Table S1. Primers used for genetic engineering of CLD-expressing constructs

Primer	Sequence 5'→3'	Enzyme
C1	GGCAGCCATATGAAGAAAGTGGTGCTGGGC	Nde Ⅰ
C2	GCCGGATCCACCTCCACCAGAGCCACCTCCGCCCGAACCGCCACCGCCAGCTAGCACCACGATGTCTAT	BamH Ⅰ
C3	GCCGGATCCACCTCCACCAGAGCCACCTCCGCCAGCTAGCACCACGATGTCTAT	BamH Ⅰ
C4	GCCGGATCCACCTCCACCAGAGCCACCTCCGCCCGAACCGCCACCGCCGGTGTCAGAGTTGGCAGTCAA	BamH Ⅰ
C5	GCCGGATCCACCTCCACCAGAGCCACCTCCGCCCGAACCGCCACCGCCCTCCACTTCACAGATGTAAGT	BamH Ⅰ
C0	CGCAAGCTTCTAAGCTAGCACCACGATGTCTAT	Hind Ⅲ
D1	ACGAAGCTTCTACGCAGGAGGGGGGTTTG	Hind Ⅲ
D2	GGTGGATCCCACCCCTGTCCCTGG	BamH Ⅰ
D3	GGTGGATCCGGCGGTGGCGGATCGGGCGGTGGCGGATCGCACCCCTGTCCCTGG	BamH Ⅰ
D4	GGTGGATCCGGCGGCGGCGGCTCGGGTGGTGGTGGTTCTGGCGGTGGCGGATCGGGCGGTGGCGGATCGCACCCCTGTCCCT	BamH Ⅰ
D5	GGTGGATCCGGCGGTGGCGGATCGGGCGGTGGCGGATCGGTGGGTGAGCTCTCAGAG	BamH Ⅰ
D6	GGTGGATCCGGCGGCGGCGGCTCGGGTGGTGGTGGTTCTGGCGGTGGCGGATCGGGCGGTGG	BamH Ⅰ
D0	GGCAGCCATATGCACCCCTGTCCCTGG	Nde Ⅰ
MF	GATCGCGCTGACTCAAGAAGATGCCTTTGGGAC	
MR	GTCCCAAAGGCATCTTCTTGAGTCAGCGCGATC	

Table S2. Schematics of CLD components

protein	CD4	Linker (aa)[a]	neck	DC-SIGN	C5' [b]	C3' [c]	D5' [d]	D3' [e]	Mr. (KD)	Note
sCD4	+	—	—	—	C1	C0			22.1	N terminal 183 aa
sDC-SIGN	—	—	—	+			D0	D1	18.4	Carbohydrate-recognition domain
C15D	+	15	—	+	C1	C2	D2	D1	40.2	
C20D	+	20	—	+	C1	C3	D3	D1	40.5	
C25D	+	25	—	+	C1	C2	D4	D1	40.9	
C35D	+	35	—	+	C1	C2	D6	D1	41.5	
mC35D	+	35	—	+	C1	C4	D6	D1	33.7	N terminal 106 aa
sC35D	+	35	—	+	C1	C5	D6	D1	31.6	N terminal 87 aa
C25ND	+	35	+	+	C1	C2	D5	D1	59.8	
C35ND	+	35	+	+	C1	C2	D6	D1	60.5	
C35NDs60c	+	35	+	+	MF	MR			60.5	

a: linker is composed of Gly4 Ser repeat.

b: CD4 5' primer;

c: CD4 3' primer.

d: DC-SIGN 5' primer;

e: DC-SIGN 3' primer.

Fig. S1. Kinetics of gp140-CLD binding. The kinetics of binding was performed on a Forte-Bio Octet Red System. Biosensors coupled with biotinylated-CN54 gp140 were immersed in different concentrations of C35NDs60c (200 nM,100 nM, 50nM, 25 nM,12.5 nM), sDC-SIGN (1200 nM, 600 nM, 300 nM, 150 nM) or sCD4 (1200 nM, 600 nM, 300 nM, 150 nM, 75 nM) for association and dissociation. The response in nm shift was recorded as a function of time (rough curves), and fitted either to a langmuir binding model or a bivalent binding model (smooth curve). One out of two independent experiments is shown.

