

Background

- Lumbosacral radiculopathy (LSR) or sciatica, is a type of low back pain (LBP) that presents a significant global health challenge. LSR can be caused by disc or vertebral damage which compresses or irritates nerve roots in the lumbar region leading to numbness or radiating pain.
- Current therapies aim to alleviate symptoms of LSR but no interventions halt or reverse degeneration.
- This study evaluated an allogeneic multi-protein platelet and plasma-derived biologic, C-1101, on human monocytes, ovine spine tissues, and a murine model of inflammatory peripheral neuropathy.

Aims

- The aims were to assess the capacity of C-1101 to 1) modulate human macrophage cytokine secretion 2) impact cytokine secretion and differential gene expression in three relevant spine cell lines (nucleus pulposus, annulus fibrosus, dorsal root ganglia) in a relevant large animal ovine model and 3) enhance analgesia in a rodent murine model of paclitaxel-induced peripheral neuropathy.

Methods

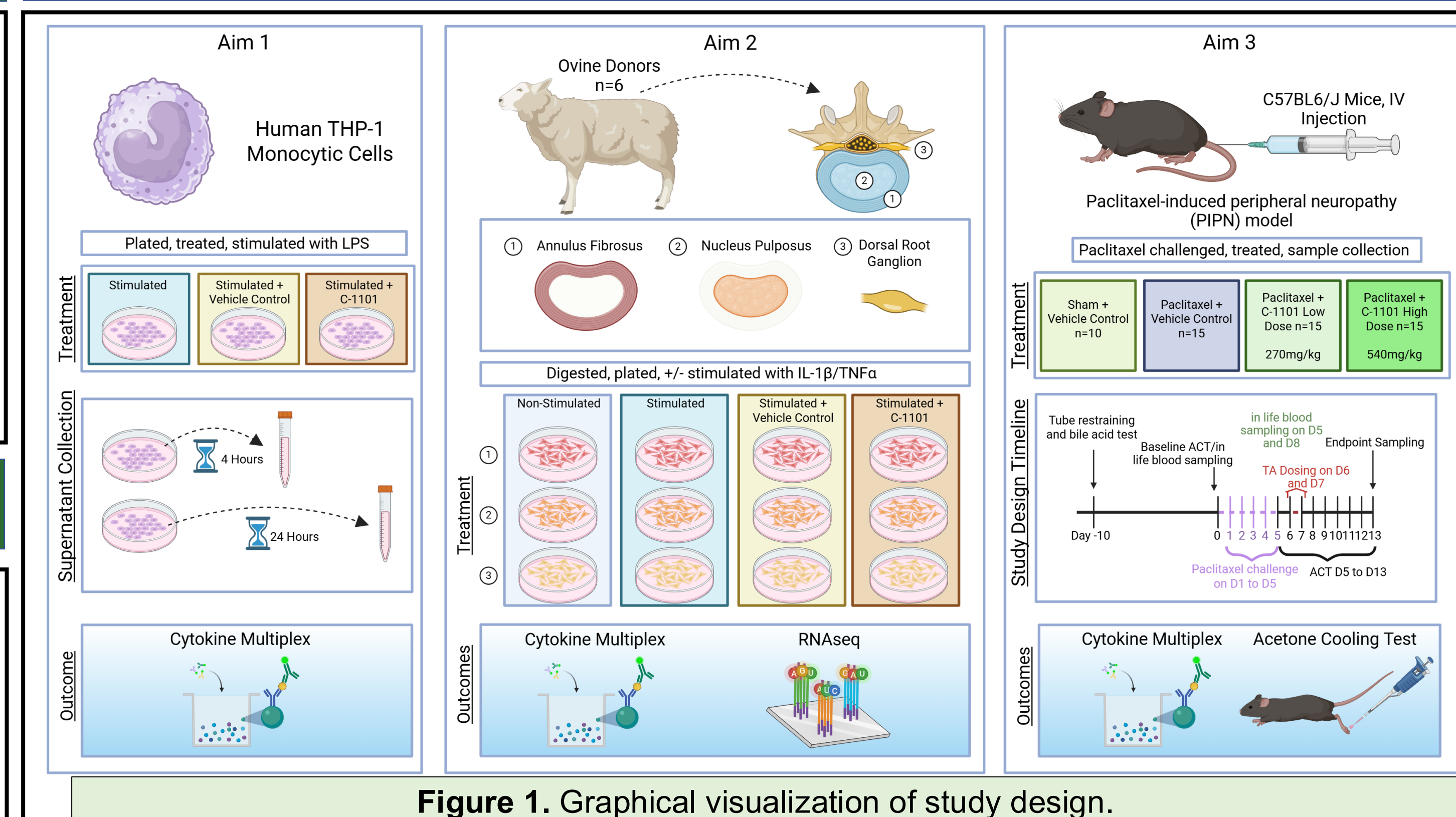


Figure 1. Graphical visualization of study design.

Results

Figure 2: C-1101 reduced inflammatory cytokine secretion from LPS stimulated human monocytic THP-1 cells

