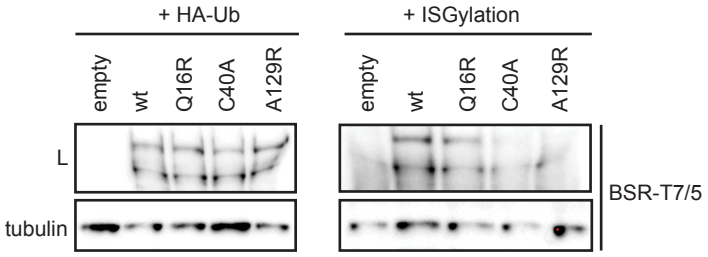

Figure S1. Cellular levels of CCHFV L protein during Ub or ISG15 overexpression, Related to Figure 2. The cellular levels of CCHFV L protein when co-expressed with HA-Ub or V5-ISG15 (and the conjugating enzymes Ube1L, UbcH8 and HERC5) in BSR-T7/5 cells were analyzed by western blotting.

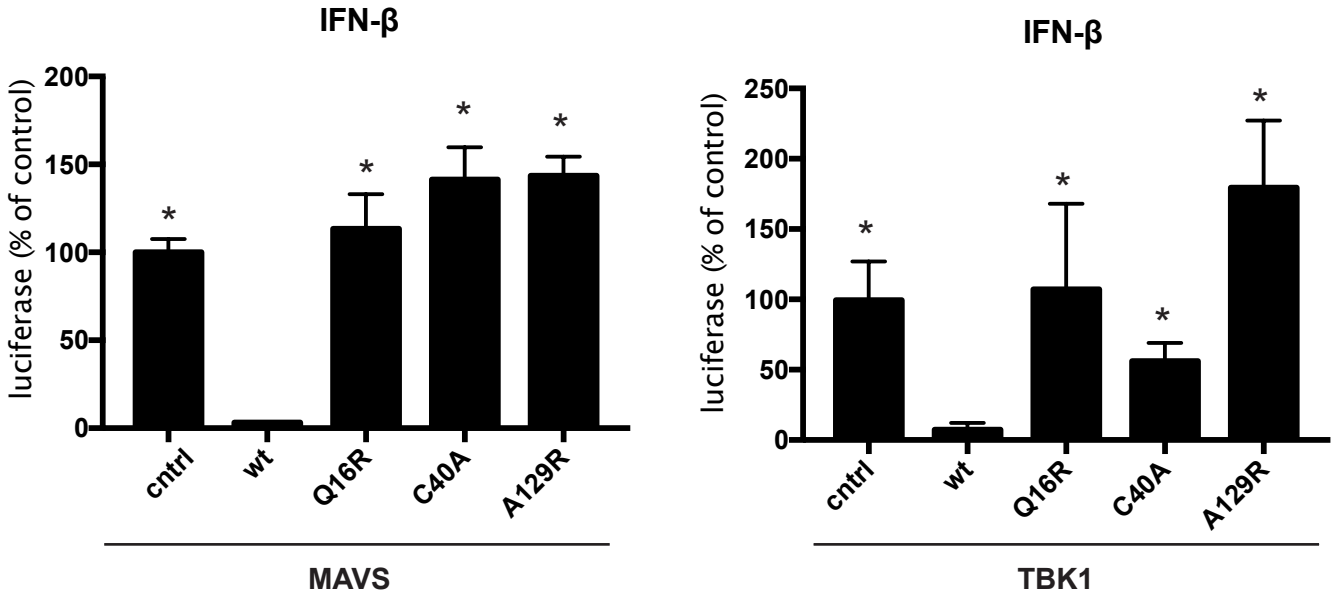

Figure S2. OTU supprssion of IFN-β reporter activation induced by MAVS or TBK1, Related to Figure 3. HEK293T cells were transfected with plasmids expressing the CCHFV OTU domain, MAVS or TBK1 to induce IFN-β expression, and a firefly luciferase reporter gene under control of the IFN-β promoter. Luciferase expression was determined as a measure of type I IFN activation. The data presented are the mean and standard deviation of a representative experiment performed using four biological replicates, *$p < 0.05$.

