cGAS-STING Pathway Limits SARS-CoV-2 Replication in ACE2+ Airway Cell Lines

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BACKGROUND

In an effort to develop alternative in vitro models for SARS-CoV-2, we previously screened several airway-derived cell lines for their permissiveness to SARS-CoV-2 infection (Puray-Chavez et al., 2021 Cell Reports). Surprisingly, we found that a number of airway-derived cell lines are unable to support SARS-CoV-2 despite expressing ACE2 at endogenous levels.

Type I/III interferons (IFNs) potently restrict SARS-CoV-2 replication through the actions of hundreds of interferon-stimulated genes (ISGs) that are upregulated upon IFN signaling. Here we report that these ACE2 positive airway cell lines are unable to support SARS-CoV-2 replication due to basally high IFN pathway activity. We show that the basally active cGAS-STING DNA sensing pathway underlies the observed IFN pathway activation in these cells which subsequently restricts SARS-CoV-2 replication.

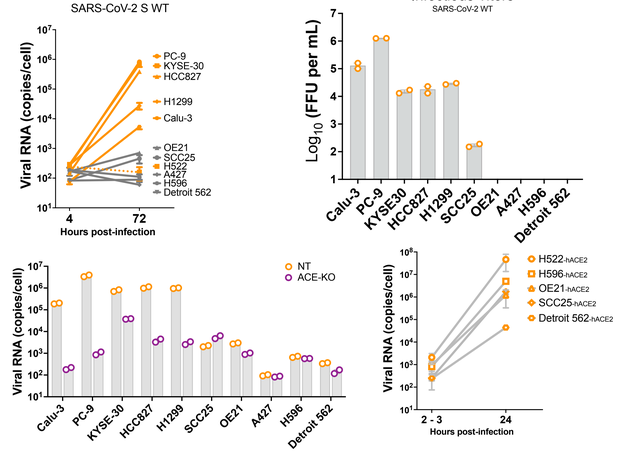
METHODS

Calu-3, H596, H522, H1299, A427, HCC827, PC-9, Detroit562, SCC25, KYSE30 and OE21 cells were infected with SARS-CoV-2 WT strain 2019-nCoV/USA-WA1/2020.

RNA sequencing was used to study the basal level of genes in the type-I IFN pathway in uninfected cells and further validated by qRT-PCR. Viral replication was detected through analysis of cell associated RNA, reporter viruses and antigen staining.

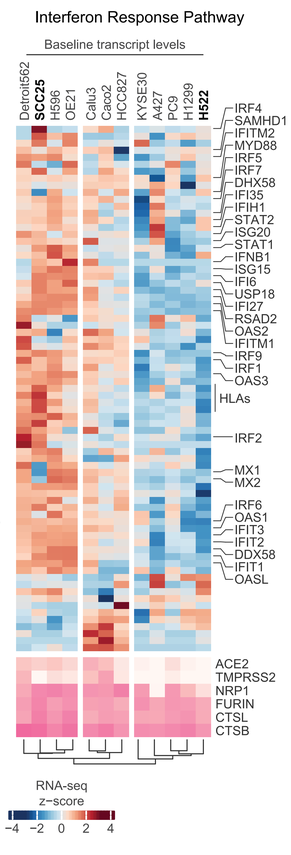
RESULTS

Some airway-derived cell lines that express endogenous levels of ACE2 do not support SARS-CoV-2 replication

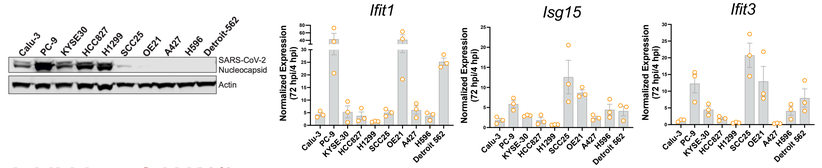


cGAS-STING-dependent activation of IFN-mediated innate immunity underlies the inability of some ACE2+ airway cell lines to support SARS-CoV-2 replication

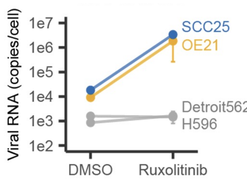
High ACE2 expressing airway-derived cell lines also express high levels of IFN pathway genes



