Validation data for A549-Dual™ KO-MDA5 cells

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Version 19K09-MM

A549-Dual™ KO-MDA5 cells were generated from A549-Dual™ cells by stable knockout of the human MDA-5 gene (melanoma-differentiation-associated gene 5, also known as Ifih1 or Helicard), which encodes the cytoplasmic RNA helicase that plays an important role in antiviral responses. The knockout of the MDA5 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): MDA-5, RIG-I and the endosomal dsRNA sensor TLR3. A549-Dual™ and A549-Dual™ KO-MDA5 cells can be used to study MDA-5 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 interferon regulatory factor (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-MDA5 cells in response to type I interferons (IFNs) and diverse PRR ligands has been assessed.

Western blot

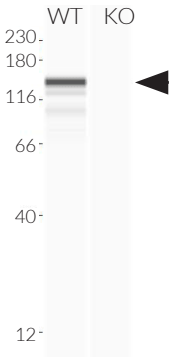
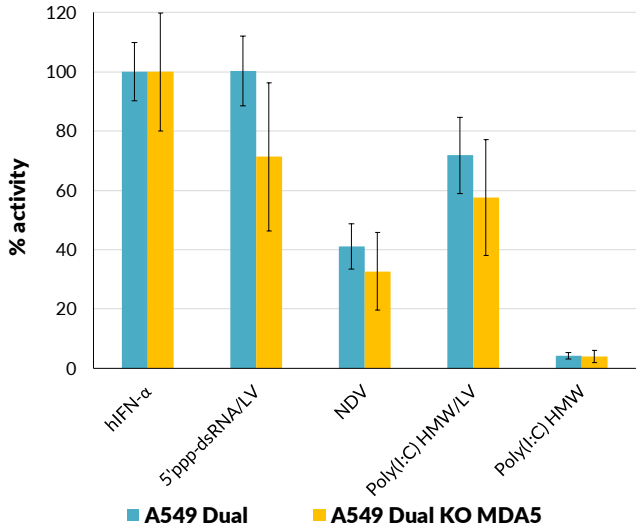


Figure 1: Validation of MDA-5 knockout by Western blot (Wes™). Analysis of lysates from the A549-Dual™ (WT) and A549-Dual™ KO-MDA-5 (KO) cells using Anti-MDA-5, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the MDA-5 protein (117 KDa).

IRF INDUCTION (Lucia luciferase reporter)



NF-κB INDUCTION (SEAP reporter)

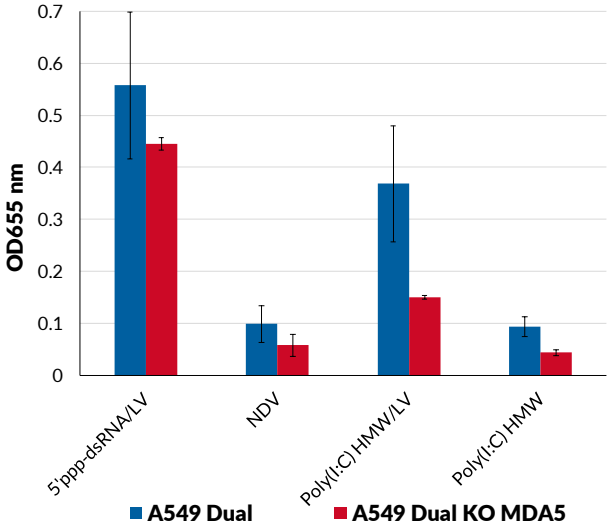


Figure 2: A549-Dual™ (parental cell line) and A549-Dual™ KO-MDA5 cells were stimulated with hIFN-α (1 x 10^4 U/ml), 5'ppp-dsRNA /LyoVec™ (1 µg/ml), inactivated Newcastle disease virus (NDV; 5 x 10^5 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/ml), poly(dA:dT)/LyoVec™ (10 ng/ml) and poly(I:C) HMW (1 µg/ml). 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. The IRF induction of each ligand is expressed relative to that of hIFN-α at 1 x 10^4 U/ml (taken as 100%). **Figure 3:** A549-Dual™ and A549-Dual™ KO-MDA5 cells were incubated with 5'ppp-dsRNA /LyoVec™ (1 µg/ml), NDV (5 x 10^6 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/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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