# Untangling the impact of CD101 on HIV-1 transmission

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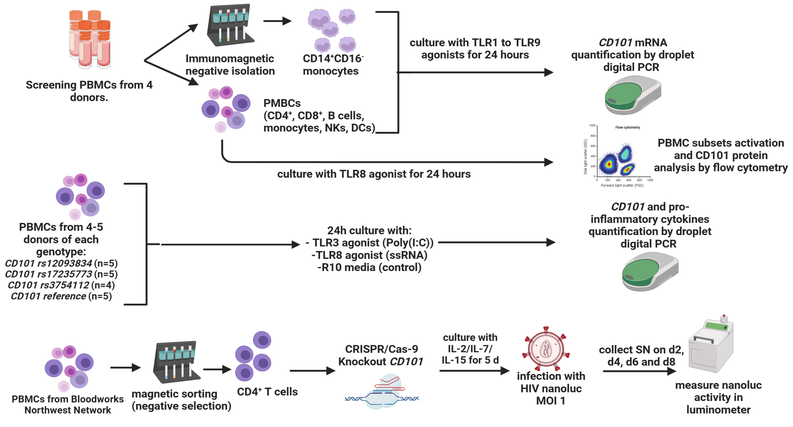
## BACKGROUND

- Five missense variants (*rs3754112, rs17235773, rs116063197, rs12093834, rs34882009*) in the Immunoglobulin-like domain of the *CD101* gene (*CD101* Ig-like variants) increase HIV acquisition risk[1], but the mechanism is unknown.
- A quarter of Africans have one or more of the five *CD101* Ig-like variants.
- CD101 inhibits T cell activation and is expressed on antigen-presenting cells (APCs) and T cells[2,3].
- We hypothesized CD101 regulates inflammatory homeostasis.
- We also explored the impact specifically of the three most frequent *CD101* Ig-like variants (*rs3754112*, *rs17235773*, *rs12093834*) on inflammatory regulation and HIV-1 susceptibility.

## METHODS (FIGURE 1)

- Toll-like receptors (TLR1 to TLR9) are stimulants of the innate immune system.
- We tested TLR agonists for effect on *CD101* mRNA levels in PBMCs and purified monocytes.
- We evaluated the effect of TLR8 agonist on *CD101* and pro-inflammatory cytokine (*IL-6*, *IFIT1* and *IFNβ1*) mRNA in PBMCs.
- We used flow cytometry to measure CD101 expression on PBMC subsets (n=4).
- We compared cells from individuals previously identified with (n=14) one of three *CD101* Ig-like variants or without (n=5) *CD101* variants (reference *CD101*) to assess the effect of *CD101* variation on PBMC responses to TLR8 stimulation.
- We also used CRISPR to knock out CD101 in primary CD4⁺ T cells to test its impact on HIV infectivity.

### Figure 1. Study design

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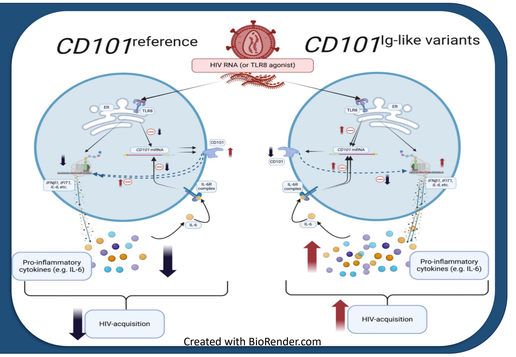
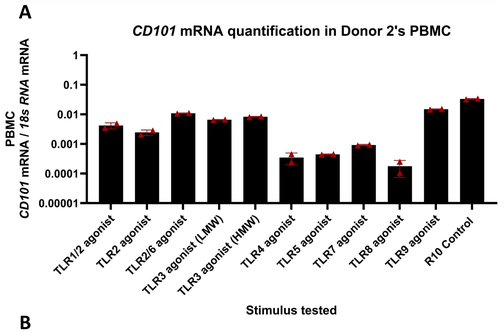
**This is the first study reporting the impact of the innate immune system stimulation via Toll-like receptors on *CD101* expression. *CD101* may affect HIV infection risk indirectly, via effects on inflammation, rather than directly on HIV replication. Candidate *CD101* variants may accentuate this effect.**



