

## **Effects of melatonin and 6-hydroxymelatonin on the cytokine expression of macrophages and endothelial cells under conditions of sepsis**

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### **Background**

Sepsis is a devastating condition that claims thousands of lives every year. Central to the pathophysiology of sepsis are mitochondrial dysfunction and oxidative stress that upregulate pro-inflammatory responses. Melatonin and its major metabolic derivative, 6-hydroxymelatonin, are powerful antioxidants and have been shown to ameliorate sepsis-induced oxidative stress. The purpose of this project was to perform a broad exploratory study to establish techniques to determine differential production of key cytokines by endothelial cells and macrophages under conditions mimicking sepsis and assess the effect of melatonin or 6-hydroxymelatonin.

### **Methods**

Human umbilical vein endothelial cells and Tamm-Horsfall protein 1 derived macrophages were cultured and exposed to lipopolysaccharide plus peptidoglycan for 24 hours to simulate sepsis. Melatonin or 6-hydroxymelatonin were added at a range of concentrations for a further 24 or 72 hours. The supernatants were collected and multiplex assays using flow cytometry were performed to quantify interleukin (IL) - 1 $\beta$ , IL-6, IL-8, IL-10 and IL-18, macrophage inhibitory factor (MIF), monocyte chemoattractant protein (MCP)-1 and tumour necrosis factor (TNF)  $\alpha$ . Cell viability was determined using acid phosphatase to investigate the effect of each drug on cell survival.

