Supplementary Information for:

Probing the Effect of Force on HIV-1 Receptor CD4

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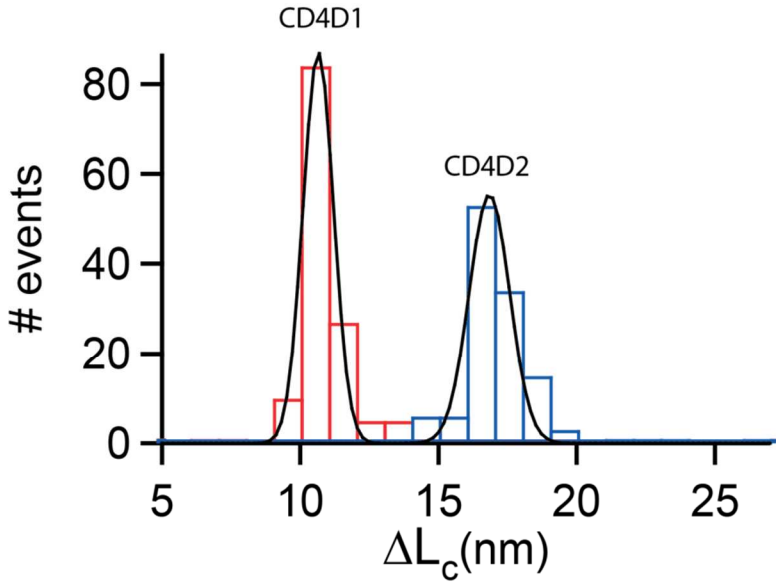
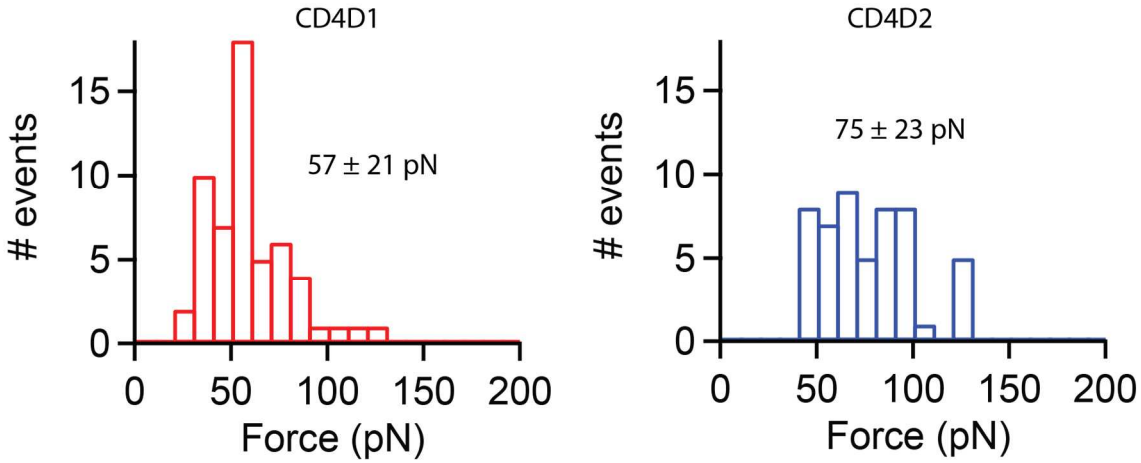
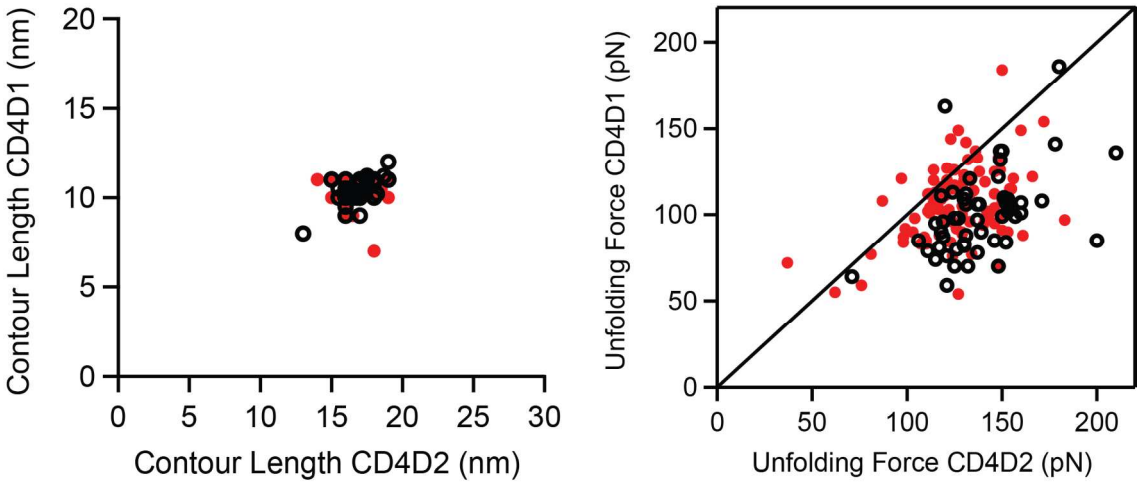


