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Functional and structural determinants of T-cell receptor mediated HIV control

Gursev Anmole, Shuguang Li, Nathan Chatron, Rachel Miller, Funsho Ogunshola, Zaza M Ndhlovu, George F Gao, and Mark A Brockman

“I have no conflicts of interest to disclose”

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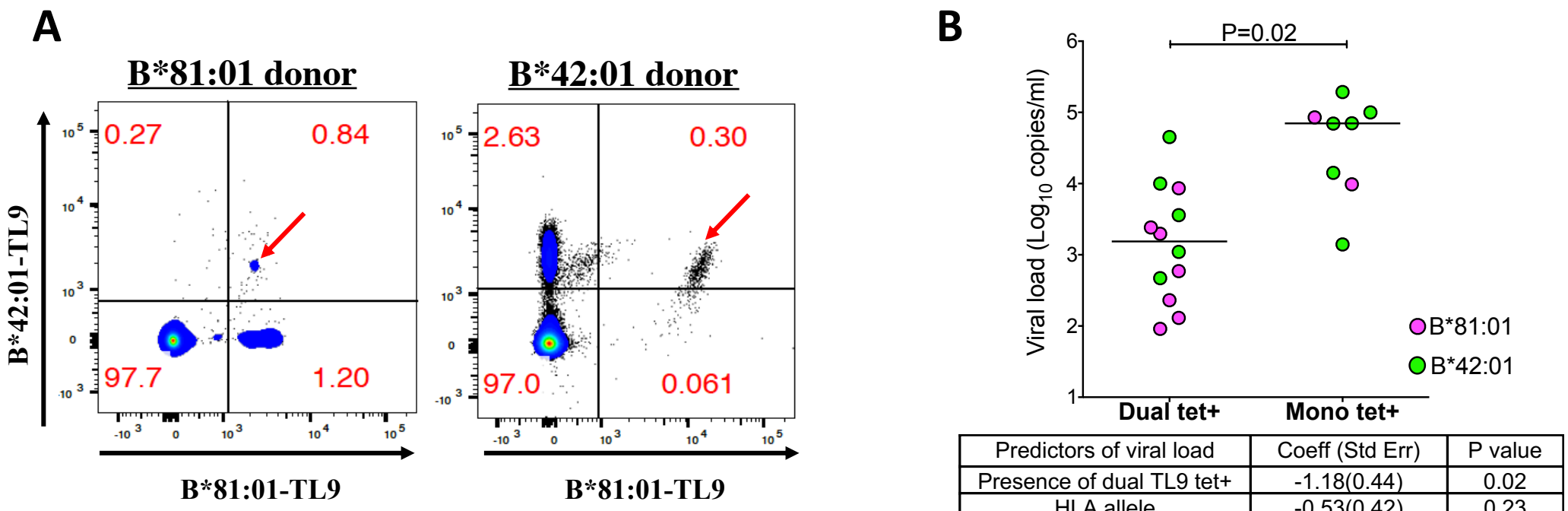
Background:

HLA-B*81:01 is associated with control of HIV-1 subtype C infection, whereas the closely related allele B*42:01 is not. The immuno-dominant epitope for both HLA alleles is Gag TL9 (TPQDLNTML). We recently observed that the presence of CD8 T cells able to recognize the HIV Gag TL9 epitope presented on both B*42 and B*1 is associated with lower viral loads. Dual-HLA reactivity was associated with TCR clonotypes that display broader recognition of viral escape variants. Here, we characterized an expanded panel of B*42 restricted TCR clones to gain further insight into mechanisms of HIV control.

