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TITLE: Identification and Targeting of Metastasis-Suppressing miRNAs in Triple-Negative Breast Cancer

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14. ABSTRACT

Background. Triple-negative breast cancer (TNBC) constitutes ~20-25% of all breast cancer cases and has the worst prognosis due to high rates of distant recurrence, high rates of metastasis and a lack of effective molecularly targeted therapies. For patients diagnosed with advanced TNBC, the median duration of survival is only 12 months, which is much shorter than for patients diagnosed with other types of breast cancer. Therefore, TNBC patients, especially those presenting with advanced stage disease, are in desperate need of novel molecularly targeted therapies. Since TNBC is heterogeneous, the molecular underpinnings have been difficult to discern, thus making it challenging to develop effective targeted therapeutics. miRNA replacement therapy is a novel type of targeted therapy that is beginning to be investigated in clinical trials as it shows great promise. The remaining hurdle, however, is uncovering the most effective method for *in vivo* delivery. One mechanism for delivery that has not been previously investigated is a liposome-like structure called a DNAsome, which is ideal for the *in vivo* delivery of small RNA molecules. We therefore hope to develop miRNA replacement therapy for the regression of existing metastases in late-stage TNBC patients using the DNAsome.
Overarching Challenge. This proposal aims to 1) revolutionize treatment regimens by replacing interventions that have life-threatening toxicities with molecularly targeted ones that are safe and effective and 2) Eliminate the mortality associated with metastatic breast cancer.
Objective/Hypothesis. miRNA dysregulation has been implicated in tumorigenesis and metastasis within various cancer types, including breast cancer. Importantly, we have shown as proof-of-principle, that *in vivo*, miR-708, an anti-metastatic miRNA, can block metastatic progression following metastatic seeding of TNBC cells. *We therefore hypothesize that there are miRNAs that initiate metastasis regression and/or block metastatic progression. We also propose that these miRNA can be delivered in vivo as part of an effective cancer treatment.* Our overall goal is use preclinical models of breast cancer to demonstrate whether miRNA replacement therapy is an effective way of initiating the regression of existing breast cancer metastases and thus reducing the high mortality rate characteristic of TNBC.
Specific Aims. 1) To identify miRNAs that are capable of initiating regression of metastases in TNBC and 2) To assess the potential of DNAsome delivered miRNAs as a metastasis regression therapeutic.
Study Design. We have identified many miRNAs that are downregulated in metastatic TNBC cell lines compared to non-metastatic but tumorigenenic breast cancer cell lines. We propose to perform an *in vivo* screen to identify if any of these miRNAs can specifically regress existing lung, brain or bone metastases. We will then identify the mRNA targets of each of the miRNAs that leads to regression of metastases at any or all of these sites. This will

15. SUBJECT TERMS
Triple negative breast cancer, metastasis, micro RNA, miRNA, epigenetic, DNAsomes, nanoparticle, Targeted therapy

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1. **INTRODUCTION:**

This proposal aims to identify miRNAs that regress established metastasis and can be delivered for the treatment of advance high-risk breast cancer patients. We will pursue our goals by identifying miRNAs that lead to the regression of existing metastases, determining the molecular mechanisms by which the identified miRNAs induce regression and exploring the potential for in vivo delivery of miRNAs for the treatment of late-stage metastatic breast cancer. The projects two specific aims are (1) To identify miRNAs that regress established metastases in breast cancer and (2) To assess the potential of DNAsome delivered miRNAs as a metastasis regression therapeutic.

The overarching goals are to 1) Revolutionize treatment regimens by replacing interventions that have life-threatening toxicities with ones that are molecularly targeted, safe and effective and 2) Lead to the elimination of mortality associated with metastasis in high-risk category of breast cancer patients.

2. **KEYWORDS:**
Triple negative breast cancer, metastasis, micro RNA, miRNA, epigenetic, DNAsomes, nanoparticle, Targeted therapy

3. **ACCOMPLISHMENTS:**
What were the major goals of the project?

Aim1: To identify miRNAs that regress established metastases in breast cancer.

Major Task 1: Identification of miRNAs that regress existing metastases in TNBC

Subtask 1: Create stable breast cancer cell lines expressing each miRNA of interest under the control of an inducible promoter. Cell lines used: MDA-MB-231-LM2, MCF7
Subtask 2: Determine whether expression of the miRNA of interest in cancer cells can regress existing lung metastases in an orthotopic mouse model of breast cancer. Total Animals = 100; SCID mice: n = 10 / miRNA expressing cell line x 10 miRNAs = 100 mice
Subtask 3: Generate breast cancer cell lines that specifically metastasize to the brain or bone that stably express each miRNA of interest under the control of an inducible promoter. Cell lines used: BoM-1833, BrM-831
Subtask 4: Determine whether expression of the miRNA of interest in cancer cells can regress existing brain and/or bone metastases in mice. Total Animals = 100
BALB/c-nu/nu nude mice: n=10 mice/miRNA expressing cell line (5 induced, 5 uninduced) x 10 miRNAs = 100 mice

Milestones: 1) Stable cells with inducible miRNAs, 2) Identification of 3-4 miRNAs that regress lung, brain and/or bone metastases in vivo. Local IRB/IACUC Approval; HRPO/ACURO Approval;

Major Task 2: mRNA target identification of miRNAs that show regression of lung, brain and/or bone metastases.

Subtask 1: Use of established algorithms to identify candidate mRNA targets of miRNAs identified in major task 1.
Subtask 2: Cloning of 3' UTRs of candidate mRNAs into a dual luciferase UTR vector.

Subtask 3: Generation of breast cancer cell line stably knocked down for the putative mRNA target(s). Cell lines used: MDA-MD-231-LM2
Subtask 4: *in vivo* analysis of whether the loss of mRNA target expression regresses established metastases. Total Animals = 100; SCID mice: n=10/miRNA or shRNA x 10 miRNAs (including scrambled controls) = 100 mice

Milestones: 1) Identification of several potential mRNA targets of the miRNA(s) of interest, 2) successful cloning of 3' UTR dual luciferase plasmids and subsequent identification of putative mRNA targets, 3) Generation of TNBC cell lines stably expressing an inducible shRNA construct for the putative mRNA target, 4) Verification that knock-down of the putative mRNA in cancer cells leads to the regression of established metastases.

Major Task 3: To evaluate the clinical significance of identified miRNAs.

Subtask 1: Use human TNBC patient samples to determine whether the miRNAs of interest are downregulated in metastatic lesions compared to the primary tumor.
Subtask 2: Use the same human TNBC patient samples as above to determine whether the mRNA targets are upregulated in metastatic lesions compared to the primary tumor.

Milestones: 1) Demonstration that the miRNAs of interest are downregulated in metastatic lesions compared to the primary tumor. 2) Demonstration that the mRNA target is upregulated in metastatic lesions compared to the primary tumor.

Aim 2: To assess the potential of DNAsome delivered miRNA as metastasis suppressing and regressing therapeutics.

Major Task 1: Synthesize DNAsomes carrying each miRNA shown to regress metastases in aim 1.
Milestone: As explained in the last report, gold nanoparticles will be utilized as carriers for delivery of miRNA.

Major Task 2: Determine whether DNAsomes carrying the miRNAs identified in aim 1 can regress in vivo metastases to the lung, brain and/or bone.

Subtask 1: Optimize the in vivo delivery of DNAsomes using PDX models of TNBC. Total Animals = 120 mice; SCID mice: n=5/group (4 doses, 3 delivery frequencies) = 60 mice/miRNA x 2 miRNAs (1 specific + 1 scrambled control) = 120 mice.
Subtask 2: Determine whether DNAsome mediated delivery of miRNAs can regress existing lung, brain and/or bone metastases. Total Animals = 100 mice; SCID mice: n=10/miRNA analyzed x 10 miRNAs = 100 mice
Subtask 3: Evaluation of toxicity in DNA-some-mediated delivery of miRNAs. Total Animals = 30 mice; Mice: n=15/group x 2 groups (DNAsome treated and untreated) = 30
Milestones: Demonstrate that miRNA replacement therapy can regress TNBC metastases in mouse models and *in vitro* toxicity will be evaluated.

