Supporting Information

Stavrou et al. 10.1073/pnas.1217399110

SI Materials and Methods

Infectivity Assays. The levels of M-MLV infection in the spleens and thymuses of the infected mice were determined by infectious center (IC) assays using a focal immunofluorescence assay (FIA) as previously described (1).

Virus Spread Assay. NR-9456 macrophages(5×10^4 per well) were seeded on a 12-well plate. Equal amounts of WT and glyco-Gag mutant Moloney murine leukemia virus (M-MLV) were used to infect the cells for 2 h at 37 °C in the presence of 2 μg/mL polybrene. The cells were washed and harvested at 24, 48, 72, and 96 h after infection (hpi). DNA from the infected cells was isolated, and quantitative PCR (qPCR) was performed using MLV env-specific primers. GAPDH primers were used for normalization.

Target Cell Assay. mCAT-1 cells were transfected using Lipofectamine 2000 with apolipoprotein B editing complex (APOBEC3)Δexon5.HA ($APOBEC^{BL6}$) and APOBEC3FL.HA ($APOBEC^{BALB}$) (2). The transfected cells were infected 16 h after transfection with equal amounts of WT and glycosylated Gag protein (glyco-Gag) mutant virus purified from APOBEC3 KO mice. DNA from infected cells was isolated at 22 hpi using the Qiagen DNeasy Blood and Tissue Kit (Qiagen), and protein extracts were prepared from the transfected cells for Western blots. PCR was performed using MLV env-specific primers described above. GAPDH primers were used for normalization: 5′-CCCCTTCA-TTGACCTCAACTACA-3′ and R′ primer 5′-CGCTCCTGGAG-GATGGTGAT-3′.

Transmission Electron Microscopy. For transmission electron microscopy (TEM) analysis, viruses were harvested from tissue culture medium (30 mL) from productively infected NIH 3T3 or Ti-6 cells. The media were passed through 0.45-μm filters and then sedimented through a 20% sucrose cushion by centrifugation at 25,000 rpm in a Beckman SW28 rotor for 1 h at 4 °C. After aspiration of the supernatants, the viral pellets were suspended and fixed in 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium codylate, pH 7.4 (Electron Microscopy Science), for 1 h at room temperature and stored at 4 °C. High-speed virus pellets were prepared in Beem capsules for thin-section TEM as previously described (3, 4) and examined and imaged in a Hitachi 7600 microscope operated at 80 kV.

1. Low A, et al. (2009) Enhanced replication and pathogenesis of Moloney murine leukemia virus in mice defective in the murine APOBEC3 gene. *Virology* 385(2):455–463.
2. Rulli SJ, Jr., et al. (2008) Interactions of murine APOBEC3 and human APOBEC3G with murine leukemia viruses. *J Virol* 82(13):6566–6575.
3. Mariner JM, McMahon JB, O'Keefe BR, Nagashima K, Boyd MR (1998) The HIV-inactivating protein, cyanovirin-N, does not block gp120-mediated virus-to-cell binding. *Biochem Biophys Res Commun* 248(3):841–845.
4. Belanger JM, et al. (2010) Characterization of the effects of aryl-azido compounds and UVA irradiation on the viral proteins and infectivity of human immunodeficiency virus type 1. *Photochem Photobiol* 86(5):1099–1108.

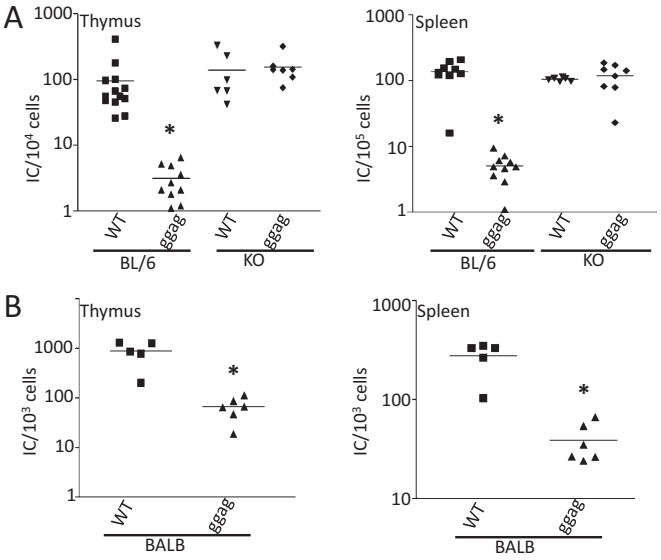

Fig. S1. MLV WT and glyco-Gag mutant virus loads in infected mice. Mice were infected with equal amounts of virus and killed at 16 dpi. (*A*) Levels of ICs in cells isolated from the spleens and thymi of C57BL/6 and APOBEC3 KO mice infected with WT and glyco-Gag mutant virus. (*B*) Virus titers (ICs) of the same viruses isolated from the tissues of BALB/c mice. Statistical significance was determined by paired Student *t* test. $*P \leq 0.005$.

