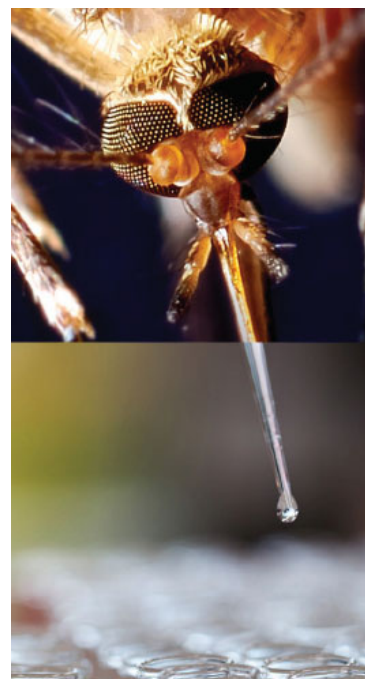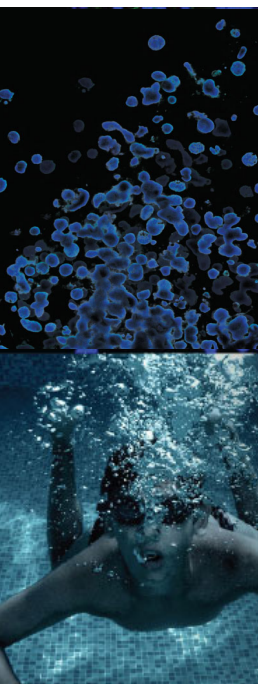# EMT Reporter Cell Lines: Migrating to Better Models of Transition

Diana Douglas, BS
Senior Biologist, ATCC

Sangeeta Kumari, MS
Senior Biologist, ATCC

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

ATCC

Agenda

- Background
  - EMT
  - EMT and cancer metastasis
- VIM-RFP reporter lines
  - HCT116 VIM RFP EMT
  - MDA-MB-231 VIM RFP MET
  - A549 VIM RFP EMT
- ECAD-EmGFP reporter lines
  - PANC-1 ECAD EmGFP MET
  - BT-474 ECAD EmGFP EMT
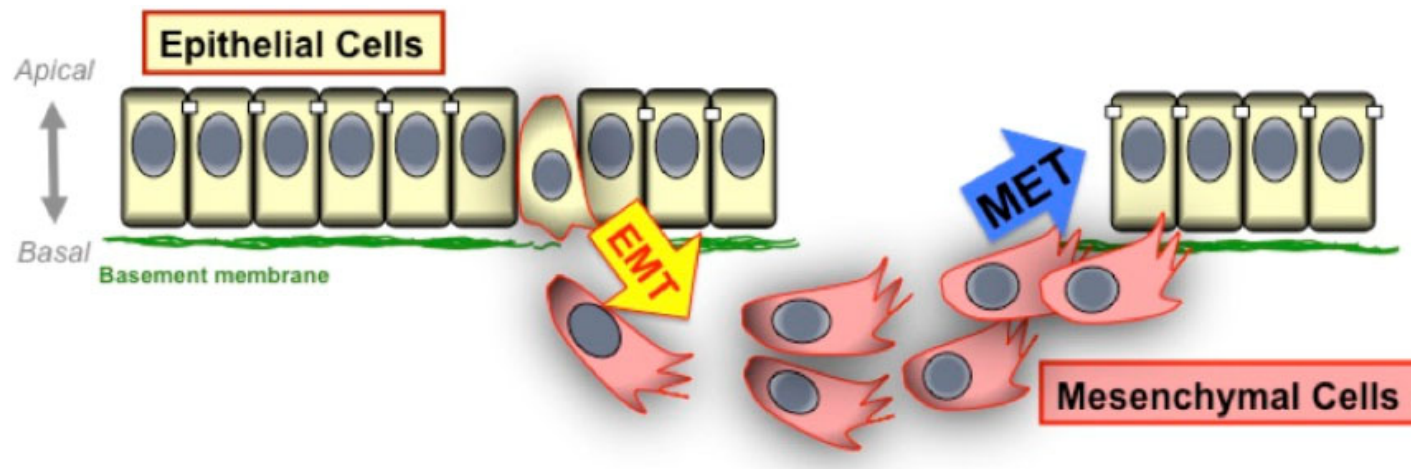 - MCF10A ECAD EmGFP EMT
- Conclusions
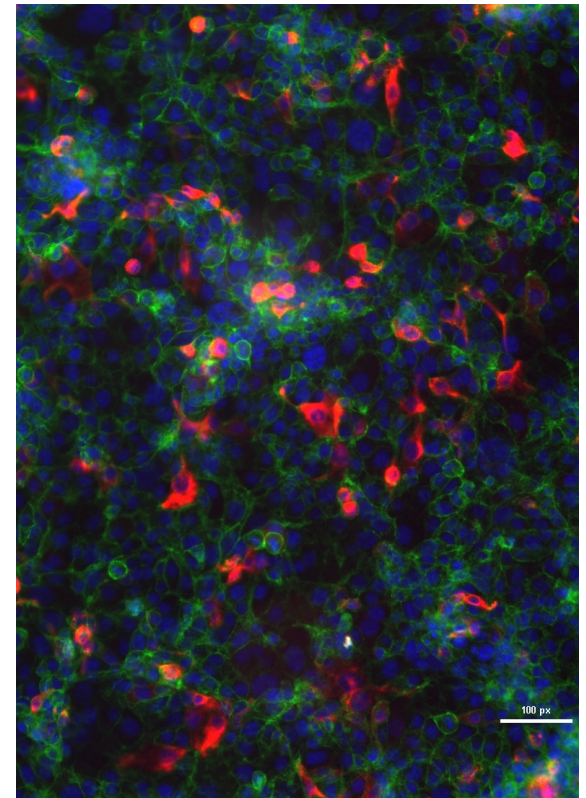
Background – EMT

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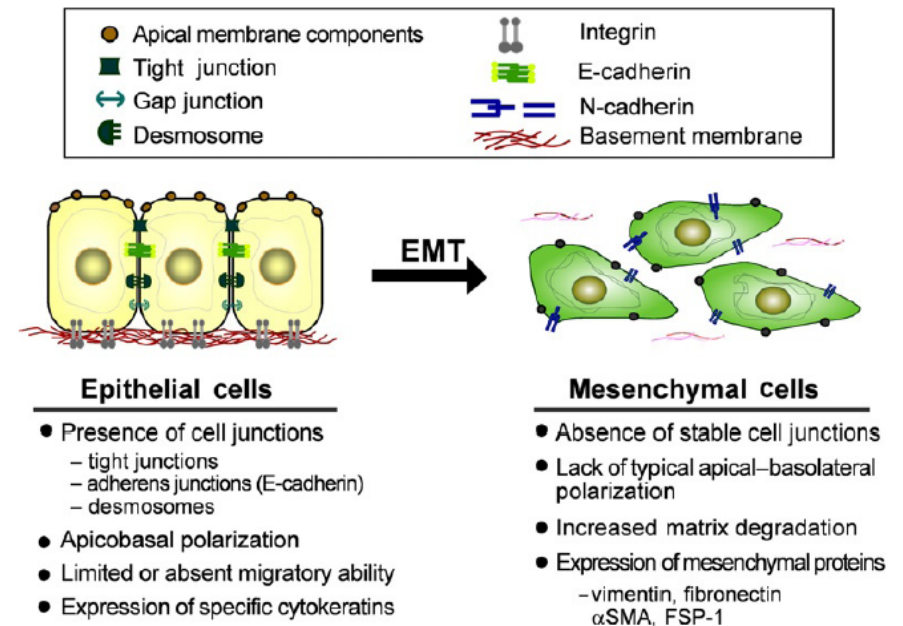
Lee et al, International Review of Cell and Molecular Biology, 2012.

EMT and MET in cancer progression

Epithelial Cells

Apical

Basal

Basement membrane

EMT

MET

Mesenchymal Cells

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

- Epithelial cancer cells reactivate EMT

- The EMT process facilitates metastatic dissemination

- "Partial EMT": the transition is a complex and multistep process
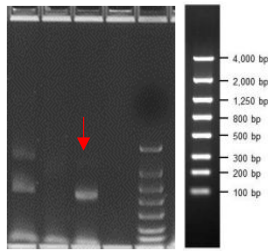
ATCC's EMT and MET reporter cell lines

- 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
A549 VIM RFP	CCL-185EMT™	Lung cancer	EMT	VIM-RFP
HCT116 VIM RFP	CCL-247EMT™	Colorectal cancer	EMT	VIM-RFP
MDA-MB-231 VIM RFP	HTB-26MET™	Breast cancer	MET	VIM-RFP
BT-474 ECAD EmGFP	HTB-20EMT™	Breast cancer	EMT	ECAD-GFP
PANC-1 ECAD EmGFP	CRL-1649MET™	Pancreatic cancer	MET	ECAD-GFP
MCF10A ECAD EmGFP	CRL-10317EMT™	Breast epithelial cells	EMT	ECAD-GFP

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Precision editing to create reporter KI alleles in cancer cell lines

WT allele

KI donor

Integrated KI allele

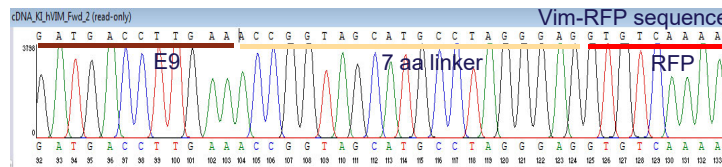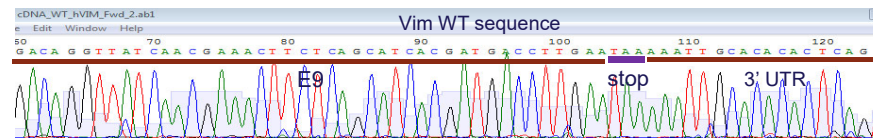= CRISPR/Cas9

Knock-in verification at the genomic level

RHA: ~ 1.0 kb

RFP Fwd
TAA

Exon8 // RFP Exon9 **VIM/RFP KI allele**

RHA Rev

C A C A A G C T C A A T G G T T A A A A A T T G C A C A C A C
CavenneRFP   VIM RHA 5' end

A G C A T A G G A A G T T G C C A A G A T A T A G G
VIM RHA 3' end   downstream of RHA sequence

LHA Fwd
TAA

Exon8 // RFP Exon9 **VIM/RFP KI allele**

RFP Rev

LHA: ~ 1.1 kb

C T C A G A T A C N G C T G G C T A A T T T G A G
VIM gDNA   VIM LHA 5' end sequence

C G T G A T A T A T A G G A T A A T T T A G T C T T
gRNA

A T G A C C T T G A A A C C G G T A G C A T G C C T A G G G A G G T G T C A A A A G G
VIM E9   7 AA linker   RFP

8

ATCC

Knock-in verification at the transcriptional and translational levels

Vimentin-RFP reporter lines

HCT16 VIM RFP: colorectal cancer
MDA-MB-231: breast cancer
A549 VIM RFP: lung cancer