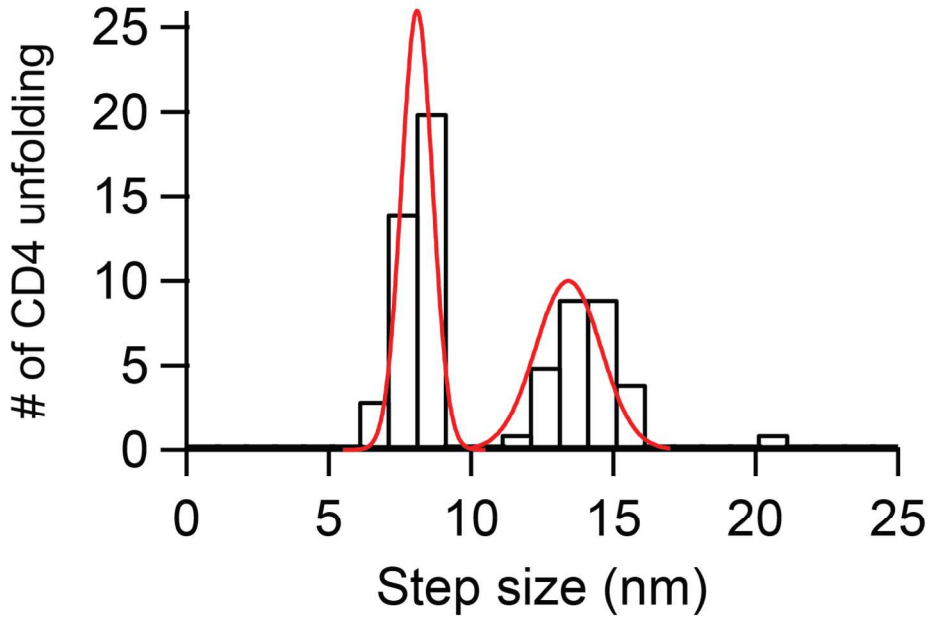
Supplementary Fig. 1. Contour length histogram of CD4D1 and CD4D2 unfolding in force-extension mode. The measured contour length is 10 ± 1 nm and 16 ± 4 nm for CD4 D1 and CD4 D2, respectively (n=257).



