b/miR-16-2 cluster, and miR-16-1/15a cluster.

32. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-16-1/15a cluster and any one of the miRNAs selected from the
15 group consisting of miR-376a, miR-93/10b cluster, let-7a, miR-10b, miR-181a, miR-9-1, and miR-99a.

33. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-10b and any one of the miRNAs selected from the group
20 consisting of miR-16-1/15a cluster, miR-212, miR-196, and miR-15b/miR-16-2 cluster.

34. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-15b/miR-161-2 cluster and any one of the miRNAs selected
from the group consisting of miR-216, miR-181a, miR-9-1, and miR-10b.

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35. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR181c and miR-9-1 or miR-216.

36. The composition of any one of claims 17-22, wherein the combination of two
30 microRNAs comprises miR-212 and miR-376a or miR-10b.

37. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises miR-9-1 and any one of the miRNAs selected from the group consisting of miR-15b/miR-16-2 cluster, miR-16-1/15a cluster, miR-324, and miR-181c.

5 38. The composition of any one of claims 17-22, wherein the combination of two microRNAs comprises let-7a and miR-16-1/15a cluster or miR-216.

39. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7c, miR-451a/451b/144/4732 cluster, and miR-324 or miR376a.

10

40. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7d, miR-181c, and miR-10b or miR-9-1.

41. The composition of any one of claims 17-22, wherein the combination of three
15 microRNAs comprises let-7e/miR-99b cluster, miR-15b/miR-16-2 cluster, and miR-181a or miR-16-1/miR-15a cluster.

42. The composition of any one of claims 17-22, wherein the combination of three
20 microRNAs comprises let-7e/miR-99b cluster, miR-16-1/15a cluster and miR-15b/miR-16-2 cluster or miR-181c.

43. The composition of any one of claims 17-22, wherein the combination of three
25 microRNAs comprises let-7e/miR-99b cluster, miR-181a, and miR-324 or miR-15b/miR-16-2 cluster.

44. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-181c, and miR-429 or miR-16-1/15a cluster.

30 45. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7e/miR-99b cluster, miR-376a, and miR-199b/3154 cluster or miR-188.

46. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7i, miR-15b/miR-16-2 cluster, and miR-451a/451b/144/4732 cluster or let-7c.

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47. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises let-7i, miR-199b/3154 cluster, and miR-10b or miR-29a.

48. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-10b, miR-15b/miR-16-2 cluster, and any one of the miRNAs selected from the group consisting miR-373, miR-211, and miR-126.

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49. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-10b, miR-373, and miR-15b/miR-16-2 cluster or miR-451a/451b/144/4732 cluster.

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50. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-10b, miR-451a/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of miR-373, miR-429, and miR-708.

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51. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-126, miR-15b/miR-16-2 cluster, and miR-10b or miR-181a.

52. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-126, miR-181a, and miR-451a/451b/144/4732 cluster or miR-15b/miR-16-2 cluster.

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53. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-126, miR-181c, and miR-451a/451b/144/4732 cluster or miR-29a.

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54. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-126, miR-29a, and miR-211 or miR-181c.

55. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-126, miR-451a/451b/144/4732 cluster, and miR-181a or miR-181c.

56. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-128b, miR-16-1/15a cluster, and miR-181c or miR-31.

57. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-128b, miR-31, and miR-24-2/27a/23a cluster or miR-16-1/15a cluster.

58. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-128b, miR-324, and miR-216 or miR-188.

59. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-16-1/15a cluster, and any one of the microRNAs selected from the group consisting of miR-216, miR-429, miR-451a/451b/144/4732 cluster, and let-7e/miR-99b cluster.

60. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-181a, and any one of the microRNAs selected from the group consisting of miR-9-1, miR-126, miR-489, let-7e/miR-99b cluster, miR-216, and miR-488.

61. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-181c, and miR-328 or miR-488.

62. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-216, and any one of the microRNAs selected from the group consisting of miR-373, miR-16-1/15a cluster, and miR-181a.

5 63. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-373, and any one of the microRNAs selected from the group consisting of miR-216, miR-9-1, and miR-10b.

64. The composition of any one of claims 17-22, wherein the combination of three
10 microRNAs comprises miR-15b/miR-16-2 cluster, miR-376a, and miR-24-2/27a/23a cluster or miR-324.

65. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-451a/451b/144/4732 cluster, and any
15 one of the microRNAs selected from the group consisting of let-7a, miR-16-1/15a cluster, miR-708, and let-7i.

66. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-488, and miR-181a or miR-181c.

20

67. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-15b/miR-16-2 cluster, miR-489, and miR-128b or miR-181a.

68. The composition of any one of claims 17-22, wherein the combination of three
25 microRNAs comprises miR-15b/miR-16-2 cluster, miR-9-1, and miR-181a or miR-373.

69. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-16-1/15a cluster, miR-181c, and any one of the microRNAs selected from the group consisting of miR-489, miR-211, let-7e/miR-99b cluster, miR-128b,
30 and miR-29a.

70. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-16-1/15a cluster, miR-216, and miR-126 or miR-15b/miR-16-2 cluster.

5 71. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-16-1/15a cluster, miR-451/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of miR-489, miR-15b/miR-16-2 cluster, and miR-328.

10 72. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-16-1/15a cluster, miR-489, and miR-181c or miR-451/451b/144/4732 cluster.

15 73. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-216, and any one of the microRNAs selected from the group consisting of miR-489, miR-15b/miR-16-2 cluster, and let-7i.

20 74. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-324, and any one of the microRNAs selected from the group consisting of miR-708, miR-31, and let-7e/miR-99b cluster.

75. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-376a, and miR-24-2/27a/23a cluster or miR-29c.

25 76. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-451a/451b/144/4732 cluster, and miR-126 or miR-128b.

30 77. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-488, and miR-15b/miR-16-2 cluster or miR-29a.

78. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181a, miR-489, and miR-15b/miR-16-2 cluster or miR-216.

79. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-29a, and any one of the microRNAs selected from the group consisting of miR-126, miR-16-1/15a cluster and miR-9-1.

80. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-29c, and miR-31 or miR-324.

81. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-31, and any one of the microRNAs selected from the group consisting of miR-328, miR-29c, and miR-99a.

82. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-324, and miR-129-2 or miR-29c.

83. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-328, and miR-15b/miR-16-2 cluster or miR-31.

84. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-376a, and miR-708 or miR-212.

85. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-451a/451b/144/4732 cluster, and any one of the microRNAs selected from the group consisting of miR-126, miR-196, and miR-9-1.

86. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-488, and miR-15b/miR-16-2 cluster or miR-132.

87. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-181c, miR-9-1, and any one of the microRNAs selected from the group consisting of miR-451a/451b/144/4732 cluster, let-7d, and miR-29a.

5 88. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-24-2/27a/23a cluster, miR-37a, and any one of the microRNAs selected from the group consisting of miR-328, miR-181a and miR-15b/miR-16-2 cluster.

10 89. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-29a, miR-199b/3154 cluster, and let-7i or let-7c.

90. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-29a, miR-9-1, and miR-181c or miR-451a/451b/144/4732 cluster.

15

91. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-31, miR-376a, and miR-16-1/15a cluster or miR-488.

20 92. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-328, miR-451a/451b/144/4732 cluster, and let-7e/miR-99b cluster or miR-16-1/15a cluster.

93. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-373, miR-451a/451b/144/4732 cluster, and miR-10b or miR-708.

25

94. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-376a, miR-451a/451b/144/4732 cluster, and let-7c or miR-9-1.

30 95. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-708, and any one of the microRNAs selected from the group consisting of miR-10b, miR-15b/miR-16-2 cluster, and miR-373.

96. The composition of any one of claims 17-22, wherein the combination of three microRNAs comprises miR-451a/451b/144/4732 cluster, miR-9-1, and any one of the microRNAs selected from the group consisting of miR-181c, miR-29a, and miR-376a.

5

97. A method for enhancing sensitivity of a cell to a chemotherapeutic agent, comprising contacting the cell with a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10.

10

98. The method of claim 97, further comprising contacting the cell with the chemotherapeutic agent.

99. The method of claim 97 or 98, wherein the cell is a cancer cell.

15

100. The method of any one of claims 97-99, wherein the combination of microRNAs are expressed from one or more recombinant expression vectors.

101. A method for treating cancer in a subject, comprising administering to the subject a combination of two microRNAs selected from the combinations set forth in Table 3 or a combination of three microRNAs selected from the combinations set forth in Table 5 or Table 10 and a chemotherapeutic agent in an effective amount.

20

102. The method of claim 101, wherein administering a combination of microRNAs comprises expressing the combination of microRNAs from one or more recombinant RNA expression vectors.

25

103. The method of claim 101 or 102, wherein the effective amount of the chemotherapeutic agent administered with the combination of microRNAs is less than the effective amount of the chemotherapeutic agent when administered without the combination of microRNAs.

30

104. The method of any one of claims 97-103, wherein the combination of microRNAs comprises a combination of microRNAs as set forth in any of claims 23-96.

105. A method for reducing cell proliferation, comprising contacting a cell with a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10.

106. The method of claim 105, wherein the cell is a cancer cell.

107. The method of claim 105 or 106, wherein the combination of microRNAs are expressed from one or more recombinant expression vectors.

108. A method of treating cancer in a subject, comprising administering to the subject a combination of three microRNAs selected from the combinations set forth in Table 7 or Table 10.

109. The method of claim 108, wherein administering a combination of microRNAs comprises expressing the combination of three microRNAs from one or more recombinant expression vectors.

110. The method of any one of claims 105-109, wherein the combination of microRNAs comprises a combination of microRNAs as set forth in any of claims 4-16.

111. A method for identifying a combination of microRNAs that enhances sensitivity of a cell to an agent, the method comprising:

contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector;

contacting the first population of cells with an agent, wherein the second population of cells is not contacted with the agent;

identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells;

comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells;

identifying a combination of two or more microRNAs that is absent from or has
5 reduced abundance in the first population of cells relative to the abundance of the same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances sensitivity a cell to the agent.

112. The method of claim 111, wherein the combinations of microRNAs that enhance
10 sensitivity of a cell to the agent are compared to the combinations of microRNAs that reduce cell proliferation to identify the combinations of microRNAs that enhance sensitivity of a cell to the agent and reduce cell proliferation.

113. A method for identifying a combination of microRNAs that enhances resistance of a
15 cell to an agent, the method comprising:

contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector;

contacting the first population of cells with an agent, wherein the second population
20 of cells is not contacted with the agent;

identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells;

comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the
25 second population of cells;

identifying a combination of two or more microRNAs that has increased abundance in the first population of cells relative to the abundance same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances resistance of a cell to the agent.

114. The method of any one of claims 111-113, wherein the agent is a cytotoxic agent.

115. The method of claim 114, wherein the cytotoxic agent is a chemotherapeutic agent.

116. The method of claim 114, wherein the chemotherapeutic agent is an anti-mitotic/anti-microtubule agent.

5

117. The method of claim 116, wherein the chemotherapeutic agent is docetaxel.

118. A method for identifying a combination of microRNAs that reduces cell proliferation, the method comprising:

10 contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector;

 culturing the first population of cells and the second population of cells such that the second population of cells is cultured for a longer duration compared to the first population of
15 cells;

 identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells;

 comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the
20 second population of cells;

 identifying a combination of two or more microRNAs that is absent from or in reduced abundance in the second population of cells but present in or in increased abundance in the first population of cells as a combination of microRNAs that reduces cell proliferation.

25 119. The method of claim 118, wherein the combinations of microRNAs that reduce cell proliferation are compared to the combinations of microRNAs that enhance sensitivity of a cell to an agent to identify the combinations of microRNAs that reduce cell proliferation and enhance sensitivity of a cell to the agent.

30 120. A method for identifying a combination of microRNAs that enhances cell proliferation, the method comprising:

contacting a first population of cells and a second population of cells with a plurality of combinations of two or more microRNAs expressed from a recombinant expression vector;

5 culturing the first population of cells and the second population of cells such that the second population of cells is cultured for a longer duration compared to the first population of cells;

identifying the combinations of two or more microRNAs in the first population of cells and the combinations of two or more microRNAs in the second population of cells;

10 comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells;

identifying a combination of two or more microRNAs that is present in or in increased abundance in the second population of cells but absent from or in reduced abundance in the first population of cells as a combination of microRNAs that enhances cell proliferation.

15 121. The method of any one of claims 111-120, wherein the microRNA expression vector is delivered to the first population of cells and/or the second population of cells by a virus.

20 122. The method of claim 121, wherein the virus is a lentivirus.

123. A method for determining a synergistic or antagonistic interaction of a combination of miRNAs on sensitivity of a cell to an agent and cell proliferation, comprising

(1) contacting a first population of cells, a second population of cells, a third population of cells and a fourth population of cells with a plurality of combinations of two or more

25 microRNAs expressed from a recombinant expression vector;

(2) (a) contacting the first population of cells with an agent, wherein the second population of cells is not contacted with the agent;

(b) culturing the third population of cells and the fourth population of cells such that the fourth population of cells is cultured for a longer duration compared to the third
30 population of cells;

(3) identifying the combinations of two or more microRNAs in the first population of cells, the second population of cells, the third population of cells and the fourth population of cells;

(4) (a) comparing the abundance of each combination of two or more microRNAs in the first population of cells to the abundance of each combination of two or more microRNAs in the second population of cells;

(b) comparing the abundance of each combination of two or more microRNAs in the third population of cells to the abundance of each combination of two or more microRNAs in the fourth population of cells;

(5) (a) (1) identifying a combination of two or more microRNAs that is absent from or has reduced abundance in the first population of cells relative to the abundance of the same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances sensitivity a cell to the agent; and

(2) identifying a combination of two or more microRNAs that has increased abundance in the first population of cells relative to the abundance same combination of two or more microRNAs in the second population of cells as a combination of microRNAs that enhances resistance of a cell to the agent

(b) (1) identifying a combination of two or more microRNAs that is absent from or in reduced abundance in the fourth population of cells but present in or in increased abundance in the third population of cells as a combination of microRNAs that reduces cell proliferation, and

(2) identifying a combination of two or more microRNAs that is present in or in increased abundance in the fourth population of cells but absent from or in reduced abundance in the third population of cells as a combination of microRNAs that enhances cell proliferation;

(6) calculating a genetic interaction score for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation;

(7) calculating an expected phenotype value for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation; and

(8) comparing the genetic interaction score for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation with the expected

phenotype value for the effect of each combination of microRNAs on sensitivity of a cell to an agent and cell proliferation, wherein a genetic interaction score greater than the expected phenotype value indicates a synergistic interaction between the microRNAs of the combination, or wherein a genetic interaction score less than the expected phenotype value indicates an antagonistic interaction between the microRNAs of the combination.

124. The method of claim 123, wherein the expected phenotype value is calculated based on the additive model or the multiplicative model.

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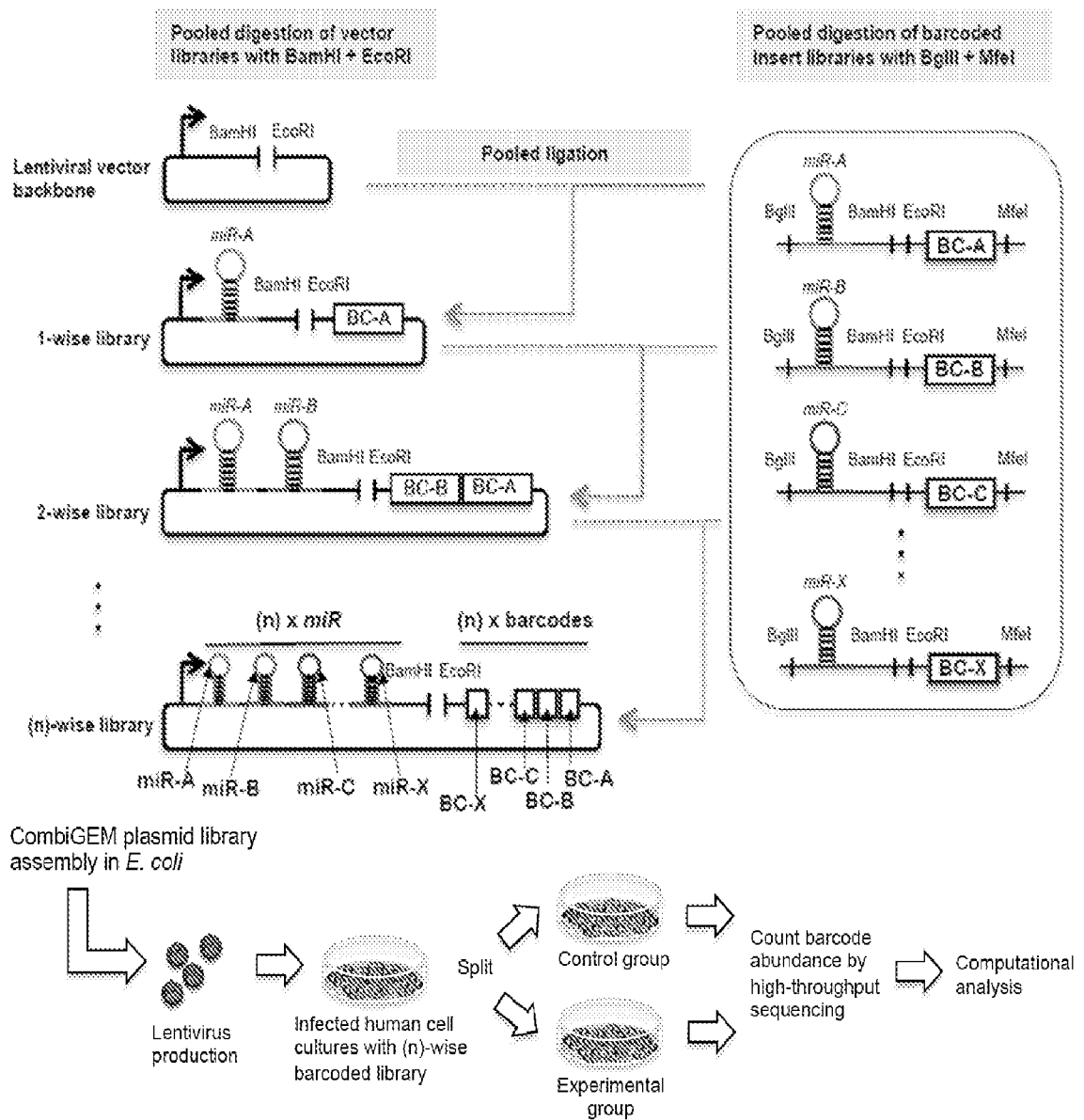


FIG. 1

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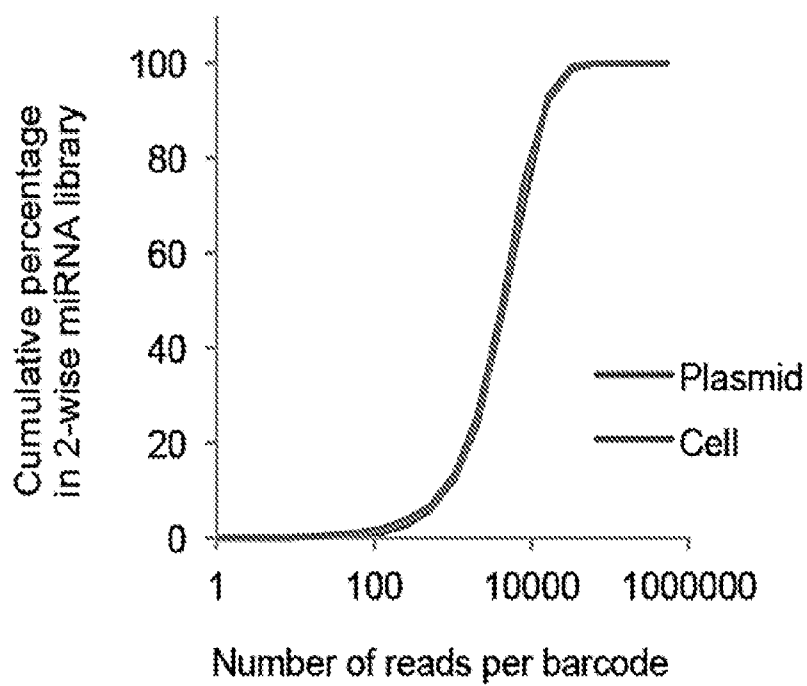


FIG. 2A

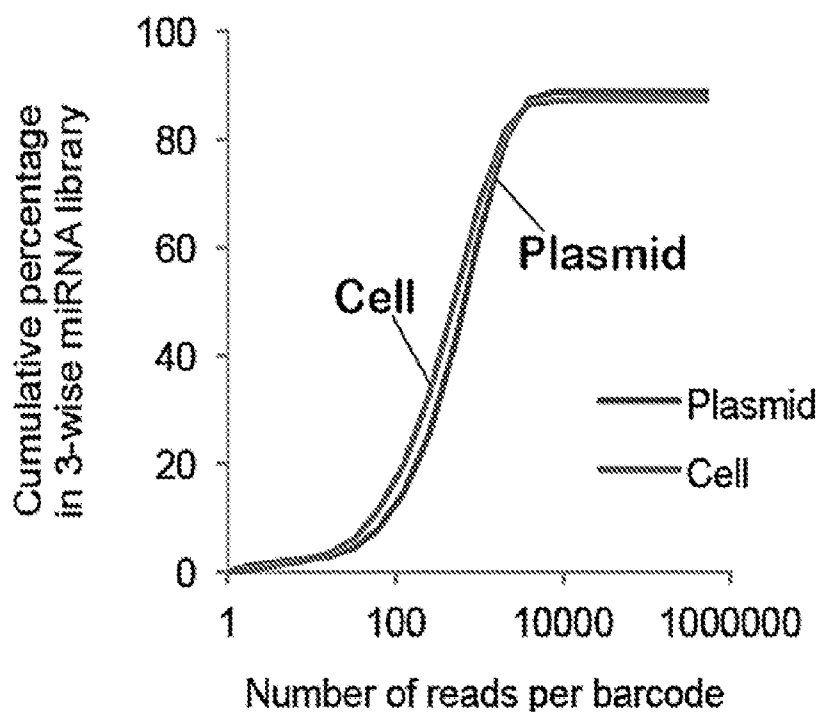


FIG. 2B

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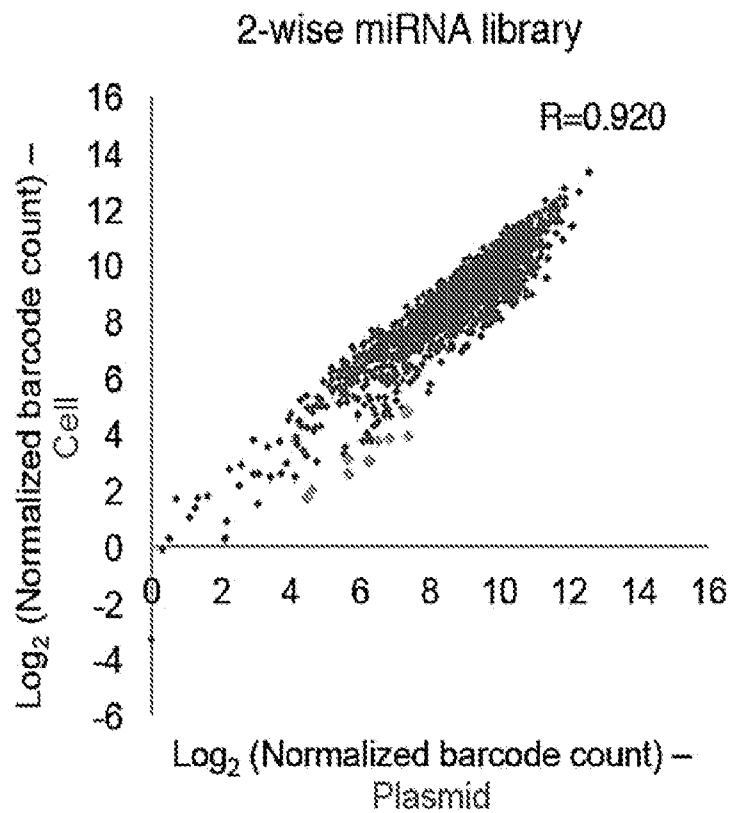


