Supporting information

Supplemental Table 1

RNA-seq analysis of transcript expression in Clone 35 cells untreated or treated with stim-SN.

Samples (s10-s15) were prepared as shown in Supplemental Fig. 4. Heatmap analysis summarizing the expression levels of each gene is provided.

Supplemental Figure 1

Inhibitory effect of supernatants from activated $CD4^+$ T cells on SARS-CoV-2 variants

(A) Clone 35 cells were cultured as indicated. Three days post-inoculation, the culture SNs were used to determine the amount of virus progeny (copies/μL) and $TCID_{50}$. (B) Clone 35 cells were cultured as in Fig. 1 legend in the presence (orange symbols) or absence (blue symbols) (N=4) of SNs (stim-SN, 10% final concentration) from activated $CD4^+$ T cells. On days 1, 2, and 3 after culture, the quantity of virus in the culture SNs was measured by qPCR.

Supplemental Figure 2

Differential inhibitory effects of supernatants from activated and resting $CD4^+$ T cells on the expansion of SARS-CoV-2 original strain

Clone 35 cells (2×10^4/well) were cultured with SARS-CoV-2 (original strain, 1×10^4 copies/μL) in the presence or absence of titrated amounts of SNs from $CD4^+$ T cells (N=4), rest (2) SN, derived from a resting culture in the presence of IL-2 alone; stim (b2) SN, derived from a stimulation culture including both anti-CD3/CD28 beads and IL-2; stim (b) SN, derived from a stimulation culture with anti-CD3/CD28 beads. After 3 days, the amount of virus in culture SNs was quantified by qPCR. The amount of virus progeny in SNs in the presence of each SN is expressed as the percentage production relative to the amount of viruses cultured in the absence of SN.

Supplemental Figure 3

Inhibitory effect of supernatants from activated $CD4^+$ T cells on SARS-CoV-2 propagation in human alveolar epithelial cells

Human alveolar epithelial cells were cultured with titrated amounts of SARS-CoV-2 (original or Delta strain) in the presence (orange symbols) or absence (blue) of stim-SN at a final concentration of 10% (N=3). After 3 days, the amount of virus in culture SNs

was quantified by qPCR. (A and C) The actual amount of viruses in SNs is shown. (B and D) The amount of virus progeny in SNs in the presence of stim-SN is expressed as the percentage production relative to the amount of viruses cultured in the absence of stim-SN. The control (the amount of viruses cultured in the absence of stim-SN) is indicated by the dotted line.

Supplemental Figure 4

Samples used for RNA-seq

(A) Clone 35 cells (1.5×10^5/well in a 12-well plate) were cultured in the presence or absence of supernatants from resting (rest-SN) or stimulation (stim-SN) cultures at final concentrations of 10% for 24 or 43 hours as shown. After culture, cells were used for total RNA preparation. (B) After the 43-hour culture, a subset of cells was harvested (samples s13-s15), washed three times, and counted. These cells were subsequently co-cultured with SARS-CoV-2 viruses (original strain, 1×10^4 copies/μL). After 3 days, the amount of virus in culture SNs was quantified by qPCR. Virus production in each culture is shown as the percentage production relative to that in cultures with freshly prepared Clone 35 cells (indicated as c35). As a positive control, stim-SN (10%) was added to fresh Clone 35 cells. (C) A heat map based on the RNA-seq data shows the

relative expression levels of representative ISGs.

Supplemental Figure 5

Measurement of type-I, -II, and -III IFNs in the supernatants from $CD4^+$ T cell culture

The concentration of IFNs in the SNs (10%) from resting- or stimulating-culture was measured using enzyme-linked immunoassay kits for IFN-α2, IFN-β, IFN-γ, and IFN-λ 3/1/2. N=3 per group. The results are plotted as OD values. The result is representative of three different experiments.

Supplemental Figure 6

Induction of ISGs in Clone 35 cells by IFN-γ

Clone 35 cells (1.2×10^5/2 mL/well in a 24-well plate) were cultured in the presence or absence of stim-SN (10% final concentration) or IFN-γ (4.6 ng/mL final concentration) for 24 hours (N=3 per group) without infection. Gene expression levels in each sample were evaluated as described in the subsection titled "Quantitative RT-PCR" of the Materials and Methods.