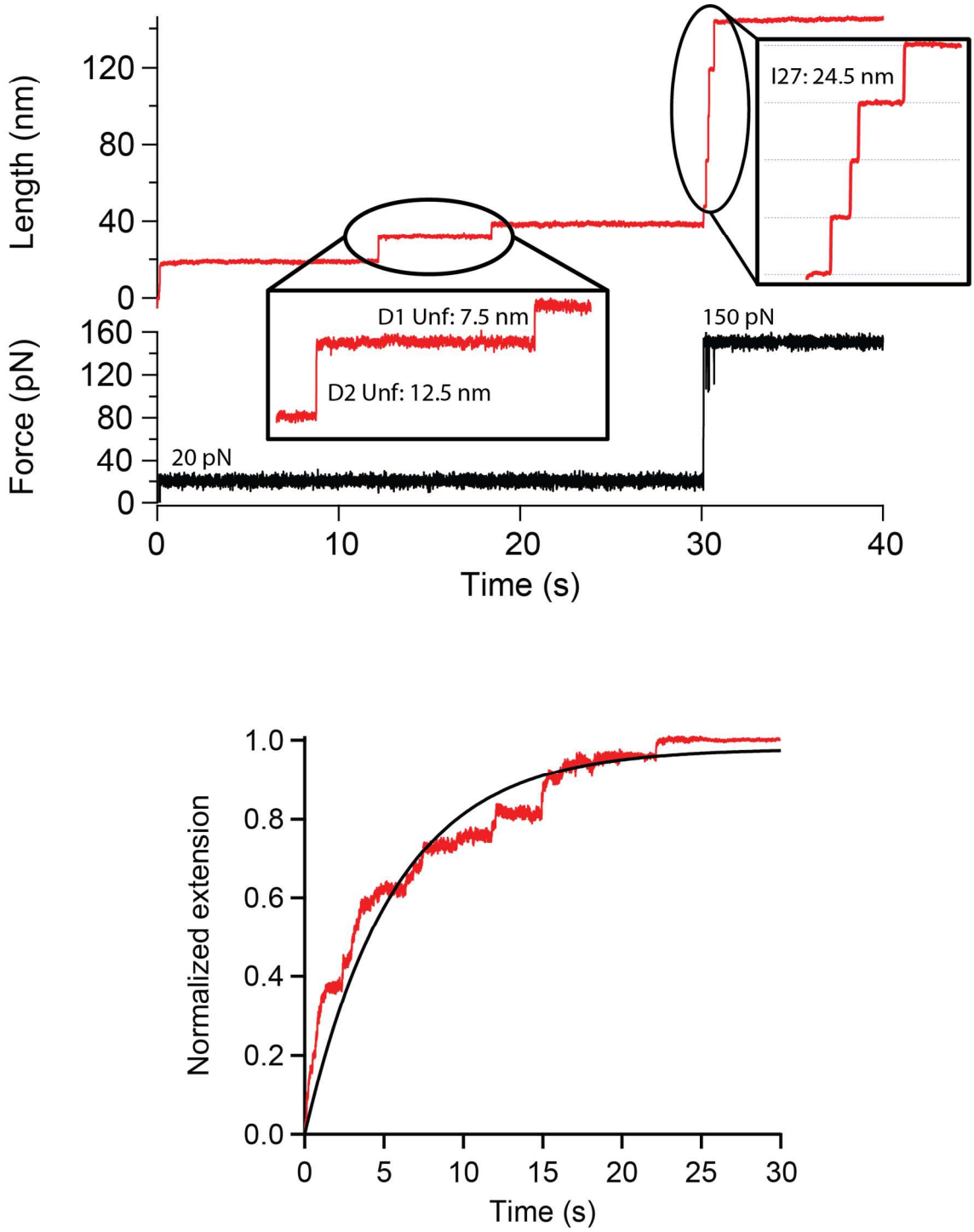
Supplementary Fig. 2. Unfolding force histograms for CD4D1 and CD4D2 at pulling speed of 10 nm/s in force-extension mode. The unfolding force decreases by 40% with respect of the unfolding force measured at 400 nm/s.



