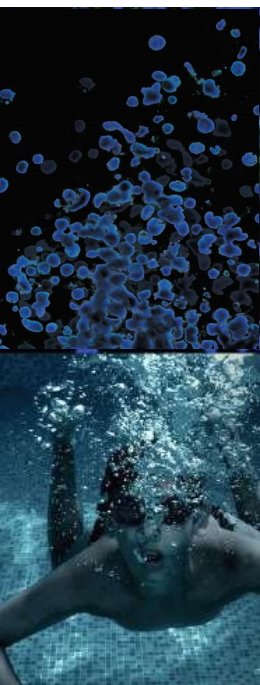# EMT reporter cell lines: Elevating biological models of metastasis

Diana Douglas, BS
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

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
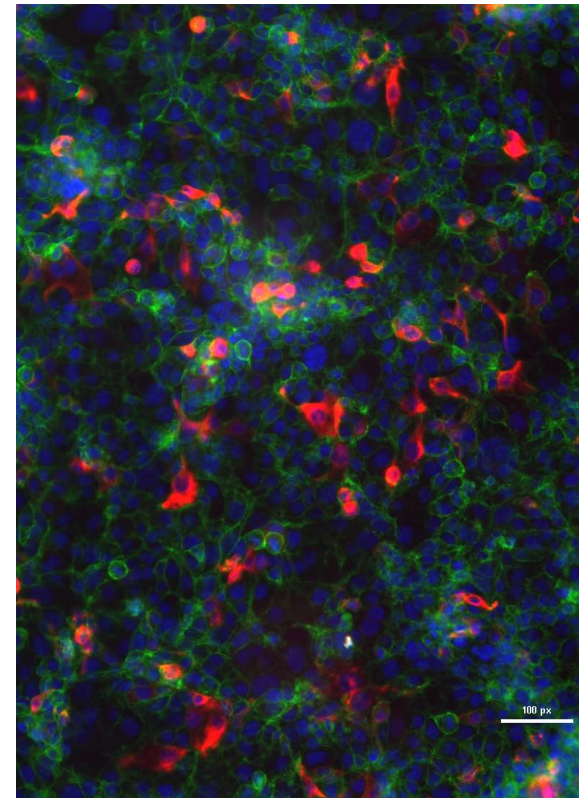
Background – EMT

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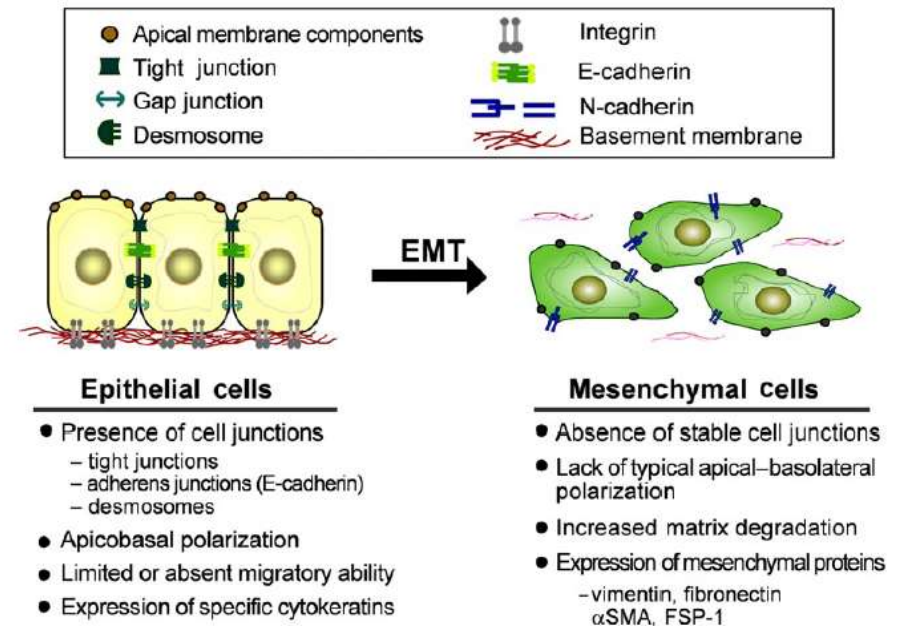
Lee et al, International Review of Cell and Molecular Biology, 2012.

