

Validation data for THP1-Dual™ KO-IRF3 cells

<https://www.invivogen.com/thp1-dual-koirf3>

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Version 19E07-NJ

THP1-Dual™ KO-IRF3 cells were generated from the THP1-Dual™ cell line through the stable knockout of the *IRF3* gene. Both alleles harbor a 70-base pair deletion in the fourth *IRF3* exon causing the loss of IRF3 expression, as verified by PCR (Figure 1) and western blot (Figure 2). 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). Lucia luciferase and SEAP activities are readily assessable in the cell culture supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution detection reagents, respectively. As expected, IRF and NF-κB responses are severely impaired when the KO-IRF3 cells are incubated with STING agonists, such as 2'3'-cGAMP, which do not require transfection to access the cytosol (Figure 3). However, differential responses are observed when using RNA agonists with transfection reagents (Figure 4). Such observations could highlight overlapping nucleic acid-sensing or regulatory mechanisms. THP1-Dual™ KO-IRF3 cells retain a full ability to respond to cytokines such as type I interferons (IFN-α and IFN-β) and TNF-α, for which signaling is IRF3-independent (Figure 4). However, their response to lipopolysaccharide (LPS), which is partially TBK1/IRF3-dependent, is decreased as compared to THP1-Dual™ cells (Figure 5).

PCR

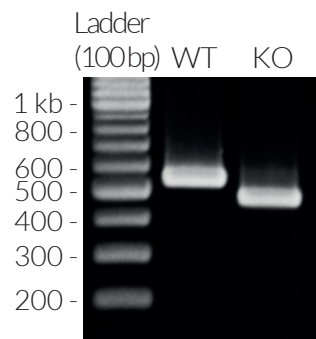


Figure 1: Validation of IRF3 knockout by PCR. Amplification of the targeted *IRF3* region in THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO) cells. THP1-Dual™ KO-IRF3 cells feature a biallelic deletion of 70 base pairs (arrow).

Western blot

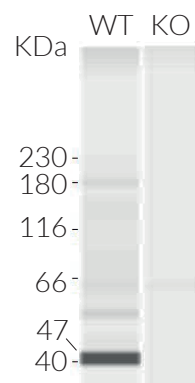


Figure 2: Validation of IRF3 knockout by western blot (WES by Protein Simple). Analysis of lysates from the THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO) using Anti-IRF3 (10 µg/ml), followed by HRP-conjugated anti-mouse secondary antibody (undiluted). The arrow indicates the expected band for the IRF3 protein (47 kDa).

Functional validation of TBK1 knockout

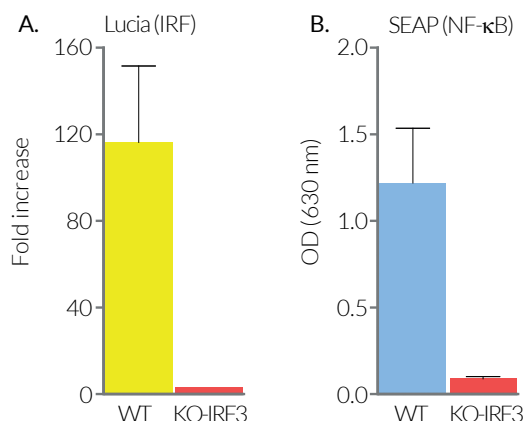


Figure 3: 2'3'-cGAMP-induced IRF and NF-κB responses in THP1-Dual™-derived cells. 2×10^5 THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO-IRF3) cells were incubated with 30 µg/ml 2'3'-cGAMP (STING agonist). After overnight incubation, IRF (A) and NF-κB (B) responses were assessed by measuring the activity of Lucia luciferase and SEAP in the supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively. Activity fold increase over non-induced cells (Lucia luciferase readout) or reading of optical density (OD) at 630 nm (SEAP readout) are shown.

TECHNICAL SUPPORT

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Evaluation of IRF and NF- κ B responses upon RNA transfection

3p-hpRNA and 5'ppp-dsRNA are two RIG-I agonists that must be complexed with a transfection reagent to enter the cytoplasm. The weak agonist 5'ppp-dsRNA loses its ability to induce IRF (Lucia) in KO-IRF3 cells when complexed with LyoVec™ or LTX (A, C). On the contrary, the IRF (Lucia) response is unexpectedly increased when using the highly potent agonist 3p-hpRNA complexed to LyoVec™ or LTX (A, C). Surprisingly, with either agonist, the NF- κ B (SEAP) response is barely affected (B, D). These data suggest that the use of different agonists and transfection reagents could highlight overlapping RNA-sensing or regulatory mechanisms.

