Validation data for A549-hACE2-TMPRSS2 cells

https://www.invivogen.com/a549-hace2tmprss2-cells

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A549-hACE2-TMPRSS2 cells were generated from the A549 lung carcinoma cell line. The A549 cell line is a well-characterized cellular model of the alveolar basal epithelial lung cells for the study of respiratory infections. ACE2 and TMPRSS2 overexpression in A549-hACE2-TMPRSS2 cells has been verified by RT-qPCR **(Figure 1)**, and ACE2 expression has been verified by cell surface staining **(Figure 2)**. Unlike their parental cell line, A549-hACE2-TMPRSS2 cells can be infected with pseudotyped lentiviral particles expressing the SARS-CoV-2 Spike protein **(Figure 3,** and data not shown**)**. As expected, lentiviral particles expressing the globally dominant Spike G614-variant have greater infectivity of A549-hACE2-TMPRSS2 cells, when compared to particles expressing the original D614 Spike protein **(Figure 3, bottom row).**

Validation of *ACE2* and *TMPRSS2* overexpression by RT-qPCR

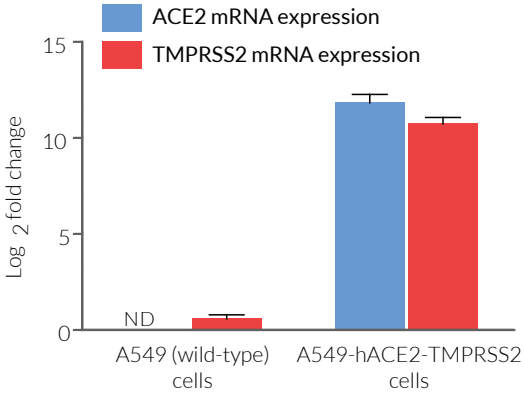

Figure 1: Human ACE2 and TMPRSS2 mRNA expression in A549-hACE2-TMPRSS2 cells. Total mRNA was extracted from ~1x10^6 A549 (wild-type) and A549-hACE2-TMPRSS2 cells. ACE2 and TMPRSS2 mRNA were amplified using quantitative RT-qPCR. Data are represented as the $\log_2$ fold change comparing ACE2 or TMPRSS2 expression to a housekeeping gene. ND: not detected.

Validation of ACE2 surface expression by FACS

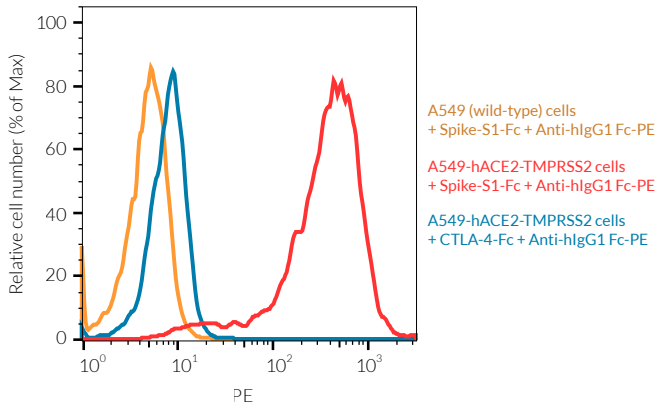

Figure 2: Surface expression of hACE2 by A549-hACE2-TMPRSS2 cells. ~2x10^5 A549 (wild-type) and A549-hACE2-TMPRSS2 cells were incubated with 1 µg of Spike-S1-Fc or CTLA-4-Fc fusion proteins for 1h at 4°C. Cells were then washed and incubated with 0.5 µg of a goat anti-hIgG1-Fc antibody coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow-cytometry.

Infection of A549-hACE2-TMPRSS2 cells by SARS-CoV-2 Spike pseudotyped lentiviral particles

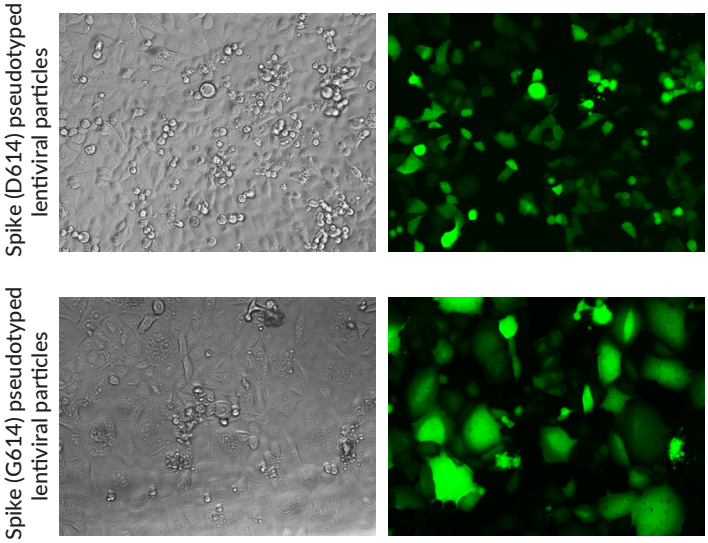

Figure 3: Infection of A549-hACE2-TMPRSS2 cells by Spike (D614 or G614) pseudotyped lentiviral particles. ~2x10^4 A549-hACE2-TMPRSS2 cells were cultured in the presence of SARS-CoV-2 Spike D614-variant (up panels) or Spike G614-variant (bottom panels) pseudotyped GFP lentiviral particles. After 72h, the transduction efficiency of the Spike pseudotyped GFP particles was evaluated by fluorescence microscopy.

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