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

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

33 Monocyte-derived dendritic cells (MoDC) express high levels of DC-SIGN (CD209) (>95%)
34 but negligible levels of langerin ($\leq 2\%$) while monocyte-derived Langerhans cells (MoLC)
35 evidenced substantial langerin expression ($\geq 20\%$) and lowered DC-SIGN levels (Fig 1a and
36 Sup. Fig. 1a). Upon treatment of both DC subtypes for 24h with interferon- α (IFN- α),
37 langerin expression was significantly increased ($\geq 60\%$) in MoLC whereas it remained very
38 low in MoDC ($\leq 2\%$) (Fig. 1a). Noteworthy, we noticed that langerin levels were barely
39 increased in MoDC treated with IFN- α , suggesting that optimal IFN- α -mediated control of
40 langerin expression required a pre-conditioning transcriptional environment, like the one set
41 during MoLC differentiation. Interestingly, among the markers screened, CD86 and CD208
42 were also positively upregulated upon IFN treatment, although at much lower levels
43 compared to langerin (Fig. 1a). The enhanced expression of HLA-ABC molecules was also
44 observed upon IFN treatment of both DC subtypes, as previously reported in lymphoid cells
45 (7). The IFN- α -mediated upregulation of langerin expression in MoLC was confirmed by
46 immunofluorescence microscopy analyses of MoLC, treated or not with IFN- α for 24h (Fig.
47 1b) and further validated by immunoblotting of MoLC lysates (Sup. Fig. 1b). To expand our

48 findings to a more relevant LC model, we isolated *ex vivo* human epidermal LC (eLC) from
49 abdominoplasties which were processed as previously described (8). Cells crawling out from
50 the epidermal layer were treated or not with IFN- α and stained with fluorescently-coupled
51 langerin and CD1a antibodies. As shown in Fig. 1c, the pool of langerin⁺ expressing cells
52 from 3 different donors was substantially increased upon IFN- α treatment. We further
53 evidenced that only type-I IFN (IFN- α 2a, IFN- α 2b, IFN- β 1a and IFN- β 1b), but not type-II
54 (IFN- γ), were able to upregulate langerin expression levels (Fig. 1d), reminiscent of the ISG
55 bone marrow stromal cell antigen-2 (BST-2 also named CD317 or tetherin) expression pattern
56 (Sup. Fig. 2). Human eLC also showed a type-I IFN-dependent increase in langerin
57 expression (Fig. 1e). Using human PBMC or isolated primary human CD4⁺ T cells in parallel
58 to autologous MoLC and MoDC, we demonstrated a broad-spectrum IFN- α -mediated
59 increase in Retinoic acid-inducible gene I (RIG-I) mRNA levels in all cell types while
60 significant IFN- α -mediated langerin mRNA upregulation was seemingly confined to MoLC
61 (Fig. 1f), as also confirmed at protein level by immunoblotting (Fig. 1g). Cells pre-treated
62 with cycloheximide (CHX), a known protein synthesis inhibitor evidenced a decrease in both
63 langerin and RIG-I protein expression (Fig. 1h). Importantly, CHX treatment did not impede
64 upregulation of langerin and RIG-I gene expression upon IFN- α administration, therefore
65 demonstrating a direct involvement of IFN- α in *de novo* langerin expression (Fig. 1i).
66 Interestingly, TLR agonists administered to MoLC induced a global lower TNF- α production
67 compared to MoDC. Yet, IFN-treated MoLC (MoLC-IFN) responded efficiently to viral-
68 mimicking TLR agonists suggesting that these cells remain endowed with efficient viral
69 sensing and subsequent antiviral response (Sup. Fig. 3). We thus compared the antiviral
70 capacity of MoDC and MoLC, in presence or absence of IFN, upon challenge with wild-type
71 HIV-1 (HIV) or VSV-G pseudotyped GFP-expressing lentivectors (Lv-GFP) able to bypass
72 langerin-mediated HIV entry restriction (5, 8). As expected, MoDC were more susceptible to
73 HIV infection than autologous MoLC while pre-treatment with type-I IFN strongly reduced

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

85 **Author contributions**

86 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
87 experimental design or provided reagents. M.A.C., G.M., J.L., M.O.I., K.F., L.P., and F.P.B.
88 carried out experiments; F.P.B. wrote the manuscript. All authors read and commented the
89 manuscript.

