SUPPLEMENTARY MATERIALS AND METHODS

Real time quantitative PCR

The RT-qPCR analysis of selected type-I IFNs related genes, IRF7 and Oas3. The RT-qPCR was performed on the Applied Biosystems StepOne^{TM} (Life Technologies) in 96-well format. The reactions were performed using fast SYBR Green PCR Master Mix (Life Technologies). IRF7 primer sequence, 5'-aagaccaacttccgctgtgc-3' (sense) and 5'-agcattgctgaggctcactt-3' (antisense); Oas3 primer sequence, 5'-tcattgacctcaaccatgat-3' (sense) and 5'-agatgccagggaggagtatg-3' (antisense). Primer sequences were chosen with the software Primer3 (http://primer3.sourceforge.net/) to detect 3 type-I IFNs related genes. The overall PCR product size was smaller than 200 bases.

ELISA assay

Mouse IFN-α expression levels in sera from mice treated with irLLC/SeV/GM cells or irLLC/SeV/GM cells plus imiquimod were measured using the VeriKineTM mouse IFN-α ELISA kit (PBL Interferon-Source, Piscataway, NJ).

***In vivo* experiments**

For prophylactic tumor vaccination assays, on day 11 before tumor challenge with 2.0 × 10^5 parental LLC cells in the right flank, C57/BL6N mice were s.c. vaccinated with the one million of the indicated tumor vaccine cells (irLLC, irLLC/SeV/GFP or irLLC/SeV/GM cells) in the left flank. Subcutaneous tumor growth was monitored with a digital caliper every 2-3 days.

Flow cytometric analysis

On days 2 and 4 after tumor challenge with LLC cells, TDLNs harvested from the indicated groups of mice (n=3-5) were homogenized by mincing in RPMI 1640 medium containing 10% FBS and filtered through a 100-μm cell strainer. Cells were stained with an anti-mouse CD11c antibody in combination with anti-mouse antibodies including anti-MHC class I-PE (28-14-8) (eBioscience, San Diego, CA), anti-CD40-PE (3/23), anti-I-A/I-E-FITC (M5.114.15.2), or anti-CCR7-Alexa488 (4B12) (all Biolegend) at a dilution of 1:25-1000 for 30 min. At 24 hours after 2^{nd} i.p. administration of anti-mouse PDCA-1 antibody, we harvested splenocytes and treated them with ammonium chloride

to lyse red blood. For pDCs detection, splenocytes were stained with anti-PDCA-1-APC (JF05-1C2.4.1) (Miltenyi Biotec, Bergisch Gladbach, Germany) and anti-CD11c-PerCPCy5.5 (N418) (eBioscience). Cells were incubated with antibodies diluted at 1:25 to 1,000 for 30 min on ice and analyzed with BD FACSCalibur flow cytometer, CellQuest software (BD Biosciences), and FlowJo software (Tree star, Ashland, OR).

SUPPLEMENTARY FIGURE LEGENDS

Figure S1.

Effective depletion of PDCA-1$^+$ cells in mouse splenocytes.

Dot plots depict PDCA-1 and CD11c expression on splenocytes in mice treated with isotype (left panel) or anti-PDCA-1 antibody (right panel).

Figure S2.

Validation of gene expression data by quantitative real time PCR.

Each column represents the relative amount of mRNAs expression level of IRF7 and Oas3.

Figure S3.

Effects of TLR ligands on body weight changes in mice s.c. treated with LLC/SeV/GM cells.

Bar graph represents mean+SEM of body weight of each experimental mouse group as indicated in Fig. 4A.

Figure S4.

Increased number of TVS-infiltrating PDCA-1$^+$ cells in mice treated with combined irLLC/SeV/GM cells and imiquimod.

At 6 hours after the first tumor vaccination, infiltrating lymphocytes in TVS were as indicated in Fig. 5A. Bar graphs show mean+SEM of number of PDCA-1$^+$ cells in TVS.

Figure S5.

Increased serum IFN-α level in mice treated with combined irLLC/SeV/GM cells and imiquimod.

IFN-α expression levels in mice sera at 6 hours after the first tumor vaccination, were measured by ELISA. Combined data from two independent experiments with similar results are shown.

Figure S6.

Prophylactic tumor vaccination using irLLC/SeV/GM cells induced significant

antitumor effects in a syngeneic mouse model.

On day 11 before s.c. tumor challenge with 2.0×10^5 parental LLC cells in the right flank of female C57/BL6N mice, mice were s.c. inoculated with HBSS (untreated), 1.0×10^6 irLLC cells, irLLC/SeV/GFP (MOI=100) cells or irLLC/SeV/GM (MOI=100) cells. Statistically significant difference on day 24 after the tumor challenge is shown between mice treated with irLLC/SeV/GM cells and other mice groups (*$P < 0.05$, **$P < 0.01$).

Figure S7.

Enhanced expression levels of maturation and co-stimulatory molecules including CCR7, CD40, MHC class I, and MHC class II in tumor cells-derived GM-CSF-sensitized DCs in TDLNs

Representative histograms depict MFI of CCR7, CD40, MHC class I, or MHC class II expression, in $CD11c^+$ DCs in TDLNs from indicated mouse groups collected on days 2 or 4 after tumor challenge.