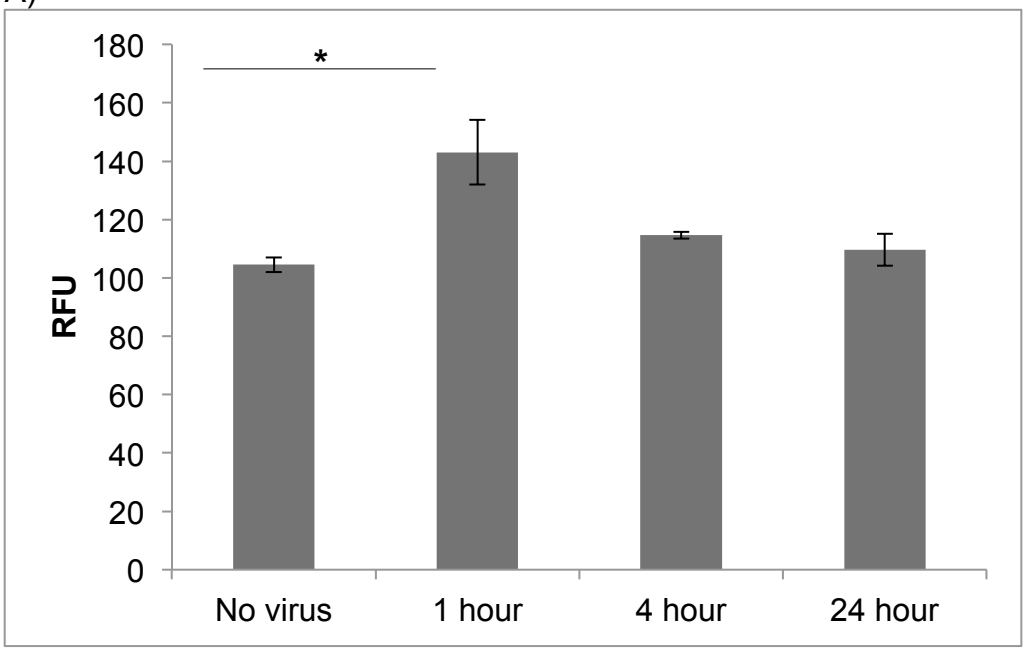
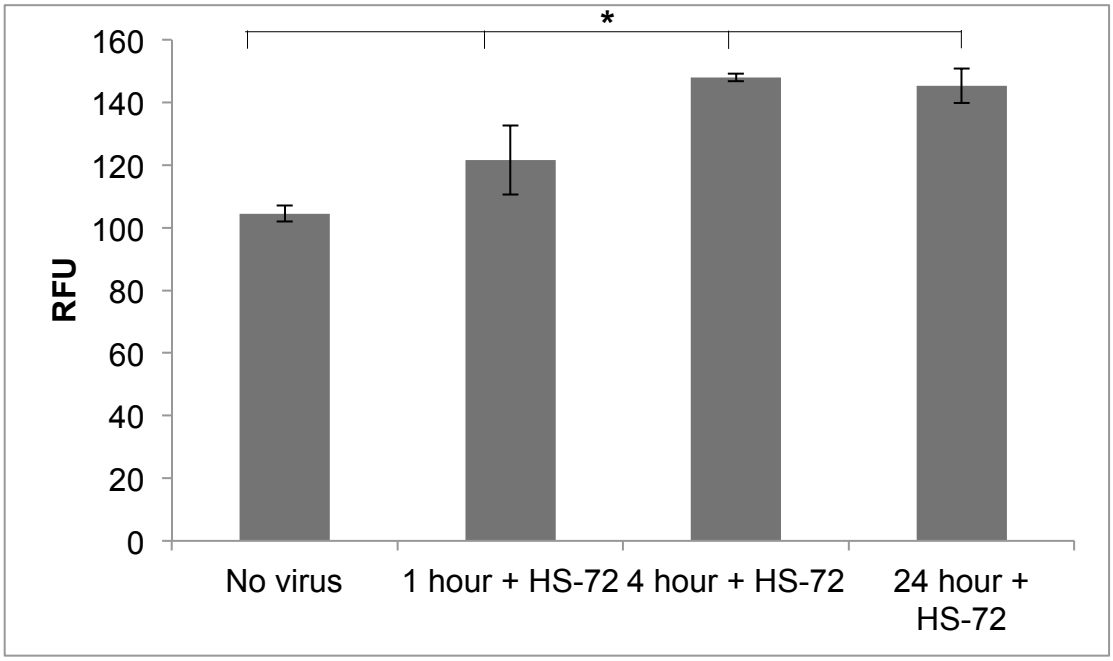
S4 Fig. related to Fig. 4.

