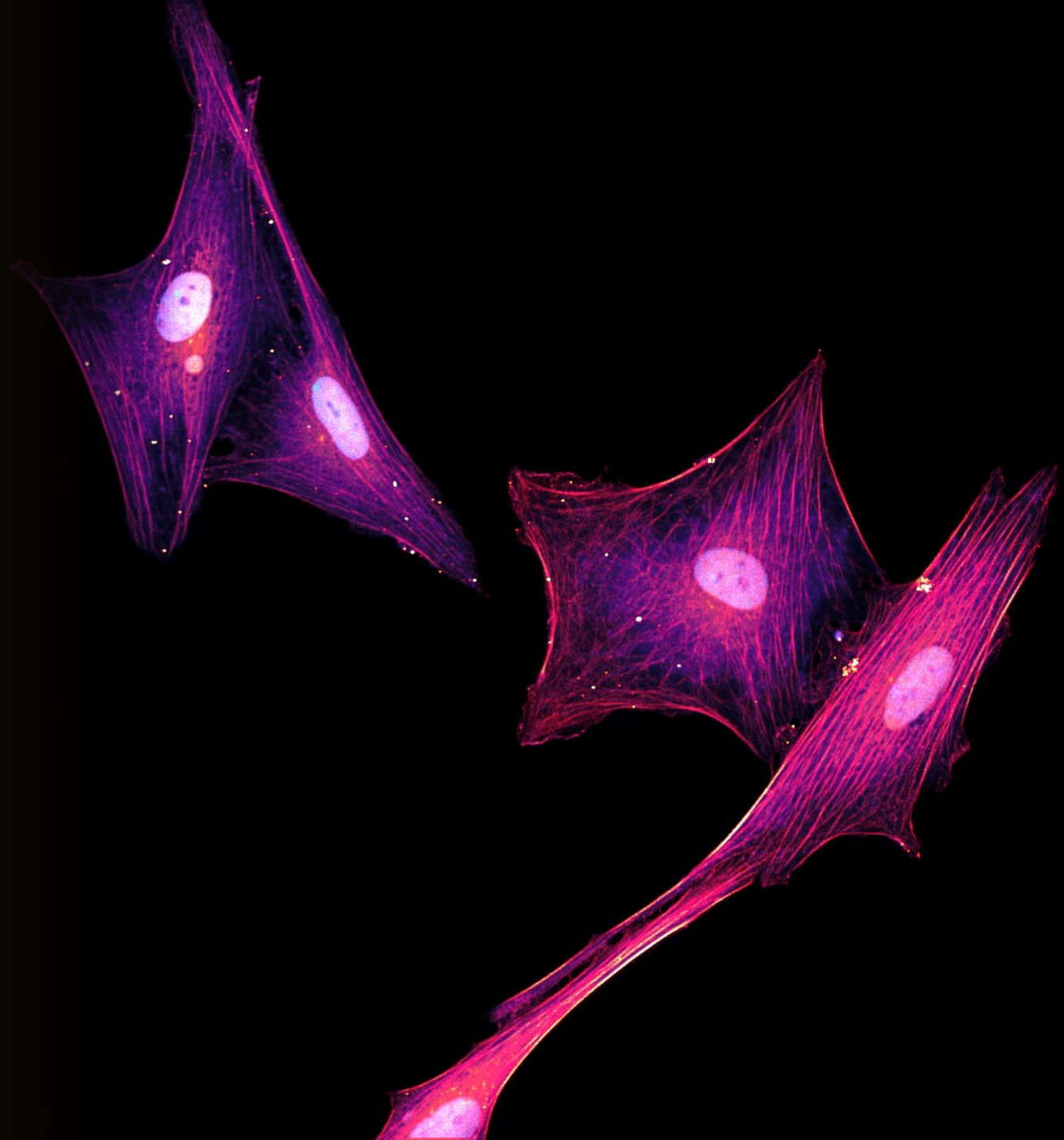




Understanding Complex Biology

CASE STUDY

Induction & Inhibition
of Fibrotic State Using
a Novel, Biologic
Therapeutic



OBJECTIVE

A client requested a custom project to measure fibrosis induction and inhibition in hepatic stellate cells (HSCs).

