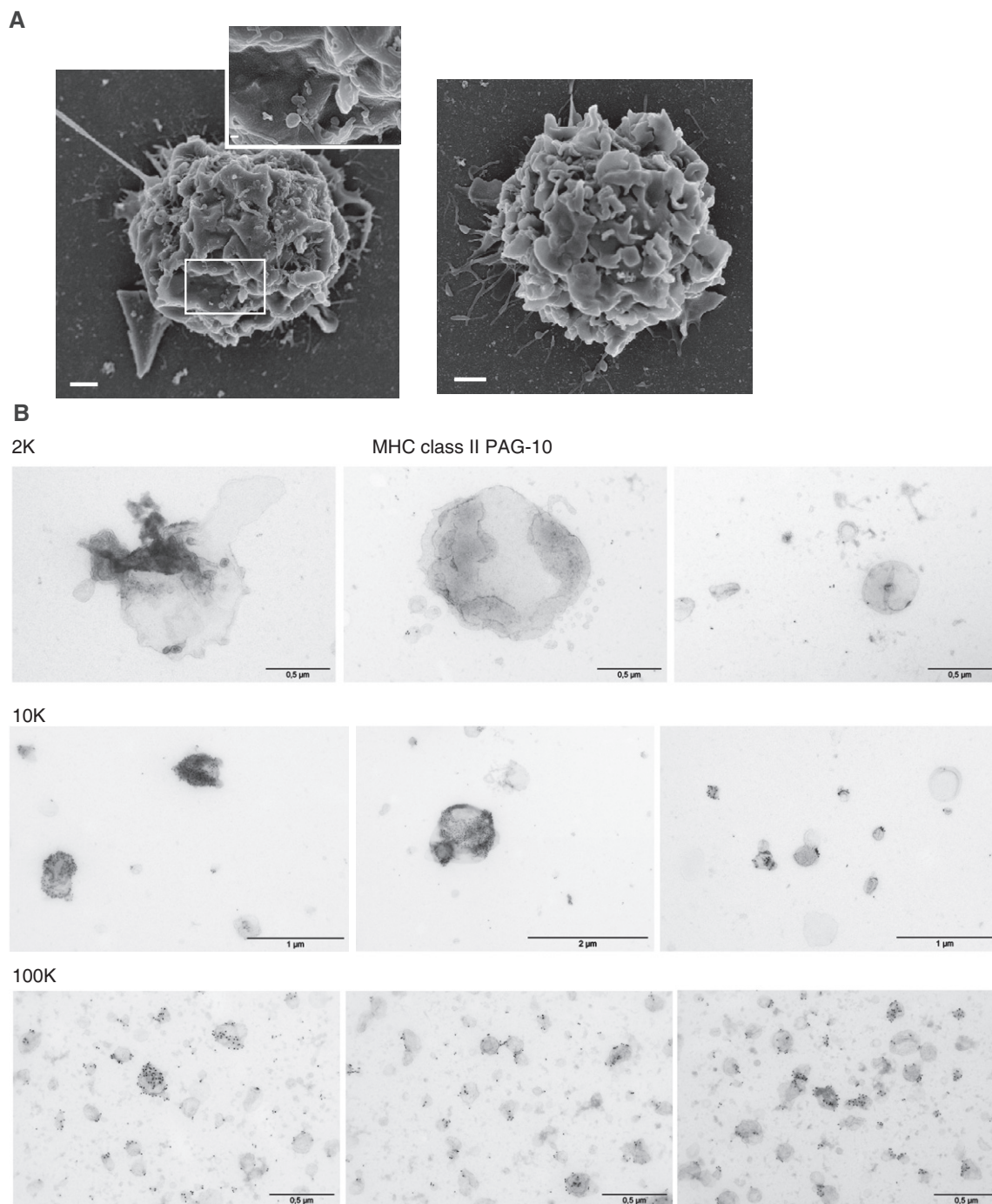


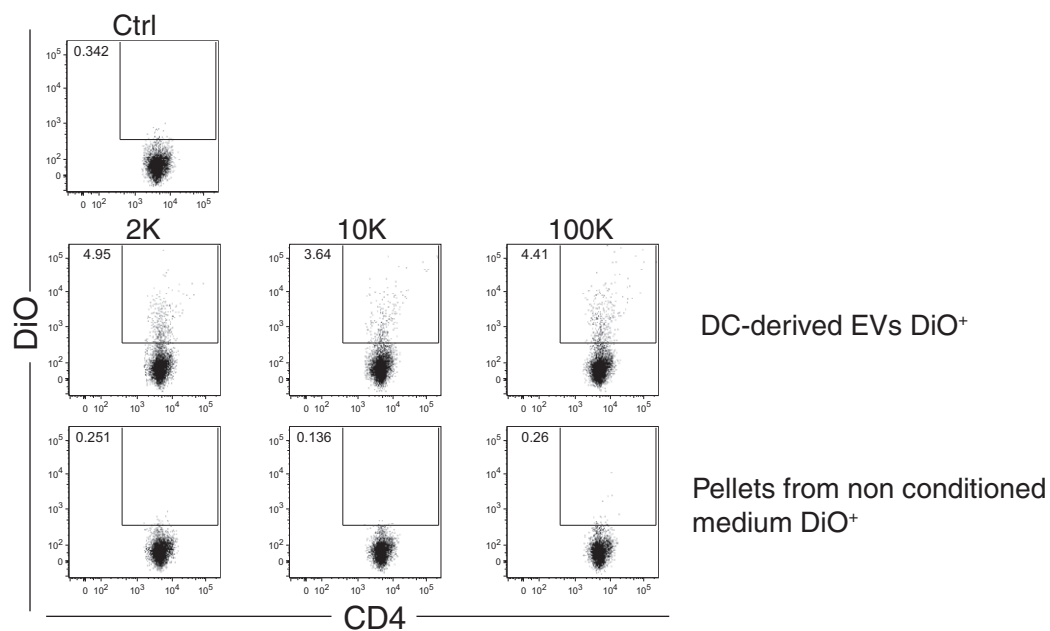
## Expanded View Figures



**Figure EV1. SEM of immature DCs and whole-mount TEM analysis of each pellet.**

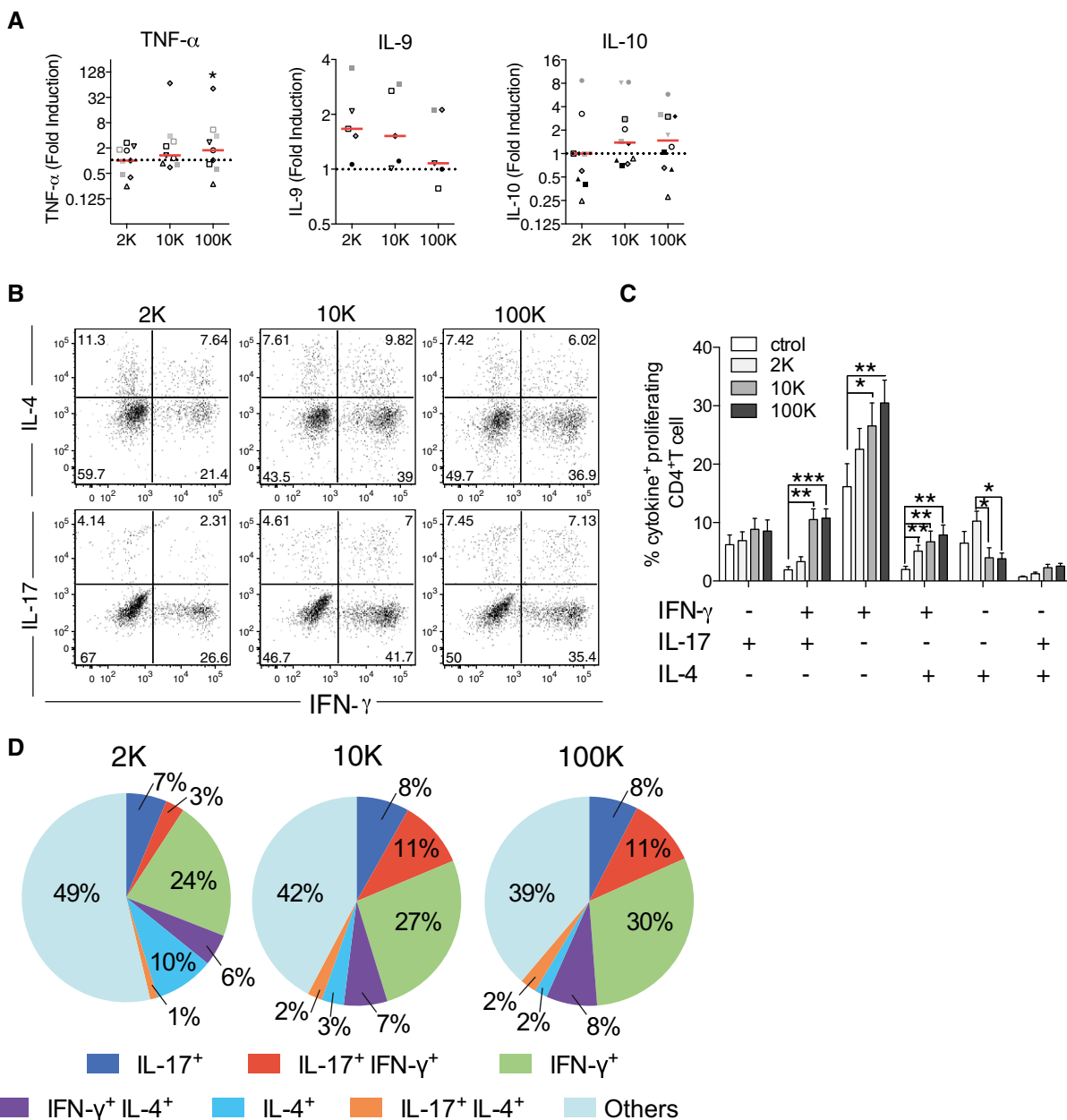
A DCs were loaded on poly-lysine-coated slides, fixed and analysed by SEM. Two DCs are shown as representative images. Scale bars = 1  $\mu$ m for the whole cell and 100 nm for the inset.