What was accomplished under these goals?

Aim 1, Major Task 1

Our laboratory had previously identified microRNAs (miRs) that are downregulated in metastatic breast cancer by next-gen miR sequencing (miR-seq) (2). From our literature review of the identified downregulated miRs, we selected 19 miRs (miR- 342-3p, 342-5p, 489, 652, 195, 196b, 34a, 365, 185, 335, 421 that have not been fully studied in the context of metastatic breast cancer (Table 1 describes five selected miRs).

Table 1. Literature Review of five selected miRNAs

miRs	mir141	mir429	mir365	mir342-5p	mir652
Fold change	-244.20	-19.07	-10.27	-27.15	-3.18
Oncomir	Colon, Prostate, SCC	Colorectal, NSCLC, HCC, Prostate	Gastric, SCC	-	-
Suppressor	Gastric, Pancreatic, Bladder	Colorectal, Pancreatic, Ovarian, Gastric	Lung, Pancreatic, Melanoma	Colon	Pancreatic
Predicted Targets	TAZ, MAP4k4, STAT4	Onecut2, Sox-2, PTEN, RASSF8, TIMP-2, TBK1, p27Kip1, c-myc	TTF-1, NRP1, SHC1, BAX	NAA10	ZEB1
References	Zuo QF, et al. 2015; Cheng H, et al. 2011; Jou AF, et al. 2015; Zhao G, et al. 2013	Tang J, et al. 2015; Sun Y, et al. 2014; Li J, et al. 2013; Chen J, et al. 2011; Wang L, et al. 2014; Lang Y, et al. 2014; Song B, et al. 2015; Ouyang Y, et al. 2015.	Guo SL, et al. 2013; Hamada S, et al. 2014; Qi J, et al. 2012; Zhou M, et al. 2013	Yang H, et al. 2016	Deng S, et al. 2015

In order to identify miRNAs specifically downregulated in metastatic breast cancer cells, we performed miR-PCR for miRNAs on a preliminary panel of non-metastatic and metastatic breast cancer cell lines (Fig. 1). From this panel, we selected top 5 miRNA which were cloned into a doxycycline (dox) inducible lentiviral construct that contains both the genes for rtTA3 and the miRNA on the same plasmid (Fig. 3A). The inducible plasmid is a miR-E based system with a variation of Tet-On 3G plasmid, which is 100-fold more sensitive to dox, and has reduced leakiness combined with a more robust expression of the miR of interest (3).

Figure 1. miRNA expression in non-metastatic and metastatic breast cancer cell lines.

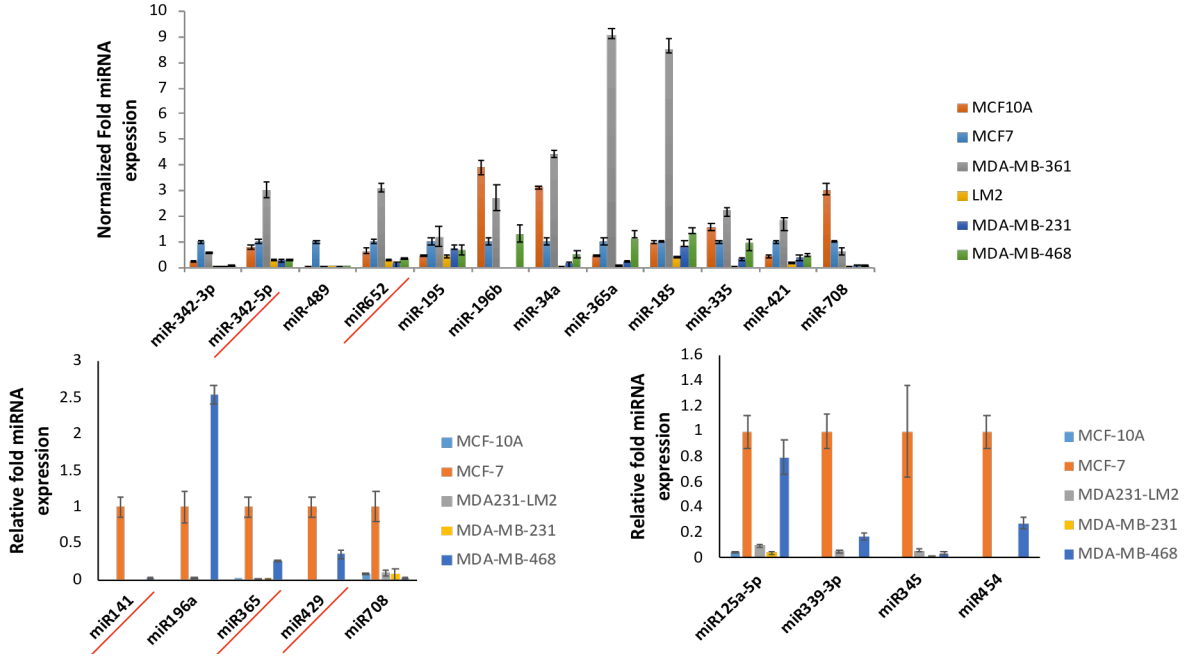


Figure 2. Expression of 5 selected miRNAs in a panel of non-metastatic and metastatic breast cancer cell lines.

