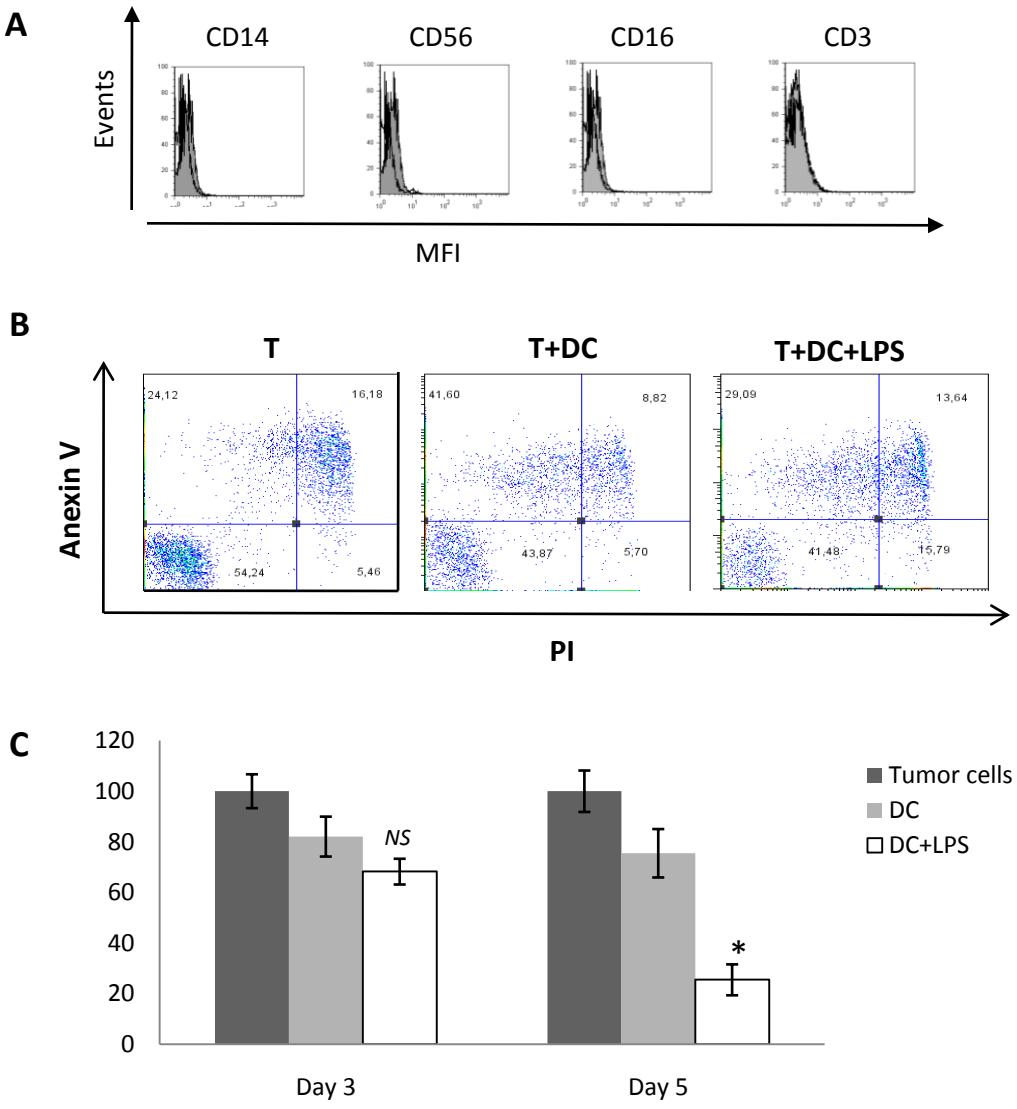
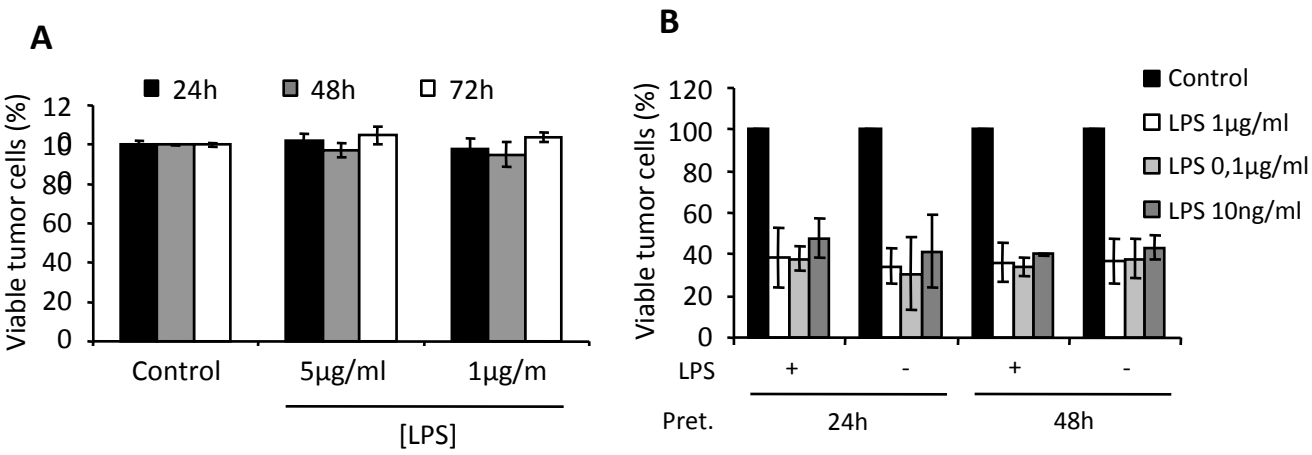
Supplemental figure 1