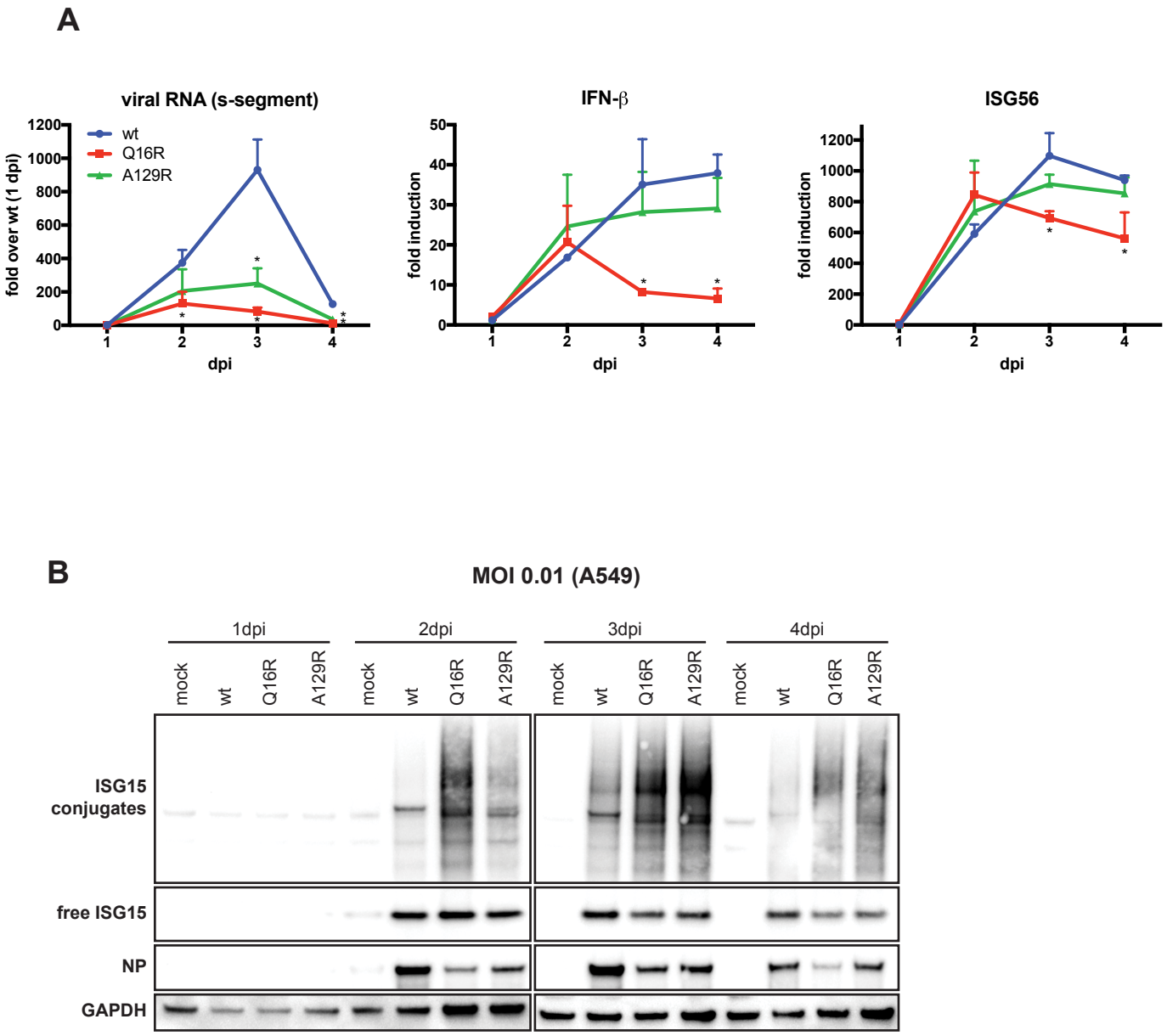

Figure S3. Immune responses in A549 cells infected with CCHFV, Related to Figure 4. A549 cells were infected at MOI 0.01 and RNA (A) and protein samples (B) were harvested at the indicated time points. RNA samples were analyzed for expression levels of viral RNA (S-segment), IFN-β and ISG56. The cell lysates were separated using SDS-PAGE and analyzed for ISG15 protein expression and conjugation levels. The data presented in (A) are the mean and standard deviation of a representative experiment performed using 3 biological replicates *p<0.05.

