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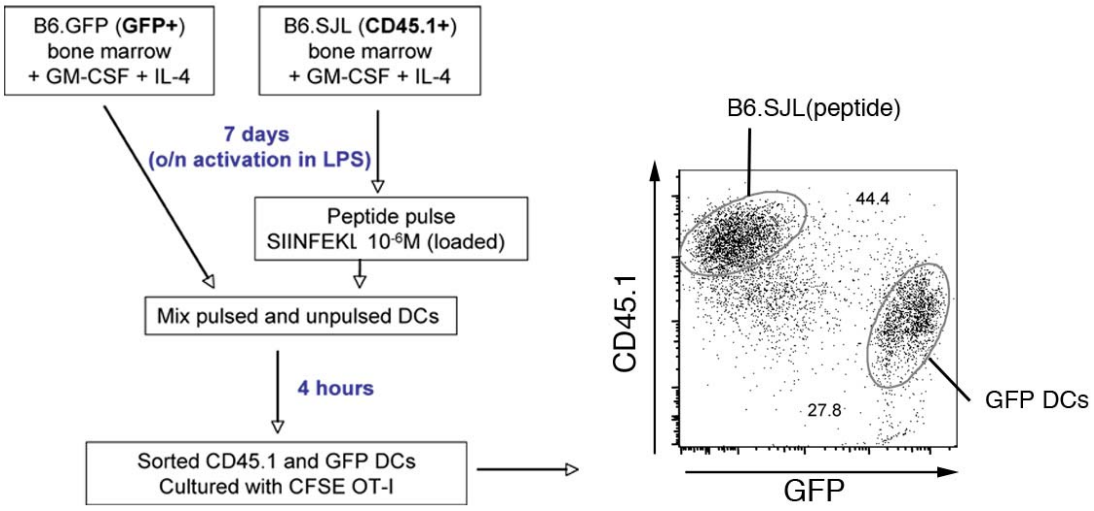
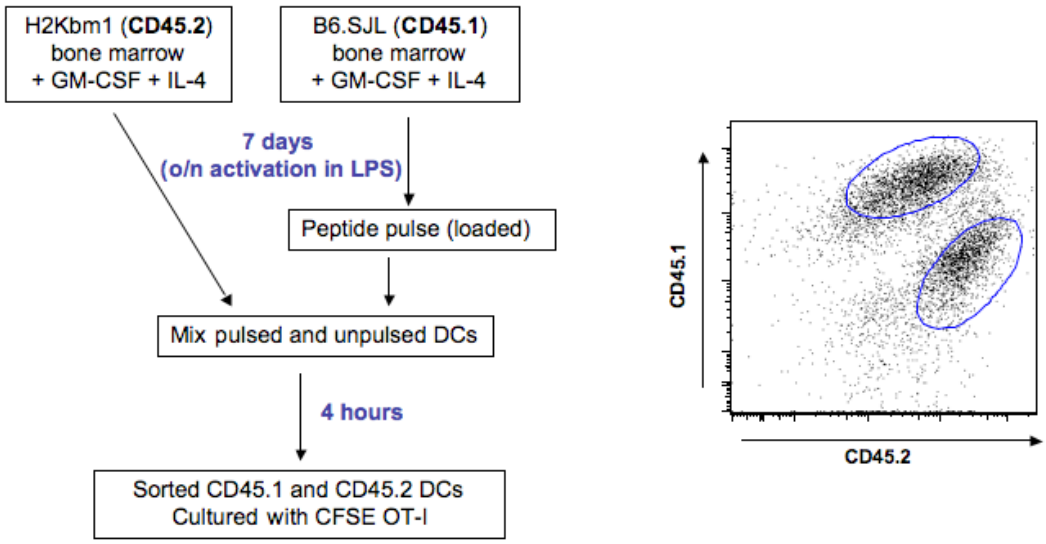
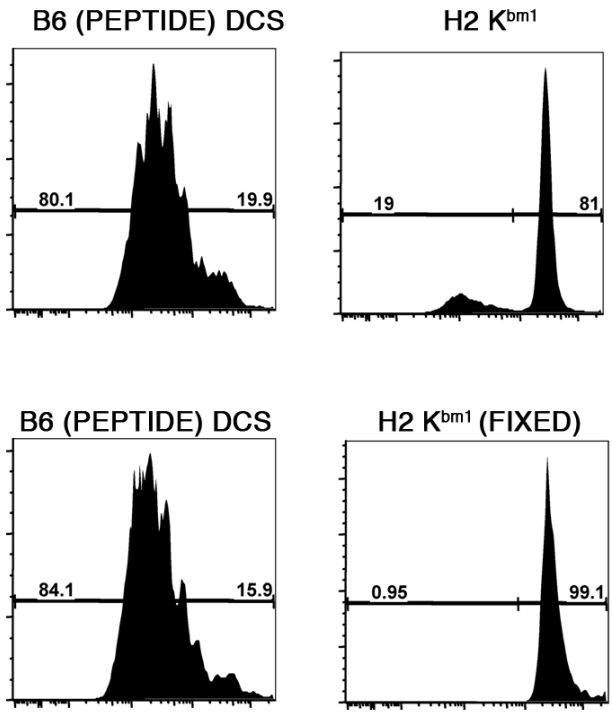


