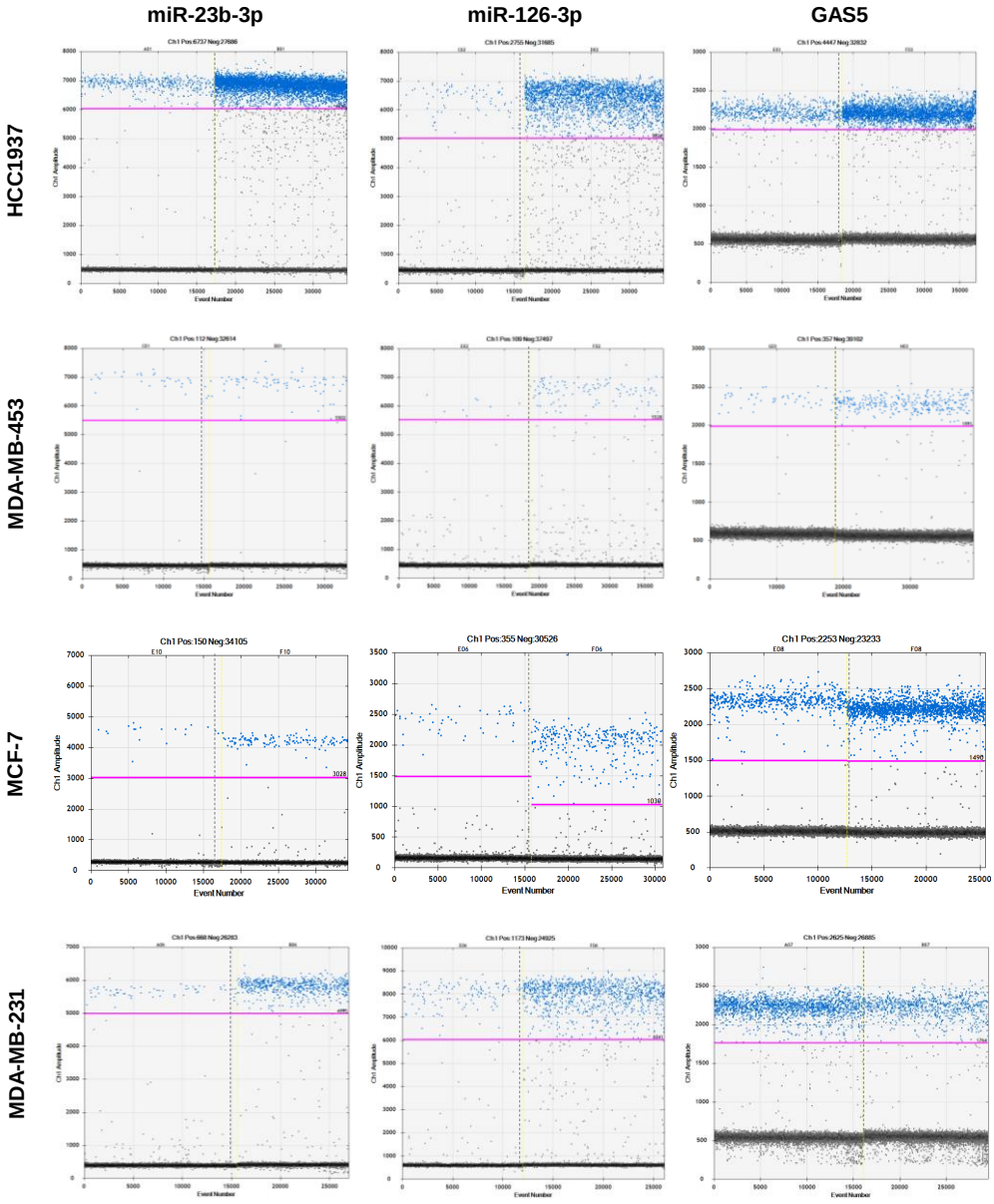
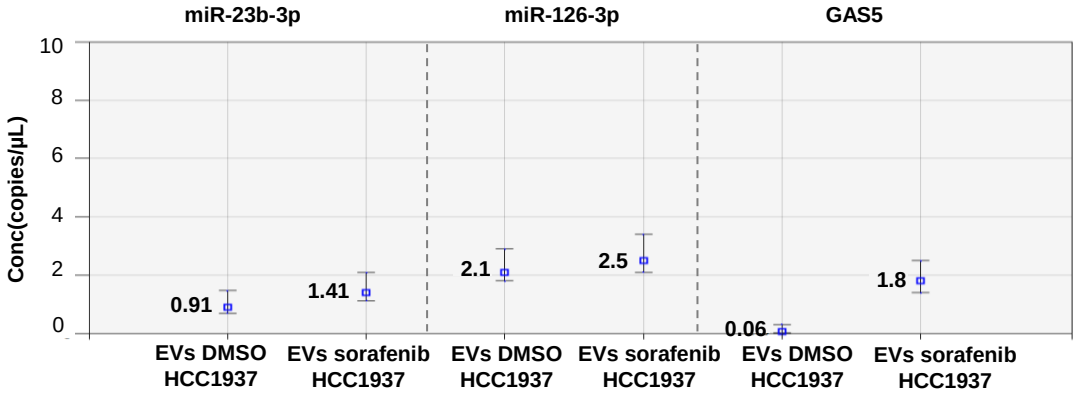
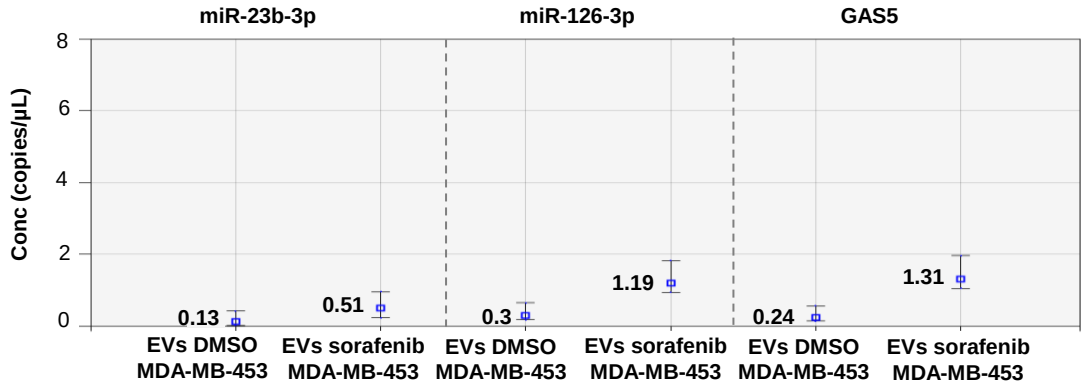