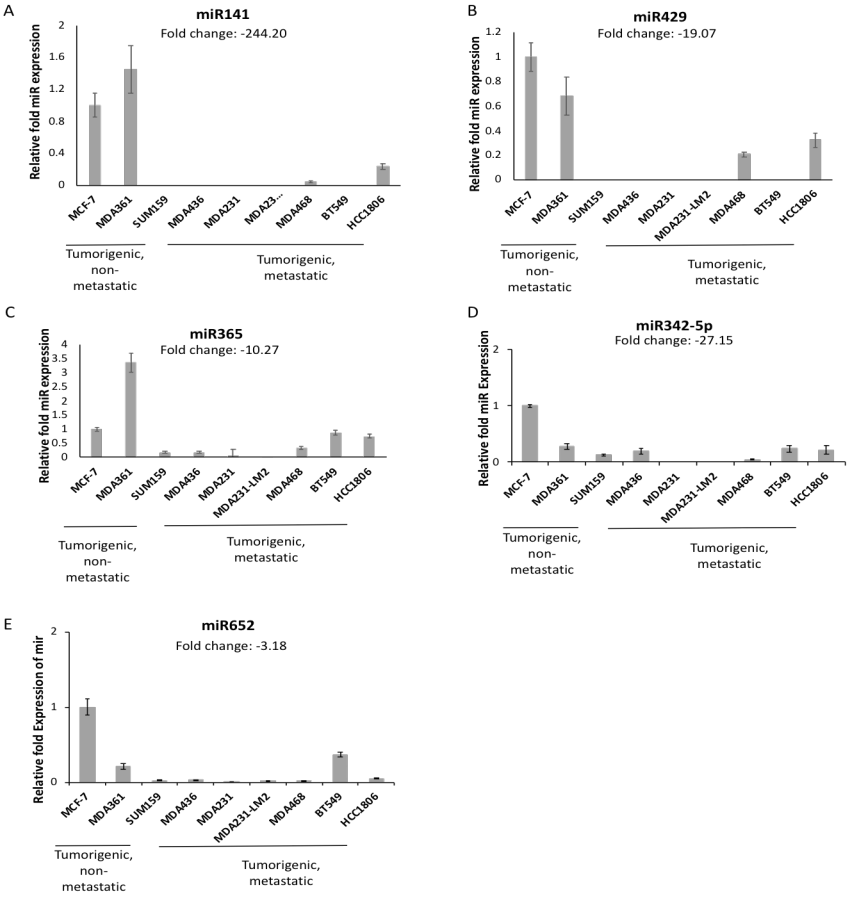
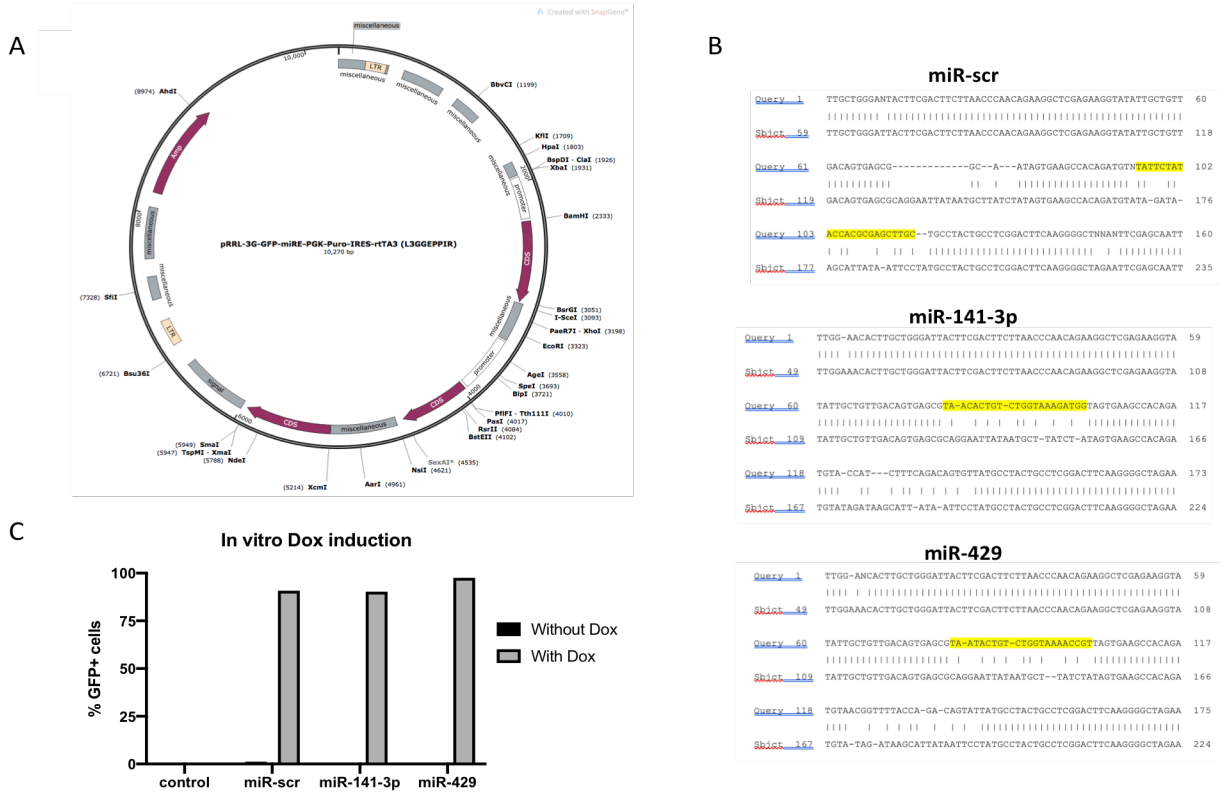


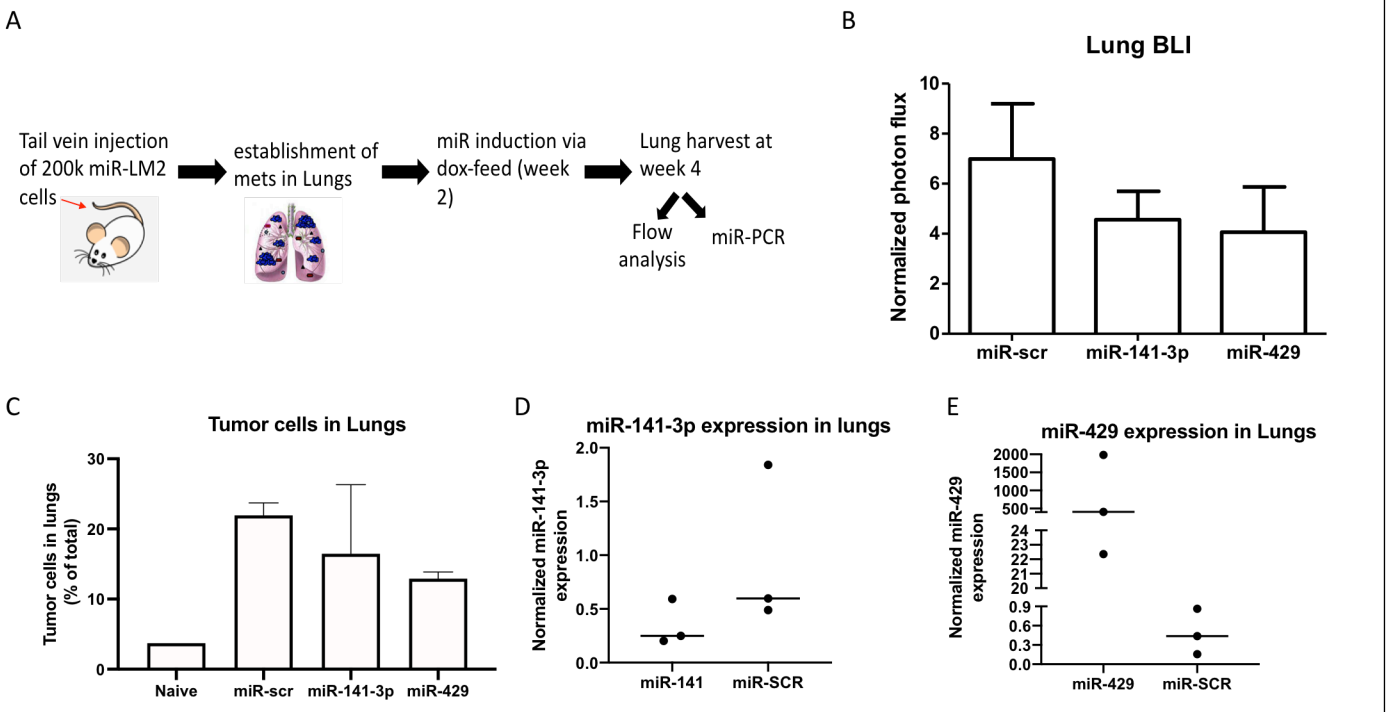
Figure 3. (A) Map of the third generation reverse tetracycline controlled transactivator 3 (rtTA3) lentiviral expression vector. (B) Inserted miR fragments in rtTA3 plasmid. (C) GFP expression is induced after 24h of treatment with doxycycline.



We were successfully able to clone miRNAs 141-3p and 429 in the dox-inducible vector (Fig. 3B). With the rtTA3 system, we were able to show that LM2 cells stably expressing the miRNAs elicited a markedly tight inducible expression in the presence of Dox (Fig. 3C).

