**Cell Reports**

**SUPPLEMENTAL INFORMATION**

# Porous silicon microparticle potentiates anti-tumor immunity by enhancing cross-presentation and inducing type I interferon response

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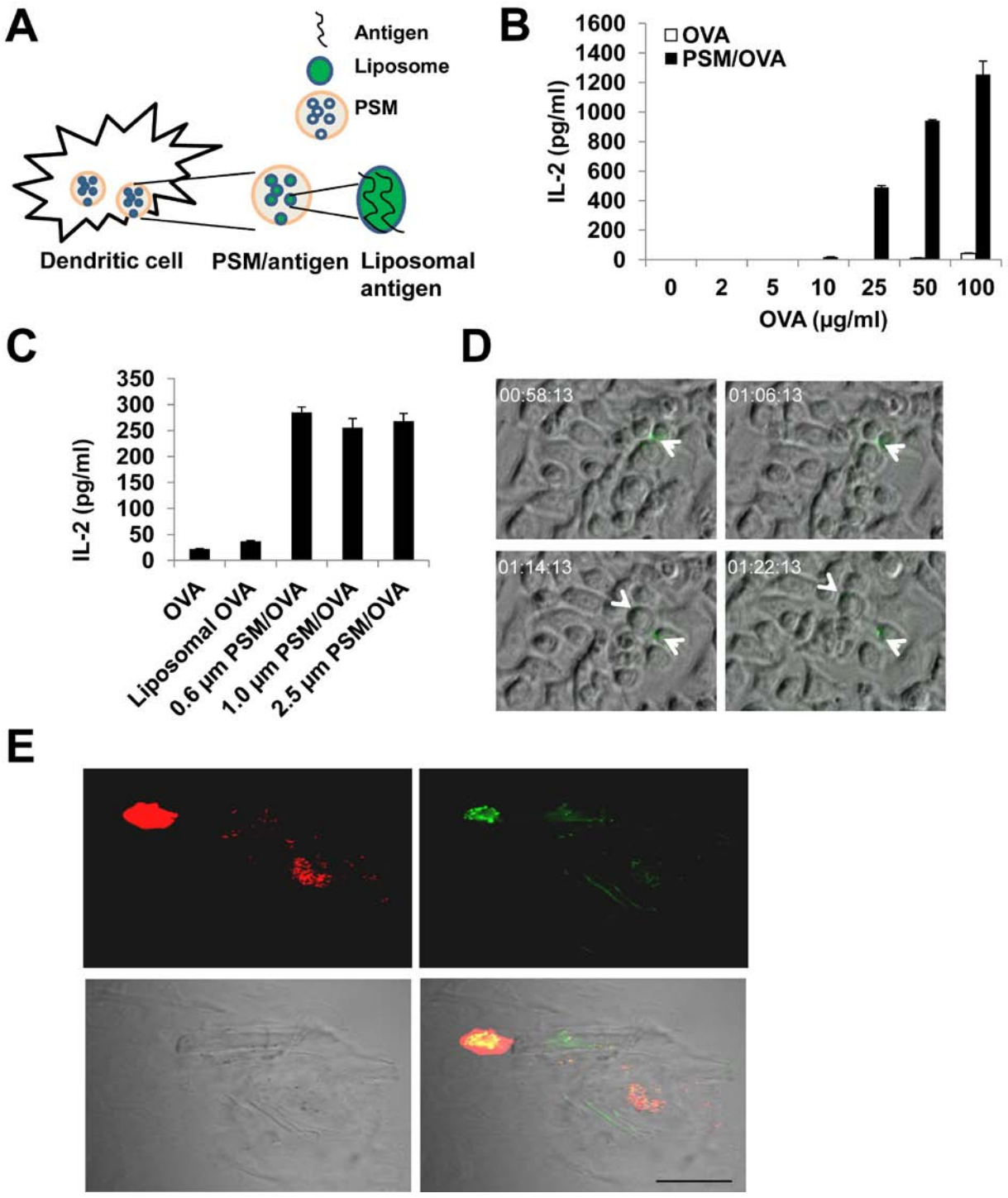


**Figure S1. PSM potentiate antigen presentation in DCs. Related to Figure 1.**

(A) A cartoon illustrating the components in a DC vaccine. The antigen is packaged into liposomes and loaded into the PSM particles that are internalized by the DCs.