Dual HLA-B*42/B*81 TL9 tetramer+ response is associated with lower viral load

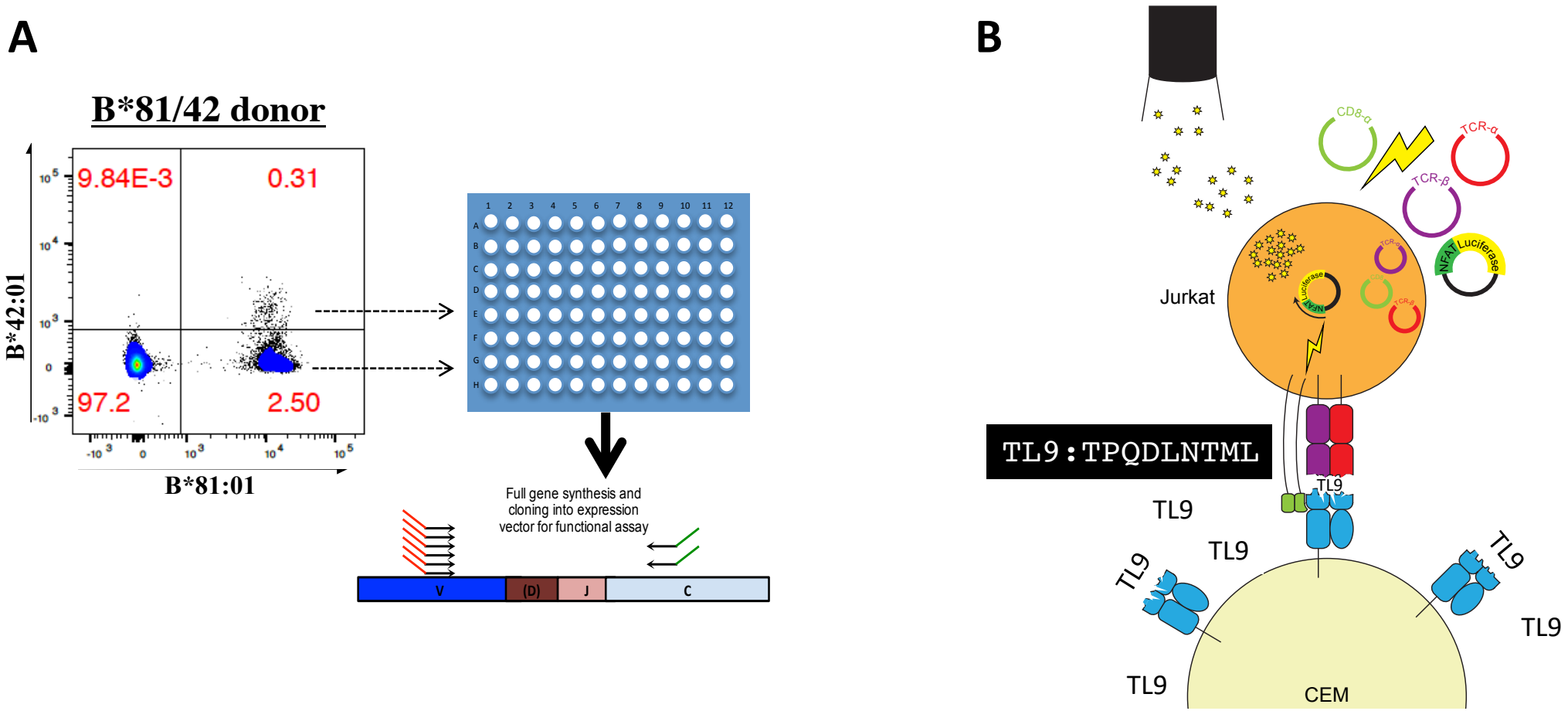


Predictors of viral load	Coeff (Std Err)	P value
Presence of dual TL9 tet+	-1.18(0.44)	0.02
HLA allele	-0.53(0.42)	0.23

Representative flow plots TL9 tetramer responses in one B*81:01 and one B*42:01 participant **(A).** Multivariable linear regression analyses that included HLA allele and presence of dual tetramer-reactive T cells as independent variables indicated dual-reactivity (p=0.02) but not HLA (p=0.23) a determinant of plasma viral load **(B).**