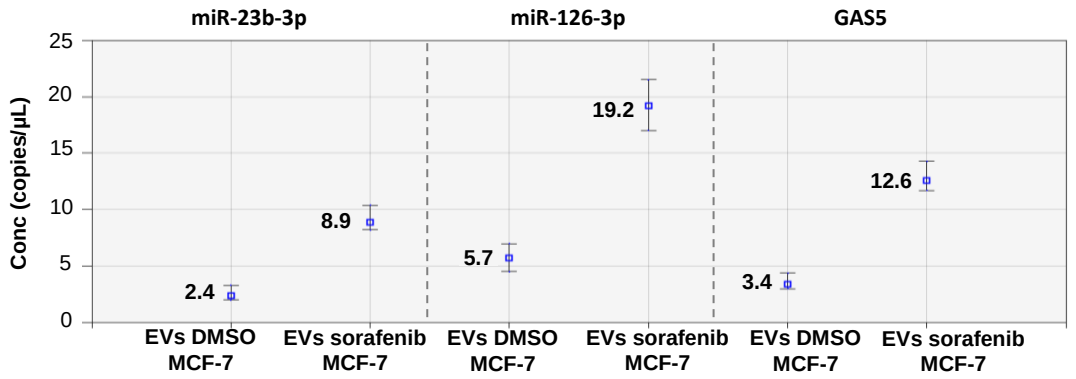
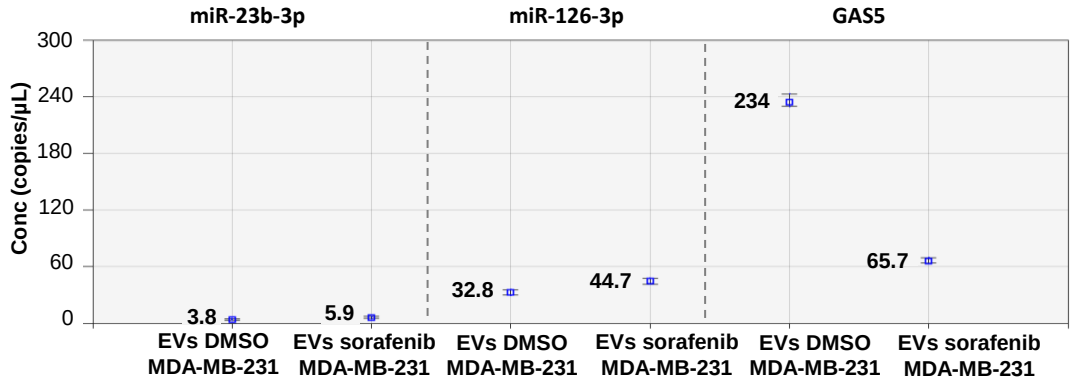