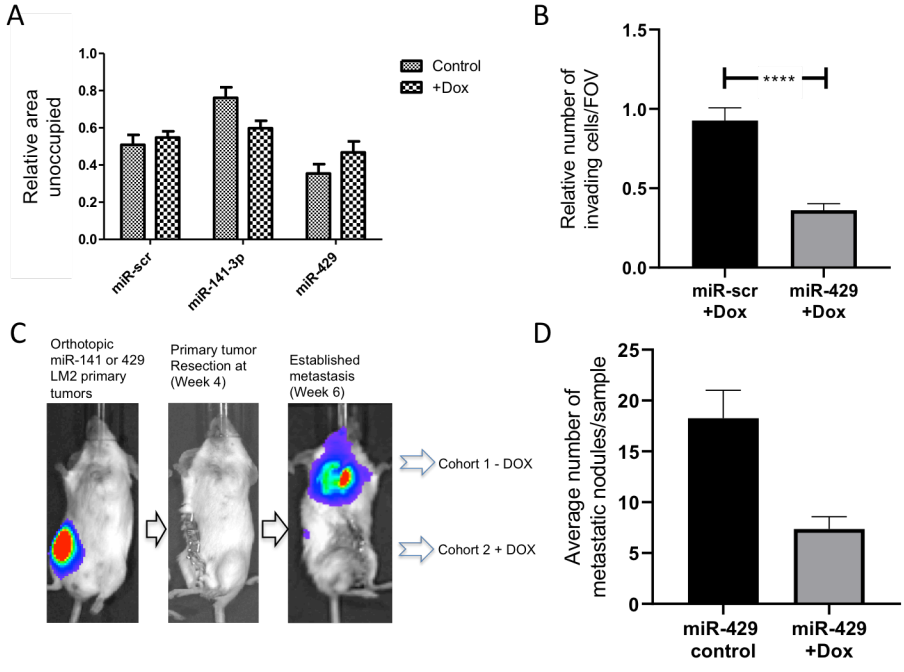
To determine if expression of the selected miRNAs in cancer cells can regress lung metastases, we injected LM2 cells with stably integrated miRNAs (see scheme Fig. 4A) in SCID mice. After lung mets were established around week 2, as determined by bioluminiscence imaging (BLI), doxicycline feed was provided to induce miRNA expression and lung mets were continuously monitored by BLI. Compared to scr controls, we observed a reduction in mets in LM2 cells expressing either miR141-3p or miR-429 (Fig. 4B). Consistent with reduction in metastatic burden, flow cytometry showed a reduction in percent mcherry+ tumor cells with miR-429 (Fig. 4C). To check effective induction of miRNAs with *dox* feed *in vivo*, we extracted RNA from lungs obtained from mice injected with miR-scr, miR-141-3p and miR-429. These were then used in miR-PCR to analyze the expression levels of miR-141-3p and miR-429, compared to miR-scr. We did not see much upregulation of miR-141-3p (Fig. 4D), whereas expression of miR-429 was significantly increased (Fig. 4E), compared to controls (miR-scr).

Figure 4. (A) *In vivo* metastasis model scheme. (B) Lung BLI at week 4 before harvest. (C) Percentage of mCherry+ tumor cells in lungs per total cells analyzed by flow. (D) miR-141-3p expression in bulk lungs analyzed by miR-PCR. (E) miR-429 expression in bulk lungs analyzed by miR-PCR.



To evaluate the impact of miRNAs *in vitro*, we used *in vitro* migration and invasion through matrigel assays. We did not see difference in *in vitro* migration in the two miRNAs after dox

Figure 5. (A) *In vitro* migration assay. (B) *In vitro* invasion defects by miR-429. (C) *In vivo* orthotopic metastasis scheme. (D) Lung nodules, determined by H&E.



induction, compared to miR-scr controls (Fig. 5A), however, *in vitro* invasion through matrigel was significantly reduced in miR-429 induced cells, compared to miR-scr controls (Fig. 5B). Using an orthotopic *in vivo* model (Fig. 5C), we identified that dox-induced miR-429 was able to reduce lung nodules in this model, compared to the control cells without dox-induction (Fig. 5D).

Changes/Problems. Cloning miRs into doxycycline (dox)-inducible lentiviral vector expressing transactivator rtTA2 had issues with the leakiness of the inducible vector, as GFP+ cells were detected in the absence of doxycycline (Dox). These GFP+ cells in the absence of Dox indicate leaky miR expression. We resolved this by cloning the miR sequences including Scrambled (Scr), miR-141-3p, and miR-429 in the third generation reverse tetracyclin-controlled transactivator 3 (rtTA3) expression vector (Fig. 3A). We had some trouble with *in vivo* expression of miR-141, as seen in miR-PCR of lungs. Therefore, we decided to pursue miR-429.

Aim 1, Major Task 2

For the initial 5 miRNAs (Table 1), we used mRNA target-predicting algorithms (Targetrank, targetscan, cbioPortal), to identify the possible overlapping genes which bind to these miRs at their respective 3'UTR. These overlapping genes have been identified in Table 2.

Table 2. Overlapping target genes from mRNA target-predicting algorithms (Targetrank, Targetscan, cbioPortal)

microRNAs →	mir141	mir429	mir365	mir342-5p	mir652
TARGETS →	ZEB1, TGFB2, MYBL1, DUSP3, CDK13	ZEB1/2, HIPK3, GMFB, WASF3, PTPN21, LHFP	UBR3, PIM1, LDHA	CPLX2, IL17REL, CCAR2, SOX12, PPARGC1A	CDC25b, PARP8

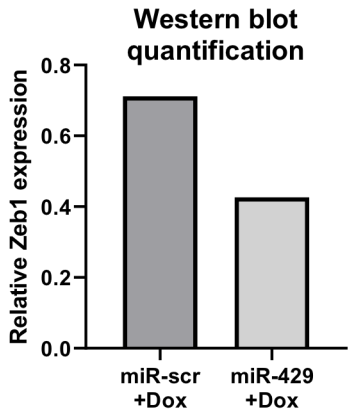
After identifying miR-429 as our potential miRNA of interest due to its encouraging roles in metastasis regression and suppression, we identified its possible downstream targets. Using algorithms including TargetScan and cBioPortal, we have identified common miR-429 targets (Fig. 6A). We were also able to confirm that miR-429 induction in TNBC cells leads to reduced Zeb1 levels (Fig. 6B). We expect that Zeb1 is the downstream mediator of miR-429 effects.

Figure 6. miR-429 targets. (A) Predicted targets of miR-429. (B) Western blot quantification of Zeb1 after miR-scr or miR-429 induction in culture.

A

TargetScan	cBioPortal
Zeb2	Zeb2
Zeb1	Zeb1
WIPF1	WIPF1
HSPA9	HSPA9
SEC23A	SEC23A
VASH2	VASH2

B

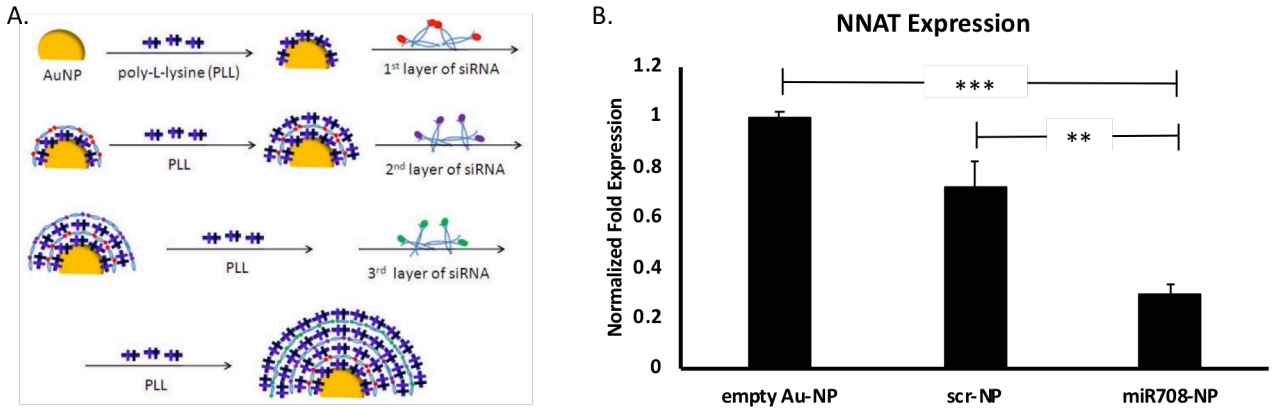


Aim 2, Major Task 1

Changes/Problems. Due to a lack in the progress in the creation of customized DNAsomes for miRNA packaging by Dr. Luo and his team, we are instead utilizing gold nanoparticles. As we had stated in our alternative approaches, if the DNAsome approach does not materialize we would consider packaging miRNAs in nanoparticles given their known success as drug delivery vehicles. Fortunately, we were able to find good options for using gold nanoparticles to encapsulate miRNAs here at Weill Cornell, utilizing the expertise of our Weill Cornell colleagues.

