Validation data for THP1-Dual™ KO-IRF5 cells

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THP1-Dual™ KO-IRF5 cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the *IRF5* gene, as verified by PCR and Western blot **(Figure 1)**. These cells feature two reporter genes allowing the simultaneous study of NF-κB- and IRF-induced responses by monitoring the SEAP (secreted embryonic alkaline phosphatase) **(Figure 2)** and Lucia luciferase activities, respectively **(Figure 3)**.

Validation of *IRF5* knockout

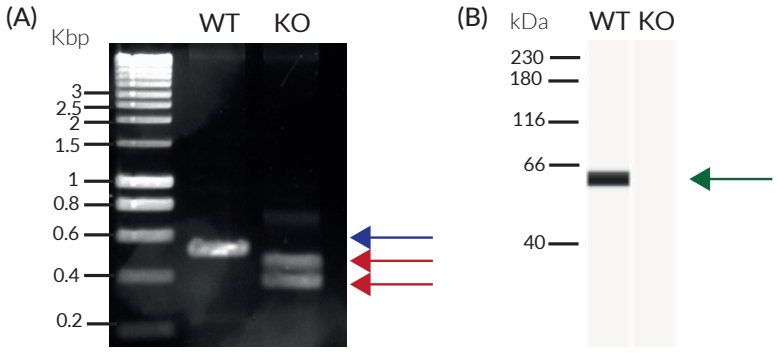

Figure 1: Validation of IRF5 KO. (A) The targeted IRF5 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-IRF5 (KO; red arrows) cells was amplified by PCR. THP1-Dual™ KO-IRF5 cells feature two different frameshift deletions for each allele, causing an early stop codon and inactivation of IRF5. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-IRF5 (KO) cells were analyzed using an anti-human IRF5 antibody (green arrow), followed by an HRP-conjugated anti-rabbit secondary antibody (WES assay). As expected a band was detected at ~56 kDa in the WT cells only.

Functional validation of *IRF5* knockout (NF-κB response)

IRF5 has been implicated downstream of several pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). As expected, NF-κB-mediated responses are significantly reduced in THP1-Dual™ KO-IRF5 upon incubation with TLR4 (LPS-EK UP) and TLR8 (CL075 and R848) agonists, when compared to their parental cell line,THP1-Dual™.

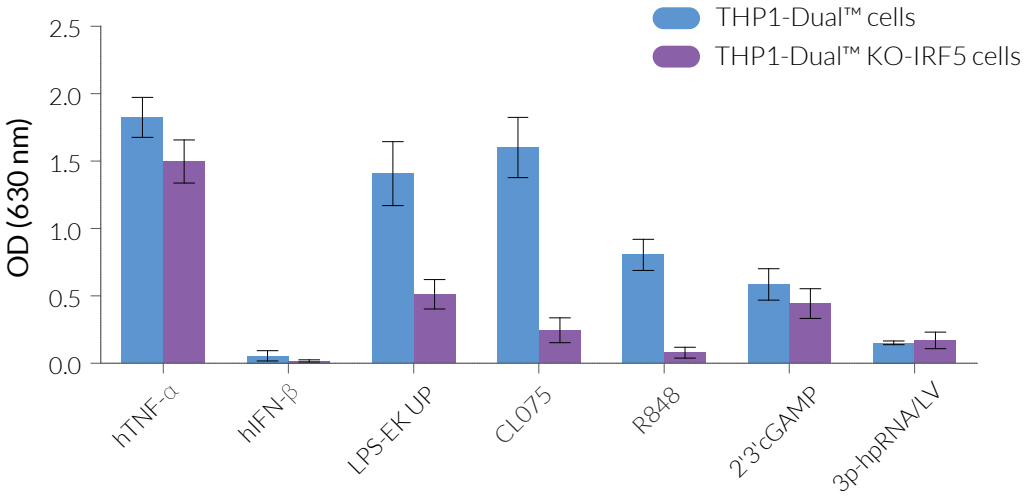

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF5 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 3 µg/ml CL075 (TLR7/8 agonist), 3 µg/ml R848 (TLR7/8 agonist), 30 µg/ml 2'3'-cGAMP (STING agonist), and 300 ng/ml 3p-hpRNA complexed with LyoVec™ (LV; RIG-I agonist). After overnight incubation, the activation of NF-κB was assessed by measuring the activity of SEAP in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM).

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Functional validation of *IRF5* knockout (IRF response)

Differentiation of THP1-Dual™ cells using phorbol 12-myristate 13-acetate (PMA) significantly increases their senitivity to IRF-inducers, such as LPS (i.e. TLR4-dependent IRF activation). There is no notable difference in the IRF-mediated responses in THP1-Dual™ KO-IRF5 cells and their parental cell line, upon incubation with TLR4, RIG-I, or STING agonists.

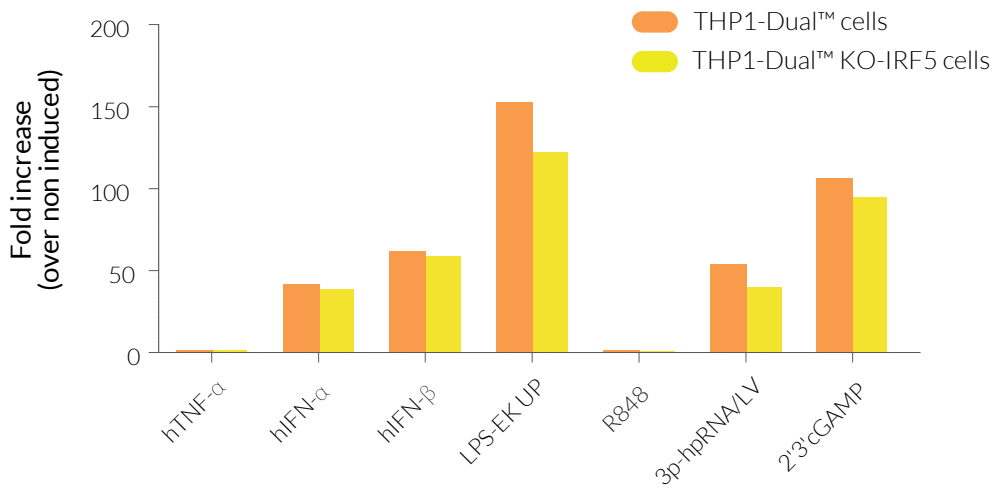

Figure 3: IRF responses in PMA-differentiated THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF5 cells were pretreated with PMA (Phorbol 12-myristate 13-acetate; 10 ng/ml for 3 hours). After 3 days recovery, the cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-α or hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 1 µg/ml R848 (TLR7/8 agonist), 300 ng/ml 3p-hpRNA complexed with LyoVec™ (LV; RIG-I agonist), and 10 µg/ml 2'3'-cGAMP (STING agonist). After overnight incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown as a fold increase over non-induced cells (Lucia luciferase readout).