EMT Reporter Models for Cancer Research: A Window into Invasion and Metastasis

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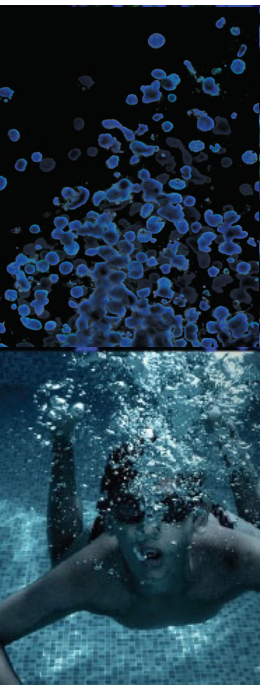
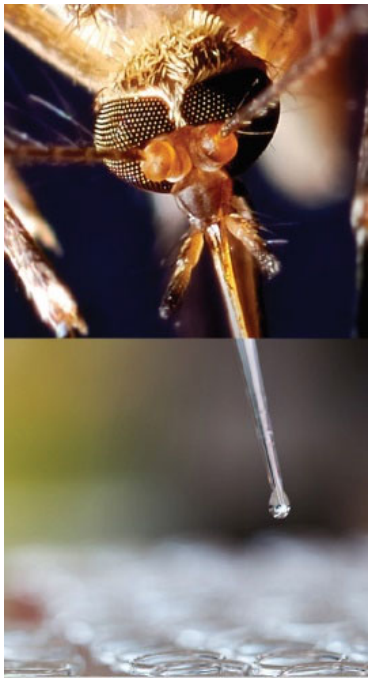
Credible Leads to Incredible™





About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viruses, and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

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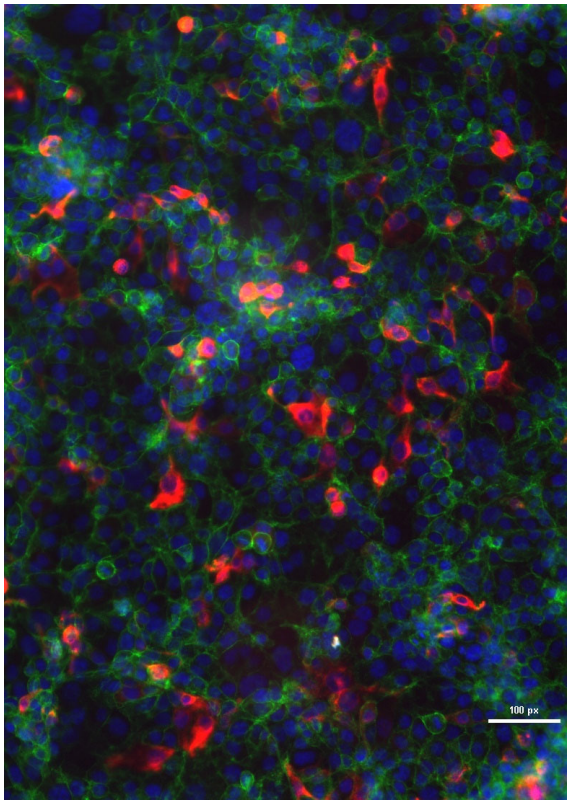
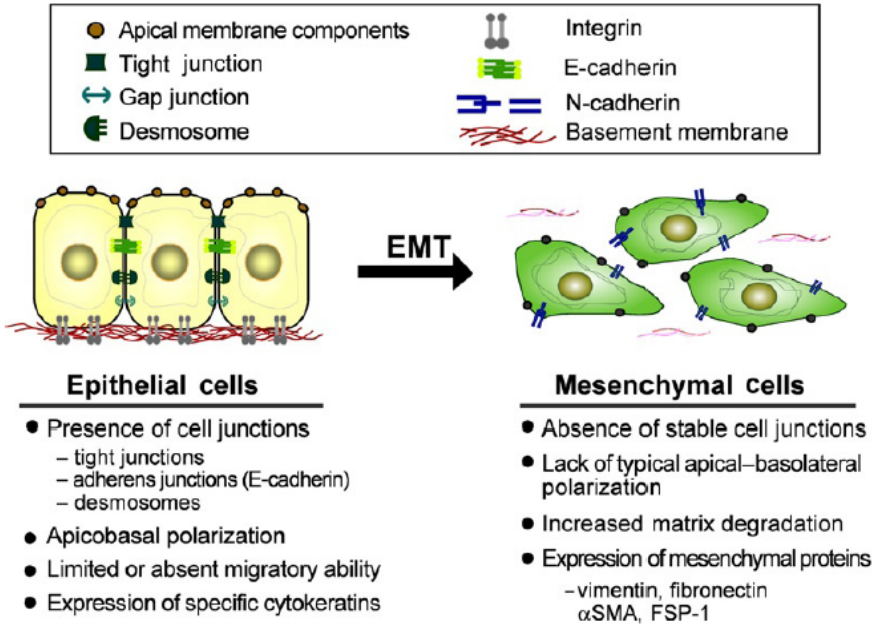
Agenda

- Background
  - EMT
  - EMT and cancer metastasis
- MCF10A ECAD EmGFP EMT
  - Gene editing strategy and confirmation
  - Morphology and growth for parental vs gene edited cells
  - Function data
- Summary



Background – EMT

- The epithelial-to-mesenchymal transition (**EMT**) is a reversible process. Epithelial cells:
  - Reduce their intercellular adhesions and proliferative capacity
  - Gain a mesenchymal phenotype with increased migratory and invasive properties
- EMT classifications and functions:
  - Implantation, embryogenesis, and organogenesis
  - Wound healing, tissue regeneration, and organ fibrosis
  - Tumor metastasis



Lee et al, International Review of Cell and Molecular Biology, 2012.

