The Fibrin-derived Peptide FX06 Protects Human Pulmonary Endothelial Cells Against the COVID-19-Triggered Cytokine Storm

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SYSTEMS BIOLOGY IRELAND

UCD DUBLIN

BACKGROUND

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been a major health emergency since 2019. Endothelial dysfunction is a hallmark of COVID-19, leading to severe illness, i.e. multi-organ failure, coagulopathy, and death[1]. FX06, a fibrin-derived natural peptide, formerly known as Bβ_{15-42}, protects the vasculature in myocardial ischemia-reperfusion in animal models[2]. Therefore, it is a promising therapeutic candidate for endothelial complications such as capillary leak in COVID-19 and other infectious diseases. The aim of this project is to investigate whether FX06 can help to prevent COVID-19 progression *in vitro*.
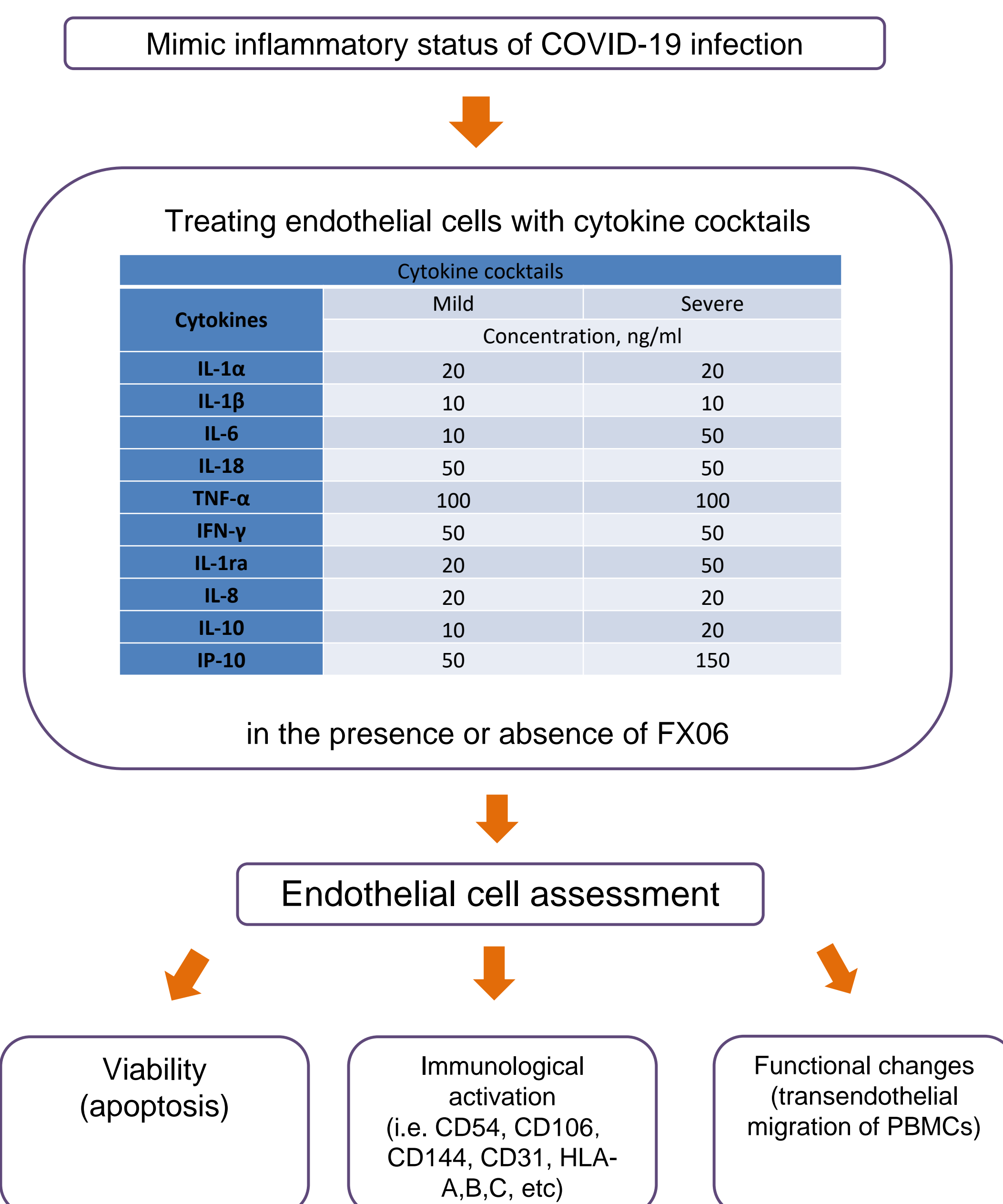
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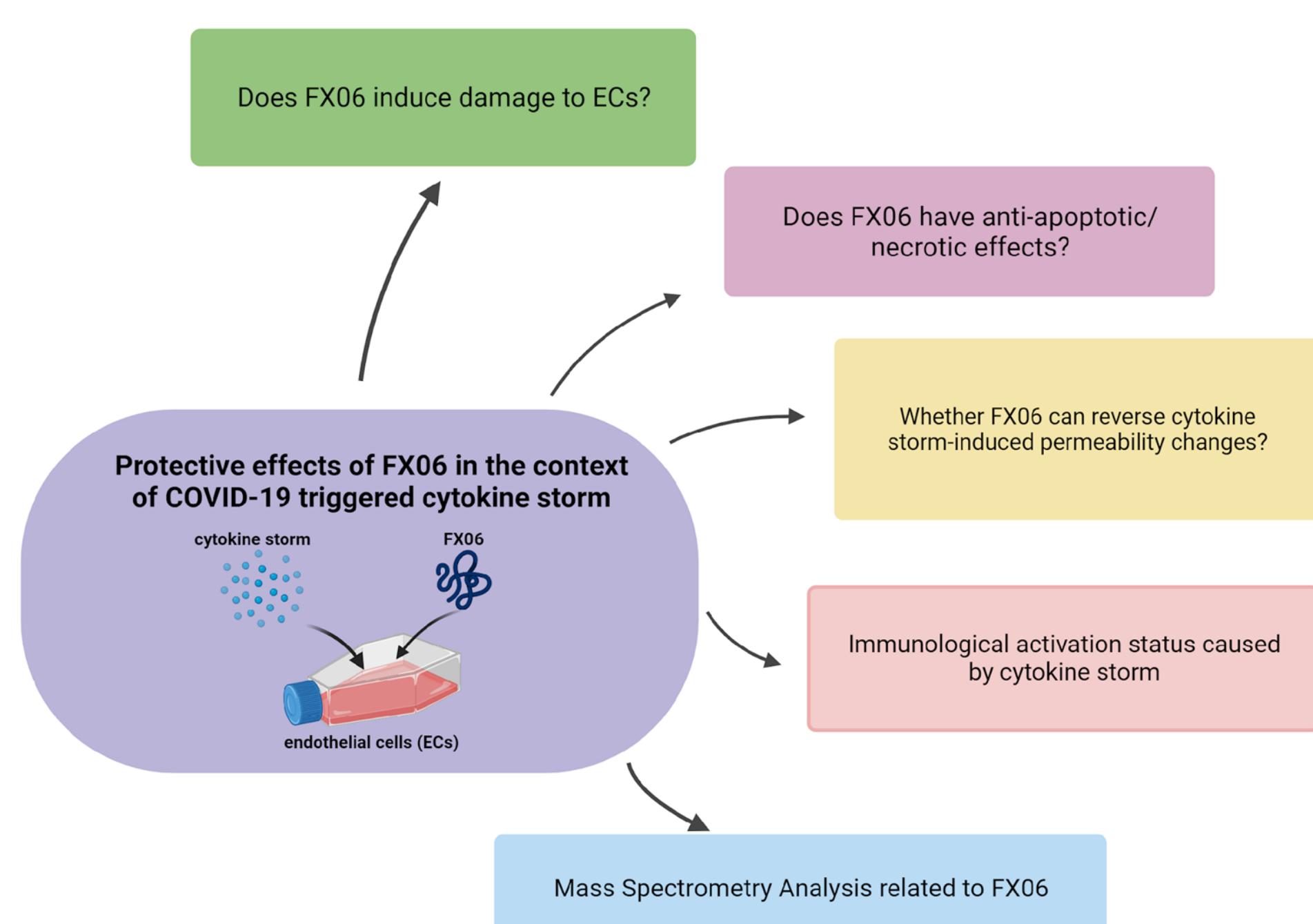
METHODS