FIG. 2C

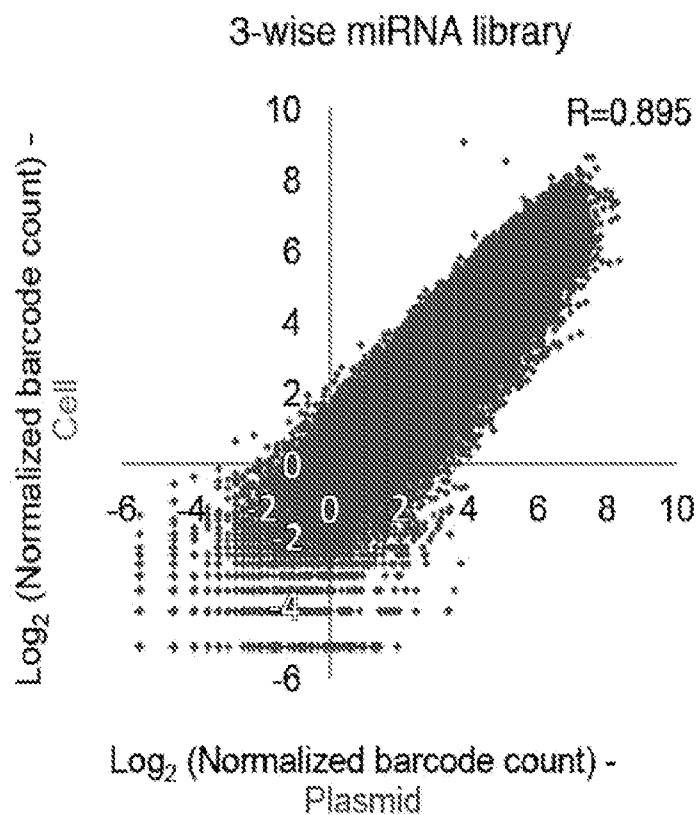


FIG. 2D

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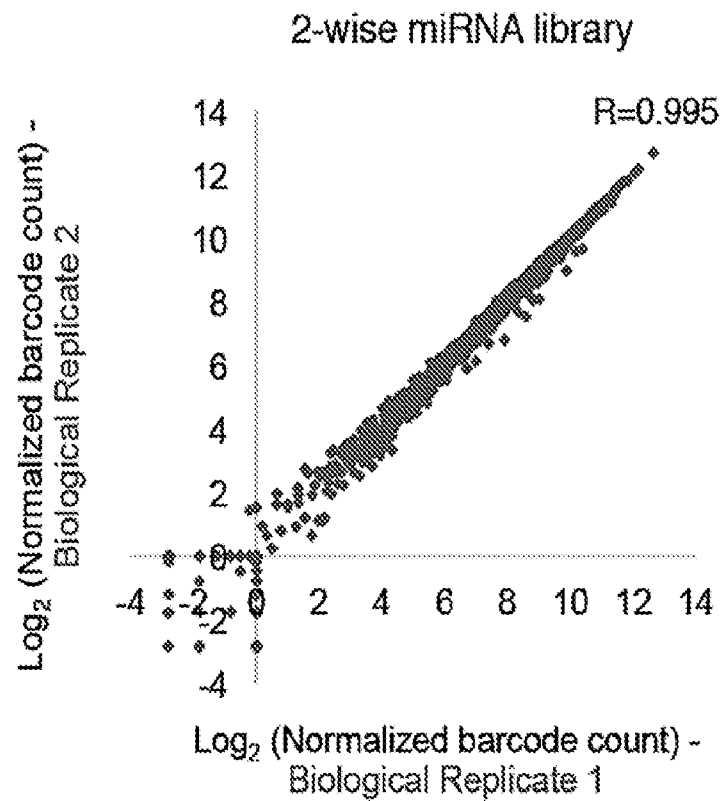


FIG. 2E

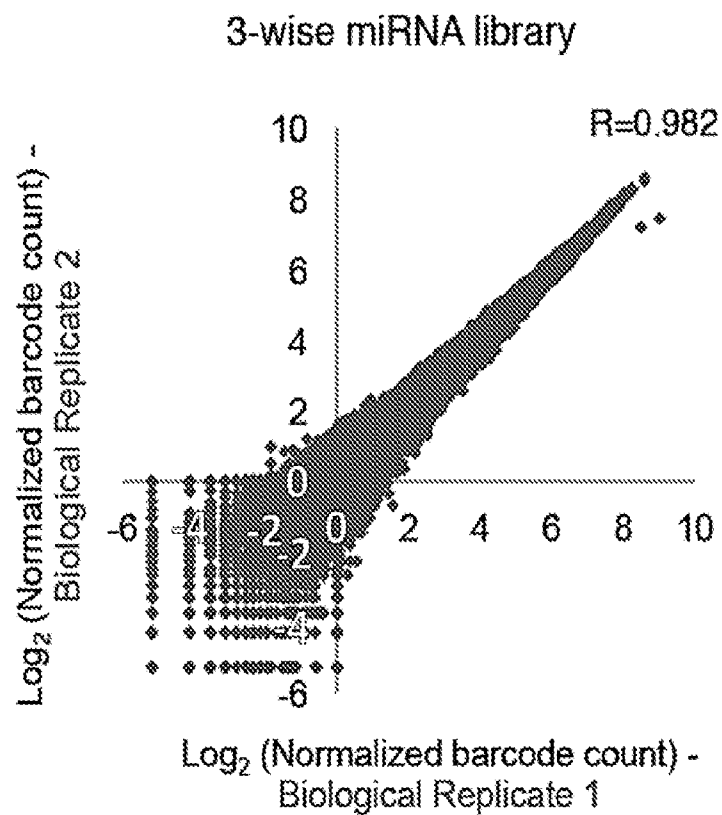


FIG. 2F

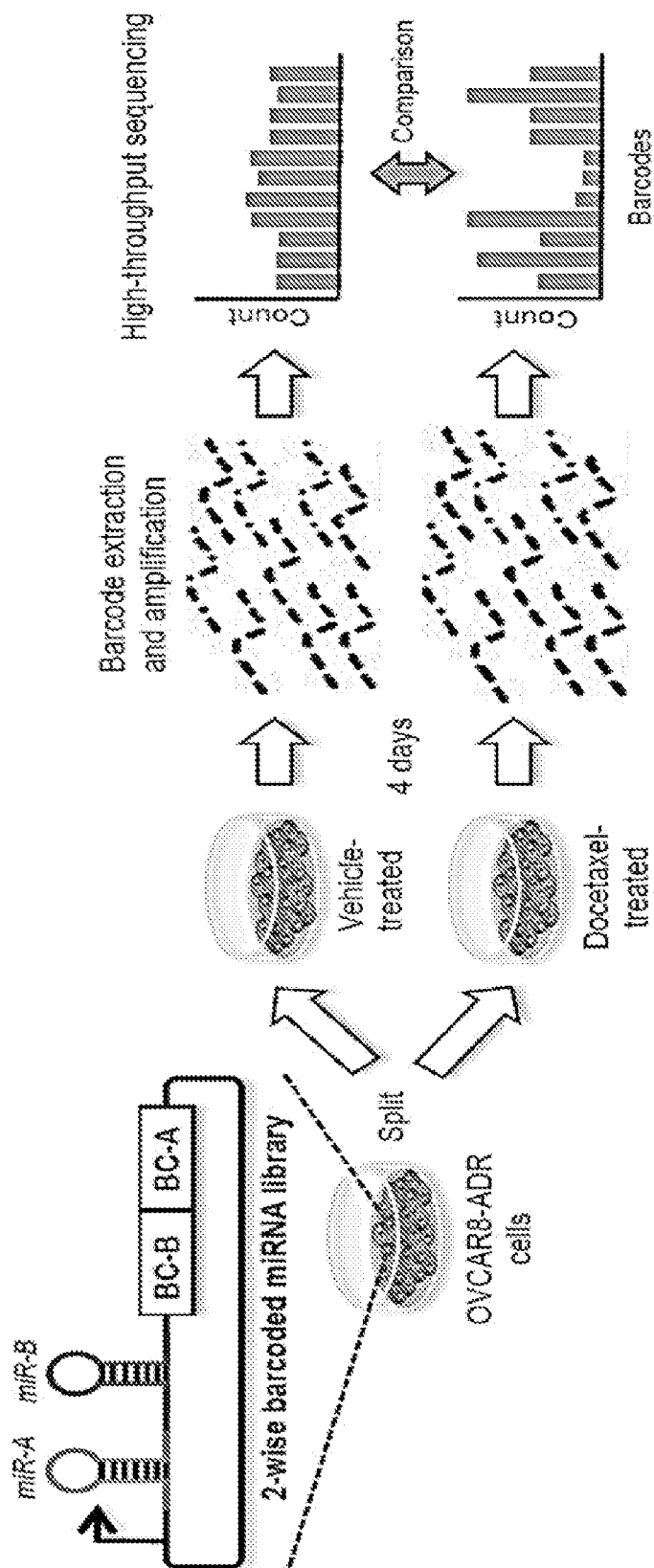


FIG. 3A

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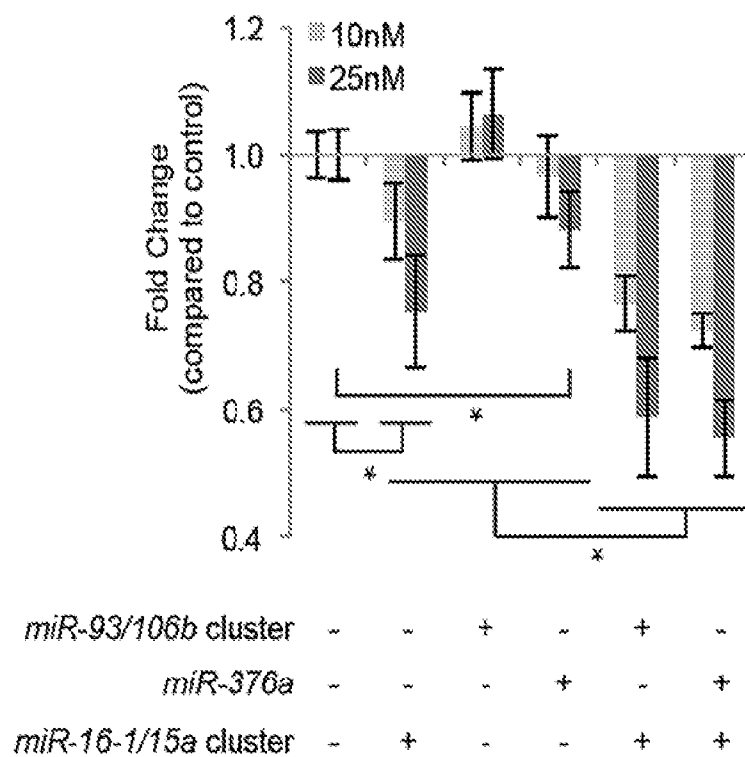
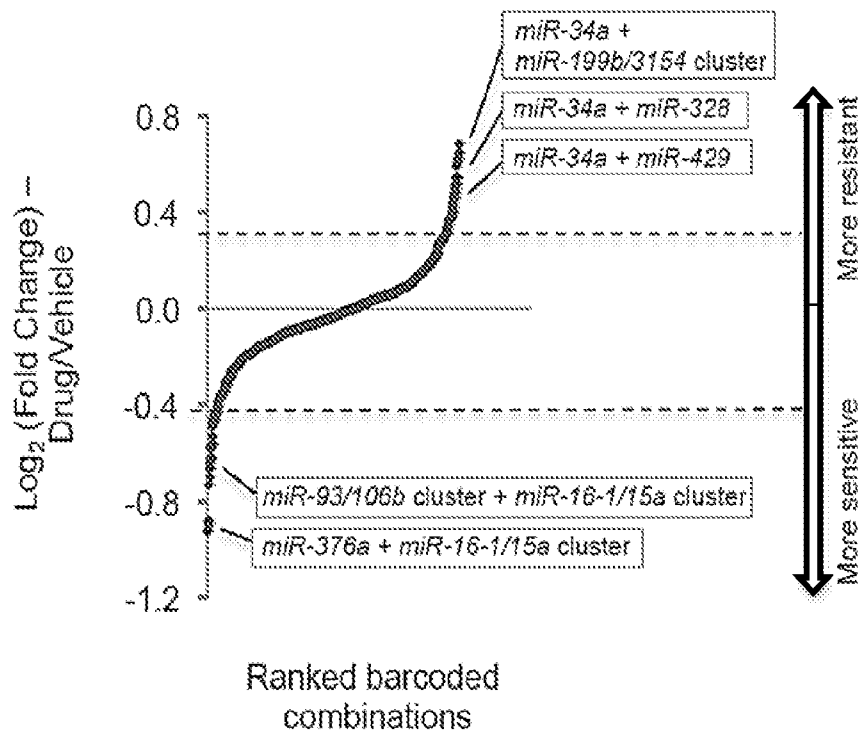


FIG. 3C

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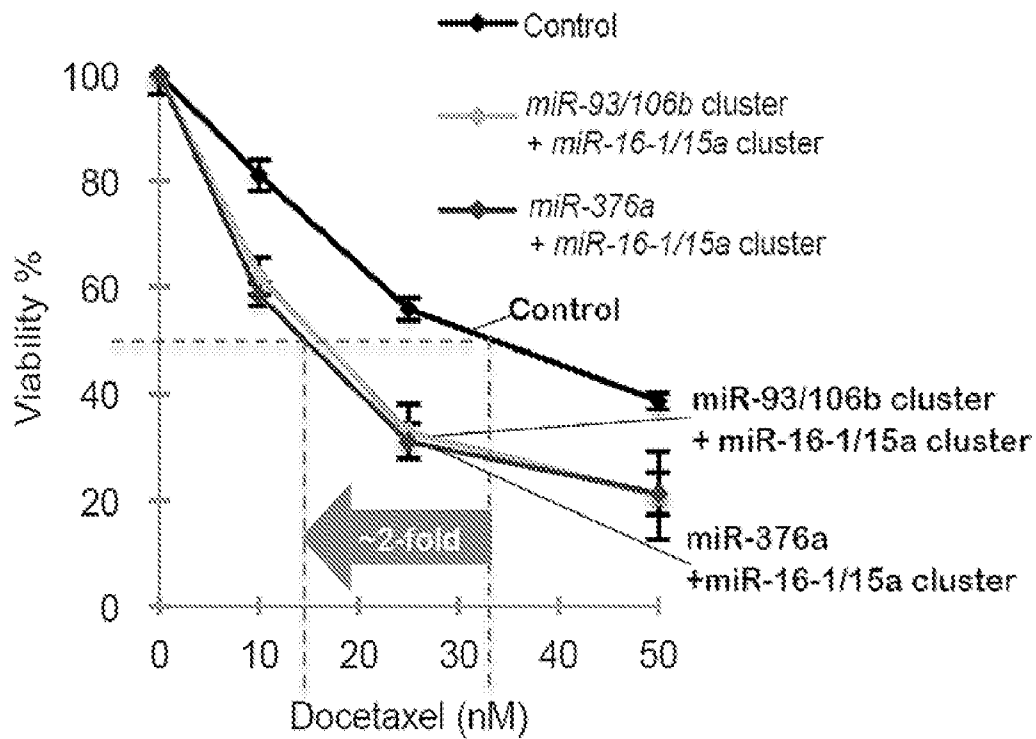


FIG. 3D

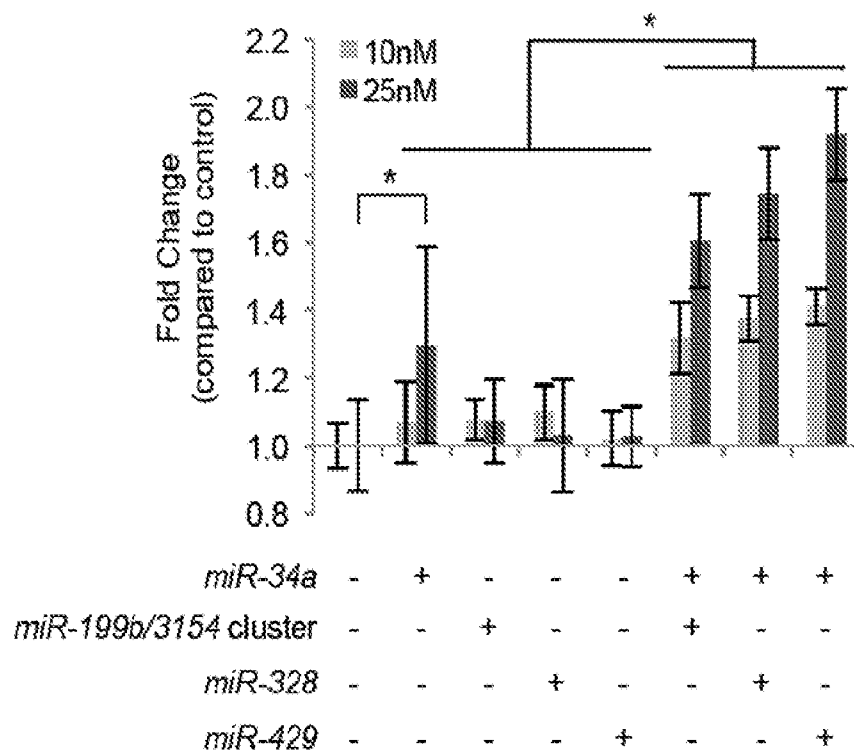


FIG. 3E

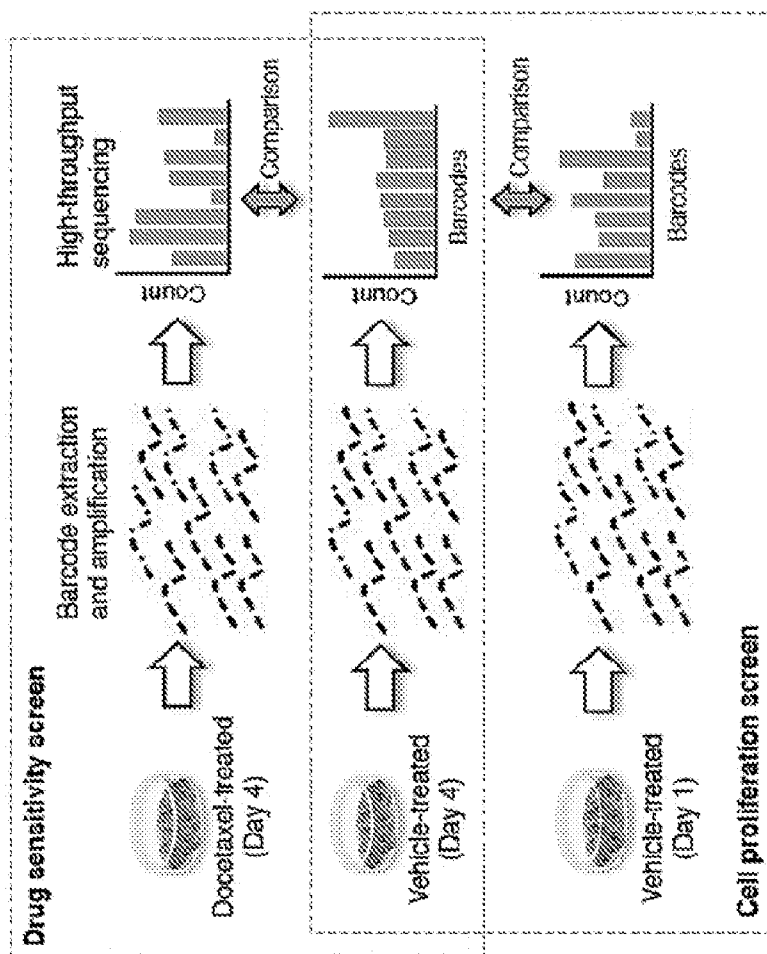
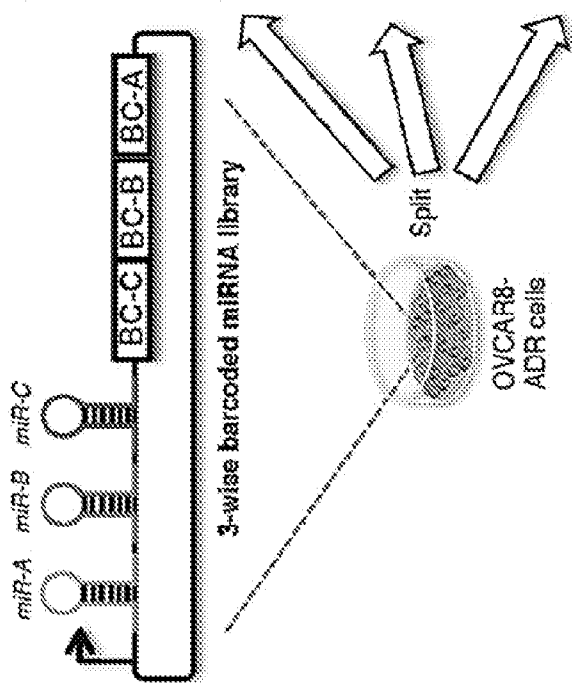


FIG. 4A



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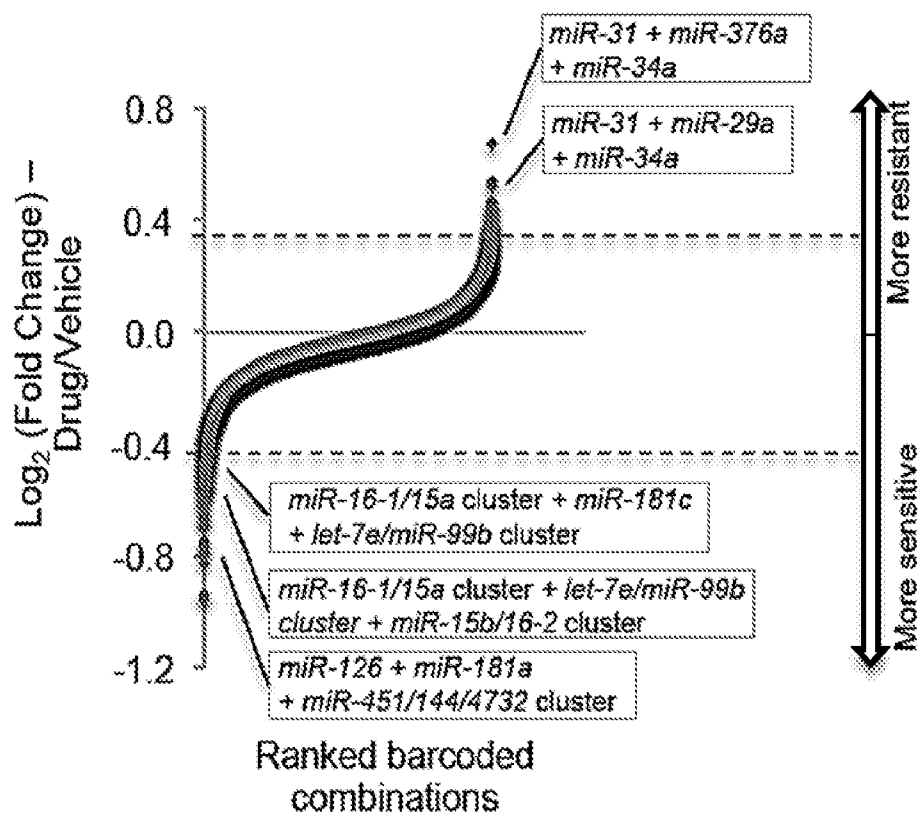


