Supplemental Material to:

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Functional characterization and STAT3 dependence of a dendritic cell-derived CD14+ population arising upon IL-10-exposed maturation

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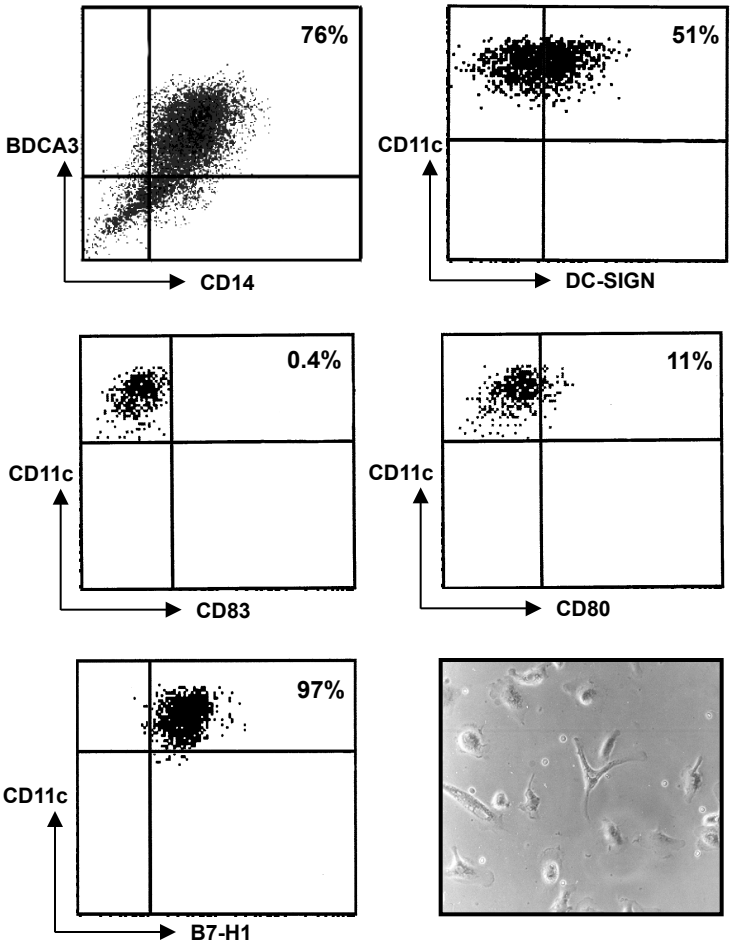
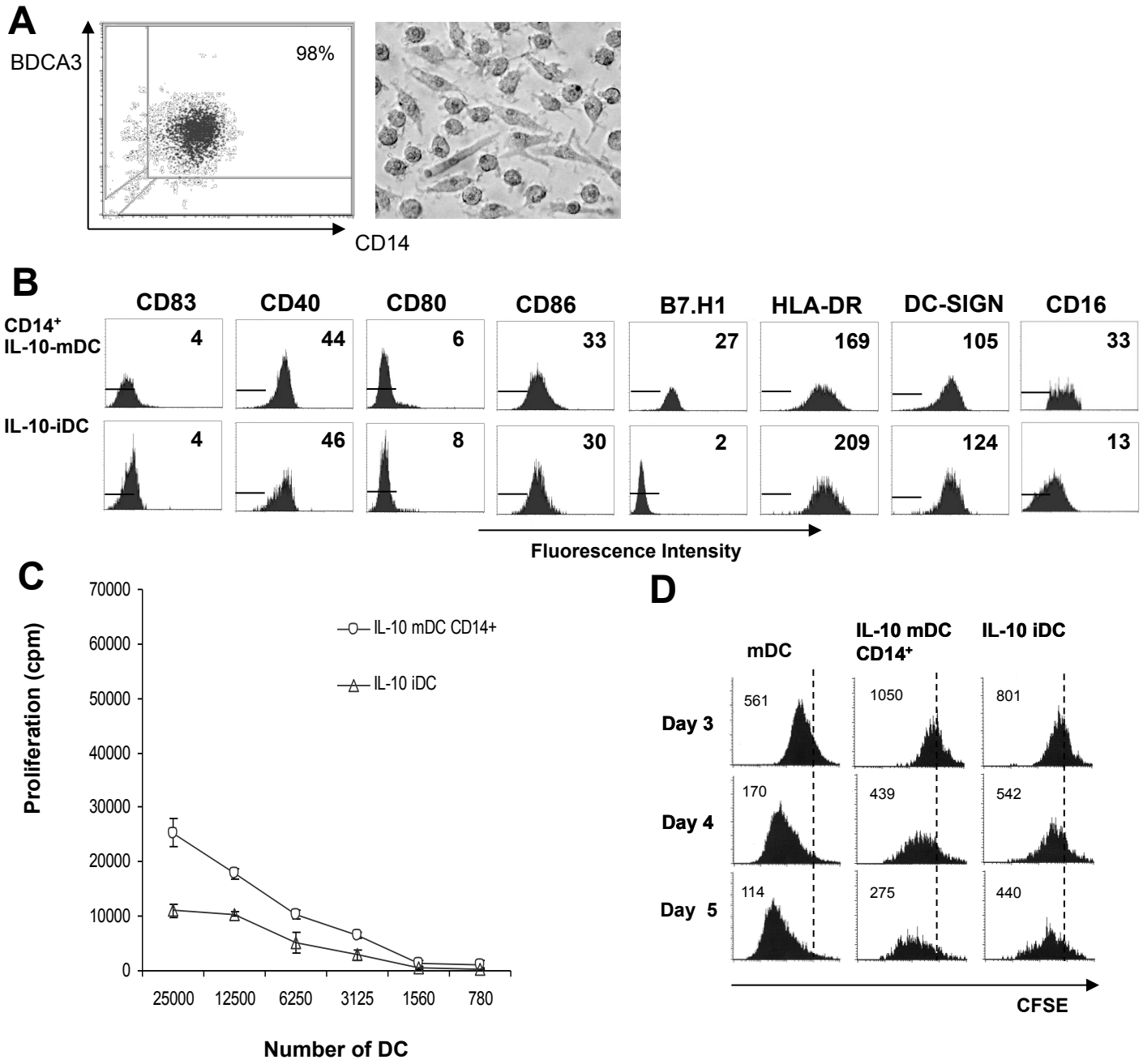