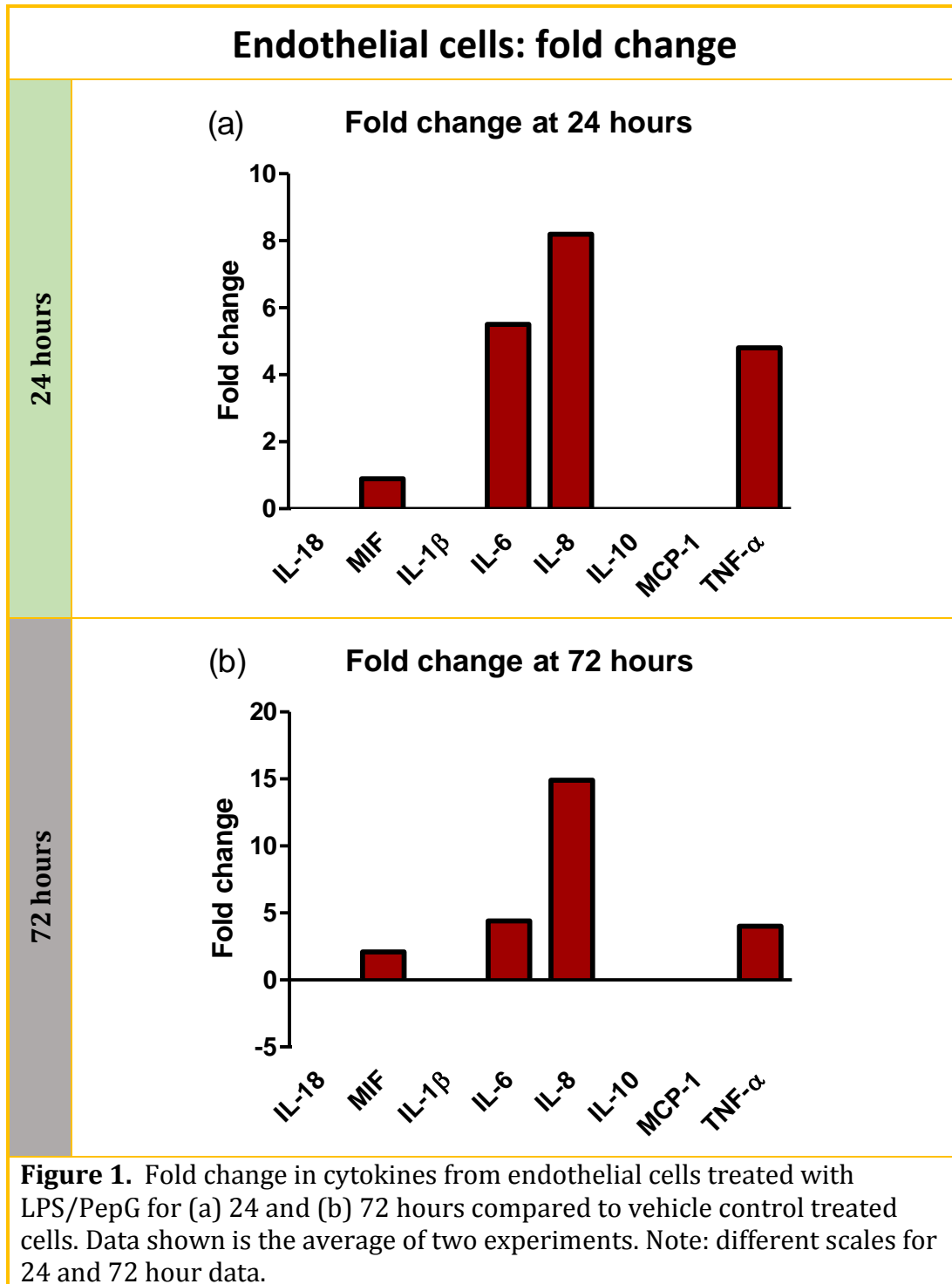
### **Results**

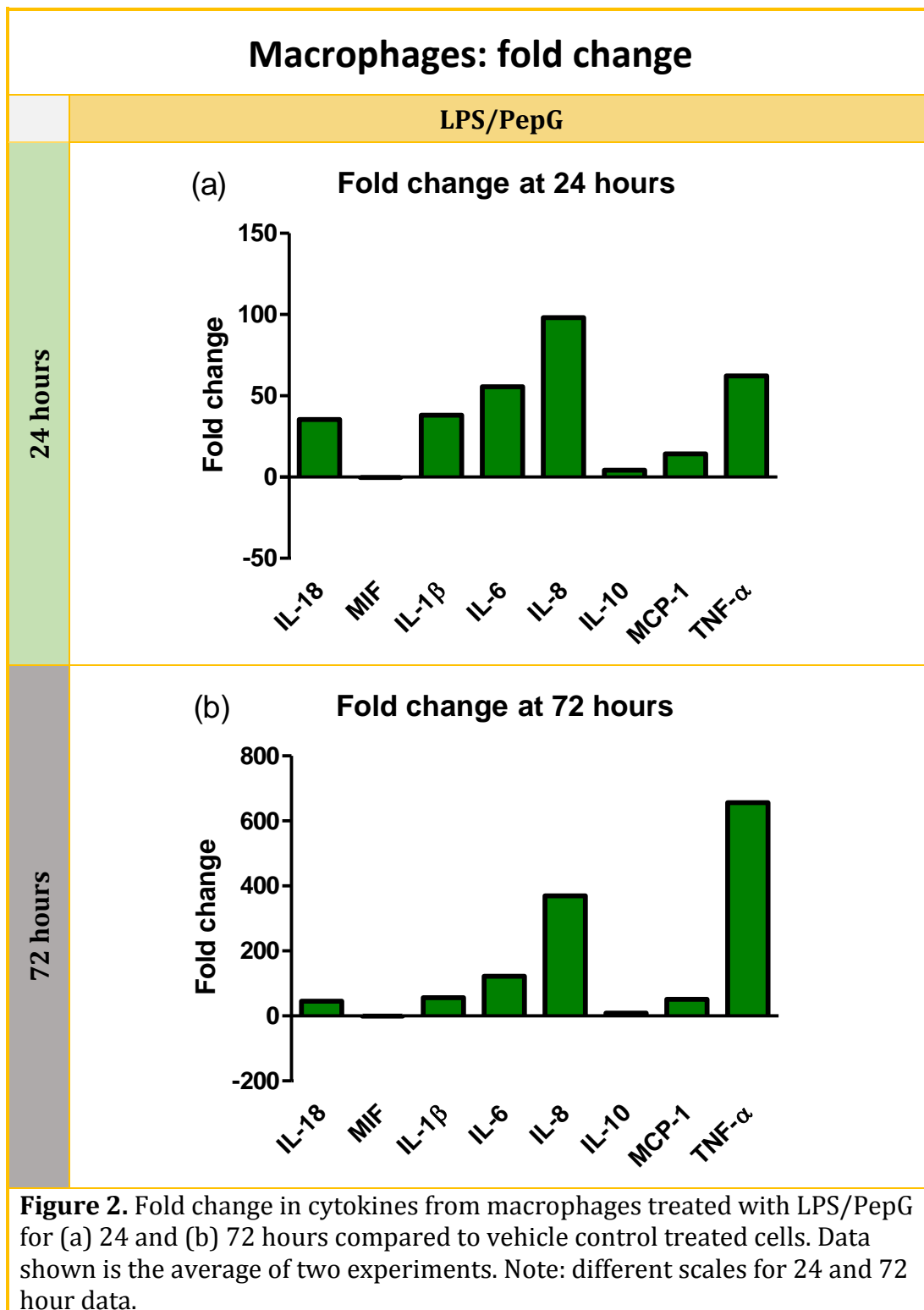
Treatment with LPS/PepG seemingly increased production of all cytokines by macrophages (apart from MIF) and only MIF, IL-6, IL-8 and TNF- $\alpha$  by endothelial cells. Melatonin and 6-hydroxymelatonin were found to have apparently similar effects with a mostly suppressive effect on the production of all the cytokines in the macrophages but only TNF- $\alpha$  in endothelial cells (Appendix 1). No changes in viability were detected with either cell type at any of the experimental or control conditions.

