Validation data for THP1-Dual™ KO-IKKε cells

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Version 21B04-ED

THP1-Dual™ KO-IKKε cells were generated from the THP1-Dual™ cell line through the stable knockout (KO) of the *IKK*ε gene, as verified by PCR and Western blot **(Figure 1)**. These cells feature two reporter genes allowing the simultaneous study of the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). Importantly, comparison to our THP1-Dual™ KO-TBK1 cells, which feature a KO of the highly similar IKK-related kinase TBK1, allows overlap and differences in signaling functions between IKKε and TBK1 to be fully elucidated **(Figure 2-3)**.

Validation of *IKK*ε knockout

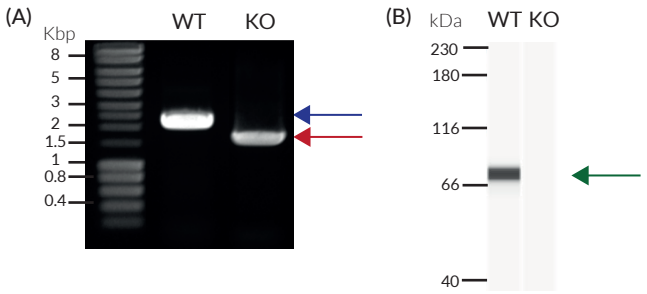

Figure 1: Validation of IKKε KO. (A) The targeted IKKε region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-IKKε (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-IKKε cells feature a frameshift deletion, causing an early stop codon and inactivation of IKKε. **(B)** Lysates from THP1-Dual™ (WT) and THP1-Dual™ KO-IKKε (KO) cells were analyzed using an anti-human IKKε antibody (green arrow), followed by a HRP-conjugated anti-rabbit secondary antibody (WES assay). As expected a band was detected at ~80 Da in the WT cells only.

Functional validation of *IKK*ε knockout (NF-κB and IRF response)

IKKε and TBK1 have both been shown to coordinate the activation of IRF3 and NF-κB in the innate immune response. Thus, as expected the NF-κB-dependent response in THP1-Dual™ KO-IKKε upon stimulation with the STING agonist, 2'3'-cGAMP, is greatly reduced and comparable to THP1-Dual™ KO-TBK1 (A). However, IKKε seems to play a lesser role in IRF-dependent STING signaling (B). Notably, THP1-Dual™ KO-IKKε cells retain a full ability to respond to (C) NF-κB- and (D) IRF-dependent cytokines such as human hTNF-α and type I interferons (hIFN-β), respectively, for which signaling is IKKε-independent.

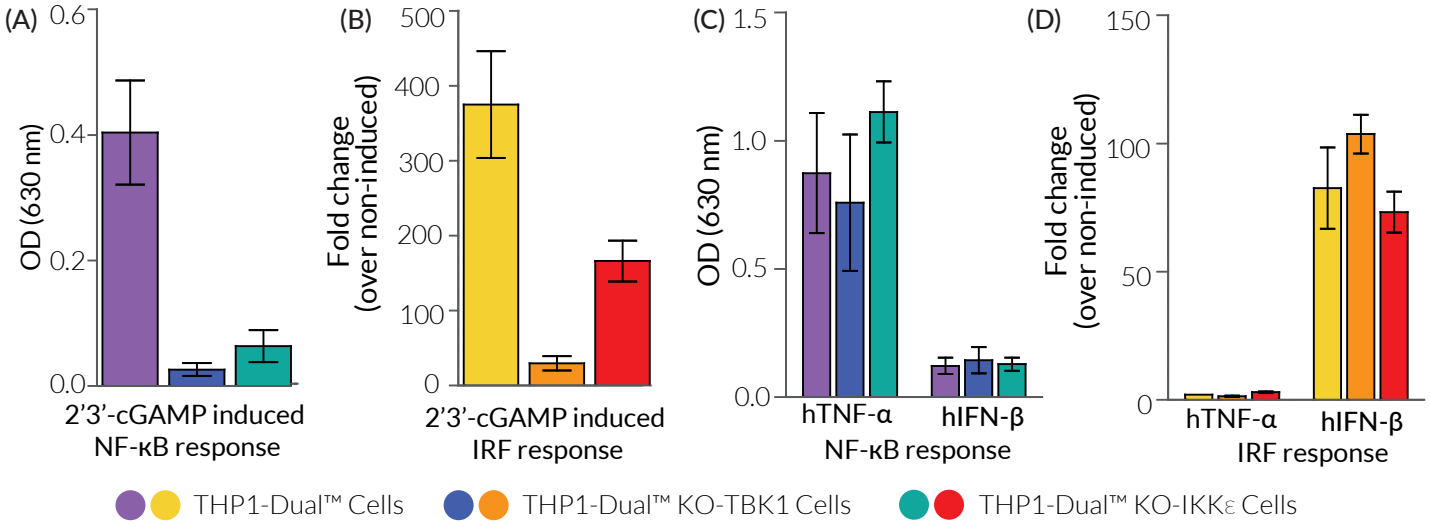

Figure 2: NF-κB and IRF responses in THP1-Dual™-derived cells. THP1-Dual™ (WT) and THP1-Dual™ KO-TBK1, and THP1-Dual™ KO-IKKε cells were incubated with (A-B) 30 µg/ml 2'3'-cGAMP (STING agonist), (C-D) 0.3 ng/ml human TNF-α (hTNF-α), or 10^4 U/ml human IFN-β (hIFN-β). After overnight incubation, the NF-κB activity was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM) (A, C). The IRF response was assessed by measuring Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are shown as a fold change (mean ± SEM) over non-induced cells (B, D).

