Supplemental Information

EXPERIMENTAL PROCEEDURES

Cell lines and antibodies

Cell lines including human embryonic kidney (HEK)-293 (CRL-1573), F9 embryonic carcinoma (CRL-1720), Mouse embryonic fibroblasts (MEF) (SCRC-1008) and NIH-3T3 (CRL-1658) cells were purchased from American Type Culture Collection. All cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 1000 U/mL penicillin and 100 mg/mL streptomycin. Ba/F3 cells were cultured in RPMI medium supplemented with 10% heat-inactivated FBS and 1.6 ng/mL of recombinant IL-3 (R&D Systems). All cells were maintained in a 37°C incubator with 5% CO_2. Antibodies used in this study include: Anti-Histone H3 (ab1791, Abcam), Anti-Histone H3 tri-methyl K9 (ab8898, Abcam), Anti-Acetyl-Histone H3 (06-599, Millipore), anti-MLV NC (National Cancer Institute serum 81S-216), anti-MLV CA (National Cancer Institute serum 79S-804) and rabbit IgG control antibody (sc-2027, Santa Cruz).

Chromatin immunoprecipitation

5×10^6 cells were crosslinked with 1% formaldehyde at room temperature for 10 min, followed by quenching with 0.125 M glycine for 5 min. Cells were lysed in 0.5 mL of ChIP lysis buffer (50 mM Tris-HCl pH 8.0, 1% SDS, 10 mM EDTA) containing Protease inhibitor cocktail (Roche) and sonicated to produce an average fragment size of 200–800 base pairs. Each immunoprecipitation was performed by incubating 30 μg of sonicated chromatin together with 4 μg of respective antibodies in ChIP dilution buffer (10 mM Tris-HCl pH 8.0, 1% Triton X-100,

0.1% SDS, 150 mM NaCl, 2 mM EDTA) overnight at 4°C. Next day, 25 µl of Protein A/G Dynabeads (Life Technologies) were added for an additional 4 h. Captured antibody-antigen complexes were washed 2 times each in ChIP low salt buffer (20 mM Tris-HCl pH 8.0, 1% Triton X-100, 0.1% SDS, 150 mM NaCl, 2 mM EDTA), ChIP high salt buffer (20 mM Tris-HCl pH 8.0, 1% Triton X-100, 0.1% SDS, 500 mM NaCl, 2 mM EDTA), ChIP LiCl buffer (10 mM Tris-HCl pH 8.0, 1% NP-40, 250 mM LiCl, 1 mM EDTA), and TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). DNA was eluted from beads in 200 µl elution buffer (TE buffer containing 1% SDS, 100 mM NaCl, 5 mM DTT), reverse crosslinked (65°C overnight), treated with RNase A (37°C, 1 h) and Proteinase K (37°C, 2 h), and purified using QIAquick PCR purification kit according to the manufacturer's instructions (Qiagen). Quantitative real-time PCRs were performed with indicated primers. The number of copies of each PCR product in both input and immunoprecipitated DNA was first determined from standard curves generated using control plasmids (pNCA-GFP for total viral DNA, and an MLV 2-LTR circle plasmid generated via overlapping PCR for circular DNA). Relative enrichment is shown as percent of input DNA calculated by dividing the number of copies from immunoprecipitated DNA by input DNA and multiplying by 100%.

