



# TissueSpec® ECM Hydrogels for predictive 3D models of human cancer

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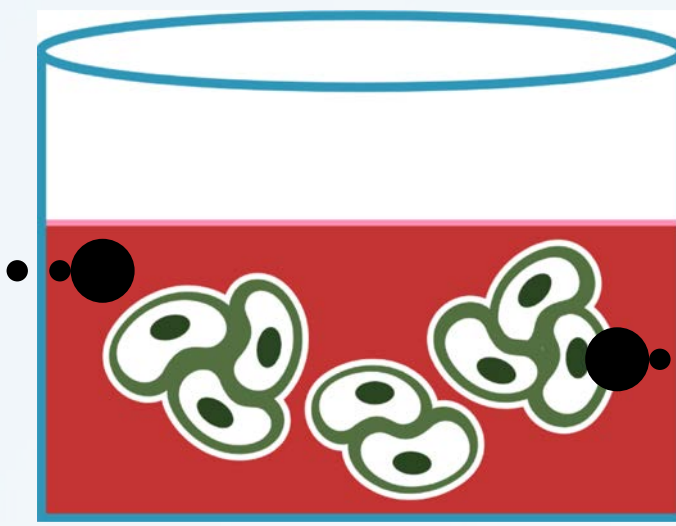


## BIOMIMETIC LUNG CANCER MODEL

*In-vitro* cancer models lack the **tissue-specific ECM** molecules & mechanics of the tumor environment, and thus fail to be predictive.

### LUNG TISSUE-DERIVED ECM HYDROGEL

TissueSpec® Lung Hydrogel



### PATIENT-DERIVED LUNG CANCER CELLS

Jacket Lung Adenocarcinoma Cells

> TissueSpec® Lung ECM Hydrogel provides the physiologic 3D lung microenvironment for patient-derived Jacket lung adenocarcinoma cells, resulting in more predictive models of human lung cancer.

> **Results:** Lung adenocarcinoma cells cultured in 3D TissueSpec® Lung ECM show significantly different malignancies, drug responses, and IC<sub>50</sub> values compared to cells cultured in Matrigel or without ECM.

## FEATURES

### > TissueSpec® Lung ECM Hydrogel

Proteomics show a unique, lung-specific signature with a consistent profile across lots.



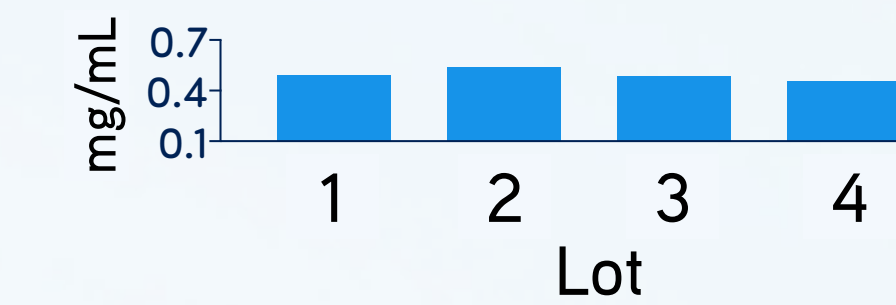
#### Mass spec profile

ECM components	Biomolecules
collagens	type I–VI, VIII, IX, XI, XVI
laminins	subunit α5, β2, γ1
glycoproteins	fibrillin 1, fibulin 5, nidogen
proteoglycans	heparan sulfate, aggrecan, hyaluronan
elastin	