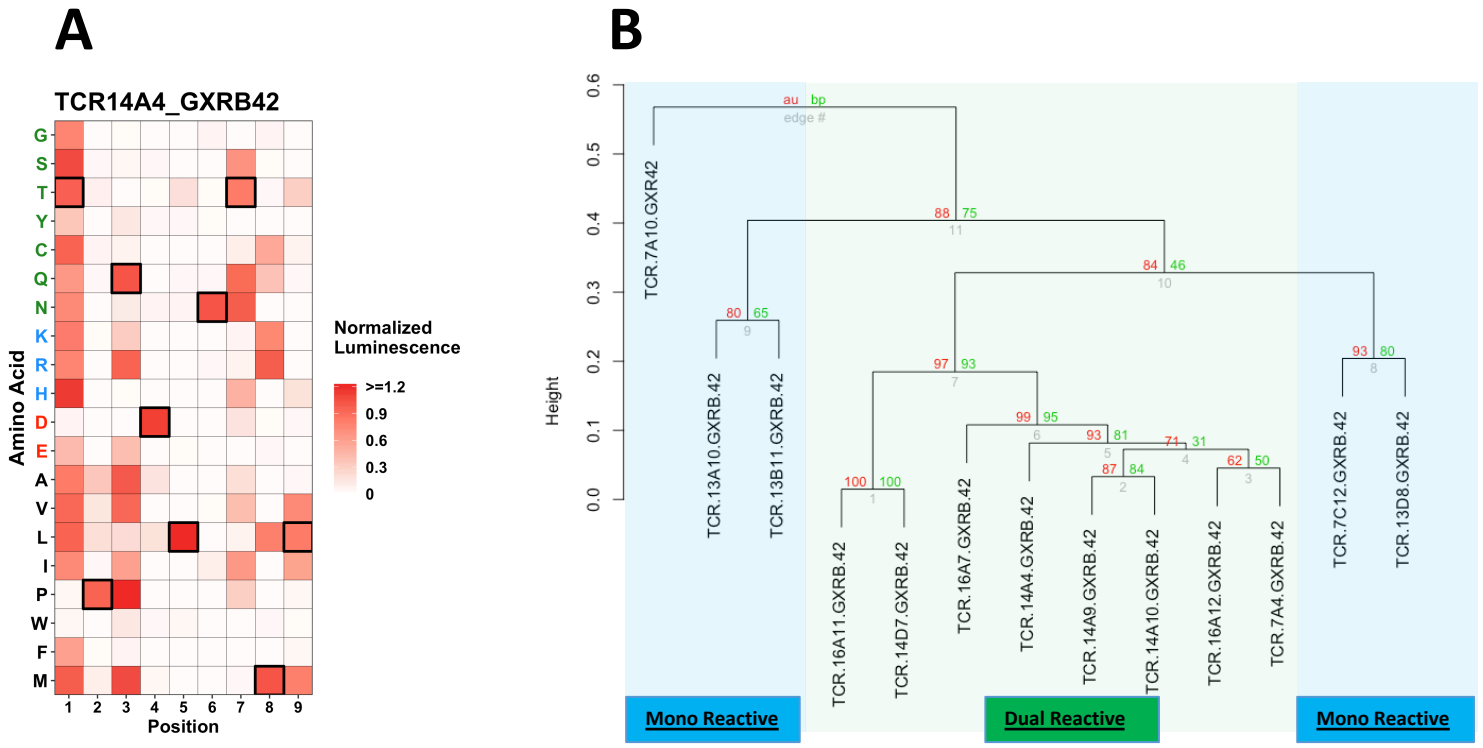
Ogunshola and Anmole et al. Nature Communications 2019.

Methodologies to isolate and functionally characterize TL9 specific TCR



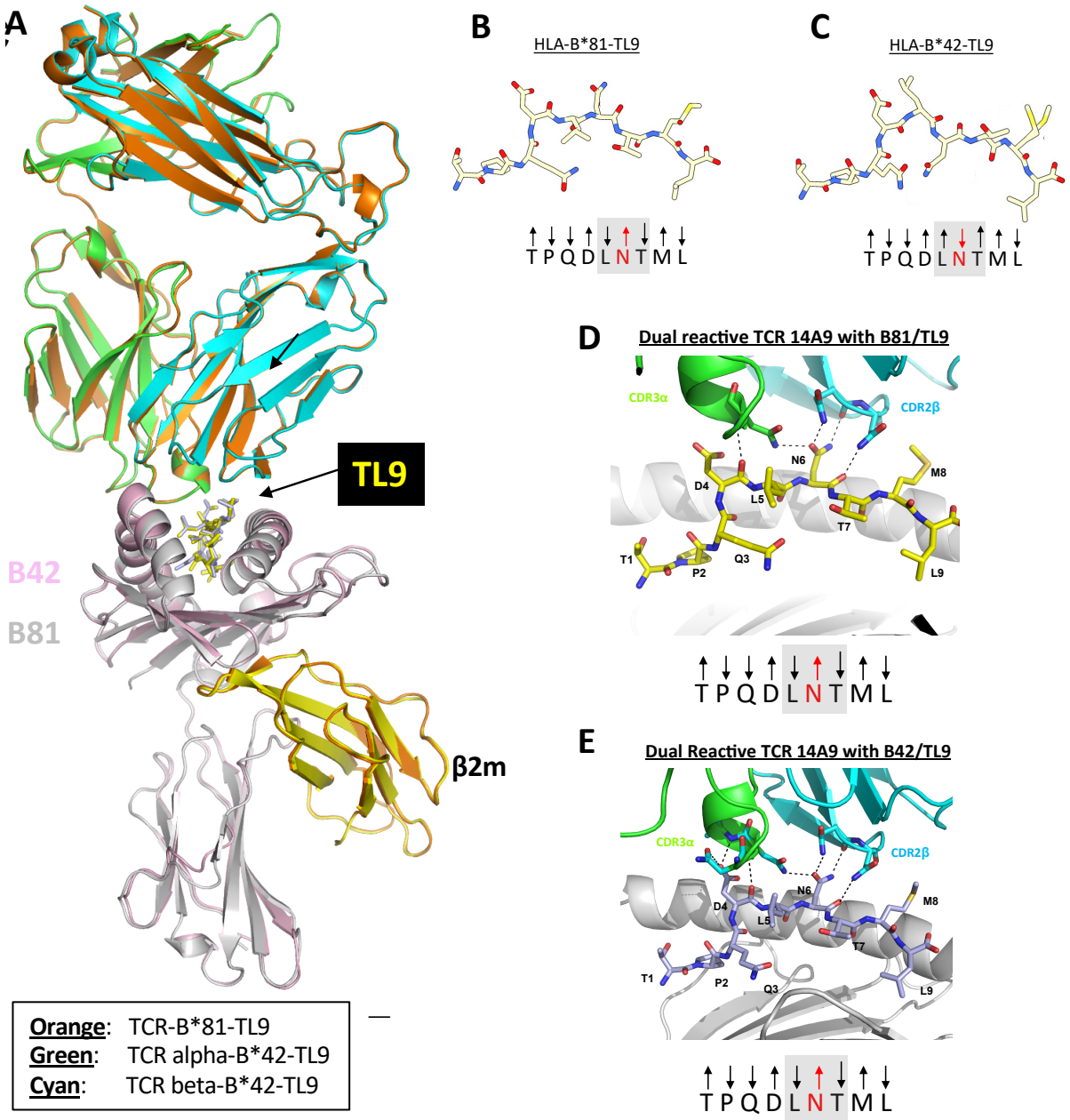
Single TL9 tetramer+ CD8 T cells were isolated by FACS from 6 HIV+ participants (3x B*81; 3x B*42) displaying dual-reactive phenotypes. TCR beta genes were amplified by RT-PCR and sequenced. Paired TCR alpha genes were amplified for selected dominant clones (**A**). Full-length alpha/beta genes were synthesized and their antigen specificity was assessed using a Jurkat T-cell based luciferase reporter method (**B**). TCR+ Jurkat "effector" cells were co-cultured with B*81 or B*42 "target" cells pulsed with either TL9, TL9 variants (180 peptides, representing all possible single amino acid TL9 mutations) or infected with HIV-1. TCR signaling was quantified by luminescence.

