Supporting Information

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SI Materials and Methods

Binding and Internalization. VSV-GPΔ was bound to cells at an approximate m.o.i. of 15 at 4 °C. After 2 h, unbound virus was washed off and cells were either fixed immediately in 2% PFA or warmed to 37 °C for 2 h to allow internalization before fixing. Cells were treated with permeabilization solution (10% supplemented calf serum, 10 mM Glycine, 10 mM Hepes, and 0.05% Saponin) for 30 min, blocked in 10% FBS for 15 min, and stained with a monoclonal Ab to GP (Lisa Hensley, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD) followed by anti-mouse AlexaFluor 488. Texas-Red conjugated phalloidin was used to visualize actin. Images were collected with a Nikon C1 laser scanning confocal unit attached to a Nikon Eclipse TE2000-E microscope. The mean pixel intensity in the green channel was determined for 10 representative cells for each condition using the ImageJ software program. For internalization samples, virions localized at the cell periphery were excluded from the analysis.

293T and Jurkat T cells were maintained, and binding of the recombinant EboV receptor binding region was performed, as described (1, 2).

Immunofluorescence. CHO K1, CHO B2, and CHO B2-α5 cells were transfected with plasmids encoding human CatL (Origene) using PEI (Polysciences). After 48 h, cells were fixed, treated for 30 min with permeabilization solution, and blocked for 15 min in 10% FBS at 4 °C. Cells were stained with Abs specific for CatL (Athens Research and Technology), EEA1 (BD Biosciences), LBPA (Jean Gruenberg, University of Geneva, Switzerland), and LAMP1-Cy3 (Abcam) in 5% BSA-PBS for 1 h at room temperature. Secondary staining was performed with anti-mouse and anti-rabbit AlexaFluor 488 and AlexaFluor 555 (Invitrogen). Coverslips were mounted using Fluoromount G (Southern Biotech) and images were collected with a Nikon C1 laser scanning confocal unit attached to a Nikon Eclipse TE2000-E microscope with a 100×, 1.45 N.A. Plan Apochromat objective. Images were quantified for overlap using the colocalization analysis tool in the EZ-C1 3.5 software platform (Nikon Instruments). Ten representative fields containing CatL positive cells for each staining condition were filtered using 10% minimum intensity thresholds for both the green and red channels. Degree of colocalization was reported as a Pearson's correlation coefficient.

1. Dube D, et al. (2008) Cell adhesion promotes ebola virus envelope glycoprotein-mediated binding and infection. *J Virol* 82:7238–7242.

2. Dube D, et al. (2009) The primed ebolavirus glycoprotein (19 kilodalton GP1,2): Sequence and residues critical for host cell binding. *J Virol* 83:2883–2891.

