SUPPLEMENTARY INFORMATION

Manuscript Type: Article

Title: Human transcription factor YY1 could upregulate the HIV-1 gene expression

Author's name: KyungLee Yu, Yu-Mi Jung, Seong-Hyun Park, Seong-Deok Lee and Ji-Chang You*

Affiliation: National Research Laboratory of Molecular Virology, Department of Pathology, The Catholic University of Korea, Seocho-guBanpo-daero 222, Seoul, 63071, Korea

Running title : YY1 could enhance the HIV-1 transcription

Keywords: Ying Yang 1 protein, Human deficiency virus type-1, Transcription activation, Long-terminal repeat region, Tat protein

***Corresponding author :**

Tel: 82-2-2258-7312, Fax: 82-2-2258-7790, E-mail:jiyou@catholic.ac.kr

MATERIALS AND METHODS

Plasmid Construction

For the expression of YY1 and truncated mutants, YY1 and truncated form were amplified by PCR. All of PCR products insert to *Bgl*II/*Kpn*I site of pCMV-HA (Clontech). To construct YY1-mCherry, YY1 amplified by PCR, digested with *Bgl*II/*Kpn*I and made blunted end by klenow DNA polymerase (New England Biolabs) then insert to *Hind*III-klenow treated pCDNA3-mCherry (a kindly provided by Dr. de Rocquigny H). To create $YY1_{T348A}$ and $YY1_{T378A}$, we used an overlap extension PCR method. For $YY1_{T348,378A}$, performed overlap PCR substituting T378 to A378 using $YY1_{T348A}$ as a template. All of primer using overlap PCR also listed in Supplement Table 1. A pNL4-3_{GFP} was kindly provided by Dr. Y.C Sung [1]. A U3/Fluc, U3R/Fluc and U3RU5/Fluc were previously described [2]. For Tat expression, Tat amplified by PCR using pNL4-3_{GFP} as a template then insert to *EcoR*I/*Eag*I site of pCMV-Myc (Clontech). All of primers used in this study are listed in Supplement Table1.

Cell Culture and stable cell line

HeLa and 293T cells were maintained in Dulbecco's modified Eagle medium (Hyclone) and MT-4 cells were maintained in RPMI-1640 medium (Hyclone), both supplemented with 10% fetal bovine serum (Tissue Culture Biologicals) and penicillin and streptomycin (Invitrogen), and incubated at 37°C in 5% CO_2. To generate MT-4_shYY1 stable cell line, 293T cell were transfected with DNA mixture of pLP1, pLP2, pLP/VSVG (Invitrogen) and shYY1 plasmid (TRCN0000019895, Sigma-Aldrich) to produce the shYY1 lentivirus. At 30 h post-transfection, lentiviral supernatant were harvested and filtered by 0.45-μm filter then transduced to MT-4 cells. Transduced cells were maintained and selected

with media containing 5μg/ml of puromycin over two weeks.

Virus production and analysis

The 293T cells ($2.5x10^5$) in 12-well plate were transfected 200ng of pNL4-3_{GFP} and 50ng of pDs-Red with 1μg protein expression plasmid or 40nM siRNA using jetPEI (Polyplus) or Lipofector-EZ (Aptabio), respectively. 24 h later, cells and viral supernatant were harvested. Cell were washed with 1xPBS and lysed in RIPA buffer supplemented with protease inhibitor cocktail (P8340, Sigma-Aldrich), then measured fluorescence of GFP and RFP by fluorometer (Infinite F200, TECAN) and subjected to western blotting. The viral supernatant filtered with 0.45-μm filter then analyzed by western blotting and p24 ELISA assay (XB-1000, Xpressbio). MT-4 cells ($1x10^5$) in 48-well plate were transfected 1μg of protein expression plasmid or 40nM of siRNA using Lipofector-EZ. 24 h later, 10ng of HIV-$1_{NL4-3GFP}$ virus generated from pNL4-3_{GFP} transfected 293T cell were transduced and incubated for three days then analyzed cell and viral supernatant as described above. GFP positive cell indicating HIV-1 infection were captured by inverted fluorescence microscope (IX71, Olympus) and counted by FACS analysis (FACS Canto II, BD bioscience).

Immunofluorescence (IF) analysis

HeLa cells ($4x10^5$) in 24-well plate inserted 12mmΦ size of cover glass (0111520, Marienfeld) were transfected with the indicated plasmids using jetPEI. IF was performed as described previously (40).

Firefly luciferase (Fluc) assay

Fluc activity was measured by Luciferase assay system (E1500, Promega) and luminometer (Infinite F200, TECAN) following the manufacturers' instructions.