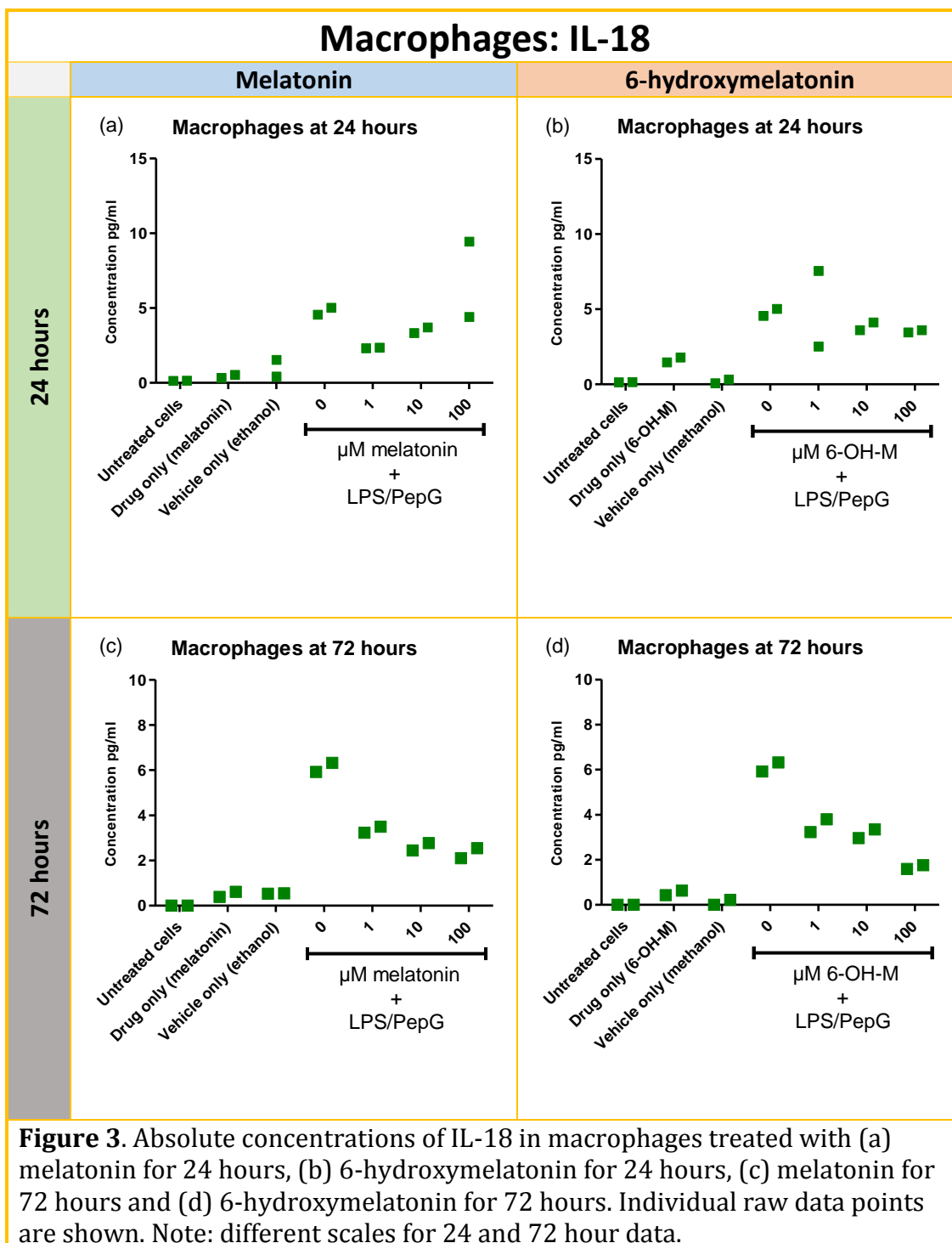
### **Conclusion**

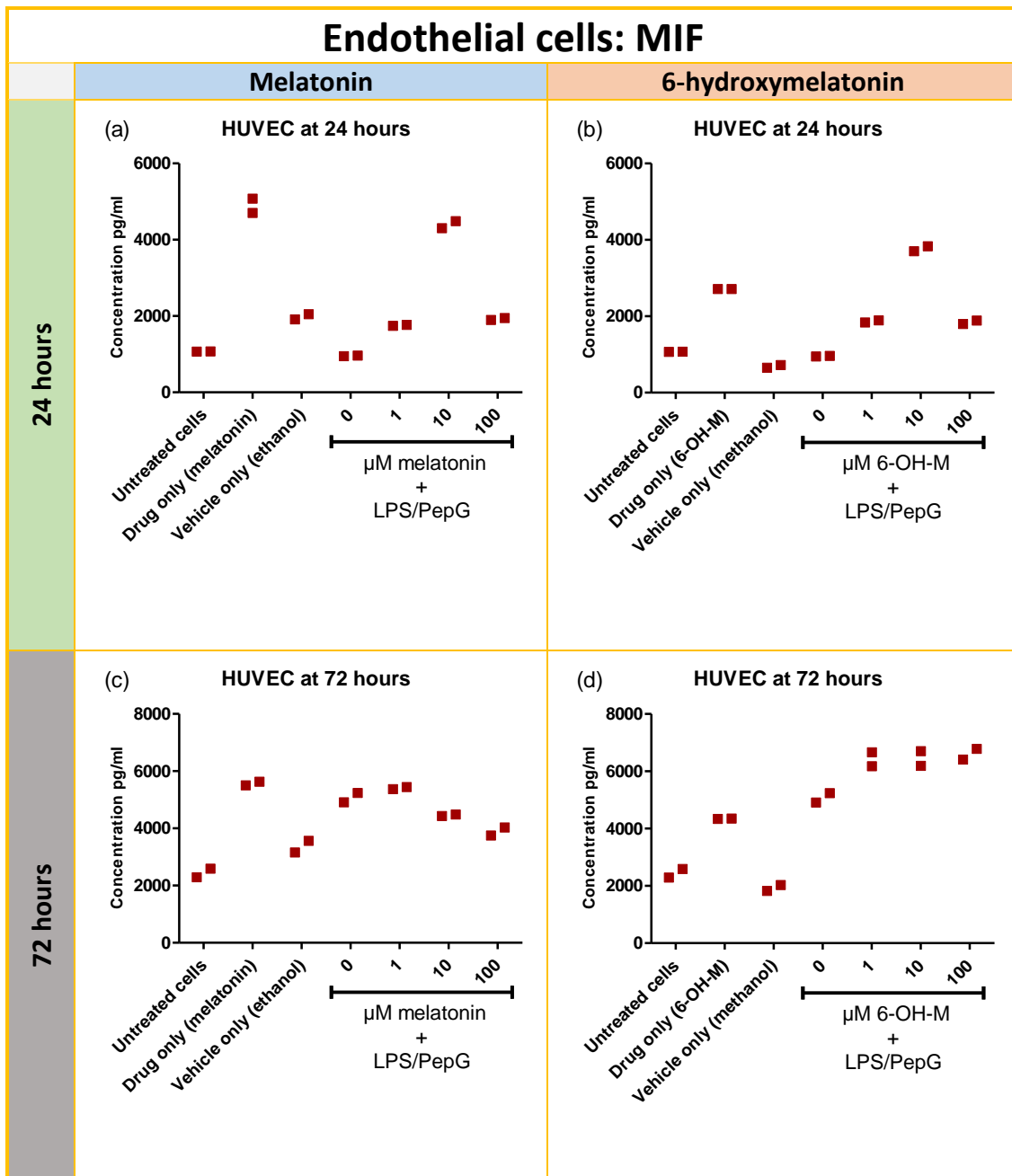
In this project the model for investigating the effects of melatonin and 6-hydroxymelatonin on the cytokine production of endothelial cells and macrophages under conditions mimicking sepsis was successfully established along with multiplex assays for determination of multiple cytokines. We have now secured a vacation scholarship to continue this work.