(B) IL-2 production by B3Z cells after co-incubation with DCs pre-treated with different concentrations of soluble OVA or PSM/OVA. DCs were incubated with OVA or PSM/OVA for 3 h, washed extensively (without fixation), and then co-incubated with B3Z cells for18 h. IL-2 levels in the cell culture media were measured with ELISA. Data are presented as mean ± S.D.
(C) IL-2 production by B3Z cells after co-incubation with DCs pre-treated with soluble OVA, liposomal OVA, or OVA loaded in different size PSM particles. DCs were pre-treated with 100 μg/ml OVA, liposomal OVA, or PSM/OVA for 3 h, followed by fixation and extensive wash, and then co-incubated with B3Z cells. IL-2 levels in culture medium were measured with ELISA. Data are presented as mean ± S.D.
(D) Inter-cellular transfer of PSM/antigen. DC2.4 cells were incubated with FITC-OVA–loaded PSM for 1 h. Unbound particles were washed away, and DCs internalized with the fluorescent PSM/FITC-OVA were added to a pre-seeded DC2.4 cell culture. Particle movement was tracked under a fluorescent microscope by video recording every 8 minutes. Snapshots demonstrating particles transferred from one cell to a neighboring cell are shown. Details can be found in **Movie S1**.
(E) *In vivo* release of PSM/antigen from injected DCs. BMDCs were incubated with PSM/FITC-OVA for 1 h. After extensive washing to get rid of free particles, DCs were labeled with CellTracker Red dye, and intravenously injected into mice. Animal tissues were harvested 24 h post-injection, and frozen sections were processed and examined under a fluorescent microscope. Some green fluorescent PSM/FITC-OVA particles had separated from the red DCs in this lung tissue section. Scale bar, 20 μm.

