www.sciencetranslationalmedicine.org/cgi/content/full/5/190/190ra79/DC1

Supplementary Materials for

FDA-Approved Selective Estrogen Receptor Modulators Inhibit Ebola Virus Infection

Lisa M. Johansen,* Jennifer M. Brannan, Sue E. Delos, Charles J. Shoemaker, Andrea Stossel, Calli Lear, Benjamin G. Hoffstrom, Lisa Evans DeWald, Kathryn L. Schornberg, Corinne Scully, Joseph Lehár, Lisa E. Hensley, Judith M. White, Gene G. Olinger*

*Corresponding author. E-mail: gene.olinger@us.army.mil (G.G.O.); ljohansen@zalicus.com (L.M.J.)

Published 19 June 2013, *Sci. Transl. Med.* **5**, 190ra79 (2013)
DOI: 10.1126/scitranslmed.3005471

The PDF file includes:

Supplemental Materials

Supplemental Figures and Legends

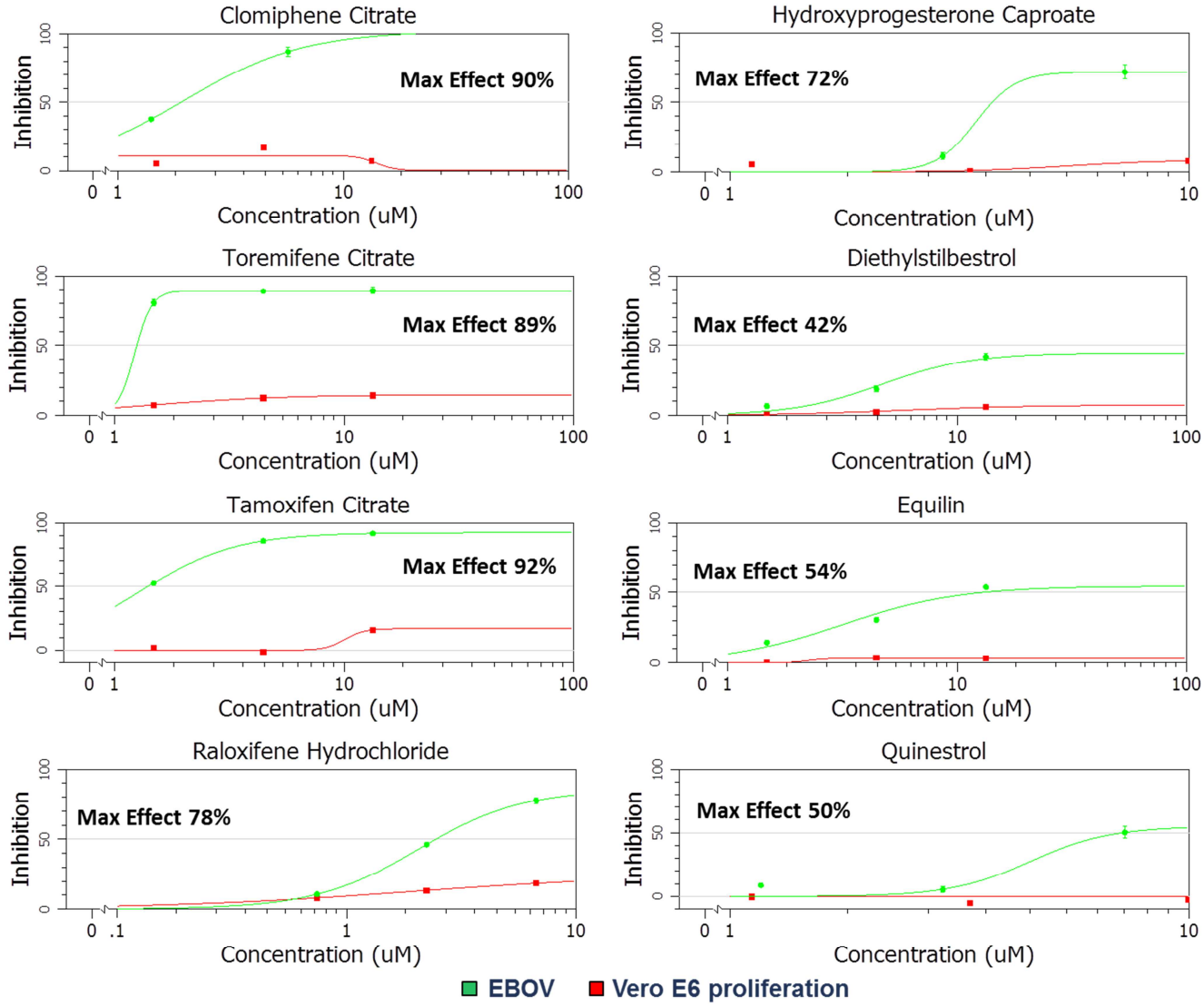

Fig S1. SERMS inhibit EBOV infection in vitro. ER antagonists and agonists that inhibit EBOV infection were identified from a screen of US and EX-US approved drugs and molecular probes using eGFP-EBOV engineered EBOV. The preliminary screen was performed using three dose points as