Supplemental Figure 7

Inhibitory effect of IFN-γ against some SARS-CoV-2 variants

(A) Clone 35 cells (5×10^5/well in a 6-well plate) were cultured in the presence or absence of titrated amounts of IFN-γ for 48 hours (pre-treatment). After culture, cells were harvested, washed three times, and counted. These cells were then co-cultured with SARS-CoV-2 viruses (original or Delta strain, 1×10^4 copies/μL). After 3 days, the amount of virus in culture SNs was measured by qPCR. (B) Virus production by the cells pre-treated with IFN-γ is shown as the percentage production relative to that by cells pre-treated with medium. N=4 per group.

Supplemental Figure 8

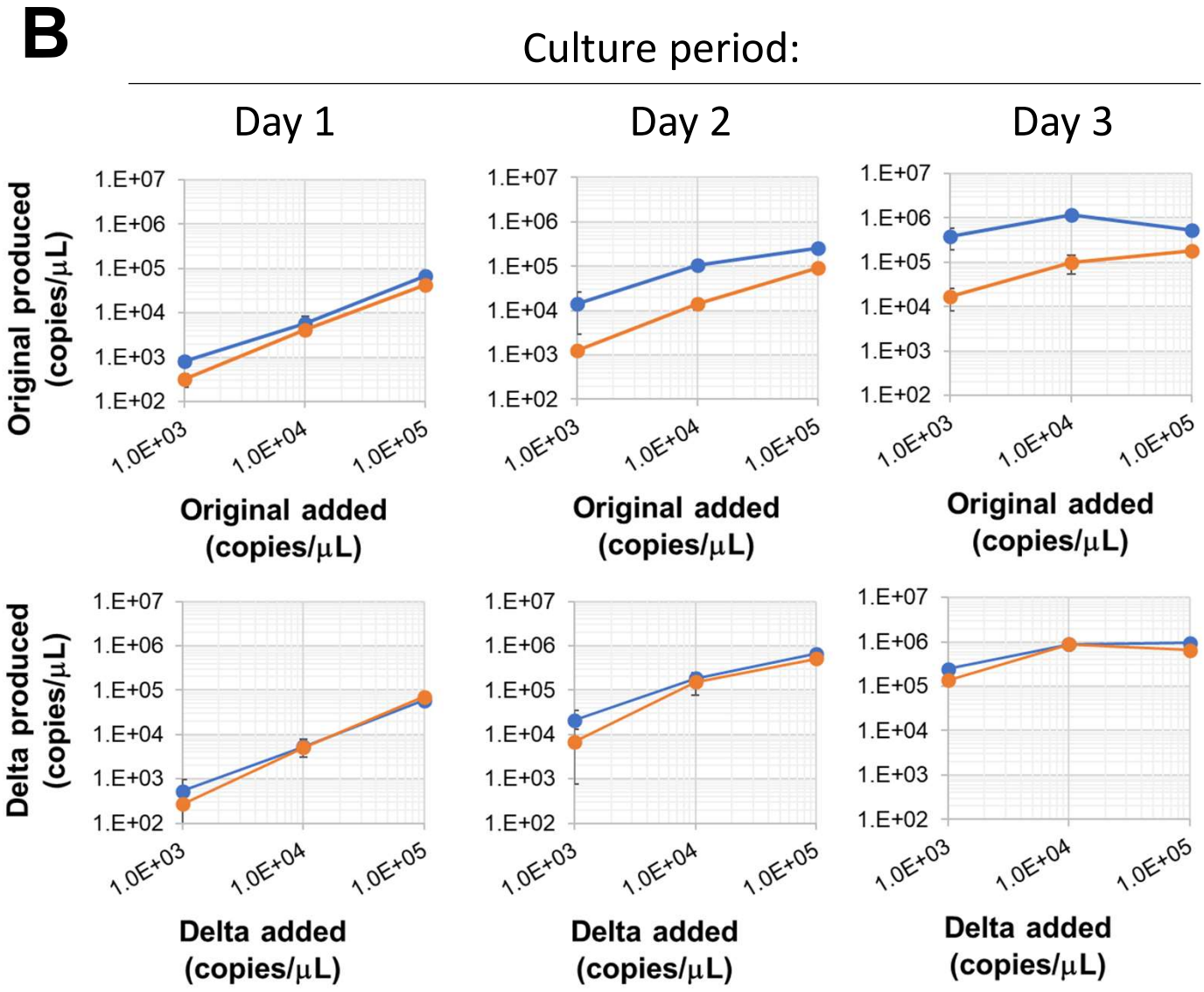
Induction of ISGs in Clone 35 cells by stim-SN in the presence of SARS-CoV-2

Clone 35 cells (indicated as c35, 1.5×10^5/well in a 24-well plate) were cultured for 24 hours in the presence or absence of stim-SN (10% final concentration), along with medium, in the presence of the SARS-CoV-2 original or Delta strain, as indicated (1×10^4 copies/μL, N=3 per group). The expression levels of various ISGs were quantitatively assessed as described in the subsection titled “Quantitative RT-PCR” in the Materials and Methods.