Northern blotting and Real-time quantitative PCR (qRT-PCR)

Total cell RNA was isolated using RNAiso Plus (TaKaRa). RNA were separated on a 0.7 % agarose gel containing 2.2 M formaldehyde then following of DIG Northern Starter Kit (Roche) manufacturer's instruction. The probe of Env (corresponded to 8368-8784 nt of pNL4-3) and RFP (corresponded to 1-400 nt of RFP) are generated by in vitro transcription using T7 polymerase. For qRT-PCR, cDNA generated using 5x M-MLV reverse transcriptase pre-mix (EBT-1511, Elpisbiotech) then ubjected to qRT-PCR using LightCycler 480 SYBR Green 1 Master, white 96-multiwell plate and LightCycler480 instrument (Roche). All of primers used in qRT-PCR are also listed in Supplement Table1.

siRNA and Antibodies

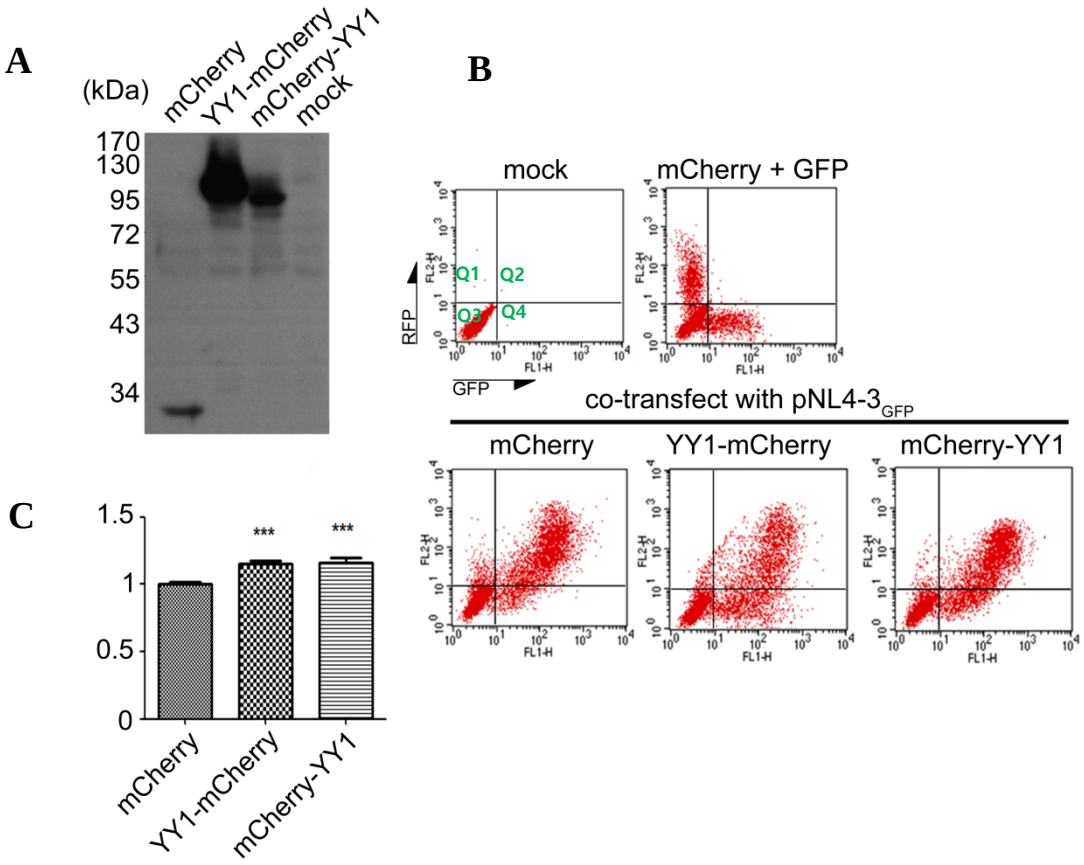
ON-TARGET*plus* Non-targeting siRNA #1 (cat: D-001810-01-05) (control siRNA), On-TARGET*plus* SMARTpool YY1 (cat: L-011796-00) and custom siTat (seguence: 5'-CUGCUUGUACCAAUUGCUAUUUU-3') were purchased from Dharmacon. Primary antibodies used in this study include the following: rabbit anti-YY1 (sc-1703, SantaCruz); rabbit anti-p55 (ThermoFisher); mouse anti-HA (H-9658, Sigma-Aldrich); rabbit anti-RFP (632496, Clontech); mouse anti-β-actin (A00702, GenScript); mouse anti-GAPDH (A01622, GenScript). Secondary antibodies included: Immunopure Goat anti-Mouse IgG (H+L) HRP conjugate (31430, ThermoFishe) and Immunopure Goat anti-Rabbit IgG (H+L) HRP conjugate (31460, ThermoFisher).

