Supplementary Material

Self-adjuvant nanovaccine delivering cytokines as a novel platform to empower glioblastoma immunotherapy

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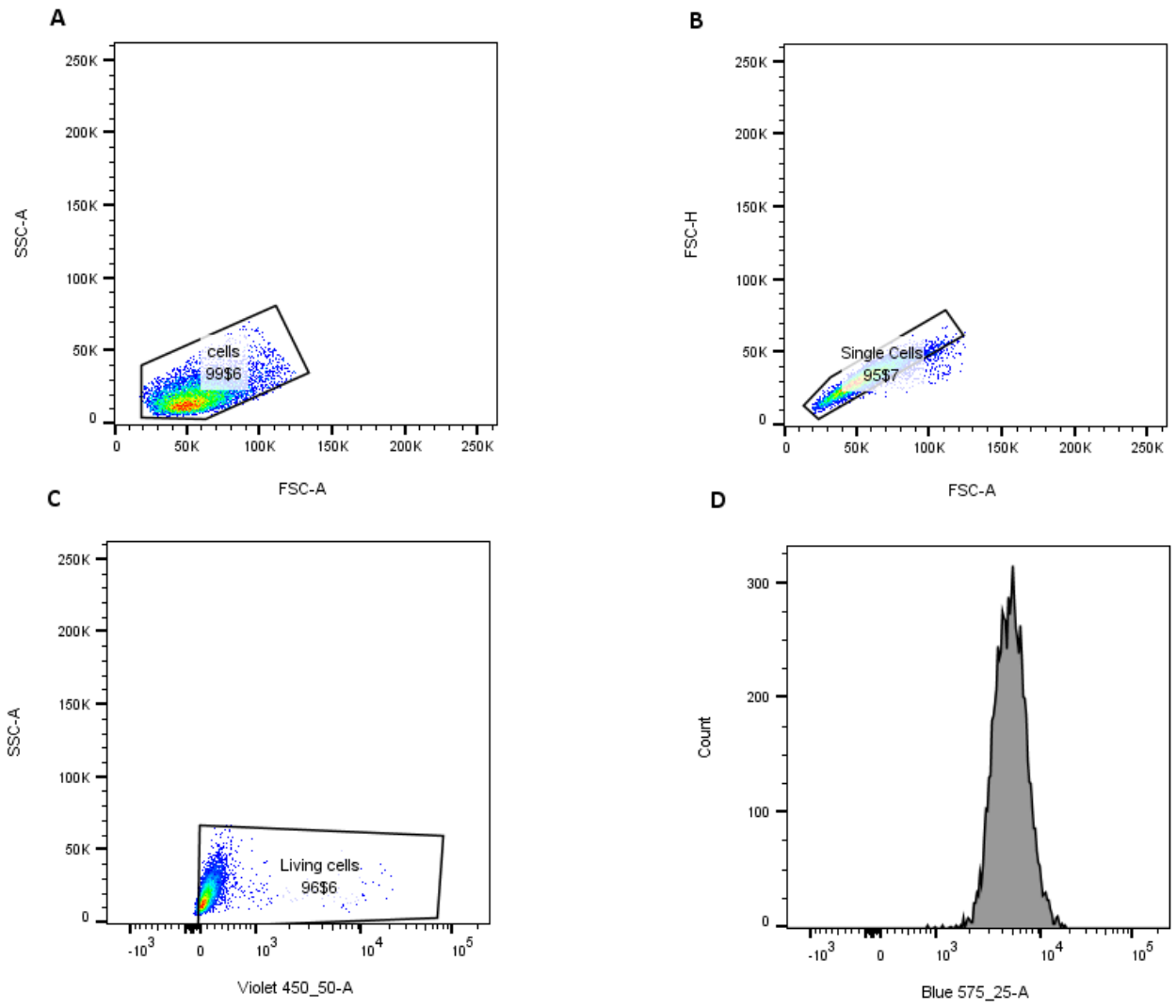

Figure S1. **Gating strategy for flow cytometry.** (A) At first, U87-MG cells were gated using forward and side scatter area (FSC-A and SSC-A, respectively) to identify the cells of interest**.** (B) Next, to remove the doublets, a new gate was performed using forward scatter height and forward scatter area (FSC-H and FSC-A, respectively). (C) To remove dead cells, LIVE/DEAD™ Fixable Violet Dead Cell Stain was used, a new gate was used for side scatter area and Fixable Violet Dead Cell. (D) Single-parameter histograms were used to identify intracellular IL-12.

