Validation data for THP1-Dual™ KO-MyD cells

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Version 23A10-AK

THP1-Dual™ KO-MyD cells are engineered from the THP1-Dual™ cell line through the verified stable knockout of the human (h)*MyD88* 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. Lucia luciferase and SEAP activities are readily assessable in the cell culture supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution detection reagents, respectively. THP1-Dual™ KO-MyD cells display significantly reduced responses to ligands that activate receptors whose signaling is MyD88-dependent, such as TLR1/2, TLR2/6, TLR4, TLR5, TLR8 and the interleukin-1 receptor (IL-1R). These cells remain responsive to ligands that activate receptors whose signaling is MyD88-independent, such as the tumor necrosis factor (TNF) receptor or NOD1 **(Figure 2)**.

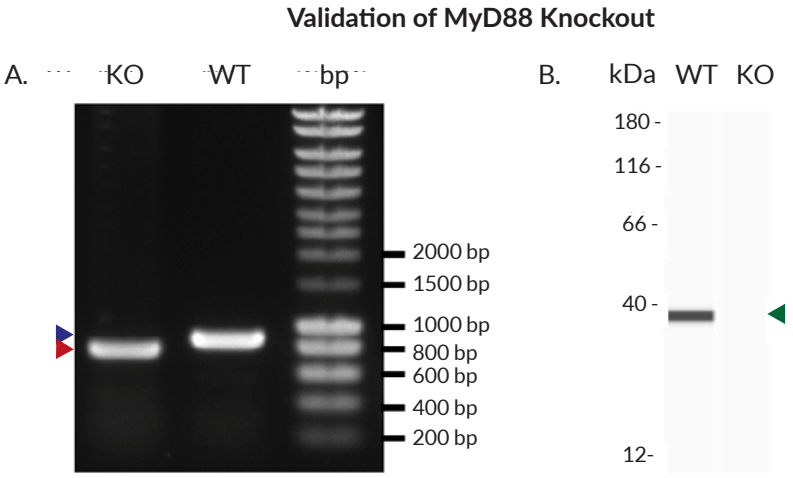

Figure 1: Validation of MyD88 knockout in THP1-Dual™ KO-MyD cells. (A) The targeted *MyD88* region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-MyD (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-MyD cells feature a biallelic deletion. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-MyD (KO) cells were analyzed by Western blot (Wes™) using an anti-human MyD88 antibody, followed by an HRP conjugated anti-rabbit secondary antibody. The green arrow indicates the expected band for the MyD88 protein (33 kDa).

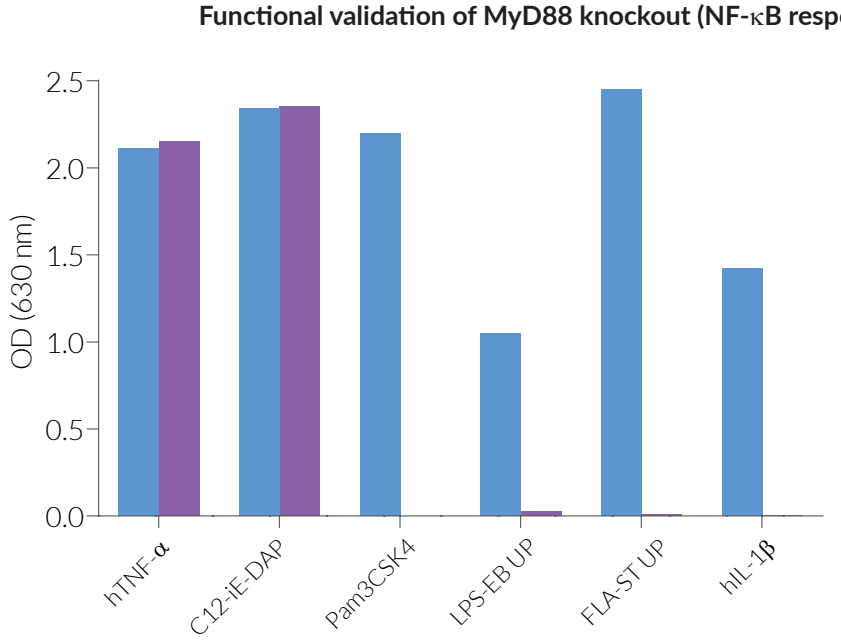

Figure2:MyD88-dependentand-independentresponses in THP1-Dual™ and THP1-Dual™ KO-MyD cells. Cells were incubated with 1 ng/ml human (h) TNF-α (MyD88-independent NF-κB-SEAP positive control), 1 µg/ml C12-iE-DAP (NOD1 ligand), 1 ng/ml Pam3CSK4 (TLR1/2 ligand), 100 ng/ml LPS-EB Ultrapure (UP; TLR4 ligand), 100 ng/ml Flagellin (FLA)-ST Ultrapure (TLR5 ligand), and 1 ng/ml IL-1β (IL-1R ligand). 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.

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