Validation data for RAW-Lucia™ ISG-KO-IRF5 cells

https://www.invivogen.com/raw-lucia-isg-ko-irf5

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Version 19K25-MM

RAW-Lucia™ ISG-KO-IRF5 (KO-IRF5) cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the *IRF5* gene, as verified by PCR **(Figure 1)** and Western blot **(Figure 2)**. These cells feature a reporter gene allowing the study of the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase. Lucia luciferase activity is readily assessable in the cell culture supernatant using the QUANTI-Luc™ detection reagent. As expected, the IRF response is strongly diminished when the KO-IRF5 cells are incubated with STING agonists, such as 2'3'-cGAMP. The IRF response is not completely abrogated because other IRFs, such as IRF3 have overlapping functions downstream in the STING signaling pathway **(Figure 3)**. However, different responses are observed when using DNA- or RNA-based agonists with varying transfection reagents **(Figure 4)**. Notably, KO-IRF5 cells retain full ability to respond to type I interferons (IFN-α and IFN-β) and lipopolysaccharide (LPS) **(Figure 5)**.

PCR

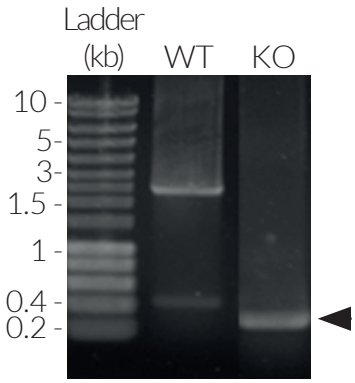

Figure 1: Validation of IRF5 knockout by PCR. Amplification of the targeted *IRF5* region in RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-IRF5 (KO) cells. RAW-Lucia™ ISG-KO-IRF5 cells feature a biallelic deletion (arrow).

Western blot

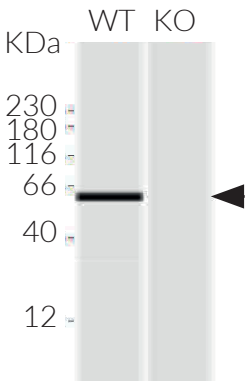

Figure 2: Validation of IRF5 knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-IRF5 (KO) cells using Anti-IRF5, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the IRF5 protein (56 KDa).

Functional validation of IRF5 knockout

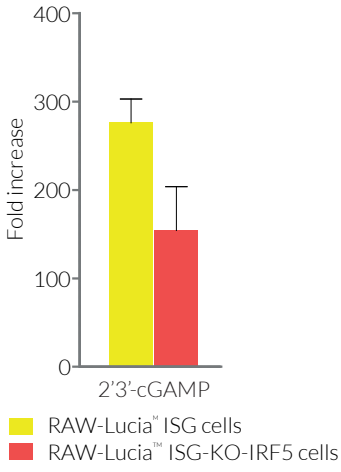

Figure 3: 2'3'-cGAMP-induced IRF response in RAW-Lucia™ ISG-derived cells. RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-IRF5 cells were incubated with 30 µg/ml 2'3'-cGAMP (STING agonist). After overnight incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using the QUANTI-Luc™ detection reagent. Activity fold increase over non-induced cells is shown.

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Evaluation of IRF reponses upon DNA or RNA transfection

DNA- and RNA-based agonists of cytosolic nucleic acid sensors must be complexed with a transfection reagent to enter the cytoplasm. G3-YSD and VACV-70 are DNA ligands that induce a potent IRF response in RAW-Lucia™ ISG cells whether complexed to LyoVec™ or LTX transfection reagents (A, B). However, we observe that the IRF response is not dimished to same extent in RAW-Lucia™ ISG-KO-IRF5 (KO-IRF5) cells, depending on the transfection reagent. While the response to both agonists is strongly impaired when using LyoVec™ (A), it is less impacted when using LTX (B). These data suggest that different transfection reagents could highlight overlapping DNA-sensing mechanisms. 3p-hpRNA and 5'ppp-dsRNA are two RIG-I agonists. The weak agonist 5'ppp-dsRNA loses its ability to induce a potent IRF response in KO-IRF5 cells when complexed with LyoVec™ or LTX (C, D). On the contrary, a differential IRF response is observed when using the highly potent agonist 3p-hpRNA with the two transfection reagents. While the IRF response to 3p-hpRNA complexed to LyoVec™ in KO-IRF5 cells is strongly diminished (C), the response to 3p-hpRNA complexed LTX remains unaltered (D). These data suggest that the use of different RNA-based agonists and transfection reagents could highlight overlapping RNA-sensing or regulatory mechanisms.