B Whole-mount EVs were stained for MHC II (PAG10) and analysed by TEM. Images are shown for the different pellets.



**Figure EV2. Flow Cytometry analysis of fluorescently labelled-EV transfer to CD4<sup>+</sup> T cells.**

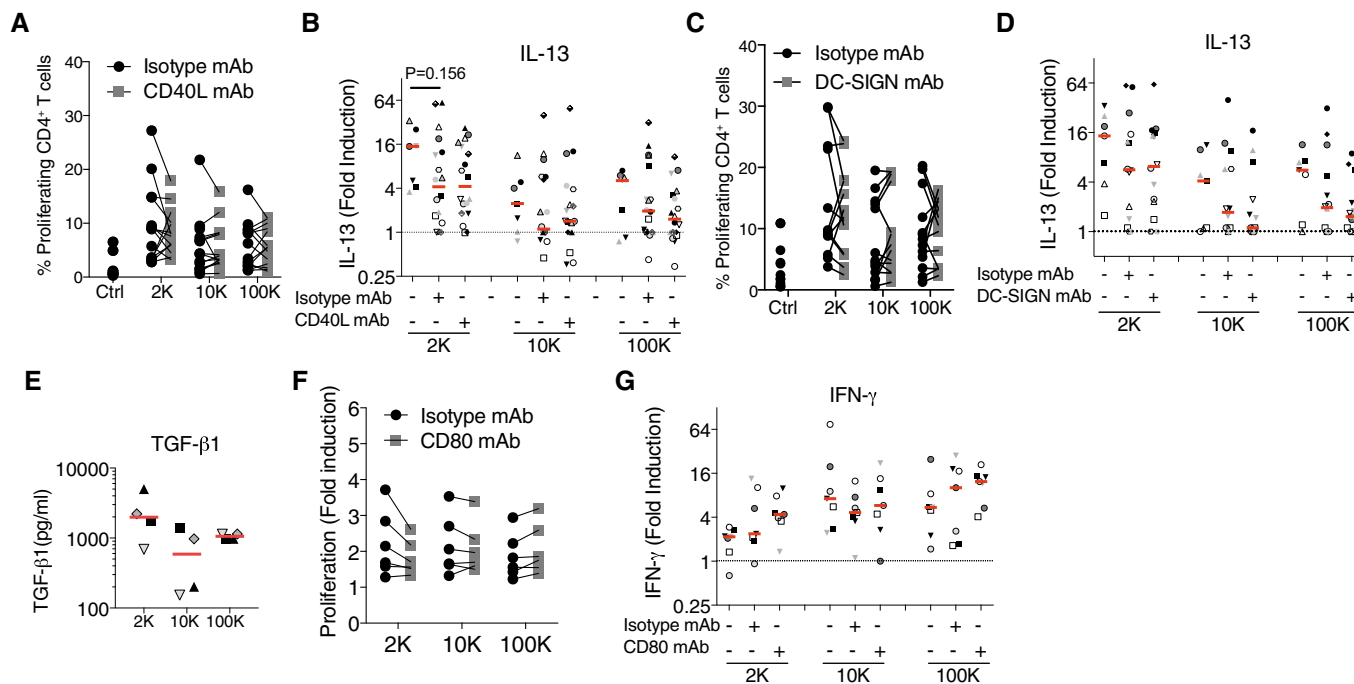
EV pellets (2K, 10K and 100K) coming from conditioned medium of DCs cultures or the pellets from non-conditioned medium (depleted of serum EVs) were labelled with DiO lipophilic dye, washed in PBS and re-ultracentrifuged three times and cultured for 18 h with allogeneic primary total CD4<sup>+</sup> T cells. Transfer of the dye was analysed by flow cytometry. Dot-plot graphs show the percentage of CD4<sup>+</sup> DiO<sup>+</sup> cells for both EV-treated and control-treated CD4<sup>+</sup> T cells.