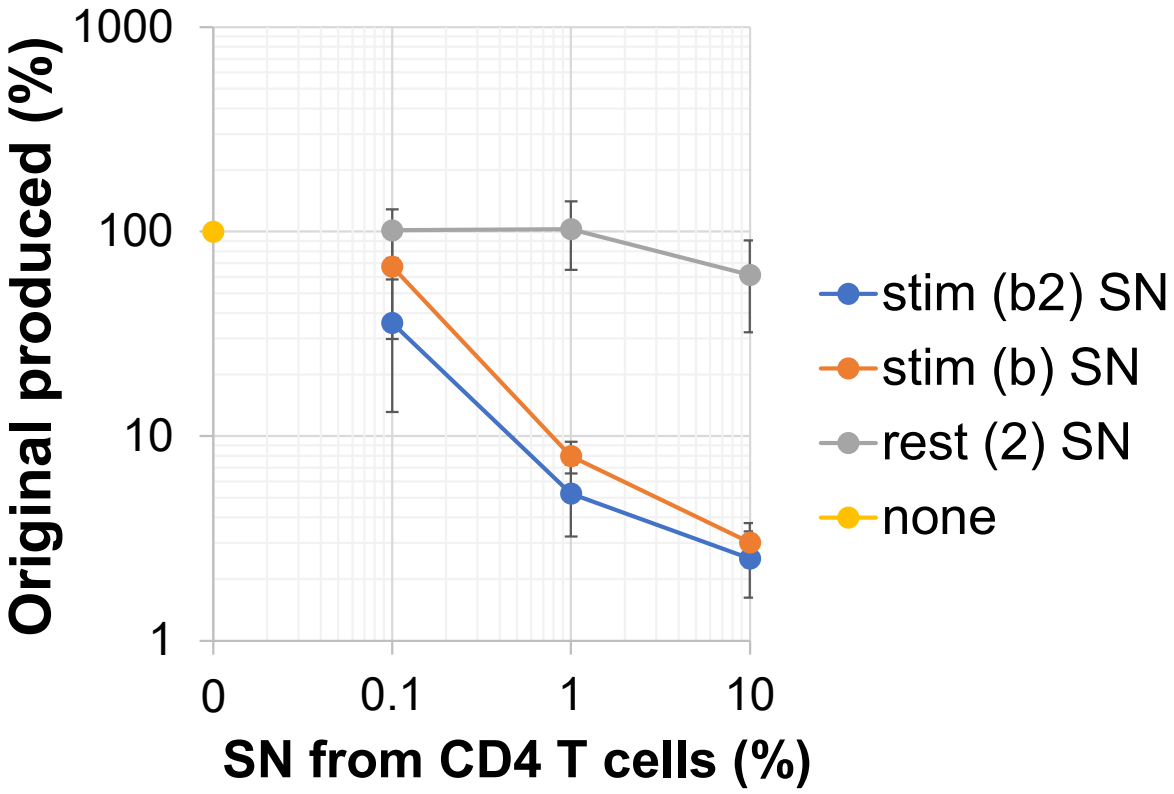
A

Clone 35 cells cultuted with:		SARS-CoV-2 in day 3-culture SNs	
SARS-CoV-2 ($1x10^4$ copies/μL)	stim-SN (10%)	copies/μL	$TCID_{50}$/50 μL
Original	(-)	$7.67 x10^5$	$3.98 x10^4$
	(+)	$4.78 x10^4$	$3.16 x10^3$
Delta	(-)	$7.31 x10^5$	$5.62 x10^4$
	(+)	$6.00 x10^5$	$4.33 x10^4$