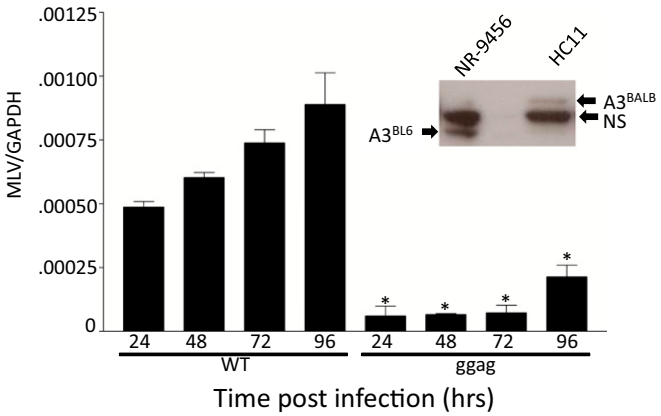

Fig. S2. Glyco-Gag counteracts APOBEC3 in a macrophage cell line. Equal amounts of WT and mutant viruses isolated from APOBEC3 KO mice were used to infect NR-9456 cells and at different times after infection, DNA was isolated and analyzed by qPCR with primers specific for M-MLV. Levels of M-MLV DNA compared with levels of GAPDH DNA from the same samples are shown. Shown is the average of three independent experiments ± SE. One-way ANOVA was used for statistical analysis; comparisons are to the WT-infected cells at each time point. *$P \leq 0.05$. *Inset* shows the expression of the APOBEC^{BL6} protein in NR-9456 and APOBEC^{BALB} in HC11 BALB/c mammary epithelial cells by Western blot with anti-mouse APOBEC3 antisera. The APOBEC3 antibody also detects a nonspecific band whose migration is intermediate between the two mA3 forms (NS).

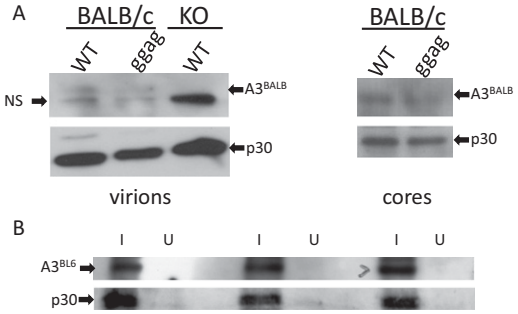

Fig. S3. APOBEC3 is packaged at similar levels in WT and glyco-Gag mutant viruses and cores in vivo. (*A*) Western blot of virions (*Left*) and cores (*Right*) isolated from APOBEC3 KO and BALB/c mice infected with WT or glyco-Gag mutant viruses. A3^{BALB}, APOBEC3^{BALB}; NS, nonspecific band. (*B*) APOBEC3 is found in the supernatants of splenic cultures from M-MLV–infected but not uninfected mice. Equal numbers of splenocytes from three uninfected BL6 mice or from three independent M-MLV–infected BL/6 mice were cultured for 48 h, and the supernatants were pelleted through 30% sucrose cushions (Materials and Methods). The pellets were resuspended in equal volumes of sample buffer and analyzed by Western blots using anti-APOBEC3 and anti-MLV antisera. I, infected; U, uninfected.

