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

https://www.invivogen.com/a549-dual-ko-rigi

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Version 19K09-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. The knockout of the RIG-I gene has been confirmed by PCR, sequencing and Western blot (figure 1). 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-kB pathway, through monitoring the activity of SEAP, and the IRF pathway, through assessing the activity of the secreted Lucia luciferase. The IRF pathway (figure 2) and NF-κB pathway (figure 3) 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.

Western blot

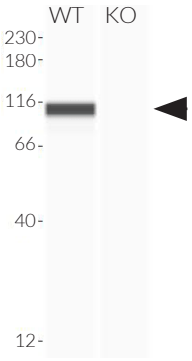
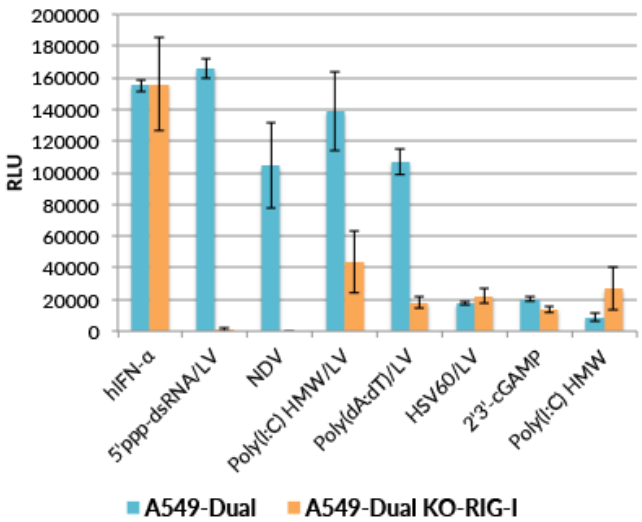


Figure 1: Validation of RIG-I knockout by Western blot (Wes™). Analysis of lysates from the A549-Dual™ (WT) and A549-Dual™ KO-RIG-I (KO) cells using Anti-RIG-I, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the RIG-I protein (102 KDa).

IRF INDUCTION (Lucia luciferase reporter)



NF-kB INDUCTION (SEAP reporter)

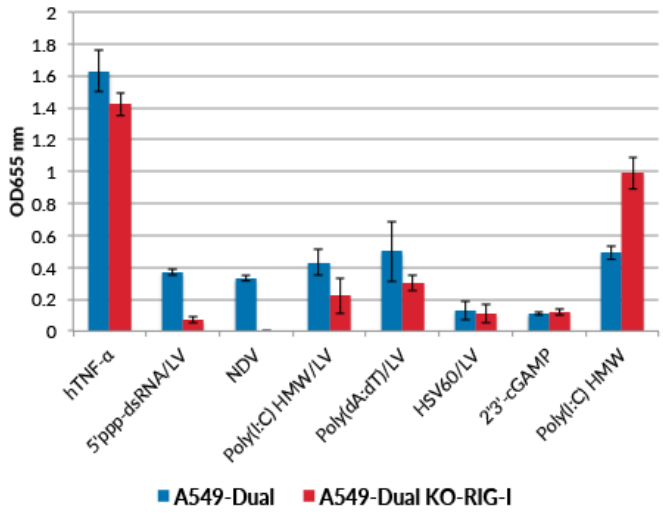


Figure 2: A549-Dual™ (parental cell line) and A549-Dual™ KO-RIG-I cells were stimulated with hIFN-α (1 x 10^4 U/ml), 5'ppp-dsRNA /LyoVec™ (1 µg/ml), inactivated NDV (1.86 x 10^5 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/ml), poly(dA:dT)/LyoVec™ (10 ng/ml), HSV60/LyoVec™ (1 µg/ml), 2'3'-cGAMP (10 µg/ml) and 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 3:** A549-Dual™ and A549-Dua™ 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-kB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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