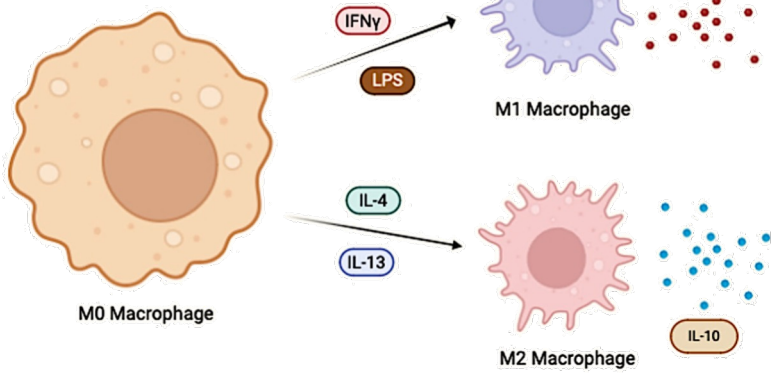


# Novel characterization of Plexin D1's role in regulating the macrophage immune response

## BACKGROUND

Macrophages are involved in the progression and prevention of **atherosclerosis** and **cancer**, the two leading causes of death.



M1 macrophages are **pro-inflammatory** and combat pathogens and tumor cells.

M2 macrophages are **anti-inflammatory** and engage in tissue repair and wound healing.

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**Plexin D1 (Plxnd1)** is a cell-surface receptor protein that plays **critical role in angiogenesis and immune cell regulation**. Plxnd1 signaling with its ligand, Sema3E, is crucial for angiogenesis.

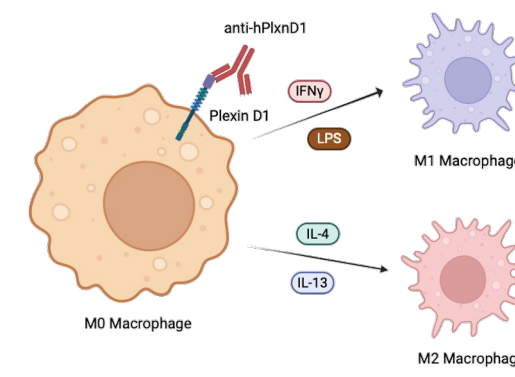
### Plxnd1 and Macrophages:

Zhang et. al (2023) observed more Plxnd1+ M1 macrophages than Plxnd1+ M2 macrophages in bifurcation lesions of atherosclerotic mice. Wanschel et. al (2013) found that Sema3E inhibits MCP-1 (CCL2) induced migration of peritoneal macrophages *ex vivo*.

**Problem:** Role of Plxnd1 in macrophages incompletely understood, **lack of targets** that modulate macrophage behavior

**Goal:** Characterize role of Plxnd1 in regulating macrophage polarization, cytokine release, migration, and phagocytosis

## METHODS



- Create **novel** macrophage polarization model (both M1 and M2 signals)
- Treat cells with Plxnd1 agonist (Sema3E) or antagonist (anti-Plxnd1 Ab)

- Analyze M0, M1, M2 markers with **Flow Cytometry**

- Analyze cytokine secretion with **ELISA (TNF $\alpha$  and IL-4)**

### Transwell Migration Assay

- **Top Chamber:** Macrophages & treatment (anti-Plxnd1 Ab)
- **Bottom Chamber:** Chemoattractant (Sema3A/C/E)

### In vitro Phagocytosis Assay

- 2-day pre-treatment of anti-Plxnd1 antibodies
- pHrodo Red E. Coli bioparticles conjugate