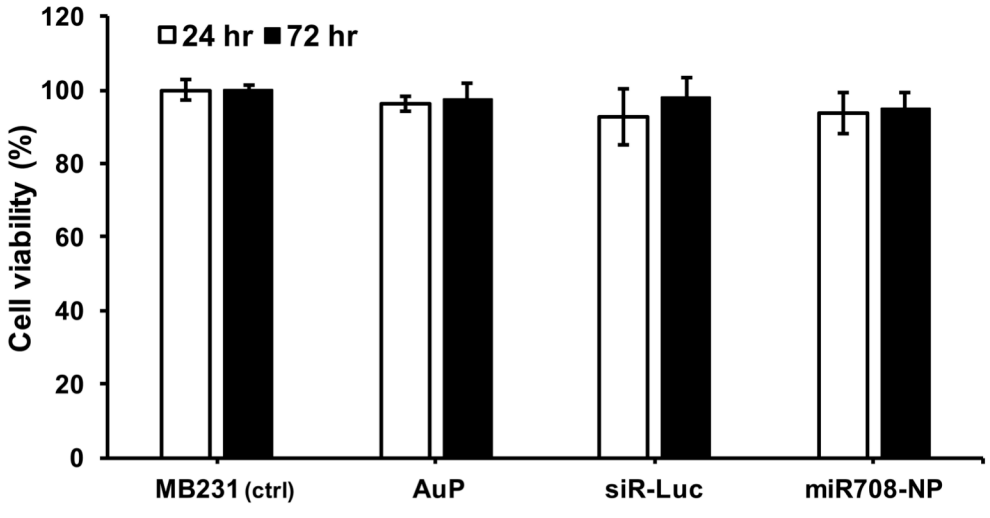
To begin evaluating the efficacy of miR-mimics as potential combination adjuvant therapeutics, we proposed a delivery method, developed by our collaborator Dr. Ching Tung, which utilizes gold nanoparticles coated with alternating layers of positively-charged (poly)- L-lysine (PLL) and negatively charged miR-oligos of interest. For our initial in vitro analysis, we used miR-708 for delivery into cells via gold nanoparticles (Fig. 7A). We used HEK293T cells, transfected them with an overexpressing plasmid for NNAT, the downstream target of miR-708. These cells were then divided into 3 different groups and treated with either empty gold nanoparticle (Au-NP), or Au-NPs coated with a scrambled miR sequence (scr), or miR-708 coated Au-NPs. After 24 hours, the cells were harvest, RNA was extracted and a qPCR was performed to evaluate the levels NNAT for the three different treatments. We observed that the cells that were treated with miR-708 Au-NPs had significantly downregulated NNAT levels, as compared to scr Au-NPs or empty Au-NPs (Fig. 7B).

Figure 7. (A) Preparation of multi-layered siRNA coated gold nanoparticles using alternative positively charged PLL and negatively charged siRNA layers (Ref. Lee et al., *Small*, 2011). (B) HEK-293T cells were transfected with NNAT. 48 hours after transfection, nanoparticles: either empty gold (empty Au-NP), or scrambled (scr-NP), or microRNA-708 (miR708-NP), were added to transfected 293T cells in triplicates. After 48 hours, RNA was extracted from the cells and NNAT expression was measured via qPCR.



After confirming the *in vitro* efficacy of nanoparticles in the delivery of miR-mimic. We used miR-708, as a prototype as it has been extensively characterized, and its target NNAT was identified and published by us previously (2). In order to ensure that nanoparticles are not cytotoxic to cells, we analyzed the *in viro* cytotoxicity of these particles layered with siRNA against luciferase, miR-708, or empty nanoparticles (AuNP), using MTT assay (Fig. 8). There was no detectable cytotoxicity observed at the doses used.

Figure 8. Cell viability evaluated after incubating cells with various NPs for 24 or 72 hours.



Next, we explored miR-708 delivery using nanoparticles *in vivo*. 1 X 10^6 LM2 cells were injected in the fourth mammary fat pad of 8-week old SCID mice (n=10/group). One cohort of mice was injected via tail vein with nanoparticles layered with scrambled sequence (scr-NP), and the other cohort was injected with miR-708 nanoparticles (miR708-NP), twice weekly at week 4 and week 5 (Fig. 9A). These nanoparticles were labeled with cy5.5 for fluorescent tracking (Fig. 9B lower), to allow us to track homing of the particles in vivo to the tumor site. At week 6, primary tumors were resected when they reached 1 cm^3, and lung metastases were allowed to develop. We observed that miR-708 delivery did not affect the primary tumor size (Fig. 9B upper, Fig. 9C), however the expression of its target, NNAT, was significantly reduced in bulk primary tumors as compared to scr cohort (Fig. 9D). We also had two additional mice as controls that did not receive any nanoparticle injections. We performed immunofluroscence imaging of bulk primary tumors to see the localization of cy5.5 in mice treated with nanoparticles versus control mice that did not receive any nanoparticle injections, and importantly specific cy5.5 signal was detected in nanoparticle-injected mice in the primary tumors Fig. 10.

Figure 9. (A) Schematic representation of the experiment design (n=10 per group). LM2 cells were injected into the fourth mammary fat pad of female SCID mice. At week 4, AuNPs layered with either with scr (scr-NP, scr: 1.58 mg/kg) or miR708 (miR708-NP, miR708: 1.58 mg/kg) oligos, and labeled with cy5.5 were injected via tail vein, twice weekly for two weeks. The mice were imaged for luminescence and fluorescence. (B) Representative luminescence (Lum) and fluorescence (Flu) images of primary tumors. (C) BLI quantification of primary tumors in the two groups. No change in primary tumor size in either group (p = 0.9) was observed. (D) Bulk primary tumors (n = 5 per group) from the mice in each group (scr-NP or miR708-NP) were lysed, and analyzed for the expression of miR-708. Primary tumors from the mice treated with miR708-NP had higher miR-708 expression (p = 0.04).