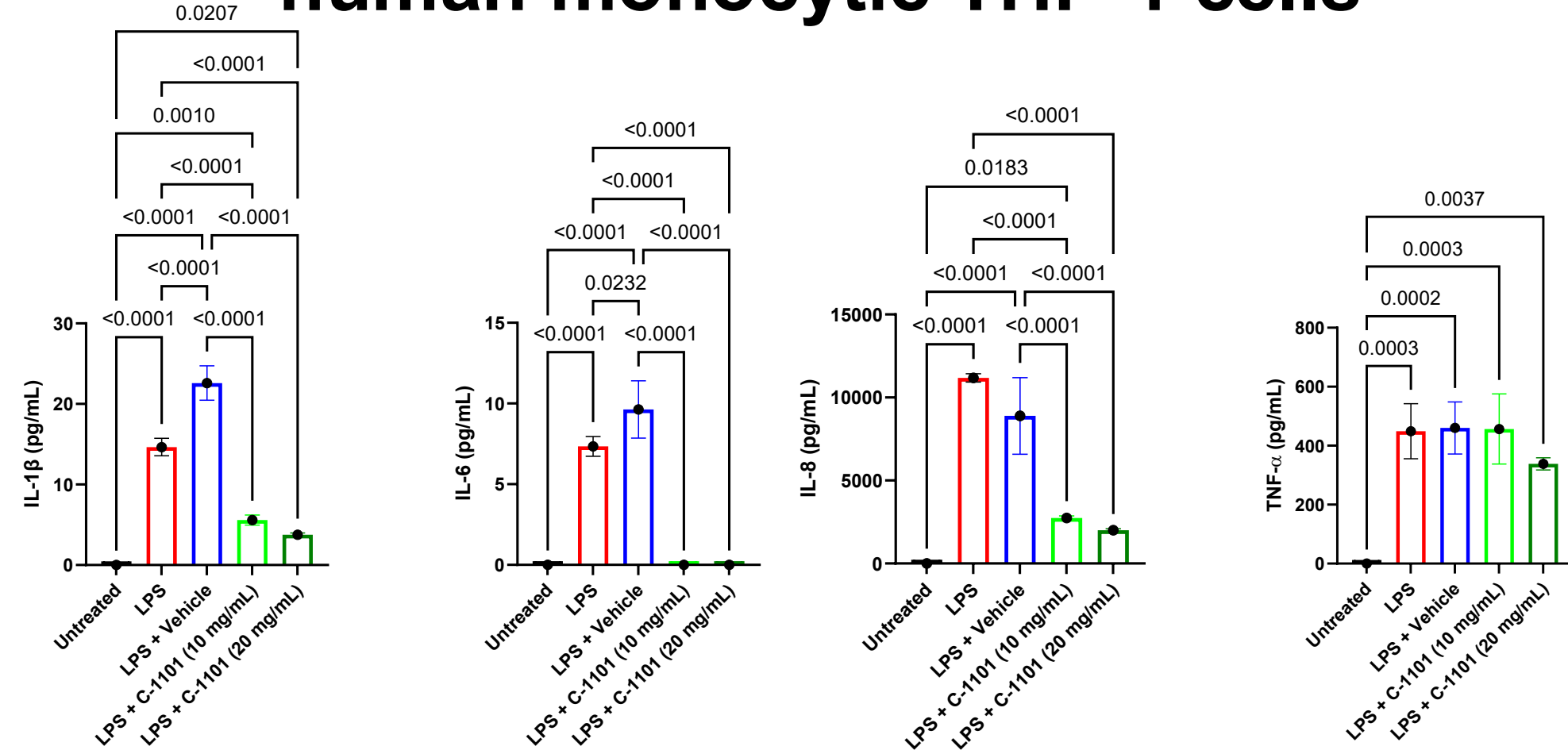


Figure 2. THP-1 human monocytic cells were pre-incubated with media, vehicle, or C-1101 (10 or 20 mg/mL), then stimulated with lipopolysaccharide (LPS, 500 ng/mL). Cytokine secretion was assessed at 24 hours.

Figure 3: C-1101 promoted cytokine secretion in inflamed ovine annulus fibrosis (AF) cells.

Figure 3 – C-1101 promoted cytokine secretion in inflamed ovine annulus fibrosis (AF) cells. Ovine AF cells were stimulated with IL-1β and TNFα (20 ng/mL) and then treated with vehicle control or C-1101 (50 mg/mL). A non-stimulated control was also used. The stimulated and treated cells were cultured for 24 hours, washed with PBS, then cultured for an additional 24 hours in complete culture media. Cell-conditioned media was collected at 24 hours and frozen at -20°C until it was thawed and analyzed by multiplex bead assay (14-plex).