- Flow Cytometry Analysis
- Immunofluorescence
- Collagen-based Transendothelial Migration (TEM) Assay Under Static And Shear Stress Conditions
- Confocal Microscope based-High Content Screening
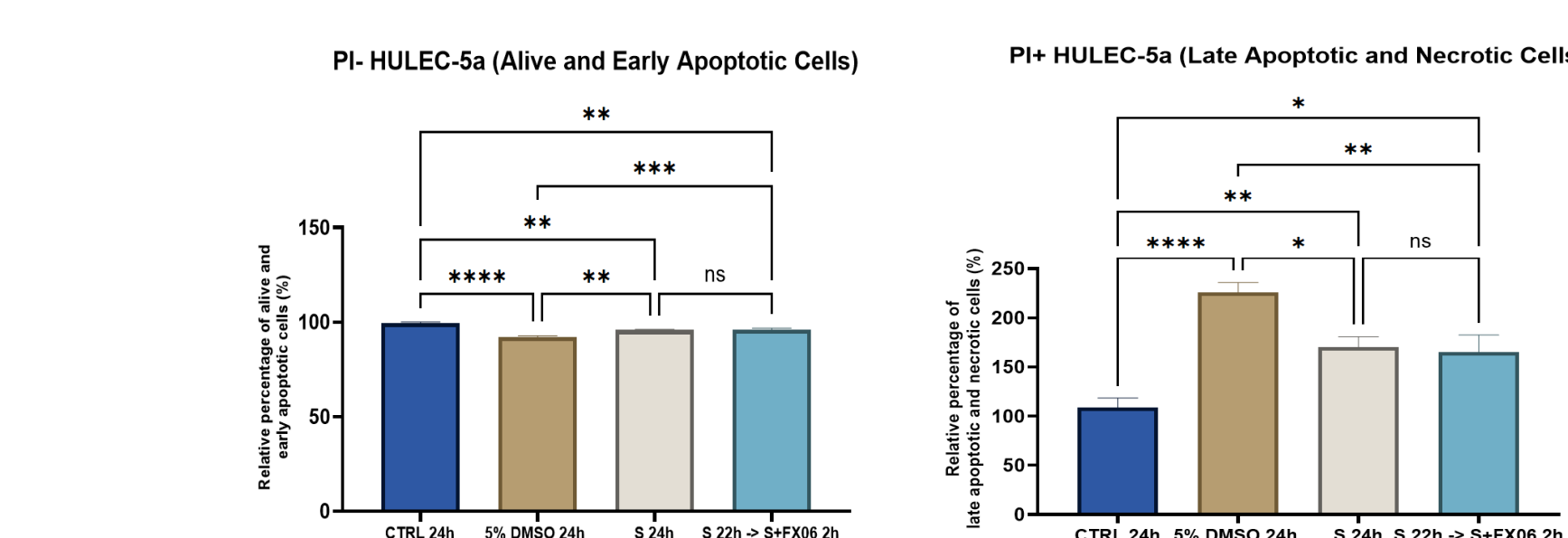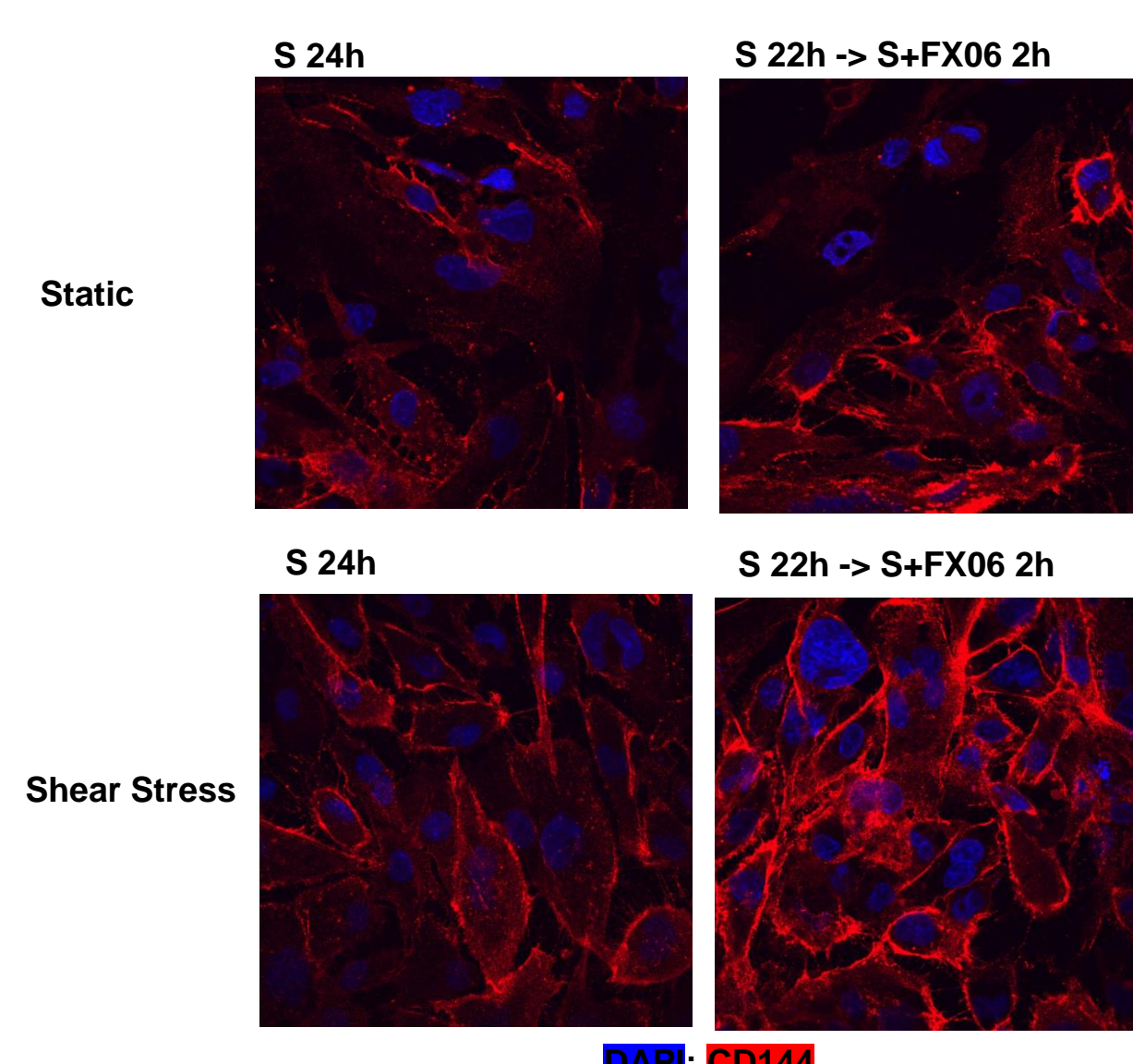
EXPERIMENT DESIGN

