Supplementary Information for

Transcutaneous immunization against cancer using a solid-in-oil nanodispersion

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1. Dispersive properties of OVA and OVA S/O in IPM

![Image of native OVA and OVA S/O dispersed in IPM]

**Figure S1.** Physical appearance of native OVA (left) and OVA S/O (right) dispersed in IPM.

2. Release profile of OVA from S/O nanodispersions.

![Graph showing OVA release over time]

**Figure S2.** Release profile of OVA from S/O nanodispersions. n = 3, data are mean ± standard deviation of the mean.
3. Skin penetration of FITC-OVA S/O nanodispersions

**Figure S3.** Plot profiles of fluorescence intensities against the depth from surface of skin after the treatment of FITC-OVA S/O (solid line), FITC-OVA PBS solution (dashed line), and PBS (dotted line).
4. Serum OVA-specific IgG levels of mice

C57BL6 mice were immunized with OVA S/O nanodispersions or s.c. injection with OVA PBS solution twice, at a one-week interval. One week after the second immunization, blood was collected from a tail vein and the production of OVA-specific serum IgG was evaluated using an enzyme-linked immunosorbent assay (ELISA), as described previously. The antibody titer was defined by the dilution factor at which the optical density of the sample was equal to that of a non-immunized sample.

Figure S4. Serum OVA-specific IgG levels of mice immunized by transcutaneous administration of OVA S/O or s.c. injection of OVA PBS solution. n = 8.
5. **Antitumor effect induced by OVA-specific cancer immunity**

The C57BL/6N mice were vaccinated with OVA S/O nanodispersions, s.c. injection or topical application of an OVA PBS solution twice, at a one-week interval. One week after the second immunization, E.G7-OVA cells \( (2.5 \times 10^6 \text{ cells}) \) were inoculated into the backs of mice. The tumor volumes of mice were evaluated every two or three days after the 5th day. Tumor volumes were calculated using the following formula: 

\[
\text{Tumor-volume (mm}^3\text{)} = (\text{major axis}) \times (\text{minor axis})^2 \times 0.5. 
\]

At day 17, mice were sacrificed and tumors were extracted.

**Figure S5.** (a) Antitumor effect induced by OVA-specific cancer immunization through transcutaneous administration of OVA PBS solution (triangles), OVA S/O (circles) and s.c. injection of OVA PBS solution (squares). Mice without immunization (diamonds) were used as controls. \( n = 4–8 \). \( ^*p < 0.05 \). (b) Representative appearance of tumors harvested from C57BL/6 mice at day 17.

**References**