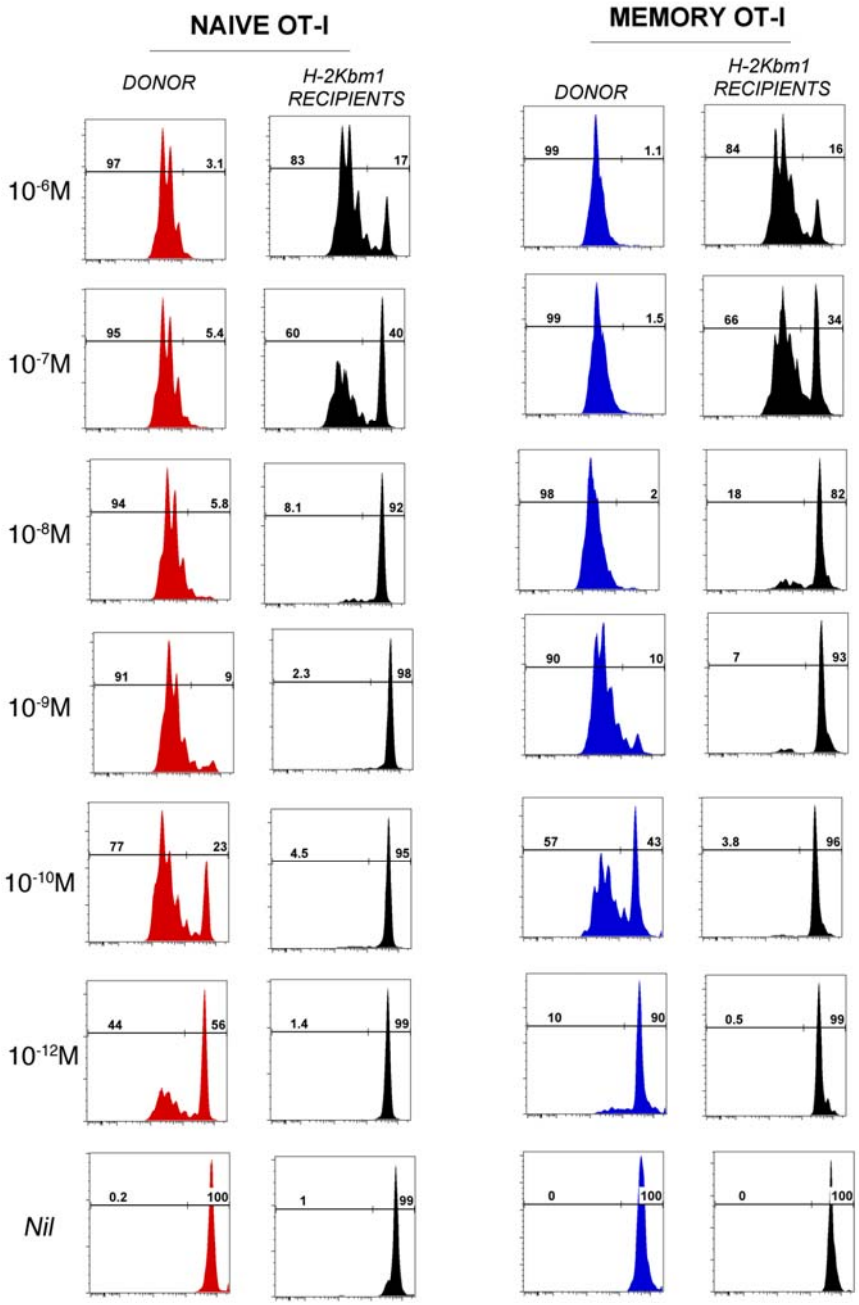
Supplementary Figure 1 Transfer of peptide loaded class I molecules between DCs. In vitro generated B6.SJL DCs were peptide pulsed and cultured with unloaded GFP+ DCs for 4 hours. DCs were then separated by cell sorting and cultured with CFSE labeled OT-I for 60 hours. Schematic diagram of experimental procedure and representative flow cytometry plot depicting the level of discrimination between donor (CD45.1) and recipient (GFP+) cells.