ATCC

HCT 116 VIM RFP EMT reporter cells display an epithelial-to-mesenchymal morphology change upon induction
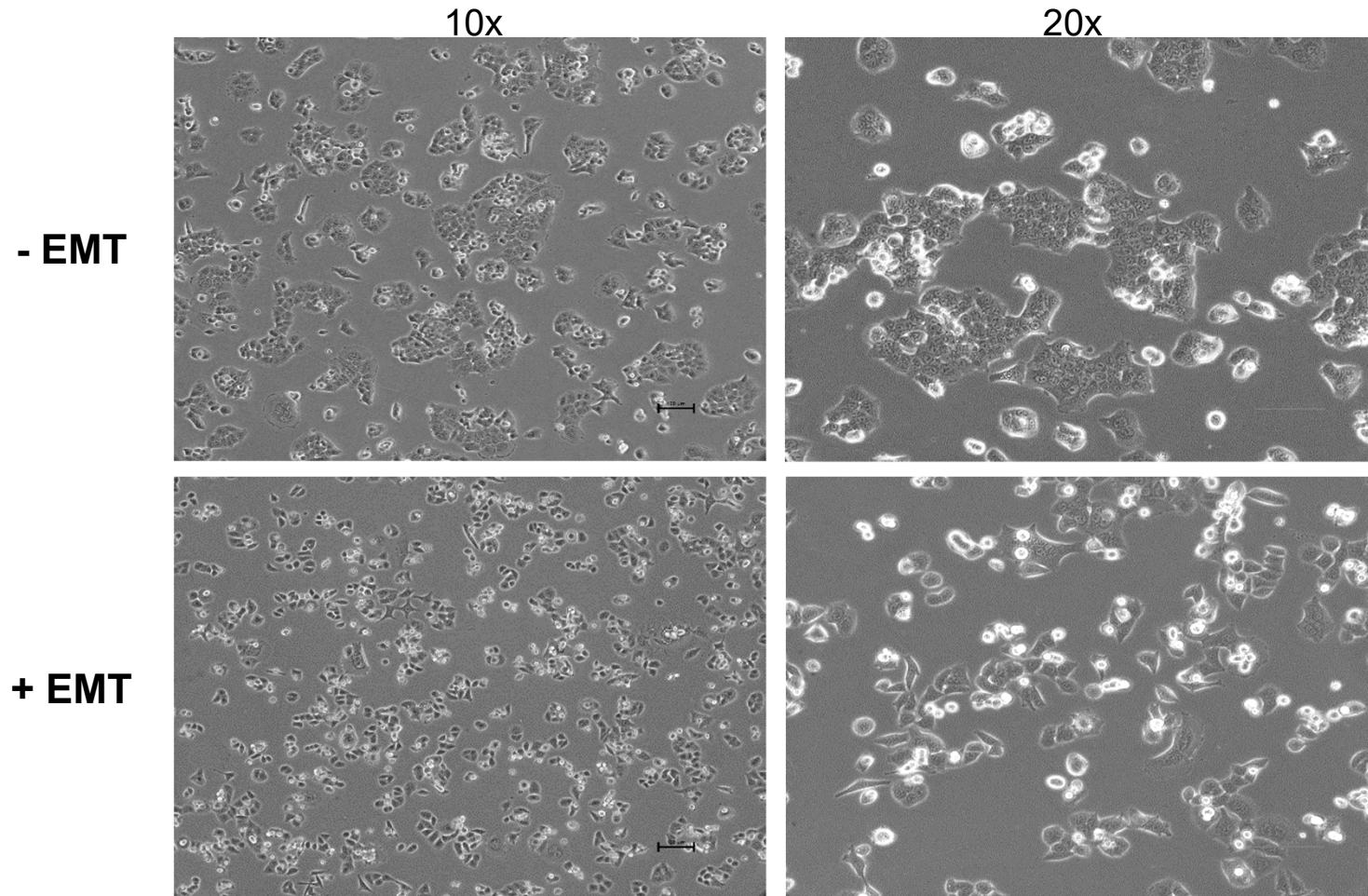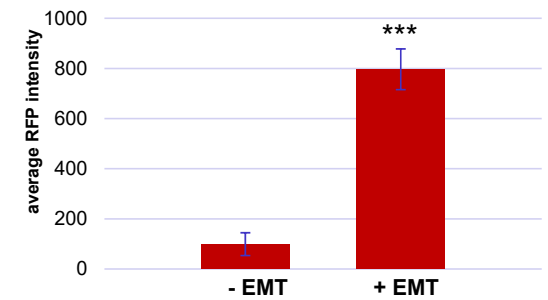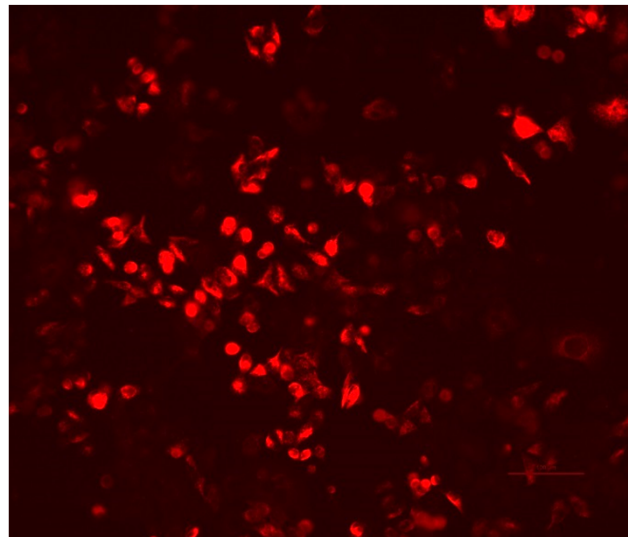
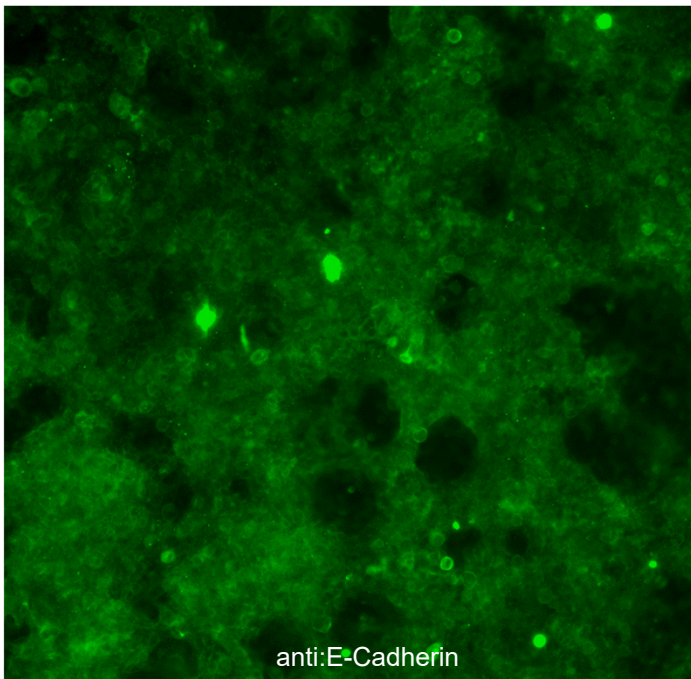# HCT116 VIM RFP reporter cells display an increase in intrinsic vimentin expression
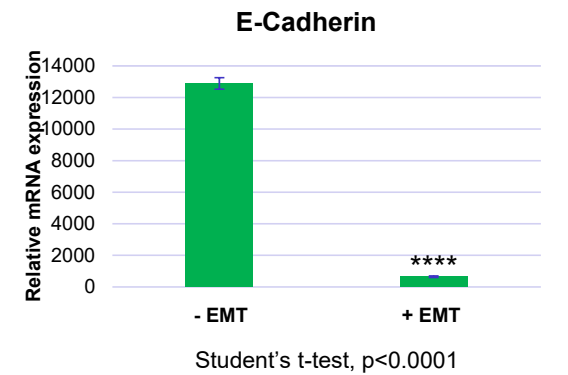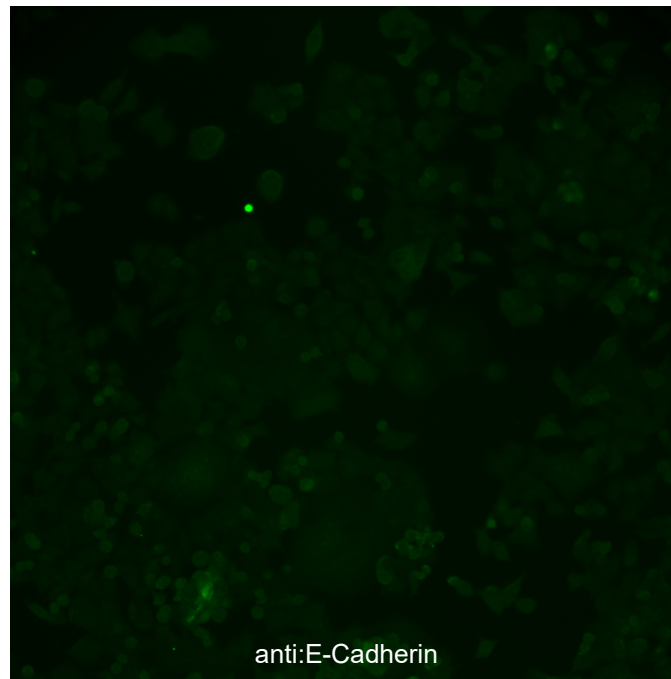
- EMT

+ EMT
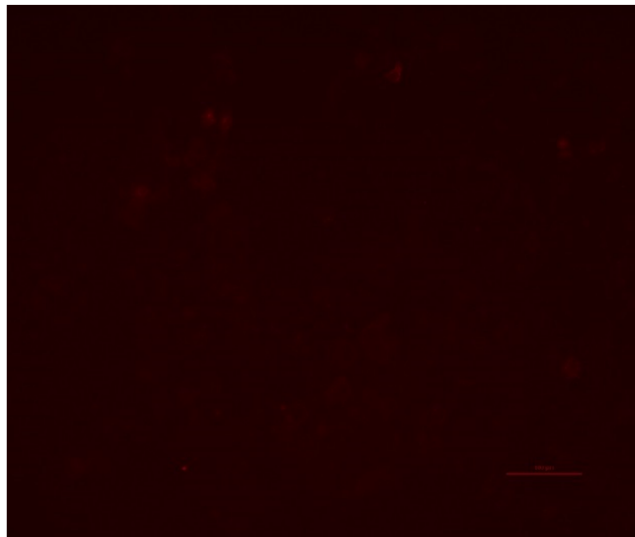
Student's t-test, p<0.001

~ 8.0 fold increase

HCT116 VIM RFP reporter cells display a decrease in the epithelial marker E-cadherin

- EMT

+ EMT

anti:E-Cadherin
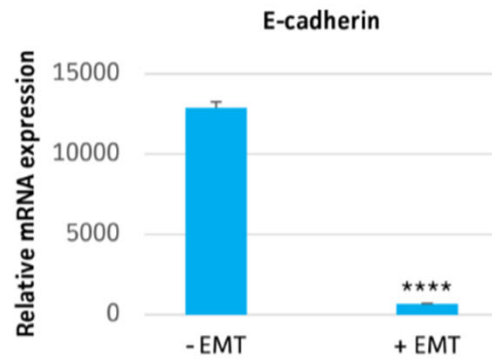

anti:E-Cadherin

E-Cadherin

Relative mRNA expression

14000
12000
10000
8000
6000
4000
2000
0

- EMT   + EMT

****

Student's t-test, p<0.0001

~ 19.8 fold decrease (95% decrease)

ATCC

HCT116 VIM RFP: quantitative analysis of EMT marker gene expression by ddPCR™
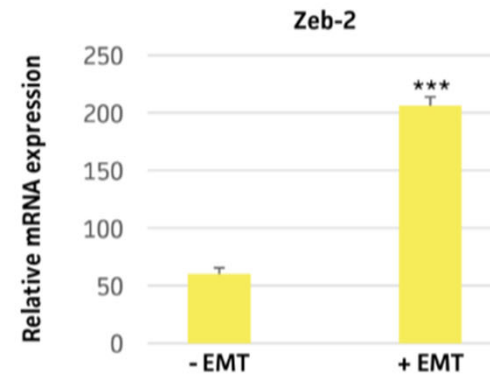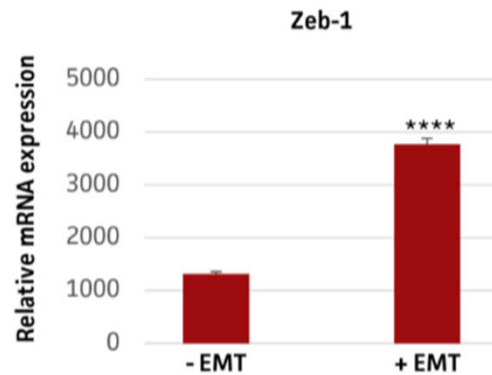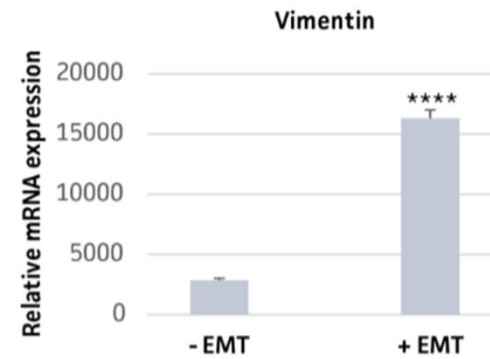
Student's t-test, ***p<0.001, ****p<0.0001