shown. Compounds were scored as active if they showed %inhibition of eGFP-EBOV of >40% with no or minimal effect on cell proliferation. The percent inhibition that the compounds inhibit eGFP-EBOV is shown in green. The effect of the compounds on uninfected Vero E6 cell proliferation (a measure of toxicity) is shown in red. Indicated is the maximum % EBOV inhibition (Max Effect) observed for the compounds.

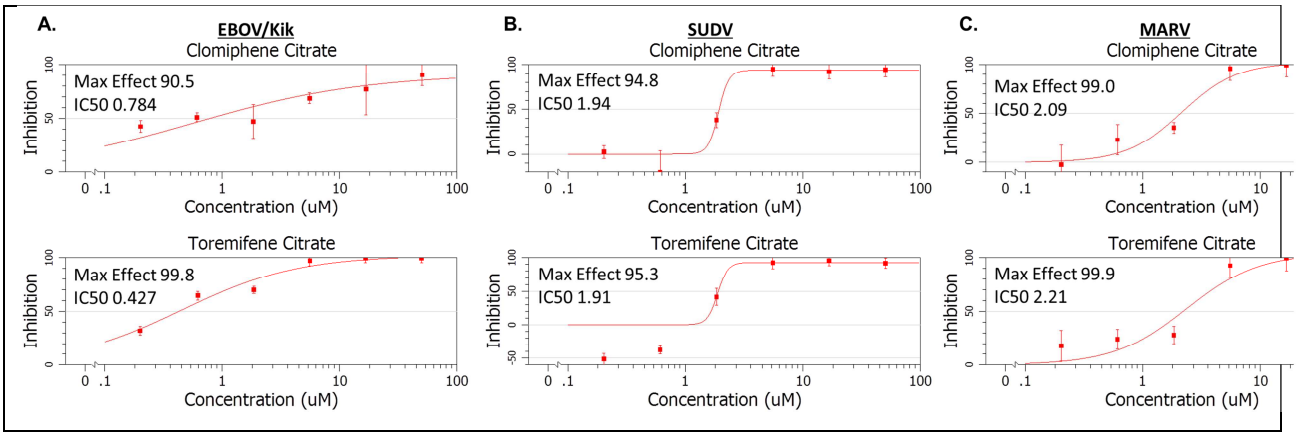

Fig S2. The activity of clomiphene and toremifene against native strains of filoviruses using qRT-PCR. Native isolates of **A)** EBOV/Kik, **B)** SUDV and **C)** MARV were used to infect Vero E6 cells and the number of genomic copies were determined by qRT-PCR. Results indicate that both clomiphene and toremifene strongly inhibit infection by these native isolates. Shown for each dose response is the Max Effect of the compound in % Inhibition (Max Effect) and the IC50 concentration in μM (IC50). Error bars are the SEM. Results are from two or more experiments with multiple internal replicates.

