Supplementary figure legends

Figure S1. RT-PCR analysis of EVs transfected with miR-335-5p. A comparison is made between EVs transfected with miR mimics vs. control (PBS with no EVs).

Figure S2. Verification of Cre expression.PCR for Cre mRNA was performed. LX2 cells (Cre negative, control cells) do not show any expression of Cre. Similarly, the EVs obtained from these cells, as well as the supernatant (Sup in the figure) that is left from conditioned media (CM) after EV extraction do not demonstrate any Cre. LX2 cells that express Cre demonstrate the Cre mRNA as shown. In addition, EVs isolated from these cells demonstrate Cre, but not the Sup (CM depleted of EVs). H2O is included as another negative control.

Figure S3. HCC cells transfected with PMSC-Lox-ds Red-Lox-eGFP-puro-WRPE. As expected, these cells demonstrate fluorescence in the red, but not the green channel.

Figure S4. Exosomal transfer from fibroblasts cells to HCC cells *in vitro.* LX2-Cre cells were added to MHCC97L-Loxp-dsRed-Loxp cells (red) for 1 week. We noted the switch in color from red to green occurring in select cancer cells. These findings argue that Cre was shuttled from LX2 cells to HCC cells. Scale bars represent 50 μm.

Supplementary materials and methods

HCC – LX2 co-culture

Same numbers of HCC cells stably infected with pCDH-EF1-MCS-IRES-GFP lentivirus and LX2 cells (5×10^5) were seeded in a T75 flask and were co-cultured for 14 days. After co-culturing, HCC cells and LX2 cells were separated by FACS sorting according based on GFP positivity.

Infection of LX2 cells with a Cre expressing lentivirus

Puro.Cre empty vector (Addgene plasmid #17408) (7.5 μg/ 10 cm plate), PxPAX2 (6 μg/10 cm plate) (Addgene), and pMD2.G vector (2 μg/ 10 cm plate) were transfected into 2, 100 mm culture dishes of 293T cells, using X-tremeGENE HP DNA Transfection reagent (Roche). Seventy-two hours after transfection, supernatant were collected. LX2 cells were plated in a T75 flask in 10ml of DMEM media containing 10% FBS and 1% Pen-Strep. Cells were grown in a 37ºC incubator at 5% CO2 to 50% confluence. LX2 cells were then transduced with virus supernatant supplemented with polybrene. After 72 hours, LX2 cells were started on treatement with puromycin (0.5 μg/ml) for 2 weeks.

miR transfection

The miR mimics used in this study were as follows: hsa-miR-335-5p, mirVana® miRNA mimic (Life Techologies Corporation, ThermoFisher, USA), Mature miRNA Sequence:UCAAGAGCAAUAACGAAAAAUGU; mir Vana miRNA mimic negative control (Life Techologies Corporation, ThermoFisher, USA). MiR-335-5p mimic or miRNA Negative Control (NSM) were transfected into HCC cells or LX2 cells using Lipofectamine RNAiMAX Reagent (ThermoFisher, USA) according to the manufacturer's instructions.

Transwell invasion assay of HCC cells

HepG2, MHCC97H, MHCC97L and Huh7 cells were first transfected with miR-335-5p mimic or NSM for 48h. Then, cells were washed three times with PBS. A number of 5×10^4 cells were suspended in 100 μl of serum-free medium and seeded onto the upper chamber of the Transwell (8-μm pore size, 6.5-mm diameter; Corning, Corning, NY, USA) in a 24-well plate. Medium with 10% FBS (200 μl) as chemoattractant was placed in the lower chambers. After 24 h of incubation, non-invading cells in the Matrigel were removed by wiping with a cotton swab. Invading cells at the transwell membrane were stained with crystal violet (Thermo Fisher Scientific). Cells from five random fields were counted at ×10 original Magnification with Image J software. The percentage of invasion was calculated as follows: Count and average the total number of cells in each of the random fields within an insert, divide this number by the area of the microscope viewing fields and then multiply this number by the entire area of the transwell insert, then the percent invasion can be calculated by dividing this number by the number of cells seeded. We multiplied this value by 100 to get a percent.