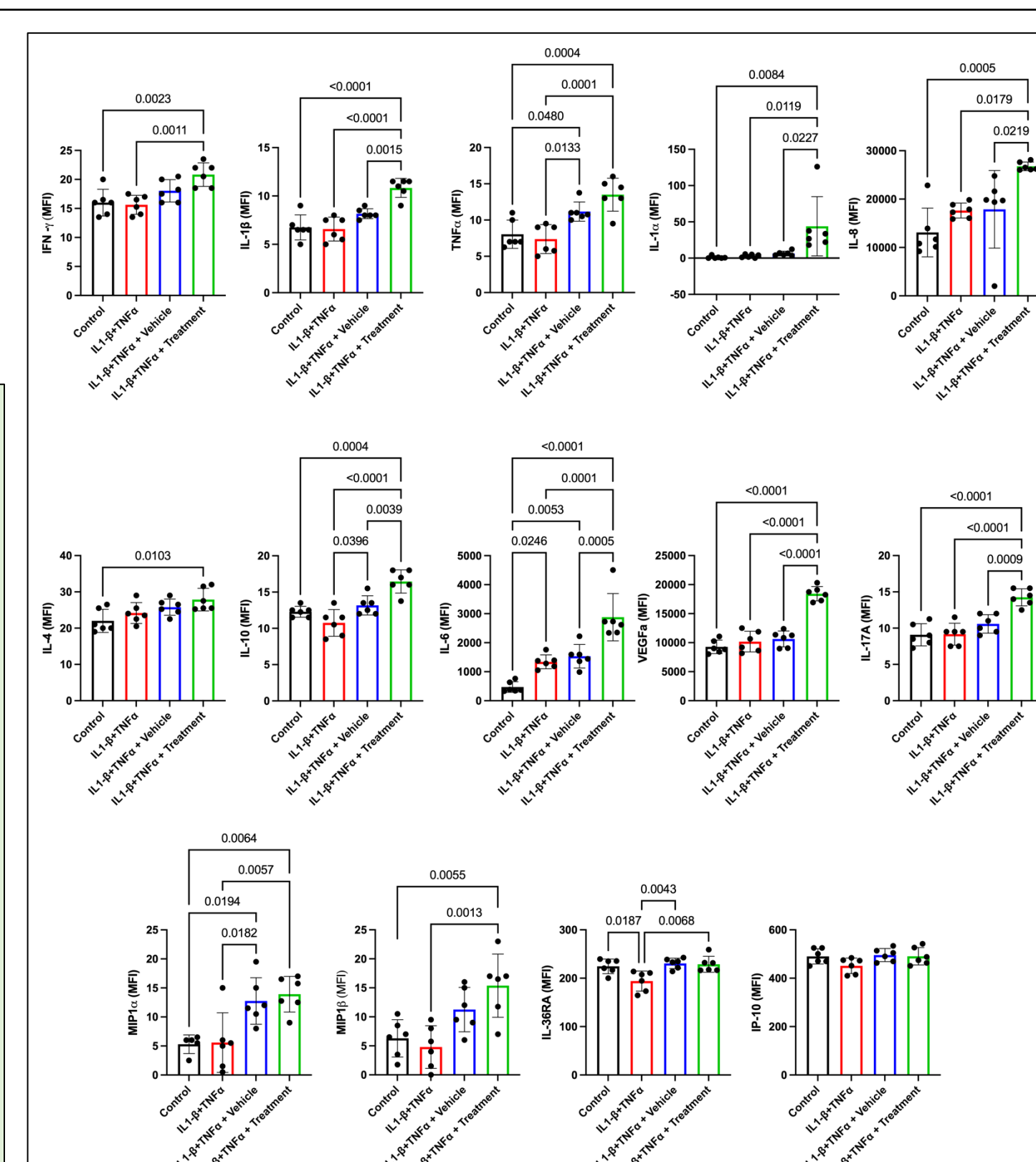


Figure 4: C-1101 elicited cytokine secretion in inflamed ovine nucleus pulposus (NP) cells

Figure 4 – C-1101 elicited cytokine secretion in inflamed ovine nucleus pulposus (NP) cells. Similar to AF cells, ovine NP cells were stimulated with IL-1β and TNFα (20 ng/mL) and then treated with vehicle control or C-1101 (50 mg/mL). A non-stimulated control was also used. The stimulated and treated cells were cultured for 24 hours, washed with PBS, then cultured for an additional 24 hours in complete culture media. Cell-conditioned media was collected at 24 hours and frozen at -20°C until it was thawed and analyzed by multiplex bead assay (14-plex).

