Figure S1

A

Gated on MHCII<sup>hi</sup> CD11c<sup>hi</sup>

<table>
<thead>
<tr>
<th>naive</th>
<th>48h, Influenza</th>
<th>48h, polyI:C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.95</td>
<td>5.36</td>
<td>3.17</td>
</tr>
<tr>
<td>52</td>
<td>45.7</td>
<td>7.81</td>
</tr>
</tbody>
</table>

B

CD11b+ CD103+ CD8+ CD4+

<table>
<thead>
<tr>
<th>12h</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.81</td>
<td>1.11</td>
<td>2.63</td>
</tr>
<tr>
<td>1.11</td>
<td>8.43</td>
<td>1.09</td>
</tr>
</tbody>
</table>

C

Sub-capsular macrophages: gating strategy

D

Gate 3

<table>
<thead>
<tr>
<th>Not infected</th>
<th>Day 1 pi</th>
<th>Day 2 pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>0.12</td>
<td>0.26</td>
</tr>
</tbody>
</table>

E

WT Migratory DC (CD11c<sup>hi</sup>MHCII<sup>hi</sup>)

<table>
<thead>
<tr>
<th>Not infected</th>
<th>Day 1 pi</th>
<th>Day 2 pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP</td>
<td>1.05</td>
<td>1.78</td>
</tr>
</tbody>
</table>

F

MLN

1  CD11c<sup>hi</sup>MHCII<sup>hi</sup>

<table>
<thead>
<tr>
<th>exclusion of LN resident DC</th>
<th>Migratory DC</th>
<th>Migratory DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11c</td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>CD4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G

Gated on total LN cells

<table>
<thead>
<tr>
<th>WT not infected</th>
<th>WT day 2 pi</th>
<th>Ccr7&lt;sup&gt;-/-&lt;/sup&gt; day 2 pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC-II</td>
<td>0.026</td>
<td>0.13</td>
</tr>
<tr>
<td>CD11b</td>
<td>0.05</td>
<td>0.022</td>
</tr>
</tbody>
</table>
**Figure S2**

(A) Flow cytometry analysis showing the percentage of CD11c<sup>high</sup>-MHC-II<sup>high</sup> migratory DCs in the lung and MLN 7 days after infection with PBS or DT.

(B) B220<sup>CD3<sup>+</sup>CD8<sup>+</sup> T cells 7 days after infection with PBS or DT. The percentage of H-2b/ASNENMETM<sup>+</sup> specific CD8<sup>+</sup> T cells was measured in the MLN and lung.

(C) Body weight loss (%) over 8 days post infection with PBS or DT.
**Figure S3**

### A

Dilution of LN single cell suspension

<table>
<thead>
<tr>
<th>Dilation</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0/2</td>
<td>0/8</td>
</tr>
<tr>
<td>Ccr7⁻/⁻</td>
<td>0/3</td>
<td>0/10</td>
</tr>
</tbody>
</table>

Detection limit < 10 viral particle

### B

- Alveolar macrophages
  - GFP
  - HA
  - DAPI

- LN CD103⁺GFP⁺ DC
  - GFP
  - HA
  - DAPI

- LN CD103⁺GFP⁺ DC
  - GFP
  - HA
  - DAPI

### C

LN CD103⁺GFP⁺ DC

- EEA1
- GFP
- DAPI

### D

Proteases

<table>
<thead>
<tr>
<th>CD103⁺</th>
<th>CD11b⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctsa</td>
<td>1</td>
</tr>
<tr>
<td>Ctsb</td>
<td>2</td>
</tr>
<tr>
<td>Ctsc</td>
<td>3</td>
</tr>
<tr>
<td>Ctsd</td>
<td>1</td>
</tr>
<tr>
<td>Ctsf</td>
<td>2</td>
</tr>
<tr>
<td>Ctsz</td>
<td>3</td>
</tr>
<tr>
<td>AEP</td>
<td>+1</td>
</tr>
</tbody>
</table>

Lysosomal markers

<table>
<thead>
<tr>
<th>CD103⁺</th>
<th>CD11b⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamp1</td>
<td>1</td>
</tr>
<tr>
<td>Lamp2</td>
<td>2</td>
</tr>
<tr>
<td>Lyz1</td>
<td>3</td>
</tr>
<tr>
<td>Lyz2</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure S4

A

Bead uptake at 3 hrs

CD11b+ CD103+

0.00 0.05 0.10 ns

Bead positive DC (% among live cells)

Migration of bead positive DC

Time after injection (hours)

12 36 60

Bead positive DC (% among live cells)