Proliferation of HCC cells in transwell co-culture conditions

LX2 cells transfected with miR-335-5p mimic or NSM were cultured for 2 days by themselves, then washed and trypsinised, centrifuged and washed 3 times with PBS. LX2 cells (1.25×10^4) were seeded into the lower well of 12 well with 0.4 μm transwell co-culture system (cat# 3460, Corning, Tewksbury, MA). For the upper chamber, 1X105 HepG2, MHCC97H, MHCC97L and Huh7 cells were seeded at the same time after trypinization and counting as above. After 4 days, the four kinds of HCC cell number were determined by counting (Countess, Invitrogen).

Transwell invasion assay of HCC cells lines co-cultured with LX2

LX2 cells were treated with miR-335-5p mimic or NSM as described above. After incubation for 48 h, 3×10^4 LX2-335 or LX2-NSM and 3×10^4 HepG2, MHCC97H, MHCC97L and Huh7 cells

were mixed together for co-culturing for 48h. Next, co-cultured cells were trypsinized and plated in serum-free medium onto the upper chambers of the Transwell (8-μm pore size, 6.5-mm diameter; Corning, Corning, NY, USA) in 24 well plates. Medium with 10% FBS (200 μl) was placed in the lower chambers as chemoattractant. After 24 h of incubation, non-invading cells in the Matrigel were removed by wiping with a cotton swab. Invading cells at the transwell membrane were stained with crystal violet (Thermo Fisher Scientific). The invasion index was calculated as above.

Scratch migration assay of HCC cells lines after co-culture with LX2
LX2 cells were treated with miR-335-5p mimic or NSM. After incubation for 48 h, the 0.4 μm transwell co-culture membrane were added in the same plate, and the HepG2, MHCC97H, MHCC97L and Huh7 cells (100,000 cells/well) were plated on the membrane. After incubation for 48 h, the HCC cells were removed by trypsinisation, counted and plated at 2×10^6 cells per well with serum-free medium in new 24-well dishes. Cells were incubated overnight yielding confluent monolayers for wounding. Wounds were made using a pipette tip (200ul) and photographs taken immediately (time zero) and 24 or 48 h after wounding. The distance migrated by the cell monolayer to close the wounded area during this time period was measured.

Spheroid generation and invasion measurement
We generated MHCC97H cell spheroidsby using the hanging drop method[14]. Briefly, we re-suspended MHCC97H for a concentration of 5×10^4 /ml. Next, we used a multi-channel pipettor to transfer 20 μl droplets of cell suspension to the inner surface of the lid of a 100 mm dish. We then quickly invested the lid and placed over the dish which contained PBS (to prevent cell medium evaporation). The hanging drop cultures were incubated at 37 °C for 72 hr to generate spheroids. Next, we collected the spheroids, mixed them with 100 μl of basement membrane material and 100 μl of cold (4 °C) type I collagen. We then pipetted 40 μl drops of the viscous mixture into the centers of wells in a 24-well plate. Plates were then placed at 37 °C in an incubator and left undisturbed for 30 min. Next, we slowly submerged these 3D cultures in 1 ml of cell culture media. Exosomes were transfected with miR-335-5p mimics or miR-NSM as above. Then, 5μg of exosomes-miR-335 or exosome-NSM per well were added in the medium on top of spheroid. Pictures were taken at time 0h, 24h, 48h, and 72h. The invasion areas were measured with Image J software. The invasion index was calculated by dividing the invasion area by the area of the 24h spheroid. * $p<0.05$ comparing with other group.

