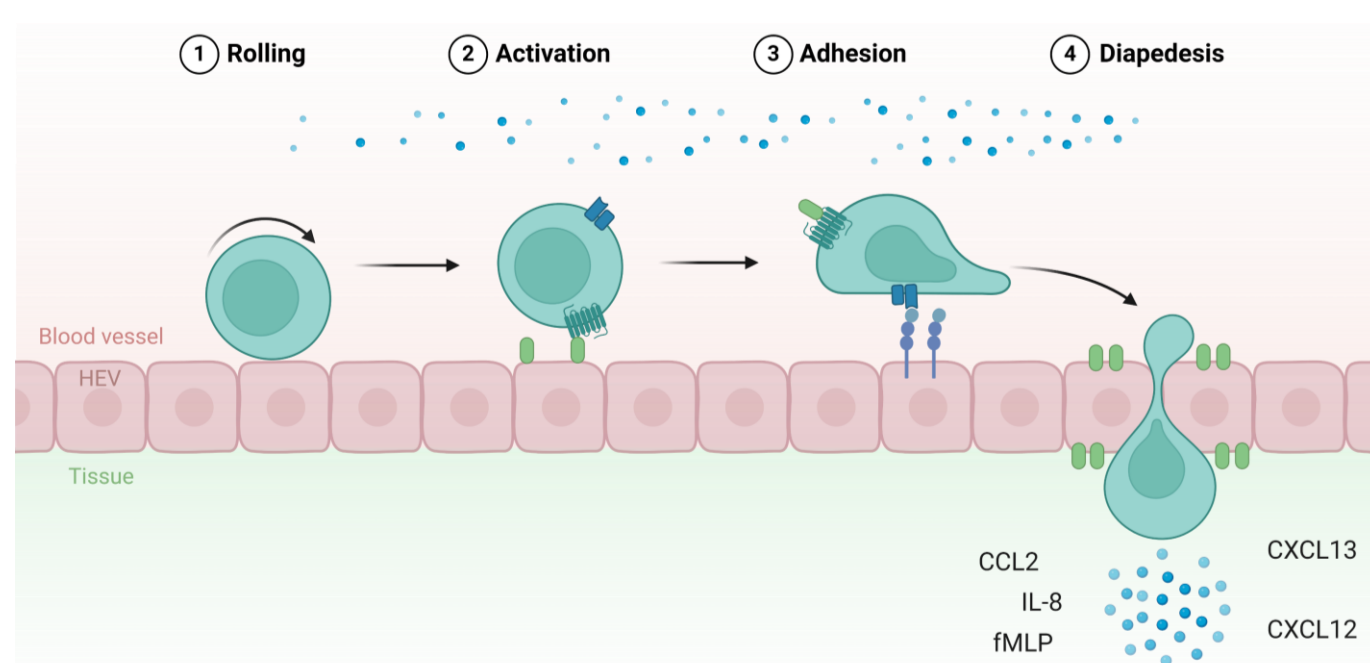


Background

- Current immunotoxicity testing guidelines rely on costly, low-throughput rodent models.
- High-throughput new approach methods (NAMs) are necessary for rapid identification of immunotoxics.
- Chemotaxis is a critical function of immune cells by which they are recruited to specific sites *in vivo* for immune cell development, response to infection, and wound healing.
- Previous work has shown that some xenobiotics are able to inhibit chemotaxis.
- We sought to develop a high-throughput assay to model chemotaxis *in vitro* using Jurkat T cells.