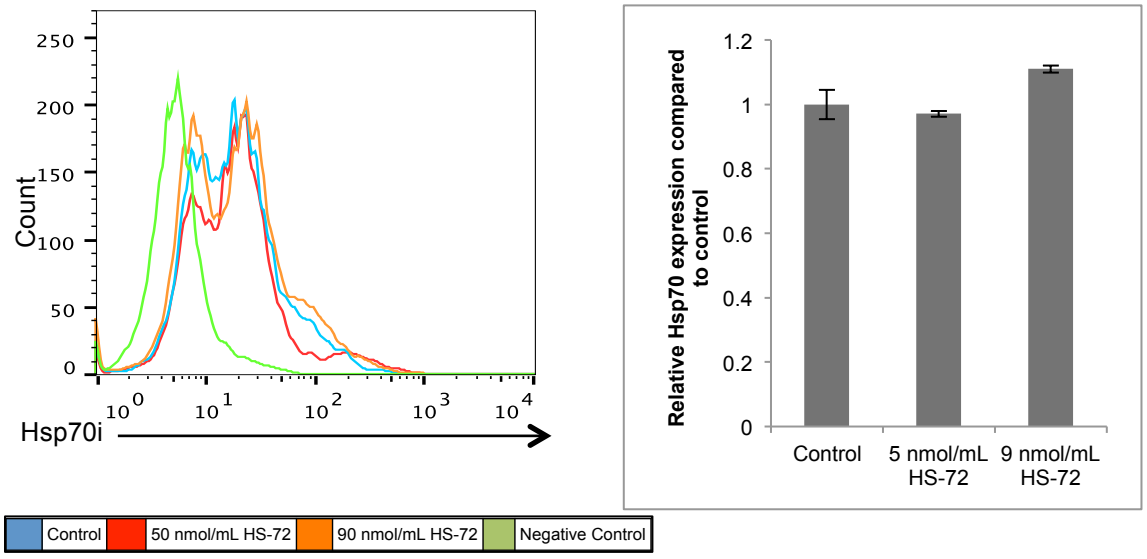
A)



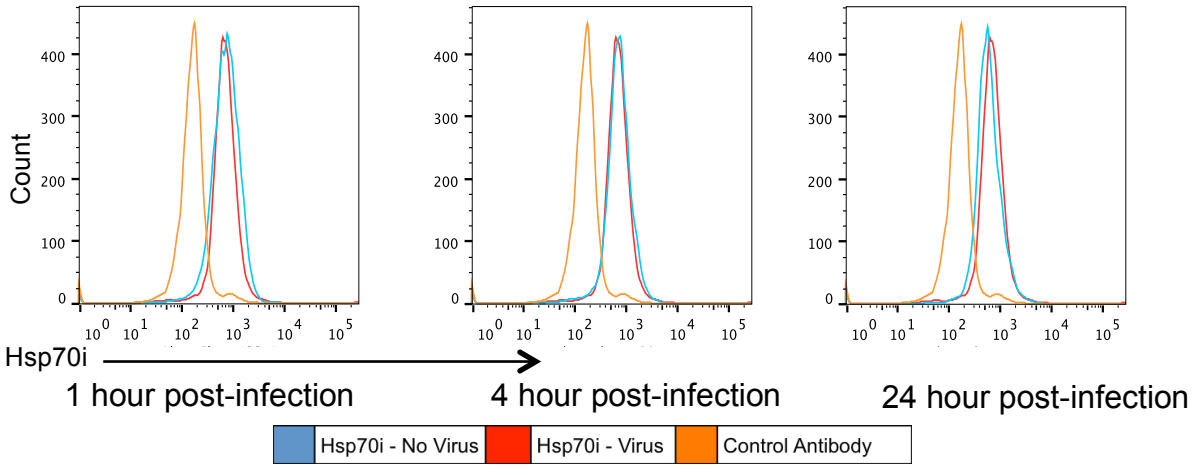
B)



