

Axatilimab Abrogates Inflammatory Cytokines and Chemokines and Interrupts the Differentiation of Monocytes to Macrophages, a Pathogenic Driver of Inflammation and Fibrosis in cGVHD

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Introduction

- The pathophysiology of chronic graft-versus-host disease (cGVHD) involves at least 3 distinct overlapping processes: tissue damage and inflammation, dysregulation of adaptive immunity leading to chronic inflammation, and fibrosis resulting in end-organ damage¹
- The role of monocyte-derived macrophages as the pathogenic driver of inflammation and fibrosis in cGVHD has been previously described²
- Colony-stimulating factor 1 receptor (CSF-1R), a member of the receptor protein tyrosine kinase family of growth factor receptors, is the primary signaling pathway regulating the development and differentiation of monocytes and macrophages, which drive fibrosis and inflammation in cGVHD³
- Axatilimab, a high-affinity, humanized immunoglobulin G4 (IgG4) monoclonal antibody that targets and blocks CSF-1R signaling, has demonstrated efficacy and safety in 2 clinical trials for patients with cGVHD^{4,5}
 - In the phase 1/2 study (NCT03604692), treatment with axatilimab led to reductions in skin macrophage density and decreases in key profibrotic macrophage cytokines⁴
 - In the pivotal, randomized, phase 2 AGAVE-201 study (NCT04710576), axatilimab 0.3 mg/kg once every 2 weeks demonstrated high response rates and was generally well tolerated in patients with cGVHD⁵
- The effects of axatilimab on monocytes and macrophages need further investigation

Objective

- To characterize the mechanism of action of axatilimab and its effects on monocytes and macrophages

Methods

In Vitro Assays

- Primary monocytes and monocyte-derived macrophages were used to evaluate the surface expression of CSF-1R by flow cytometry
- Monocytes were treated with macrophage CSF (M-CSF) and axatilimab for 24 hours and were analyzed for monocyte chemoattractant protein-1 (MCP-1) secretion by enzyme-linked immunosorbent assay (ELISA)
- In order to evaluate the effects of axatilimab on monocytes, purified CD14+ monocytes were cultured with M-CSF and treated with axatilimab for 24 or 72 hours, then were analyzed for cell viability using CellTiter-Glo (Promega, Madison, WI)
- In order to evaluate the effects of axatilimab on monocyte-derived macrophages, monocytes were differentiated into macrophages with M-CSF for 5 days, then treated with axatilimab for 72 hours with appropriate controls
- Supernatants and cell pellets from cultures were assessed for secreted protein with an inflammatory 80-plex assay (Luminex Corporation, Austin, TX) and gene expression (NanoString Technologies, Seattle, WA) profiles, respectively