Appendix 1 – Select graphs and figures

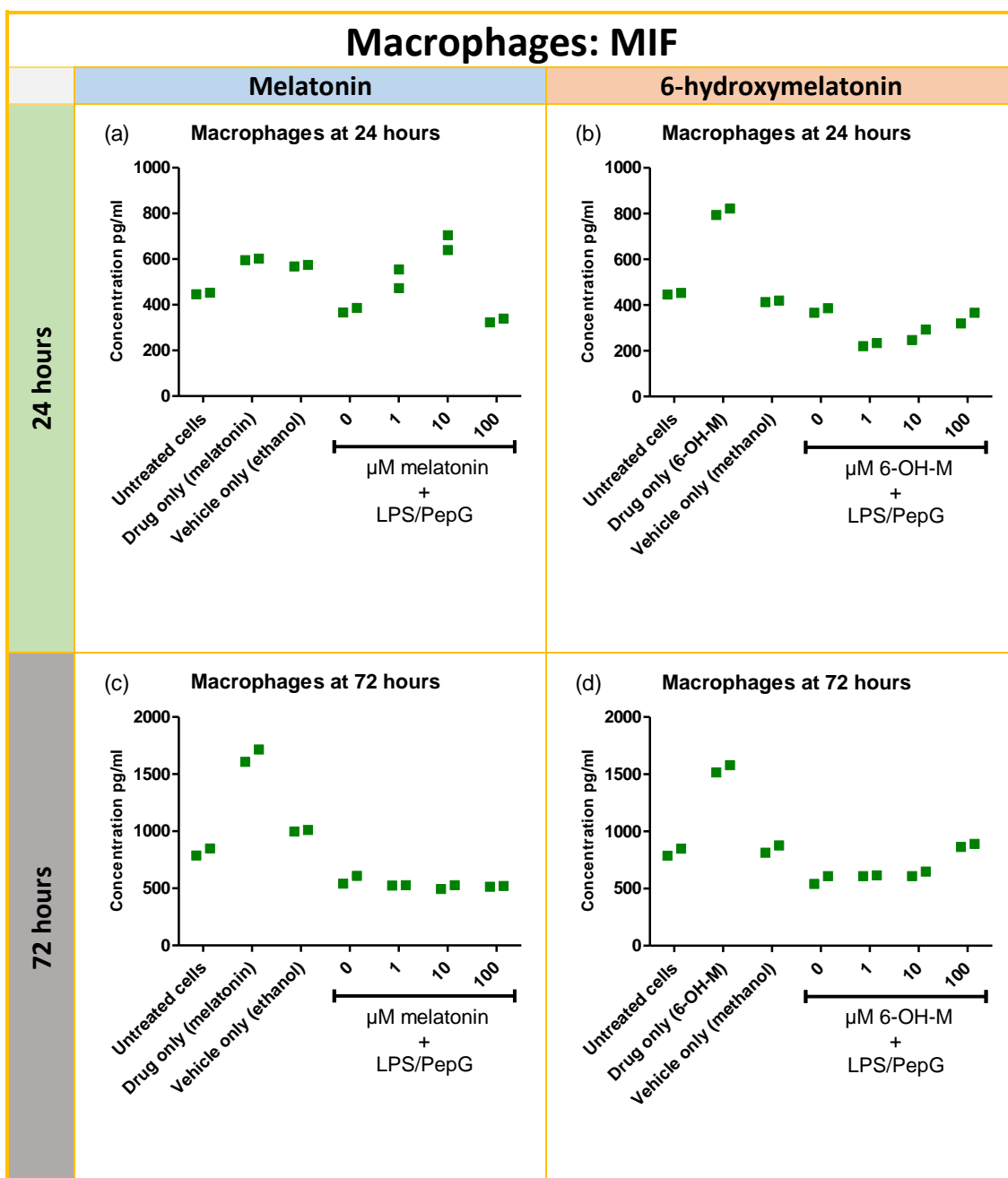




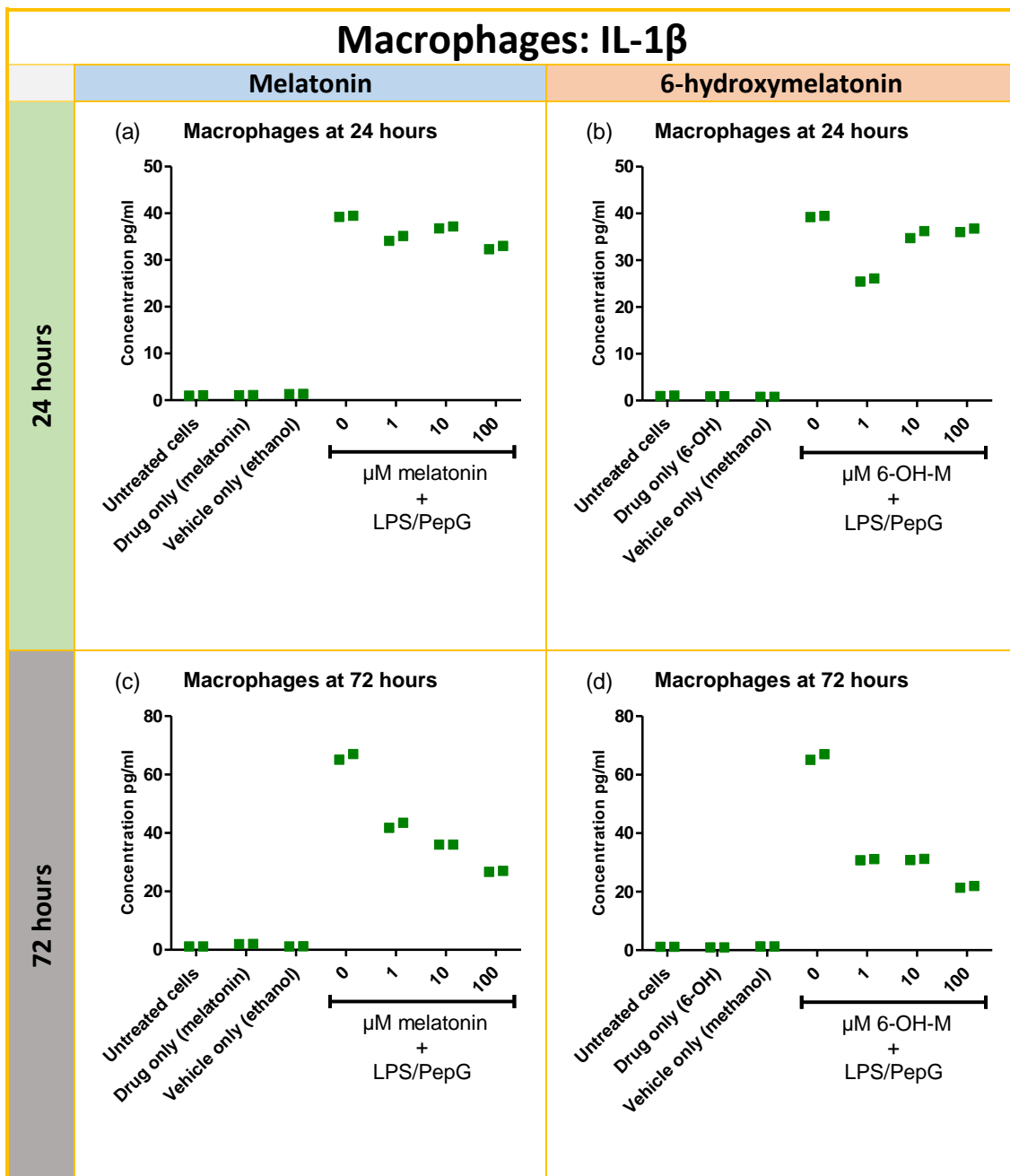




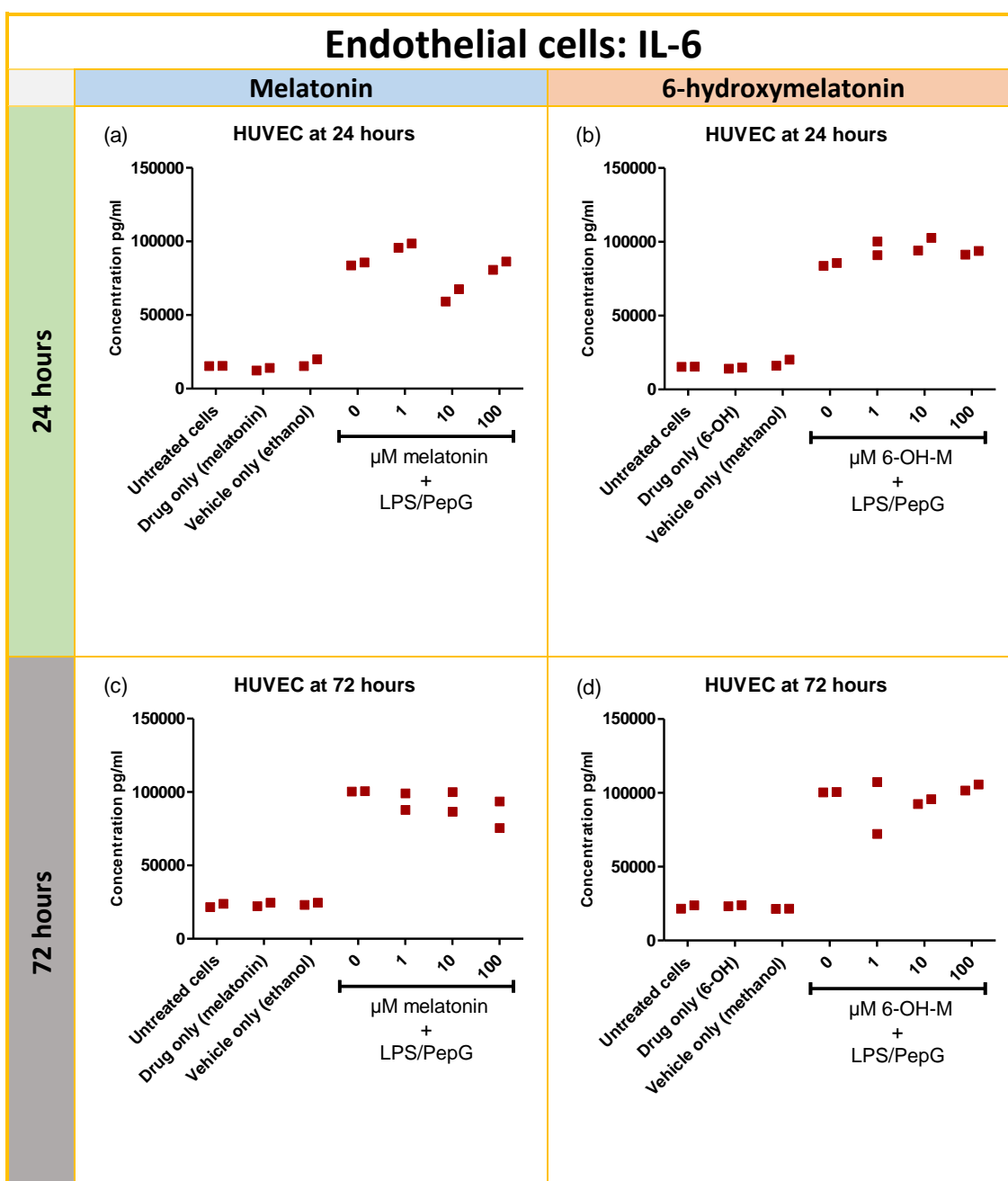
**Figure 4.** Absolute concentrations of MIF in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.



