#### Supplementary figure 1. Isotype staining.

Isotype control for Figure 1 to determine gates for CD3 staining and CD3-CD1a clusters.

## Supplementary figure 2. IL-6 and IL-8 response of skin to different pathogens and pathogenic ligands.

(A,B,C) Skin biopsies were stimulated with heat-killed or live *Candida albicans* with Amphotericin B to prevent hyphen formation or without to allow hyphen formation. After 24 hours the supernatant was collected and TNF $\alpha$ , IL-6 and IL-8 production was measured by ELISA. The values were analyzed for statistical differences by ANOVA (\*= p<0.05; \*\* = p<0.01 versus the no treatment condition).

(D,E) Skin biopsies were stimulated with *Candida albicans*, *Neisseria gonorrhea*, TNF $\alpha$ , Pam3CSK4, LTA, LPS or flagellin. After 24 hours the supernatant was collected, and IL-6 and IL-8 production was measured by ELISA. Error bars represent the standard deviation of duplicates.

### Supplementary figure 3. TNF $\alpha$ and Pam3CSK4 enhance HIV-1 transmission ex vivo.

Epidermal sheets were stimulated with TNFα, Pam3CSK4, LTA, LPS or flagellin for six hours and ex vivo transmission was determined as in Figure 1 and 3. The co-cultures were monitored by fluorescence microscopy at day 7. Lower panels show HIV-1-eGFP expression and the upper panels the overlay with bright field. A representative experiment out of two is depicted.

## Supplementary figure 4. Expression of Langerin, CD4 and CCR5 after stimulation with TNF $\alpha$ and Pam3CSK4

Epidermal sheets were stimulated with TNF $\alpha$ , Pam3CSK4. After three days the cells were harvested and stained for the expression of CD4, CCR5 and Langerin by

specific antibodies and subsequently analysed by flow cytometry. The mean fluorescence intensity of the staining is depicted. The three donors were measured in different experiments.

#### Supplementary figure 5. Pam3CSK4 activates LCs.

(A) Emigrant LCs were stimulated with Pam3CSK4 for 2 hours and stained with CD1a and counterstained with Alexa-488 and mounted on glass slides. The cells were analysed for clustering by fluorescence microscopy. Pictures are taken at a magnification of 40X. (B) LCs stimulated with Pam3CSK4 were analysed for morphologic changes by bright field microscopy. Pictures are taken at a magnification of 400X (lower panels are zoomed in).

#### Supplementary figure 6. Pam3CSK4 increases HIV-1 capture by LCs

(A-B) Emigrant LCs were stimulated with Pam3CSK4 for 1 hour and where indicated subsequently inoculated with NL4.3 BaL. After two hours, the cells were extensively washed. The cells were fixed, permeabilized and stained with HIV-1 p24 and for the LC-marker CD1a. The cells were counterstained with isotype-specific Alexa antibodies and analysed by confocal microscopy. Left panels show CD1a staining, middle panels HIV-1 p24 staining and right panels overlay. Pictures are taken at a magnification of 630X with a 4X zoom.

## Supplementary figure 7. HIV-1 captured by LCs partially co-localizes with HLA Class-I.

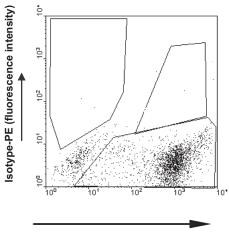
Emigrant LCs were stimulated with Pam3CSK4 for 1 hour and where indicated subsequently inoculated with NL4.3 BaL. After two hours, the cells were extensively washed. The cells were fixed, permeabilized and stained with HIV-1 p24 and HLA class-I. The cells were counterstained with isotype-specific Alexa antibodies and analysed by confocal microscopy. Left panels HLA class-I, middle panels HIV-1 p24

staining, right panels overlay. Pictures are taken at a magnification of 630X with a 4X zoom.

# Supplementary figure 8. TLR ligands induce HIV-1 transmission independent of $\mbox{TNF}\alpha$

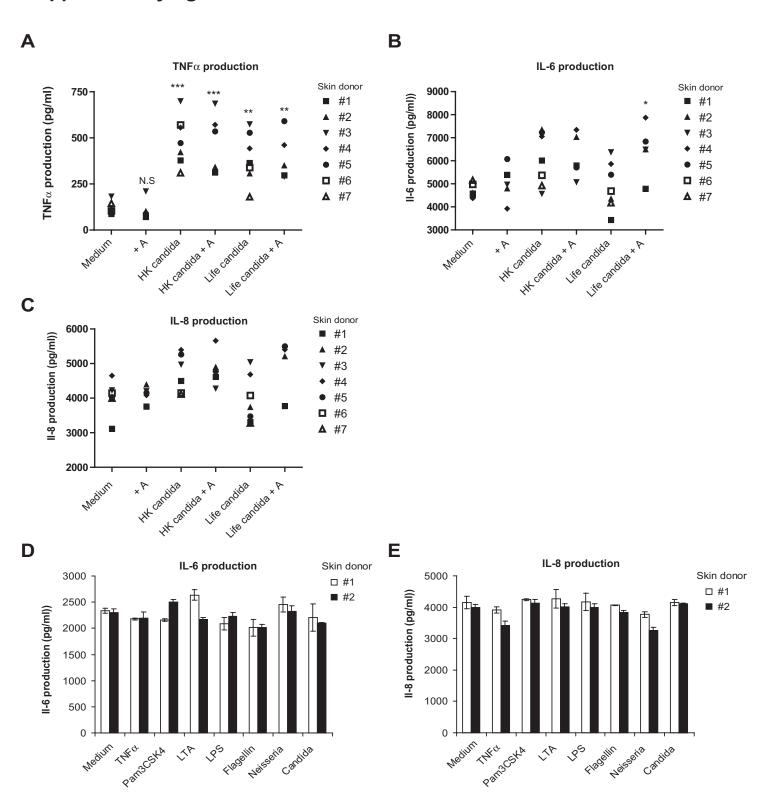
Epidermal sheets were pre-incubated with anti-TNFα for 30 minutes and subsequently stimulated with TNFα, Pam3CSK4, LTA, LPS or flagellin for six hours before HIV-1-eGFP was added. Ex vivo transmission was determined as in Figure 1. Co-cultures (day 7) were analysed for GFP expression by flow cytometry. HIV-1 transmission is depicted as percentage of CCR5<sup>+</sup> Jurkat T cells positive for GFP expression. Error bars represent the standard deviation of duplicates. A representative experiment of two donors is depicted.