Goals

1. Understand and evaluate the ability of a client-selected, secreted protein to induce a fibrotic state in healthy, human, primary HSCs.
2. Evaluate the ability of this novel, biologic therapeutic agent to inhibit fibrotic induction through measurements of F-actin and α -SMA fiber alignment, quantity, and morphology.
3. Compare the performance of the therapeutic agent with the performance of a known anti-fibrotic treatment in HSCs.



EXPERIMENTAL DESIGN

Cell Model

Primary, human, hepatic stellate cells (HSCs)

Palette

Hoechst (nuclei)

anti- α -SMA (α -SMA fibers)

Phalloidin (F-actin filaments)

Optimization

- Evaluate various doses of fibrotic inducer at various time points for induction of fibrotic phenotype.

Treatments and Timelines

- Culture cells in 384-well, imaging microplates, utilizing vendor recommended media.
- On day *in vitro* (DIV)1, serum starve cells for 24 hours prior to treatment with therapeutic agents and fibrotic inducer TGF- β on DIV2.
- Culture cell for 72 hours after treatment with TGF- β .
- Fix at 72hrs and stain cells with palette.

Deliverables

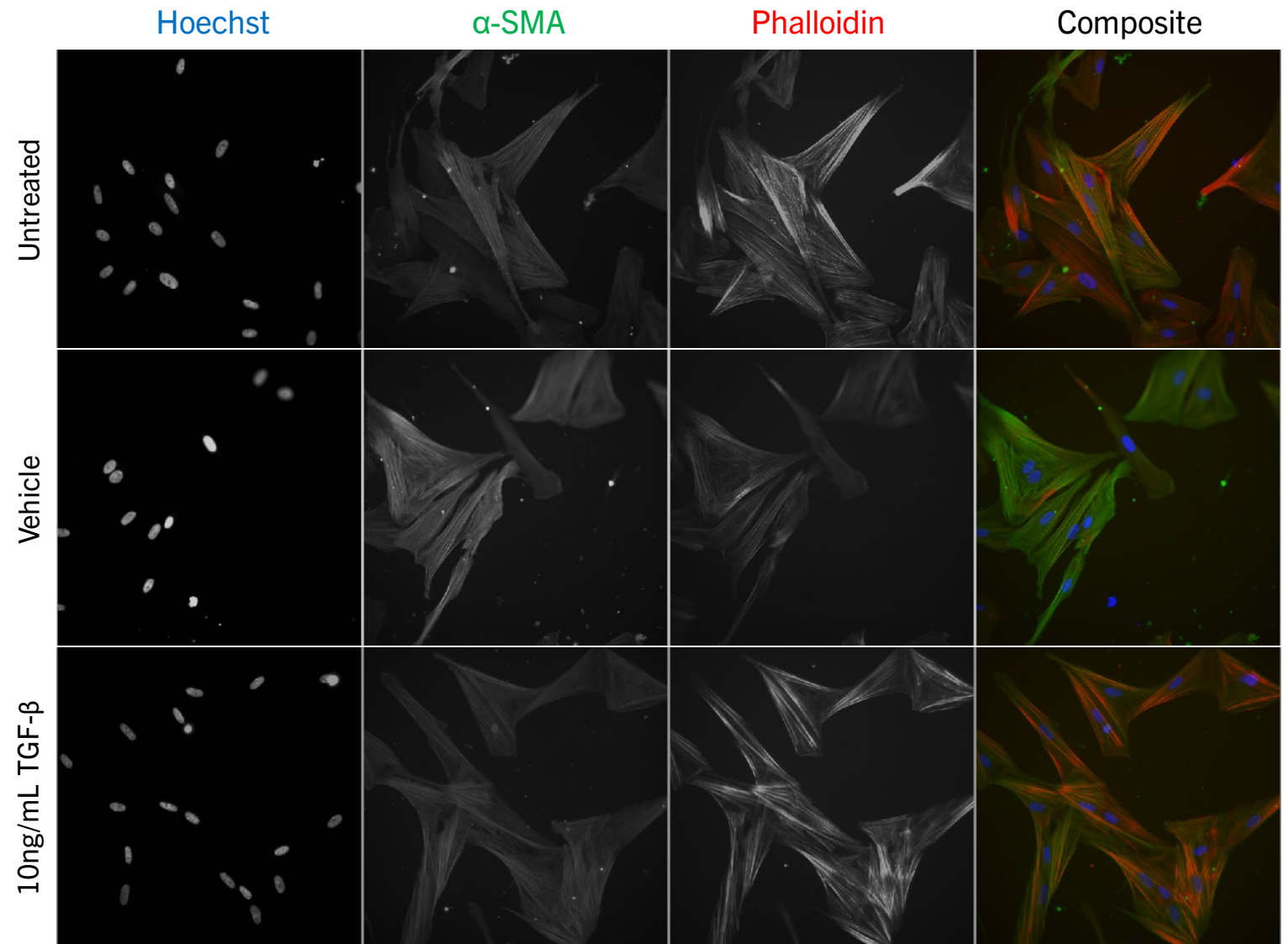
- May include cell count, fiber length, fiber intensity, fiber alignment, co-localization of f-actin with α -SMA, cell morphology, and other metrics, as appropriate for the study design.
- Reporting via a presentation-ready report to include detailed methodology, statistical analysis and IC₅₀-curve fits, where applicable. Representative images will be provided for controls and a reasonable selection of test conditions.



