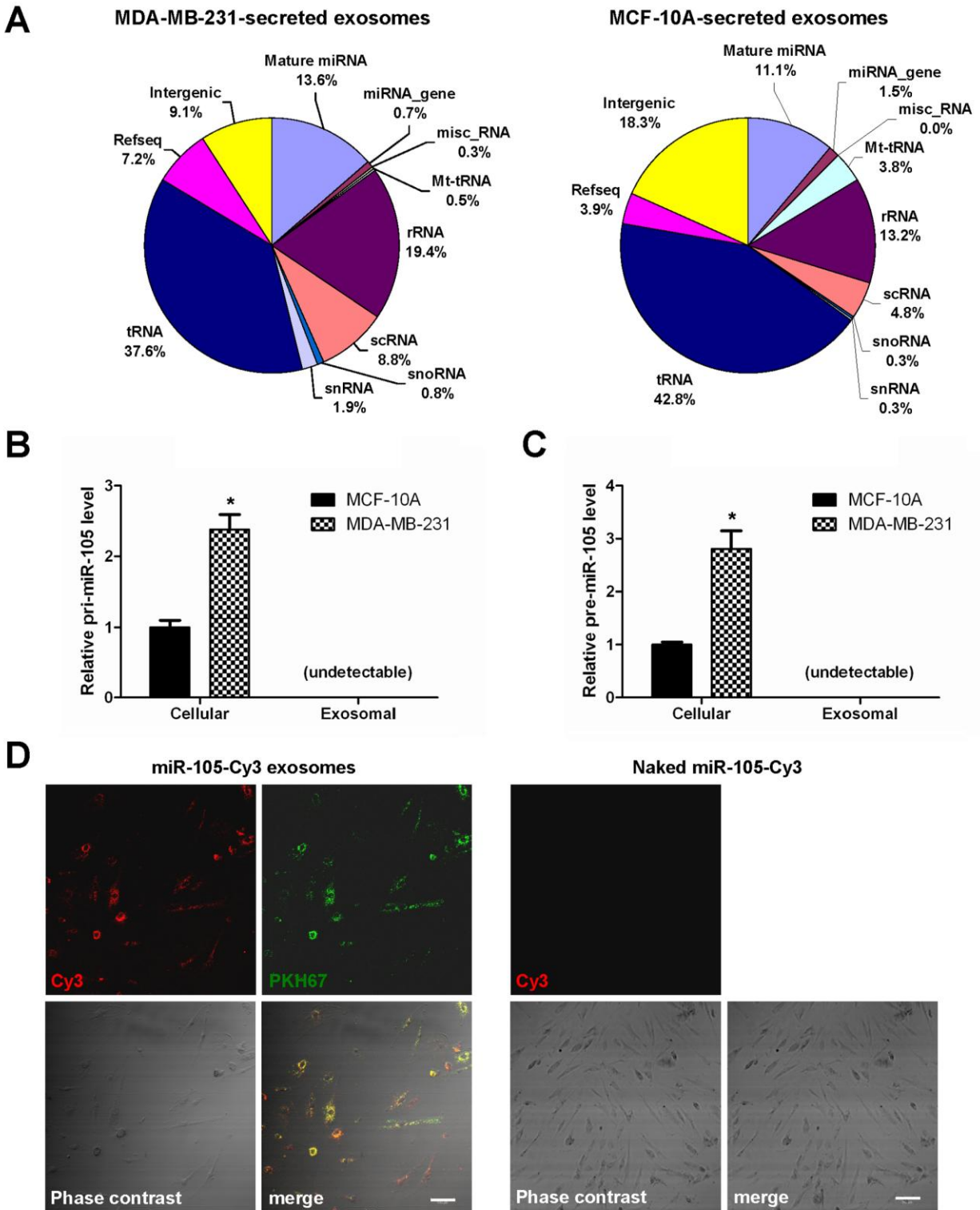
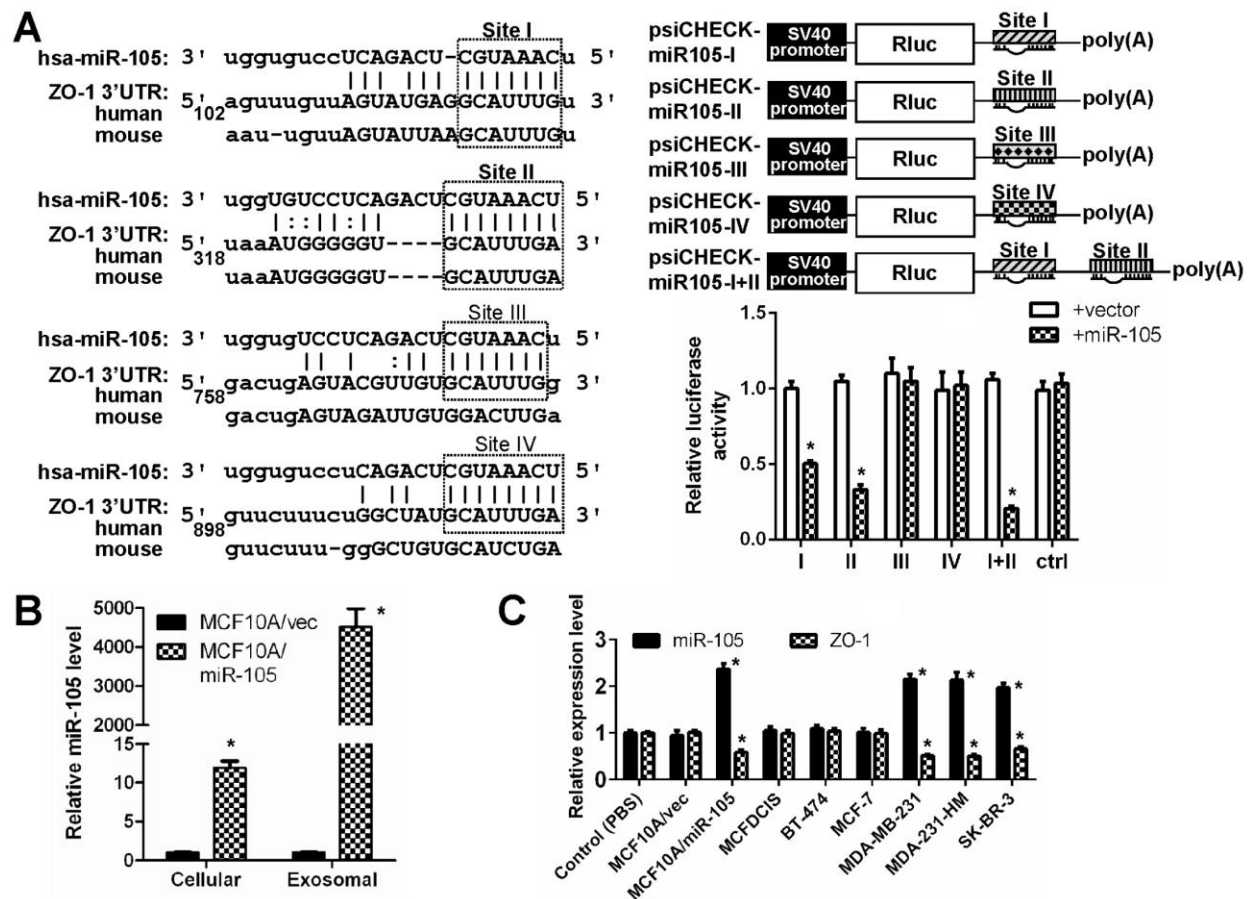