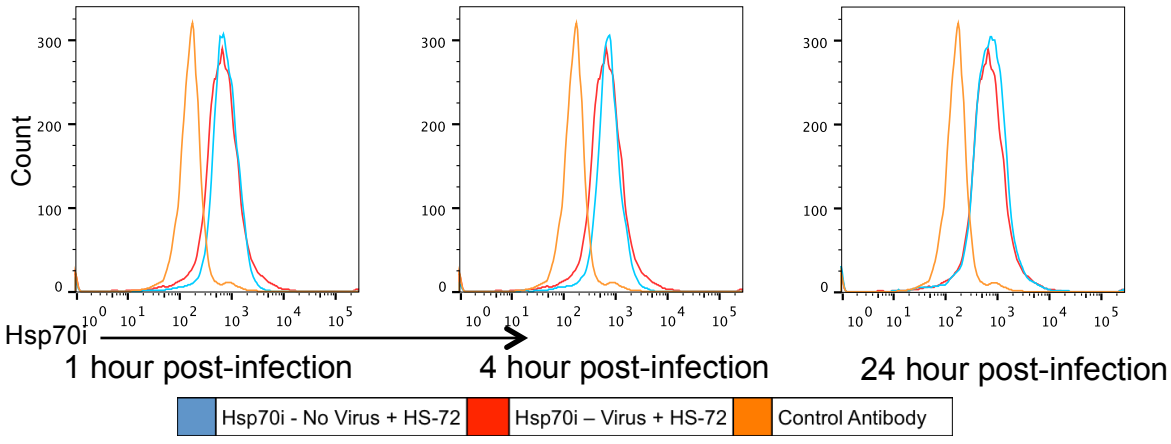
C)

