Langerin (CD207) represents a novel Interferon-Stimulated Gene in Langerhans cells

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Interferons (IFN) are warning cytokines released upon pathogen sensing. IFN control the expression of interferon-stimulated genes (ISG) which are often crucial to restrict viral infections and to establish a cellular antiviral state (1, 2). Langerin (CD207), a well-known surface receptor of Langerhans cells (LC), belongs to the C-type lectin receptor (CLR) family and constitutes a major pathogen binding receptor able to regulate both innate and adaptive immune responses (3, 4). Importantly, this CLR was reported as an antiviral receptor, notably able to bind and internalize incoming Human Immunodeficiency Virus (HIV) virions toward Birbeck granules (BG) for degradation (5, 6). However, langerin was never viewed as a contributor of interferon-mediated antiviral immune response. We now provide evidence that langerin is an ISG whose expression is upregulated upon IFN treatment in monocyte-derived and *ex vivo* human skin-isolated LC.

Monocyte-derived dendritic cells (MoDC) express high levels of DC-SIGN (CD209) (>95%) but negligible levels of langerin (≤ 2%) while monocyte-derived Langerhans cells (MoLC) evidenced substantial langerin expression (≥ 20%) and lowered DC-SIGN levels (Fig 1a and Sup. Fig. 1a). Upon treatment of both DC subtypes for 24h with interferon-α (IFN-α), langerin expression was significantly increased (≥ 60%) in MoLC whereas it remained very low in MoDC (≤ 2%) (Fig. 1a). Noteworthy, we noticed that langerin levels were barely increased in MoDC treated with IFN-α, suggesting that optimal IFN-α-mediated control of langerin expression required a pre-conditioning transcriptional environment, like the one set during MoLC differentiation. Interestingly, among the markers screened, CD86 and CD208 were also positively upregulated upon IFN treatment, although at much lower levels compared to langerin (Fig. 1a). The enhanced expression of HLA-ABC molecules was also observed upon IFN treatment of both DC subtypes, as previously reported in lymphoid cells (7). The IFN-α-mediated upregulation of langerin expression in MoLC was confirmed by immunofluorescence microscopy analyses of MoLC, treated or not with IFN-α for 24h (Fig. 1b) and further validated by immunoblotting of MoLC lysates (Sup. Fig. 1b). To expand our findings to a more relevant LC model, we isolated *ex vivo* human epidermal LC (eLC) from abdominoplasties which were processed as previously described (8). Cells crawling out from the epidermal layer were treated or not with IFN-α and stained with fluorescently-coupled langerin and CD1a antibodies. As shown in Fig. 1c, the pool of langerin^{+} expressing cells from 3 different donors was substantially increased upon IFN-α treatment. We further evidenced that only type-I IFN (IFN-α2a, IFN-α2b, IFN-β1a and IFN-β1b), but not type-II (IFN-γ), were able to upregulate langerin expression levels (Fig. 1d), reminiscent of the ISG bone marrow stromal cell antigen-2 (BST-2 also named CD317 or tetherin) expression pattern (Sup. Fig. 2). Human eLC also showed a type-I IFN-dependent increase in langerin expression (Fig. 1e). Using human PBMC or isolated primary human CD4^{+} T cells in parallel to autologous MoLC and MoDC, we demonstrated a broad-spectrum IFN-α-mediated increase in Retinoic acid-inducible gene I (RIG-I) mRNA levels in all cell types while significant IFN-α-mediated langerin mRNA upregulation was seemingly confined to MoLC (Fig. 1f), as also confirmed at protein level by immunoblotting (Fig. 1g). Cells pre-treated with cycloheximide (CHX), a known protein synthesis inhibitor evidenced a decrease in both langerin and RIG-I protein expression (Fig. 1h). Importantly, CHX treatment did not impede upregulation of langerin and RIG-I gene expression upon IFN-α administration, therefore demonstrating a direct involvement of IFN-α in *de novo* langerin expression (Fig. 1i). Interestingly, TLR agonists administered to MoLC induced a global lower TNF-α production compared to MoDC. Yet, IFN-treated MoLC (MoLC-IFN) responded efficiently to viral-mimicking TLR agonists suggesting that these cells remain endowed with efficient viral sensing and subsequent antiviral response (Sup. Fig. 3). We thus compared the antiviral capacity of MoDC and MoLC, in presence or absence of IFN, upon challenge with wild-type HIV-1 (HIV) or VSV-G pseudotyped GFP-expressing lentivectors (Lv-GFP) able to bypass langerin-mediated HIV entry restriction (5, 8). As expected, MoDC were more susceptible to HIV infection than autologous MoLC while pre-treatment with type-I IFN strongly reduced