**Figure 5.** Absolute concentrations of MIF in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.

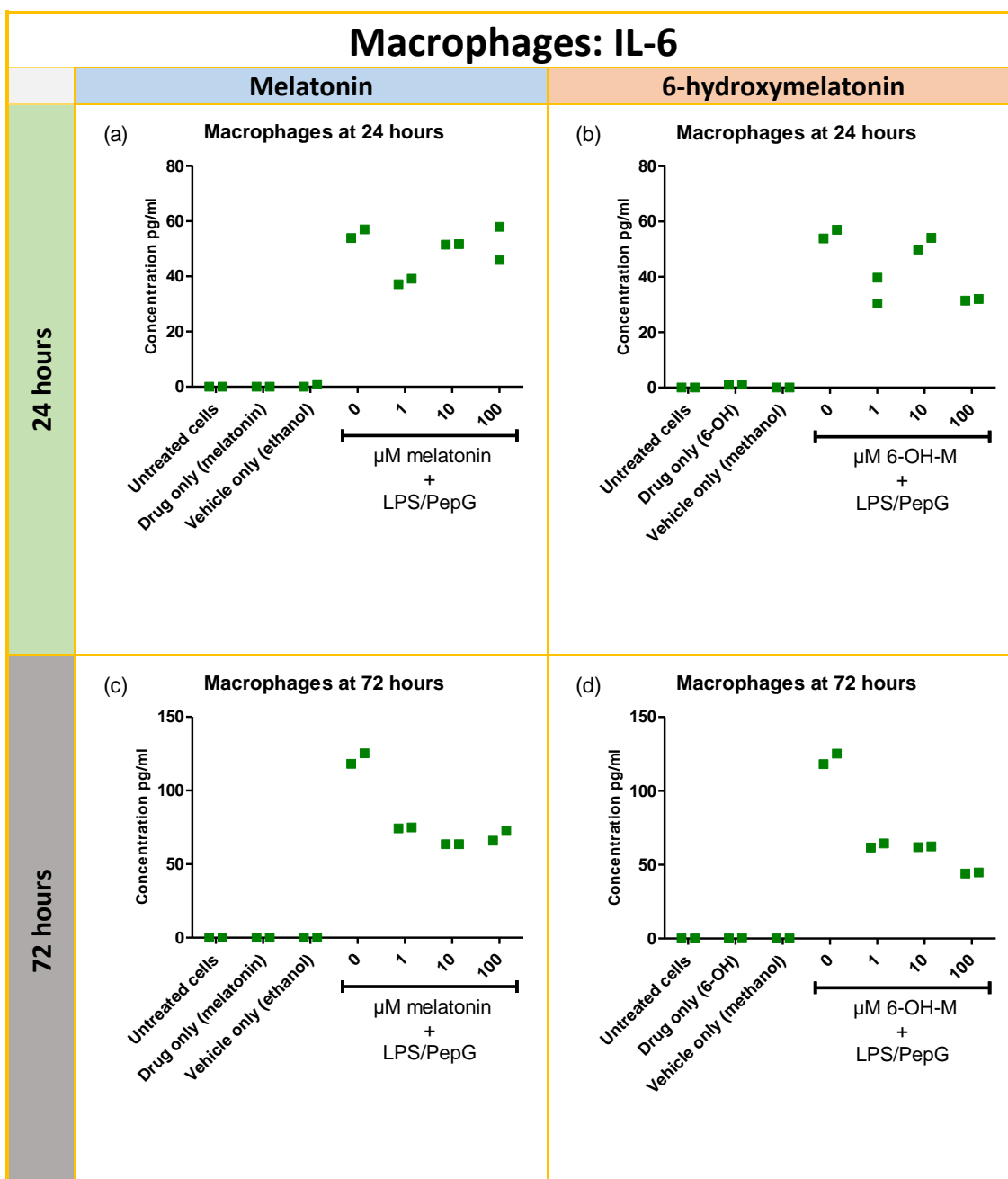


**Figure 6.** Absolute concentrations of IL-1 $\beta$  in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.

