Validation data for THP1-Dual™ KO-TREX1 cells

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Version 22I21-AK

THP1-Dual™ KO-TREX1 cells are engineered monocytes that were specifically designed to monitor TREX1 mediated signaling. They were generated from the THP1-Dual™ parental cell line through the stable biallelic knockout of the *TREX1* 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 an increase in NF-κB-mediated responses induced by 2'3'-cGAMP, and to a lesser extent by VacV70, two CDS (cytosolic DNA sensor) agonists **(Figure 2)**. In contrast, there was a slight reduction in the IRF-mediated responses between THP1-Dual™ KO-TREX1 and their parental cell line, upon incubation with Poly(dA:dT) and 2'3'-cGAMP **(Figure 3)**.

Validation of TREX1 Knockout

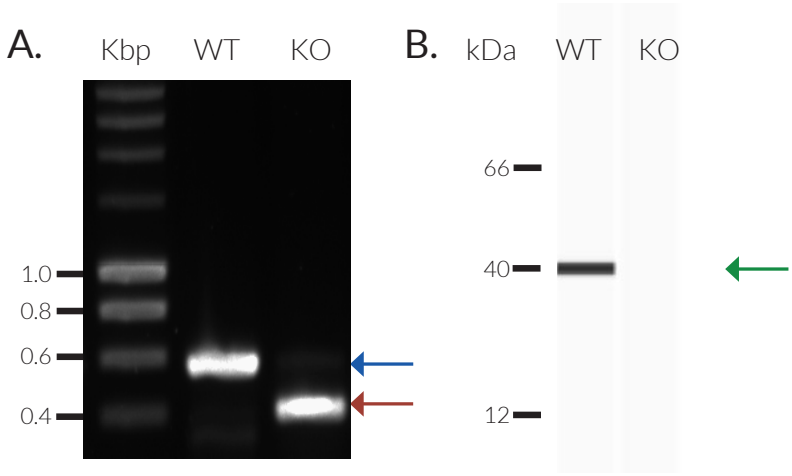

Figure 1: Validation of TREX1 KO. (A) The targeted TREX1 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-TREX1 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-TREX1 cells were generated by a biallelic deletion causing the inactivation of TREX1. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-TREX1 (KO) cells were analyzed using an anti-human TREX1 antibody, followed by an HRP-conjugated anti-rabbit secondary antibody (Wes™ system). As expected a band was detected at ~33 kDa in the WT cells only (green arrow).

Functional validation of TREX1 knockout (NF-κB response)

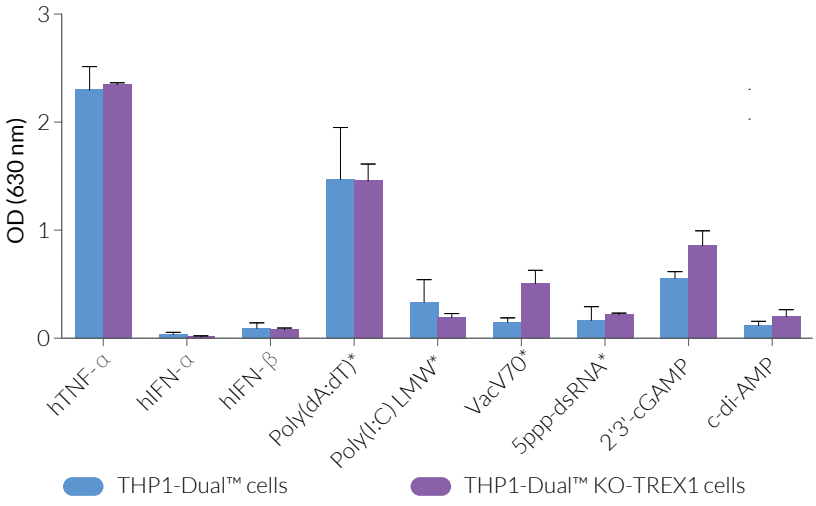

Figure 2: NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1 Dual™ KO-TREX1 cells were incubated with 10 ng/ml human (h)TNF-α (NF κB-SEAP positive control), 10 000 U/ml hIFN-α, 10,000 U/ml hIFN-β (IRF-Lucia positive control), 1 µg/ml Poly(dA:dT)* and 1 µg/ml Poly(I:C) LMW* (both PRR agonists), 1 µg/ml VacV70*(CDS agonist), 1 µg/ml 5'ppp-dsRNA* (RIG-I agonist), 30 µg/ml 2'3'-cGAMP and 30 µg/ml c-di-AMP (both STING agonists). 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 TREX1 knockout (IRF response)

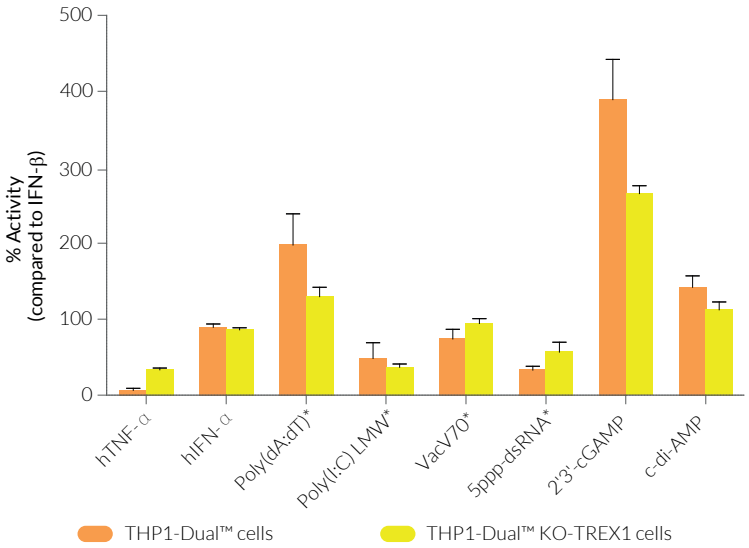

Figure 3: IRF responses in THP1-Dual™-derived cells. THP1-Dual™ and THP1-Dual™ KO-TREX1 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 10,000 U/ml hIFN-α, 1 µg/ml Poly(dA:dT)* and 1 µg/ml Poly(I:C) LMW* (both PRR agonists), 1 µg/ml VacV70*(CDS agonist), 1 µg/ml 5'ppp-dsRNA* (RIG-I agonist), 30 µg/ml 2'3'-cGAMP and 30 µg/ml c-di-AMP (both STING agonists). 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).

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