HIV infection of both DC subtypes (Fig. 1j and 1k). However, the marked antiviral effect observed in HIV-infected MoLC over HIV-infected MoDC was not evident anymore when both cell types were pre-treated with type-I IFN and challenged with Lv-GFP (Fig. 1l) as clearly indicated by a reduced fold of inhibition of infection between the cell types (Fig. 1m). Although the infection rate was seemingly higher in both cell types when exposed to Lv-GFP compared to HIV, the antiviral effect of IFN-α on Lv-GFP infection was diminished in MoLC, but not MoDC (compare Fig. 1k and 1m). This suggests the presence of a type-I IFN-inducible cell surface factor on MoLC able to limit entry of incoming HIV wild-type virions, a reported function for langerin. In conclusion, our study offers a novel aspect on the regulation of expression of the CLR langerin and extends the list of ISG as potential cellular effectors able to amplify the host antiviral response.

Author contributions

M.A.C., G.M and F.P.B. conceived the study. J.C.B., V.P, S.N. and F.P.B. helped in experimental design or provided reagents. M.A.C., G.M., J.L., M.O.I., K.F., L.P., and F.P.B. carried out experiments; F.P.B. wrote the manuscript. All authors read and commented the manuscript.

Acknowledgements

We thank Richard O.S. Karoo and members of the Spire Cardiff Hospital (Wales, UK) for providing skin samples. We are grateful to J. Paul Mitchell, Sion A. Coulman (Cardiff, Wales, UK) and all members of the Viral Trafficking, Restriction and Innate Signaling team for excellent technical help and/or critical reading of the manuscript. We are also grateful to Caroline Goujon and Olivier Moncorgé (IRIM, Montpellier, France) for the kind gift of reagents. Immunofluorescence and part of the flow cytometry acquisitions were performed at the Montpellier RIO Imaging facility (Montpellier, France). This work was supported in part by the ANRS grant N° D15236 to F.P.B. This work was also supported by President's Research Scholarship Cardiff University to M.A.C. and grants from ISSF-WT and Gates Foundation to V.P. G.M. was a recipient from a Labex EpiGenMed post-doctoral fellowship (Montpellier, France) and is currently supported by the ANRS. Funding bodies had no role in preparation of the article, study design, or interpretation of the data.

Conflict of Interest

The authors state no conflict of interest.