## SUPPLEMENTAL INFORMATION



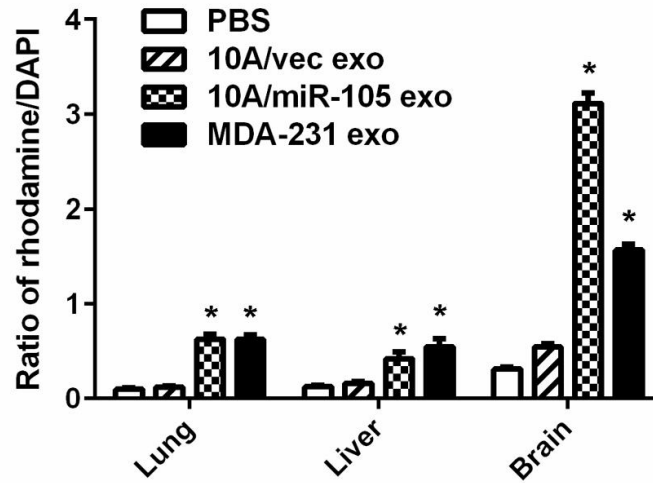
**Figure S1. Mature miR-105 in MDA-MB-231-secreted exosomes can be transferred to endothelial cells, related to Figure 2.** (A) Composition of small RNA sequences in MDA-MB-231 and MCF-10A derived exosomes. Total sequence reads obtained from deep sequencing were aligned to human genome database NCBI36/hg18. The percentage of each class of small RNAs was indicated in the pie chart. scRNA: small cytoplasmic RNA; snRNA: small nuclear RNA; snoRNA: small nucleolar RNA; mt-tRNA: mitochondrial tRNA. (B-C) Pri- and pre-miR-105 are undetectable in exosomes. Cellular and exosomal RNA of MCF-10A and MDA-MB-231 were subjected to RT-qPCR for pri-miR-105 (B) or pre-miR-105 (C). Results are presented as mean  $\pm$  SD. \* $p < 0.005$ . (D) Exosomal but not naked miR-105 is internalized by endothelial cells. PKH67-labeled exosomes secreted by MDA-MB-231 cells transfected with 2 nmol Cy3-labeled miR-105, or 1 nmol naked Cy3-labeled miR-105, were incubated with HMVECs growing on chamber slides. Fluorescent and phase contrast images were captured at 24 hr. Bar = 100  $\mu$ m.