C

Lung

Gated on MHCII-CD11c+

WT

CD11b+ CD103+

43.2 35.6

Ccr7-/−

CD11b+ CD103+

40.6 32.1

D

Lung

Gated on CD11b+ DC

CD11b+ DC

CD103+ DC

42.7 24.9

4.01e-3

68.4

6.67

63.3

MLN

Gated on CD11c+Beads+

Gated on CD45+

Gated on CD11c+Beads+

WT

Ccr7-/−

E

H-2b/ASNENMETM specific CD8+ T cells (% among live cells)

H-2b/ASNENMETM specific CD8+ T cells (abs. nb. x103)

control DT treated

control DT treated

4.01e-3

0.059

68.4

16.7

6.67

63.3
Figure S5

CD11b⁺, 12h post in vitro infection

21.3

1.12
A

Figure S6

Steady state

Viral sensors

IFN signaling

CD103+ DC

Alveolar macrophages

12 h p.i.

Viral sensors

IFN signaling

B

Lung

Influenza virus

Infected Macrophage

Infected CD103+ DC

DEATH

IFN

Protected CD103+ DC

Dying infected cell

MIGRATION

Lymph Node

Cross-presenting CD103+ DC

CD8+ T cells
Supplemental figure legends

Figure S1:

(A) Mice were infected with 10^6 pfu of NS1-GFP virus or injected with 10µg of poly(I:C) intra-nasally. Two days after infection, lung tissue MHCII^+CD11c^+ DC were analyzed by flow cytometry. In infected lungs or poly(I:C) injected lungs, infiltrating CD11b^+ DC express Ly6C (middle and right panels). The infiltrating CD11b^+Ly6C^+ DC were not found in the lungs of naive mice (left panel).

(B) Mice were infected with 10^6 pfu of NS1-GFP virus. The percentage of GFP expression in each LN DC population is represented. LN DC were analyzed by flow cytometry at the indicated time points after infection. GFP expression is restricted to lung migratory CD103^+ DC and appears as early as 12 hours after infection.

(C) Gating strategy for LN sub-capsular macrophages. LN cell suspension is analyzed by flow cytometry. LN sub-capsular macrophages are defined as CD11b^+CD169^+CD11c^−CD103^−.

(D) WT and Ccr7^−/− mice were infected with 10^6 pfu of NS1-GFP virus. LN sub-macrophages were analyzed by flow cytometry at the indicated time points after infection. Percentage of GFP expression in sub-capsular macrophages is represented. GFP is not expressed by sub-capsular macrophages one and two days after infection.

(E) WT mice are infected with 10^6 pfu of NS1-GFP virus. LN DC were analyzed by flow cytometry at the indicated time points after infection. Percentage of GFP expression in lung migratory DC is represented. GFP expression is restricted to migratory CD103^+ DC subset.

(F) Gating strategy to define LN DC populations in mice infected with influenza virus. LN cell suspension is analyzed by flow cytometry 24 hours after infection with 10^6 pfu of NS1-GFP virus. Lung migratory CD103^+ and CD11b^+ DC subsets were defined as CD11c^+MHCII^highCD4^−CD8^− DC.

(G) WT and Ccr7^−/− mice were infected with 10^6 pfu of NS1-GFP virus. Percentage of GFP expression is analyzed by flow cytometry at the indicated time points after infection in total LN cells. GFP is not expressed in LN cells of Ccr7^−/− mice two days after infection.

Figure S2:

(A) Langerin-DTR mice were injected intra-tracheally with 0.1µg of DT or PBS. One day later, the depletion of CD103^+ DC from the lung and the draining LN was assessed by flow cytometry.
(B) Langerin-DTR mice were injected intra-tracheally with DT or PBS and infected one day later with 3x10³ pfu of NS1-GFP virus. Seven days later the number of endogenous anti-NP CD8⁺ T cells was assessed in the mediastinal LN and lungs by flow cytometry. Plots show the percentage of NP-specific CD8⁺ T cells generated in DT treated mice compared to PBS treated mice.

(C) Langerin-DTR mice were injected intra-tracheally with DT or PBS and infected one day later with 3x10³ pfu of NS1-GFP virus. Body weight was measured everyday Graph shows the percentage of body weight loss in DT treated mice compared to PBS treated mice.

**Figure S3:**

(A) WT and Ccr7⁻/⁻ mice were infected with 10⁶ pfu of NS1-GFP virus. Forty-eight hours later, mediastinal LN were collected and LN single cell suspension was injected into 10 day-old embryonnated chicken eggs. The presence of virus in the allantoic fluid was measured by standard hemagglutination assay using 0.5 % chicken red-blood cells.

