flow cytometry. (C) Dyn2 K44A inhibits transferrin uptake. Vero cells expressing a Dyn2-K44A-eGFP fusion protein were pulsed with 1 μg/mL Alexa-647-labeled transferrin for the indicated times and VLP uptake was assessed by flow cytometry. (D) Effect of Dyn2 K44A expression on uptake of EBOV VLPs. Representative maximal Z projections of cells transfected with eGFP or Dyn2-K44A-eGFP and exposed to RFP-EBOV VLPs at 37˚C for 30 min are shown. Scale bars, 5 μM (E) Endogenous dynamin-2 colocalizes with EBOV VLPs. Vero cells were exposed to the indicated ligands or eGFP/RFP-VLPs for 10 min at 37˚C, and then fixed and visualized by confocal fluorescence microscopy. Endogenous dynamin was detected by staining with anti-dynamin primary antibody and an Alexa 594-conjugated secondary antibody. Scale bars, 10 μM. (A-C) Means +/-SD of three replicates are shown.

Figure 8. Effects of macropinocytosis and dynamin inhibitors on EBOV GP-dependent infection in monocytes and dendritic cells. (A) Inhibitors of macropinocytosis and dynamin reduce VSV-GPΔMuc infection in the Jaws murine dendritic cell line. Jaws cells were pre-treated with 1% DMSO, 60 μM dynasore, 25 μM EIPA, 2.5 μM rottlerin, 0.01 μM latrunculin A, 5 μM ML9, or 50 μM blebbistatin and then exposed to VSV-GPΔMuc or VSV-G. Infection was scored by counting eGFP-positive cells. (B) Effects of dynasore and EIPA on infection by VSV-GP-FL and VSV-GPΔMuc in adherent PBMCs. CD14-positive PBMCs were pre-treated with 0.5% DMSO, 60 μM Dynasore, 25 μM EIPA, or 300 μM E-64d, and then exposed to virus. Infection was scored by counting eGFP-positive cells. (A-B) Means +/-SD of three replicates are shown.

Supplementary Figure 1. Inhibitors of macropinocytosis have similar effects on viral infection mediated by EBOV GP-FL and EBOV GPΔMuc. (A) Inhibitors of macropinocytosis reduce infection by both VSV-GP-FL and VSV-GPΔMuc. Vero cells were pre-treated with 1% DMSO, 20 mM ammonium chloride, 60 μM dynasore, 25 μM EIPA, 2.5 μM rottlerin, 0.01 μM latrunculin A, 5 μM ML9, or 50 μM blebbistatin, and then exposed to VSV-GP-FL, VSV-GPΔMuc, or VSV-G. Infection was scored by counting eGFP-positive cells. (B) Inhibitors of macropinocytosis reduce VLP entry mediated by both GP-FL and GPΔMuc. Vero cells pre-treated as above were exposed to βLaM-VLPs expressing EBOV GPΔMuc or GP-FL. VLP entry was assessed by flow cytometry. (A-B) Means +/-SD of three replicates are shown.