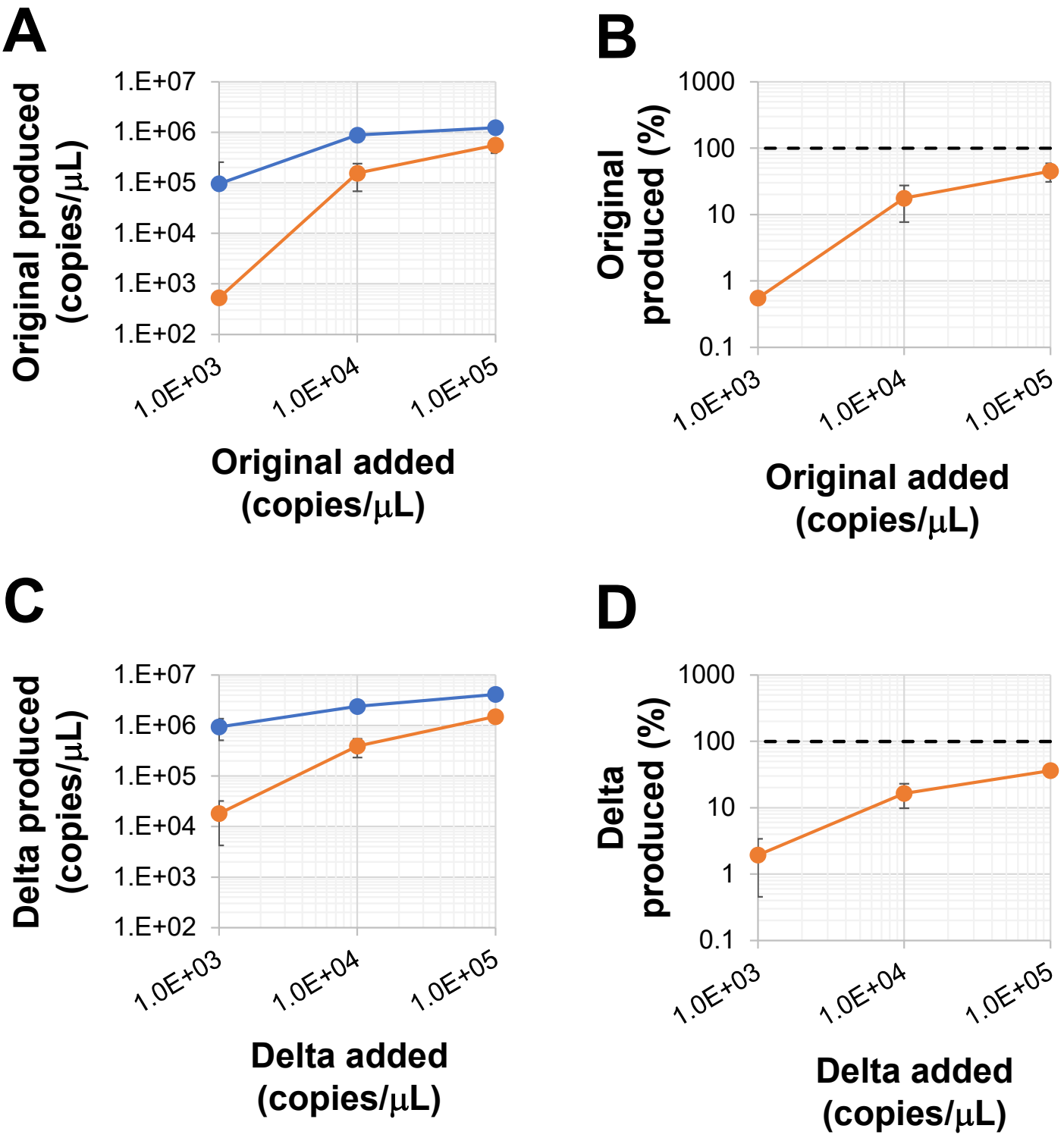
B



Supplemental Fig. 1



Supplemental Fig. 2

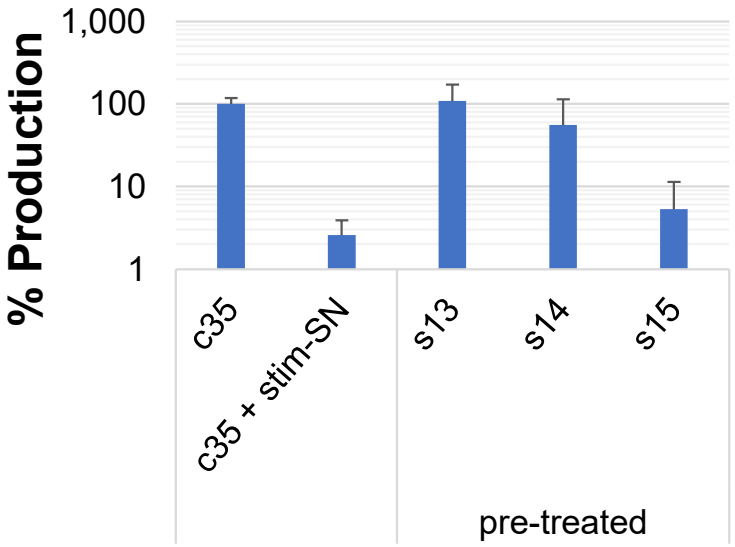


Supplemental Fig. 3