Supplementary Figure 2 Transfer of peptide loaded class I molecules between DCs. Peptide pulsed B6.SJL DCs were cultured with unloaded H2-K^{bm1} DCs. DCs were then separated by cell sorting and cultured with CFSE labeled OT-I cells for 60 hours. Shown is a schematic of the experimental procedure and a representative flow cytometry profile depicting the level of discrimination between donor (CD45.1) and recipient (CD45.2) cells.

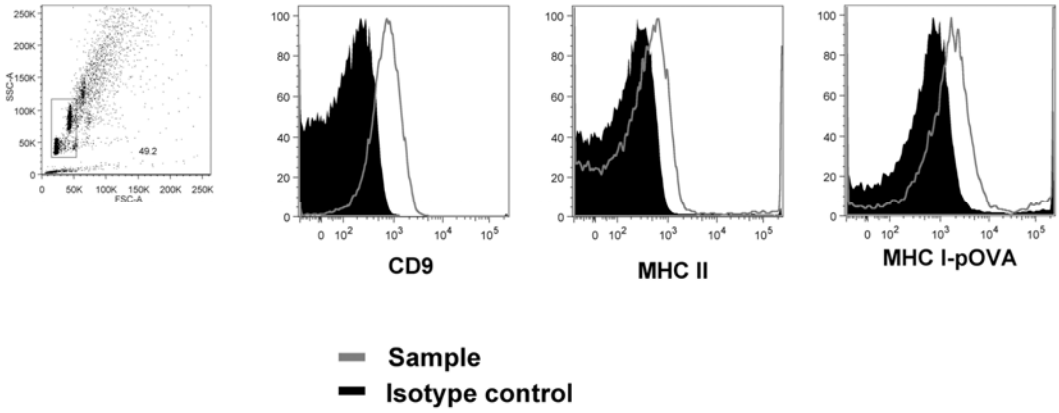


Supplementary Figure 3 Transfer of peptide loaded class I molecules between DCs. Peptide pulsed B6.SJL DCs were cultured for 4 hours with unloaded H2-K^{bm1} DCs that were or were not fixed. DCs were then separated by cell sorting and 2 $x10^4$ DCs were cultured with CFSE labeled OT-I T cells for 60 hours. Representative flow cytometry profiles are depicted.