**Table S1. MiRNAs differentially expressed and secreted by MDA-MB-231 and MCF-10A cells ( $p < 0.05$ ;  $\log_2FC > 1$  or  $< -1$ ), related to Figure 2.** Provided as an Excel spreadsheet file.

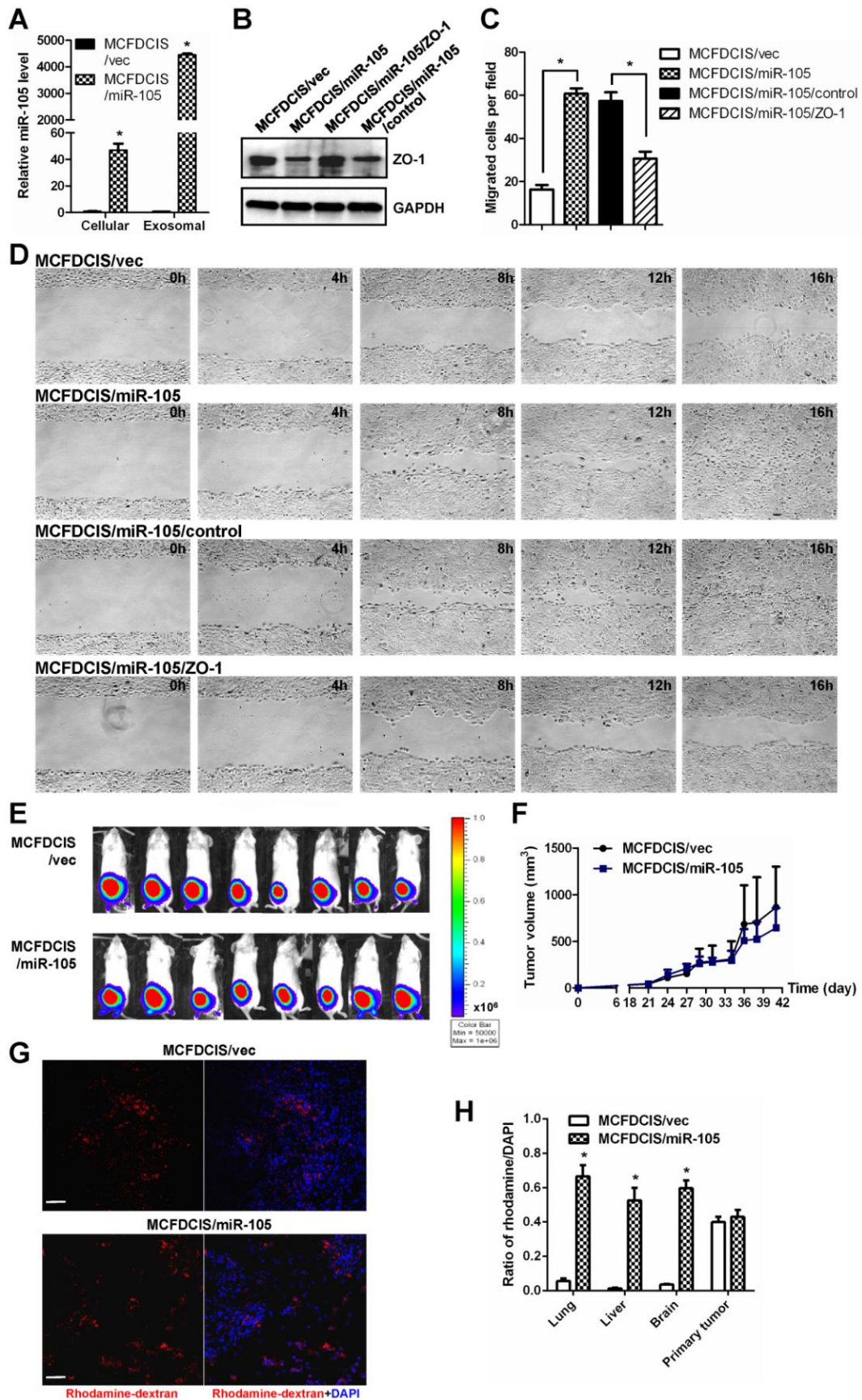


**Figure S2. ZO-1 is a target of miR-105, related to Figure 3.** (A) The predicted miR-105 binding sites (I-IV) in the 3'UTR of human ZO-1 are indicated together with the corresponding sequences in mouse ZO-1. The indicated psiCHECK luciferase reporters containing each of the predicted sites or both site I and site II, or a control reporter containing a scrambled sequence, were used to transfect HMVECs that were also infected with retrovirus expressing miR-105 or vector (as control). Luciferase activity was analyzed at 48 hr post transfection, and compared to the cells infected with the control virus. (B) MCF-10A cells stably transduced with miR-105 or vector were analyzed for miR-105 expression and secretion by RT-qPCR as in Figure 2A-B. (C) Effect of exosomes of various origins on ZO-1 expression in HMVECs. At 48 hr after exosome treatment, RNA was extracted from the recipient HMVECs and subjected to miR-105 and ZO-1

