ICAM-1-decorated extracellular vesicles loaded with miR-146a and Glut1 drive immunomodulation and hinder tumor progression in a murine model of breast cancer

Silvia Duarte-Sanmiguel¹, Ana I. Salazar-Puerta¹,², Ana Panic¹, Daniel Dodd¹,³, Carlie Francis¹, Diego Alzate-Correa¹,², Lilibeth Ortega-Pineda¹, Luke Lemmerman¹, Maria A. Rincon-Benavides¹,², Kavya Dathathreyal, William Lawrence¹,³, Neil Ott¹, Jingjing Zhang⁵, Binbin Deng⁶, Shipeng Wang¹, Sandra P. Santander⁷, David W. McComb⁶,⁸, Eduardo Reategui⁵, Andre F. Palmer⁵, William E. Carson⁹, Natalia Higuita-Castro¹,²,⁴,⁹,¹⁰, Daniel Gallego-Perez¹,²,⁴,⁹,¹⁰*

¹ The Ohio State University, Department of Biomedical Engineering, Columbus, OH 43210
² The Ohio State University, Gene Therapy Institute, Columbus, OH 43210
³ The Ohio State University, Biomedical Sciences Graduate Program, Columbus, OH 43210
⁴ The Ohio State University, Biophysics Program, Columbus, OH 43210
⁵ The Ohio State University, William G. Lowrie Department of Chemical and Biomolecular Engineering, Columbus, OH 43210
⁶ The Ohio State University, Center for Electron Microscopy and Microanalysis (CEMAS), Columbus, OH 43210
⁷ Juan N. Corpas University Foundation, Center of Phytoimmunomodulation Department of Medicine, Bogota, Colombia
⁸ The Ohio State University, Department of Materials Science and Engineering, Columbus, OH 43210
⁹ The Ohio State University, Department of Surgery, Columbus, OH 43210
¹⁰ The Ohio State University, Dorothy M. Davis Heart and Lung Research Institute, Columbus, OH 43210

*Corresponding Authors: gallegoperez.1@osu.edu and higuitacastro.1@osu.edu
Supplementary Figure 1. *In silico* functional analysis identifying signaling pathways common to miR-146a and *Glut1*. (A) Functional interacting network of miR-146a-5p and miR-146a-3p targets leads to the identification of a subgroup of interacting genes involved in *Hematological System Development and Function*. (B) Functional interacting network of *Glut1* (Slca2a1). (C) Common factors between miR-146a and *Glut1* functional interacting networks suggest that the pro-inflammatory repolarization seen in MDSCs could potentially be partially mediated by the Hypoxia-Inducible Factor (HIF)-1 regulatory pathway.
Supplementary Figure 2. TNT chip fabrication. (A) Schematic diagram showing the fabrication process for TNT devices. First, nanoscale openings are patterned and etched into the Si wafer surface using a combination of lithography and DRIE (1-4). Next, microscale reservoirs are drilled on the back side of the wafer using lithography and DRIE to gain fluidic access to the nanochannels (5-7). Lastly, microscale photoresist (PR) pillars were patterned on the nanochannel side and used as an etch mask to define the needles using SF6, which undercut the PR to form the needle (8-10). (B) SEM images of the cross-section and surface of the TNT device.
Supplemental Figure 3. Characterization of in vitro derived engineered EVs. NanoSight analysis showing the concentration (dilution factor 2X10²) and size distribution of (A) control EVs and (B) engineered EVs produced and released by the ‘donor’ cells 24 hours after transfection.
Supplemental Figure 4. Characterization of skin derived EVs after TNT treatment. (A) NanoSight analysis of the amount and average size and (B) individual plots showing the particle distribution in terms of concentration (dilution factor \(1 \times 10^2\)) and size of the EVs obtained from 1 cm\(^2\) of TNT-treated skin. (C) Western blot analysis confirmed the presence of ICAM-1 protein in the ICAM-1 decorated EVs loaded with sham, and engineered EVs (ICAM-1 decorated EVs loaded with miR-146a + Glut1) compared to control EVs (without ICAM-1), using β-actin as the internal control. RT-qPCR quantification of (D) miR-146a and (E) Glut1 loading inside engineered skin EVs compared to skin-derived control skin EVs.
Supplemental Figure 5. Co-localization analysis of iNOS expression with macrophages. Representative fluorescence micrograph of tumor tissue showing the expression of the pro-inflammatory marker iNOS (yellow), the expression of the classically activated (M1-like) macrophage marker CD68 (red), and the nuclear stain DAPI (blue). Analyses of the double positive signal of iNOS and CD68 in tumor tissue revealed a co-localization of ~70%.