Translational Neuroinflammation Assays for High-Throughput *In Vitro* Screening

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Background

Microglial cells are central mediators of neuroinflammation, and in vitro models are essential for studying their activation profiles. While lipopolysaccharide (LPS) is a widely used and potent microglial activator, it represents a partially unphysiological stimulus in the context of neuroinflammation. To address this, LPS and several cytokine cocktails were tested for they efficacy to induce neuroinflammation to better mimic the complex inflammatory environment in vitro. In this study, we thus compared the responses of murine (BV-2) and human (HMC3) microglial cell lines to different pro-inflammatory stimuli.

Material and Methods

BV-2 and HMC3 cells were stimulated with either lipopolysaccharide (LPS) or one of five different cytokine cocktails representing combinations of interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin 1-β (IL-1β). Inflammatory activation was assessed on the transcriptional and protein level 24 h after stimulation. mRNA expression was analyzed by RT-qPCR in comparison to the housekeeping gene HPRT. Expression levels relation to the unstimulated vehicle control (VC) were calculated by the ΔΔC(t) method. Cytokine release into the culture supernatant was measured by immunosorbent assays (Mesoscale Discovery, MSD).

Results

Induction of Cytokine mRNA Expression

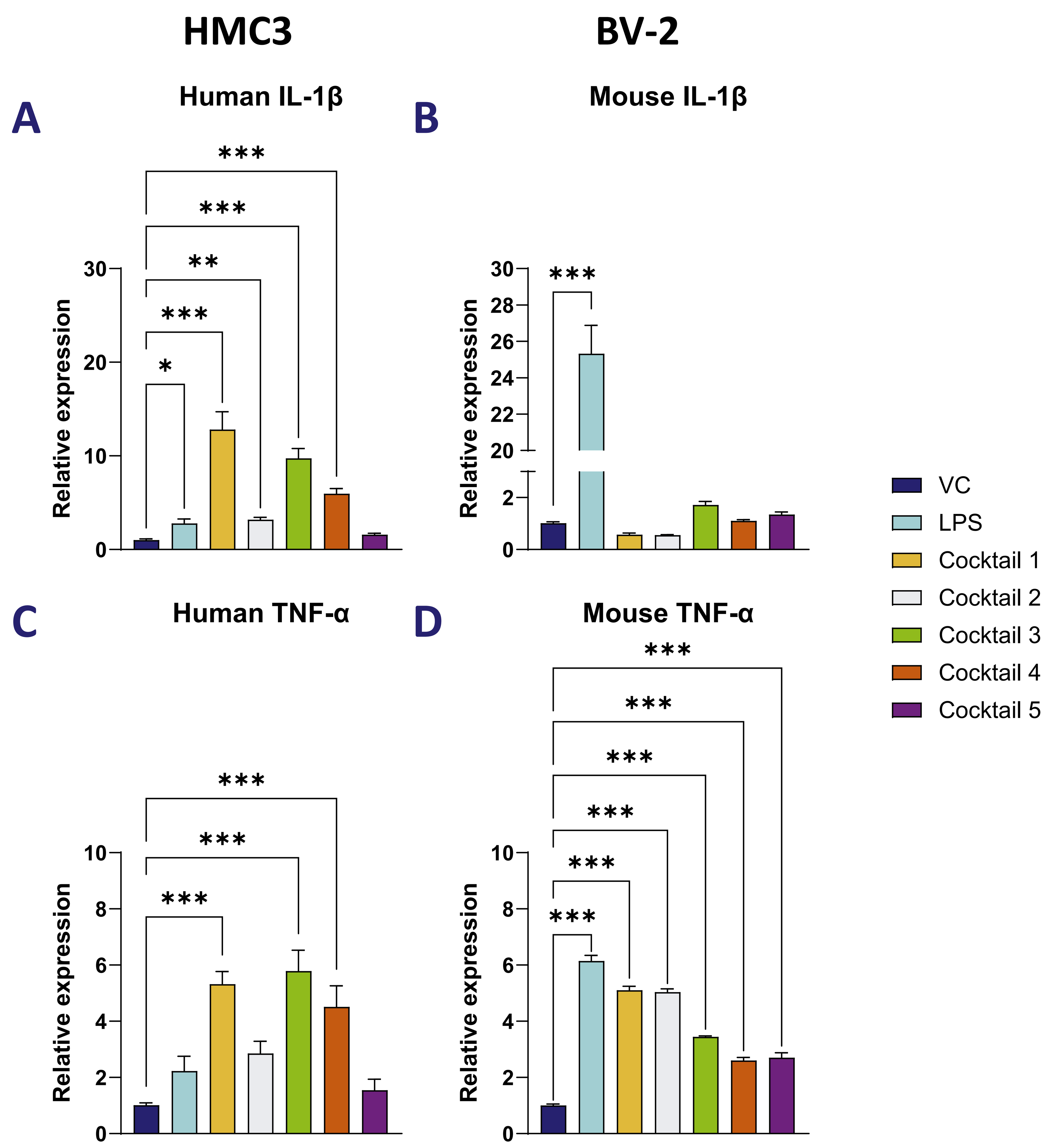

Figure 1: Effect of 24 h LPS or cytokine cocktail treatment on cytokine mRNA expression levels in human microglial HMC3 or murine microglial BV-2 cells. mRNA levels of IL-1β (**A**, **B**) and TNF-α (**C**, **D**) in HMC3 and BV-2 cells. Mean + SEM. n = 6 per group. One-way ANOVA followed by the Dunnett`s *post hoc* test versus vehicle control (VC). *p <0.05, **p <0.01 and ***p <0.001.

