SUPPLEMENTAL INFORMATION

G-Quadruplex Folding Enforces HIV-1 Transcriptional Latency via Nucleolin Recruitment

Marianna Terreri,[1] Beatrice Tosoni,[1#] Emanuela Ruggiero,[1#] Rosalba Perrone,[1,2] Paola Soldà,[1] Ilaria Frasson[1], Matteo Nadai[1], Lolita Sasset,[3] Maria Mazzitelli,[3] Alfredo Garzino-Demo,[1,4] Anna Maria Cattelan,[1,3] Sara N. Richter[1,5,6*]

[1]Department of Molecular Medicine, University of Padova, Via A. Gabelli 63, 35121 Padova, Italy.

[2]Buck Institute for Research on Aging, Novato, CA, USA

[3]Infectious Diseases Unit, Padova University Hospital, Via Giustiniani 2, 35128 Padua, Italy.

[4]Department of Microbial Pathogenesis, University of Maryland Baltimore School of Dentistry, Baltimore, Maryland, USA

[5]Microbiology and Virology Unit, Padova University Hospital, Via Giustiniani 2, 35128 Padua, Italy.

[6]Lead contact

Content

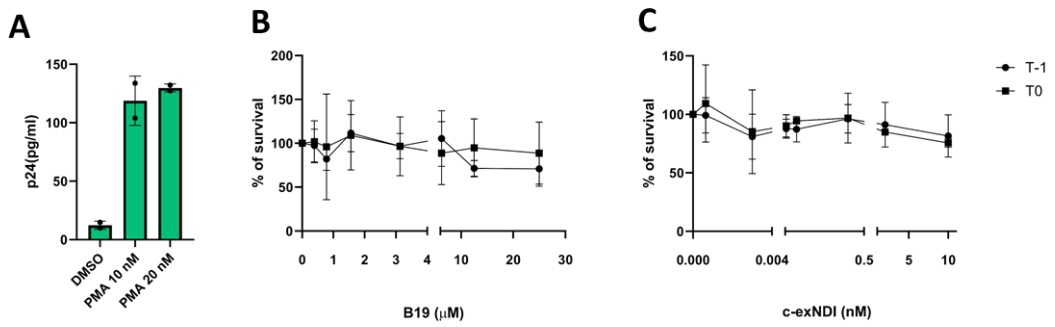

Figure S1. U1 cell line transcriptional reactivation and cytotoxicity. (A) Cells were treated with 10 nM and 20 nM PMA. Supernatants were collected 48 h post-stimulation and subjected to p24 ELISA assay. Cell cytotoxicity evaluated 72 h after B19 **(B)** or c-exNDI treatment **(C)** using the ATP-lite assay. Compounds were administered 24 h prior (T-1) or simultaneously with (T0) PMA stimulation (10 nM). All the data are represented as mean ± standard deviation (n=2).