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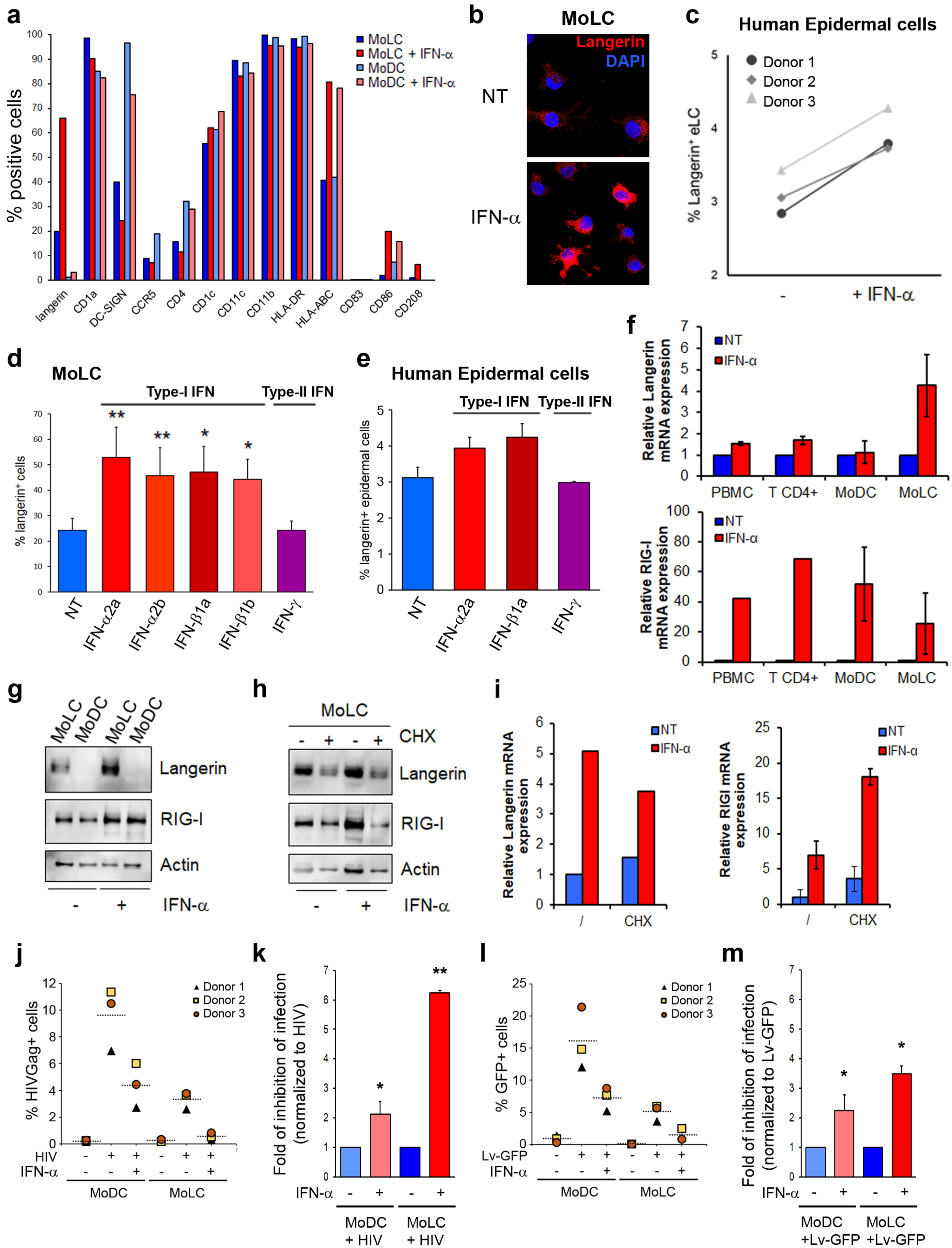
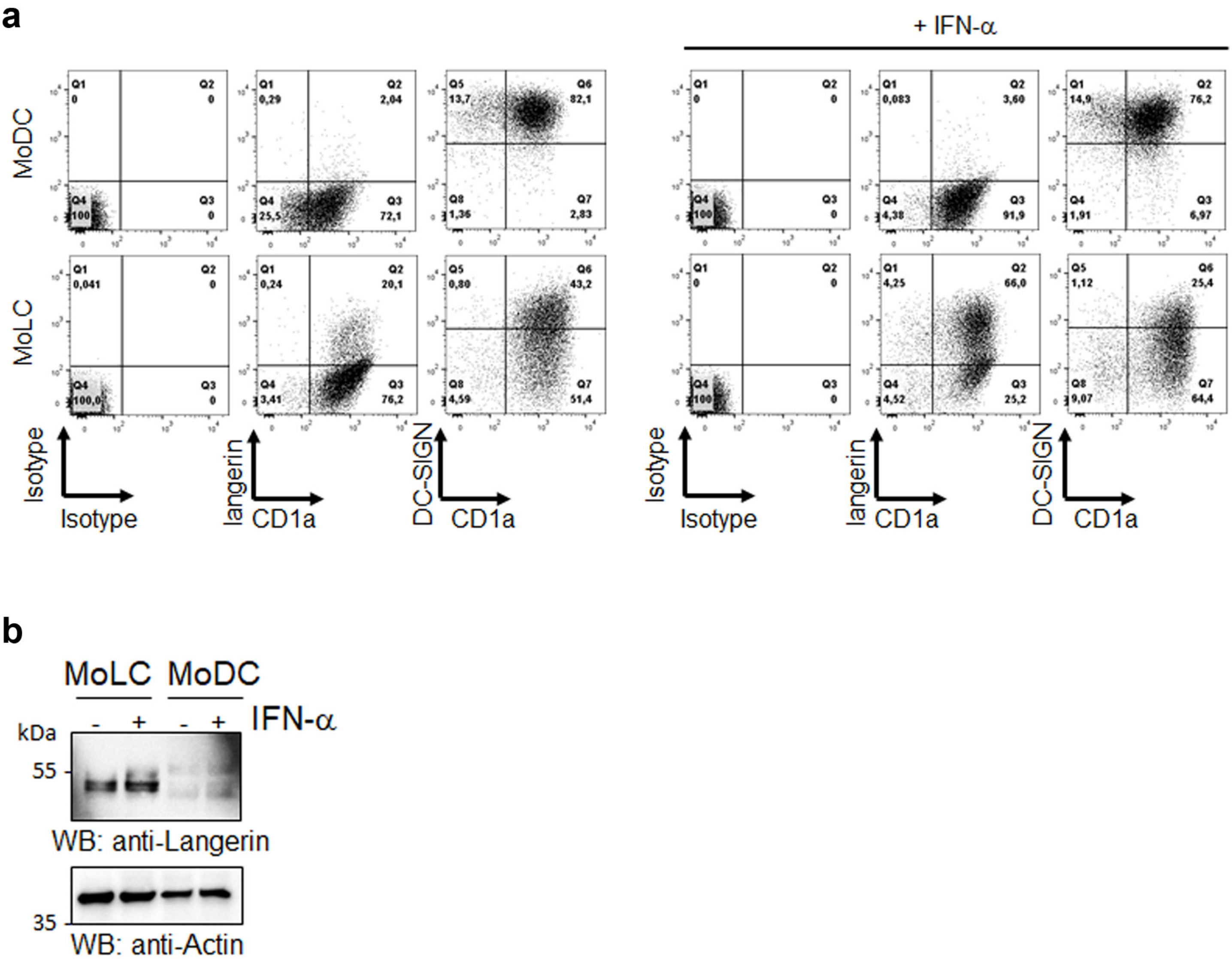
Figure legend