**Figure EV3. Release of IL-9, IL-10 and TNF- $\alpha$  and intracellular production of IL-4, IFN- $\gamma$  and IL-17 in CD4<sup>+</sup> T cells upon DC-derived EV subtypes stimulation.**

**A** IL-9, IL-10 and TNF- $\alpha$  cytokine concentration was measured in culture supernatant after 6 days of culture of total CD4<sup>+</sup> T cell with DC-derived EVs by cytometric bead array ( $n = 11$ , one symbol per donor). Red line indicates median.

**B–D** Cultured T cells were re-stimulated for 4 h with PMA and ionomycin in the presence of brefeldin A and permeabilized and stained for IFN- $\gamma$ , IL-4 and IL-17A. Proliferating cells were gated and evaluated for their single or double intracellular cytokine staining. Representative plots (B), quantification of the percentage (C) and relative proportions (D) of single or double positive proliferating CD4<sup>+</sup> T cells for each cytokine (IL-4<sup>+</sup>, IL-17<sup>+</sup>, IFN- $\gamma$ <sup>+</sup>, IFN- $\gamma$ <sup>+</sup>IL-17<sup>+</sup>, IL-4<sup>+</sup>IL-17<sup>+</sup> and IFN- $\gamma$ <sup>+</sup>IL-4<sup>+</sup>) are shown (four independent experiments,  $n = 12$  donors). In (C), the mean of 12 donors  $\pm$  SEM is shown. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  (Friedman test).

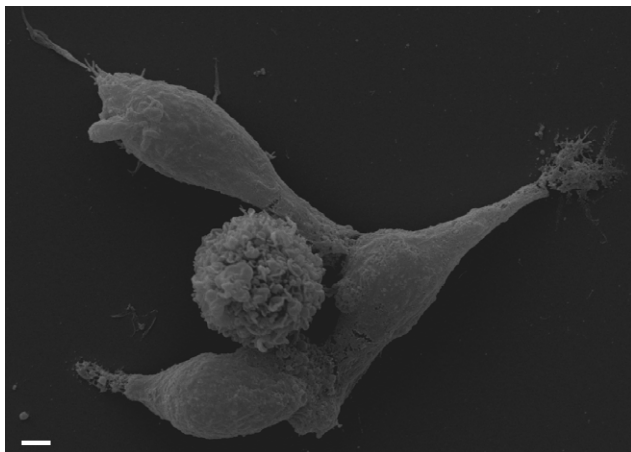


**Figure EV4. Blockade of CD40–CD40L interaction or of DC-SIGN decreases the response induced by sEVs, whereas blockade of CD80 decreases the response induced by iEVs.**

