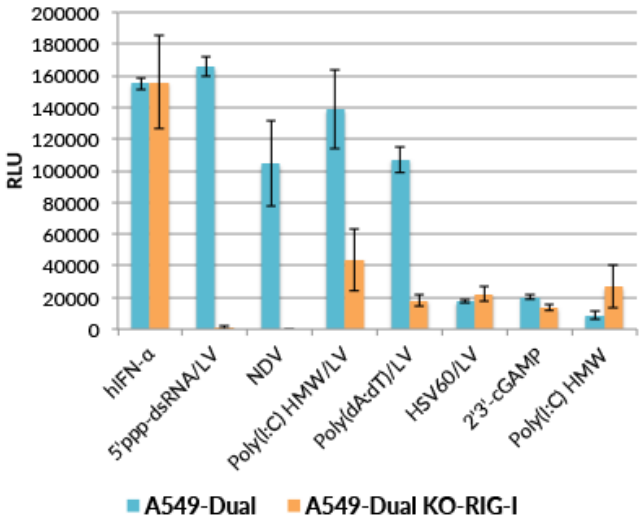
Validation data for A549-Dual™ KO-RIG-I cells

For research use only

Version # 16I21-MM

A549-Dual™ KO-RIG-I cells were generated from A549-Dual™ cells by stable knockout of the human RIG-I gene (also known as DDX58), which encodes the cytoplasmic double-stranded RNA (dsRNA) sensor RIG-I. These cells derive from the human A549 lung carcinoma cell line, which responds to ligands for the pattern recognition receptors (PRRs): RIG-I and the endosomal dsRNA sensor TLR3. A549-Dual™ and A549-Dual™ KO-RIG-I cells can be used to study RIG-I signaling. Both cell lines express two inducible reporter constructs that enable the simultaneous study of the NF-κB pathway, through monitoring the activity of SEAP, and the IRF pathway, through assessing the activity of the secreted Lucia luciferase. The IRF pathway (figure 1) and NF-κB pathway (figure 2) induction in A549-Dual™ KO-RIG-I cells in response to cytokines and diverse PRR ligands has been assessed. Interestingly, the IRF induction in response to human type I interferons (IFNs), and the NF-κB induction in response to human TNF-α, are unaffected by the knockout of the RIG-I gene. However, as expected, these cells respond weakly or do not respond to cytoplasmic double-stranded RNA (e.g. 5'ppp-dsRNA/LyoVec™ and poly(I:C)/LyoVec™) or to Newcastle disease virus (NDV), an RNA virus of the *Paramyxoviridae* family. The knockout of the RIG-I gene in these cells has been confirmed by PCR and sequencing.

IRF INDUCTION (Lucia luciferase reporter)



NF-κB INDUCTION (SEAP reporter)

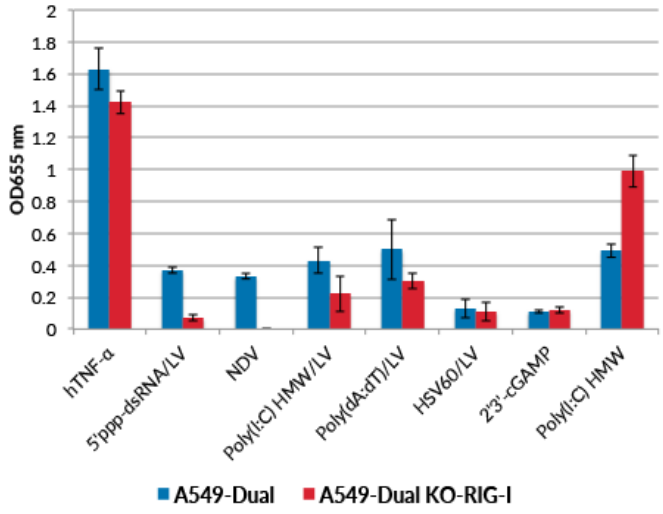


Figure 1: A549-Dual™ (parental cell line) and A549-Dual™ KO-RIG-I cells were stimulated with hIFN-α (1 x 10^4 U/ml), 5'ppp-dsRNA /LyoVec™ (1 µg/ml), inactivated NDV (1.86 x 10^5 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/ml), poly(dA:dT)/LyoVec™ (10 ng/ml), HSV60/LyoVec™ (1 µg/ml), 2'3'-cGAMP (10 µg/ml) and poly(I:C) HMW (1 µg/ml). After a 24h incubation, IRF activation was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent.

Figure 2: A549-Dual™ and A549-Dual™ KO-RIG-I cells were incubated with human TNF-α (1 ng/ml), 5'ppp-dsRNA /LyoVec™ (1 µg/ml), NDV (5 x 10^6 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/ml), poly(dA:dT)/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (100 ng/ml), HSV60/LyoVec™ (1 µg/ml), 2'3'-cGAMP (10 µg/ml) and poly(I:C) HMW (1 µg/ml). After a 24h incubation, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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