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103

104 **Conflict of Interest**

105 The authors state no conflict of interest.

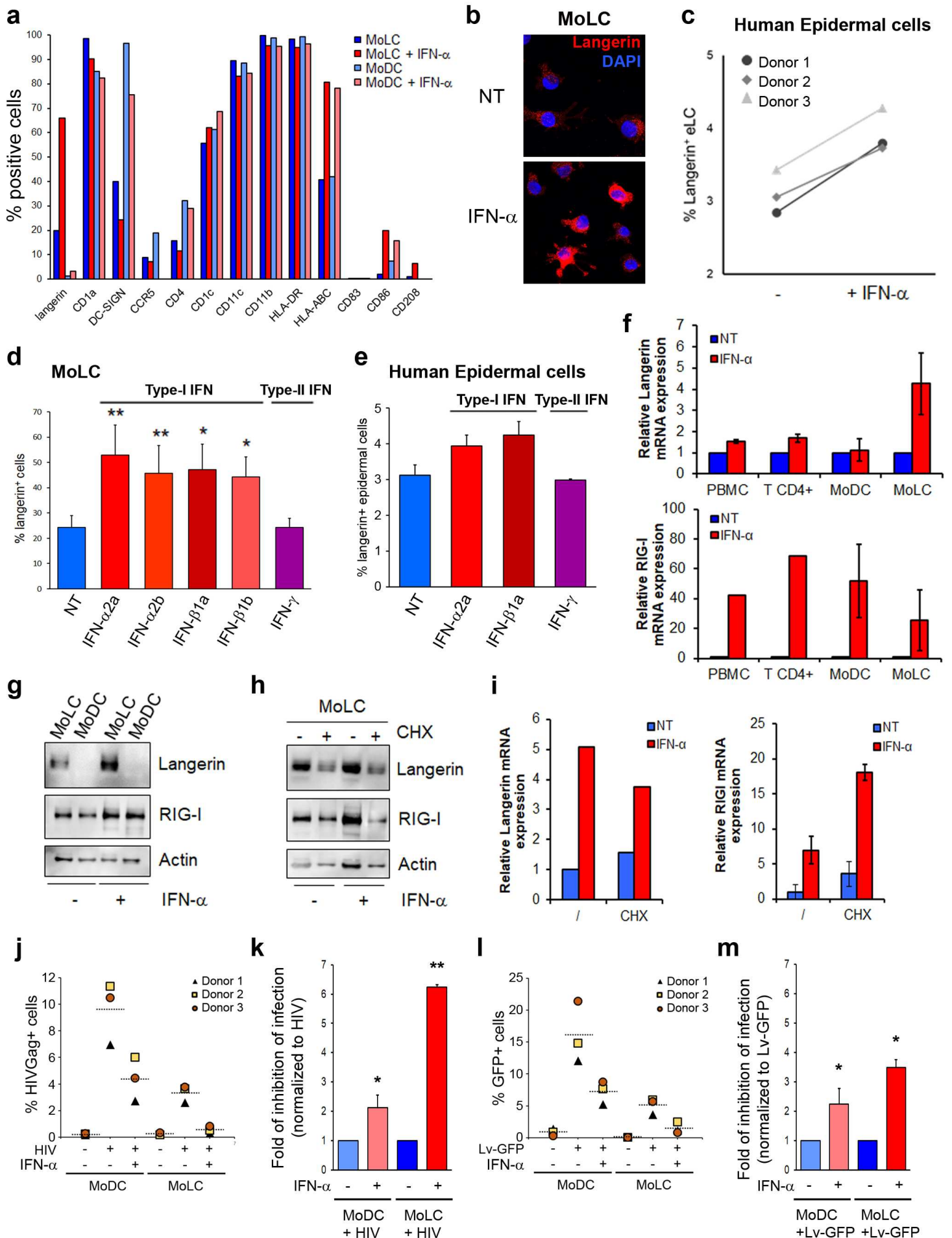
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126 **Figure legend**