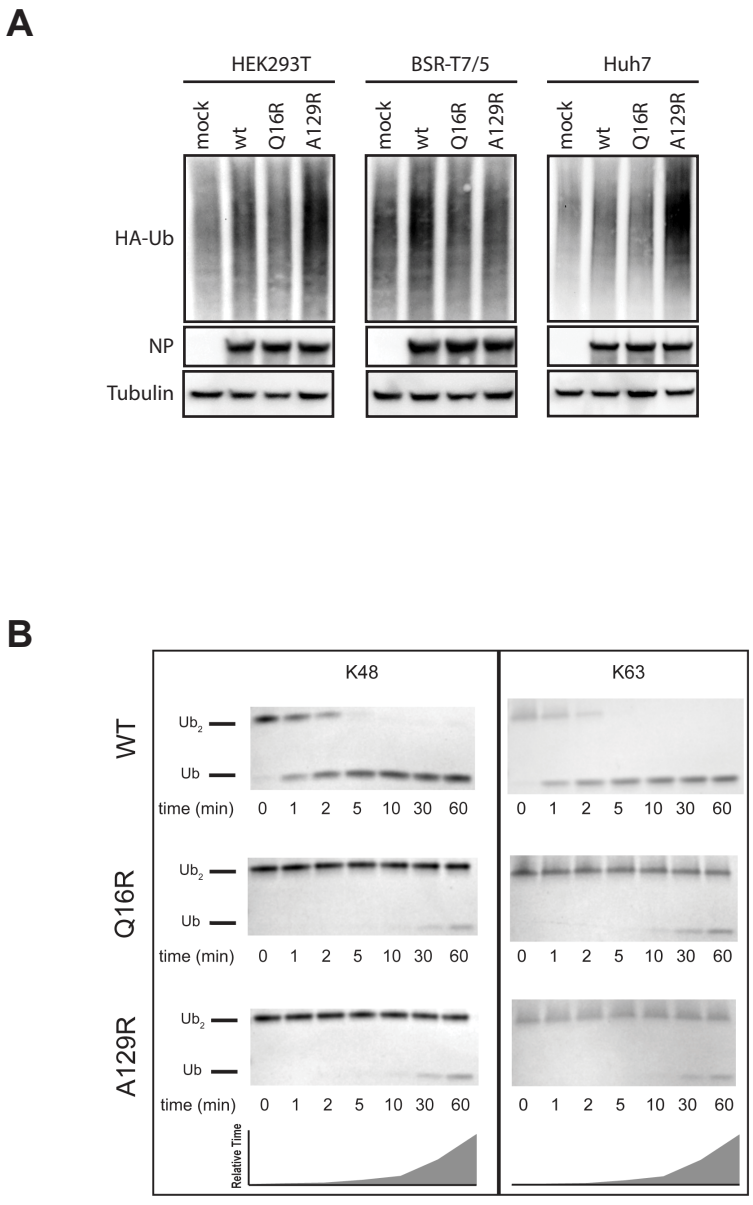

Figure S4. Protein ubiquitination in infected cells and OTU activity on di-Ub substrates, Related to Figure 5 and 6. (A) General levels of HA-tagged ubiquitinated proteins in CCHFV-infected cells. The indicated cell lines were infected with CCHFV (MOI 5) and 4 h post infection transfected with HA-Ub. Cell lysates were harvested 24 h post infection, and HA-Ub levels were assessed by western blot. (B) CCHFV OTU activity on K48- and K63-linked Ub chains. OTU activity towards K48- or K63-linked poly-ubiquitin chains was assessed in vitro by incubating the OTU mutants with di-Ub substrates and analyzing Ub levels using Coomassie staining after separation using SDS-PAGE.