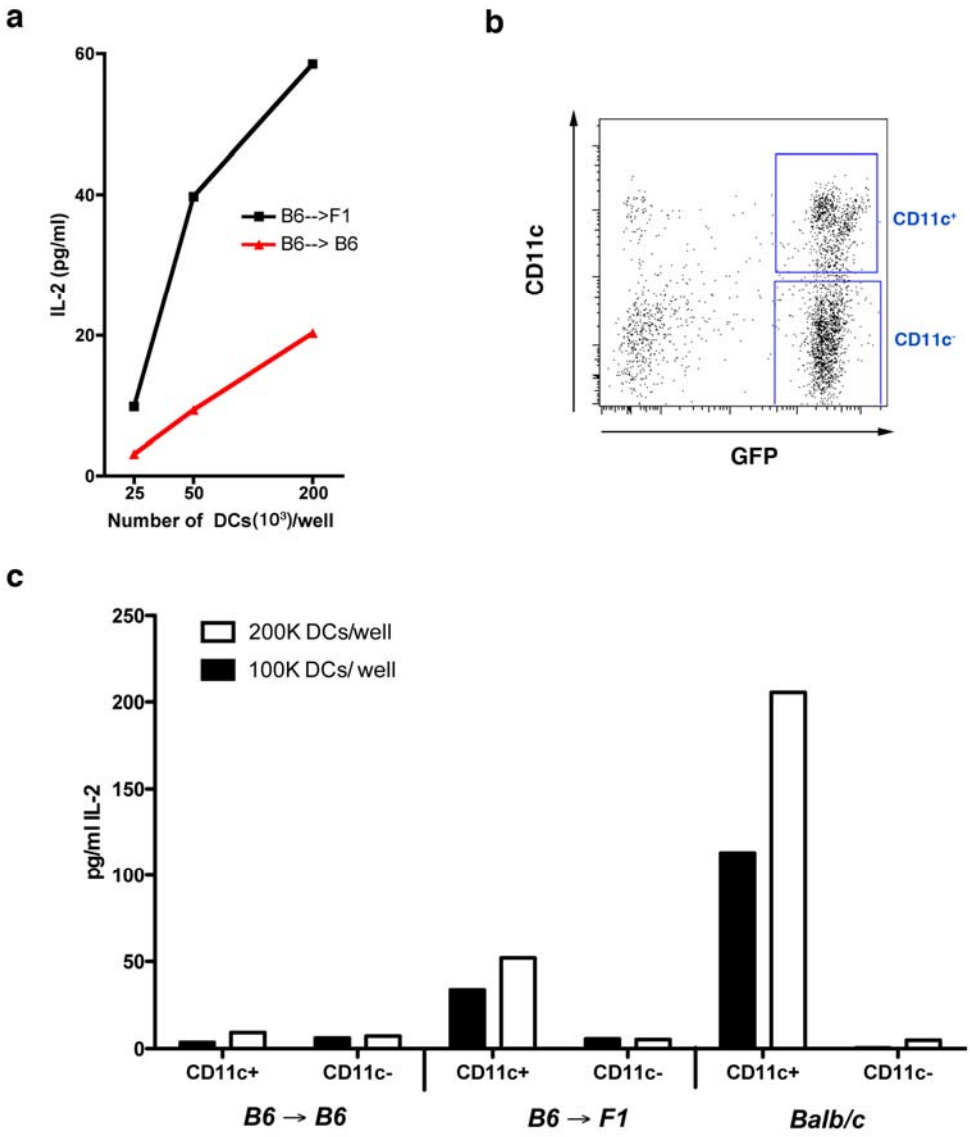


Supplementary Figure 4 Transfer of peptide loaded class I molecules between DCs in vitro.

In vitro generated B6.GFP DCs were pulsed with varying concentrations of SIINFEKL peptide and cultured with unloaded H2-K^{bm1} DCs. DCs were separated by cell sorting and 4 x 10^4 cells were cultured with CFSE labeled naïve or memory OT-I cells for 60 hours. Representative flow cytometry profiles are depicted.



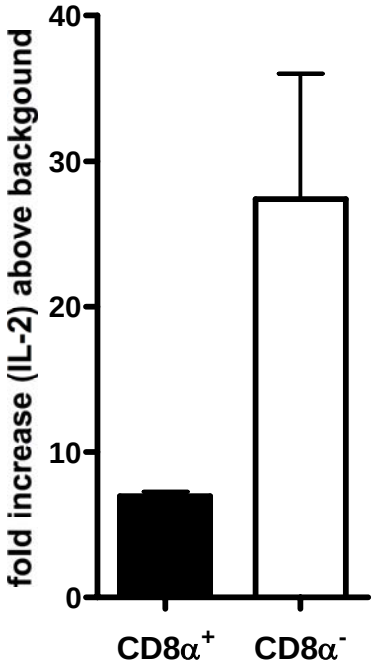
Supplementary Figure 5 Phenotype of exosomes recovered from DC supernatants
Exosomes recovered from the supernatants of peptide pulsed B6 DCs were attached to beads and stained for CD9, MHC II and MHC I/OVA. Filled histograms represent isotype control staining.