#### Key components (µg/mL)

collagens	400 – 530
elastin	40 – 50
GAGs	3 – 5

#### Collagen



### > Jacket Lung Adenocarcinoma Cells (Cellaria)

Demonstrate consistent growth, form tumorspheres under low-attachment conditions

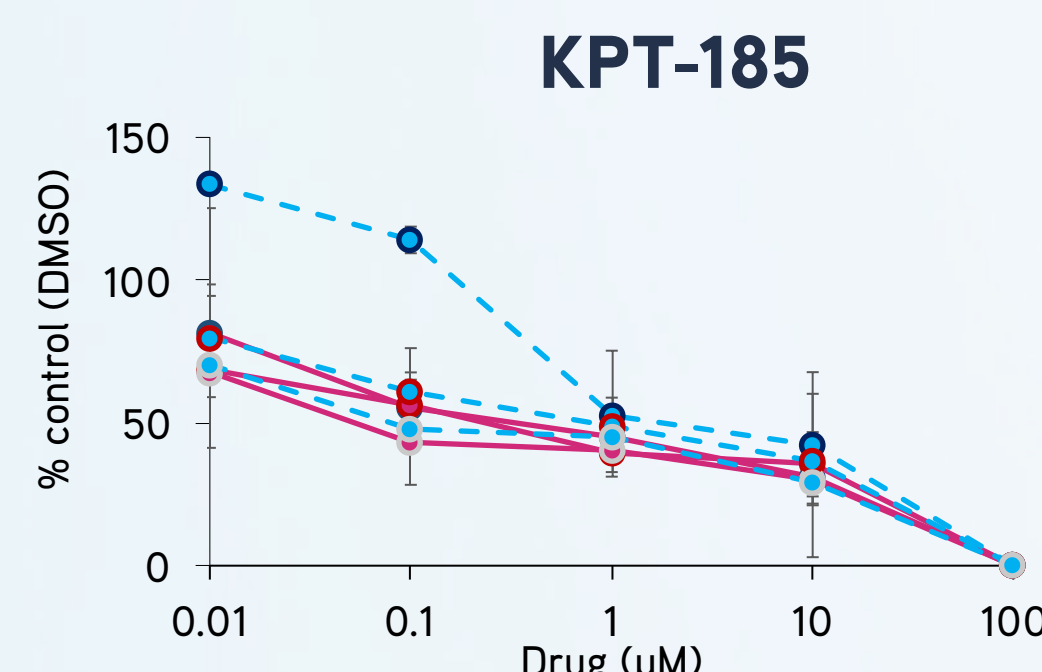
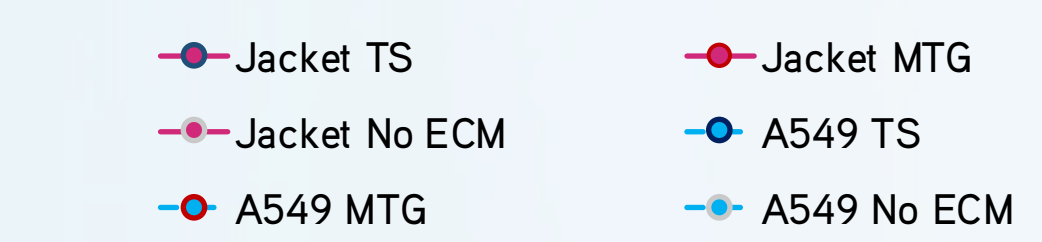
#### Epithelial phenotype



#### Gene mutations

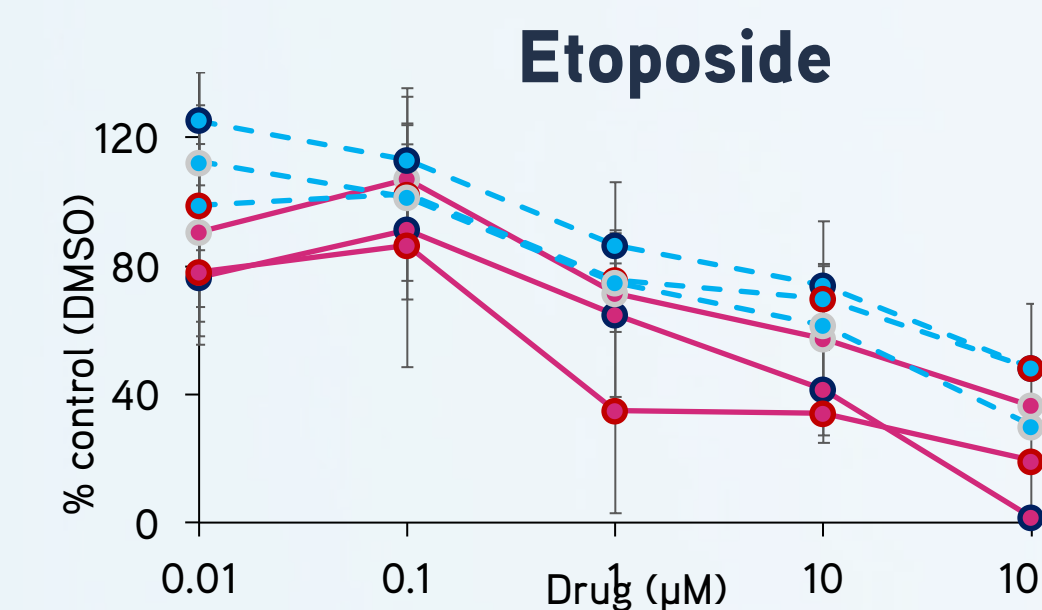
Gene	Alteration	Freq. (%)
Androgen rec.	F814V	100
KRAS	G12A	100
MSH6	G477S	32
MUTYH	V493M	62
TP53	S215G	100

