Supporting Information

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SI Methods

Quantification of mBST-2 Expression. 43D and 17-5 cells were treated with different concentrations of mouse IFN-α for 24 h. Total cellular RNAs (1 μg) were reverse-transcribed with the qScript cDNA synthesis kit (Quanta Biosciences). The cDNAs were subjected to real-time PCR with Power SYRB Green PCR Master Mix (Applied Biosystems) and primers for mBST-2 (forward primer 5′- tcaggagtccctggagaaga and reverse primer 5′-atggagctgccagagttcac). GAPDH cDNA with appropriate primers was also amplified for normalization. The results are expressed as relative mBST-2 RNA levels compared to those in the untreated cells.

Confocal Microscopy. Confocal microscopy was performed as described in ref. 1 with slight modifications. The 43D and 17-5 cells were plated on glass coverslips 1 day before fixation. The cells were incubated with ice-cold PBS or 0.5% Triton X-100 for 2 min at 4 °C with gentle shaking. Afterward the cells were immediately overlaid with 4% paraformaldehyde and incubated for 20 min at RT. After washing with PBS and blocking with 3% BSA, 1% Triton X-100 in PBS, antibodies were added. The cells were stained with fluorescent-conjugated secondary antibodies followed by mounting with Vectashield mounting medium (Vector Laboratories). The images were analyzed on a confocal laser scanning microscope LSM710 with the image analyzing software Zen 2008 (Carl Zeiss).

1. Beer C, Pedersen L, Wirth M (2005) Amphotropic murine leukaemia virus envelope protein is associated with cholesterol-rich microdomains. *Virol J* 2:36.
2. Kuznetsov YG, et al. (2002) Atomic force microscopy investigation of fibroblasts infected with wild-type and mutant murine leukemia virus (MuLV). *Biophys J* 83: 3665–3674.
3. Low A, et al. (2007) Mutation in the glycosylated gag protein of murine leukemia virus results in reduced in vivo infectivity and a novel defect in viral budding or release. *J Virol* 81:3685–3692.

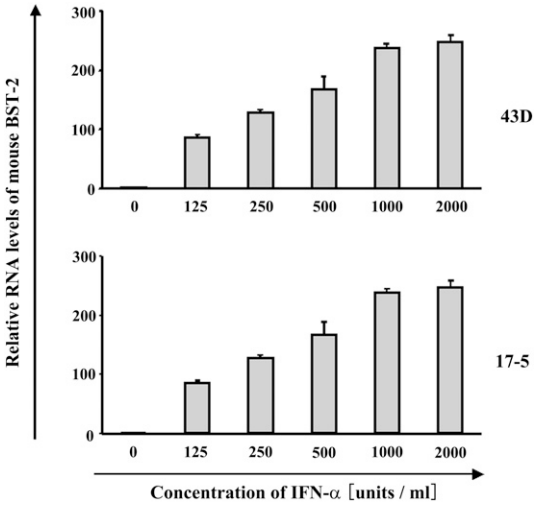

Fig. S1. IFN induces mouse BST-2 in both 43D and 17-5 cells. 43D and 17-5 cells were treated with different concentration of mouse IFN-α for 24 h. Total RNA extracted from the cells was subjected to real-time RT-PCR for quantification of mouse BST-2 and GAPDH. Expression levels of mouse BST-2 was normalized by GAPDH, and the results were expressed as relative RNA levels of mBST-2 to those in the untreated cells.