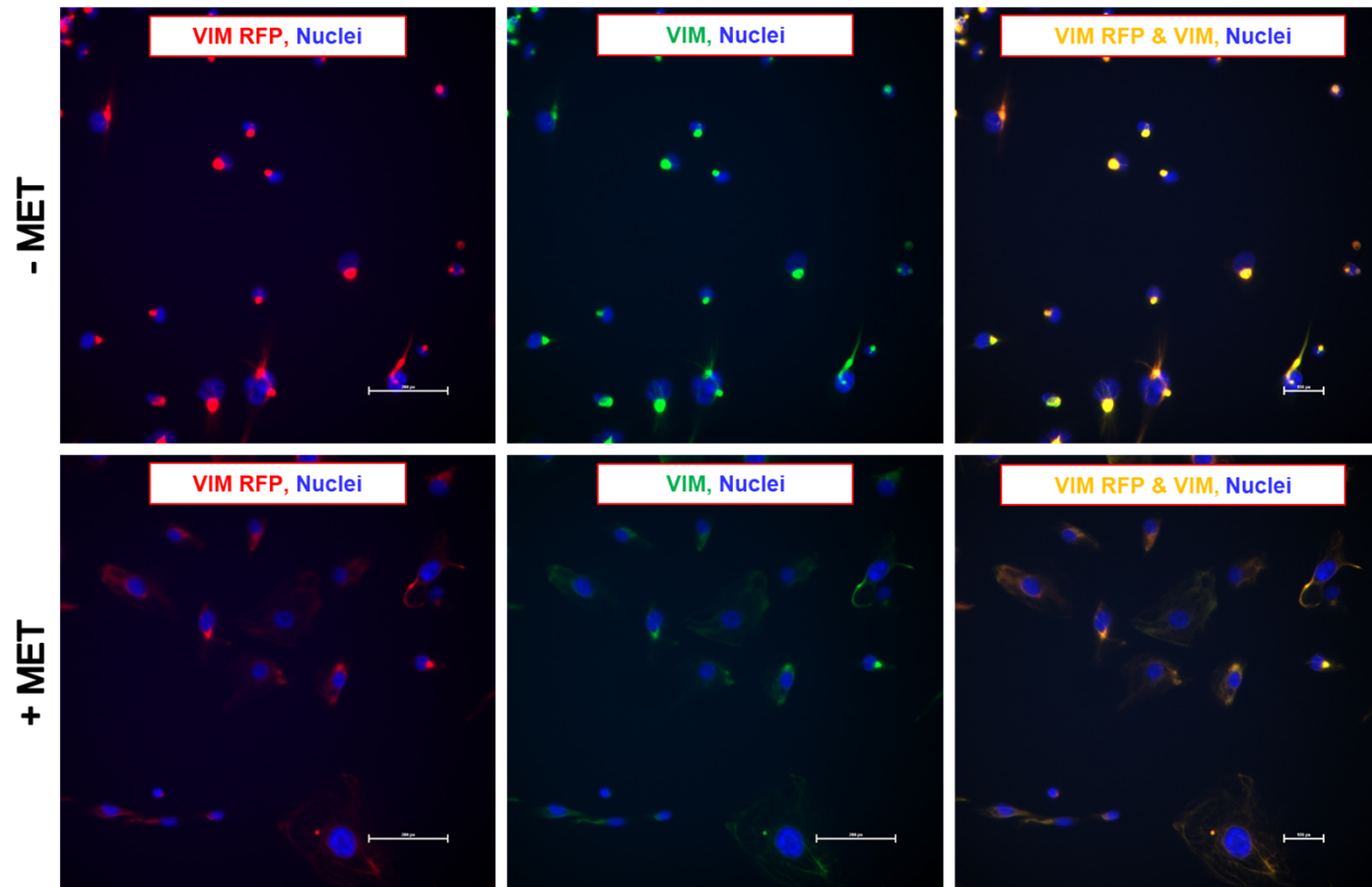# MDA-MB-231VIM RFP MET cells display a morphology change upon MET induction
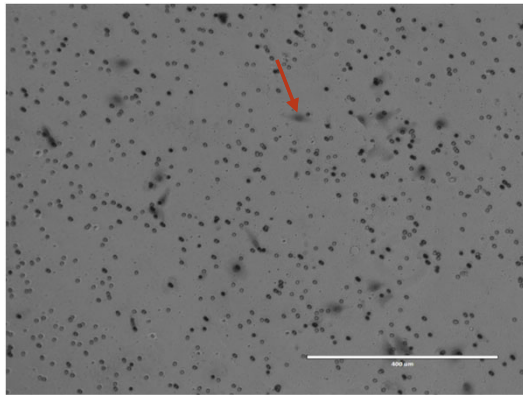
-MET
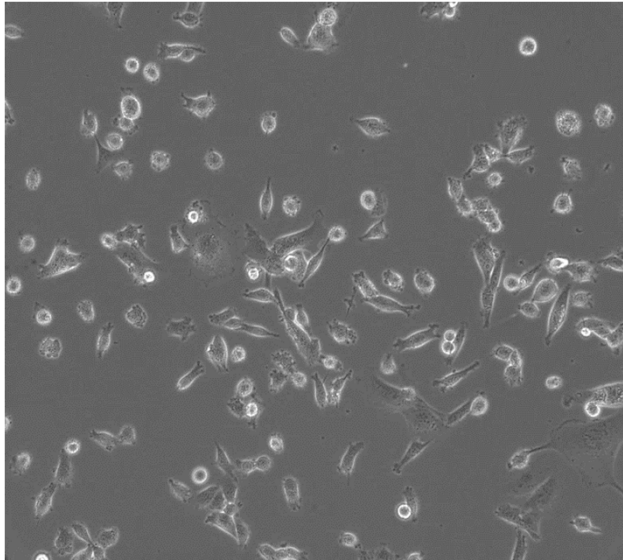
+MET
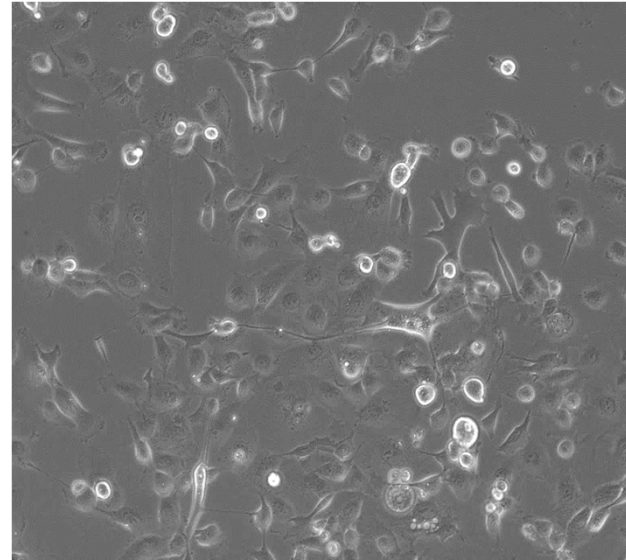
MDA-MB-231 VIM RFP MET cells display a decrease in the intrinsic mesenchymal marker vimentin
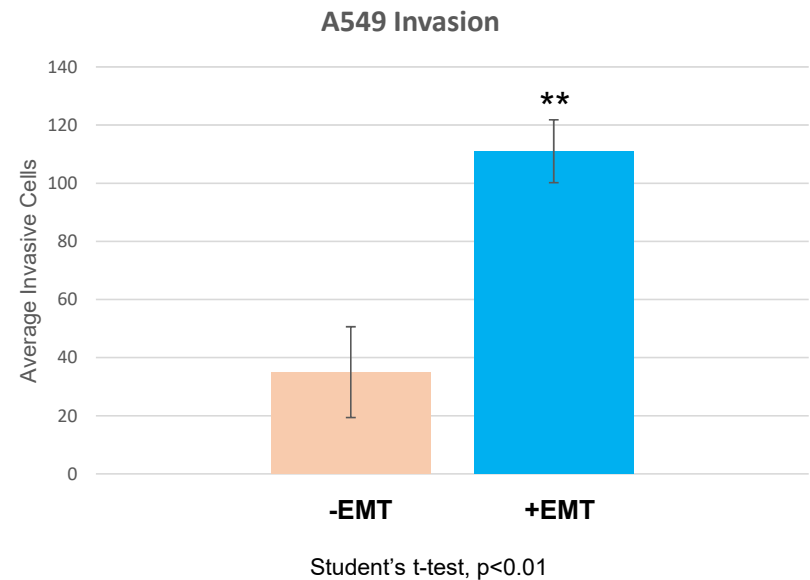
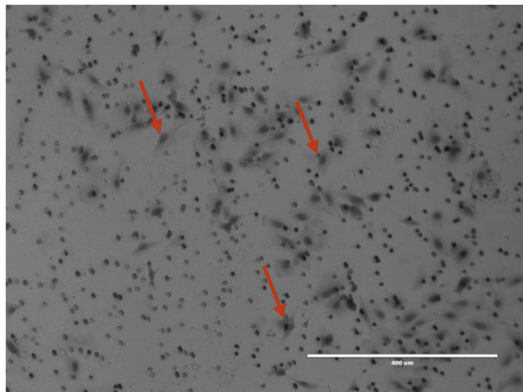# A549 VIM RFP EMT cells display an increase in invasive capacity upon EMT induction
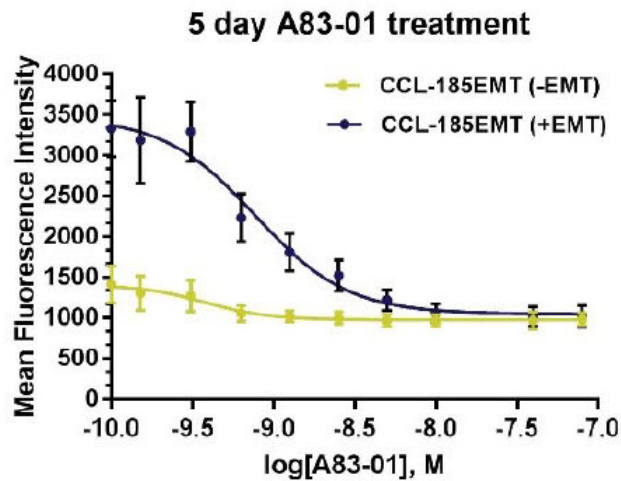
-EMT

+EMT

A549 Invasion
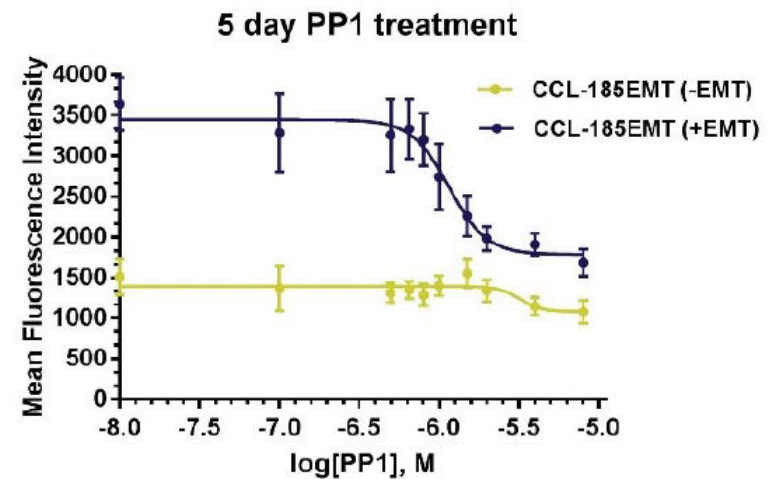

Student's t-test, p<0.01

~3 fold increase in invasive cells upon EMT induction

Treatment of A549 VIM RFP EMT cells with small molecule EMT inhibitors effectively blocks EMT
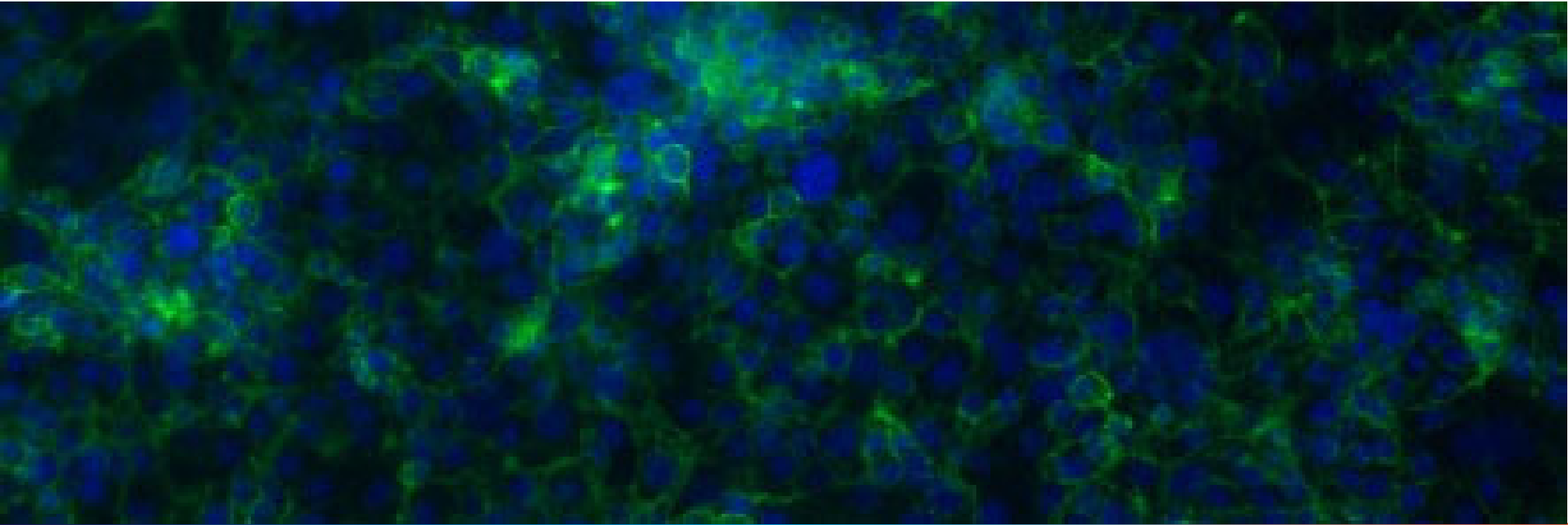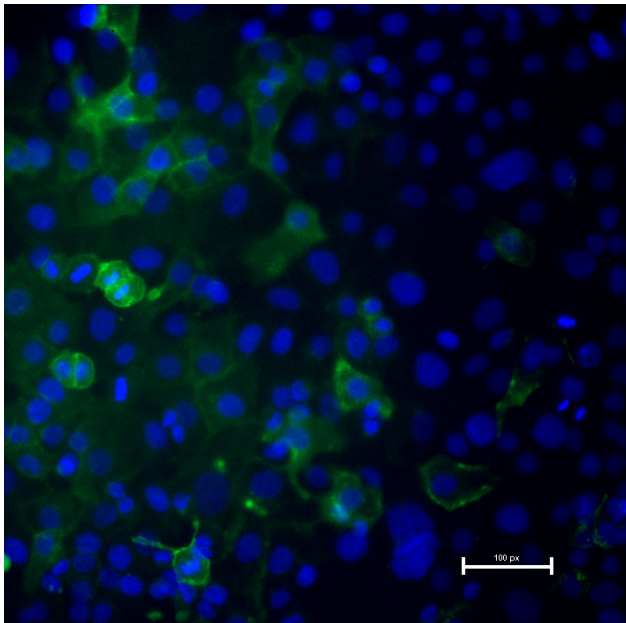
E-cadherin-EmGFP reporter lines

