Validation data for RAW-Lucia™ ISG-KO-IFI16 cells

For research use only

Version # 16C15-MM

RAW-Lucia™ ISG-KO-IFI16 cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of murine ortholog of IFI16, p204 (also known as IFI204, PYHIN2). These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express several CDSs, including IFI16. The knockout of the IFI16 gene in these cells has been confirmed by PCR (see figure 1) and sequencing. Biological activity has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (see figure 2).

PCR AMPLIFICATION

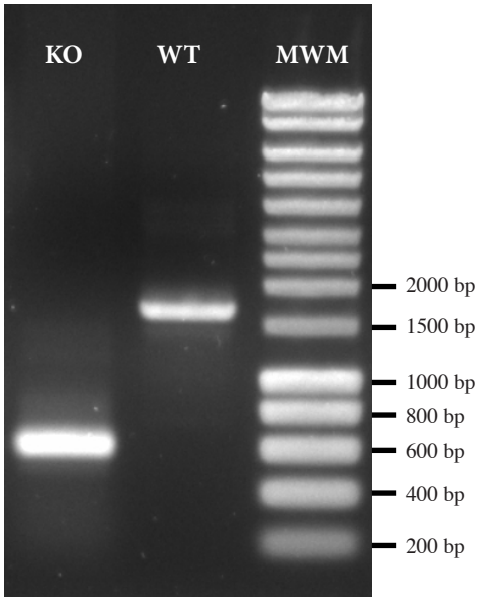

Figure 1: PCR amplification of the targeted region in the RAW-Lucia™ ISG-KO-IFI16 (KO) and RAW-Lucia™ ISG (WT) cells. MWM = molecular weight marker

IRF INDUCTION (Lucia luciferase reporter)

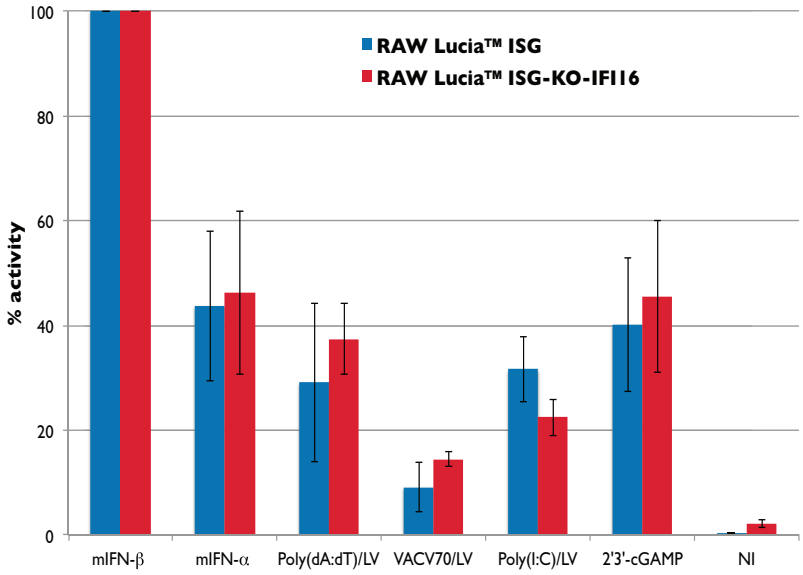

Figure 2: Stimulation of RAW-Lucia™ ISG-KO-IFI16 and RAW-Lucia™ ISG cells (parental cell line) with poly(dA:dT)/LyoVec™ (1 µg/ml), VACV70/LyoVec™ (1 µg/ml), poly(I:C)/LyoVec™ (1 µg/ml), and 2'3'-cGAMP (3 µg/ml). Mouse IFN-α ($1x10^4$ U/ml) and IFN-β ($1x10^4$ U/ml) serve as positive controls. Non-induced cells (NI) have been included as a negative control. 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 mIFN-β at $1x10^4$ U/ml (taken as 100%).

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