Validation data for THP1-Dual™ KO-TRIF Cells

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Version 22L12-AK

THP1-Dual™ KO-TRIF cells were generated from the THP1-Dual™ cell line through the verified stable knockout of the human (h)*TRIF* gene (**Figure 1**). These cells also 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. 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, the prior differentation treatment using PMA (Phorbol 12-myristate 13-acetate) does not rescue the IRF-response in LPS-stimulated TRIF-KO cells in comparison to their parental cell line THP1-Dual™ **(Figure 2)**. The NF-κB response is slightly dimished in PMA-differentiated THP1-Dual™ KO-TRIF cells upon incubation with TLR4-specific ligands such LPS and CRX-527. Of note, the remaining NF-κB-dependent signal to TLR4 ligand stimulation is most likely due to the more dominant activation of the TLR4-MyD88 signaling pathway **(Figure 3)**.

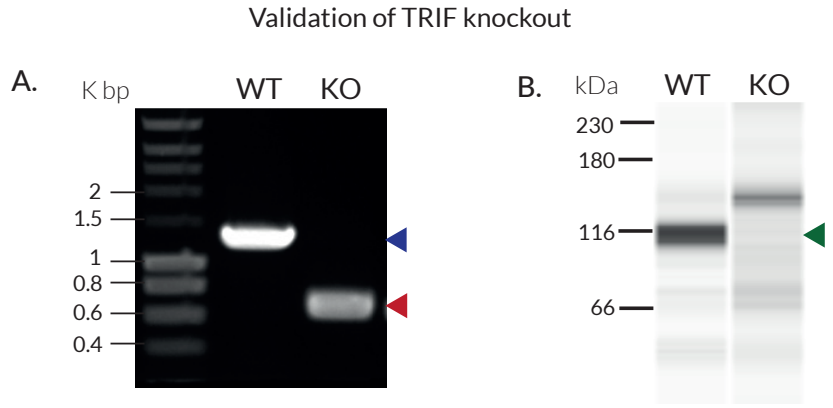

Figure 1: Validation of TRIF KO. (A) The targeted TRIF region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-TRIF (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-TRIF cells feature a frameshift deletion, causing an early stop codon and inactivation of TRIF. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-TRIF (KO) cells were analyzed using an anti-human TRIF antibody (green arrow), followed by an HRP-conjugated anti-rabbit secondary antibody. As expected a band was detected at ~100 kDa in the WT cells only.

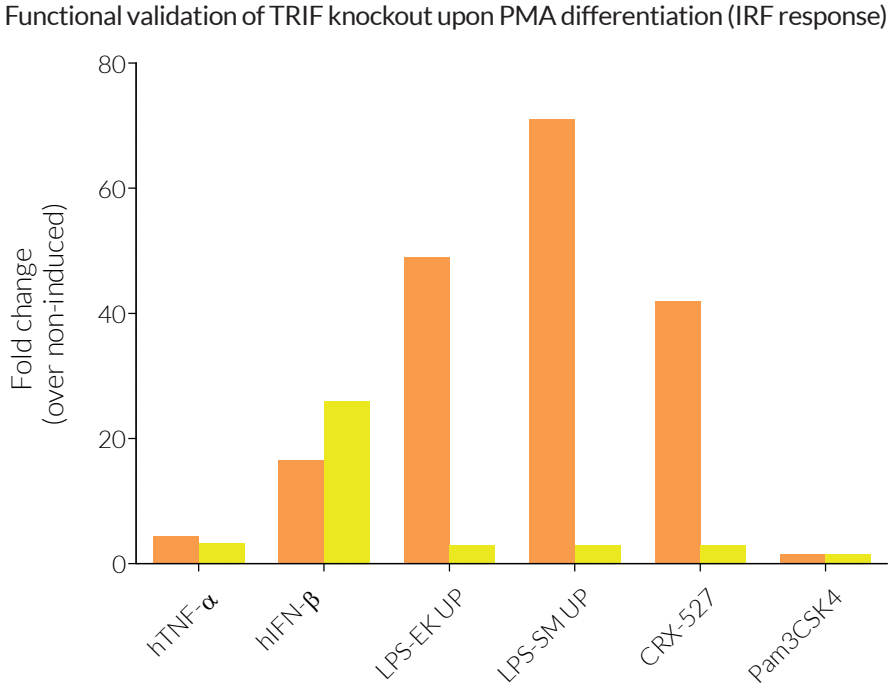

Figure 2: IRF responses in PMA-differientated THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TRIF cells were pretreated with PMA (Phorbol 12-myristate 13-acetate; 10 ng/ml for 3 hours). After 3 days recovery, the cells were incubated with 10 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 30 ng/ml LPS-EK Ultrapure (UP), 30 ng/ml LPS-SM-UP, 30 ng/ml CRX-527 and 10 ng/ml Pam3CSK4 (TLR2/1 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).

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Functional validation of TRIF knockout upon PMA differentiation (NF-κB response)

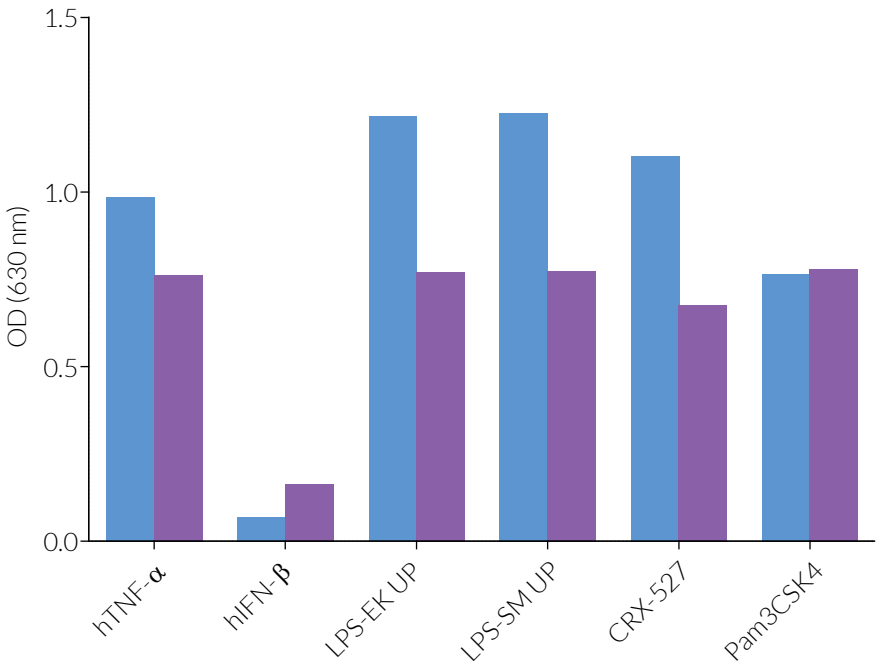

Figure 3: NF-κB responses in PMA-differentiated THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TRIF cells were pretreated with PMA; 10 ng/ml for 3 hours). After 3 days recovery, the cells were incubated with 10 ng/ml human (h) TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 30 ng/ml LPS-EK Ultrapure (UP), 30 ng/ml LPS-SM UP, 30 ng/ml CRX-527 and 10 ng/ml Pam3CSK4 (TLR2/1 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.

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