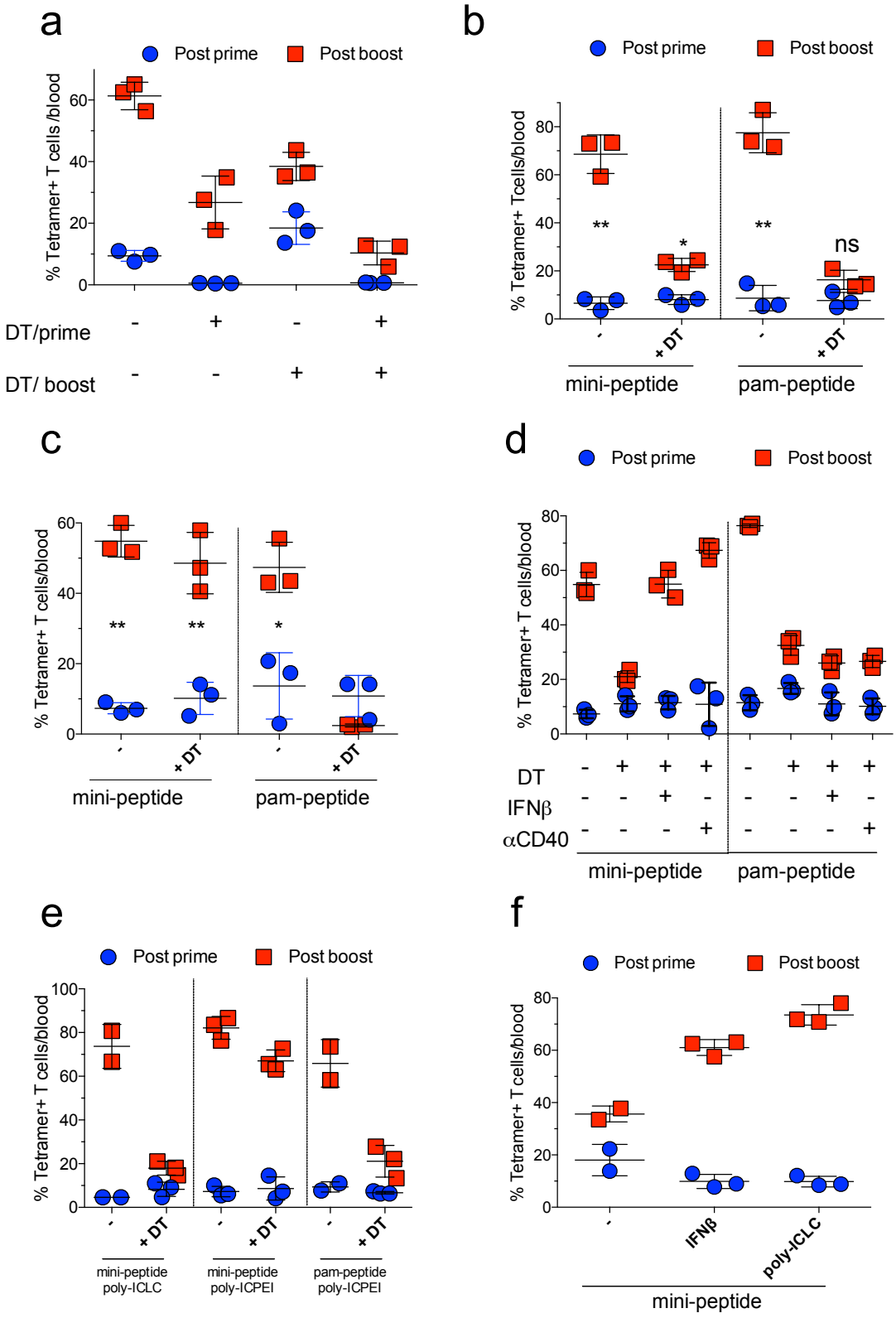
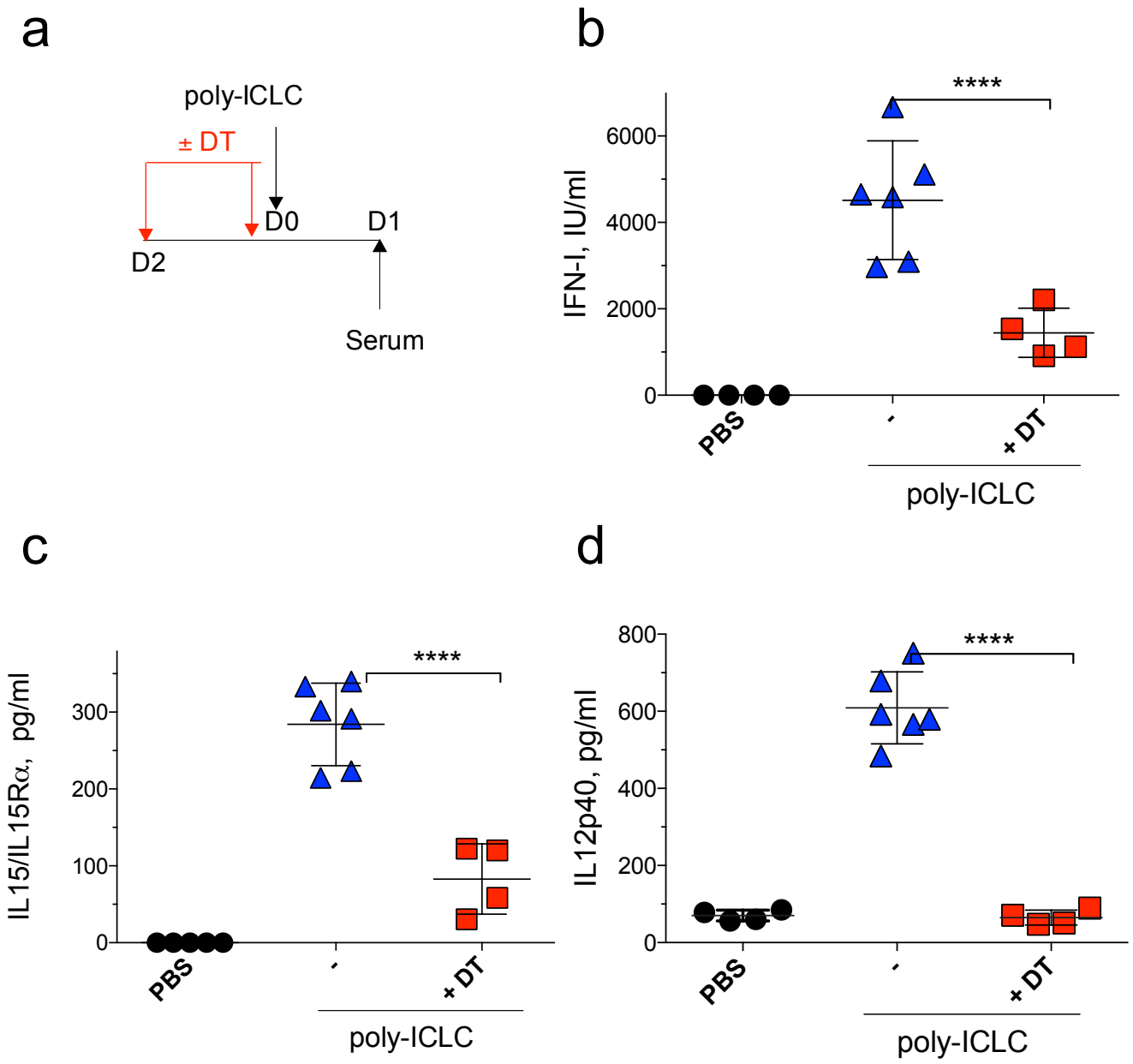
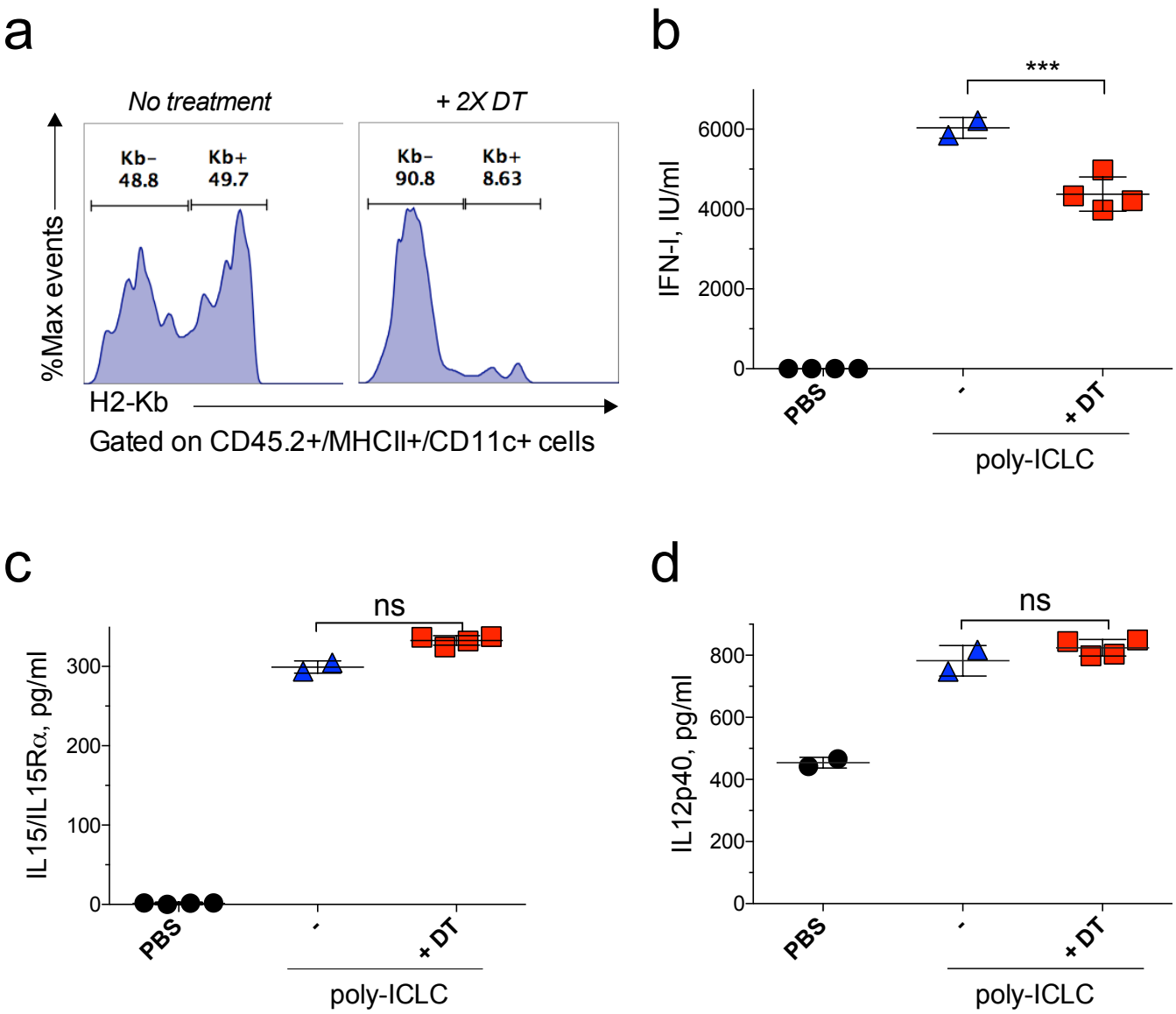


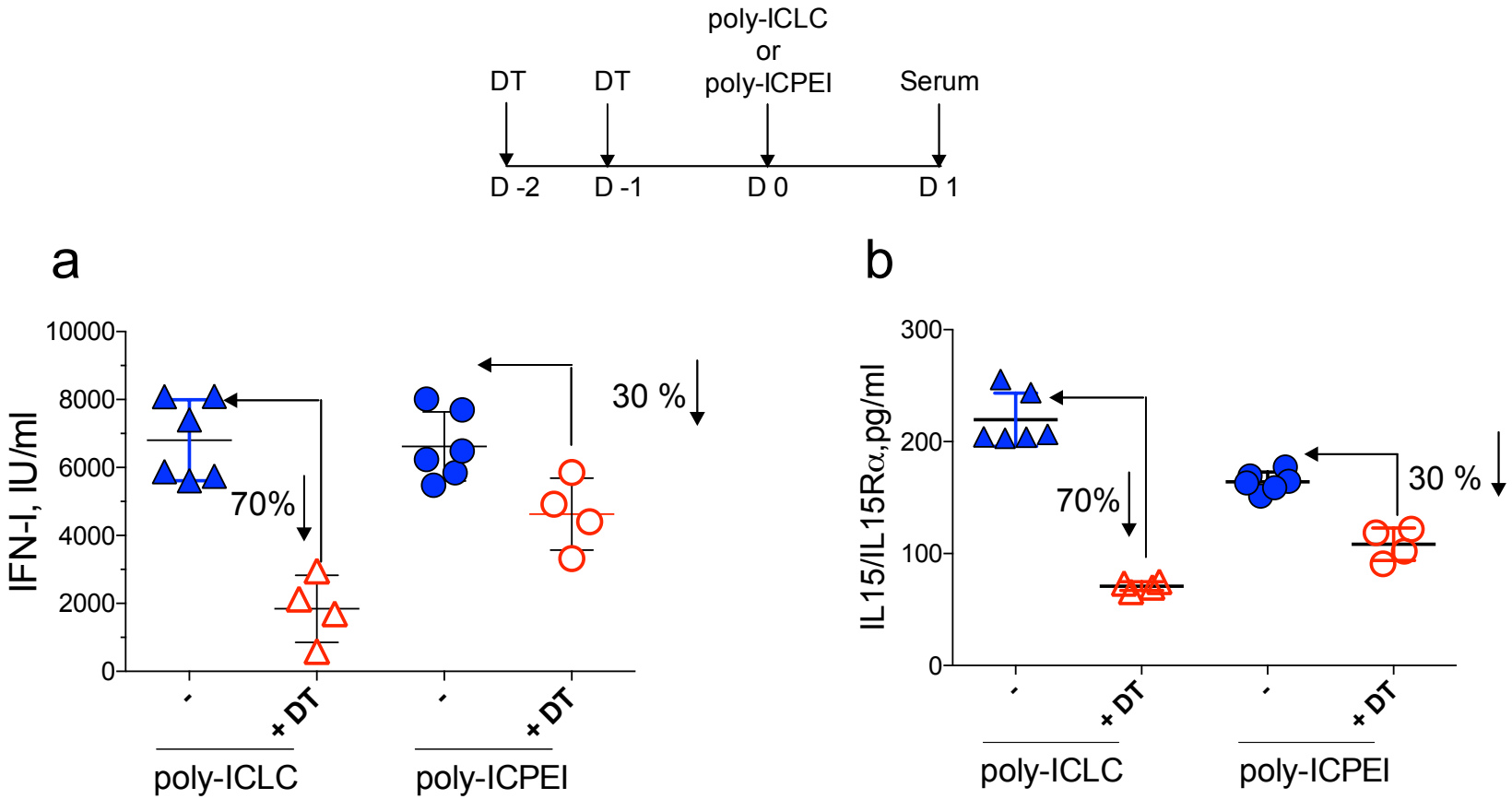
Supplemental Fig. 1: Role of DCs for T cell expansion induced by peptide vaccination. **a-f** Percentages of tetramer+ T cells (OT-I cells) among the total CD8 