REPRESENTATIVE IMAGES

72hrs, Fibrotic Inducer TGF- β

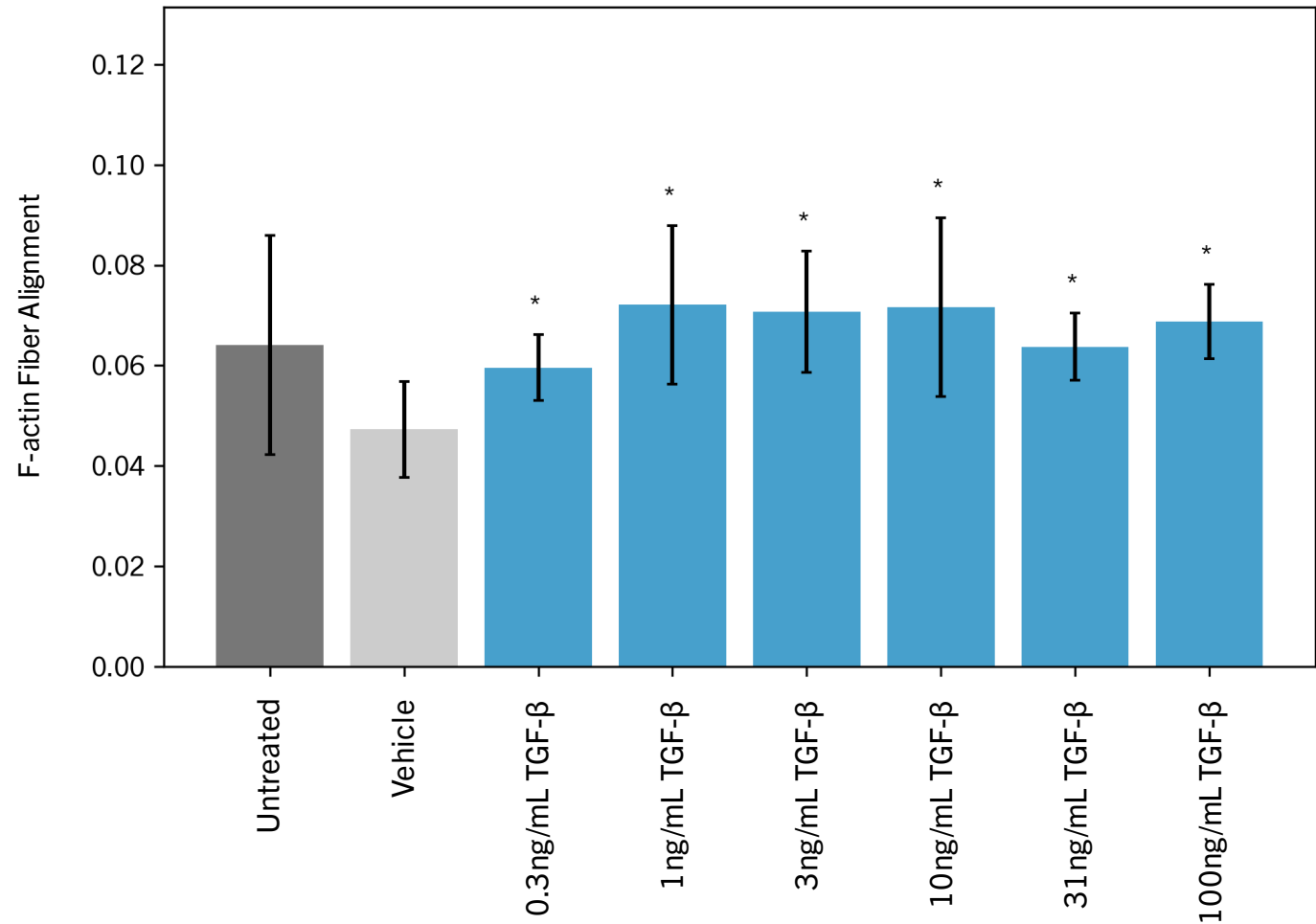
Representative images of HSCs 72hrs after treatment with fibrotic inducer TGF- β .



