Figure S1

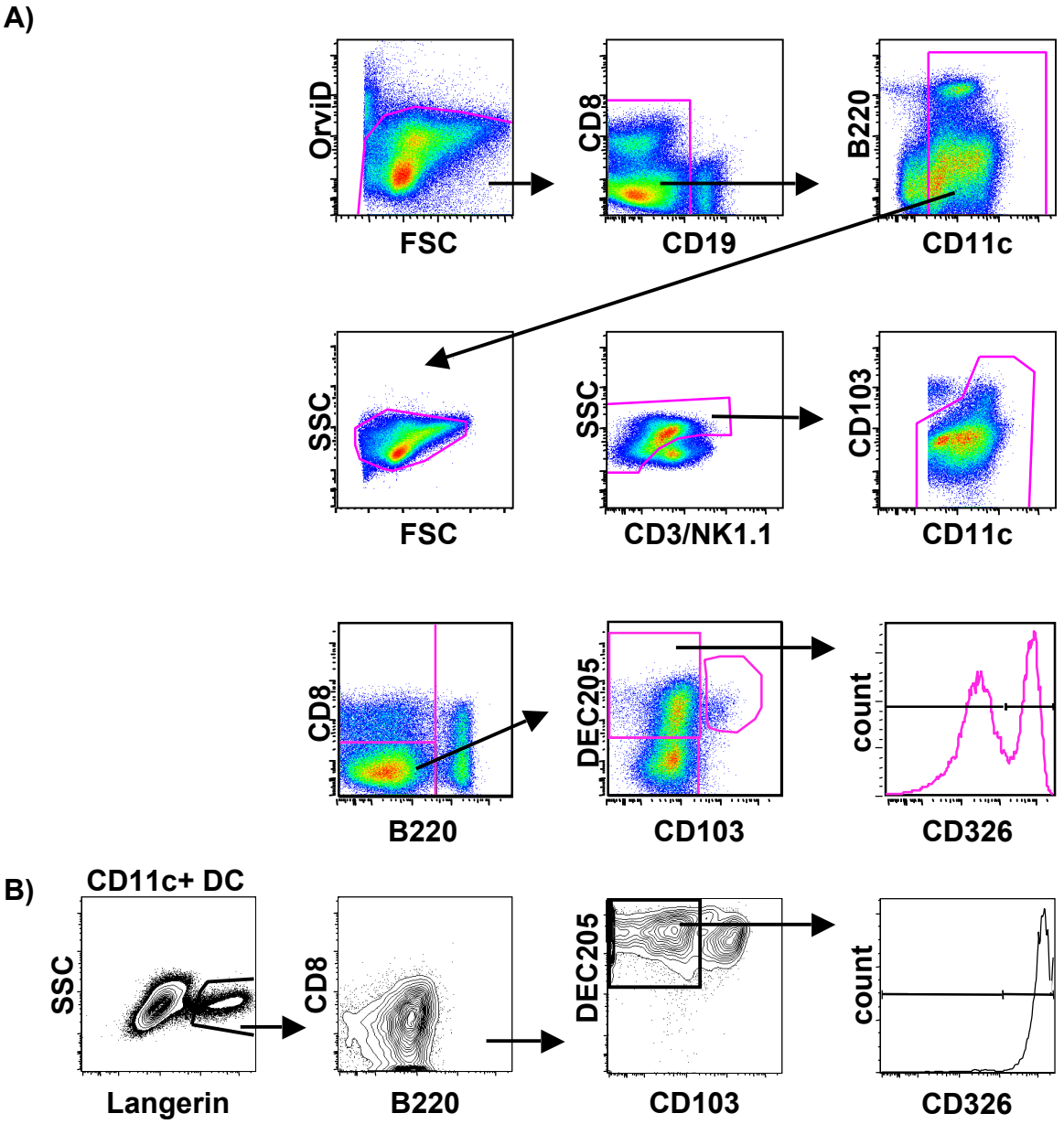

Figure S1: Phenotypic characterization of DC subpopulations. DLNs were harvested and pooled 48h post immunization with OVA-conjugate. (A) Alternative gating strategy to purify CD11c+ DCs. Dead cells of positively (CD11c+) enriched DCs were excluded using the viability dye LIVE/DEAD® Fixable Orange Dead Cell Stain (OrViD). Remaining T, NK and B cells were further excluded by CD3, NK1.1 and CD19 staining, respectively. DCs were categorized by the basis of CD11c+ expression. DC subsets were described by their expression of CD8, B220, CD103, DEC205 and CD326. (B) To analyze the expression of langerin on all DC subsets, an intracellular staining with AF488-labeled Langerin was added to the DC staining panel. Langerin+ CD11c+ cells were overlaid on the DC gating tree to display only Langerin-expressing DC populations. These data show that langerin is not expressed on CD8+ DCs or CD8-DEC205+CD103-CD326- dermal DCs. By contrast, Langerin is expressed on CD8-DEC205+CD103-CD326+ (epidermal LC) and CD8-DEC205+CD103+ dermal DCs.

