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Supporting Information

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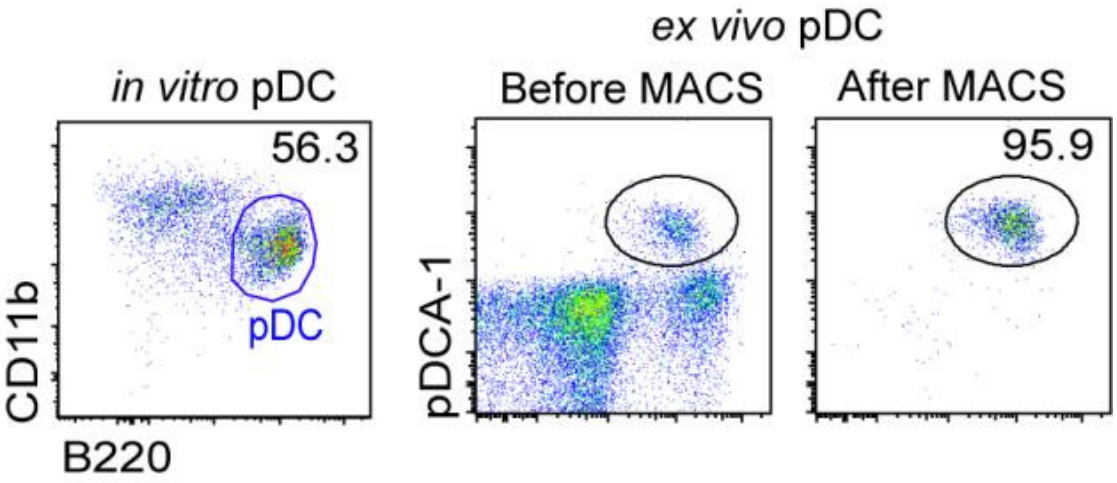
Plasmacytoid dendritic cells induce tolerance predominantly by cargoing antigen to lymph nodes

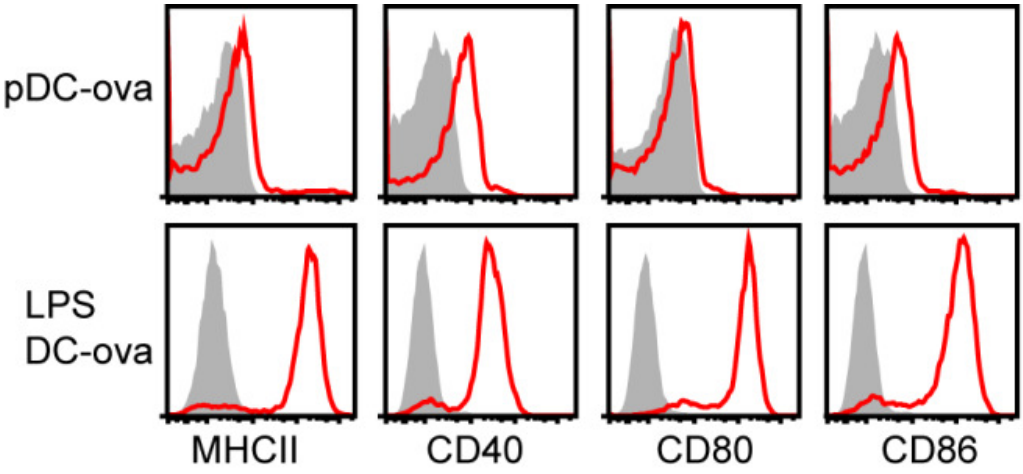
Supplemental Figure 1

Generation of pDCs. In vitro pDCs: BM cells isolated from the femur and tibia of mice were cultured in medium supplemented with cell culture supernatant of Flt-3L–secreting B16FL tumor cells. After 8 days of culture, pDCs were sorted from a mixed population of DCs using depicted markers. pDCs were also purified from cell isolates of LNs and spleen of mice bearing Flt-3L over-expressing tumors. Ex vivo pDCs: Cell isolates had a defined population of pDCA-1^{high} and CD11c^{int} pDCs. The pDCs were purified by negative MACS isolation and were consistently pure (>90%). In some cases of low purity, pDCs were further enriched by positive selection by FACS using antibodies against the indicated markers (data are representative of at least 5 experiments).