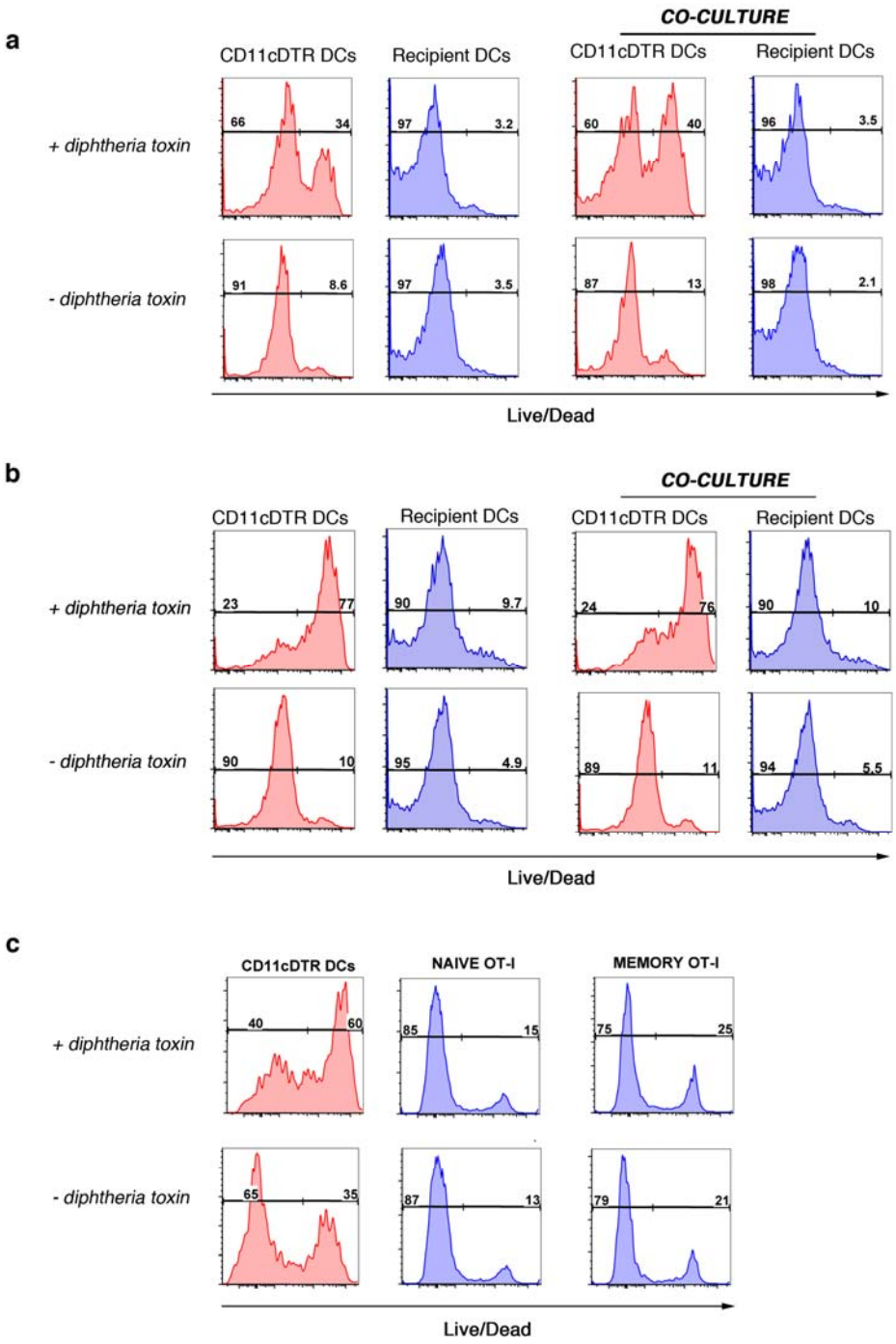
Supplementary Figure 6 The ability to acquire functional peptide loaded MHC I in vivo is restricted to CD11c$^+$ DCs.

(**a**) B6.GFP→B6 and B6.GFP→F_1 mice were infected with LCMV and on day 3 p.i. CD11c+ cells were sorted from the spleen and cultured with an L^d-NP_{118} specific hybridoma. The amount of IL-2 produced was determined by ELISA.

(b, c) B6.GFP→B6, B6.GFP→F_1, and Balb/c mice were infected with LCMV and on day 2 p.i. CD11c+ and CD11c- GFP+ cells were sorted from T and B cell depleted spleens and cultured with L^d-NP_{118} hybridoma for 48 hours. The amount of IL-2 produced was determined by ELISA. (**b**) Representative flow cytometry plot of sorted populations. (**c**) Data is representative of 3 independent experiments and depicts the mean amount of IL-2 produced.