Figure S2

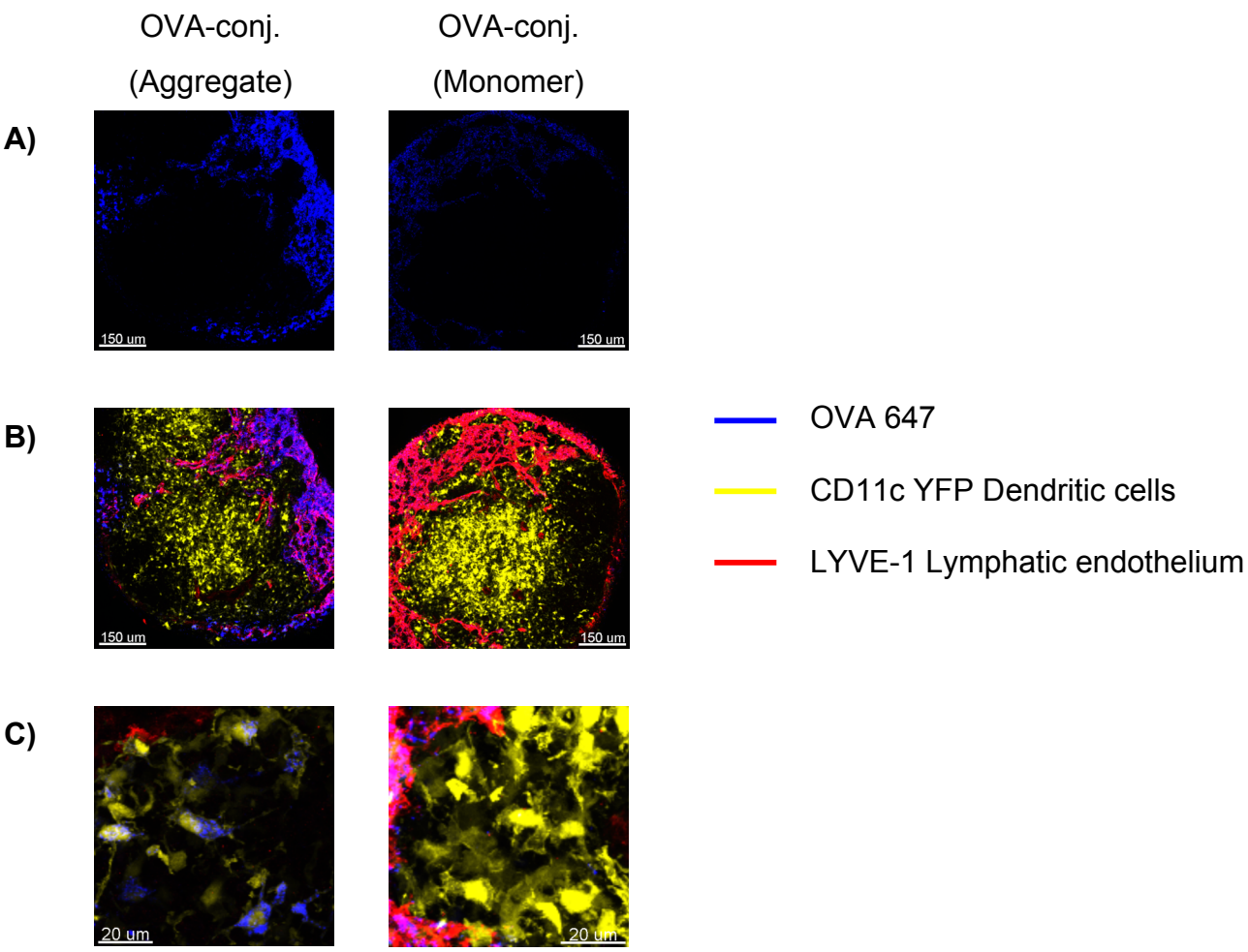

Figure S2: Aggregated OVA accumulates in the medullary sinus, is acquired by resident DCs, and shuttled by migratory DCs. Footpads of CD11cYFP mice were injected with AF647-labeled aggregated or monomeric OVA-conjugate. 6h later popliteal LNs were harvested, sectioned and analyzed by confocal microscopy. (A) Comparison of signal intensity acquired at identical settings of aggregated vs. monomeric AF647-labeled OVA (blue). (B) and (C) co-localization of OVA (blue), LYVE-1 (red) and CD11c (yellow) at different magnifications (20x/63x). Data are representative of at least two independent experiments.