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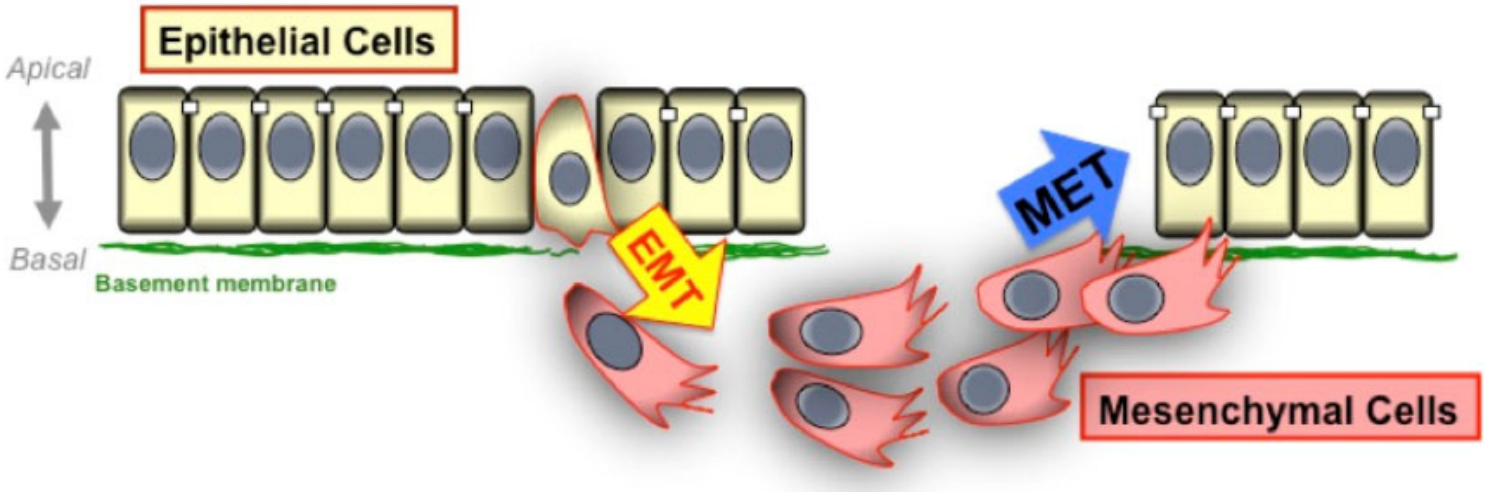
EMT and MET in cancer progression



http://murraylab.biosciences.uom.org.au/

- The EMT process facilitates metastatic dissemination
- ATCC EMT models: **MCF10A, BT-474, A549, HCT116**
- ATCC MET models: **PANC-1 & MDA-MB231**

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ATCC's EMT and MET reporter cell lines

- We have developed EMT and MET reporter cell lines for use as a platform in drug screening and to learn more about the EMT/MET pathway and how it relates to cancer progression
- In these cell lines, commonly used EMT marker genes (VIM or ECAD) are tagged with a fluorescent protein to allow real-time tracking of cellular status

Designation	ATCC® No.	Tissue type/disease	EMT or MET	Marker	Availability
MCF10A ECAD EmGFP	CRL-10317EMT™	Breast epithelial cells	EMT	ECAD-GFP	Available
PANC-1 ECAD EmGFP	CRL-1649MET™	Pancreatic cancer	MET	ECAD-GFP	Available
BT-474 ECAD EmGFP	HTB-20EMT™	Breast cancer	EMT	ECAD-GFP	Available
A549 VIM RFP	CCL-185EMT™	Lung cancer	EMT	VIM-RFP	Available
HCT116 VIM RFP	CCL-247EMT™	Colorectal cancer	EMT	VIM-RFP	Available
MDA-MB-231 VIM RFP	HTB-26MET™	Breast cancer	MET	VIM-RFP	Available

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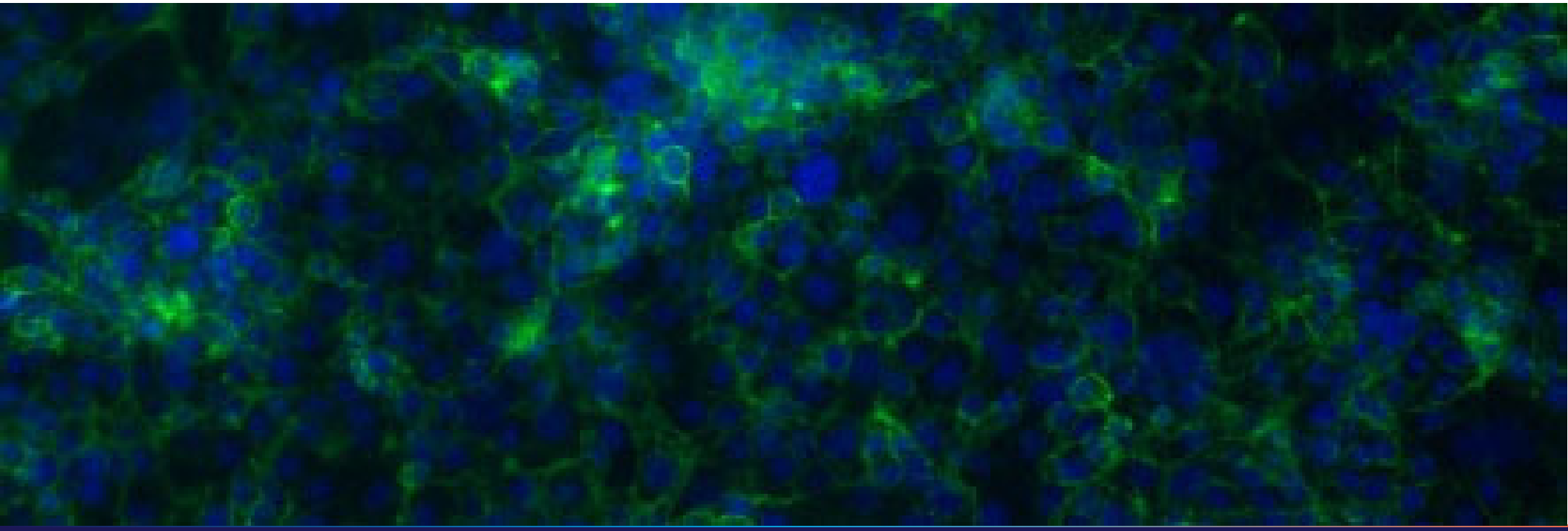