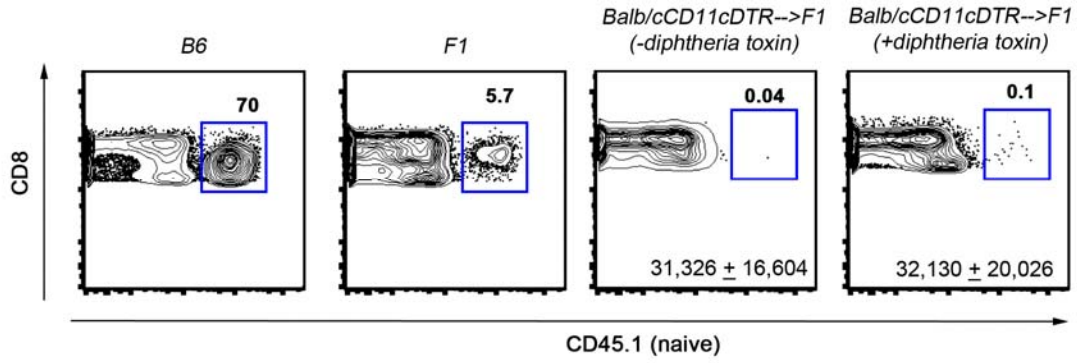


Supplementary Figure 7 CD8α$^-$ DCs are more efficient than CD8α$^+$ DCs at antigen presentation via cross-dressing.
B6.GFP→B6 and B6.GFP→F_1 mice were infected with LCMV and on day 2 p.i. CD11c+CD8α$^+$ and CD11c+CD8α$^-$ cells were sorted from the spleen and cultured with an L^d-NP_{118} specific hybridoma. The amount of IL-2 produced was determined by ELISA. Depicted is the average fold increase of IL-2 produced in the presence of DCs isolated from B6.GFP→F_1 mice over the amount produced using DCs from B6.GFP→B6 mice. Data is pooled from 2 independent experiments.



Supplementary Figure 8 No detectable transfer of functional diphtheria toxin receptor between DCs in vitro.

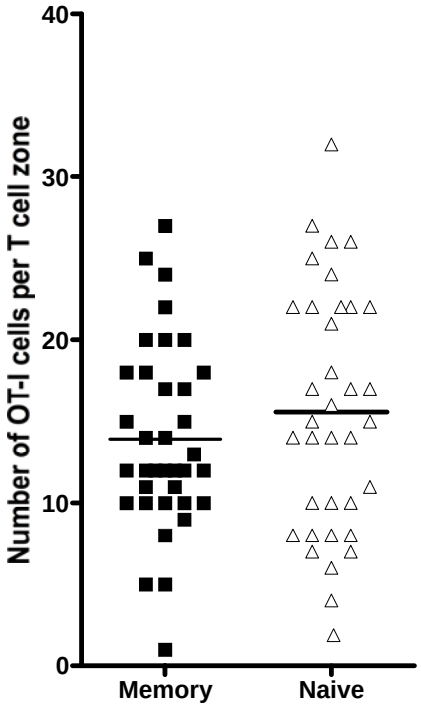
In vitro generated CD11cDTR DCs were peptide pulsed and cultured with unloaded recipient DCs for 4 hours. DCs were then cultured in the presence or absence of 2 μg/ml diphtheria toxin. The amount of toxicity was measured (**a**) 7 hours and (**b**) 24 hours later by staining cells with LIVE/DEAD dye. Depicted are representative flow cytometry profiles. (**c**) In vitro generated B6 CD11cDTR DCs were peptide pulsed and cultured with naïve or memory OT-I.CD45.1+ T cells in the presence or absence of 2 μg/ml diphtheria toxin. Survival was measured 24 hours later by staining cells with LIVE/DEAD dye. Depicted are representative flow cytometry profiles.



Supplementary Figure 9 Cross-dressed DCs in VSV infected mice fail to stimulate naive CD8$^+$ T cells

B6, F_1(Balb/c x B6) or Balb/c.CD11cDtr → F_1(Balb/c x B6) mice were seeded with naïve OT-I.CD45.1 T cells prior to infection with VSV-OVA. Balb/c.CD11cDtr → F_1 chimeras were treated with 100 ng of Diphtheria toxin daily or were left untreated. On day 7 p.i. the spleens were recovered and the proportion of OT-I cells of the total CD8$^+$ T cell population was determined by flow cytometry.