FIG. 4B

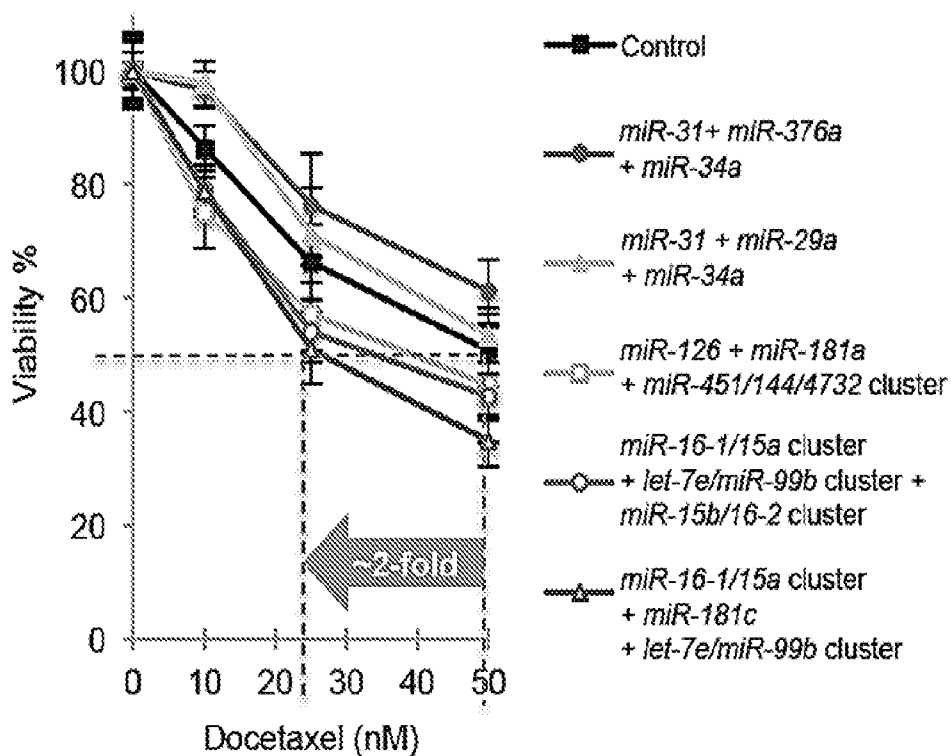


FIG. 4C

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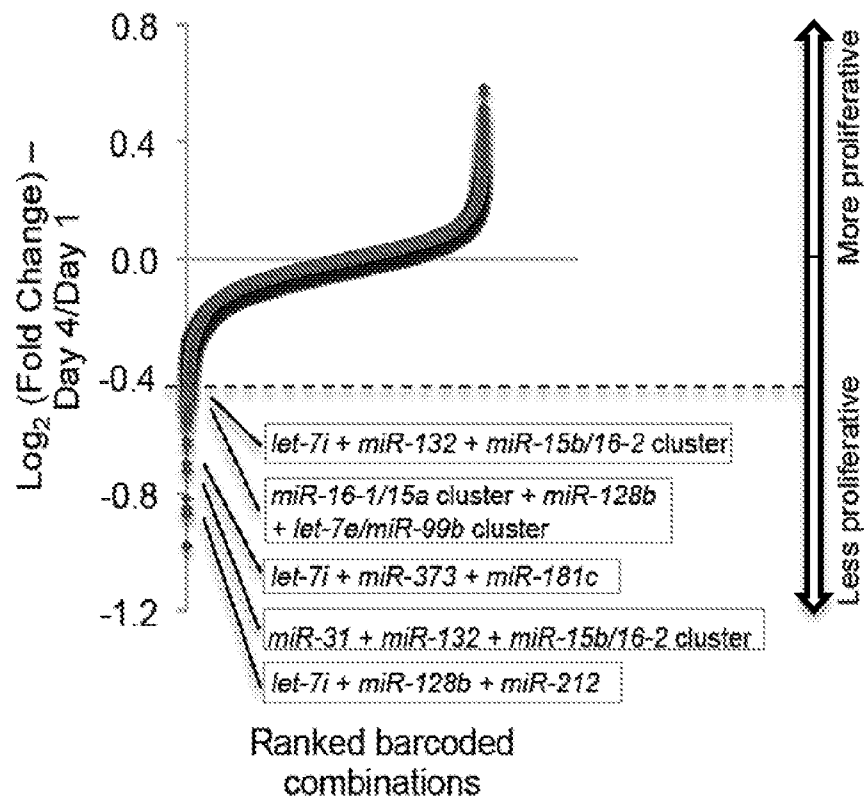


FIG. 4D

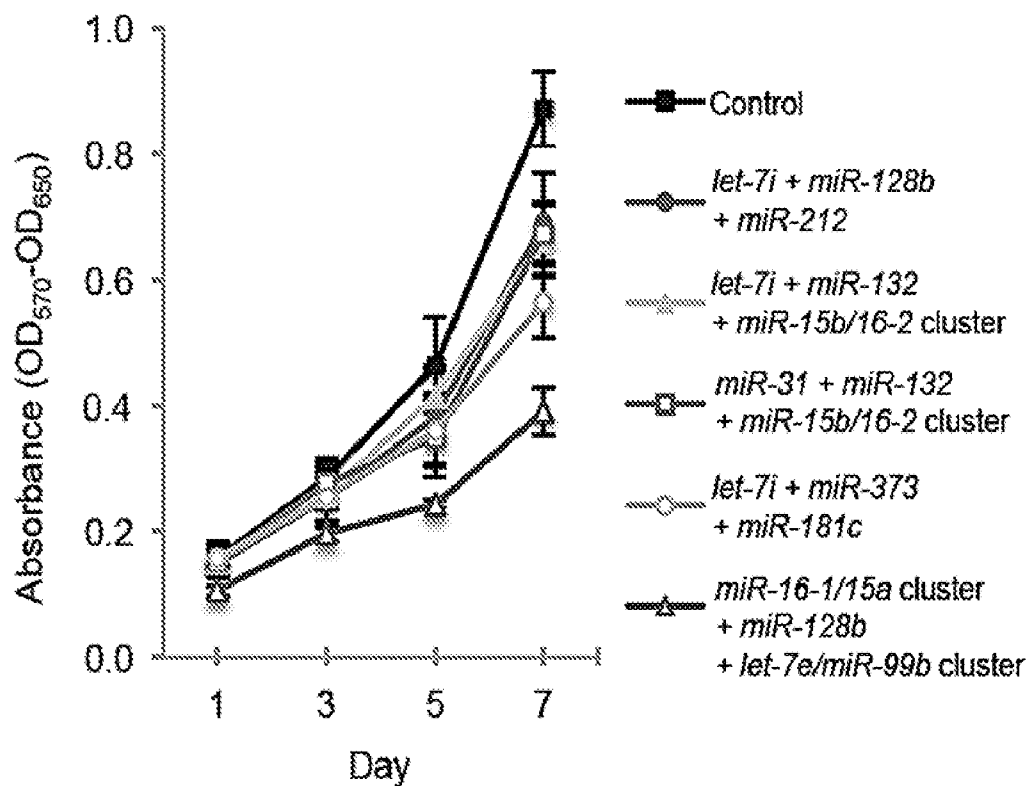


FIG. 4E

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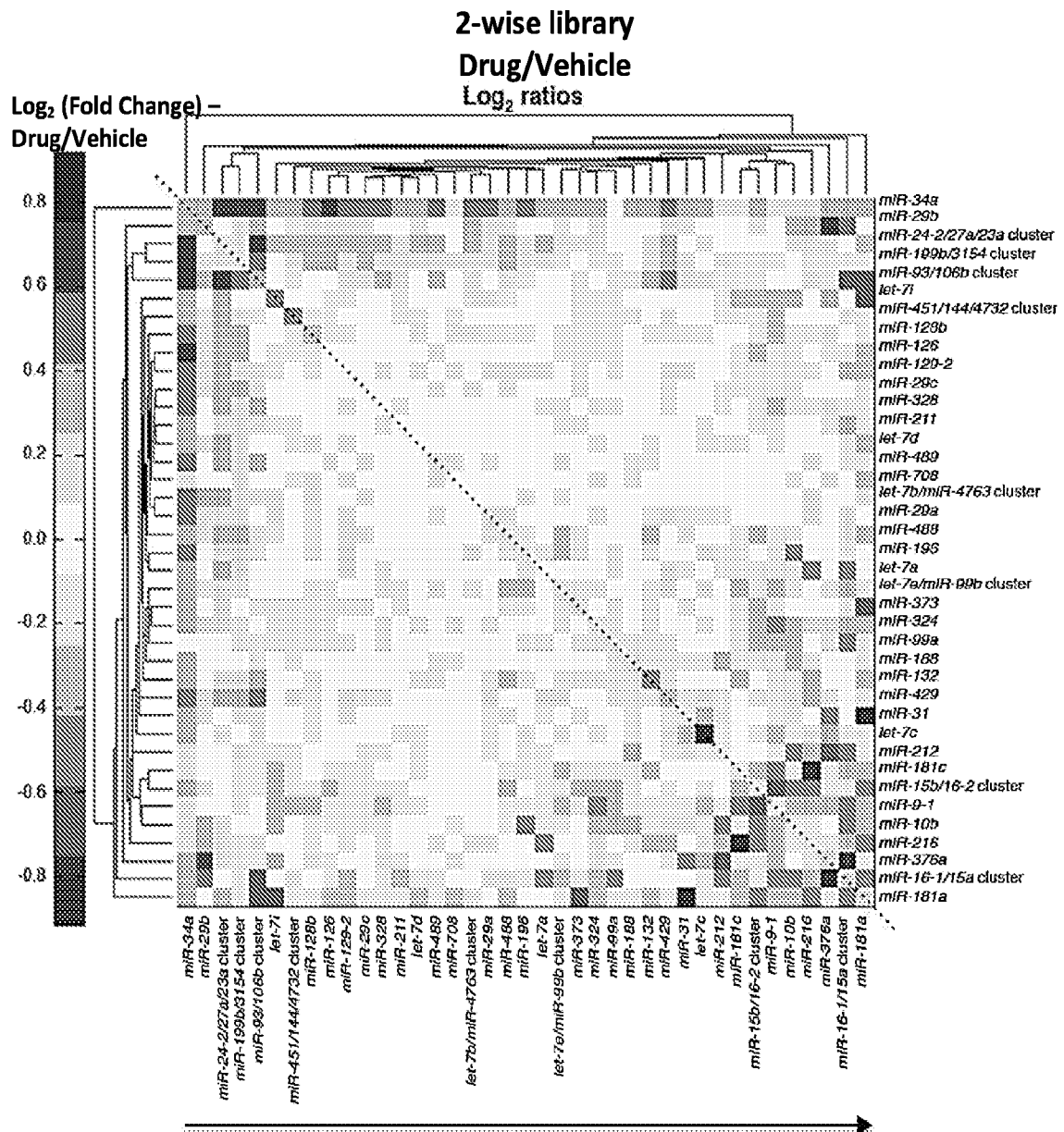


FIG. 5A

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Genetic interaction map

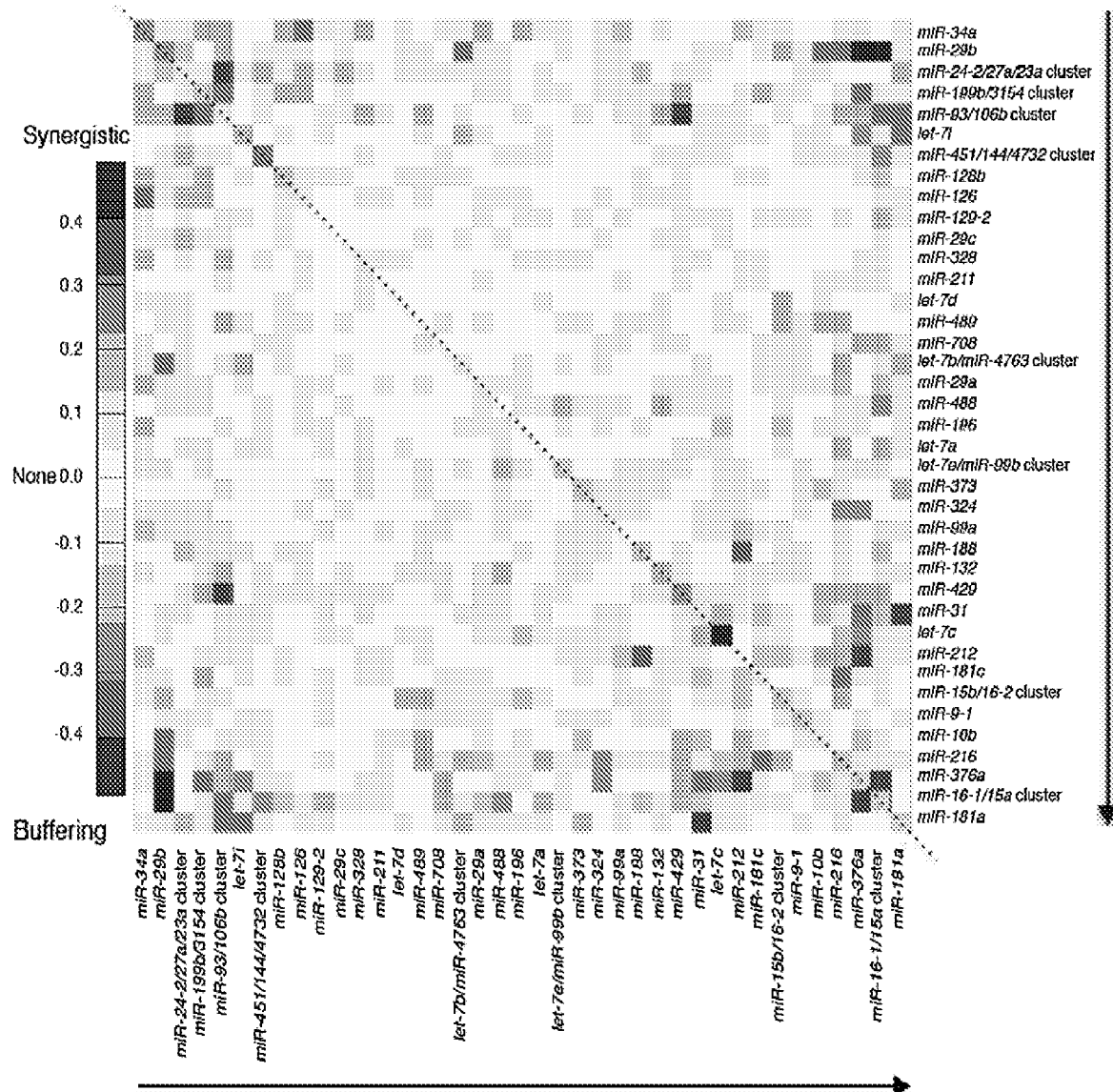


FIG. 5A (Continued)

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3-wise library Drug/Vehicle

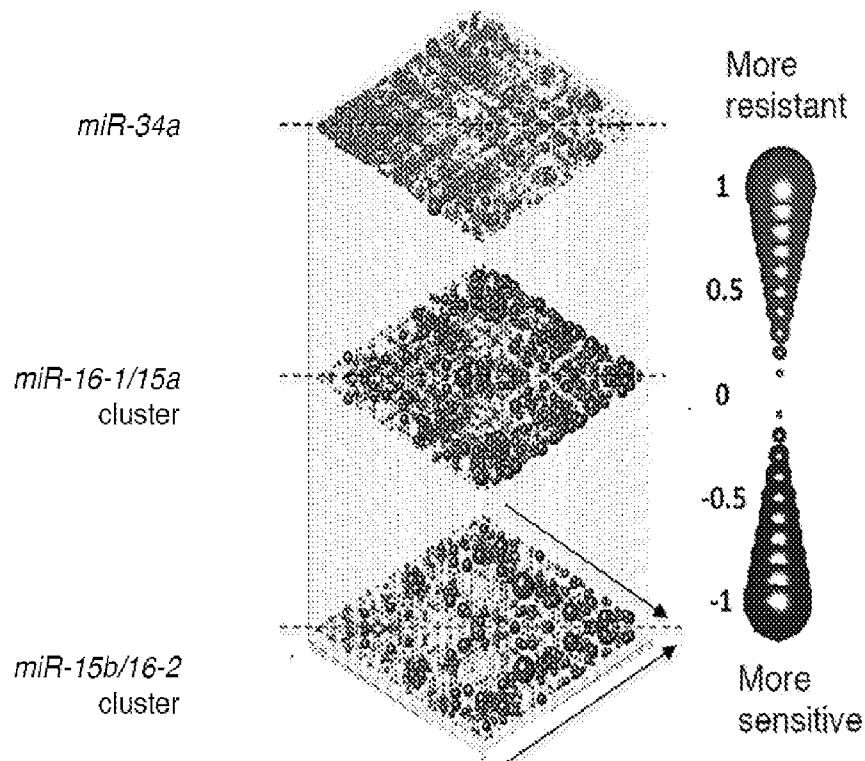


FIG. 5B

3-wise library Day 4/Day 1

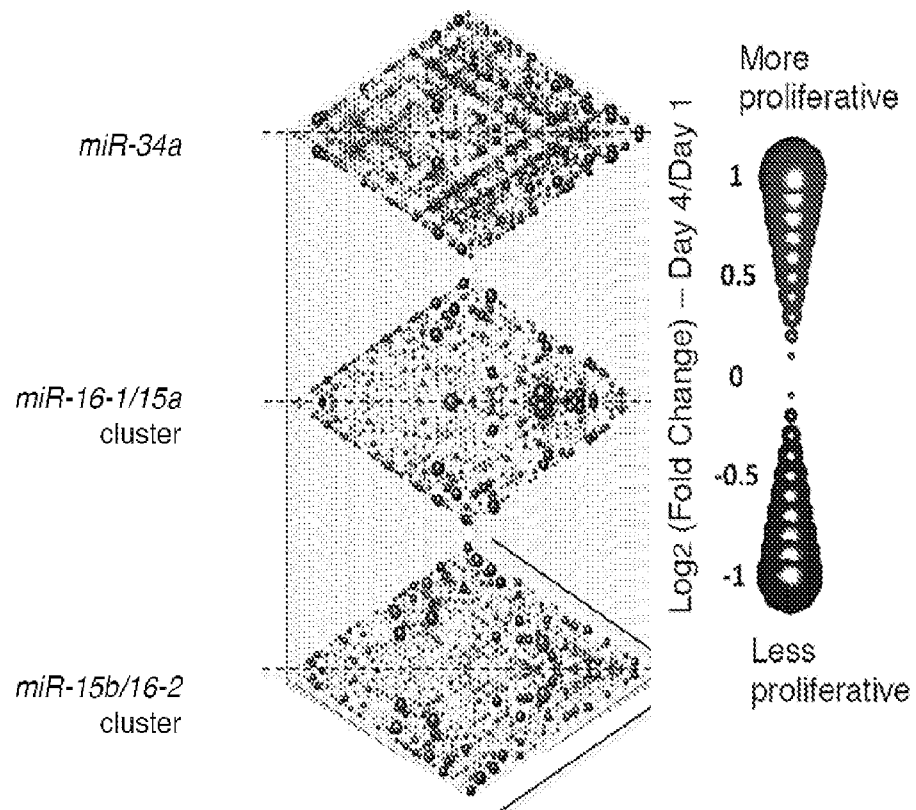


FIG. 5C

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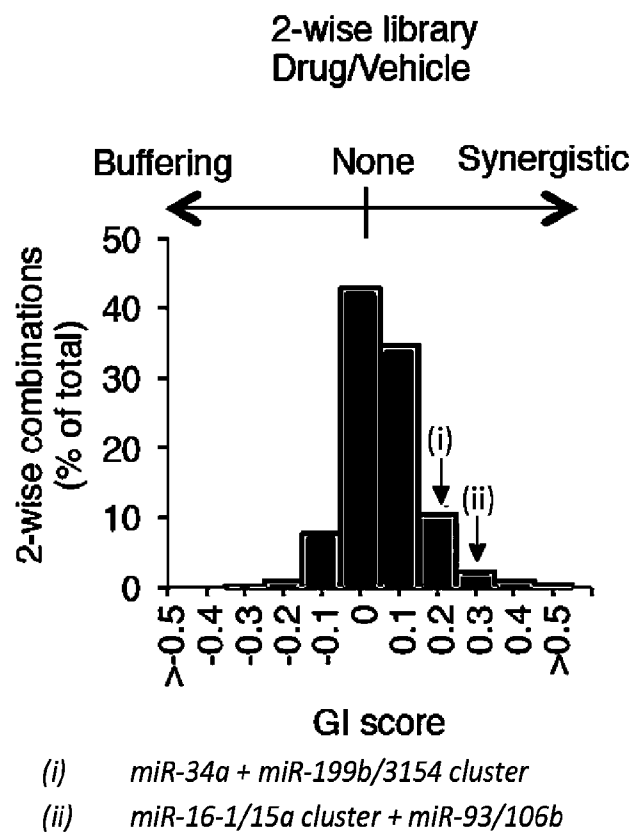


FIG. 5D

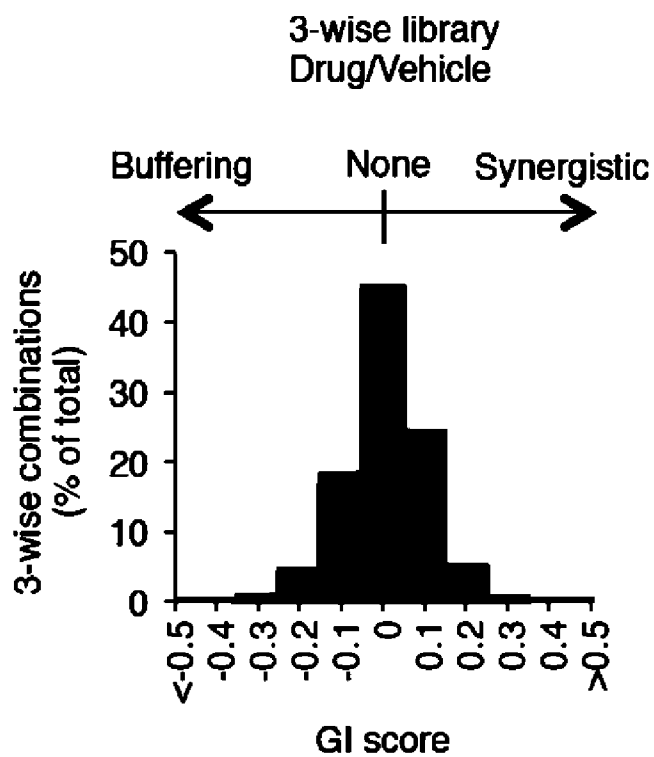
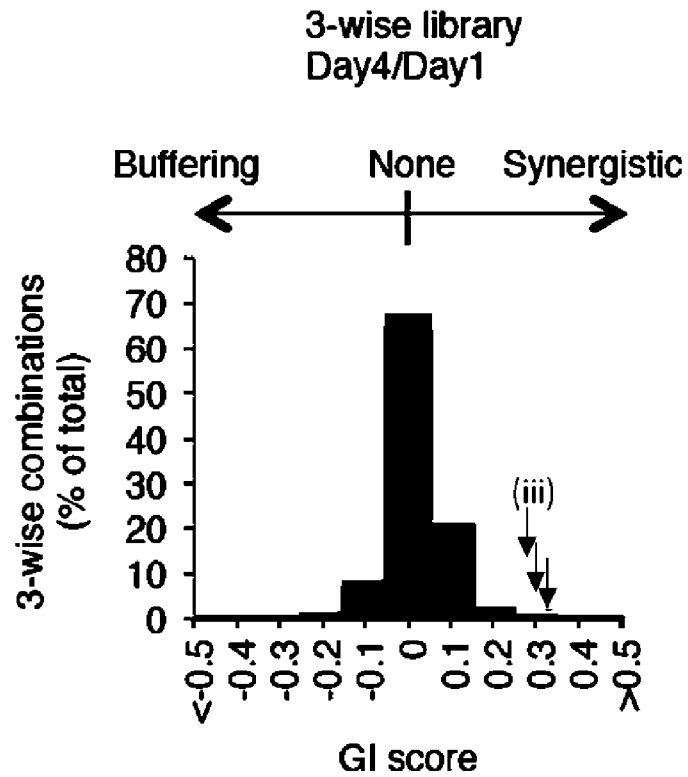


