Supporting Information

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SI Materials and Methods

Cell Lines. The NCI-60 panel of tumor lines was purchased from NCI Developmental Therapeutics Program. This panel of cells as well as the Huh-7 cells (JCRB0403) were maintained in RPMI (Gibco BRL) containing 5% FBS (HyClone) with 1% penicillin/streptomycin at 37 °C and 5% CO_2. HEK 293T cells and derivative lines, M2 and H3, as well as Vero cells were maintained in DMEM (Gibco BRL) containing 10% FBS and 1% penicillin/streptomycin. Airway epithelia were isolated from human or porcine trachea or bronchi and grown at the air-liquid interface as described previously (1). All polarized airway epithelial cells used were well differentiated (>2 wk old; resistance >1,000 Ohm × cm^2) into ciliated, nonciliated, basal, and goblet cells. Procedures used for procurement of human airway tissue have been approved by the Institutional Review Board of the University of Iowa.

Production of Pseudovirions. FIV and VSV pseudovirions were produced as previously described (2–4). Briefly, VSVΔG-eGFP virions bearing either EBOV glycoprotein or the G glycoprotein were produced in HEK 293T cells by transfection of the glycoprotein-expressing plasmid followed 24 h later with VSVΔG-eGFP virion transduction. Newly pseudotyped virions were collected for 48–72 h and supernatant was filtered through a 0.45 microfilter. Pseudovirion titers were assessed on Vero cells.

FIV pseudovirions were produced by transfection of three plasmids into HEK 293T cells that have been described previously (4). The three plasmids included a psi-sequence containing FIV that expressed β-gal, a plasmid bearing the FIV gag and pol genes, and a plasmid expressing a viral glycoprotein gene (EBOV GP, Marburgvirus GP, VSV-G or Lassa fever virus GP). Supernatant containing virus was collected at 24, 48, and 72 h posttransfection, filtered, and concentrated 200-fold by 16-h centrifugation at 7,000 × *g* in a Sorval GSA rotor. Transduction was determined by either staining for β-gal activity using X-Gal or the Galacto-Light (Applied Biosystems) detection kit as per manufacturer's instructions.

Production of Recombinant Vesicular Stomatitis Virus. Recombinant, replication-competent VSV was produced as previously described (5). Briefly, the EBOV glycoprotein was cloned into the genome of a recombinant VSV (Indiana). The recombinant genome contained the eGFP gene in place of VSV-G. EBOV GP was cloned in front of eGFP. Infectious virus was generated as described (5). All virus stocks were titered in highly permissive Vero cells 4 d following infection. Infectious titers were ~5×10^{10} infectious units/mL.

Production of Biologically Cloned TIM-1–Expressing HEK 293T Cells. HEK 293Ts were transfected with TIM-1 using a PEI transfection protocol. Cell populations that expressed TIM-1 were enriched by incubating cells with TIM-1 monoclonal antibody ARD5 followed by μMACS Protein G MicroBeads (Miltenyi Biotec) as per manufacturer instructions. Selected cells were grown to confluence and enriched again for TIM-1 expression. This process continued until the population was roughly 20% TIM-1^+. The cells were stained with ARD5 primary antibody and a FITC-conjugated anti-mouse secondary antibody. Single cells were then sorted into 96-well plates using a FACS Aria at the University of Iowa Flow Cytometry Facility. High, medium, and low TIM-1–expressing populations, as well as TIM-1^{null} clonal populations, were obtained.

Flow Cytometric Analysis of TIM-1 Surface Expression. Live cells (8×10^5) were lifted from plates with 5 mM EDTA in PBS, washed to remove all EDTA, and incubated with either (*i*) 0.8 μg/mL of polyclonal goat anti-human TIM-1 antisera (R&D Systems) or normal goat sera or (*ii*) 5 μg/mL of ARD5 mAb or an IgG2a isotype control for 2 h at 4 °C in PBS containing 5% FCS. Cells were washed and incubated with donkey α-goat or α-mouse Cy5 conjugated secondary (Jackson ImmunoResearch) for 30 min on ice. Cells were washed extensively, and TIM-1 expression was evaluated using flow cytometry.

