Validation data for THP1-Dual™ KO-STING cells

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Version 19K24-MM

THP1-Dual™ KO-STING cells are engineered monocytes that were specifically designed to monitor STING-mediated signaling. They were generated from THP1-Dual™ cells by stable knockout of the STING 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 STING gene in these cells has been confirmed by PCR (figure 1) and Western blot (figure 2). Biological activity has been assessed using the Lucia luciferase reporter assay to monitor interferon regulatory factor (IRF) induction. THP1-Dual™ KO-STING cells exhibit no detectable response to cytosolic DNA and cyclic dinucleotides (CDNs) while retaining the ability to respond to type I interferon (figure 3).

PCR AMPLIFICATION

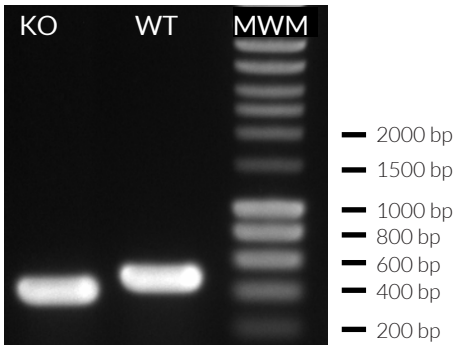

Figure 1: Validation of STING knockout by PCR. Amplification of the targeted region in the THP1-Dual™ KO-STING (KO; biallelic deletion) and THP1-Dual™ cells (WT; endogenously express the HAQ hSTING variant). MWM = molecular weight marker.

WESTERN BLOT

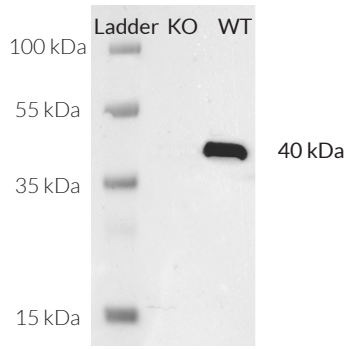

Figure 2. Validation of STING knockout by Western blot. Analysis of lysates (40 µg) from the THP1-Dual™ KO-STING (KO) and THP1-Dual™ cells (WT) using Anti-hSTING-IgG (0.2 µg/ml), followed by HRP-conjugated goat anti-mouse IgG1 secondary antibody (1:3000 dilution).

IRF INDUCTION (Lucia luciferase reporter)

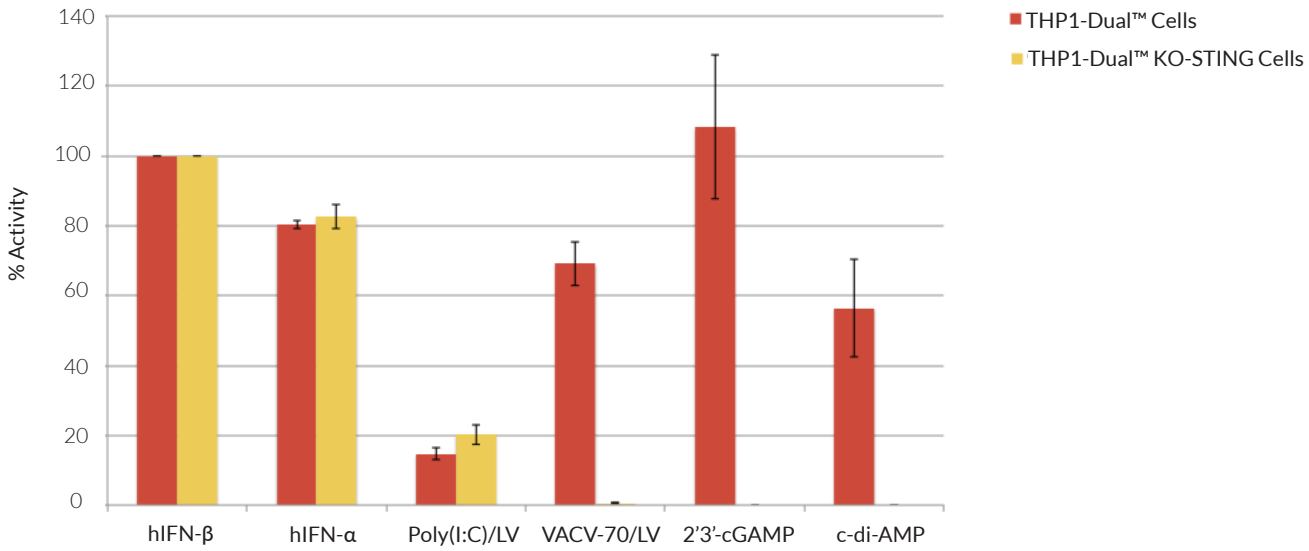

Figure 3: IRF responses in THP1-Dual™ and THP1-Dual™ KO-STING cells to various stimuli. Cells were incubated with Poly(I:C) LMW/LyoVec™ (100 ng/ml), VACV-70/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml), and c-di-AMP (10 µg/ml). Human IFN-α and IFN-β ($1x10^4$ U/ml) serve as positive controls. After a 24h 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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