FIG. 5E

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(iii) *miR-16-1/15a cluster + miR-128b + let-7e/miR-99b cluster*

FIG. 5F

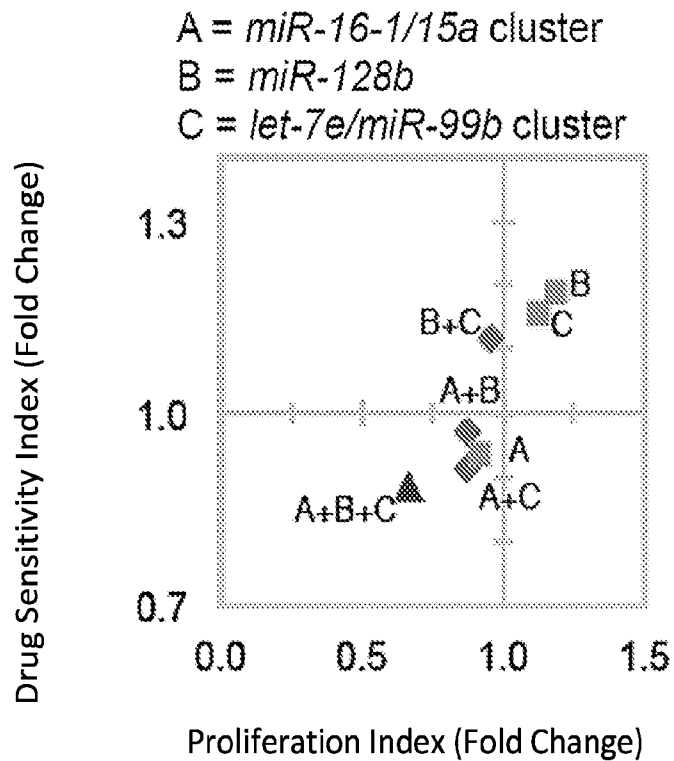


FIG. 6A

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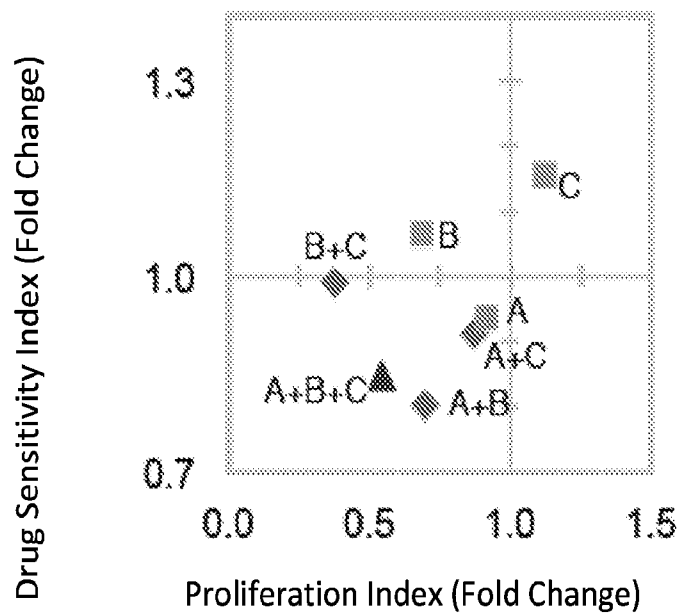
A = *miR-16-1/15a* clusterB = *miR-181c*C = *let-7e/miR-99b* cluster

FIG. 6B

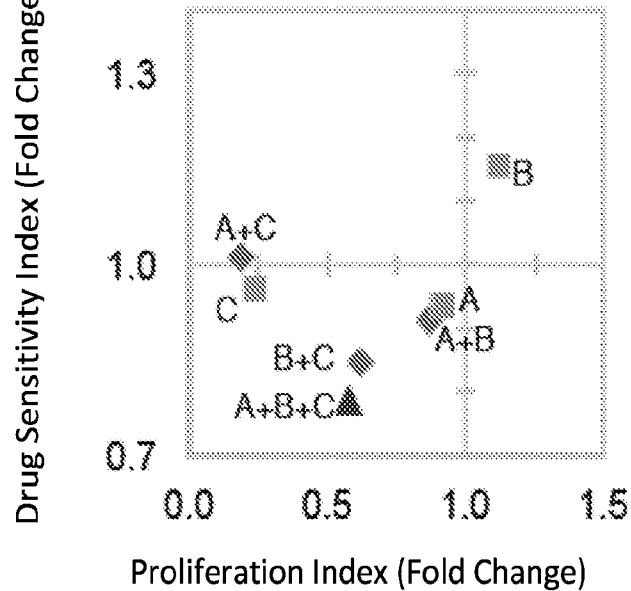
A = *miR-16-1/15a* clusterB = *let-7e/miR-99b* clusterC = *miR-15b/16-2* cluster

FIG. 6C

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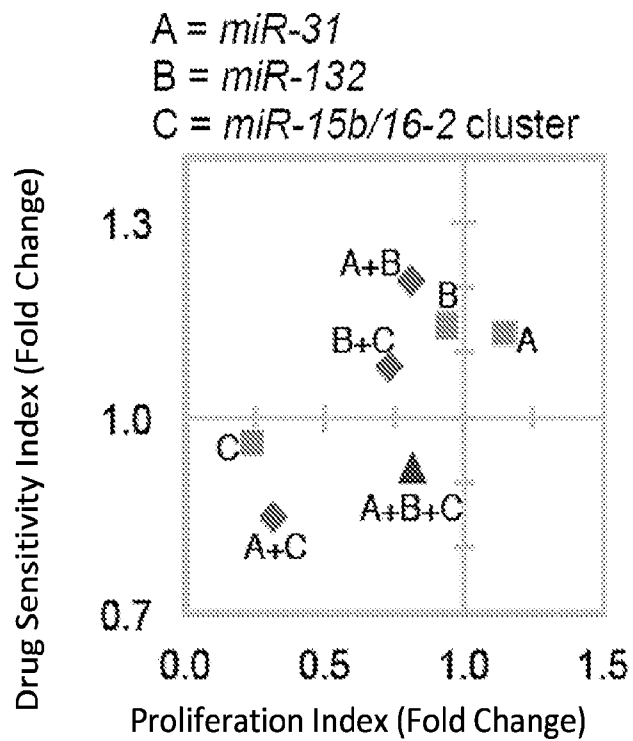


FIG. 6D

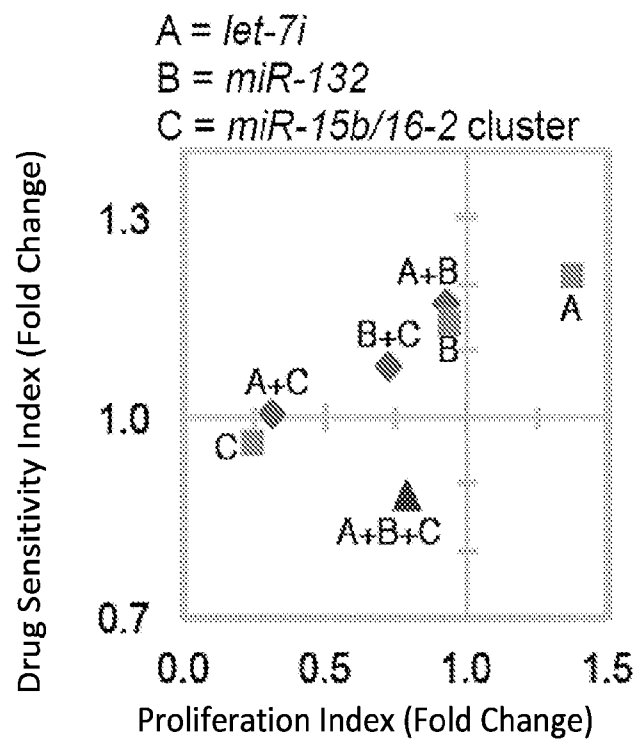


FIG. 6E

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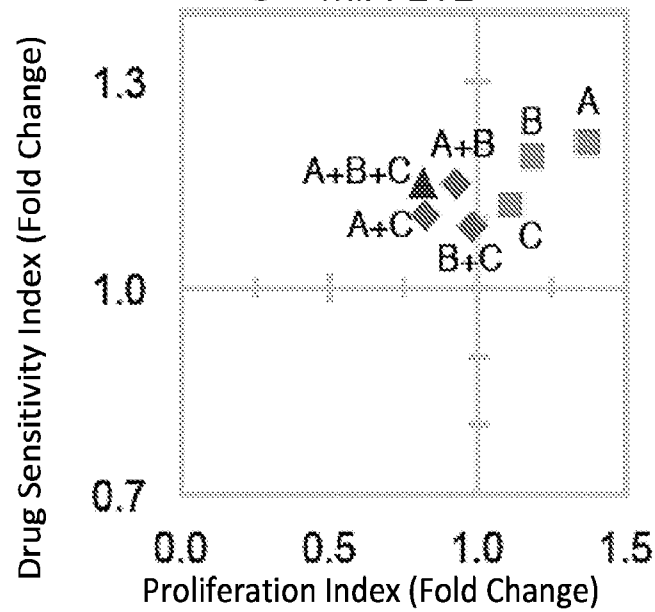
A = *let-7i*B = *miR-128b*C = *miR-212*

FIG. 6F

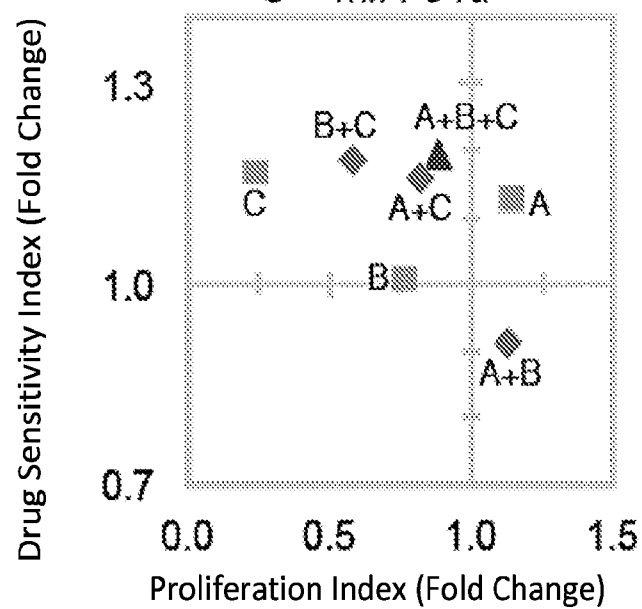
A = *miR-31*B = *miR-29a*C = *miR-34a*

FIG. 6G

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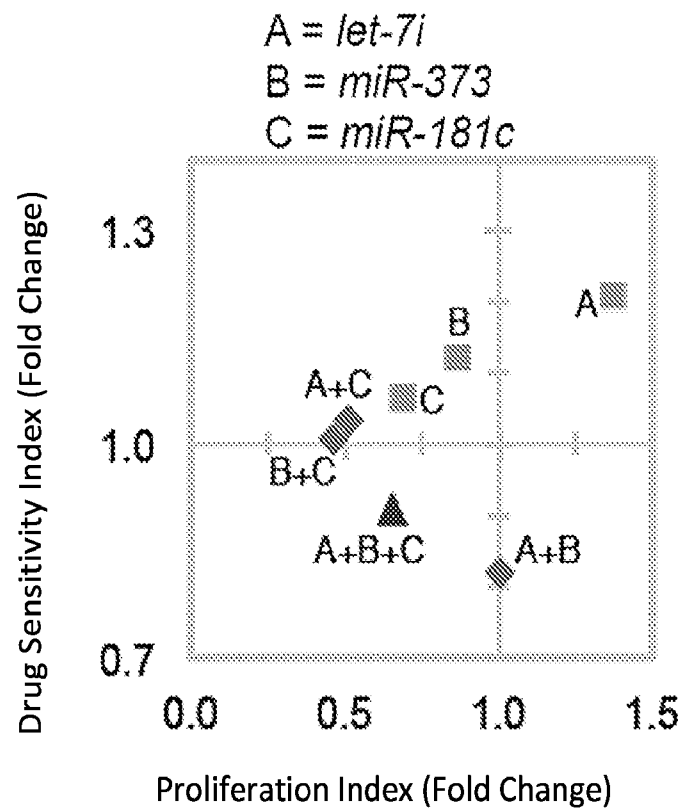


FIG. 6H

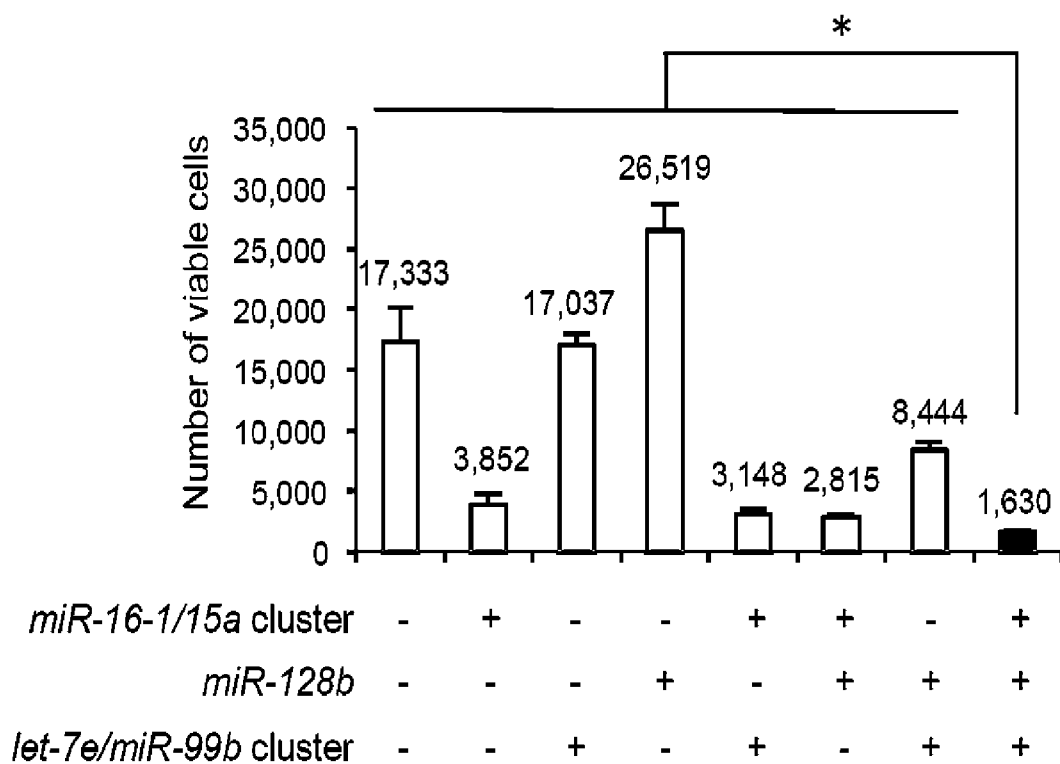


FIG. 6I

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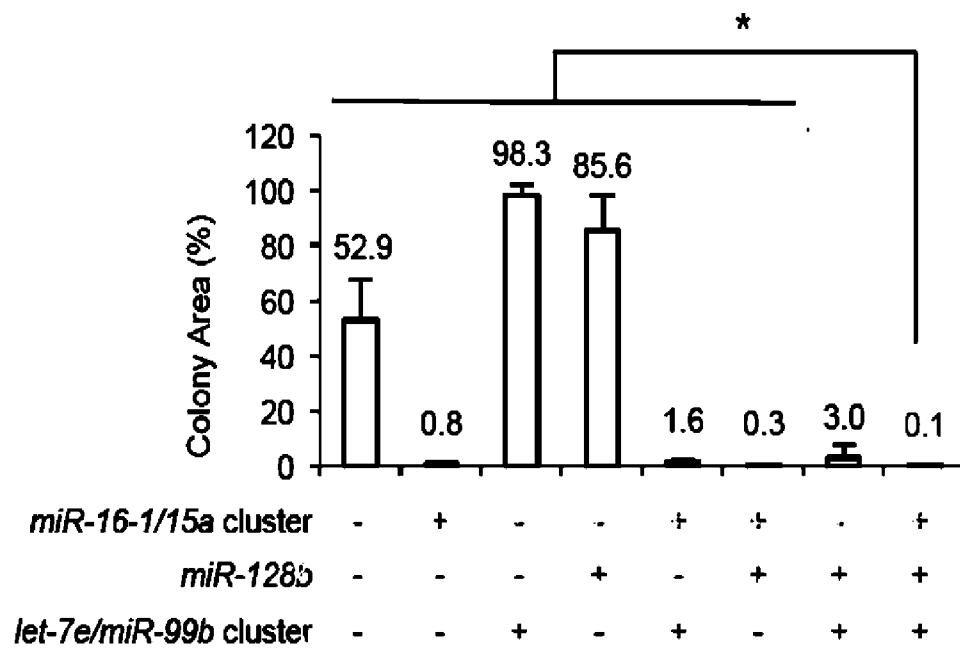


FIG. 6J

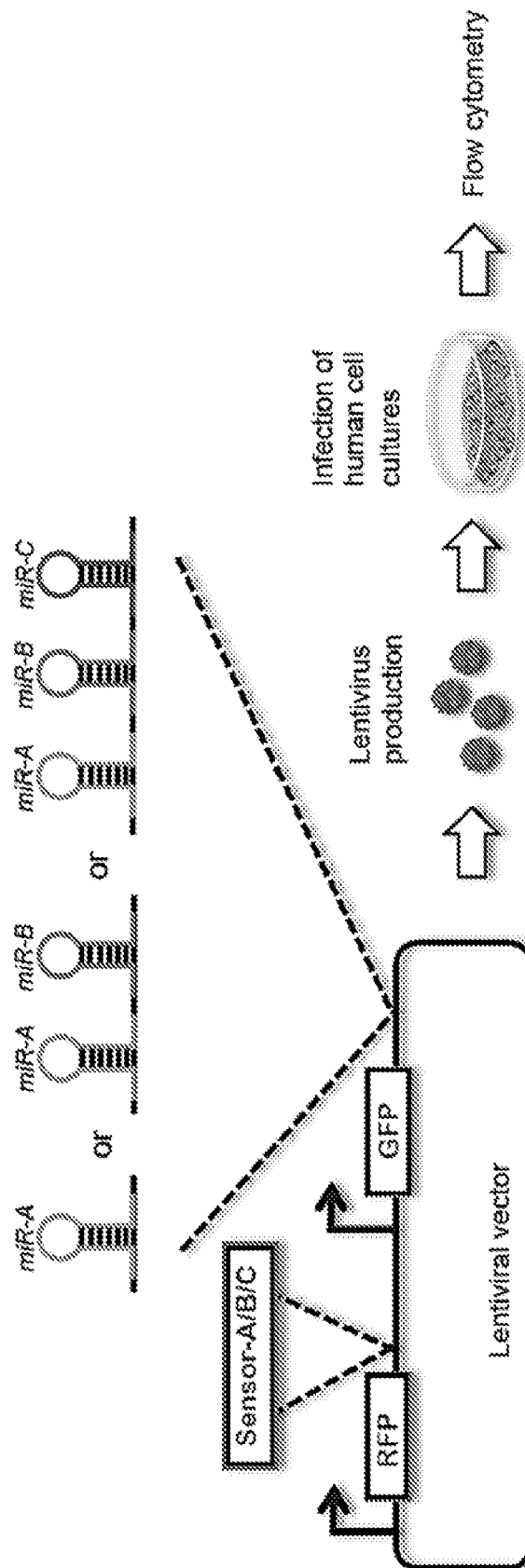


FIG. 7A

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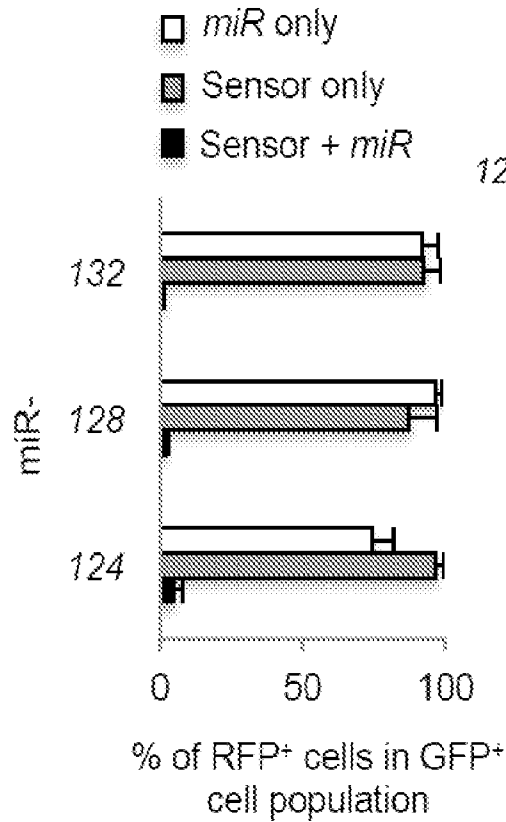