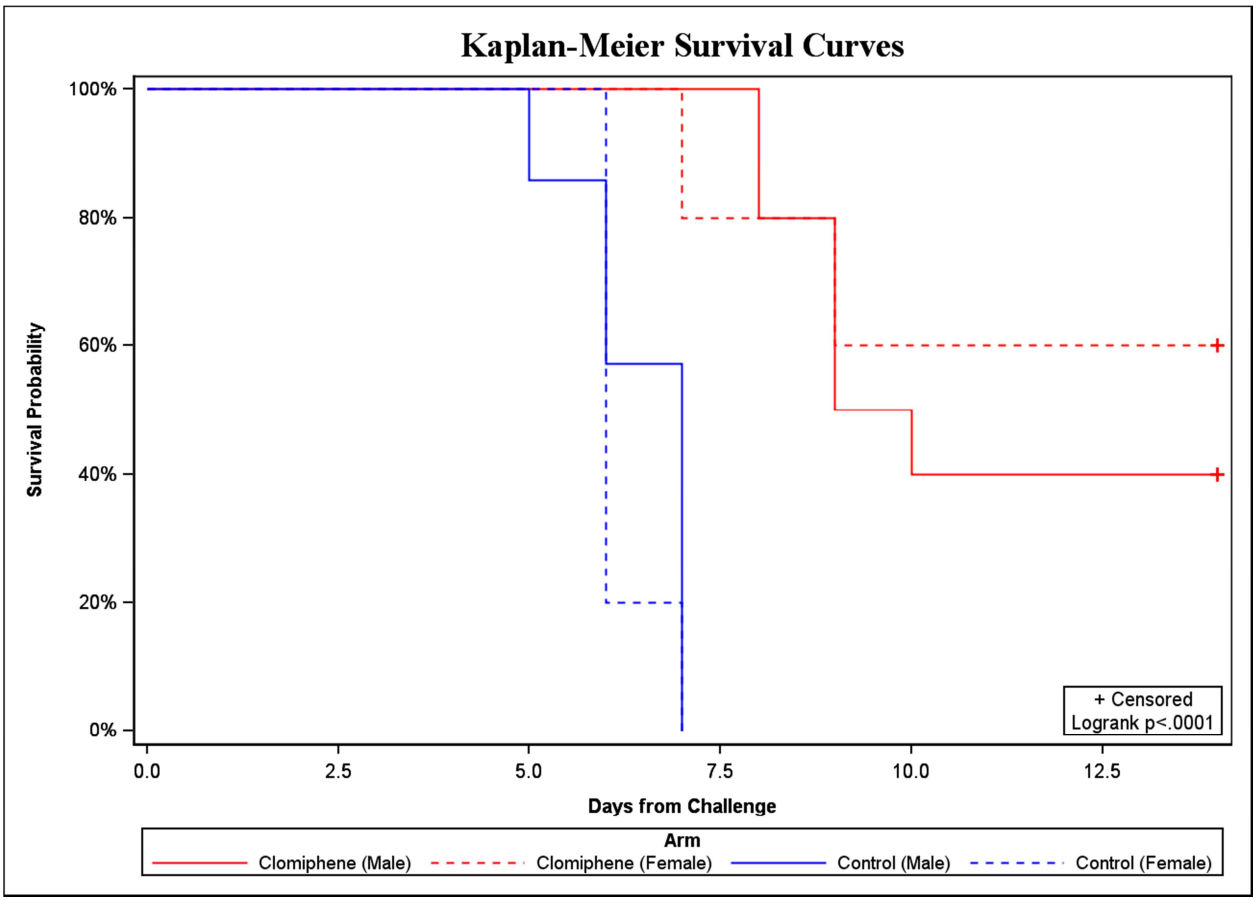

Fig S3. Kaplan-Meier survival curves for clomiphene in both male and female mice infected with a mouse-adapted Ebola virus. In this study, C57BL/6 mice male and female (5 to 8 weeks old) were exposed to a viral target dose of 1000 pfu. One hour after exposure mice were treated with 60 mg/kg clomiphene or vehicle as described in the Material and Methods for 10 days. Mice were dosed on days 0, 1, 3, 5, 7, and 9. The male vehicle control group (N=7) is shown as a solid blue line, the male clomiphene treated group (N=10) is shown as a solid red line. The female vehicle control group (N=5) is shown as a dashed blue line and the female clomiphene treated group is shown as a dashed red line (N=5). Kaplan-Meier curves were used to determine if there were statistically significant differences between the male and female treatment groups. The Kaplan-Meier curves were evaluated based on the log-rank test for overall homogeneity. Pairwise comparisons were also made using the log rank test with a post hoc Tukey-Kramer adjustment for multiple testing. Time to death was analyzed by analysis of variance comparing only those mice which succumbed prior to study end. A model fitting time to death using gender, treatment, and gender×treatment interaction was evaluated in order to determine if gender×treatment interaction had an effect on time to death. Due to small sample size,