Supplementary Figure 1. Fluorescent intensity of the droplets after amplification of miR-23b-3p, miR-126-3p and GAS5 using the ddPCR technology. The individual lanes correspond to EV_{DMSO} or $EV_{sorafenib}$. X axis, number of droplets with fluorescence; Y axis, fluorescence intensity detected in the FAM-channel (blue dots, positive); pink line, threshold; grey dots, droplets with background fluorescence of non-incorporated probes (negative). Results are representative of at least two independent experiments.

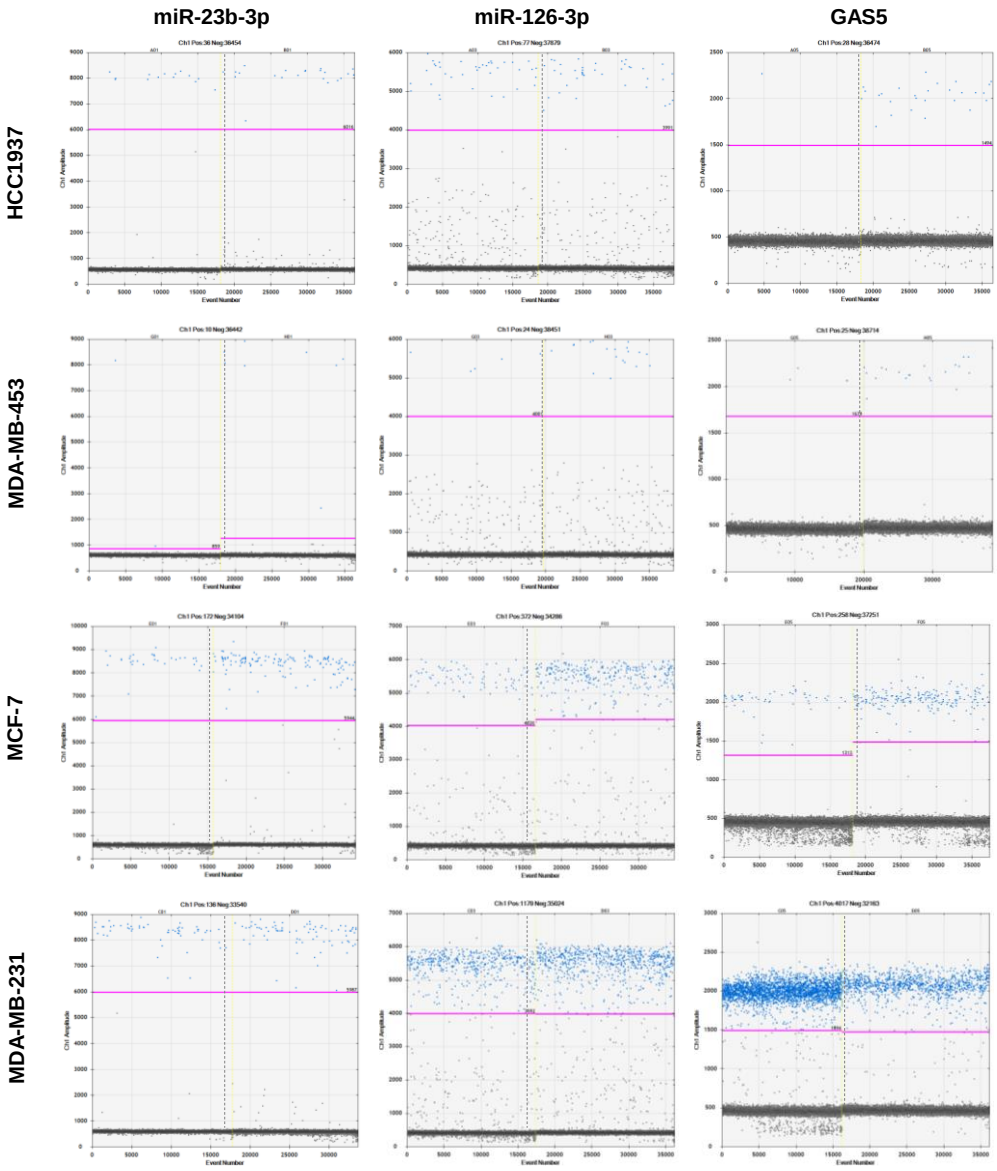




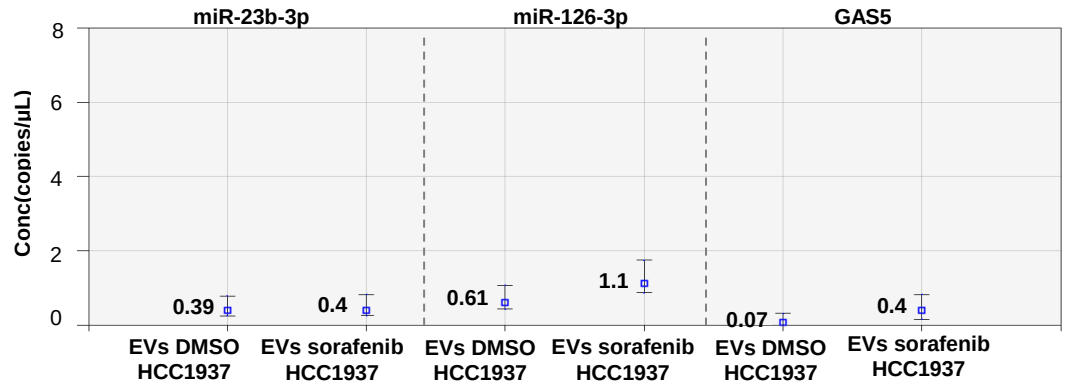


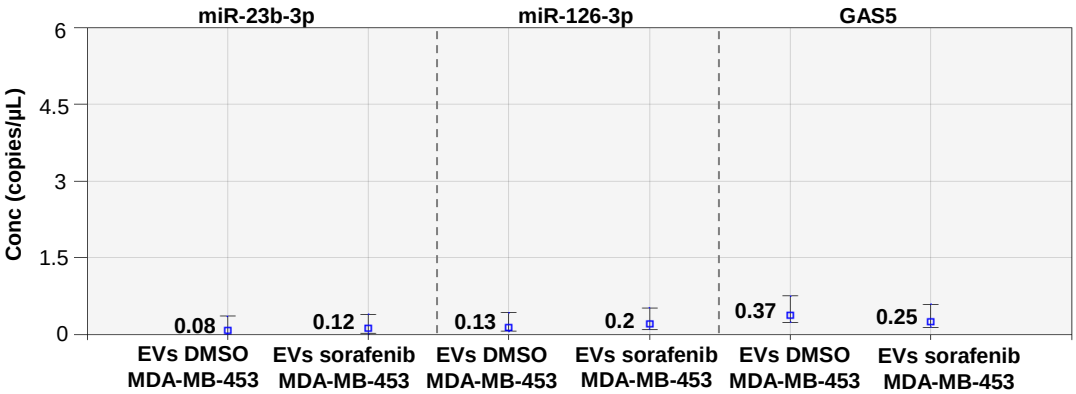