FIG. 7B

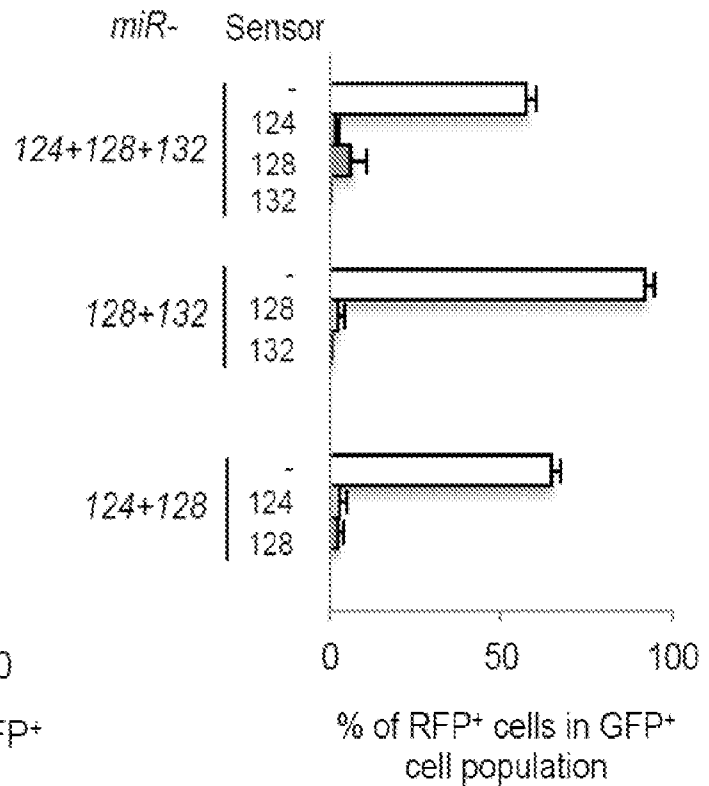


FIG. 7C

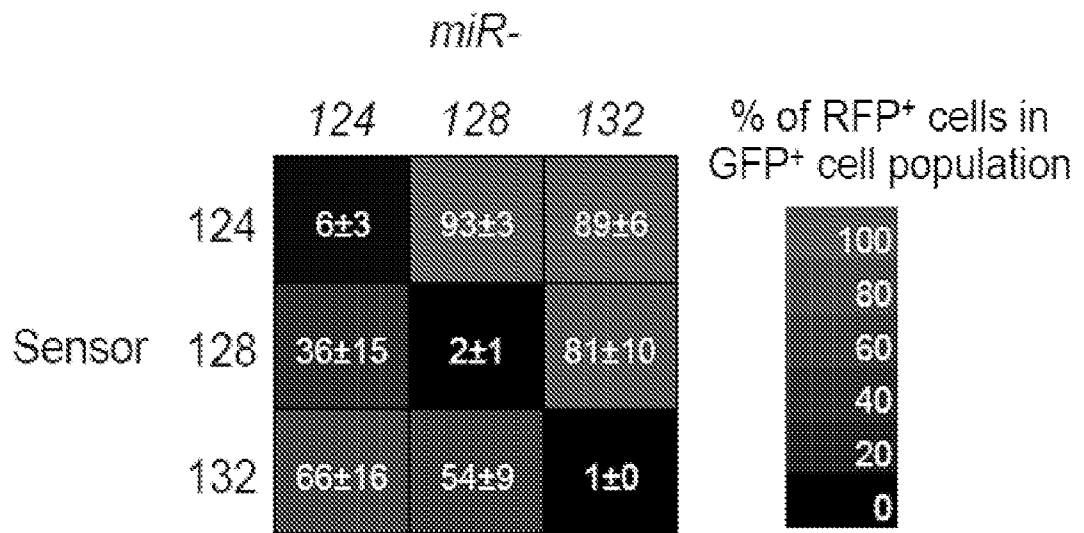


FIG. 7D

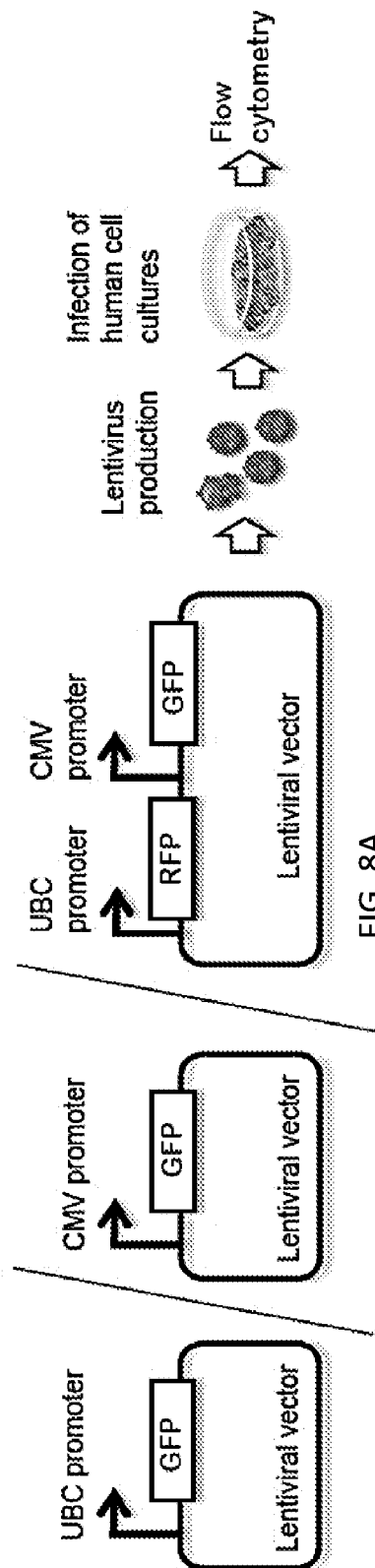


FIG. 8A

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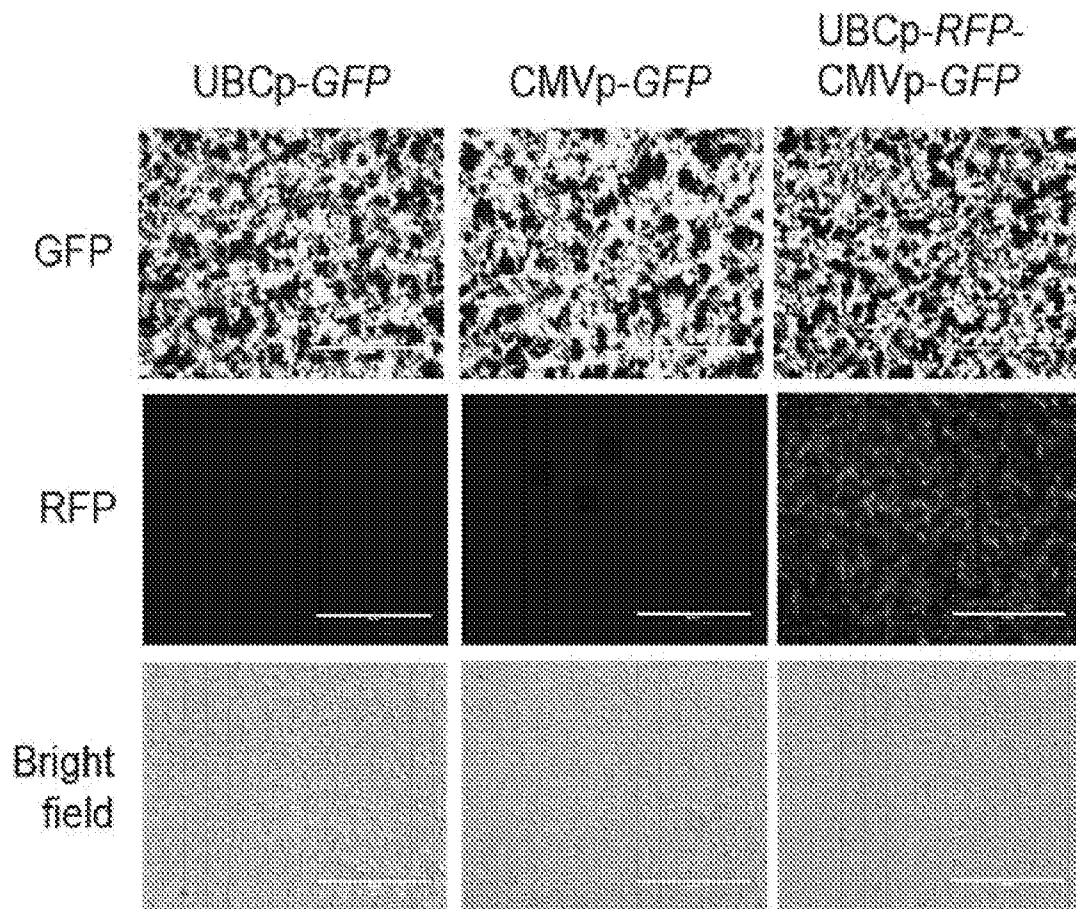


FIG. 8B

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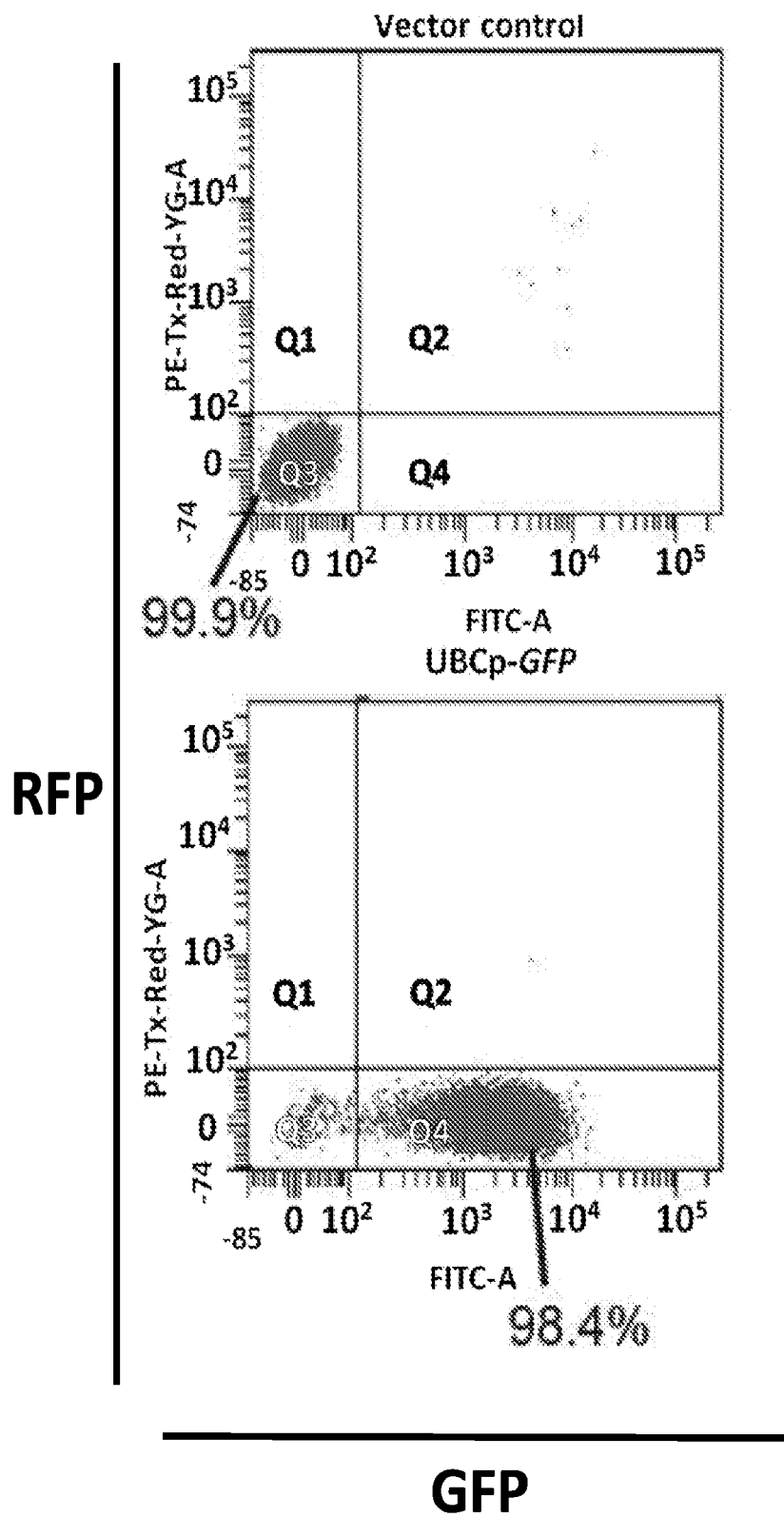


FIG. 8C

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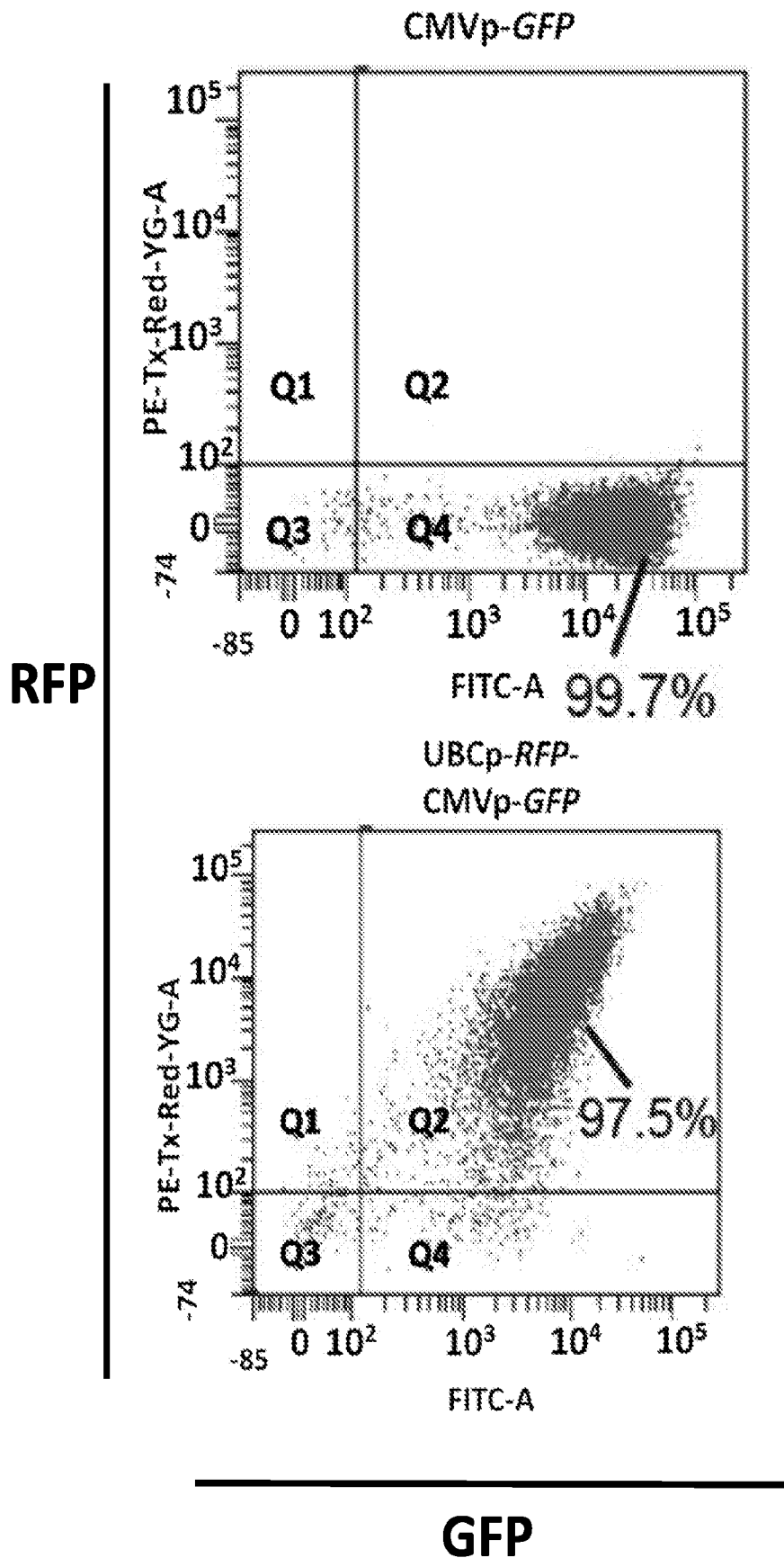


FIG. 8C (Continued)

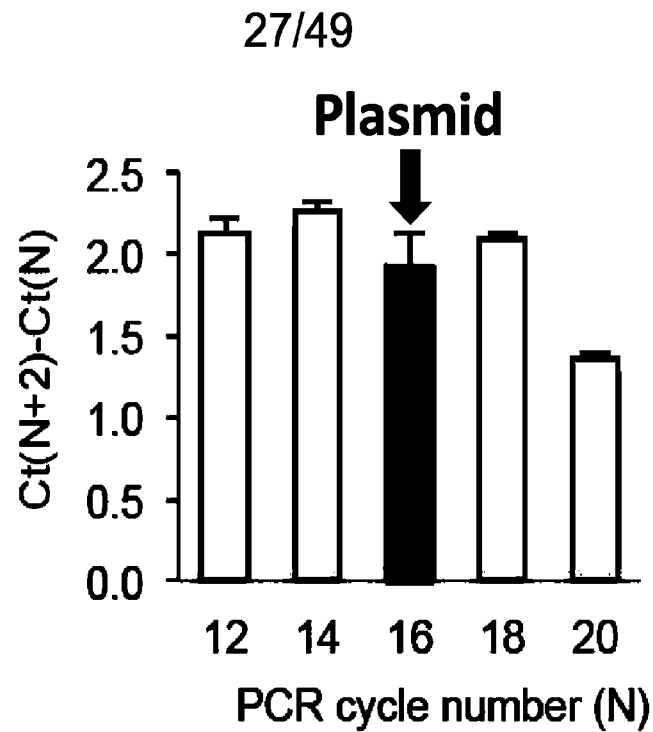


FIG. 9A

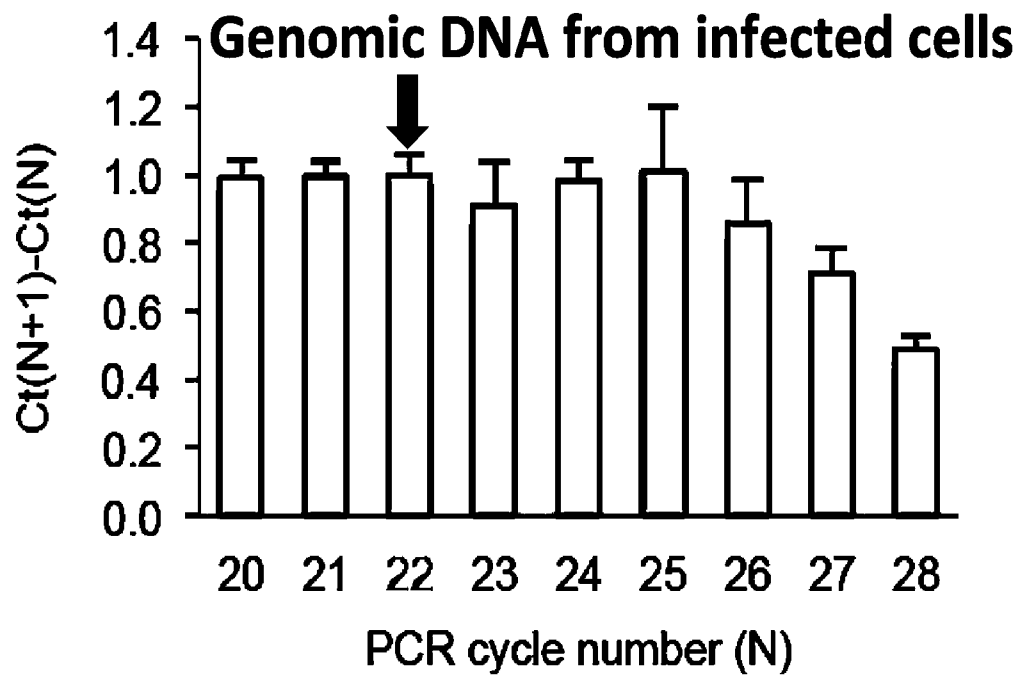


FIG. 9B

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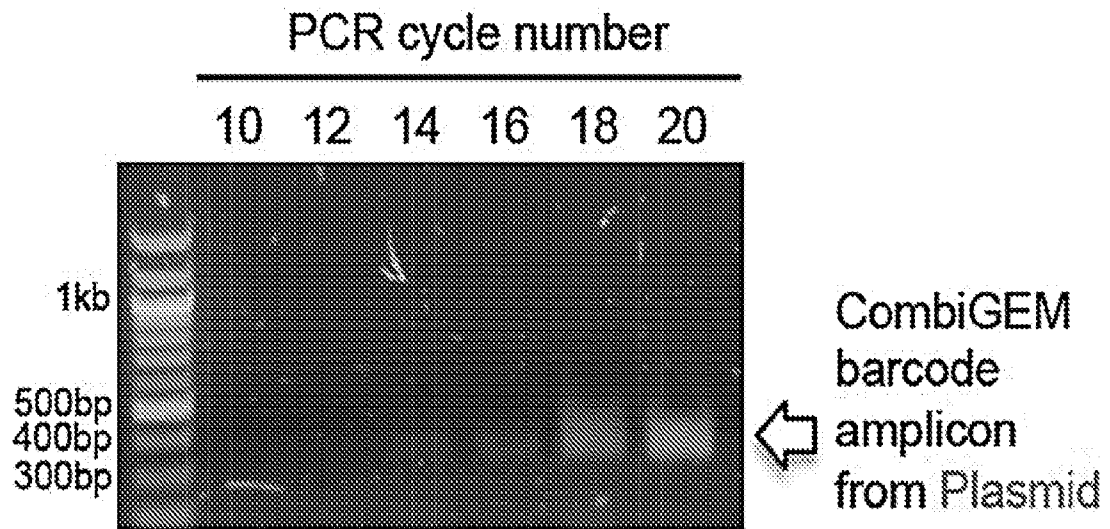