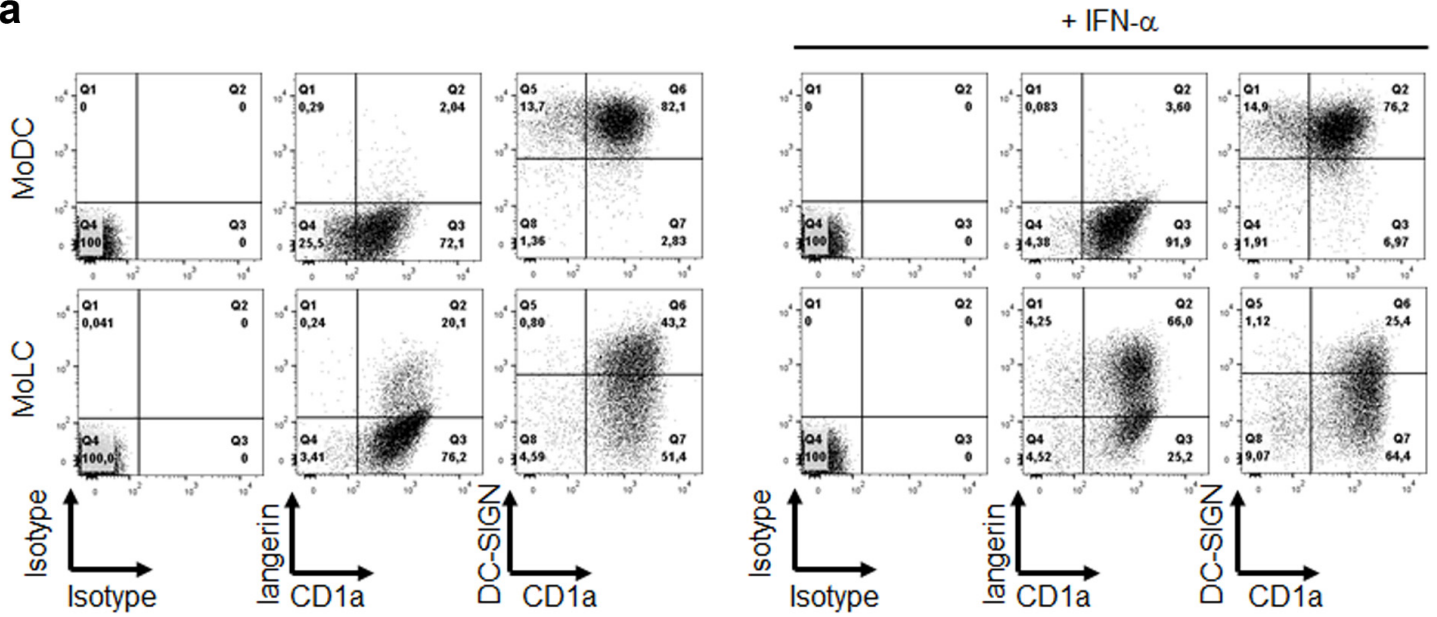
127 (a) Interferon-mediated modulation of cell surface markers in human primary monocyte-
128 derived DC (MoDC) and LC (MoLC), pre-treated or not for 24h with 10^3 U/ml of IFN- α 2a.
129 (b) MoLC, treated or not with IFN- α 2a for 24h, were spotted on coverslips, fixed,
130 permeabilized and stained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