Supplementary Table 1

Name of primer	Sequences of primer	Restriction enzyme
YY1 F	5’-AAAGATCTCTATGGCCTCGGGC-3’	*Bgl* II
YY1 R	5’-CAGGTACCTCACTGGTTGTTT-3	*Kpn* I
155 F	5’-AAAAGATCTCTATGGCGGCCGGCAAGA-3’	*Bgl* II
296 F	5’-GGAGATCTCTATGATAGCTTGCCCTCATAA -3’	*Bgl* II
154 R	5’-CAAGGTACCTCACGCCACGGTGACCA-3’	*Kpn* I
200 R	5’-AAAGGTACCTCACGGGTCGGCGCCGCC-3’	*Kpn* I
295 R	5’-CAGGTACCTCATGTTCTTGGAGCAT-3’	*Kpn* I
K 348A F	5’-CATGCCGGAGAGAAGCCC-3’	-
K 348A R	5’-TTCTCTCCGGCATGAACCAGTT-3’	-
K 378A F	5’-GCCGGAGACAGGCCC-3’	-
K 378A R	5’-GCCTGTCTCCGGCATGGATT-3’	-
Tat F	5’- AAAGAATTCGGATGGAGCCAGTAGAT -3’	*EcoR* I
Tat R	5’- AAACGGCCGCTATTCCTTCGGGCCTGT -3’	*Eag* I
Name of primer	**Sequences of primer for qRT-PCR**	
Gag qRT F	5’-GCAGCCATGCAAATGTTAAAAGAG-3’	
Gag qRT R	5’-TCCCCTTGGTTCTCTCATCTGG-3’	
RFP qRT F	5’-GAGATCCACAAGGCCCTGAA-3’	
RFP qRT R	5’-GGAGTCCACGTAGTAGTAGCC-3’	

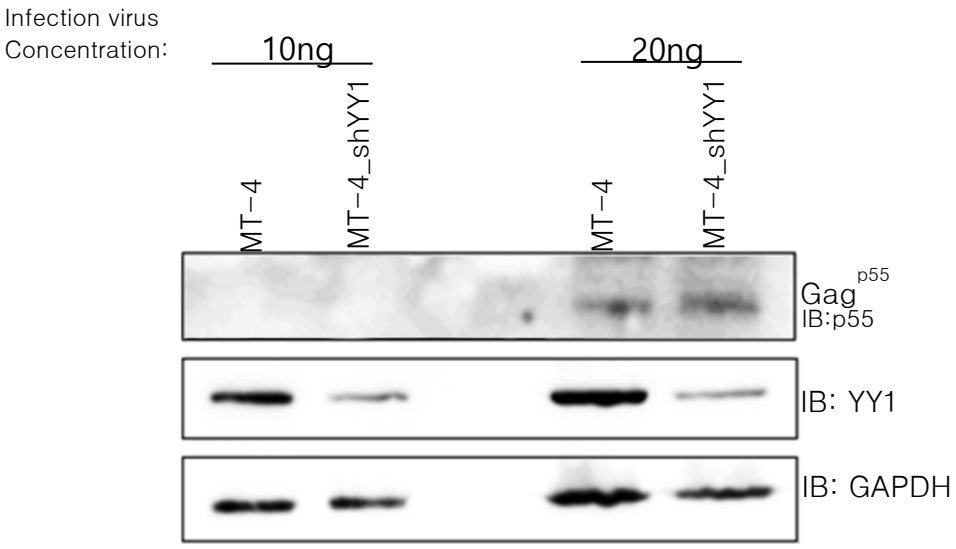
Primers used in this study. F: Forward. R:Reverse. Restriction enzyme sites are underlined.

Supplementary Figure 1



Supplementary 1. YY1 increases the GFP expression derived by HIV-1 LTR. (A) Confirmation of mCherry, YY1-mCherry, mCherry-YY1 expression by western blot (B) FACS analysis. mCherry + GFP; separately transfected mCherry and pNL4-3$_{GFP}$ plasmid then mixed when cells were harvested. FACS result shown that Red and Green fluorescent detected in Q1 and Q4 region, respectively. Co-transfected cell; each mCherry or mCherry tagged YY1 plasmid were transfected with pNL4-3$_{GFP}$. Dual positive cells (mCherry^{+} GFP^{+}) were detected in Q2 region. (C) GFP mean fluorescent value gained from Q2 region of (B).

Supplementary Figure 2



Supplementary 2. YY1 knock-down does not affect virus entry. $1x10^5$ cell of MT-4 and MT-4_shYY1 cells were infected 10 and 20ng of HIV-$1_{NL4-3GFP}$, then cell were harvested and lysed at three hours post-infection. The same quantity of protein was loaded to SDS-PAGE, then immuno-blotted with indicated antibodies.

References

1. Lee AH, Han JM, Sung YC (1997) Generation of the replication-competent human immunodeficiency virus type 1 which expresses a jellyfish green fluorescent protein. Biochem Biophys Res Commun 233, 288-292
2. Lee SD, Yu KL, Park SH, Jung YM, Kim MJ, You JC (2018) Understanding of the functional role(s) of the activating transcription factor 4(atf4) in hiv regulation and production. BMB Rep 51, 388-393