E-cadherin-EmGFP reporter lines

MCF10A ECAD EmGFP: breast epithelial cells

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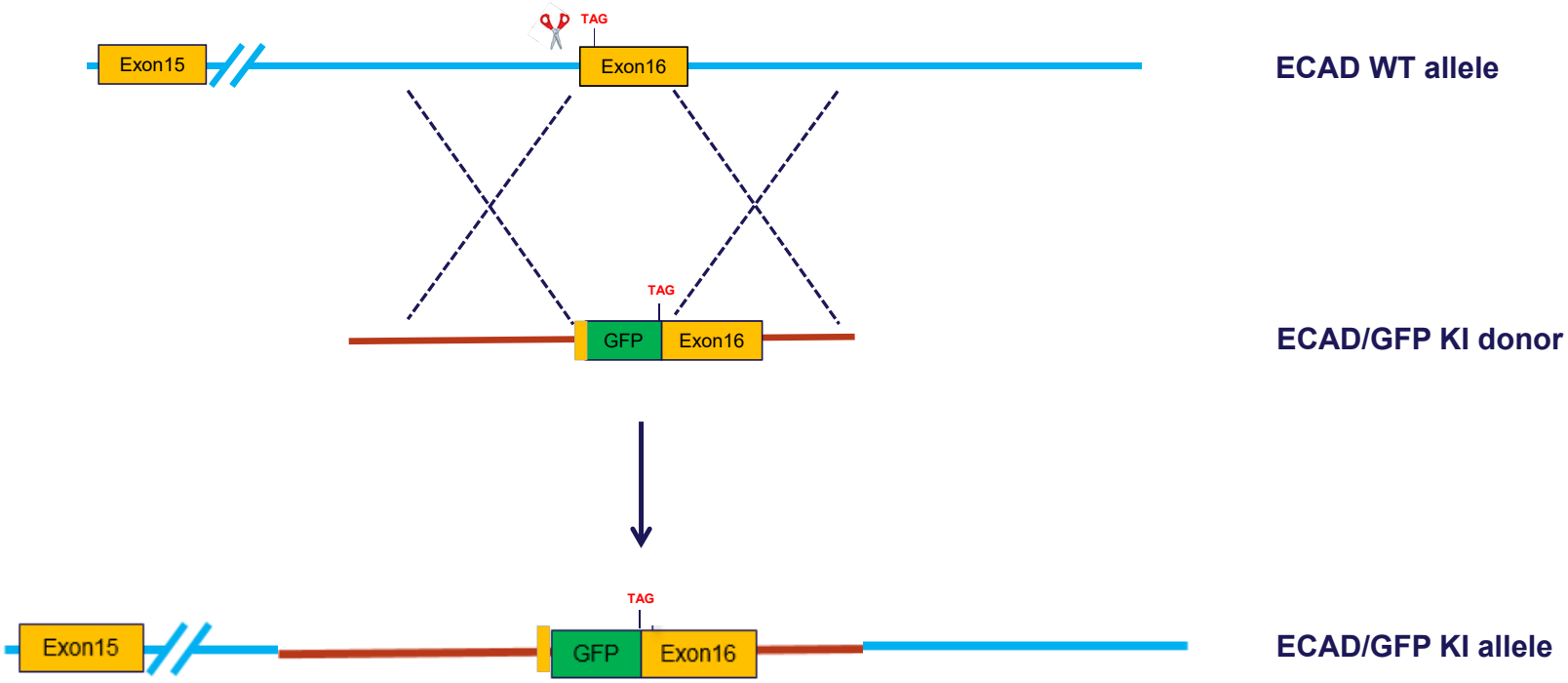
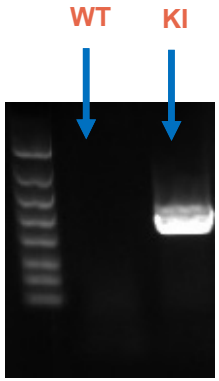
Generation of E-cadherin-EmGFP Knock-In Allele



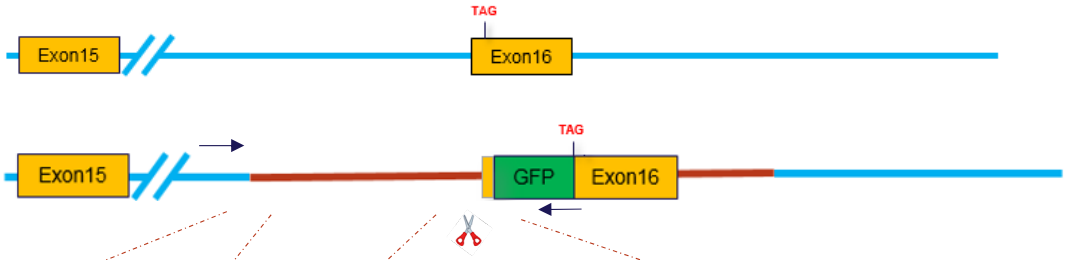
= CRISPR/Cas9

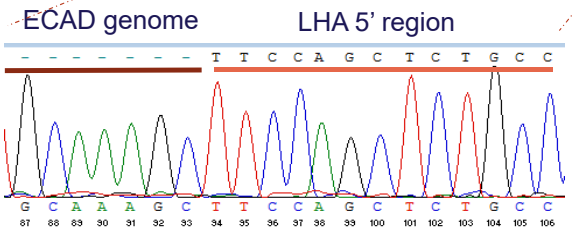
GFP= EmGFP (Emerald Green Fluorescent Protein)

Knock-in verification at the genomic level

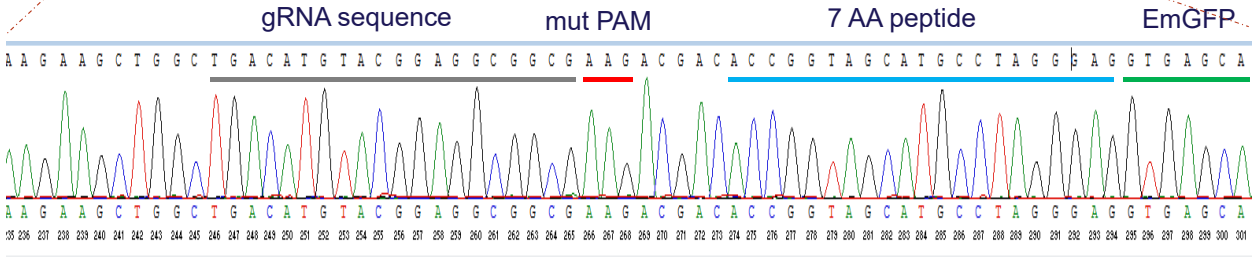


LHA~ 0.8 kb



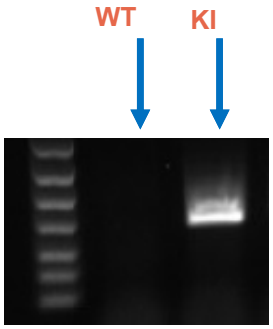


LHA junction

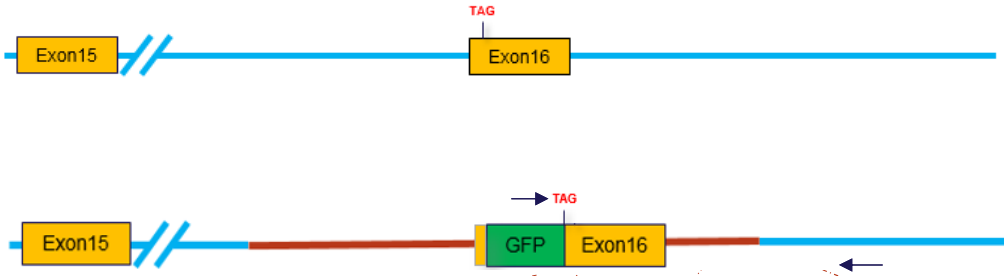


LHA/EmGFP junction

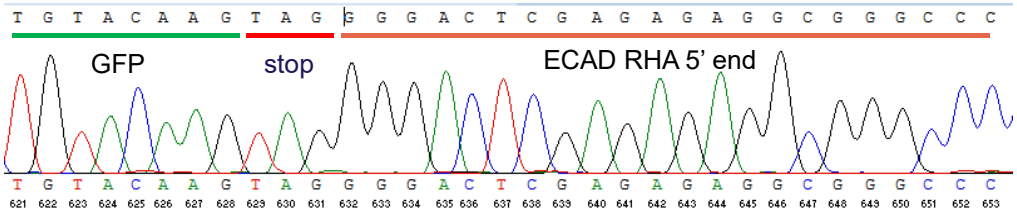
Knock-in verification at the genomic level