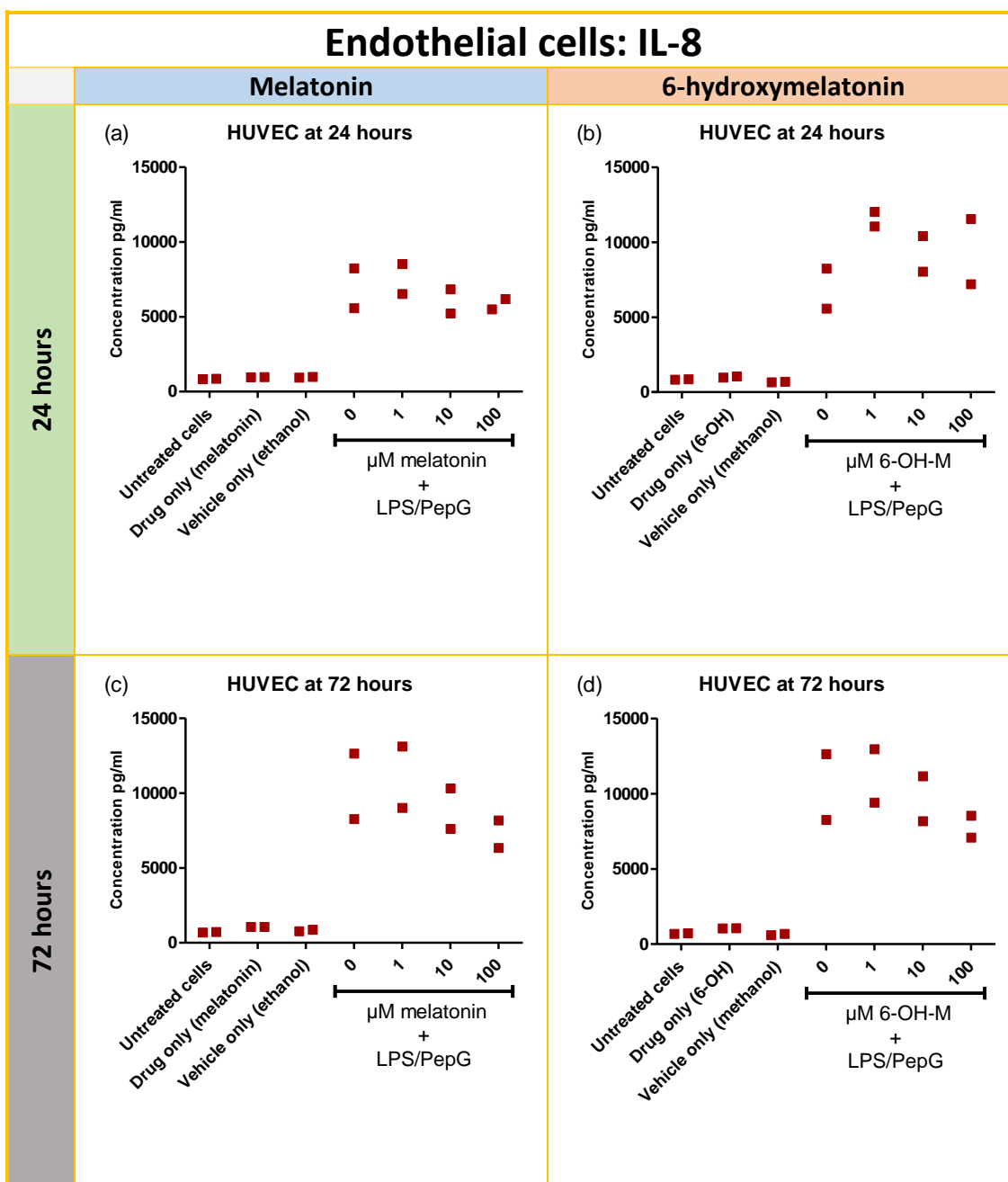


**Figure 7.** Absolute concentrations of IL-6 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown.

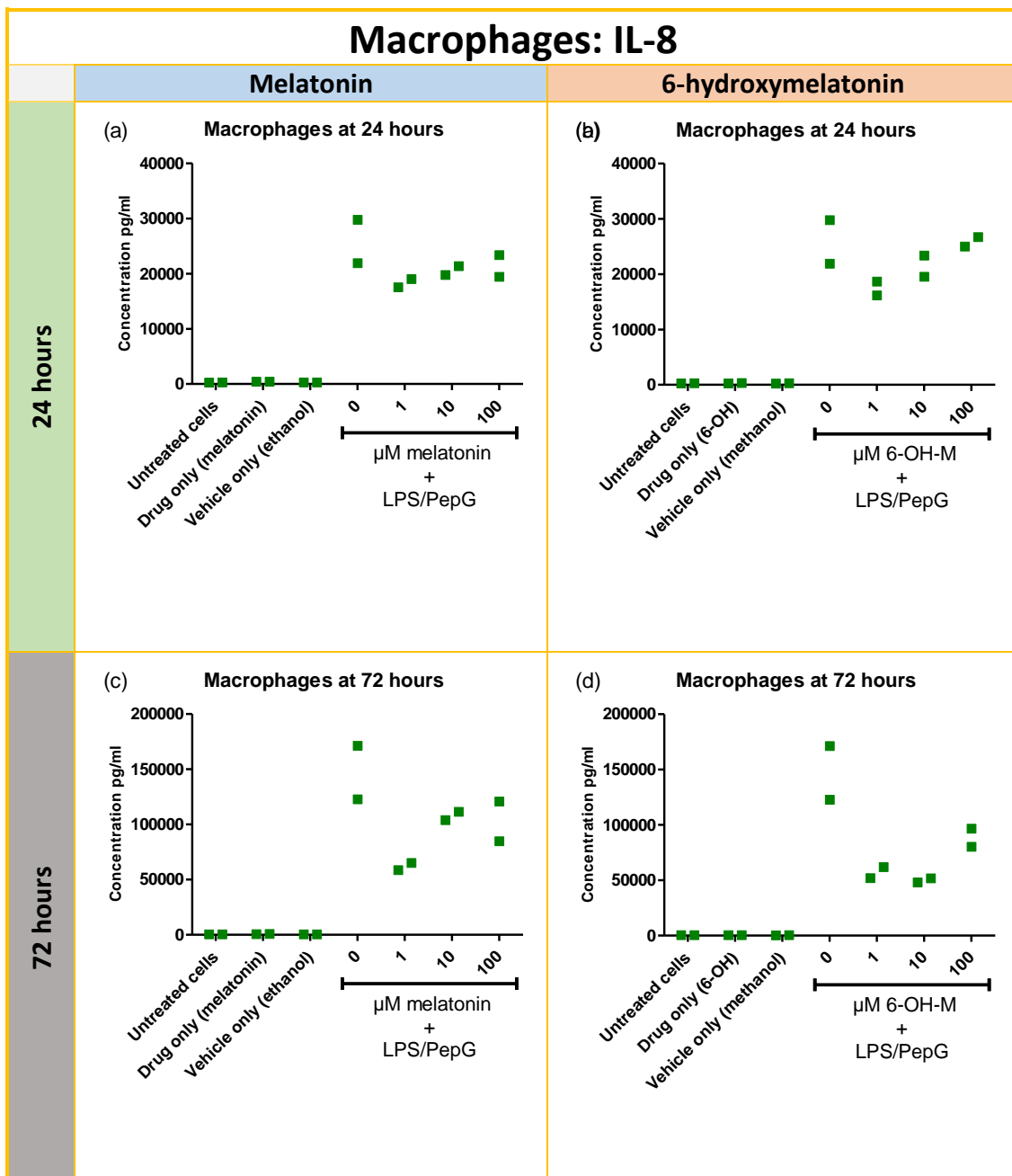




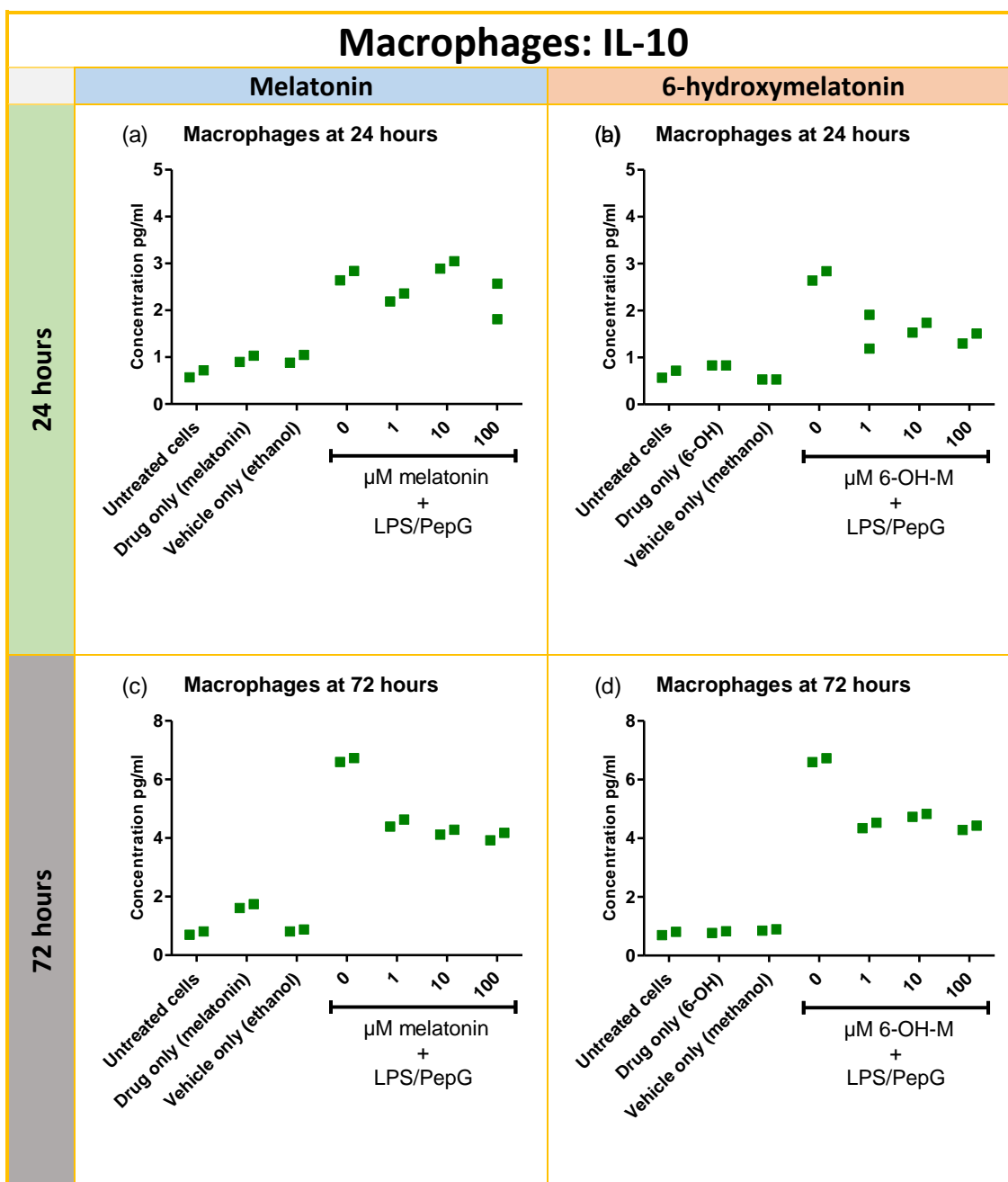
