

Supplemental Figure Legends

Fig. S1. pDCs pulsed with anti-Siglec-H-OVA plus CpG *in vitro* expressed MHCI-OVA complexes recognized by anti-H-2K^b-SIINFEKL. Freshly isolated pDCs were untreated or pulsed with anti-Siglec-H-OVA and CpG, washed extensively and then co-cultured with freshly isolated cDCs as in Fig 3C. Expression of surface MHCI-OVA (H-2K^b-SIINFEKL) on gated pDCs was shown. Data are representative of three independent experiments.

Fig. S2. Batf3-dependent cDC1s played a critical role in pDC-mediated cross-priming *in vivo*. **(A to C)** Increasing pDCs and cDCs in Batf3^{-/-} mice led to increased proliferation of naive OTI cells. WT, Batf3^{-/-} and Flt3L-treated Batf3^{-/-} mice were immunized with anti-Siglec-H-OVA plus CpG (n=4) following adoptive transfer of naive OTI CD8 T cells, and spleen and LN cells were examined for pDCs/cDCs and cross-priming. The percentages of pDCs and cDCs in spleen were increased after Flt3L treatment as shown in (A). The percentages of Thy1.1⁺ OTI cells out of total CD8 T cells are depicted in (B), and the percentages of Thy1.1⁺ OTI cells that had undergone >5 cycles of proliferation in (C). **(D)** WT but not Batf3^{-/-} cDCs were able to induce OTI cell proliferation by WT pDCs *in vitro*. WT pDCs pulsed with anti-Siglec-H-OVA plus CpG were co-cultured with naive OTI cells (1x10⁵ cells). 2x10⁴ WT or Batf3^{-/-} cDCs were added as indicated. Percentages of proliferated cells (CFSE^{low}) out of total OTI cells were shown. **(E)** Batf3^{-/-} cDCs were efficient in acquiring functional MHCI-antigen complexes (H-2K^b-SIINFEKL) from cross-presenting pDCs. WT pDCs pulsed with anti-Siglec-H-OVA plus CpG were cultured with WT or Batf3^{-/-} cDCs, and subjected to flow cytometry. Expression of MHCI-OVA (H-2K^b-SIINFEKL) on gated cDCs on day 5 were shown, and the percentages of MHCI-OVA⁺ cells were similar in WT CD8⁺ cDC1s (4.4/12=37%) and WT CD8⁻ cDC2s (22/61=36%).

Data are representative of at least two independent experiments. NS= $P>0.05$, * $P<0.05$, ** $P<0.01$, and *** $P<0.001$.

Fig. S3. Antigen-presenting pDCs conferred cDCs expression of functional MHCI-antigen complexes to prime naive CD8 T cells through a cell contact-independent mechanism. **(A)** pDCs induced cDCs to prime naive OTI cells through a cell contact-independent mechanism. OTI cells were co-cultured with WT cDC in the inner chambers, and various numbers of pDCs pulsed with anti-Siglec-H-OVA plus CpG were added to the inner chambers or out chambers as indicated. Percentages of proliferated (CFSE^{low}) Thy1.1⁺ OTI cells out of total OTI cells were shown. **(B)** pDC culture supernatants transferred antigens to naive WT cDCs resulting in their expression of MHCI-OVA complexes (H-2K^b-SIINFEKL). cDCs were cultured alone or with culture supernatants of pulsed WT pDCs, and subjected to flow cytometry. Expression of MHCI-OVA on cDCs was shown. Data shown are representative of two or more independent experiments.

Fig. S4. Exosomes from pDCs targeted through siglec-H with a TLR7 agonist and a tumor antigen similarly primed antigen-specific CD8 T cells with bystander cDCs. **(A)** Exosomes from WT pDCs cultured with anti-Siglec-H-OVA plus R848 induced cDC-dependent priming of naive OTI CD8 T cells. WT pDCs were cultured with anti-Siglec-H-OVA plus R848 and isolated exosomes were added to OTI cells in the presence or absence of naive WT cDCs, and subjected to flow cytometry. Percentages of total proliferated cells (CFSE^{low}) and proliferated IFN- γ ⁺ cells (CFSE^{low}IFN- γ ⁺) out of total OTI cells were shown. **(B)** Exosomes from pDCs targeted with anti-Siglec-H-hgp100 mediated cDC-dependent priming of gp100-specific CD8 T cells. WT pDCs were cultured with with anti-Siglec-H-hgp100 plus CpG and isolated exosomes were added to

gp100-specific CD8 T (pme1-1) cells in the presence or absence of naive WT cDCs, and subjected to flow cytometry. Percentages of total proliferated cells (CFSE^{low}) and proliferated IFN- γ ⁺ cells (CFSE^{low}IFN- γ ⁺) out of total pme-1 cells were shown. Data shown are representative of two independent experiments.