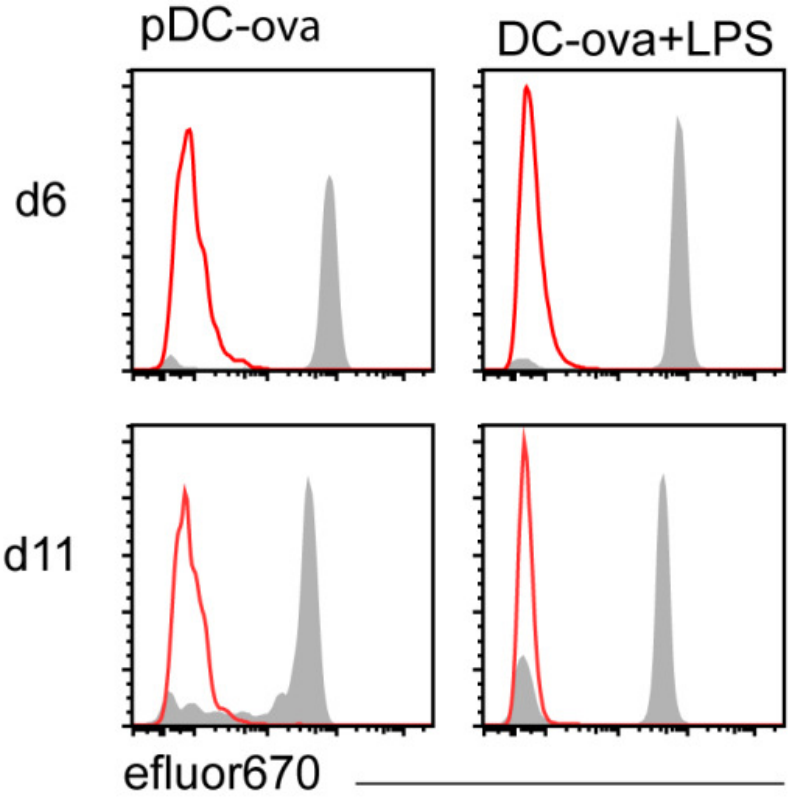
Supplemental Figure 2

Phenotype of in vitro generated DCs. DCs, as depicted, were analyzed for the expression of MHCII and co-stimulatory molecules by FACS. Shaded curves represent isotype controls. Results from one representative of at-least 5 experiments are shown.



