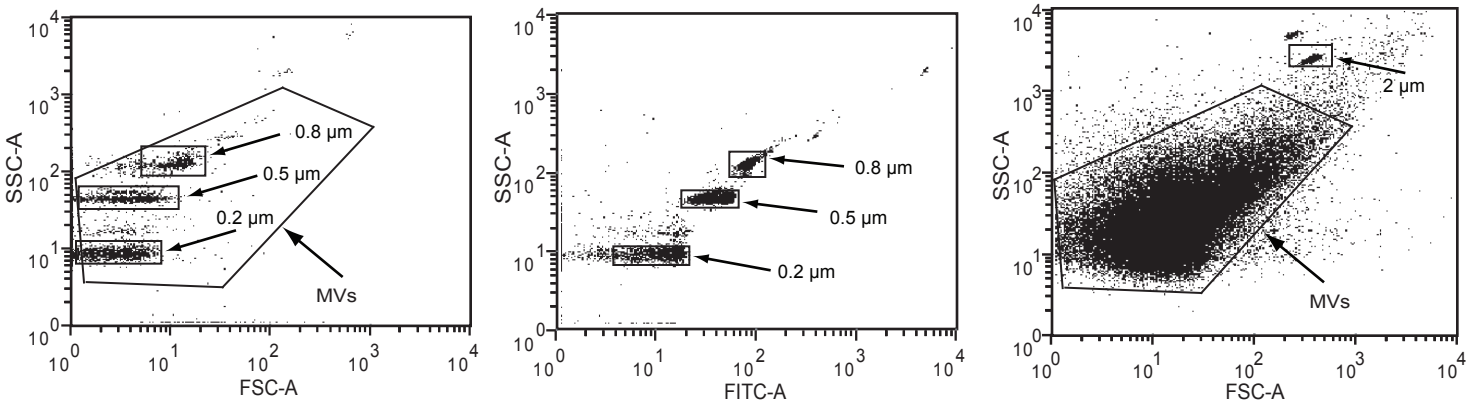
A.



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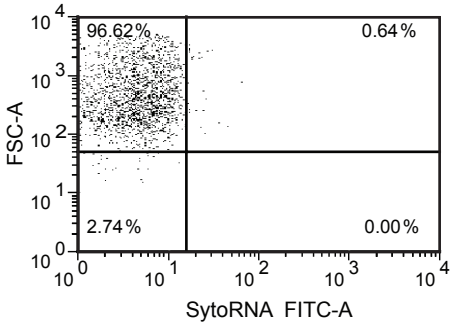
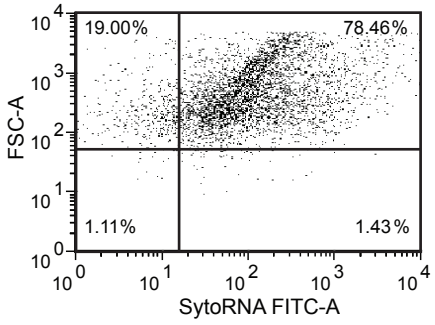
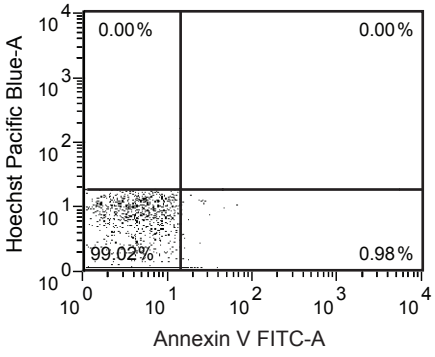


GM-CSF-generatated Microvesicles Non-stained

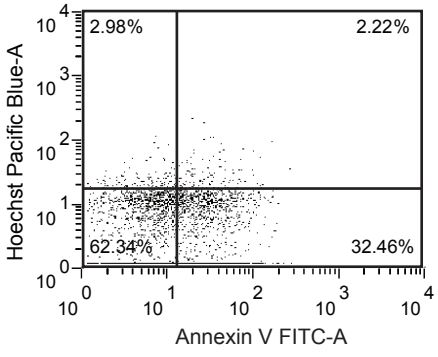


GM-CSF-Generated Microvesicles SytoRNA Stained

C.



