Supplementary Information

Id2 expression delineates differential checkpoints in the genetic program of CD8α$^+$ and CD103$^+$ dendritic cell lineages

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Supplementary Table

Supplementary Table I. Comparative relationship between *in vivo* and *in vitro* DC subsets and sorting regime

Group	DC Subset	*In Vivo*	*In Vitro*
1	**Bone marrow progenitor**	Not sorted *in vivo*	$CD11c^{+}/CD103^{-}/Id2\text{-}GFP^{-}/CD45RA^{-}$
2	**Immature pDC**	Not sorted *in vivo*	$CD11c^{+}CD103^{-}Id2\text{-}GFP^{-}CD45RA^{int}$
3	**Mature pDC**	$CD11c^{+}CD45RA^{high}$	$CD11c^{+}CD103^{-}Id2\text{-}GFP^{-}CD45RA^{hi}$
4	**CD8α^{+} DC**	$CD11c^{+}CD103^{-}CD8^{+}CD205^{int}$ (LN) $CD11c^{+}CD45RA^{-}CD4^{-}CD8\alpha^{+}$ (spleen)	$CD11c^{+}CD103^{-}Id2\text{-}GFP^{+}CD45RA^{int}Sirp\text{-}\alpha^{-}$
5	**CD4 DCs**	$CD11c^{+}CD45RA^{-}CD8^{-}CD4^{+}$	$CD11c^{+}CD103^{-}Id2\text{-}GFP^{+}CD45RA^{-}Sirp\text{-}\alpha^{+}$
	DN DCs	DN DC Id2-GFP ^{int}: $CD11c^{+}CD45RA^{-}CD4^{-}CD8^{-}Id2\text{-}GFP^{int}$ DN DC Id2-GFP^{high}: $CD11c^{+}CD45RA^{-}CD4^{-}CD8^{-}Id2\text{-}GFP^{high}$	
	Langerhans cells	$CD11c^{+}CD103^{-}CD8^{-}Id2\text{-}GFP^{high}$ $CD205^{high}$	
	Dermal DCs	$CD11c^{+}CD103^{-}CD8^{-}Id2\text{-}GFP^{high}$ $CD205^{int}$	
6	**CD103 DC**	$CD11c^{+}CD8^{-}Id2\text{-}GFP^{high}$ $CD103^{high}$	$CD11c^{+}CD103^{+}Id2\text{-}GFP^{high}$

Supplementary Figures

Supplementary Figure 1. Expression of Id2-GFP in pDCs. Dot plots show the analysis of pDCs in indicated tissues. DCs from *Id2*$^{gfp/gfp}$ (green line) and wild-type (black line) mice were enriched from (**A**) thymus, (**B**) spleen, (**C**) peripheral LNs and (**D**) mesenteric LNs and stained with antibodies against surface markers as described in Experimental Procedures. cDCs were identified by their high expression of CD11c and absence of CD45RA while pDCs were identified by their intermediate expression of CD11c and CD45RA. Data show representative profiles and gating strategy for total live cells enriched from lymphoid tissues. Each experiment was performed two to five times with similar results.

Supplementary Figure 2. Specificity controls for CD103 expression in *in vitro* DC subsets. Bone marrow cells were isolated from femurs of C57BL/6 and *Itgae*$^{-/-}$ (CD103-deficient) mice. Single cell suspensions were depleted of red blood cells then cells were cultured at a density of 1.5×10^6 cells/ml in the presence of 100 ng/ml recombinant Flt3L for 5 days. Cells were stained with mAb recognizing CD11c, CD45RA and biotinylated anti-CD103 or anti-Armenian Hamster IgG followed by SA-APC/Cy7. Antibodies recognizing CD3ε, CD19 and NK1.1 were used to exclude non-DC lineages. Samples were analyzed by flow cytometry and dead cells were excluded by staining with propidium iodide. Profiles are gated on CD11c$^+$ cells and are representative of at 3 similar experiments (n=7 per group).