differences in overall survival between groups were evaluated using Fisher's exact test. At study day 28, 40% of the male mice and 60% of the female clomiphene treated mice had survived. The male animals treated with clomiphene had a mean time to death of 8.83 days compared with 8 days for the female treated mice. The gender treatment interaction was not significant ($p=0.4425$) indicating no gender differences in response to clomiphene treatment. There was however, a highly significant difference between clomiphene and control with an estimated 2.1 day difference in time to death and a logrank $p< 0.0001$. The significance of the survival between clomiphene treated (male plus female) and control groups (male plus female) using the Fisher's exact test indicated a $p=0.0081$. Taken together these results indicate no gender differences in response to clomiphene treatment and a significant survival benefit with the treatment.

Overall, survival will vary by study, due to the dose of the virus exposure. Animals are given a target exposure dose of 1000 pfu where the actual dose of challenge varies study to study.

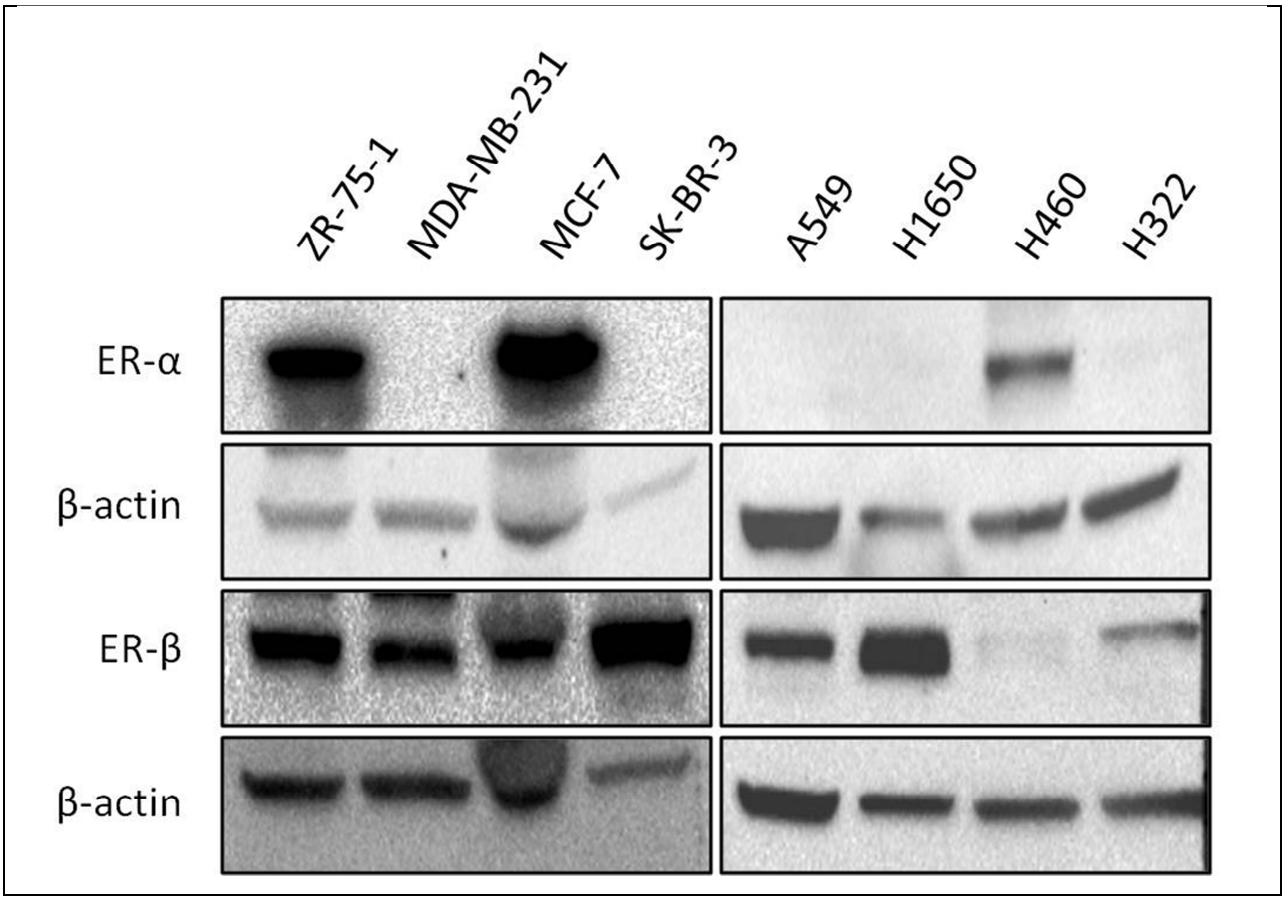

Fig S4. Western blot analysis of ER-α and ER-β expression in cell line panel. This cell line panel was used for infections highlighted in Fig 5. Results show the cell lines have varying expression of ER-α and ER-β. ZR-75-1and MCF-7 express both receptors. MDA-MD-231, SK-BR-3, A549, H1650 and H322 express ER-β only. The H460 cell line expresses ER-α only.