Mimic inflammatory status of COVID-19 infection

Treating endothelial cells with cytokine cocktails

Cytokines	Cytokine cocktails	
	Mild	Severe
	Concentration, ng/ml	
IL-1α	20	20
IL-1β	10	10
IL-6	10	50
IL-18	50	50
TNF-α	100	100
IFN-γ	50	50
IL-1ra	20	50
IL-8	20	20
IL-10	10	20
IP-10	50	150

in the presence or absence of FX06

Endothelial cell assessment

Viability (apoptosis)

Immunological activation (i.e. CD54, CD106, CD144, CD31, HLA-A,B,C, etc)

Functional changes (transendothelial migration of PBMCs)

RESULTS

FX06 prevents TEM of PBMCs under static and shear stress conditions
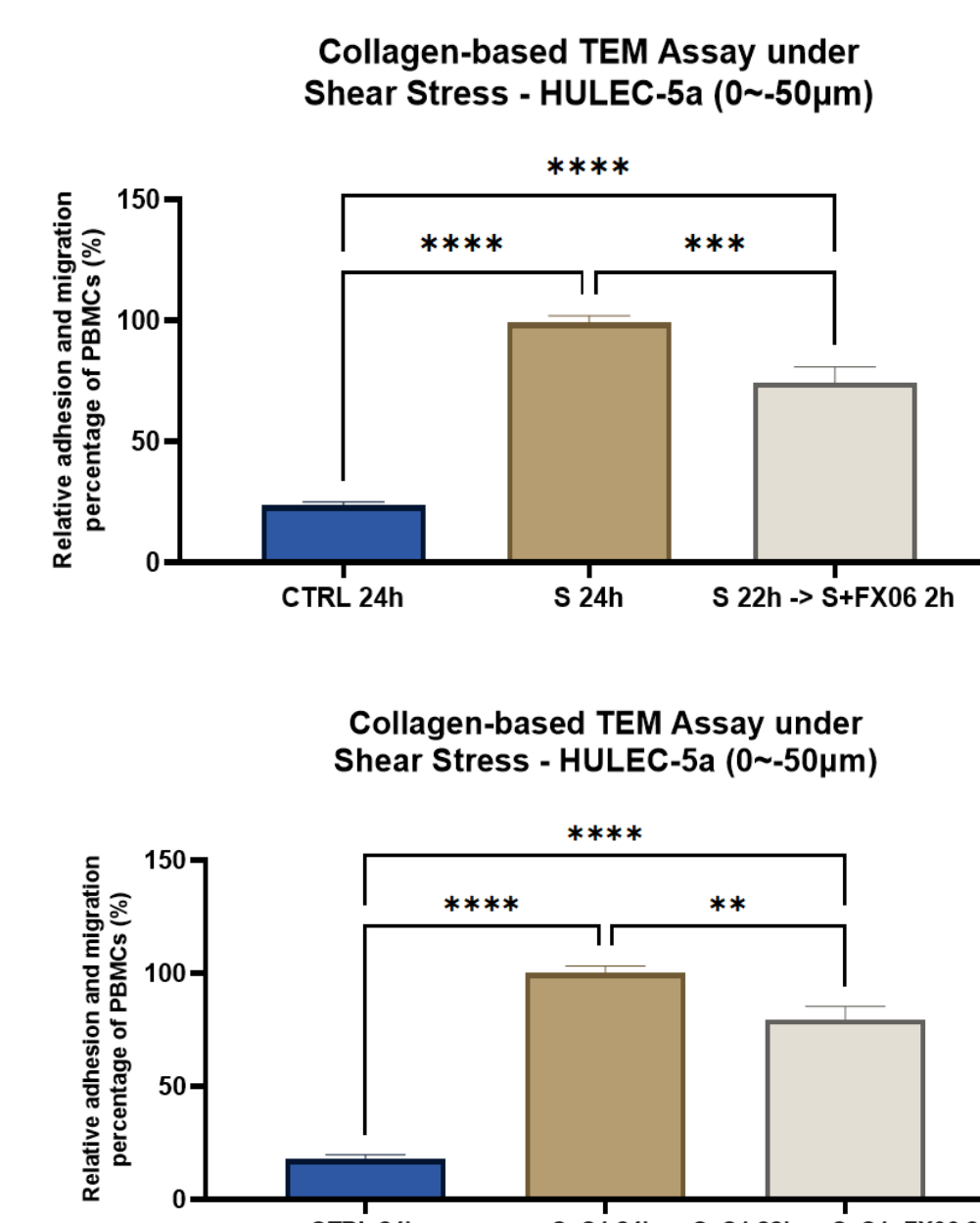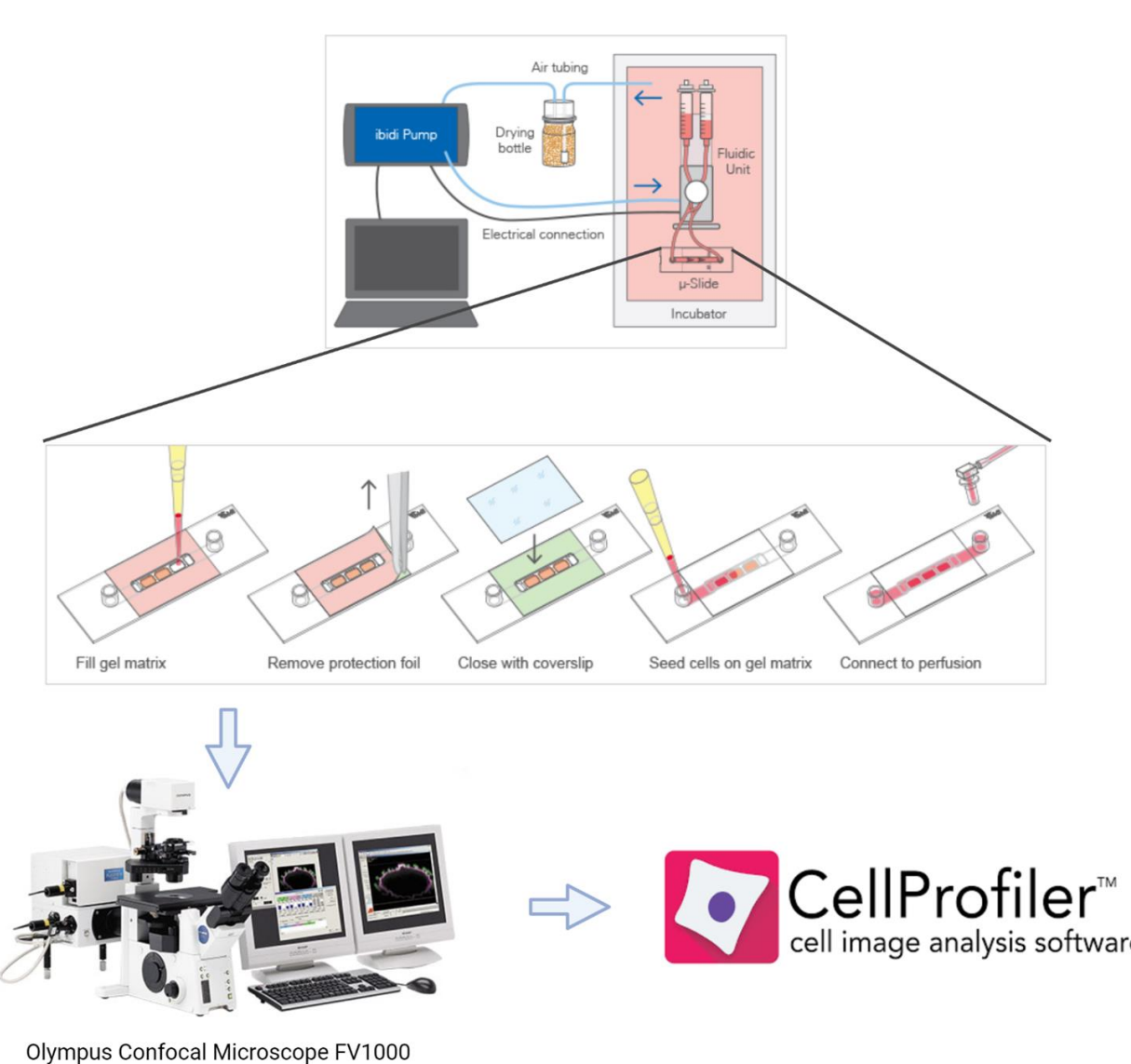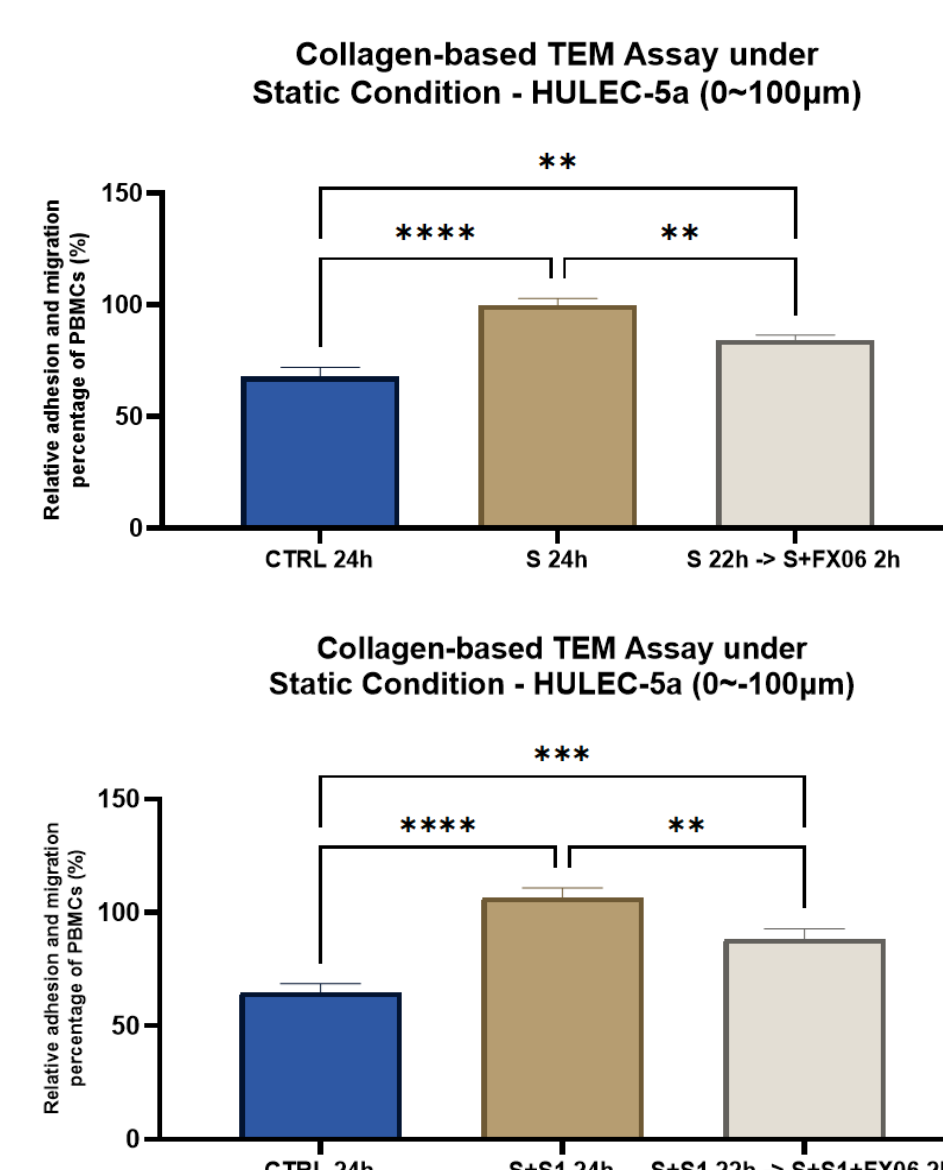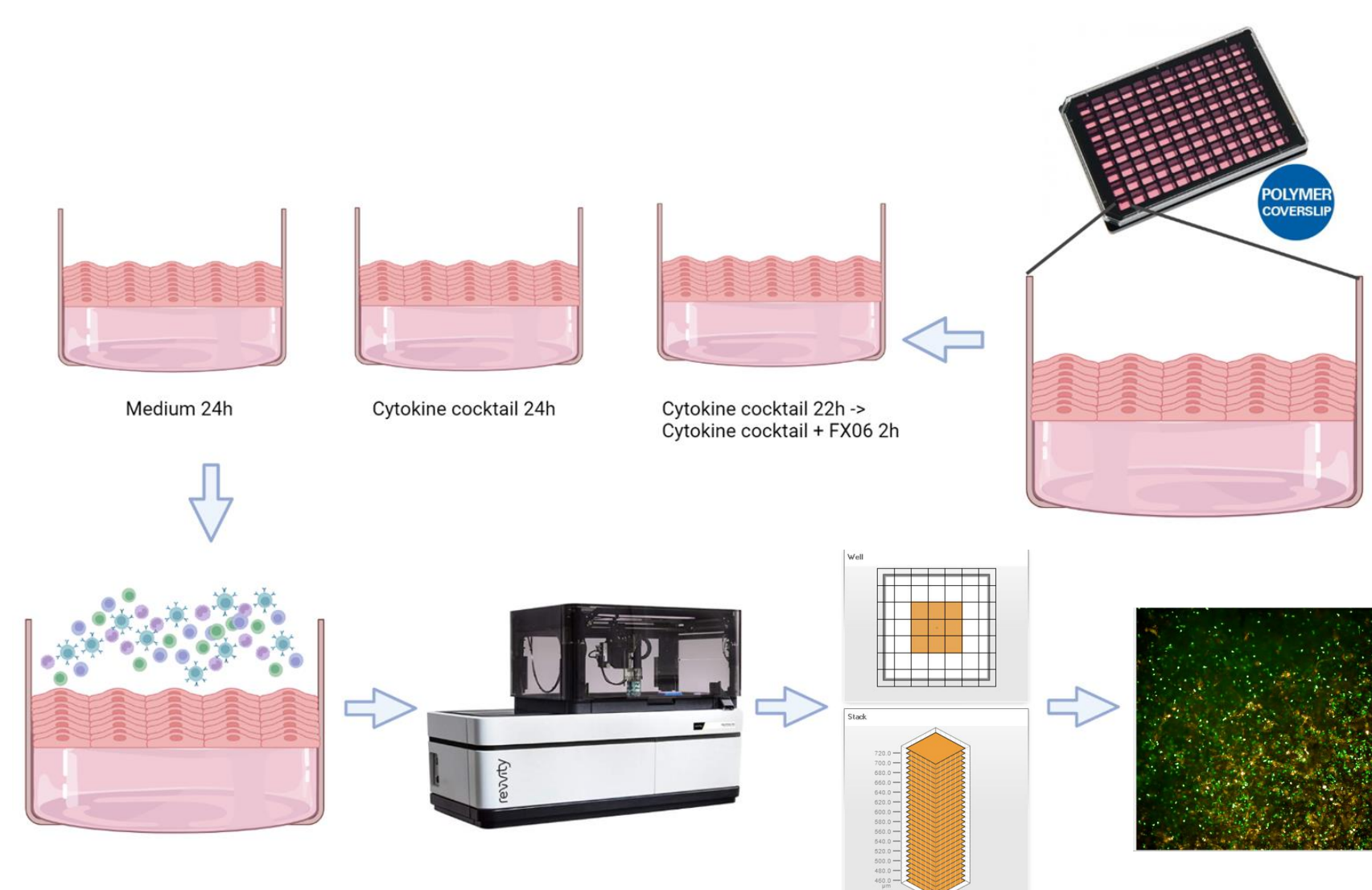
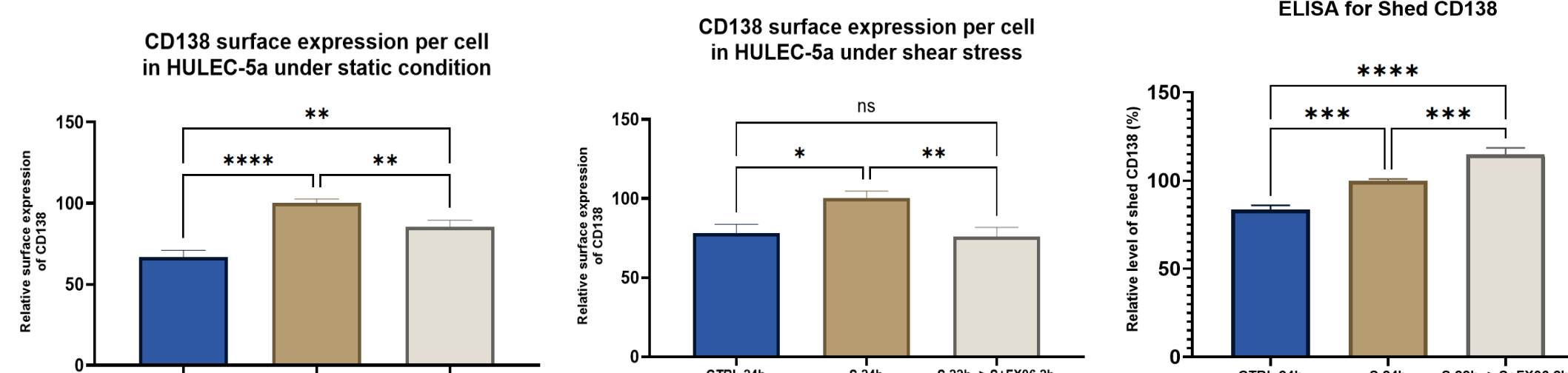**FX06 does not protect the endothelium from cytokine-induced cell death**

