Validation data for A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells

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

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Version 21C17-NJ

A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells were generated from the A549-Dual™ KO-RIG-I lung carcinoma cell line through stable integration of the human *ACE2* and *TMPRSS2* genes.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-Dual™ hACE2-TMPRSS2 and A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells has been verified by RT-qPCR **(Figure 1)**, and ACE2 expression has been verified by cell surface staining **(Figure 2)**. The biallelic deletion of the *RIG-I* gene has been verified by PCR **(Figure 3)**. A549-Dual™ KO-RIG-I hACE2-TMPRSS2 and their parental cell line are highly permissive to infection with pseudotyped lentiviral particles expressing the SARS-CoV-2 Spike (G614) protein **(Figure 4)**. As expected upon nucleic acid stimulation, the IRF activity in A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells is abolished when compared to A549-Dual™ hACE2-TMPRSS2 cells **(Figure 5A)**. On the contrary, both cell lines display similarly low to no NF-κB activity in response to nucleic acid stimulation **(Figure 5B)**.

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

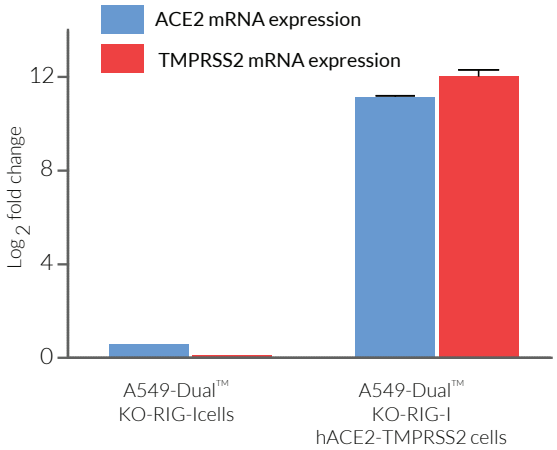

Figure 1: Human ACE2 and TMPRSS2 mRNA expression in A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells.
Total mRNA was extracted from ~1x10^6 A549-Dual™ hACE2-TMPRSS2 and A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells. ACE2 and TMPRSS2 mRNA were amplified using quantitative RT-qPCR. Data are represented as the $\log_2$ fold change comparing ACE2 or TMPRSS2 expression to a housekeeping gene.

Validation of ACE2 surface expression by FACS

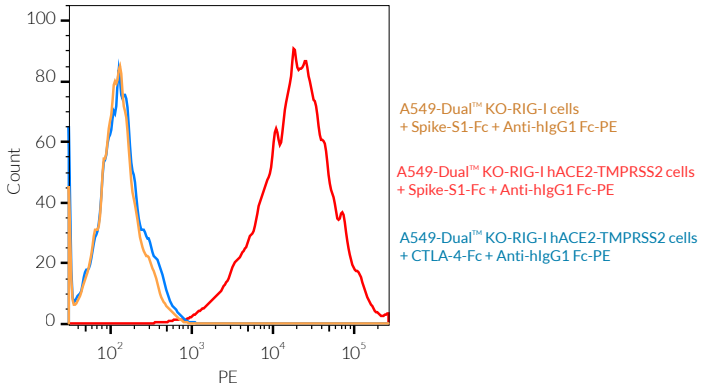

Figure 2: Surface expression of hACE2 by A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells.
~2x10^5 A549-Dual™ KO-RIG-I and A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells were incubated with 1 µg of Spike-S1-Fc or CTLA-4-Fc fusion proteins for 1h at 4°C. Cells were then washed and incubated with 0.5 µg of a goat anti-hIgG1-Fc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

Validation of *RIG-I* knock-out by PCR

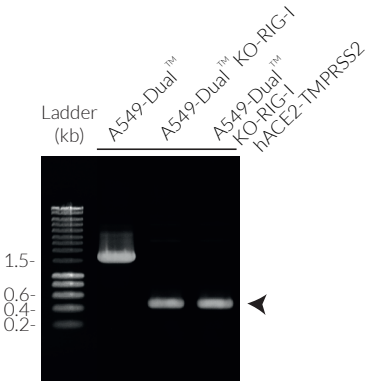

Figure 3: Validation of RIG-I KO in A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells.
The targeted *RIG-I* gene in A549-Dual™ (WT), A549-Dual™ KO-RIG-I, and A549-Dual™ KO-RIG-I ACE2-TMPRSS2 cells was amplified by PCR. A549-Dual™ KO-RIG-I, and A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells feature a biallelic deletion (arrow).

