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

https://www.invivogen.com/raw-lucia-isg-ko-cgas

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Version 19K09-MM

RAW-Lucia™ ISG-KO-cGAS cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the cGAS gene. These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express several CDSs, including cGAS. The knockout of the cGAS gene in these cells has been confirmed by PCR (see figure 1), sequencing and Western blot (figure 2). Biological activity has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (see figure 3). RAW-Lucia™ ISG-KO-cGAS cells respond to interferons (e.g. IFN-α and IFN-β), cyclic dinucelotides (e.g. 2'3'-cGAMP) and transfected poly(I:C). However, as expected, they respond very poorly to transfected DNA, such as poly(dA:dT)/LyoVec™ and VACV70/LyoVec™.

PCR amplification

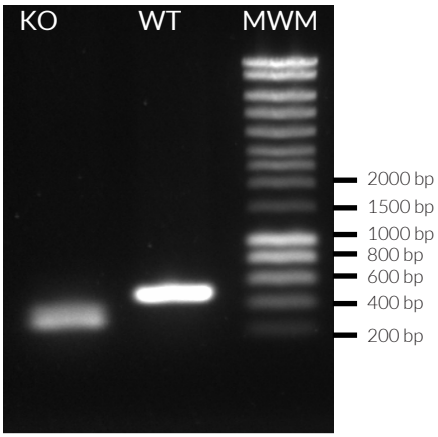

Figure 1: PCR amplification of the targeted region in the RAW-Lucia™ ISG-KO-cGAS (KO) and RAW-Lucia™ ISG (WT) cells. MWM = molecular weight marker

Western blot

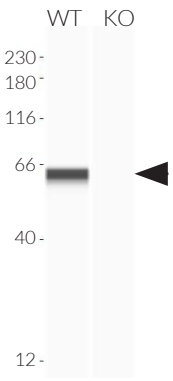

Figure 2: Validation of cGAS knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ (WT) and RAW-Lucia™ ISG-KO-cGAS (KO) cells using Anti-cGAS, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the cGAS protein (59 KDa).

IRF INDUCTION (Lucia luciferase reporter)

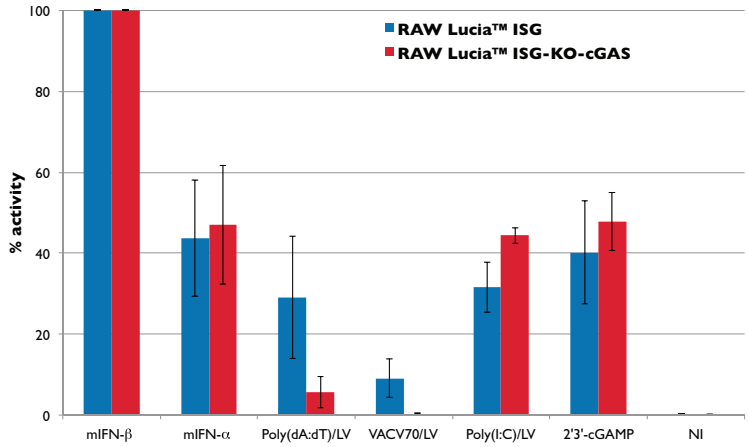

Figure 3: Stimulation of RAW-Lucia™ ISG-KO-cGAS and RAW-Lucia™ ISG cells with poly(dA:dT)/LyoVec™ (1 µg/ml), VACV70/LyoVec™ (1 µg/ml), poly(I:C)/LyoVec™ (1 µg/ml), and 2'3'-cGAMP (3 µg/ml). Mouse IFN-α (1x10^4 U/ml) and IFN-β (1 x 10^4 U/ml) serve as positive controls. Non-induced cells (NI) have been 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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