Figure 1

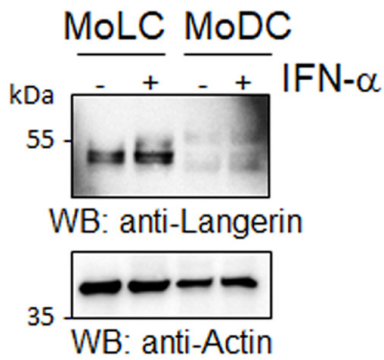


Supplemental Figure 1

a

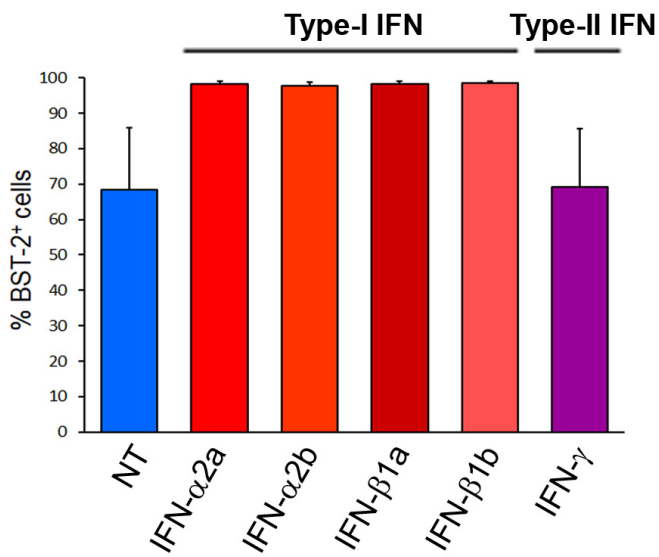
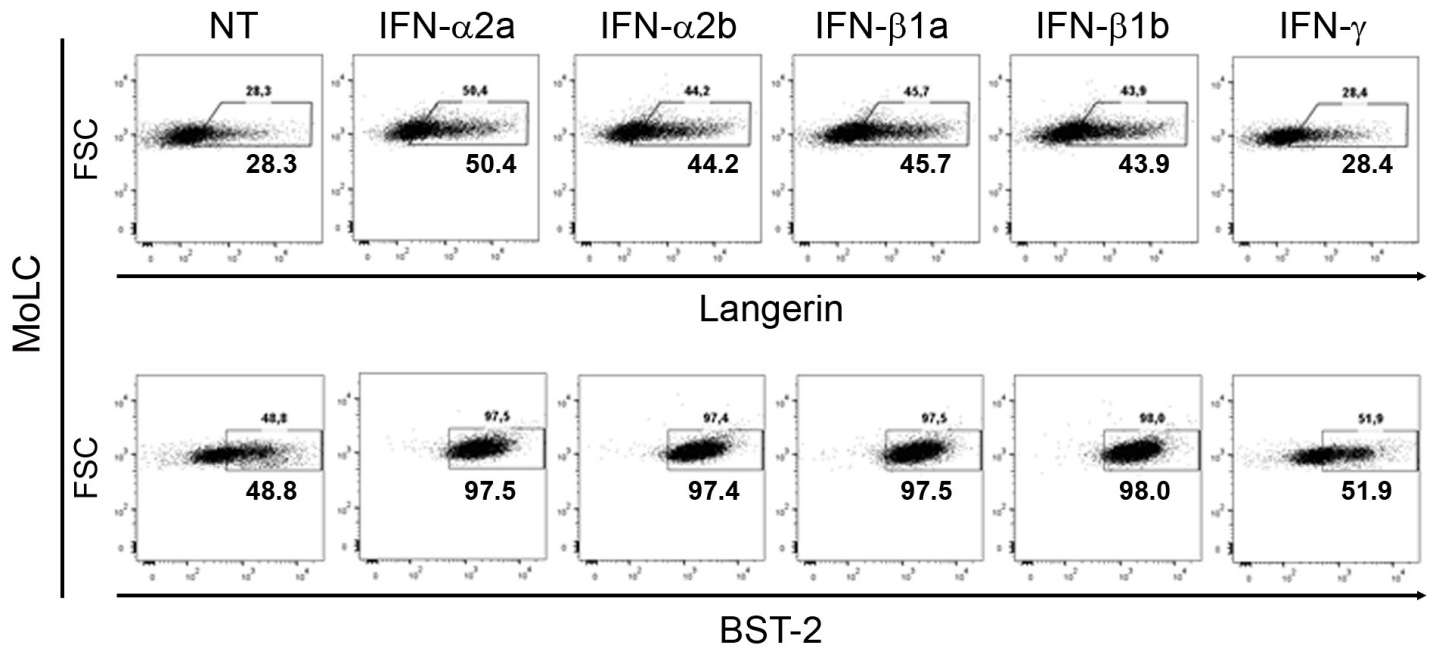