population in blood 7 days after the prime and the boost of the experiments in **Fig. 1** (shown following the same order). Results are presented as individual mice (each symbol) with the mean ± SD for each group.



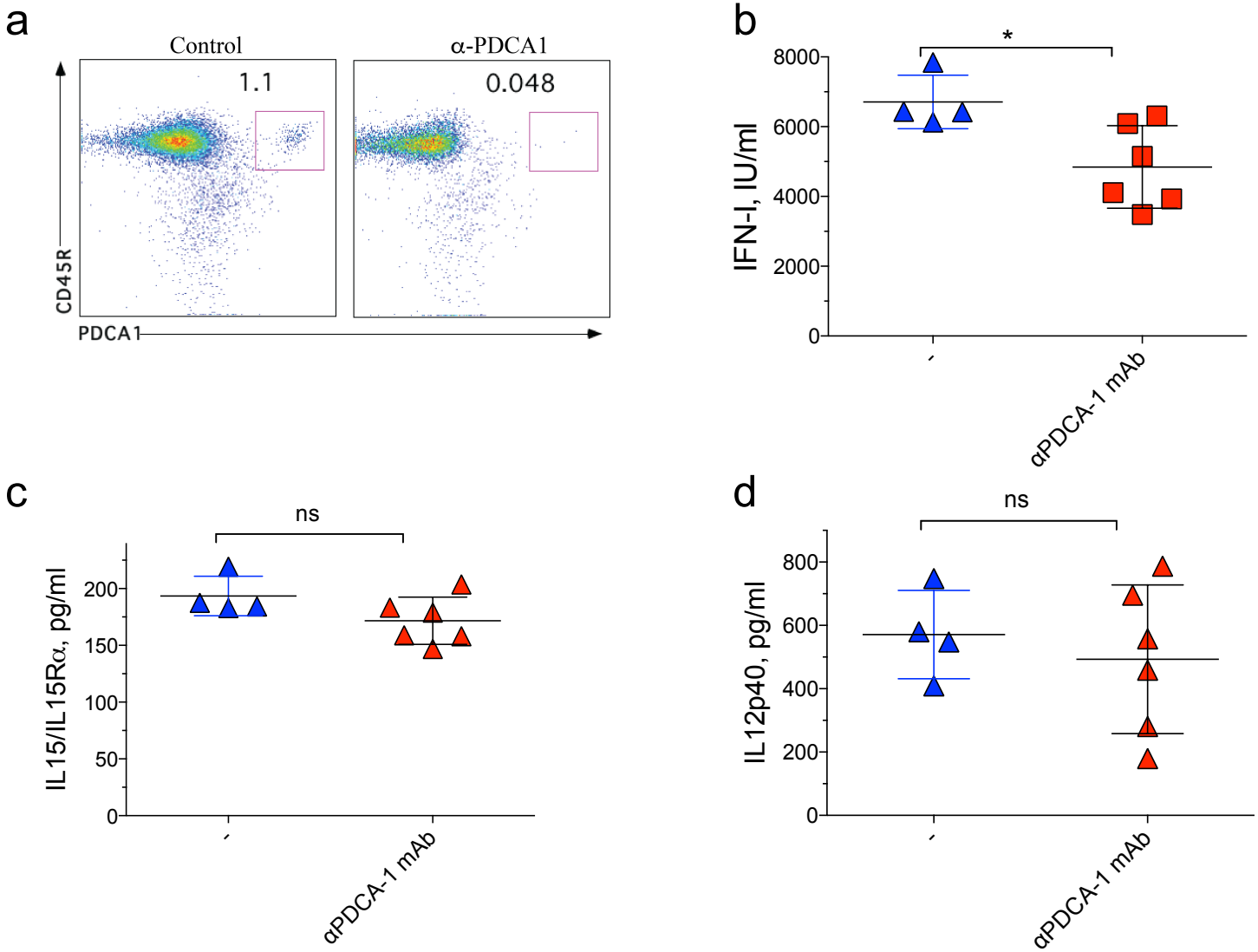
Supplemental Fig. 2: DC depletion diminishes cytokine production by poly-ICLC. **a,** CD11cDTR BM chimeric mice were treated or not with diphtheria toxin (DT) and were injected with 50 µg poly-ICLC (i.v.) **b-d**, 18 h later the levels of IFN-I, soluble IL15/IL15Rα complexes and IL12p40 were measured in serum. Results are presented as individual mice (each symbol) with the mean ± SD for each group.