## DRUG RESPONSES

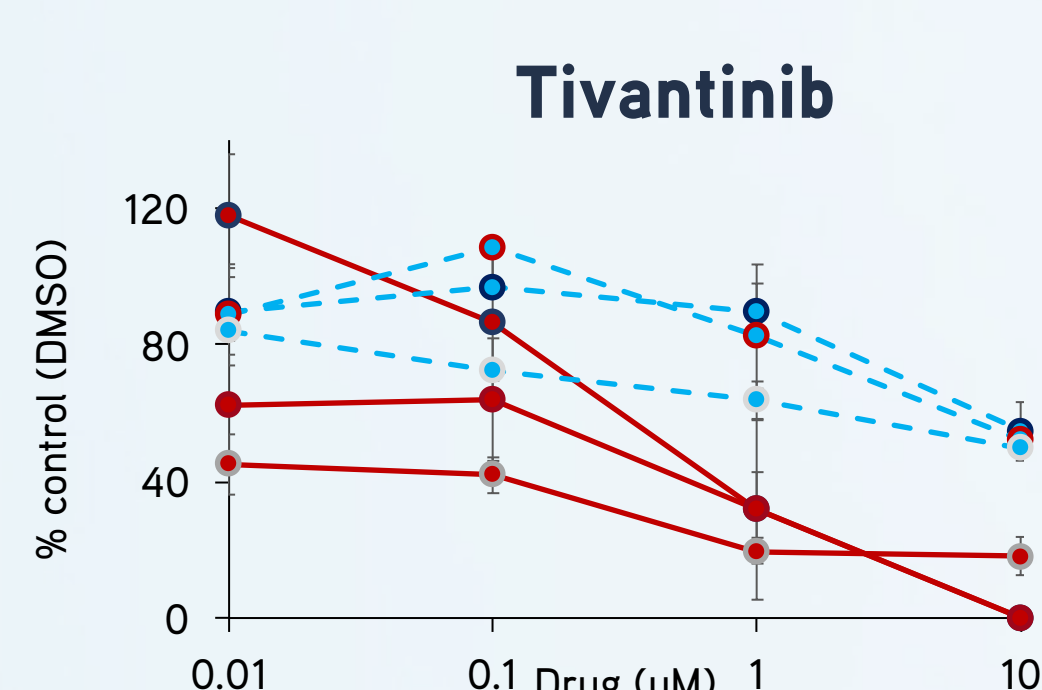


> **Methods:** 5000 cells were cultured in TissueSpec® Lung Hydrogel, Matrigel, or tissue culture plastic (no ECM).

After 24 hours, drugs reconstituted in DMSO were added. Cells that received DMSO only were cultured as controls. Cells were cultured for 72 hours, then MTT reagent was added and incubated for 4 hours.



Cell number was normalized to average cell number of DMSO control for various doses. IC<sub>50</sub> calculations (non-linear fit, GraphPad).

