Validation data for THP1-Dual™ KO-RIG-I cells

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Version 21C25-NJ

THP1-Dual™ KO-RIG-I cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the *RIG-I* 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 or severely diminished in THP1-Dual™ KO-RIG-I cells upon incubation with 3p-hpRNA, a RIG-I-specific ligand, or with Poly(I:C) HMW, a RLR inducer **(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 *RIG-I* knockout

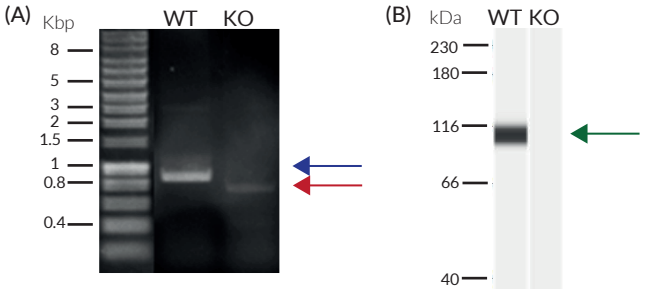

Figure 1: Validation of RIG-I KO.
(A) The targeted MAVS region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-RIG-I (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-RIG-I cells feature a frameshift deletion, causing an early stop codon and inactivation of RIG-I. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-RIG-I (KO) cells were analyzed using an anti-human RIG-I antibody (green arrow), followed by a HRP-conjugated anti-rabbit secondary antibody (WES assay). As expected, a band was detected at ~102 Da in the WT cells only.

Functional validation of *RIG-I* knockout (IRF response)

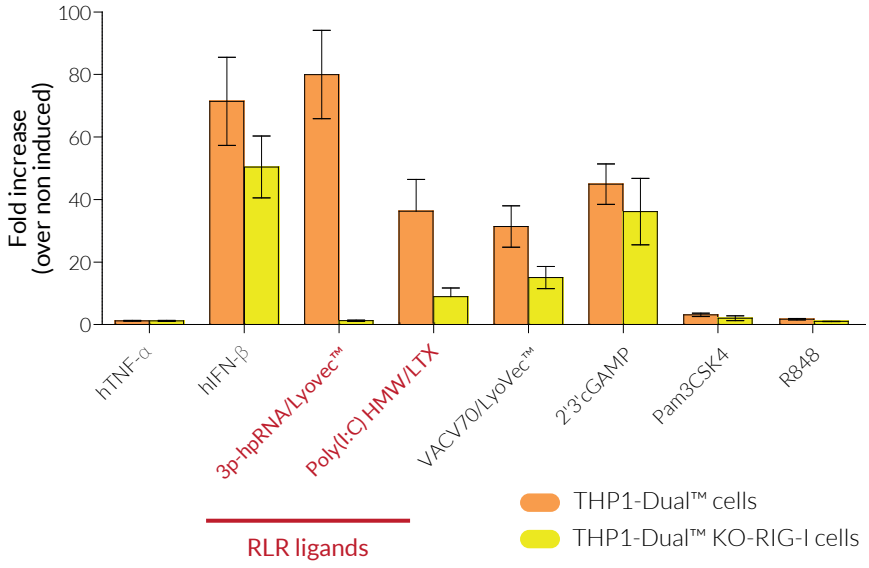

Figure 2: IRF responses in THP1-Dual™-derived cells.
THP1-Dual™ (WT) and THP1-Dual™ KO-RIG-I cells were incubated with 1 ng/ml human TNF-α (hTNF-α), or 10^4 U/ml human IFN-β (hIFN-β), 300 ng/ml 3p-hpRNA/Lyovec, 1 µg/ml Poly(I:C) HMW/LTX, 1 µg/ml VACV-70/Lyovec, 3 µg/ml 2'3'-cGAMP, 3 ng/ml Pam3CSK4, or 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 *RIG-I* knockout (NF-κB response)

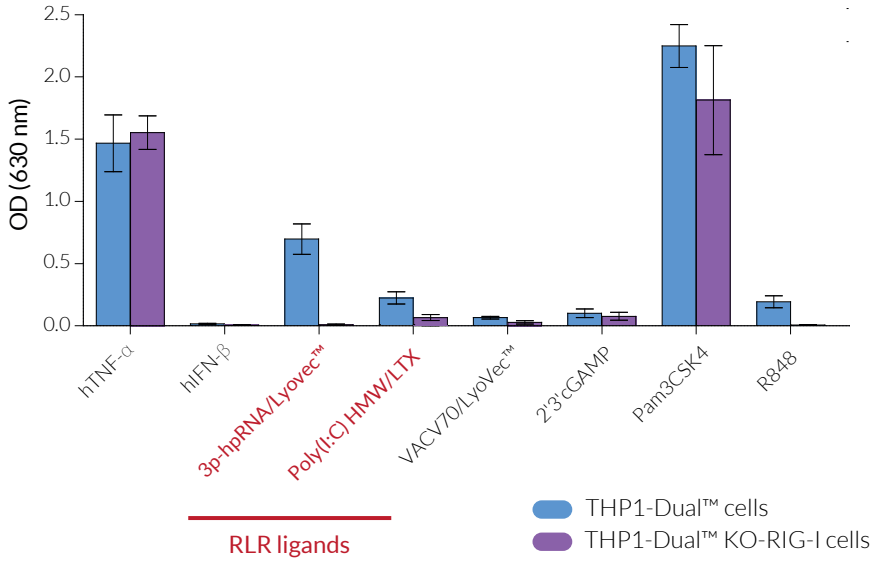

Figure 3: NF-κB responses in THP1-Dual™-derived cells.
THP1-Dual™ (WT) and THP1-Dual™ KO-RIG-I cells were incubated with 1 ng/ml human TNF-α (hTNF-α), or 10^4 U/ml human IFN-β (hIFN-β), 300 ng/ml 3p-hpRNA/Lyovec, 1 µg/ml Poly(I:C) HMW/LTX, 1 µg/ml VACV-70/Lyovec, 3 µg/ml 2'3'-cGAMP, 3 ng/ml Pam3CSK4, or 1 µg/ml R848. After overnight incubation, the NF-κB 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 ± SEM).