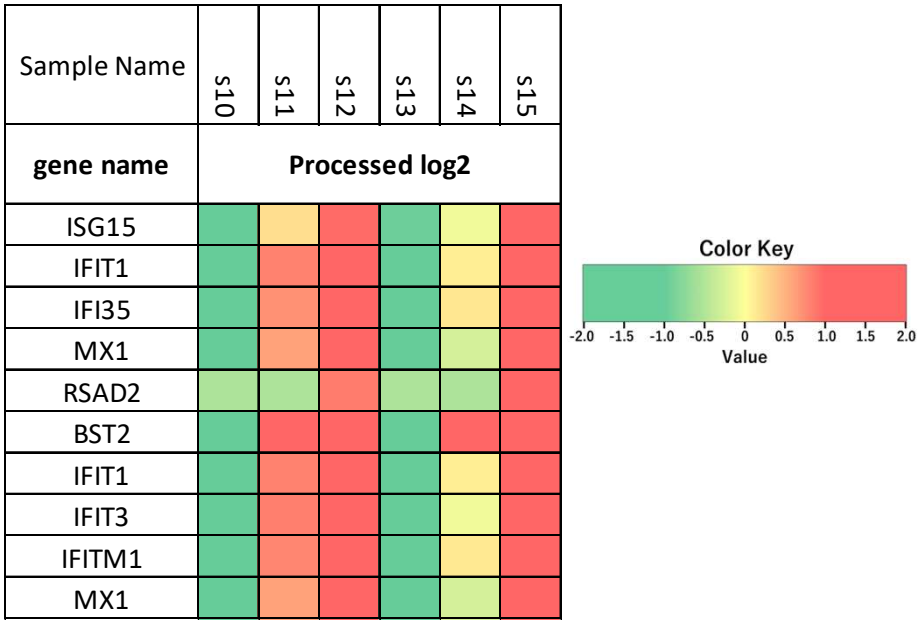
A

Clone 35 cells pre-treated with:	Pre-treatment period:	
	24-hr	43-hr
none	s10	s13
rest-SN (10%)	s11	s14
stim-SN (10%)	s12	s15