EMT and MET in cancer progression

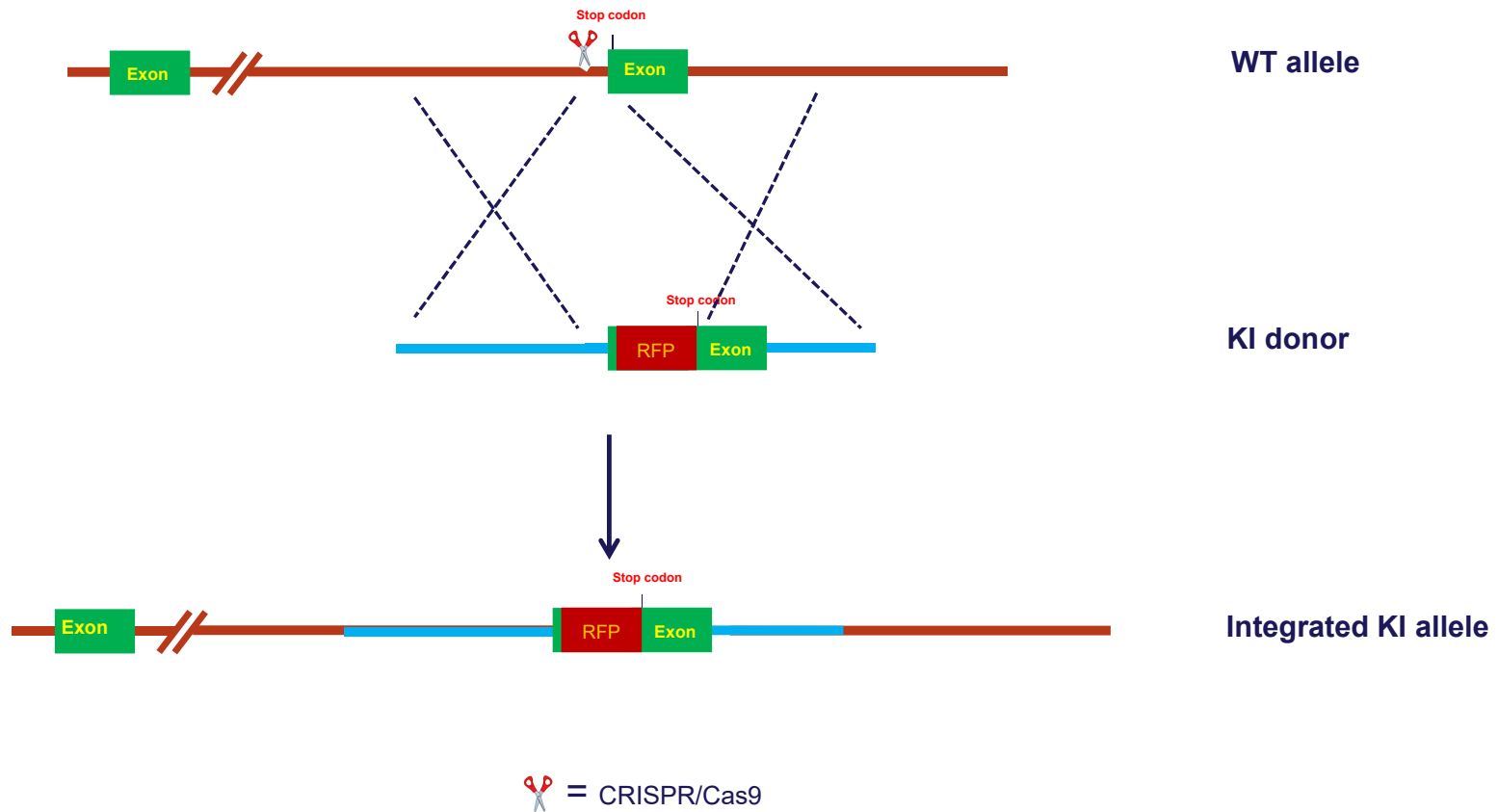http://murraylab.biosciences.uom.org.au/
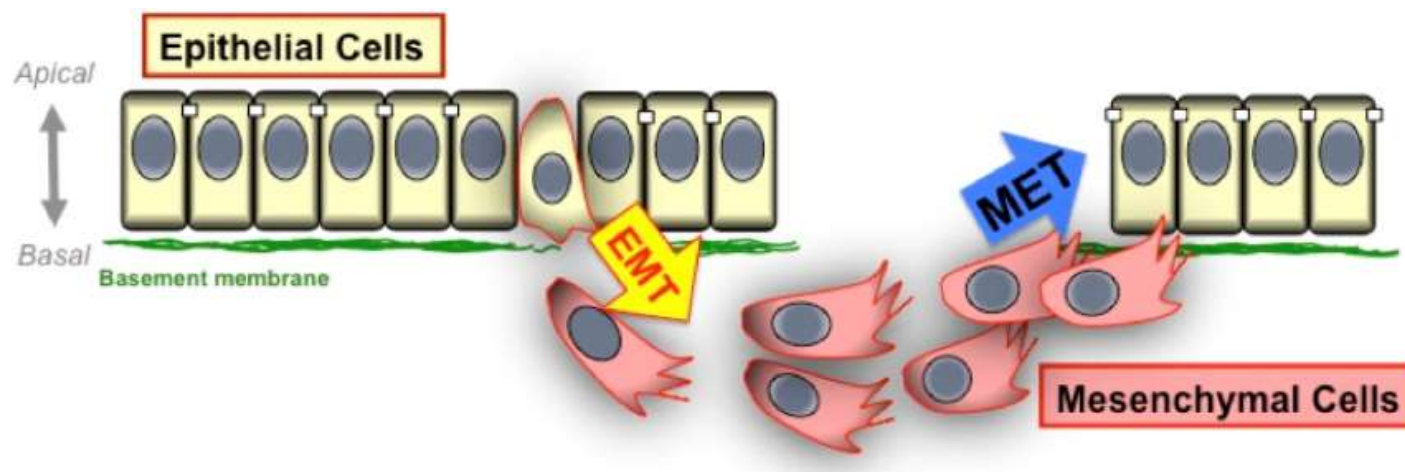
- Epithelial cancer cells reactivate EMT