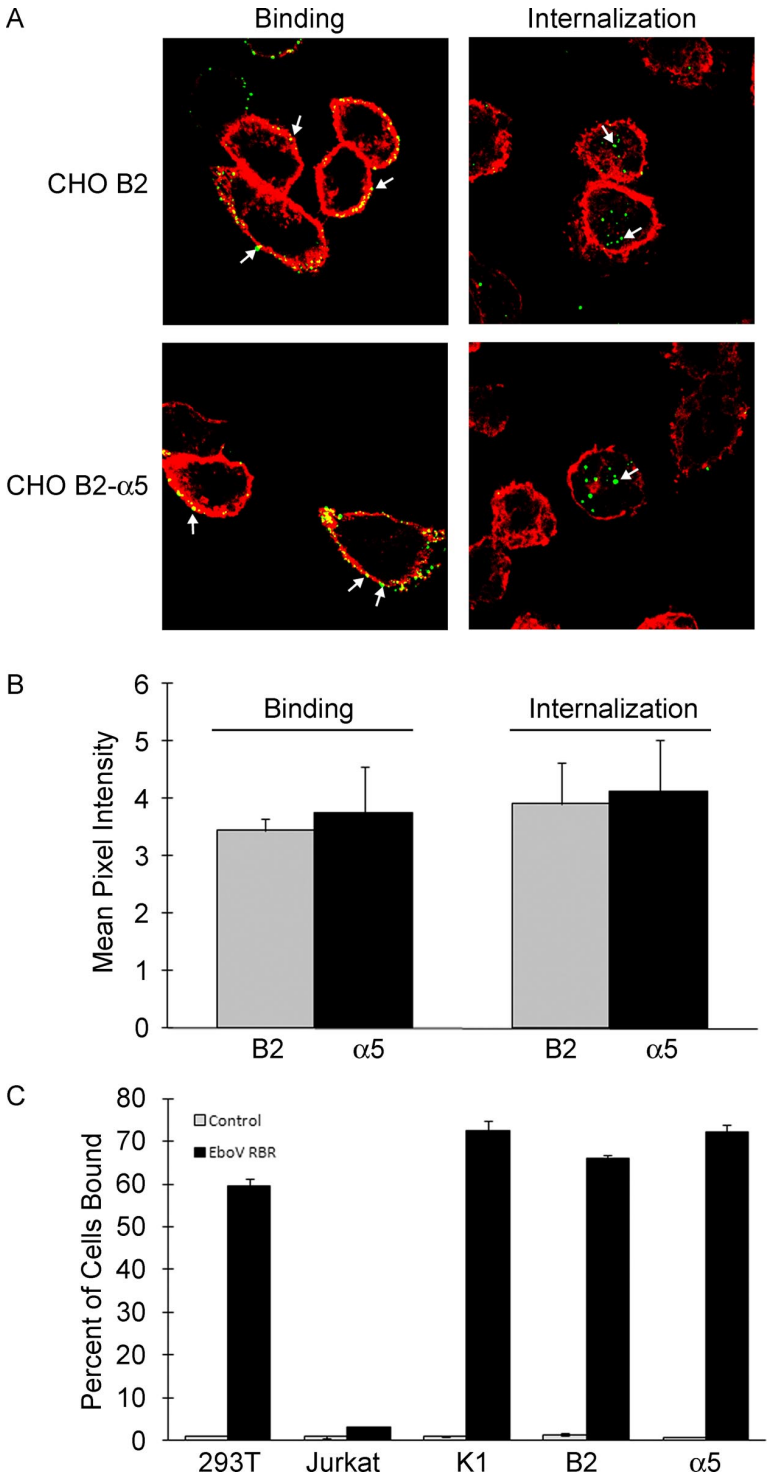

Fig. S1. $\alpha5\beta1$ integrin is not required for EboV GP-mediated binding or internalization. (*A*) VSV-GPΔ was bound to cells at 4°C and either fixed immediately (Binding) or warmed to 37°C to allow internalization (Internalization) before fixing. Cells were permeabilized and stained with anti-EboV GP antibody (green) and phalloidin (red). These are larger images of the same panels shown in Fig. 3*B*. (*B*) The mean pixel intensity in the green channel was calculated for 10 cells of each sample. For internalization samples, virions localized at the cell periphery were excluded from the analysis. Error bars indicate SEM. (*C*) Recombinant EboV RBR or rabbit Fc (Control) was bound to 293T (susceptible to EboV GP-mediated infection), Jurkat (refractory to EboV GP-mediated infection), CHO K1 (K1), CHO B2 (B2), and CHO B2-$\alpha5$ ($\alpha5$) cells, stained with AlexaFluor 488 conjugated Protein A, and analyzed by flow cytometry. Averages of duplicate samples are shown with error bars indicating SEM. The experiment has been conducted three times with the same results.