Supplementary Figure 3. *In vitro* DC subsets have distinct differentiation characteristics. (**A**) *In vitro* generated *Id2*$^{gfp/gfp}$ DCs were fractionated into 6 populations (as shown in Figure 4A) by FACs sorting on day five of culture. (**B**) Cells

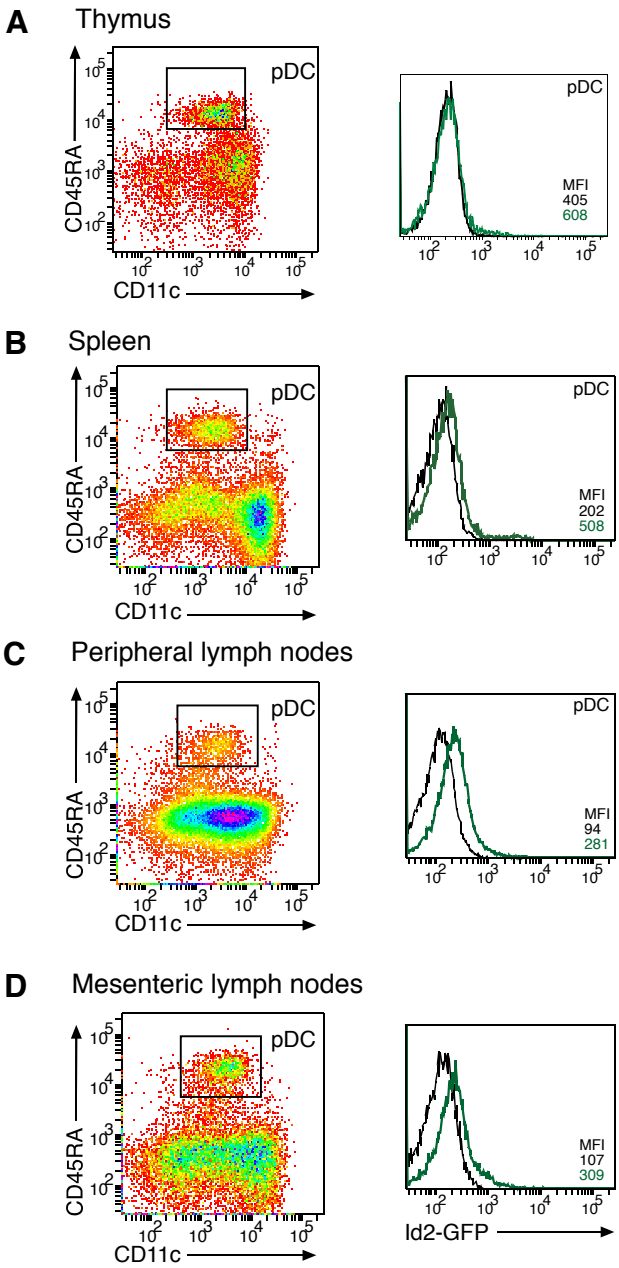
were then re-cultured for a further three days in Flt3L-conditioned medium (recovered from the same cells on day five), then stained for CD11c, CD45RA, CD103 and Sirp-α and analyzed by flow cytometry to determine the survival and phenotype of the differentiated cells. Data are representative of three similar experiments.

Supplementary Figure 4. Immature pDCs exhibit similar activation behaviour to mature pDCs *in vitro*. Bone marrow cells from *Id2*$^{gfp/gfp}$ mice were cultured at a density of 1.5 × 10^6 cells/ml in the presence of 100 ng/ml recombinant Flt3L for 5 days. Cultures were then stimulated with 1 μg/ml LPS, 25 μg/ml poly I:C or 500 ng/ml CpG for 18h then stained with mAb recognizing CD11c, CD45RA, Sirp-α and CD103 and CD80 (16-10A1) or MHC class II (M5/114) to monitor activation status. NK cells, B cells and T cells were excluded using negative against anti-NK1.1, anti-CD19 and anti-CD3ε mAbs. Data are representative of at least three experiments with similar results.