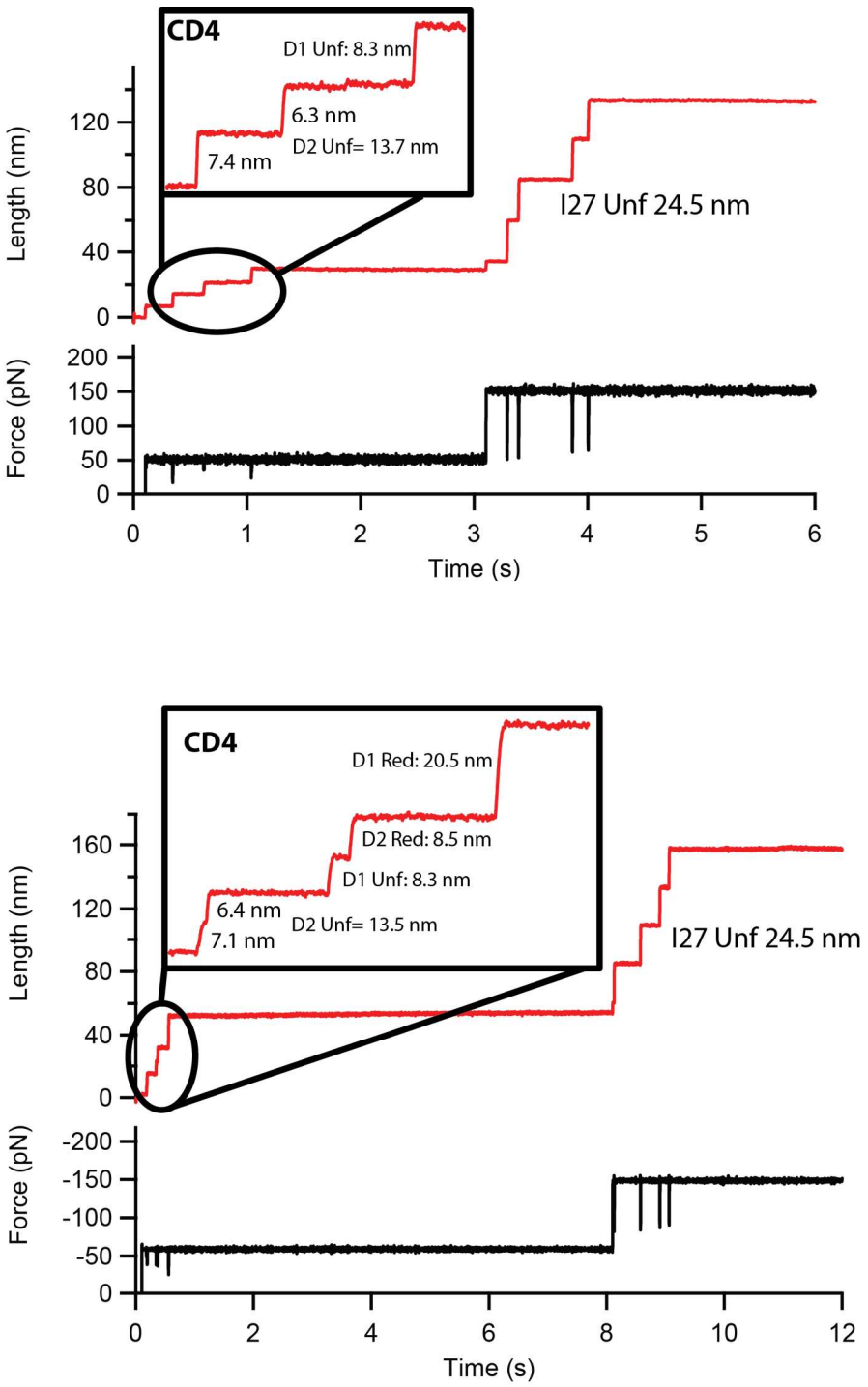
Supplementary Fig. 3. Comparative plots of contour length (left) and unfolding force (right) for CD4D1 and CD4D2. Data in the absence and presence of Ibalizumab, red dots and black circles, respectively. The plot of unfolding forces demonstrates that the unfolding force of CD4D2 is generally higher than that of CD4D1 even though the unfolding of CD4D2 generally occurs prior to the unfolding of CD4D1.



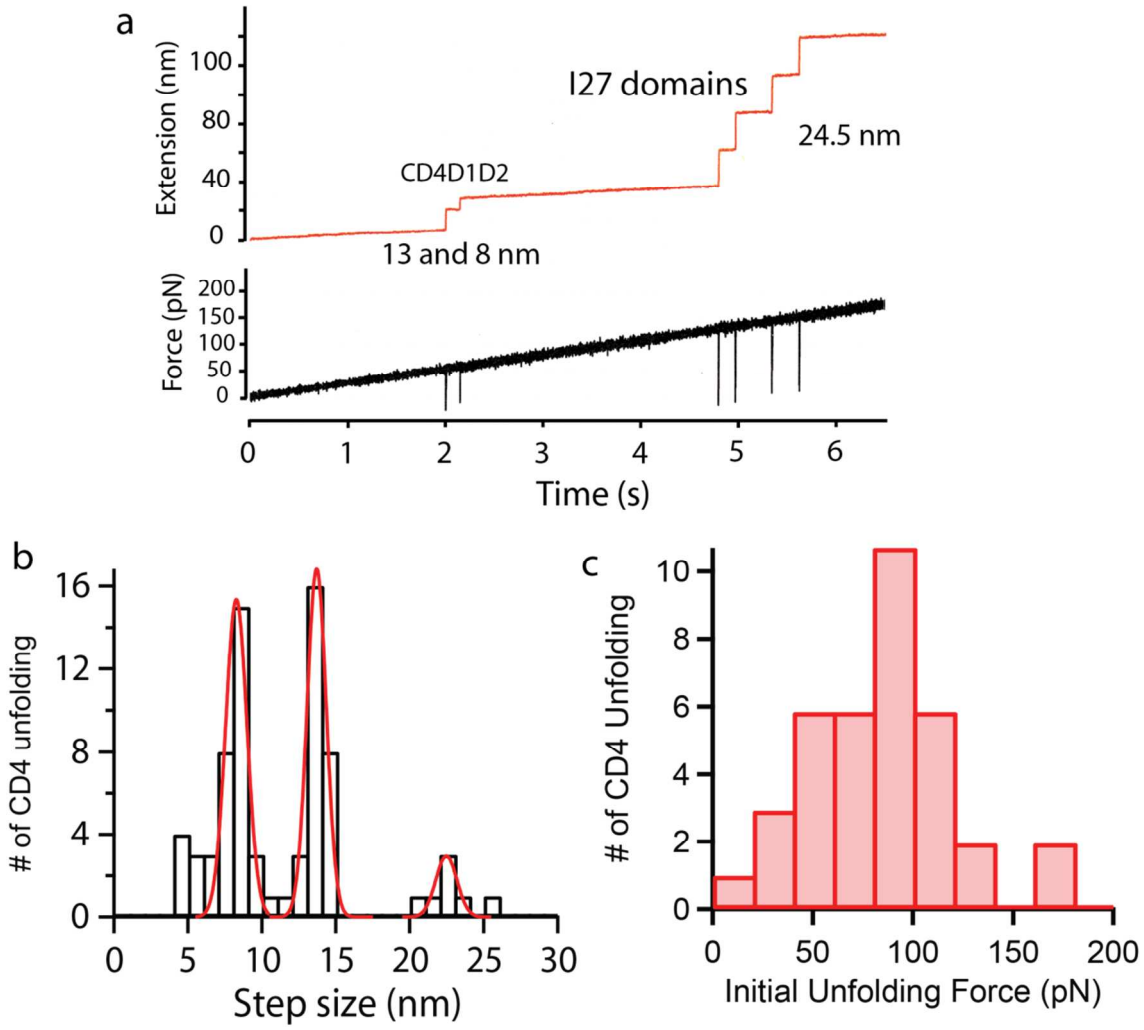
Supplementary Fig. 4. Step size histogram for the unfolding of CD4D1D2 at constact force of 50 pN. The measured step size at this force is 8.1 ± 0.8 nm and 13.4 ± 1.6 nm for CD4D1 and CD4D2 respectively (n=66).