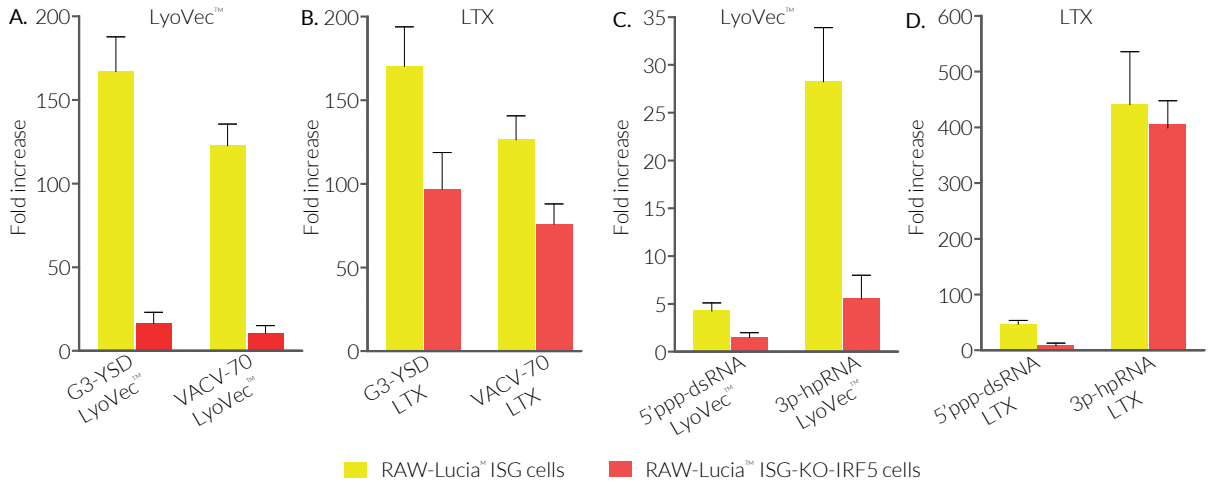

Figure 4: DNA- or RNA-induced IRF response in RAW-Lucia™ ISG-derived cells. RAW-Lucia™ ISG or RAW-Lucia™ ISG-KO-IRF5 cells were transfected with 1 µg/ml G3-YSD, 1 µg/ml VACV-70 (A, B), 1 µg/ml 3p-hpRNA, or 1 µg/ml 5'ppp-dsRNA (C, D) complexed with Lyovec™ (A, C) or LTX (B, D). After overnight incubation, the IRF response was assessed by measuring bioluminescent activity of the Lucia luciferase in the supernatant using QUANTI-Luc™. Activity fold increase over non-transfected cells is shown.

Evaluation of IRF reponses upon type I interferon or LPS induction

RAW-Lucia™ ISG-KO-IRF5 cells retain a full ability to respond to type I interferons (IFN-α and IFN-β) for which signaling is IRF5-independent and driven by STAT1/STAT2/IRF9. Similarly, the IRF response to lipopolysaccharide (LPS), which is mediated by TLR4/MyD88 signaling remains unaltered in RAW-Lucia™ ISG-KO-IRF5 cells, which suggests that IRF5 is not implicated or that IRF5 is fully redundant with other IRFs such as IRF3.

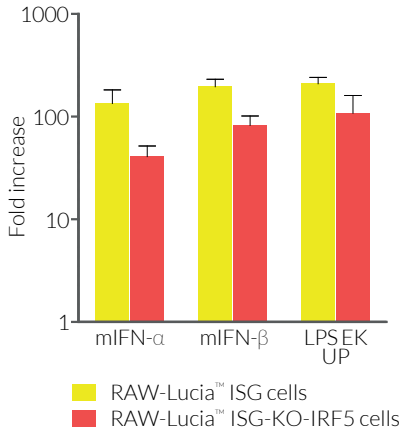

Figure 5: Type I interferon-or LPS-induced IRF response in RAW-Lucia™ ISG-derived cells. RAW-Lucia™ ISG or RAW-Lucia™ ISG-KO-IRF5 cells were incubated with 10^4 U/ml mouse IFN-α (mIFN-α), 10^4 U/ml mouse IFN-β (mIFN-β), or 1 µg/ml LPS EK Ultrapure (UP). After overnight incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using the QUANTI-Luc™ detection reagent. Activity fold increase over non-induced cells is shown.

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