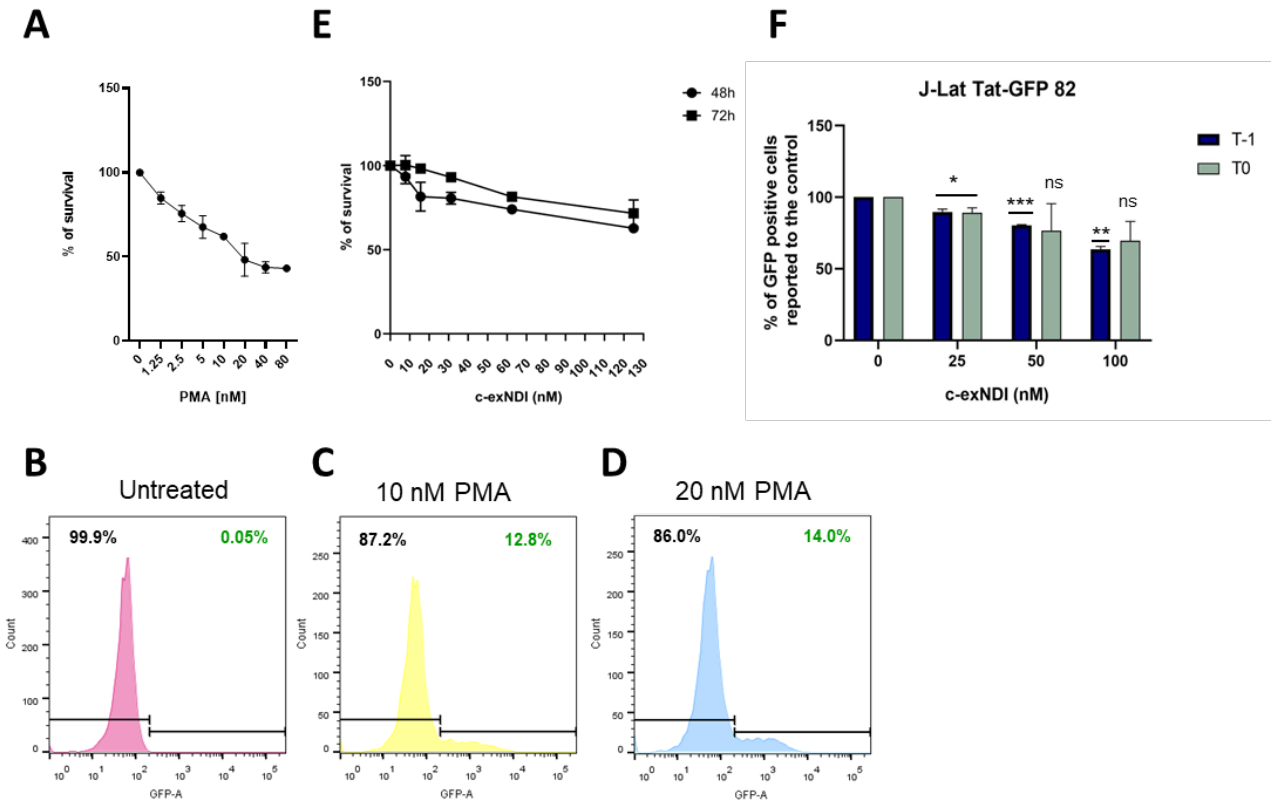

Figure S2. Transcriptional reactivation of J-Lat Tat-GFP 82 cell line. (A) Cytotoxicity of increasing concentrations of PMA evaluated 24 h post treatment. Data are represented as mean ± standard deviation (n=2). Histograms represent the percentage of GFP positive cells of the untreated sample **(B)** and the sample treated with 10 nM **(C)** or 20 nM PMA **(D).** Percentage values of inactive (black) and active (green) cells are reported**. (E)** Cytotoxicity of c-exNDI on J-Lat Tat-GFP 82 cell line evaluated 48 h and 72 h post treatment. Data are reported as a percentage of the absorbance of the treated sample reported to the untreated control and are represented as mean ± standard deviation (n=3). **(F)** Bar graphs show the percentage of GFP fluorescent cells representing reactivated J-Lat Tat-GFP 82 cells at increasing c-exNDI concentrations. All samples have been treated with PMA 10 nM and data are calculated as the percentage of GFP fluorescence of the treated and stimulated samples normalized on the stimulated untreated control. C-exNDI was administered 24 h before (T-1, violet bars) or **s**imultaneously with (T0, grey bars) PMA stimulation. Data are represented as the mean of n=2 experiments ± standard deviation. Statistical significance was calculated using t-test. Significant p value < 0.05 (*) and p-value < 0.001 (***) are indicated.