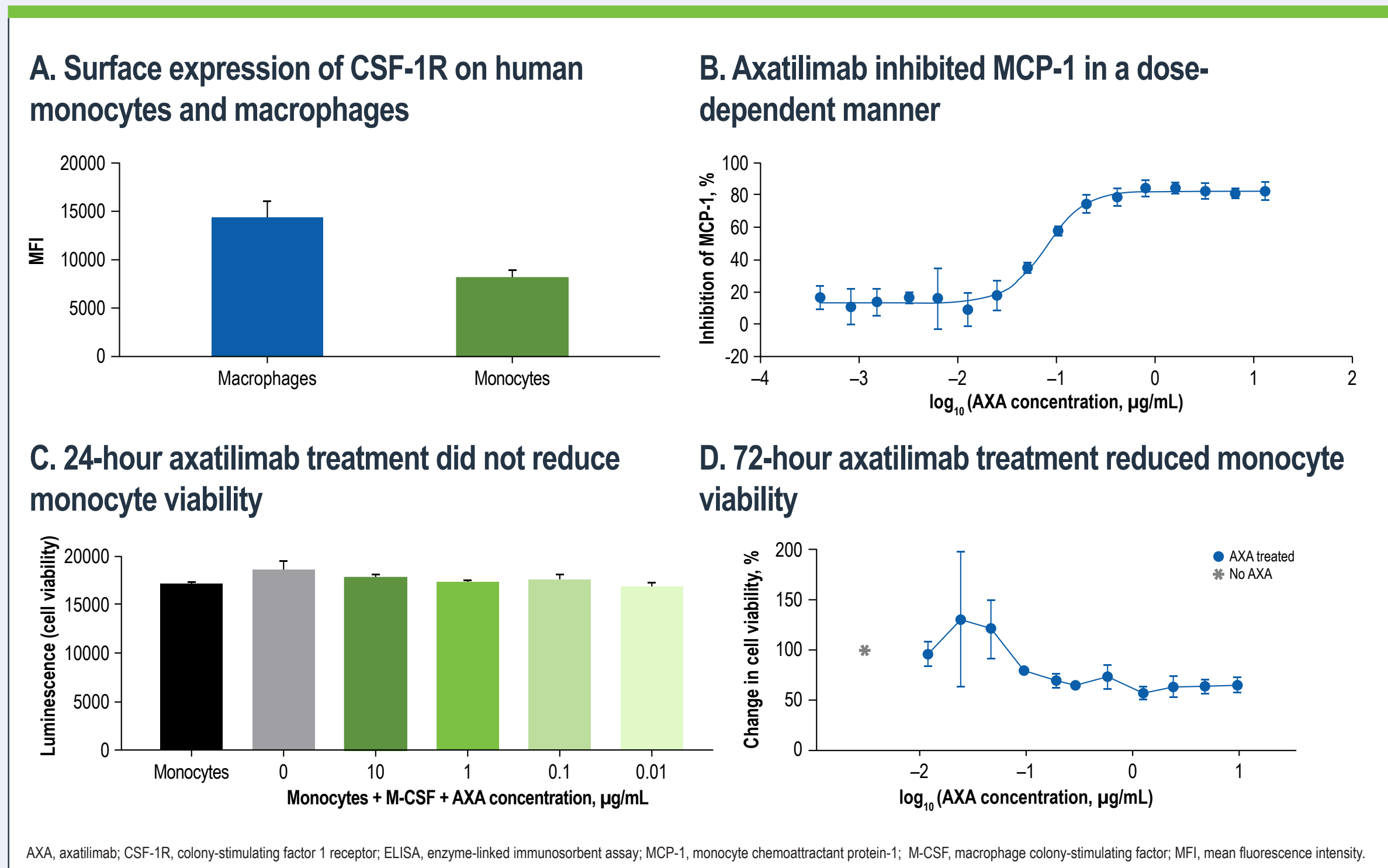
In Vivo Study

- A human CSF-1/CSF-1R knock-in mouse model (Biocytogen, Waltham, MA) was treated with axatilimab 0.3 mg/kg or 30 mg/kg for 4 days
- A flow cytometry panel was used to assess immune cell populations in blood after axatilimab treatment

Results

- CSF-1R is expressed on the surface of human primary macrophages and monocytes (**Figure 1A**)
- Shorter axatilimab treatment (24 h) did not impair monocyte viability and resulted in the inhibition of MCP-1 (**Figure 1B–C**)
 - MCP-1 is a key driver of tumor growth factor beta (TGF-β) and a mediator of immune cell chemotaxis and fibrosis
- Longer-duration axatilimab treatment (72 h) of human monocytes resulted in impaired cell survival (**Figure 1D**)

Figure 1. CSF-1R Surface Expression and MCP-1 Inhibition in Human Primary Monocytes and Macrophages



- Axatilimab treatment of human primary monocytes led to the dose-dependent modulation of secreted inflammatory cytokine and chemokine proteins (**Figure 2A–B**) and reduced the expression of genes associated with inflammation and fibrosis (**Figure 3A–B** and **Table 1**)

Figure 2. Effects of Axatilimab Treatment on Levels of Secreted Inflammatory Proteins in Human Primary Monocytes