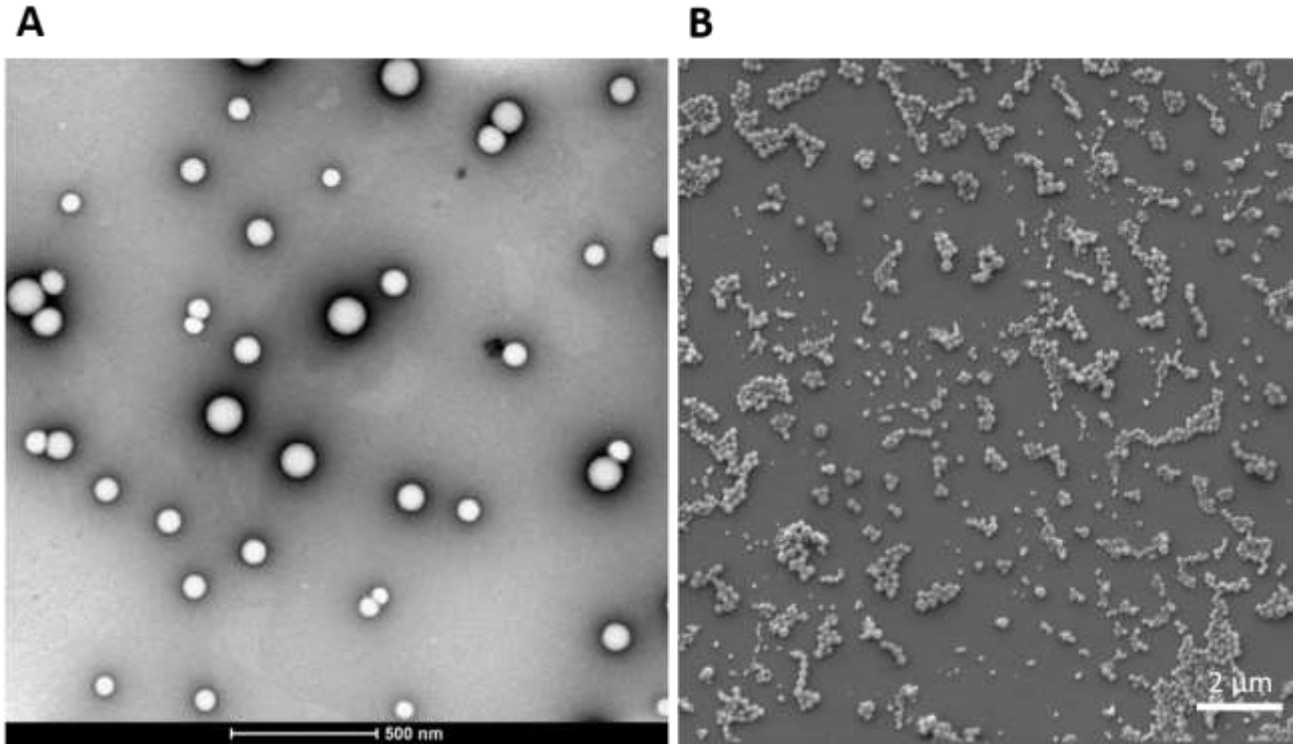

Figure S2. Morphology of Empty PLGA NP. The morphology of the Empty PLGA NP was analyzed by (E) transmission electron microscopy (TEM) and (F) scanning electron microscopy (SEM). Scale bar of TEM microphotographs: 500nm. Scale bar of SEM microphotographs: 2 µm.

Table S1. Information about the primers used for Real-time qRT-PCR. FW: Forward. RV: Reverse.

Primers	Gene symbol	Gene name	Sequence (5' – 3')	Product length	Efficiency
IL-6	IL6	Interleukin 6	FW:CCACTCACCTCTTCAGAACG	25	93%
			RV:CATGTTACTCTTGTTACATGTCTCC		
IL-8	CXCL-8	C-X-C motif chemokine ligand 8	FW: GAGAAGTTTTTGAAGAGGGCTGA	91	90%

			RV: GCTTGAAGTTTCACTGGCATCT		
Housekeeping	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	FW: GTCGGAGTCAACGGATTTGG	147	82%
			RV: GCCATGGGTGGAATCATATTGG		
	YWHAZ	Tyrosine 3-monooxygenase/tryptoph an 5-monooxygenase activation protein zeta	FW: GCTGGTGATGACAAGAAAGGGAT	120	90%
			RV: GTTAAGGGCCAGACCCAGTC		