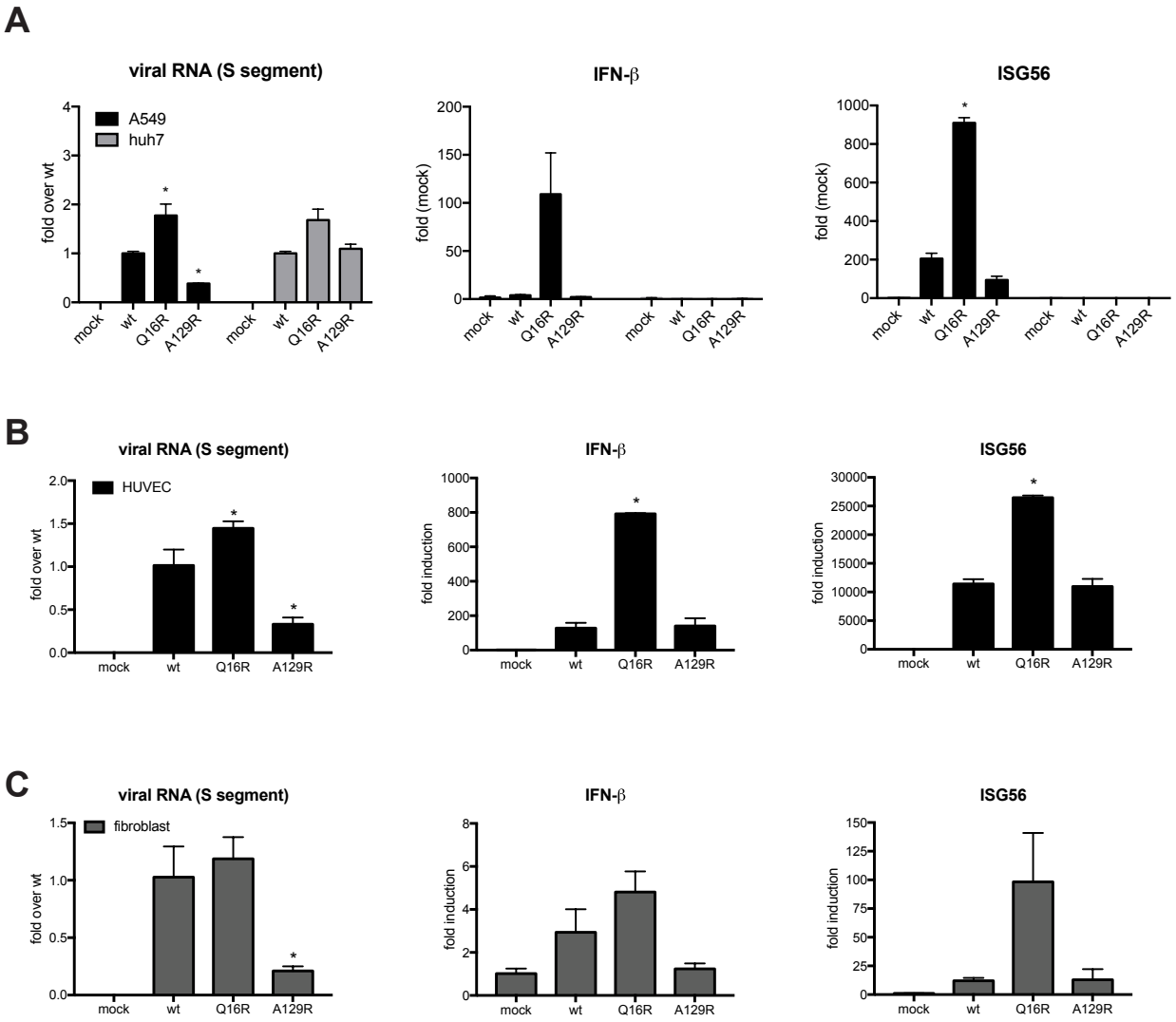

Figure S5. Immune responses in CCHFV-infected cells, Related to Figure 6. (A) A549 and Huh7 cells were infected with CCHFV (MOI 5) and samples were harvested 24 h post infection. RNA transcript levels were analyzed using qRT-PCR. (B) HUVECs and (C) fibroblasts were infected with CCHFV (MOI 7.5), and samples were harvested 14 h post infection. RNA transcript levels were analyzed using qRT-PCR. The data presented are the mean and standard deviation of a representative experiment performed using duplicate (A) or three biological replicates (B,C), *$p < 0.05$.