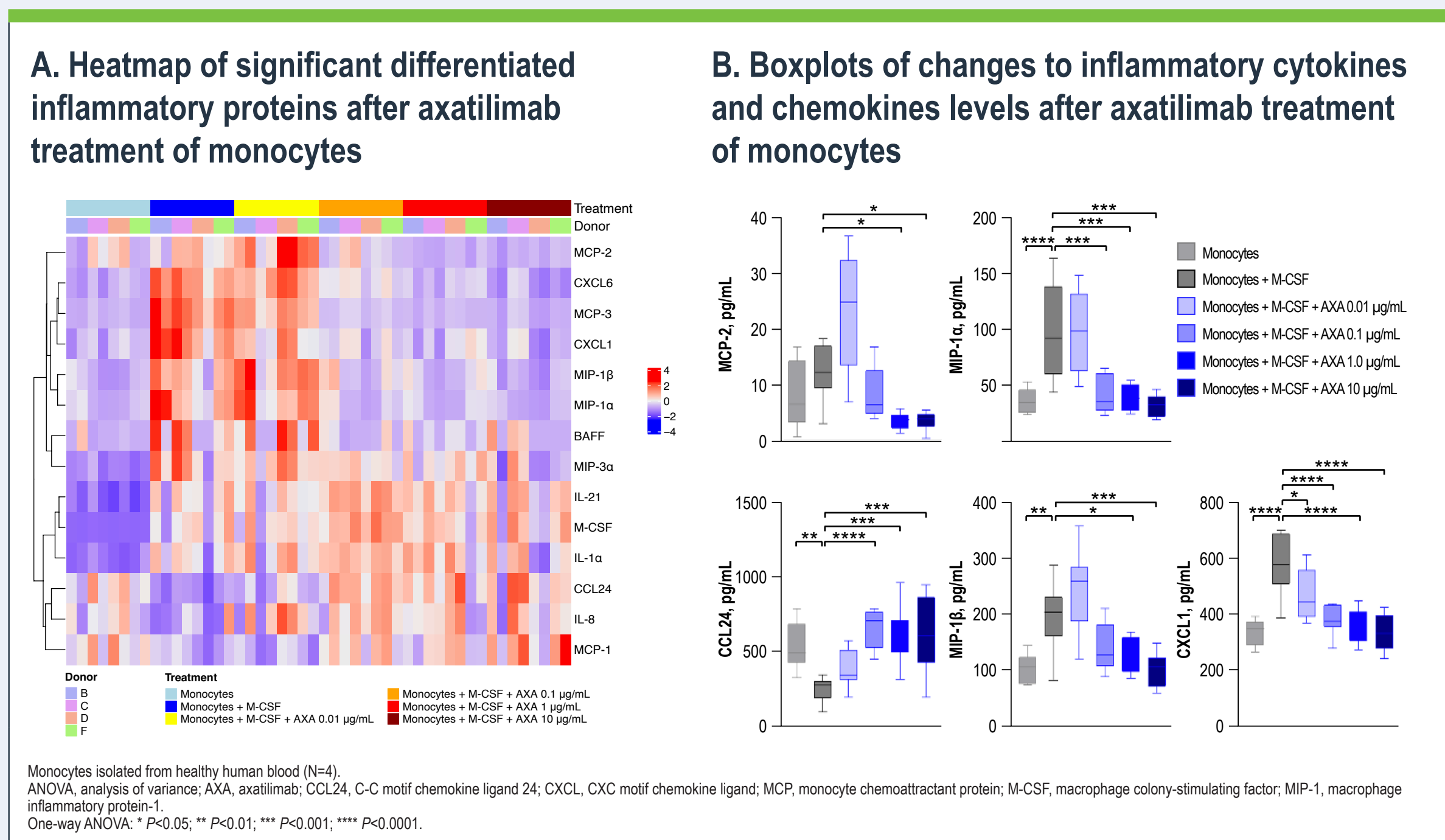


Figure 3. Effects of Axatilimab Treatment on the Expression of Genes Associated With Inflammation and Fibrosis in Human Primary Monocytes