FIG. 9C

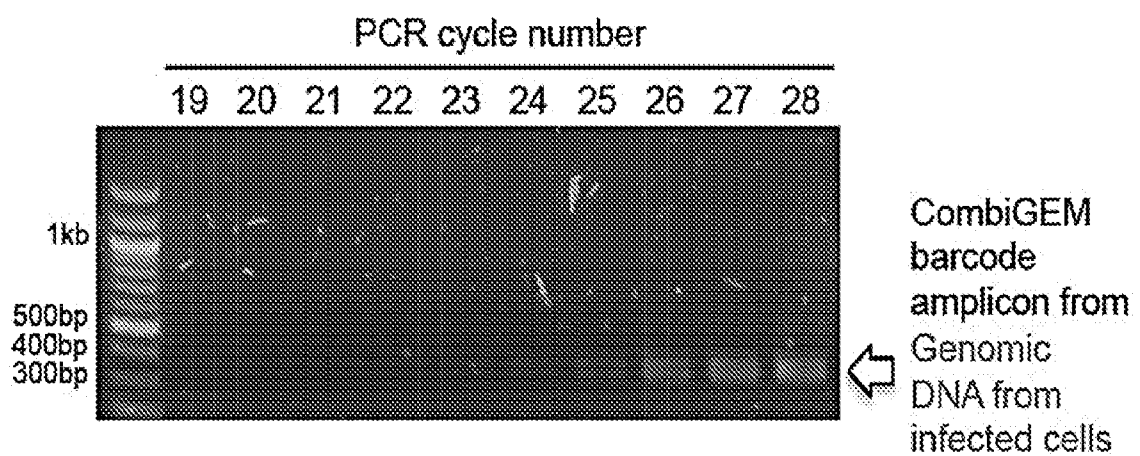


FIG. 9D

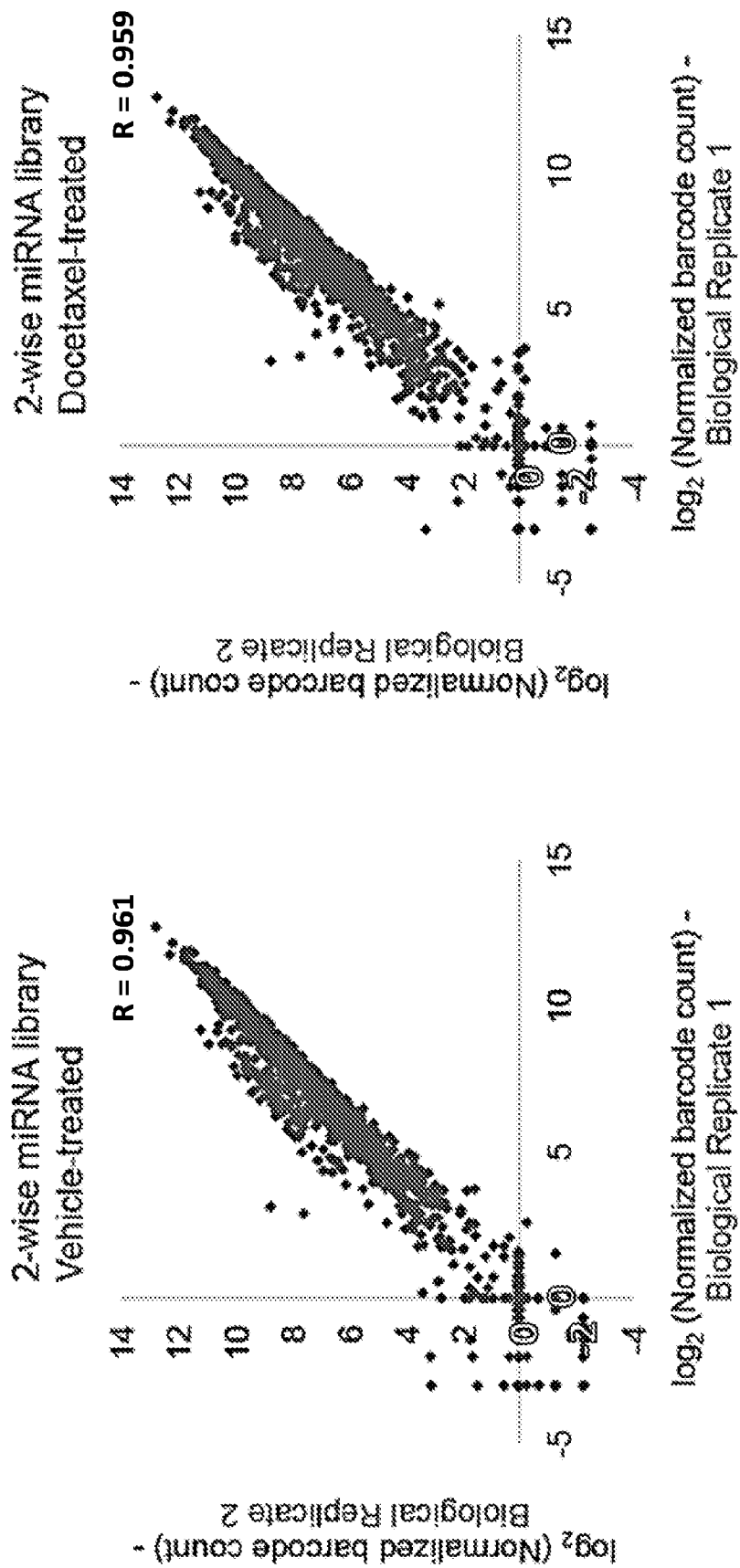


FIG. 10A

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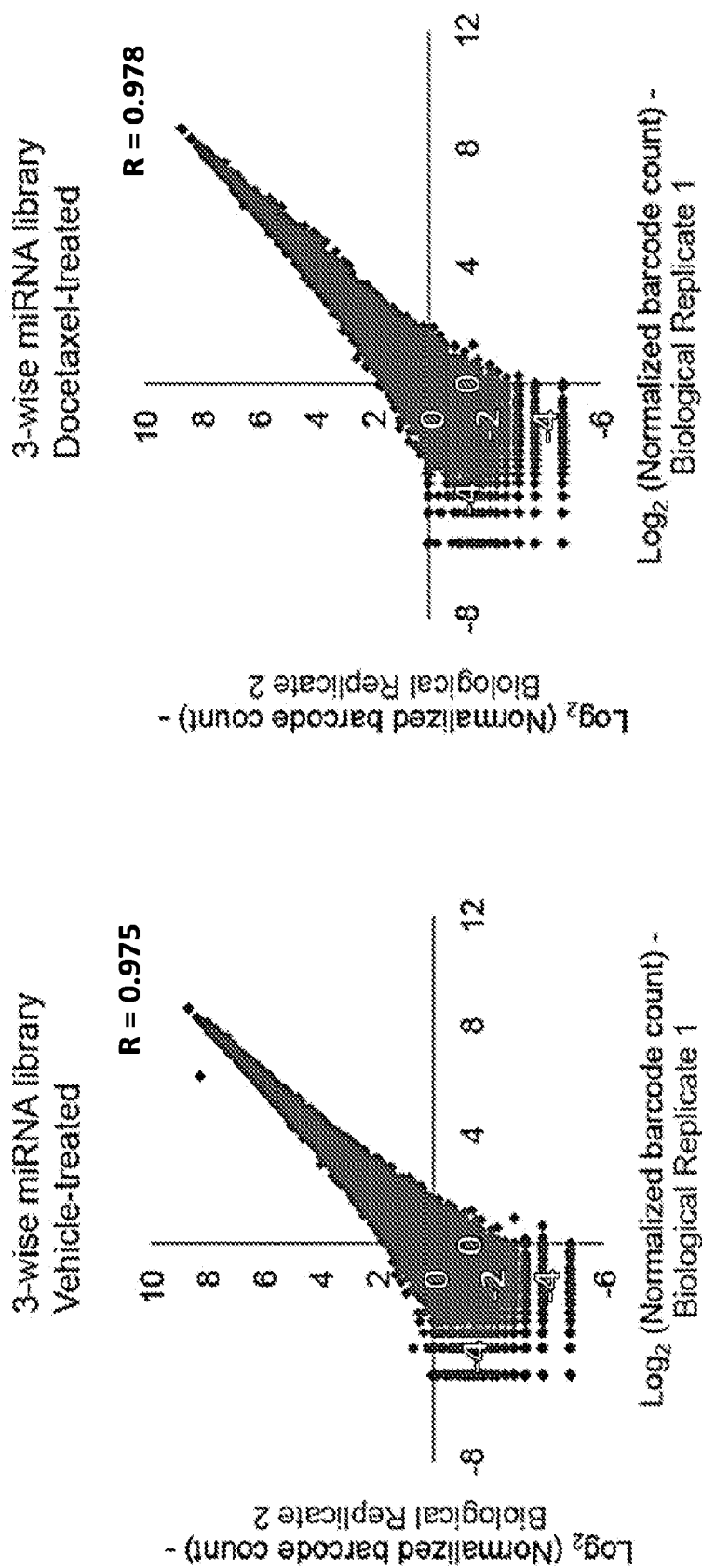
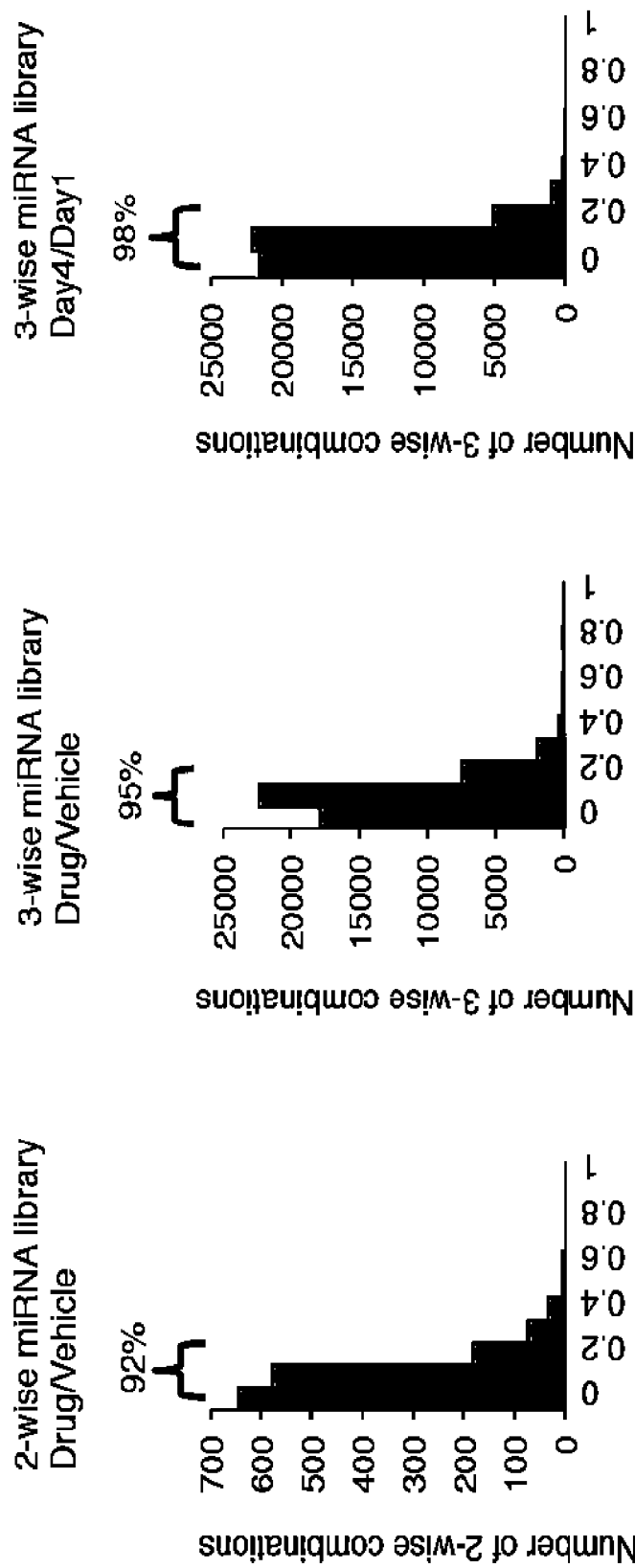


FIG. 10B



CV for different
arrangements

FIG. 11A

CV for different
arrangements

FIG. 11B

CV for different
arrangements

FIG. 11C

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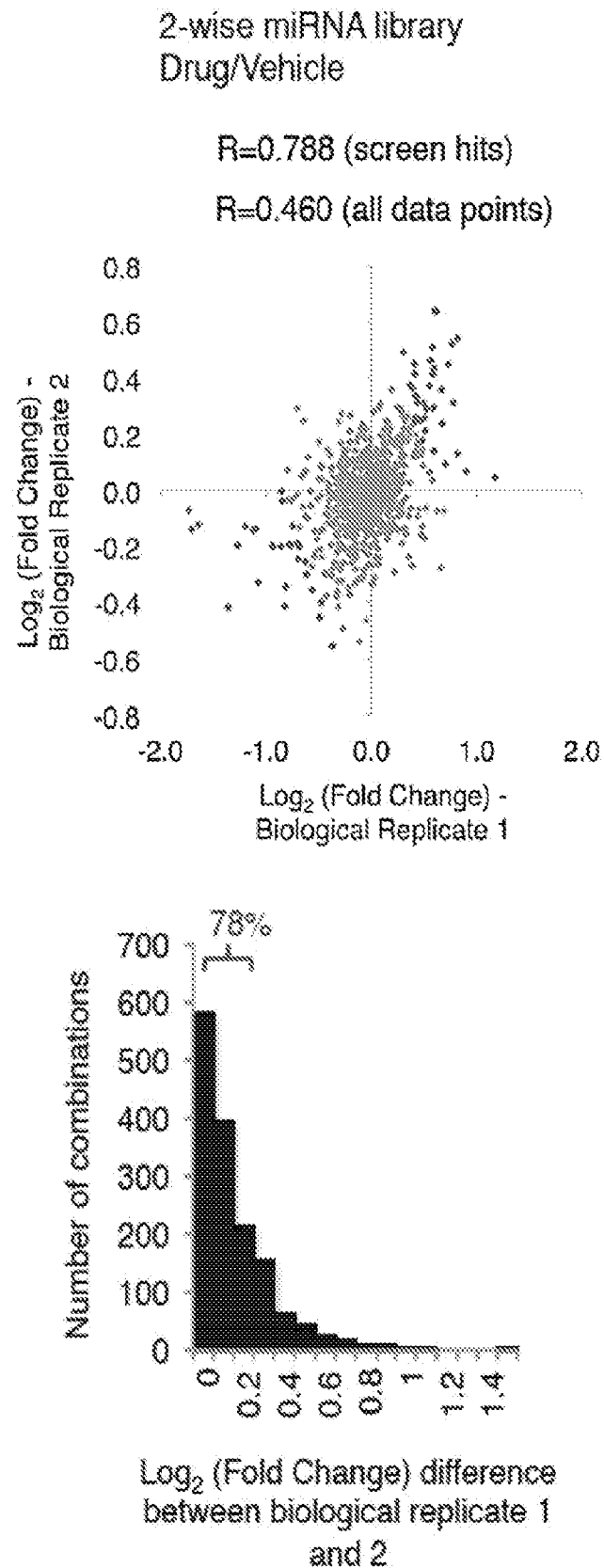


FIG. 12A

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3-wise miRNA library

Drug/Vehicle

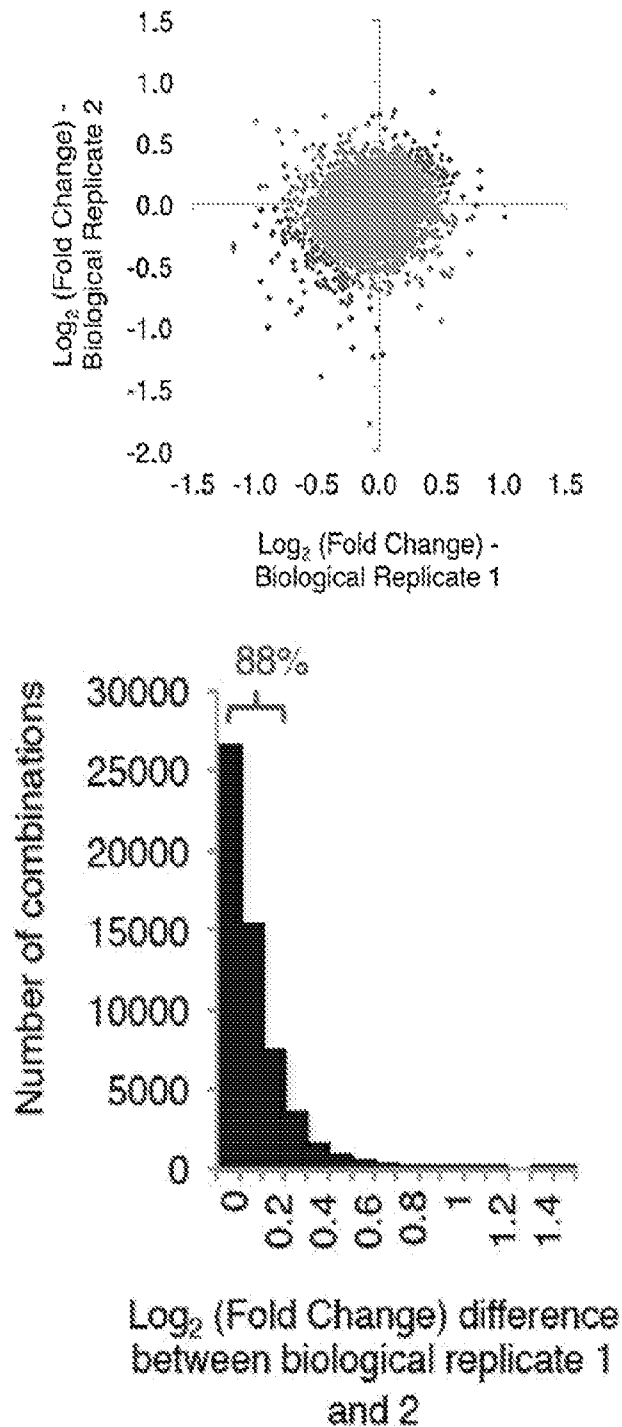
 $R=0.636$ (screen hits) $R=0.258$ (all data points)

FIG. 12B

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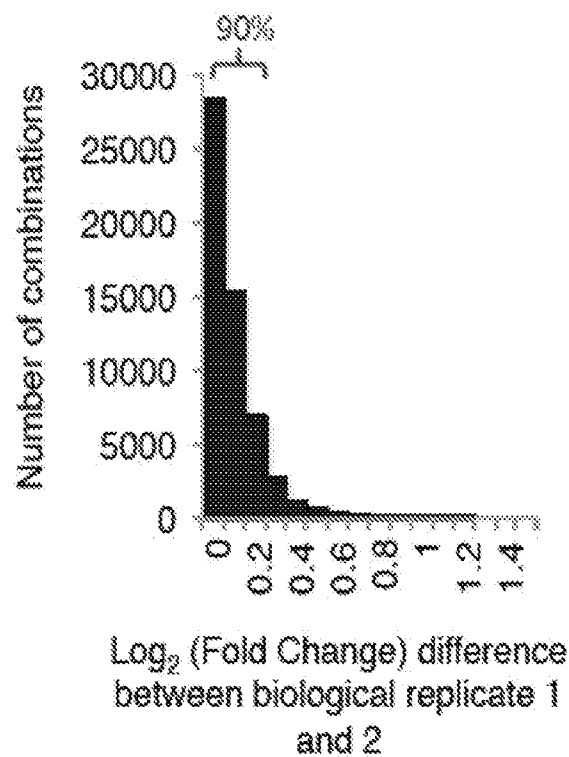
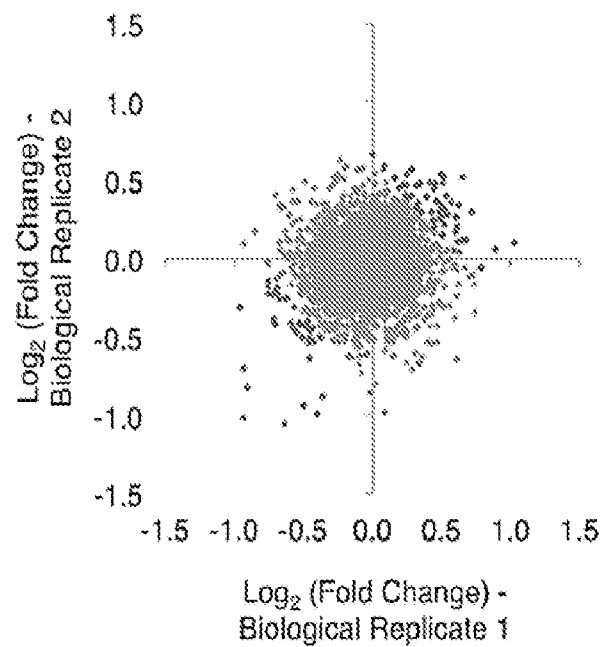
3-wise miRNA library
Day4/Day1 $R=0.750$ (screen hits) $R=0.168$ (all data points)

FIG. 12C

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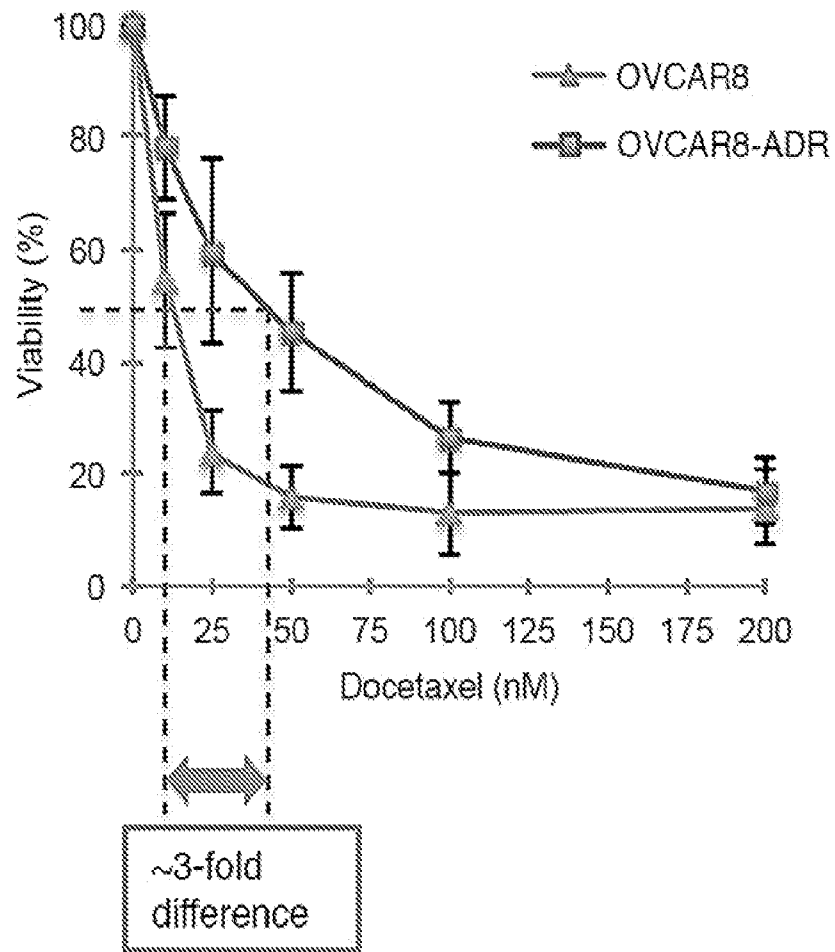


FIG. 13

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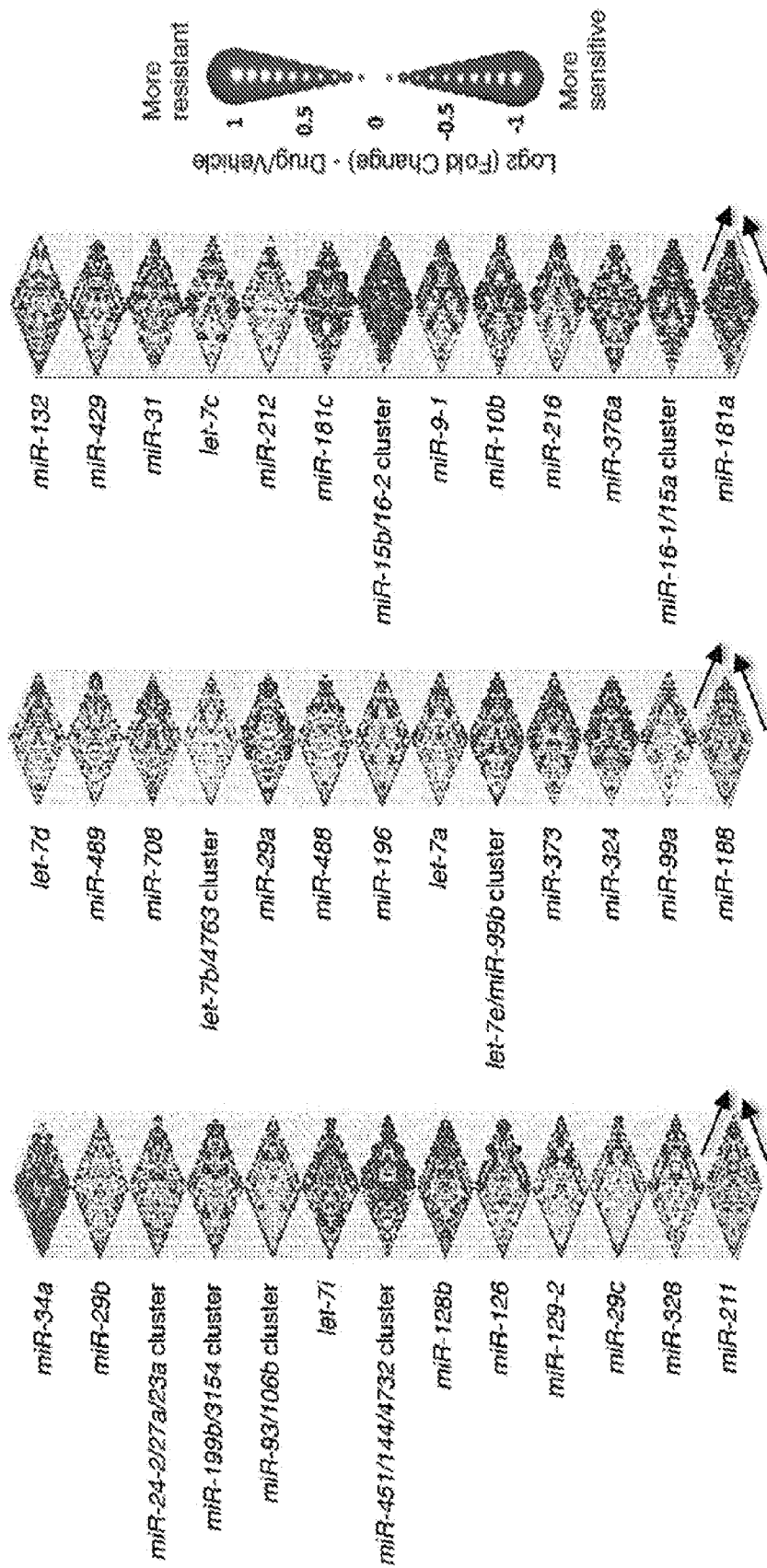