(a) Interferon-mediated modulation of cell surface markers in human primary monocyte-derived DC (MoDC) and LC (MoLC), pre-treated or not for 24h with 10^3 U/ml of IFN-α2a. **(b)** MoLC, treated or not with IFN-α2a for 24h, were spotted on coverslips, fixed, permeabilized and stained with langerin antibodies. Nuclei were stained with DAPI. **(c)** Epidermal walkout cells treated or not with IFN-α2a for 24h, were analyzed for langerin expression levels upon staining and flow cytometry analysis. (**d**) Graph representing langerin$^+$ MoLC untreated or treated for 24h with IFN-α2a or IFN-α2b or IFN-β1a or IFN-β1b or IFN-γ (all at 10^3 U/ml). (**e**) Same experiment as above but with epidermal walkout cells treated as indicated. (**f**) RT-qPCR analyses of langerin and RIG-I mRNA expression in indicated cells treated or not with IFN-α2a for 8h (n=2). (**g**) Lysates from MoDC and MoLC treated or not with IFN-α2a for 24h were immunoblotted with langerin and RIG-I antibodies. Loading was controlled with anti-actin (n=2). MoLC pretreated or not with 10µM of cycloheximide (CHX) for 1h, were stimulated for 24h to analyze indicated protein expression levels by immunoblotting (**h**) or 8h to analyze indicated transcripts levels by RT-qPCR (**i**). MoDC or MoLC were incubated or not with 10^3 U/ml IFN-α2a for 24h prior to challenge with HIV-1-R5 viruses or Lv-GFP for 72h. Cells were analysed for HIV-Gag (**j**) or GFP (**l**) expression by flow cytometry and represented in a graph in which means of HIV-Gag$^+$ cells and GFP$^+$ cells are represented by a dotted horizontal segment (n=3). The fold of inhibition of HIV infection (**k**) or Lv-GFP transduction (**m**) were represented in graphs with data normalized to each untreated cell type (n=3).