SFig.1. Phenotype and morphology of DC migrated from healthy donor skin explants, after intradermal delivery of IL-10, over the course of two days of culture. Cells were gated on characteristic high Forward and Side Scatter properties prior to marker analysis. Microphotograph shows typical stretched macrophage-like morphology (400x magnification). Healthy human skin specimens were obtained after informed consent and signing of a "non-objection statement" at the time of hospital admission from patients undergoing corrective breast or abdominal plastic surgery at the VU University medical center (Amsterdam, The Netherlands) in accordance with the "Code for Proper Use of Human Tissues" as formulated by the Dutch Federation of Medical Scientific Organizations (www.fmwv.nl). 50 ng IL-10 was injected intradermally in a total volume of 20 µl serum-free medium whereafter 6 mm punch biopsies were taken. The biopsies were transferred to a 48-well plate containing 1 ml IMDM supplemented with 5% human pooled serum (HPS) (Sanquin), sodium penicillin, streptomycin sulfate, L-glutamine, and 2-ME. DC were allowed to migrate from the biopsies for 2 days and were subsequently harvested and phenotypically characterized by FACS analysis.



SFig. 2. CD14+ IL-10 mDC are phenotypically, morphologically and functionally similar to immature MoDC differentiated in the presence of IL-10 (IL-10-iDC). A) IL-10-iDC are CD14+BDCA3+ and have a macrophage-like morphology (400x magnification). B) Phenotypic (FACS) profile of IL-10-iDC vs CD14+ IL-10-mDC. Note differences in CD16, and most notably, B7-H1 levels. C) Mixed leukocyte reactivity instigated by IL-10-iDC vs CD14+ IL-10-mDC, means with SEM are shown from 3 experiments. D) Proliferative anergy induction by both IL-10-iDC and CD14+ IL-10-mDC. After 8 days of allo-stimulation of T cells with mDC, IL-10-iDC or CD14+ IL-10-mDC, they were pulsed with CFSE and re-stimulated by anti-CD3 and –CD28 mAbs; T cell proliferation was followed over time by CFSE dilution. The dotted line indicates CFSE levels of non-stimulated T cells. Data from one representative experiment out of three are shown.

Supplementary Table 1 Employed qRT-PCR primers

Target	Forward primer (5'-3')	Reverse primer (5'-3')
IDO	TATTCAAGGCAATGCAAATG	TGGGTTCACATGATCGTGG
IL4Rα	GTTCCTGGACCTGCTCGG	AGGGCATGTGAGCACTCG
IL6R	TTCCCAGGAGTCCCAGAAG	GCTGCAAGATTCCACAACC
STAT3	CGGCGTCCAGTTCACTACT	GCTGCCGTTGTTGGATTCT
TGFß	TCCTGGCGATACCTCAGC	GAGCAGTGGGCGCTAAGG
HIF1α	CGTTCCTTCGATCAGTTGTC	TCAGTGGTGGCAGTGGTAGT
MMP3	TGGATGCCGCATATGAAG	CAGAAATGGCTGCATCGA
MMP9	TACTGTGCCTTTGAGTCCG	TTGTCGGCGATAAGGAAG
VEGF A	AAGGAGGAGGGCAGAATCAT	CCAGGCCCTCGTCATTG