PANC-1 ECAD EmGFP: pancreatic cancer
BT-474 ECAD EmGFP: breast cancer
MCF10A ECAD EmGFP: breast epithelial cells

ATCC®

PANC-1 ECAD EmGFP MET reporter cells display a morphology change upon induction
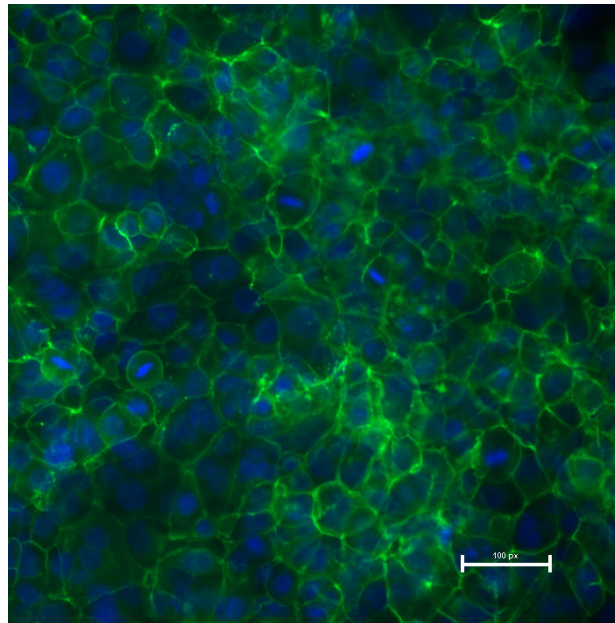

10x   20x

- EMT

+ EMT

ATCC
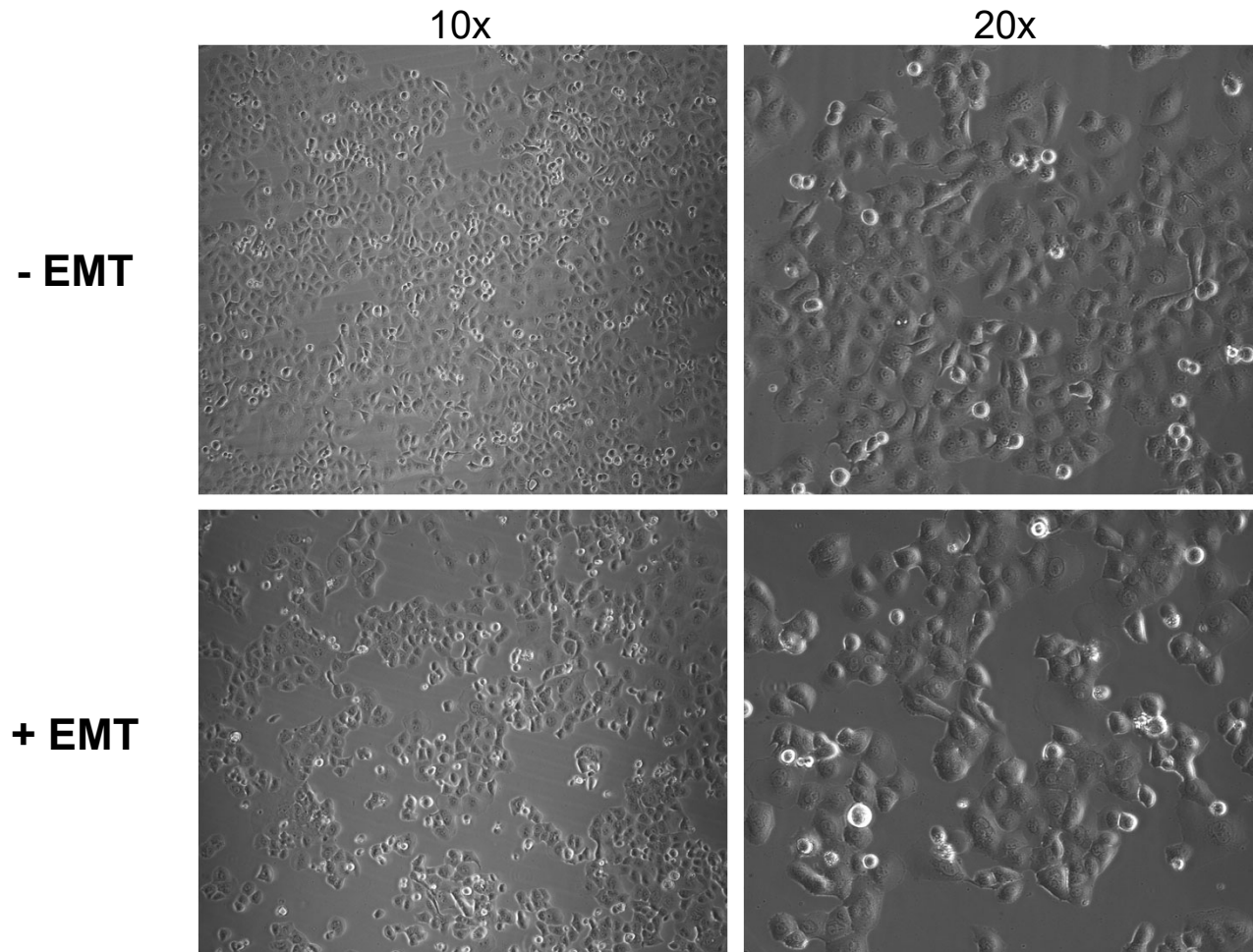
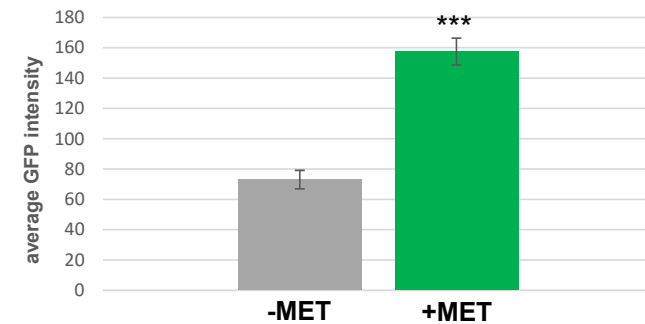# PANC-1 ECAD EmGFP MET cells display an increase in intrinsic E-cadherin expression

- MET

+ MET (Pre-miR-200)

PANC-1 clone B3 ECAD expression

Student's t-test, p<0.001

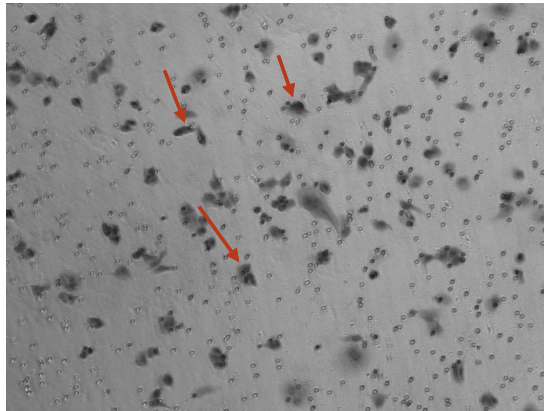**Increase in ECAD expression upon MET induction > 2-fold**

PANC-1 ECAD EmGFP cells display a decrease in the mesenchymal marker SNAIL

-MET   +MET
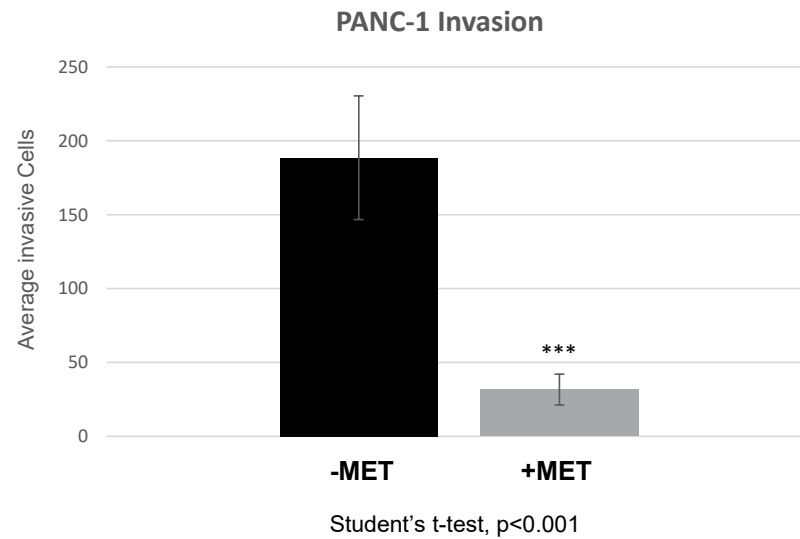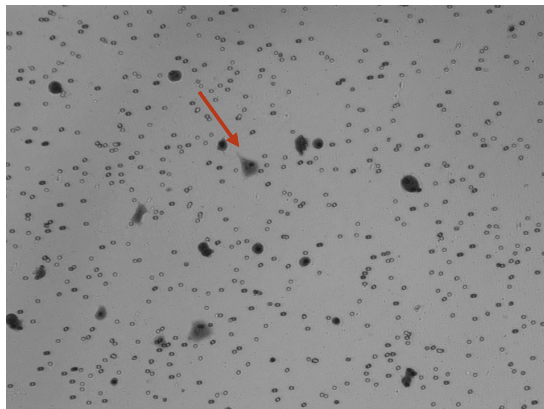
SNAIL

SNAIL + DAPI

PANC-1 clone B3 SNAIL expression

average anti-SNAIL intensity

***

-MET   +MET

Student's t-test, p<0.001

~60% decrease in SNAIL upon induction

ATCC®
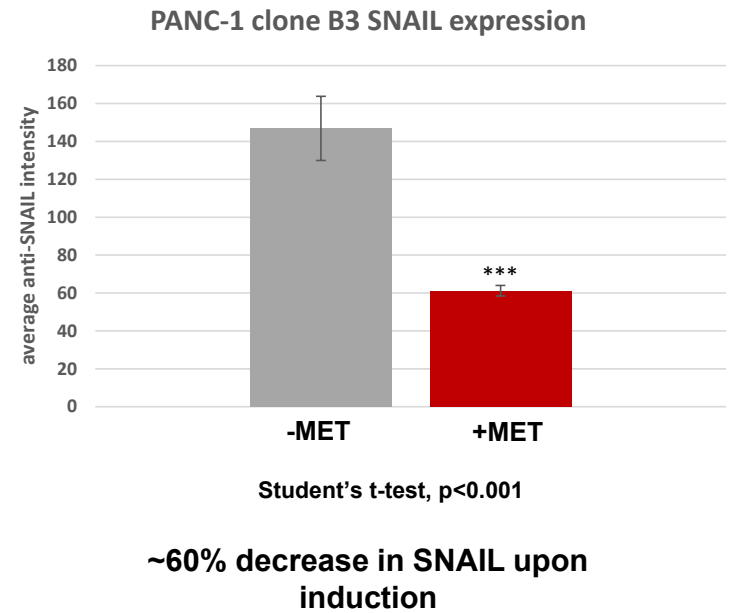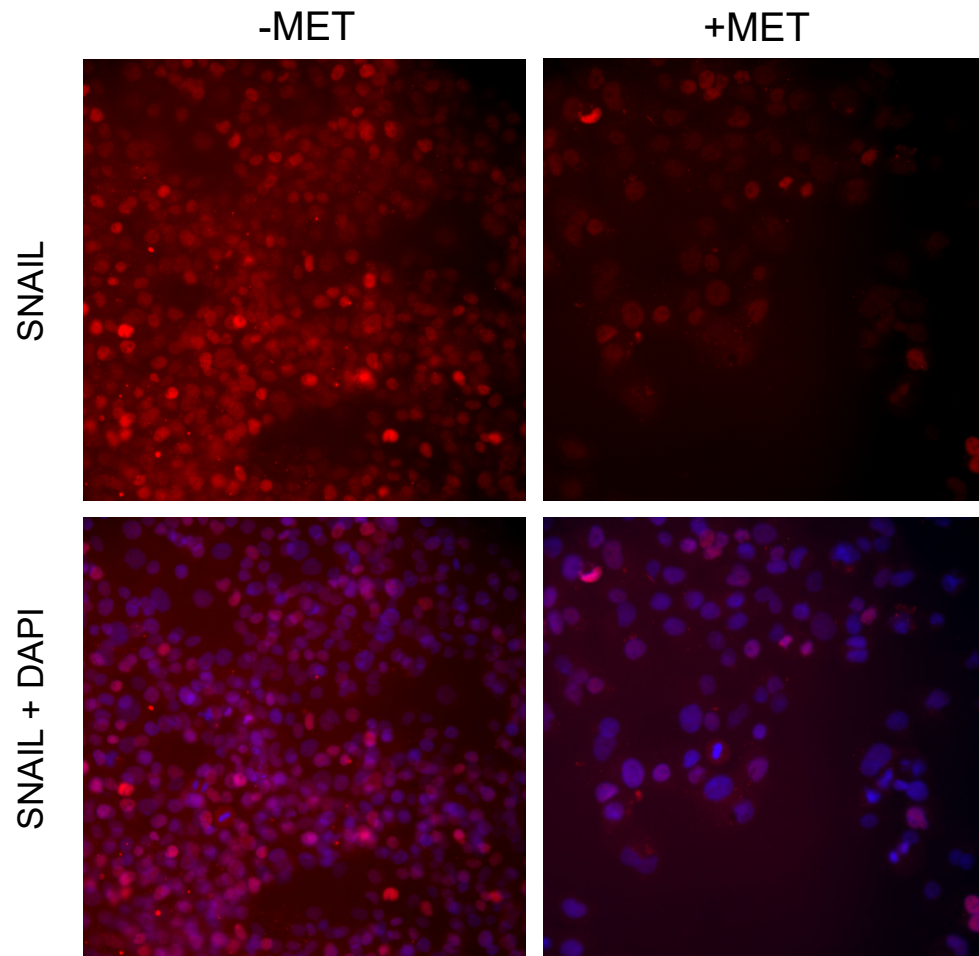
PANC-1 ECAD EmGFP cells show a decreased invasive capacity after EMT