b



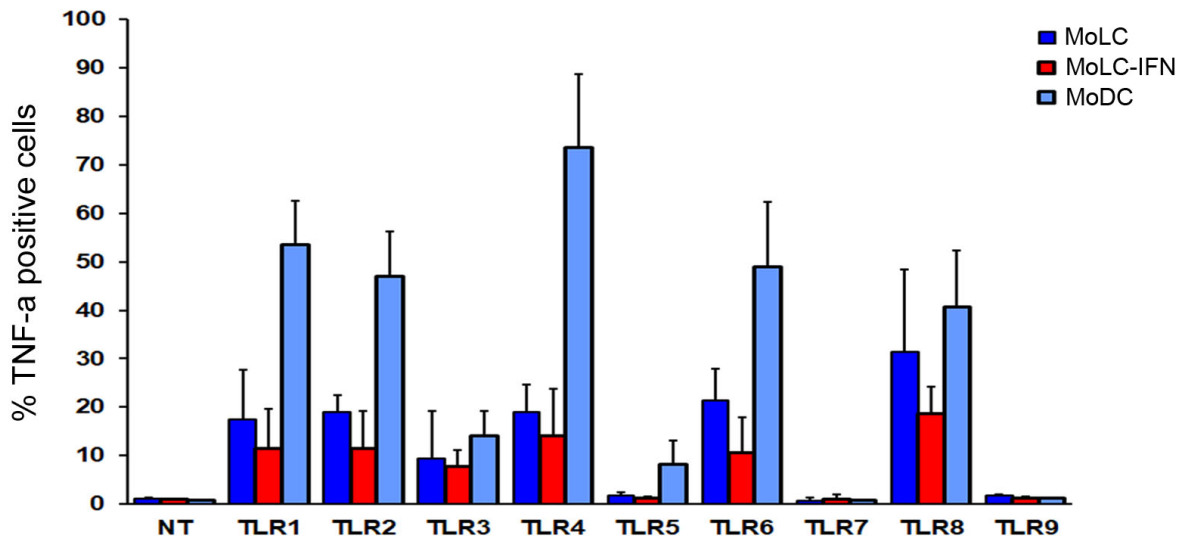
Legend. (A) Human primary monocyte-derived DC (MoDC) and LC (MoLC), pre-treated or not for 24h with 10^3 U/ml of IFN- α 2a, were analyzed by flow cytometry for the expression of CD1a, DC-SIGN and langerin. (B) Lysates from MoDC and MoLC pre-treated or not with IFN- α 2a for 24h were immunoblotted with 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- α 2a (10^3 U/ml) or IFN- α 2b (10^3 U/ml) or IFN- α 1a (10^3 U/ml) or IFN- α 1b (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-coupled antibodies 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- α 2a (MoLC+IFN), were stimulated for 20h with the indicated 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.