

Supplementary Figure 1. Additional sh.RNA cell line data. (A) Western blot analysis performed on sh.RNA cell lines to determine the relative knockdown of the indicated target genes following lentiviral transduction and puromycin selection. Pr TMEM16F, cells transfected with a plasmid encoding TMEM16F. (B) Functional assays demonstrating sh.RNA targeting TMEM16F reduces PtdSer translocation following the addition of ionophore A23187. Gate shown was used to compare expression levels. (**C**) shRNA-mediated knockdown of XKr8 does not reduce surface expression of PS following infection with EBOV. Images of cells transduced with lentiviral vectors encoding scrambled shRNA are provided in Figure 1A; confocal microscopy analyses of all cell lines were performed at the same time. Scale bar = $30 \,\mu$ m. (**D**) Flow cytometry analysis of annexin V staining in EBOV-infected cells. *** P<0.001, n.s: not significant (Student's t-test). Panels A – C, representative of 3 independent experiments, panel D, representative averages from triplicate samples of one of 5 independent experiments.



Supplementary Figure 2. Annexin V staining on EBOV-infected sh.RNA cell lines. (A) Annexin V staining on EBOV-infected sh.RNA cell lines. (B) Gating strategy used to determine viral infectivity and annexin V expression. Cells were gated away from debris based on FSC vs SSC. Plots for GFP versus SSC were used to determine the percentage of infected (GFP⁺) cells. For comparison of multiple samples in one plot, data was converted to histograms. Panels A, B are representative of one out of three independent experiments.



Supplementary Figure 3. **Development of virion surface protein detection assay.** (**A**-**D**) Examples of purified VLP characterization by imaging flow cytometry. (**A**) VLPs stained for GP: bright field (channel 1: BF), EBOV GP (channel 2: GP), viral membrane (channel 3: VM) and annexin V (channel 11); M, merged images based on GP, viral membrane and annexin V. (**B**-**D**) Histograms showing relative GP expression (**B**), viral membrane dye via staining with PKH26 (**C**) and annexin V (**D**).