Figure S1, related to Figure 1

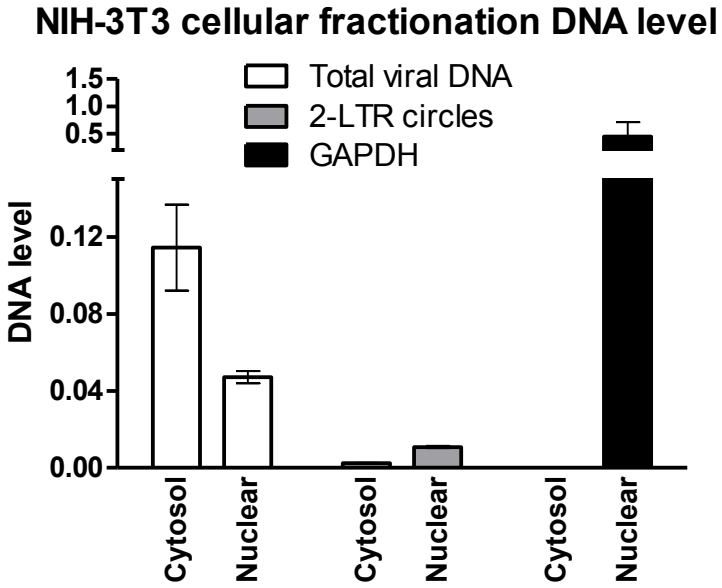

Figure S1, related to Figure 1.
NIH-3T3 cells infected with WT MLV-GFP virus were subjected to nuclear/cytoplasmic fractionation at 12 h post infection, followed by qPCR analysis of viral DNA forms in the two cellular fractions. DNA levels shown are calculated as exponential base 2 of PCR threshold cycle number (Ct), normalized to nuclear GAPDH. Results shown are means ± SDs from two independent experiments performed in duplicate.

Figure S2, related to Figure 1

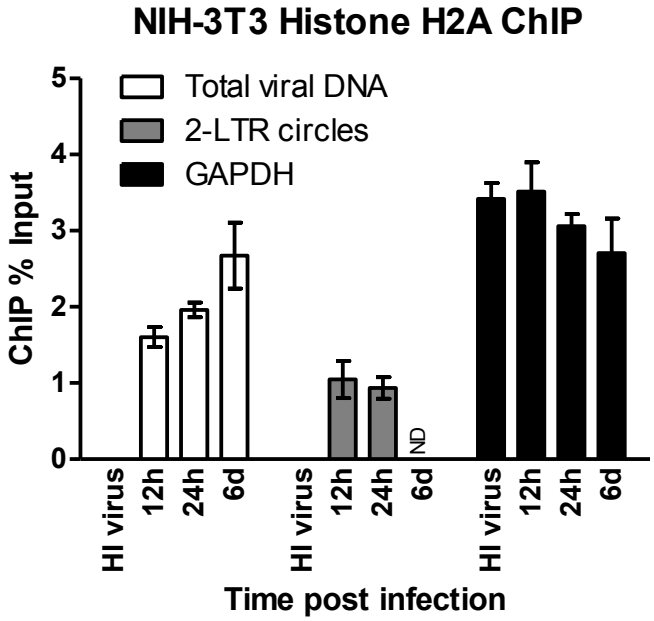

Figure S2, related to Figure 1.
Histone H2A chromatin immunoprecipitation (ChIP) analysis of chromatin harvested at indicated time points following MLV-GFP infection of NIH-3T3 cells. ChIP data is presented as % of input DNA, calculated by dividing the ChIP copy number for each gene target by the copy number from input DNA and multiplying by 100%. Results shown are means ± SEMs from three independent experiments performed in duplicates. ND denotes not determined.

Figure S3, related to Figure 4F-G

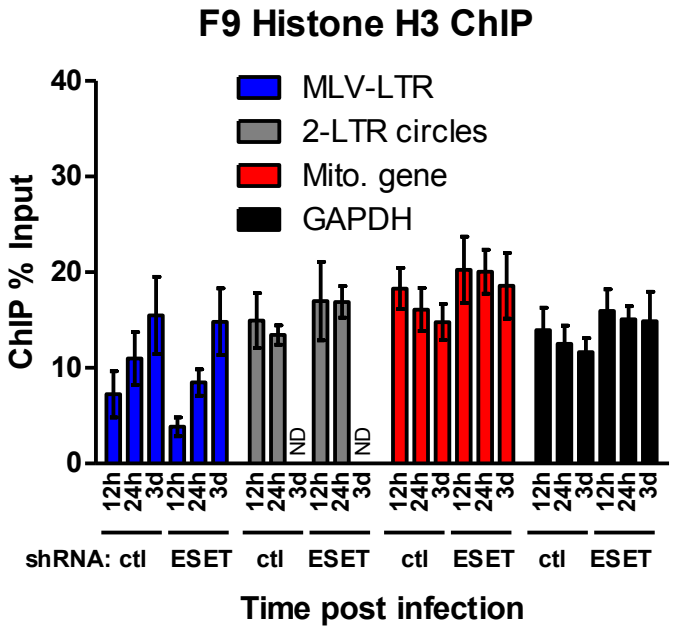

Figure S3, related to Figure 4F-G.
Histone H3 ChIP analysis of chromatin harvested at indicated time points following MLV-GFP infection of F9 cells. ChIP data is presented as % of input DNA as described above. Results shown are means ± SEMs from three independent experiments performed in duplicates. ND denotes not determined.

