Validation data for RAW-Lucia™ ISG-KO-TBK1 cells

https://www.invivogen.com/raw-lucia-isg-ko-tbk1

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Version 19K15-MM

RAW-Lucia™ ISG-KO-TBK1 cells were generated from the RAW-Lucia™ ISG cell line, which is derived from the murine RAW 264.7 macrophage cell line, through the stable knockout of the TBK-1 gene which has been confirmed by Western blot (figure 1). These cells express an interferon regulatory factor (IRF)-inducible secreted reporter gene, Lucia luciferase. As a result, RAW-Lucia™ ISG-KO-TBK1 and RAW-Lucia™ ISG cells can be used to study the role of TBK-1 by the monitoring IRF-induced Lucia luciferase activity (figure 2). Of note, the response of RAW-Lucia™ ISG-KO-TBK1 cells to murine type I interferons (IFNs) is unaffected by the knockout of the TBK-1 gene. As expected, RAW-Lucia™ ISG-KO-TBK1 cells do not respond to cyclic dinucelotides or to transfected double-stranded DNA, such as VACV70/LyoVec™.

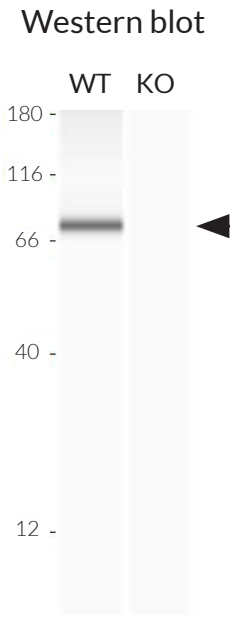

Figure 1: Validation of TBK-1 knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ (WT) and RAW-Lucia™ ISG-KO-TBK1 (KO) cells using Anti-TBK-1, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the murine TBK-1 protein (83 kDa).

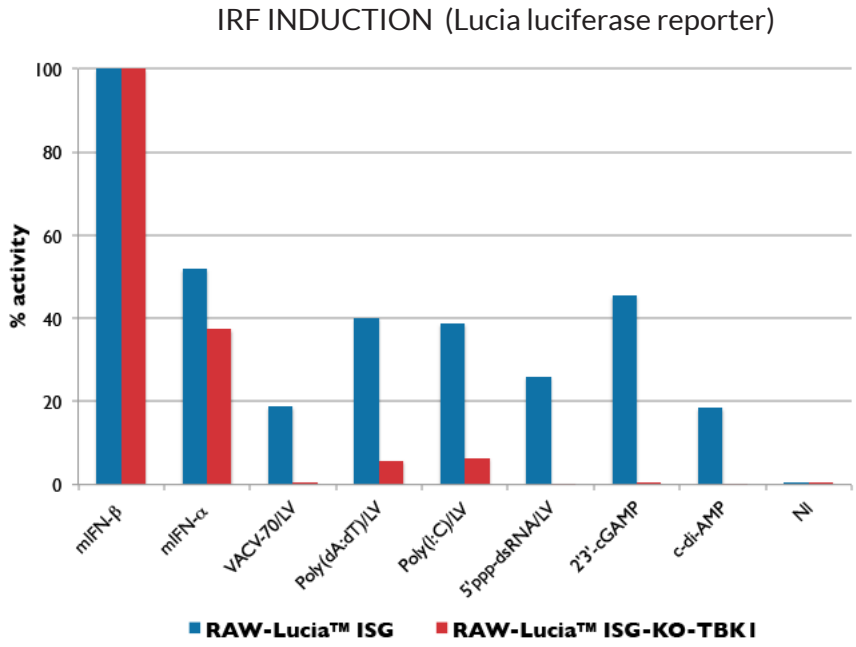

Figure 2: Response of RAW-Lucia™ ISG-KO-RIG-I cells to various stimuli. RAW-Lucia™ ISG-KO-TBK1 and RAW-Lucia™ ISG cells (parental cell line) were incubated with VACV70/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (1 µg/ml), poly(I:C) HMW/LyoVec™ (1 µg/ml), 5'ppp-dsRNA/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml) and c-di-AMP (3 µg/ml). Mouse IFN-α (1 x 10^4 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 1 x 10^4 U/ml (taken as 100%).

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