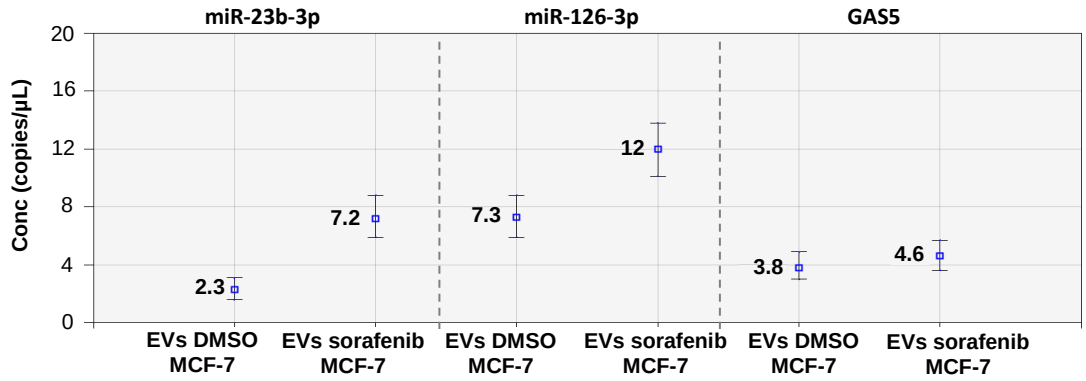
Supplementary Figure 2. Levels of miR-23b-3p, miR-126-3p, and GAS5 encapsulated in the EVs derived from HCC1937, MDA-MB-453, MCF-7, and MDA-MB-231 breast cancer cells were determined using ddPCR technology. EVs were obtained by ultracentrifugation. Treatment with sorafenib caused dysregulation of the selected ncRNAs levels. The highest fold increase (F.I.) were detected in $EV_{sorafenib}$ released by MDA-MB-453 cells (F.I. miR-23b-3p=3.9; F.I. miR-126-3p=4; F.I. GAS5=5.4) with only one exception represented by $EV_{sorafenib}$ from HCC1937 where GAS5 had an F.I. of 30. The error bars indicate 95% Poisson confidence interval.