- The EMT process facilitates metastatic dissemination

- "Partial EMT": the transition is a complex and multistep process
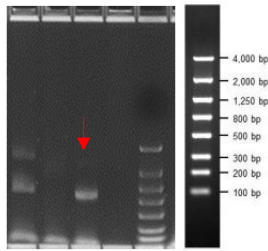
ATCC®

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

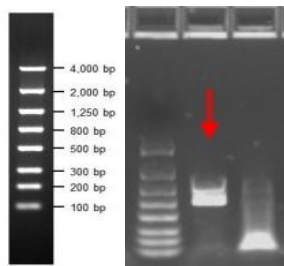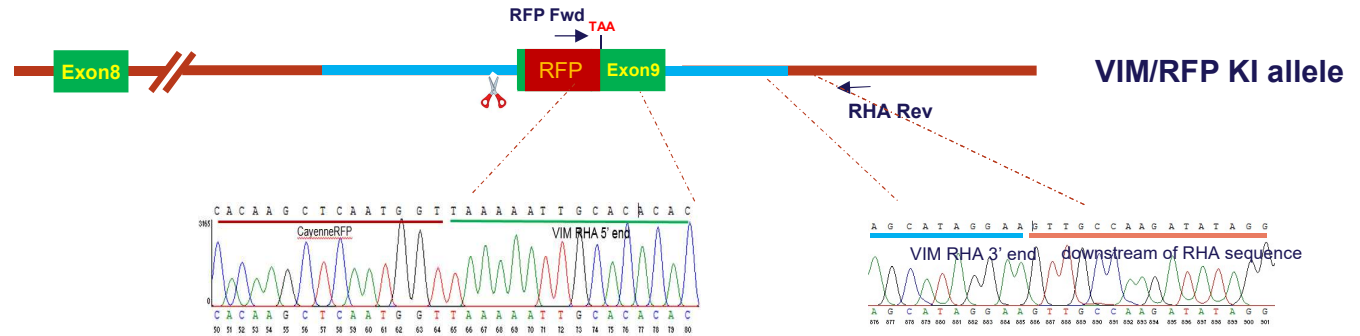| Designation	ATCC® No.	Tissue type/disease	EMT or MET	Marker	Availability
A549 VIM RFP	CCL-185EMT™	Lung cancer	EMT	VIM-RFP	Available
HCT116 VIM RFP	CCL-247EMT™	Colorectal cancer	EMT	VIM-RFP	Available
MDA-MB-231 VIM RFP	HTB-26MET™	Breast cancer	MET	VIM-RFP	Available
BT-474 ECAD EmGFP	HTB-20EMT™	Breast cancer	EMT	ECAD-GFP	Available Q4 2019
PANC-1 ECAD EmGFP	CRL-1649MET™	Pancreatic cancer	MET	ECAD-GFP	Available Q1 2020
MCF10A ECAD EmGFP	CRL-10317EMT™	Breast epithelial cells	EMT	ECAD-GFP	Available Q1 2020