Figure 1

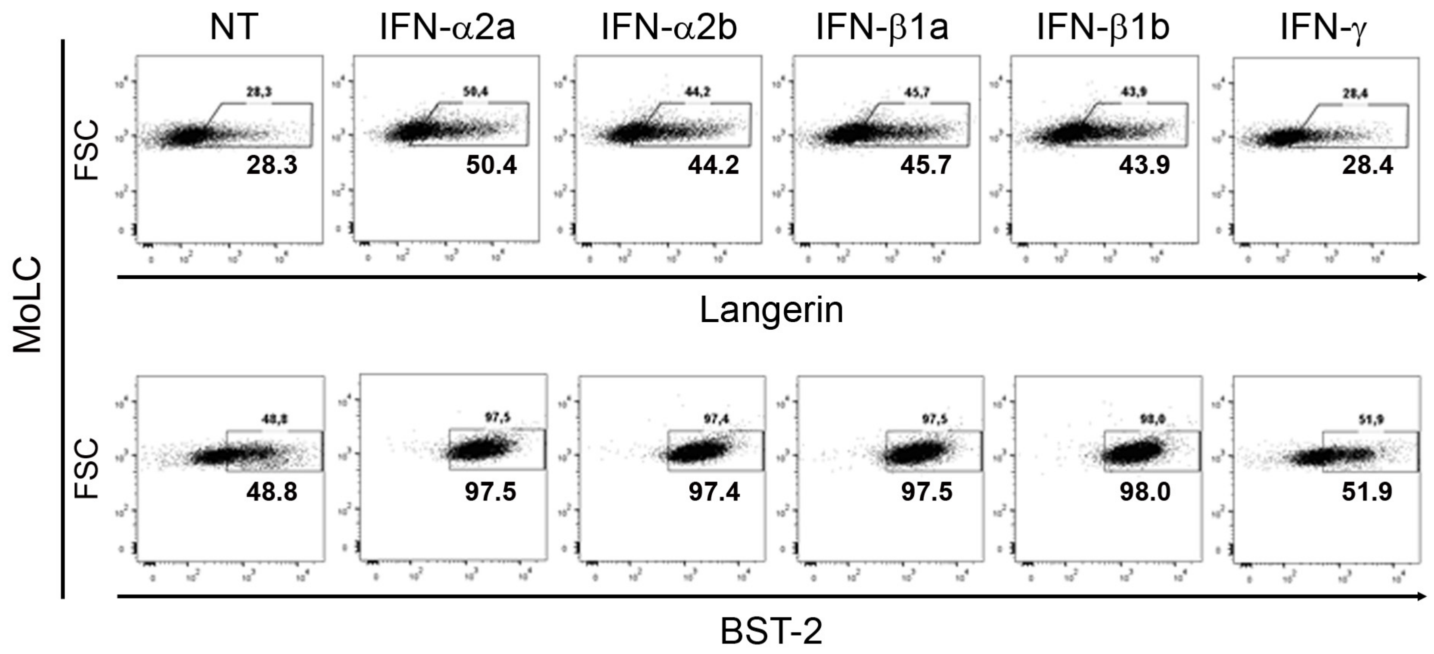

a
% positive cells
MoLC
MoLC + IFN-α
MoDC
MoDC + IFN-α
langerin
CD1a
DC-SIGN
CCR5
CD4
CD1c
CD11c
CD11b
HLA-DR
HLA-ABC
CD83
CD86
CD208
b
MoLC
Langerin
DAPI
NT
IFN-α
c
Human Epidermal cells
Donor 1
Donor 2
Donor 3
% Langerin+ eLC
-
+ IFN-α
d
MoLC
Type-I IFN
Type-II IFN
% langerin+ cells
NT
IFN-α2a
IFN-α2b
IFN-β1a
IFN-β1b
IFN-γ
e
Human Epidermal cells
Type-I IFN
Type-II IFN
% langerin+ epidermal cells
NT
IFN-α2a
IFN-β1a
IFN-γ
f
Relative Langerin mRNA expression
NT
IFN-α
PBMC
T CD4+
MoDC
MoLC
Relative RIG-I mRNA expression
g
MoLC
MoDC
MoLC
MoDC
Langerin
RIG-I
Actin
IFN-α
h
MoLC
CHX
Langerin
RIG-I
Actin
IFN-α
i
Relative Langerin mRNA expression
NT
IFN-α
/
CHX
Relative RIGI mRNA expression
j
% HIVGag+ cells
Donor 1
Donor 2
Donor 3
HIV
IFN-α
MoDC
MoLC
k
Fold of inhibition of infection (normalized to HIV)
IFN-α
MoDC + HIV
MoLC + HIV
l
% GFP+ cells
Donor 1
Donor 2
Donor 3
Lv-GFP
IFN-α
MoDC
MoLC
m
Fold of inhibition of infection (normalized to Lv-GFP)
IFN-α
MoDC +Lv-GFP
MoLC +Lv-GFP