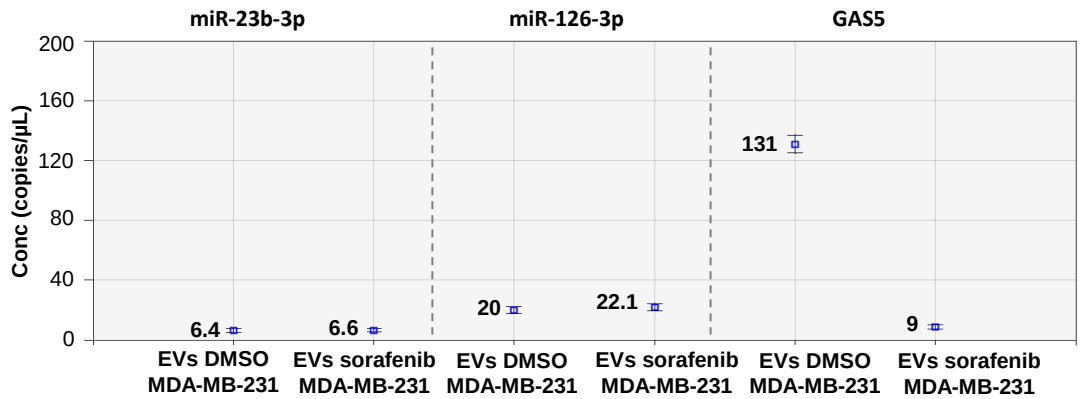


Supplementary Figure 3. Fluorescent intensity of the droplets after amplification of miR-23b-3p, miR-126-3p and GAS5 using the ddPCR technology. The individual lanes correspond to EV_{DMSO} or $EV_{sorafenib}$. EVs were obtained by ultracentrifugation. X axis, number of droplets with fluorescence; Y axis, fluorescence intensity detected in the FAM-channel (blue dots, positive); pink line, threshold; grey dots, droplets with background fluorescence of non-incorporated probes (negative). Results are representative of at least two independent experiments.