Figure S4, related to Figure 6

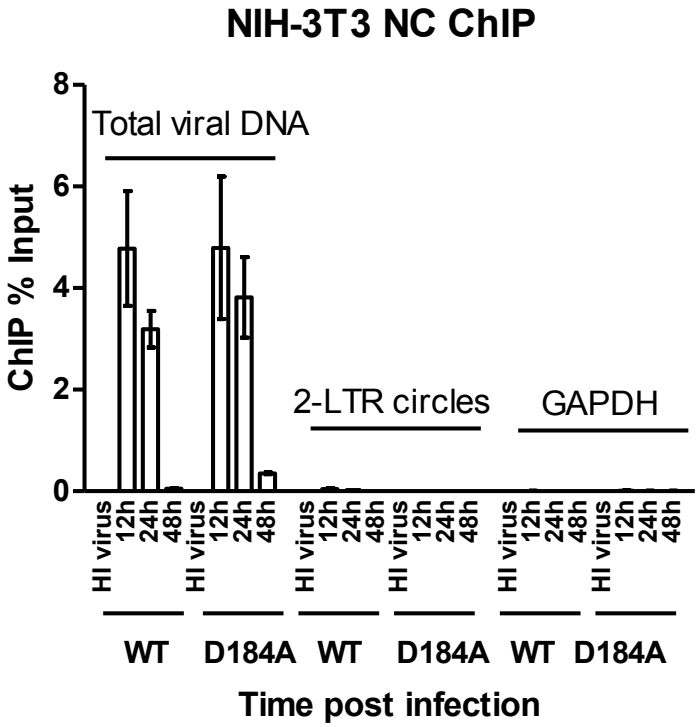

Figure S4, related to Figure 6.
Nucleocapsid (NC) ChIP analysis of chromatin harvested at indicated time points following WT or D184A-IN MLV-GFP infection of NIH-3T3 cells. ChIP data is presented as % of input DNA as described above. Results shown are means ± SDs from two independent experiments performed in duplicates. ND denotes not determined. HI virus denotes heat inactivated virus.

Figure S5, related to Figure 6

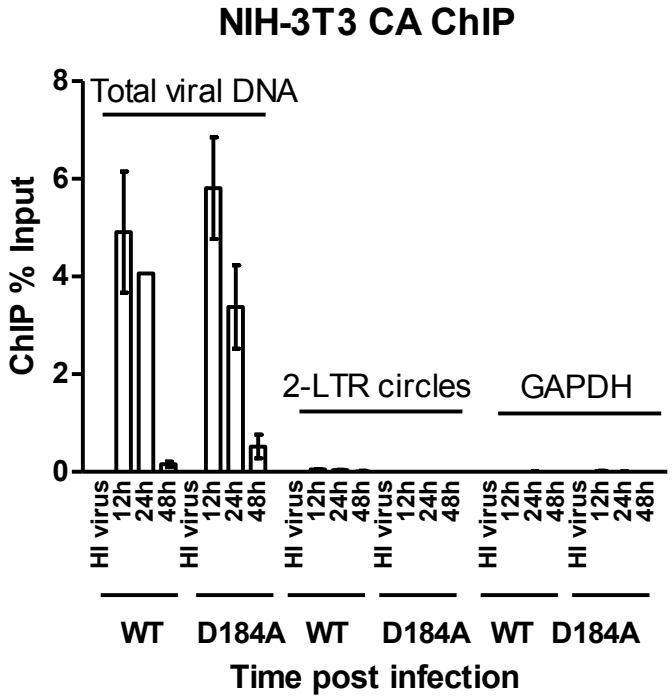

Figure S5, related to Figure 6.
Capsid (CA) ChIP analysis of chromatin harvested at indicated time points following WT or D184A-IN MLV-GFP infection of NIH-3T3 cells. ChIP data is presented as % of input DNA as described above. Results shown are means ± SDs from two independent experiments performed in duplicates. ND denotes not determined. HI virus denotes heat inactivated virus.