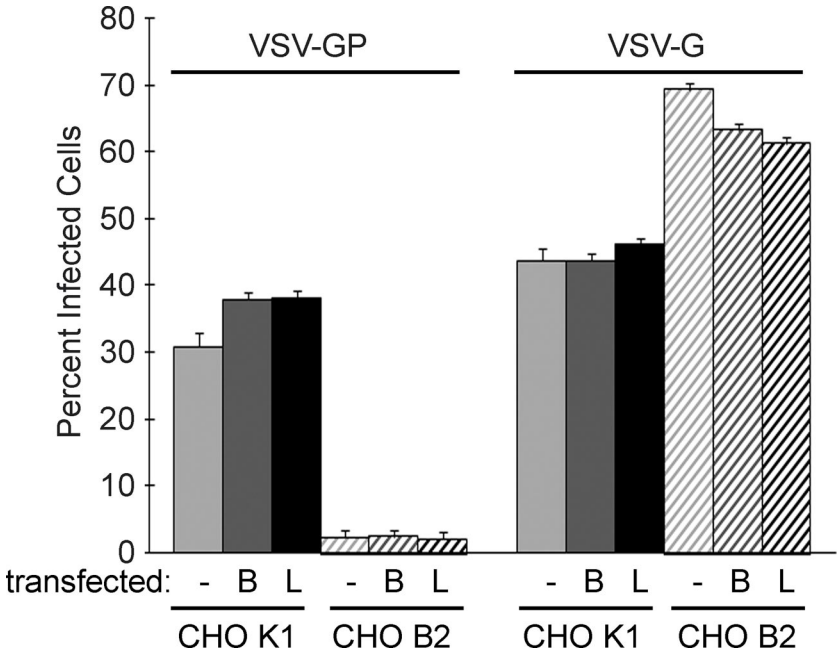

Fig. S2. Overexpression of CatB or CatL does not rescue infection in the CHO B2 cells. CHO K1 and CHO B2 cells were either left untransfected (−) or transfected with plasmids encoding human CatB (B) or CatL (L). 48 h post-transfection, the cells were infected with VSV-GP or VSV-G and the percentage of infected cells was measured by flow cytometry. Averages of duplicate samples from a single experiment are shown with error bars indicating SEM.

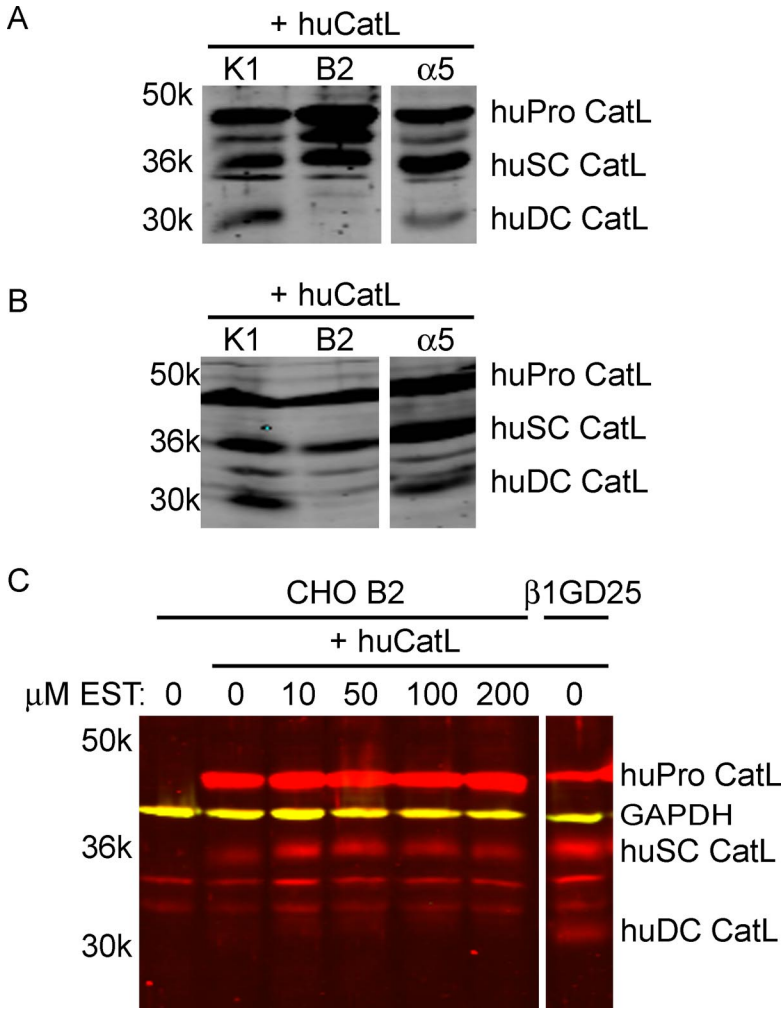

Fig. S3. The DC form of CatL is not visualized when cells are lysed in low pH or boiling sample buffer or treated with a cysteine protease inhibitor. CHO cells transfected with a plasmid encoding human CatL were lysed in lysis buffer adjusted to pH 6.0 (100 mM sodium acetate, 1mM EDTA, 1% Triton X-100) (*A*) or with boiling sample buffer (50 mM Tris, pH 6.8, 1% SDS, 5% sucrose, 1% β-mercaptoethanol, 0.01% bromophenol blue) (*B*) and analyzed by SDS-PAGE and immunoblotting for CatL. Panels shown in each part are from the same blot and same exposure; gaps indicate where lanes were removed. (*C*) CHO B2 cells were mock-treated or treated with increasing concentrations of the cysteine protease inhibitor E64d (EST; Calbiochem) for 24 h before lysing and immunoblotting for CatL. $\alpha5\beta1$ integrin-expressing $\beta1$GD25 cells were analyzed in the same gel as a positive control for DC CatL expression.