Figure S1

miR-335

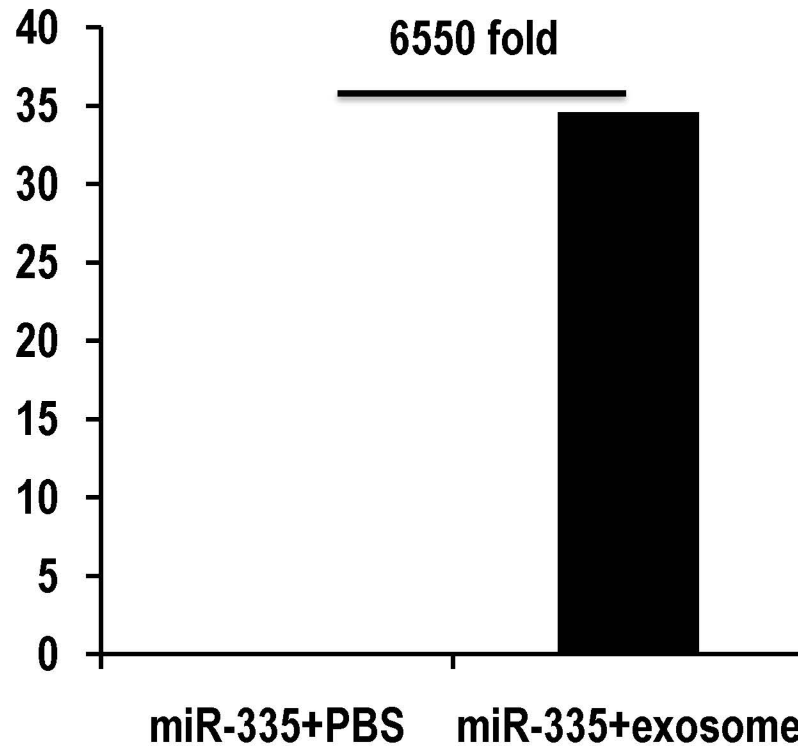


Figure S2

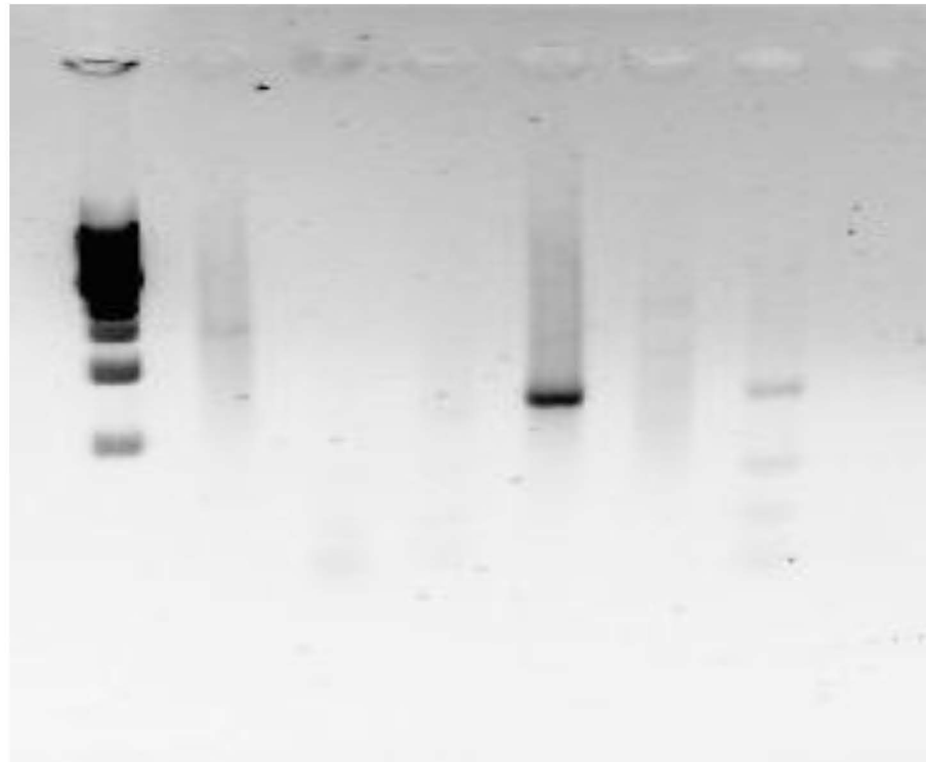
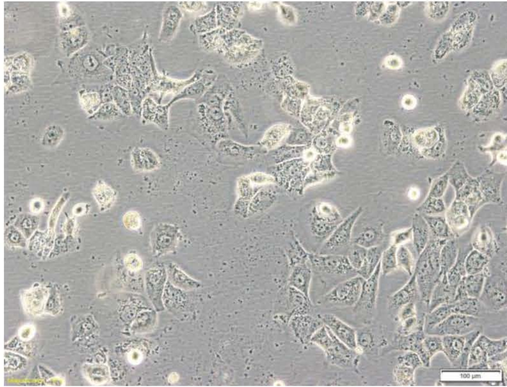
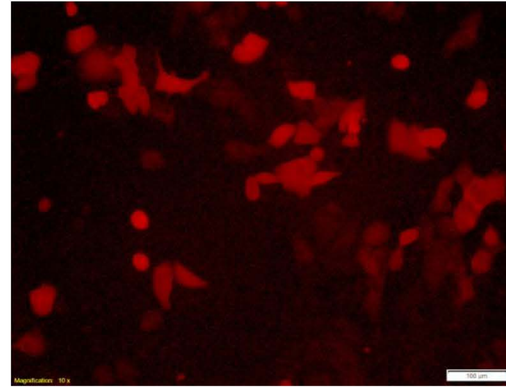


