Validation data for RAW-Lucia™ ISG-KO-IRF1 cells

https://www.invivogen.com/raw-lucia-isg-ko-irf1

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RAW-Lucia™ ISG-KO-IRF1 (KO-IRF1) cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the *IRF1* 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. Because IRF1 and other IRF-family members bind to closely related and overlapping ISRE motifs in ISG-promoters, RAW-Lucia™ ISG-KO-IRF1 cells and their RAW-Lucia™ ISG parental cells display similar induction of the Lucia luciferase reporter upon incubation with 3p-hpRNA (a RIG-I agonist) or 2'3'-cGAMP (a STING agonist). However, the responses to lipopolysaccharide (LPS), a TLR4 agonist, and to mIFN-β and mIFN-γ are dimished in the KO-IRF1 cell line as compared to the parental cell line **(Figure 3)**. This may be partly explained by the fact that IRF1 contributes to the amplification of TLR4- and IFN-induced cellular responses.

PCR

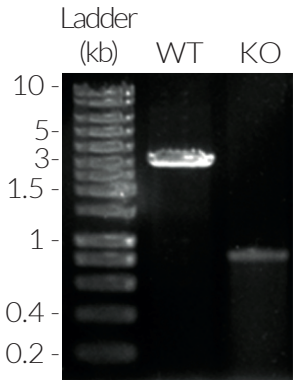

Figure 1: Validation of IRF1 knockout by PCR. Amplification of the targeted *IRF1* region in RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-IRF1 (KO) cells. RAW-Lucia™ ISG-KO-IRF1 cells feature a biallelic deletion (arrow).

Western blot

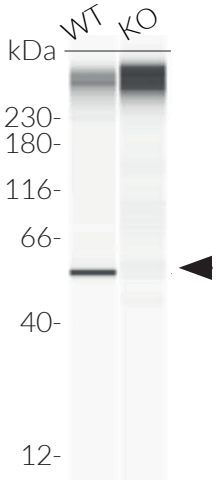

Figure 2: Validation of IRF1 knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-IRF1 (KO) cells using Anti-IRF1, followed by a HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the IRF1 protein (56 KDa).

Functional validation of IRF1 knockout

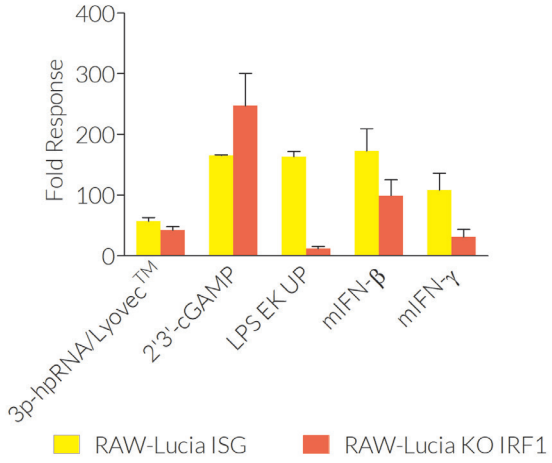

Figure 3: IRF response in RAW-Lucia™ ISG-derived cells. RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-IRF1 cells were incubated with 1µg/ml 3p-hpRNA/Lyovec™ (RIG-I agonist), 30 µg/ml 2'3'-cGAMP (STING agonist). 10 ng/ml LPS EK Ultrapure (TLR4 agonist), 10 ng/ml mIFN-β or mIFN-γ. After overnight incubation, the 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.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com