QUANTITATIVE DATA

72hrs, F-actin Fiber Alignment

Treatment of HSCs with TGF- β increased fiber alignment, compared to untreated or vehicle-treated cells.

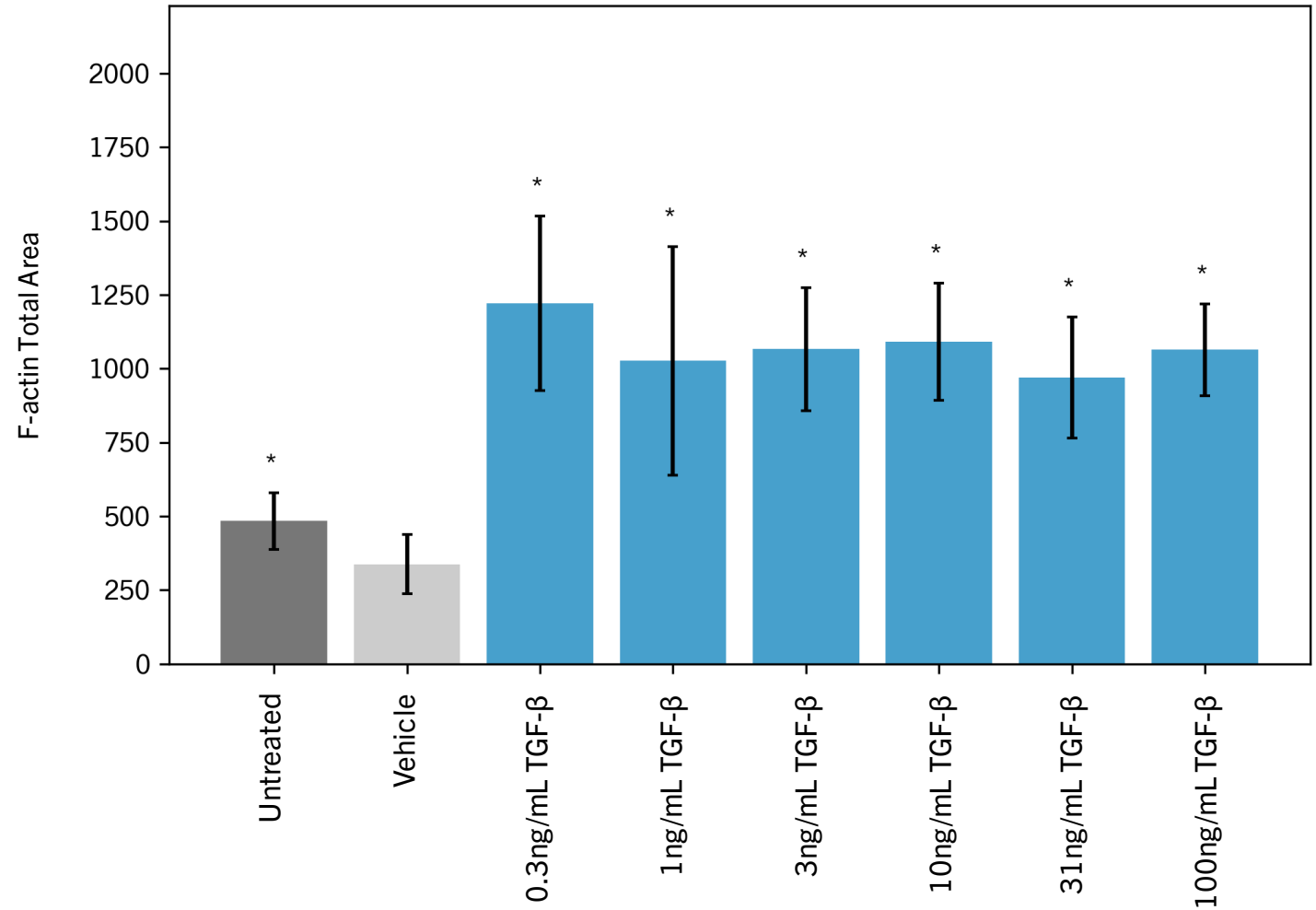


*p-values calculated against vehicle.

QUANTITATIVE DATA

72hrs, F-actin Total Area

Treatment of HSCs with TGF- β increased total area of F-actin, compared to untreated or vehicle-treated cells.