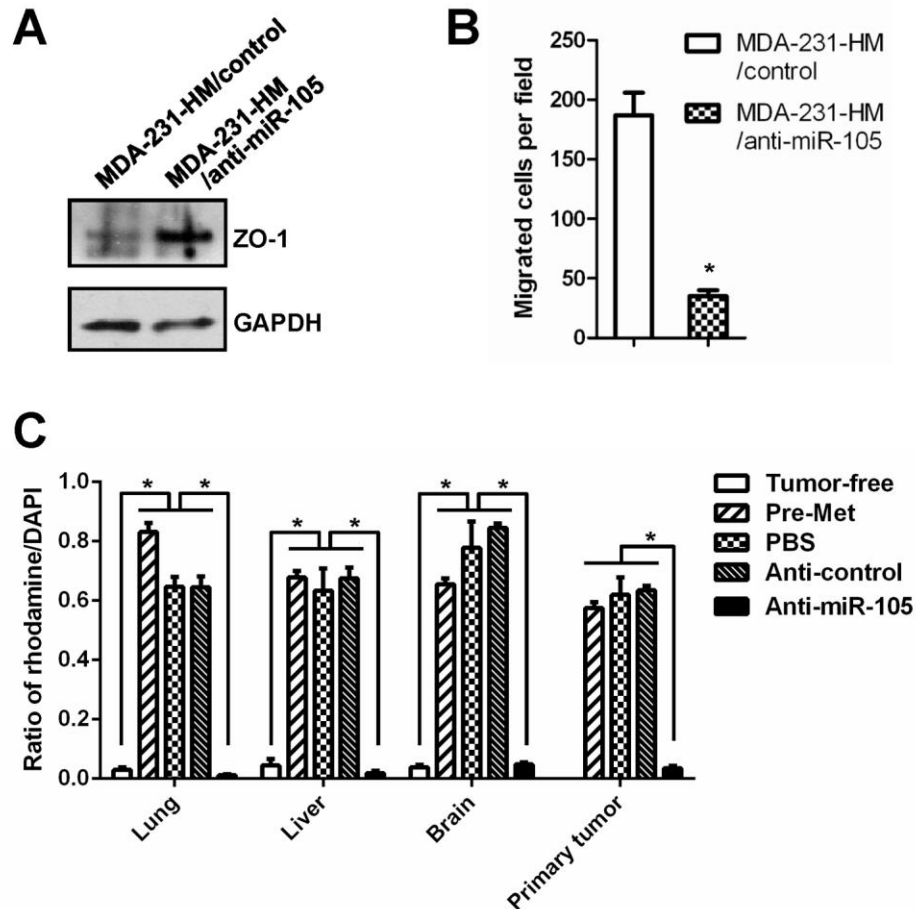
RT-qPCR using U6 and 18S as the corresponding internal reference. Data was compared to the control (PBS) treatment. Results are presented as mean  $\pm$  SD. \* $p < 0.005$ .



**Figure S3. Cancer-secreted miR-105 induces vascular permeability *in vivo*, related to Figure 4.** Quantified data of the *in vivo* vascular permeability assay in Figure 4C (n = 3). Levels of rhodamine-dextran fluorescence in tissues were quantified using ImagePro 6.3 software and normalized to the levels of DAPI. Results are presented as mean  $\pm$  SD. \*p < 0.005 compared to the white columns (controls).