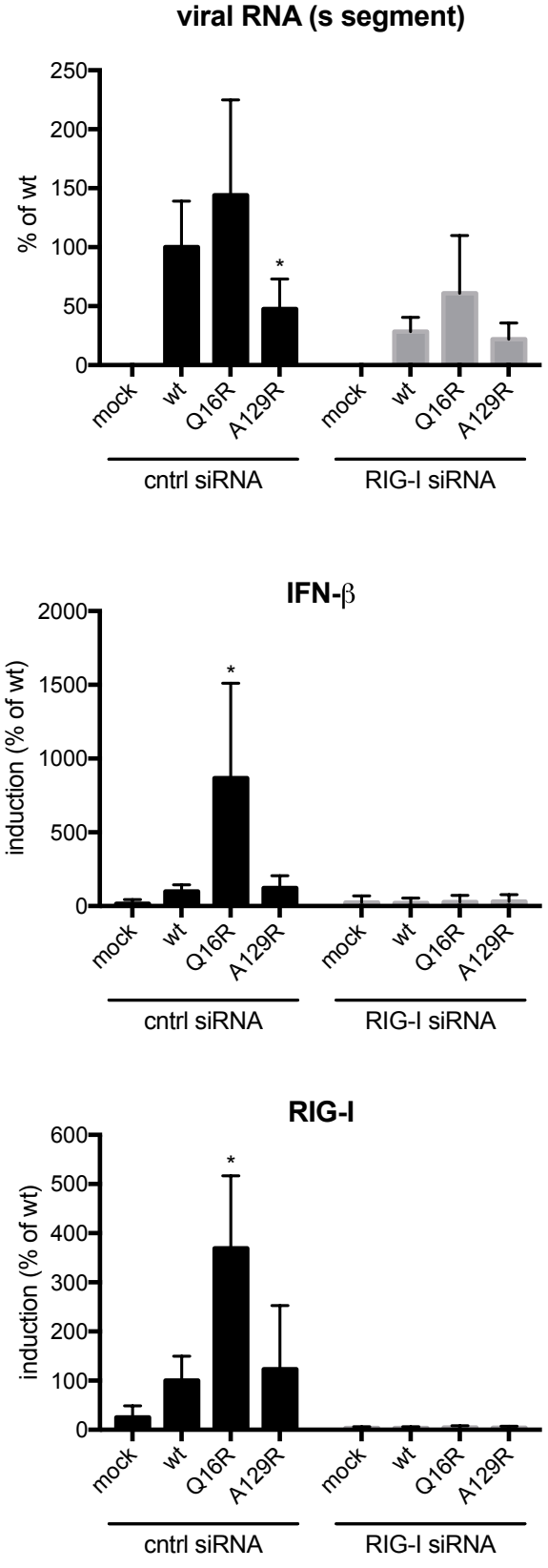

Figure S6. CCHFV infection of RIG-I depleted A549 cells, Related to Figure 6. A549 cells were transfected with siRNA targeting RIG-I, and infected with CCHFV (MOI 5) 2 days later. Samples were harvested 20 h post infection and RNA levels were analyzed using qRT-PCR. The data are presented as mean values ± SD of 2 independent experiments. Statistical differences were determined relative to the wild-type, *$p < 0.05$, ns = not significant.

