

Validation data for THP1-Dual™ KO-MAVS cells

<https://www.invivogen.com/thp1-dual-ko-rlr>

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Version 21C25-NJ

THP1-Dual™ KO-MAVS cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the MAVS gene, as verified by PCR and Western blot (Figure 1). These cells feature two reporter genes allowing the simultaneous study of the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). As expected, the IRF and NF-κB responses are abolished in THP1-Dual™ KO-MAVS cells upon incubation with 3p-hpRNA, a RIG-I-specific ligand (Figures 2-3). These cells retain the ability to respond to other NF-κB activating ligands such as recombinant human TNF-α, as well as IRF-activating ligands such as type I interferons (IFNs) (Figures 2-3).

Validation of MAVS knockout

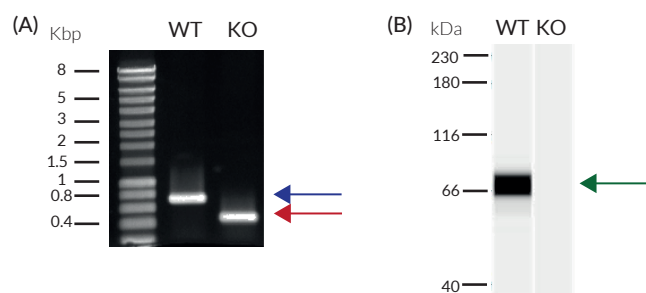


Figure 1: Validation of MAVS KO.

(A) The targeted MAVS region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-MAVS (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-MAVS cells feature a frameshift deletion, causing an early stop codon and inactivation of MAVS. (B) Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-MAVS (KO) cells were analyzed using an anti-human MAVS antibody (green arrow), followed by a HRP-conjugated anti-rabbit secondary antibody (WES assay). As expected, a band was detected at ~80 Da in the WT cells only.

Functional validation of MAVS knockout (IRF response)

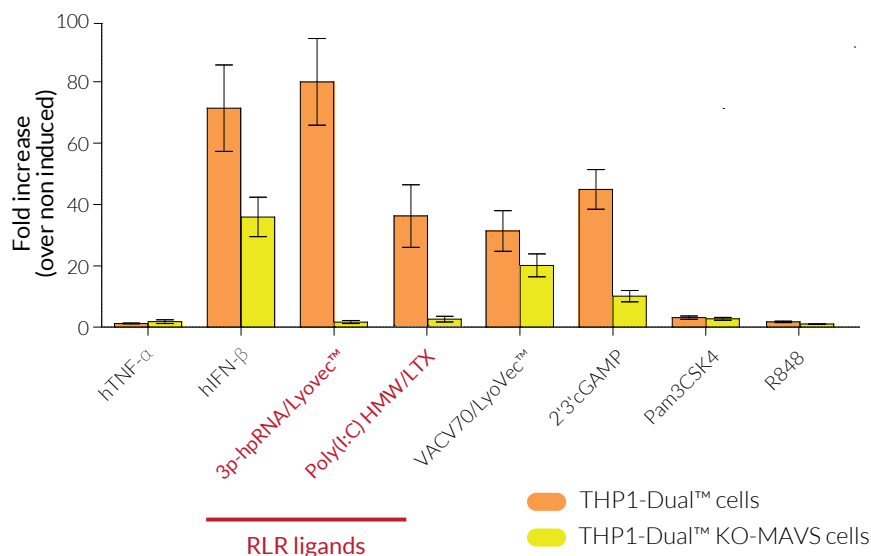


Figure 2: IRF responses in THP1-Dual™-derived cells.

THP1-Dual™ (WT) and THP1-Dual™ KO-MAVS cells were incubated with 1 ng/ml human TNF-α (hTNF-α), or 10⁴ U/ml human IFN-β (hIFN-β), 300 ng/ml 3p-hpRNA/Lyovec, 1 µg/ml Poly(I:C) HMW/LTX, 1 µg/ml VACV-70/Lyovec, 3 µg/ml 2'3'-cGAMP, 3 ng/ml Pam3CSK4, or 1 µg/ml R848. After overnight incubation, the IRF response was assessed by measuring Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are shown as a fold change (mean ± SEM) over non-induced cells.

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Functional validation of MAVS knockout (NF- κ B response)

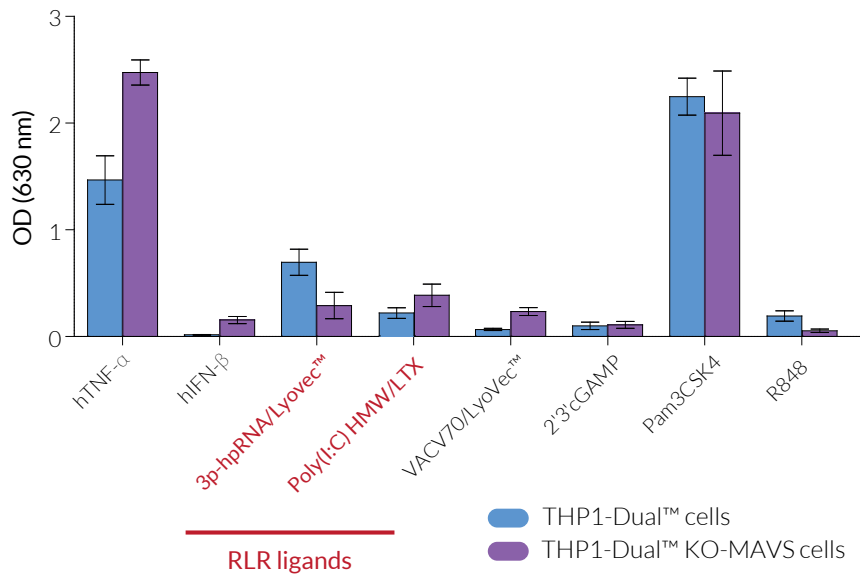


Figure 3: NF- κ B responses in THP1-Dual™-derived cells.

THP1-Dual™ (WT) and THP1-Dual™ KO-MAVS cells were incubated with 1 ng/ml human TNF- α (hTNF- α), or 10^4 U/ml human IFN- β (hIFN- β), 300 ng/ml 3p-hpRNA/Lyovec, 1 μ g/ml Poly(I:C) HMW/LTX, 1 μ g/ml VACV-70/Lyovec, 3 μ g/ml 2'3'-cGAMP, 3 ng/ml Pam3CSK4, or 1 μ g/ml R848. After overnight incubation, the NF- κ B activity was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean \pm SEM).

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