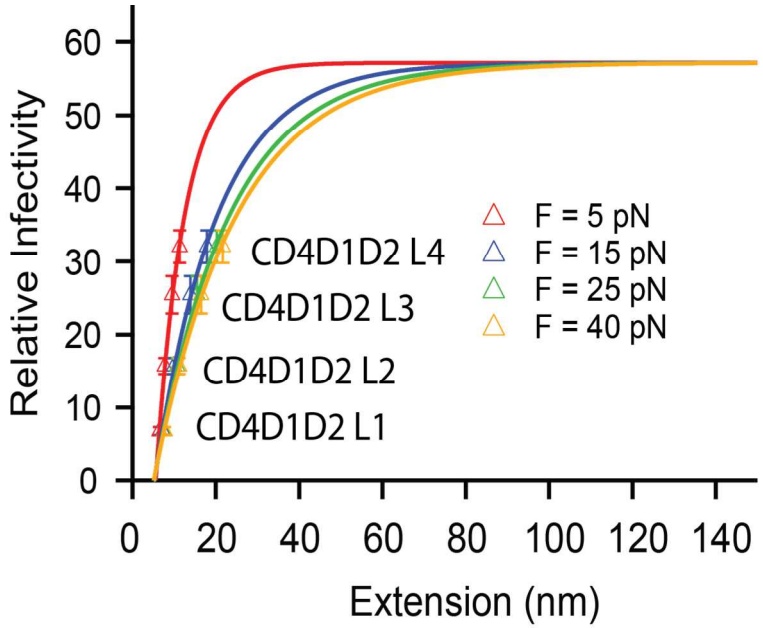
Supplementary Fig. 5. Force-clamp experiments at 20 pN pulling force. Upper panel: A pulse of 20 pN force triggers the mechanical extension of CD4D1D2. A second pulse of force of 150 pN is applied to monitor the unfolding of I27. Bottom panel: Summed, averaged and normalized traces of CD4D1D2 unfolding at 20 pN (n=23). A single exponential fit renders an unfolding rate of $0.19 \pm 0.06\ s^{-1}$.



Supplementary Fig. 6. Detection of mechanical intermediates in the unfolding of CD4D2. The unfolding of this domain is detected in two steps of ~7.2 and ~6.4 nm. This phenomenon is observed in ~5% of the total number of traces at forces of 50-60 pN, but it could be more significant at lower forces. The upper panel is in the absence of any reducing agent and the lower panel in the presence of 10μM human thioredoxin, triggering the reduction of both disulfide bonds in CD4 domains.