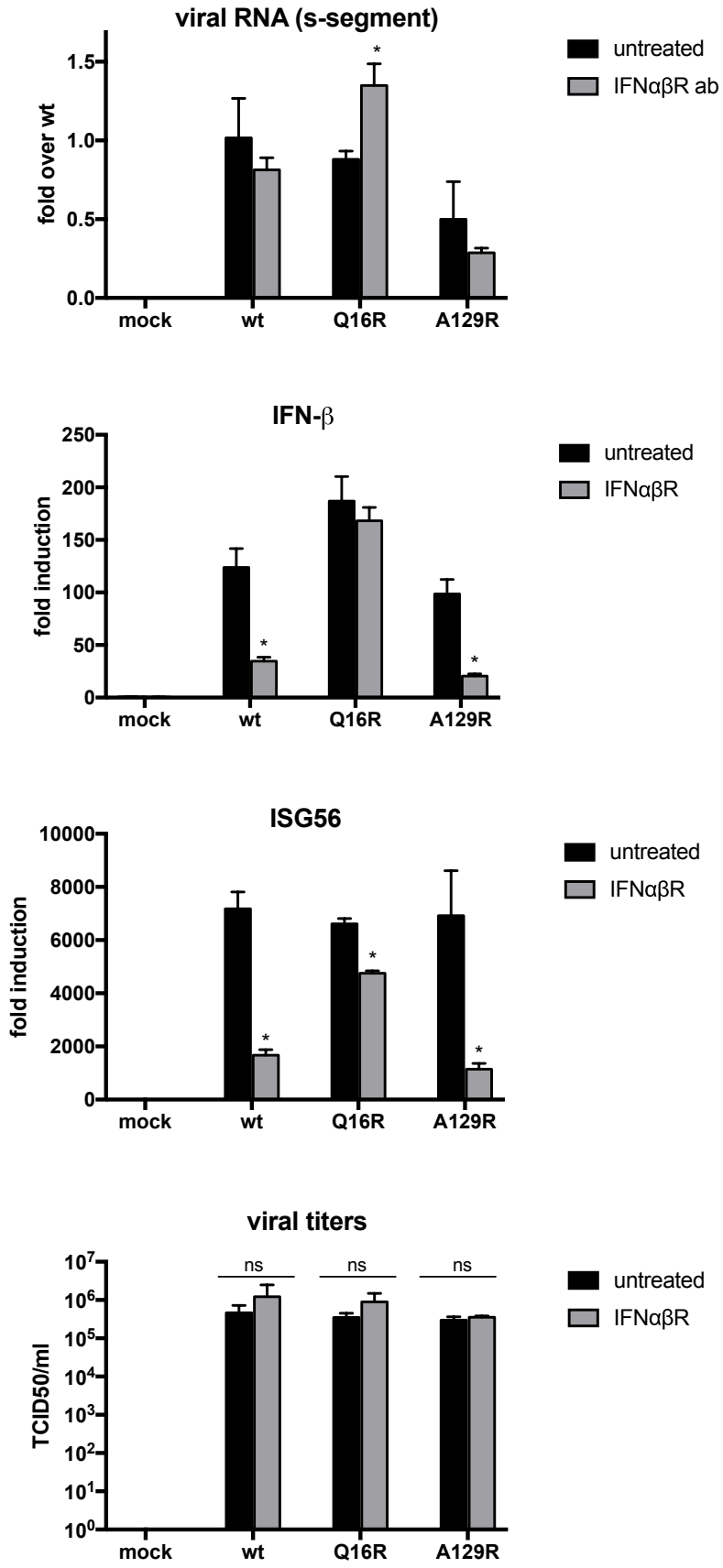

Figure S7. Effects of an IFN signaling inhibitor on CCHFV-mediated immune activation in primary cells, Related to Figure 6. HUVEC cells were infected with CCHFV (MOI 5) and treated with the indicated antibodies 1 hour post infection. Samples were harvested 20 h post infection, and RNA transcript levels were analyzed using qRT-PCR. The data are presented as mean values ± SD of a representative experiment performed using triplicate biological replicates, *$p < 0.05$, ns = not significant.