Supporting Information

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Materials and Methods

Tissue Culture and Transfections. HeLa cells were propagated in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. CEMx174, A3.01, H9, and Jurkat cells were maintained in RPMI medium 1640 containing 10% FBS. For transfection of HeLa cells, cells were grown in 25-cm^2 flasks to ≈80% confluence (≈3 × 10^6 cells). Cells were transfected using Lipofectamine Plus (Invitrogen) following the manufacturer's recommendations. A total of 5 μg of plasmid DNA per 25-cm^2 flask (5 × 10^6 cells) was used. Total amounts of transfected DNA were kept constant in all samples of each experiment by adding empty-vector DNA as appropriate. Cells were harvested 24 h after transfection. For IFNα induction of Bst-2, cells were cultured before cell lysis for 24 h in the presence of 0.1–10 ng/mL IFNα-2c [a generous gift from Kathryn C. Zoon (National Institute of Allergy and Infectious Diseases, Bethesda, MD)].

Preparation of Monocyte-Derived Macrophages (MDM) and Stimulation with IFNα. Human monocytes from normal donors were prepared as described in ref. 1 by countercurrent centrifugal elutriation using a Beckman System. Elutriated monocytes were >99% viable as determined by trypan blue exclusion and were >95% pure as determined by morphological analysis using Giemsa stain of representative cytocentrifuge preparations. All reagents used in the preparation and culture of monocytes were free of detectable endotoxin as determined by the *Limulus* amebocyte assay (2). Monocytes were precultured as adherent cell monolayers by using a modification of the procedure of Lazdins *et al.* (3). Briefly, 4 × 10^6 cells were suspended in 2 mL of high glucose (4.5 g/L) DMEM (GIBCO) supplemented with 50 units/mL penicillin, 50 μg/ml streptomycin (GIBCO), 2 mM L-glutamine (GIBCO), 1 mM sodium pyruvate (GIBCO), and 10% pooled human serum (PHS). Monocytes were cultured in complete DMEM supplemented with 10% PHS for 6–7 days to allow differentiation into MDM as reported in ref. 4. IFNα-2c was added as indicated in the main text, and cells were cultured for 24 h. Cells were then washed twice with PBS and lysed in 300 μL of sample buffer and processed for immunoblotting (50 μL each).

Virus Preparation. Virus stocks for infection were prepared by transfecting 293T cells with appropriate plasmid DNAs. Virus-containing supernatants were harvested 24 h after transfection. Cellular debris was removed by centrifugation (3 min, 3,000 × *g*), and clarified supernatants were filtered (0.45 μm) to remove residual cellular contaminants. For immunoblot analysis of viral proteins, virus-containing supernatants (5 mL) were concentrated by ultracentrifugation through 4.5 mL of 20% sucrose in PBS as described in ref. 5.

Immunoblotting. For immunoblot analysis of intracellular proteins, whole-cell lysates were prepared as follows. Cells were washed once with PBS, suspended in PBS (400 μL per 10^7 cells), and mixed with an equal volume of sample buffer [4% SDS, 125 mM Tris·HCl (pH 6.8), 10% 2-mercaptoethanol, 10% glycerol, and 0.002% bromophenol blue]. Proteins were solubilized by boiling for 10–15 min at 95 °C. Residual insoluble material was removed by centrifugation (2 min, 15,000 rpm in an Eppendorf Minifuge). For immunoblot analysis of virus-associated proteins, concentrated viral pellets were suspended in a 1:1 mixture of PBS and sample buffer and boiled. Cell lysates and viral extracts were subjected to SDS/PAGE; proteins were transferred to PVDF membranes and reacted with appropriate antibodies as described in the main text. Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences) and visualized by enhanced chemiluminescence (ECL; Amersham Biosciences).

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4. Miyagi E, *et al.* (2008) APOBEC3G-independent reduction in virion infectivity during long-term HIV-1 replication in terminally differentiated macrophages. *Virology* 379:266–274.
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Fig. S1. Cell-type-specific variation of Bst-2 cell surface expression. (*A*) Cell surface expression of Bst-2 in 293T and HeLa cells. 293T cells (*Left*) and HeLa cells (*Right*) were either left untreated or were treated for 24 h with 1 ng/mL IFNα. Cells were then stained for Bst-2 and subjected to FACS analysis as described in *Materials and Methods*. (*B*) Cell surface expression of Bst-2 in T cell lines. Jurkat cells, A3.01 cells, H9 cells, and CEMx174 cells were analyzed by FACS as in *A*. The color code is the same as in Fig. 3*B*.

Fig. S2. Vpu does not cause cell surface downmodulation of Bst-2 in acutely infected H9 cells. (*A*) H9 cells were infected with HIV-1 virus stocks, and virus replication was monitored as described in Fig. 3*A*. (*B*) Portions of the H9 cells were removed on days 6 and 8 for Bst-2 cell surface staining followed by staining for intracellular p24 as described in *Materials and Methods*. The day 6 (*Top*) and day 8 (*Bottom*) samples are shown.