Supplemental figure 1. *Ex vivo* generated monocyte-derived DC becomes tumor cytotoxic after activation with LPS at day 5

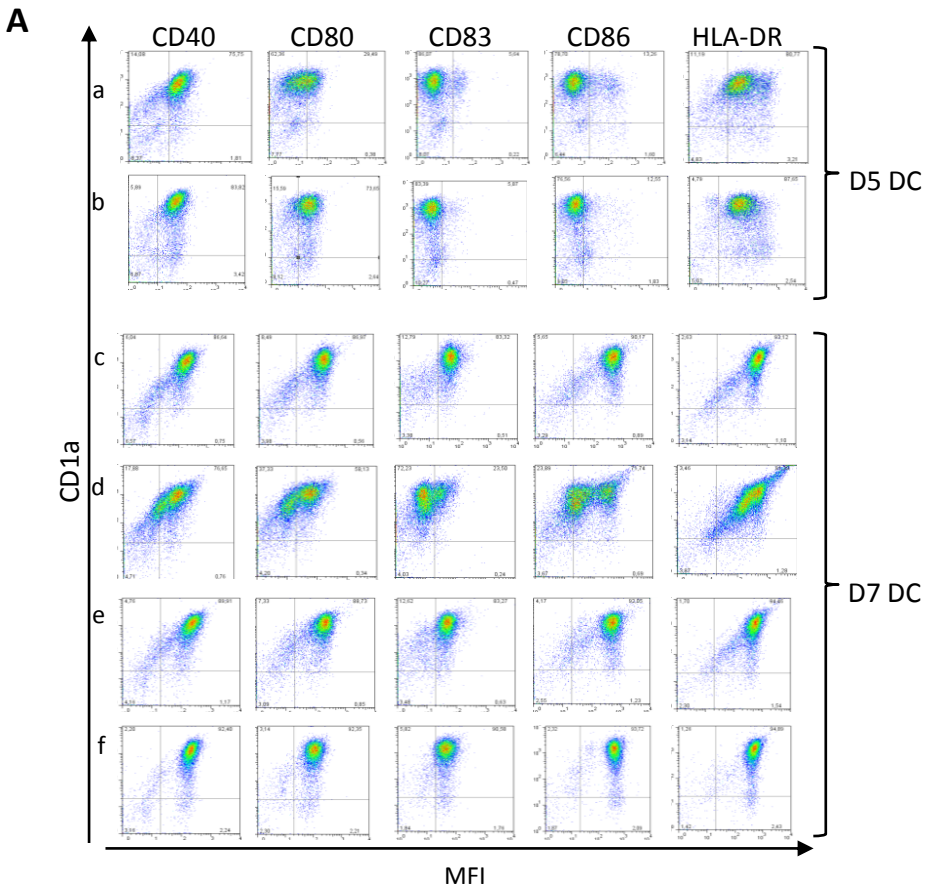
A: Flow cytometry analysis of monocyte-derived DC obtained on day 5**.** Filled gray histograms correspond to isotype controls. **B:** T lymphocytes were cultured alone (T), or with day 5 monocyte-derived DC from healthy donors with (DC+LPS) or without LPS (DC) (DC:T ratio = 5:1). After 48 h, the cells were stained with anti-CD3 Ab and with annexin V-FITC/propidium iodide (PI). The percentage of PI^-/Annexin-V^+ (apoptotic) or PI^+/Annexin-V^+ (necrotic) T cells was determined after gating on CD3 positive cells. **C:** HT29 tumor cells were cultured with day 3 or day 5 immature monocyte-derived DC in the presence or absence of LPS. Tumor cell viability was assessed after 48 h. Supplemental Figure 1A-C: Results are representative of 6 experiments performed with DC generated from 6 healthy volunteers.