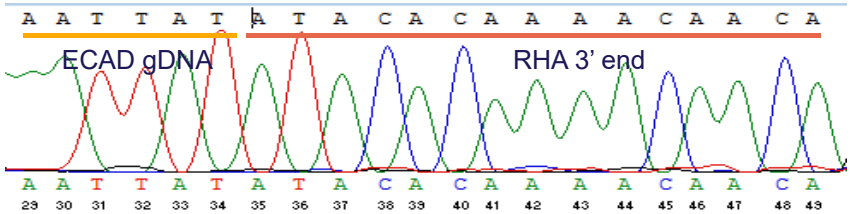
RHA~ 0.6 kb



ECAD WT allele



EmGFP/RHA junction



RHA junction region

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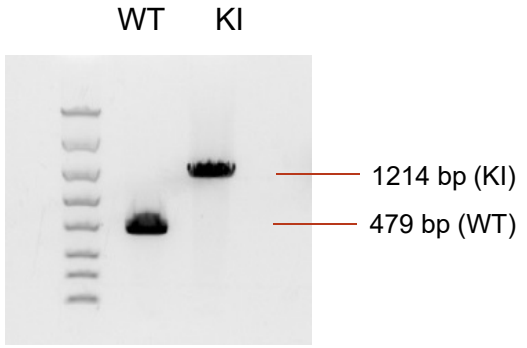
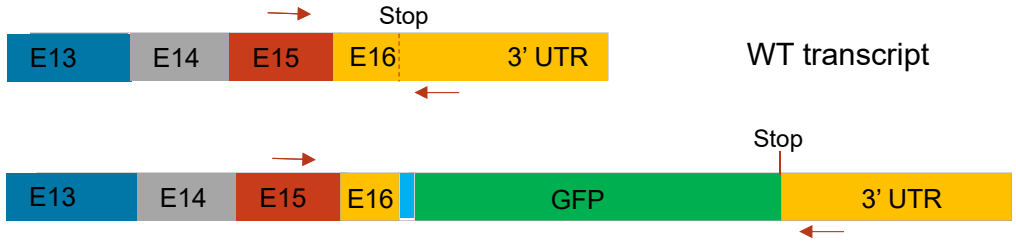
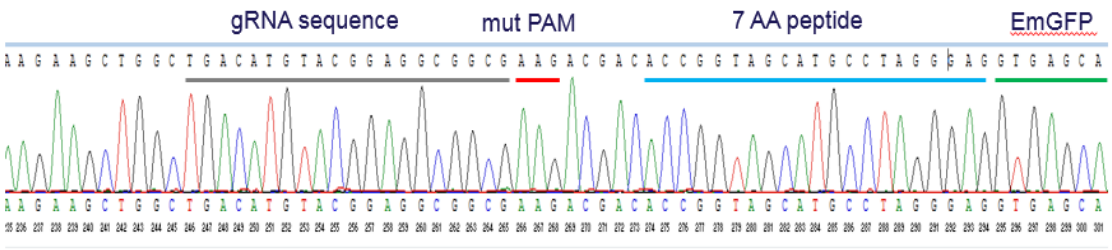
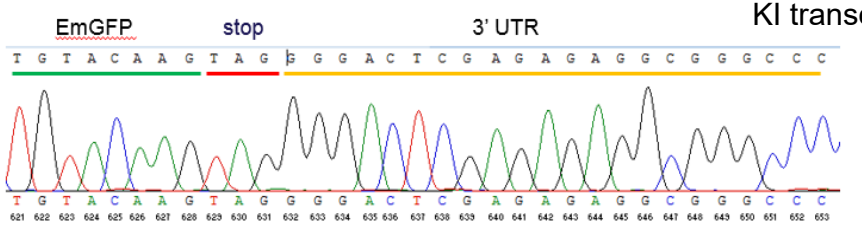
Knock-in verification at the transcriptional and translational levels







