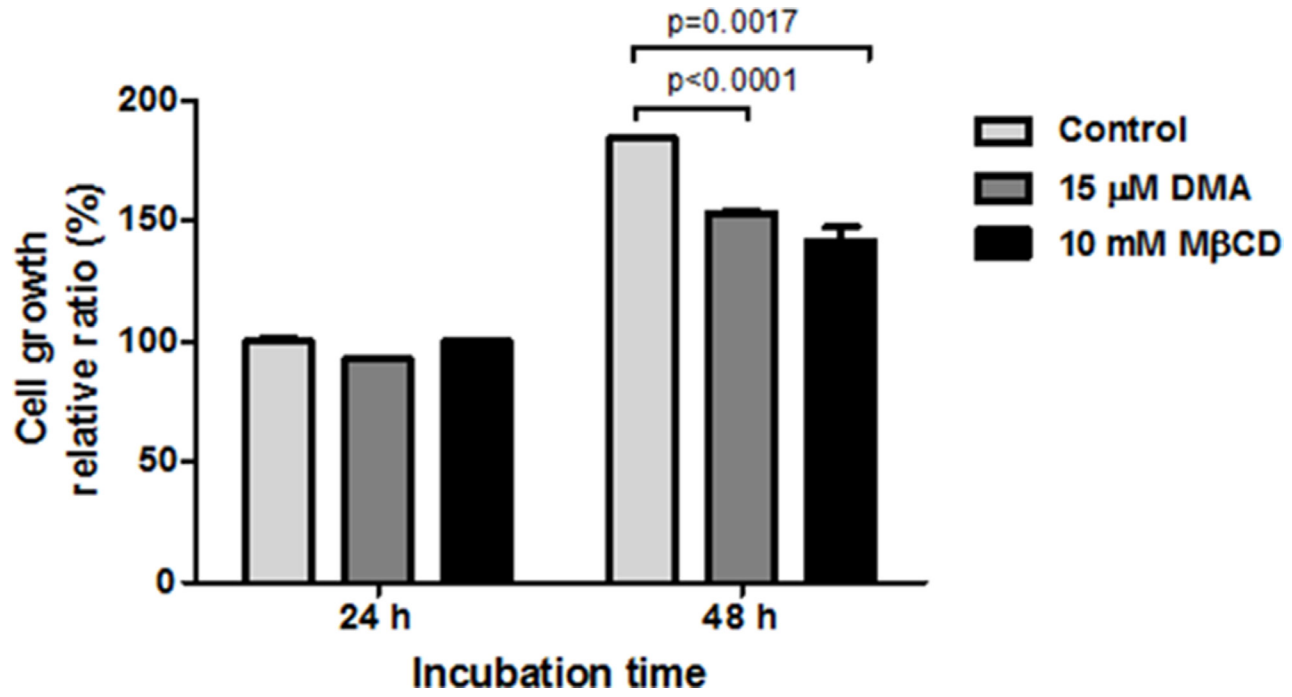
# Breast cancer cell-derived exosomes and macrophage polarization are associated with lymph node metastasis