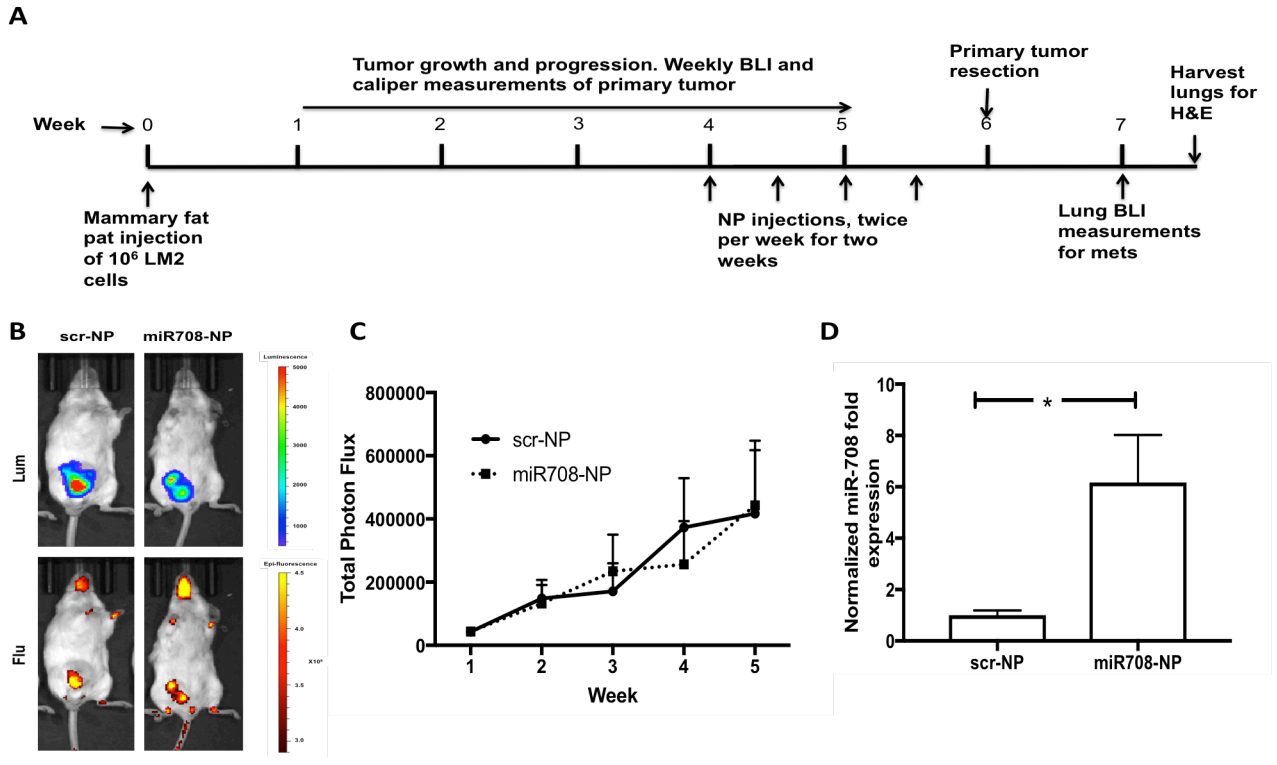
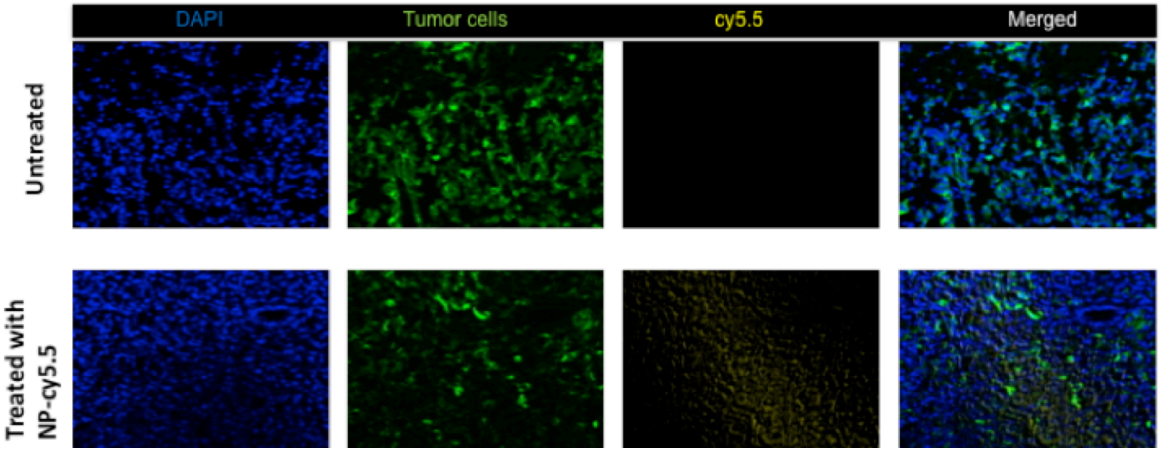


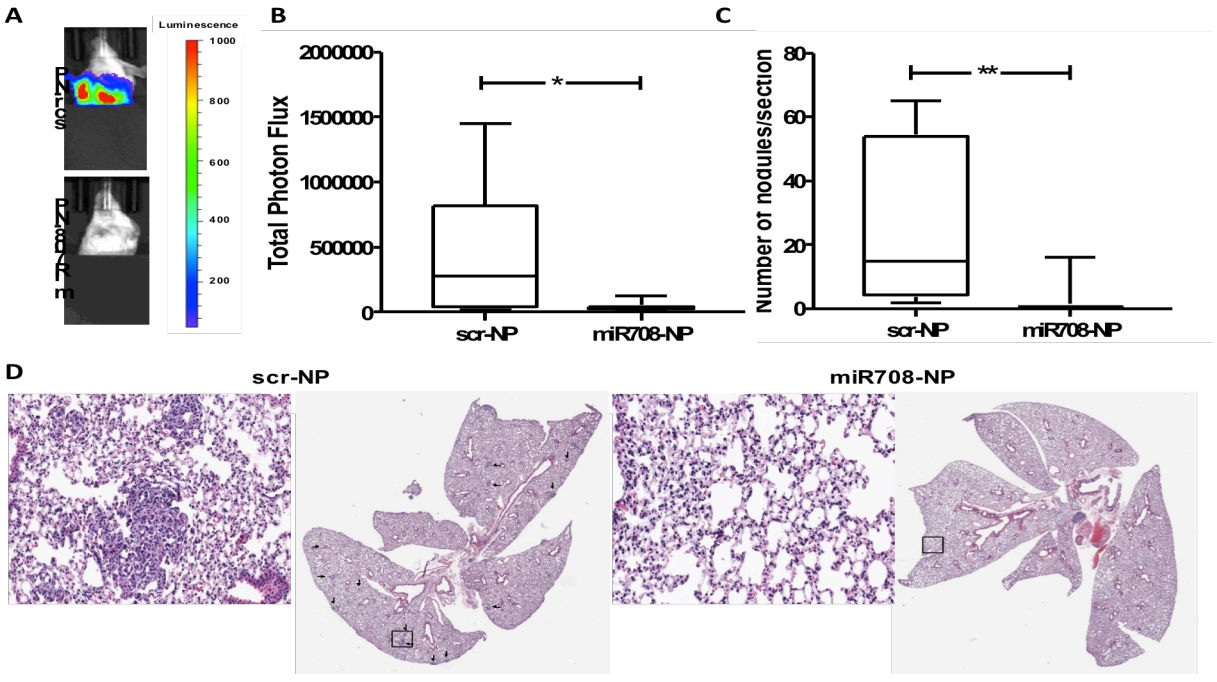
Figure 10. Immunofluorescence of cy5.5 in bulk primary tumors in mice treated with AuNPs and labeled with cy5.5. Untreated mice were used as controls.



Aim 2, Major Task 2

To explore the consequences of nanoparticle-mediated mir-708 delivery on breast cancer progression *in vivo*, we analyzed the lung metastases developed in mice after primary tumor resection. We found that in the scr-NP group, mice developed significantly more lung metastases as compared to miR708-NP group (Fig. 11A-B). H&E analysis showed reduced lung nodules in miR-708 treated group (Fig.

Figure 11. (A-B) Primary tumors were resected at 1cm^3. The mice (n = 10 per group) were monitored for lung metastasis by BLI, one week after resection. There was a significant reduction in total photon flux from lungs of mice treated with miR708-NP (P = 0.03). (C-D) 1.5 weeks after resection, mice were sacrificed, and lungs were harvested for H&E analysis. Representative images of lung metastases (black arrows point to mets in lungs) and the quantification of the two cohorts, scr-NP (n=7 per group) and miR708-NP (n=8) (p = 0.003).