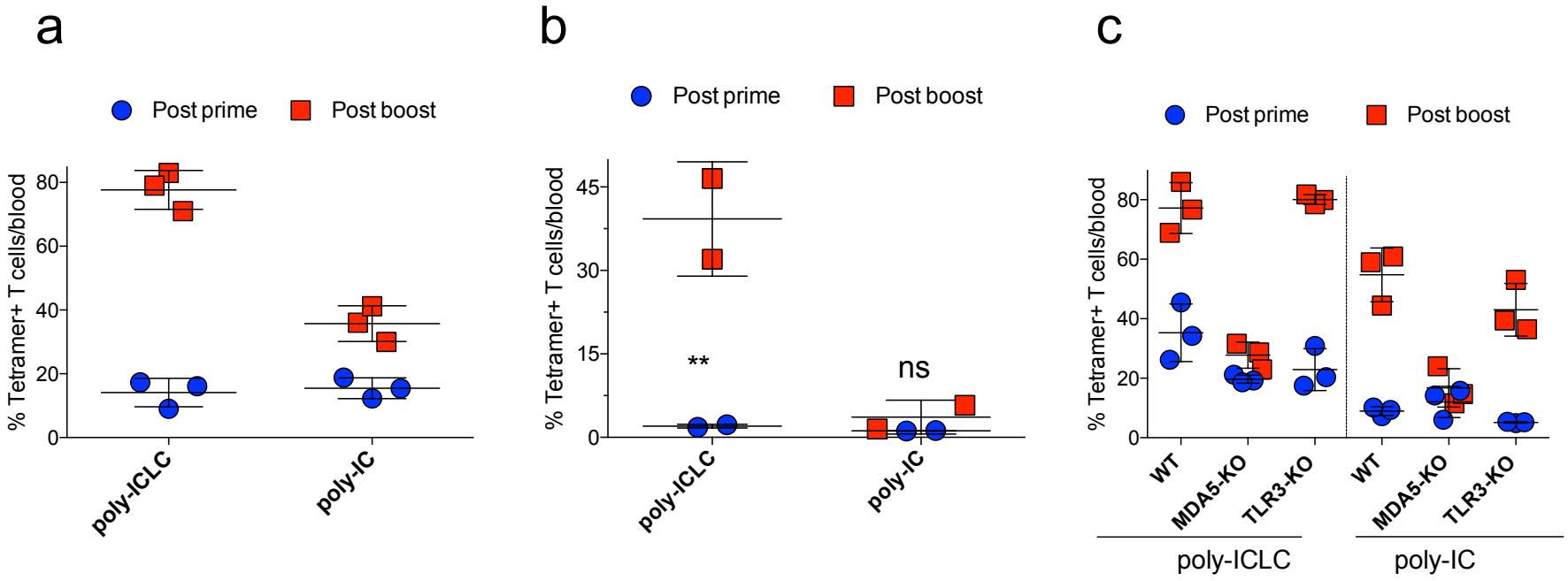
Supplemental Fig. 3: Cytokine production by DCs from mixed BM (CD11cDTR+β2M-KO) chimeras. a, Flow cytometry analysis showing efficient depletion of MHC-I+ CD11c+ spleen cells in double BM chimeras after 2 DT doses (right panel). **b-d,** Mixed BM reconstituted mice were stimulated as described in Supplemental Fig. 2a and 18 h later the levels of IFN-I, IL15/IL15Rα complexes and IL12p40 were measured in serum. Results are presented as individual mice (each symbol) with the mean ± SD for each group.