HLA-B*42+ patient derived dual reactive TCR are functionally distinct from mono reactive TCR and show cross-reactivity to naturally occurring HIV escape variants



(A) Representative heat map summarizing cross reactivity profile of a dual reactive TCR 14A4 towards TL9 variants spanning all 20 amino acids substituted from TL9 positions 1-9. Intensity of heat map indicates strength of signaling relative to WT TL9 (boxes). The On the y-axis are amino acids and the x- axis are the positions 1-9. This analysis was conducted on 8 dual-B*42/B*81 reactive and 5 mono-B*42 reactive TCR clones. Reactivity profiles were subjected to hierarchical clustering, shown using pvclust with 5000 iterations **(B)**. Dual reactive TCR cluster more closely with one another compared to mono reactive TCR. (**C**) For TCR cross reactivity analysis, we focused on testing TCR cross reactivity to variants present at a frequency of >10% in the total single amino acid variant sequence space in HIV LANL. Dual reactive TCR recognize a larger proportion of naturally occurring TCR. Note, B*81 derived TCR were additionally tested and showed high amounts of escape variant recognition.

Dual reactive TCR induce TL9 on HLA-B*42 into a HLA-B*81 like conformation



Orange: TCR-B*81-TL9
Green: TCR alpha-B*42-TL9
Cyan: TCR beta-B*42-TL9

Crystal structures were solved for the tripartite complex of TCR clone 14A9 (dual-reactive B*42-derived clone) bound to either B*81/TL9 or B*42-TL9. An overlay of these structures illustrates that the TCR engages both antigens using an identical mechanism **(A).** In the absence of TCR, the TL9 peptide adopts a different orientation when bound to HLA-B*81 (**B**) verses B*42 (**C**) based off Kloverpris et al *JVI* 2012. The direction of the arrows below each figure indicates that the residue is exposed to TCR (up) or buried in the HLA binding groove (down). Of note, TL9 positions 5,6 and 7 differ between the 2 HLA types . (**C**) Notably, in the TCR-bound structure TL9 is presented by B*42 in a "B*81-like" conformation, with residue N6 exposed and forming critical interactions with TCR (**D and E**). Interestingly, the TCR beta chain engages the peptide using its CDR2 region (indicated in Cyan).

Conclusions:

- Gag TL9-specific T cell responses in B*81 and B*42 expressing individuals contained dual HLA-reactive cells that were associated with lower viremia
- B*81-derived and dual-reactive B*42-derived TCR recognized a greater proportion of HIV escape variants in TL9, suggesting that these TCR clonotypes display enhanced ability to control HIV
- Dual reactive TCR induce conformational change in the TL9 peptide bound to B*42, adopting a "B*81-like" structure
- This work highlights the impact of TCR functional diversity on CD8 T cell-mediated control of HIV and presents a novel strategy to isolate and comprehensively characterize antigen specific TCR.
- Cross-reactive TCR may serve as novel immune-based therapeutics to detect and eliminate latent HIV reservoirs