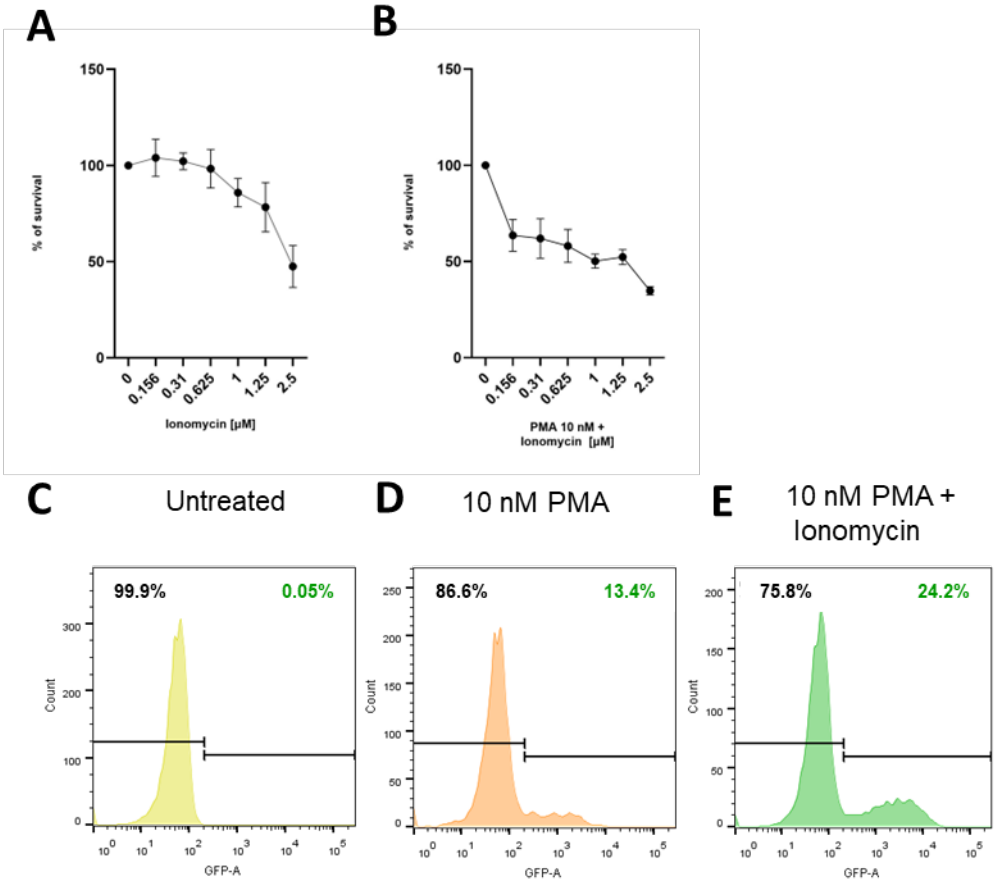

Figure S3. Effect of Ionomycin on LTR reactivation. Cytotoxicity on J-Lat Tat-GFP 82 of Ionomycin at indicated concentrations, in the absence **(A)** or presence **(B)** of 10 nM PMA, measured 24 h after treatment. Data are reported as a percentage of the absorbance of the treated sample reported to the untreated control. All the data are reported as a mean ± standard deviation. Histograms display the percentage of GFP positive cells of the untreated sample **(C)** and the sample treated with 10 nM PMA **(D)** or 10 nM PMA and 1 µM Ionomycin **(E)**. Percentage values of inactive (black) and active (green) cells are reported.

