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Supplementary Information for

Yin Yang 1 is a potent activator of HTLV-1 LTR-driven gene expression via RNA binding

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Supplementary Information Text

Methods

Plasmids

A plasmid encoding HA-tagged wild-type YY1 was a generous gift from Dr. Shi Yang (Harvard). To generate HTLV-1 LTR luciferase reporter constructs, full-length HTLV-1 LTR and desired truncation mutants were PCR amplified and cloned into the HindIII site of the pGL3-Basic vector (Promega) via Sequence and Ligation Independent Cloning (SLIC) (1). To generate HTLV-1 R-SV40 chimeric promoter constructs, portions of the HTLV-1 R were PCR amplified and cloned into XhoI (R upstream of TSS), HindIII (R downstream of TSS) or BamHI (R downstream of 3' polyA signal) sites of pGL3-promoter using SLIC. Generation of YY1 DNA binding site mutants was accomplished using GeneArt® site-directed mutagenesis kit (Invitrogen) according to the manufacturer's instructions. YY1 truncation mutants were PCR amplified and cloned into BamHI/XhoI sites of the pCDNA3-Flag vector (Invitrogen) using SLIC. His-tagged YY1 for recombinant protein expression in E. coli was previously described (2). All mutations and cloning were verified by DNA sequencing. Cloning primers are available upon request.

Immunoblotting

HEK-293 cells were lysed on ice for 20 mins in RIPA buffer supplemented with protease inhibitor cocktail (Roche), followed by centrifugation at 16,100 x g for 10 mins at 4°C. Clarified lysates were subjected to SDS-PAGE and transferred to nitrocellulose membrane (Bio-rad). Membranes were blocked for 1 hr at room temperature with Buffer for Fluorescent Western Blotting (Rockland), followed by incubations with either anti-HA (Clone16B12, 1:5000, Covance), anti-YY1 (C20, 1:1000, Santa Cruz), anti-Tubulin (Clone DM1A, 1:5000, Sigma), anti-His (MMS-156P, 1:5000, Covance), or anti-Flag M2 (1:5000, Sigma) for 1 hr at room temperature. Donkey anti-mouse secondary antibodies conjugated to IRDye 680 (1:20000, LI-COR) were then added for 30 mins and all blots were subsequently developed using the Odyssey Infrared Imaging System (LI-COR).

Electrophoretic Mobility Shift Assay (EMSA)

EMSA were performed exactly as previously described (2). Briefly, 5'-Biotin-labeled double-stranded DNA probes were incubated with 500 ng of purified YY1 protein in EMSA binding buffer (10 mM Tris, pH 7.5, 50 mM KCl, 1 mM DTT, 2.5% glycerol, 5 mM $MgCl_2$, 50 ng/μl Poly (dI-dC), 0.05% Nonidet P-40) at room temperature for 30 min. DNA-protein complexes were resolved on 6% DNA retardation gels (Novex) and developed using the LightShift Chemiluminescent EMSA kit (Pierce) according to the manufacturer's instructions. All EMSA probes are listed in Supplementary Table 3.

