Validation data for THP1-Dual™ KO-SAMHD1 Cells

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Version 22I21-AK

THP1-Dual™ KO-SAMHD1 cells were generated from the THP1-Dual™ cell line through the stable biallelic knockout of the *SAMHD1* gene, as verified by PCR and western blot **(Figure 1, A and B)**. Additionally, these cells 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 **(Figures 2 and 3)**. We observe a reduction of NF-κB-mediated responses induced by LPS-EK, and to a lesser extent by LPS-SM, two TLR4 agonists **(Figure 2)**. There is no notable difference in the IRF-mediated responses between THP1-Dual™ KO-SAMHD1 and their parental cell line, upon incubation with TLR4, TLR7/8, CDS, RIG-I, or STING agonists **(Figure 3)**.

Validation of SAMHD1 knockout

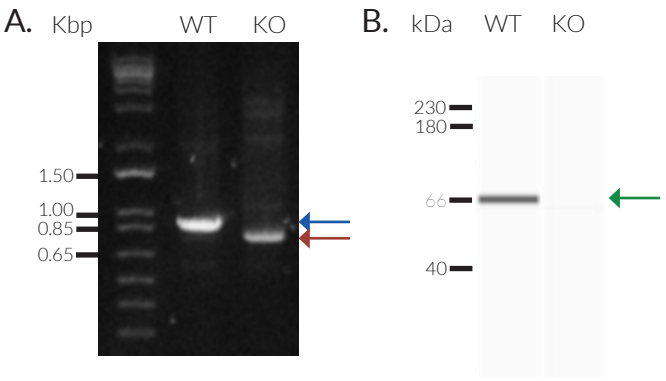

Figure 1: Validation of SAMHD1 KO. (A) The targeted SAMHD1 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-SAMHD1 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-SAMHD1 cells were generated by a biallelic deletion causing the inactivation of SAMHD1.
(B) Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-SAMHD1 (KO) cells were analyzed using an anti-human SAMHD1 antibody, followed by an HRP-conjugated anti-rabbit secondary antibody (JESS™ system). As expected a band was detected at ~70 kDa in the WT cells only (green arrow).

Functional validation of SAMHD1 knockout (NF-κB response)

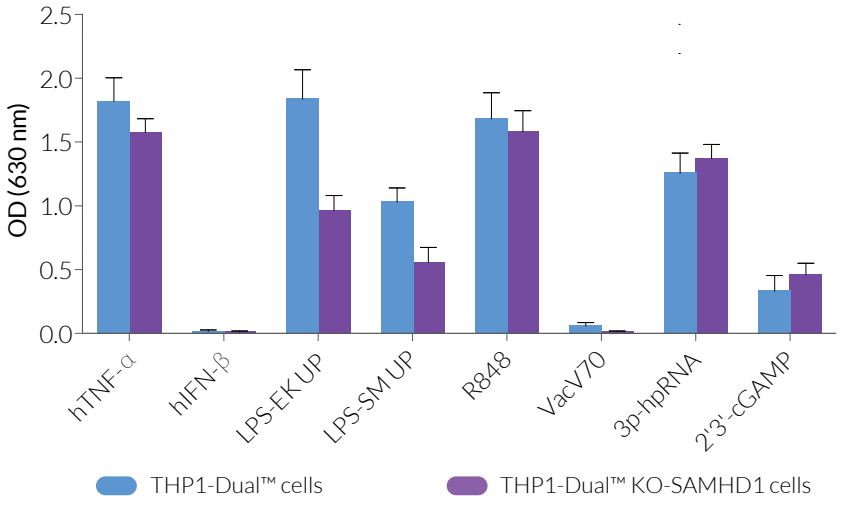

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1 Dual™ KO-SAMHD1 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 100 ng/ml LPS-EK Ultrapure and 100 µg/ml LPS-SM Ultrapure (both TLR4 agonists), 10 µg/ml R848 (TLR7/8 agonist), 1 µg/ml VacV70 complexed with LyoVec™ (CDS agonist), 1 µg/ml 3p-hpRNA complexed with LyoVec™ (RIG-I agonist) and 30 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 SAMHD1 knockout (IRF response)

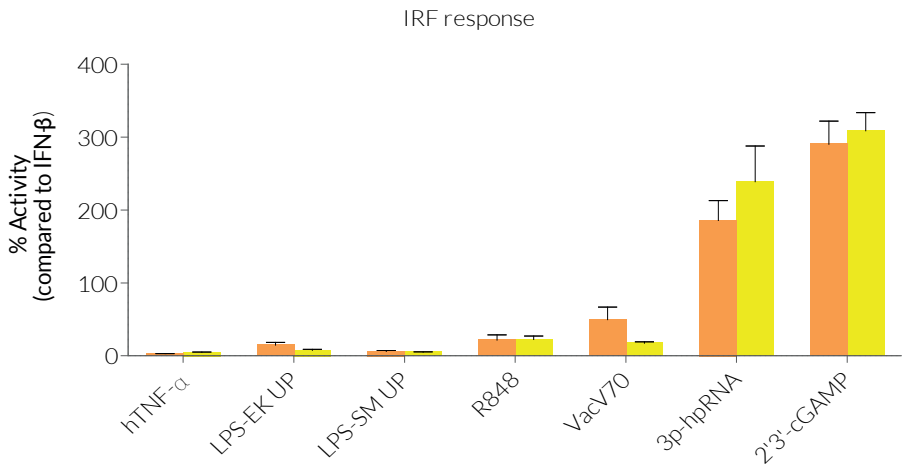

Figure 3: IRF responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-SAMHD1 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 100 ng/ml LPS-EK Ultrapure and 1 µg ml LPS-SM Ultrapure (both TLR4 agonists), 10 µg/ml R848 (TLR7/8 agonist), 1 µg/ml VacV70 complexed with LyoVec™ (CDS agonist), 1 µg/ml 3p-hpRNA complexed with LyoVec™ (RIG-I agonist) and 30 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™. The IRF induction of each ligand is expressed relative to that of hIFN-β at $1x10^3$ U/ml (mean ± SEM).