siRNA-Mediated Knockdown of TIM-1. Vero cells or Caco-2 cells (2×10^5) were seeded in a six-well format overnight. Before transfection, media was replaced with serum-free DMEM for 1 h. Cells were transfected with a 100 nM solution of *TIM-1* siRNA (Santa Cruz Biotech) or firefly *luciferase* siRNA (Ambion) using Lipofectamine 2000 (Invitrogen). FITC-conjugated siRNA was included as a transfection control. Cells were lifted and distributed into 48-well plates 24 h following transfection. TIM-1 silencing was verified 48 h following transfection by immunostaining the cells for surface expression of TIM-1. RNAi-treated cells were transduced with EBOV GP pseudotyped VSV or VSV-G pseudotyped VSV at an MOI of 0.01. Data are presented as number of eGFP$^+$ cells in the indicated experimental wells divided by the number of eGFP$^+$ cells in the control wells.

Ability of Ectopic TIM-1 Expression to Enhance EBOV Pseudovirion Transduction/Infection. HEK 293T or NCI-H522 cells (4×10^5) were seeded in a six-well format. Human TIM-1 and TIM-3 (Origene) expression plasmids were transfected into cells using Lipofectamine 2000 (Invitrogen). Cells were lifted and plated into a 48-well plate at 24 h following transfection. TIM-1 surface expression was determined by flow cytometry as described above. TIM-3 expression was verified using 0.8 μg/mL of goat α-human TIM-3 (R&D Systems). For the HEK 293T studies, control, TIM-1–, and TIM-3–expressing cell lines were transduced with FIV/β-gal pseudotyped with either EBOV GP or VSV G at an MOI of 0.01. Transduction was determined 24 h later by analyzing β-gal expression by Galacto-Light. The ability of ARD5 mAb to block transduction was performed as described above. For the NCI-H522 studies, transfected cells were infected with serial 10-fold dilutions of infectious EBOV GP VSV for 1, 3, or 5 d. Infected cells were lifted from the plates, washed, fixed in 3.7% formaldehyde, and evaluated for eGFP positivity by flow cytometry.

Generation and Purification of Soluble EBOV GP-His Trimers and Thermolysin Processing. EBOV GPΔO/pcDNA3.1 was modified to express EBOV GP1/GP2ΔTM fused to a T4 phage foldon sequence, followed by a thrombin cleavage site and 6× His tag, using standard cloning techniques (6). HEK 293T cells were transfected with the construct and 24 h later washed twice with PBS. The media was then replaced with Opti-MEM (Invitrogen). Supernatant was collected through 72 h following transfection and concentrated by ammonium sulfate precipitation followed by filtering through a 150,000 molecular weight cutoff (MWCO) protein concentrator (89921; Thermo Scientific). Concentrated, purified trimer was bound to HisPur Ni-NTA resin (88221; Thermo Scientific) and eluted with imidazole. For purification of processed soluble EBOV GP, thermolysin (0.50 mg/mL) was added to trimerized EBOV GP bound to HisPur NI-NTA resin for 30 min at 37 °C in thermolysin buffer [40 mM Hepes + 40 mM Mes + 50 mM NaCl + 0.1 mM $CaCl_2$ (pH 7.5)]. Beads were

washed, and processed trimer was eluted according to the manufacturer (Thermo Scientific). Imidazole was removed using a 7,000 MWCO Zeba desalting column (89882; Thermo Scientific). Concentration of EBOV GP was determined by measuring the A_{280} with a NanoDrop (Thermo Scientific).

EBOV GP-His Trimer Binding to Cells. Equimolar amounts of trimerized, unprocessed, or thermolysin-processed wild-type EBOV GP or K114A/K115A mutant GP were bound to TIM-1$^+$ H3 cells in binding buffer (PBS + 200 mM NaCl + 1 mM EGTA) at 4 °C for 2 h. Unbound protein was removed with three washes with cold binding buffer. Cells were lysed, and bound GP was determined via immunoblotting using rabbit anti-GP1 antisera.