Both, LPS and various cytokine cocktails, significantly induce mRNA expression of IL-1β and TNF-α in human HMC3 cells, while in murine BV-2 cells, LPS induces a higher mRNA expression of IL-1β compared to cytokine cocktails (Figure 1). Cytokine secretion levels vary between human HMC3 and murine BV-2 cells; for instance, LPS strongly induces IL-6 secretion in murine BV-2 cells but not as much in human HMC3 cells. Different cytokines are secreted at varying levels depending on whether LPS or specific cytokine cocktails are used as stimulus. Only IP-10 secretion shows a similar pattern between HMC3 and BV-2 cells, even though the absolute values differ quite strongly (Figure 2).

Results

Induction of Cytokine Secretion

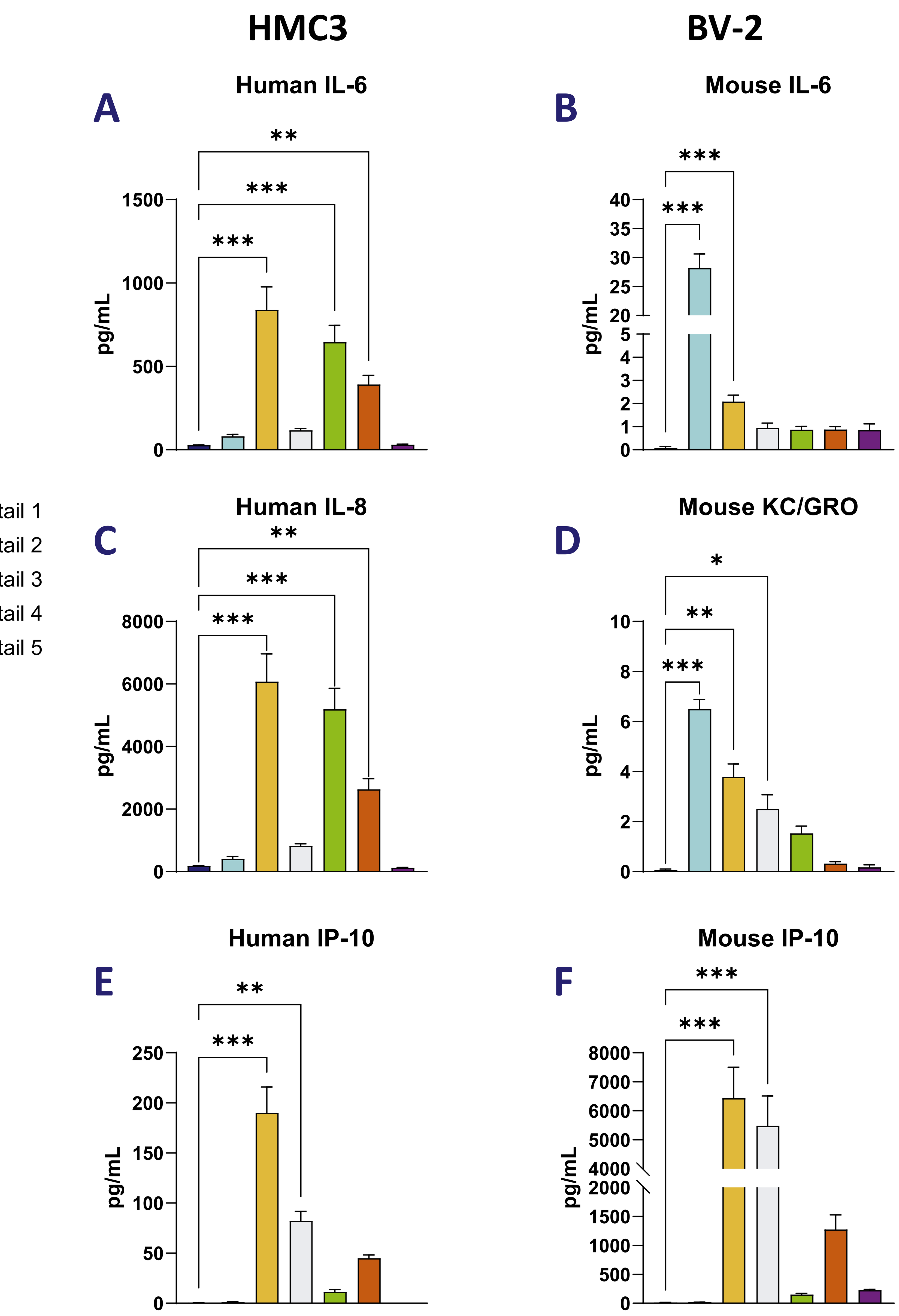

Figure 2: Effect of 24 h LPS or cytokine cocktail treatment on cytokine secretion in human microglial HMC3 or murine microglial BV-2 cells. Secreted levels of IL-6 (**A**, **B**), IL-8 (**C**), murine homologue KC/GRO (**D**), and IP-10 (**E**,**F**) in respective cell lines. Mean + SEM. n = 6 per group. One-way ANOVA followed by the Dunnett`s *post hoc* test or Kruskal-Wallis test followed by Dunn's multiple comparison test versus vehicle control (VC). *p <0.05, **p <0.01 and ***p <0.001.

Conclusion

These findings underline the importance of stimulus selection and highlight species-specific differences in microglial activation, which are critical to consider when translating *in vitro* findings to human pathophysiology.

For more information about the models please visit: www.scantox.com
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