ATCC®

Precision editing to create reporter KI alleles in cancer cell lines

Stop codon

Exon // Exon — WT allele

Stop codon

RFP Exon — KI donor

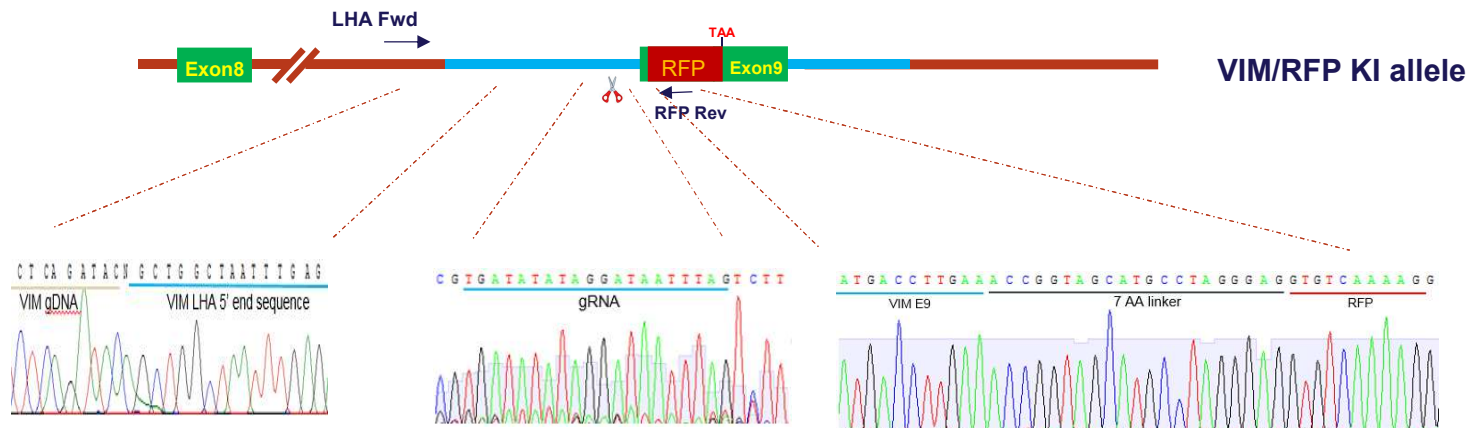Stop codon

Exon // RFP Exon — Integrated KI allele

✂ = CRISPR/Cas9

Knock-in verification at the genomic level

RHA: ~ 1.0 kb
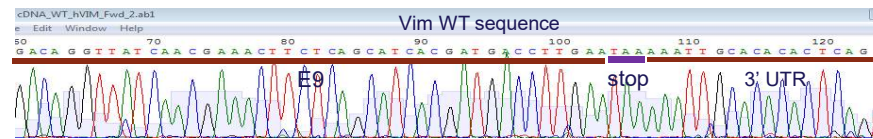
RFP Fwd

Exon8 // RFP Exon9 — VIM/RFP KI allele

RHA Rev

CACAAGCTCAATGGTTAAAAATTGCACACAC
CayenneRFP   VIM RHA 5' end

AGCATAGGAA GTTGCCAAGATATAGG
VIM RHA 3' end   downstream of RHA sequence

LHA Fwd

Exon8 // RFP Exon9 — VIM/RFP KI allele

RFP Rev

CTCAGATACGCTGGCTAATTTGAG
VIM gDNA   VIM LHA 5' end sequence

CGTGATATATAGGATAATTTAGTCTT
gRNA

ATGACCTTGAAACCGGTAGCATGCCTAGGGAGGTGTCAAAAGG
VIM E9   7 AA linker   RFP

LHA: ~ 1.1 kb

8

Knock-in verification at the transcriptional and translational levels

WT transcript

RFP KI transcript

WT transcript

RFP KI transcript

anti-Vimentin

Vimentin-RFP

anti-Vimentin & Vimentin-RFP