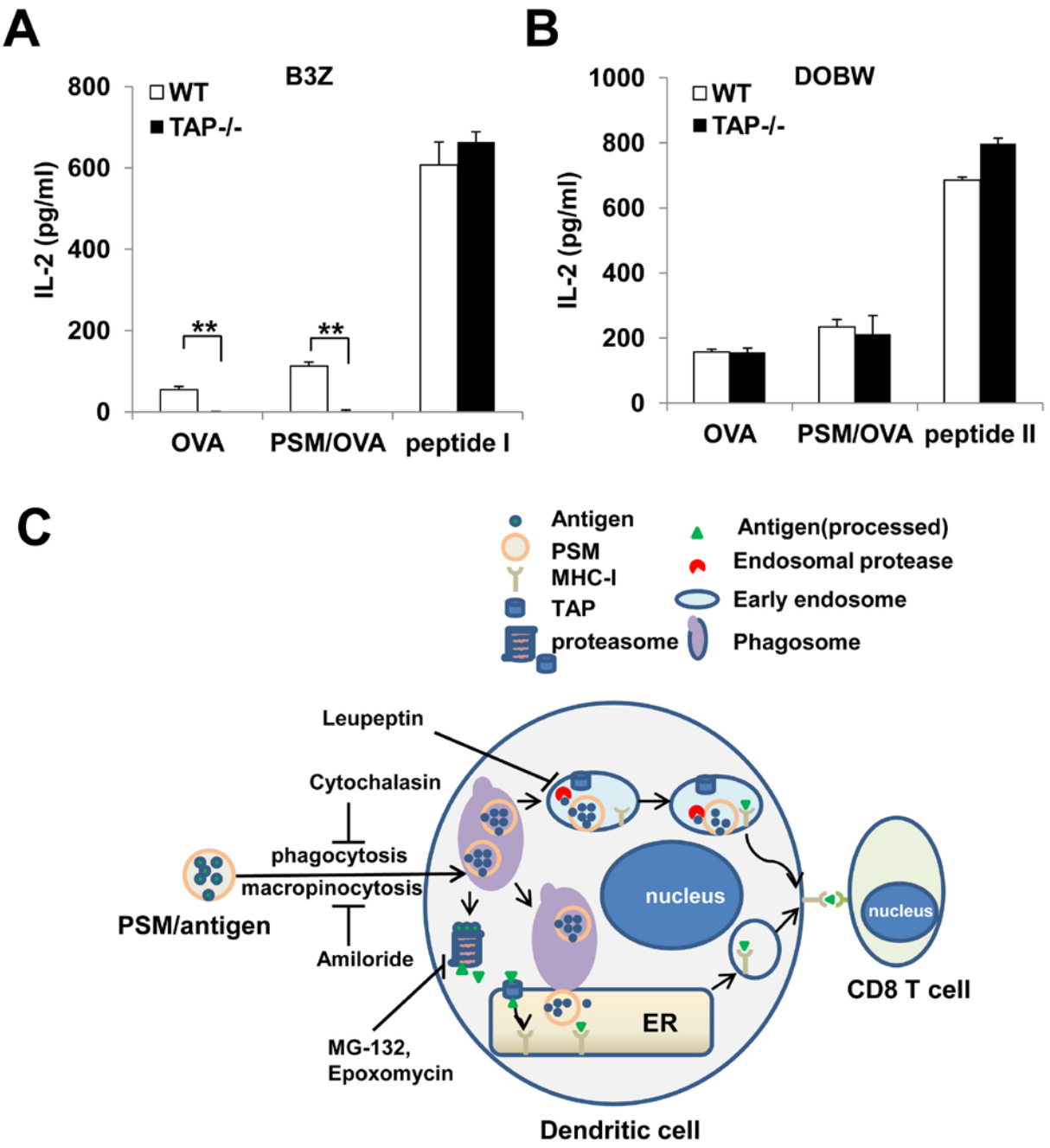


**Figure S2. Cross-presentation of PSM/antigen in DCs. Related to Figure 2.**

(A) IL-2 production by B3Z cells after co-incubation with WT or TAP$^{-/-}$ DCs pretreated with OVA, PSM/OVA or peptide I (OVA 257-264, 100 ng/ml). **, $P$<0.01.
(B) IL-2 production by DOBW cells after co-incubation with WT or TAP$^{-/-}$ DCs pretreated with OVA, PSM/OVA or peptide II (OVA 323-339, 100 ng/ml). Data are presented as mean ± S.D.
(C) A schematic illustration showing PSM/antigen internalization, antigen processing and presentation from DCs to CD8 T cells.