KI transcript

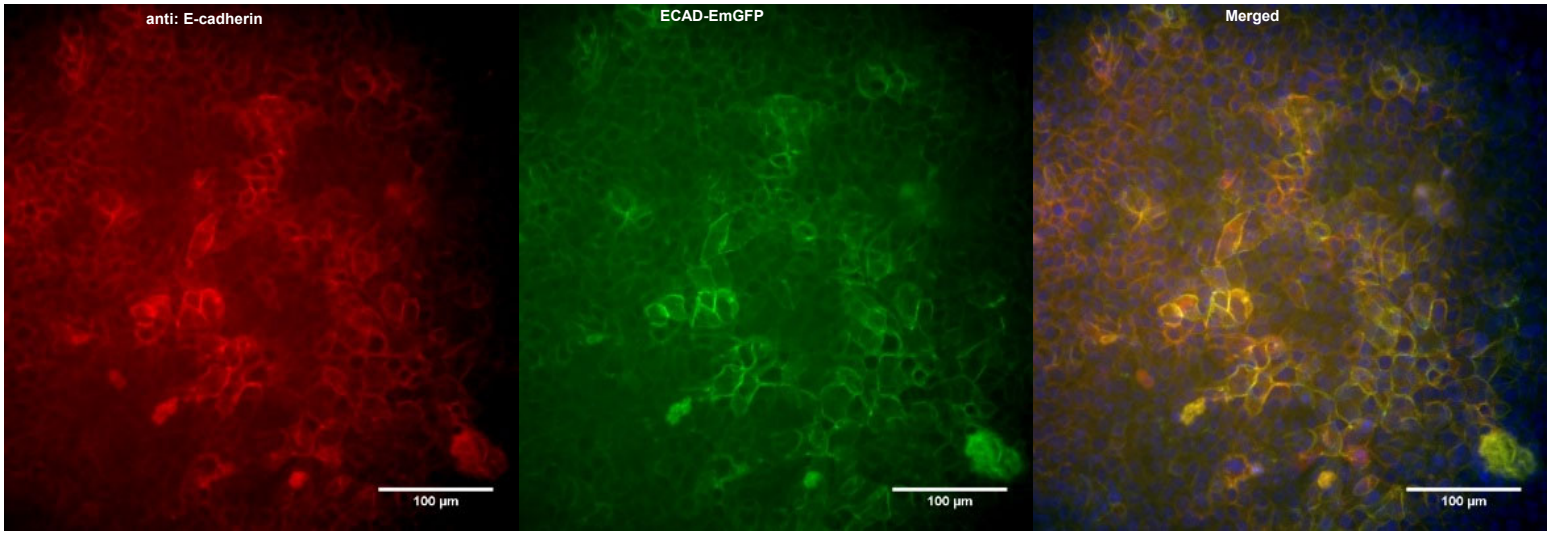


Knock-in verification at the transcriptional and translational levels

Anti-Ecadherin

Endogenous ECAD-EmGFP

Co-localization of ECAD-GFP expression



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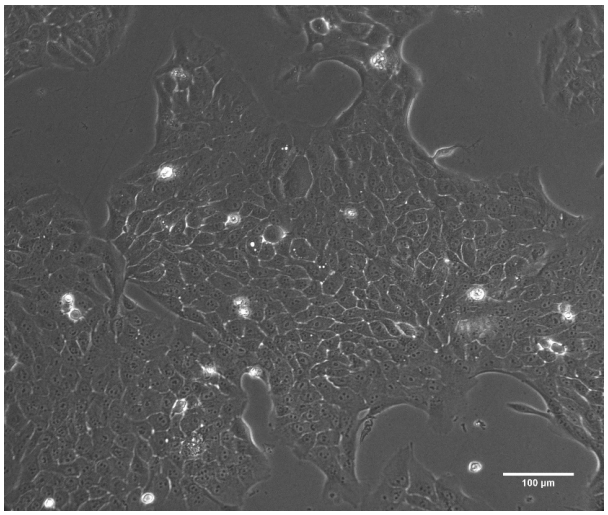
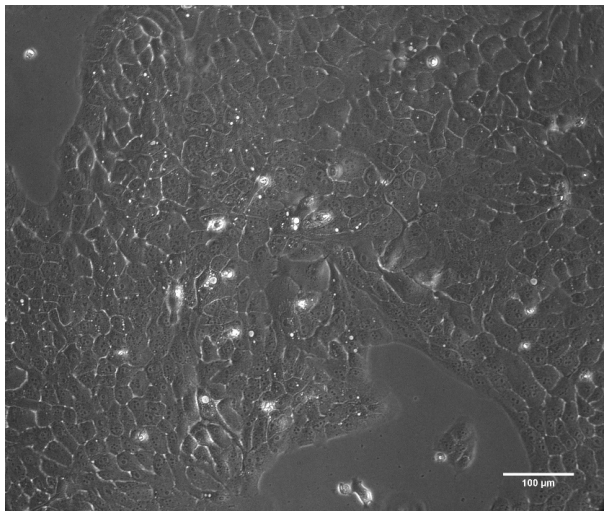
Morphology of parental and gene edited MCF 10A cells

MCF 10A WT

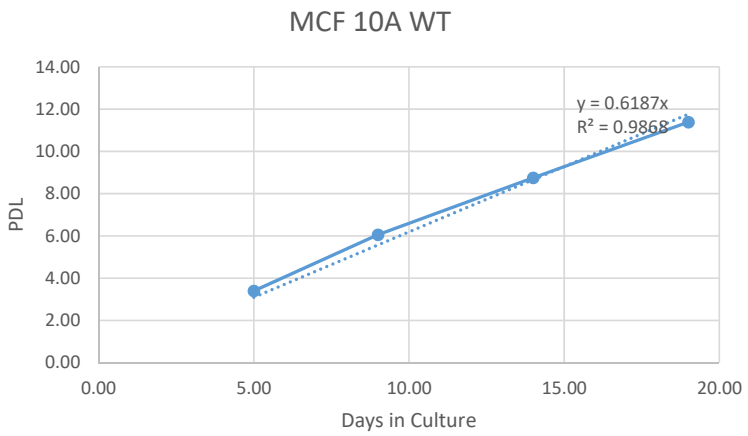


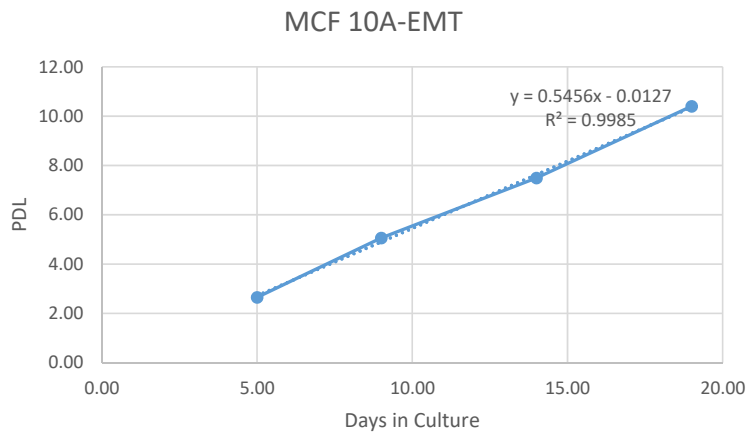
MCF 10A EMT



Growth media: Same as parental

Growth rate of parental and gene edited MCF 10A





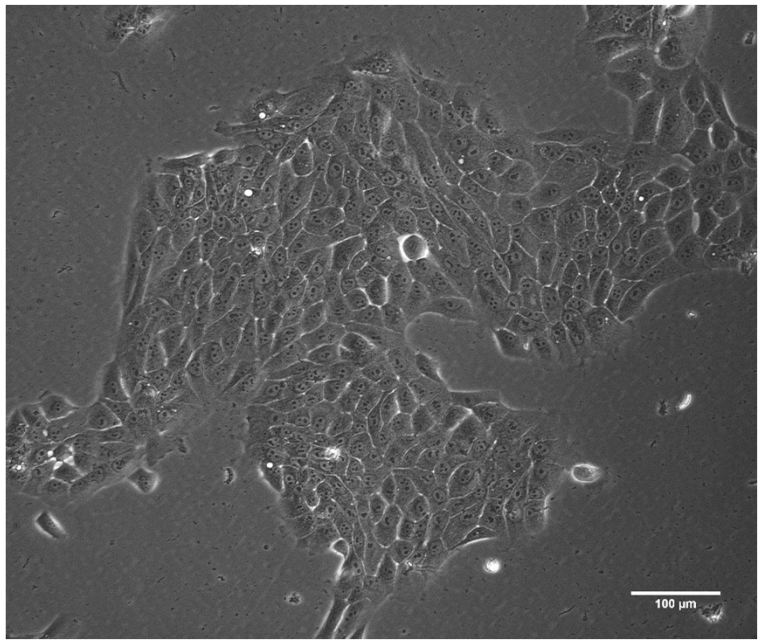
Population doubling time for MCF 10A WT = 42hrs