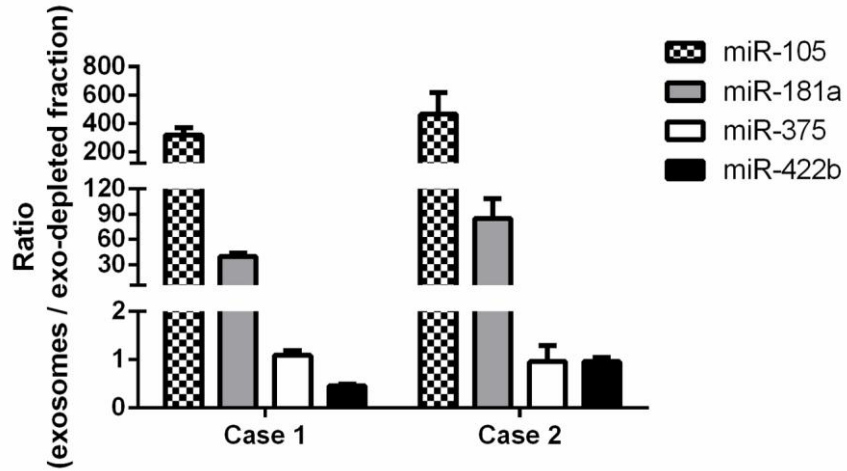


**Figure S4. MiR-105 overexpression in poorly metastatic BC cells promotes metastasis, related to Figure 5.** (A) MCFDCIS cells stably transduced with miR-105 or vector were analyzed for miR-105 expression and secretion by RT-qPCR. Data was normalized to levels of U6 (for cellular miR-105) or miR-16 (for exosomal miR-105). (B) Transduced MCFDCIS cells with or without transfection with a ZO-1 expression plasmid or vector control were analyzed by Western blot at 48 hr post transfection. (C) The indicated cells were subjected to transwell migration assay and cells that had migrated within 8 hr were quantified from triplicate wells. (D) Confluent monolayers of MCFDCIS cells treated as indicated were wounded with a pipette tip. Wound closure was monitored at the indicated time points. (E) The week 3 BLI of the mice bearing MCFDCIS xenograft tumors shown in Figure 5A (n = 8). (F) Growth curves of MCFDCIS xenograft tumors. Tumor volume determined in the two groups of mice shown in Figure 5A did not show significant difference (n = 8). (G) Vascular permeability in primary MCFDCIS xenograft tumors. *In vivo* vascular permeability was determined in MCFDCIS xenograft tumors (n = 3) as in Figure 5D. Representative images are shown. No significant difference is observed between the two groups. Bar = 100  $\mu$ m. (H) Quantified data of the *in vivo* vascular permeability assays in Figures 5D and S4G (n = 3). Levels of rhodamine-dextran fluorescence in tissues were quantified using ImagePro 6.3 software and normalized to the levels of DAPI. \*p < 0.005 compared to the white columns (controls). Results are presented as mean  $\pm$  SD.



**Figure S5. MiR-105 inhibition suppresses metastasis and restores vascular integrity *in vivo*, related to Figure 6.** (A) MDA-231-HM cells transfected with an anti-miR-105 compound or control compound were analyzed by Western blot. (B) The transfected MDA-231-HM cells were subjected to transwell migration assays. Cells that had migrated within 8 hr were quantified from triplicate wells. (C) Quantified data of *in vivo* vascular permeability assays in Figure 6F (n = 4). Levels of rhodamine-dextran fluorescence in tissues were quantified using ImagePro 6.3 software and normalized to the levels of DAPI. Results are presented as mean  $\pm$  SD. \*p < 0.005.





**Figure S6. Circulating miR-105 predominantly exists in exosomes, related to Figure 7.**

Exosomes isolated from two cases of BC patient sera as well as the corresponding exosome-depleted serum fractions were subjected to RNA extraction and RT-qPCR of selected miRNAs.

The ratio of miRNA level in the exosomes to that in exosome-free fraction was shown. Results are presented as mean  $\pm$  SD.

**Table S2. Clinical characteristics of all patients, related to Figure 7.**

	<b>Cohort for serum study</b>	<b>Cohort for tumor study</b>	<b>Cohort for serum-tumor correlation</b>
	<b>n (%)</b>	<b>n (%)</b>	<b>n (%)</b>
<b>Patient (n)</b>	38	20	18
<b>Age (Mean <math>\pm</math> SD)</b>	51 $\pm$ 9	54 $\pm$ 9	54 $\pm$ 9
<b>ER<sup>+</sup></b>	26 (68)	13 (65)	11 (61)
<b>ER<sup>-</sup></b>	12 (32)	7 (35)	7 (39)
<b>PR<sup>+</sup></b>	20 (53)	10 (50)	9 (50)
<b>PR<sup>-</sup></b>	18 (47)	10 (50)	9 (50)
<b>HER2<sup>+</sup></b>	12 (32)	6 (30)	6 (33)
<b>HER2<sup>-</sup></b>	26 (68)	14 (70)	12 (67)
<b>Stage II</b>	15 (39)	9 (45)	7 (39)
<b>Stage III</b>	23 (61)	11 (55)	11 (61)