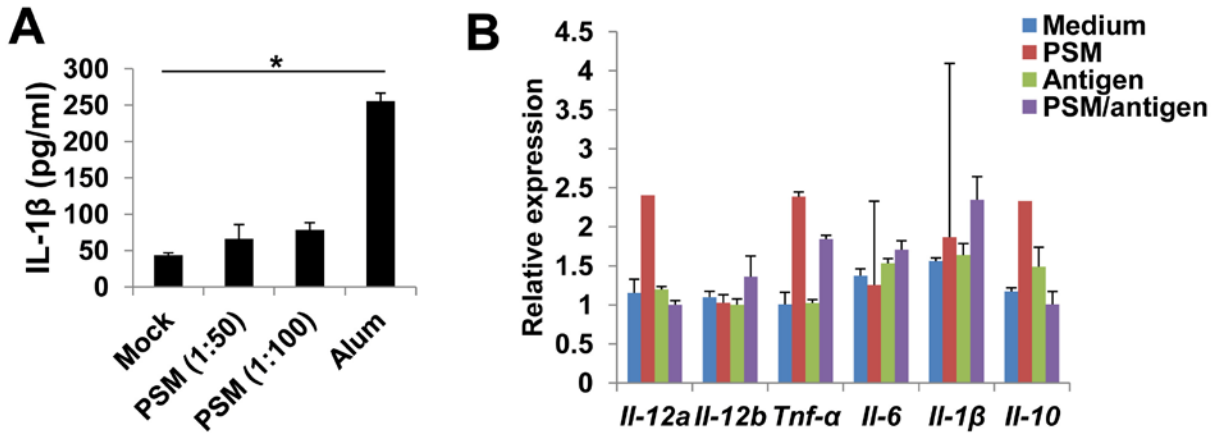


**Figure S3. PSM did not induce pro-inflammatory response in DCs. Related to Figure 3.**

(A) Quantification of IL-1β release from BMDCs 4 h after co-incubation with PSMs or Alum. BMDCs were primed with 50 ng/ml LPS overnight prior to co-incubation with particles. Data are presented as mean ± S.D. *, $P<0.05$.
(B) Quantification of mRNA levels of pro-inflammatory cytokines in BMDCs 3 h after incubation with the indicated agents. Data are presented as mean ± S.D.