Figure S3

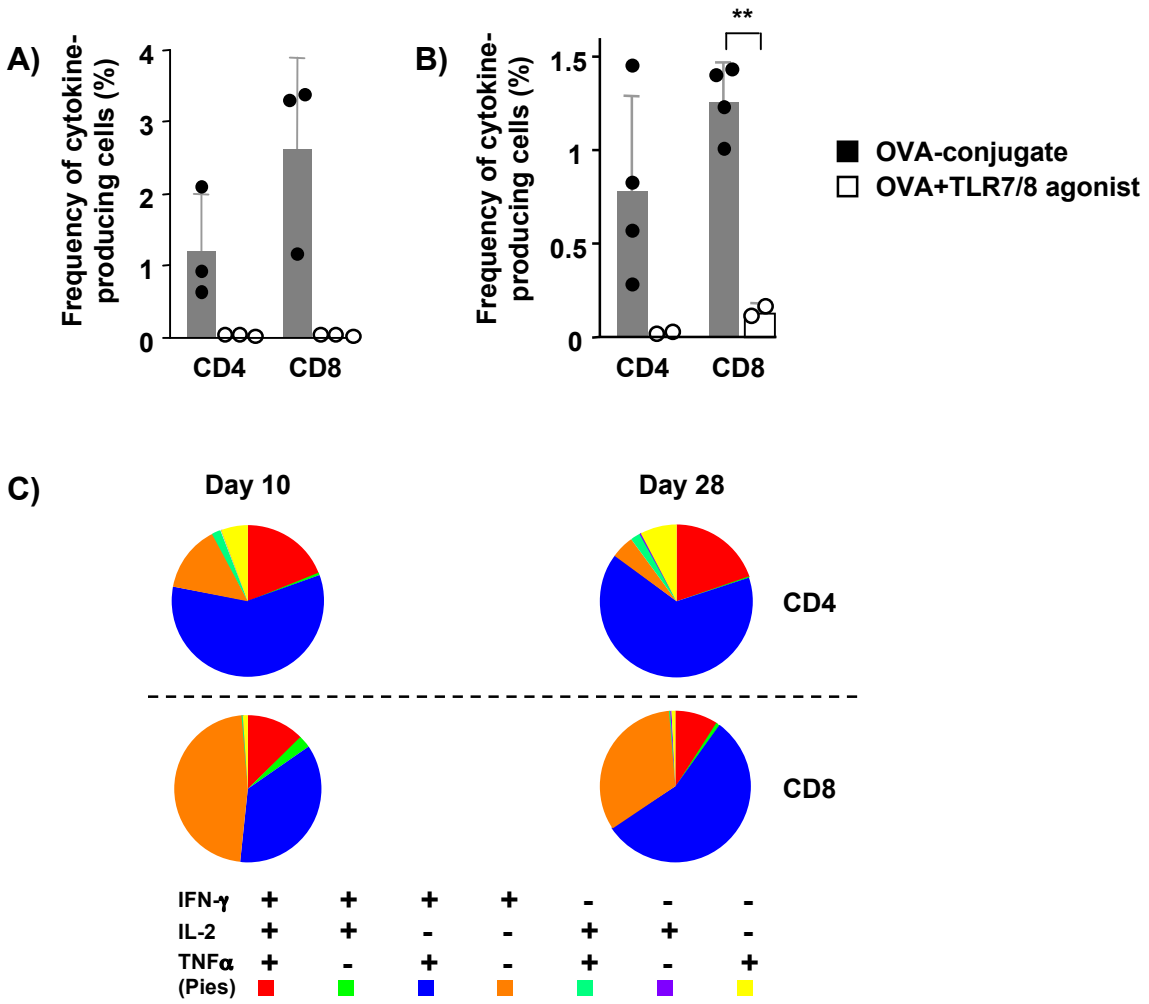

Figure S3: OVA-conjugate induces durable memory responses. Mice (n=7) were immunized s.c. with 20μg OVA plus free TLR7/8 agonist or the OVA-conjugate vaccine. (A and B) The frequency of OVA-specific IFN-γ, IL-2 or TNFα producing CD4+ or CD8+ T cells was assessed in spleen 10 (A) and 28 (B) days after the first immunization. (C) Pie charts represent the proportion of OVA-specific CD4+ or CD8+ T cells producing IFN-γ, IL-2, or TNFα alone or in any combination. (**, $p < 0.01$).

Figure S4

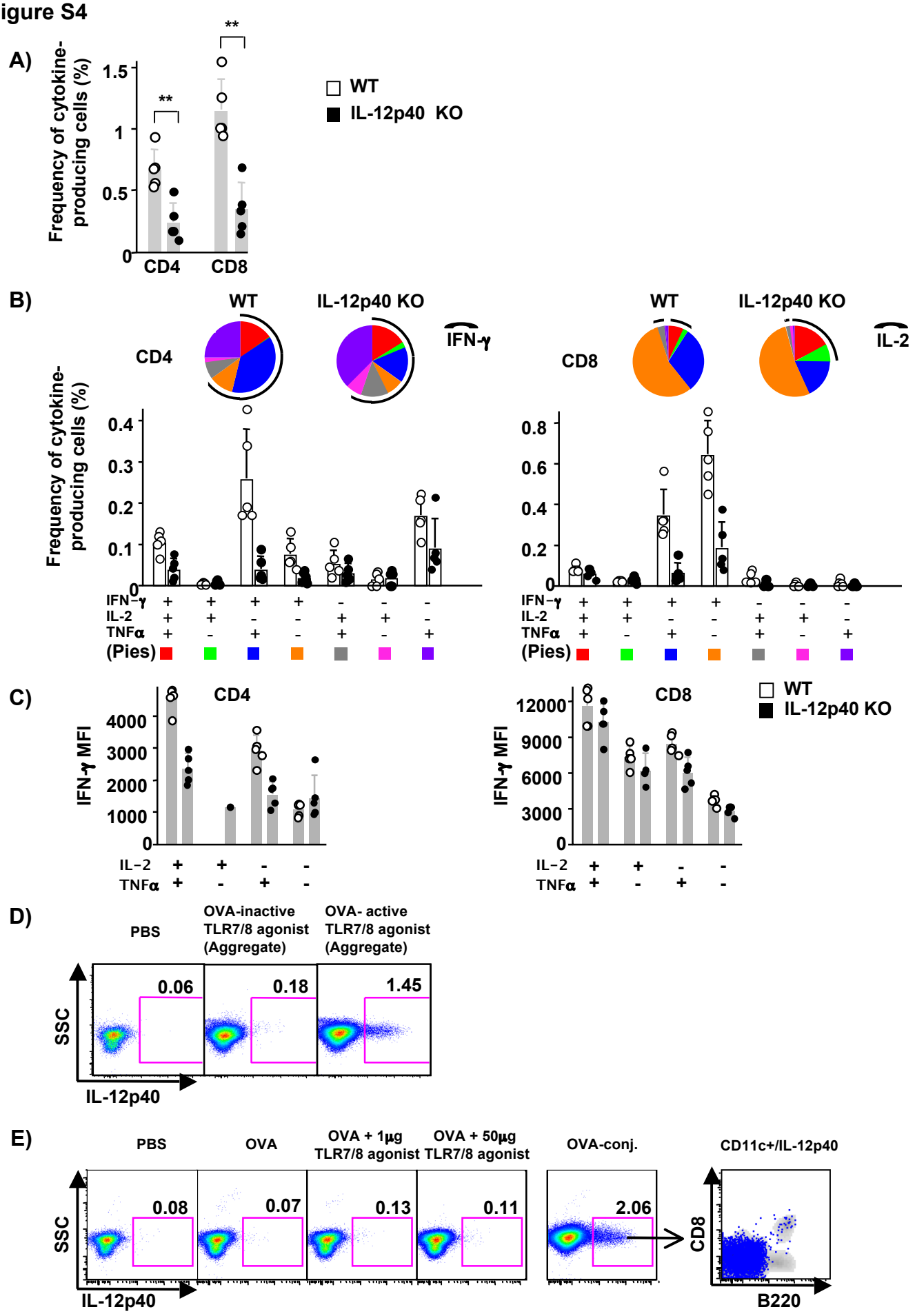
A)
Frequency of cytokine-producing cells (%)
1.5
1
0.5
0
**
**
CD4
CD8
WT
IL-12p40 KO
B)
WT
IL-12p40 KO
CD4
IFN-γ
Frequency of cytokine-producing cells (%)
0.4
0.3
0.2
0.1
0
IFN−γ + + + + - - -
IL-2 + + - - + + -
TNFα + - + - + - +
(Pies)
WT
IL-12p40 KO
CD8
IL-2
0.8
0.6
0.4
0.2
0
IFN−γ + + + + - - -
IL-2 + + - - + + -
TNFα + - + - + - +
(Pies)
C)
CD4
IFN-γ MFI
4000
3000
2000
1000
0
IL−2 + + - -
TNFα + - + -
CD8
IFN-γ MFI
12000
9000
6000
3000
0
IL−2 + + - -
TNFα + - + -
WT
IL-12p40 KO
D)
PBS
OVA-inactive TLR7/8 agonist (Aggregate)
OVA- active TLR7/8 agonist (Aggregate)
0.06
0.18
1.45
SSC
IL-12p40
E)
PBS
OVA
OVA + 1μg TLR7/8 agonist
OVA + 50μg TLR7/8 agonist
OVA-conj.
CD11c+/IL-12p40
0.08
0.07
0.13
0.11
2.06
SSC
IL-12p40
CD8
B220