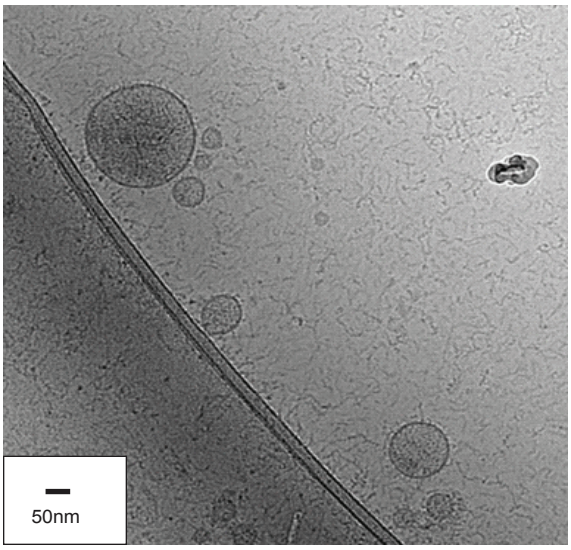
GM-CSF-generatated Microvesicles Non-stained



GM-CSF-Generated Microvesicles Annexin V and Hoechst Stained

Supplemental Figure 1

A.



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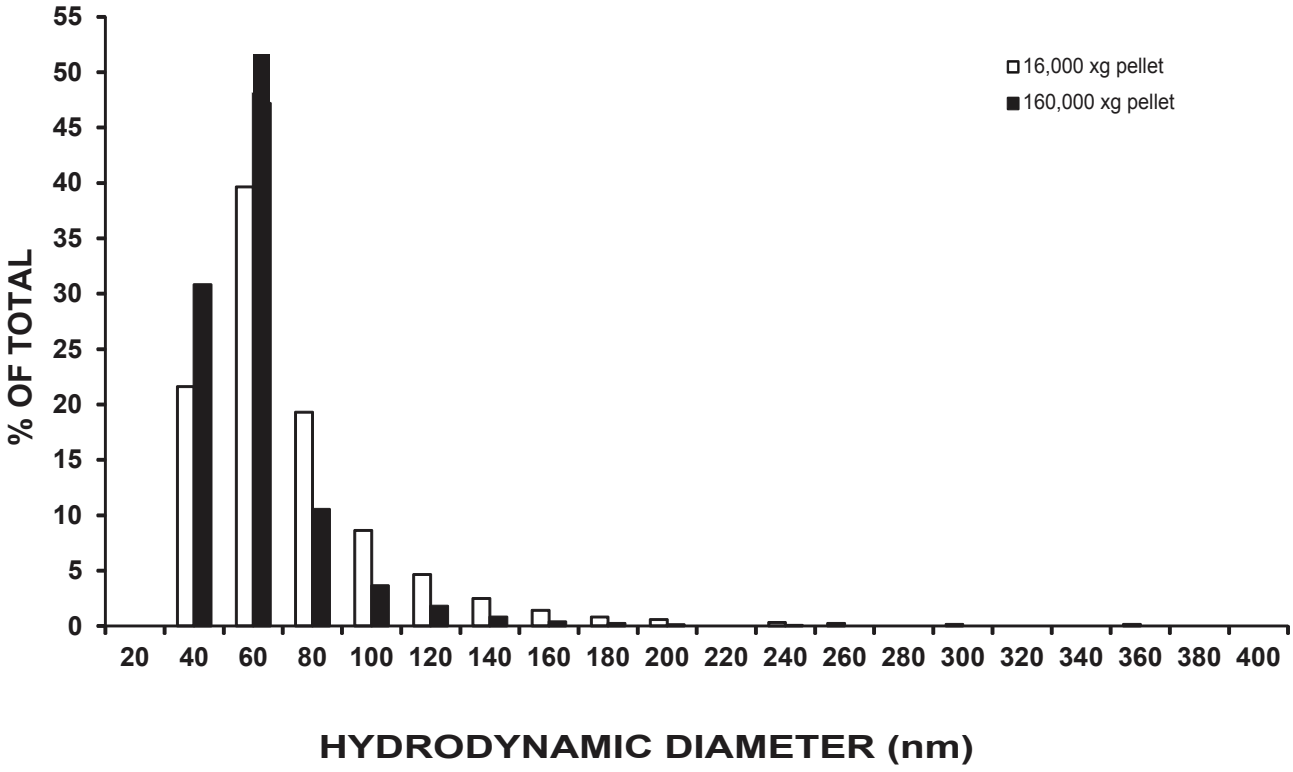
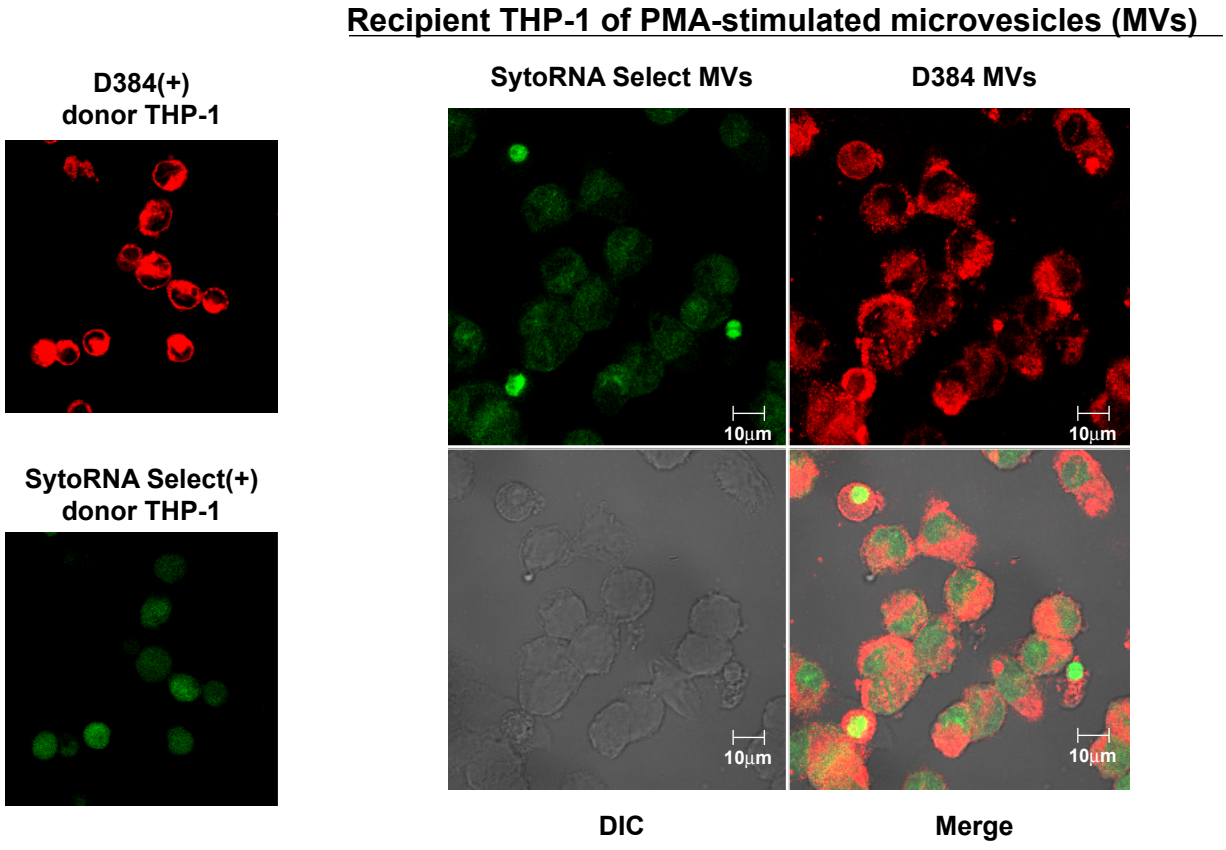


Supplemental Figure 2

A.



