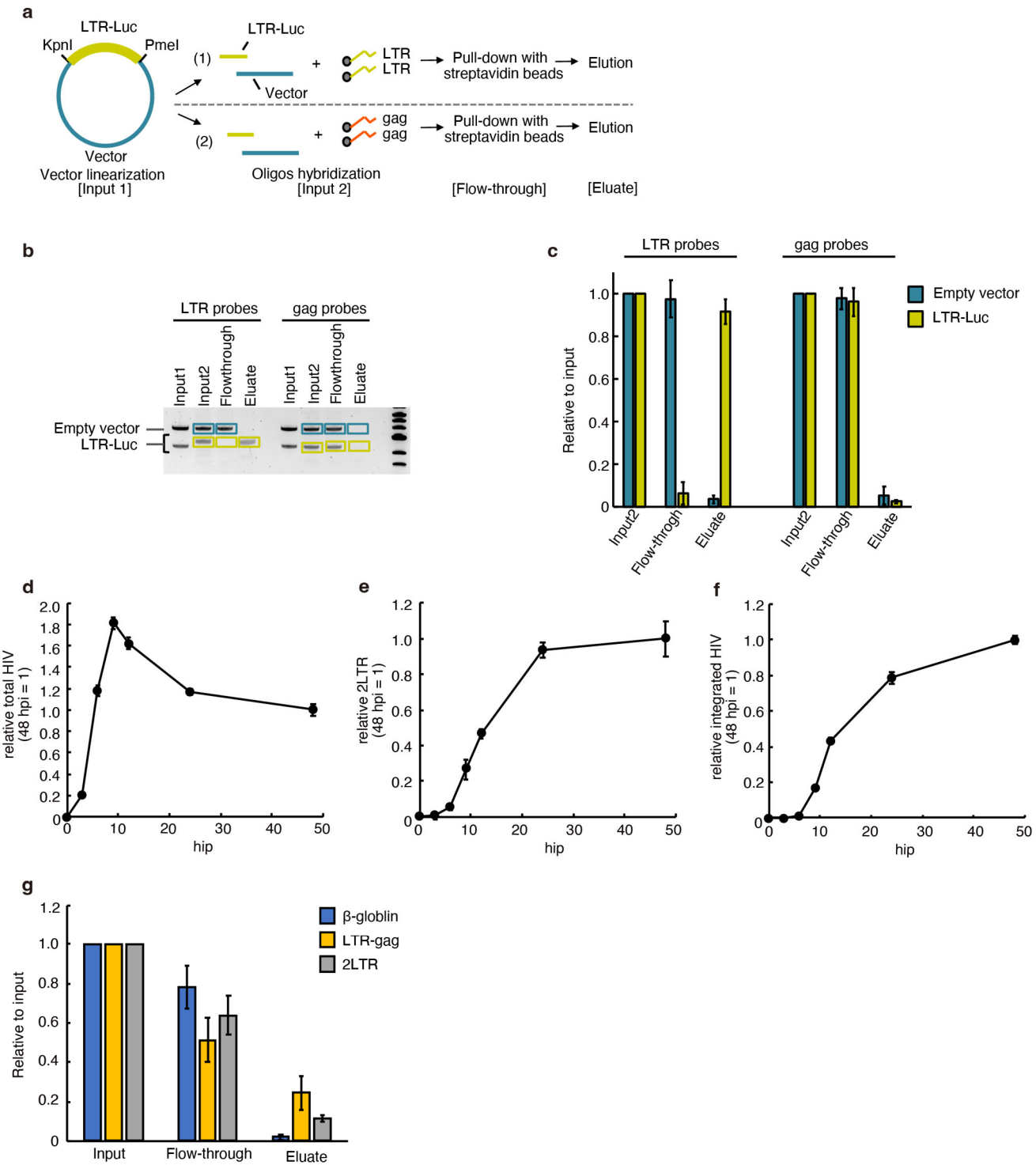


Extended Data Fig 1. Optimization of PICh method to the isolation of unintegrated HIV-1 DNA in vitro and in infected cells.