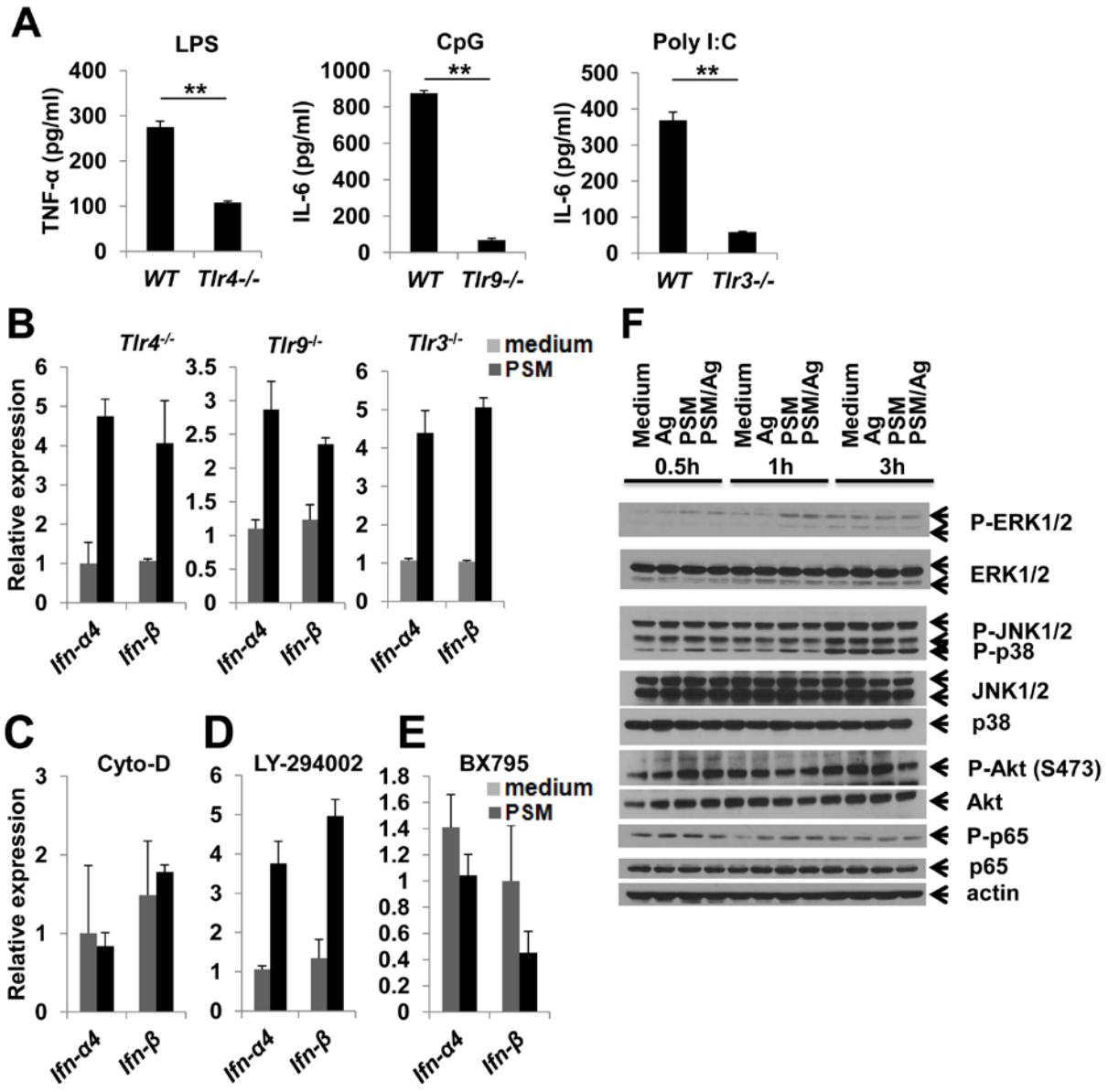


**Figure S4. PSM induced activation of IFN-I signaling in DCs. Related to Figure 4.**

(A) Quantification of TNF-α and IL-6 production by BMDCs from WT, *Tlr4*$^{-/-}$, *Tlr9*$^{-/-}$and *Tlr3*$^{-/-}$ mice 18h after treatment with LPS (TLR4 ligand, 200 ng/ml), CpG (TLR9 ligand, 1 μg/ml), or poly I:C (TLR3 ligand, 20 μg/ml). Data are presented as mean ± S.D. **, $P<0.01$.

(B) PSM-induced *Ifn-α4* and *Ifn-β* gene expression in BMDCs was not affected by TLR deficiency. BMDCs isolated from wild-type or knockout mice were co-incubated with PSM, and cells were harvested for IFN-I gene expression analysis.

(C-E) PSM-induced *Ifn-α4* and *Ifn-β* gene expression in BMDCs pre-treated with chemical inhibitors. Cyto-D (cytoschalatin D) is a phagocytosis inhibitor; LY-294002 is a PI3K inhibitor; and BX-795 is an inhibitor for IKKi/TBK1.
(F) Western blot analysis on activation status of innate immune signaling pathways in DCs after treatment with medium only, antigen (Ag), PSM, or PSM/antigen.

