Validation data for THP1-Dual™ KO-IRF1 cells

https://www.invivogen.com/thp1-dual-ko-irf1

For research use only

Version 21I23-NJ

THP1-Dual™ KO-IRF1 cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the *IRF1* gene, as verified by PCR and 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. There are no notable differences in the NF-κB- and IRF-mediated responses in THP1-Dual™ KO-IRF1 cells when compared to their parental cell line **(Figures 2 and 3)**.

Validation of *IRF1* knockout

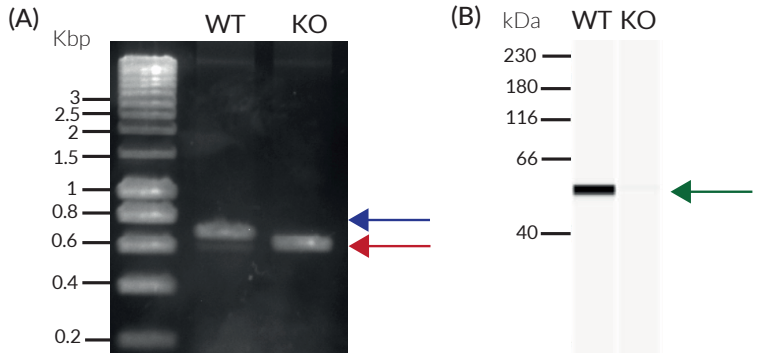

Figure 1: Validation of IRF1 KO. (A) The targeted IRF1 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-IRF1 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-IRF1 cells feature a frameshift deletion, causing an early stop codon and inactivation of IRF1. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-IRF1 (KO) cells were analyzed using an anti-human IRF1 antibody (green arrow), followed by an HRP-conjugated anti-rabbit secondary antibody (WES™ assay). As expected a band was detected at ~48 kDa in the WT cells only.

Functional validation of *IRF1* knockout (NF-κB response)

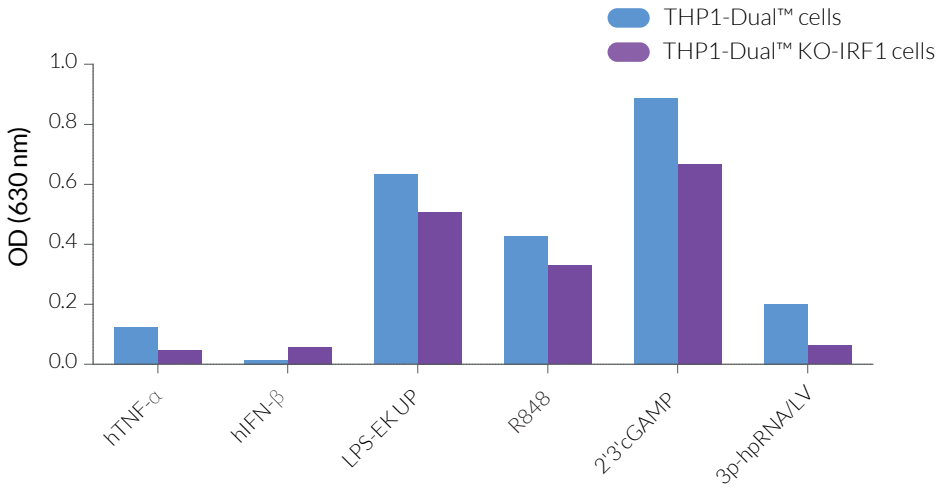

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF1 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 3 µg/ml R848 (TLR7/8 agonist), 300 ng/ml 2'3'-cGAMP (STING agonist), and 30 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).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com

Functional validation of *IRF1* knockout (IRF response)

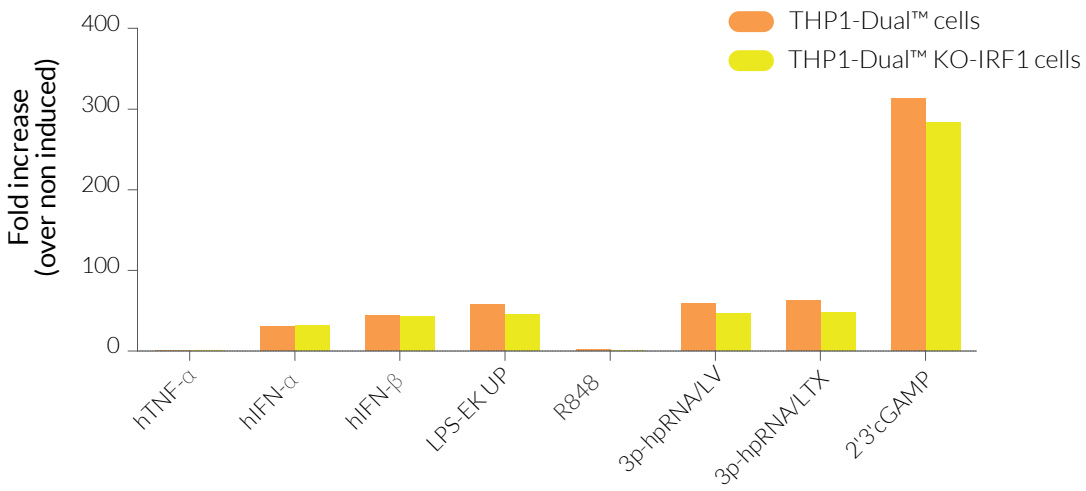

Figure 3: IRF responses in PMA-differentiated THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-IRF1 cells were pretreated with PMA (Phorbol 12-myristate 13-acetate; 10 ng/ml for 3 hours). After 3 days recovery, the cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 3 µg/ml R848 (TLR7/8 agonist), 300 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).