

Supplementary Information

Metabolic Click-labeling of Interleukin-10 Enhances Immunomodulatory Potential and Wound Healing Properties of Mesenchymal Stem Cell-derived Extracellular Nanovesicles

Hee Gyeong Ko^{1,2}, Yun A Kim^{1,2}, Jun Kwon^{1,2}, So Won Jeon^{1,2}, Jong Sang Yoon^{1,2}, Min-Ho Kang^{1,2}, Ju-Ro Lee^{3,4}, and Han Young Kim^{1,2,*}

¹Department of Biotechnology, The Catholic University of Korea, Bucheon-si, Gyeonggi-do, Republic of Korea ²Department of Biomedical-Chemical Engineering, The Catholic University of Korea, Bucheon-si, Gyeonggi-do, Republic of Korea ³Center for Systems Biology, Massachusetts General Hospital Research Institute, Boston, MA, USA ⁴Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

*To whom correspondence should be addressed:

Prof. Han Young Kim

E-mail: hy0408@catholic.ac.kr

Supplementary information figures

Gene	Forward (5' =>3')	Reverse (5' => 3')
Human Sequences (qRT-PCR)		
Ang-1	GTG CTC ACG TGG CTC GAC TAT A	GCA CAG CAA GCT CAG CAG TT
FGF2	CCA CCT ATA ATT GGT CAA AGT GG	GAA ACG AGG GAG AAA GGA TGG A
HGF	GAC GCA GCT ACA AGG GAA CA	AAA AGC TGT GTT CGT GTG GT
GAPDH	CCA CTC CTC CAC CTT TGA	ACC CTG TTG CTG TAG CCA
Bcl-2	CCG CAT CAG GAA GGC TAG AG	CTG GGA CAC AGG CAG GTT CT
BAX	TCA GGA TGC GTC CAC CAA GAA G	TGT GTC CAC GGC GGC AAT CAT CTT

Gene	Forward (5' =>3')	Reverse (5' => 3')
Mouse Sequences (qRT-PCR)		
TNF- α	GAT CGG TCC CCA AAG GGA TG	CCA CCT GGT GGT TTG TGA GTG
IL-6	AGC TAC CTG GAG TAC ATG AAG A	GTG ACT CCA GCT TAT CTC TTG GT
Arg-1	CCA CAC TGA CTC TTC CAT TCT T	CCA CAC TGA CTC TTC CAT TCT T
β -actin	GGC TAT ATT CCC CTC CAT CG	CCA GTT GGT AAC AAT GCC ATG T
IL-10	GCT CTT GCA CTA CCA AAG CC	CTG CTG ATC CTC ATG CCA GT
IL-1 β	GCC ACC TTT TGA CAG TGA TGA G	GAC AGC CCA GGT CAA AGG TT
HGF	CAA CGC GGA TGG TTT ATT AC	CCA TAA TCT CCC TCA CAT GG
FGF2	AAG CGG CTC TAC TGC AAG AAC G	CCT TGA TAG ACA CAA CTC CTC TC

Fig S1. List of qRT-PCR primers of human and mouse-specific genes.

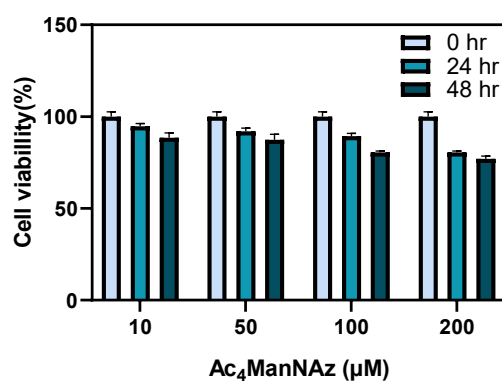


Fig. S2. Cytotoxicity test of MSC under treatment of Ac₄ManNAz. Cytotoxicity of MSCs measured until 48 h post-treatment of various concentrations of Ac₄ManNAz.

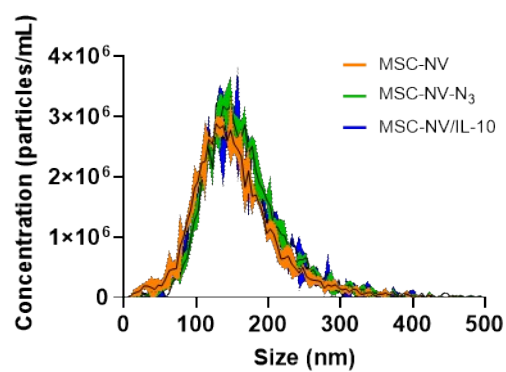


Fig. S3. Particle size and number of MSC-NV, MSC-NV-N₃, and MSC-NV/IL-10, as measured by nanoparticle tracking analysis (NTA).

