Validation data for B16-Blue™ ISG-KO-STING cells

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Version 25A22-AK

B16-Blue™ ISG-KO-STING cells were generated from the B16-Blue™ ISG cell line, a murine B16 melanoma-derived cell line, through stable knockout of the murine STING gene. STING (stimulator of interferon genes), alternatively known as MPYS, TMEM173, MITA and ERIS, is a direct sensor of cyclic dinucleotides (CDNs). The knockout of the STING gene in these cells has been confirmed by PCR, sequencing and Western blot (figure 1). B16-Blue™ ISG-KO-STING and B16-Blue™ ISG cells can be used for the study of the STING signaling pathway and for the detection of bioactive murine types I interferons (IFNs) by monitoring the activation of the JAK/STAT pathway. Both cell lines express the secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE. The induction of the interferon regulatory factors (IRF) pathway in B16-Blue™ KO-STING and B16-Blue™ ISG cells in response to CDNs and type I IFNs has been assessed. Unlike the parental cells, B16-Blue™ ISG-KO-STING cells do not respond to canonical or non-canonical CDNs while retaining the ability to respond to type I IFNs (figure 2).

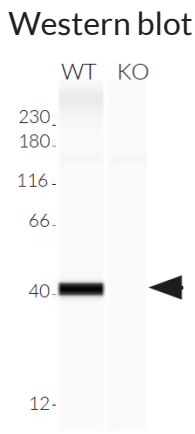

Figure 1: Validation of STING knockout by Western blot (Wes™). Analysis of lysates from the B16-Blue™ (WT) and B16-Blue™ KO-STING (KO) cells using Anti-STING, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the STING protein (43 kDa).

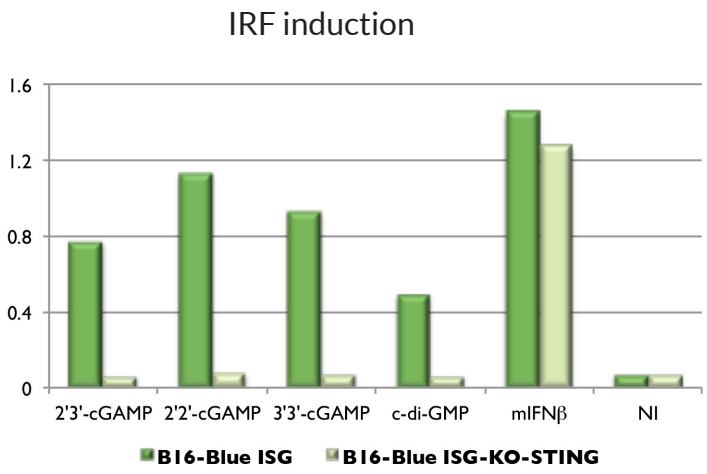

Figure 2: Response of B16-Blue ISG and B16-Blue ISG-KO-STING to CDNs and IFN-β. Cells were stimulated with 30 µg/ml of the cyclic dinucleotides, and 10^3 U/ml of mIFN-β. Cells were not permeabilized. After 24h incubation, the levels of IRF-induced SEAP were determined using QUANTI-Blue™ Solution.

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