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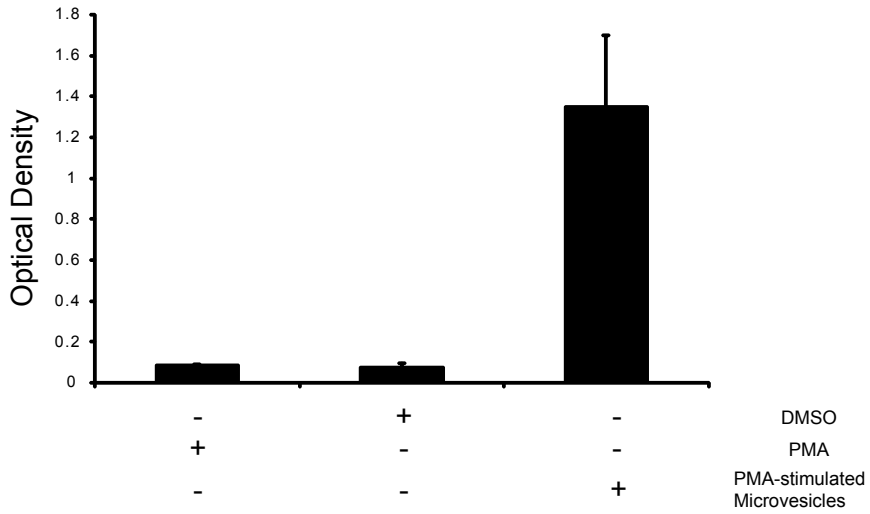
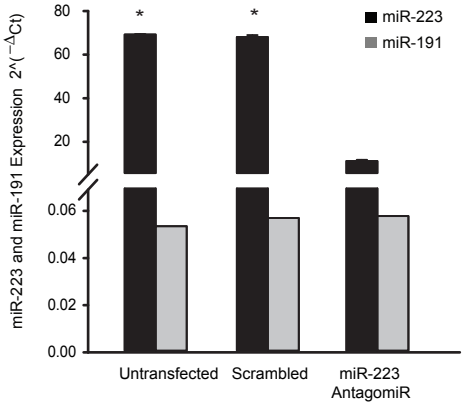


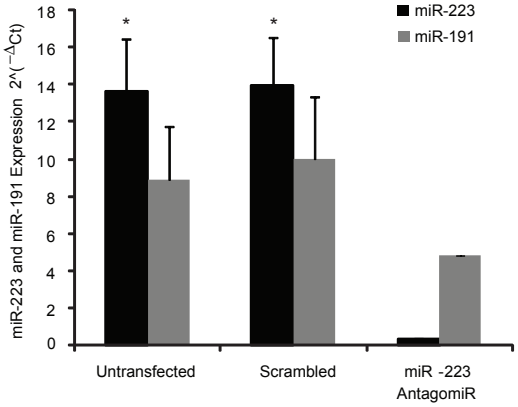
Supplemental Figure 3

A. THP-1



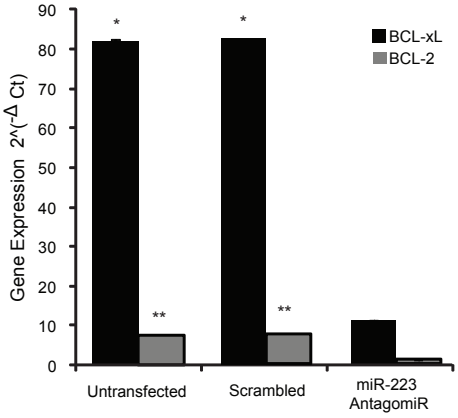
* P<0.05 compare to miR-223 antagomiR for miR-223 expression.

B. Monocytes



* P<0.05 compare to miR-223 antagomiR for miR-223 expression.

