



SUPPLEMENTARY FIG. S7. (A) Quantitation of Fig. 4D—Incubation of DC culture supernatants with α CTLA-4-coated beads blocks subsequent uptake of CFSE-labeled EV by unlabeled DC. Incubation of DC culture supernatants with α CTLA-4-coated beads blocked the subsequent uptake of CFSE-labeled EV by unlabeled DC in a titratable manner. At an antibody concentration of 5 μ g/mL, 26% fewer recipient DC internalized CFSE⁺ EV (* P < 0.05), whereas at an antibody concentration of 50 μ g/mL, 35% fewer recipient DC internalized CFSE⁺ EV (** P < 0.01). Staining of CD11c internal control was not statistically different between groups. Error bars = \pm SD of three independent experiments. (B) Quantitation of S6A—DC-EV CTLA-4 physiologically binds B7. In three experiments, incubation of unlabeled recipient DC with cell culture supernatants derived from CFSE-labeled DC for 20 min at 4°C along with α CD80 and α CD86 flow-qualified antibodies and 0.1% sodium azide resulted in no change in B7 MFI using 1% or 10% supernatant (as well as no detectable uptake of CFSE⁺ vesicles); however, in 100% supernatant, CD80 MFI and CD86 MFI were both significantly reduced as CFSE uptake quintupled. There was no statistical difference in CD11c MFI among any of the dilutions. * P < 0.05. Error bars = \pm SD. (C) Quantitation of S6B—DC-EV CTLA-4 physiologically binds B7. In three experiments, preclearance of CFSE-labeled DC culture supernatants with α CTLA-4 beads for 1 h before incubation of unlabeled DC for 20 min at 4°C along with α CD80 and α CD86 flow-qualified antibodies and 0.1% sodium azide resulted in a titratable and statistically significant increase in B7 MFI with no concomitant increase in CD11c MFI, while percentage of CFSE⁺ cells dropped 45%. * P < 0.05. Error bars = \pm SD. (B, C) Dashed line, percentage of CFSE⁺ cells.