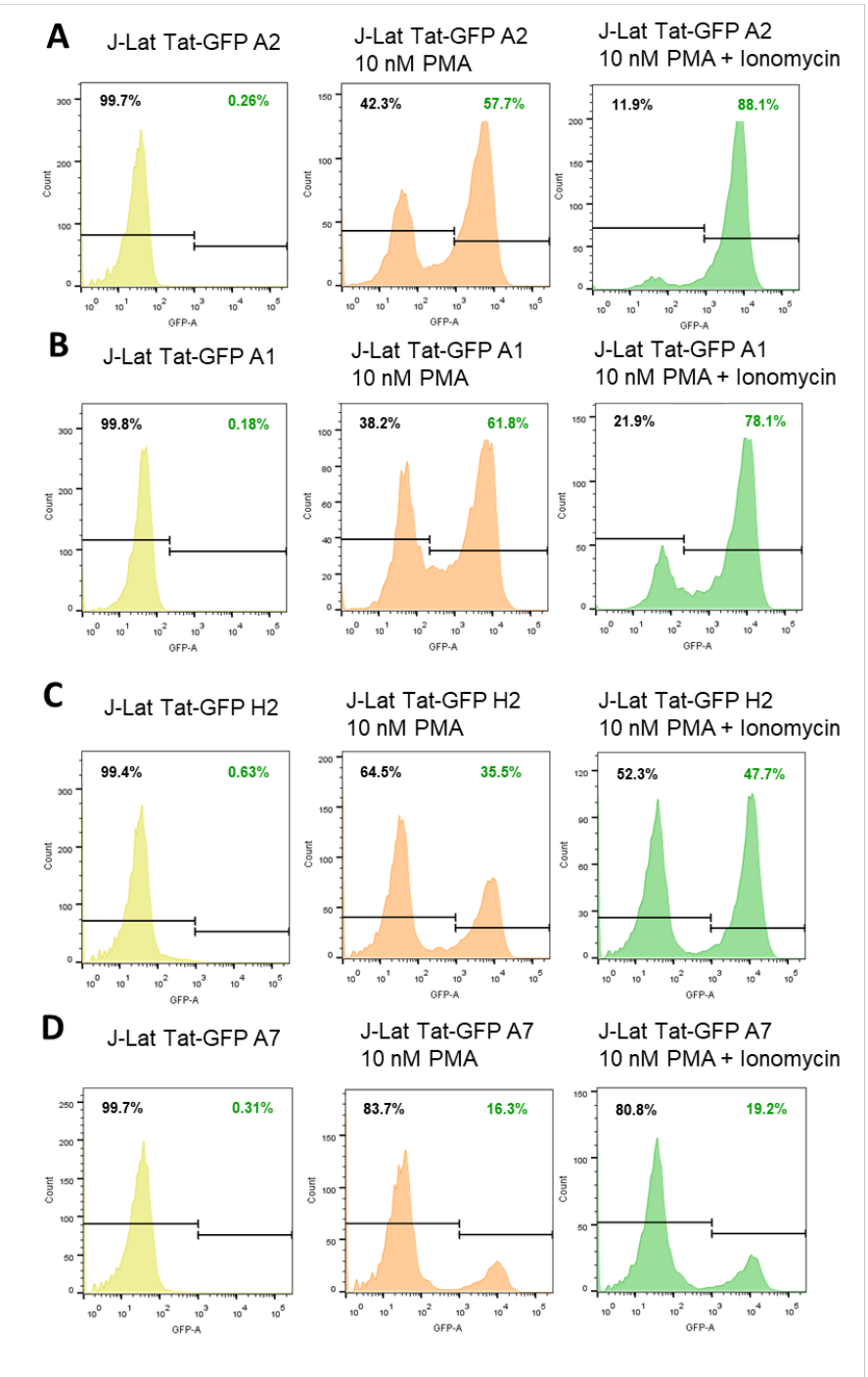

Figure S4. Transcriptional reactivation of J-Lat Tat-GFP cell lines measured by FACS. Histograms display the transcriptional reactivation induced by PMA and Ionomycin in **(A)** J-Lat Tat-GFP A2, **(B)** J-Lat Tat-GFP A1, **(C)** J-Lat Tat-GFP H2, **(D)** J-Lat Tat-GFP A7 cell lines. Percentage values of inactive (black) and active (green) cells are reported.

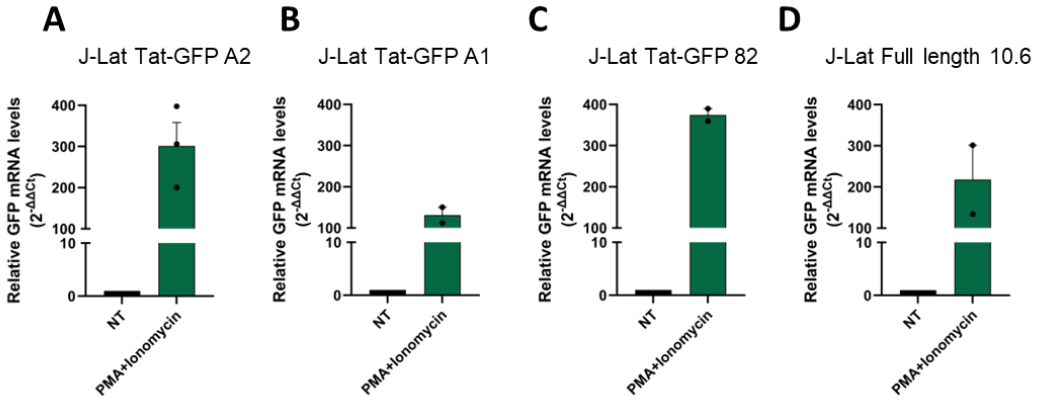

Figure S5. Transcriptional reactivation of J-Lat Tat-GFP cell lines measured by GFP expression. Relative GFP expression reported to the housekeeping gene *gapdh* in J-Lat cell lines untreated (NT, black bars) and treated with 10 nM PMA and 1 µM Ionomycin (green bars). All the data are reported as mean ± standard deviation of almost two replicates.

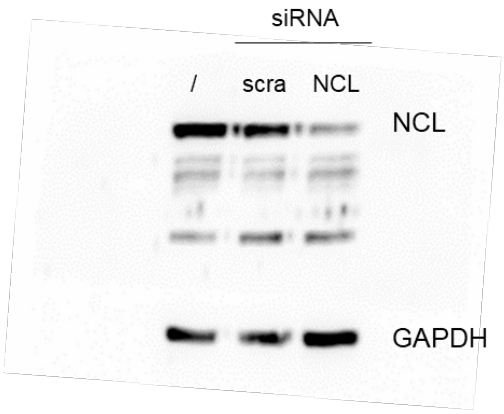

Figure S6. Western blot for the evaluation of NCL silencing for fluorescence analysis. Protein extracts were obtained from J-Lat Tat-GFP A2 cells untreated or treated with 5 μM scrambled siRNA and with 5 μM α-NCL siRNA. GAPDH was used as housekeeping gene.