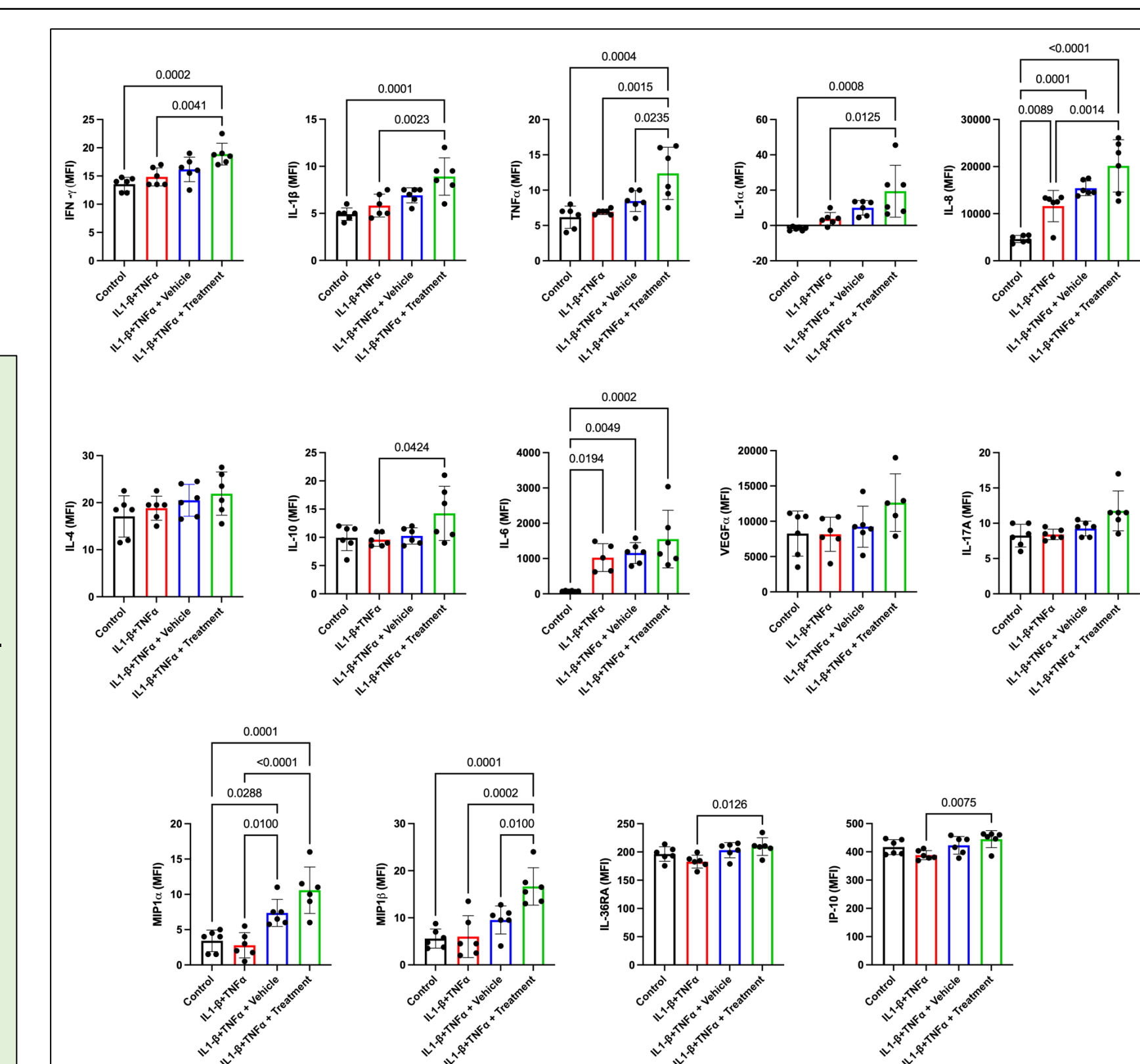


Figure 5: C-1101 altered differential gene expression and inflammatory pathways in ovine spine cells