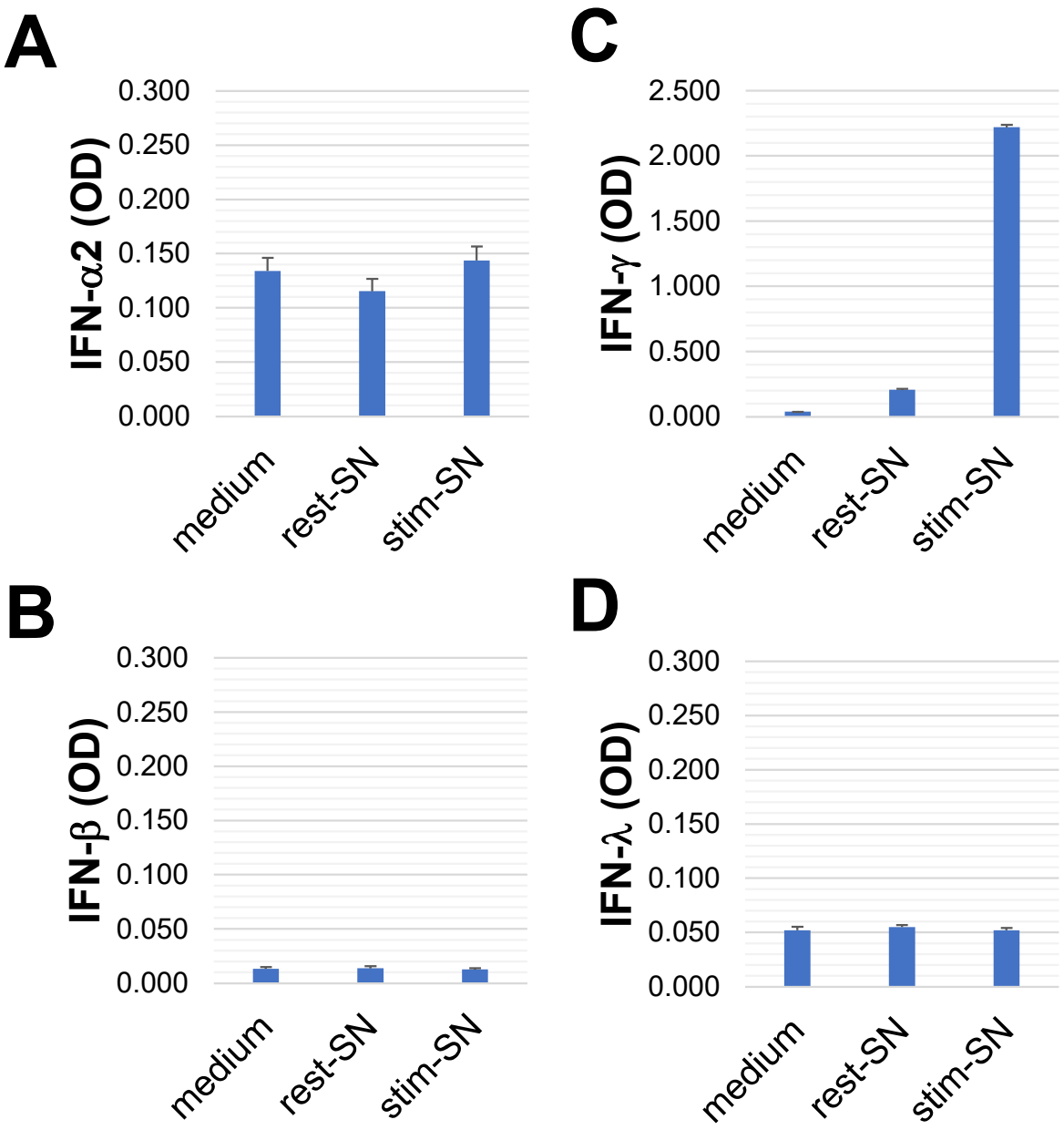
B



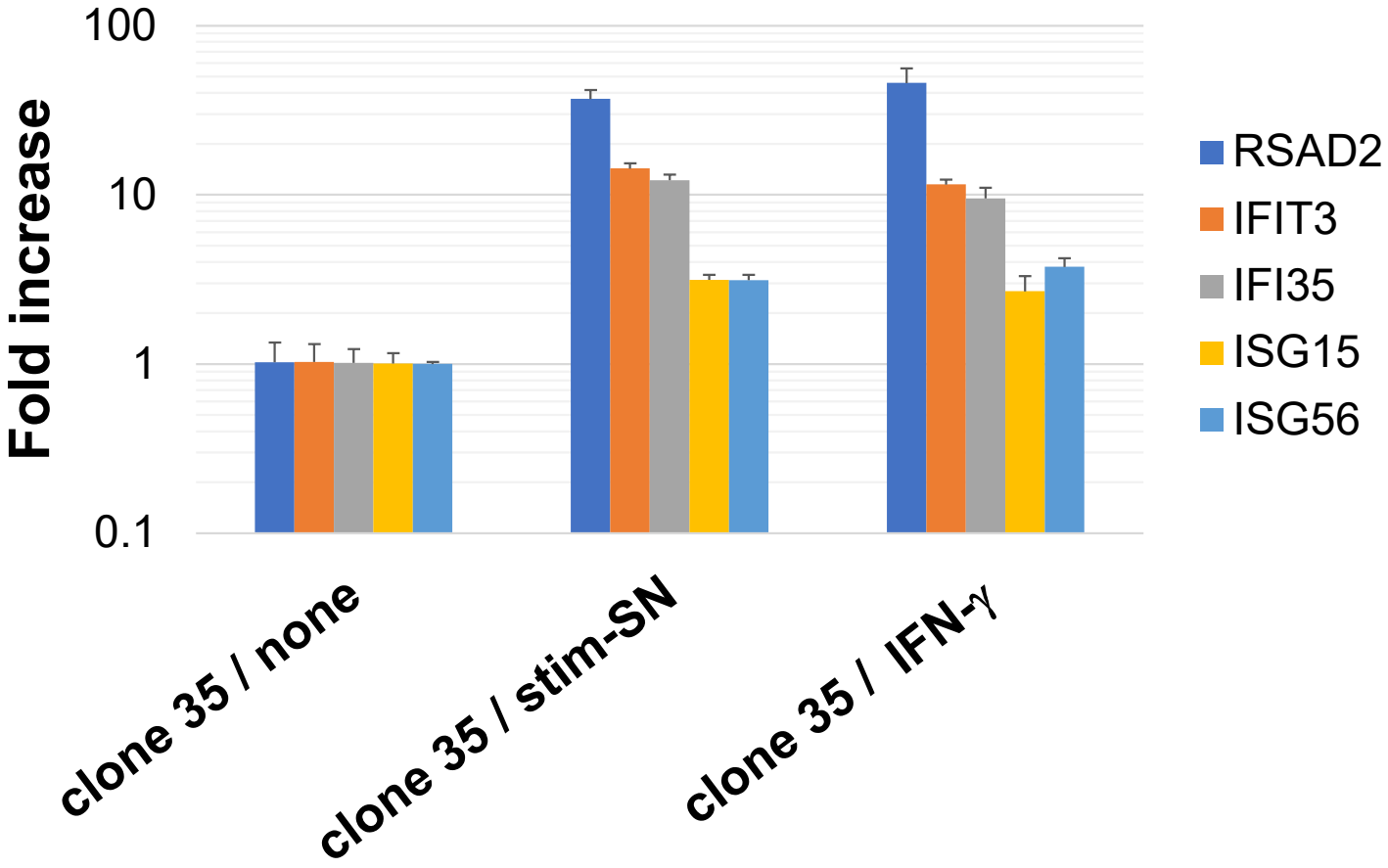
C



Supplemental Fig. 4