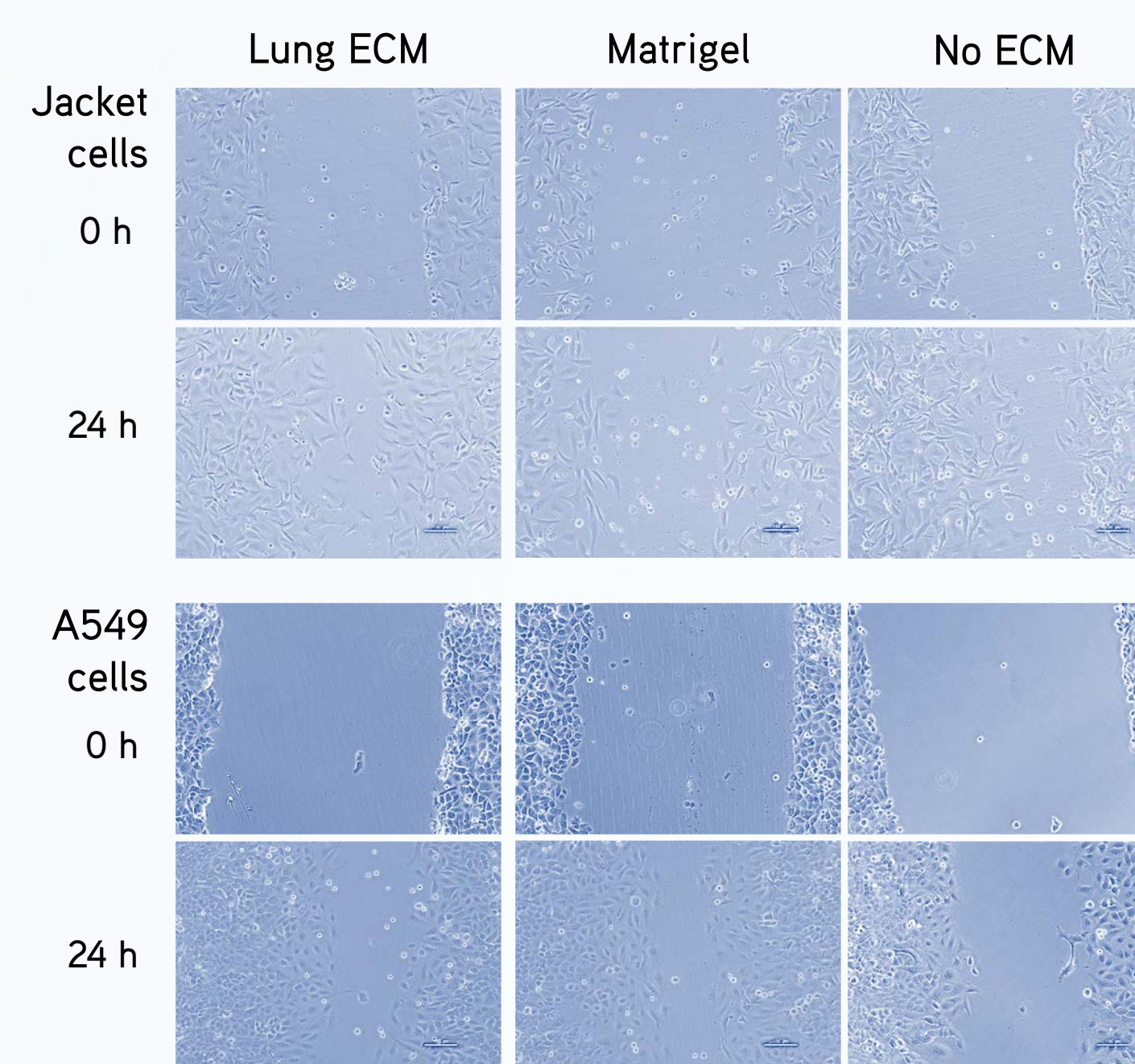


> **Results:** Cells cultured in TissueSpec® Lung Hydrogel exhibited distinct drug resistance profiles which may indicate a more predictive physiological response. Jacket cells exhibited lower IC<sub>50</sub> values compared to A549 cells.