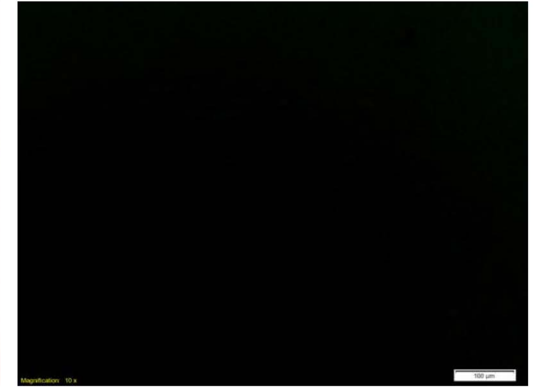
Figure S3

White channel | Red channel | Green channel

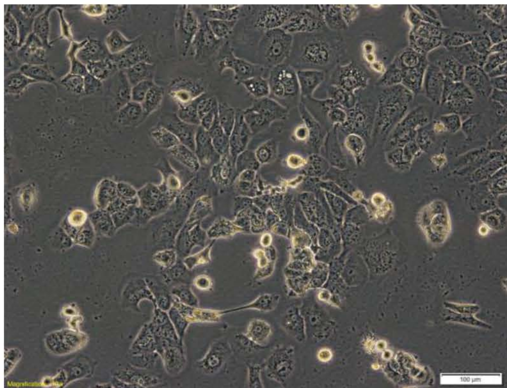
MHCC97L-Lox-dsRed-Lox

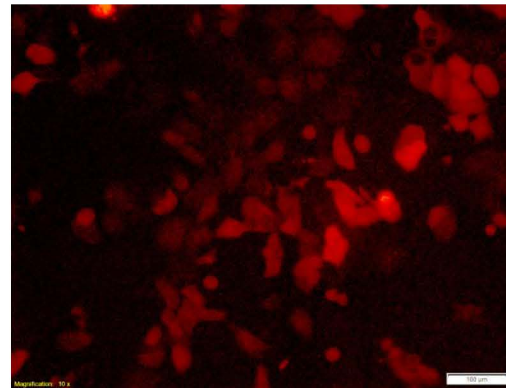




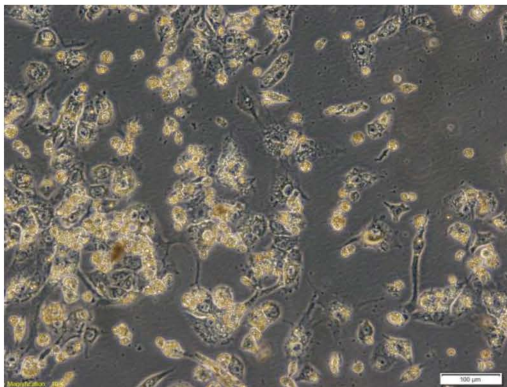


MHCC97H-Lox-dsRed-Lox





HepG2-Lox-dsRed-Lox



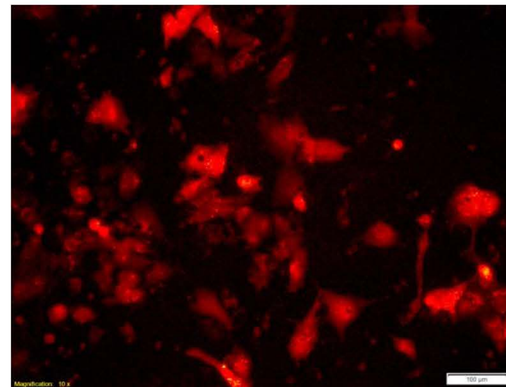


Figure S4