Population doubling time for MCF 10A-EMT= 45hrs

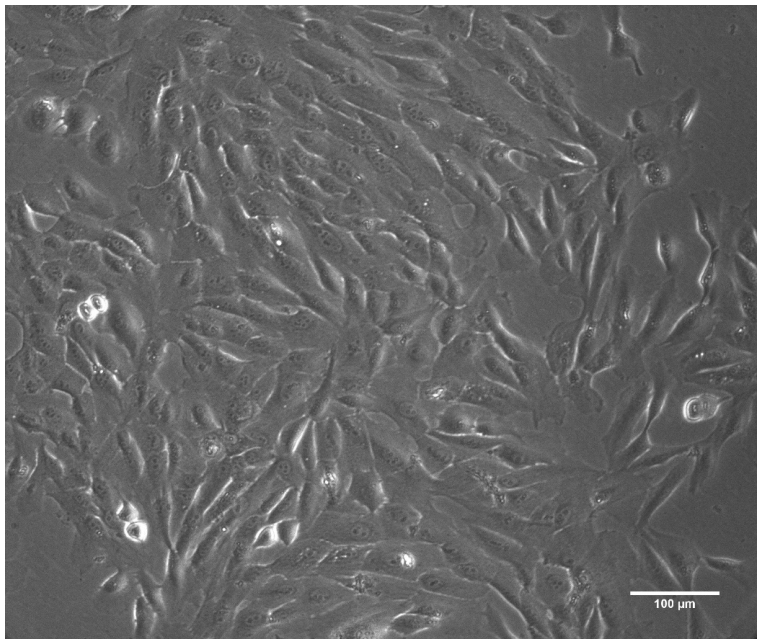
Difference in growth=7%

MCF 10A EMT reporter cells display morphology change upon induction

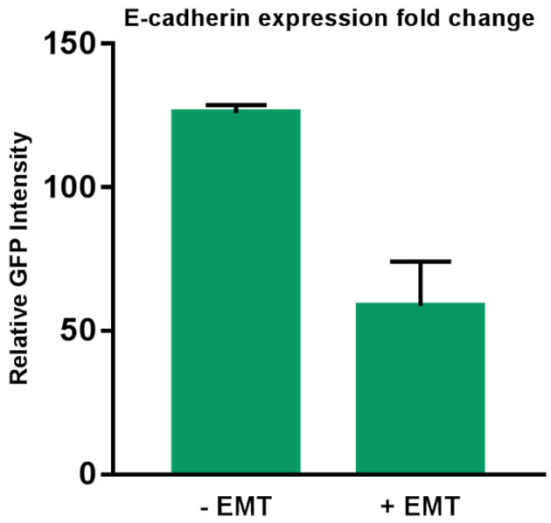
- EMT (control)

