Validation data for THP1-Dual™ KO-IRF7 cells

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THP1-Dual™ KO-IRF7 cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the *IRF7* gene, as verified by Western blot **(Figure 1)**. These cells feature two reporter genes allowing the simultaneous study of of NF-κB- and IRF-induced responses by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. We observe an impact of the IRF-7 knock-out on NF-κB-mediated responses **(Figure 2)**, and no effect on IRF-mediated responses **(Figure 3)**, in THP1-Dual™ KO-IRF7 cells upon incubation with TLR agonists (LPS-EK, R848) when compared to the THP1-Dual™ cells.

Validation of *IRF7* knockout

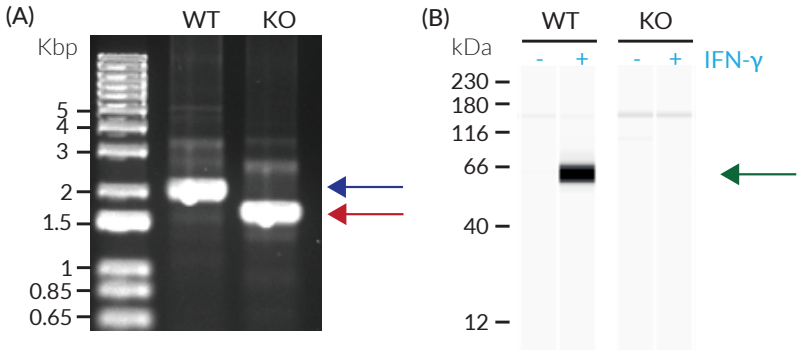

Figure 1: Validation of IRF7 KO. (A) The targeted IRF7 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-IRF7 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-IRF7 cells feature a frameshift deletion, causing an early stop codon and inactivation of IRF7. **(B)** THP1-Dual™ (WT) and THP1-Dual™ KO-IRF7 (KO) cells were treated with 20 ng/ml interferon (IFN)-γ to induce the expression of IRF7. Lysates from non-treated (-) and treated (+) WT and KO cells were analyzed using an anti-human IRF7 antibody, followed by an HRP-conjugated anti-rabbit secondary antibody (WES™ assay). As expected, a band was detected in the IFN-γ treated WT cells only (green arrow). The predicted molecular weight of IRF7 is ~66 kDa.

Functional validation of *IRF7* knockout (NF-κB response)

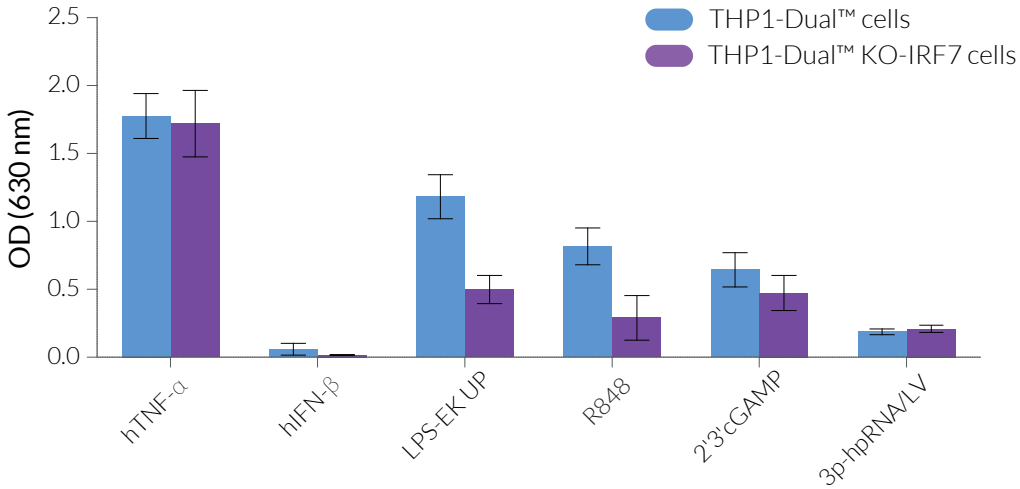

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF7 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 3 µg/ml R848 (TLR7/8 agonist), 30 µg/ml 2'3'-cGAMP (STING agonist), and 1 µg/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 *IRF7* knockout (IRF response)

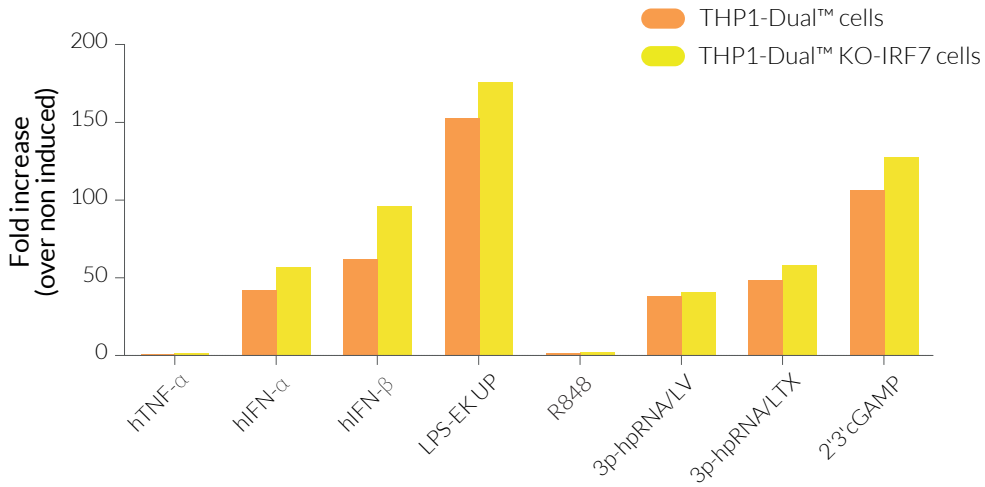

Figure 3: IRF responses in PMA-differentiated THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF7 cells were pretreated with PMA (Phorbol 12-myristate 13-acetate; 10 ng/ml for 3 hours). After 3 days recovery, the cells were incubated with 1 ng/ml human (h) TNF-α (NF-κB-SEAP positive control), 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), 100 ng/ml 3p-hpRNA complexed with either LyoVec™ (LV) or LTX (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).