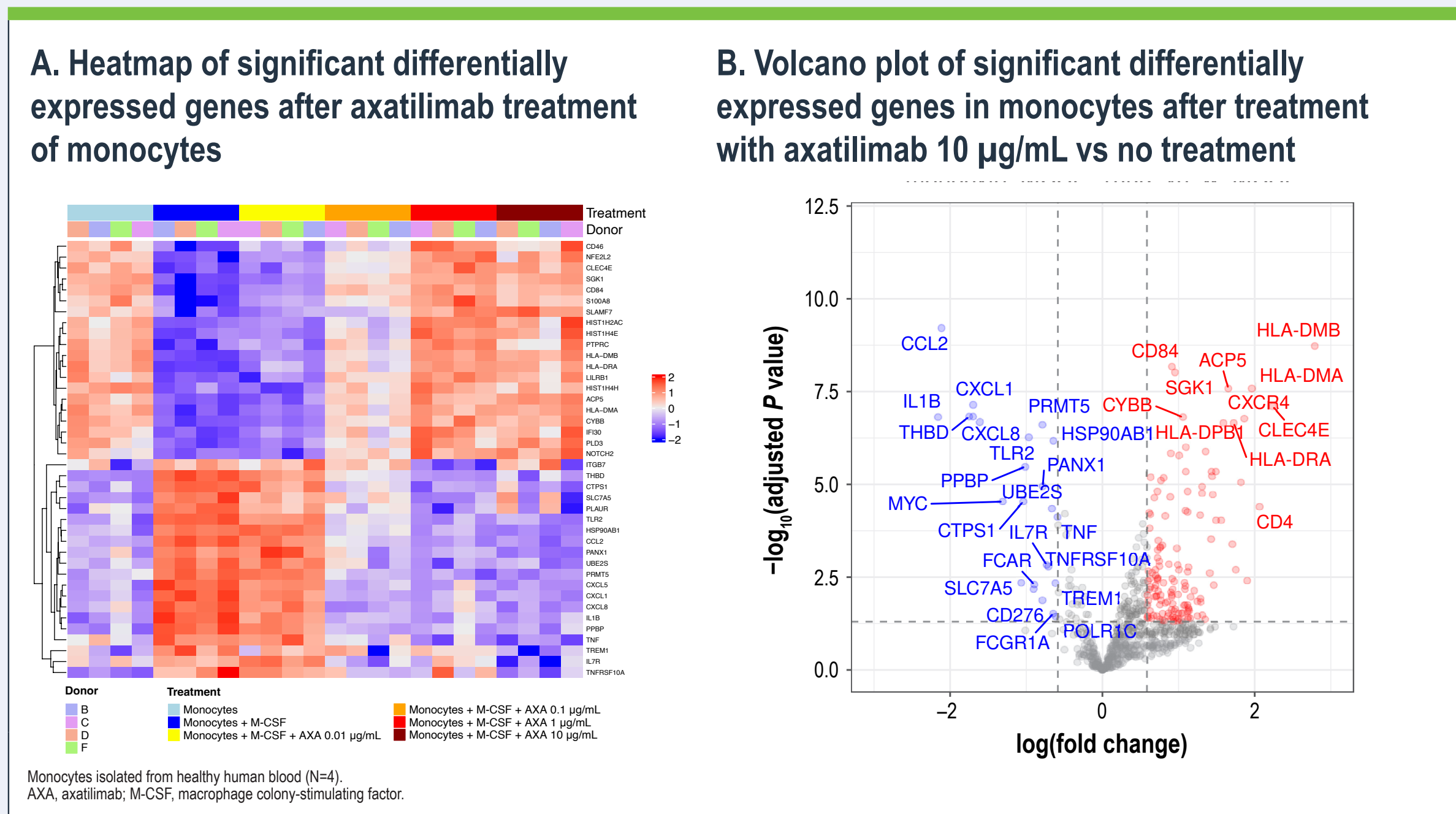


Table 1. Downregulated Signaling Pathways in Monocytes After Treatment With Axatilimab

Comparison	Pathways	P value	Adjusted P value	NES	Enrichment
AXA 10 µg/mL vs no treatment	HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.000006	0.00023	–2.06	Downregulated
	HALLMARK_MYC_TARGETS_V1	0.000021	0.00045	–2.43	Downregulated
	HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.0007	0.01	–2.16	Downregulated
AXA 1 µg/mL vs no treatment	HALLMARK_MYC_TARGETS_V1	0.00012	0.005	–2.29	Downregulated
	HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.00036	0.008	–1.82	Downregulated
AXA 0.1 µg/mL vs no treatment	HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.00024	0.01	–1.86	Downregulated
	HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.00062	0.013	–2.28	Downregulated
	HALLMARK_MYC_TARGETS_V1	0.001	0.017	–2.18	Downregulated

AXA, axatilimab; NES, network enhancement-based similarity.