**Table S3. Clinical information of patients in the serum study, related to Figure 7.**

<b>Patient</b>	<b>ER</b>	<b>PR</b>	<b>HER2</b>	<b>Stage</b>	<b>Yr. Dx.</b>	<b>Age Dx.</b>	<b>Progression</b>
1	+	-	-	2	2003	61	Y
2	+	-	+	2	2006	69	Y
3	-	-	-	2	2007	34	Y
4	+	+	-	3	2010	46	Y
5	-	-	-	3	2010	50	Y
6	+	+	+	2	2006	61	Y
7	+	+	+	3	2009	62	Y
8	+	-	-	3	2006	47	Y
9	+	-	+	3	2006	69	Y
10	-	+	-	3	2008	51	Y
11	+	-	-	3	2006	50	Y
12	-	-	-	3	2008	47	Y
13	-	-	-	2	2008	31	Y
14	+	+	+	3	2008	39	Y
15	+	+	+	3	2008	58	Y
16	-	-	+	3	2009	32	Y
17	+	+	-	3	2007	64	N
18	+	+	-	3	2006	53	N
19	+	-	-	3	2008	53	N
20	+	-	-	3	2007	63	N
21	-	-	+	3	2007	49	N
22	+	+	-	2	2006	50	N
23	-	+	-	2	2007	49	N
24	-	-	-	2	2007	51	N
25	+	+	+	2	2008	49	N
26	+	+	-	3	2006	53	N
27	+	+	-	2	2006	51	N
28	+	+	-	2	2007	50	N
29	+	+	-	3	2007	47	N
30	+	+	-	2	2007	54	N
31	+	-	-	3	2007	63	N
32	+	+	-	3	2007	64	N
33	+	+	-	2	2007	54	N
34	+	+	-	2	2008	49	N
35	-	-	+	2	2006	32	N
36	+	+	-	3	2006	47	N
37	-	-	+	3	2006	50	N
38	-	-	+	3	2007	53	N

**Table S4. Clinical information of patients in the tumor study, related to Figure 7.**

<b>Patient</b>	<b>ER</b>	<b>PR</b>	<b>HER2</b>	<b>Stage</b>	<b>Yr. Dx.</b>	<b>Age Dx.</b>	<b>Progression</b>
1	+	-	+	2	2006	69	Y
2	+	-	-	2	2010	59	Y
3	+	+	-	3	2010	46	Y
4	-	-	-	3	2010	50	Y
5	+	+	+	2	2006	61	Y
6	-	-	-	3	2008	47	Y
7	-	-	-	2	2008	31	Y
8	+	-	+	3	2006	69	Y
9	-	+	-	3	2008	51	Y
10	+	+	+	3	2009	62	Y
11	+	+	-	3	2007	64	N
12	+	+	-	2	2006	44	N
13	+	+	-	3	2006	53	N
14	+	-	-	3	2008	53	N
15	+	-	-	3	2007	63	N
16	-	-	+	3	2007	49	N
17	+	+	-	2	2006	50	N
18	-	+	-	2	2007	49	N
19	-	-	-	2	2007	51	N
20	+	+	+	2	2008	49	N

**Table S5. Clinical information of patients in the serum-tumor correlation study, related to Figure 7.**

<b>Patient</b>	<b>ER</b>	<b>PR</b>	<b>HER2</b>	<b>Stage</b>	<b>Yr. Dx.</b>	<b>Age Dx.</b>	<b>Progression</b>
1	+	-	+	2	2006	69	Y
2	+	+	-	3	2010	46	Y
3	-	-	-	3	2010	50	Y
4	+	+	+	2	2006	61	Y
5	-	-	-	3	2008	47	Y
6	-	-	-	2	2008	31	Y
7	+	-	+	3	2006	69	Y
8	-	+	-	3	2008	51	Y
9	+	+	+	3	2009	62	Y
10	+	+	-	3	2007	64	N
11	+	+	-	3	2006	53	N
12	+	-	-	3	2008	53	N
13	+	-	-	3	2007	63	N
14	-	-	+	3	2007	49	N
15	+	+	-	2	2006	50	N
16	-	+	-	2	2007	49	N
17	-	-	-	2	2007	51	N
18	+	+	+	2	2008	49	N

## **Supplemental Experimental Procedures**

**Clinical specimens.** Human specimens were obtained from voluntarily consenting patients at the City of Hope Medical Center (Duarte, CA) under institutional review board-approved protocols. For circulating miR-105 analysis, 38 patients with stage II–III BC at the time of registration were included, including 16 who developed systemic recurrence while on study and 22 who had not developed systemic relapse. The two groups were balanced for age, treatment regimen, and sample collection time. The mean follow-up is 4.2 years for the group with disease progression and 4.7 years for the group without at the time of analysis. Exosomes were isolated from serum, and RNA was extracted using TRIZOL LS reagent (Life Technologies; Grand Island, NY) and subjected to reverse transcription-quantitative PCR (RT-qPCR) for miRNAs as described (Wu et al., 2012). For the evaluation of miR-105 and ZO-1 expression using tumor specimens, a cohort including 20 stage II–III BC patients (10 developed systemic recurrence later with a mean follow-up of 3.5 years and 10 did not with a mean follow-up of 4.7 years) that partially overlaps with the serum cohort was studied. Among these, 18 patients had paired serum and tumor specimens available. The clinical information is summarized in Tables S2-5. The BC tissue array (Figure 7G) was purchased from US Biomax (Cat #BR1505a; Rockville, MD).