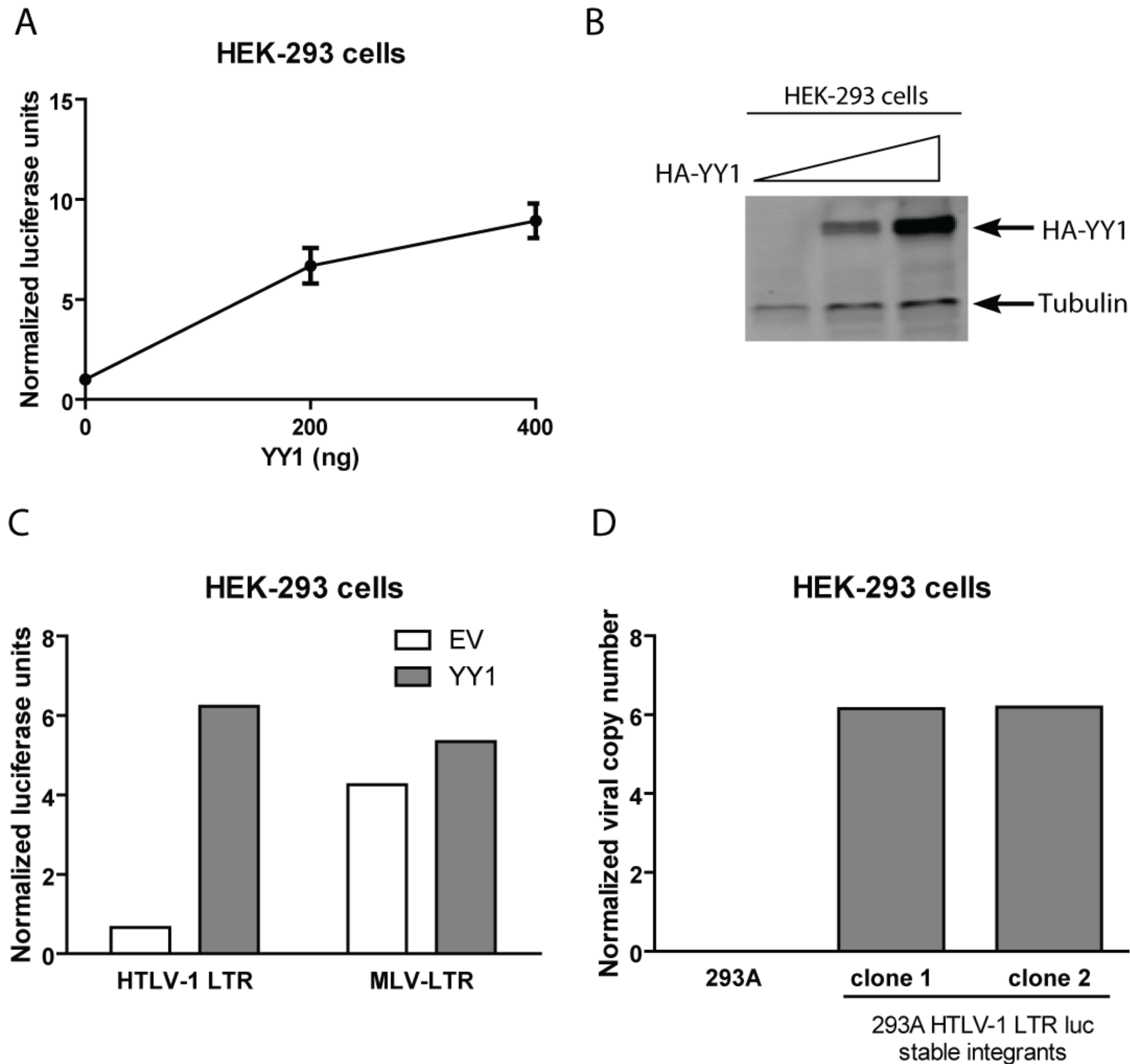
Nuclear Run-on assay

10 million HEK-293 cells cotransfected with plasmid encoding HA-YY1 and LTR luciferase reporter plasmids were subjected to nuclear run-on assay using biotin labeling and magnetic bead capture exactly as described (3).

Chromatin immunoprecipitation (ChIP) assay

ChIP assays were performed as described (4). Briefly, cells were cross-linked with 1% formaldehyde, followed by quenching with 0.125 M glycine for 5 min, lysed, and sonicated to produce an average DNA fragment size of 200–800 bp. Immunoprecipitations were performed by incubating 30 μg of sonicated chromatin with 1 μg respective antibodies overnight, and Protein A/G Dynabeads were added for an additional 4 hr. Captured antibody-antigen complexes were washed repeatedly, and DNA was eluted from the beads. qPCRs were performed with specific primers listed in Supplementary Table 1. Relative enrichment was calculated by determining the bound/input value using the $2^{-\Delta\Delta CT}$ method (5).

Fig. S1.