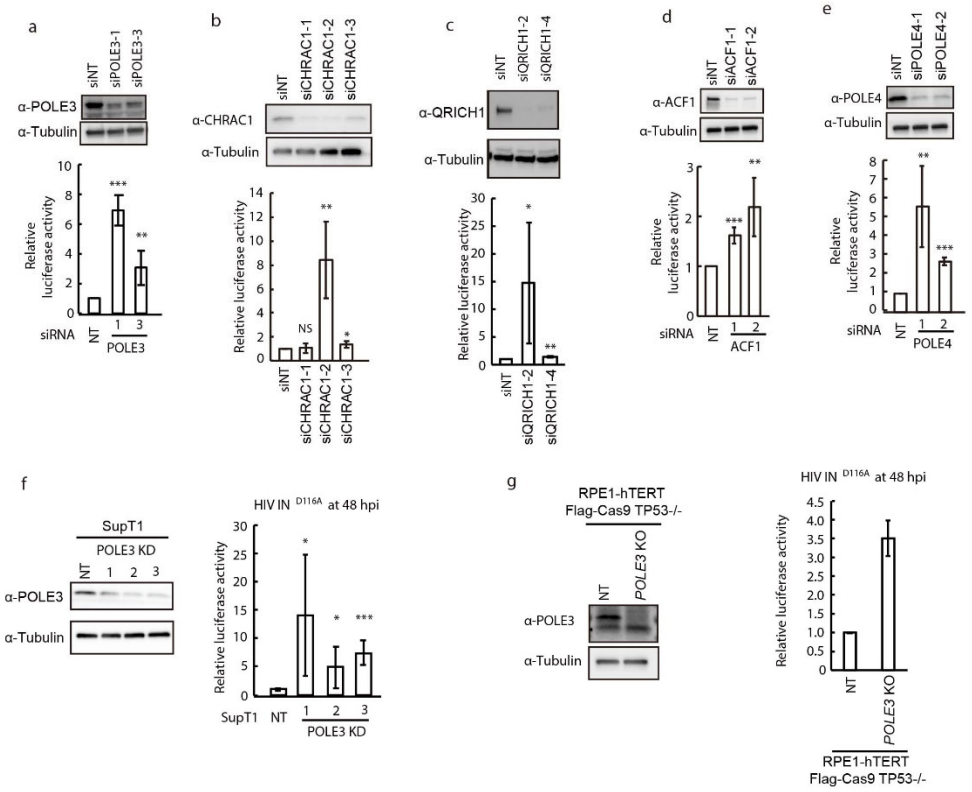
(a) Outline of the plasmid pull-down assay using LTR-luc plasmid. Linearized plasmid was mixed with PICh probes specific for the LTR (1) or Gag (2) conjugated to spacer and desthiobiotin. After hybridization, hybrids are captured using streptavidin beads and eluted with biotin.

(b) Each fraction (input, flow-through, and eluate) were analyzed by agarose gel electrophoresis and EtBr staining.