11C-D).
After obtaining the anti-metastasis phenotype, it was important to characterize this phenotype further in the context of tumor stem cells as it has been reported that the metastatic cells exhibit stem cell phenotyes. Therefore, in a pilot approach we harvested the tumor tissues for further investigation
We isolated GFP+ cy5.5+ tumor cells from bulk primary cohorts in mice from the two groups (miR708-NP and scr-NP), above by FACS, and performed a mammosphere assay with these sorted tumor cells (Fig. 12A). We observed that the mammosphere formation ability of tumors cells isolated from miR-708 treated cohort was significantly reduced when compared to scr treated cohort (Fig. 12B). Tumor stem cells can be labeled with a reporter system consisting of SOX2/OCT4 response elements (SORE6), with destabilized form of the mCherry fluorescent protein (dsmCherry), in a lentiviral backbone (Fig. 12C upper), obtained from Wakefied group (NIH). This reporter system was induced in LM2 cells, and utilized for our experiments. SORE6+ cells have a higher mammosphere formation/tumor initiating capacity (Fig. 12C lower) and higher migration *in vitro* (Fig. 12D) than SORE6- cells. We analyzed the inherent expression levels of miR-708 and NNAT in SORE6- and SORE6+ cells (Fig. 12E), since the mammosphere formation in tumor cells obtained from primary tumors treated with miR-708 was lower than scr cohort. We observed that SORE6+ cells had significantly higher miR-708 and lower NNAT expression. Also, when treated with miR-708 layered nanoparticles, the *in vitro* migration in SORE6+ is significantly reduced (Fig. 12F). Hence, the effect of miR-708 deliver in breast cancer may have a specifically affect this subset of cancer cells that are known to be more pluripotent and more invasive.

To examine in vivo cytotoxic effect of NP-based delivery system, AuP or cy5.5 conjugated scr-NP were injected via tail vein in 4T1 breast tumor bearing Balb/cJ mice. Although biodistribution via optical imaging revealed the presence of some NPs in major organs of mice treated with scr-NP (Fig. 13A-B), no toxicity was observed after treatment of both AuP and scr-NP. Histopathologic studies showed that there was no morphological evidence of injury to the heart, liver, kidneys, spleen, or bone marrow (Fig. 13C). Next, to assess acute toxicity, we performed a blood biochemical analysis for the liver, renal, and muscle parameters in AuP and scr-NP injected mice. None of the treatments changed the number or morphology of blood cells (Fig. 13D). We also performed serum biochemistry to evaluate organ functions of the animals. No renal, hepatic, or muscle toxicity was observed in the animals treated with AuP and scr-NP, compared to the untreated cohort. The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH) (for liver injury), creatine kinase (CK), lactate dehydrogenase (LDH) (for muscle injury), and urea (for renal function), as well as the concentration of Troponin I (for heart function), were comparable to the results observed in control mice (Fig. 13E-F). Bone marrow smear test was also performed, and there were no overt abnormalities after treatment (data not shown). Overall, our results showed that our NP-mediated delivery system displays minimal host toxicity, thus conferring a great potential for inhibiting TNBC progression.

Figure 12. (A) miR-708 overexpression in LM2 cells leads to a significant reduction in migration as compared to scr delivery in the same cells (p = 0.005). (B) Reporter construct for SOX2/OCT4 (1) (upper panel); *in vitro* mammospheres formed by SORE6+ population is significantly higher than SORE6- cells (p = 0.03) (lower panel). (C) A more migratory population (SORE6+) was detected using a SOX2/OCT4 reporter construct in SORE6+ cells (p = 0.005). (D) SORE6+ and SORE6- cells were FACS sorted and analyzed for miR-708 (p = 0.03) and NNAT (p = 0.01) levels by qPCR. SORE6+ cells have inherently higher NNAT levels, with lower expression of miR-708 as compared to SORE6- cells. (E) After treatment with either scr-NP or miR708-NP, migration in SORE6+ cells was reduced (p = 0.03), whereas SORE6- cells remained unaffected (p = 0.9). (F) Mammosphere formation by sorted SORE6+ populations from *in vitro* cells in culture decreased (p = 0.011) after treatment with miR708-NP, whereas sorted SORE6- cells remained unaffected (p = 0.9).

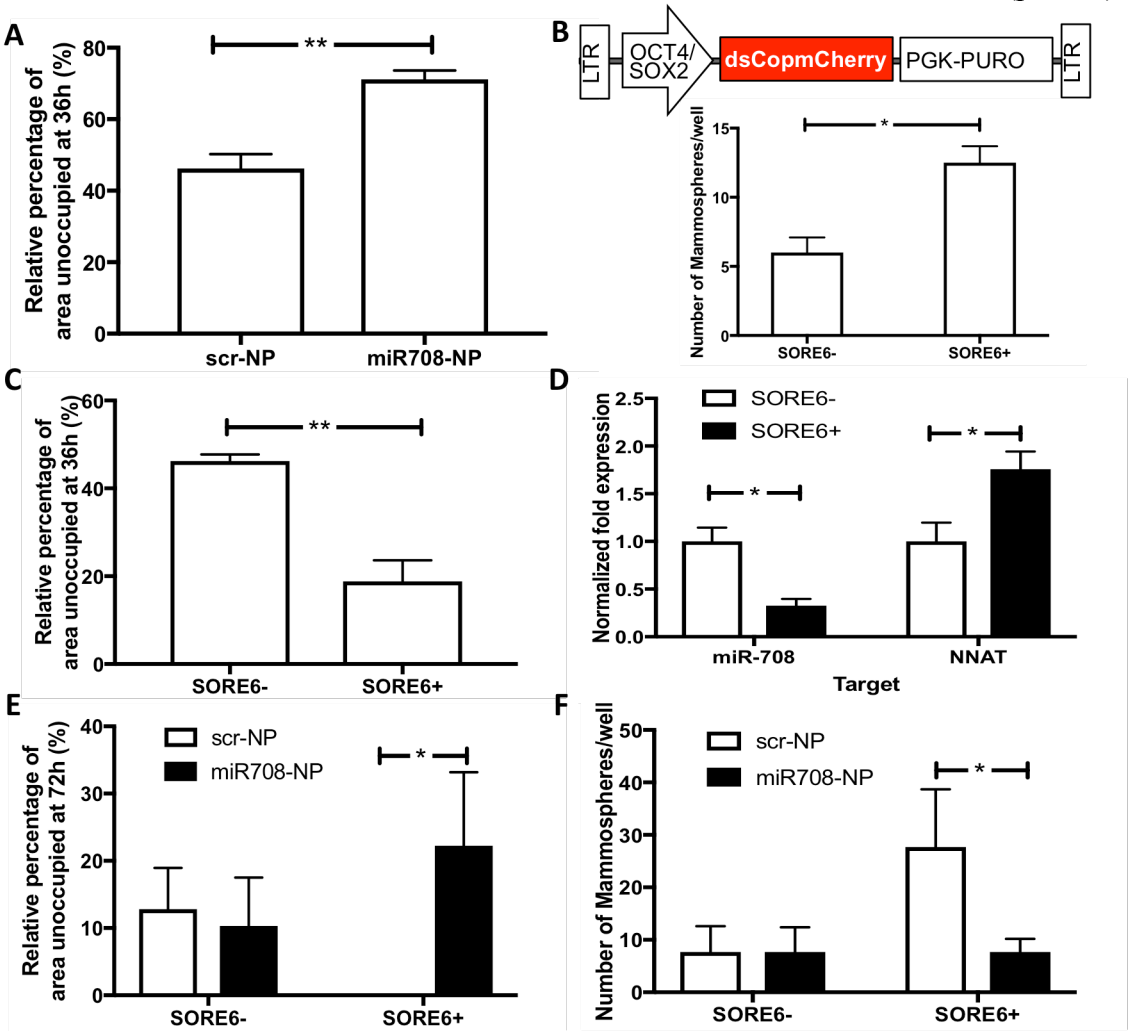
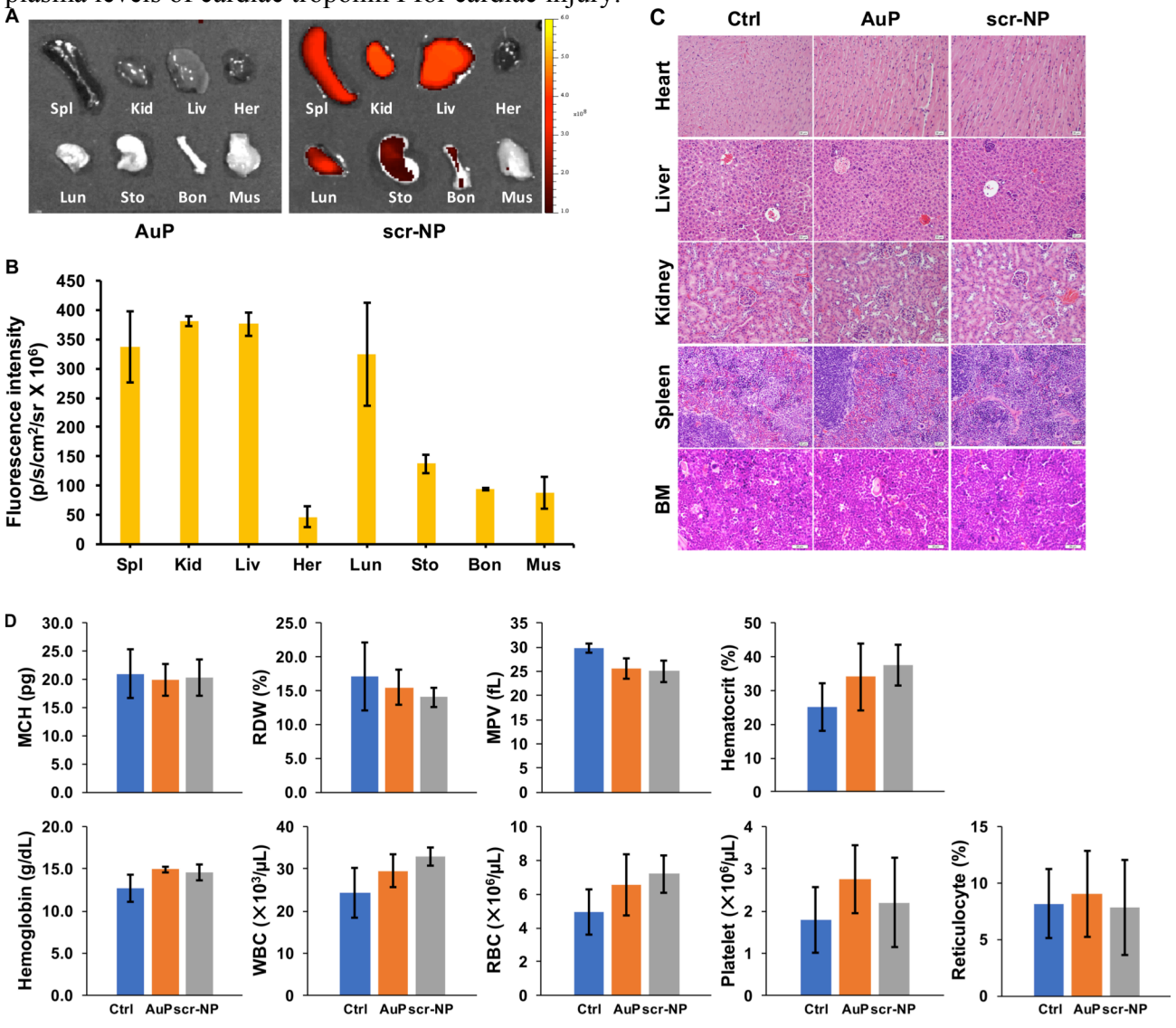


