

Ex vivo Activation of Dendritic Cells via Coacervate-mediated Exogenous Delivery of Tumor Cell Lysates

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Supplemental Information

Experimental section

Method

Cross-presentation in BMDC using BMDC-based ELISA

The expression of MHCI-restricted AH-1 peptide on BMDCs was also evaluated by our customized BMDC-based ELISA. After 48 h of stimulation using various TCL formulations, BMDCs were fixed with 4 % paraformaldehyde for 15 min at RT, quenched using 1 % hydrogen peroxide with wash buffer (0.05 % Tween-20) for 25 min at RT. to consume the endogenous peroxidase activity, and blocked using 5 % BSA with PBS for 2 hours at RT. After that, the cells were labeled with rabbit anti-AH-1 peptide (gp70 H2-Ld-restricted epitope) IgG and goat anti-rabbit IgG conjugated horseradish peroxidase (HRP). Then, TMB chromogen substrate solution and a stop solution (2N H₂SO₄) was sequentially added. OD at 450 nm and 570 nm were detected using a microplate reader and BMDCs were lysed using RIPA buffer for detection of DNA amount. The amount of DNA was quantified by using Picogreen assay (Thermo fisher scientific) following manufacturer protocol. Then, displayed amount of MHCI-restricted AH-1 peptide was calculated as, MHCI-restricted AH-1 peptide =

$$\frac{OD \text{ of MHCI - restricted AH - 1 peptide amount}}{F.I \text{ of DNA amount}} \quad (\text{pg}/\mu\text{g})$$

Result

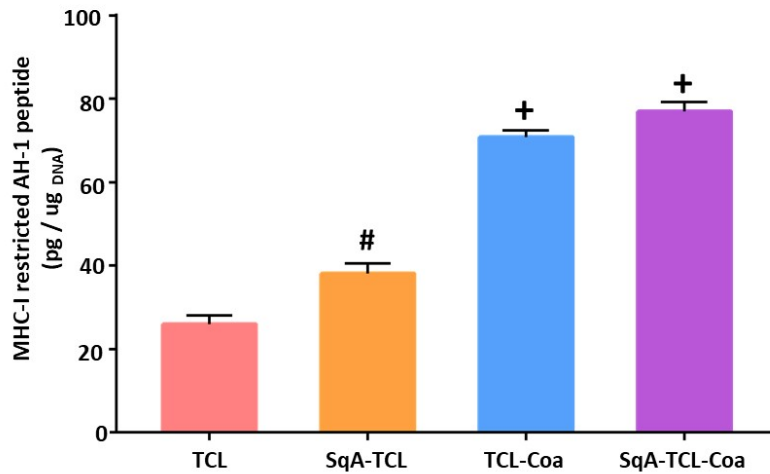


Figure S1. Quantification of cross-presented MHC-I restricted AH-1 peptide on BMDCs using BMDC-based ELISA. (# : indicate the statistical difference between non-treated TCL and SqA treated TCL group (TCL vs SqA-TCL or TCL-Coa vs SqA-TCL-Coa), + : indicate the statistical difference between bolus cargo and Coa embedded cargo (TCL vs TCL-Coa or SqA-TCL vs SqA-TCL-Coa, ($p < 0.05$))

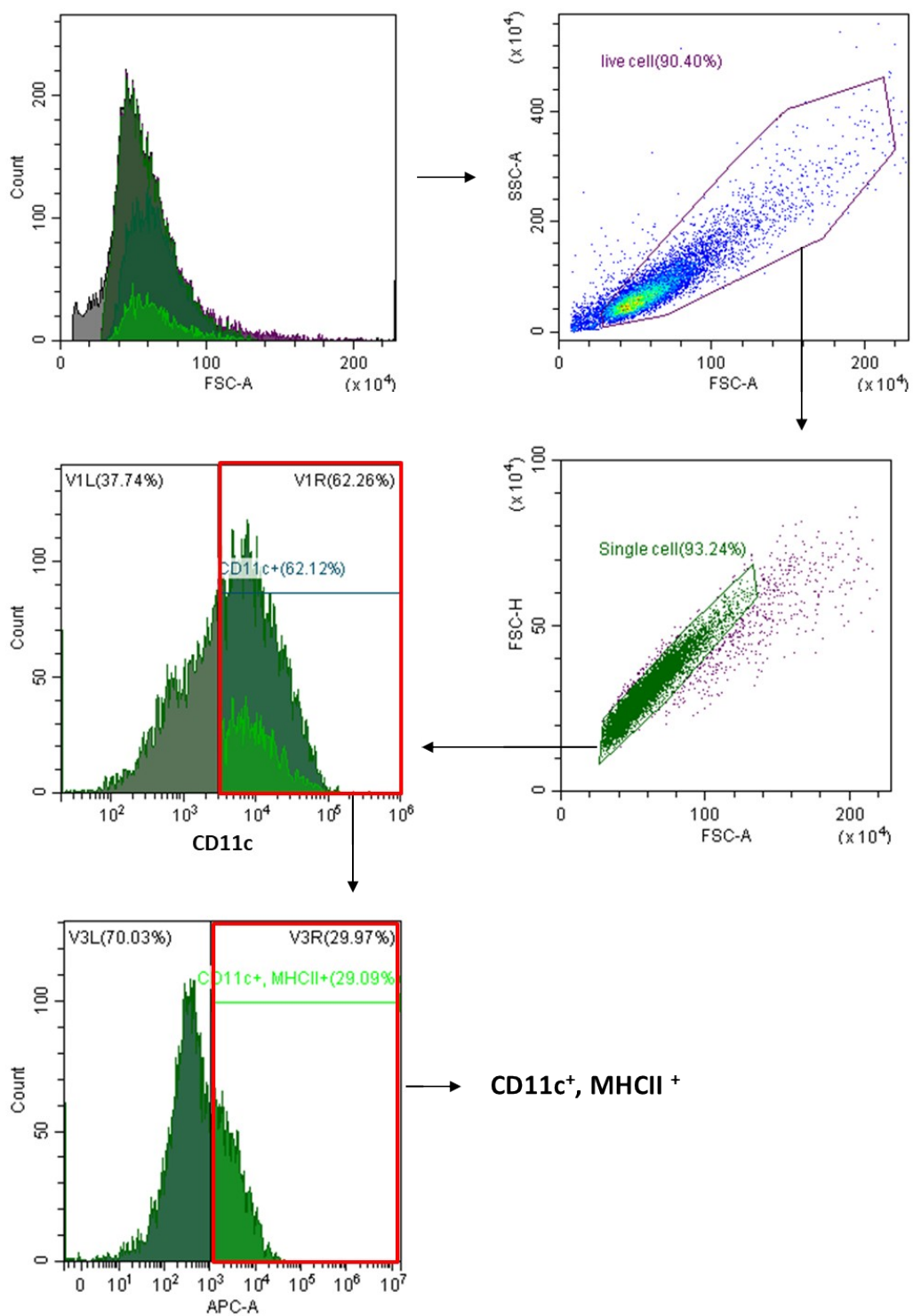


Figure S2. Flow cytometry gating strategy