Validation data for THP1-Dual™ KO-TLR8 Cells

https://www.invivogen.com/thp1-dual-ko-tlr8

For research use only

Version 21B04-ED

THP1-Dual™ KO-TLR8 cells were generated from the THP1-Dual™ cell line through the verified stable knockout of the TLR8 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**).

Validation of *TLR8* knockout

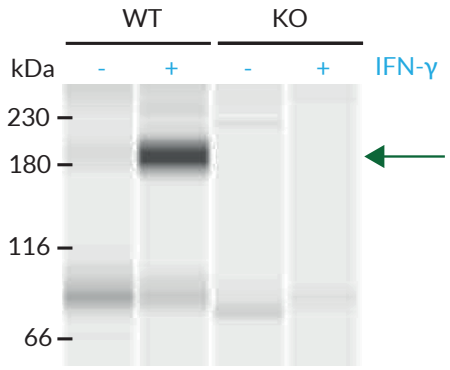

Figure 1: Validation of TLR8 KO. PMA-differentiated THP1-Dual™ (WT) and THP1-Dual™ KO-TLR8 (KO) cells were treated with 20 ng/ml interferon(IFN)-γ to induce the expression of TLR8. Lysates from non-treated (-) and treated (+) WT and KO cells were analyzed using an anti-human TLR8 antibody, followed by 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 TLR8 is ~120 kDa.

Functional validation of TLR8 knockout (NF-κB response)

TLR8 recognizes microbial RNA degradation products, which ultimately leads to MyD88-dependent activation of NF-κB. As expected, the NF-κB response is abolished in THP1-Dual™ KO-TLR8 cells upon incubation with both synthetic ligands (R848 & CL075) and single-stranded (ss) RNA (ORN06 & ssRNA40) when compared to the THP1-Dual™ cells. No notable differences are observed for the other ligands tested.

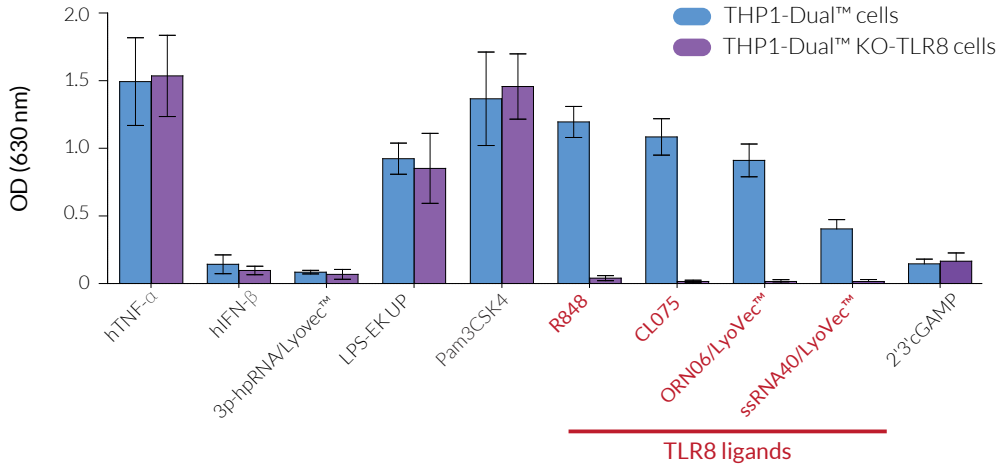

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TLR8 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1 µg/ml LPS-EK Ultrapure (UP; TLR4),0.1 ng/ml Pam3CSK4 (TLR2 agonist), 10 µg/ml R848 (TLR7/8 agonist), 10 µg/ml CL075 (TLR7/8 agonist), 5 µg/ml ORN06/LyoVec™ (TLR8 agonist), 5µg/ml ssRNA40/LyoVec™ (TLR8 agonist) and 10 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).

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 Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com

Functional validation of TLR8 knockout (IRF response)

TLR8 has been shown to activate IRF-dependent signaling. THP1-Dual™ (parental cell line) displays a slight increase in the IRF-dependent secretion of Lucia lucierfase upon activation with TLR8-specific agonists (in red). Importantly, this response is abolished in THP1-Dual™ KO-TLR8 cells. Additionally, the response of known ligands to signal in an IRF-dependent manner, such as 2'3'-cGAMP (STING agonist) and human interferon β (hIFN-β) was confirmed to be unchanged between THP1-Dual™ and THP1-Dual™ KO-TLR8.

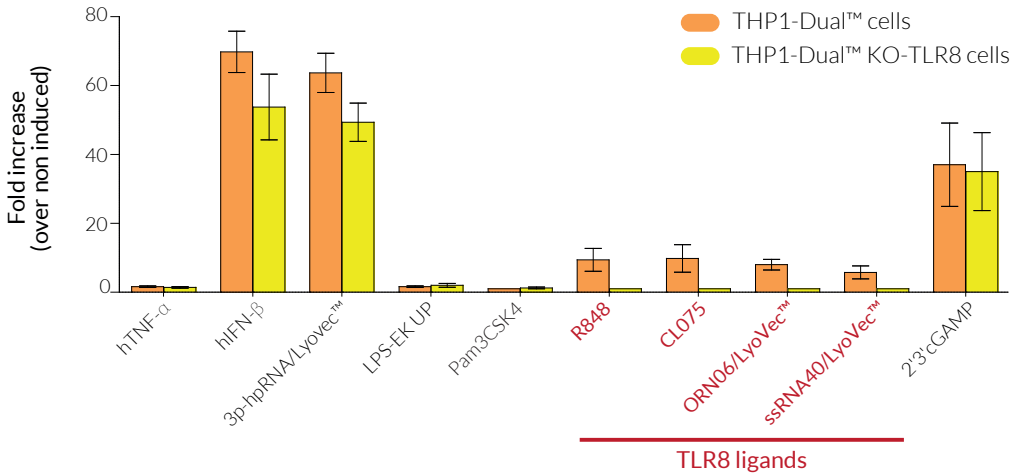

Figure 3: IRF responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TLR8 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x 10^4 U/ml hIFN-β (IRF-Lucia positive control), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1 µg/ml LPS-EK Ultrapure (UP; TLR4),0.1 ng/ml Pam3CSK4 (TLR2 agonist), 10 µg/ml R848 (synthetic TLR7/8 agonist), 10 µg/ml CL075 (synthetic TLR7/8 agonist), 5 µg/ml ORN06/LyoVec™ ('natural' TLR8 agonist), 5µg/ml ssRNA40/LyoVec™ ('natural' TLR8 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 change (mean ± SEM) over non-induced cells.

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 Hong Kong: +852 3622-3480
E-mail: info@invivogen.com