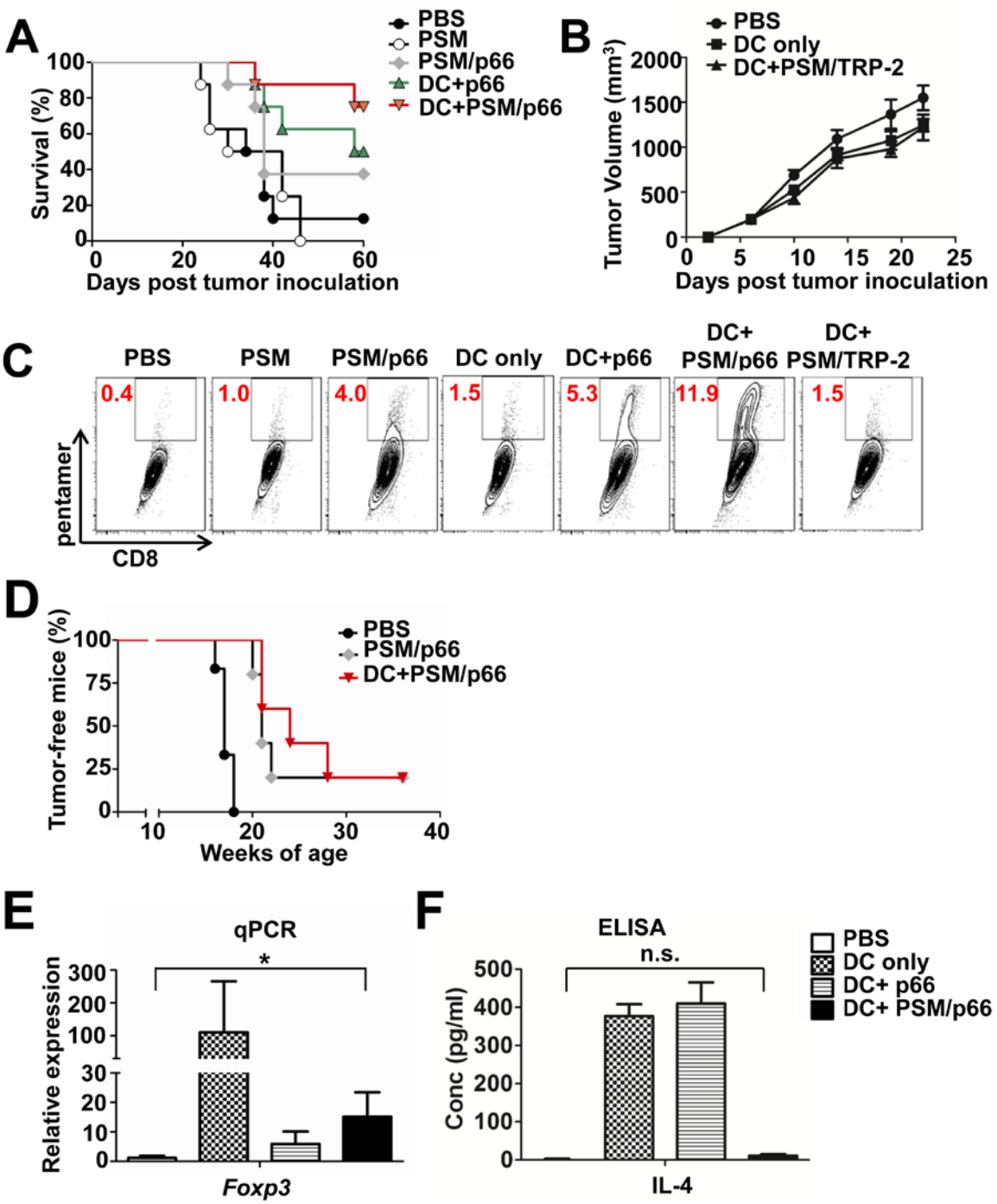


**Figure S5. PSM/antigen-primed DC vaccine stimulated intra-tumor immunity and inhibited tumor growth. Related to Figure 5.**

(A) Kaplan-Meier plot on survival of TUBO tumor-bearing mice after treatments with the indicated agents (n= 8/group). This study corresponds to results in Figure 5A.

(B) TUBO tumor growth in mice (n = 8/group) treated with PBS, DC only, or DC+PSM/TRP2. Balb/c mice were inoculated with TUBO cells in the mammary gland fat

pad ($0.5X10^6$ cells per mouse). Four days later, the mice were treated *i.v.* with PBS, DCs only, or DCs primed with PSM/TRP-2. Tumor sizes were recorded in the next 4 weeks. Tumor volume is presented as mean ± SEM.

(C) Percentage of intra-tumor antigen-specific CD8 T cells in different treatment groups. Balb/c mice bearing TUBO tumors were treated with the indicated agents. Tumors were harvested 10 days after treatment, and processed for single cells. They were then stained with HER2 p66 pentamer and anti-CD8 antibody, and analyzed by flow cytometry. Percentage of p66-specific CD8 T cells in total intra-tumor CD8 T cells is shown in red.

(D) Percentage of tumor-free mice in the post-treatment animals (n= 5/group). This study corresponds to results in Figure 5B.

(E) Quantitative RT-PCR analysis on *Foxp3* mRNA levels in tumor tissues isolated 10 days after treatments with the indicated agents. Data are presented as mean ± S.D. *, $P<0.05$.

(F) ELISA measurement on IL-4 levels in supernatant of primary tumor cell culture. Cells were established from tumors in different groups 10 days after treatment. Data are presented as mean ± S.D. n.s., not statistically significant.