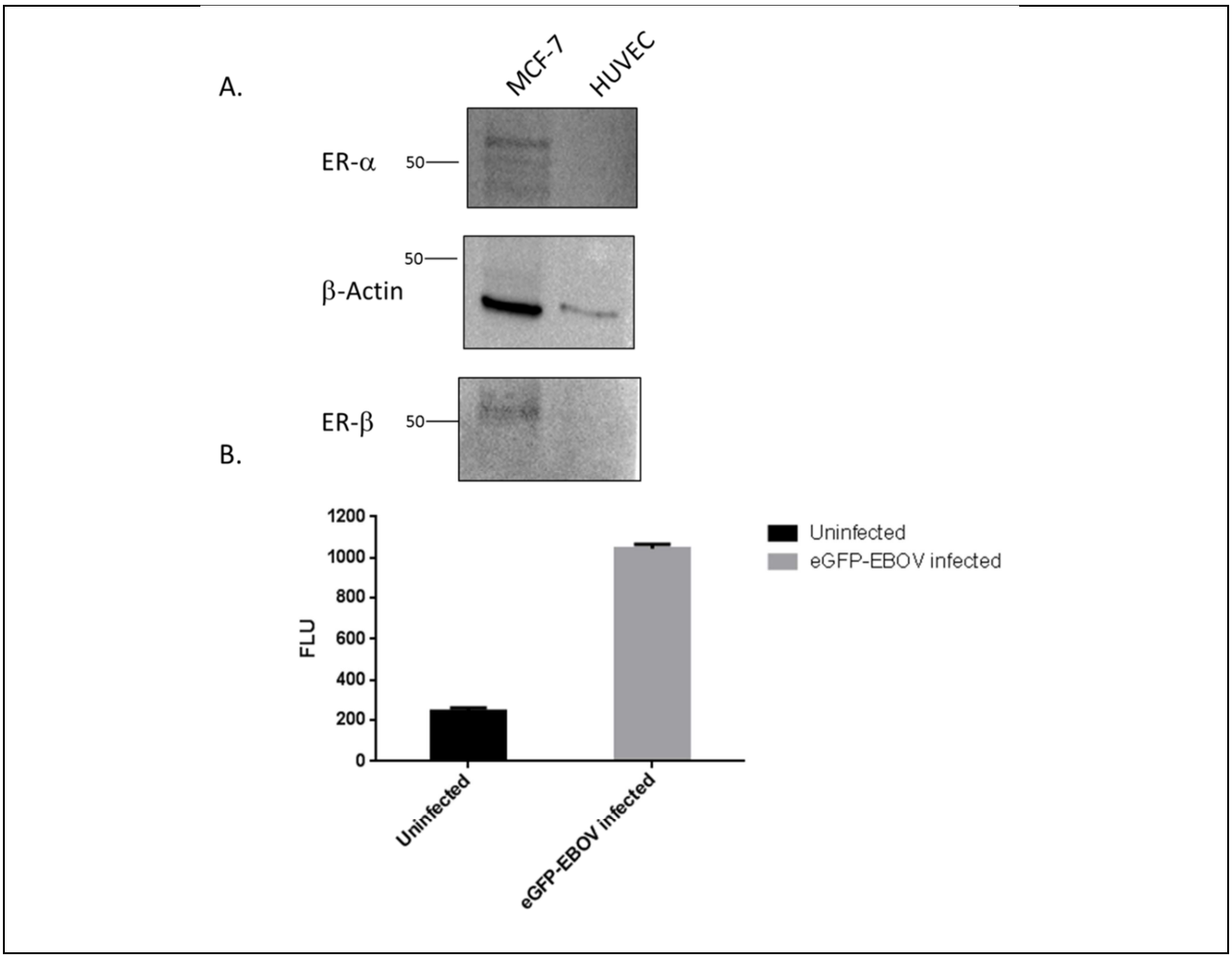

Fig S5. Activity of clomiphene and toremifene in HUVECs. **A)** Western blots showing that HUVEC cells do not express ER-α or ER-β. MCF-7 lysate was used as a positive control for ER-α and ER-β expression. **B)** Raw fluorescent counts (FLU) from HUVEC cells uninfected and infected with eGFP-EBOV indicated HUVEC cells can be infected. The infection conditions were the same conditions as used in Fig 5. Taken together, results indicate that eGFP-EBOV infection is not dependent on the expression of ER-α or ER-β.