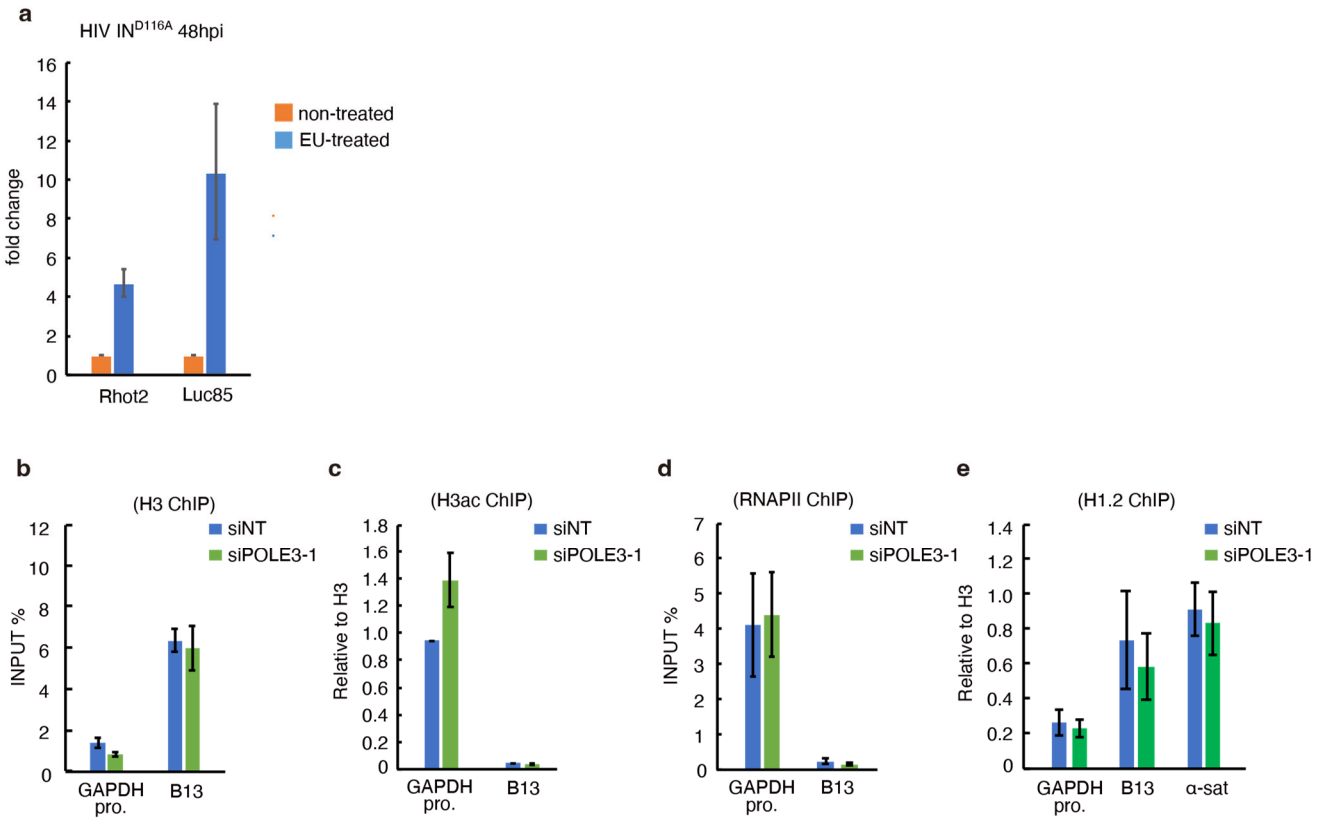
(c) Quantification of target (LTR-luc) capture represented as percent DNA relative to input 2.

(d-f) SupT1 cells were infected with a VSV-G pseudotyped HIV-Luc at a MOI of 0.8. Cells were harvested at 3, 6, 9, 12, 24 and 48 hpi. Total HIV DNA (d), 2LTR circles (e) and integrated DNA (f) were analyzed by qPCR. Data are represented as quantification relative to the value at 48 hpi. The mean values ± SD of three independent experiments are plotted.

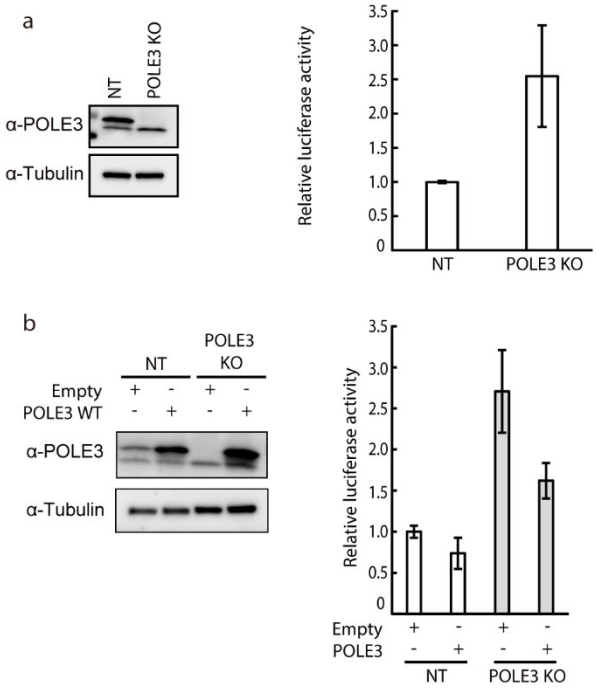
(g) Genome capture of genomic DNA from SupT1 cells infected with HIV-Luc for 9 hrs. Genomic DNA was hybridized with probes designed for PICh, captured on streptavidin beads and eluated with biotin. Quantitative PCR analysis of input, flow through and eluate fractions using indicated primers. Results are represented as quantification relative to input. The mean values ± SD of four independent experiments are plotted.