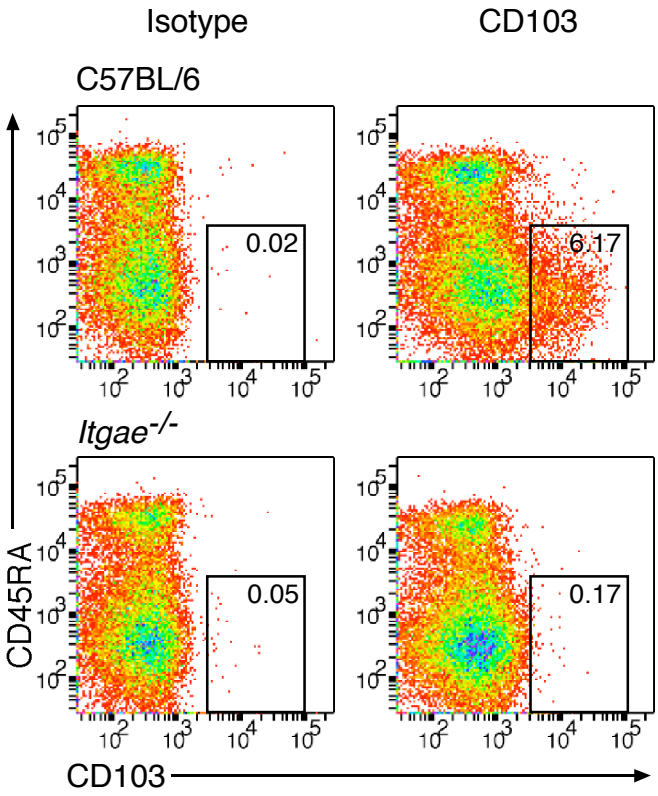
Supplementary Figure 5. *In vitro* cross-presentation in Flt3L-stimulated cultures is limited to CD103-expressing Id2-GFP^{high} DCs. *In vitro* generated *Id2*$^{gfp/gfp}$ DCs were flow cytometrically sorted eight days after initiation of cell culture according to their expression of CD103, CD45RA, Sirp-α and Id2-GFP and analyzed for their ability to cross-present cell-associated OVA to CFSE-labeled OVA-specific CD8$^+$ T cells (upper panels). The ability of these subsets of present exogenous antigen to CFSE-labeled OVA-specific CD4$^+$ T cells was evaluated as a control (lower panels). Data are representative of four independent experiments. T cell proliferation was analysed in 1-3 replicates for each DC subset/responder population for each experiment.

Supplementary Figure 6. Analysis of DC subsets in *Id2*$^{gfp/gfp}$, *Id2*$^{gfp/gfp}$ × *Batf3*$^{-/-}$ and *Id2*$^{gfp/gfp}$ × *Irf-8*$^{-/-}$ mice. DCs were enriched from spleen and peripheral LNs from *Id2*$^{gfp/gfp}$, *Id2*$^{gfp/gfp}$ × *Batf3*$^{-/-}$ and and *Id2*$^{gfp/gfp}$ × *Irf-8*$^{-/-}$ strains then stained with antibodies against CD11c, CD4, CD45RA, CD8α or CD103, as described in the Materials and Methods. Samples were analyzed by flow cytometry and dead cells were excluded by staining with propidium iodide. Spleen and LNs were pooled from two to three mice in each group. Dot plots are gated on total CD11c^{+} cells showing pDCs (Id2^{-}) and cDCs (Id2^{+}). Percent and Id2-GFP MFI of CD8α^{+} or CD103^{+} cells are shown. Histograms are gated on CD11c^{+} cDCs and CD8α MFI of gated cells are shown (grey, *Id2*$^{gfp/gfp}$; red, *Id2*$^{gfp/gfp}$ × *Batf3*$^{-/-}$; *Id2*$^{gfp/gfp}$ × *Irf-8*$^{-/-}$, blue). Data are representative of at least two to four similar experiments.