Cell type	Substrate	IC <sub>50</sub> (µM)		
		KPT-185	Etoposide	Tivantinib
Jacket	TissueSpec® Lung Hydrogel	0.4	3.8	0.52
	Matrigel	0.2	0.7	0.20
	Plastic (no ECM)	0.08	17.9	0.01
A549	TissueSpec® Lung Hydrogel	2.8	70.3	11.7
	Matrigel	0.8	61.1	9.8
	Plastic (no ECM)	0.1	19.0	5.5

## MIGRATION & INVASION

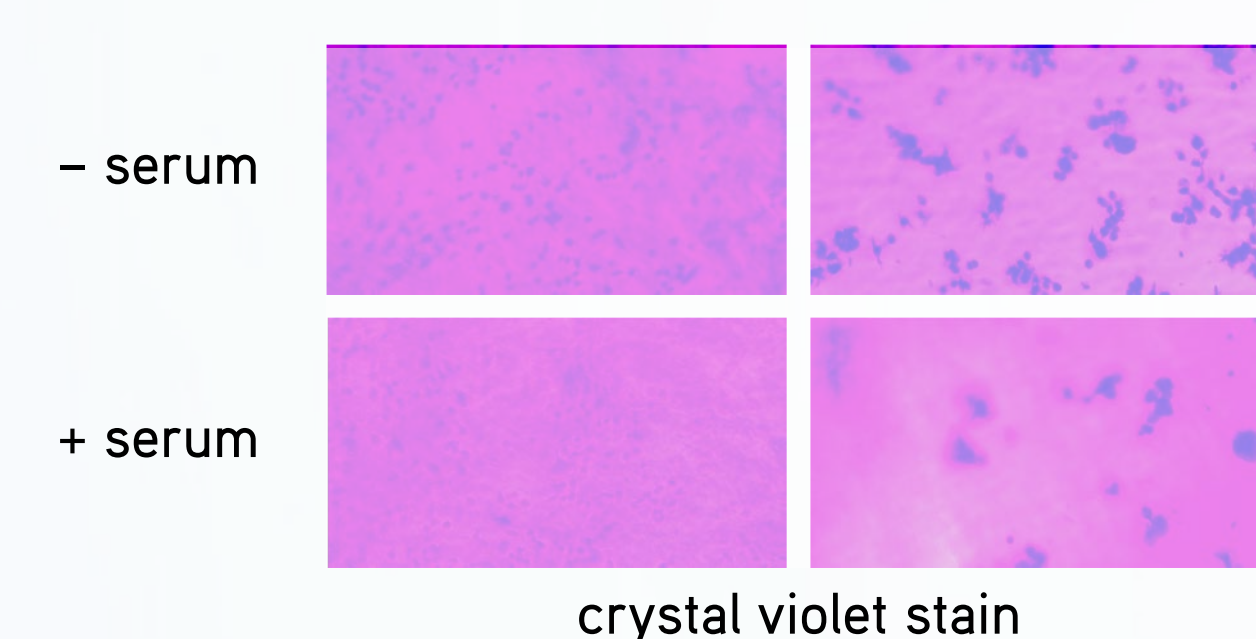
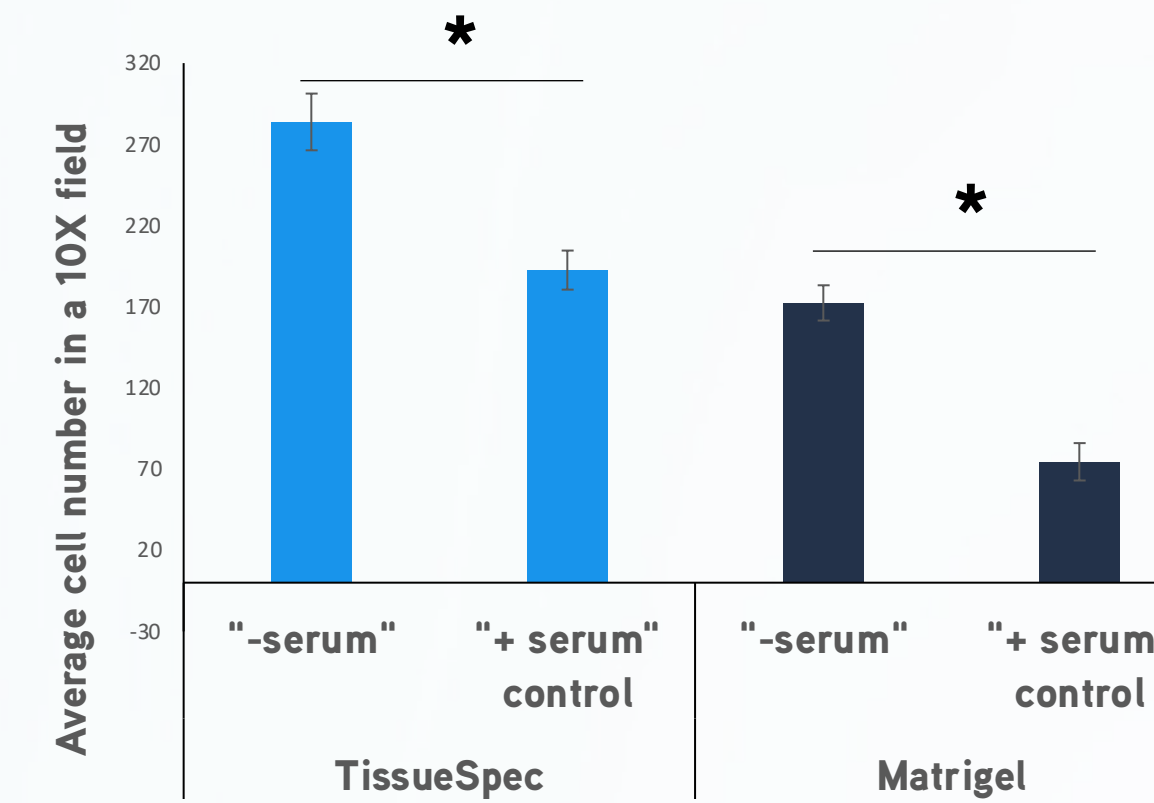
### Migration assay