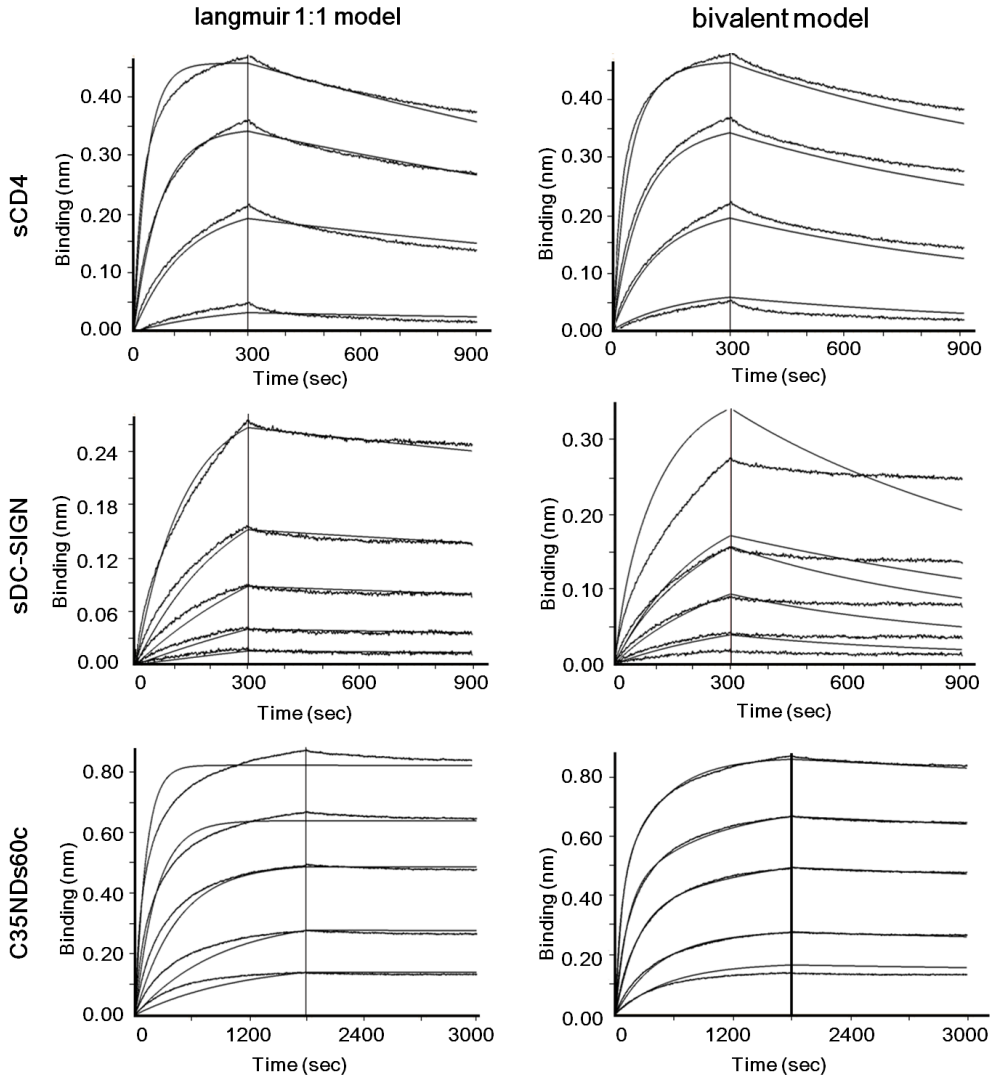

Fig.S2. CLD-gp140 interaction in the presence of anti-CD4 or/and anti-DC-SIGN antibodies. The binding assay was performed on a Forte-Bio Octet Red System. C35NDs60c-immobilized sensors were incubated in running buffer supplemented with antibodies against CD4 or/and DC-SIGN as association buffer for 1800 seconds. Association was keep performing in the presence (black curve) or absence (grey curve) of 5 μg/ml CN54 gp140 for another 1800 seconds. Dissociation was subsequently performed in running buffer for 1800 seconds. Anti-CD4 antibody: RPA-T4, 20μg/ml. Anti-DC-SIGN antibodies: 507 + 526, 20μg/ml each. One out of two independent experiments is shown.

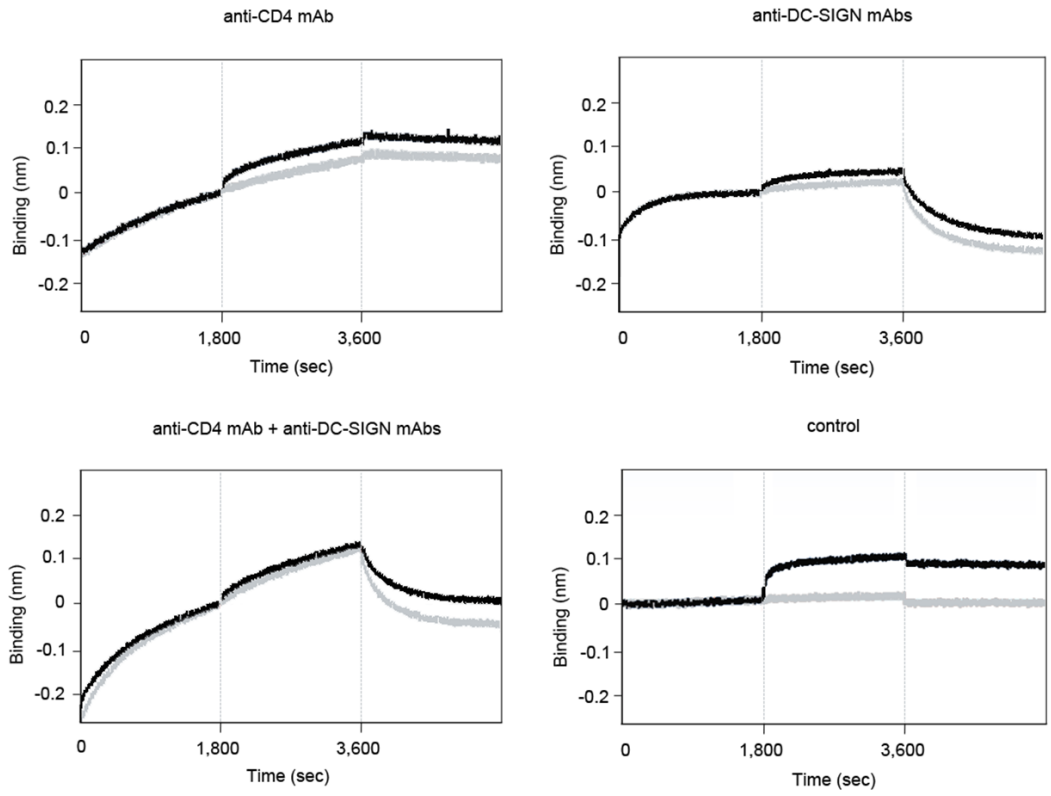

Fig. S3. Dose-response anti-HIV activity of CLD in cell lines and primary cells. BaL Env-pseudotyped HIV-1 was pre-treated with serially diluted C35NDs60c or a comination of sCD4 and sDC-SIGN for 1 h before the addition to (a) U87-CD4.CCR5 cells, (b) Raji/DC-SIGN cells and (c) iMMDCs. (a) Following another 48 h culture, cells were lysed and luciferase acitivity was measured. (b and c) Following another 2 h culture, cells were extensively washed and either lysed for capture assay or cocultured with U87-CD4.CCR5 cells for transfer assay. The concentration ratio at each diltuion point: C35NDs60c:sCD4:sDC-SIGN=1:1:1. Data shown are mean ± SD of two independent experiments, with each condition performed in triplicate. Medium alone was defined as 100%.