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## RESULTS

### Macrophage Polarization and Cytokine Release

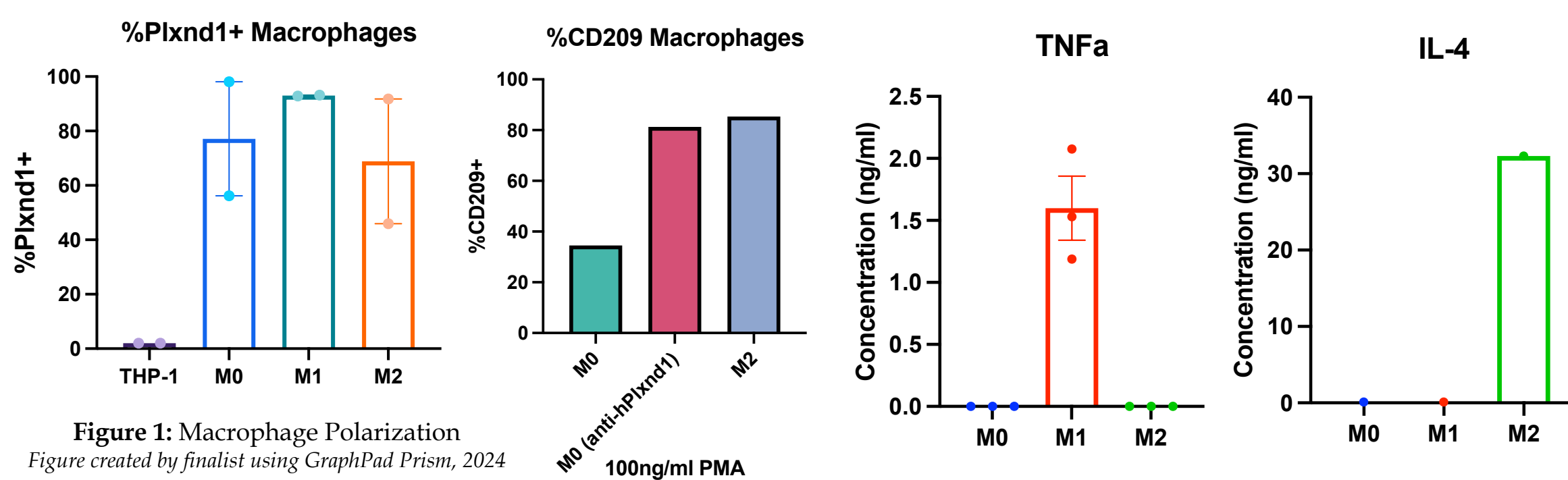


Figure 1: Macrophage Polarization

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Figure 1: Plxnd1 is upregulated on M0, M1, M2 macrophages and Plxnd1 blocking promotes M2 macrophage polarization

- Plxnd1 blocking increased percentage of CD209-expressing cells in M0 macrophages to similar level as M2
- TNF $\alpha$  and IL-4 secreted by M1 and M2 macrophages, respectively