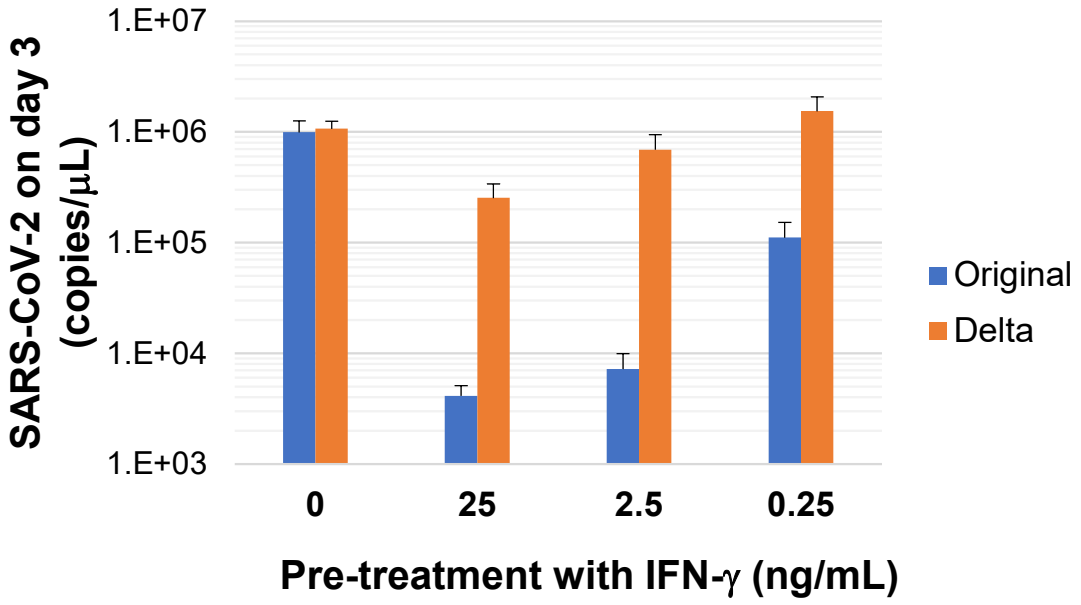


Supplemental Fig. 5

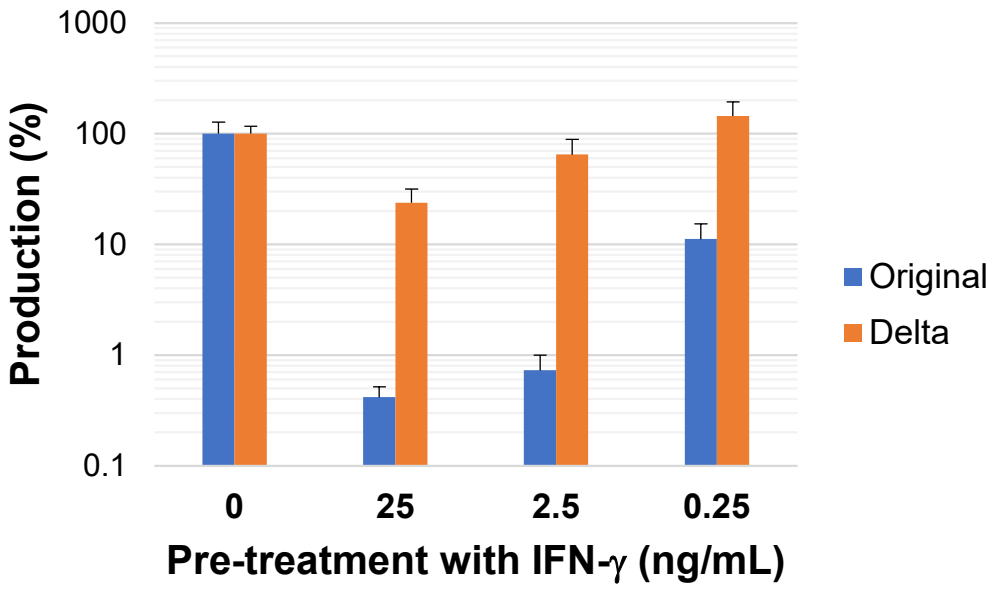