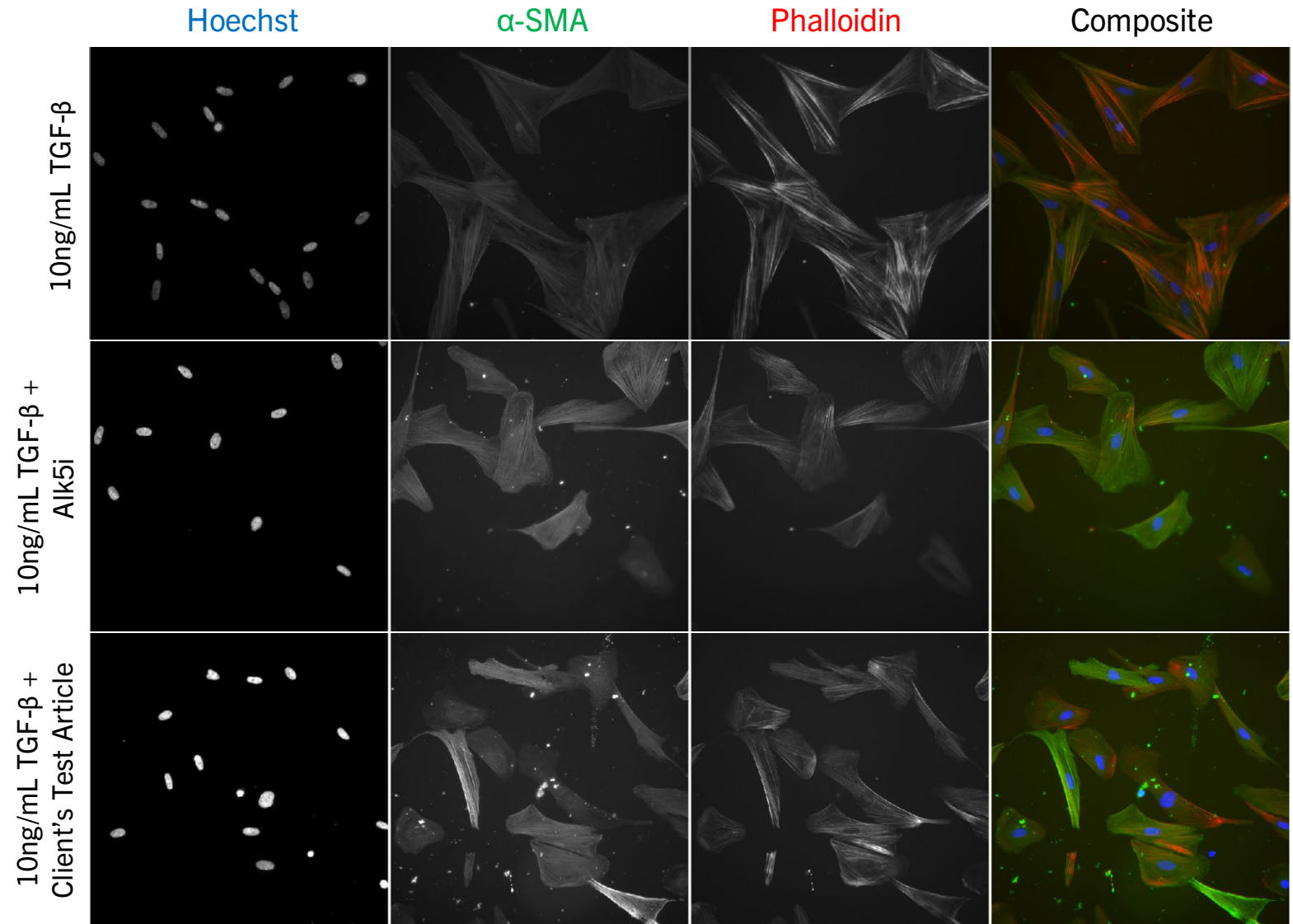


**p*-values calculated against vehicle.

REPRESENTATIVE IMAGES

72hrs, Alk5i & Client's Test Article

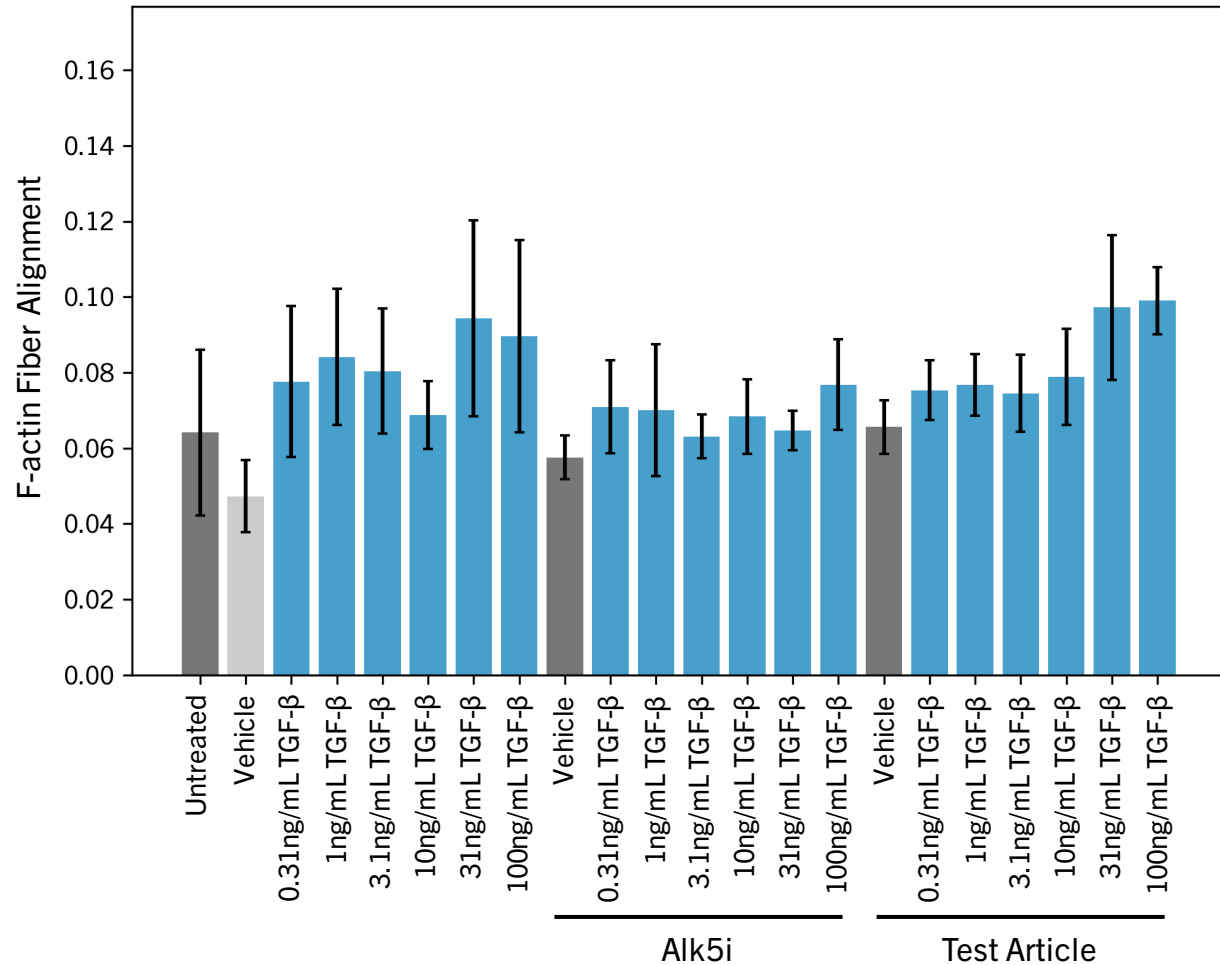
Representative images of HSCs pre-treated with Alk5i and client's test article, 72hrs after treatment with TGF- β .



QUANTITATIVE DATA

72hrs, F-actin Fiber Alignment

Neither Alk5i nor the client's test article significantly prevented the increase in fiber alignment in HSCs induced by TGF- β treatment.