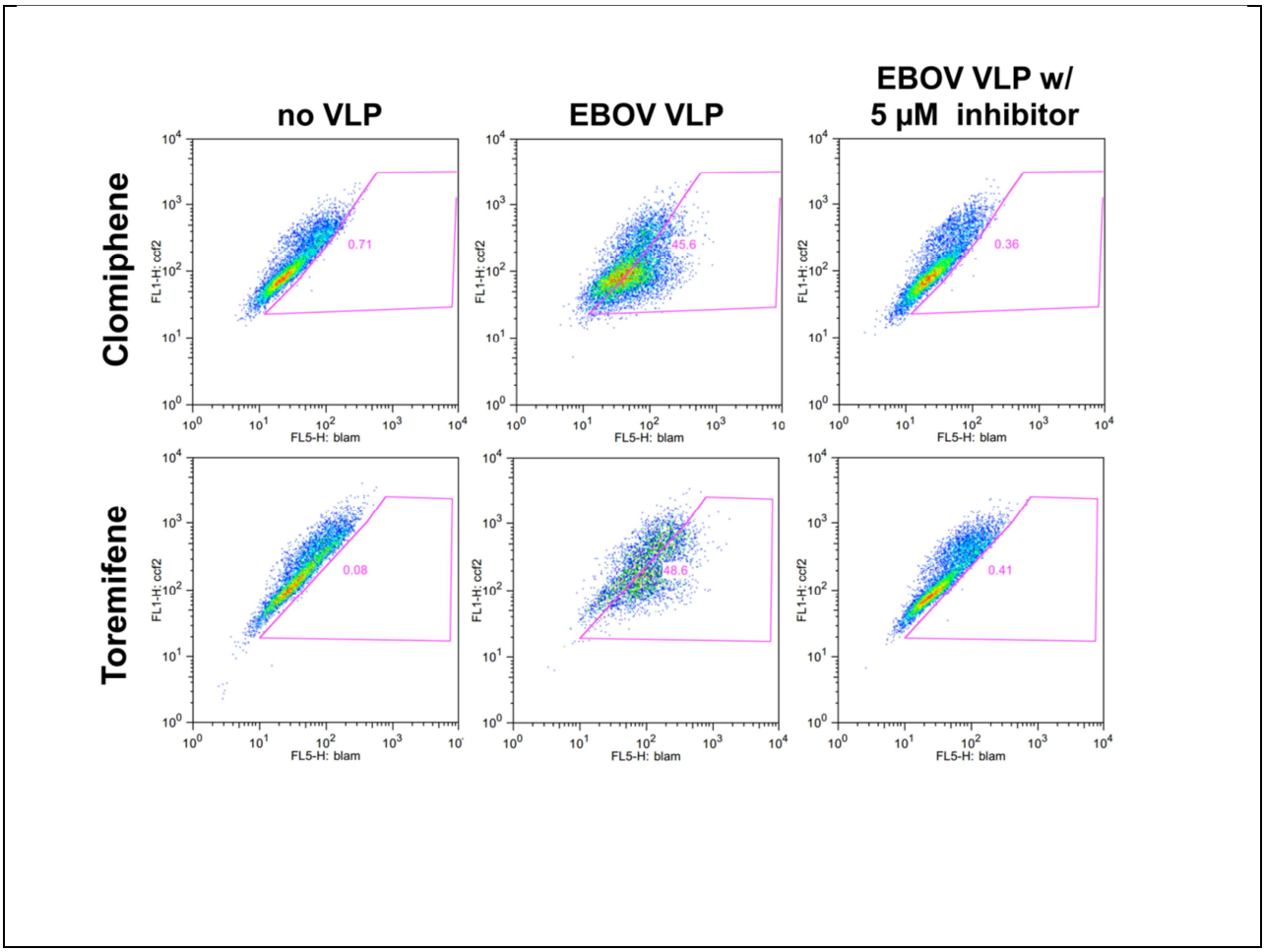

Fig S6. Representative flow cytometry plots from experiments conducted for Fig. 6. After gating for live SNB19 cells based on their forward vs. side scatter profiles, the fluorescent emissions of the cells in the FL1 (ccf2) and FL5 (blam) channels were plotted relative to each other. FL1 emission denotes degree of substrate (ccf2) loading by cells and FL5 emission denotes degree of substrate cleavage (by beta lactamase VP40). Examples of VLP-GP entry both with and without inhibitors are shown. A gate (magenta) was drawn based on the no VLP sample, and used to quantitate cells that exhibited a positive shift in the FL5 channel (signifying VLP entry in those cells). For each sample, between 5000-10,000 cells were collected. Three samples were analyzed for each condition. Data were analyzed and plots were generated using the FlowJo software package.