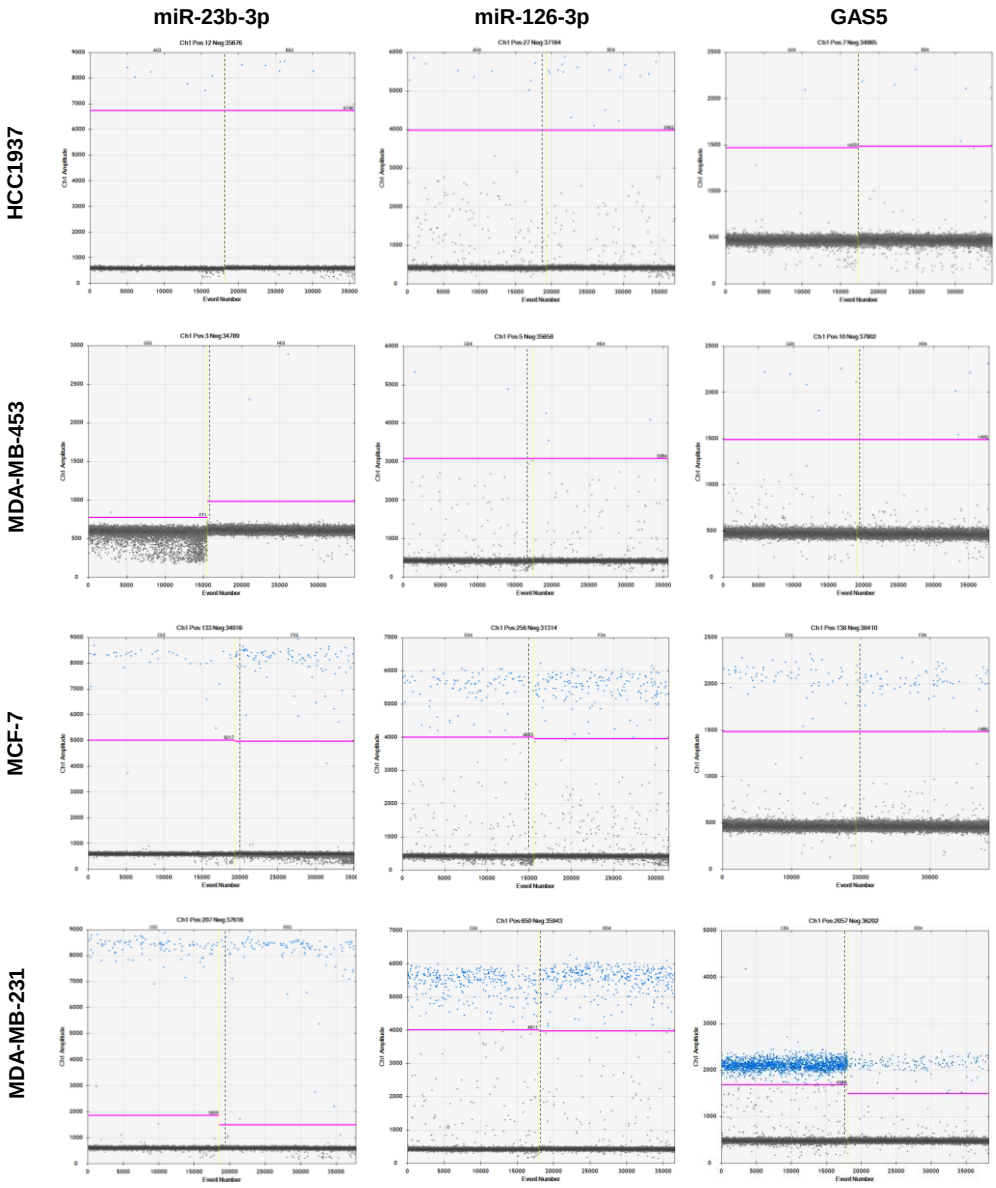








Supplementary Figure 4. Levels of miR-23b-3p, miR-126-3p, and GAS5 encapsulated in the EVs derived from HCC1937, MDA-MB-453, MCF-7 and MDA-MB-231 breast cancer cells were determined using ddPCR technology. EVs were obtained by immunoprecipitation. Treatment with sorafenib caused dysregulation of the selected ncRNAs levels. The highest fold increase (F.I.) were detected in $EV_{sorafenib}$ secreted by HCC1937 cells (F.I. miR-23b-3p=1; F.I. miR-126-3p=1.8; F.I. GAS5=5.7) with only one exception represented by $EV_{sorafenib}$ from MCF-7 where *miR-23b-3p* had an F.I. of 3.1. The error bars indicate 95% Poisson confidence interval.



Supplementary Figure 5. Fluorescent intensity of the droplets after amplification of miR-23b-3p, miR-126-3p, and GAS5 using the ddPCR technology. The individual lanes correspond to EV_{DMSO} or $EV_{sorafenib}$. Evs were obtained by immunoprecipitation. X axis, number of droplets with fluorescence; Y