CD138 – A potential marker for therapeutic response?

The Role of RhoA in Maintaining the Integrity of ECs

124 kDa Vinculin
22 kDa RhoA

CTRL   S   SF2   SF6   F2   F6

FX06 restores continuous VE-cadherin/CD144 distribution on EC

Static — S 24h — S 22h -> S+FX06 2h
Shear Stress — S 24h — S 22h -> S+FX06 2h

DAPI; CD144
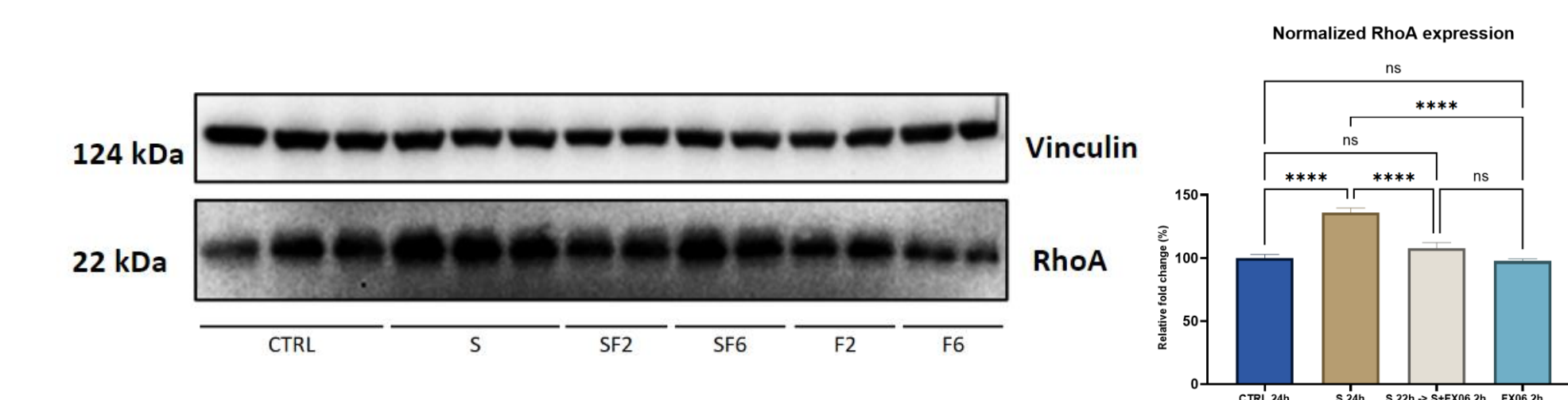
Protective Effect of FX06 on Reducing Cytotoxicity of CD8+ T Cells