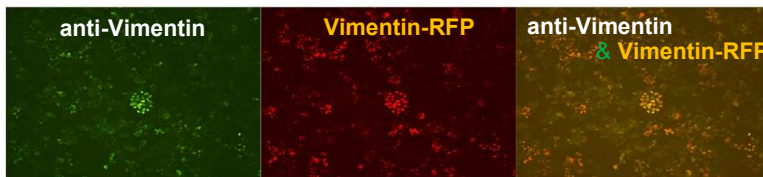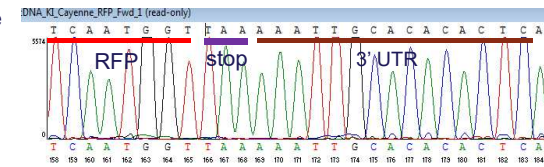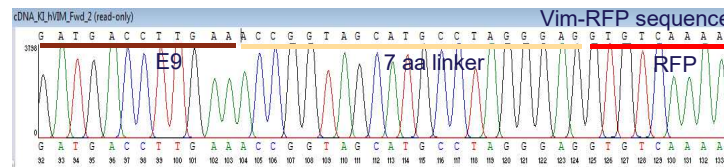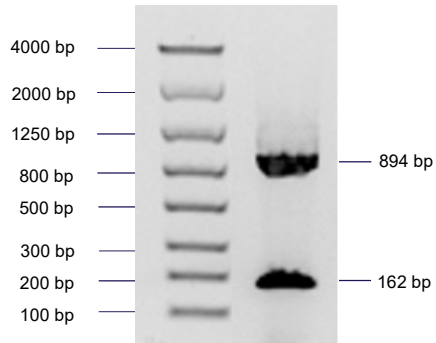
ON/OFF Target	Locus	Sequence	# mismatches	Score	Gene	ontarget	
ON		CTAAATTATCCTATATATCACGG	0	100	None	True	
OT1	chr3:+138434072	CTTGATTATCCTATATATCACGG	2	5.1	None	False	√
OT2	chr4:+26013990	CTAAACTCTGCTATATATCAGGG	3	1.4	None	False	√
OT3	chrX:+98243253	CGTCATTTCCTATATATCAAGG	4	1.3	None	False	√
OT4	chr16:+69339888	AATAATTATGCTATATATCAGGG	4	1.3	None	False	√
OT5	chr4:+160836913	TTTTATTATTCTATATATCATGG	4	1.3	None	False	√
OT6	chr2:-14364834	CTAATATAGCCTATATATCAGGG	3	0.9	None	False	√
OT7	chr10:-15151953	TTAACCTATGCTATATATCACGG	4	0.8	NM_004808	False	√
OT8	chrX:-100600804	TTAACTTACCCTGTATATCAGGG	4	0.4	NM_004085	False	√
OT9	chr7:+91788412	TCCAATTCTCCTATATTTCTTGG	4	0.3	NM_001161528	False	√
OT10	chr14:-103805277	CTAAAGTATCATATATCTAAGG	4	0.1	NM_001969	False	√
OT11	chr17:-6364831	CTACATGATCCTTTACATCACGG	4	0.1	NM_031220	FALSE	√