### Macrophage Migration

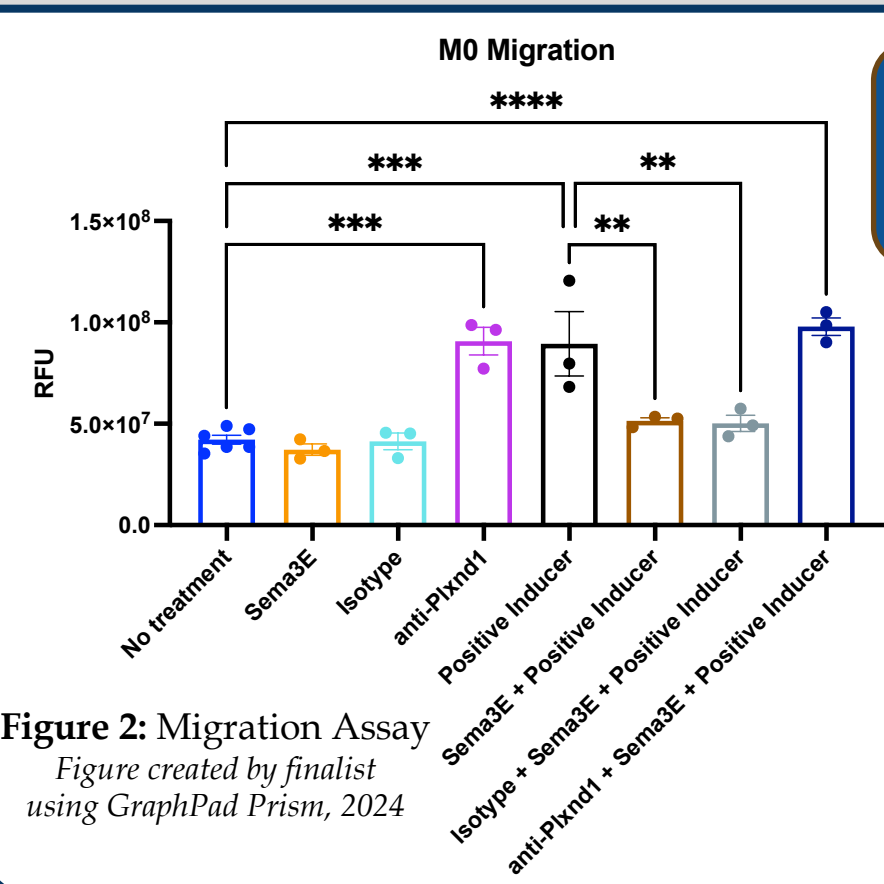


Figure 2: Migration Assay

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Figure 2: Plxnd1 blocking induces migration, while Plxnd1 activation immobilizes macrophages

- Plxnd1 blocking increases M0 migration by two-fold
- Plxnd1 activation via Sema3E immobilizes macrophages
- Sema3A is a potent chemoattractant in M0 and M2 macrophages (dose-dependent response, data not shown)

### Macrophage Phagocytosis

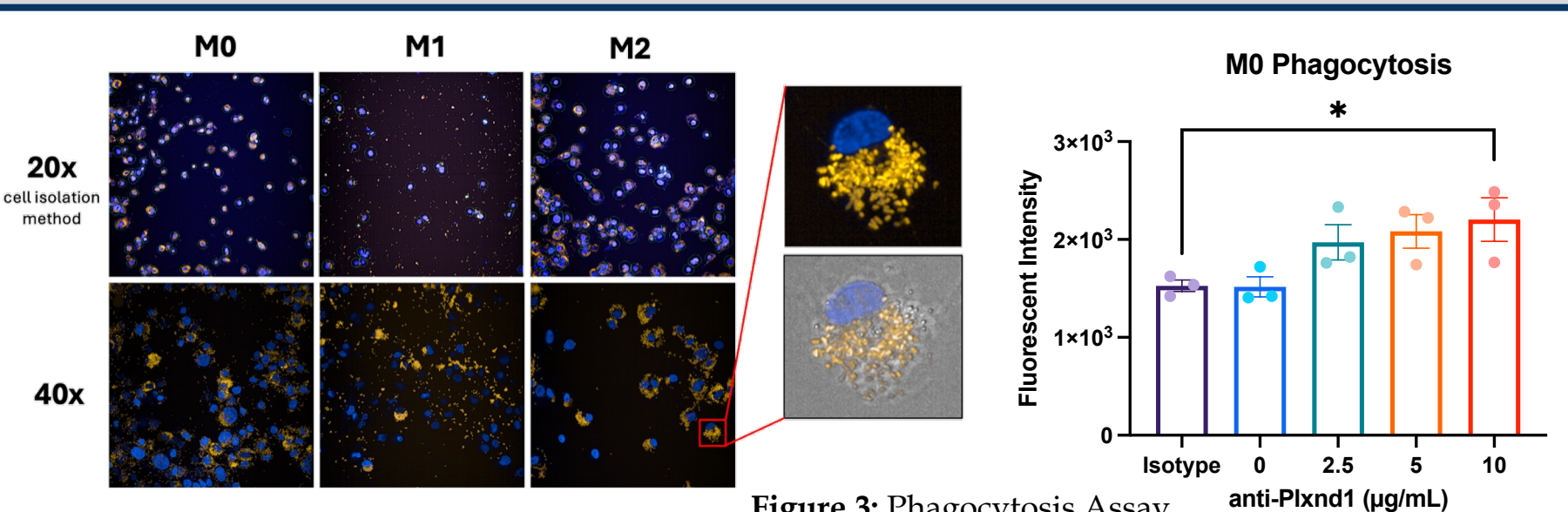


Figure 3: Phagocytosis Assay

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- 10 $\mu$ g/mL anti-Plxnd1 antibody sufficient to boost phagocytosis in M0 macrophages

Figure 3: Plxnd1 blocking promotes phagocytosis in resting-state (M0) macrophages in dose-dependent manner

## DISCUSSION

**Impact:** Plxnd1 is crucial for the macrophage immune response.

Plxnd1 is highly expressed on THP-1 derived macrophages.