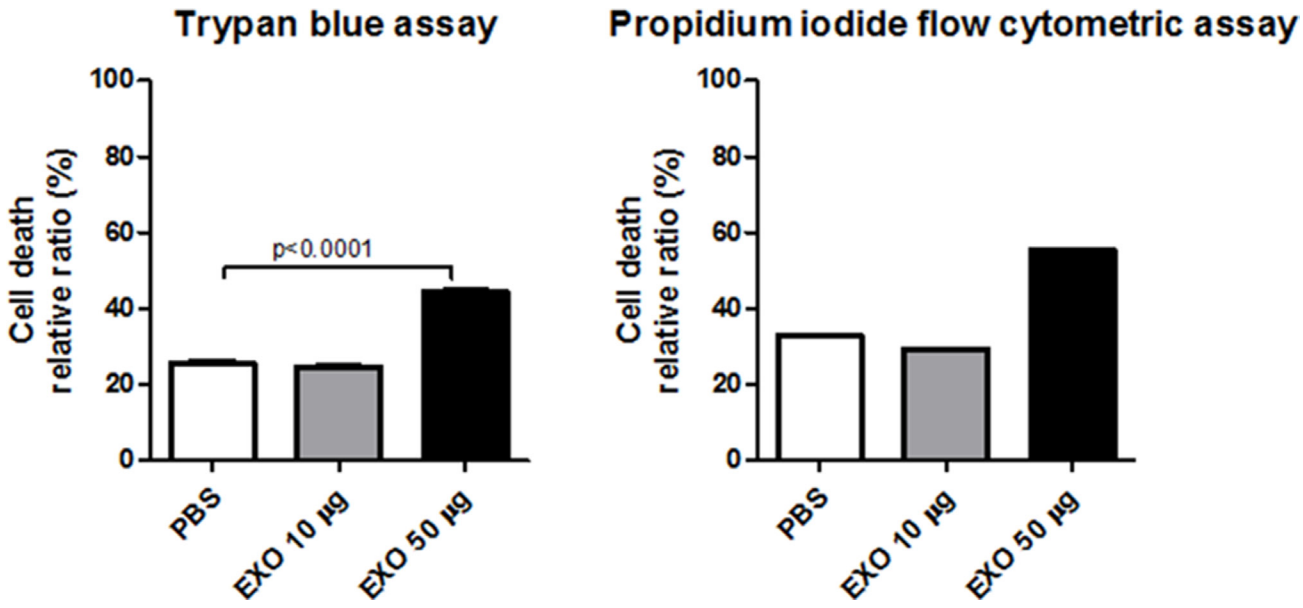
## SUPPLEMENTARY MATERIALS

**Supplementary Table 1: Specific primer sequence for real-time RT-PCR**

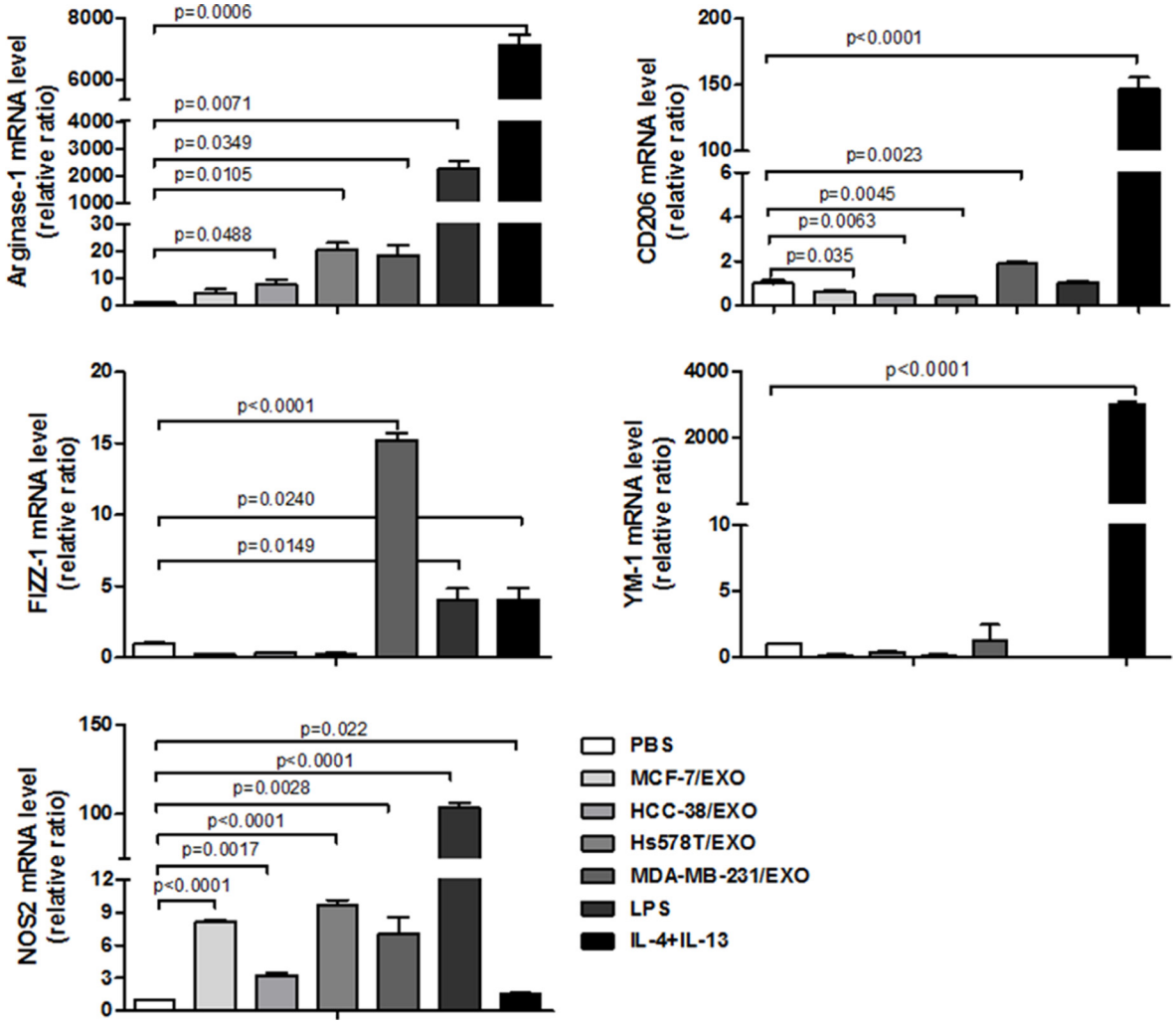
| Gene | | Sequence (5′ -> 3′) |
|---|---|---|
| NOS2 | Forward | CAGAGGACCCAGAGACAAGC |
| | Reverse | TGCTGAAACATTTCCTGTGC |
| Arginase-1 | Forward | GGAATCTGCATGGGCAACCTGTGT |
| | Reverse | AGGGTCTACGTCTCGCAAGCCA |
| CD206 | Forward | TTCGGTGGACTGTGGACGAGCA |
| | Reverse | ATAAGCCACCTGCCACTCCGGT |
| FIZZ-1 | Forward | TCCAGTGAATACTGATGAGA |
| | Reverse | CCACTCTGGATCTCCCAAGA |
| YM-1 | Forward | GGGCATACCTTTATCCTGAG |
| | Reverse | CCACTGAAGTCATCCATGTC |
| β-actin | Forward | TTCCTGGGCATGGAGTCCTGTGG |
| | Reverse | CGCCTAGAAGCATTTGCGGTGG |