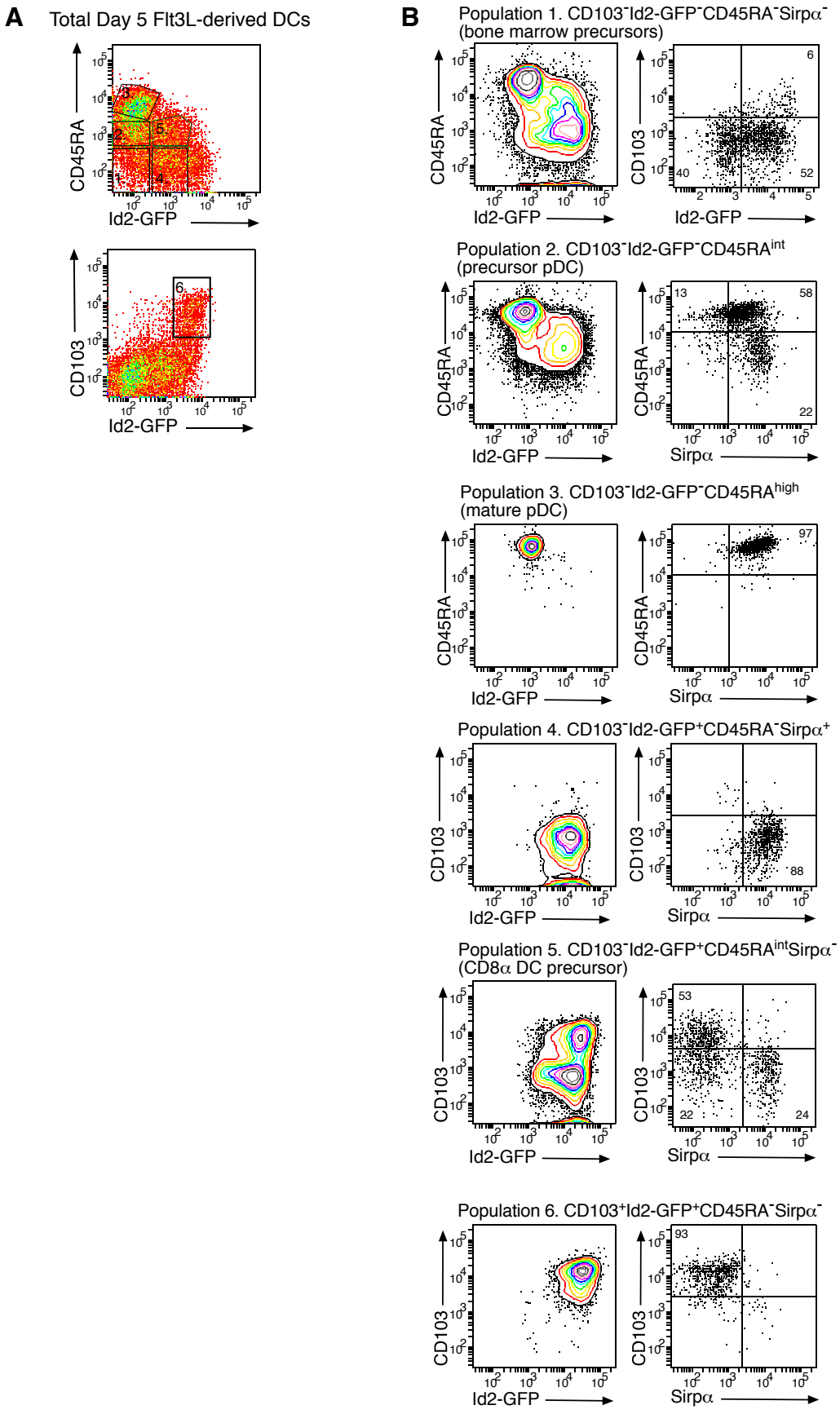
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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