Soluble Protein Competition Assay. A total of 5×10^5 H3 cells were transfected with 3 μg of an empty vector, soluble EBOV GP-His, or TIM-1/Fc expression plasmid. Transfected cells were seeded at 3×10^4 the next day and transduced with EBOV GP or LFV GP VSV pseudovirions 48 h after transfection at a MOI of 0.02. eGFP$^+$ cells were quantified with flow cytometry at 24 h following tranduction. Data are expressed as a percent of the empty vector control.

TIM-1/Fc Binding to EBOV GP1 and TIM-4–Expressing Cells. A total of 2.5 μg of purified TIM-1/Fc was incubated with HEK 293T cells transfected with an empty vector, EBOV GP, or TIM-4–expressing plasmids as previously described (7). Cells were lifted from the plate with 5 mM EDTA in PBS. TIM-1/Fc binding was determined with a Cy5-conjugated goat anti-human IgG (Jackson ImmunoResearch) and assayed by flow cytometry.

Immunohistochemistry and Confocal Microscopy. Well-differentiated human and porcine epithelial cell cultures were rinsed with PBS, fixed in 2% paraformaldehyde for 15 min, and rinsed three times with PBS (5 min each). The cells were incubated for 1 h with SuperBlock (Pierce) and 0.1% Triton X-100. Cells were again rinsed three times with 1× PBS for 5 min each time. Cells were incubated with 100 μL polyclonal goat anti–Tim-1 antibody (0.2 μg/mL; R&D Systems) or goat sera control diluted 1:50 in SuperBlock. Cells were again rinsed three times with PBS for 5 min each. Alexa 488 anti-goat secondary antibody (0.2 μg/mL in SuperBlock) was applied for 1 h at room temperature. Counterstains were anti-β tubulin Cy-3 cilia stain (0.5 μg/mL in PBS, applied for 45 min at room temperature) and To-Pro-3 nuclear stain (1 μM in PBS, applied for 10 min at room temperature). Images were captured with a Bio-Rad Radience 2100 multiphoton confocal microscope and analyzed using ImageJ software.

TIM-1 Antibody Inhibition of Binding and Entry. To test the ability of the anti–TIM-1 monoclonal antibodies to block EBOV pseudovirion binding, Vero cells were incubated with 0.5 μg/mL of each mAb for 1 h at 4 °C in a 96-well format. Unbound antibody was removed and cells were incubated with equivalent quantities of EBOV GP FIV for 2 h at 4 °C. Unbound virus was removed and cells were lysed in PBS + 1% SDS. Cell lysates were separated by SDS/PAGE, transferred to nitrocellulose, and immunoblotted for FIV capsid (FIV antisera kindly provided by Edward Hoover, Colorado State University, Fort Collins, CO) and monoclonal anti-human β-actin antibody (Abcam).

To test the ability of the antibodies to inhibit EBOV transduction, the indicated cell lines were plated at 4×10^4/well in a 48-well plate overnight. Cells were incubated with 0.5 μg/mL of the indicated mAb for 1 h at 4 °C. Cells were then shifted to 37 °C and transduced with EBOV GP pseudotyped VSV or VSV-G pseudotyped VSV at an MOI of 0.01. Transduced cells were assessed for eGFP expression at 24 h following transduction. Data are presented as the number of eGFP$^+$ cells in the presence of each mAb divided by the number of eGFP$^+$ cells in the isotype control.

ARD5 Inhibition of EBOV Infection. The infectious EBOV studies were performed at the University of Texas-Galveston BSL-4 facility. Cells were pretreated for 30 min with ARD5 or isotype control (0.5 μg/mL). The cells were then challenged with fivefold serial dilutions of either a replication competent EBOV or VSV (Indiana). Both viruses express eGFP upon infection. Virus stocks were removed and media containing either the mAb or isotype control was replaced for the duration of the study. At 28 h following infection, cells were photographed (epifluorescence image shown). EGFP-expressing cells were enumerated with ImageJ software using the analyze particles function. The proportion of cells infected within the linear portion of the virus titration was calculated relative to untreated cells and expressed as a percentage ± SD of EBOV GP-dependent transduction.