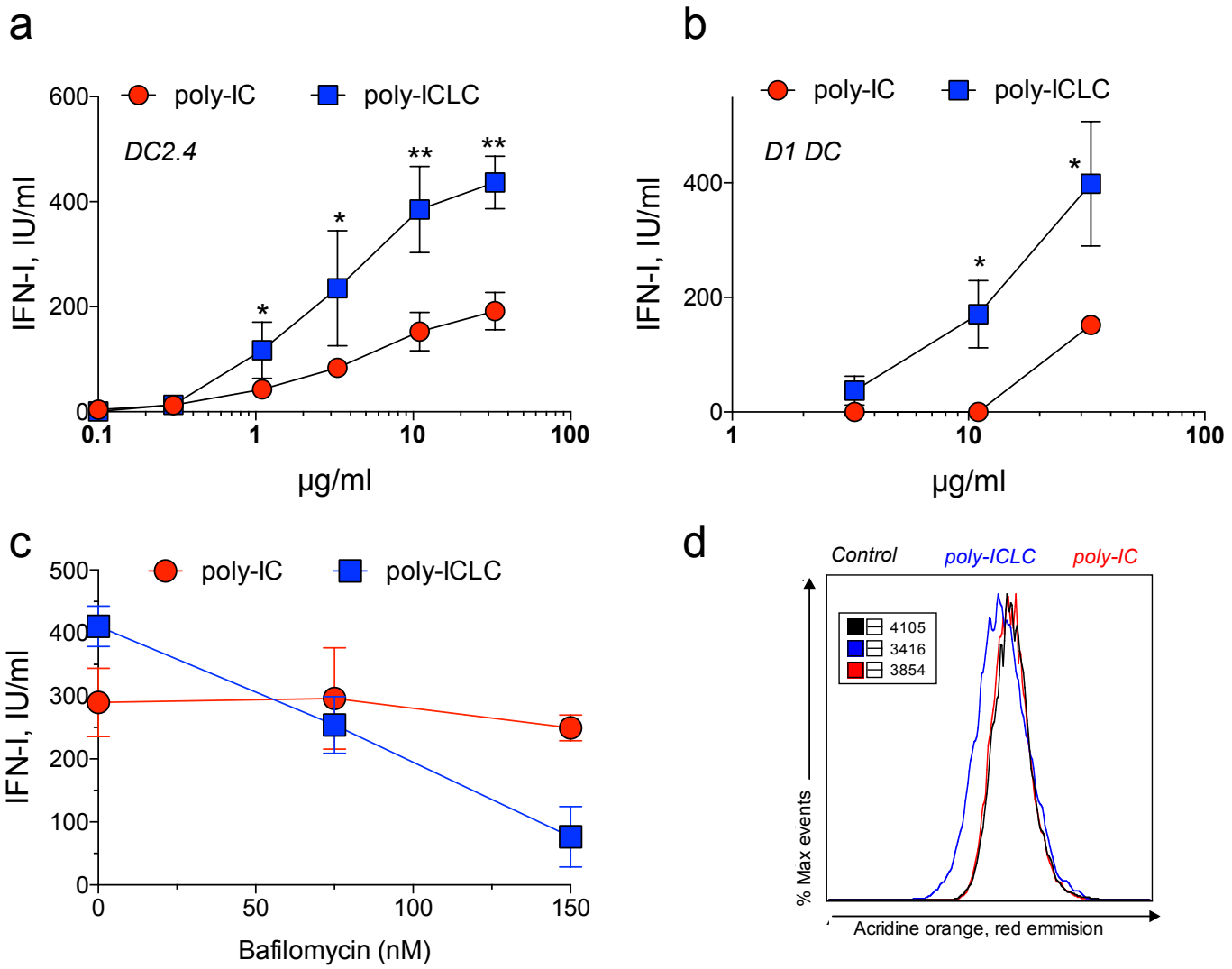
Supplemental Fig. 4: Poly-ICPEI induces IFN-I production in the absence of DCs.
a-b, DC (CD11c+ cells)-depleted mice (+DT) or control (-DT) mice were injected with 50 µg poly-ICLC or 50 µg poly-ICPEI and 18 h later IFN-I and IL15/IL15Rα complexes levels were measured in serum. Results are presented for individual mice (each symbol) with the mean ± SD for each group. Percentages denote the reduction of IFN-I and IL15/IL15Rα induced by DC depletion.



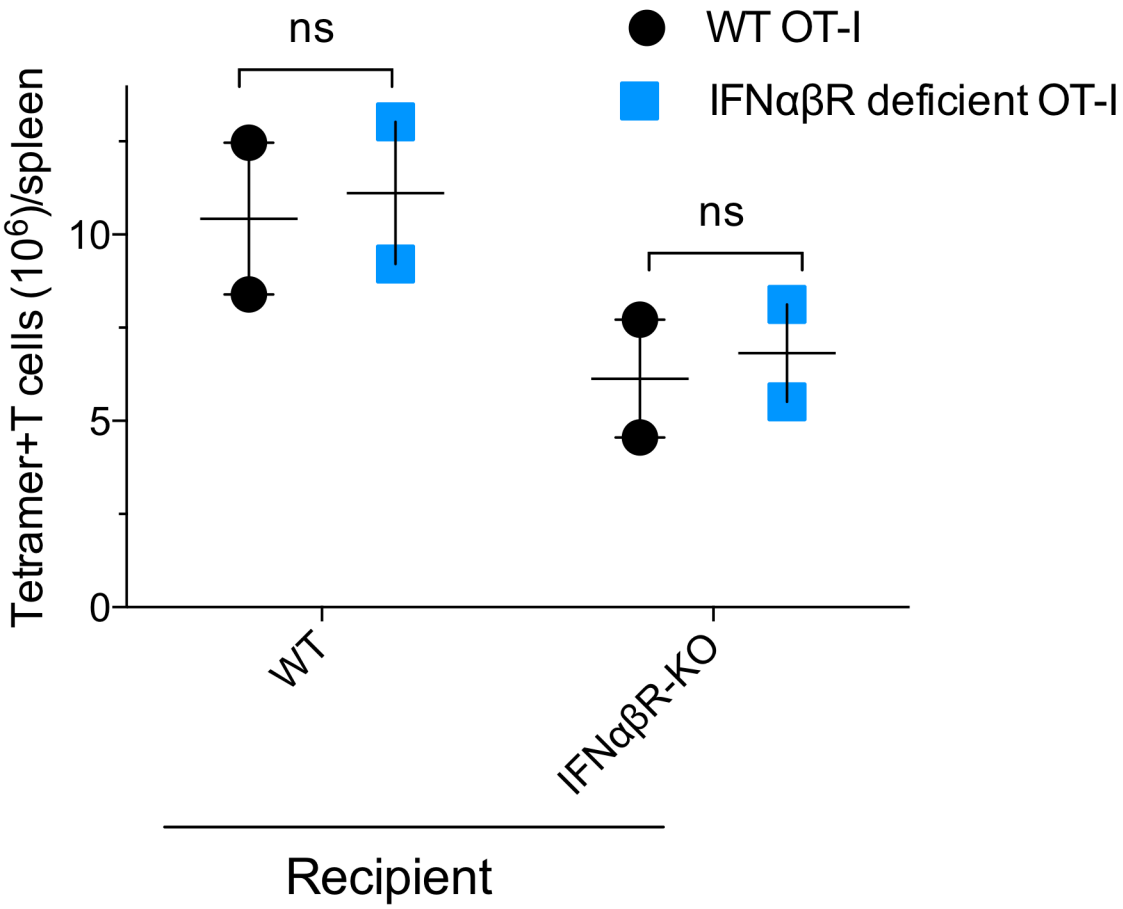
Supplemental Fig. 5: Cytokine production by poly-ICLC can occur in the absence of plasmacytoid DCs (pDCs). a, Percentages of pDCs (B220/CD45R+, PDCA-1+) in WT mice injected with 400 μg of αPDCA-1 mAb (clone BX444) or PBS, gated on the MHC-II+ population and using a different αPDCA-1 mAb (clone eBio927). **b-d,** Production of IFN-I, IL15/IL15Rα complexes and IL12p40 in serum 18 h after poly-ICLC injection. Results are presented as individual mice (each symbol) with the mean ± SD for each group.