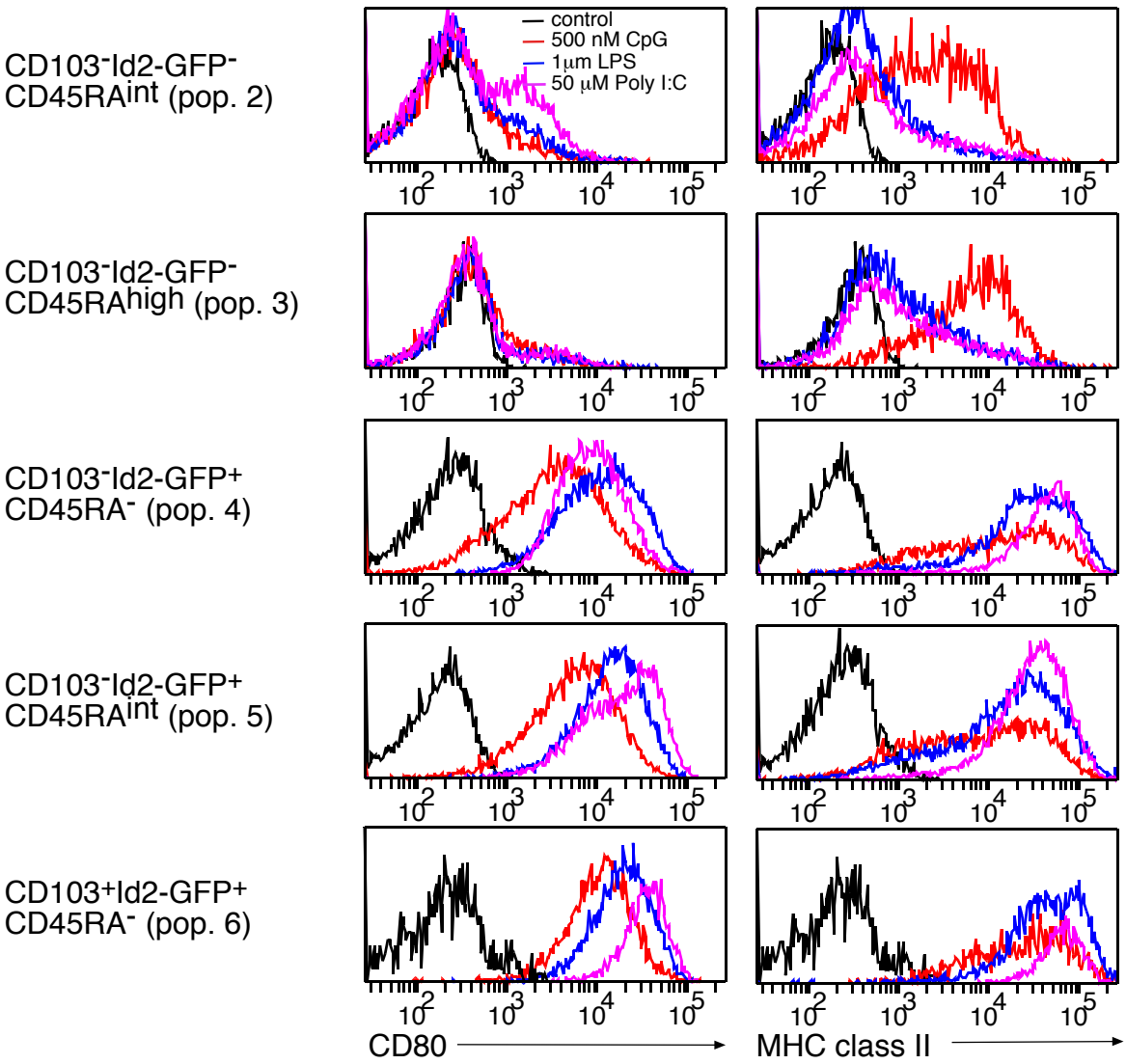
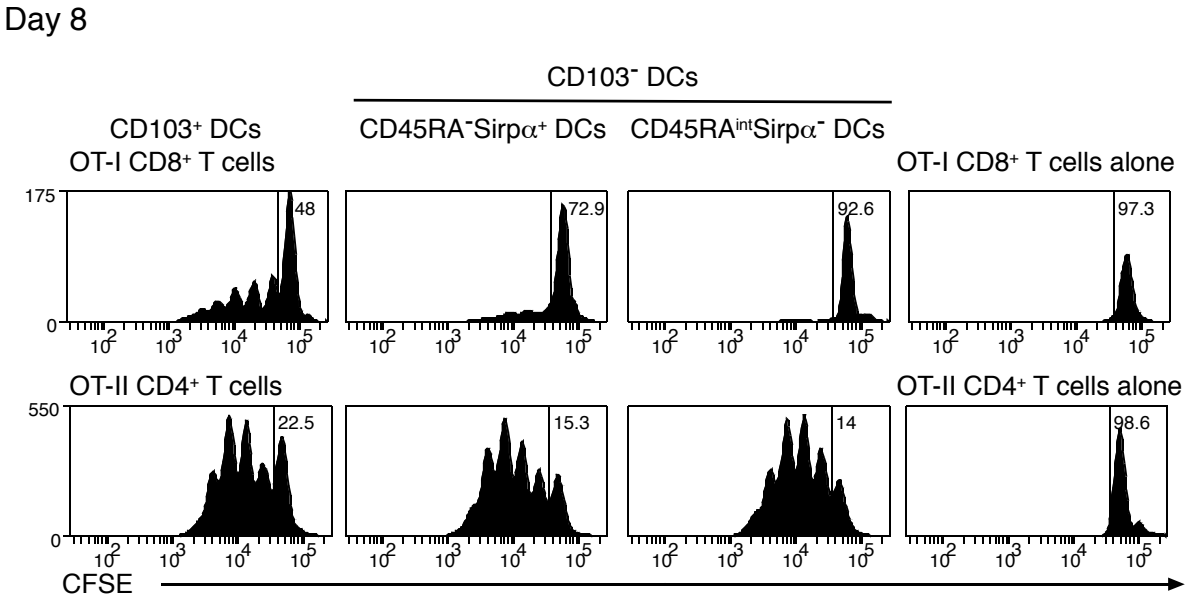


Supplementary Figure 5



Supplementary Figure 6