> **Methods:** Cells were cultured on a surface coated with NativeCoat™ Lung ECM, Matrigel, or uncoated. Initially, a scratch wound was made using a 200 µL micropipette tip. After 24 hours, cells migrated into scratched areas.

> **Results:** Jacket and A549 cells display migration when cultured on surfaces coated with NativeCoat™ Lung ECM, a physiologic lung ECM coating suitable for assessing cancer cell migration.

### Invasion assay



> **Methods:** Cells were cultured on transwell inserts coated with TissueSpec® Lung ECM or Matrigel. Media with 10% serum was added to the lower compartment, and media without serum (or +serum as control) was added to the upper compartment.

After 24 hours, cells were scratched/ removed from hydrogel surfaces, inserts were stained with crystal violet, and 5 random 10x fields were quantified.

> **Results:** A549 cells cultured on TissueSpec® Lung Hydrogel exhibit greater motility & invasiveness than cells cultured on Matrigel. \*p<0.05.

## BENEFITS



### Physiologically relevant

TissueSpec® Lung ECM Hydrogel contains the full milieu of proteins & growth factors present in the native lung



### More accurate, predictive results

TissueSpec® ECM Hydrogel provides ideal conditions for maintaining cell phenotype, leading to more accurate results compared to other substrates



### Standardized experiments

TissueSpec® ECM Hydrogel demonstrates consistent composition profiles across different lots, resulting in reproducible studies