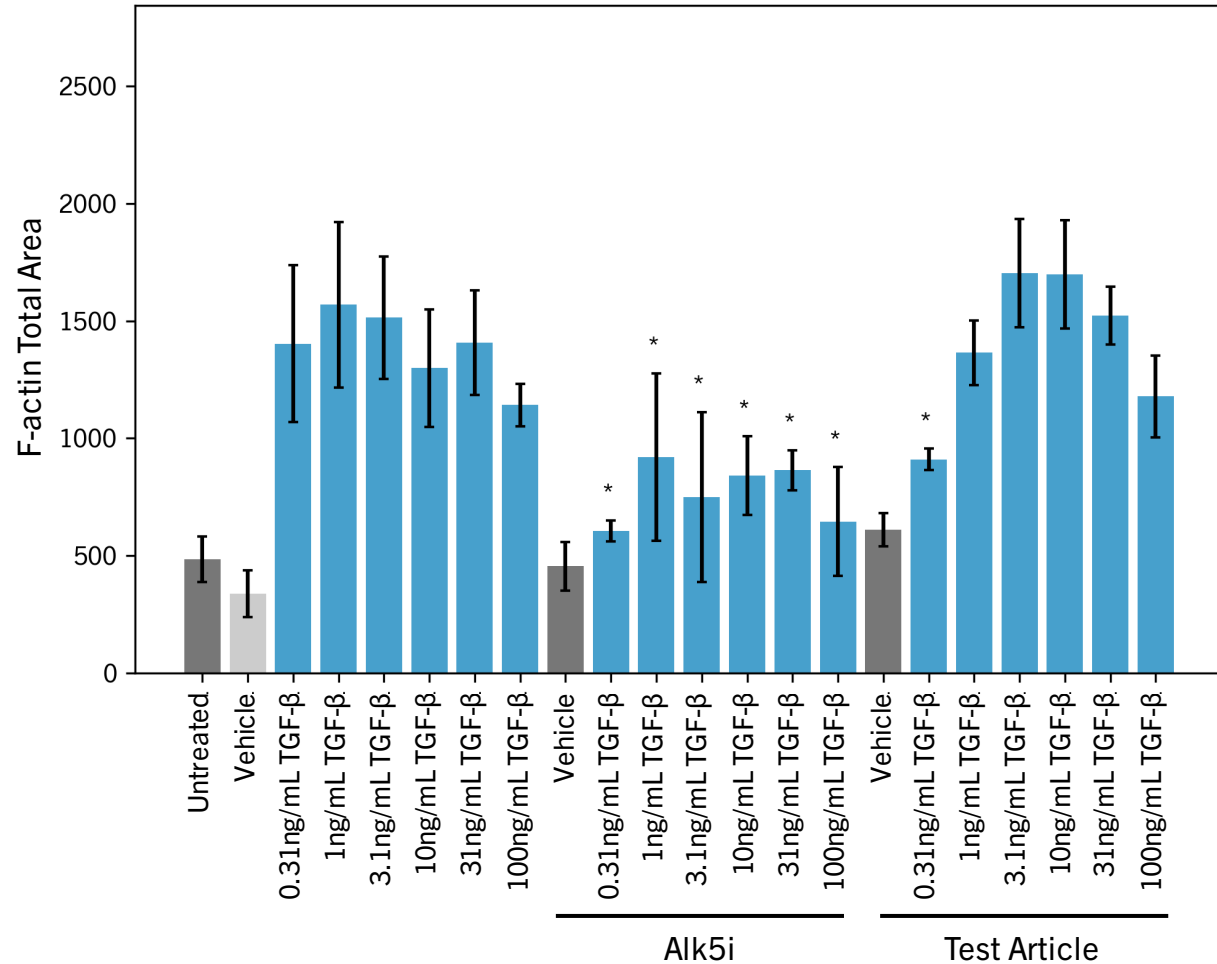
**p*-values calculated against vehicle.

QUANTITATIVE DATA

72hrs, F-actin Total Area

Alk5i significantly inhibited the increase in F-actin fiber area induced by TGF- β treatment.

The lowest tested dose of the client's test article significantly inhibited the increase in F-actin fiber area induced by TGF- β treatment.



*p-values calculated against vehicle.

SUMMARY

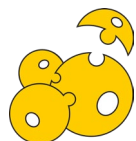
1. Treatment with TGF- β induced fibrosis in HSCs, with the strongest fibrotic measurements at 72hrs after treatment.
2. The client-provided, biologic therapeutic agent showed mixed, anti-fibrotic properties in both a time- and dose-dependent manner.
3. The client-provided, therapeutic agent did not show greater efficacy than reference inhibitor Alk5i.



ADDITIONAL RESOURCES

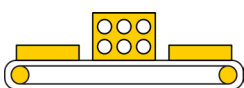
PhenoVista's Services

We develop assays in close collaboration with our clients to ensure that your specific questions will be answered. You can choose from a range of services to select the best fit for your needs. For more information, visit <https://phenovista.com/assay-services>



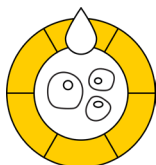
Custom Assay Services

Custom assays to answer your specific, complex biological questions.



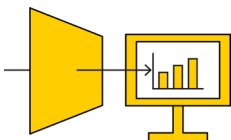
Ready-2-Go Assay Services

Defined assay offerings across a range of disease and therapeutic areas.



Cell Painting

Compare your compounds' effects against those of reference compounds.



Imaging & Analysis

Send us plates of fixed & stained cells, and we'll send you data.

Learning Library

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