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

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Version 19K10-MM

HEK-Blue™ ISG KO-STING cells were generated from the HEK-Blue™ ISG cell line through the stable knockout of the STING gene which has been confirmed by PCR, sequencing and Western blot (figure 1). Biological activity has been assessed by measuring the levels of interferon regulatory factors (IRF)-induced SEAP (secreted embryonic alkaline phosphatase) reporter activity (figure 2). HEK-Blue™ ISG cells respond strongly to non-canonical CDNs namely 2'3'-cGAMP but do not respond to canonical CDNs such as 3'3'-cGAMP and its analog cAIMP. Interestingly, fluorinated or bis-phosphorothioate analogs such as 3'3'-cGAMP Fluorinated or 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) induce a strong IRF induction. Of note, HEK-Blue™ ISG cells respond poorly to cytosolic DNA such as intracellular Poly(dA:dT). As expected, HEK-Blue™ ISG KO-STING cells do not respond to any of the CDNs tested (figure 2). Both HEK-Blue™ ISG KO-STING and HEK-Blue™ ISG cells display a robust response to human type I interferons (IFNs).

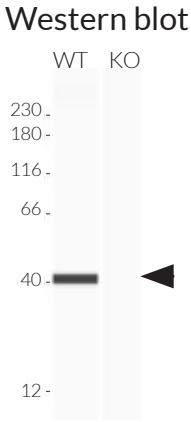

Figure 1: Validation of STING knockout by Western blot (Wes™). Analysis of lysates from the HEK-Blue™ ISG (WT) and HEK-Blue™ ISG-KO-STING (KO) cells using Anti-STING, followed by an HRP-conjugated anti-mouse secondary antibody. The arrow indicates the expected band for the human STING protein (42 KDa).

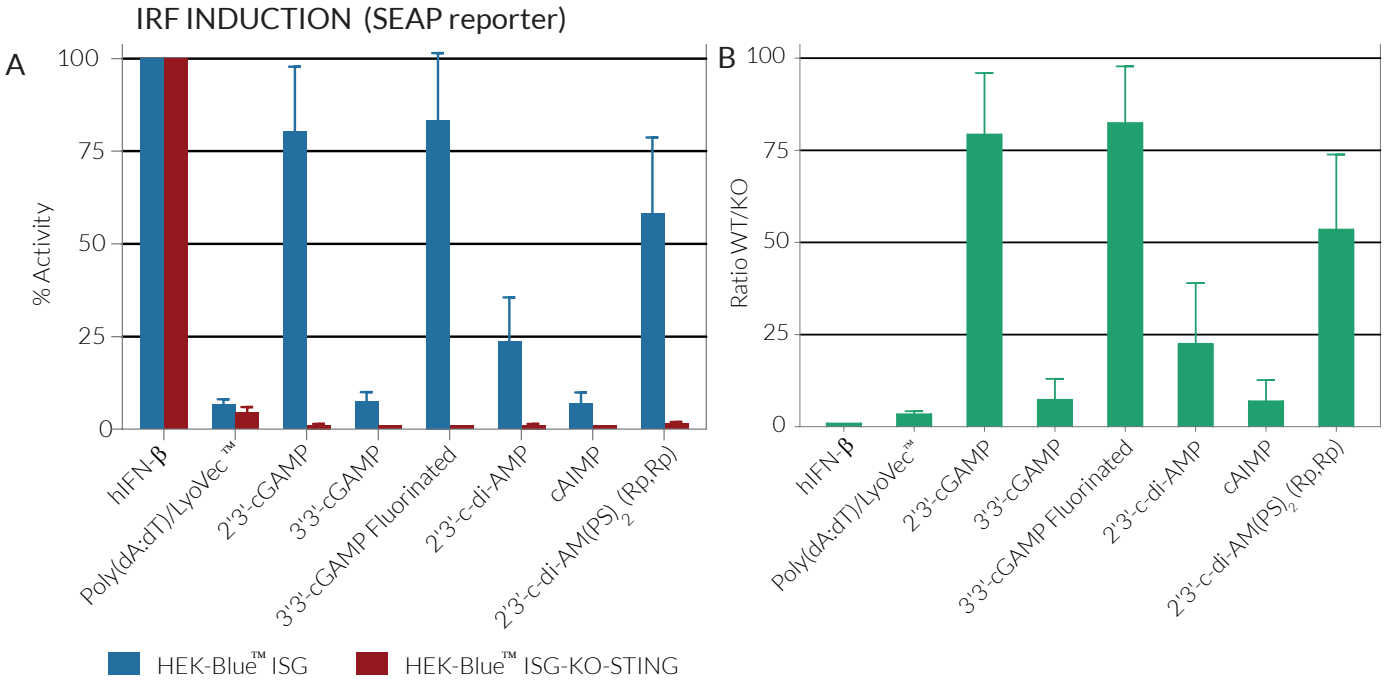

Figure 2. Response of HEK-Blue™ ISG and HEK-Blue™ ISG KO-STING cells to various CDNs, cytosolic dsDNA and IFN-β. A) HEK-Blue™ ISG KO-STING and HEK-Blue™ ISG cells (wild-type cell line) were stimulated with 1 x 10^3 U/ml human IFN-β, 1 µg/ml poly(dA:dT)/LyoVec™, 30 µg/ml 2'3'-cGAMP, 3'3'-cGAMP, 3'3'-cGAMP Fluorinated, 2'3'-c-di-AMP, cAIMP, and 2'3'-c-di-AM(PS)$_2$ (Rp,Rp). After 24h incubation, IRF activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. The IRF induction of each ligand is expressed as % activity relative to that of human IFN-β at 1 x 10^3 U/ml (taken as 100%). **B)** The difference in activity between the two cell lines is expressed as the ratio WT/KO, which was obtained by dividing each value in figure 2A for HEK-Blue™ ISG (WT) cells by the corresponding value for HEK-Blue™ ISG KO-STING cells.

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