Validation data for THP1-Dual™ KI-mSTING cells

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Version 19E17-MM

THP1-Dual™ KI-mSTING cells were generated from the THP1-Dual™ KO-STING cell line through the stable knockin of the murine *STING* (mSTING) gene. These cells feature two reporter genes allowing the simultaneous study of the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). 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™ KI-mSTING cells are heterozygous, containing a functional mSTING allele and a truncated non-functional human STING (hSTING) allele, as verified by PCR **(Figure 1)** and Western blot **(Figure 2)**. As expected, these cells exhibit a robust response to STING agonists such as 2'3'-cGAMP and 3'3'-cGAMP **(Figure 3)**. In addition, they respond to DMXAA (5,6-Dimethylxanthenone 4-acetic acid), a potent activator of murine STING, that does not activate hSTING.

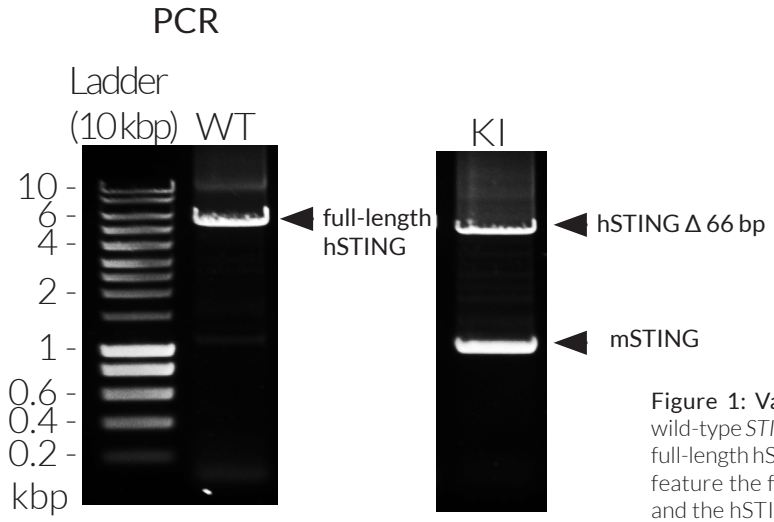

Figure 1: Validation of mSTING knockin by PCR. Amplification of the wild-type *STING* gene in THP1-Dual™ cells (WT), which endogenously express full-length hSTING (~5300 bp), and THP1-Dual™ KI-mSTING cells (KI) which feature the full-length intronless mSTING gene (~1000 bp; bottom arrow) and the hSTING gene with a 66-base pair deletion (~5234 bp; top arrow).

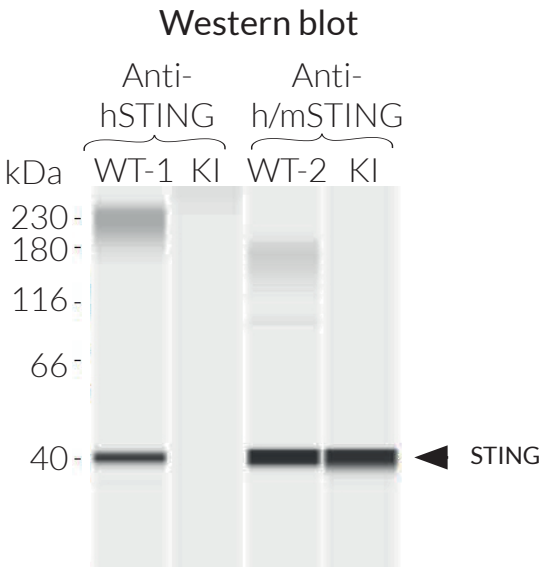

Figure 2: Validation of mSTING knockin by Western blot (WES by Protein Simple). Lysates from THP1-Dual™ (WT-1), THP1-Dual™ KI-mSTING (KI) and RAW-Dual™ (WT-2; which endogenously express wild-type mSTING) cells were analyzed using Anti-hSTING (1:50 dilution) and Anti-h/mSTING (1:50 dilution). This was followed by HRP-conjugated anti-mouse secondary antibody (undiluted). The arrow indicates the expected size for the human and murine STING protein (40 kDa).