EBOV Transduction of Airway Epithelium. Airway epithelia were incubated with 0.5 μg/mL of ARD5 or isotype control for 30 min at room temperature. Treated cells were incubated with EBOV GP pseudotyped FIV/β-gal at an MOI of 5. Virus and antibody were removed 5 h later, and cells were incubated for three additional days. Virus entry was determined by assaying for β-gal activity with Galacto-Light (Applied Biosystems) per manufacturer's instructions.

Statistical Analyses. All differences between the two groups were analyzed by a two-tailed Student's *t* test.

1. Karp PH, et al. (2002) An in vitro model of differentiated human airway epithelia. Methods for establishing primary cultures. *Methods Mol Biol* 188:115–137.
2. Takada A, et al. (1997) A system for functional analysis of Ebola virus glycoprotein. *Proc Natl Acad Sci USA* 94:14764–14769.
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7. Meyers JH, et al. (2005) TIM-4 is the ligand for TIM-1, and the TIM-1–TIM-4 interaction regulates T cell proliferation. *Nat Immunol* 6:455–464.

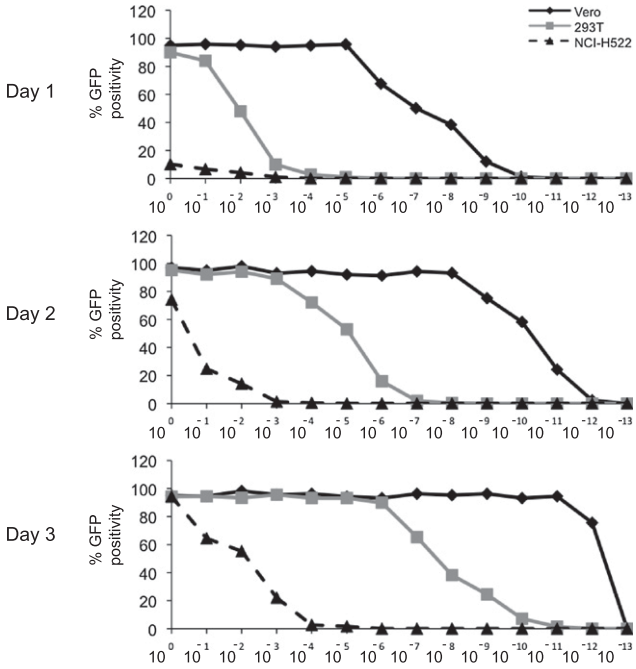

Fig. S1. EBOV permissivity of cell lines used in this study. Equal quantities of the indicated cell lines were plated and infected with 500 μL of 10-fold serial dilution of infectious EBOV GP ΔO VSV. Titers in Vero cells were the basis for the MOIs provided in the other figure legends. Infection was evaluated at days noted by detection of eGFP by flow cytometry.