Methods

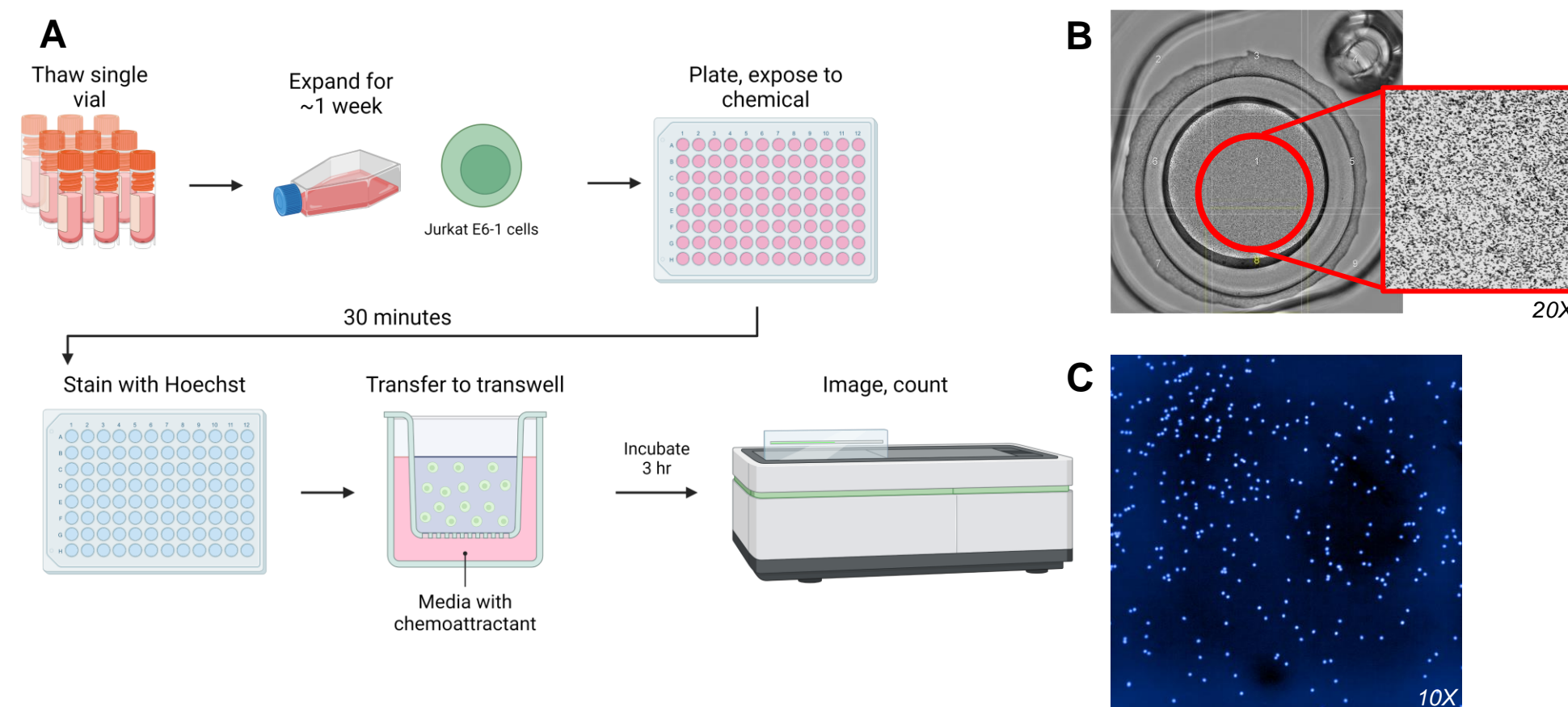


Figure 1. Workflow of *in vitro* chemotaxis experiments. (A) Jurkat T cells (a CD4⁺ T cell line) were thawed, expanded, and plated into a 96 well plate for exposure. After exposure, they were stained with a nuclear dye and transferred to the top chamber of a transwell plate for the chemotaxis assay. CXCL12 was loaded into the bottom chamber of the transwell plate to stimulate chemotaxis. After 3 hrs, the cells were imaged on an Opera Phenix high-content imager. (B) Image of a transwell plate with insert present. Inset is zoomed in on the transwell membrane to show 3-µm pores at 20X. (C) Representative image of Jurkat T cells imaged at 10X.