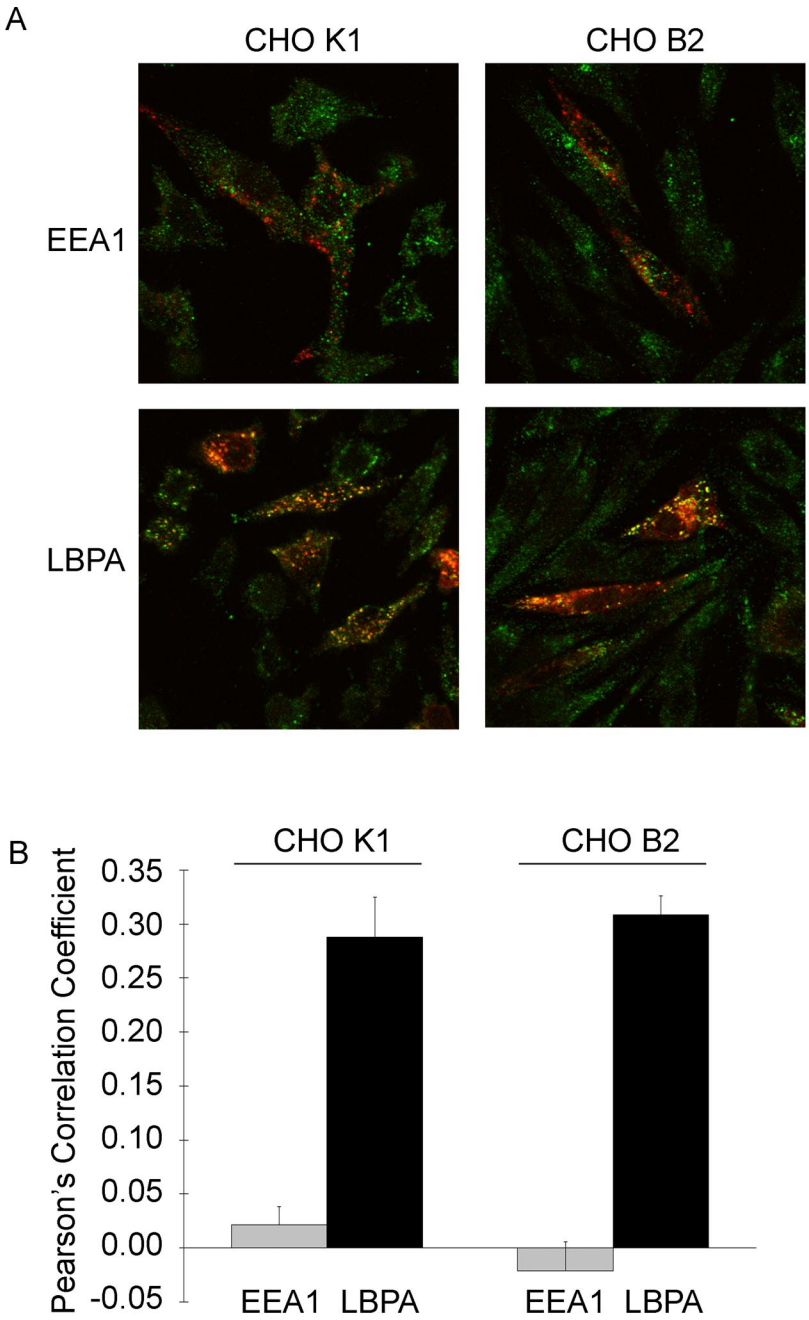

Fig. S4. CatL colocalizes with the late endosome marker LBPA in both the CHO K1 and CHO B2 cells. (*A*) CHO K1 and CHO B2 cells were transfected with a plasmid encoding human CatL and stained with Abs specific for CatL (red) and the early endosome marker EEA1 (green) or the late endosome marker LBPA (green) to determine the degree of colocalization (yellow). (*B*) Ten representative fields for each condition were quantified and degree of colocalization of CatL with EEA1 or LBPA was determined using the Pearson's correlation coefficient. Error bars indicate SEM.

