

Supplemental Information

Supplemental Figure Legends

Supplemental Figure S1. High SCAMP5 expression by human pDCs

Legend: Flow cytometry analysis of SCAMP5 expression in PBMCs of healthy subjects (n=8). **(A)** Gating strategy. To identify viable pDCs, we identified lymphocytes, single cells, viable cells (fixable viability dye) and we selected cells negative for lineage markers (CD3, CD14, CD16, CD20) and positive for pDC markers (CD123, CD303). Non-pDC PBMCs were identified by selecting lymphocytes, single cells, viable cells and lineage-marker positivity to include – amongst others – T cells, Monocytes, NK cells and B cells. **(B)** SCAMP5 expression as measured by MFI. **(C)** Histogram of SCAMP5 expression in negative controls (unstained and FMO samples), PBMCs and pDCs. * P value <0.05.

Abbreviations: A: Area; FMO: fluorescence minus one (SCAMP5); FSC: forward scatter; H: Height; IFN: interferon; MFI: median fluorescent intensity; PBMC: peripheral blood mononuclear cell; pDC: plasmacytoid dendritic cell; SCAMP5: secretory carrier membrane protein 5; SSC: side scatter.

Supplemental Figure S2. Cellular location of IFN α , SCAMP5 and CD123 in non-activated pDCs

Legend: ImageStream analyses of unstimulated (medium control) pDCs from n=4 healthy subjects. Representative images of cellular location of IFN α (green), SCAMP5 (red) and CD123 (blue) in medium-control pDCs, including composite images of SCAMP5-IFN α (yellow=colocalized) and SCAMP5-CD123 (purple=colocalized).

Abbreviations: IFN: interferon; pDC: plasmacytoid dendritic cell; SCAMP5: secretory carrier membrane protein 5.

Supplemental Tables

Supplemental Table S1. Antibodies

	Target	Fluorochrome	Cat no.	Company
Flow Cytometry	SCAMP5	Unconjugated	NBP2-13284	Novus Biologicals
	Rabbit IgG	FITC	111-095-003	Jackson
	Fixable Viability Dye	eFluor 780	65-0865-14	eBioscience
	CD3	PB	2102155	Sony Biotechnology
	CD14	PB	325616	Biolegend
	CD16	PB	558122	BD
	CD20	PB	2111640	Sony Biotechnology
	CD123	PE	130-113-326	Miltenyi
	CD303	APC	130-090-905	Miltenyi
	ImageStream	CD123	PE-Cy7	306010
Rabbit IgG		AF647	A32795	Invitrogen
SCAMP5		Unconjugated	NBP2-13284	Novus Biologicals
IFN α		PE	130-092-601	Miltenyi

Supplemental Table S2. ImageStream data

Donor	Status	IFN α +	IFN α		CD123	
		(% of pDC, cell count)	BDS (mean)	BDS ≥ 2 (% of pDC)	BDS (mean)	BDS ≥ 2 (% of pDC)
HC 1	Medium control	0% (0)	n.a.	n.a.	0.66	0.2
	Activated	31 % (146)	2.04	41.8	0.65	0.0
HC 2	Medium control	0% (0)	n.a.	n.a.	0.68	0.2
	Activated	29% (87)	1.94	47.1	0.63	0.0
HC 3	Medium control	0% (0)	n.a.	n.a.	0.74	0.0
	Activated	11% (119)	1.99	46.2	0.80	0.4
HC 4	Medium control	0% (0)	n.a.	n.a.	0.75	0.1
	Activated	28% (462)	1.87	39.2	0.78	0.2

Abbreviations: BDS: bright detail similarity; HC: healthy control; IFN: interferon; pDC: plasmacytoid dendritic cell.