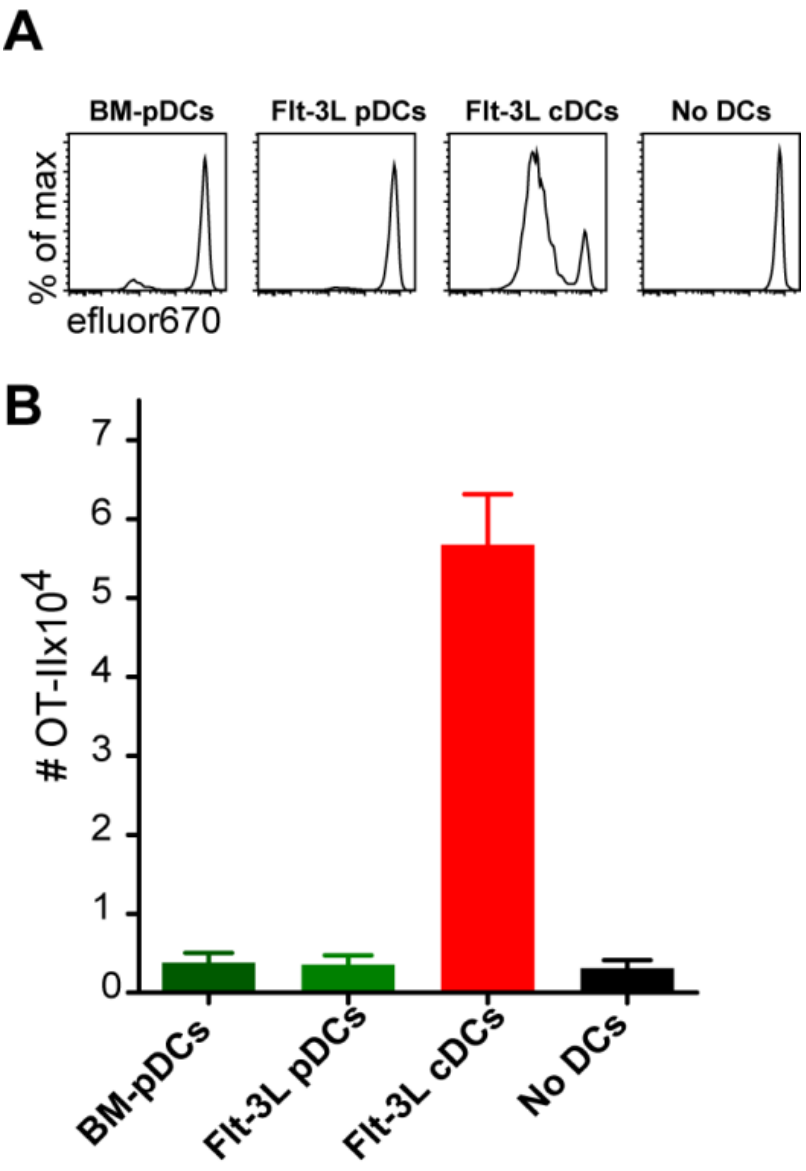
Supplemental Figure 3

pDCs induce abortive proliferation. Proliferation profiles of i.v. transferred OT-II cells isolated from popliteal LNs of wt mice at the time points indicated, that i.l received the indicated DC type at day 0. On the day of adoptive transfer mice were given an oral gavage of FTY720 and from the next day mice received FTY720 in drinking water until they were sacrificed. OT-II cells were gated as $DAPI^-$ $CD45.1^+$ $CD4^+$. Shaded curves depict control proliferation profiles of OT-II cells isolated form non-draining inguinal LN of the same mouse. Results of one representative of 5-8 LNs from 3-6 experiments are shown.