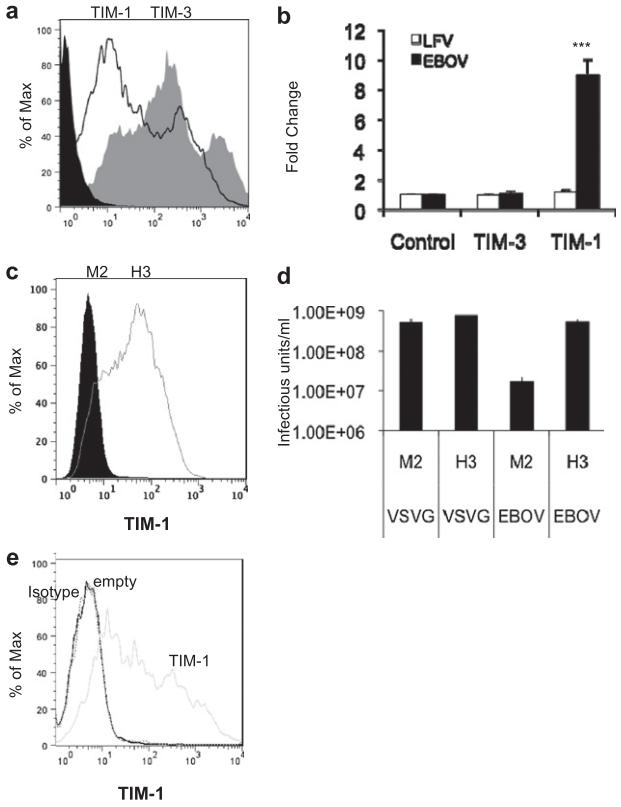

Fig. S2. TIM-1 specifically enhances EBOV entry. (*A* and *B*) Ectopic expression of TIM-1 enhances EBOV transduction. (*A*) Cell-surface protein expression at 48 h following transfection. HEK 293T cells were transfected with an empty plasmid (filled black histogram) or a plasmid expressing hTIM-1 (unfilled black histogram) or hTIM-3 (gray histogram). Cells were immunostained for TIM-1 or TIM-3 expression. (*B*) Transduction of the transfected cells by reverse transcriptase normalized EBOV GP (filled bars) or Lassa fever virus GP (unfilled bars) pseudotyped FIV at 48 h following transduction. Data are presented as fold increase in entry over cells transfected with empty vector. Transduction was evaluated by β-gal activity. (*C*) Detection of TIM-1 expression in biologically cloned HEK 293T lines. TIM-1^{-} M2 are shown in black filled histogram and TIM-1^{+} H3 are shown as gray line. (*D*) Infectious titers of VSV (Indiana) or recombinant EBOV GP ΔO VSV in M2 or H3 cells. (*E*) Expression of cell surface TIM-1 following transient transfection of NCI-H522 cells. Cells were transfected with an empty plasmid or a TIM-1–expressing plasmid and immunostained with either isotype control mAb or mouse α-human TIM-1 mAb ARD5 48 h later. Histograms show cells transfected with empty vector immunostained with ARD5 (solid black line); cells transfected with TIM-1 immunostained with isotype control (dotted gray line); and cells transfected with TIM-1 and immunostained with ARD5 (light gray solid line). About 55% of the cells transfected with the TIM-1 plasmid are expressing surface TIM-1. ***$P < 0.0001$.

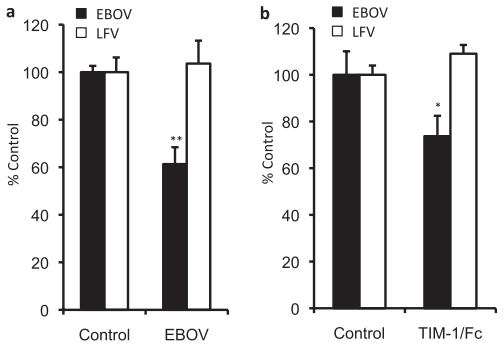

Fig. S3. Soluble, trimerized EBOV GP and TIM-1/Fc inhibit EBOV GP VSV transduction. (*A*) H3 cells transfected with an empty vector (control) or soluble EBOV GP-His. (*B*) H3 cells transfected with an empty vector (control) or soluble TIM-1/Fc. Cells were transduced with the indicated pseudovirions 48 h following transfection (MOI = 0.02). EGFP^{+} cells were analyzed by flow cytometry. Data are expressed as a percent of the empty vector control. *$P < 0.05$, **$P < 0.001$.