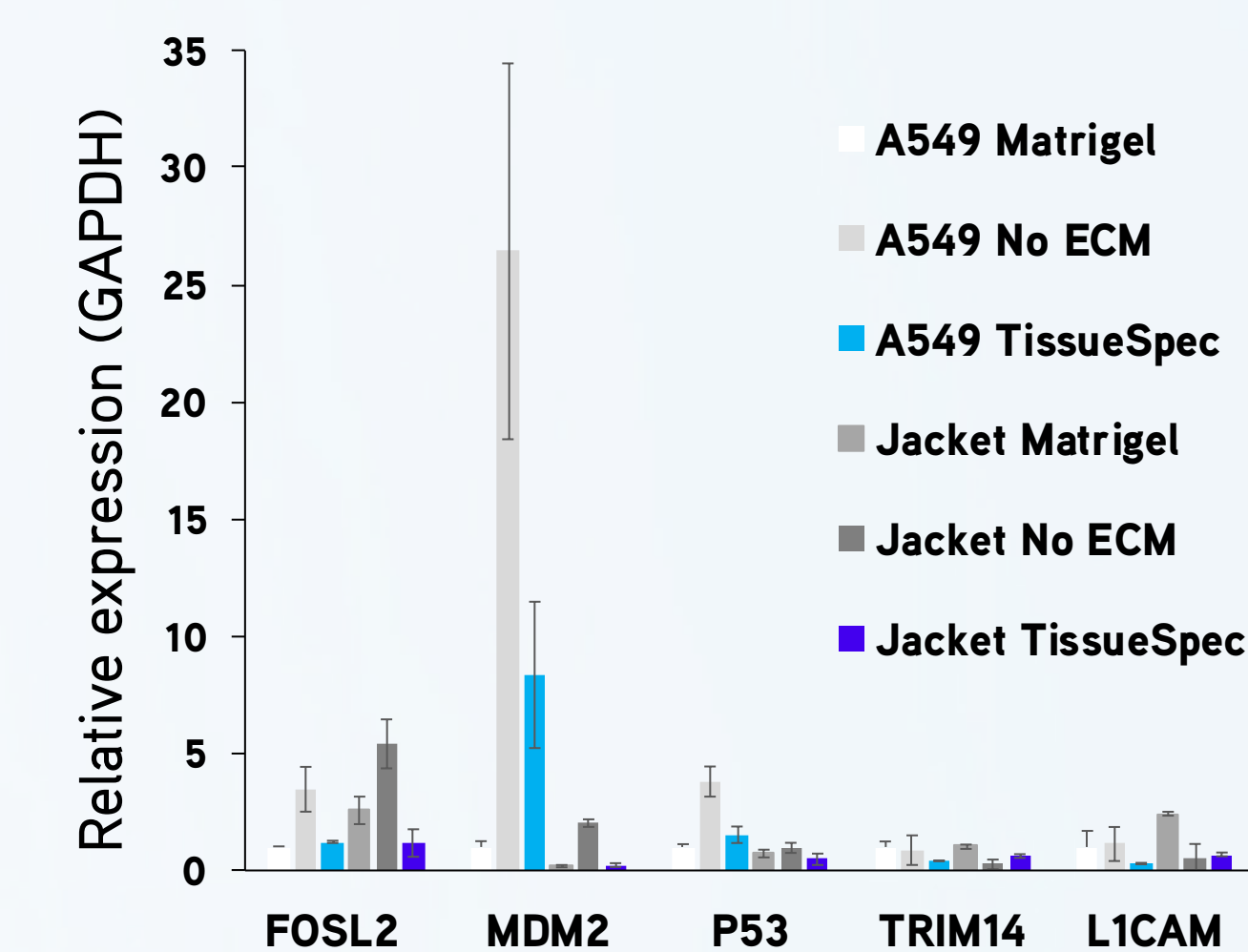


### Clinically translatable

TissueSpec® Lung ECM Hydrogel facilitates downstream clinical translation because they contain tissue-specific ECM from medical grade swine tissues.