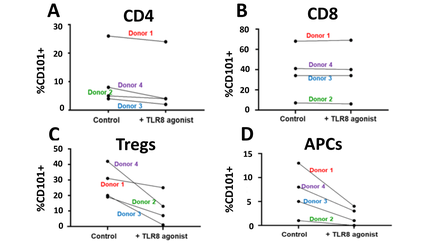

## RESULTS

### Figure 2. *CD101* mRNA expression in PBMC and isolated monocytes following TLR1 to TLR9 agonist stimulations



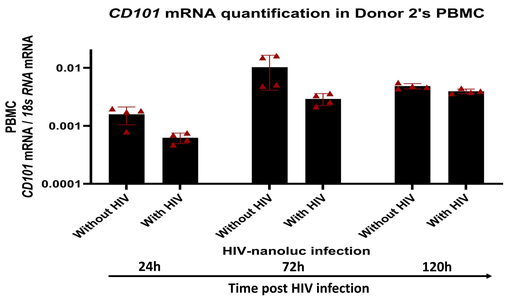

TLR 2, 4, 5, 7 and 8 stimulation reduced *CD101* mRNA expression in PBMC and monocytes. The strongest effect was from HIV ssRNA (a TLR8 agonist) (-77.0% in PBMC, -90.9% in monocytes; n=4).

### Figure 3. CD101 protein is downregulated in CD25⁺ CD127$^{low}$ $T_{regs}$ and CD3⁻ CD14⁻HLA⁻DR⁺ APCs in PBMC



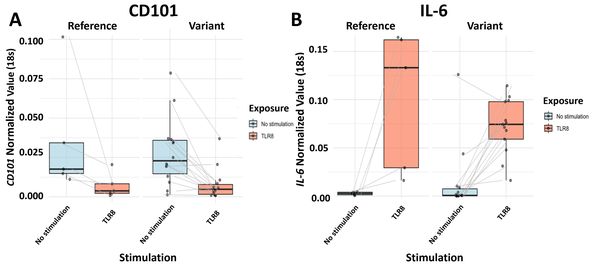

CD25⁺ CD127$^{low}$ $T_{regs}$ and HLA⁻DR⁺ APCs were most sensitive to CD101 downregulation upon TLR8 stimulation (-41.4% and -63.0%, respectively; n=4), in contrast to CD4⁺ and CD8⁺ T cells. However, CD101 downregulation was much weaker if $T_{regs}$ were purified before stimulation (-8.1%; n=5) (data not shown).

### Figure 4. *CD101* mRNA expression in PBMC post HIV-nanoluc infection




Challenge with infectious HIV also reduced *CD101* mRNA (-49.1%; n=4).

### Figure 5. Inflammatory cytokine increases following TLR8 agonist stimulation




For PBMC genotyped for CD101, following TLR8 stimulation, we observed a significant decrease for *CD101* (74.1% +/- 22.5%; n=17), in 17 out of 19 donors, and a significant increase of *IL-6* (13,277%, n=17).

### Table 1. *CD101* mRNA (non-normalized) reductions upon TLR8 stimulation in cells from people with a *CD101* variant and reference

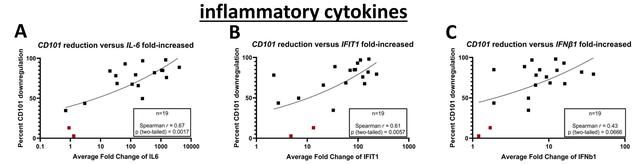
| Incubation period | 6-hour CD101 (copies/µL) | | | 24-hour CD101 (copies/µL) | | |
|---|---|---|---|---|---|---|
| Characteristic | Difference | 95% CI | p-value | Difference | 95% CI[1] | p-value |
| Reference | -22 | -34, -9.8 | <0.001 | -13 | -18, -6.9 | <0.001 |
| Ig-like CD101 | -30 | -43, -17 | <0.001 | -32 | -44,-20 | <0.001 |
| Comparing Reference and Ig-like CD101 | -8.0 | -26, 9.8 | 0.4 | -19 | -33, -5.6 | 0.006 |