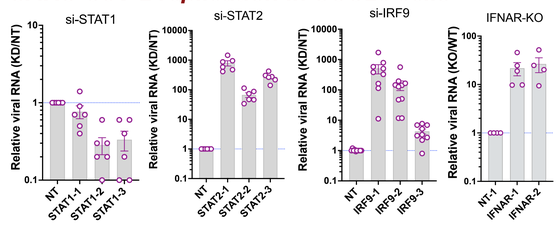
SARS-CoV-2 induces ISG expression despite lack of spreading infection



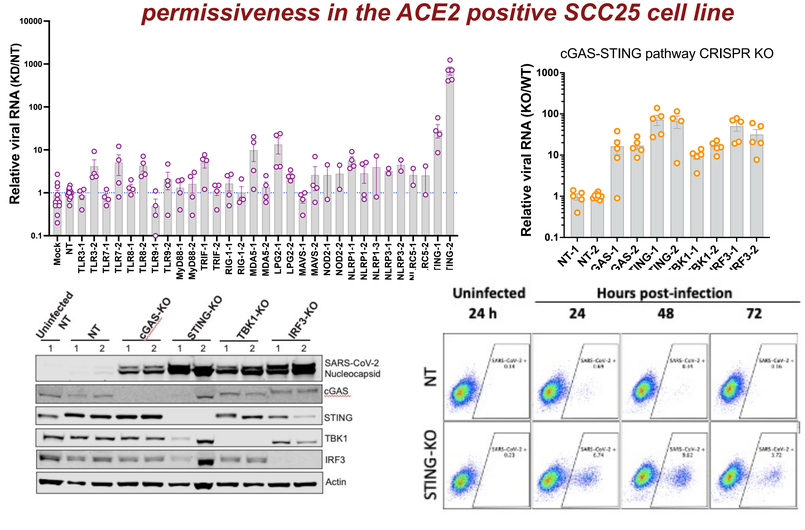
Inhibition of JAK1/2 restores SARS-CoV-2 infection



Basally active type I IFN pathway suppresses SARS-CoV-2 replication in SCC25 cells

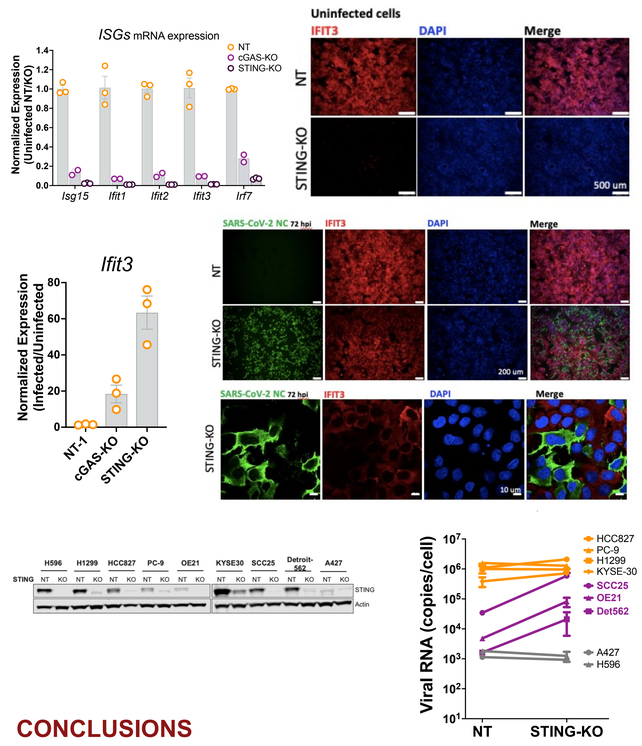


A basally active cGAS-STING pathway underlies the lack of permissiveness in the ACE2 positive SCC25 cell line



RESULTS

Cont. A basally active cGAS-STING pathway underlies the lack of permissiveness in ACE2 positive cells



CONCLUSIONS

- ✓ACE2 positive non-permissive cell lines express high basal levels of interferon-stimulated genes due to STING-cGAS pathway activation.
- ✓Knockout of cGAS-STING-mediated immune response rescue replication of SARS-CoV-2 in SCC25, OE21 and Detroit infected cells.
- ✓These findings may explain the complex SARS-CoV-2 pathogenesis *in vivo* as it shows that factors independent of ACE2 can define cellular tropism.

ADDITIONAL KEY INFORMATION

Key words: **STING, ACE2, SARS-CoV-2, type I/III IFN.**

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