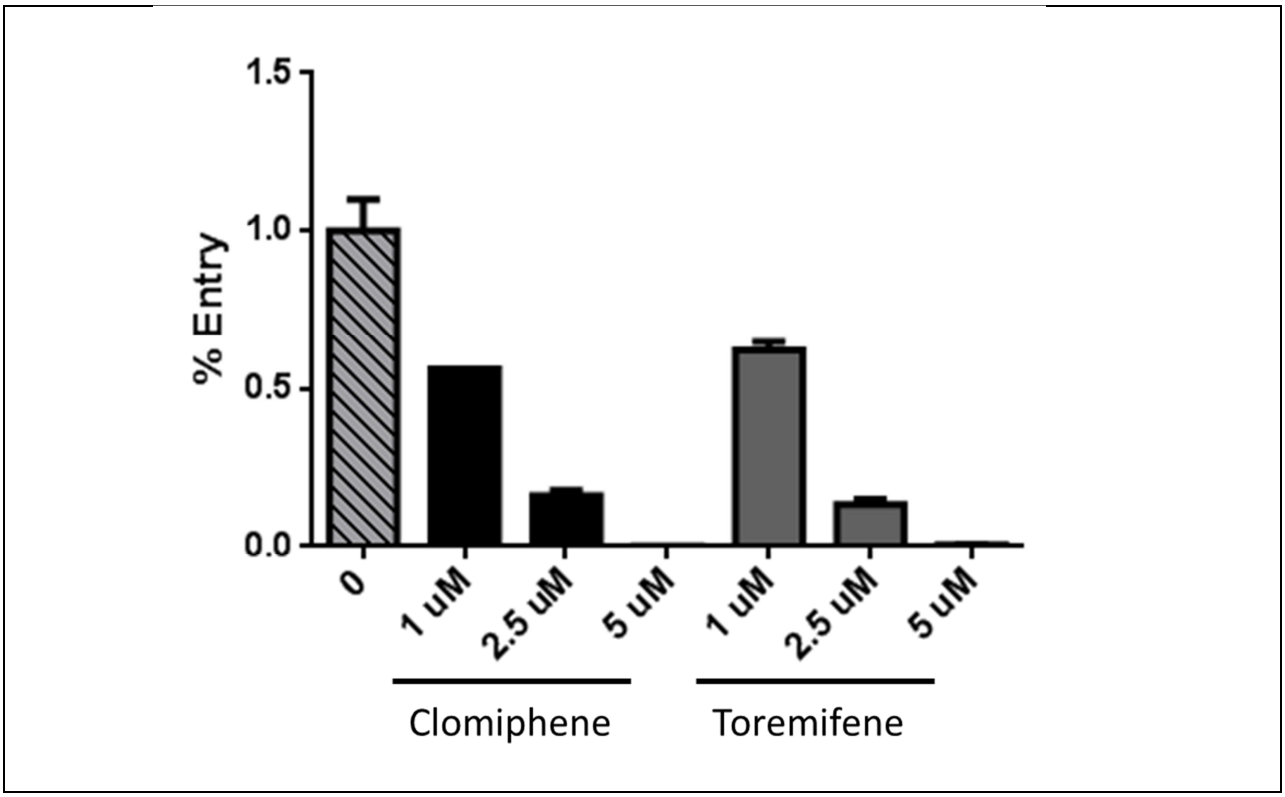

Fig S7. Effect of clomiphene and toremifene treatment on entry of VLPs bearing GP from SUDV. Results show that all of the concentrations evaluated for clomiphene and toremifene inhibited entry of VLPs containing the SUDV $GP_{1,2}$. The inhibition observed was similar to inhibition of EBOV VLP entry shown in Fig 6. Error bars represent the standard error for three replicates.

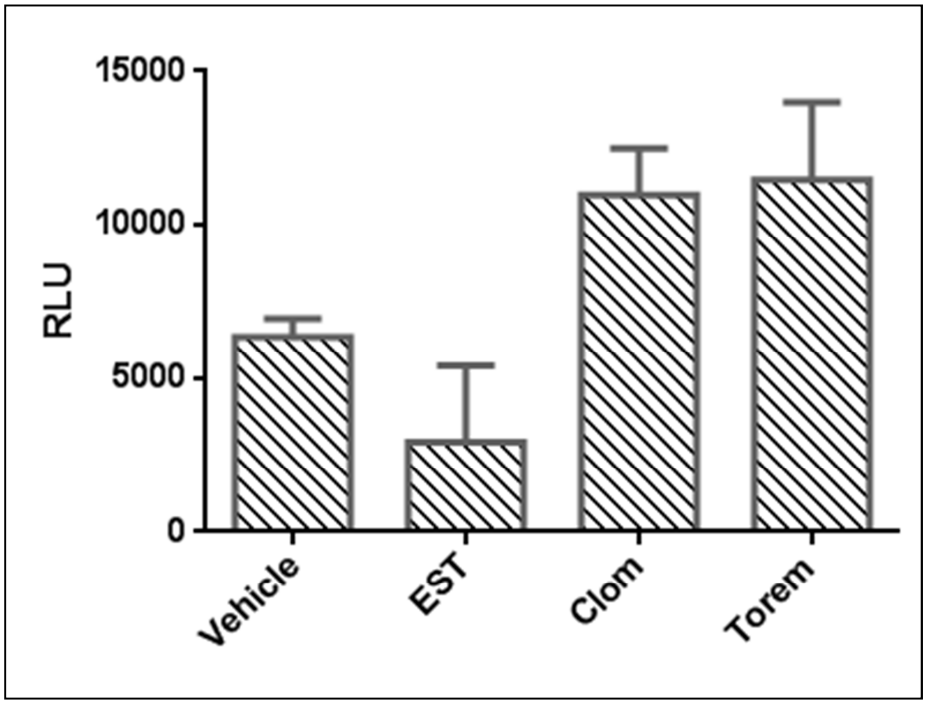

Fig S8. Cathepsin L processing at 18 hours. Clomiphene (5 μM) and toremifene (0.8 μM) were evaluated, as described in the Materials and Methods section, for their effects on cathepsin B (CatB) and cathepsin L (CatL) activity (singly and combined) in SNB19 cells. EST is a cysteine protease inhibitor that was included as a positive control for the assay. Data in Fig 7 show a 1.5 h time point. This graph shows the results for CatL at 18 h (because the raw signals for cathepsin L were low at the 1.5 h time point). Results indicate that clomiphene and toremifene do not inhibit Cat-L activity.