Supplemental Figures:

Fig. S1

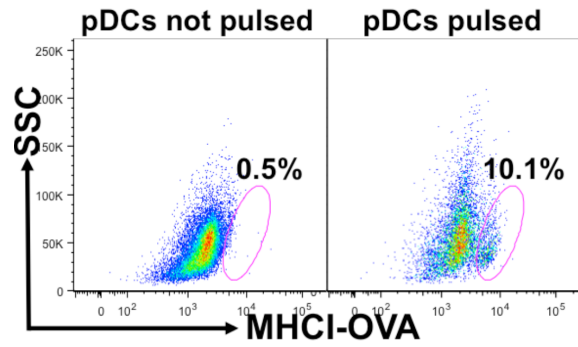


Fig. S2

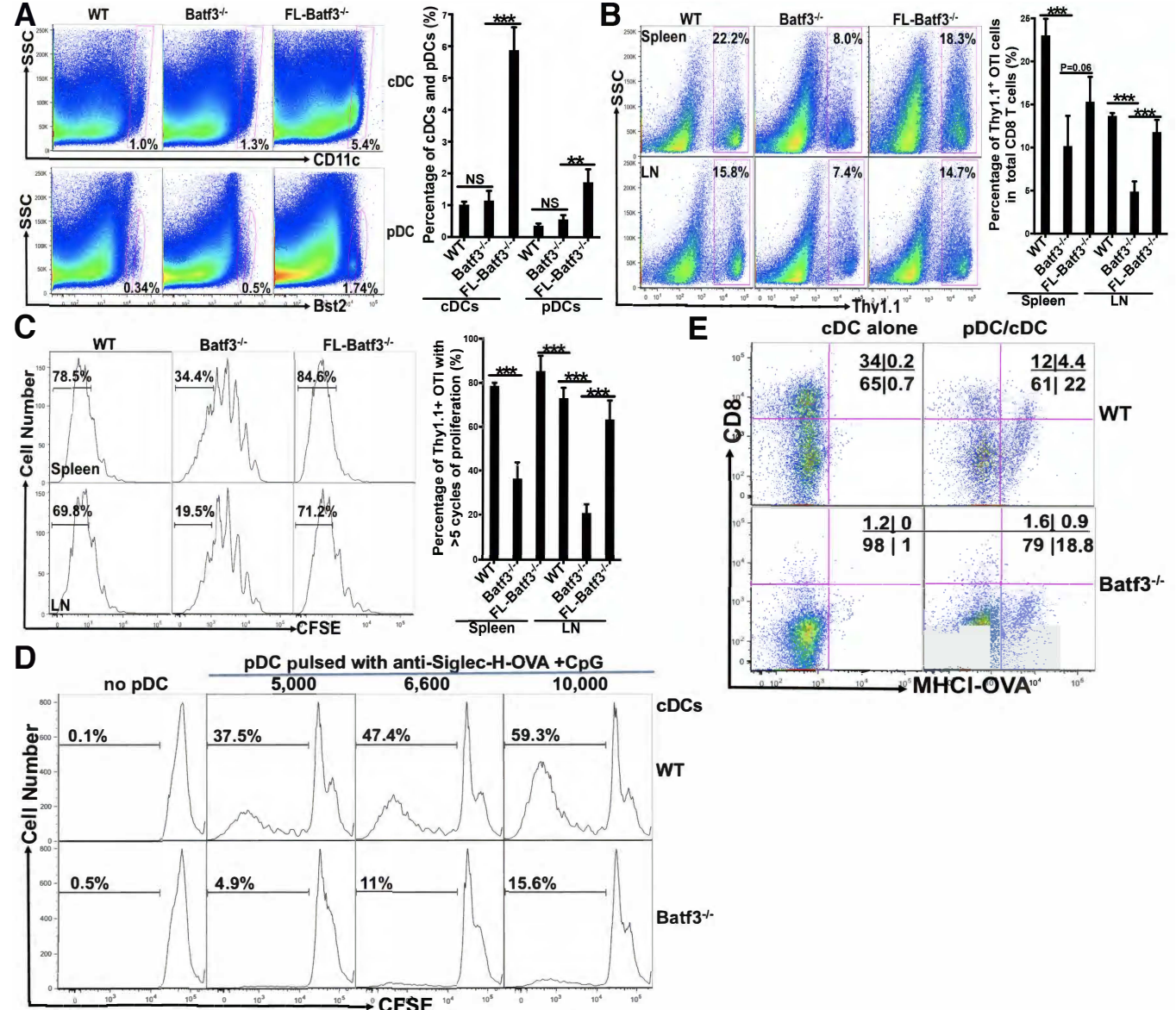


Fig. S3

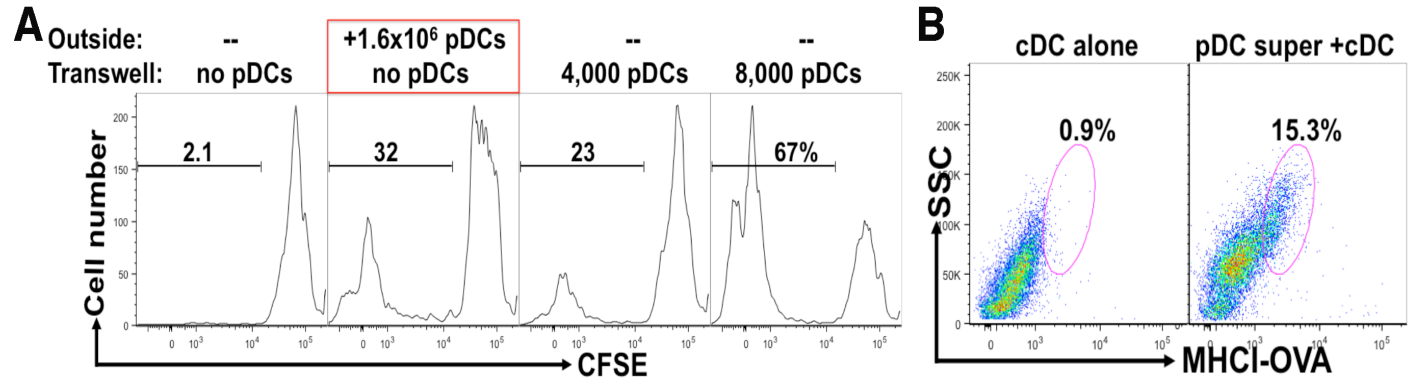


Fig. S4