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Evaluation of IRF and NF-κB reponses upon RNA transfection

3p-hpRNA is an agonist of RIG-I that must be complexed with a transfection reagent to enter the cytoplasm. Upon stimulation with 3p-hp-RNA complexed to LTX, the NF-κB-dependent response in THP1-Dual™ KO-IKKε is barely affected, similiar to THP1-Dual™ KO-TBK1 (A). However, when complexed to LyoVec™, a reduction in activity is noted in THP1-Dual™ KO-IKKε cells only (C). Interestingly, with either transfection reagent (B: LTX or D: LyoVec™), the IRF-dependant response of THP1-Dual™ KO-IKKε is comparable to the parental cell line THP1-Dual™. This is noticably different to THP1-Dual™ KO-TBK1, where the IRF-response is unexpectedly increased. This opposing observation between IKKε and TBK1 in the IRF-dependent response to RNA ligands may suggest that in the absence of TBK1, IKKε homodimers play a greater role in the RNA-sensing signaling pathway.

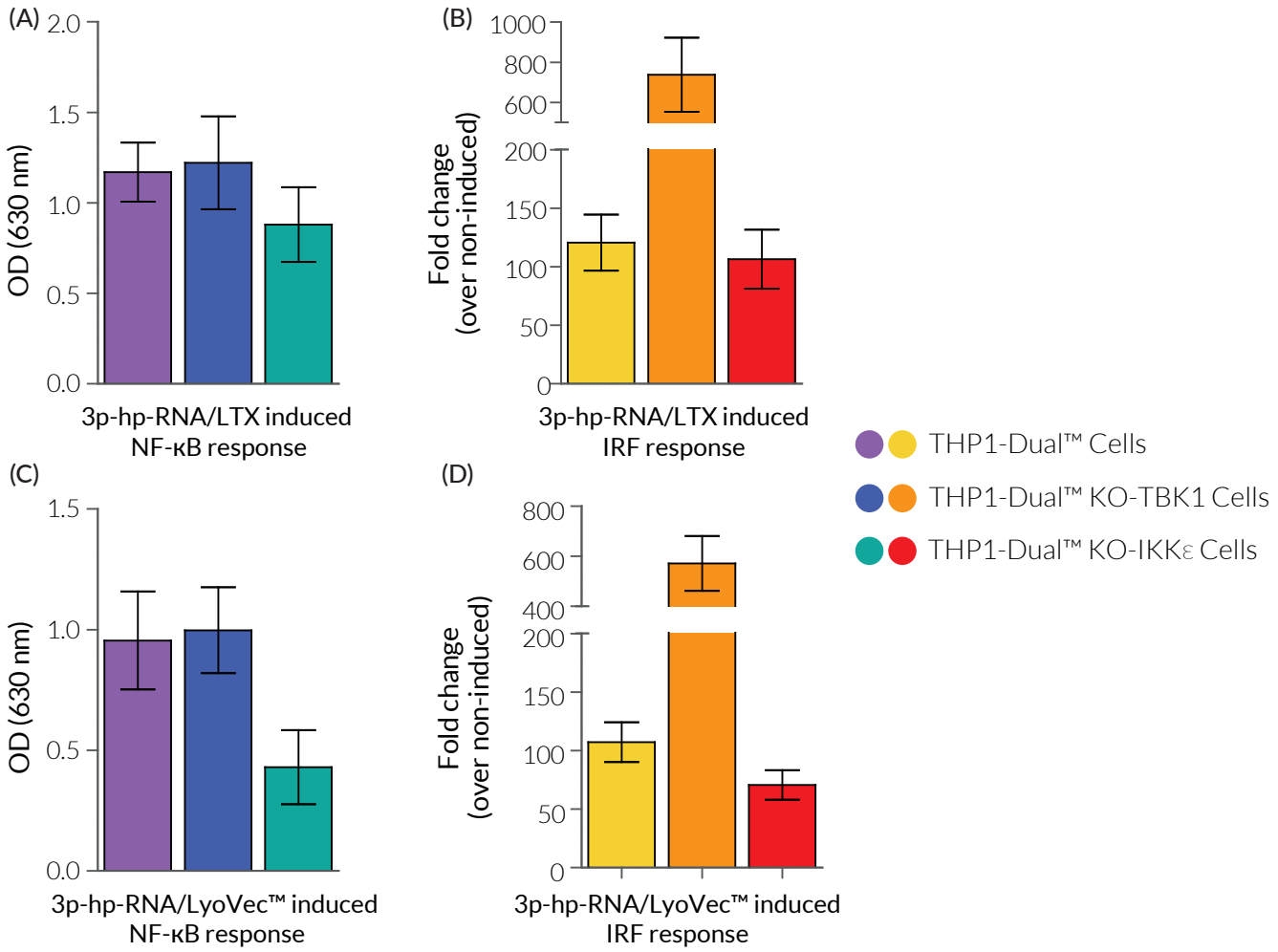

Figure 3: RNA-induced IRF and NF-κB responses in THP1-Dual™-derived cells. THP1-Dual™, THP1-Dual™ KO-TBK1, or THP1-Dual™ KO-IKKε cells were transfected with 1 µg/ml 3p-hpRNA complexed with LTX (A, B) or Lyovec™(C, D). After overnight incubation, the NF-κB activity i was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM) (A, C). The IRF response was assessed by measuring Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are shown as a fold change (mean ± SEM) over non-transfected cells (B, D).

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