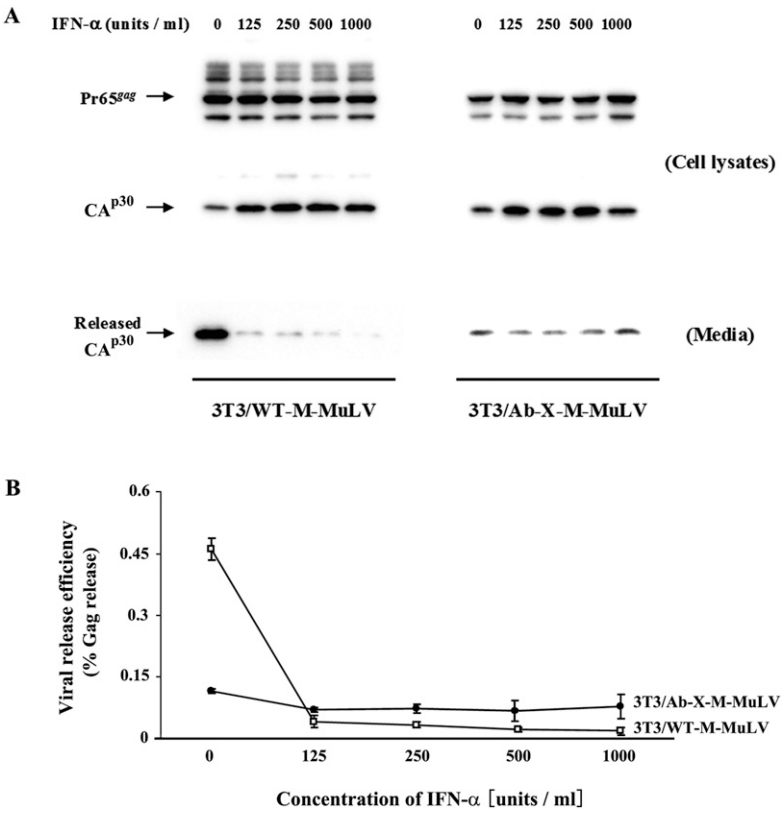

Fig. S2. Effect of IFN-α on virus release from the NIH 3T3 cells freshly infected with WT-M-MuLV and Ab-X-M-MuLV. (*A*) NIH 3T3 cells were infected with WT-M-MuLV (3T3/WT-M-MuLV) or Ab-X-M-MuLV (3T3/Ab-X-M-MuLV) isolated from each 43D and 17-5 cells. The cells were treated with different concentrations of mouse IFN for 24 h, after which media were replaced, and the cells and released viruses were collected 6 h after incubation. The same portions of cells and released viruses were subjected to the Western blots with anti-CA^{p30}. (*B*) Virus release efficiency from 3T3/WT-M-MuLV and 3T3/Ab-X-M-MuLV cells was calculated (means ± SD from two replicate experiments).

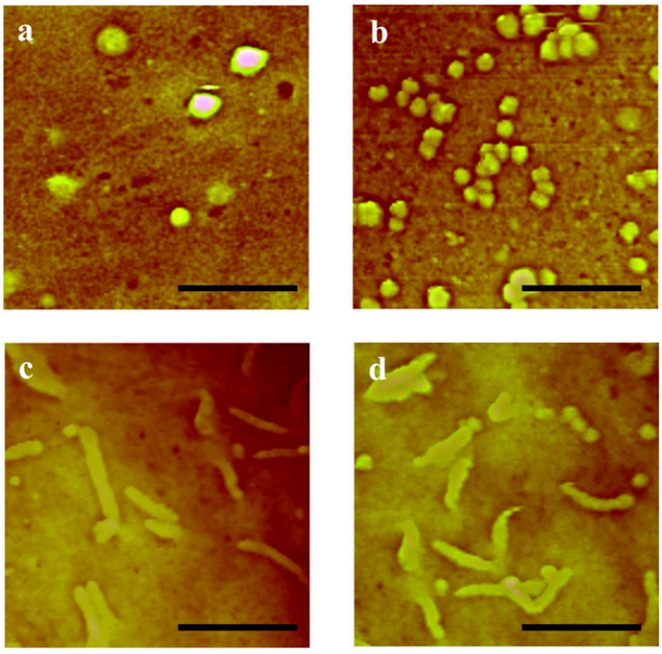

Fig. S3. Analyses of viral particles on the cell surface by atomic force microscopy (AFM). AFM procedures for virus-infected cells were described in ref. 2. Cells were grown on glass coverslips, fixed with 1% glutaraldehyde in PBS and postfixed with a 1% solution of osmium tetroxide in double-distilled H_2O. 43D cells (*A* and *B*) and 17-5 cells (*C* and *D*) with (*B* and *D*) or without (*A* and *C*) 500 U/mL IFN-α for 24 h. 43D cells show exclusively spherical virus particles on the cell surface (2, 3); some 17-5 cells show spherical particles, some show tube-like structures, and some show a combination of both (shown here). (Scale bars: 1 μm.)