Supplemental Fig. 6

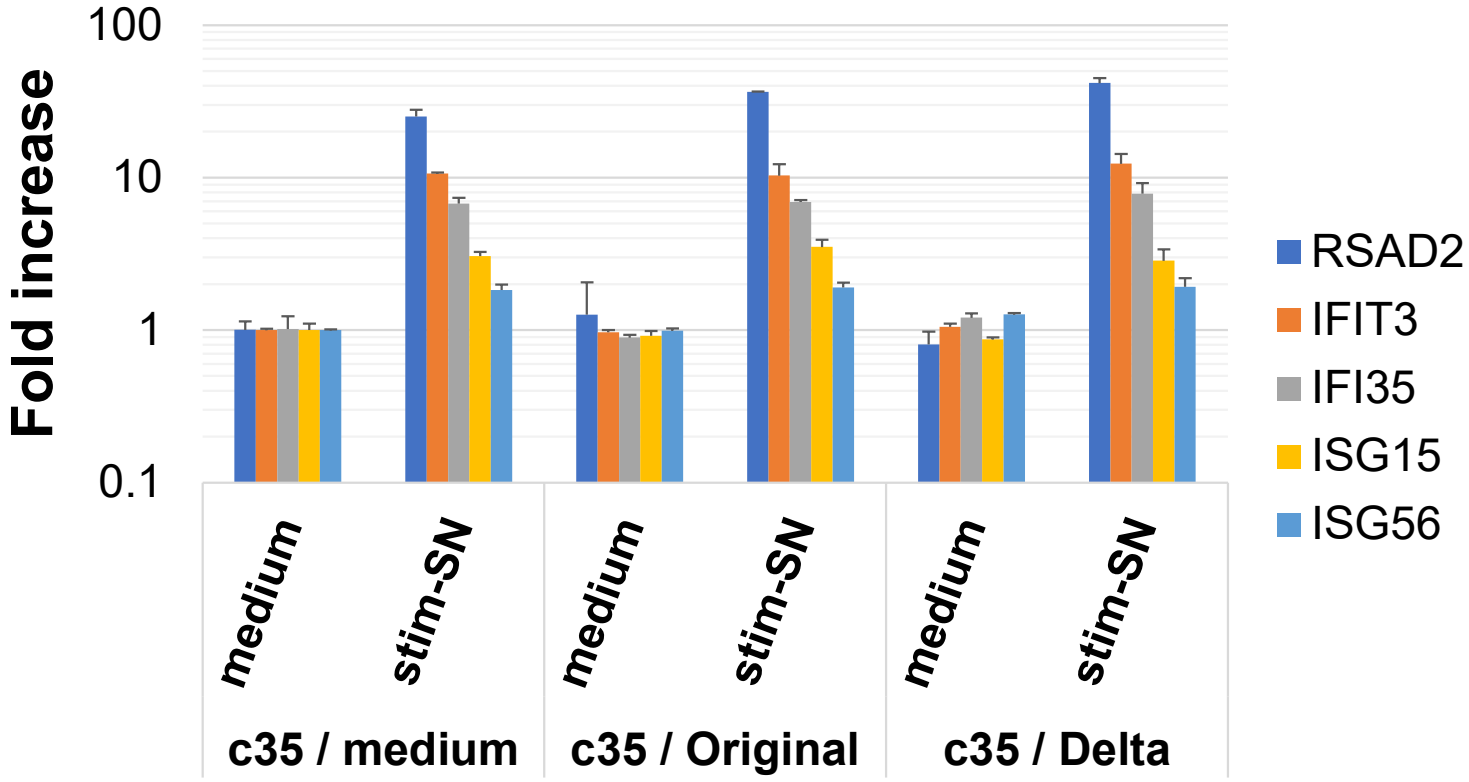
A



B



Supplemental Fig. 7



Supplemental Fig. 8