Validation data for THP1-Dual™ KO-IRF3 cells

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THP1-Dual™ KO-IRF3 cells were generated from the THP1-Dual™ cell line through the stable knockout of the *IRF3* gene, 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, different responses are observed when using RNA agonists with transfection reagents **(Figure 4)**. THP1-Dual™ KO-IRF3 cells retain the 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

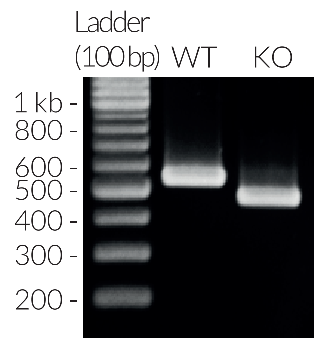

Figure 1: Validation of IRF3 knockout by PCR. Amplification of the targeted *IRF3* region in THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO) cells. THP1-Dual™ KO-IRF3 cells feature a biallelic deletion of 70 base pairs (arrow).

Western blot

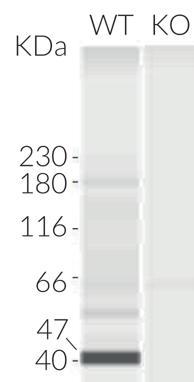

Figure 2: Validation of IRF3 knockout by Western blot (Wes™). Analysis of lysates from the THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO) using Anti-IRF3 (10 μg/ml), followed by HRP-conjugated anti-mouse secondary antibody (undiluted). The arrow indicates the expected band for the IRF3 protein (47 Da).

Functional validation of IRF3 knockout

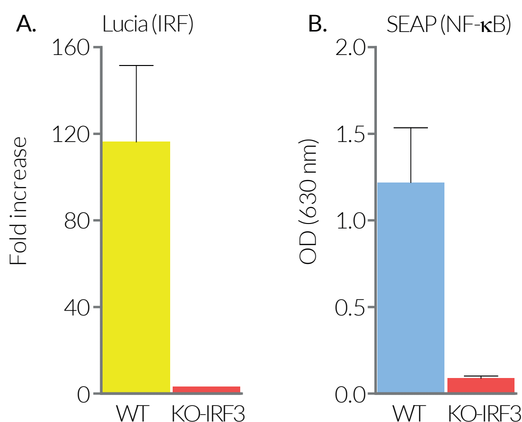

Figure 3: 2'3'-cGAMP-induced IRF and NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ (WT) and THP1-Dual™ KO-IRF3 (KO-IRF3) cells were incubated with 30 μg/ml 2'3'-cGAMP (STING agonist). After overnight incubation, IRF (A) and NF-κB (B) responses were assessed by measuring the activity of Lucia 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.

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Evaluation of IRF and NF-κB reponses 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-κB (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.