Figure 13. (A-B) The biodistribution study. The fluorescence was measured by optical imaging (Xenogen) one week after treatment of AuP or cy5.5 conjugated scr-NP (n = 3 per group). (Spl = spleen, Kid = kidney, Liv = liver, Her = heart, Lun = lung, Sto = stomach, Bon = bone, Mus = muscle). (C) Histopathologic evaluation of the major organs after any treatment. (BE = bone marrow). The scale bar represents 20 μm. (D) Whole blood biochemistry indexes. (MCH = mean corpuscular hemoglobin, RDW = red cell distribution width, MPV = mean platelet volume, WBC = white blood cell, RBC = red blood cell). (E) Serum analyte activity for liver (ALT, AST, GLDH) or muscle injury (CK, LDH). (ALT = alanine aminotransferase, AST = aspartate aminotransferase, GLDH = glutamate dehydrogenase, CK = creatine kinase, LDH = lactate dehydrogenase). (F) The plasma levels of cardiac troponin I for cardiac injury.



What opportunities for training and professional development has the project provided?
Opportunities for training and professional development on the project include the mentorship of post-doctoral associates to help advance their careers.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
Our study has the potential to establish the potential of new miRNAs as an attractive approach for treatment of metastatic breast cancer including TNBC using a novel delivery method.

How were the results disseminated to communities of interest?
A paper was accepted for publication in early 2019:

Ramchandani D, Lee SK, Yomtoubian S, Han MS, Tung CH, Mittal V. (2019) Nanoparticle Delivery of miR-708 Mimetic Impairs Breast Cancer Metastasis. Mol Cancer Ther. 2019 Mar;18(3):579-591.

Also, in 2018, 2019, and 2020, Dr. Mittal and Dr. Ramchandani have given invited seminars at the PSOC of Cornell University (Ithaca, NY) and the Keystone Symposium (Galveston, TX).

What was the impact on other disciplines?
Progress in identifying novel miRNAs as therapeutic agents for metastatic TNBC is likely to attract many investigators across disciplines in breast cancer research and result in rapid advancements towards finding a potential therapy.

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. CHANGES/PROBLEMS:
Nothing to report

6. PRODUCTS:

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	*Vivek Mittal (PD/PI - WCM) – 11% Effort*
Project Role:	*PD/PI*
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	*1.59*
Contribution to Project:	*Dr. Mittal oversees all aspects of the proposal as PD/PI. FYI: Dr. Mittal was a Mentor to Dr. Havel on the project from 04/15/16 – 07/15/16 and assumed the role of PD/PI from 07/16/16 – onwards, after Dr. Havel left on 07/15/16 to pursue other opportunities at a non-academic institute. Dr. Mittal remained the PD/PI for the remainder of the proposal.*
Funding Support:	

Name:	*Dan Luo (Co-PI – Cornell Univ) – 2.75% Effort (Only during 04/15/2016 – 09/30/2017)*
Project Role:	*Co-PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*0.33*
Contribution to Project:	*Lead optimization efforts of DNAsomes for miRNA packaging. Dr. Luo's role on the project ended on 09/30/2017*
Funding Support:	

Name:	*Divya Ramchandani, PhD (Post-Doc - WCM) – 50% Effort*
Project Role:	*Post-Doc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Ramchandani has performed all miRNA manipulations and has worked with Weill Cornell collaborators to optimize gold nanoparticles for miRNA packaging and in vivo delivery.*
Funding Support:	

Name:	*Sharrell Lee (Technician - WCM) – 15% effort*
Project Role:	*Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.8*

Contribution to Project:	*Ms. Lee has assisted Dr. Ramchandani in the in vivo work.*
Funding Support:	

Name:	*Dong Wang (Post-Doc- Cornell Univ) – 14% effort (Only during 04/15/2016 – 09/30/2017)*
Project Role:	*Post-Doc*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1.69*
Contribution to Project:	*Thomas has assisted Dr. Luo in the the synthesis and characterization of the DNAsome carriers. Ended 09/30/17*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Mittal received a two-year research grant W81XWH-19-1-0286 from the United States Dept of Defense CDMRP entitled, "Targeting tumor intrinsic immunosuppressive mechanisms to enhance efficacy of immune checkpoint blockade in lung cancer." Dates are from 08/01/2019 – 07/31/2021. He is spending 10% effort (1.2 calendar months).

Dr. Mittal received a two-year research grant UG3CA244697 from the NIH NCI entitled, "Intercepting progression from pre-invasive to invasive lung adenocarcinoma." Dates are from 09/25/2019 – 08/31/2021. He is spending 10% effort (1.2 calendar months).

What other organizations were involved as partners?
Organization Name: Cornell University
Location of Organization: Ithaca, NY
Partner's contribution to the project: Collaboration – Cornell University was a subawardee on the award during the period 04/15/16 – 09/30/17.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES:

Nothing to report

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