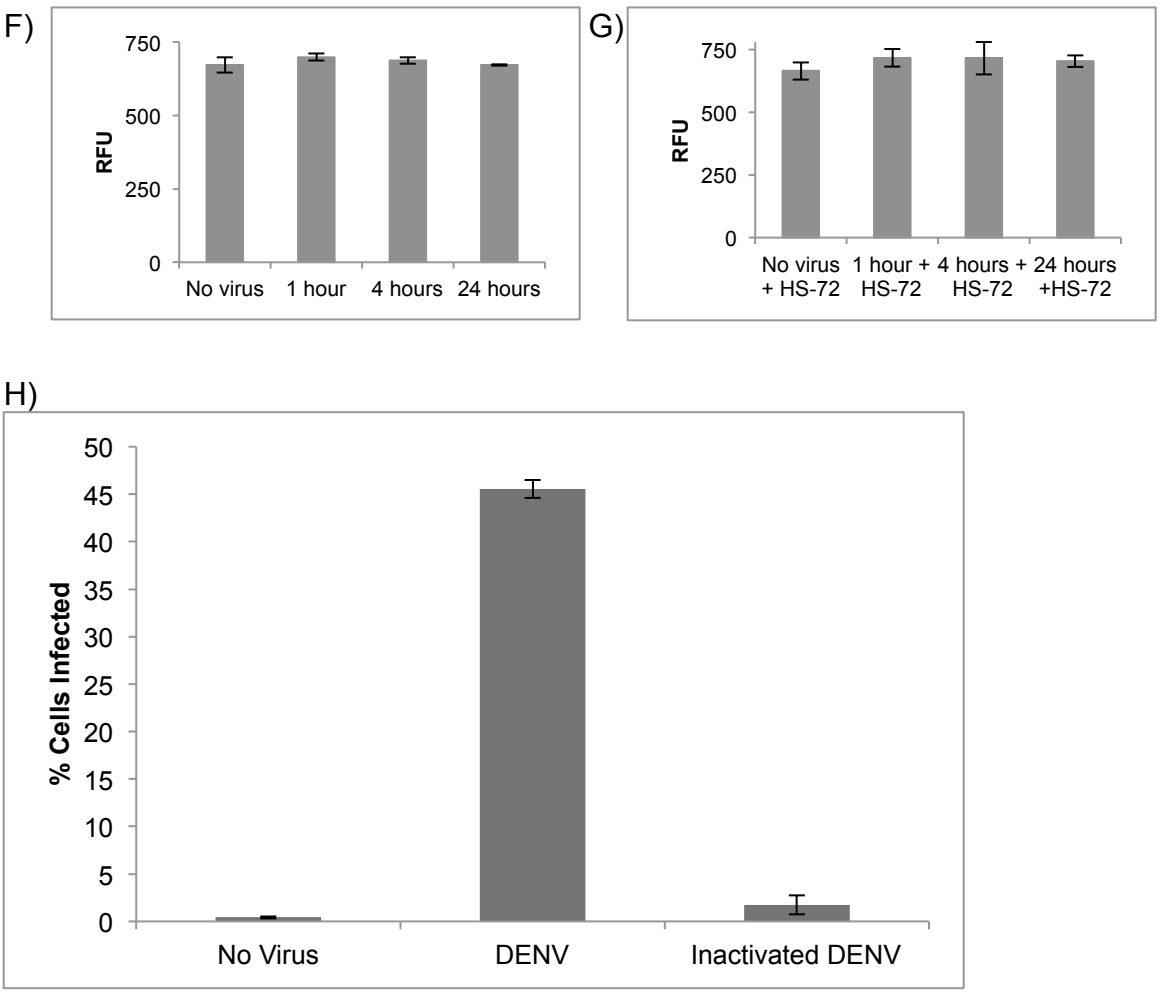

Count
Hsp70i
Relative Hsp70 expression compared to control
Control
5 nmol/mL HS-72
9 nmol/mL HS-72
Control
50 nmol/mL HS-72
90 nmol/mL HS-72
Negative Control

D)


Count
Hsp70i
1 hour post-infection
4 hour post-infection
24 hour post-infection
Hsp70i - No Virus
Hsp70i - Virus
Control Antibody

E)


Count
Hsp70i
1 hour post-infection
4 hour post-infection
24 hour post-infection
Hsp70i - No Virus + HS-72
Hsp70i – Virus + HS-72
Control Antibody



S4 Fig. Hsp70i localizes to the cell surface immediately following successful binding and infection by DENV and is maintained on the cell surface by HS-72 treatment.

A) Quantification of histogram shown in Fig. 4A. Hsp70i surface expression was determined at the indicated time points post-infection, showing a significant increase 1 hour post-infection compared to uninfected cells. While, Hsp70i surface expression is not significantly different from uninfected cells by 4 and 24 hours post-infection. U937+DC-SIGN cells were infected with DENV and at the indicated time points post infection, cells were processed for flow cytometry and incubated with a Hsp70i antibody and a fluorescent secondary antibody. Fluorescence was measured, which was used to determine Hsp70i surface expression. RFU: relative fluorescence units. (Mean ± SEM. *, $p<0.05$ compared to no virus).

B) Quantification of histogram shown in Figure 4B. Hsp70i surface expression was determined in HS-72 (75 nmol/ml) treated cells at the indicated time points post-infection, showing a significant increase 1 hour, 4 hours, and 24 hours post-infection compared to uninfected cells. This indicates that inhibiting Hsp70i ATPase activity and inducing a conformational change with HS-72 maintains Hsp70i on the cell surface following DENV. Cells infected and analyzed as

described in (A). RFU: relative fluorescence units. (Mean ± SEM. *, $p<0.05$ compared to no virus).
C) Treating uninfected U937+DC-SIGN cells with HS-72 did not significantly change the surface expression of Hsp70i. This indicates that the observed change in Hsp70i localization to the surface is related to DENV infection and not HS-72 treatment alone. U937+DC-SIGN cells were treated with HS-72 for 24 hours, upon which time cells were incubated with a Hsp70i antibody and a fluorescent secondary antibody. Fluorescence was measured, which was used to determine Hsp70i surface expression. The graph on the right represents quantification of the histogram. (Mean ± SEM).
D) Hsp70i surface expression was determined in cells treated with inactivated DENV, which showed no change compared to uninfected cells. This suggests that binding and subsequent infection of U937+DC-SIGN cells mediates the observed change in Hsp70i localization. Cells were treated with inactivated DENV, which was inactivated by heating at 55°C for 30 minutes. At the indicated time points post infection, cells were processed for flow cytometry and incubated with a Hsp70i antibody and a fluorescent secondary antibody. Control antibody is a non-specific antibody. Fluorescence was measured, which was used to determine Hsp70i surface expression.
E) Hsp70i surface expression was determined in HS-72 (75 nmol/ml) treated cells with inactivated DENV, which showed no change compared to uninfected cells. This indicates that binding and subsequent infection of U937+DC-SIGN cells mediates the observed change in Hsp70i localization in HS-72 treated cells. DENV inactivated and cells treated and analyzed as described in (D). Fluorescence was measured, which was used to determine Hsp70i surface expression.
F) Quantification of histograms shown in S3D Fig. RFU: relative fluorescence units. (Mean ± SEM).
G) Quantification of histograms shown in S4E Fig. RFU: relative fluorescence units. (Mean ± SEM).
H) Inactivated DENV does not infect U937+DC-SIGN cells. DENV was inactivated by heating at 55°C for 30 minutes. Inactivated DENV and infectious DENV were then added to cells and 24 hours post-infection cells were processed for flow cytometry. An antibody for the DENV E protein coupled with a fluorescent secondary antibody was used to determine cells positive for DENV infection. (Mean ± SEM).