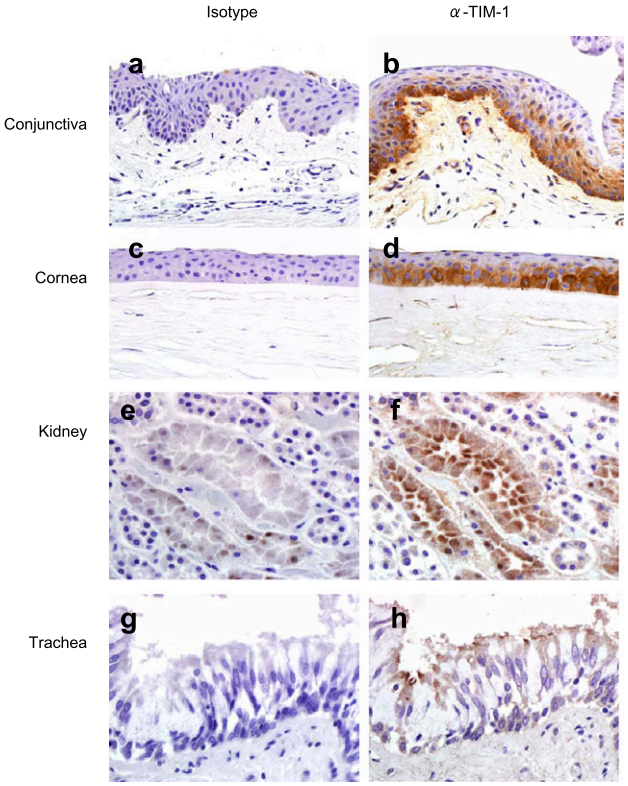

Fig. S4. TIM-1 is expressed on epithelium of tissues relevant to EBOV entry and pathogenesis. Formalin-fixed, paraffin-embedded primary human tissues. (*A* and *B*) Conjunctiva, (*C* and *D*) cornea, (*E* and *F*) kidney, and (*G* and *H*) trachea were immunostained with equivalent concentrations of either an isotype control antibody (*A*, *C*, *E*, and *G*) or TIM-1 monoclonal antibody clone 219211 (*B*, *D*, *F*, and *H*; R&D Systems) followed by application of a commercial secondary reagent kit (Dako Envision Mouse HRP). TIM-1 expression on kidney tubular epithelium is well established and serves as a positive control.

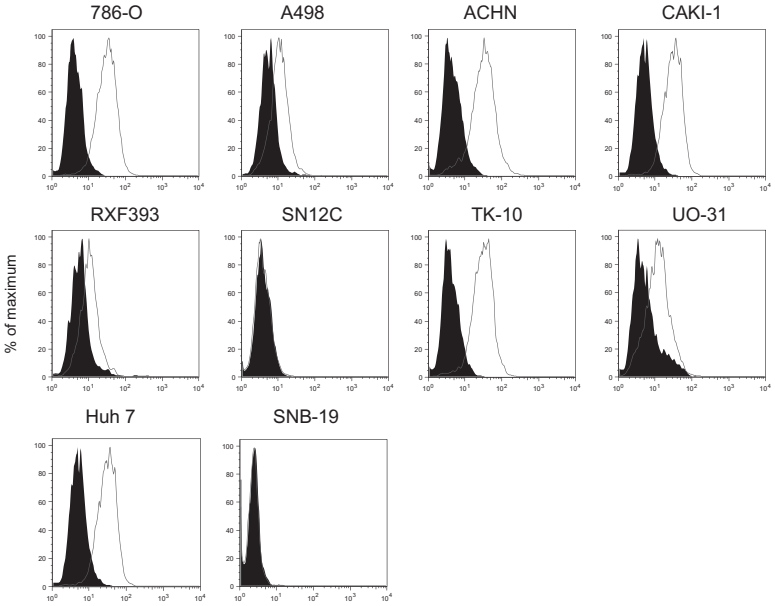

Fig. S5. TIM-1 surface expression on cell lines evaluated for the role of TIM-1 in EBOV infection. The indicated cell lines were incubated with 0.2 μg/mL of goat anti–TIM-1 antisera or normal goat sera for 3 h on ice, followed by incubation with Cy5-conjugated rabbit anti-goat antisera. Filled histograms represent isotype control staining, and the unfilled histograms indicate TIM-1 antibody-treated populations.

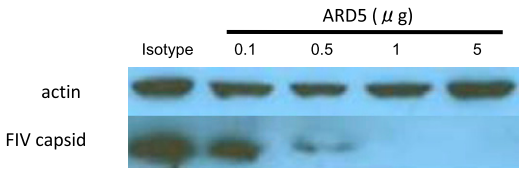

Fig. S6. ARD5 inhibition of EBOV binding to Vero cells is dose dependent. Vero cells were incubated with increasing amounts of ARD5 for 1 h at 4 °C. Cells were then incubated with equal quantities of FIV pseudotyped with EBOV GP ΔO for an additional hour at 4 °C. Unbound virus was removed by washing extensively, and cell lysates were probed for FIV capsid and β-actin by immunoblotting.