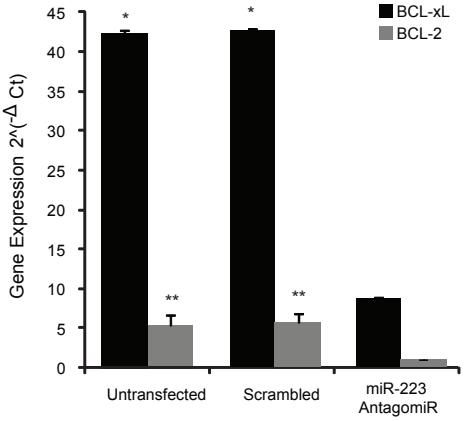
C. THP-1



* P<0.002 compare to miR-223 AntagomiR.

** P<0.002 compare to miR-223 AntagomiR.

D. Monocytes



* P<0.001 compare to miR-223 AntagomiR.

** P<0.001 compare to miR-223 AntagomiR.

Supplemental Figure 4

Supplemental Figure 1. Characterization of GM-CSF-stimulated microvesicles. (A) Using the LSRII flow cytometry, FITC conjugated polystyrene beads (0.2 – 0.8 μm) were visualized by FSC vs SSC (*left panel*) and FITC vs SSC (*middle panel*) and indicated by arrows to each designated gated region. A gate encompassing all the boxes was drawn and designated as microvesicles (MVs). All subsequent analyses used events present in this gate. In the *right panel*, a representative plot of microvesicles from GM-CSF-derived macrophages (n=6) and collected using a 16,000x g centrifugation is shown. (B) Microvesicles were collected from GM-CSF treated cells that were either were left unstained or stained with SytoRNA Select FITC. Events in the MV gated region (A) were further analyzed by flow cytometry, as indicated. Shown is a representative plot from three individual donors. (C) The isolated microvesicles were unstained or stained with Annexin V FITC and Hoechst to quantifying the presence of phosphatidylserine and DNA, respectively. Events in the MV gated region as shown in (A) were further analyzed as indicated. Shown is a representative plot from three different donors. The GM-CSF-stimulated microvesicles were 71.58 ±2.70% negative for Annexin V (average ±S.E.M, n=3) and 6.87 ±1.52% positive for DNA (average ±S.E.M, n=3).

Supplemental Figure 2. Differential centrifugations results in similar microvesicle size distribution. (A) Representative cryo-TEM images of microvesicles secreted from PMA-treated THP-1 cells spun at centrifuged at 160,000× g. Both small (30-100 nm) and large (>200 nm) microvesicles are apparent. Scale bar = 50 nm. (B) Dynamic light scattering (DLS) number-weighted distributions of the hydrodynamic diameter of THP-1 cell-secreted microvesicles obtained from samples centrifuged at 16,000× g (open bars) and 160,000× g (filled bars). The relative % of microvesicle diameters > 200 nm is less than 1.44% (16,000× g samples) and 0.19% (160,000× g samples).

Supplemental Figure 3. PMA-stimulated microvesicles bind THP-1 cells and stimulate monocyte adherence. (A) Donor THP-1 cells were stained with D384 and SytoRNA Select then treated with PMA. The PMA-stimulated microvesicles were collected and added to either THP-1 cells. Representative confocal image of THP-1 cells from three independent experiments reveal the uptake of the microvesicles. (B) Monocytes were treated with PMA-stimulated microvesicles. After 48 hours, adherent cells possessing macrophage-like phenotype were stained with crystal violet. Dye uptake was measured at 550 nm.

Supplemental Figure 4. Decrease expression of *miR-223* and survival genes in cells transfected with *miR-223* antagomiR. THP-1 cells or freshly isolated monocytes were transfected with an antagomiR to *miR-223* and cultured in the presence of PMA or GM-CSF, respectively. After 18 hours, the cells were analyzed for miRNA and gene expression. Expression of *miR-223* but not *miR-191* was decreased in the transfected (A) THP1 cells and (B) monocytes. AntagomiR-transfected (C) THP-1 cells and (D) monocytes stimulated with PMA or GM-CSF, respectively, were analyzed for gene expression as indicated. The average relative copy number is shown ±S.E.M from three independent experiments.