# Validation data for THP1-Dual™ KO-TLR4 Cells

https://www.invivogen.com/thp1-dual-ko-tlr4

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Version 22L06-AK

THP1-Dual™ KO-TLR4 cells were generated from the THP1-Dual™ cell line through the biallelic knockout (KO) of the *TLR4* 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, respectively. Upon stimulation with TLR4-agonists, we observe the complete loss of NF-κB-mediated responses when compared to the parental THP1-Dual™ cells **(Figure 2)**.The prior differentation treatment using PMA (Phorbol 12-myristate 13-acetate) does not rescue the IRF-response in the TLR4-KO cells in comparison to their parental cell line THP1-Dual™ **(Figure 3).**

Validation of TLR4 knockout

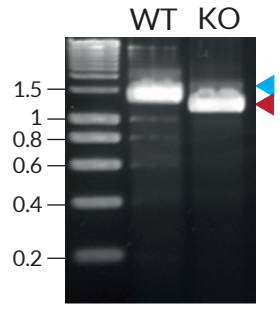


**Figure 1: PCR validation of TLR4 KO.** The targeted TLR4 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-TLR4 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-TLR4 cells feature a frameshift deletion, causing an early stop codon and inactivation of TLR4.

Functional validation of TLR4 knockout (NF-κB response)

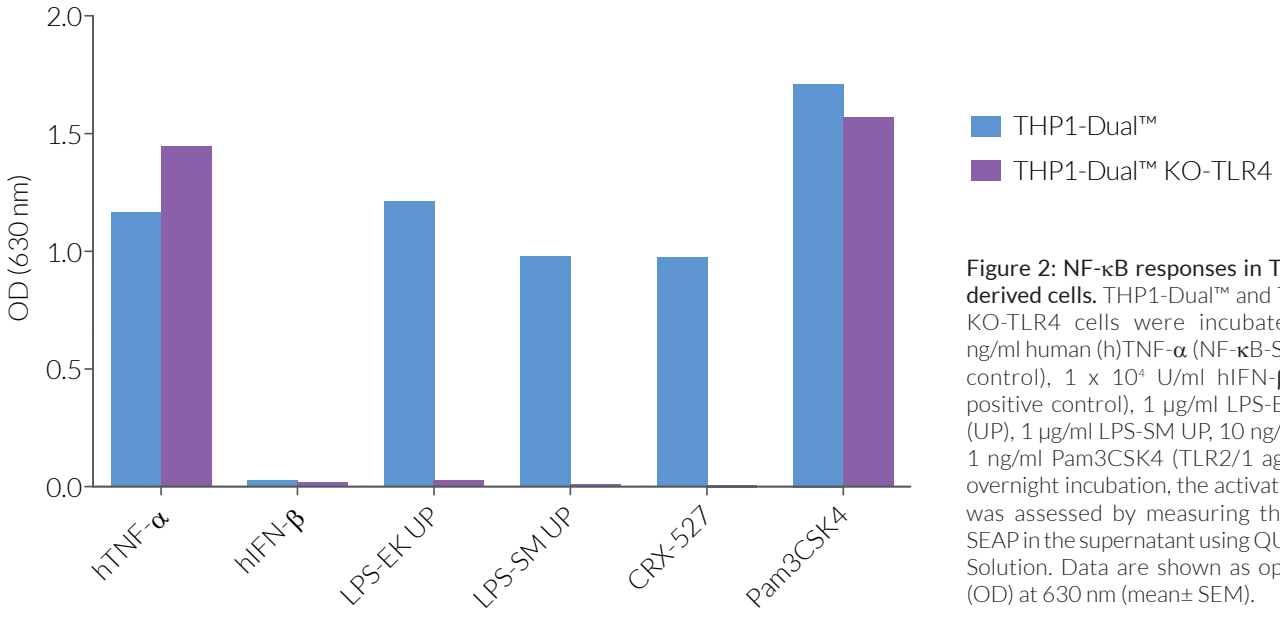


**Figure 2: NF-κB responses in THP1-Dual™-derived cells.** THP1-Dual™ and THP1-Dual™ KO-TLR4 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x $10^4$ U/ml hIFN-β (IRF-Lucia positive control), 1 µg/ml LPS-EK Ultrapure (UP), 1 µg/ml LPS-SM UP, 10 ng/ml CRX-527, 1 ng/ml 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 (mean± SEM).

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Functional validation of TLR4 knockout (IRF response) after PMA treatment

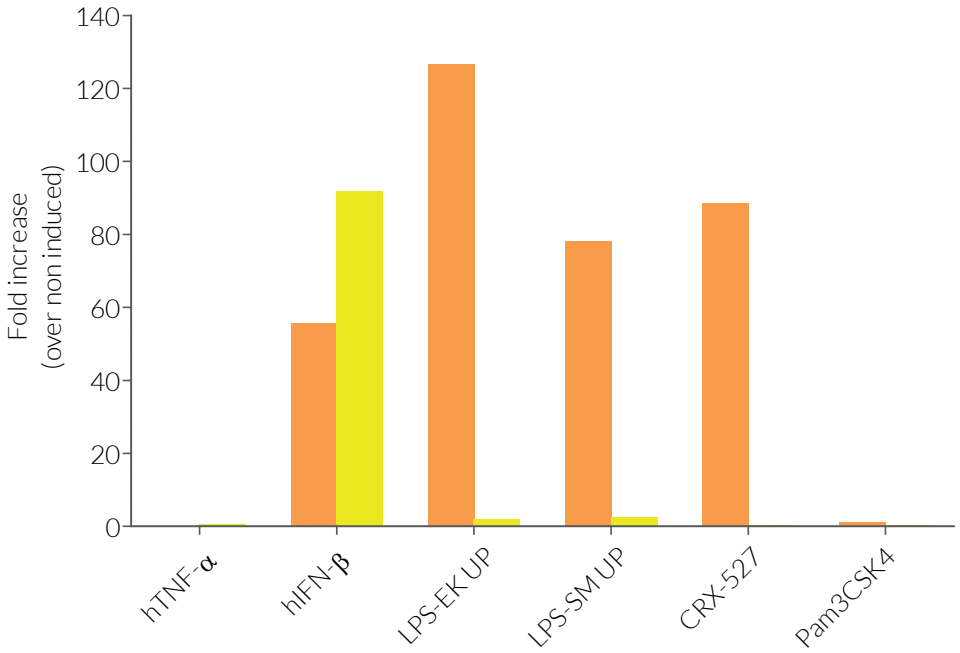


**Figure 3: IRF responses in THP1-Dual™-derived cells after PMA treatment.** THP1-Dual™ and THP1-Dual™ KO-TLR4 cells were pre-treated with PMA (Phorbol 12-myristate 13-acetate; 10 ng/ml for 3 hours). After 3 days of recovery, they incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x $10^4$ U/ml hIFN-β (IRF-Lucia positive control), 1 µg/ml LPS-EK UP, 1 µg/ml LPS-SM UP, 10 ng/ml CRX-527 and 1 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).