axis, fluorescence intensity detected in the FAM-channel (blue dots, positive); pink line, threshold; grey dots, droplets with background fluorescence of non-incorporated probes (negative). Results are representative of at least two independent experiments.

Supplementary Table 1

Cell line	miR-23b-3p	FI/ FD*	miR-126-3p	FI/ FD*	GAS5	FI/ FD*
HCC1937	↑	19.8	↑	24.4	↑	6.2
MDA-MB-453	↑	2.7	↑	4.7	↑	5.5
MCF-7	↑	4.7	↑	7.4	↑	3.3
MDA-MB-231	↑	5	↑	2.6	↓	-1.2

Fold increase/ decrease was calculated as the *ratio* between (copies/µL) $EV_{sorafenib}$ / (copies/µL) EV_{DMSO} derived from HCC1937, MDA-MB-453, MCF-7, and MDA-MB-231 breast cancer cells. EVs were obtained using Total Exosome Isolation Reagent (from cell culture media) (Thermo Fisher Scientific).

Supplementary Table 2

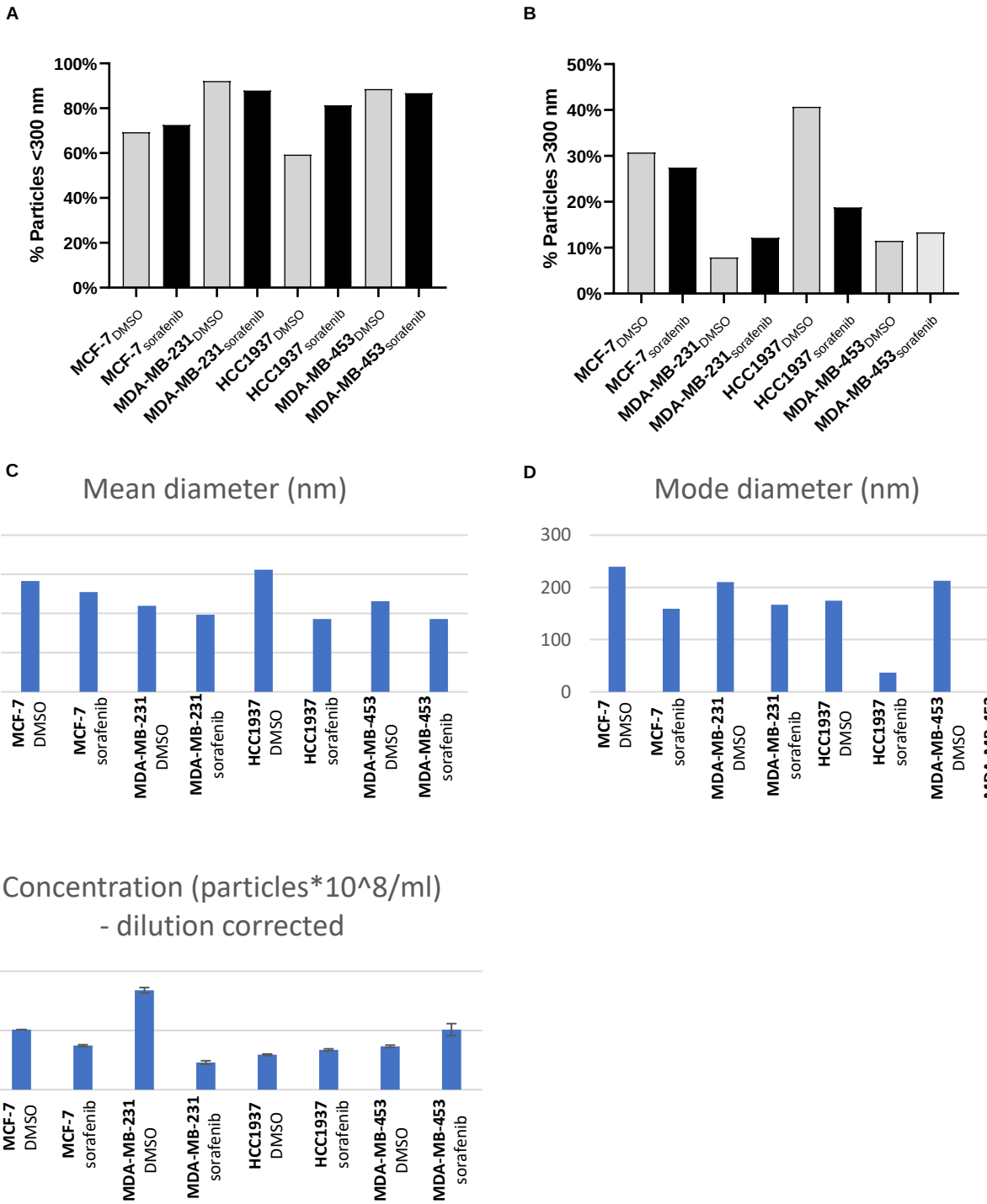
Cell line	miR-23b-3p	FI/ FD*	miR-126-3p	FI/ FD*	GAS5	FI/ FD*
HCC1937	↑	1.5	↑	1.2	↑	30
MDA-MB-453	↑	3.9	↑	4	↑	5.4
MCF-7	↑	3.7	↑	3.4	↑	3.7
MDA-MB-231	↑	1.5	↑	1.4	↓	-3.5