Supplementary Figure 1. YY1 transactivates reporter expression from HTLV-1 LTR in a dose dependent manner, but has no effect on reporter expression from MLV-LTR (referring to Fig.1)

(A) HEK-293 cells were transfected with empty vector (EV, or 0ng YY1) or increasing amounts of YY1 DNA together with HTLV-1 LTR firefly luciferase and HSV-TK renilla luciferase control plasmid. Relative luciferase expression was calculated by first dividing the firefly luciferase signal by the renilla luciferase signal in a given cell, and the resulting ratio was then normalized to the ratio obtained from EV-transfected cells (set to 1). Results shown are means ± SDs from two independent experiments performed in duplicate.

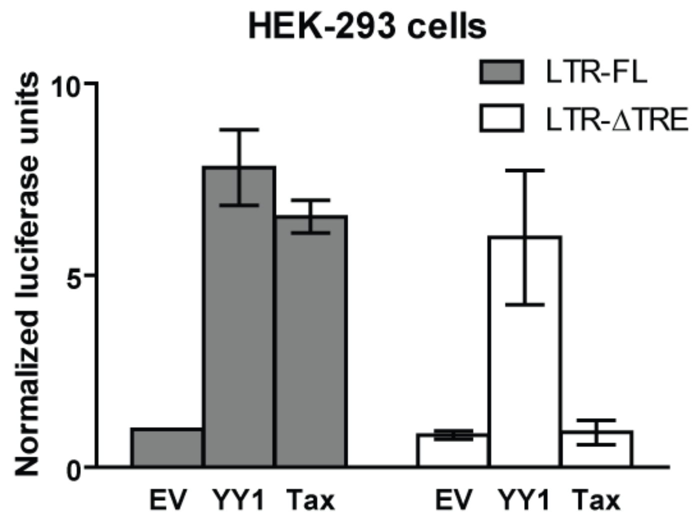
(B) HA-YY1 overexpression confirmed with western blotting using an Anti-HA antibody. Tubulin serves as a loading control.

(C) Similar assay as in Figure 1B using an MLV-LTR firefly luciferase construct. A representative experiment is shown.

(D) HEK-293 cells were co-transfected with HTLV-1 LTR luciferase DNA together with a neomycin resistant plasmid followed by selection with G418 for 2 weeks. Individual G418-resistant clones were isolated, after which genomic DNA was extracted and real-time quantitative PCR was carried out using primers specific for HTLV-1 LTR. The extent of viral integration (relative viral copy number) was quantified using the $2^{-\Delta\Delta CT}$ method by normalizing the HTLV-1 LTR signal to the single copy GAPDH gene.

Fig. S2.

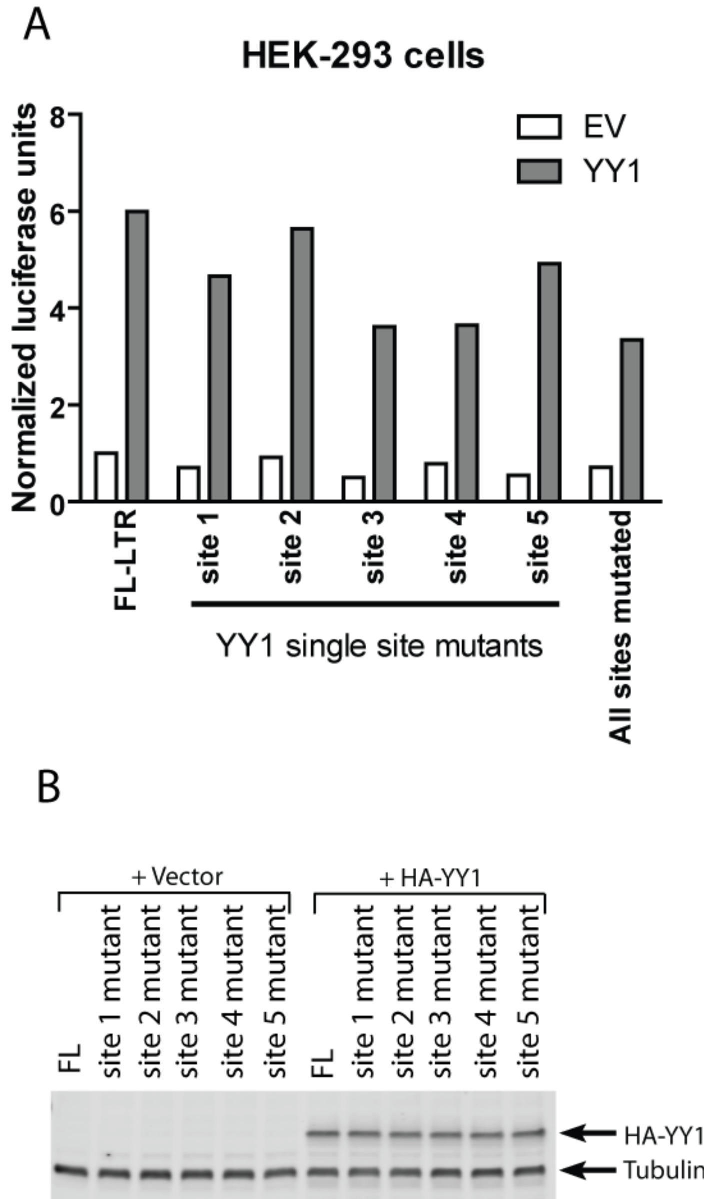
Supplementary Figure 2 (referring to Fig. 3)