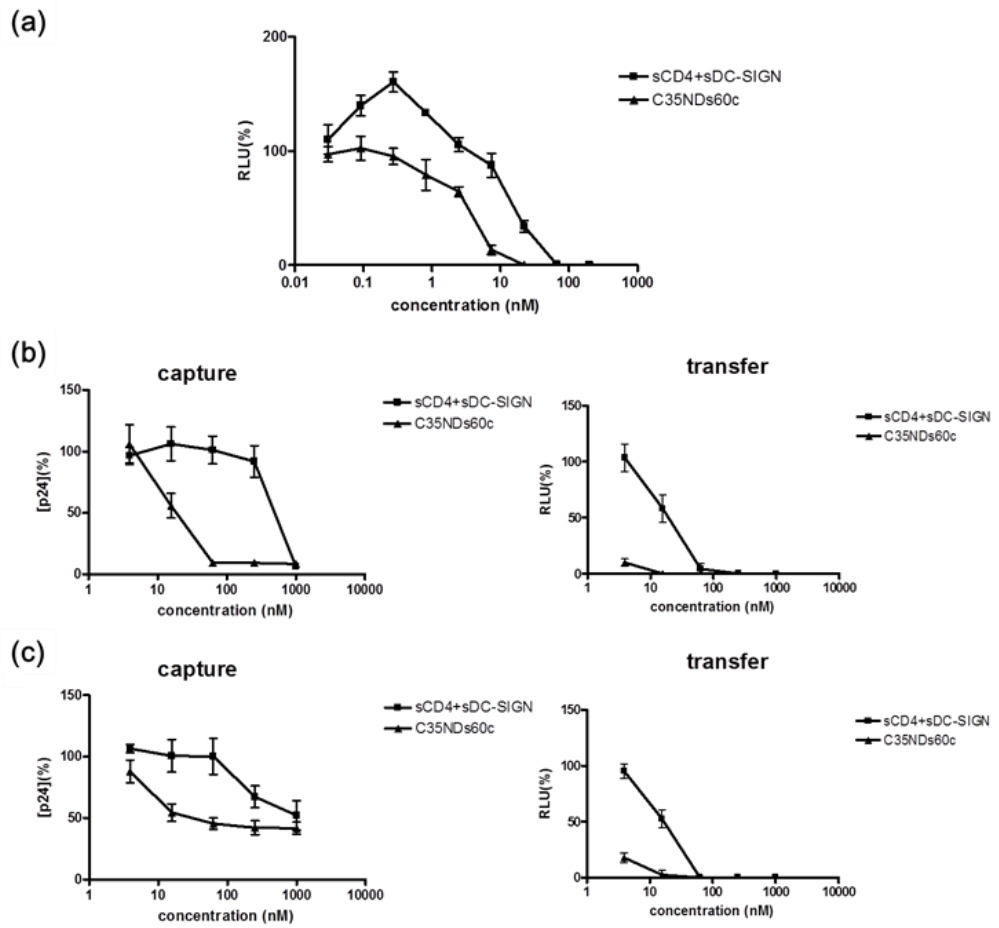

Fig. S4. Dose-response anti-HIV activity of CLD in human cervical tissues. Explants were pre-incubated with serially diluted C35NDs60c, sCD4 or sDC-SIGN for 1 h before exposure to HIV-1_{BaL} for 2 h. Explants were then extensively washed and cultured for nine days. Data shown are p24 antigen (mean ± SD) released from cultured explants at day 9. p24 in the absence of inhibitor was defined as 100%, and was 2.15 ng/ml. Data are representative of two independent experiments, with each condition performed in triplicate.