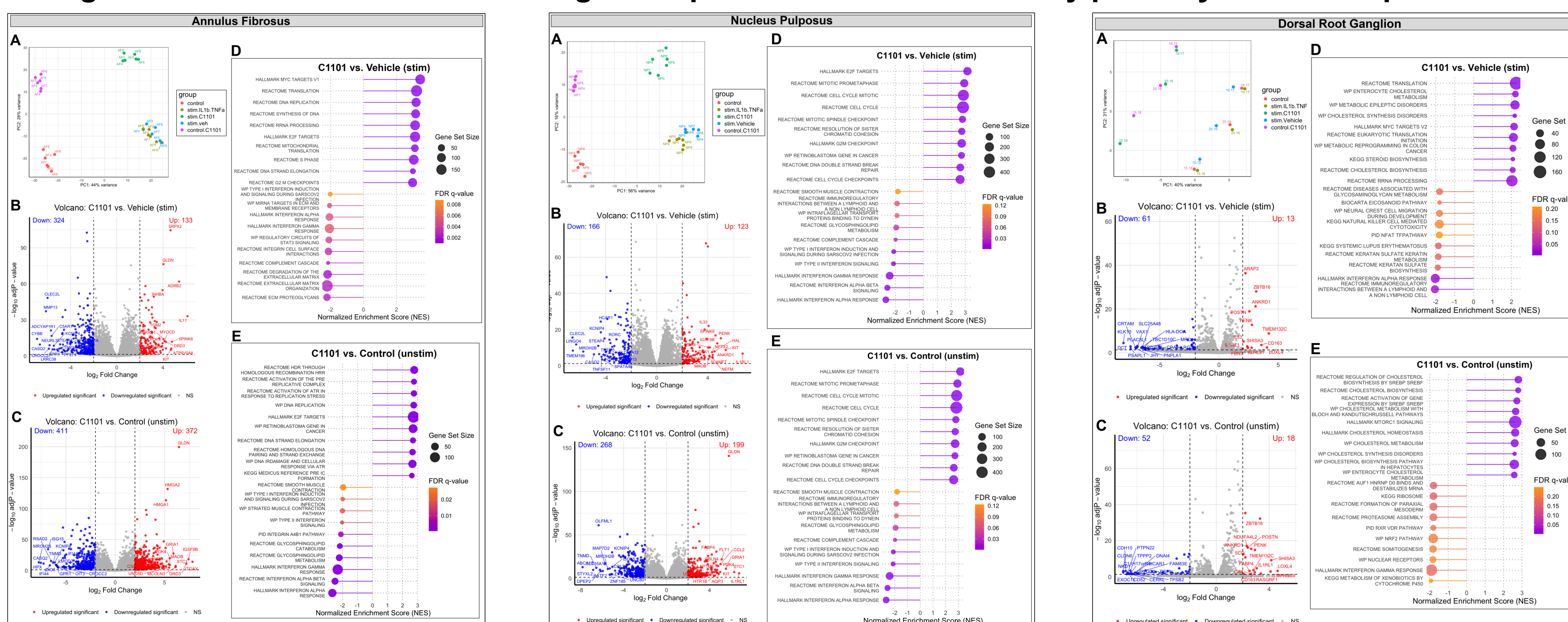


Figure 5 – Effects of C1101 treatment on transcriptome of AF, NP, dorsal root ganglia (DRG) cells. A) PCA (principal component analysis) plot of AF, cells from each treatment group. Number labels show, and biological downreg. Top 50 significant upregulated and downregulated gene names are labeled. B) Volcano plot of differential expression results comparing unstimulated C-1101 treated cells to unstimulated untreated cells. C) Volcano plot of differential expression results comparing unstimulated C-1101 treated cells to unstimulated untreated control cells. D) GSEA (gene set enrichment analysis) results. Top 20 most upregulated and downregulated pathways comparing C-1101 (stimulated) to vehicle (stimulated). X-axis shows normalized enrichment score, color scale for FDR adjusted p-value and number of genes mapped to pathway shown in circle sizes. Pathways used for analysis include Hallmark, Reactome, PID, KEGG and wikipathways. E) GSEA results comparing unstimulated C-1101 treated cells to unstimulated untreated control cells. Top 20 pathways shown.

Figure 6: C-1101 reduced allodynia and resulted in transient elevation of serum IL-1β and IL-10 in a murine model of peripheral neuropathy