### **Supplementary figure 1.**

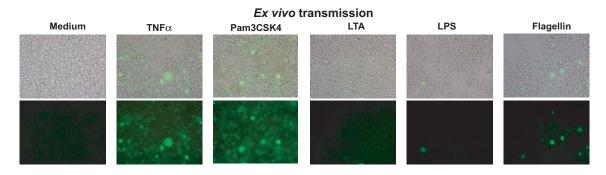


CD1a (fluorescence intensity)

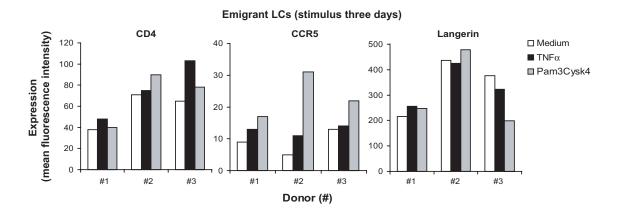
#### **Supplementary figure 2.**



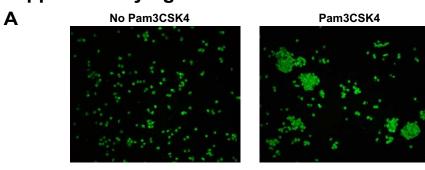
#### Supplementary figure 3.

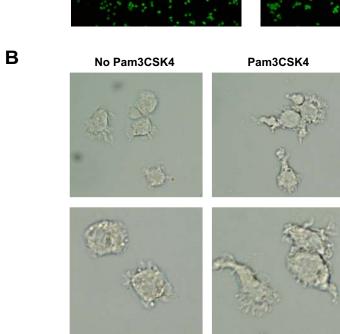


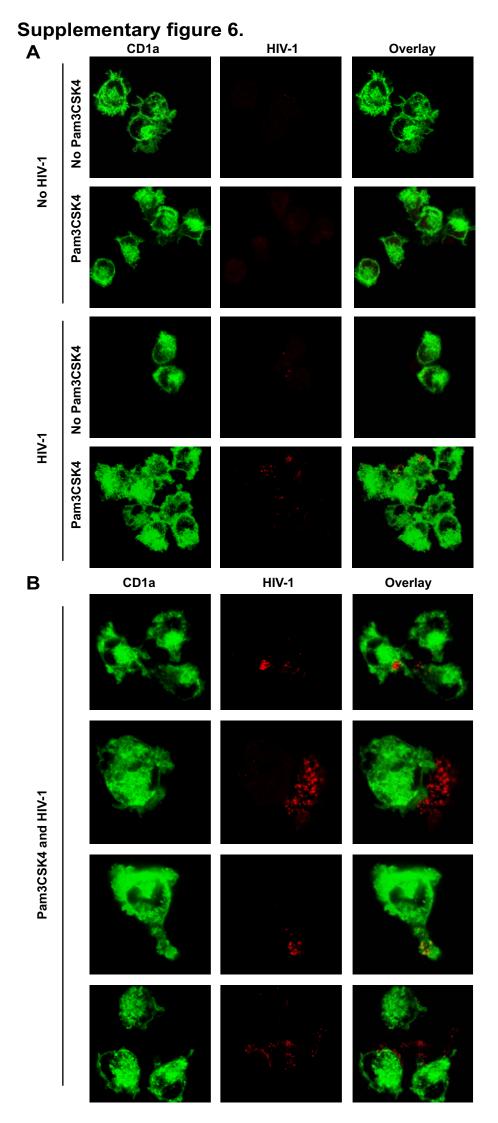
### Supplementary figure 4.



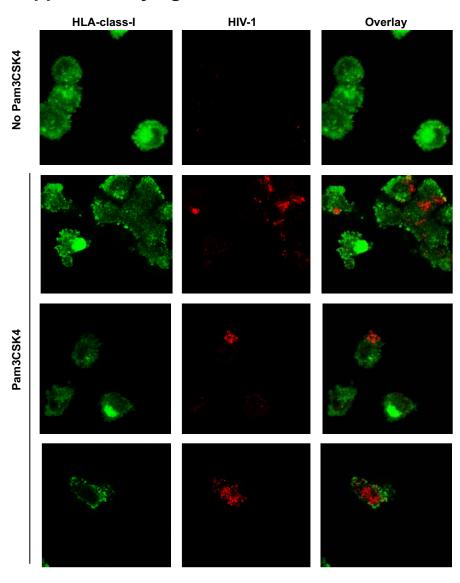
### **Supplementary figure 5.**







### Supplementary figure 7.



### **Supplementary figure 8.**

