Arginine Insertion and Loss of N-linked Glycosylation Site in HIV-1 Envelope V3 Region Confer CXCR4-tropism

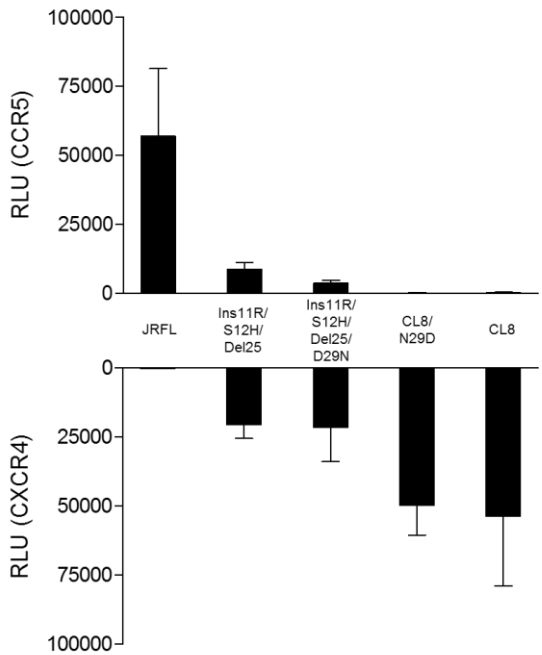
Kiyoto Tsuchiya[1], Hirotaka Ode[2,3], Tsunefusa Hayashida[1,4], Junko Kakizawa[1], Hironori Sato[2], Shinichi Oka[1,4] and Hiroyuki Gatanaga[1,4]

[1]AIDS Clinical Center, National Center for Global Health and Medicine
1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

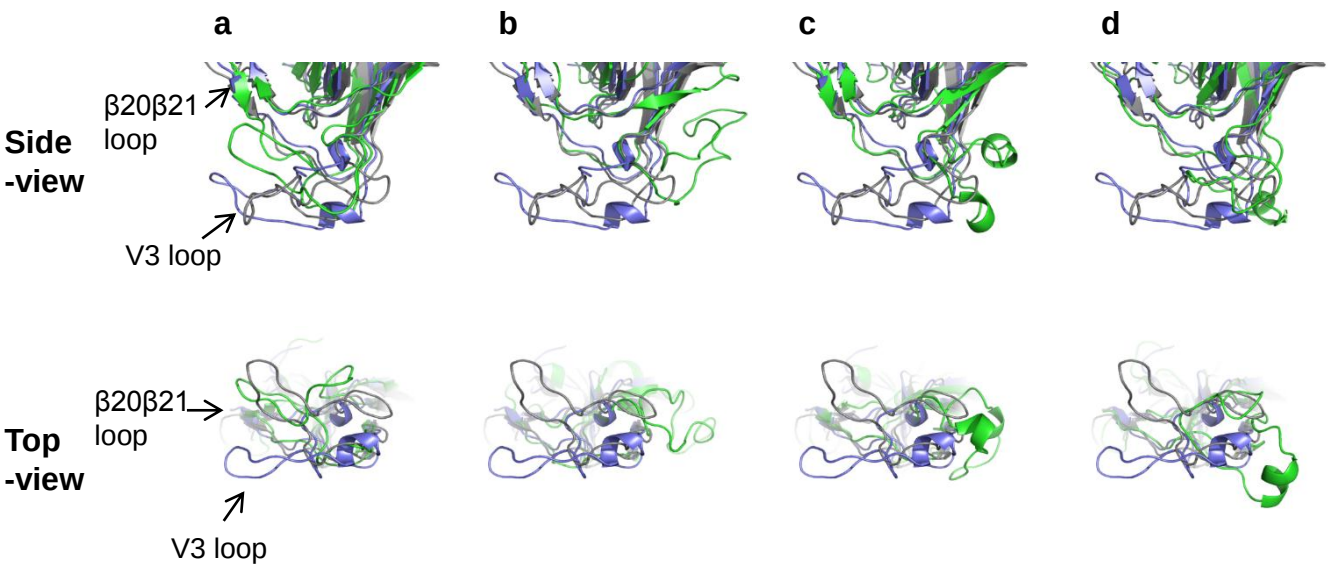
[2]Pathogen Genomics Center, National Institute of Infectious Diseases
4-7-1 Gakuen, Musashimurayama-shi, Tokyo 208-0011, Japan