Fold increase/ decrease was calculated as the *ratio* between (copies/µL) $EV_{sorafenib}$ / (copies/µL) EV_{DMSO} derived from HCC1937, MDA-MB-453, MCF-7, and MDA-MB-231 breast cancer cells. EVs were obtained by ultracentrifugation.

Supplementary Table 3

Cell line	miR-23b-3p	FI/ FD*	miR-126-3p	FI/ FD*	GAS5	FI/ FD*
HCC1937	=	1	↑	1.8	↑	5.7
MDA-MB-453	↑	1.5	↑	1.5	↓	-1.5
MCF-7	↑	3.1	↑	1.6	↑	1.2
MDA-MB-231	=	1	↑	1.1	↓	-14.5

Fold increase/ decrease was calculated as the *ratio* between (copies/µL) $EV_{sorafenib}$ / (copies/µL) EV_{DMSO} derived from HCC1937, MDA-MB-453, MCF-7, and MDA-MB-231 breast cancer cells. EVs were obtained by immunoprecipitation.

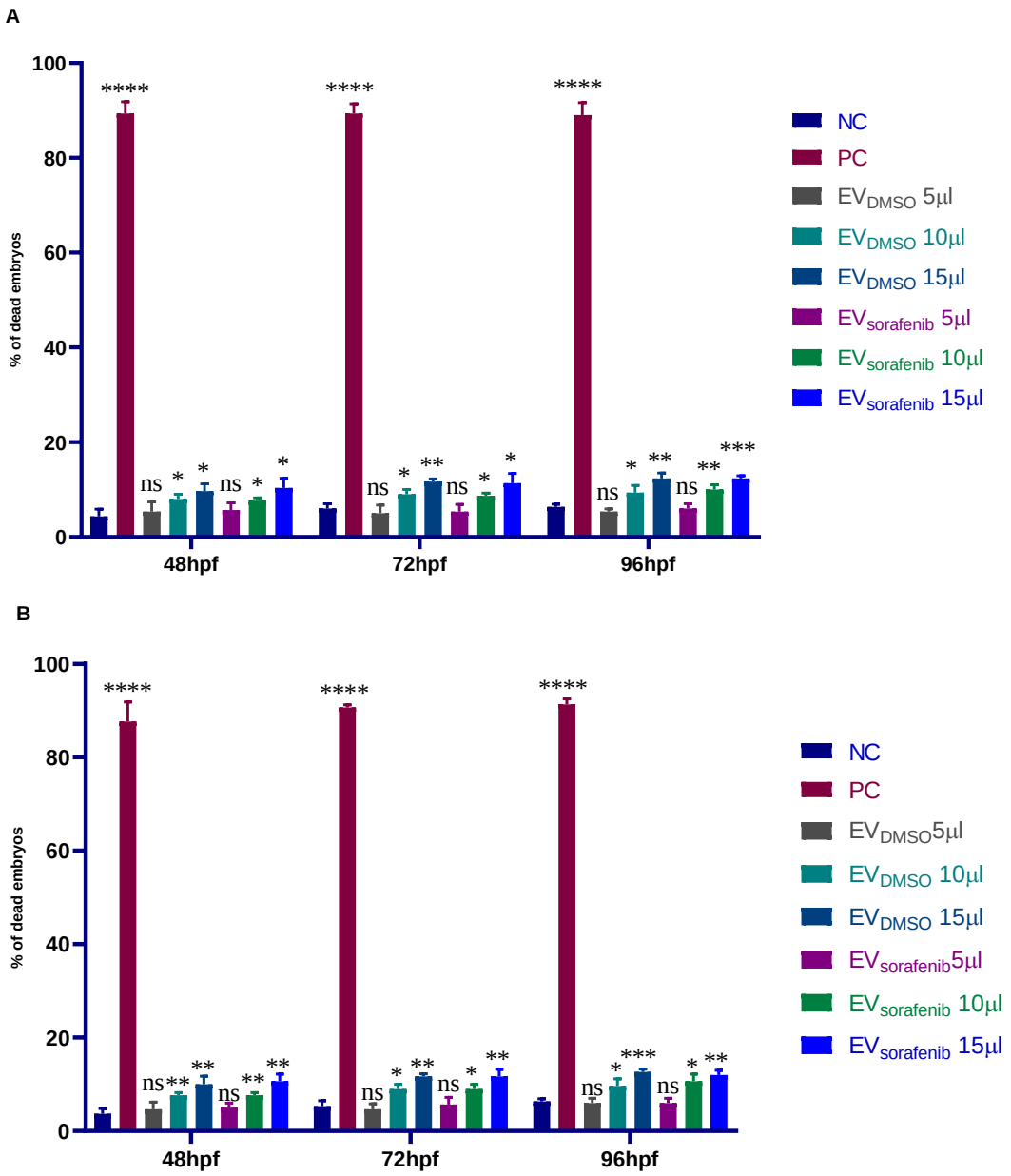


Supplementary Figure 6. Nanoparticle tracking analysis (NTA) of EV_{DMSO} and $EV_{sorafenib}$ derived from MCF-7, MDA-MB-231, HCC1937, and MDA-MB-453 cells. EVs recovered from the cell lines treated with DMSO showed heterogeneous size-distribution profiles. Quantification of the relative abundance of particles **A** <300 nm and **B** those >300 nm in diameter EVs. **C, D** Sorafenib treatment caused significant size shifts in favor of larger particle subpopulations, as demonstrated by the general increase in the mean/mode diameter ratio. **E** The concentration of the EVs was in the range of 108/ml.

Supplementary Table 4

Sample name (EVs)	Mean diameter (nm)	Mode diameter (nm)	Raw concentration (particles*10^8/ml)	SD (particles*10^8/ml)	Concentration (particles*10^8/ml) – dilution corrected	Mean/Mode diameter ratio	% Particles <300 nm	% Particles >300 nm
MCF-7$_{DMSO}$	283	239.7	1.69	0.0112	5.07	1.181	69.29	30.71
MCF-7$_{sorafenib}$	254.6	159.1	1.24	0.0798	3.72	1.600	72.58	27.42
MDA-MB-231$_{DMSO}$	219.8	210.1	2.8	0.23	8.4	1.046	92.14	7.86
MDA-MB-231$_{sorafenib}$	197	166.9	0.765	0.147	2.295	1.180	87.84	12.16
HCC1937$_{DMSO}$	312	174.7	0.983	0.065	2.949	1.786	59.31	40.69
HCC1937$_{sorafenib}$	185.9	36.7	1.12	0.0896	3.36	5.065	81.25	18.75
MDA-MB-453$_{DMSO}$	231.4	212.9	1.22	0.102	3.66	1.087	88.52	11.48
MDA-MB-453$_{sorafenib}$	185.6	78.5	1.69	0.51	5.07	2.364	86.69	13.31

Nanoparticle tracking analysis (NTA) of EVs derived from DMSO or sorafenib-treated MCF-7, MDA-MB-231, HCC1937 and, MDA-MB-453 cancer cells showed heterogeneity in terms of size-distribution profiles and concentration.



Supplementary Figure 7. Dose curve of EVs on wild-type (AB) strain zebrafish embryos. Preliminary experiments were conducted to evaluate the EVs optimum concentration on AB strain zebrafish embryos. The percentage of dead embryos treated with three different concentrations of **A** EV_{DMSO} or $EV_{sorafenib}$ derived from MDA-MB-453 and **B** EV_{DMSO} or $EV_{sorafenib}$ derived from MCF-7, was evaluated at 3 time-points (48hpf; 72hpf; 96hpf); Unpaired t-test was used to compare Ev treatment *versus* negative control; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Both experiments were conducted in triplicate with 25 embryos for each experimental point (n=25). NC, negative control, PC, positive control.

Supplementary Figure 8. Within one day post-injection, a severe phenotype and pericardial edema, indicating toxicity caused by breast cancer cells microinjection, were observed in approximately 90-95% of the injected fish. The fish injected with MDA-MB-453 BC cells experienced mortality between days 2-3 post-injection. Scale bars represent 500 µm for magnification of 20x.