

Validation data for HEK-Blue™ KO-TIFA cells

<https://www.invivogen.com/ko-alk1-tifa-cells>

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Version 19L20-ED

HEK-Blue™ KO-TIFA cells were generated from the HEK-Blue™ Null1-v cell line through the stable knockout of the TIFA gene as verified by qPCR (Figure 1). These cells feature a reporter gene allowing for the study of the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase). The activity of SEAP is abolished when the HEK-Blue™ KO-TIFA cells are incubated with ADP-Heptose, however is unimpaired when activated by human (h)TNF-α and hIL-1β (Figure 2).

Validation of TIFA knockout by qPCR

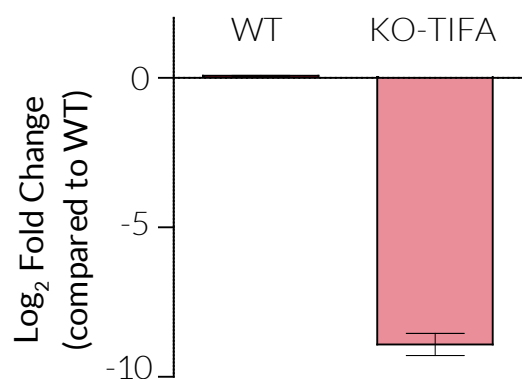


Figure 1: Validation of TIFA knockout by quantitative PCR (qPCR). The targeted TIFA region in HEK-Blue™ Null1-v (WT) and HEK-Blue™ KO-TIFA (KO-TIFA) cells was amplified by qPCR. HEK-Blue™ KO-TIFA cells display a negative fold change in TIFA gene expression compared to WT.

Functional validation of TIFA knockout

The binding of ADP-β-d-manno-heptose (ADP-Heptose), a metabolic intermediate in lipopolysaccharide (LPS) biosynthesis, to ALPK1 stimulates its essential kinase activity, which in turn phosphorylates and activates TIFA. Ultimately, this results in a TIFA-dependent NF-κB response. As expected, the HEK-Blue™ KO-TIFA cells (KO-TIFA) do not respond to ADP-Heptose (red), when compared to the parental HEK-Blue™ Null1-v cells (WT). Importantly, they retain the full ability to respond to human (h)TNF-α (purple) and hIL-1β (yellow), which also activate the NF-κB pathway.

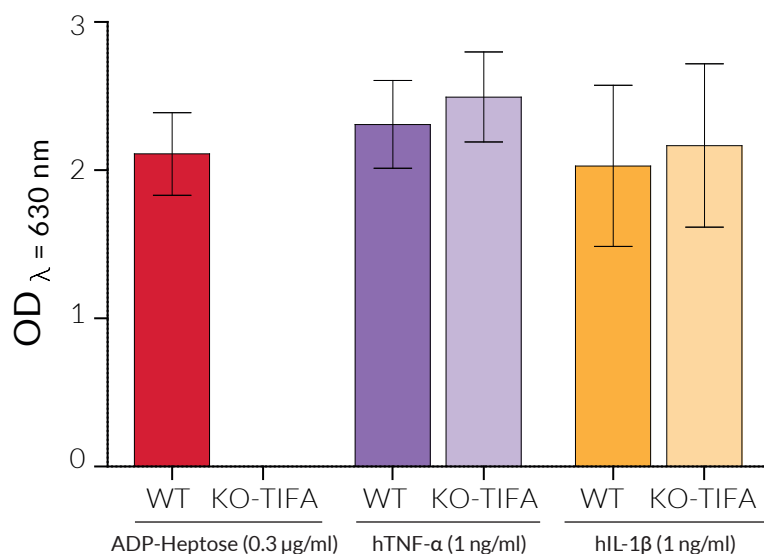


Figure 2: NF-κB response of HEK-Blue™ KO-TIFA cells. HEK-Blue™ Null1-v (WT) and HEK-Blue™ KO-TIFA (KO-TIFA) cells were incubated with 0.3 μg/ml ADP-Heptose, 1 ng/ml human (h)TNF-α, and 1 ng/ml hIL-1β in HEK-Blue™ Detection, a cell culture medium for SEAP detection. After overnight incubation, the NF-κB response was assessed by measuring the activity of SEAP in the supernatant. Data are presented as optical density (OD) at 630 nm (mean ± SEM).

TECHNICAL SUPPORT

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