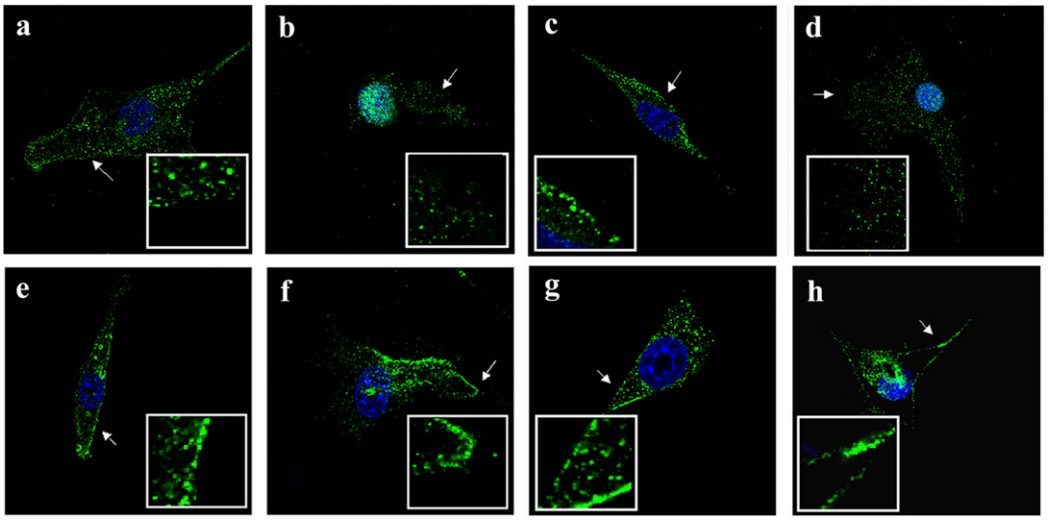

Fig. S4. Localization of CD71 and Caveolin in lipid rafts. 43D cells (*A*, *B*, *E*, and *F*) and 17-5 cells (*C*, *D*, *G*, and *H*) were incubated with ice-cold PBS (*A*, *C*, *E*, and *G*) or 0.5% Triton X-100 (B, *D*, *F*, and *H*) followed by fixation with paraformaldehyde and then incubation with anti-CD71 (*A*–*D*) or anti-Caveolin (*E*–*H*) antibodies. The antigens and nuclei were visualized by secondary antibodies conjugated with Alexa 488 and DAPI (to visualize the nuclei). The regions pointed by arrows were enlarged in the insets.

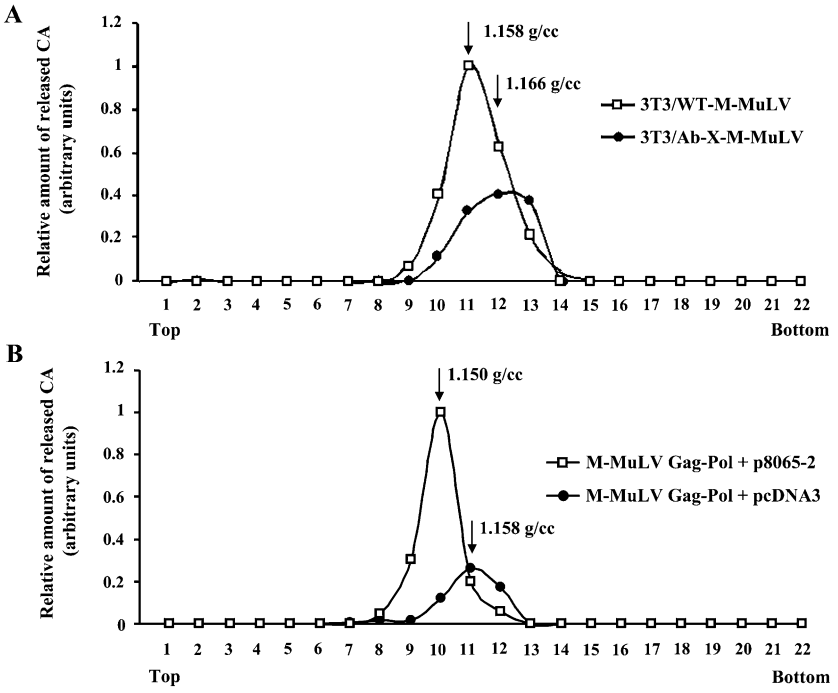

Fig. S5. Analyses of the density in viral particles released from NIH 3T3 cells infected with WT-M-MuLV and Ab-X-M-MuLV. (*A*) NIH 3T3 cells were infected with WT-M-MuLV (3T3/WT-M-MuLV) or Ab-X-M-MuLV (3T3/Ab-X-M-MuLV) released from each 43D and 17-5 cells. (*B*) 293T cells were transiently transfected with the M-MuLV Gag-Pol expression vector with or without p8065-2. Viruses harvested from the infected or transiently transfected cells were subjected to density gradient centrifugation, and distribution of CA^{p30} was analyzed by Western blots and densitometry.