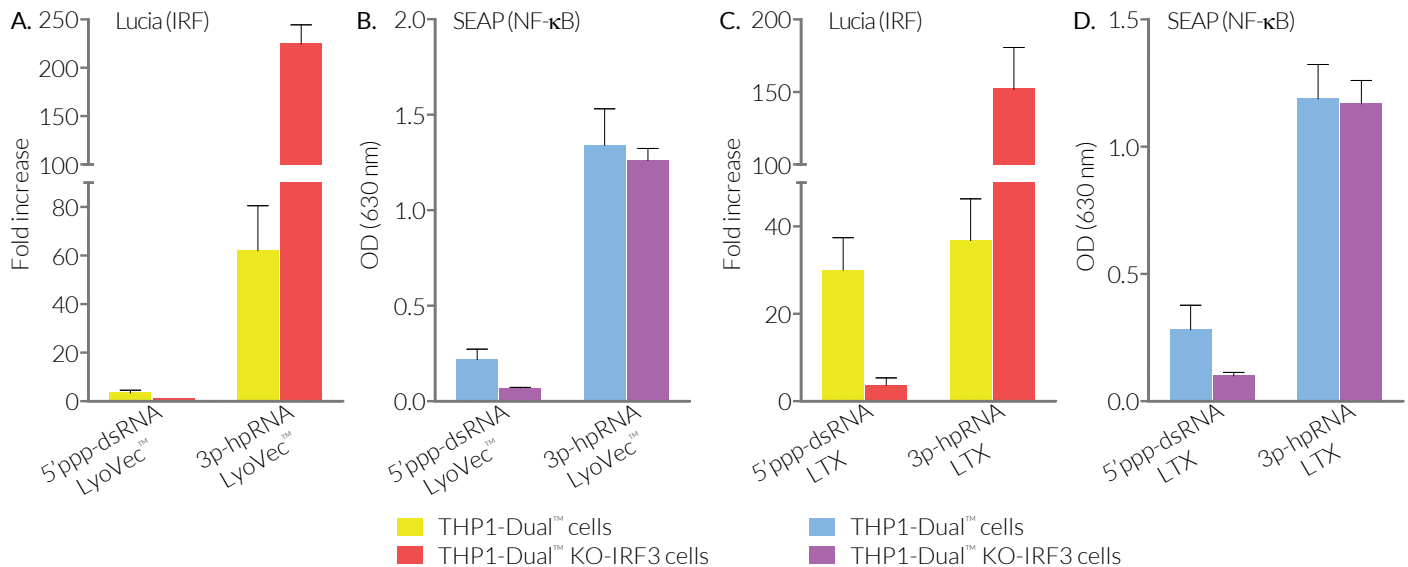


Figure 4: RNA-induced IRF and NF- κ B responses in THP1-Dual™-derived cells. 2×10^5 THP1-Dual™ or THP1-Dual™ KO-IRF3 cells were transfected with 1 μ g/ml 3p-hpRNA or 1 μ g/ml 5'ppp-dsRNA complexed with LyoVec™ (A, B) or LTX (C, D). After overnight incubation, IRF response was assessed by measuring bioluminescent activity of the Lucia luciferase in the supernatant using QUANTI-Luc™. Activity fold increase over non-transfected cells is shown (A, C). The NF- κ B activity in THP1-Dual™-derived cells was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Reading of optical density (OD) at 630 nm is shown (B, D).

Evaluation of IRF and NF- κ B responses upon cytokine or LPS induction

THP1-Dual™ KO-IRF3 cells retain a full ability to respond to cytokines such as type I interferons (IFN- α and IFN- β) and TNF- α , for which signaling is IRF3-independent and driven by STAT1/STAT2/IRF9 and NF- κ B, respectively. Interestingly, the IRF (Lucia) response to type I IFNs is increased, suggesting a partial negative regulation operated by IRF3 or by an upstream signaling molecule, such as TBK1. As expected, the NF- κ B (SEAP) response to lipopolysaccharide (LPS), which is partially TBK1/IRF3-dependent, is decreased as compared to the response in THP1-Dual™ cells.

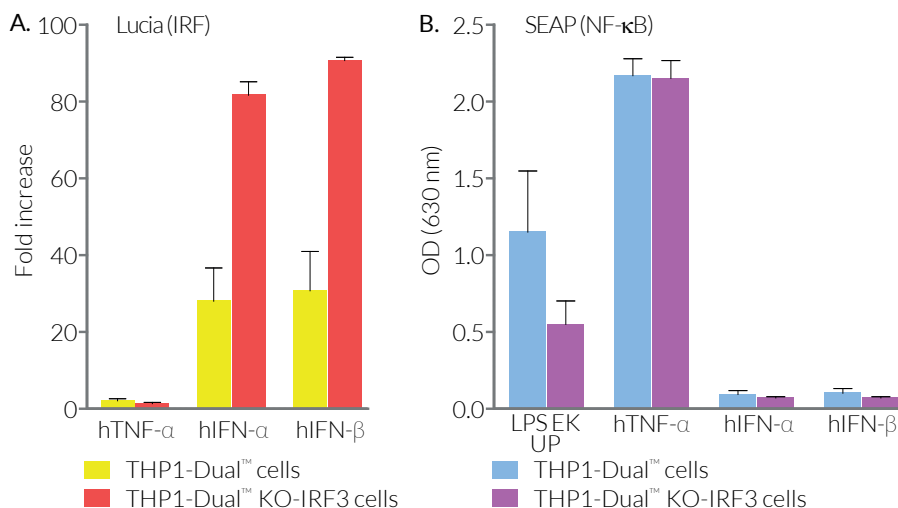


Figure 5: Cytokine or LPS-induced IRF and NF- κ B responses in THP1-Dual™-derived cells. 2×10^5 THP1-Dual™ or THP1-Dual™ KO-IRF3 cells were incubated with 10 ng/ml human TNF- α (hTNF- α), 10^4 U/ml human IFN- α (hIFN- α), 10^4 U/ml human IFN- β (hIFN- β), or 1 μ g/ml LPS EK Ultrapure (UP). After overnight incubation, IRF (A) and NF- κ B (B) responses were assessed by measuring the activity of Lucia luciferase and SEAP in the supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively. Activity fold increase over non-induced cells (Lucia luciferase readout) or reading of optical density (OD) at 630 nm (SEAP readout) are shown.

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