Validation data for HEK-Blue™ KO-ALPK1cells

https://www.invivogen.com/ko-alpk1-tifa-cells

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Version 19L20-ED

HEK-Blue™ KO-ALPK1 cells were generated from the HEK-Blue™ Null1-v cell line through the stable knockout of the *ALPK1* 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-ALPK1 cells are incubated with ADP-Heptose, however is unimpaired when activated by human (h)TNF-α and hIL-1β **(Figure 2)**

Validation of *ALPK1* knockout by qPCR

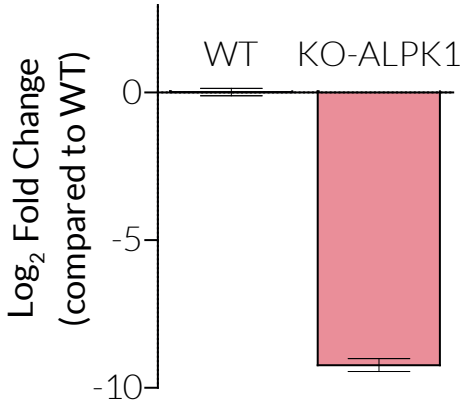

Figure 1: Validation of *ALPK1* knockout by quantitative PCR (qPCR). The targeted *ALPK1* region in HEK-Blue™ Null1-v (WT) and HEK-Blue™ KO-ALPK1 (KO-ALPK1) cells was amplified by qPCR. HEK-Blue™ KO-ALPK1 cells display a negative fold change in *ALPK1* gene expression compared to WT.

Functional validation of *ALPK1* knockout

Activation of the ALPK1-dependent NF-κB response is triggered by the binding of ADP-β-d-manno-heptose (ADP-Heptose), a metabolic intermediate in lipopolysaccharide (LPS) biosynthesis, to ALPK1. As expected, the HEK-Blue™ KO-ALPK1 cells (KO-ALPK1) 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.

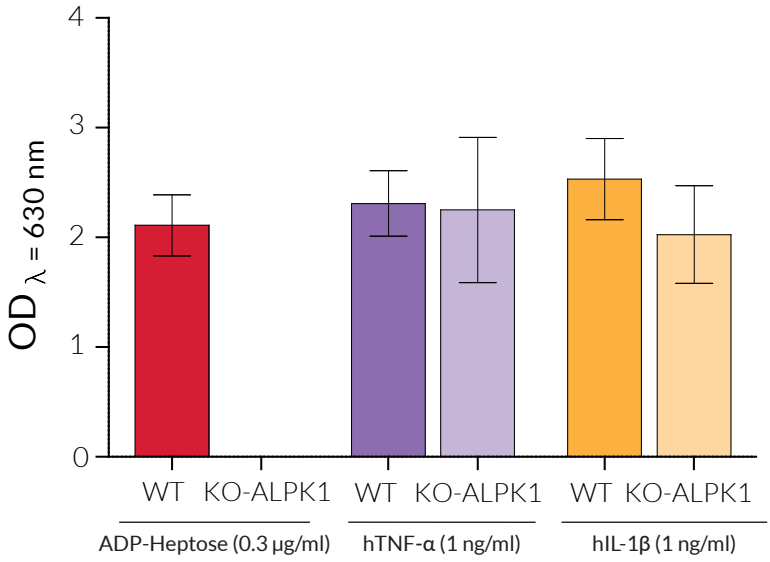

Figure 2: NF-κB response of HEK-Blue™ KO-ALPK1 cells. HEK-Blue™ Null1-v (WT) and HEK-Blue™ KO-ALPK1 (KO-ALPK1) cells were incubated with 0.3 µg/ml ADP-Heptose, 1 ng/ml human (h)TNF-α, and 1 ng/ml hIL-1β in HEK-Blue™ Detection, a cell culture medium for SEAP detection. After overnight incubation, the 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).

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