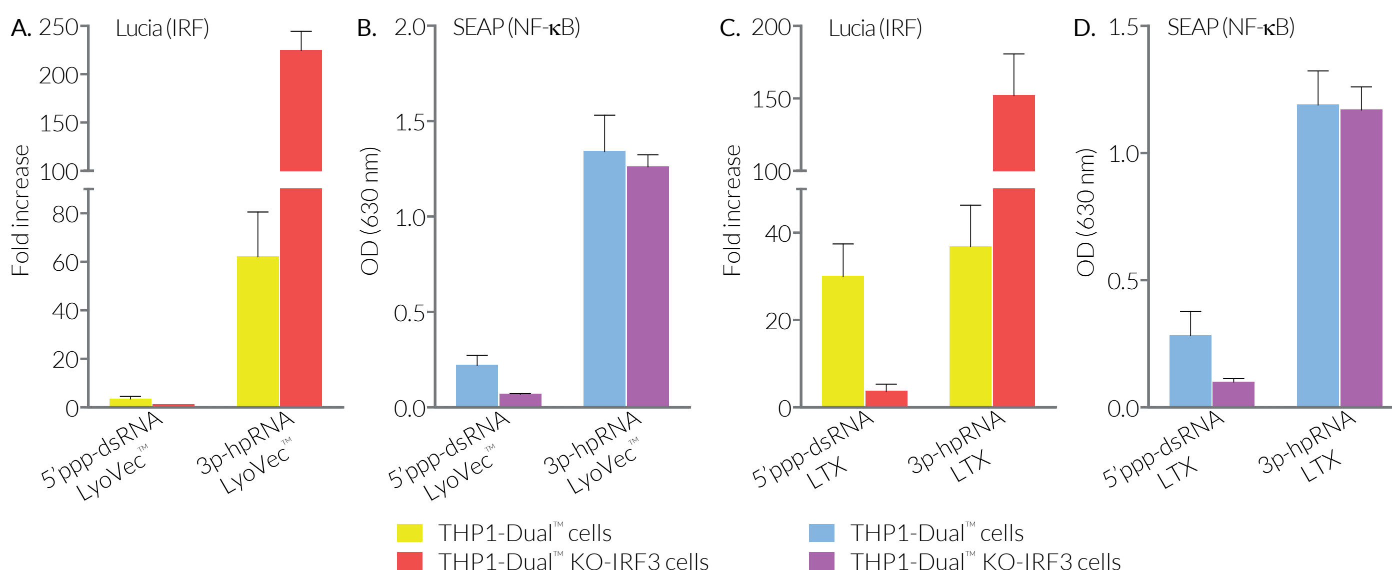

Figure 4: RNA-induced IRF and NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ or THP1-Dual™ KO-IRF3 cells were transfected with 1 µg/ml 3p-hpRNA or 1 µg/ml 5'ppp-dsRNA complexed with Lyovec™ (A, B) or LTX (C, D). After overnight incubation, IRF response was assessed by measuring bioluminescent activity of the Lucia luciferase in the supernatant using QUANTI-Luc™. Activity fold increase over non-transfected cells is shown (A, C). The NF-κB 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-κB reponses 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-κB, 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-κB (SEAP) response to lipopolysaccharide (LPS), which is partially TBK1/IRF3-dependent, is decreased as compared to the response in THP1-Dual™ cells.

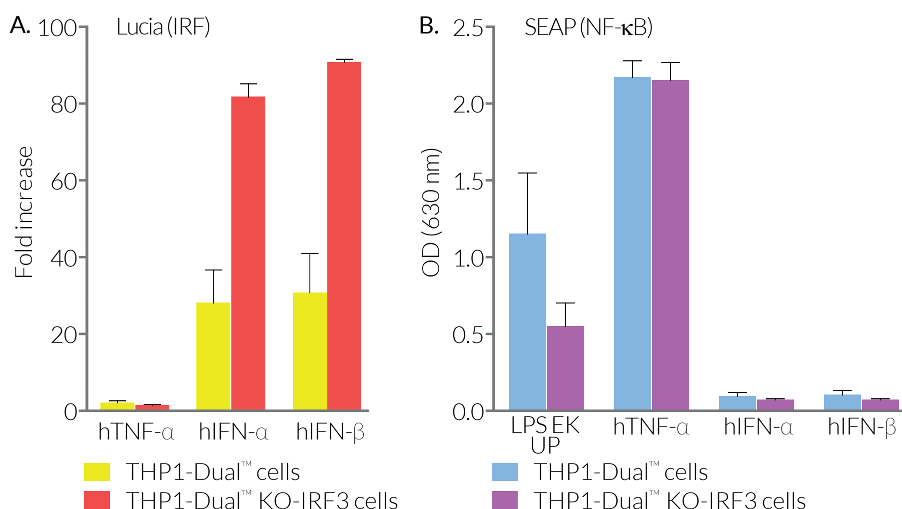

Figure 5: Cytokine or LPS-induced IRF and NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ or THP1-Dual™ KO-IRF3 cells were incubated with 10 ng/ml human TNF-α (hTFN-α), 10^4 U/ml human IFN-α (hIFN-α), 10^4 U/ml human IFN-β (hIFN-β), or 1 µg/ml LPS EK Ultrapure (UP). After overnight incubation, 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.

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