-MET
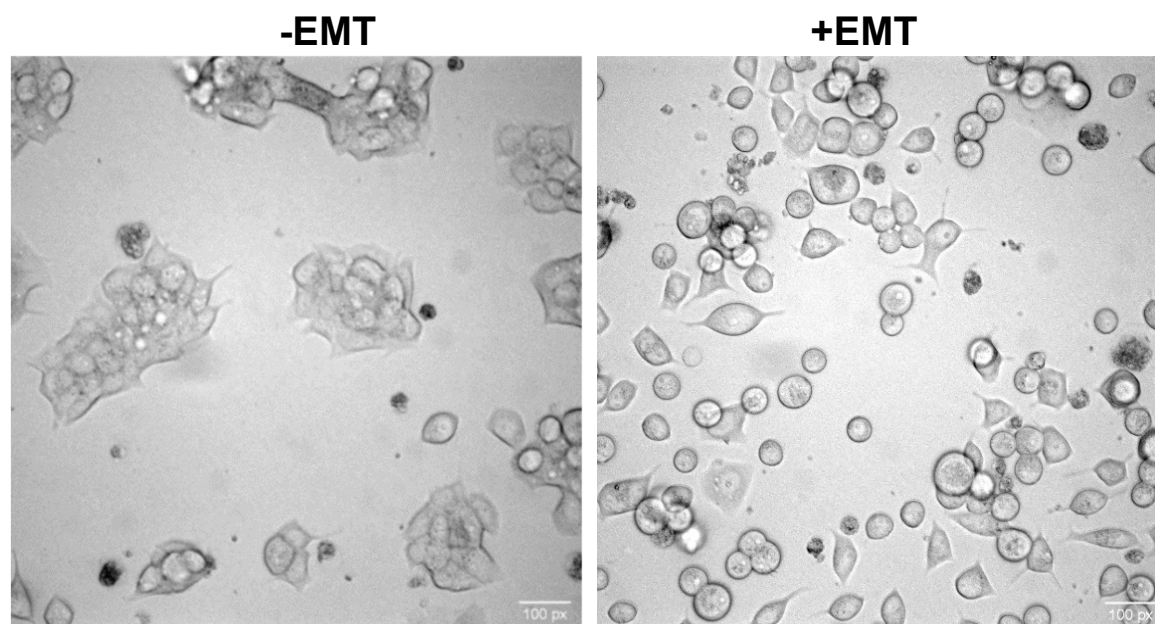

+MET

PANC-1 Invasion

Student's t-test, p<0.001

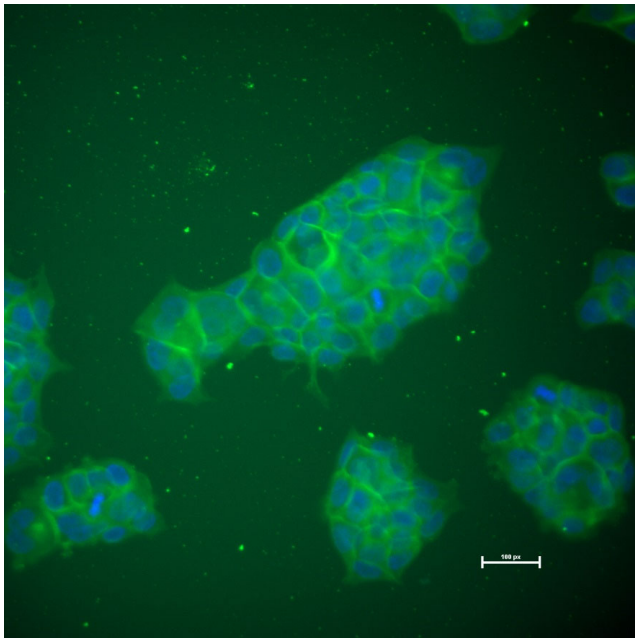**~6 fold decrease in invasive cells upon MET induction**

ATCC

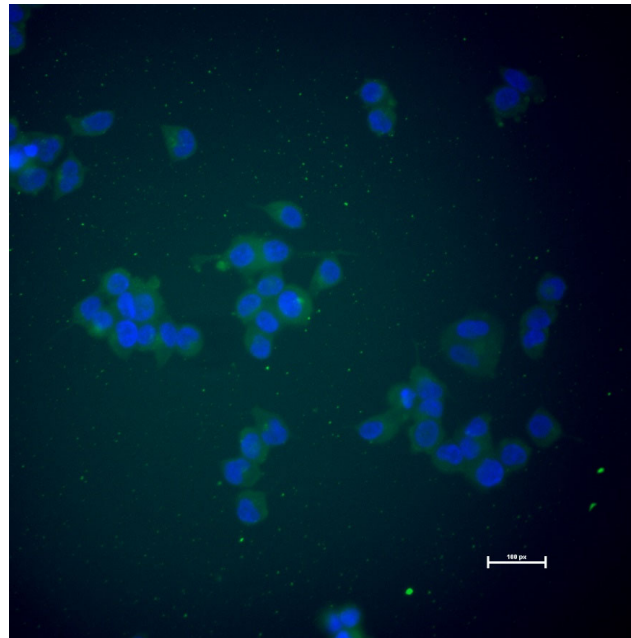# BT-474 ECAD EmGFP EMT cells display a morphology change upon EMT induction

-EMT

+EMT

ATCC

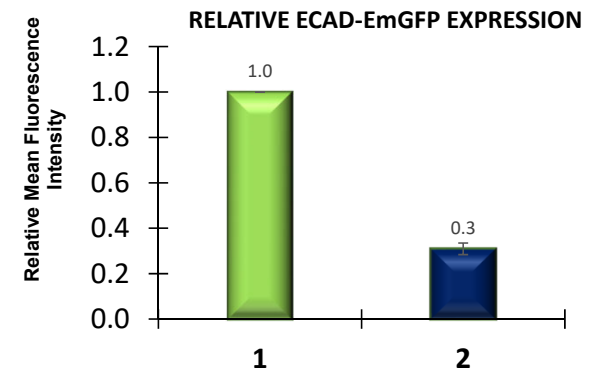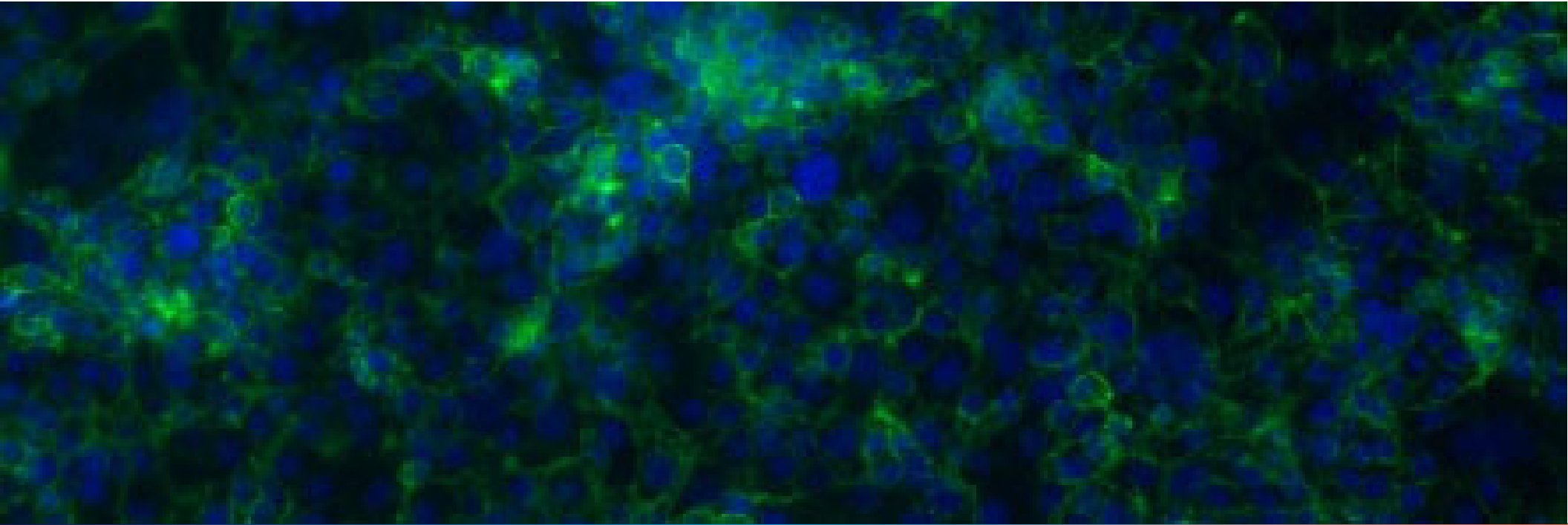# BT-474 ECAD EmGFP EMT display a decrease in endogenous E-cadherin expression