Supplementary Figure 2. Elimination of Tax responsive element (TRE) in the U3 region of the HTLV-1 LTR has no effect on YY1 mediated transactivation (referring to Fig. 3)
Similar assay as Figure 1B using wild-type (WT) or mutant LTR lacking the TRE (LTR-ΔTRE). Results shown are means ± SDs from two independent experiments performed in duplicate.

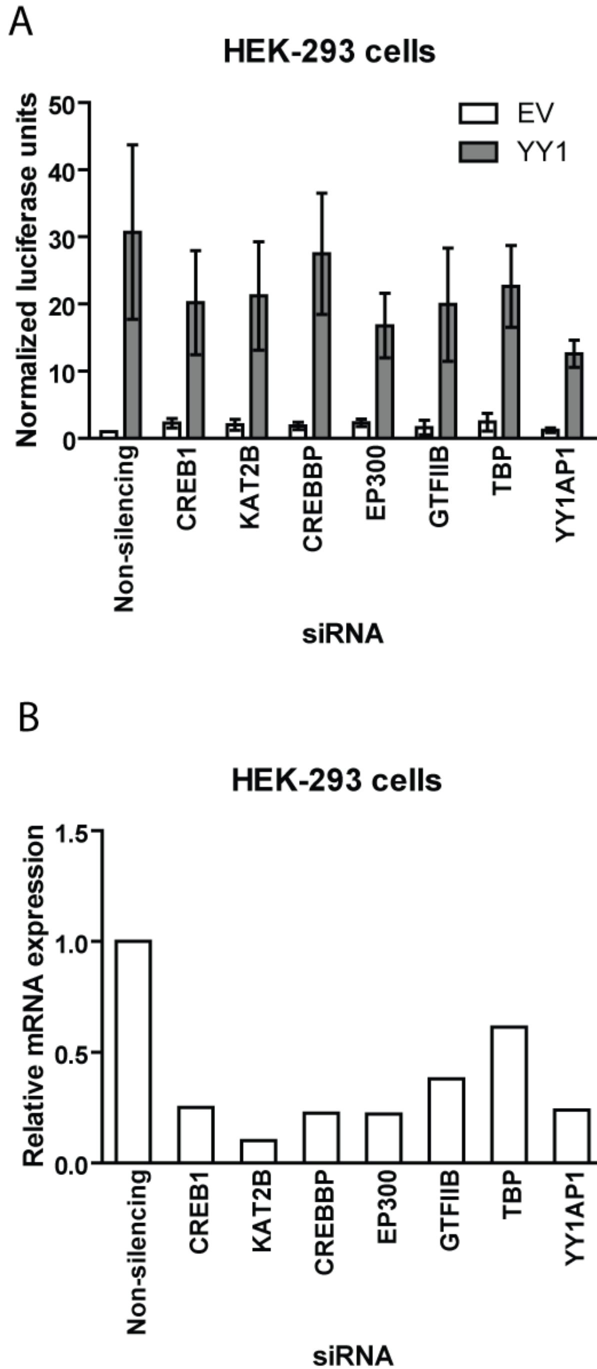
Fig. S3.