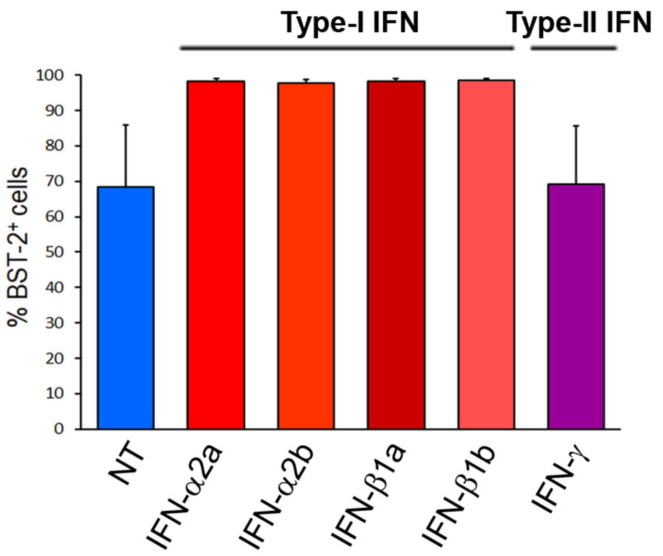
Supplemental Figure 1



Legend. (**A**) Human primary monocyte-derived DC (MoDC) and LC (MoLC), pre-treated or not for 24h with **10**^{3} U/ml of IFN-α2a, were analyzed by flow cytometry for the expression of CD1a, DC-SIGN and langerin. (**B**) Lysates from MoDC and MoLC pre-treated or not with IFN-α2a for 24h were immunoblotted with langerin antibodies. Loading was controlled with anti-actin. This experiment is representative of 3 donors.

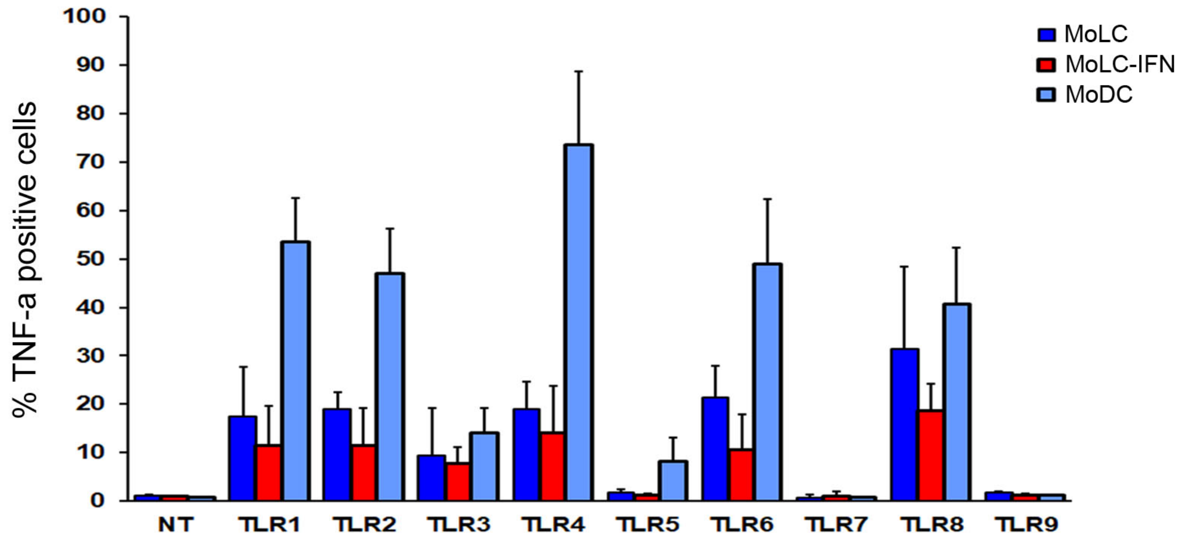
Supplemental Figure 2





Legend. MoLC were left untreated or treated for 24h with IFN-α2a (10^3 U/ml) or IFN-α2b (10^3 U/ml) or IFN-α1a (10^3 U/ml) or IFN-α1b (10^3 U/ml) or IFN-γ (10^3 U/ml). Cells were fixed washed and stained with langerin (upper dot-plots) or BST-2 (lower dot-plots) fluorophore-coupledantibodies and analyzed by flow cytometry. Experiments as above from 4 different donors were analyzed for BST-2+ cells and represented on a graph.

Supplemental Figure 3



Legend. MoDC or MoLC, treated or not with IFN-α2a (MoLC+IFN), were stimulated for 20h with the indicate**d** TLR agonists. Cells were fixed, permeabilized and stained with anti-TNF-α-FITC antibodies for 45 min. Cells were washed and processed for flow cytometry analysis. Pooled TNF-α production data from 3 different donors (n=3) are represented in a graph. NT indicates non treated cells.