-EMT

+EMT

ECAD, Nuclei

ECAD, Nuclei

RELATIVE ECAD-EmGFP EXPRESSION

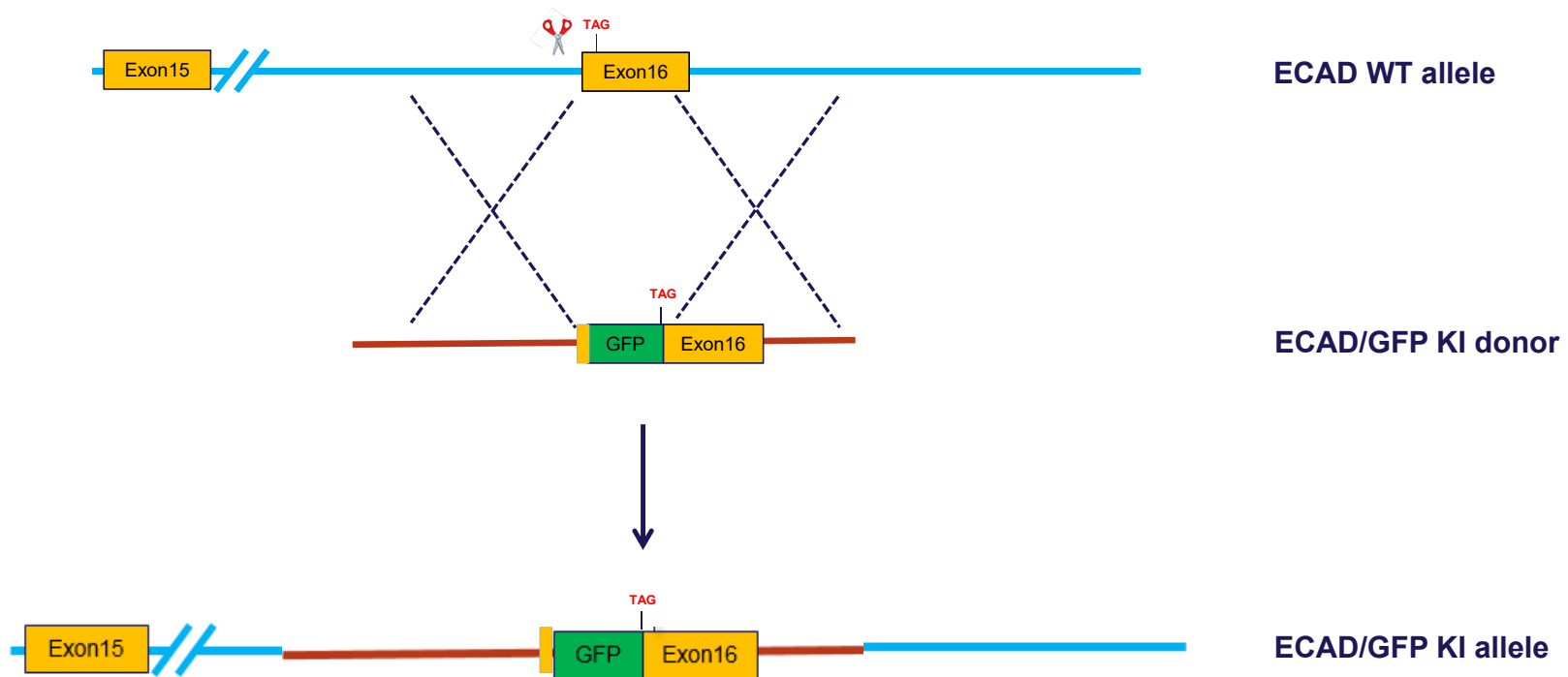Relative Mean Fluorescence Intensity

1.0

0.3

1

2

ATCC

E-cadherin-EmGFP reporter lines

MCF10A ECAD EmGFP: breast epithelial cells

ATCC

Generation of E-cadherin-EmGFP Knock-In Allele

ECAD WT allele

ECAD/GFP KI donor
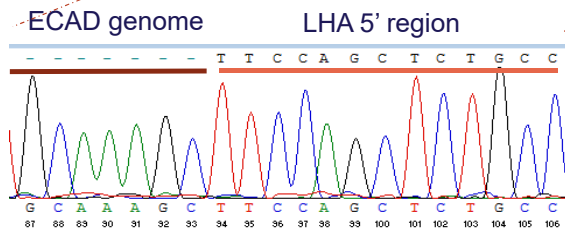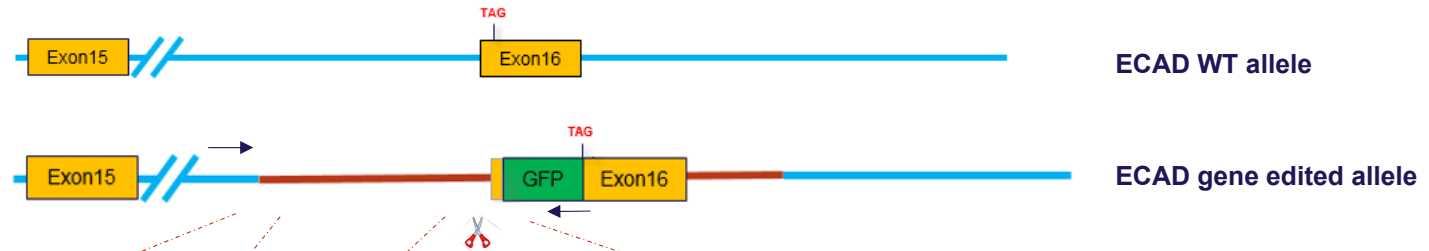
ECAD/GFP KI allele

✂ = CRISPR/Cas9   GFP= EmGFP (Emerald Green Fluorescent Protein)

ATCC®

Knock-in verification at the genomic level

WT   KI

LHA~ 0.8 kb

ECAD WT allele

ECAD gene edited allele

Exon15   Exon16   TAG

Exon15   GFP   Exon16   TAG

ECAD genome   LHA 5' region

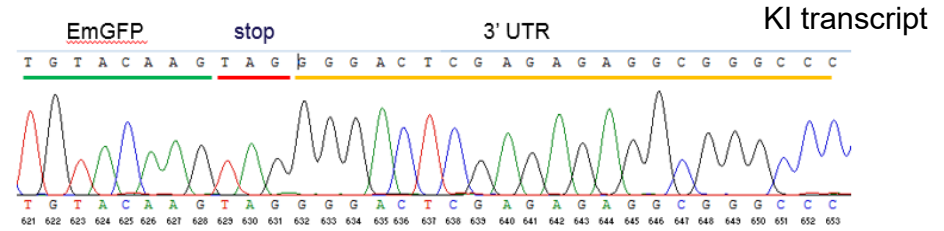gRNA sequence   mut PAM   7 AA peptide   EmGFP
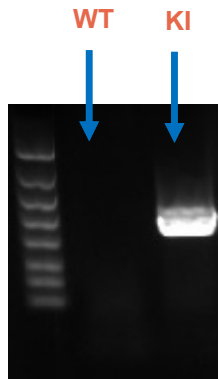
LHA junction

LHA/EmGFP junction
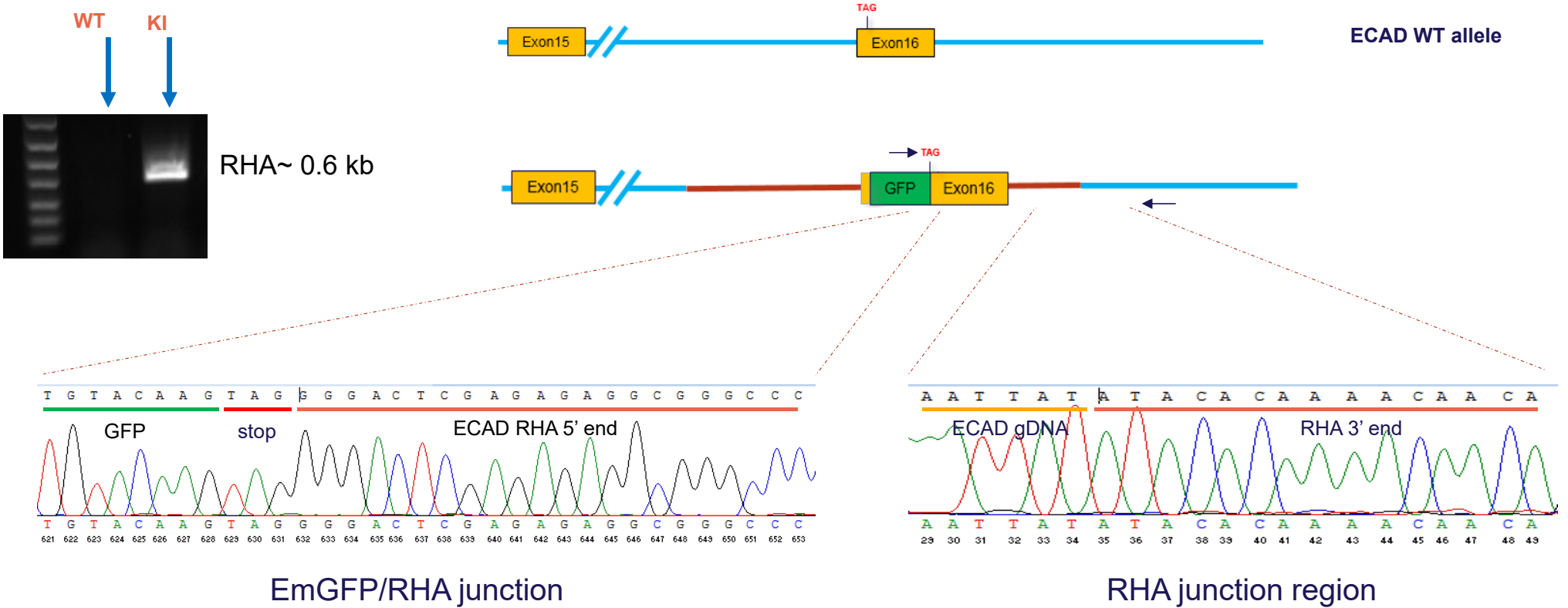
ATCC

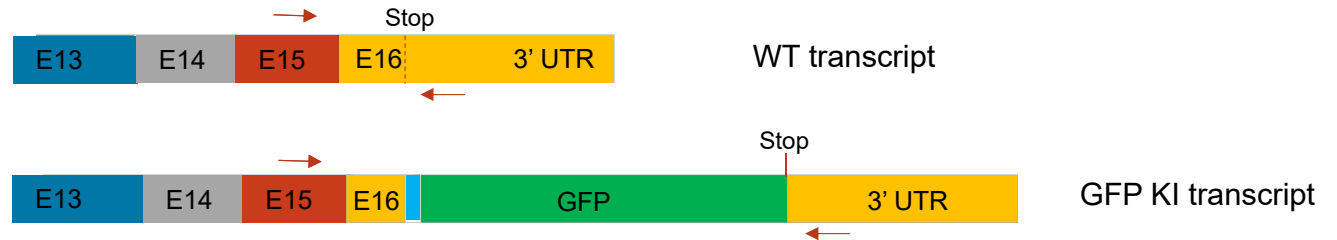# Knock-in verification at the genomic level

RHA~ 0.6 kb

ECAD WT allele

EmGFP/RHA junction

RHA junction region

Knock-in verification at the transcriptional and translational levels

WT   KI

1214 bp (KI)
479 bp (WT)

E13 E14 E15 E16 3' UTR   Stop   WT transcript

E13 E14 E15 E16 GFP 3' UTR   Stop   GFP KI transcript

gRNA sequence   mut PAM   7 AA peptide   EmGFP

AAGAAGCTGGCTGACATGTACGGAGGCGGCGAAGACGACACCGGTAGCATGCCTAGGGAGGTGAGCA

EmGFP   stop   3' UTR   KI transcript
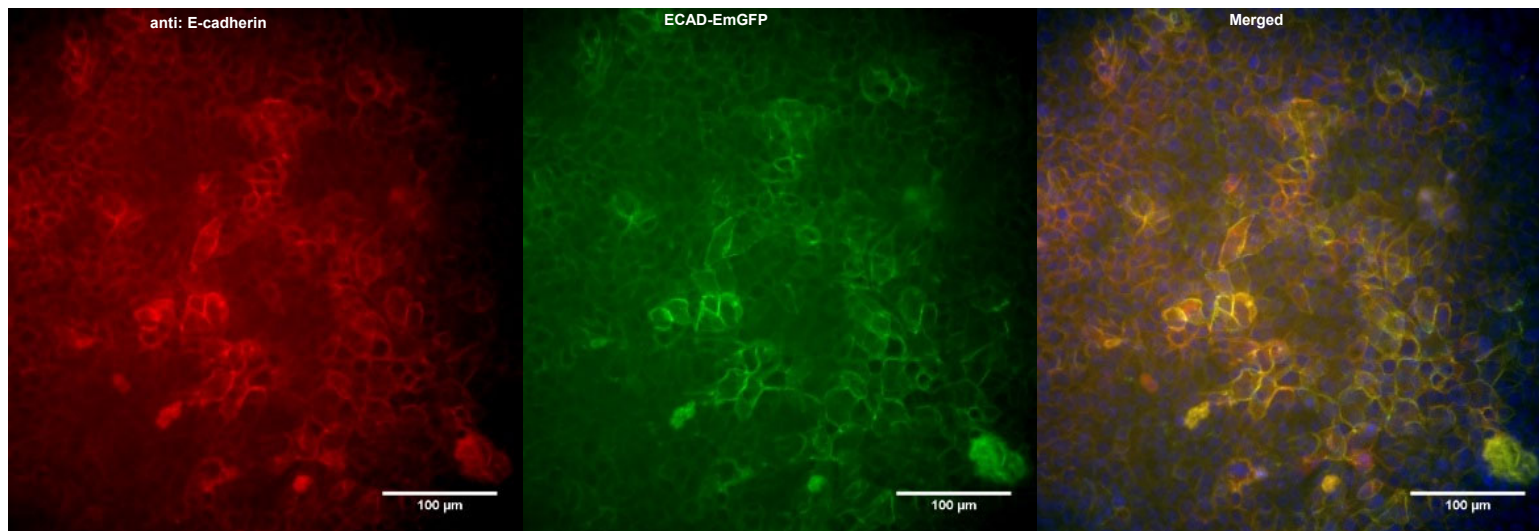
TGTACAAGTAGGGGACTCGAGAGAGGCGGGCCC