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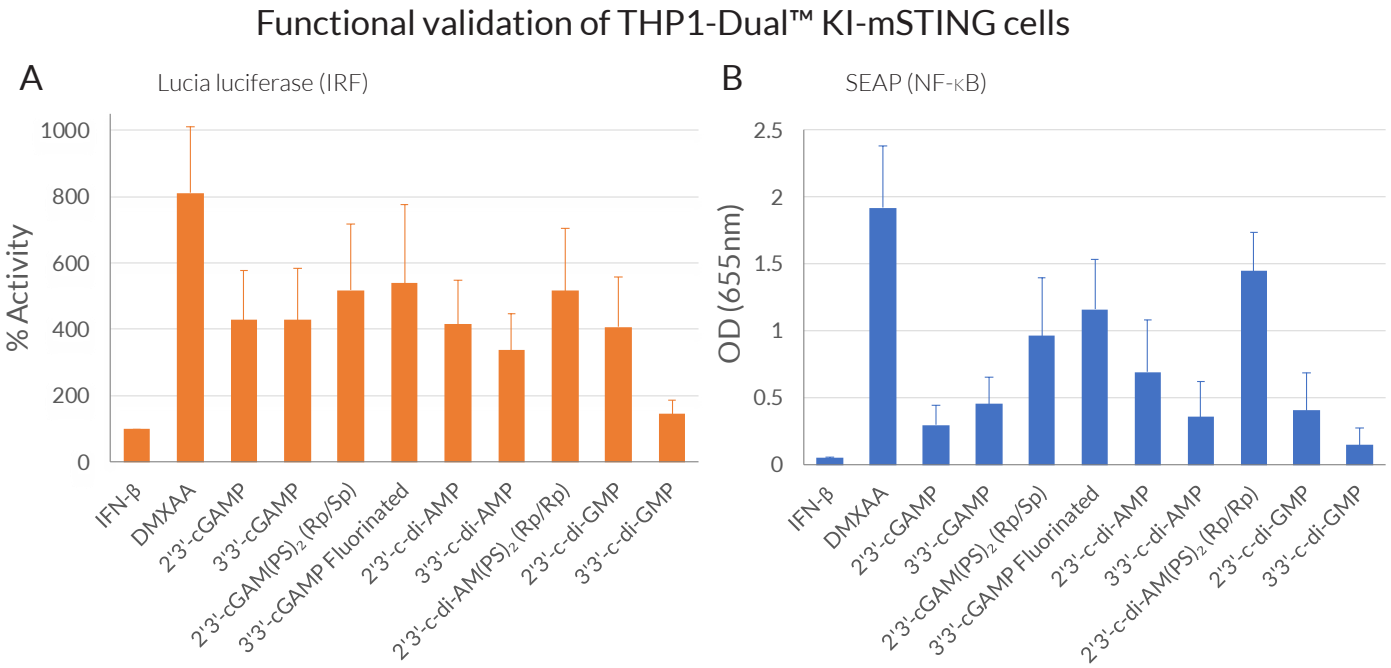

Figure 3: CDN- and DMXAA-induced IRF and NF-κB responses in THP1-Dual™ KI-mSTING cells. Cells were stimulated with human IFN-β (1 x 10^4 U/ml), DMXAA (100 µg/ml), 2'3'-cGAMP (30 µg/ml), 3'3'-cGAMP (30 µg/ml), 2'3'-cGAM(PS)$_2$ (Rp/SP) (30 µg/ml), 3'3'-cGAMP fluorinated (30 µg/ml), 2'3'-c-di-AMP (30 µg/ml), 3'3'-c-di-AMP (30 µg/ml), 2'3'-c-di-AM(PS)$_2$ (Rp/SP) (30 µg/ml), 2'3'-c-di-GMP (30 µg/ml) and 3'3'-c-di-GMP (30 µg/ml). **A)** After 24 hours, 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%). **B)** After 24 hours, NF-κB activation was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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