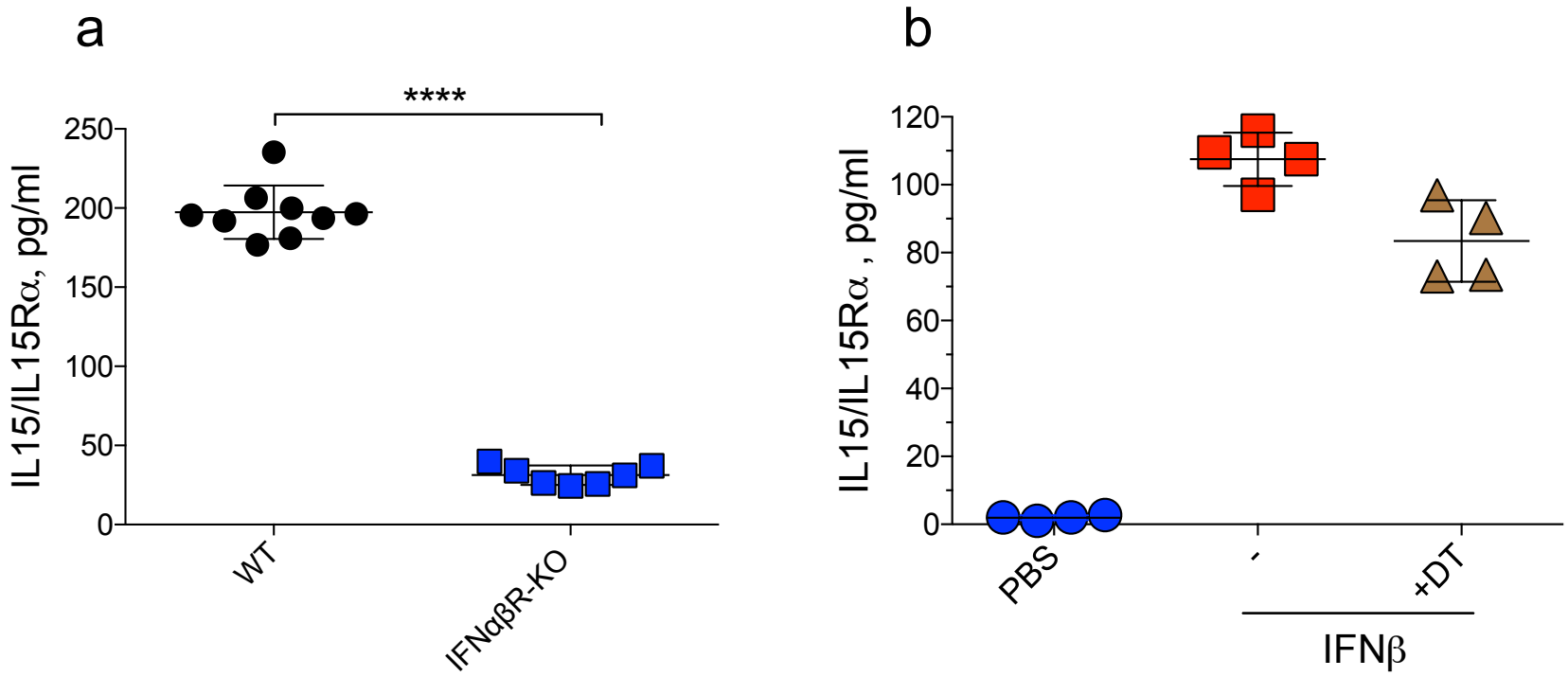
Supplemental Fig. 6: Differences in poly-IC formulations affect CTL responses. a-c, Percentages of tetramer+ CD8+ T cells (OT-I cells) among the total CD8 population in blood 7 days after the prime and boost of mice described in **Fig. 3a-c** (shown following the same order). Results are presented as individual mice (each symbol) with the mean ± SD for each group.