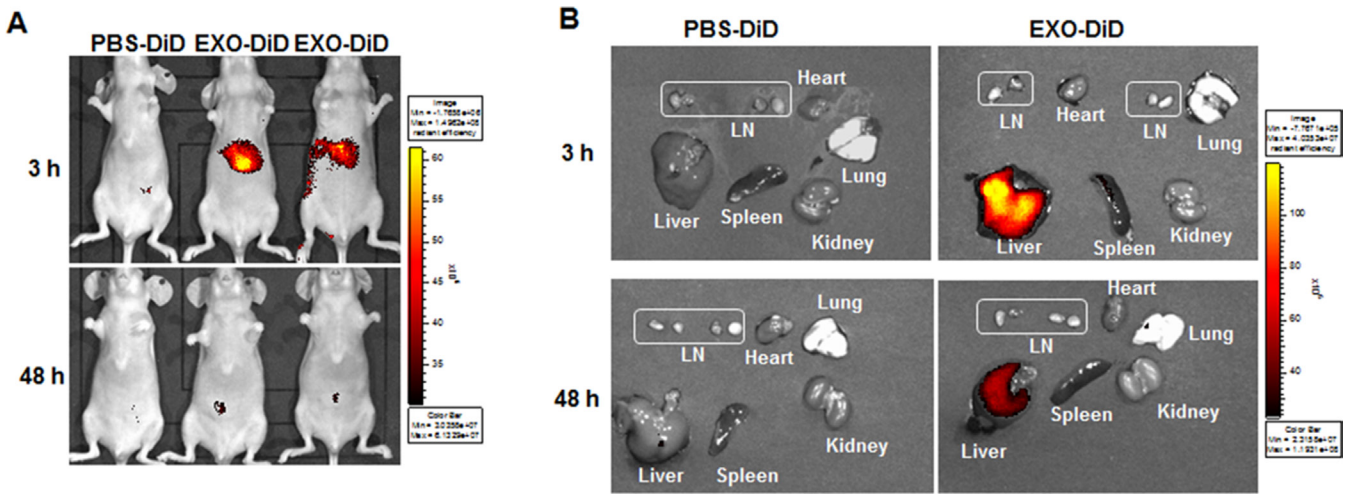

**Supplementary Figure 1: TNBC cell proliferation is suppressed by blocking exosome releasing and uptake.** Proliferation of MDA-MB-231 cells treated with exosome-releasing inhibitor 5-(N, N-dimethyl) amiloride hydrochloride (DMA, 15 μM) or exosome-uptake inhibitor methyl-β-cyclodextrin (MβCD, 10 mM) for 24 to 48 hours was evaluated by MTT assay.