Extended Data Fig 2. CHRAC1 and QRICH1 are not involved in unintegrated HIV-1 DNA silencing.
(a-e) Expression of POLE3 (a), CHRAC1 (b), QRICH (c), ACF1 (d) and POLE4 (c) proteins in cells treated with indicated siRNA were analyzed by immunoblotting with indicated antibodies. Luciferase assay in KD cells were performed at 48 hpi. Results are represented as luciferase expression relative to NT siRNA, and the mean ± SD values of 2 independent experiments in triplicate are plotted.
(f) Impact of knockout of POLE3 in SupT1 cells on uniHIV-1 DNA silencing. Expression of POLE3 proteins in POLE3 KO cells were analyzed by immunoblotting with indicated antibodies. Luciferase assays in POLE3 KO cells were performed at 48 hpi. Results are represented as luciferase expression relative to NT sgRNA, and the mean ± SD values of two independent experiments in triplicate are plotted.
luciferase expression relative to non-targeting (NT) siRNA, and the mean ± SD values of 6 independent experiments are plotted.
(e-f) Effect of POLE3 KO on uniHIV DNA silencing in RPE-1-hTERT Flag-Cas9 TP53-/- cells. POLE3 expression from whole cell extracts were analyzed by immunoblotting with anti-POLE3 antibody at the day of infection (e). Luciferase assays were performed at 48 hpi (f). Results are represented as luciferase expression relative to non-targeting (NT) sgRNA, and the mean ± SD values of three independent experiments in triplicate are plotted.
* P<0.05, ** P<0.01 independent Student's t-test.