Representative flow cytometry plots of 4 mice per group. Numbers in right corner represent the mean total number of cells ± sem.



Supplementary Figure 10 Quantitation of the number of memory and naïve T cells in the spleen

Naïve (CD45.1) and memory (GFP+) OT- I T cells were adoptively transferred into mice prior to infection with VSV-OVA. On day 2 post infection the ratio of naïve to memory T cells in a T cell area was counted from histological sections. 12 random T cell zones from 3 separate mice were counted.

Materials and Methods:

Mice

C57BL/6 (H-2b), BALB/c (H-2d), B6.C-H2-K^{bm1}/ByJ (H2K^{bm1}), C57BL/6-Tg (UBC-GFP)30Scha/J(uGFP), B6.SJL-Ptprc^{a}Pep3^{b}/BoyJ (CD45.1), F_1 [Balb/c x C57BL/6], TAP-deficient (B6.129S2-*Tap1*^{tm1Arp}/J) mice and CD11c-diphtheria toxin receptor (DTR) (Itgax-DTR/enhanced GFP) mice on a Balb/c and B6 background were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in specific pathogen-free conditions in the animal facilities at the University of Washington (Seattle, WA). OT-I TCR transgenic mice congenic for Ly5.1 or GFP+ were bred and maintained in the same facilities. All experiments were done in accordance with the Institutional Animal Care and Use Committee guidelines.

Generation of bone marrow derived DCs

Bone marrow flushed from tibias and femurs of mice was re-suspended at 1 x 10^6 cells/ml in RPMI 1640 supplemented with 2.5 mM HEPES, 5.5 x 10^{-5} M mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin, 5 mM glutamine, 10% FBS, 10 ng/ml GM-CSF and 10 ng/ml IL-4 (PeproTech). Cells were incubated at 37°C with 7% CO_2 cultured for 6 days with a media change on day 3. Maturation of the DCs was induced by adding LPS (Sigma-Aldrich) at 1 µg/ml during the last 20 hours of culture. Dendritic cells loaded with peptide were cultured for 45 minutes at 37^{o}C with 10^{-6} M $OVA_{257–264}$ peptide. DCs were fixed with 4% paraformaldehyde for 10 minutes at room temperature prior to extensive washing.

Isolation of naïve and memory T cells

OT-I.CD45.1 or OT-I.GFP cells were purified from pooled spleen and LN preparation from transgenic OT-I mice by depletion of non-CD8+ cells using a MACs CD8 enrichment kit (Miltenyi Biotec) following manufacturers instructions. Cells were labeled with 2.5 µM CFSE [5-(and-6)-carboxyfluorescein diacetate succinimidyl ester].

Generation of memory OT-I involved the in vitro activation of OT-I cells using peptide pulsed splenocytes, followed by the transfer of 5 x 10^6 cells into naïve recipients. Mice were rested for 20 days prior to re-isolation of OT-I cells from the spleen. For adoptive

transfer experiments mice received 10^6 naïve and memory OT-I T cells via tail vein injection.

In vitro MHC I transfer experiments

Bone marrow derived DCs loaded with OVA peptide (donors) were mixed at a 1:1 ratio with unloaded DCs (recipient) for 4 hours at 37°C with 7% CO_2. For experiments using Transwells, donor peptide loaded DCs were plated into the upper chamber of a 5 µm Transwell plate (Costar, Corning, NY) while recipient unloaded DCs were cultured in the lower chamber. Recipient and donor DCs were separated by cell sorting and cultured at graded numbers with 2 x 10^4 CFSE labeled naïve OT-I.CD45.1 T cells in 200 µl of RPMI 1640 containing 10% FCS, 50 µM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin in 96-well U-bottom plates (Costar, Corning, NY). Cultures were analyzed for proliferation after 60 h.

Virus infections

Mice were infected i.n. with 5 x 10^4 PFU of a recombinant vesicular stomatitis virus that expresses GFP and a secreted form of OVA[1]. The lymphocytic choriomeningitis virus (LCMV) Armstrong 53b was grown and titered as described previously[2]. Mice were infected i.p. with 2 x 10^5 PFU of LCMV Armstrong.