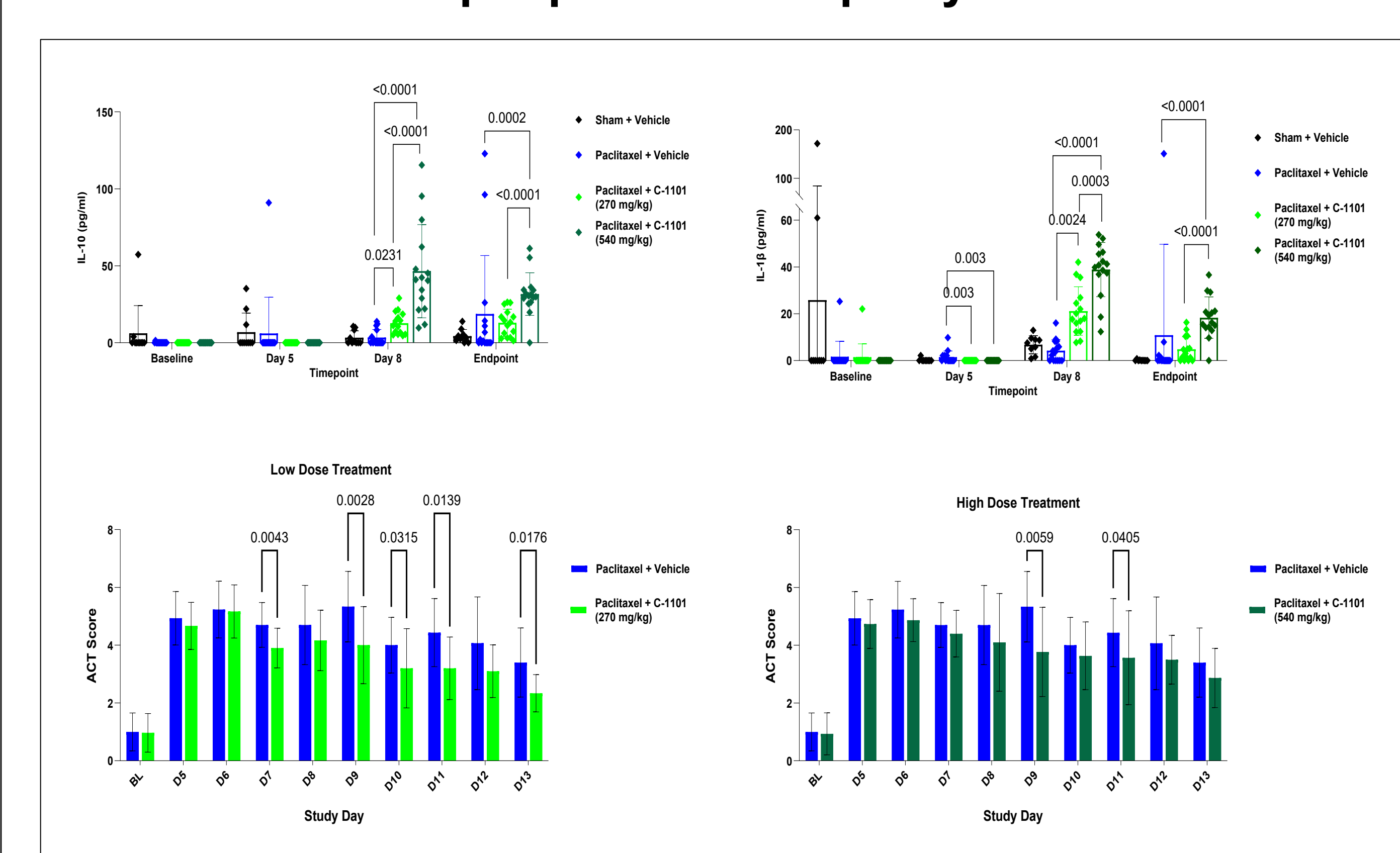


Figure 6 – C-1101 reduced allodynia and induced anti- and pro-inflammatory cytokine secretion in murine model of peripheral neuropathy. Both low (270 mg/kg) and high (540 mg/kg) dose C-1101 reduced paclitaxel-induced allodynia vs. vehicle by ACT score. Cytokine analyses revealed C-1101 and IL-10 was transiently elevated in C-1101 treatment group on day 8 and endpoint (day 13).

Discussion

- Aim 1:** C-1101 elicited an anti-inflammatory response from human macrophages following LPS stimulation.
- Aim 2:** C-1101 induced a mixed anti- and pro-inflammatory response in AF and NP cells. In an inflamed *in vitro* environment, and to a lesser extent from dorsal root ganglia cells. Transcriptomic analyses indicated downregulation in key inflammatory (namely interferons), extracellular matrix degradation and cell signaling pathways.
- Aim 3:** Both low and high dose C-1101 treatment reduced paclitaxel-induced allodynia evidenced by ACT score, but elicited transient increase of IL-1β, IL-10.
- Pleiotropic activity observed may reflect timing of administration in disease course and condition of the recipient environment in which it was administered.
- Further *in vivo* preclinical evaluation of the mechanism of action and potential efficacy is planned to support the ongoing clinical trial (NCT07264270).