Changes of Cell Morphology
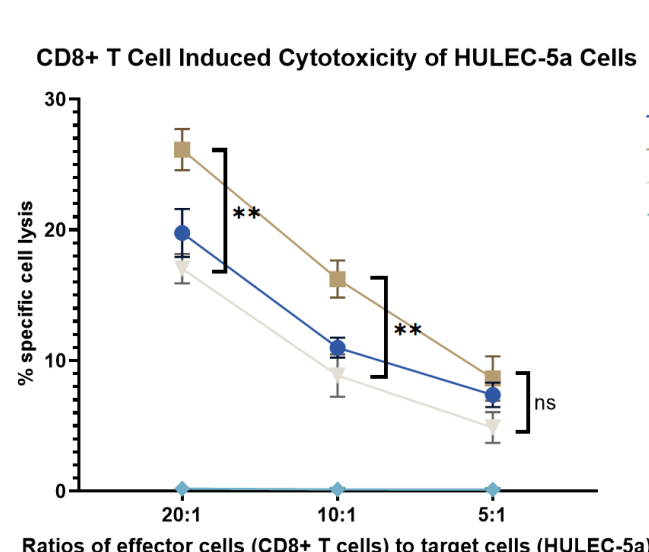
FX06 alters protein expression to protect the endothelium
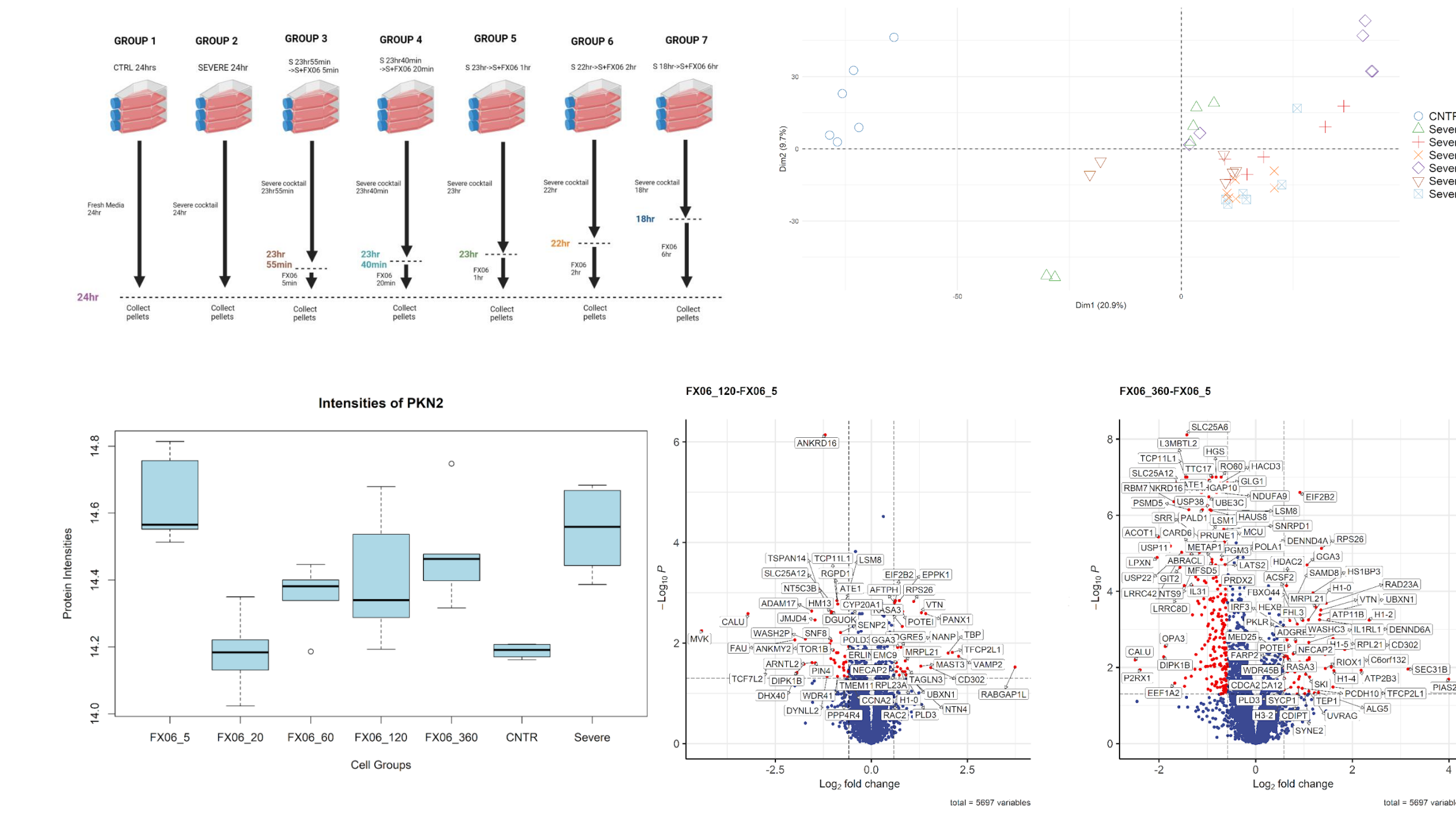
FUTURE PLANS

- Comprehensive flow cytometric analysis of pulmonary EC phenotype;
- Validation regarding the role of PKN2 in Collagen-based TEM assays under static and shear stress conditions;
- Investigation of the interaction of F-action and VE-Cadherin;
- Identification of signal transduction pathways in EC responsible for the protective effects of FX06 on TEM of PBMCs.

COVend

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