Exosome isolation and presentation assay

Exosomes were isolated following the procedure described in detail by Thery et al.,[3]. Briefly, exosomes were recovered from the supernatant of mature bone marrow derived DCs cultured for 12 hours with 1µM SIINFEKL peptide in exosome depleted media (RPMI 1640 containing 10% exosome free FBS, 50 µM 2-ME, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin). Exosomes were purified by filtration on 0.22-µm pore filters, followed by ultracentrifugation at 100 000g. Exosomes were coated on beads for FACS analysis as described in detail elsewhere [3]. In vitro T-cell stimulation assays using exosomes were performed in 96-well plates with 2 x10^4 mature bone marrow derived DCs, and 2 x 10^4 naïve CFSE labeled OT-I.CD45.1 T cells. T cell proliferation was measured after 60 hours.

Visualizing membrane transfer

DCs were labeled with the lipophilic probe, l'dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Dil; Molecular probes) or the thiol-reactive chloromethyl probe 5-chloromethylfluorescein diacetate (CMFDA; Invitrogen) at a concentration of 0.5µg/ml following manufactures instructions. A mixture of DC labeled with Dil or CMFDA were introduced into an ibiTreat, 35 mm µ-Dish (Ibidi GmbH, Germany). Cells were imaged with a DeltaVision Live Cell microscope. Images were collected every 4 min for each of the illumination conditions. The time-lapse sequences were integrated into movies and observed.

NP_{118} hybridoma and presentation assay

T cell hybridoma specific for the L^d restricted $NP_{118-226}$ LCMV immunodominant epitope were prepared by fusing T cells from LCMV primed Balb/c mice with the fusion partner, BWZ.36/CD8+ [4].

For analysis of antigen presentation to NP_{118} specific hybridoma cells (L^d restricted), 1 x 10^5 DCs were cultured with 1 x 10^5 hybridomas in a U-bottom 96 well plate. Secretion of interleukin-2 by the NP hybridoma was measured by enzyme linked immunosorbent assay 48 hours after culture.

Bone marrow chimeras

Chimeric mice were generated by irradiation of recipient F_1[BALB/c x C57BL/6] mice with 1000 cGray. 5 x 10^6 T cell- depleted Balb/cCD11cDTR or B6uGFP bone marrow cells were injected intravenously. The mice were allowed to reconstitute for 8 weeks before use.

Diphtheria toxin and NK depletion treatment

Mice were injected i.p. with 100 ng of diphtheria toxin daily for the duration of the experiment. To prevent rejection of the OT-I T cells in the BALB/c CD11cDTR→F_1 mice animals were treated with 200 µg of anti-NK1.1 antibody daily for the duration of the experiment. Treatment of diphtheria toxin and anti-NK1.1 commenced 48 hours prior to infection.

For in vitro diphtheria toxin treatment of cultured cells 2 µg/ml of diphtheria toxin was added to media and cell viability was measured at the indicated times using a LIVE/DEAD cell viability assay following manufacture's instructions (Invitrogen, Life Technologies)

Flow cytometry

Single cell suspensions were prepared from spleens by mechanical disruption. Cells were stained for 30 min on ice with the appropriate cocktail of monoclonal antibodies and washed with PBS with 1% BSA. The following conjugated monoclonal antibodies were obtained from BD or eBioscience: anti CD8α, CD45.1, and CD11c. Cells were analyzed on a FACSCanto II using Flowjo software (Tree Star).

DCs isolation and cell sorting

Spleens were enzymatically digested for 20 min with continuous mechanical disruption in 6 ml of collagenase type 3 (Worthington) (3mg/ml in RPMI 1640 supplemented with 2% FCS). CD11c+ cells were enriched by depletion of CD19+ and CD3+ cells. Cells were sorted on BD FACs Aria II.

Immunohistochemstry

Tissues were fixed in 4% paraformaldehyde, frozen in O.C.T, cut, and stained with the following antibodies: anti-CD11c, anti-B220 and anti-CD45.1 (A20) purchased from BioLegend. Slides were mounted with Vectashield containing 4', 6-diamidino-2-phenylindole (DAPI; Vector Laboratories). Images were acquired using a fluorescence microscope and were analysed using Adobe Photoshop.

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