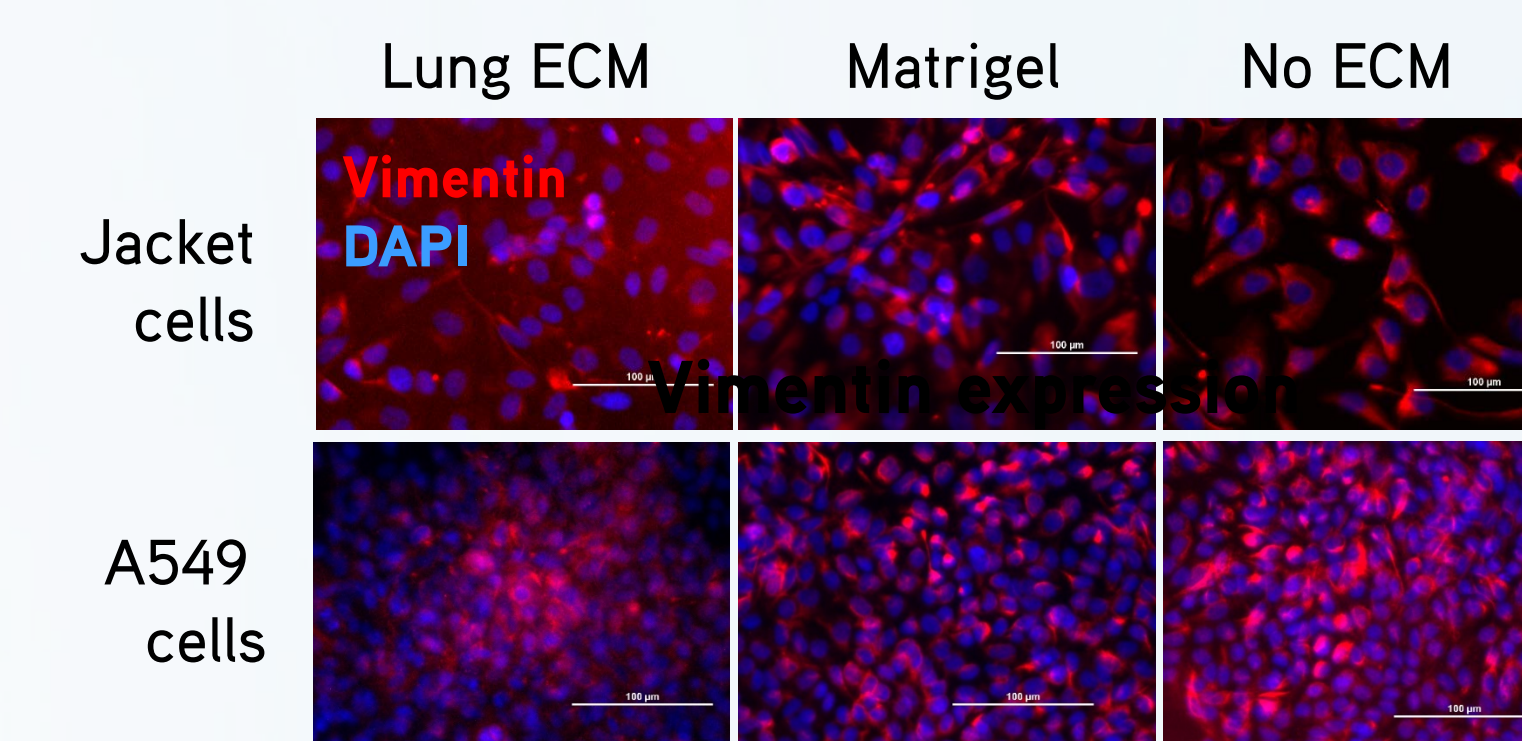
## LUNG CANCER-RELATED GENES

### Cancer-related gene expression



> **Methods:** Cells were cultured in 3D TissueSpec® Lung ECM, Matrigel, or on 2D tissue culture plastic (no ECM) for 7 days. Gene expression was normalized to GAPDH using the 2<sup>-ΔΔCt</sup> relative to A549 cells cultured in Matrigel.

> **Results:** Cells that were cultured in TissueSpec® Lung ECM Hydrogel exhibited lower cancer-related gene expression than cells cultured in Matrigel or on tissue culture plastic with no ECM.



> **Results:** A549 and Jacket cells cultured in TissueSpec® Lung ECM Hydrogel exhibited lower Vimentin expression level and abundance than cells cultured in Matrigel or on tissue culture plastic with no ECM.

## SUMMARY

We demonstrate that TissueSpec® ECM Hydrogels are physiologic and suitable for 3D models of human cancer.



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