[1]CI = Confidence Interval

In linear-regression model, *CD101* mRNA reductions at 24 hours of TLR8 stimulation were greater in cells from people with any *CD101* Ig-like variant than *CD101* reference (β (variant)=-32 vs. β(reference)=-13, interaction p=0.006. Note: when *CD101* mRNA levels were normalized to *RPP30*, β (variant)=-35 vs. β(reference)=-23, interaction p=0.2), and when *CD101* mRNA levels were normalized to *18s RNA*, β (variant)=-0.02811 vs. β(reference)=-0.00701, interaction p=0.535) (data not shown).

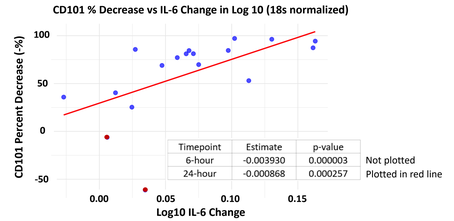
## RESULTS

### Figure 6. CD101 mRNA reduction is inversely correlated to pro-inflammatory cytokines



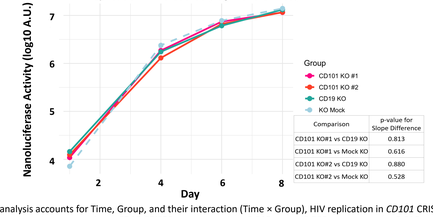

For PBMC genotyped for CD101, following TLR8 stimulation, we observed significant mRNA increases for *IFNβ1* (mean 11.1-fold), *IL-6* (644.4-fold), and *IFIT1* (83.8-fold), and a significant decrease for *CD101* (74.1% +/- 22.5%; n=17), in 17 out of 19 donors. In each panel, the line indicates the non-linear regression between the variables shown. The samples denoted in red (included in the correlation) correspond to two donors who failed to mount a pro-inflammatory response and did not downregulate *CD101* mRNA following TLR8 agonist stimulation.

### Figure 7. *IL-6* mRNA increases with *CD101* mRNA reduction




Following TLR8 stimulation, we used linear regression with generalized estimating equations to account for replicates of measurements of CD101 expression for each person. We plotted with blue symbols the means of the changes observed in *CD101* and IL-6 mRNA for a given participant. Samples in red denote those two donors that failed to mount a pro-inflammatory response and did not downregulate *CD101* mRNA following TLR8 agonist stimulation (their data were not included in the regression analysis).

### Figure 8. HIV replication in *CD101* CRISPR-knock-out (KO) CD4⁺ T cells and mock controls




Using linear model analysis accounts for Time, Group, and their interaction (Time × Group), HIV replication in *CD101* CRISPR-knocked-out CD4⁺ T cells was comparable to mock controls, CD19 CRISPR-knocked-out (p=0.813 and p=0.880) and mocks (p=0.616 and p=0.528).

## CONCLUSIONS

- *CD101* is downregulated in PBMCs in response to TLR8 (Fig. 2, Table 1) and HIV (Fig. 4).
- In PBMC, $T_{regs}$ and APCs show greater *CD101* downregulation compared to CD4⁺ and CD8⁺ T cells (Fig. 3).
- When *CD101* downregulation occurs, it is inversely related to the proinflammatory response (Fig. 5, Fig. 6 and Fig. 7). However, we have not shown a causal relationship.
- HIV replication is not affected by *CD101* KO (Fig. 8).
- Our results suggest that *CD101 variation* may affect HIV infection risk indirectly, via effects on inflammation, rather than directly on HIV replication. Candidate *CD101* variants may accentuate and prolong this effect.

## REFERENCES

*1. PLoS Pathog.* 2017 Nov 6;13(11):e1006703. 2. *Cell Rep Med 2021* Jun 15;2(6):100322. 3. *J. Immunol. 1998*;161:209–217

## ACKNOWLEDGEMENTS

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