**Figure 8.** Absolute concentrations of IL-6 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.



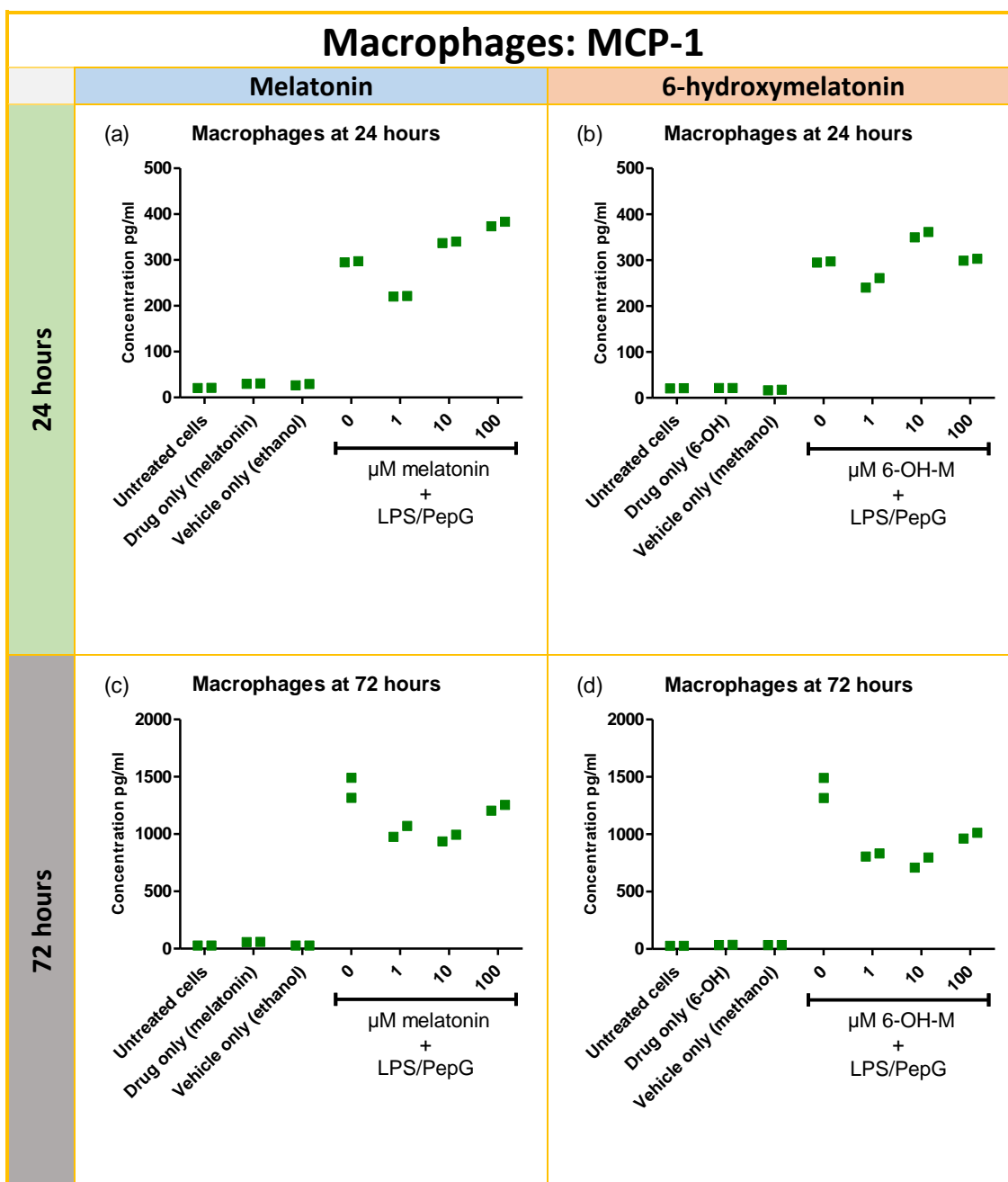
**Figure 9.** Absolute concentrations of IL-6 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown.



