Role of the *pol* gene enhancer in HIV-1 transcription and replication in myeloid infected cells

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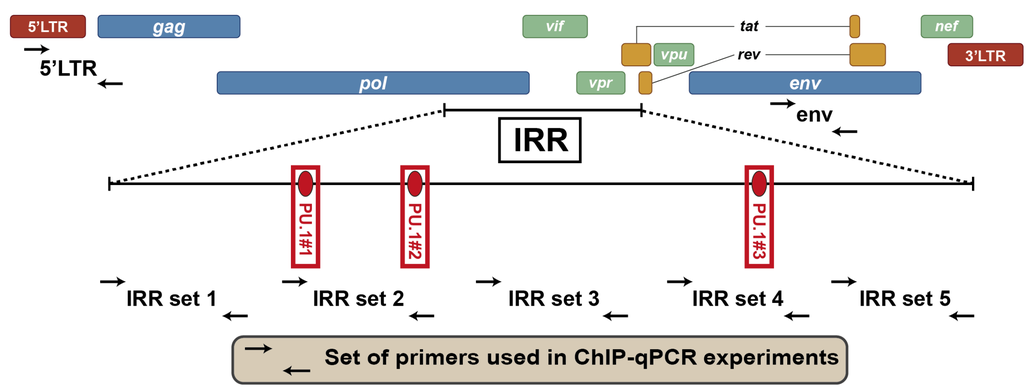
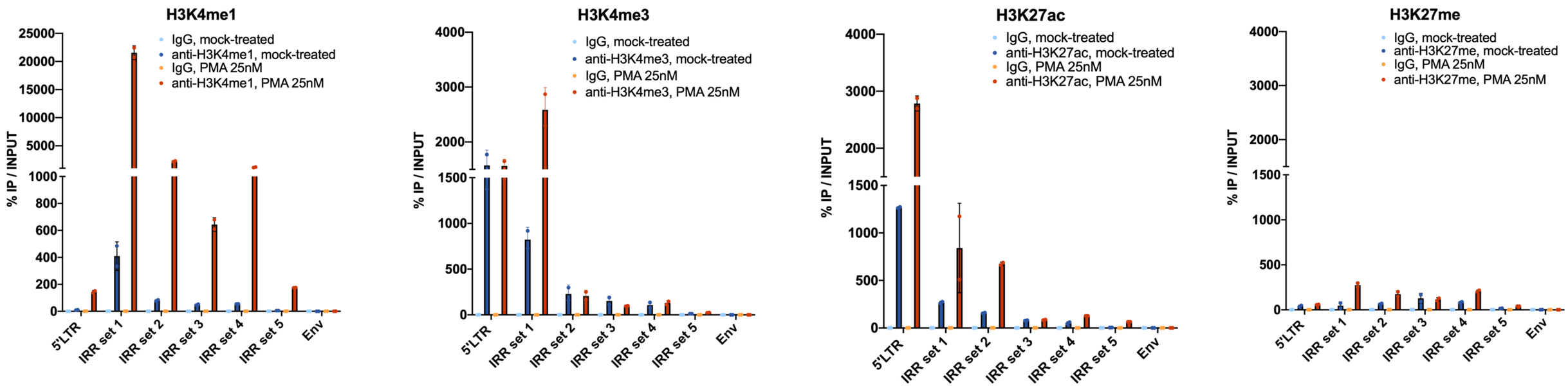
Background

There is increasing evidence for the physiological relevance of myeloid HIV-1 reservoirs such as brain microglia and urethral macrophages. However, the molecular mechanisms of HIV-1 gene expression in myeloid infected cells are still poorly understood. The HIV-1 intragenic *cis*-regulatory region (IRR) located in the *pol* gene exhibits an enhancer activity on the 5'LTR promoter. The IRR possesses multiple binding sites for various cellular transcription factors (TF). Here, we characterized several binding sites for the myeloid-specific PU.1 TF, known to be a pioneer factor inducing the opening of heterochromatin in enhancers. We studied the functional involvement of these PU.1 binding sites in the IRR-mediated control of HIV-1 gene expression in monocytes/macrophages.