- Axatilimab treatment of human monocyte-derived macrophages led to dose-dependent inhibition of secreted inflammatory cytokine and chemokine proteins (**Figure 4**) and reduced expression of genes associated with inflammation and fibrosis (**Figure 5A–B** and **Table 2**)
- Notably, axatilimab inhibited proinflammatory cytokines (IL-1β, TNF), immune cell chemoattractants (MIP-1α, MIP-1β), transcription factor NF-κB, and profibrotic cytokines (MCP-1, IL-8); all of these mediators play crucial roles in inflammatory and fibrotic conditions including cGVHD

Figure 4. Effects of Axatilimab Treatment on Levels of Secreted Inflammatory Proteins From Human Primary Monocyte-Derived Macrophages

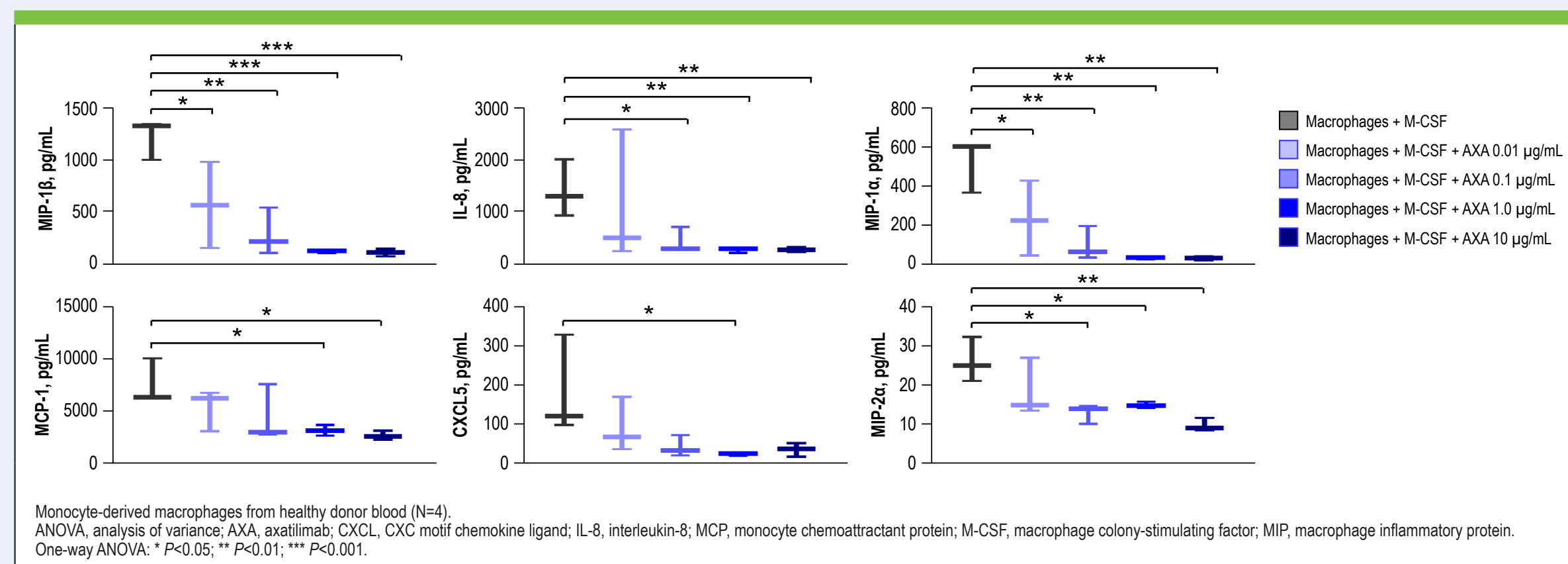


Figure 5. Effects of Axatilimab Treatment on Genes Associated With Inflammation and Fibrosis in Human Primary Monocyte-Derived Macrophages