Figure S6, related to Figure 7.

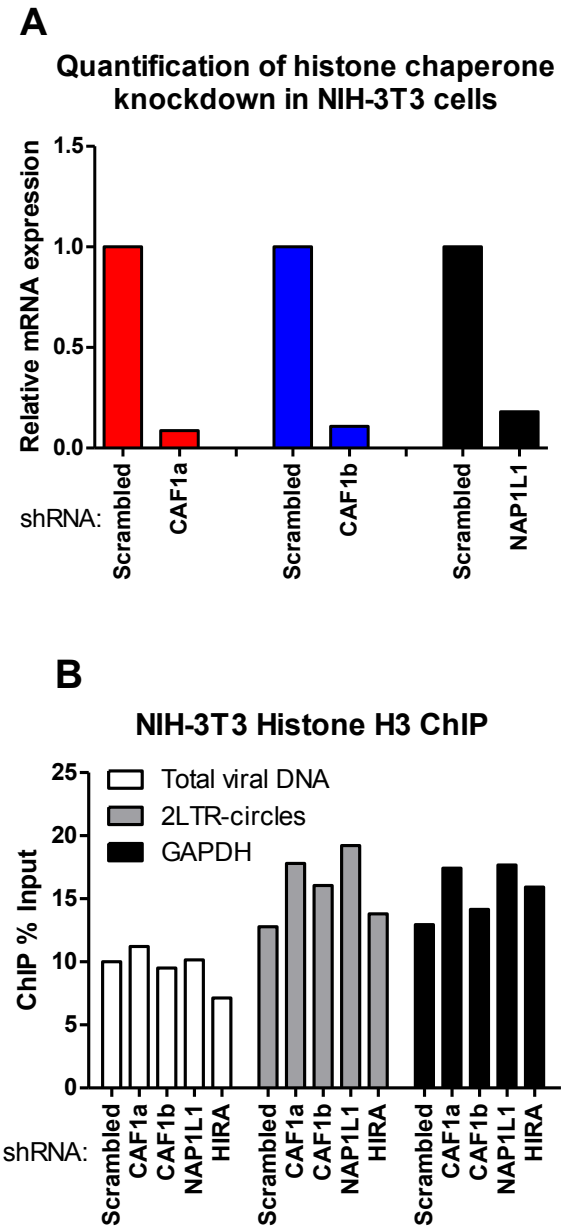

Figure S6, related to Figure 7.
(A) Stable NIH-3T3 knockdown cells were created using shRNA and mRNA levels were determined by RT-qPCR, normalized to GAPDH, and presented relative to scrambled shRNA control cells.
(B) Histone H3 ChIP analysis of chromatin harvested at 24 h following MLV-GFP infection of NIH-3T3 cells depleted of various histone chaperones. ChIP data is presented as % of input DNA, calculated by dividing the ChIP copy number for each gene target by the copy number from input DNA and multiplying by 100%.

Table S1, related to Figures 1, 3, 4, and 5.

Primer	Sequence (5'-3')
Total viral DNA (GFP)-F	AAGCTGACCCTGAAGTTCATCTGC
Total viral DNA (GFP)-R	CTTGTAGTTGCCGTCGTCCTTGAA
MLV-LTR-F	AGGGTCTCCTCTGAGTGATTGACT
MLV-LTR-R	TCGGACAGACACAGATAAGTTGCT
MLV 2-LTR circles-F	AGGGTCTCCTCTGAGTGATT
MLV 2-LTR circles-R	ATGGTGTGTGGAGGAGTATAAAG
M-PMV 2-LTR circles-F	TCCTCCAGGTTCCTACTGTT
M-PMV 2-LTR circles-R	ACGGAGAAGAACCAGGAAATAC
GAPDH-F	ACCTTTAGCCTTGCCCTTT
GAPDH-R	ACATCACCCCCATCACTCAT
Mito. gene (Mitochondrial RNA polymerase)-F	AGACACCTGCTGCCCTATGT
Mito. gene (Mitochondrial RNA polymerase)-R	GCTCCATCCCAGTGCTTTAC

Table S1, related to Figures 1, 3, 4, and 5.
ChIP qPCR primers used in this study. Primers F are forward, primers R are reverse.