Plxnd1 blocking promotes anti-inflammatory macrophage polarization, induces migration, and boosts phagocytosis.

### Future Studies:

Investigate findings in disease-relevant setting (atherosclerosis or tumor model)

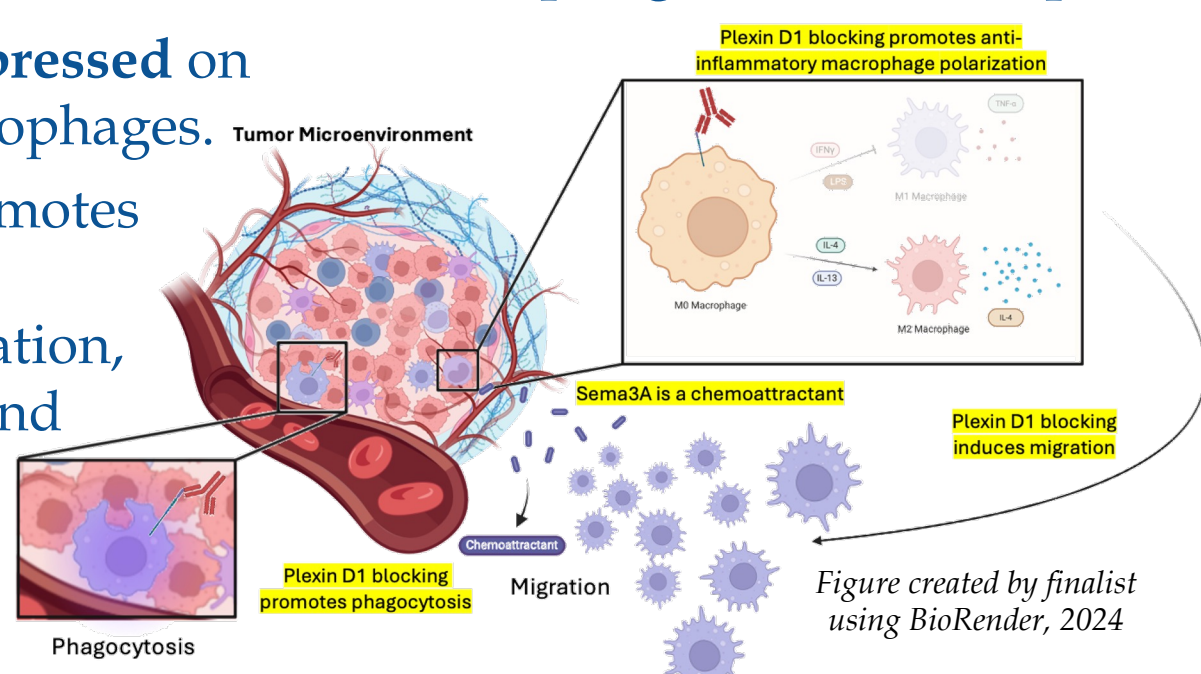


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### Therapeutic Impact

Plxnd1 might ameliorate atherosclerosis by promoting anti-inflammatory macrophage polarization and macrophage egress from plaque

## SUMMARY

**Problem:** Lack of targets to modulate macrophage activity for treatment of atherosclerosis and cancer

**Goal:** Determine the role of Plexin D1 in regulating macrophage behavior and activity

**Results:** Plexin D1 blocking is sufficient to modulate macrophage polarization, migration, and phagocytosis

**Impact:** Plexin D1 is a novel modulator of macrophage behavior

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