[3]Clinical Research Center, National Hospital Organization Nagoya Medical Center
4-1-1 Sannomaru, Naka-ku, Nagoya 460-0001, Japan

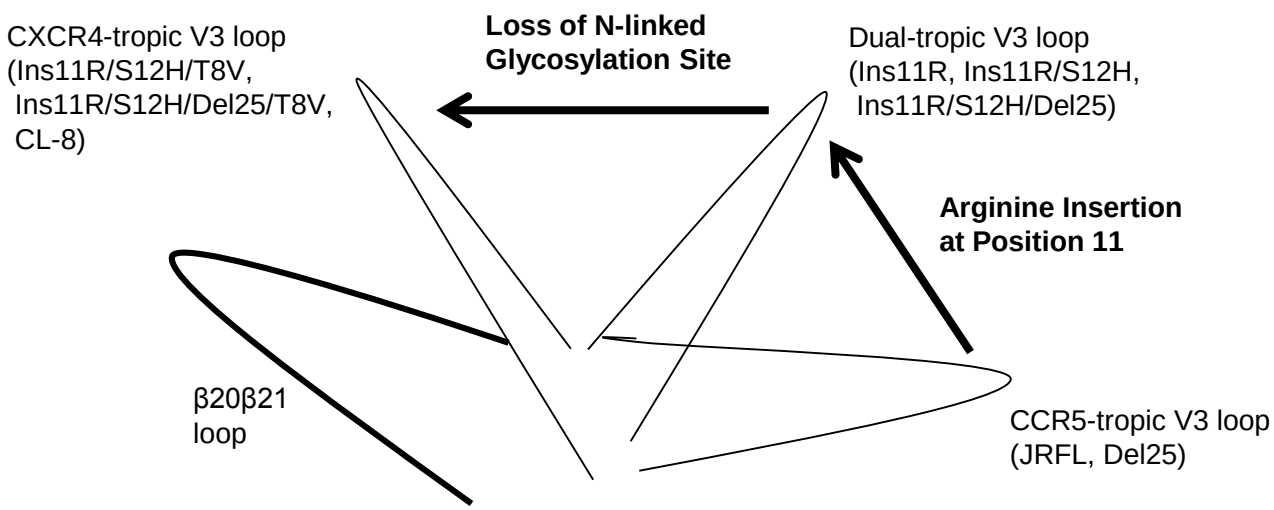
[4]Center for AIDS Research, Kumamoto University
2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan



Supplementary Figure S1. Effects of D29N in cell-cell fusion assay. Cell-cell fusion assay was performed using Env-expressing 293T cells and $CD4^+$ and $CCR5^+/CXCR4^+$ COS-7 cells. Data are mean±SD values in relative luminescent unit (RLU) of six experiments (performed in duplicate and repeated three times).



Supplementary Figure S2. Structural models of V3 loops of Ins11R/S12H/Del25-derived mutants. MD simulations were performed for the HIV-1 Ins11R/S12H/Del25 gp120 outer domain with N5Y (a), K10I (b), Y22H (c), or V26G (d) substitution in V3 loop. Structures were constructed and superimposed as described in Fig. 3 caption. (a) Superimposition of N5Y (green), Ins11R/S12H/Del25 (gray), and CL8 (navy). (b) Superimposition of K10I (green), Ins11R/S12H/Del25 (gray), and CL8 (navy). (c) Superimposition of Y22H (green), Ins11R/S12H/Del25 (gray), and CL8 (navy). (d) Superimposition of V26G (green), Ins11R/S12H/Del25 (gray), and CL8 (navy).



Supplementary Figure S3. Schematic summary of V3 loop orientation and tropism. CCR5-tropic V3 loop was placed in the opposite direction from the β20-β21 loop. Arginine insertion at position 11 changed the V3 loop orientation and conferred dual-tropism. Loss of N-linked glycosylation site further changed the V3 loop orientation into the same direction with the β20-β21 loop and altered it CXCR4-tropic.