Supplementary Fig. 7. (a) Force-ramp experiments of the polyprotein $(I27)_2$-CD4D1D2-$(I27)_2$. Typical force-ramp trace is shown in the upper panel. **(b)** Histogram pf length for the unfolding of CD4D1D2. Two dominants peaks at 8.3 ±1.1 nm for CD4D2 and 13.7 ± 1 nm for CD4D1 are observed. A less prominet peak at 22.5 ± 1.0 corresponds to the simultaneous unfolding of CD4D1 and CD4D1 (n=72). **(c)** Histogram of initial unfolding force of the tandem CD4D1D2 in force-ramp mode (n=37)

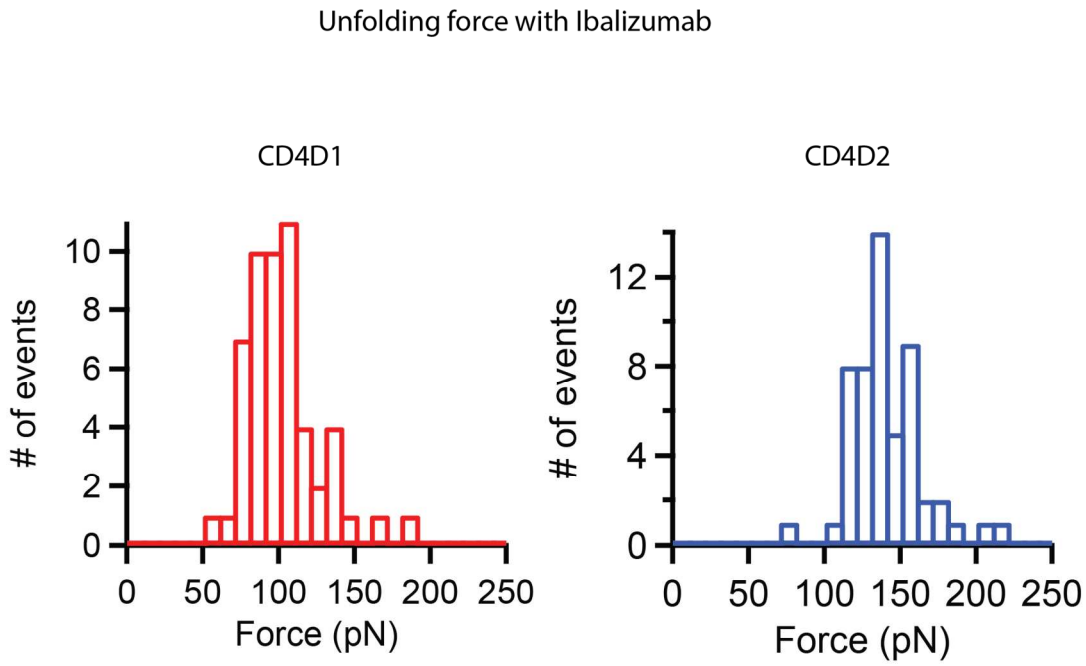


Supplementary Fig. 8. Plot of relative infectivity of HIV-1 and extension of CD4-linker constructs at different forces determined from the Freely Jointed Chain of polymer elasticity at different pulling forces. In this plot we assumed CD4D1 and CD4D2 to be folded and with extensible linkers. We also assumed the infectivity of the CD4 wild type form to be the maximum possible infectivity and therefore it represents and asymptote at ~57 RLU. In this plot is reflected the effect of force in the linkers since we consider that CD4D1 and CD4D2 are folded. As we observe, there is a drastic change in infectivity and extension between 5 to 15 pN. Nevertheless, CD4D1 and CD4D2 can unfold over time under the effect of a mechanical force. This situation can also happen for all four domains in CD4WT form. A more accurate situation is represented in figures 2c and 2d, where all different possible scenarios for domain unfolding are considered.

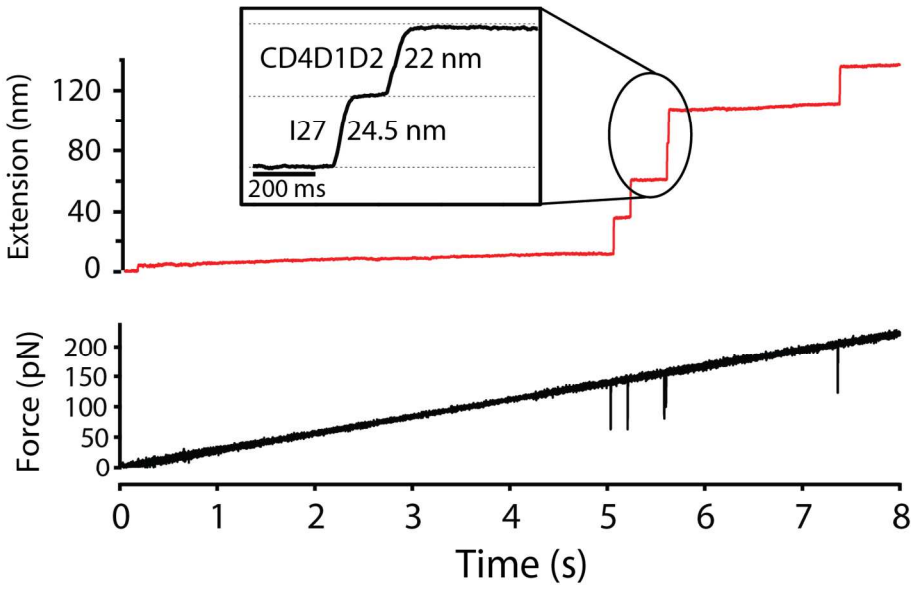
Supplementary Table 1. Fitting parameters obtained using the empirical correlation (see main text for description of parameters):