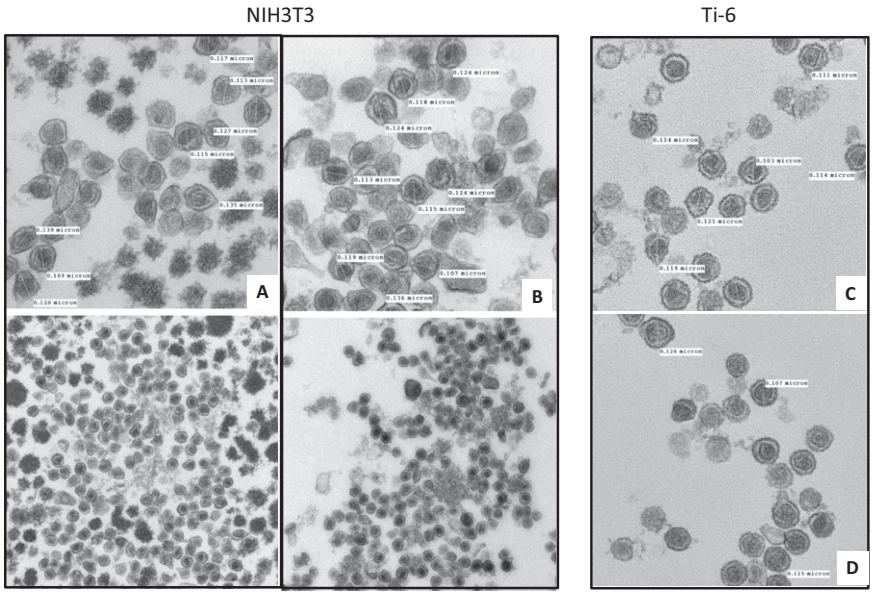

Fig. S4. TEM of M-MLV preparations. (*A*) WT M-MLV released from infected NIH 3T3 fibroblasts were fixed, stained, and analyzed by TEM. A representative field is shown, with diameters of individual particles measured. Bottom panel shows a field at lower magnification, with more particles evident; there were few particles of larger size (200–500 nm) lacking internal cores that would be indicative of exosomes. The fuzzy electron-dense material represents either virions that were stained so densely that all detail was lost or chromatin fragments and were not seen in other virion preparations. (*B*) Glyco-gag mutant M-MLV from NIH 3T3 fibroblasts are shown at low and high magnification. (*C*) WT M-MLV released from productively infected Ti-6 lymphocytes. (*D*) Glyco-Gag mutant M-MLV from infected Ti-6 cells.

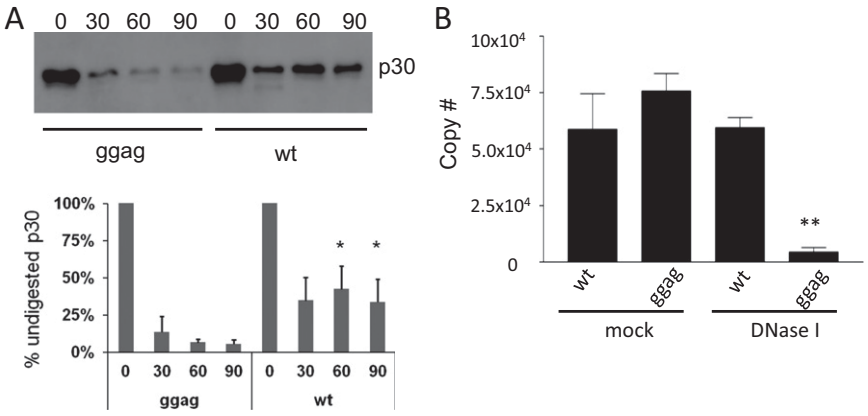

Fig. S5. Increased sensitivity of glyco-Gag mutant viruses to trypsin and DNaseI digestion. (*A*) MLV core fractions obtained from sucrose gradients such as those shown in Fig. 2*A* were pooled. Equal amounts of glyco-Gag mutant and WT cores were digested with 100 ng trypsin (5 ng/μL) at 37 °C for the times indicated and subjected to the Western blot analysis with anti-p30 antibodies, and the signals were quantified by densitometry. A representative blot for one experiment is shown at the top of the figure. Graphical representation of the results of three independent experiments are shown at the bottom of the figure; the percent digested for mutant vs. WT cores was statistically significant at 60 and 90 min (*$P \leq 0.05$ by Welch's *t* test). (*B*) Endogenous reverse transcription (EnRT) asays were performed with WT and glyco-Gag mutant virions in the presence or absence of DNase I and harvested at 6 h. Shown is the average of three independent experiments ± SE. Statistical significance was determined by one-way ANOVA. **$P \leq 0.01$.