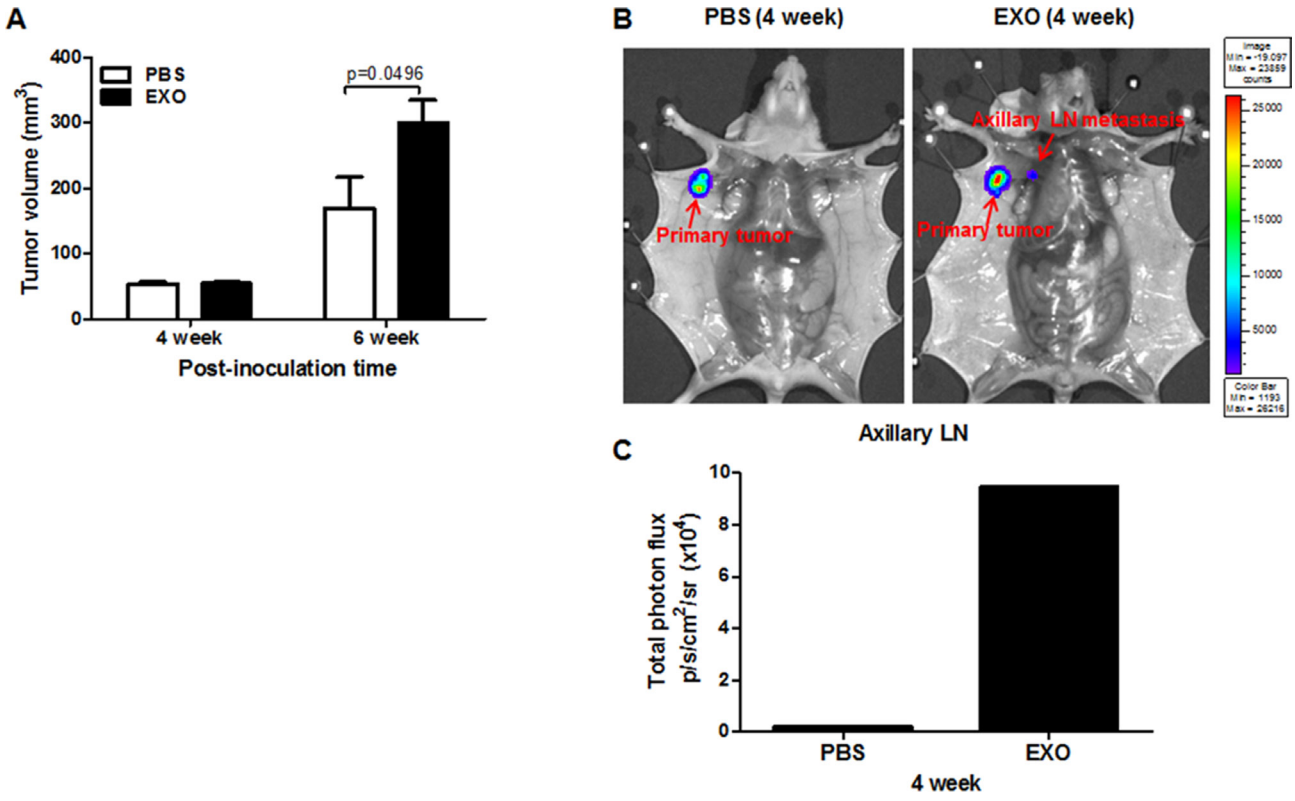

**Supplementary Figure 2: High concentration of cancer-derived exosomes leads to the cytotoxicity of RAW264.7 cells.** Cytotoxic effect in RAW264.7 cells treated with cancer exosomes (10 and 50 μg/mL) for 24 to 48 hours was investigated by trypan blue and propidium iodide flow cytometric assays.




**Supplementary Figure 3: Investigation of M1/M2 polarization markers in RAW264.7 cells treated with different breast cancer exosomes.** Arginase-1, CD206, FIZZ-1, YM-1, and NOS2 expressions in RAW264.7 cells treated with 10 µg/mL of diverse breast cancer-exosomes (MCF-7, HCC-38, Hs578T, and MDA-MB-231), LPS (100 ng/mL) for 24 to 48 hours, or a complex of IL-4/IL-13 (20 ng/mL) for 4 days were investigated by real-time RT-PCR.




**Supplementary Figure 4: Biodistribution of TNBC cell–derived exosomes.** (**A**) Follow-up *in vivo* and *ex vivo* imaging of RFP-tagged exosomes (EXO) labeled with DiD in a mouse after intravenous injection. (**B**) *Ex vivo* imaging of RFP-tagged exosomes labeled with DiD in diverse tissues, including LN, heart, liver, spleen, lung, and kidney.




**Supplementary Figure 5: Early detection of axillary LNs metastasis in xenograft tumor mice intravenously injected with TNBC cell–derived exosomes.** (**A**) Xenograft tumor volume in mice administered with PBS-derived or TNBC cell–derived exosomes at 4 weeks and 6 weeks after the injection of TNBC cells. (**B** and **C**) Representative BLI and total photon flux (mean ± S.E.) of axillary LN area in tumors from mice intravenously injected with PBS or exosomes at 4 weeks.