Figure S4: Priming of OVA-specific T cells requires IL-12p40. B6 and IL-12p40KO mice (n=5/group) were immunized as described above. (A-C) One week after the second immunization splenocytes were analyzed for cytokine producing T cells.

(A) Total frequency of IFN-γ, IL-2 or TNFα producing OVA-specific CD4+ and CD8+ T cells. (B) Pie charts represent the relative contribution of multifunctional T cells making IFN-γ, IL-2 or TNFα alone or in any combination. The black arc highlights the total proportions of IFN-γ producing CD4+ T cells or IL-2 producing CD8+ T cells.

(C) Mean fluorescence intensity (MFI) of IFN-γ staining of cytokine producing OVA-specific CD4+ and CD8+ T cells. (D) IL-12p40 production of CD11c+ DCs 48 h after immunization with an aggregated formulation of OVA linked with either inactive or active TLR7/8 agonist. (E) Comparison of IL-12p40 production of CD11c+ DCs 48h after immunization of BALB/c mice with either OVA-conjugate (active TLR7/8 agonist) or OVA plus different doses of free TLR7/8 agonist; CD11c+ IL-12p40+ cells from mice that were immunized with OVA-conjugate (blue dots) were overlaid on total CD11c+ cells (gray). (**, $p<0.01$, data are representative of three independent experiments).