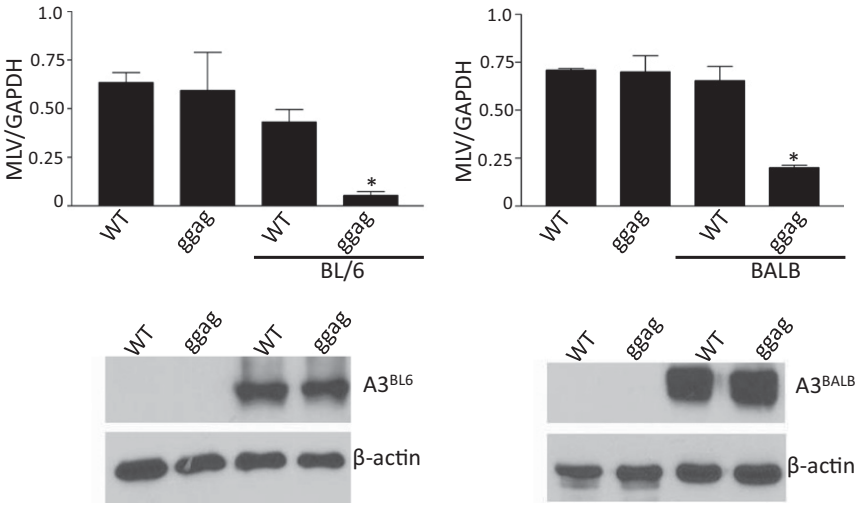

Fig. S6. APOBEC3 expressed in target cells inhibits infection by glyco-Gag mutant but not WT virus. qPCR analysis of DNA isolated from 293/MCAT-1 cells transfected with the APOBEC3$^{BL/6}$ or APOBEC3^{BALB} expression vector and then infected with WT or mutant virus. Shown is the average of three independent experiments ± SE. Statistical significance was determined by one-way ANOVA. *$P \leq 0.05$. Western blots of extracts prepared from parallel cultures analyzed with antibody to the HA tag on the APOBEC3 proteins are shown below the graphs.

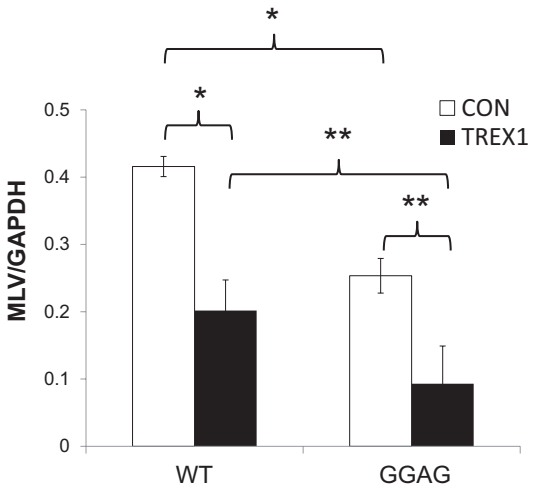

Fig. S7. Viral RNA in glyco-Gag–deficient viruses is more rapidly degraded after infection. RNA from the control or TREX1 siRNA-treated cells was isolated at 4 h after infection with WT or glyco-Gag mutant virus and analyzed by reverse-transcribed RT-qPCR for MLV RNA, using *env* primers. Shown is the average of two experiments with three technical replicates each. Statistical significance was determined by paired Student *t* test. *$P \leq 0.04$; **$P \leq 0.03$.

Table S1. Representative levels of virion RNA and IC units for different preparations of virus from spleens and NIH 3T3 cells

Virus	Cell/tissue	IC/mL	Virus/mL	Infectivity/particle
WT	spleen	2.38E+05	1.43E+08	1.66E−03
		4.42E+05	2.62E+08	1.69E−03
		2.21E+05	1.76E+08	1.26E−03
WT	3T3	1.00E+05	6.00E+08	1.67E−04
		1.30E+05	4.80E+08	2.71E−04
ggag	spleen	7.90E+05	7.30E+08	1.08E−03
		5.00E+05	5.50E+08	9.09E−04
		1.10E+05	3.60E+08	3.06E−04
ggag	3T3	6.71E+04	5.22E+07	1.29E−03
		1.33E+05	3.33E+08	3.99E−04
		2.23E+05	1.27E+08	1.76E−03

Each preparation of sucrose cushion–pelleted virus was subjected to RT-qPCR and the number of viruses estimated from the amount of virus-specific RNA. Each preparation was also titered on NIH 3T3 cells to determine the infectivity of each preparation.