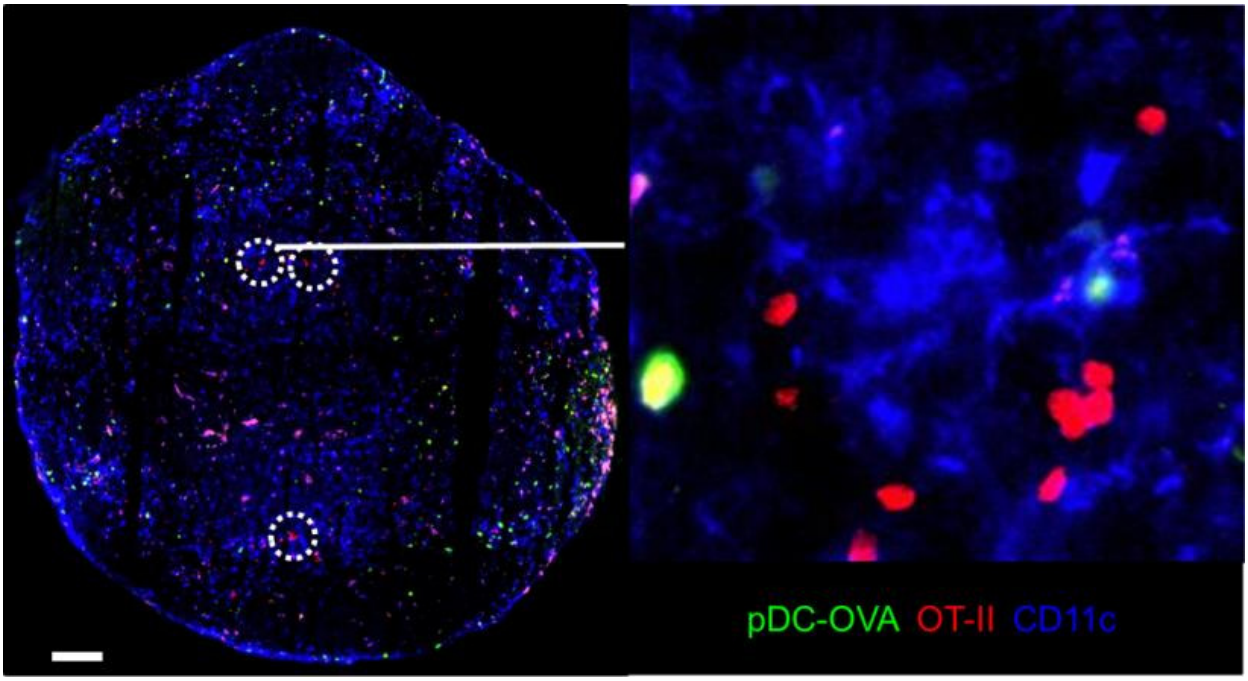
Supplemental Figure 4

BM-pDCs cannot prime CD4 T cells. Proliferation profiles of OT-II cells that were cultured with indicated DC type loaded with ovalbumin, 4 days earlier. The last plot (L-R) represents negative control wherein OT-II cells were cultured without any DCs. Plots are gated on all live DAPI- CD45.1+ CD4+ OT-II cells. B, Number of OT-II cells was determined by putting a fixed number of fluorescent latex beads in every sample (data derived from 2 experiments comprising 7-8 replicates for each group).



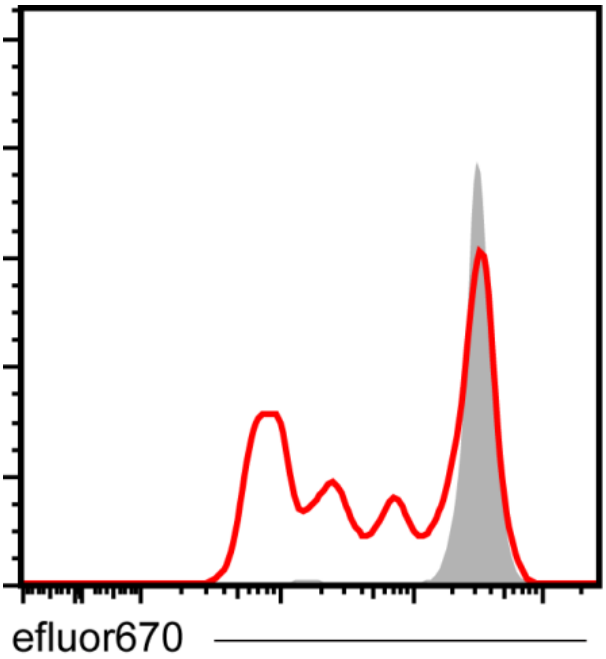
Supplemental Figure 5

pDCs transfer antigen to CD11c$^+$ DCs. Immunohistology of popliteal LN of wt mice that were sacrificed 18 hours after receiving i.v. TAMRA labelled OT-II cells (red). and GFP$^+$ ova loaded pDCs. injection of resting GFP$^+$ pDCs (green) i.l.. OT-II cells clustered around endogenous CD11c+ DCs as determined by anti-CD11c staining. Scale bar, 100µm. Results from one representative sections from 7 LNs from 2 independent experiments are shown.



Supplemental Figure 6

Transfer of membrane bound antigen. Proliferation profile of i.v. transferred OT-II cells isolated from popliteal LNs of wt mice which received by i.l transfer $CD4^+$ T cells from act-m-ova donors, 6 days earlier. On the day of adoptive transfer mice were given an oral gavage of FTY720 and from the next day mice received FTY720 in drinking water until they were sacrificed. OT-II cells were gated as $DAPI^-$ $CD45.1^+$ $CD4^+$. Shaded curves depict control proliferation profiles of OT-II cells isolated form non-draining inguinal LN of the same mouse. Results of one representative of 8 LNs from 2 independent experiments are shown.



Supplemental Movie 1.

Wt recipients received TAMRA-labeled OT-II cells (red) by i.v. injection and resting, ova-loaded GFP^+ pDCs (pDC-ova, green) or LPS-stimulated, ova-loaded GFP^+ DCs (LPS DC-ova) by i.l. adoptive transfer. Popliteal/brachial LNs were excised 12-18 h later and interactions between OT-II cells and DCs were imaged for one hour by two-photon microscopy. Time lapse recordings are maximum intensity projections along the z axis (top view) through image stacks comprising a volume of 125 x 125 x 30 μm (X x Y x Z). Cell tracks, depicting the path for the last 10 min are color coded for displacement over time (blue=0 μm; red=20 μm). One representative of 3-6 time-lapse recordings for each group is shown. Scale bar, 10 μm.

Supplemental Movie 2.

Wt recipients received TAMRA-labeled OT-II cells (red) by i.v. and resting, ova-loaded GFP+ pDCs (pDC-ova, green) by i.l. adoptive transfer. Time lapse recordings were made as in supplemental movie 1. Cell tracks are not shown. One representative of 6 time-lapse recordings is shown. Scale bar, 10 μm.