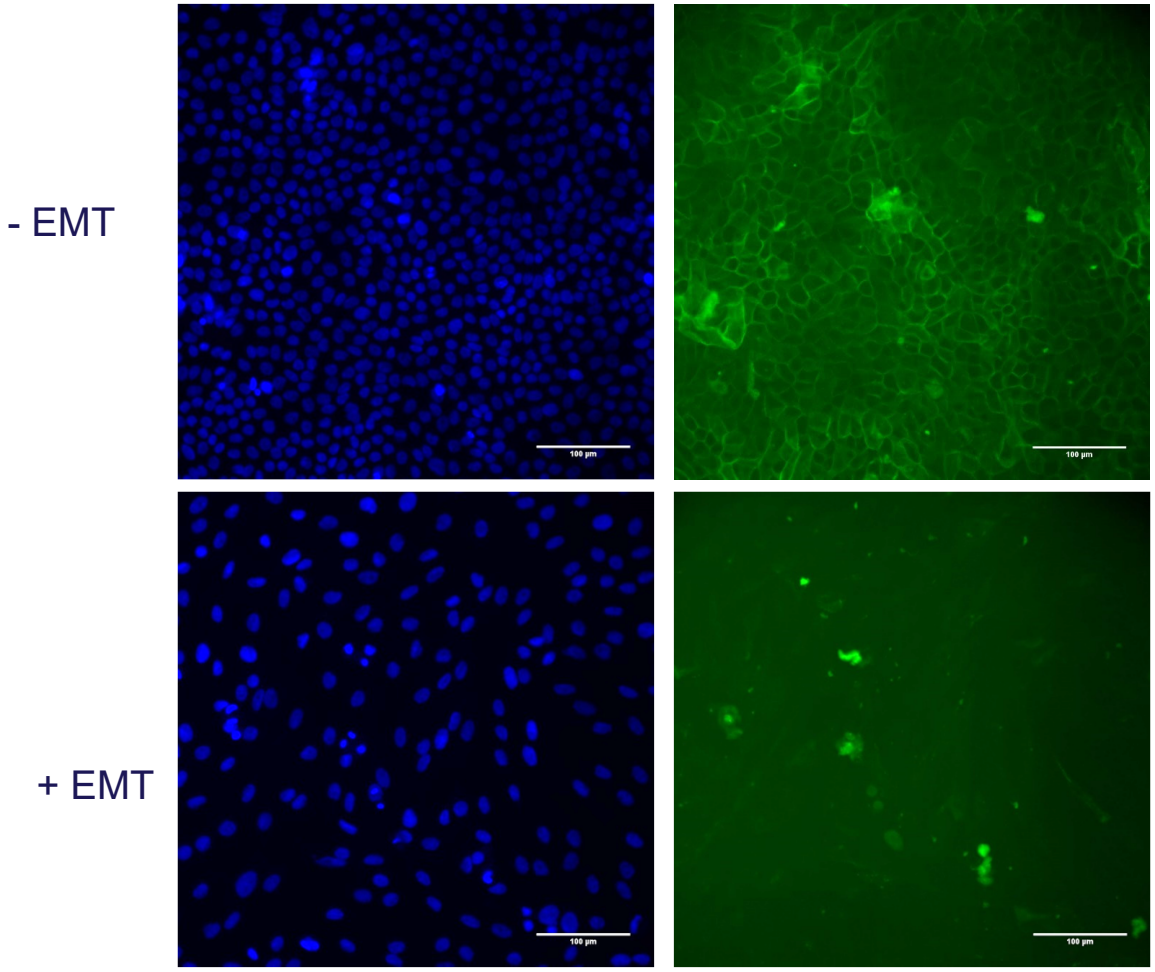


+ EMT

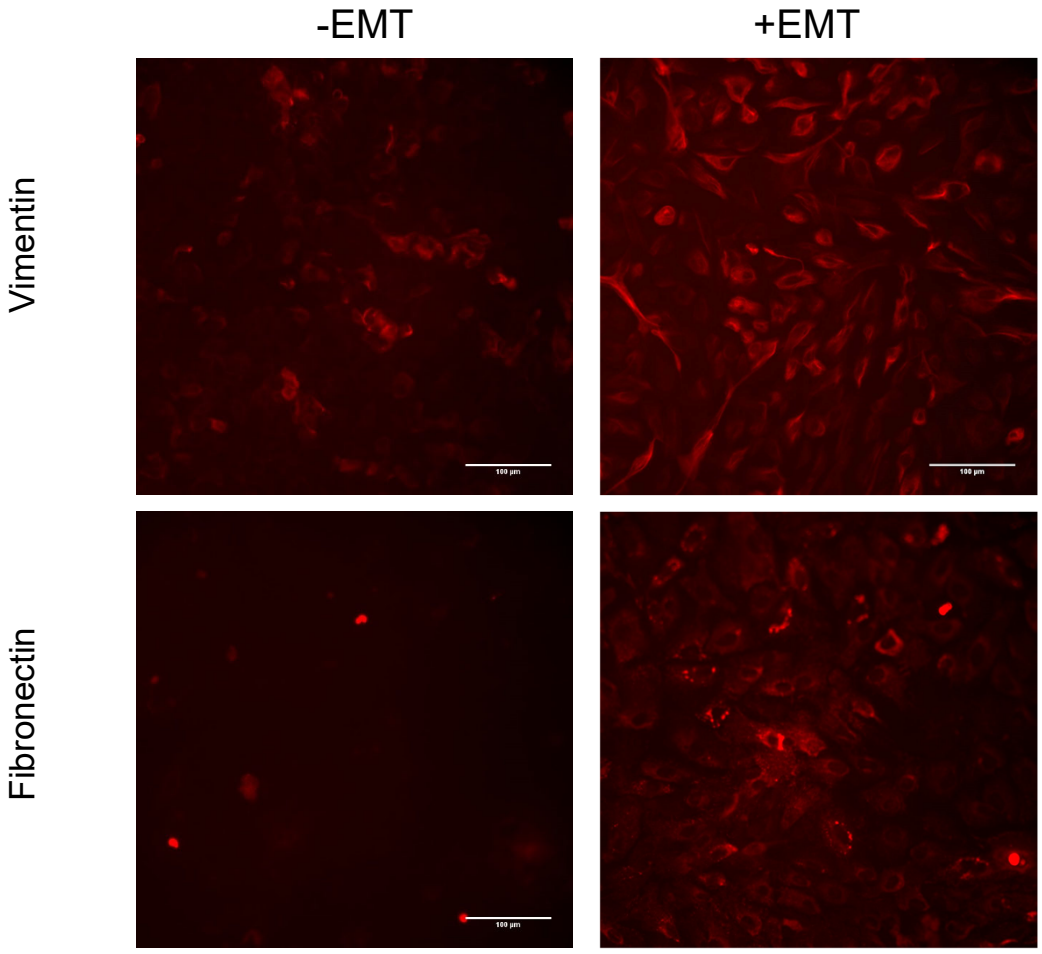


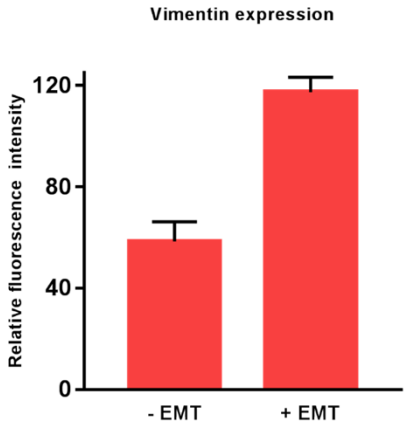
Bio-functional Data Supporting Product:
Decrease in Ecad-EmGFP expression upon EMT (2 fold)