**Cells, plasmids and viruses.** Human BC cell lines and MCF-10A were obtained from American Type Culture Collection (Manassas, VA) and cultured as recommended. Primary HMVECs (from adult dermis) were purchased from Life Technologies (Grand Island, NY) and cultured according to the manufacturer's instructions. MCF10DCIS.com (MCFDCIS) cells were purchased from Asterand (Detroit, MI). The MDA-231-HM cells were generated in our lab through explant culture of a spontaneous meningeal metastasis of MDA-MB-231 cells from an immunocompromised mouse. To construct the ZO-1 3'UTR reporter plasmids, annealed

oligonucleotides encompassing the putative miR-105 binding sites indicated in Figure S2A or a scrambled control sequence were inserted into the *XhoI/NotI* sites of psiCHECK-2 vector (Promega; Madison, WI) downstream of the *Renilla* luciferase gene. For miR-105 overexpression, the hsa-mir-105-1 gene was cloned by PCR using primers 5'-TGTTTGCCTCCTTCTTCGTC and 5'-ACAGGAACAAATGGCTTTGG, and constructed into the *BamHI/SalI* sites of pBABE-Puro or pBABE-GFP retroviral vector. The human ZO-1 expression plasmid was kindly provided by Dr. Alan Fanning (University of North Carolina at Chapel Hill). Transfection, reporter assays, production of viruses, as well as infection and selection of transduced cells were carried out as previously described (Wang et al., 2006; Wang et al., 2011). MiRIDIAN miR-105 mimic and hairpin inhibitor as well as their corresponding negative controls were purchased from Thermo Fisher Scientific (Waltham, MA). MiR-105 labeled with Cy3 at the 3' end was synthesized by Integrated DNA Technologies (Coralville, IA). The RNA polymerase II inhibitor 5,6-dichloro-1- $\beta$ -D-ribofuranoside (DRB) and VEGF were purchased from Sigma (St. Louis, MO).

**Exosome purification and EM.** For exosomes secreted by cultured cell lines, conditioned media (CM) was first prepared by incubating cells grown at sub-confluence in growth media containing exosome-depleted FBS (prepared by overnight ultracentrifugation at 100,000  $\times$ g at 4°C) for 48 hr, and pre-cleared by centrifugation at 500  $\times$ g for 15 min and then at 10,000  $\times$ g for 20 min. Exosomes were isolated by ultracentrifugation at 110,000  $\times$ g for 70 min, and washed in PBS using the same ultracentrifugation conditions. When indicated, DiI (1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Sigma) was added into the PBS at 1  $\mu$ M and incubated for 20 min before the washing spin, followed by an additional wash to remove the excess dye. Exosome labeling with PKH67 (Sigma) was performed following the manufacturer's procedures.

The pelleted exosomes were resuspended in ~100  $\mu$ l of PBS, and subjected to EM, cell treatment, or RNA extraction by *mirVana* miRNA isolation kit (Life Technologies; for small RNA extraction in Figure 1E) or TRIZOL LS (for all other experiments). For EM, exosomes were fixed with 2% paraformaldehyde, loaded on 200-mesh Formvar-coated grids, and then contrasted and embedded as described in (They et al., 2006). The grids were observed under an FEI Tecnai12 transmission electron microscope equipped with a CCD camera. For cell treatment, 2  $\mu$ g of exosomes (equivalent to those collected from  $\sim 5 \times 10^6$  producer cells) based on protein measurement using Bradford protein assay (Bio-Rad; Hercules, CA) were added to  $2 \times 10^5$  recipient cells.

**RNA extraction, RT-qPCR, Western blot analysis, and IF.** These procedures were performed as described previously (Tsuyada et al., 2012; Wang et al., 2011; Yu et al., 2010). Primers used in qPCR are: 5'-ATGGAGGAAACAGCTATATGGGA and 5'-CCAAATCCAAATCCAGGAGCC for *TJP1* (ZO-1), and 5'-CTACCACATCCAAGGAAGGCA and 5'-TTTTTCGTCACTACCTCCCCG for 18S rRNA (as internal control for ZO-1). The hsa-mir-105-1 gene cloning primers were used for pri-miR-105 qPCR, and primers for mature miR-105, U6 (as internal control for intracellular miR-105), and pre-miR-105 were purchased from Qiagen (Valencia, CA). An annealing temperature of 55°C was used for all primers. ZO-1, occludin, and VE-cadherin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), CD31 antibody from eBioscience (San Diego, CA), and GAPDH antibody from Cell Signaling (Danvers, MA).