$$I(x) = I_0 - A\exp\left\{\frac{x(F)}{x_0}\left(\tanh^{-1}\left(\frac{Fa}{k_BT}\right) - \frac{k_BT}{Fa}\right)\right\}$$

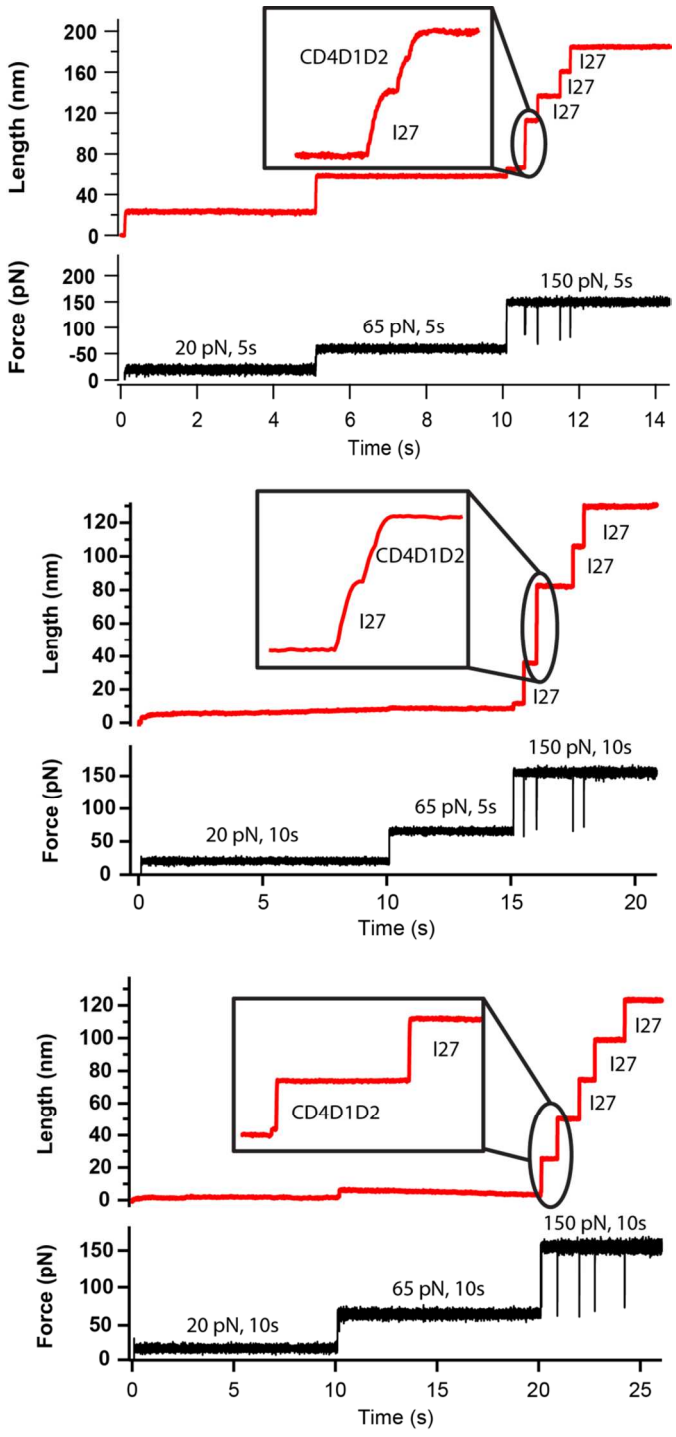
F (pN)	***A* (Infectivity units RLU)**	**x_0 (nm)**
5	128.7	6.86
15	81.5	14.9
25	76.3	17.9
40	74.1	19.7