(B) Mice were infected with 10⁶ pfu of NS1-GFP virus. Alveolar macrophages (upper panel) were purified from the lung 12 hours after infection. CD103⁺GFP⁺ (middle panel) and CD103⁺GFP⁻ DC (lower panel) were purified from the MLN, 48 hours after infection. Cells were stained at the cell surface with anti-HA Ab and analyzed by confocal microscopy (63x, zoom 2.4).

(C) Mice were infected with 10⁶ pfu of NS1-GFP virus. CD103⁺GFP⁺ were purified from the MLN 48 hours after infection. Cells were permeabilized prior to staining with anti-EEA1 antibody. Stained cells were spun into cytospin slides and analyzed by microscopy (63x) using an axioplan microscope.

(D) CD11b⁺ and CD103⁺ migratory DC subsets were double sorted from the MLN of naïve mice and analyzed by affymetrix gene chip arrays. Heat maps represent relative mRNA transcript levels in CD103⁺ DC and CD11b⁺ DC. Data are representative of 3 experiments (n=3). Red represents high relative expression, while blue represents low relative expression.

**Figure S4:**

(A) Mice were injected intra-tracheally with 1µm fluorescent latex beads mixed to 10µg of poly(I:C). Three hours later, lungs were collected and uptake of fluorescent beads was measured by flow cytometry in each lung DC subset. Dot plots show that CD103⁺ and CD11b⁺ DC subsets capture similar amount of fluorescent beads.
Mice were injected intra-tracheally with 1µm fluorescent latex beads mixed to 10µg of poly(I:C). Migration of bead positive lung DC to the MLN was measured by flow cytometry at different time-points after injection. Lung migratory CD103⁺ and CD11b⁺ DC transport latex beads from the lung tissue to the draining LN with different kinetics.

WT or Ccr7⁻/⁻ mice were injected with 1µm fluorescent beads mixed with 10µg of poly(I:C). Eighteen hours after injection, both lung tissue CD11b⁺ and CD103⁺ DC have captured beads. Beads reached the draining LN only in WT mice and were absent from the draining LN of Ccr7⁻/⁻ mice. In addition, beads were always associated with lung tissue migratory CD103⁺ and CD11b⁺ DC in WT mice.

Langerin-DTR mice treated or not with DT one day earlier, were injected intra-tracheally with 1µm latex beads coated with the influenza protein NP and mixed with 10µg of poly(I:C). Seven days later, we measured the priming of anti-NP endogenous CD8⁺ T cells in MLN (percentage and absolute numbers are shown in the plots). We found that DT injection in langerin-DTR mice abolished NP specific CD8⁺ T cell response.

**Figure S5:**
Lung CD11b⁺ DC were purified from naïve mice and infected in vitro with NS1-GFP virus (10 MOI). Twelve hours after infection, CD11b⁺ DC were stained with anti-HA Ab or the corresponding isotype control and analyzed by flow cytometry.

**Figure S6:**
(A) Deep sequencing analysis of alveolar macrophages and CD103⁺ lung DC purified from lungs of naïve mice or mice infected 12 hours earlier with 10⁶ pfu of NS1-GFP virus. Absolute values of mRNA transcript are represented.
(B) Model representing the kinetic of influenza virus infection and viral antigen transport by CD103⁺ DC to the draining MLN for antigenic presentation to naïve cytotoxic T cells. This model suggests that upon influenza virus infection, lung tissue CD03⁺ DC develop an antiviral state that promote their survival in infected lungs and their ability to capture virally infected cells. In contrast, lung tissue DC infected with influenza virus die in the lungs and do not migrate to the draining LN. Lung tissue CD103⁺ DC protected from viral infection migrate to the draining LN charged with infected cells stored in the early endosomal compartment and cross-present viral antigens to T cells and promote viral specific CD8⁺ T cell immune response.
Supplemental method

**Latex beads coating.** 50µl of 1µ latex beads (polysciences) were washed in PBS and incubated for 6 h with glutaraldehyde 8% at room temperature. After several washes, beads were then incubated overnight with 0.5 mg/ml of soluble NP. The fixation reaction was stopped by a 30 minutes incubation in ethanolamine 0.2M. NP coated beads were then washed and diluted 1:4 in PBS and mixed with 10µg of polyI:C prior to intra-tracheal injection in the mice.

**Microarray analysis.** A Gaussian Mixture Model (GMM) was used to determine thresholds consistent with a control-based approach. GMM is an Expectation-Maximization algorithm, the aim of which is to optimize the likelihood that a set of data points is generated by a mixture of Gaussian distributions. In this case, the MATLAB software “fit” function with parameter “gauss3” was used to model the observed chip-wide expression distribution profile of all non-control probe sets, such that each Gaussian component of the mixture corresponded to a different source of signal (i.e., background and genuine expression). Threshold for greater-than-chance expression were empirically defined as values above which there is an equal probability that the signal is part of either distribution.