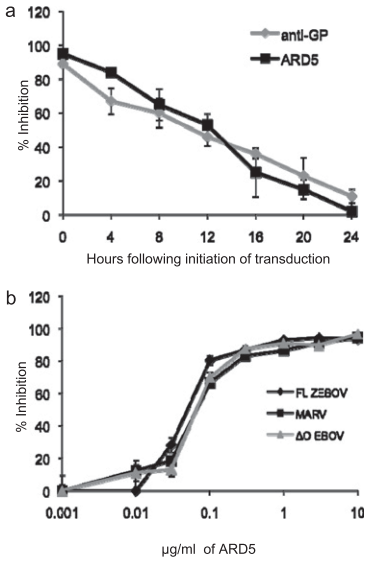

Fig. S7. ARD5 inhibits early steps of EBOV transduction and full-length EBOV and MARV pseudovirion transduction into Vero cells. (*A*) Vero cells were seeded in a 48-well plate. At the initiation of the experiment, cells were transduced with EBOV GP ΔO VSV pseudovirions (MOI = 0.05). At the time points noted, 0.5 μg/mL of ARD5 or 0.1 μg/mL of goat anti-EBOV GP1 antisera was added. Equivalent quantities of either normal goat sera or mouse IgG2a were used as control treatments. Transduction was evaluated 24 h later by eGFP expression. Shown is the ability of the antibody or antisera to inhibit EBOV transduction relative to controls. (*B*) Vero cells were incubated with the indicated amount of ARD5 for 1 h at 4 °C. Treated cells were transduced with VSV pseudotyped with the indicated viral glycoproteins (MOI = 0.05). Transduction was evaluated 24 h later by eGFP expression. Full-length EBOV (diamond), EBOVΔO (triangle), and full-length MARV glycoprotein (square) transduction levels are shown.

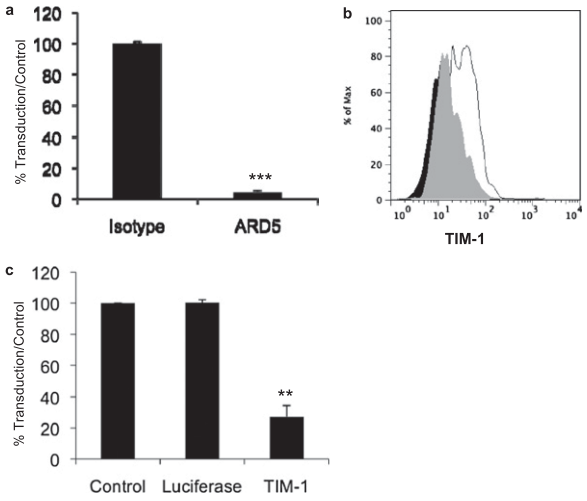

Fig. S8. TIM-1 expression is required for optimal EBOV transduction of the gastrointestinal tract cell line Caco-2. (*A*) Equal numbers of Caco-2 cells were preincubated for 1 h at 4 °C with either ARD5 or isotype control (0.5 μg/mL). Cells were shifted to 37 °C and transduced with VSV pseudotyped with either EBOV GP ΔO or VSV G. Transduction was evaluated 24 h later by eGFP expression. (*B*) Surface expression of TIM-1 on Caco-2 cells transfected with a 100 nM of either *luciferase* or *TIM-1* siRNA as assessed at 48 h by flow cytometry. Filled black histogram represents *TIM-1* RNAi-transfected cells incubated with isotype control antibody; unfilled black histogram shows TIM-1 expression in *luciferase* siRNA-transfected cells and incubated with mouse anti–TIM-1 mAb ARD5; gray histogram shows TIM-1 expression in *TIM-1* siRNA-transfected cells incubated with anti–TIM-1 mAb. (*C*) Transduction of siRNA-treated Caco-2 cells. At 48 h following transfection, cells were transduced with VSV pseudotyped with EBOV GP ΔO. Transduction was evaluated 24 h later by eGFP expression. $^{**}P < 0.001$, $^{***}P < 0.0001$.