30

ATCC
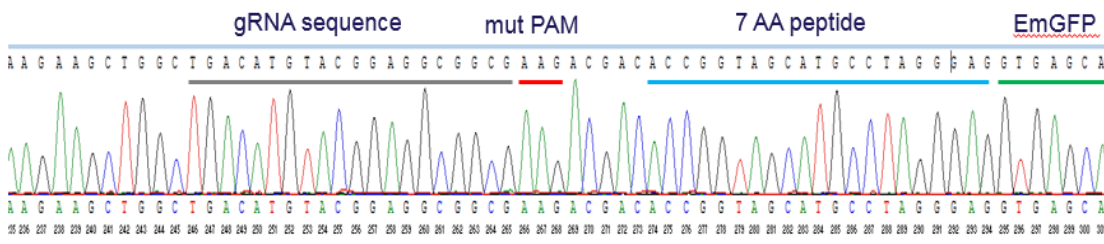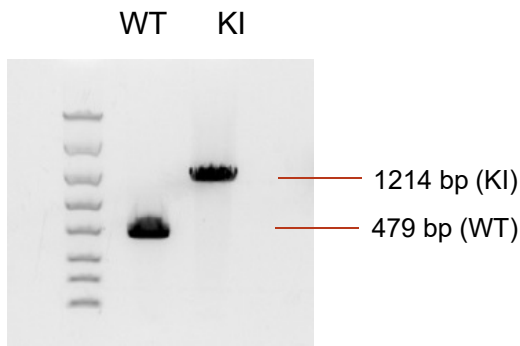
Knock-in verification at the transcriptional and translational levels
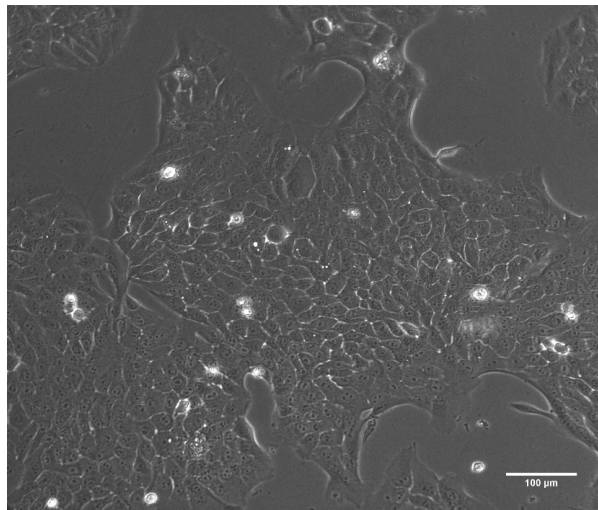
Anti-Ecadherin

Endogenous
ECAD-EmGFP

Co-localization of ECAD-GFP
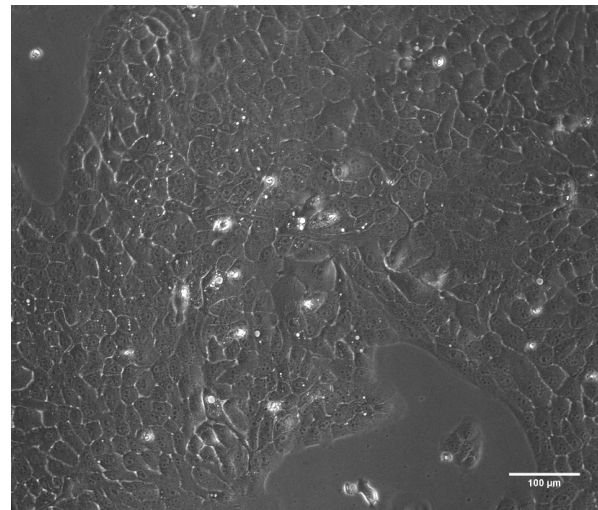expression

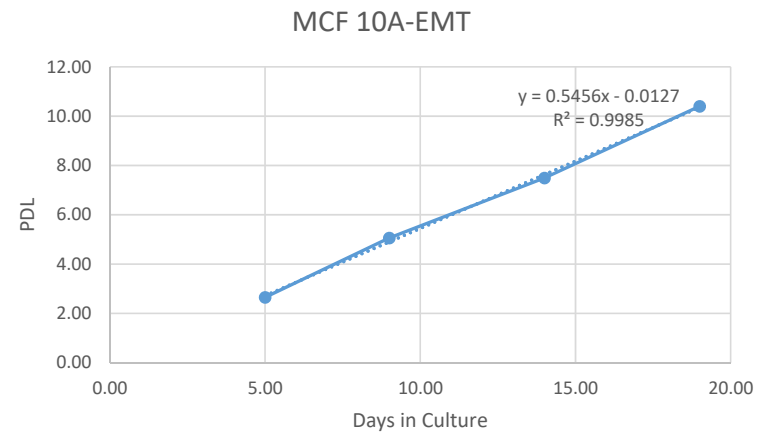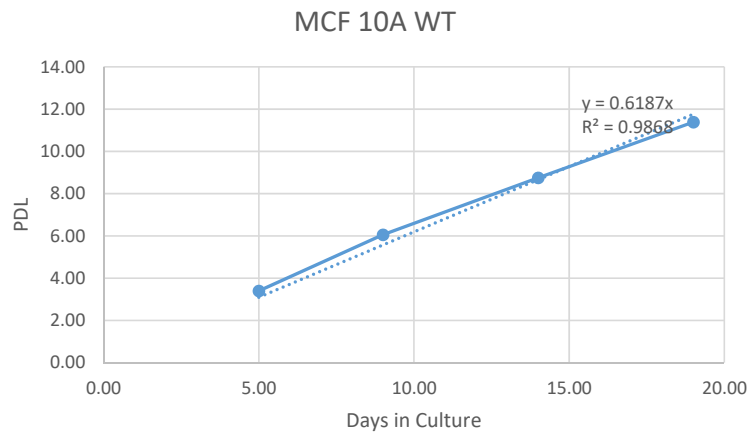# Morphology of parental and gene edited MCF 10A cells

MCF 10A WT   MCF 10A EMT

Growth media: Same as parental

Growth rate of parental and gene edited MCF 10A

MCF 10A WT

$y = 0.6187x$
$R^2 = 0.9868$

PDL (y-axis): 0.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00, 14.00
Days in Culture (x-axis): 0.00, 5.00, 10.00, 15.00, 20.00

MCF 10A-EMT

$y = 0.5456x - 0.0127$
$R^2 = 0.9985$

PDL (y-axis): 0.00, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00
Days in Culture (x-axis): 0.00, 5.00, 10.00, 15.00, 20.00

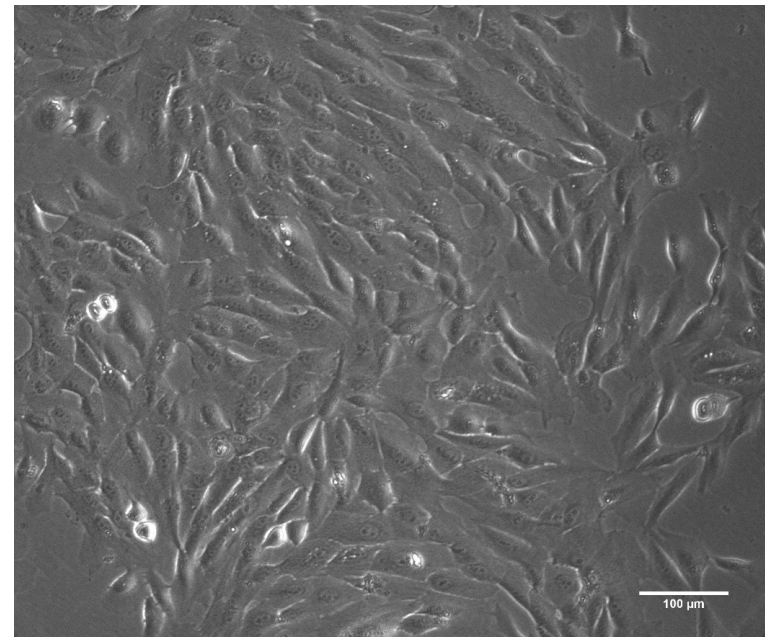Population doubling time for MCF 10A WT = 42hrs   Population doubling time for MCF 10A-EMT= 45hrs

Difference in growth=7%

ATCC

MCF 10A EMT reporter cells display morphology change upon induction
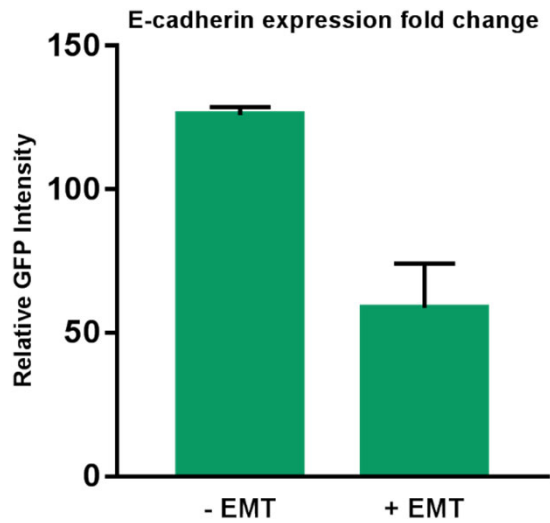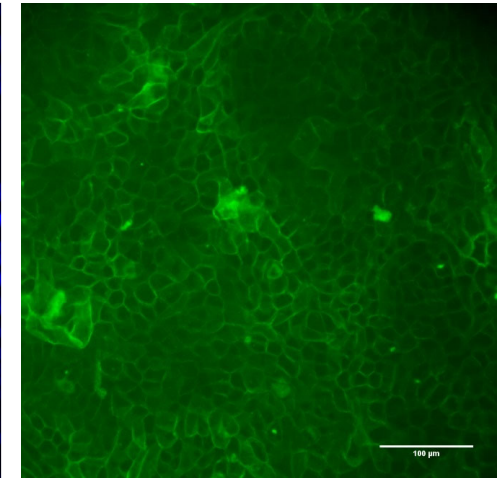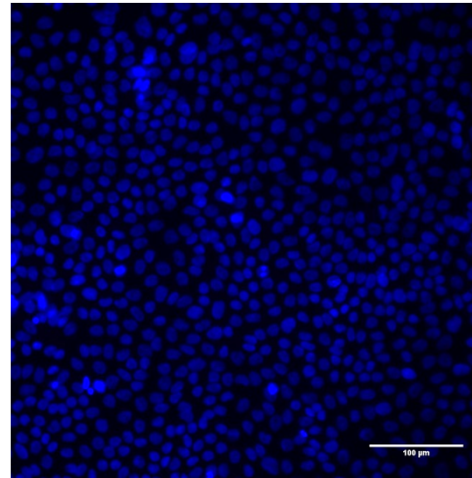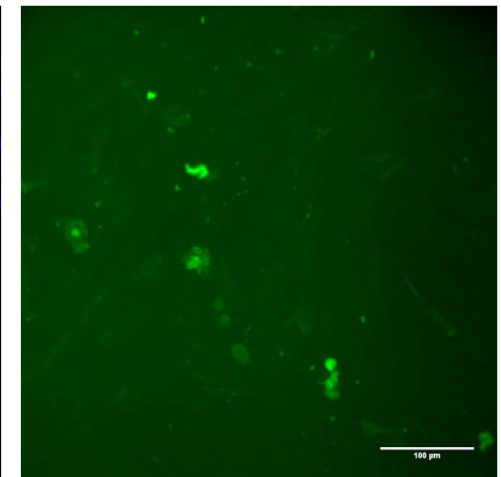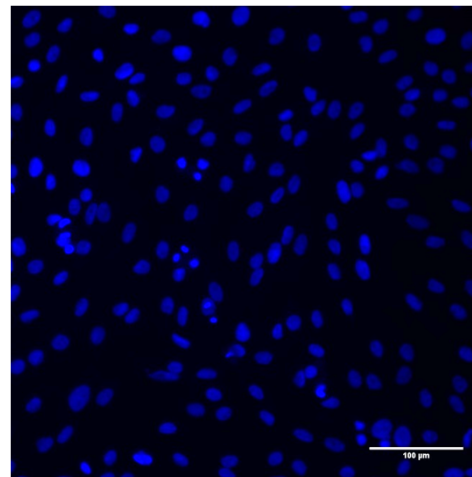
- EMT (control)

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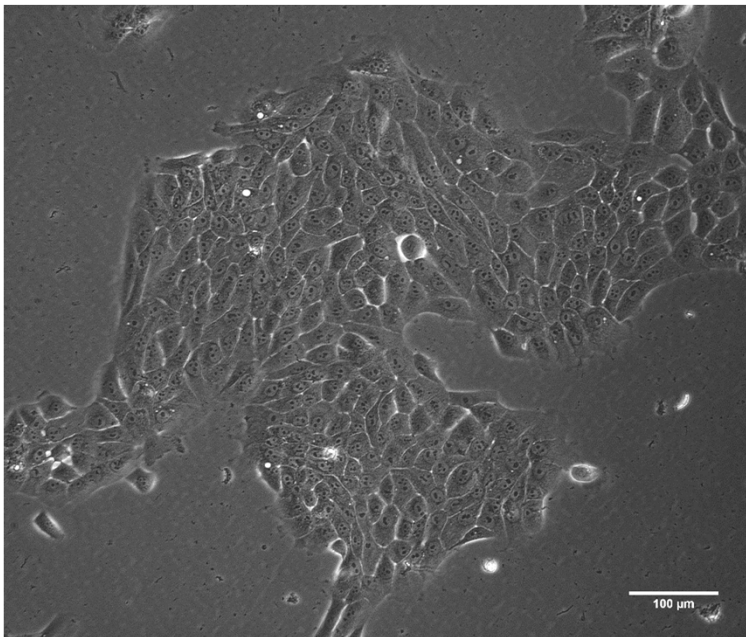
Bio-functional Data Supporting Product:
Decrease in Ecad-EmGFP expression upon EMT (2 fold)