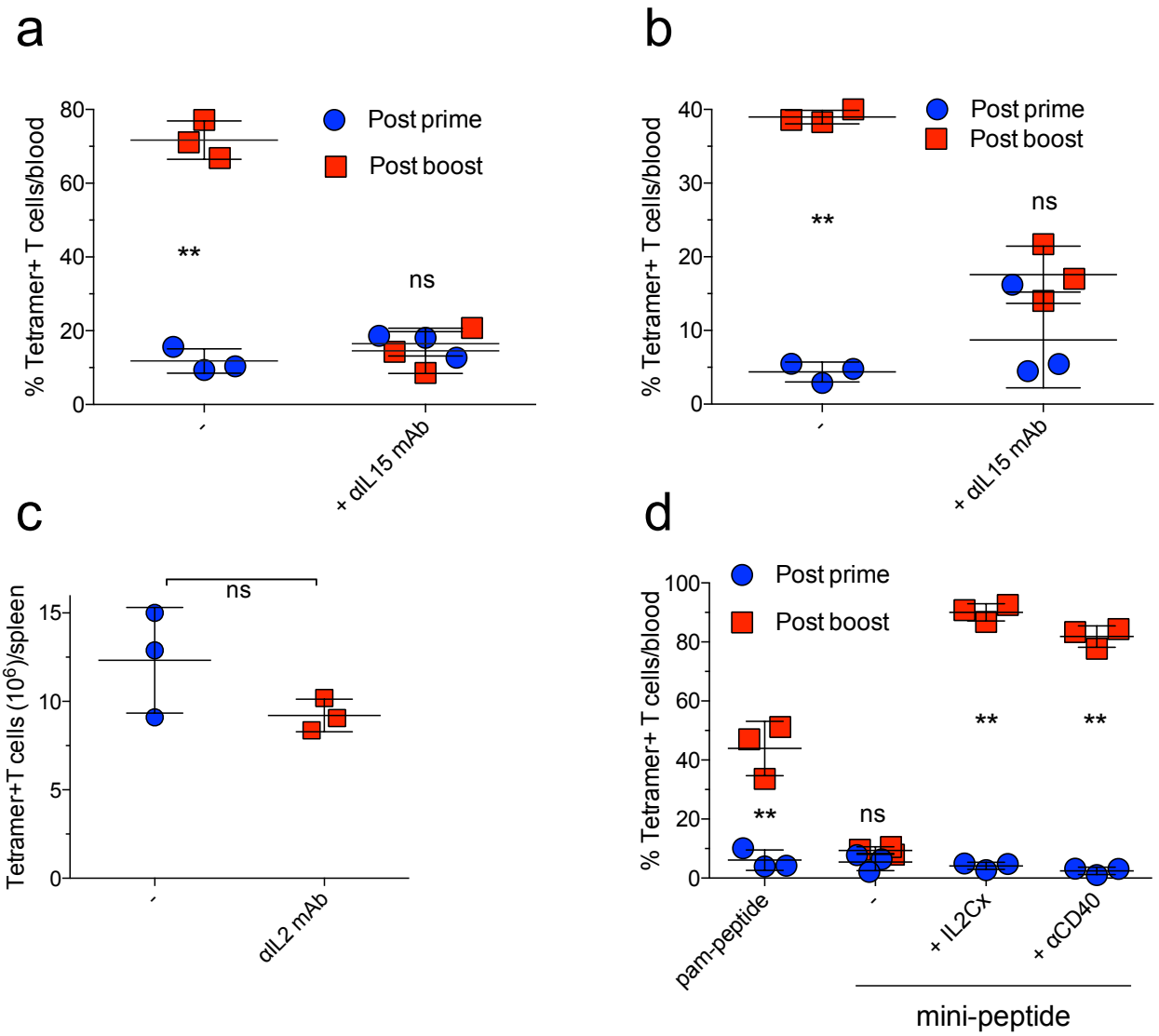
Supplemental Fig. 7: Poly-ICLC stimulates MDA5 in DCs via the proton sponge effect. **a-b,** Immortalized DC lines, DC2.4 and D1 DC were stimulated with serial dilutions of poly-IC or poly-ICLC and IFN-I production was evaluated 18 h later. **c,** The CD8αDC cell line was incubated with bafilomycin A1 6 h before stimulation with poly-IC (100 µg/ml) or poly-ICLC (25 µg/ml) and the production of IFN-I was quantified. **d,** DC2.4 cells were stained with acridine orange, were incubated with 50 µg poly-IC or poly-ICLC and the decrease of fluorescence emission at 600–650 nm reflecting endosomal escape (pH increase) was assessed 18 h later.



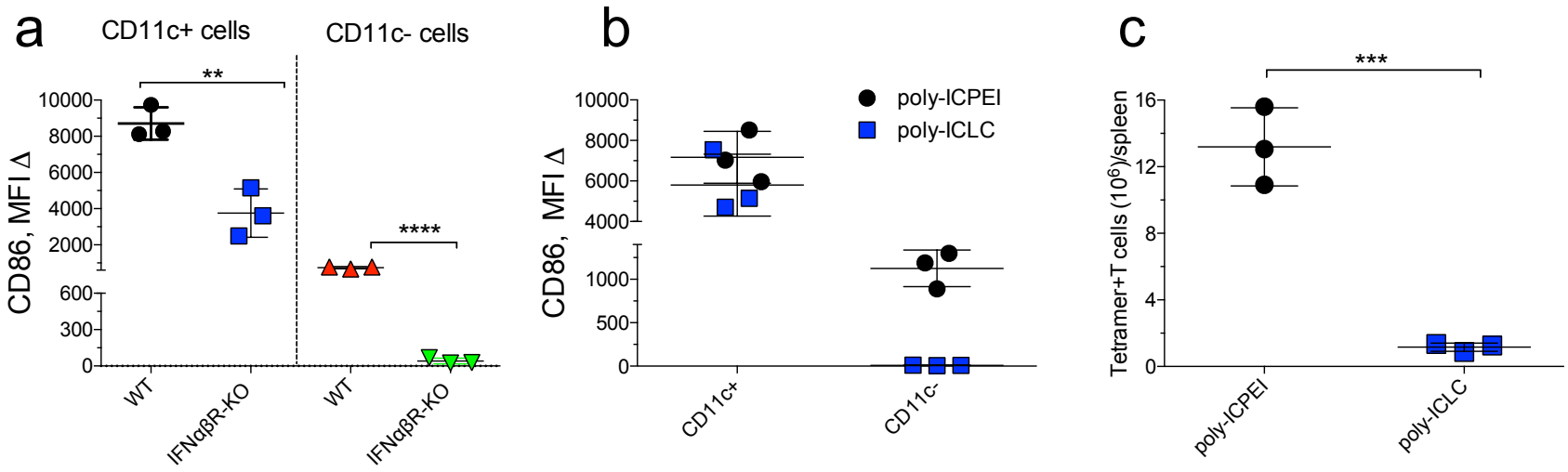
Supplemental Fig. 8: Peptide + poly-ICLC vaccines are effective in expanding low numbers of T cells independently of their expression of IFNαβR. WT and IFNαβR-KO mice received equal numbers (1000 each) of IFNαβR-KO OT-I cells (CD45.1+/CD45.2+) and WT OT-I cells (CD45.1+/CD45.2-) followed by pam-Ova + poly-ICLC prime-boost vaccination administered 14 days apart and numbers of the OT-I populations in spleens obtained 7 days after the boost are shown.