T cells migrated to CXCL12 in a biphasic manner

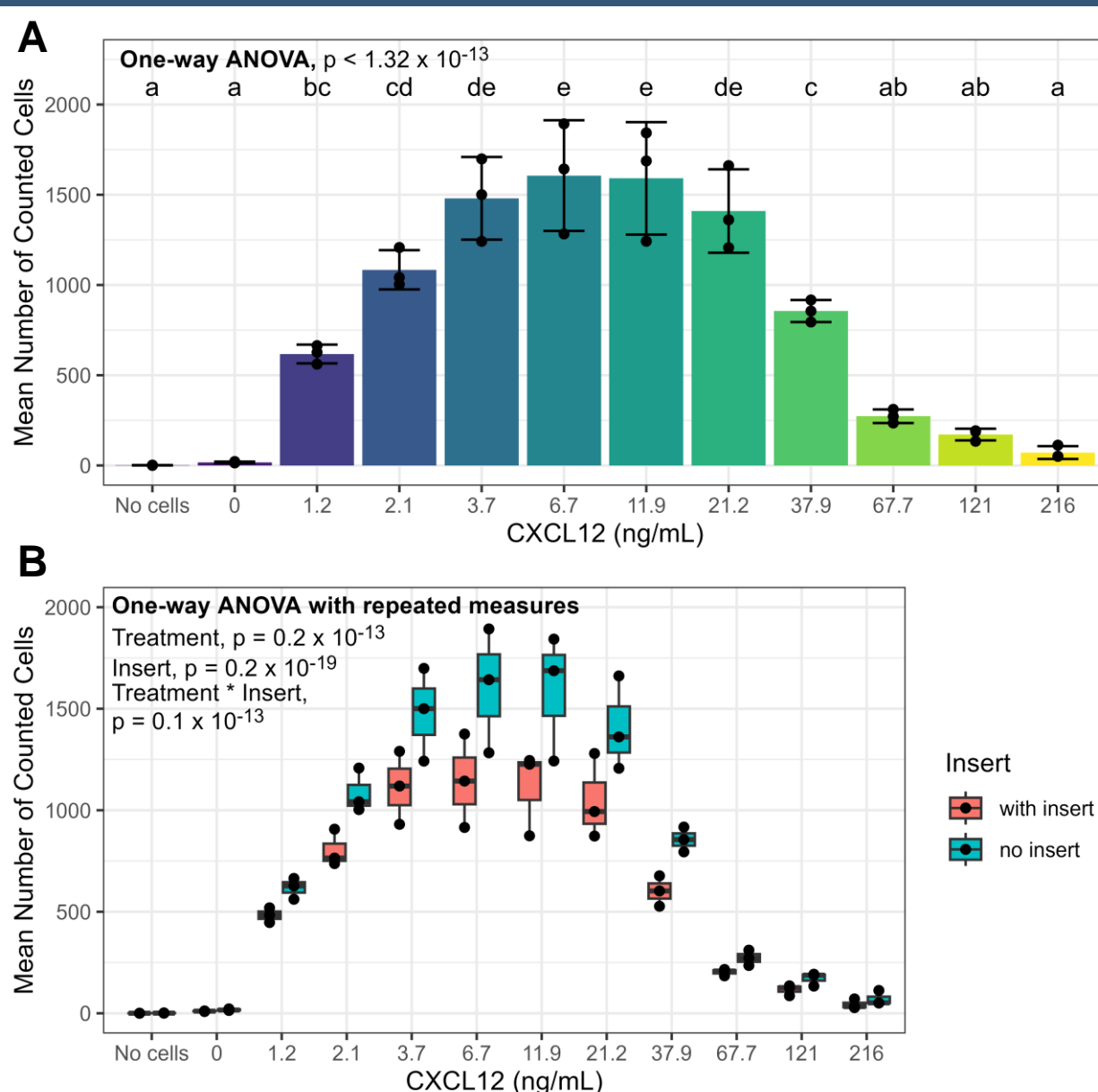


Figure 2. T cells respond in a biphasic concentration response towards CXCL12, as expected. (A) 200,000 T cells were loaded on the top chamber of a transwell membrane with 3-µm pores while different concentrations of CXCL12 were loaded in the bottom chamber. (B) Images were taken with and without the transwell insert present for comparison.