Bio-functional Data Supporting Product:
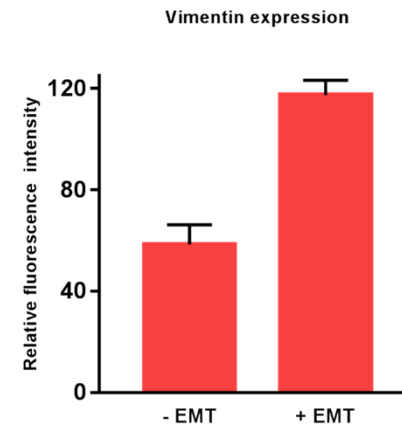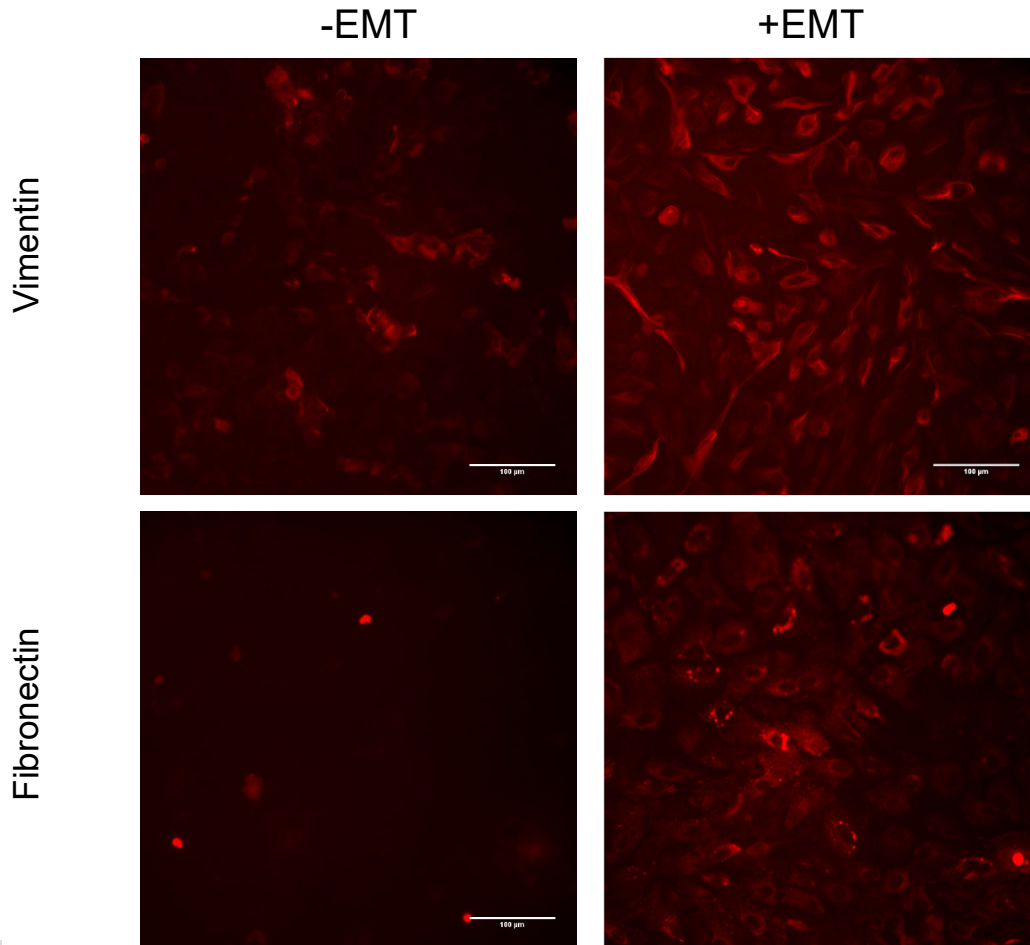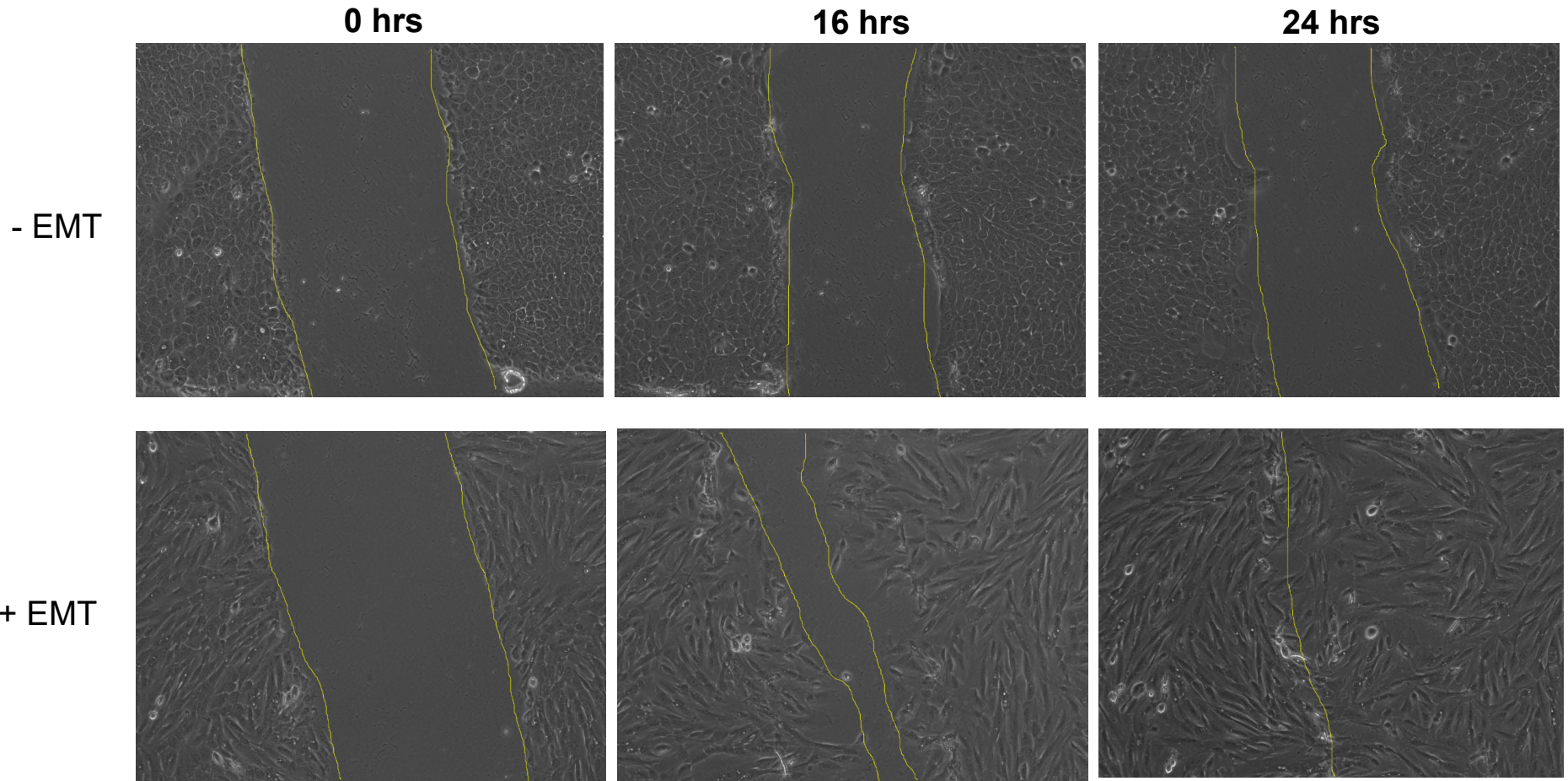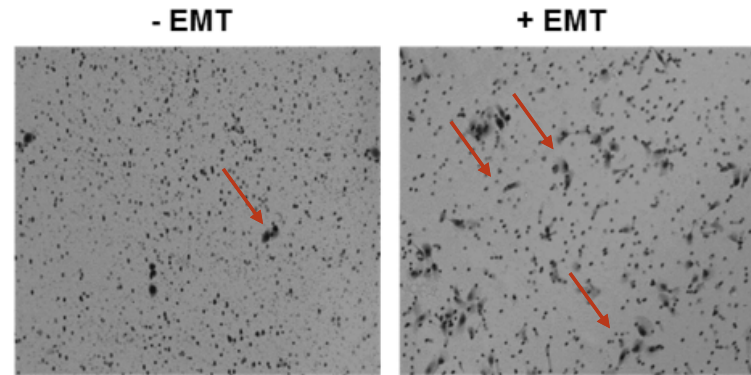## ≥ 50% increase in 2nd EMT marker (Vimentin and Fibronectin) expression upon EMT
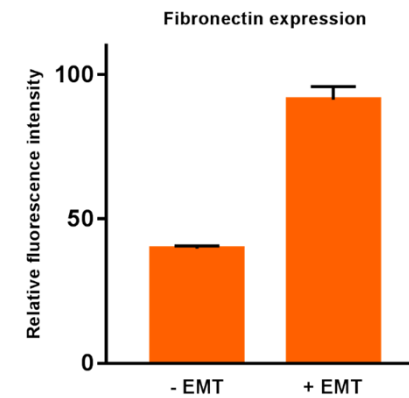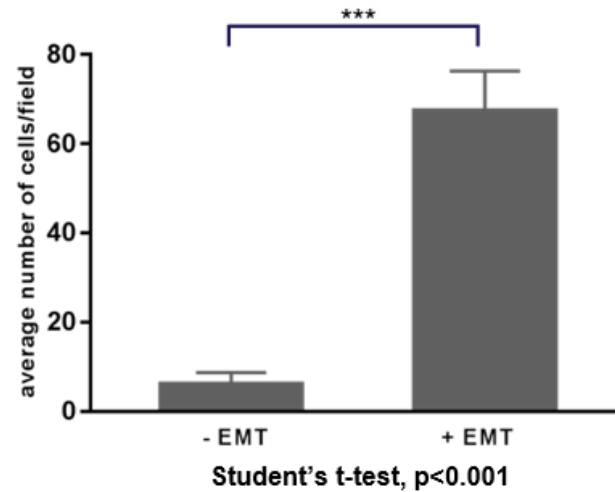
Bio-functional Data Supporting Product:
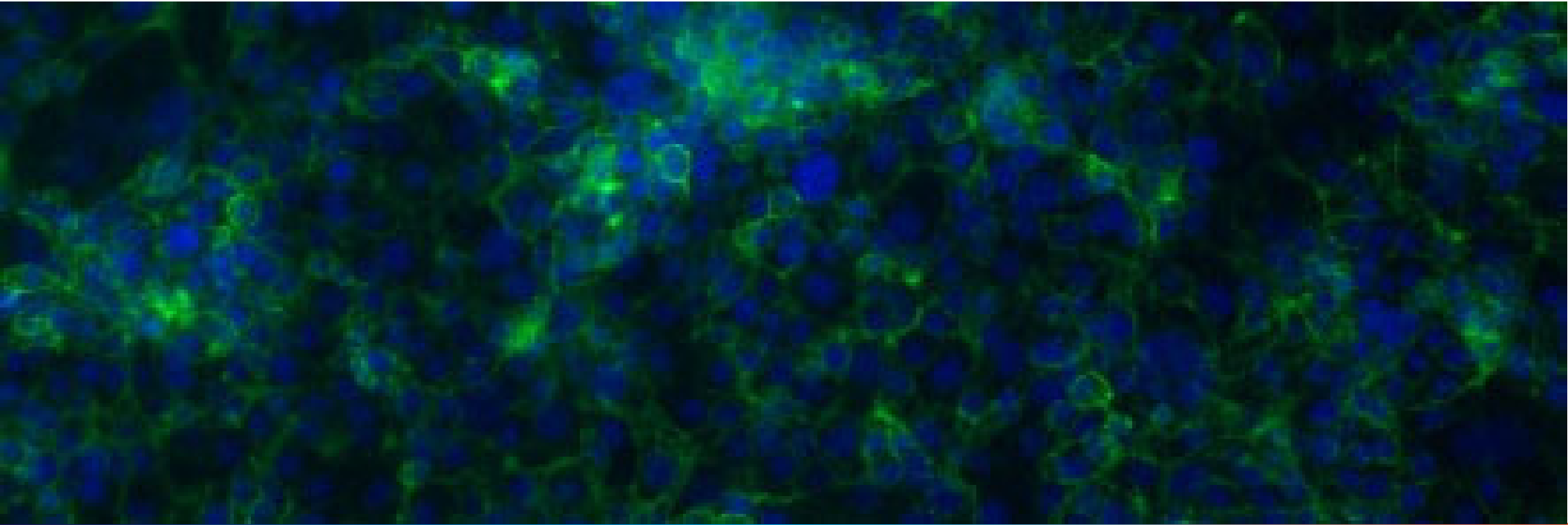## MCF10A EMT cells show a significant increase in motility after EMT induction

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

Approximately 10 fold increase in migration upon EMT induction

Summary/Conclusions

Conclusion

- ATCC scientists have created a number of EMT/MET reporter cell lines using CRISPR/Cas9 gene editing

- The EMT reporter lines have been thoroughly verified and validated at the genomic, transcriptional, and translational levels, as well as with in-depth induction/transition assays and bio-functional characterization

- These cell lines can be used to monitor cellular status changes in real time or as a platform for drug development

www.atcc.org/EMT

100 px

ATCC