FIG. 14A

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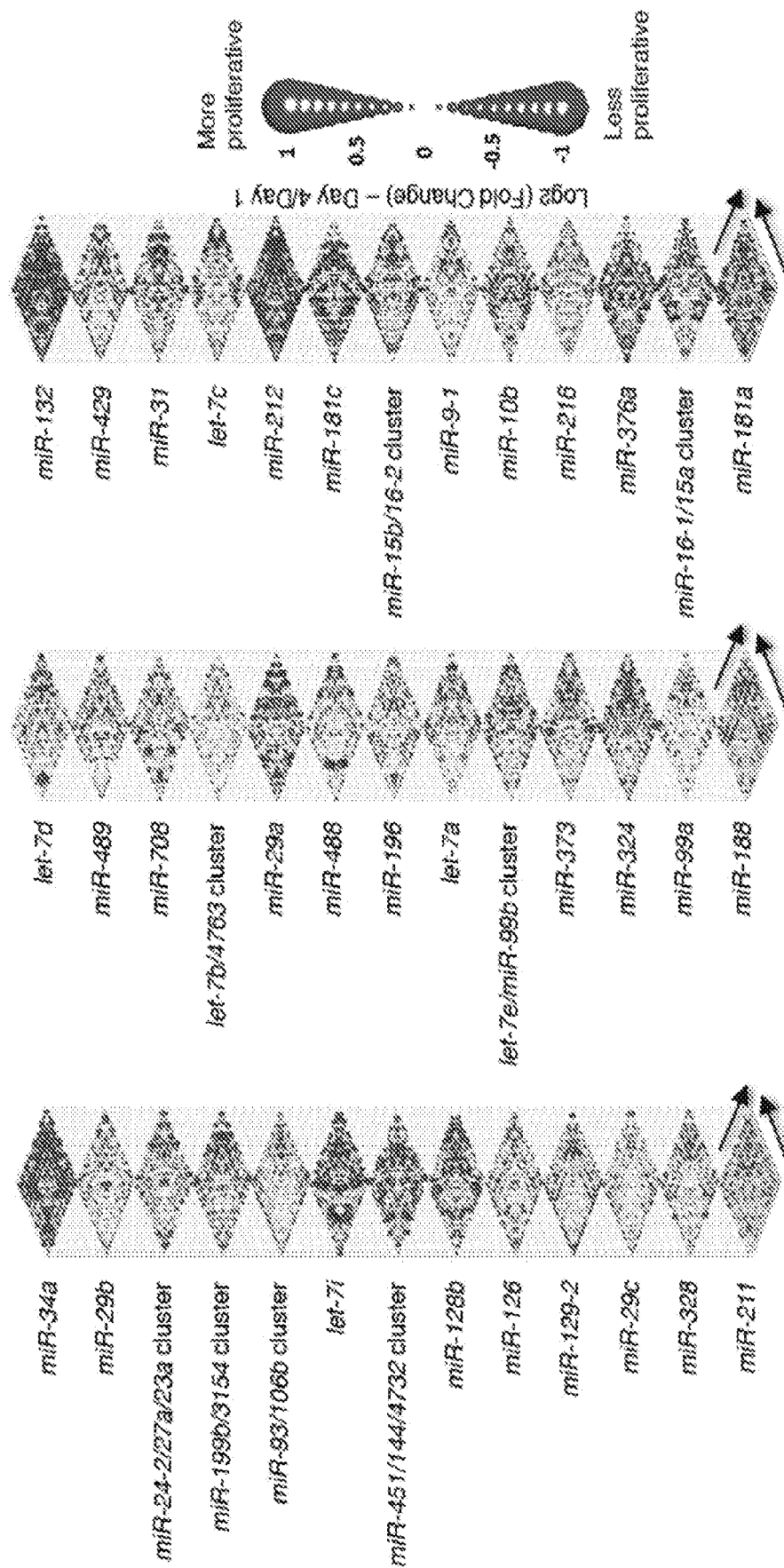


FIG. 14B

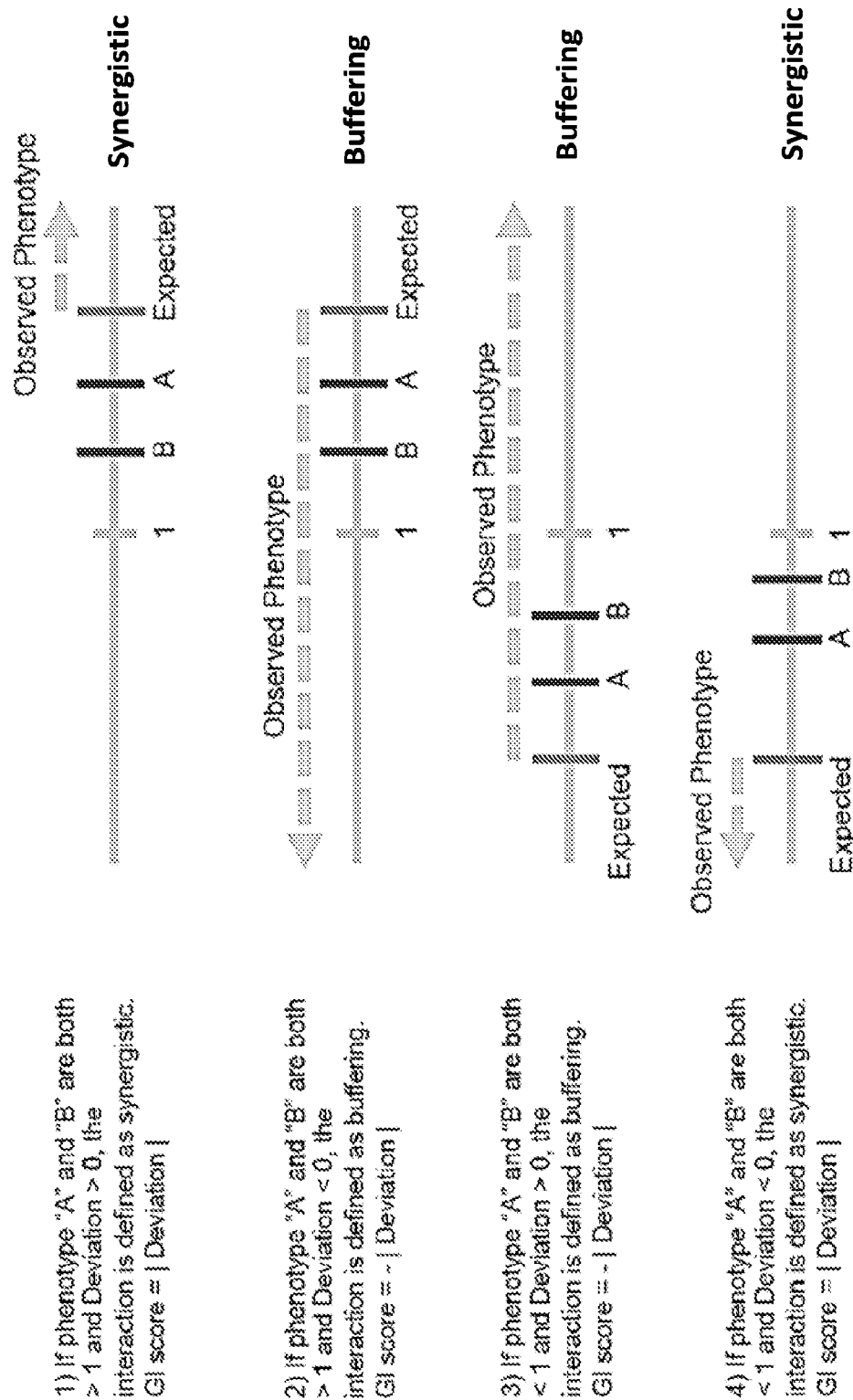


FIG. 15

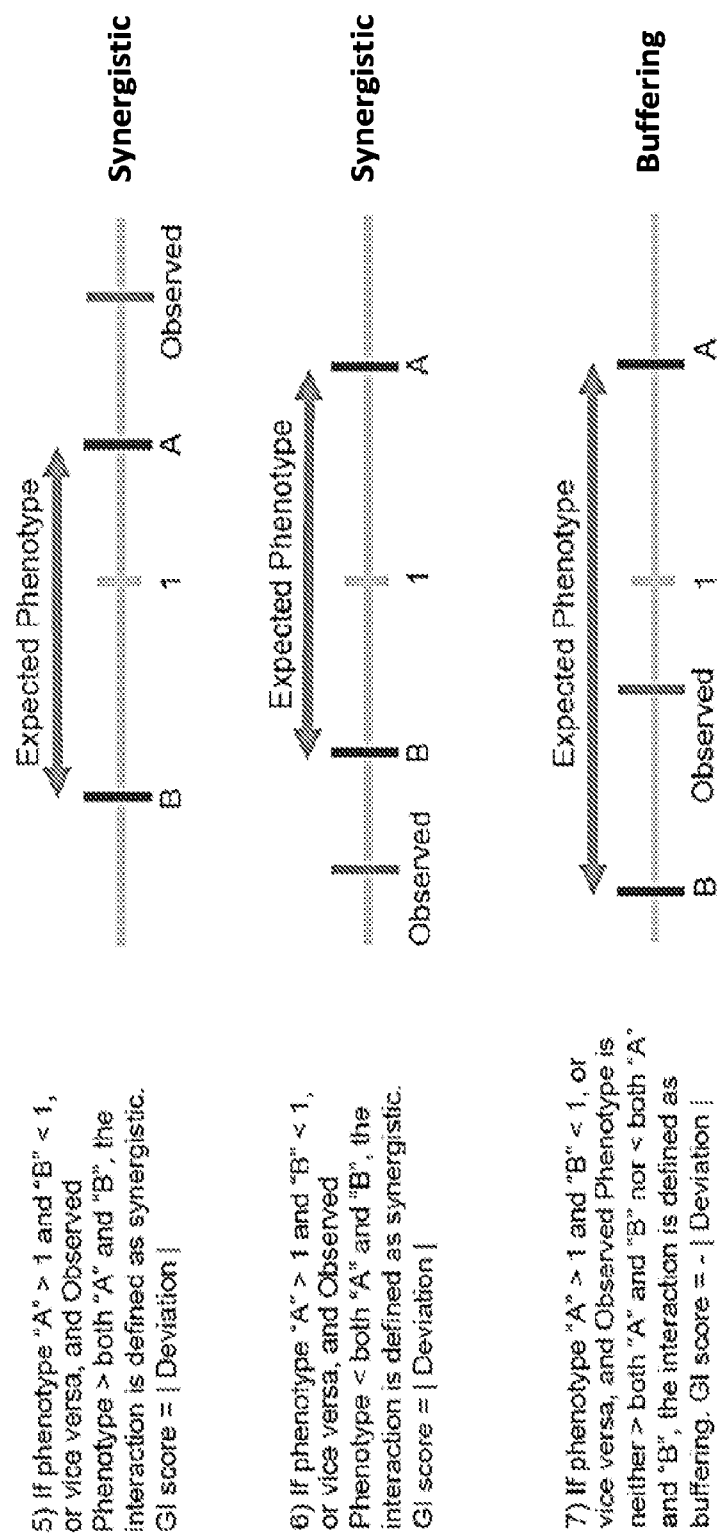
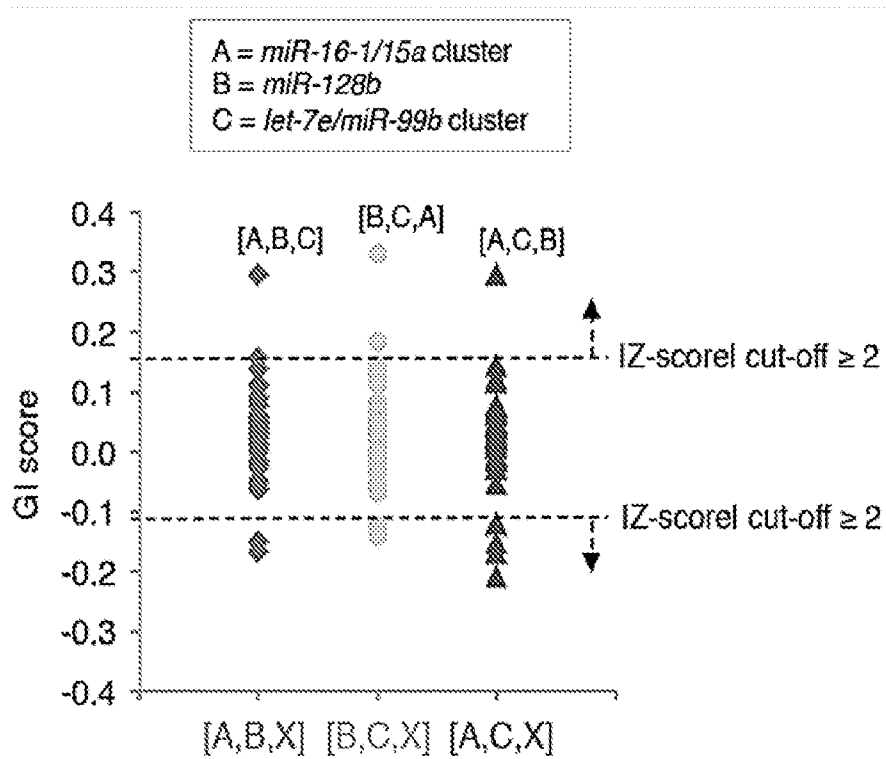


FIG. 15 (Continued)

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3-wise miRNA combinations

FIG. 16A

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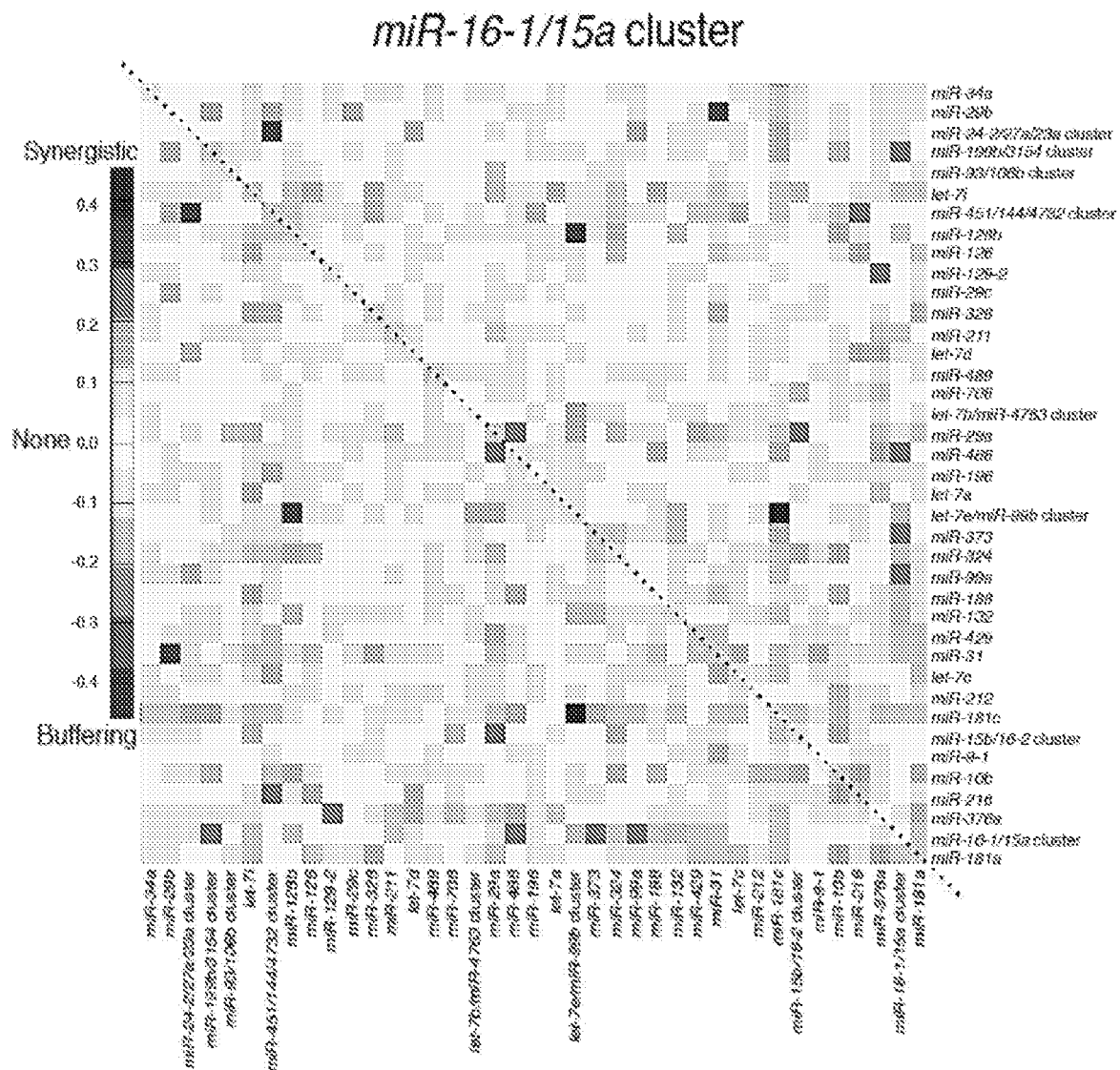


FIG. 16B

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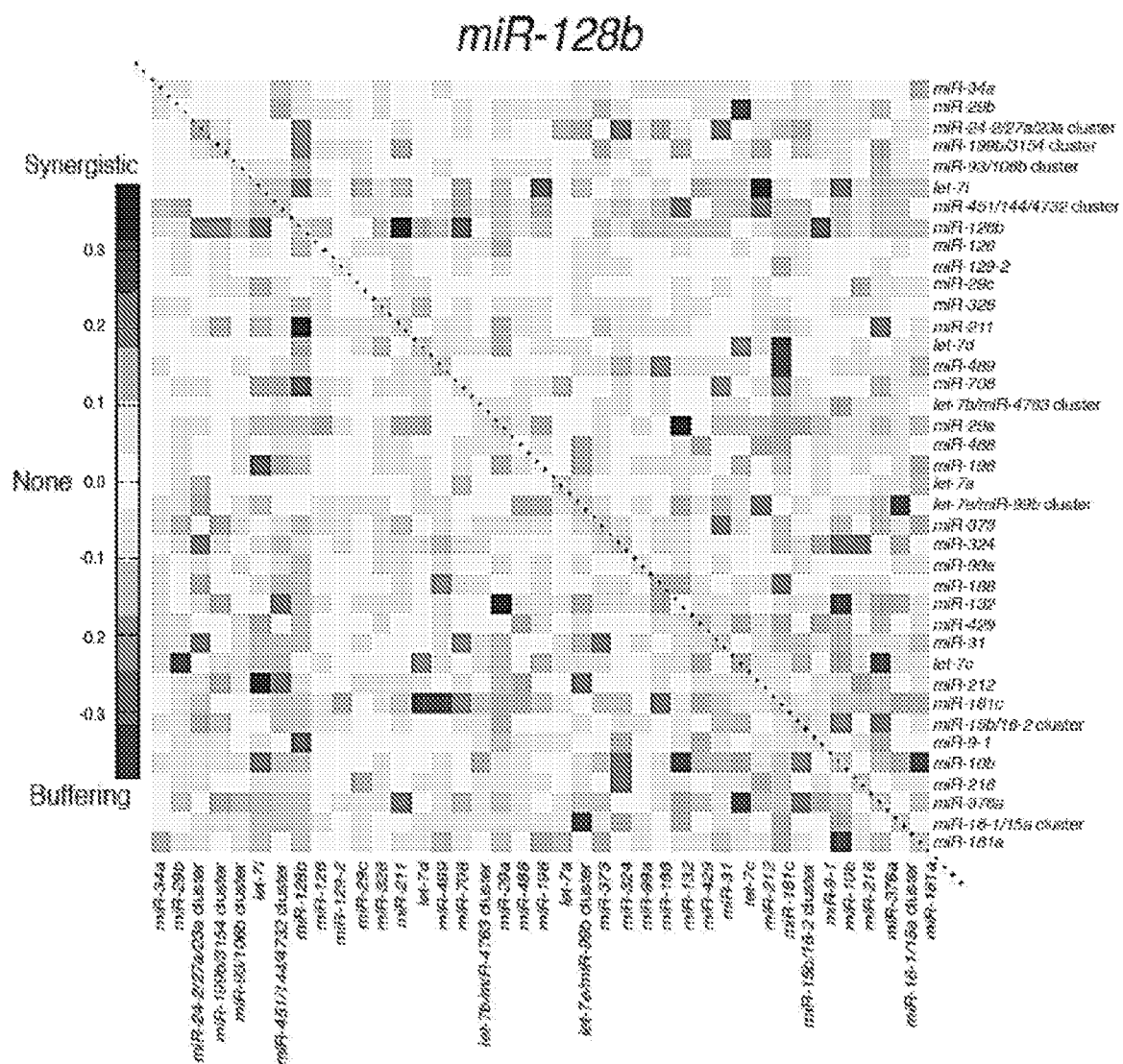


FIG. 16C

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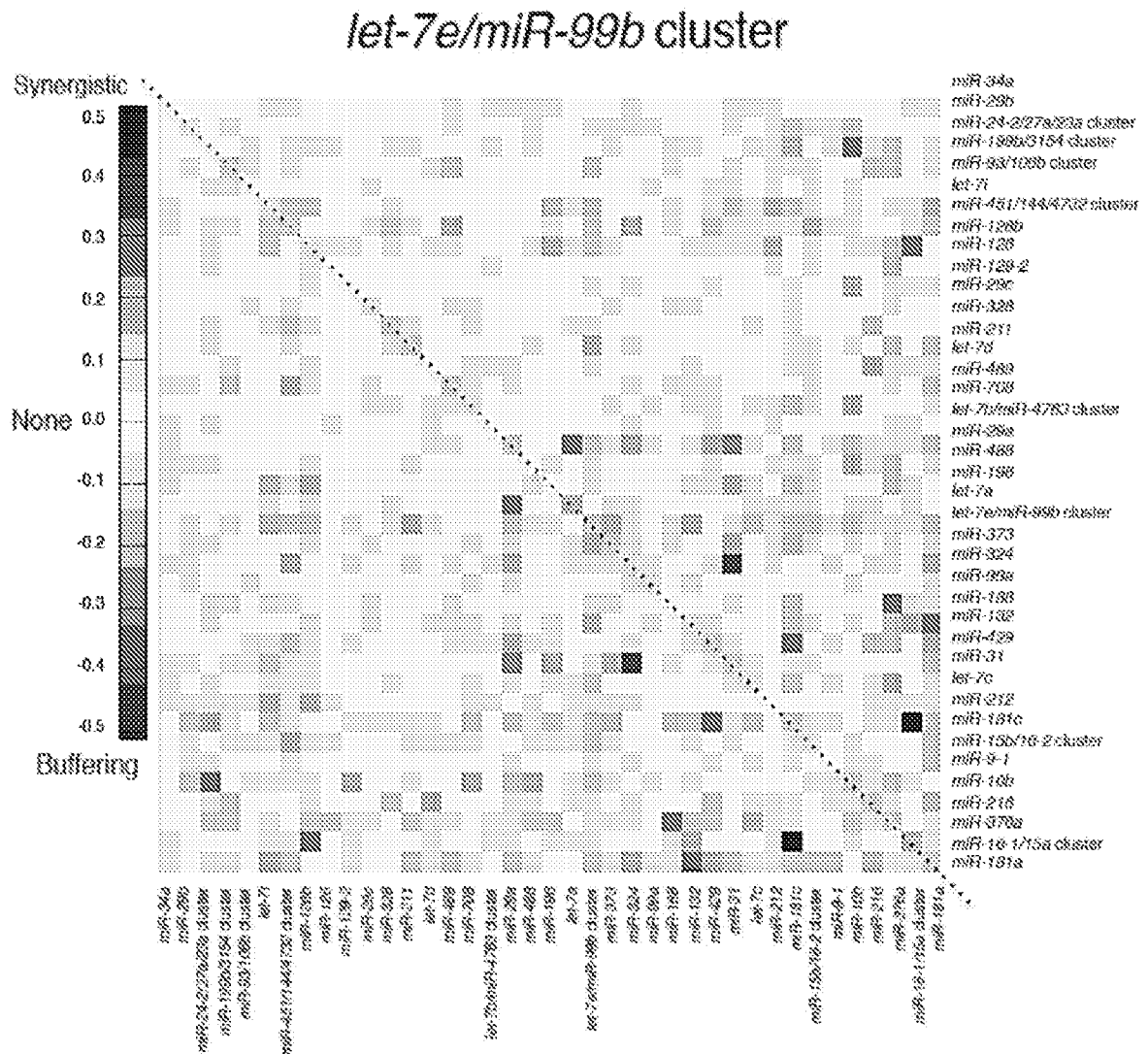