**Table S1.** *In vivo* uptake of PSM by immune cells in mouse peripheral blood. Related to **Figure 1**.

| | **0 min** | **10 min** | **60 min** |
|---|---|---|---|
| **Immature monocytes** | 0% | 3.21% | 7.24% |
| **Mature monocytes** | 0% | 1.36% | 1.81% |
| **Neutrophils** | 0% | 0.57% | 0.64% |
| **Dendritic Cells** | 0% | 4.48% | 12.33% |
| **Macrophages** | 0% | 1.2% | 1.35% |

Percentage was calculated as number of PSM positive cells in total number of the specified cell type ($CD115^{+}Ly6C^{high}$ Immature monocytes, $CD115^{+}Ly6C^{low}$ mature monocytes, $Gr1^{+}CD11b^{+}$ neutrophils, $F4/80^{+}CD11b^{+}$ macrophages, $CD11c^{+}CD11b^{+}$ dendritic cells) at different time points after intravenous administration of PSM. One of two representative experiments is shown.

**Movie S1**. Inter-cellular transfer of PSM/antigen. Related to **Figure 1**. DC2.4 cells were incubated with FITC-OVA–loaded PSM for 1 h. Unbound particles were washed away, and DCs internalized with the fluorescent PSM/FITC-OVA were added to a pre-seeded DC2.4 cell culture. Particle movement was tracked under a fluorescent microscope by video recording every 8 minutes.

## SUPPLEMENTAL EXPERIMETNAL PROCEDURES

**Cell lines.** The immature C57BL/6 DC line DC2.4 was kindly provided by Dr. Kenneth. L. Rock (University of Massachusetts Medical School, Worcester, MA). DOBW cells, a T–T hybridoma against OVA (323–339)/I-$A^d$ complex, were kindly provided by Dr. Clifford V. Harding (Case Western Reserve University, Cleveland, OH). B3Z cells, T–T hybridomas against OVA (257–264)/H-$2K^b$ complex, were obtained from Dr. Nilabh Shastri (UC Berkeley, Berkeley, CA). All the DC and T cell lines were maintained in RPMI 1640 medium containing 10% FBS, 1 mM pyruvate, 2 mM L-glutamine, 50 µM 2-β-mercaptoethanol, and antibiotics. TUBO cells were provided by Drs. Wei-Zen Wei (Wayne State University, Detroit, MI) and Guido Forni (University of Torin, Italy).

**BMDCs.** We used 6–10 week old female mice to isolate bone marrow-derived DCs. Briefly, femur and tibia from mice hinder legs were collected and bone marrow cells were flushed out with 1% FBS-containing PBS using a syringe. Cells were treated briefly with ACK lysis buffer (Lonza Inc.) to removed red blood cells, and then resuspended into RPMI1640 medium with 10% FBS, antibiotics, 55 µM β-mercaptoethanol. Cells were grown with supplement of recombinant murine GM-CSF and IL-4 (20 ng/ml). Cell culture medium was refreshed every other day. Non-adherent cells were harvested as immature BMDCs. For *in vivo* studies, BMDCs were stimulated with 100 ng/ml LPS overnight for maturation, and then primed with soluble antigen or PSM/antigen for 3 h before *i.v.* injection into mice.

**Transmission electron microscopy.** Transmission electron microscopy (TEM) analysis was performed by the TEM facility core at MD Anderson Cancer Center (Houston, Texas). BMDCs isolated from C57BL/6 mice were seeded on 24-well plates at a density of 1X $10^6$ cells and cultured for 24 h at 37°C. The culture dish was washed twice with PBS, and then cells were pulsed with PSM/OVA for 20 min at 37°C. Cells were washed twice with PBS and incubated for an additional 2 h in culture media at 37°C. Cells were fixed in 4% paraformaldehyde. After fixation samples were washed and treated with 0.1 % cacodylate buffered tannic acid, post fixed with 1% buffered osmium tetraoxide for 30 minutes and stained with 1% uracyl acetate. Samples were then dehydrated in increasing concentration of ethanol and embedded in Poly-bed 812 medium. Samples were polymerized in a 60°C oven for 2 days. Ultrathin sections were cut in a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uracyl acetate and lead citrate in a Leica EM Stainer and examined in a JEM 1010 transmission electron microscope (JOEL, USA. Inc, Peabody, MA).