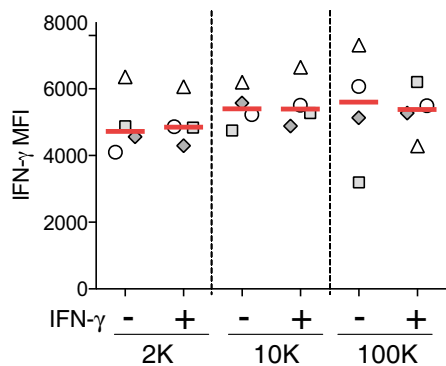
- A, B DC-derived EVs (from  $8 \times 10^6$  secreting cells) were cultured with total CD4<sup>+</sup> T cells pre-incubated with blocking antibodies against CD40L. Proliferation (A), secretion of IL-13 for the 2K, 10K and 100K (B) were evaluated after 6 days ( $n = 7-18$ , each symbol represents a DC-EV:T cell donor combination). *P*-value was calculated using a Wilcoxon signed-rank test.
- C, D DC-derived EVs (from  $8 \times 10^6$  secreting cells) were pre-incubated with blocking antibodies against DC-SIGN for 30 min and then cultured with total CD4<sup>+</sup> T cells. Proliferation (C), and secretion of IL-13 for the 2K, 10K and 100K (D) were evaluated after 6 days ( $n = 7-14$ , each symbol represents a DC-EV:T cell donor combination).
- E The presence of TGF-β1 in the 2K, 10K and 100K derived from  $10 \times 10^6$  cells was quantified by a high sensitivity ELISA ( $n = 4$ , one symbol per donor).
- F, G DC-derived EVs (from  $2 \times 10^6$  secreting cells) were pre-incubated with blocking antibodies against CD80 for 30 min and then cultured with total CD4<sup>+</sup> T cells. Proliferation of CD4<sup>+</sup> T cells was measured as the fold induction of the absolute cell number of each treatment to the absolute number of unstimulated CD4<sup>+</sup> T cells at the end of the culture (F). Secretion of IFN-γ for the CD4<sup>+</sup> T cells stimulated with the 2K, 10K and 100K is shown (G) ( $n = 7$  DC-EV:T cell combinations, one symbol per each).

Data information: (B, D, E and G) Red line indicates the median.

A



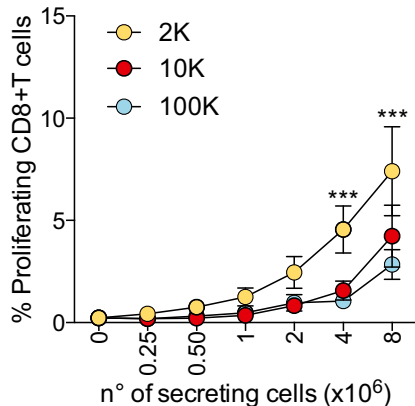
B



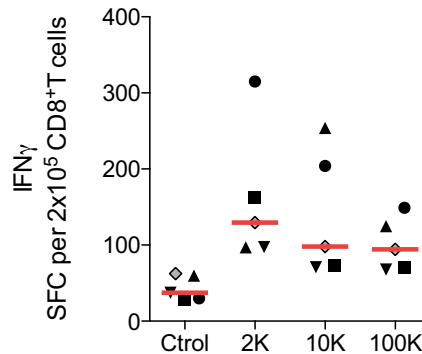
**Figure EV5. Analysis of IFN-γ incubated DCs and their EVs.**

A IFN-γ-treated DCs were loaded on poly-lysine-coated slides, fixed and analysed by SEM. Four DCs are shown as representative images. Scale bar = 1 μm.  
 B The different pellets were analysed by direct flow cytometry, to measure the presence of IFN-γ on EV surface. EVs were immunostained for IFN-γ and analysed as for Fig 1D. Quantification of the MFI is shown (Gmean specific antibody–Gmean isotype antibody) (n = 4, one symbol per donor). Red line indicates median.

A



B



**Figure EV6. CD8<sup>+</sup> T-cell activation induced by DC-derived EVs.**

A Total CD8<sup>+</sup> T-cell proliferation was evaluated after 6 days of culture with different amounts of allogeneic DC-derived EVs. Proliferation was calculated by dilution of Cell Trace Violet dye (n = 5 donors, mean ± SEM is shown). \*\*\*P < 0.001 2K compared to 10K and 100K (Friedman test).  
 B EVs purified from DCs incubated with an MHC class I-restricted CEF viral peptides were cultured with total autologous CD8<sup>+</sup> T cells on ELISPOT plates coated with IFN-γ antibodies. After 48 h, the ELISPOT plates were developed and the number of spots per well was counted. The results are expressed as mean number of SFC/ 2 × 10<sup>5</sup> CD8<sup>+</sup> T cells (n = 5 donors, one symbol per donor). Red line indicates median.