FIG. 16D

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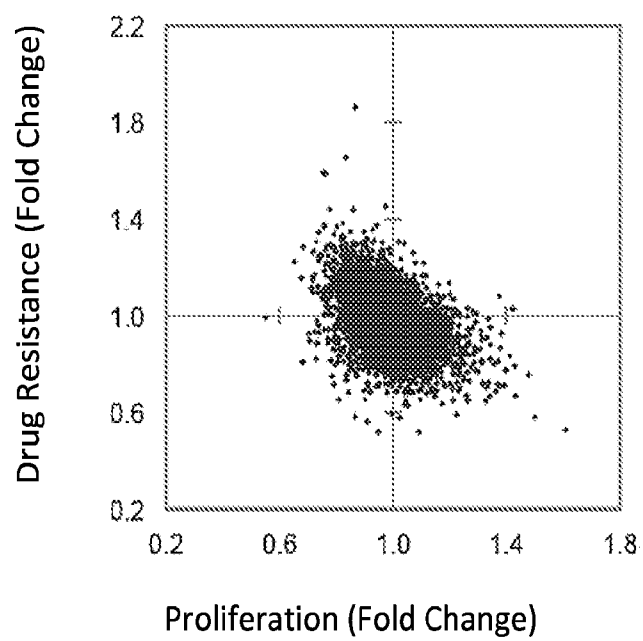


FIG. 17

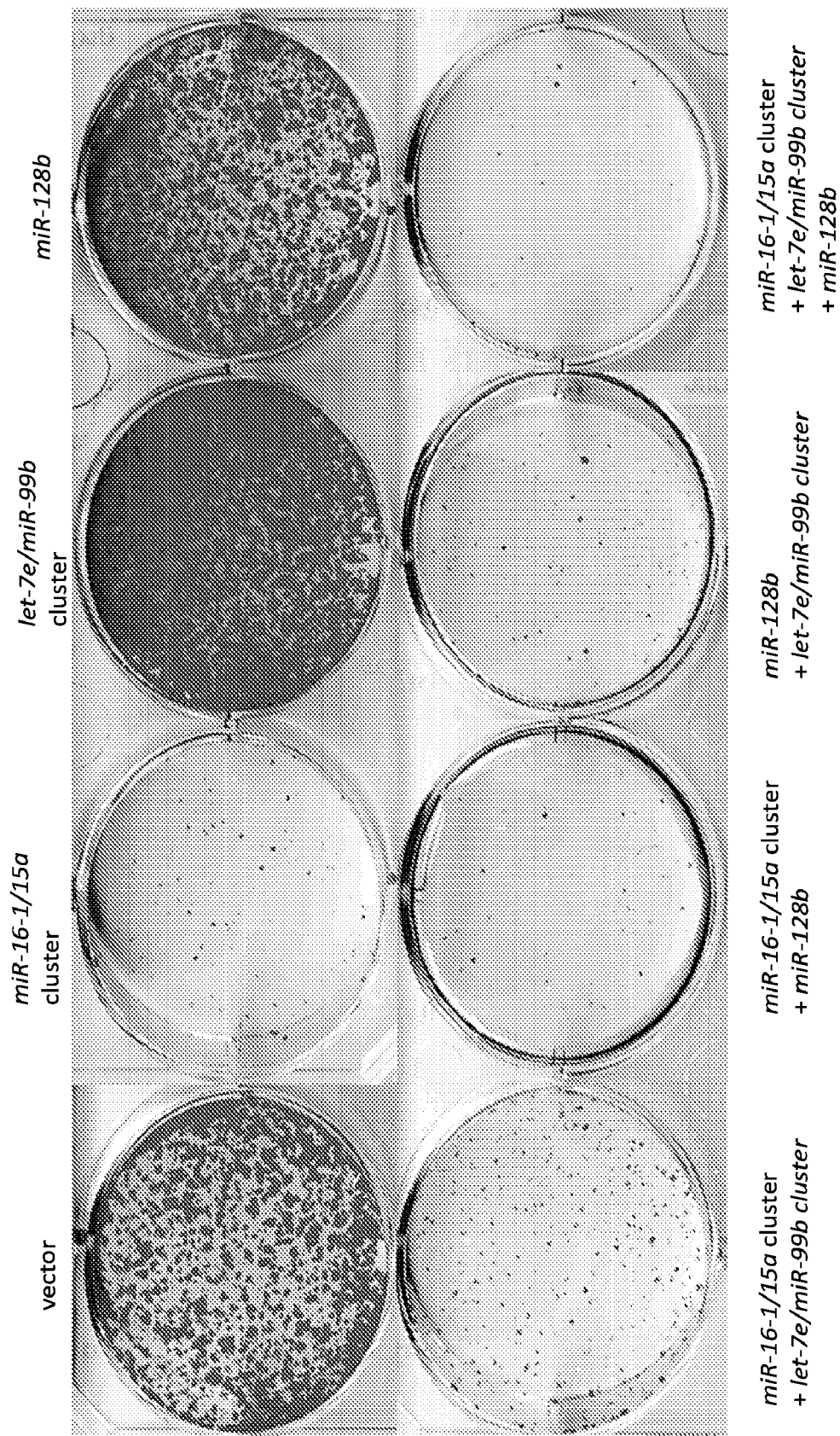


FIG. 18A

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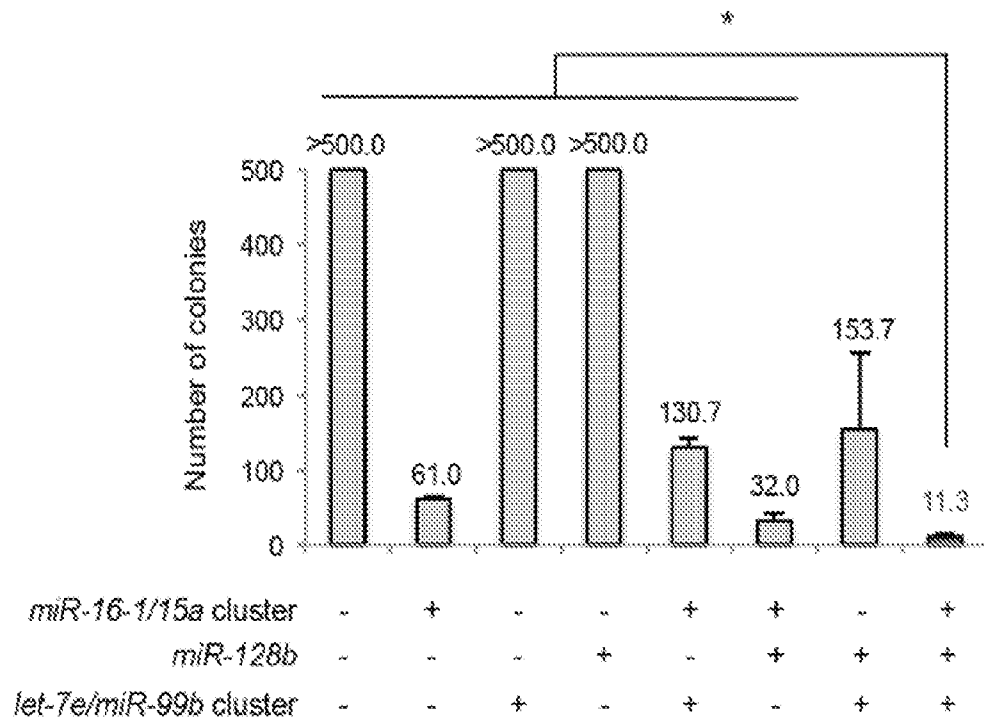


FIG. 18B

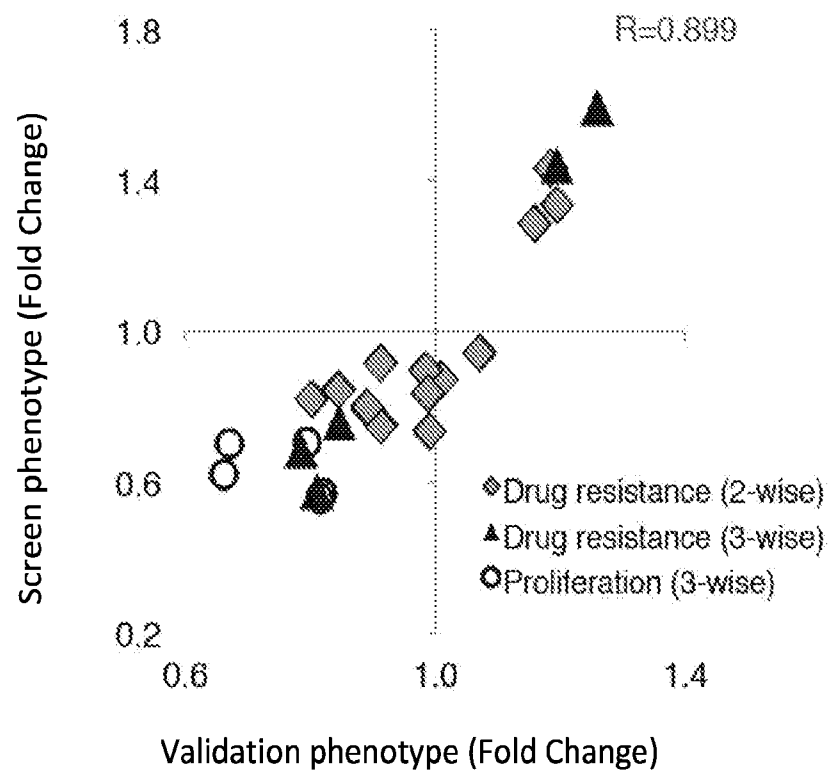


FIG. 19

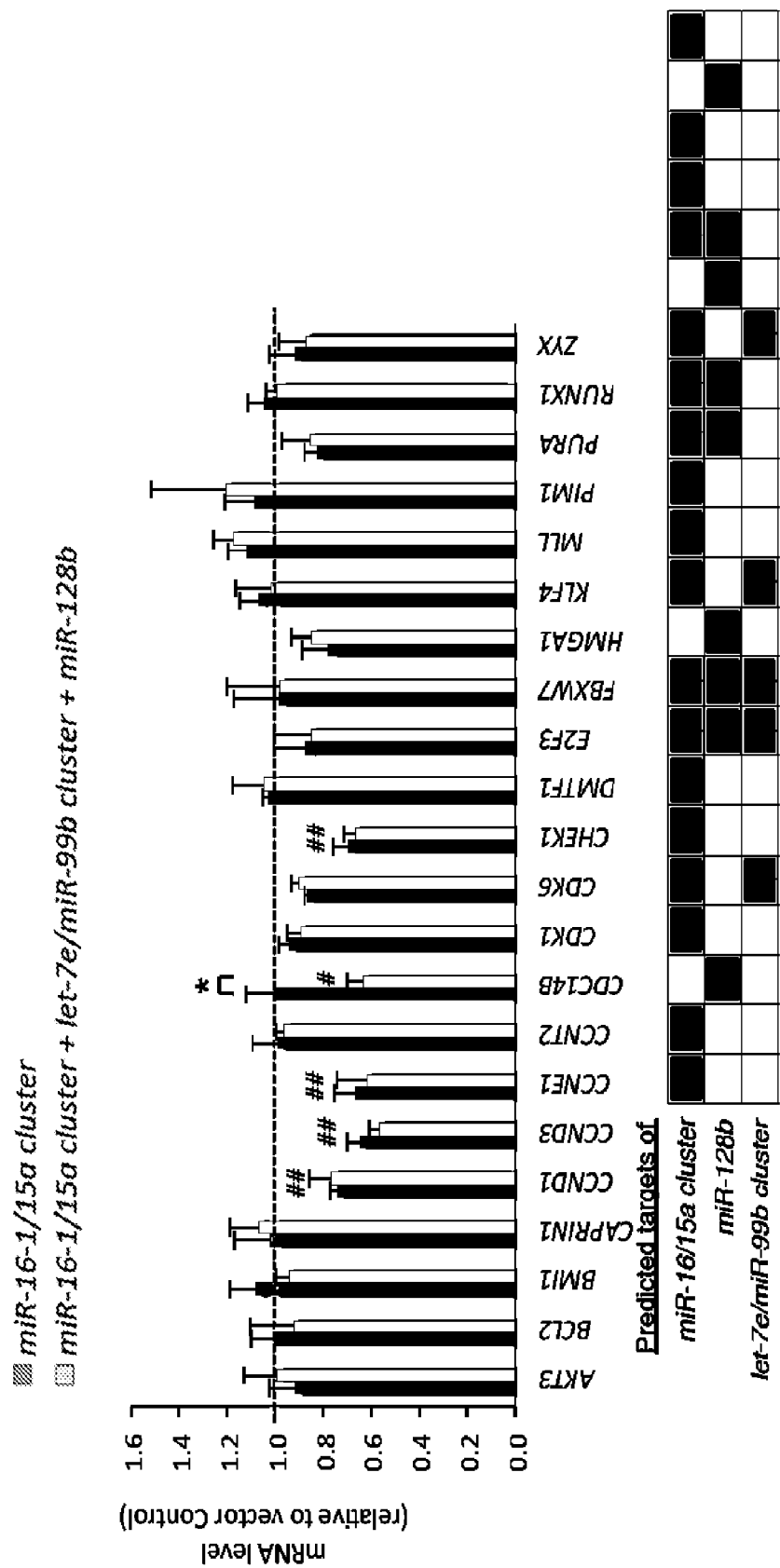


FIG. 20A

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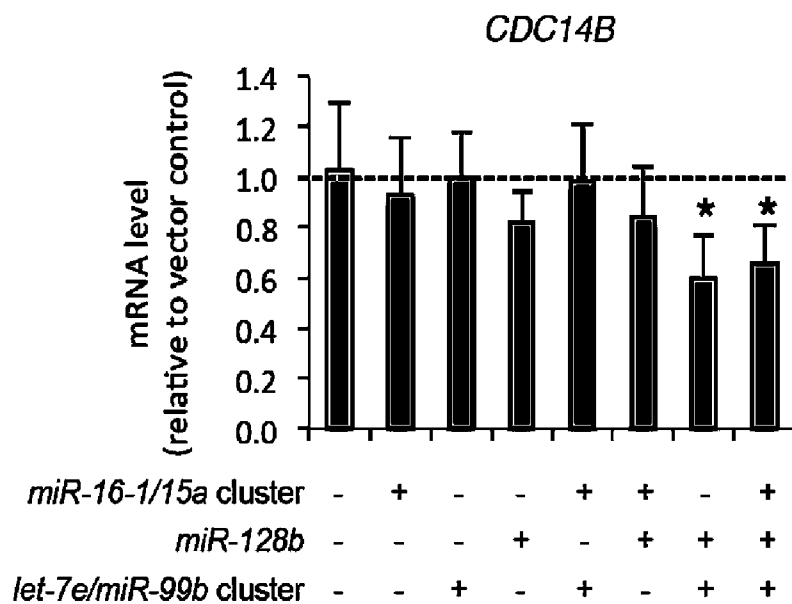


FIG. 20B

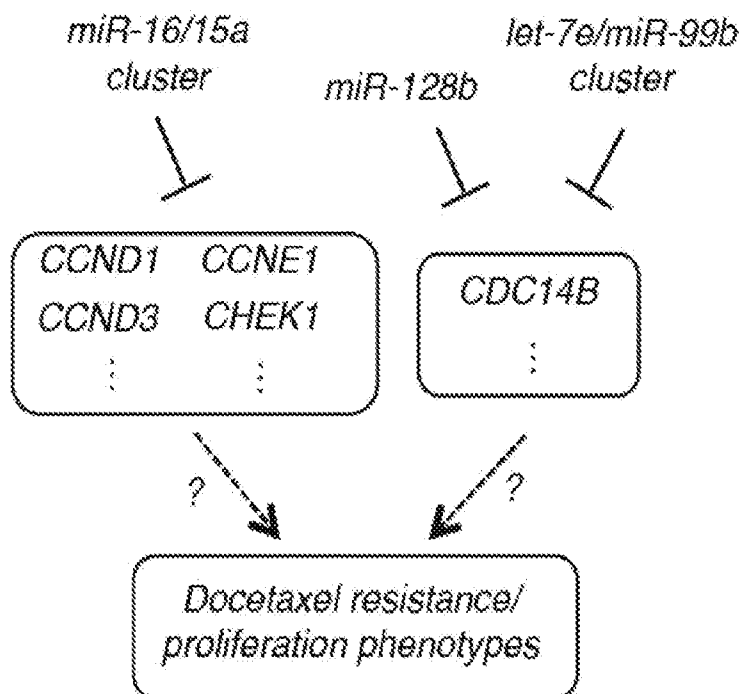


FIG. 20C

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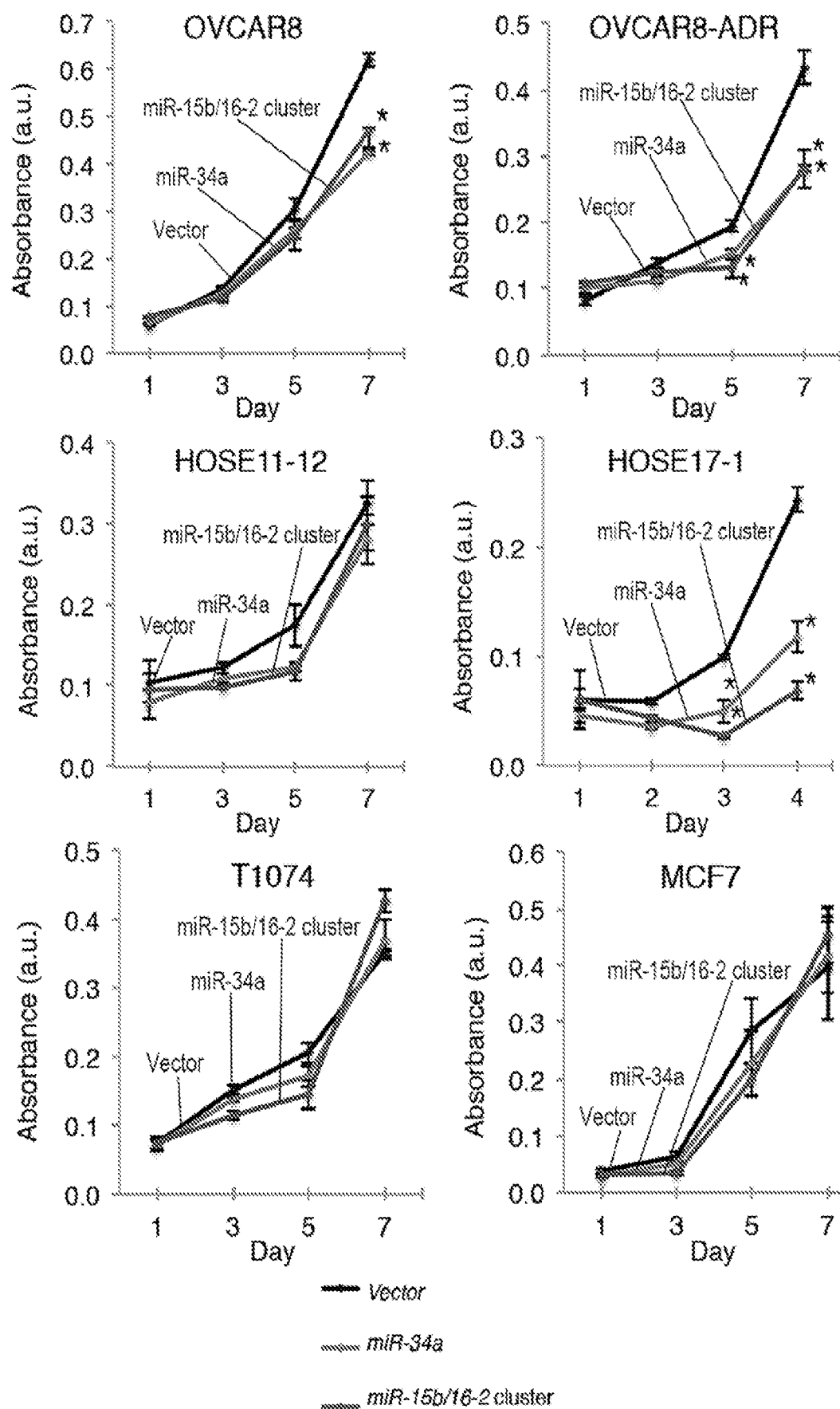


FIG. 21

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/012844

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

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

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

EP0-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YASUTO KINOSE ET AL.: "The role of microRNAs in ovarian cancer", BIOMED RESEARCH INTERNATIONAL, vol. 2, no. 7, 1 January 2014 (2014-01-01), pages 9-11, XP055264888, US	111-124
A	ISSN: 2314-6133, DOI: 10.1056/NEJMoal209026 the whole document	1-110
Y	----- US 2014/051744 A1 (THE REGENTS OF THE UNIVERSITY OF COLORADO, A BODY CORPORATE [US]) 20 February 2014 (2014-02-20)	111-124
A	the whole document ----- -/--	1-110



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

14 April 2016

Date of mailing of the international search report

25/04/2016

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Authorized officer

Macchia, Giovanni

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/012844

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOREN T. ET AL.: "MicroRNAs and their target messenger RNAs associated with ovarian cancer response to chemotherapy", GYNECOLOGIC ONCOLOGY, ACADEMIC PRESS, LONDON, GB, vol. 113, no. 2, 1 May 2009 (2009-05-01), pages 249-255, XP026035175, ISSN: 0090-8258, DOI: 10.1016/J.YGYNO.2009.01.014 [retrieved on 2009-02-23]	111-124
A	the whole document	1-110

A	WO 2014/005042 A2 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US]) 3 January 2014 (2014-01-03) the whole document	1-124

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/012844

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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			CA 2877962 A1 03-01-2014
			CN 104508130 A 08-04-2015
			EP 2867361 A2 06-05-2015
			HK 1207886 A1 12-02-2016
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