Supplementary Figure 3 (referring to Fig. 4)



Supplementary Figure 3. Elimination of YY1 DNA binding sites throughout the HTLV-1 LTR has no effect on YY1 mediated transactivation (referring to Fig. 4)
(A) Similar assay as Figure 1B using YY1 DNA binding site mutants. A representative experiment is shown.
(B) HA-YY1 overexpression confirmed with western blotting using an Anti-HA antibody. Tubulin serves as a loading control.

Fig. S4.



Supplementary Figure 4. Depletion of known YY1 coactivators of transcription using RNAi did not abolish YY1 mediated HTLV-1 transactivation (referring to Fig. 7)
(A) Similar assay as Figure 1B in cells transfected with siRNAs targeting known YY1 coactivators of transcription. Results shown are means ± SEs from three independent experiments performed in duplicate.

(B) Total RNA prepared from HEK-293 cells transfected with siRNAs outlined in (A) was subjected to qRT-PCR to assess efficiency of knockdown. Levels of individual transcripts were first normalized using the $2^{-\Delta\Delta CT}$ method to the value obtained for the alpha-tubulin gene; the obtained values were then normalized to the values obtained from HEK-293 cells transfected with non-silencing siRNA (set to 1). A representative experiment is shown.

Supplementary Table 1
Potential YY1 DNA binding sites in HTLV-1 LTR

YY1 binding site	Sequence	Location in LTR	Extent of match with YY1 consensus sequence (VDCCATNWY)
1	ACCCATTTC	U3 sense strand	8/9
2	TGCCATGAA	U3 antisense strand	7/9
3	AGCCATATG	U3 antisense strand	8/9
4	CGCCATCCA	R sense strand	7/9
5	GCCCATTCT	U5 sense strand	7/9

Supplementary Table 2
List of primers used for qRT-PCR

Primer target	Sequence
Alpha-tubulin Forward	ACCTTAACCGCCTTATTAGCCA
Alpha-tubulin Reverse	ACATTCAGGGCTCCATCAAATC
Luciferase 5' Forward	GCTGGAGAGCAACTGCATAA
Luciferase 5' Reverse	GTGATGTCCACCTCGATATGTG
Luciferase middle Forward	CGCATGCCAGAGATCCTATT
Luciferase middle Reverse	AGACGACTCGAAATCCACATATC
Luciferase 3' Forward	CGGAAAGACGATGACGGAAA
Luciferase 3' Reverse	CGGTACTTCGTCCACAAACA
HTLV-1 R for RIP Forward	TCCGCCGTCTAGGTAAGTT
HTLV-1 R for RIP Reverse	AAGCAGGGTCAGGCAAAG

Supplementary Table 3
List of oligonucleotides used for EMSA (only forward strand shown)

Primer target	Sequence
AAV-P5	GATCCGTTTTGCGACATTTTGCGACACA
MLV-PBS	GGGGGCTCGTCCGGGATCGGGAGACCCC
HTLV-1 R1 (YY1 DNA binding site is underlined)	GCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGT
HTLV-1 R2	TCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCC
HTLV-1 R3	GCCGTCTAGGTAAGTTCAGAGCTCAGGTCGAGACCGGGCCTT
HTLV-1 R4	TGTCCGGCGCTCCCTTGGAGCCTGCCTAGACTCAGCCGGC
HTLV-1 R5	TCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTGCG
HTLV-1 R1 YY1 DNA binding scrambled mutant (note that CCA is mutated to TTG)	GCCCTACCTGAGGCCGTTGTCCACGCCGGTTGAGTCGCGT
HTLV-1 R1 YY1 DNA binding deletion mutant (note that TCCA is deleted)	GCCCTACCTGAGGCCGCCACGCCGGTTGAGTCGCGT

References

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