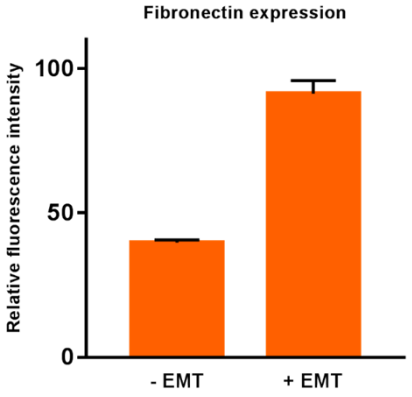




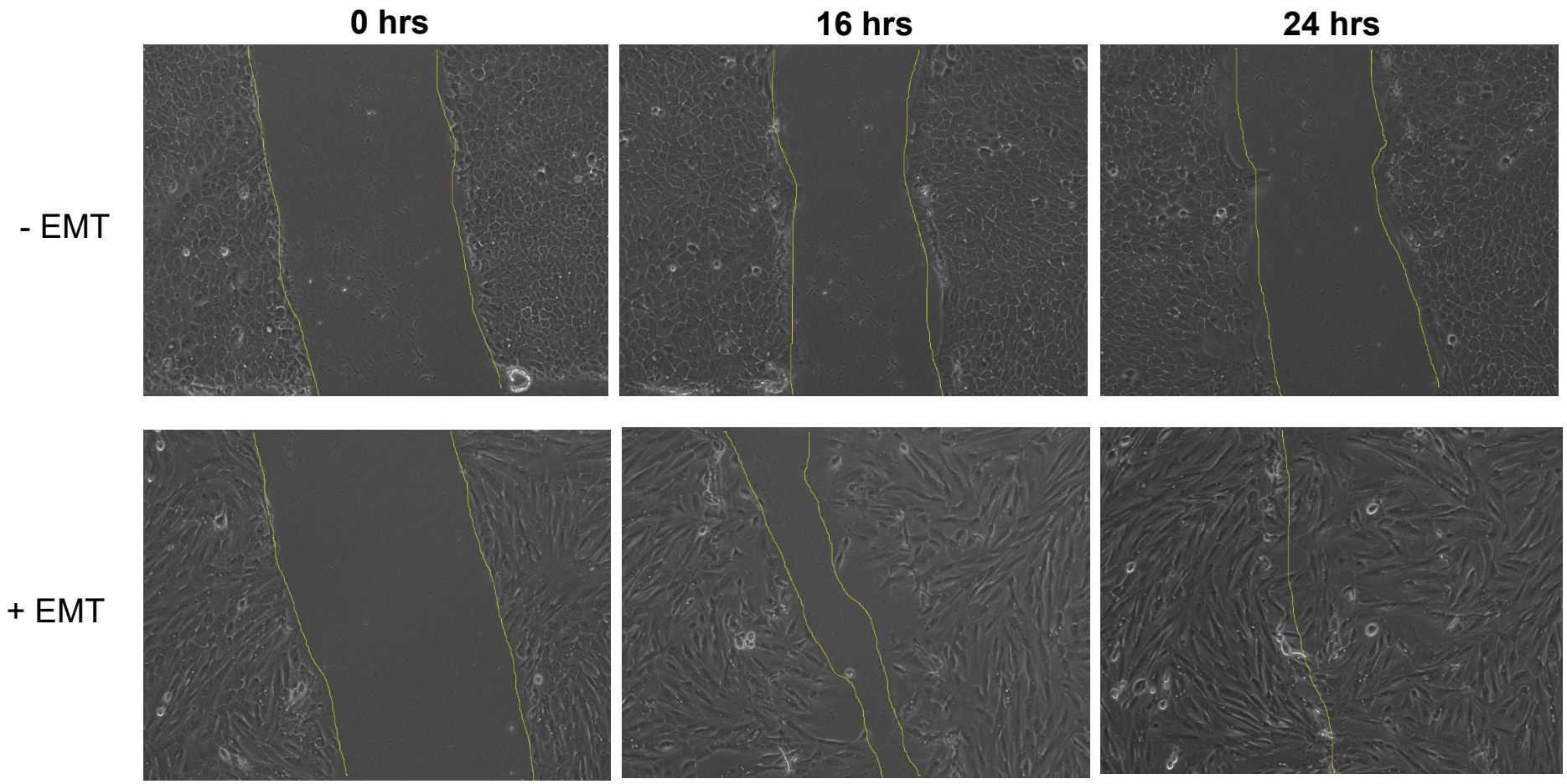
Bio-functional Data Supporting Product:
≥ 50% increase in 2nd EMT marker (Vimentin and Fibronectin) expression upon EMT



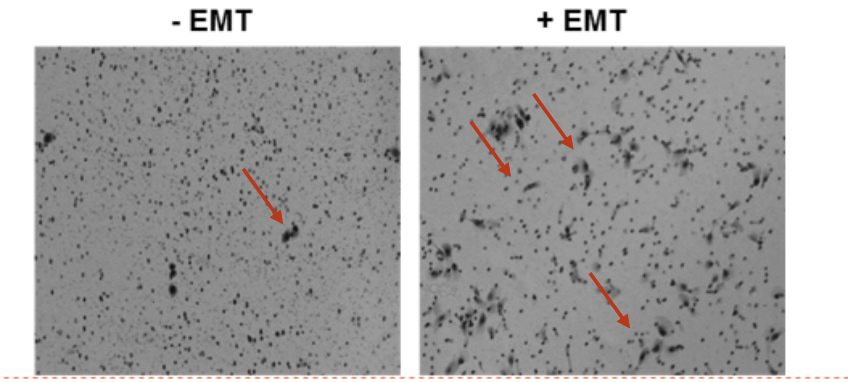


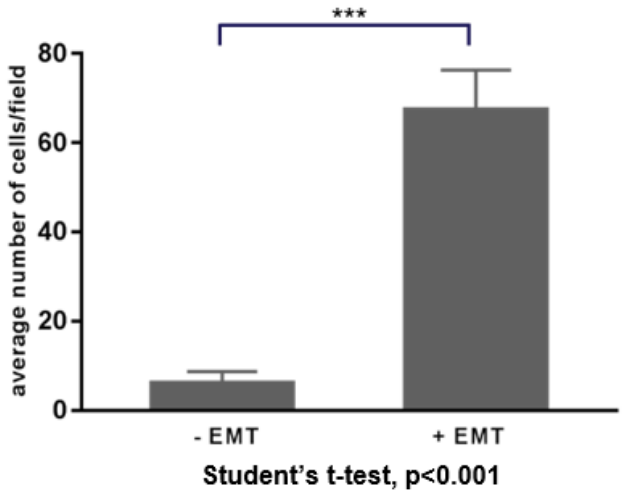


Bio-functional Data Supporting Product:
MCF10A EMT cells show a significant increase in motility after EMT induction



MCF10A ECAD EmGFP EMT cells show a significant increase in a migration assay





Approximately 10 fold increase in migration upon EMT induction

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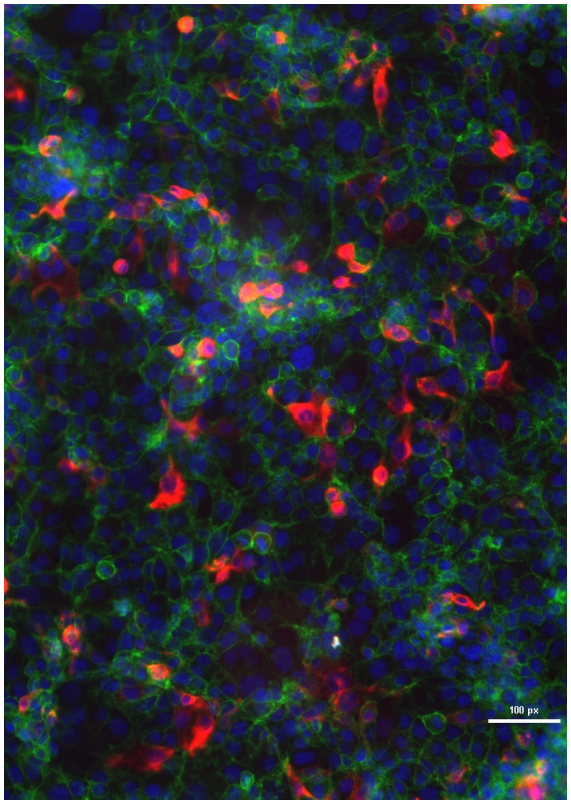
Summary

- We have created a breast epithelial, MCF 10A EMT reporter cell line
- Ecadherin, an epithelial marker is tagged to GFP using CRISPR/Cas9 gene editing technology
- Verified and validated: genomic, transcriptional, and translational levels
- Characterized with in-depth induction/transition assays
- MCF 10AEMT can be used to monitor cellular status changes in real time or as a platform to study EMT



www.atcc.org/EMT

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- Poster
- Infographic
- Decals

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