**TEER, endothelial permeability, and 3D vascular sprouting assays.** Treated HMVEC monolayers grown on 0.3 cm<sup>2</sup> polyethylene terephthalate transwell filters (0.4- $\mu$ m pore size; BD Biosciences; Franklin Lakes, NJ) were analyzed for TEER using an EVOM2 voltohmmeter



(World Precision Instruments; Sarasota, FL). Unit area resistance was calculated following the manufacturer's instructions. Three filters were used for each treatment. The permeability of treated HMVEC monolayers grown on transwell filters (0.4- $\mu\text{m}$  pore size; BD Biosciences) was assessed by the passage of rhodamine B isothiocyanate-dextran (average MW  $\sim$ 70,000; Sigma). Briefly, rhodamine-dextran was added to the top well at 20 mg/ml, and the appearance of fluorescence in the bottom well was monitored by measuring 40  $\mu\text{l}$  medium aliquots in a time course using a SpectraMax microplate reader (Molecular Devices; Sunnyvale, CA) at 544 nm excitation and 590 nm emission. Vascular sprouting assay was performed as described using microcarrier-beads coated with endothelial cells and embedded in 3D fibrin gel (Newman et al., 2011). Briefly, HMVECs were coated on microcarrier-beads in EGM2 medium for 24 hr, followed by embedding the beads into 3D fibrin gel in a 24-well plate, and overlaid with normal human lung fibroblasts in 300  $\mu\text{l}$  of EGM2 medium. The cells were incubated at 37°C with 5%  $\text{CO}_2$ .

**Wound closure, transwell migration, and trans-endothelial invasion assays.** Wound closure and transwell migration assays were performed as previously described (Wang et al., 2006). For trans-endothelial invasion assay, pre-treated HMVECs were plated and allowed to reach confluence for 3 days on transwell filters (3- $\mu\text{m}$  pore size; BD Biosciences). GFP-labeled MDA-231-HM cells (with pBABE-GFP retrovirus) were seeded into transwell inserts at 50,000 cells per well. After 10 hr, cells on the top side of inserts were scraped off and the transwell filters were examined under an IX81 inverted fluorescent microscope (Olympus; Center Valley, PA) for invading GFP<sup>+</sup> cells.

**Animals.** All animal experiments were approved by the institutional animal care and use committee at City of Hope. For exosome treatment experiments, exosomes secreted by

MCF10A/vec, MCF10A/miR-105, or MDA-MB-231 cells, or PBS (as control), were intravenously injected into the tail vein of 6-week-old female NSG mice (2  $\mu$ g exosomes per injection; two injections per week). After 5 injections, mice were either sacrificed for tissue collection and assessments, or subjected to intracardiac injection of  $1 \times 10^5$  luciferase-labeled MDA-MB-231 cells. For xenograft experiments, luciferase-labeled cells ( $2 \times 10^5$  MCFDCIS or MDA-231-HM) were injected into the No. 4 mammary fat pad of 6-week-old female NSG mice. Weekly bioluminescence imaging (BLI) was carried out using a Xenogen system (Caliper Life Sciences; Alameda, CA). When tumors became palpable, tumor volume was assessed by caliper measurements using the formula  $(\text{width}^2 \times \text{length})/2$  ( $\text{mm}^3$ ). For the miR-105 intervention study, mice were divided into 3 groups for treatment with PBS, an anti-miR-105 compound or a control compound of a sequence that does not target any miRNA. The control and miR-105 targeted compounds had the same chemical modification pattern, chimeric 2'-fluoro and 2'-methoxyethyl modifications on a phosphorothioate backbone (Davis et al., 2006) and were synthesized at Regulus Therapeutics (San Diego, CA). At first, compounds (25 mg/kg) were intraperitoneally injected daily for 5 days starting at day 3 after cancer cell implantation, and then twice weekly until the end of experiment. The same compounds were also used *in vitro* to transfect MDA-231-HM cells in Figure S5A&B. For *in vivo* vascular permeability assay, 100 mg/kg rhodamine-dextran (average MW  $\sim$ 70,000) was intravenously injected 3 hr before a transcardiac perfusion was carried out to remove the excess dye. Tissues were formaldehyde-fixed and paraffin-embedded for hematoxylin and eosin (H&E) stain or embedded in Tissue-Tek O.C.T. Compound (Sakura; Torrance, CA) to make frozen blocks for sectioning and fluorescent microscopy. For serum miRNA analysis, blood was collected through cardiac puncture and fractionated by centrifugation, and serum RNA was extracted using TRIZOL LS.

**ISH and IHC.** IHC staining was performed as previously reported (Tsuyada et al., 2012). ISH was performed in formaldehyde-fixed, paraffin-embedded tissue sections using miRCURY LNA™ microRNA ISH optimization kit and the miR-105 detection probe (Exiqon; Woburn, MA) by following the manufacturer's protocol. Probes for U6 and a scrambled sequence were used as positive and negative controls, respectively. IHC staining was performed as previously reported (Tsuyada et al., 2012) using human Ki-67 (Clone MIB-1; Dako; Carpinteria, CA), cleaved caspase-3 (Cat #9661; Cell Signaling), and ZO-1 antibodies (Cat #sc-10804; Santa Cruz Biotechnology). Stained slides were scored according to intensity of staining (-: 0; +: 1; ++: 2; and +++: 3) and percentage of the cells of interest staining positive for each antigen (0%: 0; 1~29%: 1; 30~69%: 2; and  $\geq 70\%$ : 3). The intensity score was multiplied by the percentage score to obtain a final score, which was used in the statistical analyses.