Figure S5

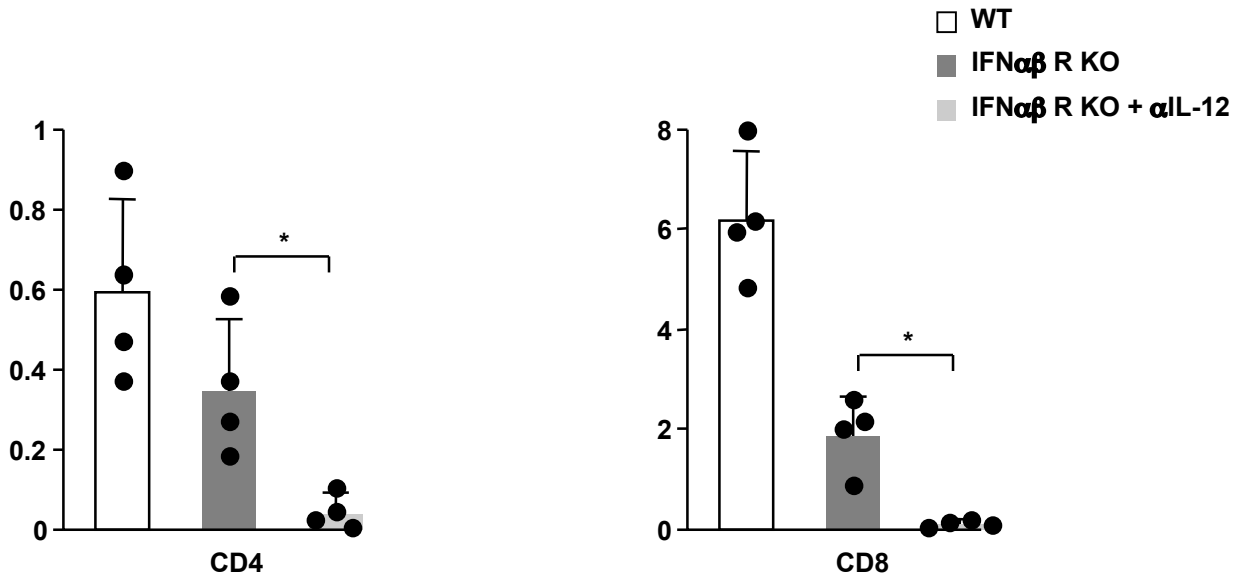

Figure S5: Priming of OVA-specific T cells requires IL-12 and Type I IFN. B6 and IFNαβ R KO mice (n=4/group) were immunized once with 20μg OVA-conjugate vaccine. IFNαβ R KO mice were treated i.p. with 1mg αIL-12 24h before immunization. The total frequency of IFN-γ, IL-2 or TNFα producing OVA-specific CD4+ and CD8+ T cells was accessed three weeks after immunization. (*, $p<0.05$: data are representative of three independent experiments).