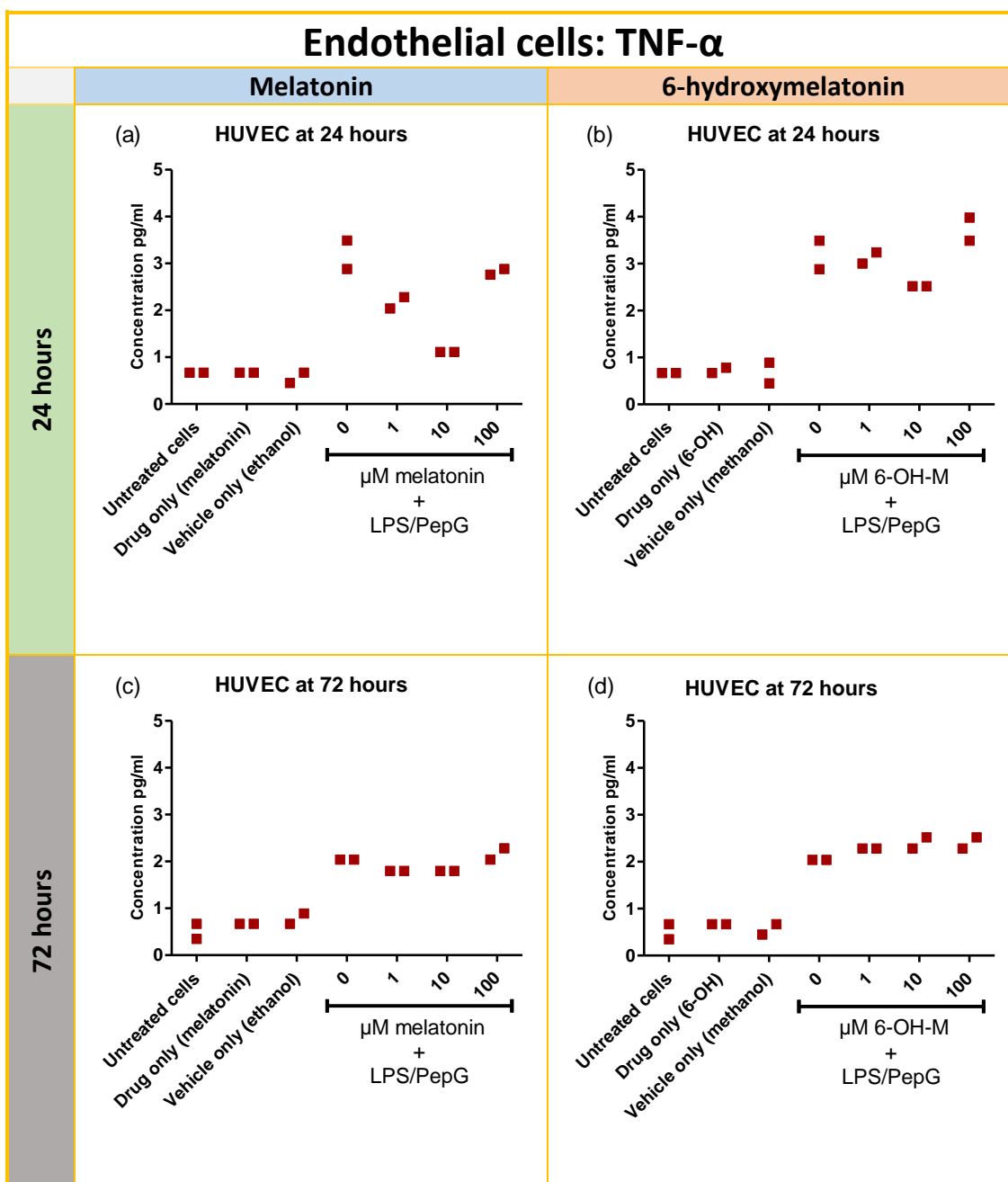
**Figure 10.** Absolute concentrations of IL-8 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.



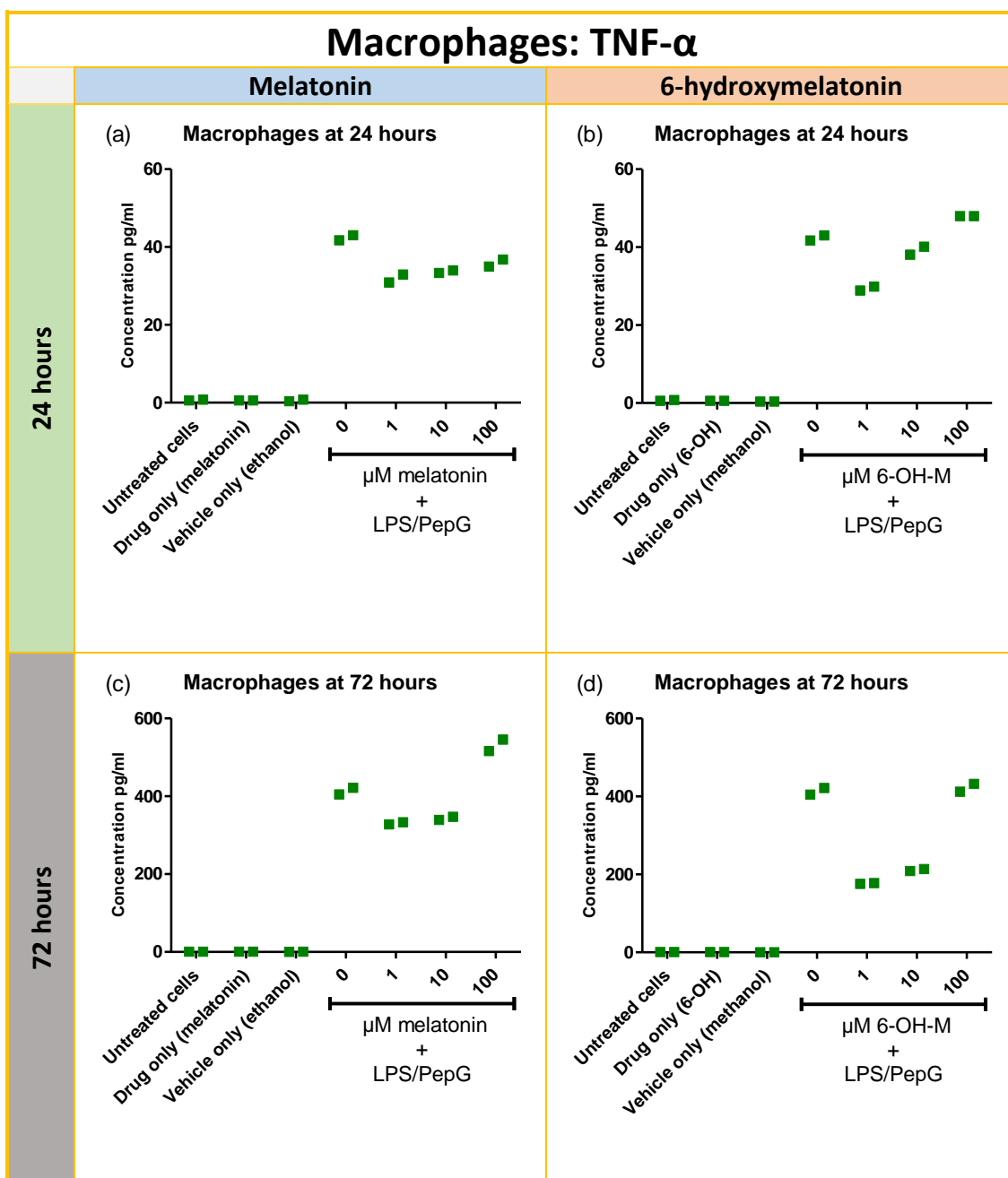
**Figure 11.** Absolute concentrations of IL-10 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.



**Figure 12.** Absolute concentrations of MCP-1 in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.



**Figure 13.** Absolute concentrations of TNF- $\alpha$  in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown.



**Figure 14.** Absolute concentrations of TNF- $\alpha$  in macrophages treated with (a) melatonin for 24 hours, (b) 6-hydroxymelatonin for 24 hours, (c) melatonin for 72 hours and (d) 6-hydroxymelatonin for 72 hours. Individual raw data points are shown. Note: different scales for 24 and 72 hour data.