Validation data for A549-Dual™ KO-MAVS cells

http://www.invivogen.com/a549-dual-ko-mavs

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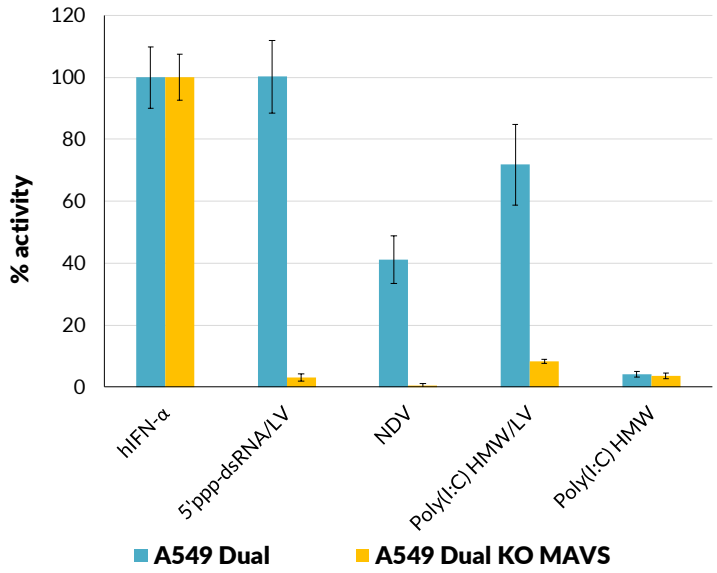
Version # 17H30-MM

A549-Dual™ KO-MAVS cells were generated from A549-Dual™ cells by stable knockout of the human MAVS gene (also known as IPS-1, CARDIF, VISA), which encodes the adaptor protein MAVS that plays a critical role in the immune response to viral infection. These cells derive from the human A549 lung carcinoma cell line, which responds to ligands for the pattern recognition receptors (PRRs): RIG-I, MDA-5 and the endosomal dsRNA sensor TLR3. A549-Dual™ and A549-Dual™ KO-MAVS cells can be used to study MAVS 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 1) and NF-κB pathway (figure 2) induction in A549-Dual™ KO-MAVS cells in response to type I interferons (IFNs) and diverse PRR ligands has been assessed. Interestingly, the IRF induction in response to human IFNs was unaffected by the knockout of the MAVS 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 MAVS gene in these cells has been confirmed by PCR and sequencing.

IRF INDUCTION (Lucia luciferase reporter)



NF-κB INDUCTION (SEAP reporter)

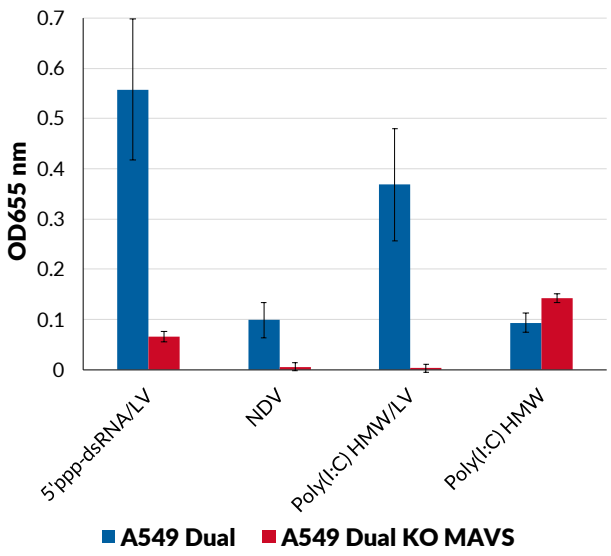


Figure 1: A549-Dual™ (parental cell line) and A549-Dual™ KO-MAVS cells were stimulated with hIFN-α (1 x 10^4 U/ml), 5'ppp-dsRNA/LyoVec™ (1 µg/ml), inactivated NDV (5 x 10^5 U/ml), poly(I:C) HMW/LyoVec™ (100 ng/ml), poly(dA:dT)/LyoVec™ (10 ng/ml) and poly(I:C) HMW (1 µg/ml). After a 24h incubation, IRF 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 2: A549-Dual™ and A549-Dual™ KO-MAVS 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.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com