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Infection of A549-Dual™ -derived cells by SARS-CoV-2 Spike (G614) pseudotyped lentiviral particles

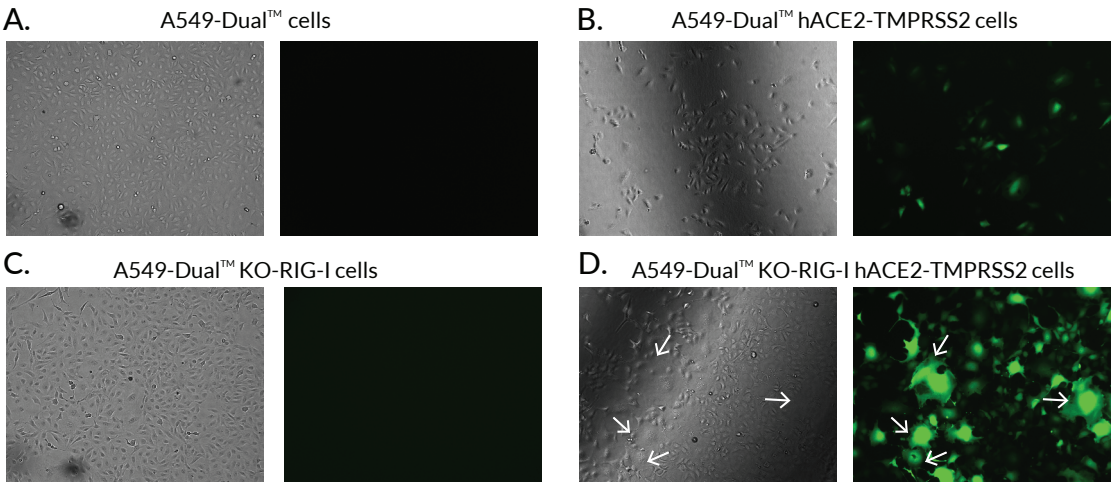

Figure 4: Infection of A549-Dual™ -derived cells by Spike (G614) pseudotyped lentiviral particles. ~1x10^4 **(A)** A549-Dual™ , **(B)** A549-Dual™ hACE2-TMPRSS2, **(C)** A549-Dual™ KO-RIG-I, and **(D)** A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells were cultured in the presence of SARS-CoV-2 Spike G614-variant pseudotyped GFP lentiviral particles. After 72h, the transduction efficiency of the Spike pseudotyped GFP particles was evaluated by fluorescence microscopy. Syncytia are indicated with white arrows.

Functional validation of IRF and NF-κB reporter systems in A549-Dual™ KO-RIG-I ACE2-TMPRSS2 cells

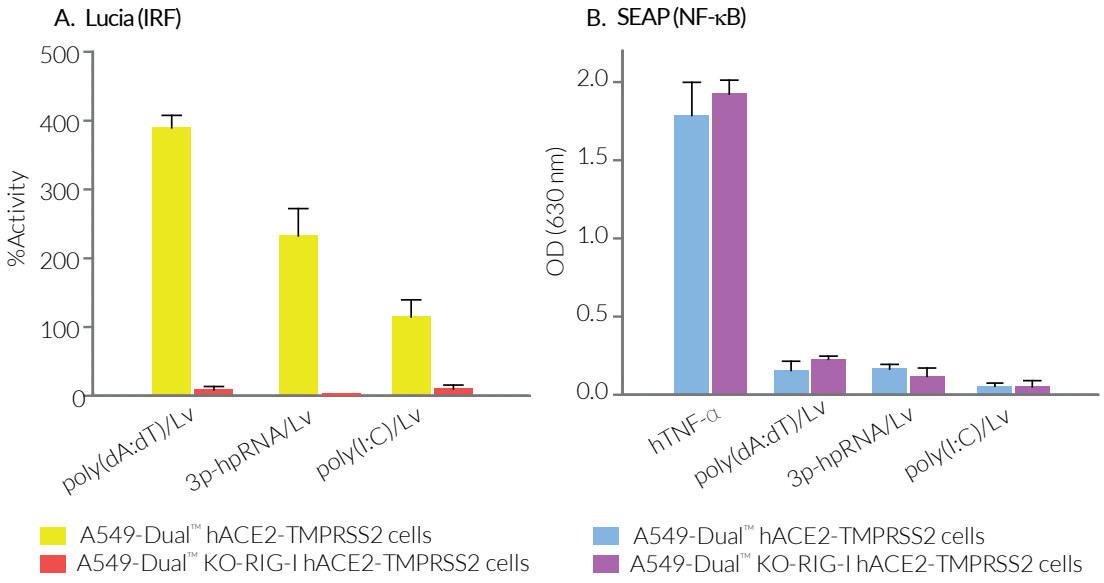

Figure 5: Activation of A549-Dual™ -derived cells. 5x10^5 A549-Dual™ hACE2-TMPRSS2 or A549-Dual™ KO-RIG-I hACE2-TMPRSS2 cells were incubated with 100 ng/ml poly(dA:dT) complexed with LyoVec™, 100 ng/ml 3p-hpRNA complexed with LyoVec™, 100 ng/ml poly(I:C) HMW complexed with LyoVec™, or 10ng/ml hTNF-**α**. After overnight incubation, IRF responses were assessed by measuring the Lucia luciferase bioluminescent activity in the supernatant using QUANTI-Luc™. Activity normalized on response upon incubation with 10^3 U/ml hIFN**β** is shown **(A)**. The NF-κB activity in A549-Dual™-derived cells was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Reading of optical density (OD) at 630 nm is shown **(B)**.

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