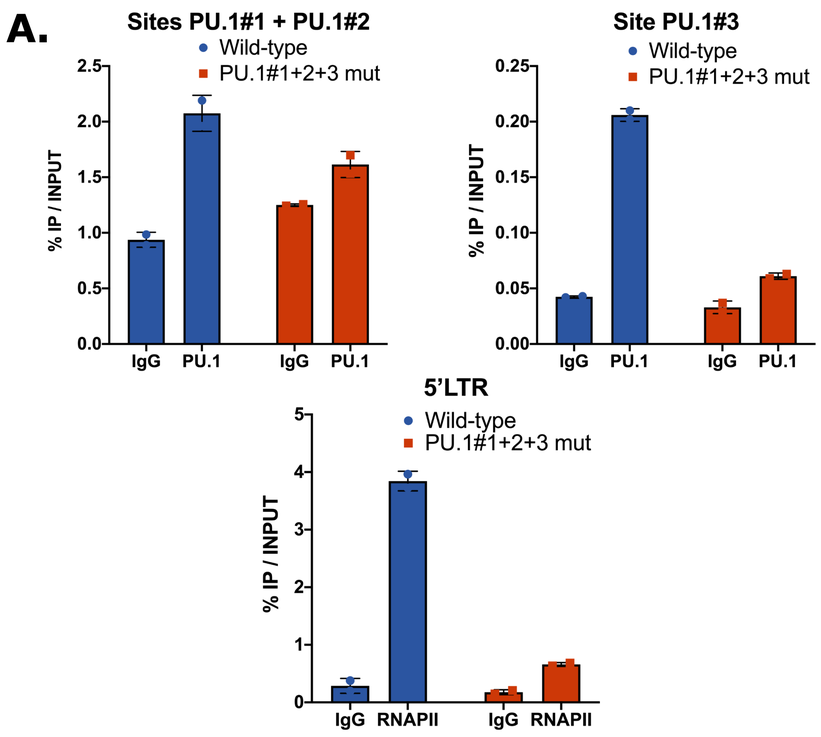


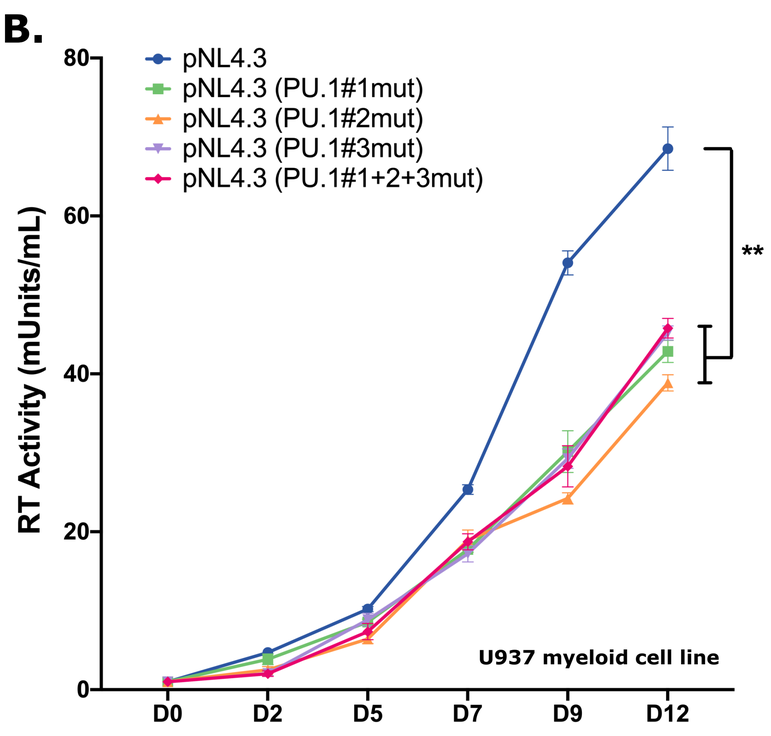
Presence of enhancer-related histone modifications in the IRR

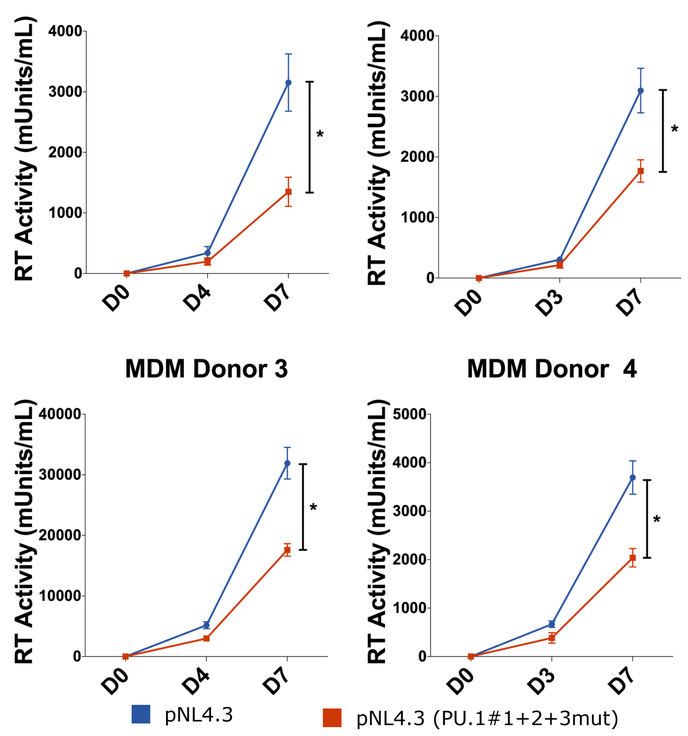


Characterization of the epigenetic profile of the IRR. Chromatin was prepared from the HIV-1-latently infected U1 cell line and was immunoprecipitated with specific antibodies directed against different histone post-translational modifications (H3K4me1, H3K4me3, H3K27ac and H3K27me), against the cellular transcription factor PU.1 or with an IgG as background measurement. The ChIP-qPCR sets of primers we used are indicated. Results are presented as histograms indicating percentages of immunoprecipitated DNA compared to the input DNA (% IP/INPUT).