Specific inhibitors blocked chemotaxis of T cells

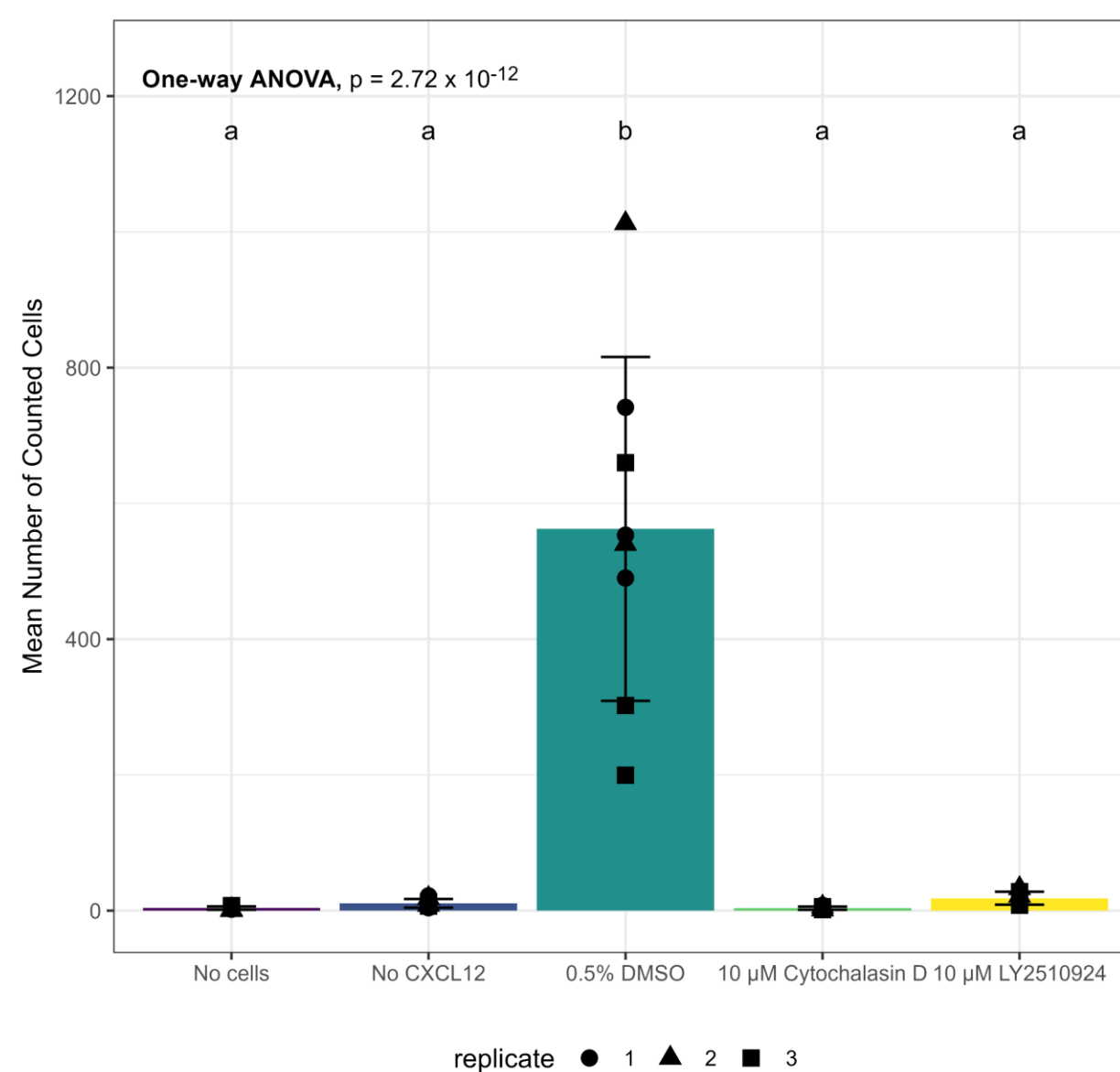


Figure 3. Chemotaxis was inhibited by two inhibitors. 200,000 T cells were loaded on the top chamber of a transwell membrane with 3-µm pores while a single concentration of CXCL12 (10.9 ng/mL) was loaded in the bottom chamber. Across 8 plates, controls were employed to assess assay performance. Cells were pre-treated with inhibitors for 30 minutes prior to stimulation of chemotaxis.

