Validation data for THP1-Dual™ KO-cGAS cells

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Version 19K16-MM

THP1-Dual™ KO-cGAS cells were generated from THP1-Dual™ cells by stable knockout of the cGAS gene which has been confirmed by Western blot (figure 1). These cells derive from the human monocytic cell line THP-1, which is often used for the study of DNA-sensing pathways. THP1-Dual™ and THP1-Dual™ KO-cGAS cells can be used to study cGAS signaling. Both cell lines express two inducible reporter constructs that enable the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP (secreted embryonic alkaline phosphatase), and the IRF (interferon regulatory factor) pathway, by assessing the activity of the secreted Lucia luciferase. The IRF induction response of these cells to different ligands has been assessed (figure 2). RAW-Lucia™ ISG-KO-cGAS cells respond to interferons (e.g. IFN-α and IFN-β) and cyclic dinucelotides (e.g. 2'3'-cGAMP). However, as expected, they respond very poorly to transfected DNA, such as VACV70/LyoVec™ and poly(dA:dT)/LyoVec™. The NF-κB response of THP1-Dual™ KO-cGAS cells is unaffected by the knockout of the cGAS gene (figure 3).

Western blot

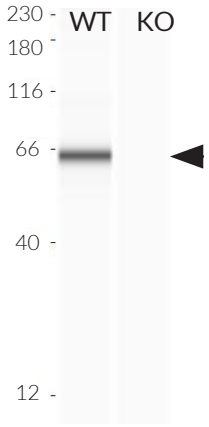
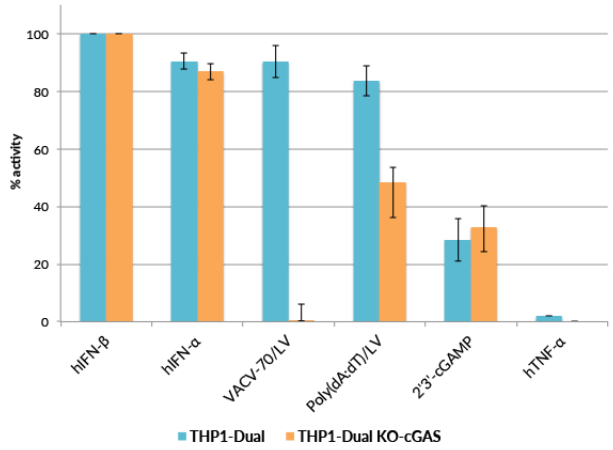


Figure 1: Validation of cGAS knockout by Western blot (Wes™). Analysis of lysates from the THP1-Dual™ (WT) and THP1-Dual™ KO-cGAS (KO) cells using Anti-cGAS, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the human cGAS protein (59 KDa).

IRF Induction (Lucia luciferase reporter)



NF-κB Induction (SEAP reporter)

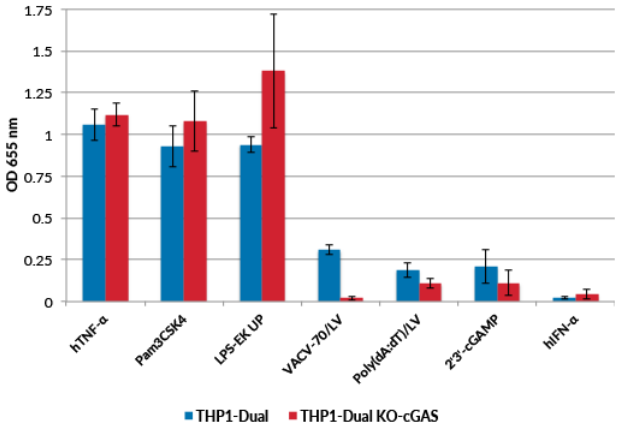


Figure 2: Stimulation of THP1-Dual™ (parental cell line) and THP1-Dual™ KO-cGAS cells with VACV70/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (100 ng/ml), and 2'3'-cGAMP (1 µg/ml). Human IFN-α (1 x 10^4 U/ml) and IFN-β (1 x 10^4 U/ml) serve as positive controls. Human TNF-α (10 ng/ml) has been included as 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 hIFN-β at 1 x 10^4 U/ml (taken as 100%).

Figure 3: THP1-Dual™ and THP1-Dua™ KO-cGAS cells were incubated with TNF-α (0.1 ng/ml), Pam3CSK4 (0.1 ng/ml; TLR1/2 ligand), LPS-EB Ultrapure (100 ng/ml; TLR4 ligand), VACV70/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (100 ng/ml), 2'3'-cGAMP (3 µg/ml) and hIFN-α (1 x 10^4 U/ml). After a 24h incubation, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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