Figure S6

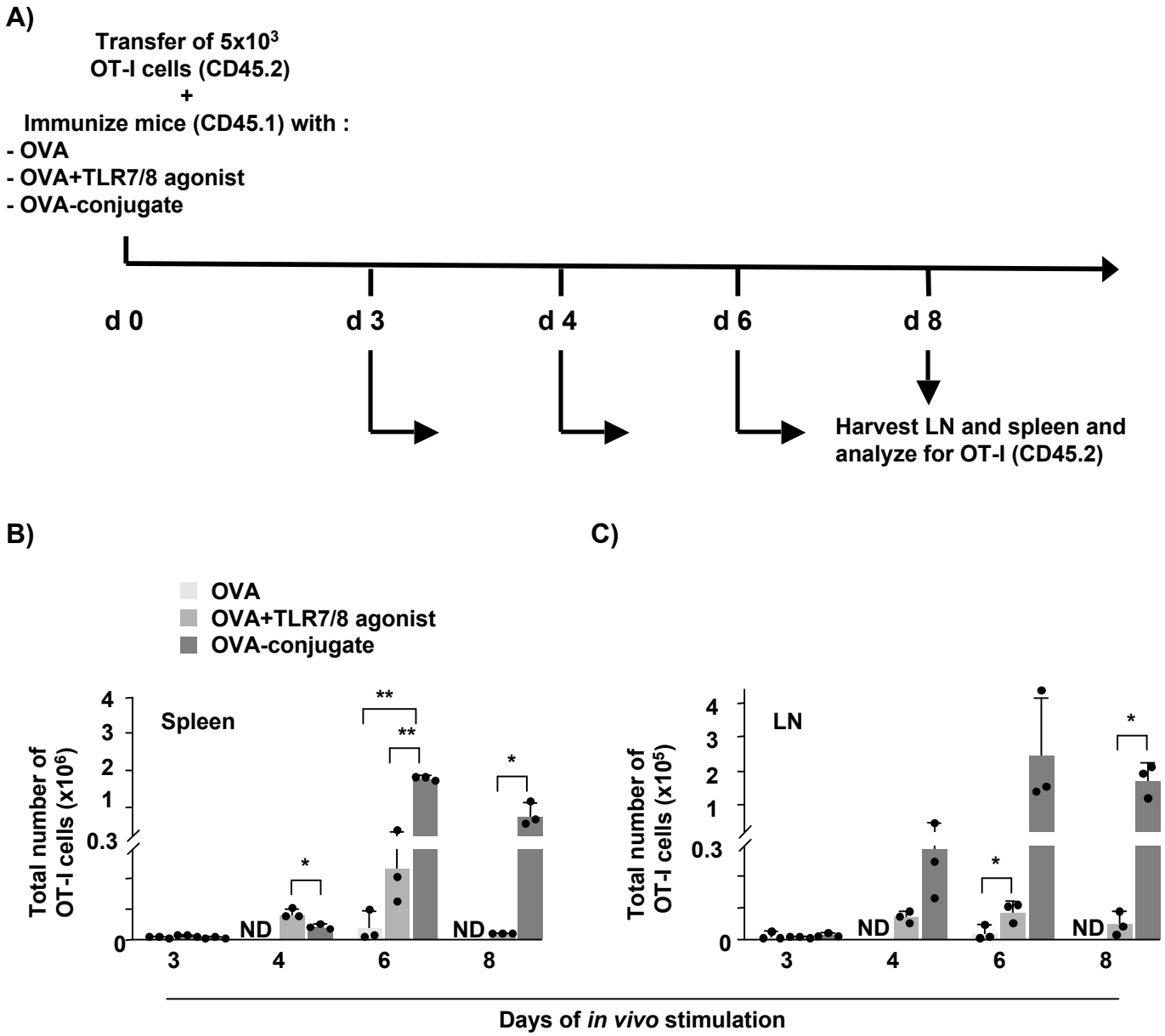

Figure S6: Lack of T cell fitness after priming with OVA plus free TLR7/8 agonist. (A) OT-I cells (CD45.2) were transferred into congenic mice (CD45.1) and immunized either with OVA, OVA plus free TLR7/8 agonist or the OVA-conjugate vaccine. Total numbers of recovered OT-I cells in (B) spleen or (C) lymph nodes after indicated days of *in vivo* stimulation are shown. (*, $p < 0.05$; **, $p < 0.01$; ND=not done; n=3, data are representative of two independent experiments)

Figure S7

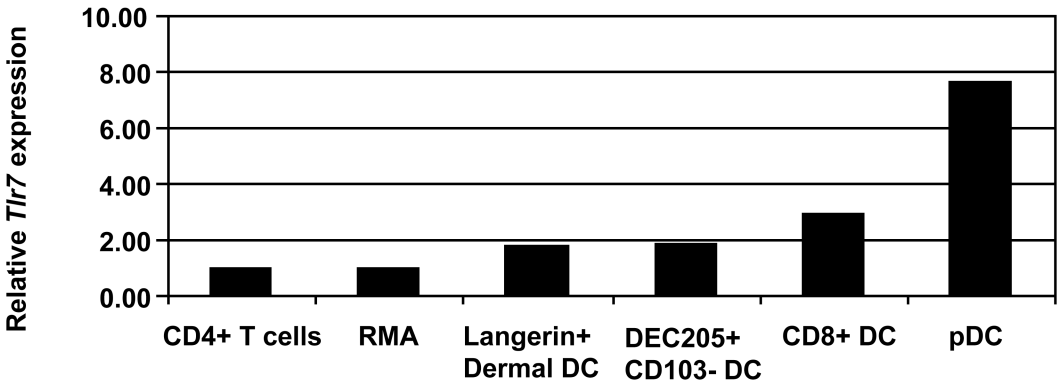

Figure S7: *Tlr7* is expressed in pDCs of naïve LNs. PLNs of naïve mice were pooled and the indicated DC subsets were sorted into RNAlater. RMA cell line was used as a negative controls. RNA was extracted and a quantitative RT-PCR was performed. B2M was used to normalize the RNA input levels. Data is shown as fold increase using RMA cell line expression as baseline.