30-min chemical exposure did not inhibit chemotaxis

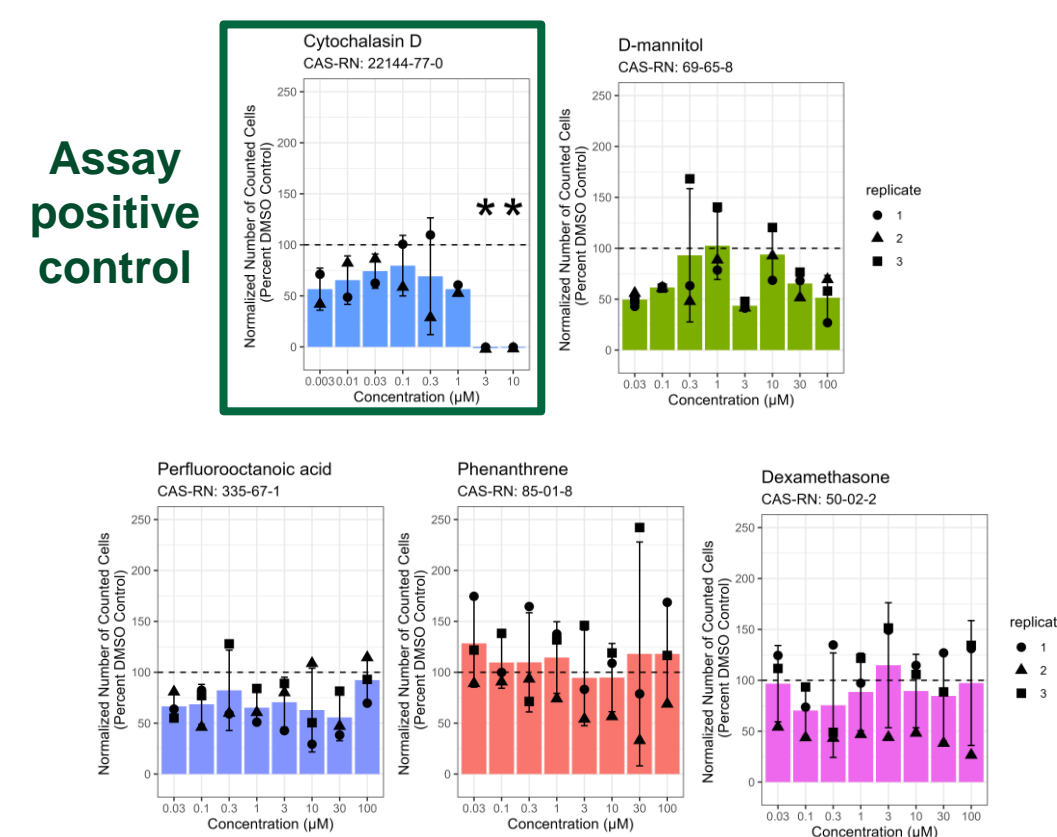


Figure 4. Select chemicals did not inhibit chemotaxis. 200,000 T cells were loaded on the top chamber of a transwell membrane with 3-µm pores while a single concentration of CXCL12 (10.9 ng/mL) was loaded in the bottom chamber. Cells were pre-treated for 30 minutes prior to stimulation of chemotaxis.

Jurkat cells successfully migrate towards CXCL12, and this migration can be inhibited by known inhibitors. Future studies will continue to optimize conditions for this assay for this cell line and others.