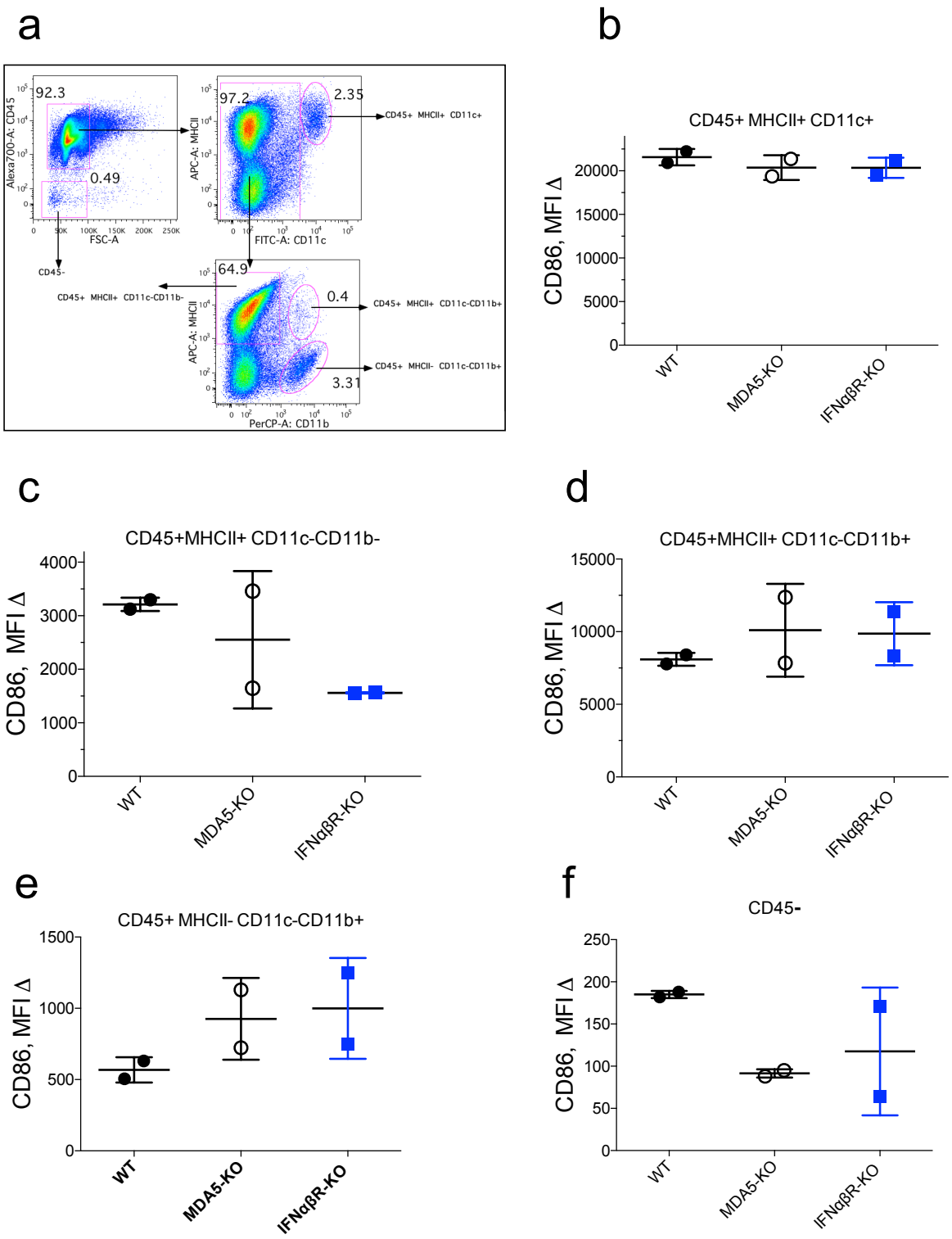
Supplemental Fig. 9: Poly-ICLC and IFN-I can generate IL15 in vivo. **a,** WT or IFNαβR-KO mice were injected (i.v.) with 50 μg poly-ICLC and 18 h later the production of IL15/IL15Rα complexes was measured in serum. **b,** CD11cDTR chimeric mice were depleted (or not) of DCs by 2 sequential DT injections (2 days apart) followed by 2 doses of IFN-β (10,000 IU/mouse, 12 h apart) and 6 h later levels of soluble IL15/IL15Rα complexes in sera were measured. Control mice injected with PBS instead of IFN-β were also DC depleted.



Supplemental Fig. 10: IFN-I signaling leads to IL15 production enabling CTL expansion. a-b, Percentage of tetramer+ CD8+ T cells (OT-I cells) among the total CD8 population in blood 7 days after prime and boost of the mice described in **Fig. 6c-d** (shown following the same order). **c,** WT mice received 10^5 naïve OT-I cells followed by 2 doses of pam-Ova + poly-ICLC vaccine administered 14 days apart. Some mice received 300 µg αIL2 mAb (JES6-5H4) on days 14, 16 and 18 and the numbers of OT-I cells in spleens were evaluated. **d,** Percentages of tetramer+ CD8+ T cells (OT-I cells) among the total CD8 population in blood after prime and boost of mice described in **Fig. 6f.** Results are presented as individual mice (each symbol) with the mean ± SD for each group.



Supplemental Fig. 11: Upregulation of CD86 expression in npAPCs may support T cell expansion in the absence of IFN-I signaling. a, WT or IFNαβR-KO mice were injected (i.v.) with 50 μg poly-ICLC and 18 h later the levels of CD86 were evaluated in CD11c+/MHC-II+ and CD11c-/MHC-II+ spleen cells. **b,** IFNαβR-KO mice were injected (i.v.) with 50 μg poly-ICPEI or poly-ICLC and 18 h later the levels of CD86 expression in CD11c+/MHC-II+ and CD11c-/MHC-II+ spleen cells were measured. **c**, IFNαβR-KO hosts received 10^5 naïve IFNαβR-KO OT-I cells and a pam-Ova + poly-ICLC vaccine prime followed 14 days later with boosts with either mini-Ova + poly-ICLC or mini-Ova + poly-ICPEI. The number of OT-I cells in spleens was evaluated 7 days after the boost. Results for **a-b** are presented as the increase of mean fluorescence intensity (MFI Δ) of CD86 surface expression in individual mice (each symbol) with the mean ± SD for each group, and for **c,** numbers of tetramer+ OT-I T cells per spleen in individual mice (with the mean ± SD).



Supplemental Fig. 12: Anti-CD40 mAb treatment upregulates CD86 expression. a-f, WT, MDA5 or IFNαβR-KO mice were injected with 100 μg αCD40 mAb and 18 h later, CD86 expression was assessed in different spleen cell subsets based on the gating strategy described in **a**. Results are presented for individual mice (each symbol) with the mean ± SD for each group.