Validation data for THP1-Dual™ KO-TLR2 Cells

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THP1-Dual™ KO-TLR2 cells were generated from the THP1-Dual™ cell line through the verified stable knockout of the TLR2 gene (**Figure 1**). These cells feature two reporter genes allowing the simultaneous study of the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase)(**Figure 2**) and the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase (**Figure 3**). Lucia luciferase and SEAP activities are readily assessable in the cell culture supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution detection reagents, respectively.

Validation of *TLR2* knockout

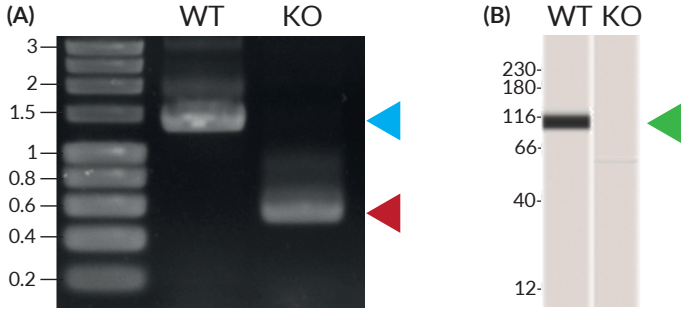

Figure 1: Validation of TLR2 KO. (A) The targeted TLR2 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-TLR2 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-TLR2 cells feature a frameshift deletion, causing an early stop codon and inactivation of TLR2. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-TLR2 (KO) cells were analyzed using an anti-human TLR2 antibody (green arrow), followed by an HRP-conjugated anti-rabbit secondary antibody. As expected a band was detected at ~90 kDa in the WT cells only.

Functional validation of TLR2 knockout (NF-κB response)

TLR2 forms a heterodimer on the cell surface with its co-receptor, TLR1 or TLR6, which is crucial for signaling and ligand specificity. Ultimately, TLR2 signaling leads to MyD88 and MAL/TIRAP-dependent activation of pro-inflammatory transcription factors such as NF-κB. As expected, the NF-κB response is abolished in THP1-Dual™ KO-TLR2 cells upon incubation with TLR2-specific ligands such as Pam3CSK4 (TLR1/2), FSL-1 (TLR2/6), and heat killed *Listeria monocytogenes* (HKLM; TLR2/6) when compared to the THP1-Dual™ cells (Figure 1), with no notable difference for the other ligands tested.

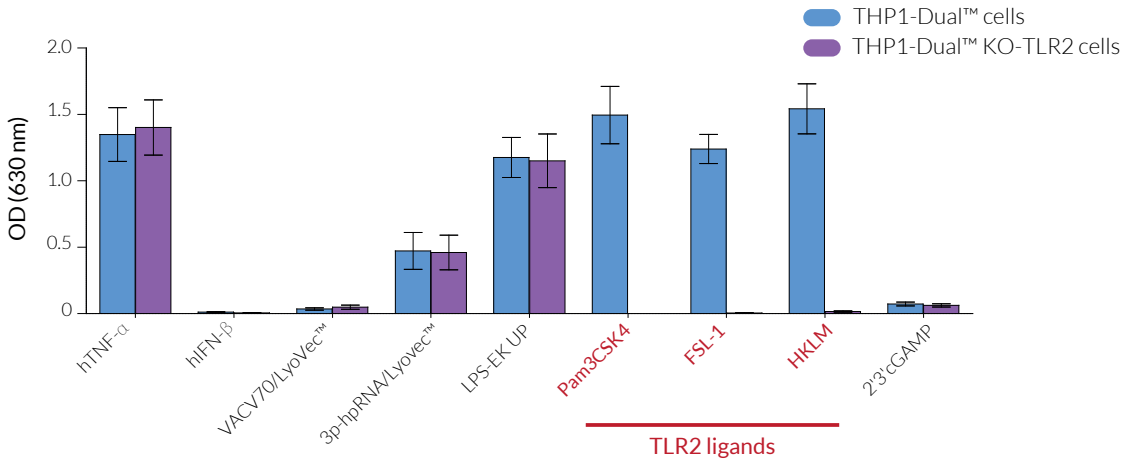

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TLR2 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 1 µg/ml VACV70/LyoVec™ (CDS ligand), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1 µg/ml LPS-EK Ultrapure (UP; TLR4),1 ng/ml Pam3CSK4 (TLR2/1 agonist), 0.3 ng/ml FSL-1 (TLR2/6 agonist), 10^7 c/ml HKLM (TLR2 agonist), and 3 µg/ml 2'3'-cGAMP (STING 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 TLR2 knockout (IRF response)

As expected, due to TLR2 not directly signaling through an IRF-dependent pathway, the secretion of Lucia lucierfase was unimpaired in THP1-Dual™ KO-TLR2 when tested across a range of ligands. Additionally, the response of ligands that signal in an IRF-dependent manner, such as 2'3'-cGAMP (STING agonist) and human interferon β (hIFN-β) was confirmed to be unaltered between the parental cell line, THP1-Dual™, and THP1-Dual™ KO-TLR2.

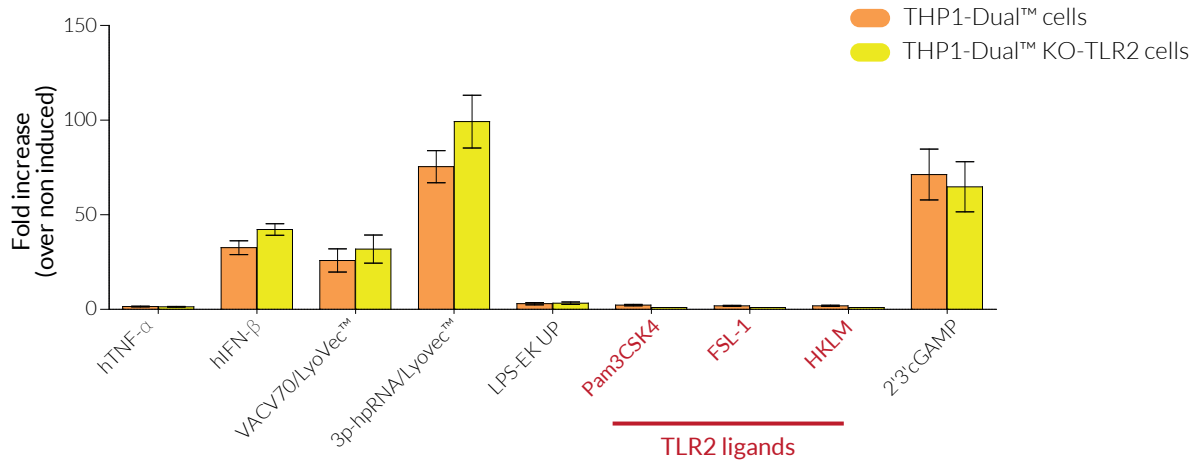

Figure 3: IRF responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TLR2 cells were incubated with 0.3 ng/ml human (h) TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 1 µg/ml VACV70/LyoVec™ (CDS ligand), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1 µg/ml LPS-EK Ultrapure (UP; TLR4), 1 ng/ml Pam3CSK4 (TLR2/1 agonist), 0.3 ng/ml FSL-1 (TLR2/6 agonist), 10^7 c/ml HKLM (TLR2 agonist), and 3 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).