Validation data for THP1-Dual™ MD2-CD14 KO-TLR4 Cells

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

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Version 22L01-AK

THP1-Dual™ MD2-CD14 KO-TLR4 cells were generated from THP1-Dual™ cells as a control cell line for the THP1-Dual™ MD2-CD14-TLR4 cells, through a biallelic knockout of the human (h)*TLR4* gene **(Figure S1)**. Additionally, they express two co-adaptors important for lipopolysaccharide (LPS) signaling, MD-2 and CD14. They 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 smooth and rough LPS, we observe the complete loss of NF-κB-mediated responses when compared to the parental THP1-Dual™ cells **(Figure 1)**. There is no notable difference in the IRF-mediated responses between THP1-Dual™ MD2-CD14 KO-TLR4 cells and their parental cell line **(Figure 2)**. However, there are strong NFκB and IRF responses to both smooth and rough LPS in the cell line overexpressing TLR4, THP1-Dual™ MD2-CD14-TLR4 **(Figures 1&2)**.

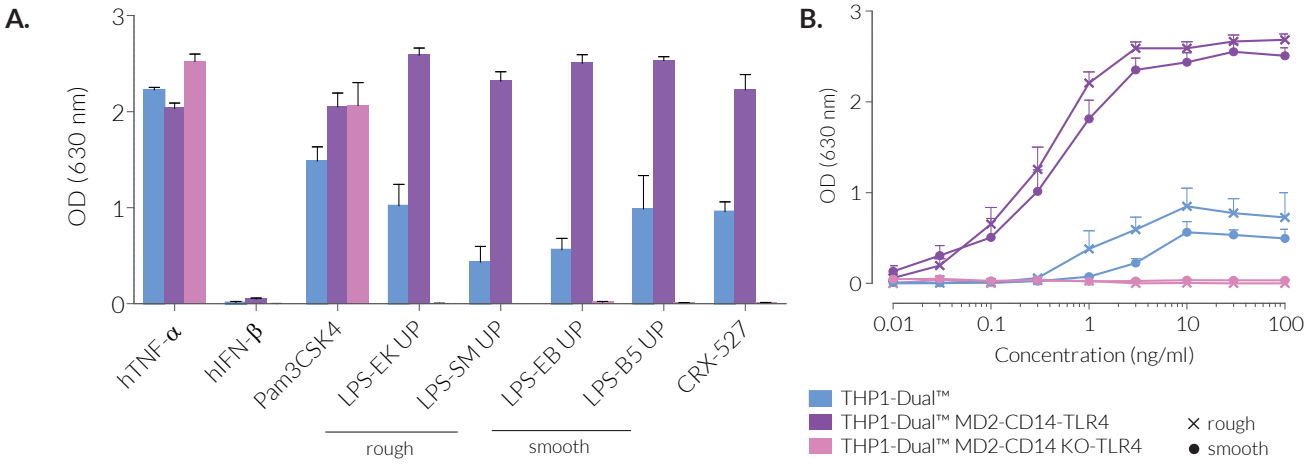

Figure 1: NF-κB responses in THP1-Dual™-derived cells. A. The cells were incubated with a variaty of rough and smooth LPS: LPS-EK Ultrapure (UP) (rough; 10 ng/ml), LPS-SM UP (rough; 10 ng/ml), LPS-EB UP (smooth; 10 ng/ml), LPS-B5 UP (smooth; 1 ng/ml) and CRX-527 (synthetic; 10 ng/ml). Human (h)TNF-α (10 ng/ml) and hIFN-β (1x10³ U/ml) were used as a NF-κB-SEAP positive control and as a IRF positive control, respectively. The TLR2-TLR1 agonist Pam3CSK4 (10 ng/ml) served as a functional control. **B.** The cells were incubated with increasing concentration of rough (LPS-EK UP) and smooth (LPS-EB UP) LPS. After 24 hours, 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).

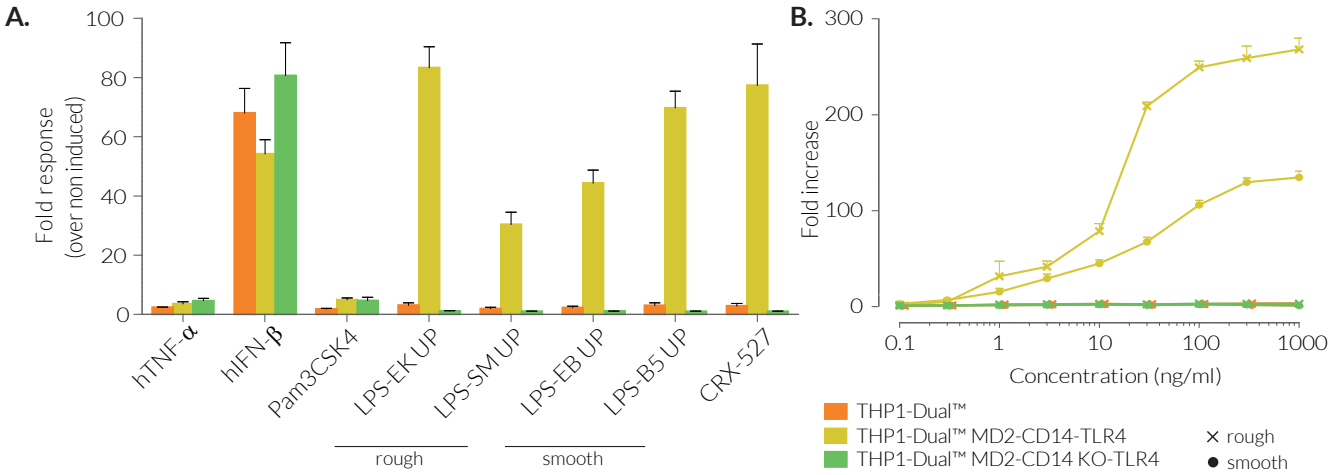

Figure 2: IRF responses in THP1-Dual™-derived cells. The cells were treated as described above *(see figure 1)*. After 24 hours, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

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Validation of TLR4 knockout

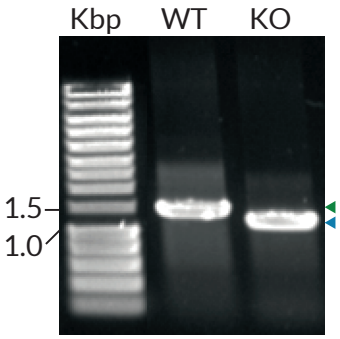

Figure S1: Validation of TLR4 KO. The targeted TLR4 region in parental THP1-Dual™ cells (WT; green arrow) and THP1-Dual™ MD2-CD14 KO-TLR4 (KO; blue arrow) cells was amplified by PCR. THP1-Dual™ MD2-CD14 KO-TLR4 cells were generated by a biallelic deletion causing the inactivation of hTLR4.