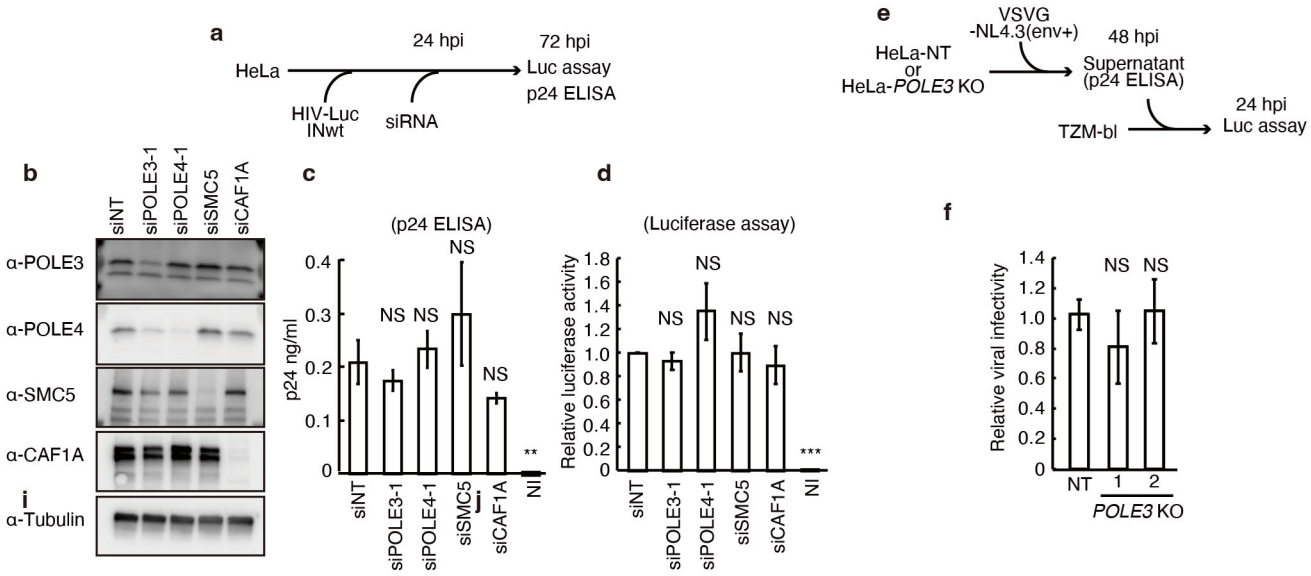


Extended Data Fig 3. Nascent HIV RNA IP and ChIP qPCR assays on human genomic region.
(a) Enrichment of EU labeled RNAs synthesized in Hela cells infected with VSV-G pseudotyped HIVLuc IN^{D116A} at 48hpi. RNAs were extracted from HeLa cells treated with or without EU. Labeled RNAs were biotinylated, and purified with streptavidin conjugated beads. Enrichment of labeled RNAs are presented as fold change over non-labeled condition. The mean ± SD values of 2 independent experiments in triplicate are plotted.
(b-e) ChIP assay using NT (blue) and POLE3 (green) KD HeLa cells infected with VSV-G pseudotyped HIV-Luc IN^{D116A}. ChIP assays were performed at 48 hpi, using anti-H3 (b), anti-H3ac
(c), anti-RNAPII (d), anti-H1.2 (e) antibodies, corresponded to Fig 3b-e. qPCR analysis was performed using specific primers for GAPDH promotor, B13, and α-satellite. The mean ± SD values of three independent experiments are plotted. The mean ± SD values of at least 3 independent experiments are plotted.



Extended data Fig 4. POLE3 KO HeLa-P4 cells

(a) Impact of knockout of POLE3 in HeLa-P4 cells on uniHIV-1 DNA silencing. Expression of POLE3 proteins in KO cells were analyzed by immunoblotting with indicated antibodies. Luciferase assays in POLE3 KO cells were performed at 48 hpi. Results are represented as luciferase expression relative to NT sgRNA, and the mean ± SD values of at least 3 independent experiments in triplicate are plotted.
(b) Exogenous POLE3 expression in POLE3 KO cells silences uniHIV-1 DNA. POLE3 KO HeLa-P4 cells stably expressing exogenous POLE3 wt, were infected with VSVG pseudotyped HIV-Luc IN^{D116A}. Whole cell extracts were analyzed by immunoblotting with indicated antibodies. Luciferase activity was analyzed at 48 hpi. Results are represented as luciferase expression relative to NT sgRNA of 3 independent experiment in triplicate.



Extended Data Fig 5. POLE3 depletion has no effect on late step of viral production

(a-d) Effect of POLE3, POLE4, SMC5, and CAF1A KD on viral release. HeLa cells were infected with VSV-G pseudotyped HIV-Luc, and treated with siRNAs at 24 hpi. The scheme of the experiment was shown in (a). Expression of POLE3, POLE4, SMC5, and CAF1A were analyzed by immunoblotting at 72 hpi (b). Viral release was assessed by p24 antigen ELISA in the supernatant (c), and luciferase assay (d) was performed at 72 hpi, the mean ± SD values of 4 independent experiments are plotted. The results showed that POLE3 KD has no effect on viral release and expression from integrated HIV-1, similar to POLE4, SMC5 and CAF1A KD.

(e-f) Infectivity of HIV-1 virions produced in POLE3 KO HeLa cells. HeLa NT or POLE3 KO cells were infected with a VSV-G pseudotyped NL4.3 (env+), and culture supernatant containing produced virions was collected at 48hpi. The infectivity of virions produced in POLE3 KO and control cells were measured in TZM-bl cells. The scheme of the experiment was shown in (e). HIV infectivity corresponded to the ratio of luminescence to the quantity of p24 CA detected in the supernatant was plotted (f). Results are represented as quantification relative to NT control cells, the mean ± SD values of 2 independent experiments in triplicate are plotted.

* P<0.05, ** P<0.01, *** P<0.001 independent Student's t-test.

The results showed that HIV-1 viruses produced in POLE3 KO cells were as infectious as those produced in control cells.