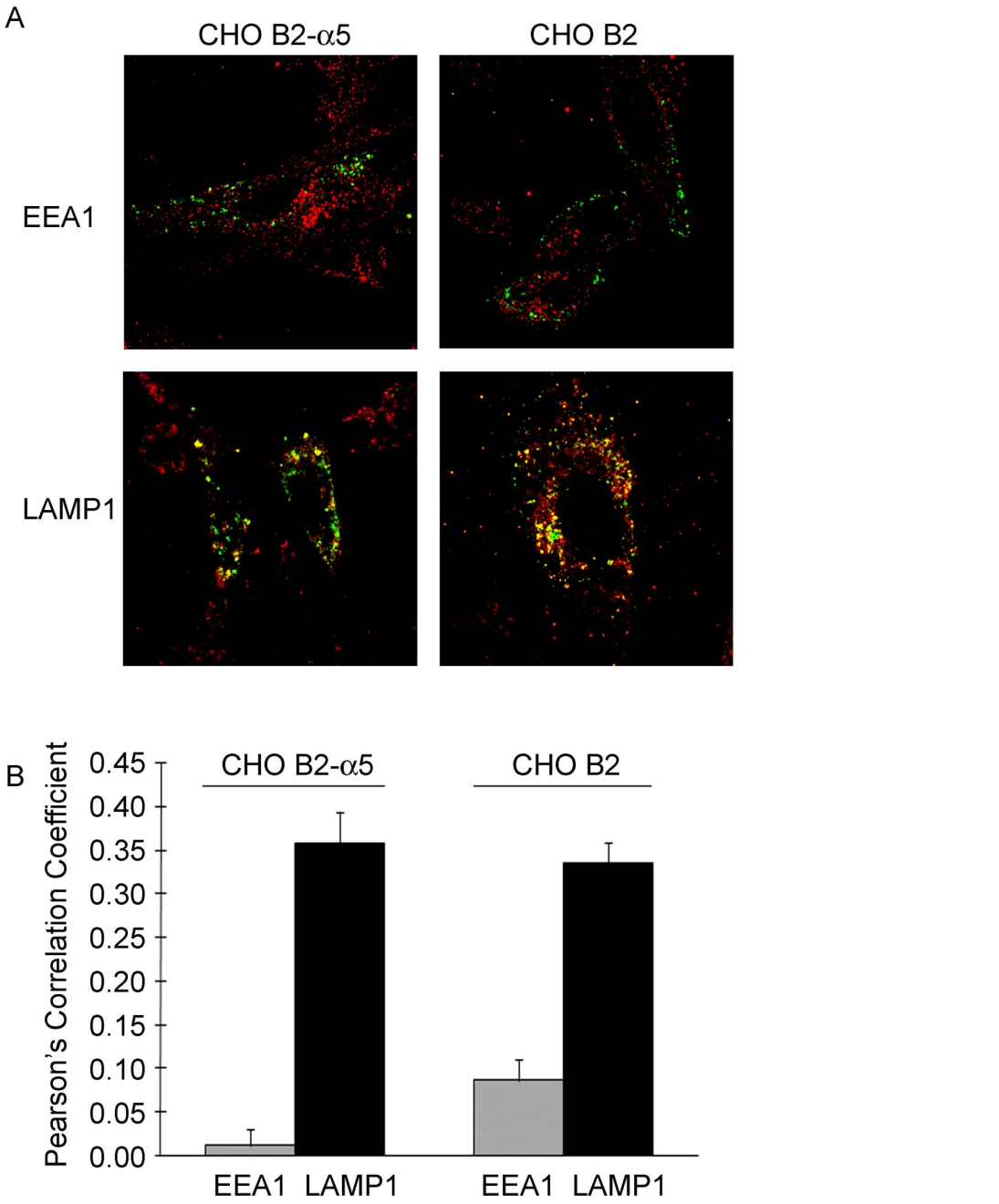

Fig. S5. CatL colocalizes with the lysosome marker LAMP1 in both the CHO B2-α5 and CHO B2 cells. (*A*) CHO B2-α5 and CHO B2 cells were transfected with a plasmid encoding human CatL and stained with Abs specific for CatL (green) and the early endosome marker EEA1 (red) or the lysosome marker LAMP1 (red) to determine the degree of colocalization (yellow). (*B*) Ten representative fields for each condition were quantified and degree of colocalization of CatL with EEA1 or LAMP1 was determined using the Pearson's correlation coefficient. Error bars indicate SEM.

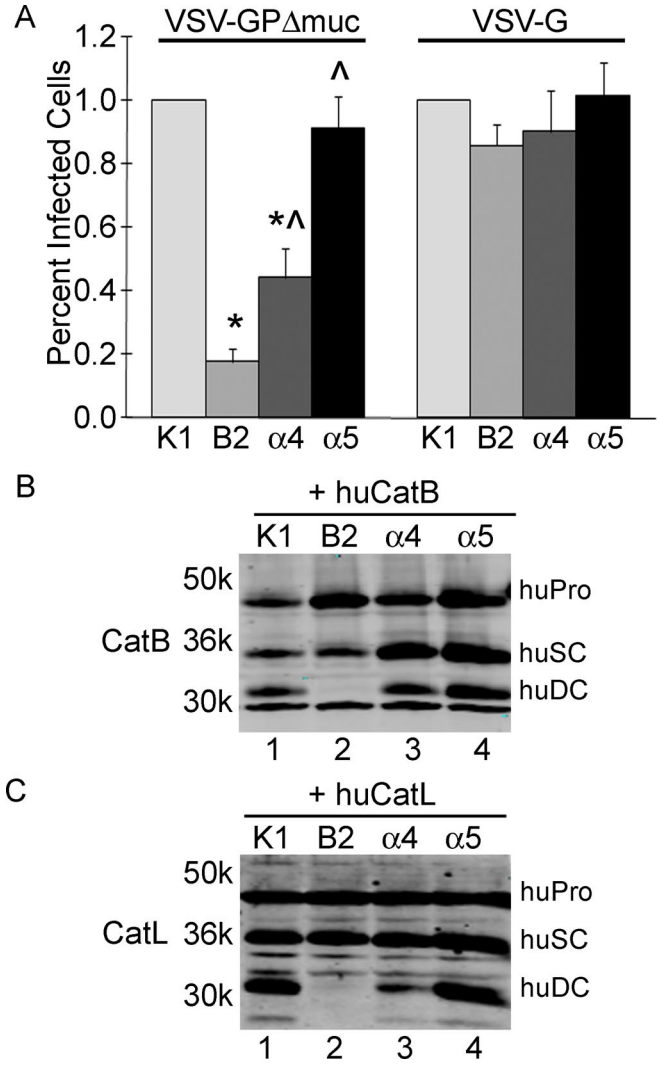

Fig. S6. Expression of $\alpha4\beta1$ partially rescues VSV-GP infection and DC CatB and CatL expression in CHO B2 cells. (*A*) CHO K1, CHO B2, CHO B2-α4 (C. Wu, University of Pittsburgh, Pittsburgh, PA), and CHO B2-α5 cells were infected with VSV-GPΔmuc or VSV-G and the percentage of infected cells was measured by flow cytometry. Results shown are the averages of normalized data from 10 experiments, including 3 experiments that are also included in the data shown in Fig. 1*B*. The average percent infection in the CHO K1 cells was 43% for VSV-GPΔmuc and 53% for VSV-G. Error bars indicate SEM. *, $P \leq 0.0002$ relative to CHO K1 cells. $\wedge$, $P \leq 0.02$ relative to CHO B2 cells. (*B* and *C*) CHO cells transfected with human CatB (*B*) or human CatL (*C*) were lysed and analyzed by immunoblotting for CatB or CatL as indicated. Blot shown in *C* is the same as shown in Fig. 4*C*, but with the lane containing the CHO B2-α4 cell lysate included (this lane was removed in Fig. 4). huPro, huSC, and huDC indicate the proform, SC form and DC form of overexpressed human CatB and CatL.