Effects of selected mutations abolishing PU.1 binding to the intragenic PU-boxes

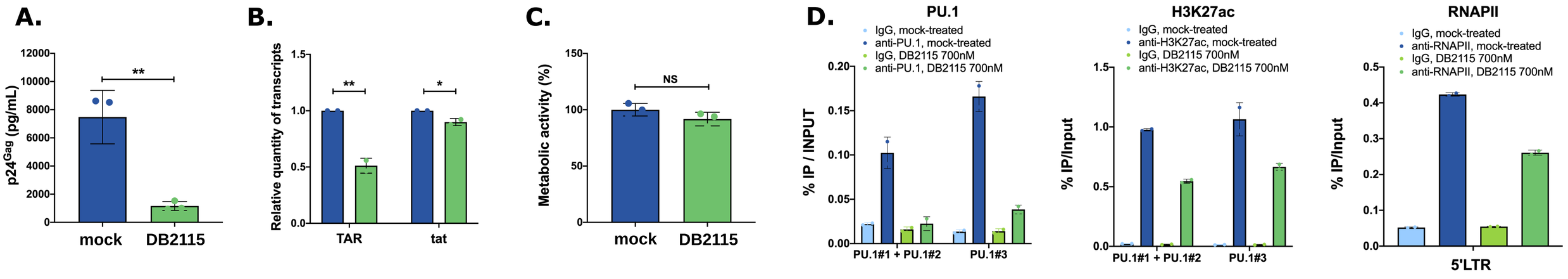






Intragenic PU.1 binding sites positively regulate HIV-1 transcriptional activity and HIV-1 replication in myeloid contexts of infection. (A) The uninfected myeloid U937 cell line was infected with either wild-type or PU.1-mutated HIV-1 particles. Chromatin prepared from these U937 infected cells was immunoprecipitated with specific antibodies directed against the cellular transcription factor PU.1, RNA polymerase II (RNAPII) or with an IgG as background measurement. Results are presented as histograms indicating percentages of immunoprecipitated DNA compared to the input DNA (% IP/INPUT). Means ± SEM of three independent experiments are shown. **(B)** U937 cells or **(C)** monocyte-derived macrophages (MDMs) were infected with equivalent amounts of p24 concentration of HIV-1 NL4.3-GFP particles containing a genomic RNA in a wild-type form or mutated in intragenic PU.1 binding sites (alone or in combination) and the infections were followed in a time-course manner. Viral production was quantified by measuring the RT viral protein activity in the culture supernatants by SG-PERT assays (Vermeire J. *et al*., PLoS One, 2012). Statistics in A, B and D were performed with an unpaired student's T-test, where * p ≤ 0.05 and ** p ≤ 0.01.

Pharmacological approach using the heterocyclic dication DB2115



DB2115 is a pharmacological compound that inhibits PU.1 binding and induces a decrease in HIV-1 transcription and replication. (Panels A, B and C) The HIV-1-latently infected myeloid cell line OM10.1 was either mock-treated (■) or treated with DB2115 (■) (a DNA minor groove binder that selectively inhibits PU.1 binding). **(A)** Viral production was quantified, by measuring p24 antigen concentration in the culture supernatent. **(B)** Total RNA was extracted, retrotranscribed and cDNA was quantified by RT-qPCR using primers detecting the initiated (TAR) or the elongated (tat) viral transcripts. **(C)** Metabolic activity was assessed by the colorimetric test WST-1. **(D)** Chromatin prepared from OM10.1 cells (mock-treated or treated with DB2115) were immunoprecipitated with specific antibodies directed against the histone mark H3K27ac, the cellular transcription factor PU.1, RNA polymerase II (RNAPII) or with an IgG as background measurement. Results are presented as histograms indicating percentages of immunoprecipitated DNA compared to the input DNA (% IP/INPUT). Means ± SEM of three independent experiments are shown. Statistics in A, B and C were performed with an unpaired student's T-test, where * p ≤ 0.05, ** p ≤ 0.01 and NS = not significant.

Conclusions

- **We identify the myeloid-specific transcription factor PU.1 as binding to several sites in the intragenic *cis*-regulatory region of HIV-1.**
- **We demonstrate that the intragenic PU.1 binding sites are important for viral replication and that they exhibit transcription enhancing activity on the 5'LTR promoter.**

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