Validation data for THP1-Dual™ KO-TLR8 cells

https://www.invivogen.com/thp1-dual-htlr7-htlr8

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Version 23C24-AK

THP1-Dual™ KO-TLR8 cells were generated from the THP1-Dual™ cell line through the verified stable knockout (KO) of the *TLR8* gene **(Figure 1)**. These these cells express two reporter genes allowing the simultaneous study of the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase), and the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase. Due to the KO of the endogenous TLR8, THP1-Dual™ KO-TLR8 cells do not respond to TLR8-specific ligands, when compared to their parental cell line THP1-Dual™ **(Figures 2 & 3)**. Of note, as THP-1 cells express endogenous levels of various TLRs, THP1-Dual™-derived cells respond to the cognate ligands including Pam3CSK4, LPS, or flagellin.

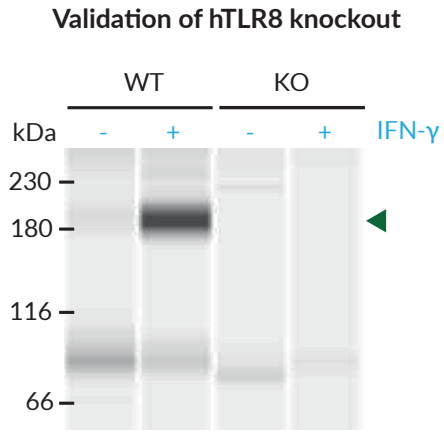

Figure 1. Validation of TLR8 KO. PMA-differentiated THP1-Dual™ (WT) and THP1-Dual™ KO-TLR8 (KO) cells were treated overnight with 20 ng/ml interferon(IFN)-γ to induce the expression of TLR8. Lysates from non-treated (-) and treated (+) WT and KO cells were analyzed using an anti-human TLR8 antibody, followed by an HRP-conjugated anti-rabbit secondary antibody (JESS™). As expected, a band was detected in the IFN-γ treated WT cells only (green arrow).

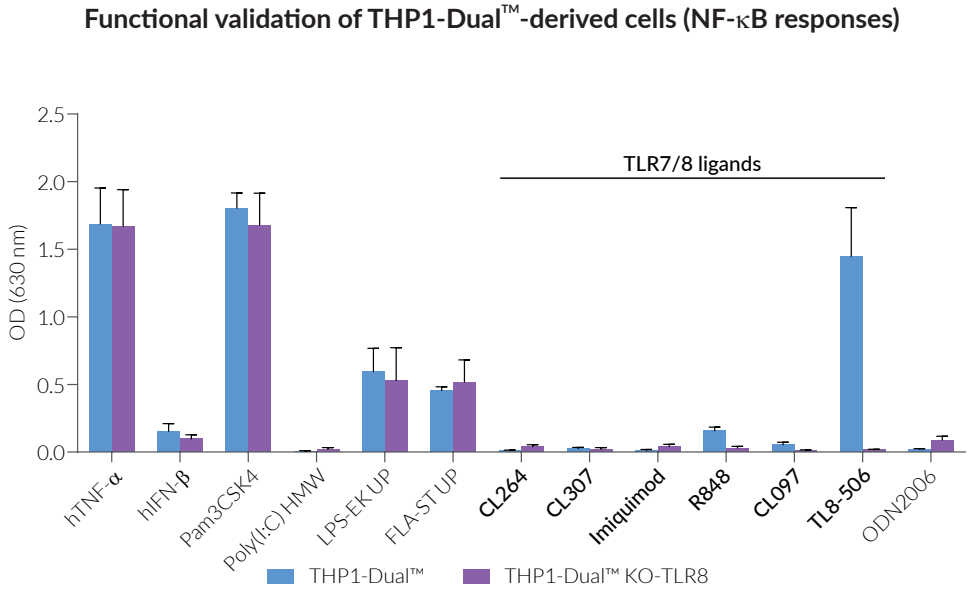

Figure 2. NF-κB responses in THP1-Dual™ -derived cells. . THP1-Dual™ and THP1-Dual™ hTLR7 KO-TLR8 cells were incubated for 24 hours with various TLR agonists: Pam3CSK4 (TLR2 ligand, 10 ng/ml), Poly(I:C) HMW (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 1 ng/ml), FLA-ST UP (TLR5 ligand, 1 µg/ml), **CL264** (TLR7 ligand, 1 µg/ml), **CL307** (TLR7 ligand, 1 µg/ml), **Imiquimod** (TLR7 ligand, 10 µg/ml), **R848** (TLR7/8 ligand, 1 µg/ml), **CL097** (TLR7/8 ligand, 1 µg/ml), **TL8-506** (TLR8 ligand, 1 µg/ml), and ODN 2006 (TLR9 ligand, 10 µg/ml). Human TNF-α (1 ng/ml) was used as an NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 630 nm (mean ± SEM).

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Functional validation of THP1-Dual™-derived cells (IRF responses)

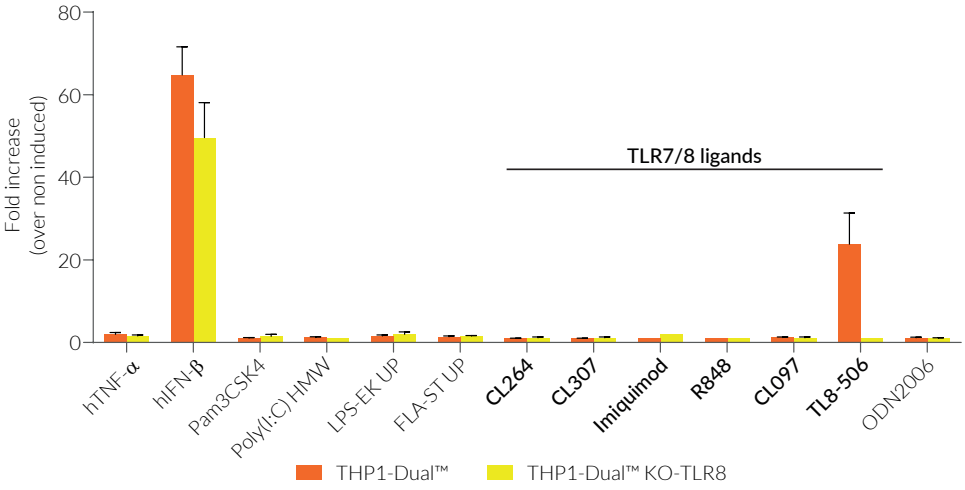

Figure 3. IRF responses in THP1-Dual™ -derived cells. THP1-Dual™ and THP1-Dual™ KO-TLR8 cells were treated as described above. Human IFN-β (1000 U/ml) was used as an IRF-positive control. After 24h incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

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