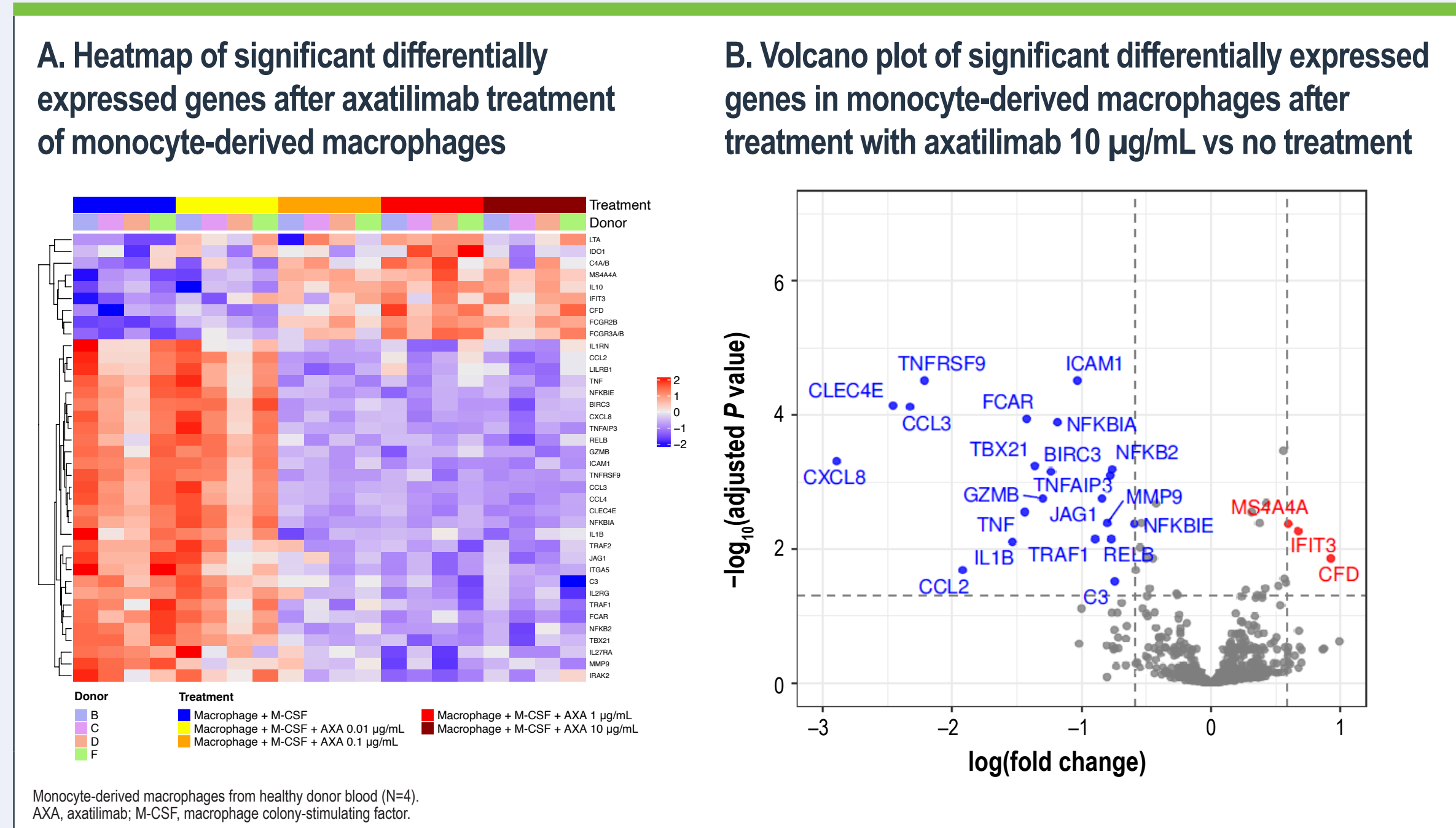


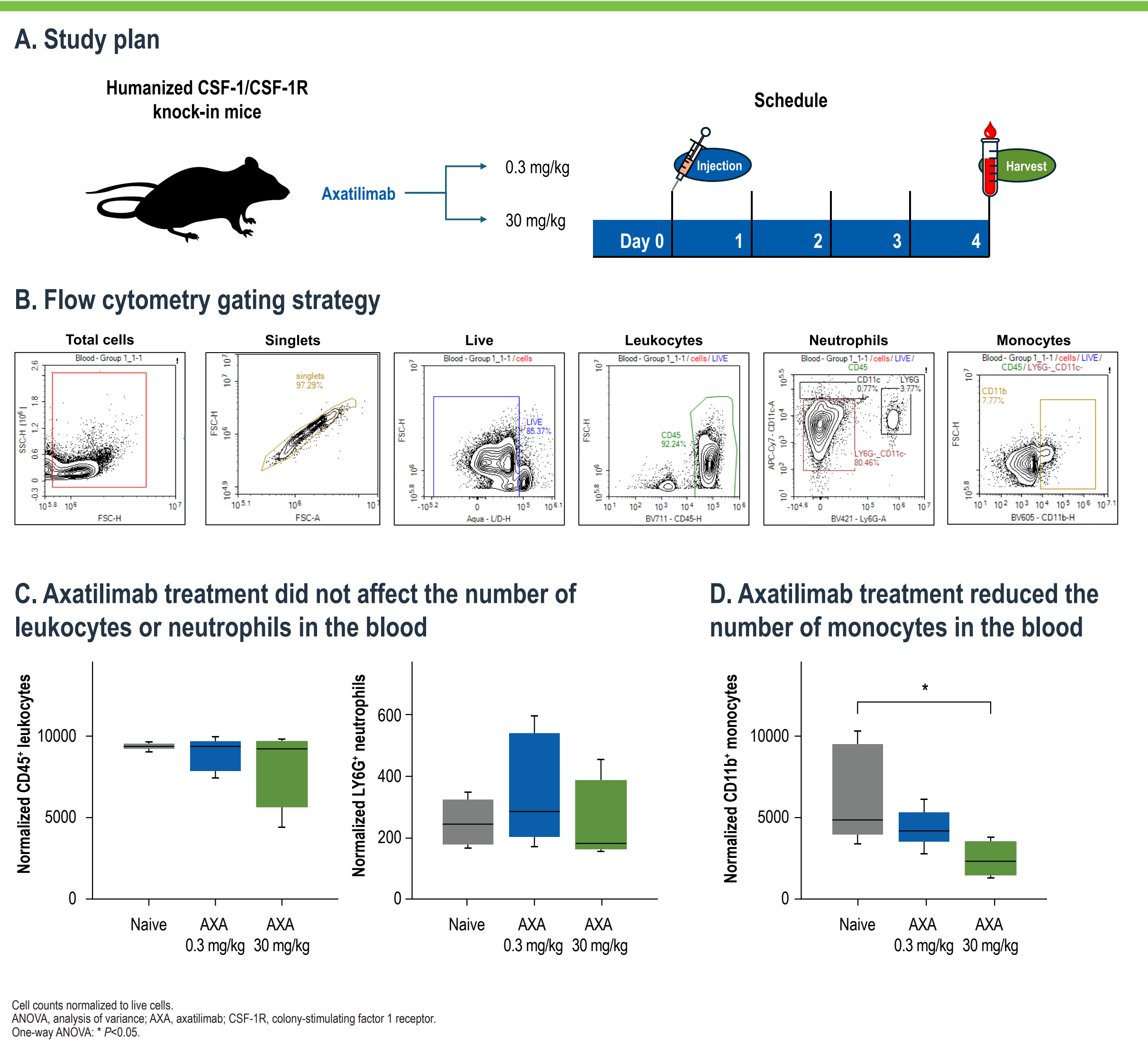
Table 2. Downregulated Signaling Pathways in Monocyte-Derived Macrophages After Axatilimab Treatment

Comparison	Pathways	P value	Adjusted P value	NES	Enrichment
AXA 10 µg/mL vs no treatment	HALLMARK_TNFA_SIGNALING_VIA_NFKB	<0.00000	0.000001	–2.23	Downregulated
AXA 1 µg/mL vs no treatment	HALLMARK_TNFA_SIGNALING_VIA_NFKB	<0.00000	<0.00000	–2.4	Downregulated
	HALLMARK_INFLAMMATORY_RESPONSE	0.00015	0.003	–1.9	Downregulated
	HALLMARK_KRAS_SIGNALING_UP	0.002	0.03	–1.79	Downregulated
AXA 0.1 µg/mL vs no treatment	HALLMARK_TNFA_SIGNALING_VIA_NFKB	<0.00000	0.000005	–2.15	Downregulated
	HALLMARK_INFLAMMATORY_RESPONSE	0.001	0.027	–1.69	Downregulated

AXA, axatilimab; NES, network enhancement-based similarity.

- Axatilimab treatment of human CSF-1/CSF-1R knock-in mice led to reductions in the number of monocytes in the blood without reducing the total leukocyte or neutrophil populations (**Figure 6A–D**)

Figure 6. Effects of Axatilimab Treatment on Human CSF-1/CSF-1R Knock-In Mouse Model



Conclusions

- Taken together, these data suggest that blocking of CSF-1/CSF-1R signaling by axatilimab reduces inflammatory and fibrosis-associated genes and protein profiles in monocytes and monocyte-derived macrophages
- In a human CSF-1/CSF-1R knock-in mouse model, axatilimab reduced the number of monocytes in the peripheral blood with no reductions in leukocyte or neutrophil populations

Acknowledgments

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