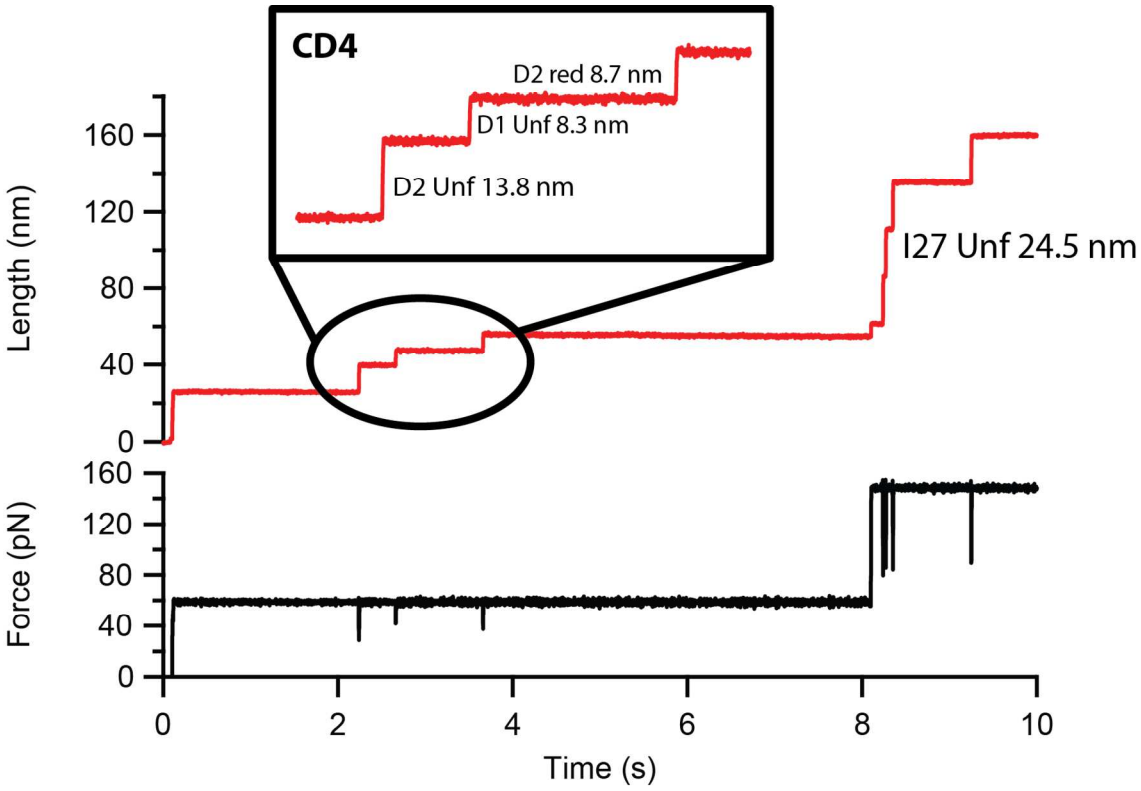
Supplementary Fig. 9. Unfolding force histograms for CD4D1 and CD4D2 in the force-extension mode and in the presence of antibody Ibalizumab. We measured and unfolding force of 100 ± 27 pN for CD4D1 (n=53) and 138 ± 23 pN for CD4D2 (n=53).



Supplementary Fig. 10. Force-ramp trace of $(I27)_2$-CD4D1D2-$(I27)_2$ in the presence of Ibalizumab. The unfolding of CD4D1D2 occurs within the unfolding of I27 modules indicating that the unfolding of the tandem CD4D1D2 occurs at a similar unfolding force than I27 modules. This phenomenon is not observed in the absence of Ibalizumab.



Supplementary Fig. 11. Force-clamp traces of $(I27)_2$-CD4D1D2-$(I27)_2$ with three pulses of force, 20, 65 and 150 pN and different times in the presence of Ibalizumab. In the three examples CD4D1D2 unfolds at 150 pN and both domains unfold almost concomitantly due to the elevated force.



Supplementary Fig. 12. Force-clamp trace of $(I27)_2$-CD4D1D2-$(I27)_2$ in the presence of 10 μM human Trx. The unfolding of CD4D1D2 is observed prior de reduction of CD4D2 disulfide bond. The reduction of CD4D1 disulfide bond is not observed in this occasion. We suspect that both disulfide bonds have different force dependence. Disulfide bond reduction by Trx enzymes has been previously shown to be force dependent [3].

REFERENCES

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