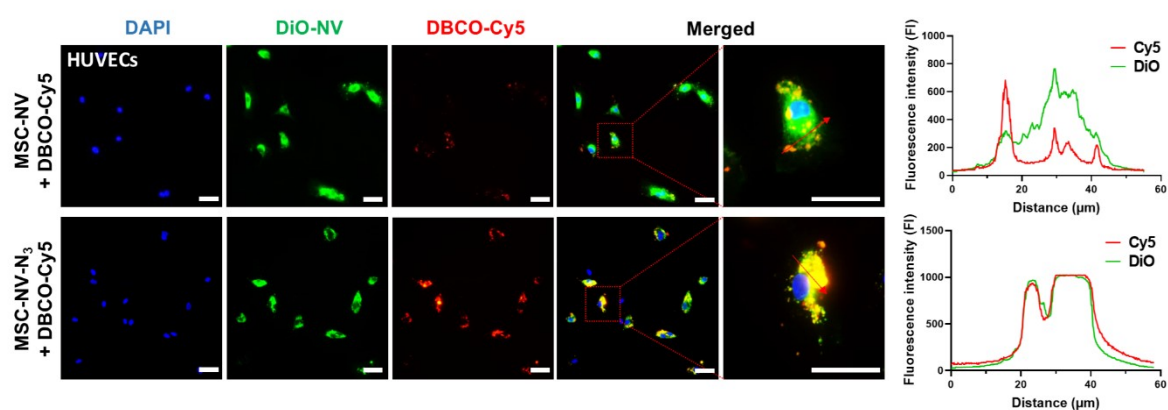


Fig. S4. Fluorescence microscope images (left) and fluorescence intensity profiles (right) of DiO-labeled NV (green) and DBCO-Cy5 (red) in HUVECs treated with MSC-NV or MSC-NV-N₃. Both NVs were reacted with DBCO-Cy5 and washed before treatment. Scale bar: 50 μm.

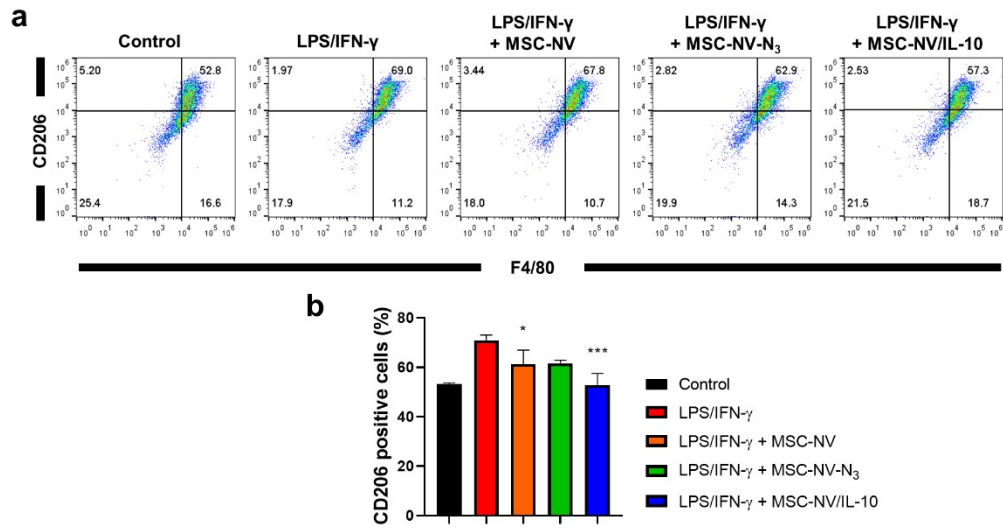


Fig. S5. (a) Representative plots showing the population of F4/80-positive and CD206-positive cells. (b) Quantification of CD206-positive BMDMs (n=3). p versus LPS/IFN- γ . Treatment of PBS served as control. Data are shown as mean $\pm$ SD with significance at *p < 0.05, **p < 0.01, ***p < 0.005, and ****p < 0.001.