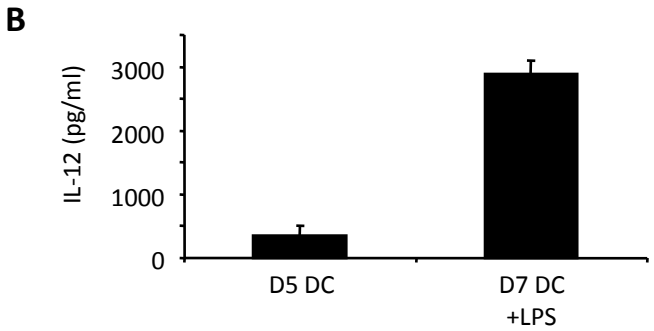
Supplemental figure 2



Supplemental figure 2. LPS does not directly affect tumor cell survival
A: HT29 tumor cells were cultured for 24 h, 48 h or 72 h alone (Control) or with LPS (5 µg/ml or 1 µg/ml) and cell survival was determined as described in materials and methods. **B:** HT29 tumor cells were pre-treated (+) or not (-) for 24 h or 48 h with LPS (1 µg/ml, 0.1 µg/ml or 10 ng/ml). Then LPS was washed out and the cells were cultured for 48 h with LPS activated monocyte-derived dendritic cells and tumor cell survival was assessed. Supplemental figures 2A and 2B are representative of 3 experiments performed in triplicate.

Supplemental figure 3

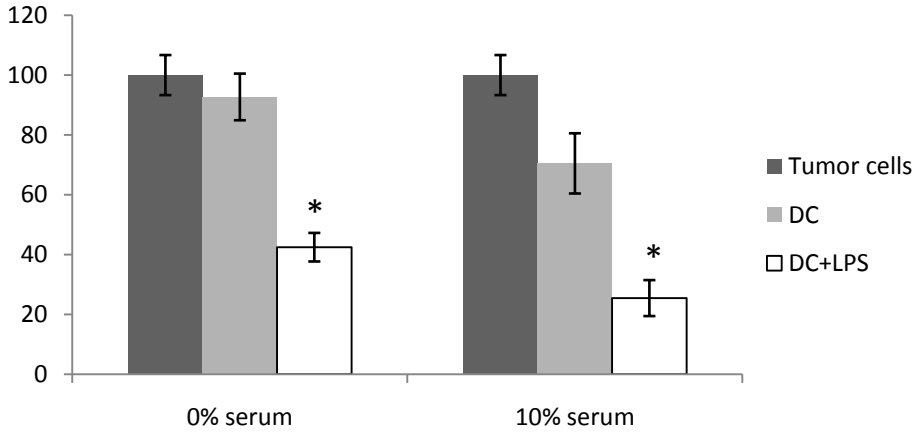




Supplemental figure 3. Phenotypic and functional characteristics of human monocyte-derived DC from healthy donors and from cancer patients

A: Phenotype of day 5 human monocyte-derived DC (D5 DC) generated from healthy donors (a) or from cancer patients (b). Phenotype of day 7 DC generated from patients and healthy donors (D7 DC) after maturation with LPS (100 ng/ml) for 48 h (c), with IFN-γ (1000 UI/ml) for 48 h (d), with TNF-α (20 ng/ml) + poly I:C (50 µg/ml) for 48 h (e), or with IL-1β (25 ng/ml) + IL-6 (10 ng/ml) + TNF-α (50 ng/ml) + PGE2 (1 µg/ml) for 48 h (f). Cells were stained with the indicated Ab and analysed by flow cytometry. **B:** IL-12 production was evaluated by ELISA in the supernatant of day 5 DC culture alone (D5 DC) or day 5 DC treated with LPS (100 ng/ml) for 48 h (D7 DC+LPS). Supplemental figure 3A-B: Results are representative of 6 experiments performed with DC generated from 6 healthy volunteers and 6 cancer patients.

Supplemental figure 4



Supplemental figure 4. hKDC can be generated in clinically relevant conditions
Immature day 5 DC generated in serum free RPMI 1640 (0% serum) or in RPMI 1640 supplemented with 10% FBS (10% serum) were assessed for their tumor killing activity after activation with LPS for 48 h. These results are representative of 3 independent experiments performed in triplicates.