Validation data for THP1-Dual™ KO-IFI16 cells

https://www.invivogen.com/thp1-dual-koifi16

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Version 23J04-AK

THP1-Dual™ KO-IFI16 cells are engineered monocytes that were specifically designed to study the role of IFI16. They were generated from THP1-Dual™ cells by stable knockout of the IFI16 gene. They derive from human THP-1 monocytes, a cell line often used to study DNA sensing pathways as they express all the cytosolic DNA sensors identified so far (with the exception of DAI). The knockout of the IFI16 gene in these cells has been confirmed by PCR, Western blot **(Figure 1)** and sequencing. Biological activity has been assessed using the Lucia luciferase reporter assay to monitor interferon regulatory factor (IRF) induction **(Figure 2)**.

VALIDATION OF IFI16 KNOCKOUT (KO)

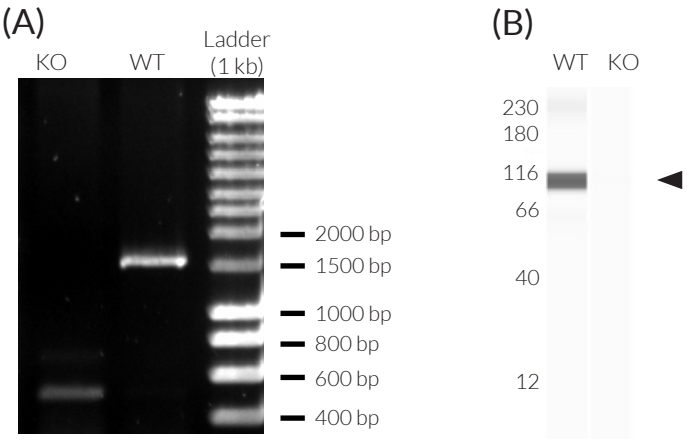

Figure 1: Validation of *IFI16* knockout in THP1-KO-IFI16 cells. **(A)** The targeted IFI16 region in THP1-null (WT) and THP1-KO-IFI16 (KO) cells was amplified by PCR. THP1-KO-IFI16 cells feature a biallelic deletion. **(B)** Lysates from THP1-null (WT) and THP1-KO-IFI16 (KO) cells were analyzed by Western blot (Wes™) using an anti-human IFI16 antibody, followed by an HRP-conjugated anti-mouse secondary antibody.

FUNCTIONAL VALIDATION OF THP1-KO-IFI16 CELLS

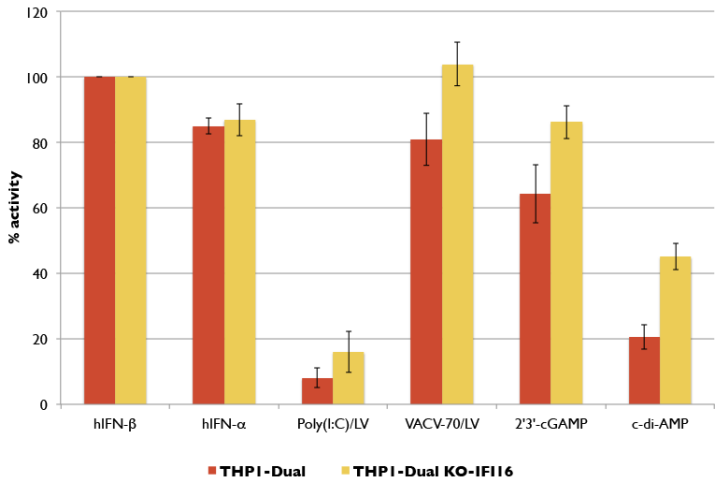

Figure 2: IRF responses in THP1-Dual™ and THP1-Dual™ KO-IFI16 cells to various stimuli. Cells were incubated with Poly(I:C) LMW/LyoVec™ (1 µg/ml), VACV-70/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml), and c-di-AMP (10 µg/ml). Human IFN-α and IFN-β ($1x10^4$ U/ml) serve as positive controls. After a 24h incubation, IRF activation was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. The IRF induction of each ligand is expressed relative to that of hIFN-β at $1x10^4$ U/ml (taken as 100%).

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