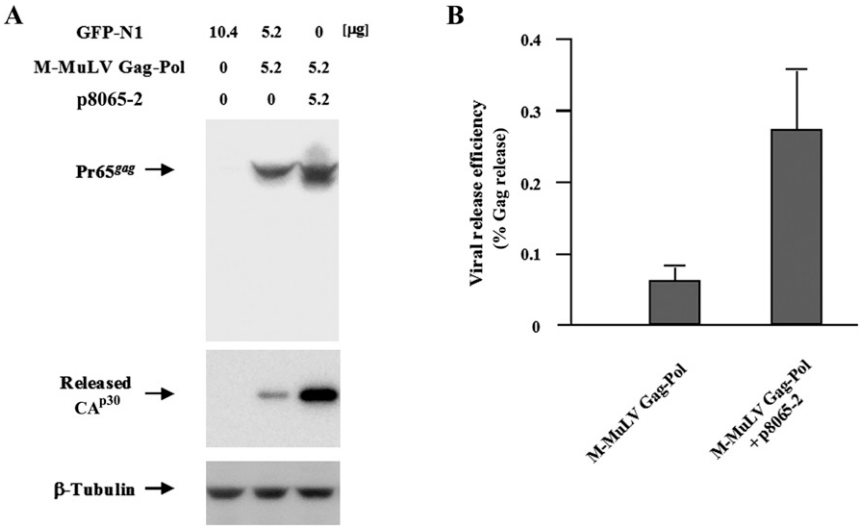

Fig. S6. gPr80 increases the efficiency of M-MuLV Gag release without Env. The M-MuLV Gag-Pol expression vector was transfected with or without p8065-2 into 293T cells. (*A*) The cells and media were harvested 48 h after transfection, and the same portions of cells and viruses were analyzed by Western blots with anti-CA^{p30} and anti-β-Tubulin antibodies. (*B*) The viral release efficiencies were quantified (means of four experiments, ±SD).

Table S1. Relative cholesterol in viral particles released from WT- and Ab-X-M-MuLV-infected cells

Exp.	Cells	Total cholesterol in sup, μg	Total CA in sup, p30 unit	Total cellular cholesterol, μg	Total cellular protein, mg	% cholesterol in released virus	Cholesterol in released virus / CA protein, μg/p30 unit	Cholesterol content in WT-M-MuLV / Ab-X-M-MuLV
	NIH 3T3	0.199	—	4.774	0.311	—	—	—
1	43D	1.196	631	3.161	0.253	23.5	0.001620	2.240
	17-5	0.486	466	3.248	0.283	9.0	0.000723	
2	43D	1.996	1151	6.848	0.513	18.6	0.001427	2.352
	17-5	0.745	612	8.596	0.595	4.0	0.000607	
3	43D	2.799	1342	10.122	0.664	17.8	0.001701	2.411
	17-5	1.095	1078	7.283	0.487	9.1	0.000706	
							Ave.	2.334 ± 0.087
1	3T3/WT-M-MuLV	0.553	267	3.279	0.218	10.4	0.001500	2.548
	3T3/Ab-X-M-MuLV	0.293	228	3.691	0.236	3.4	0.000589	
2	3T3/WT-M-MuLV	0.599	342	1.957	0.150	19.5	0.001454	2.496
	3T3/Ab-X-M-MuLV	0.421	443	3.647	0.323	6.3	0.000583	
							Ave	2.522 ± 0.037

The cholesterol contents of viral supernatants released from 43D and 17-5 cells, and from NIH 3T3 cells freshly infected with virus from these cells, were determined as described in *Materials and Methods*. Supernatant from uninfected NIH 3T3 cells was processed in the same manner, and the amount of cholesterol in sucrose gradient fractions equivalent to the virus fractions is shown in the top row of the table; 4.0% of total cholesterol was present in these fractions, and presumably represented released vesicles. This value was subtracted from the percentages of total cholesterol in the viral supernatants for all experiments; the corrected percent total cholesterol in released virus is shown. These percentages were used to calculate the amount of total cholesterol in the released virus. The amount of cholesterol in released virus relative to viral protein (as measured by Western blots and densitometry) is shown in the next-to-last column. For each experiment the CA proteins for the two viruses were quantified in the same Western blot, so relative amounts of viral protein could be compared. However, between experiments the absolute values of the p30 units were not necessarily the same. The ratios of cholesterol contents in Ab-X-M-MuLV to WT-M-MuLV from three (43D and 17-5 cells) and two (freshly infected NIH 3T3 cells) experiments are shown. The differences in cholesterol contents between 43D and 17-5 ($P < 0.005$) and between 3T3/WT-MuLV and 3T3/Ab-X-MuLV ($P < 0.05$) viruses were significant by Welch's twotailed *t* test.