9
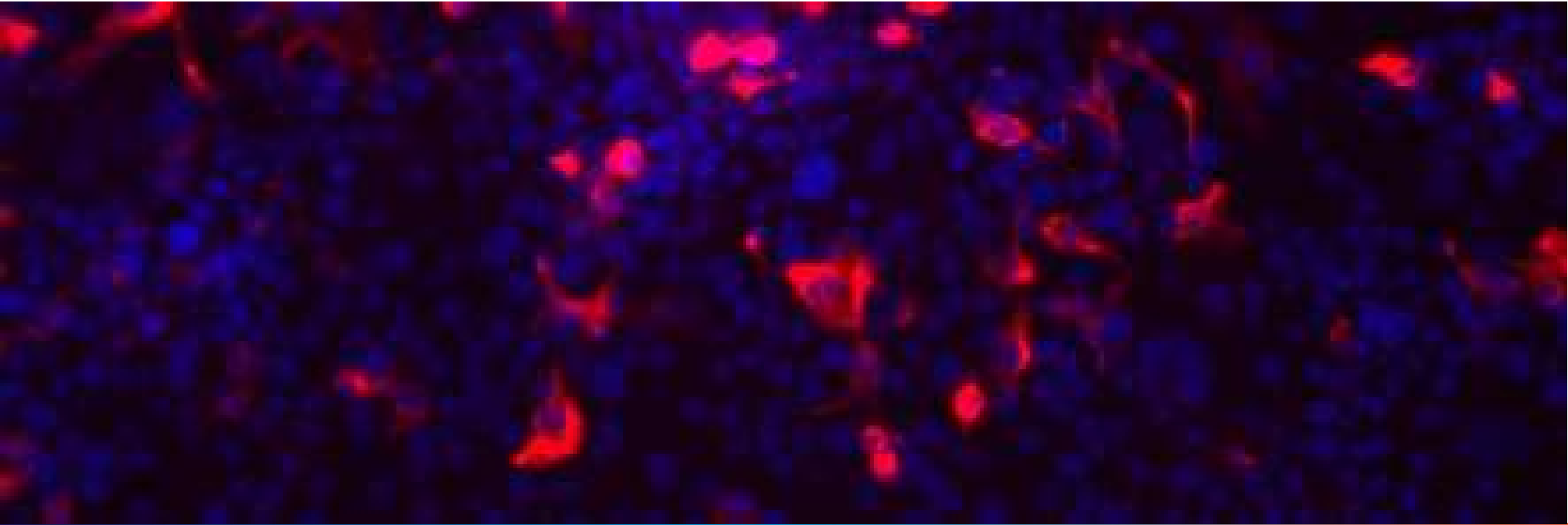
Vimentin-RFP reporter lines

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

ATCC

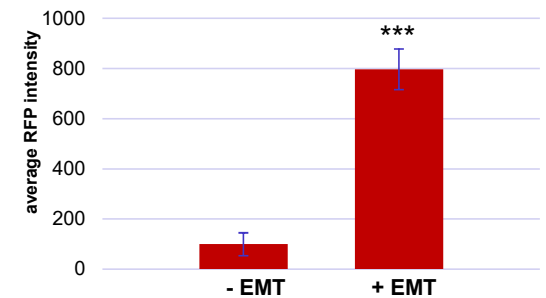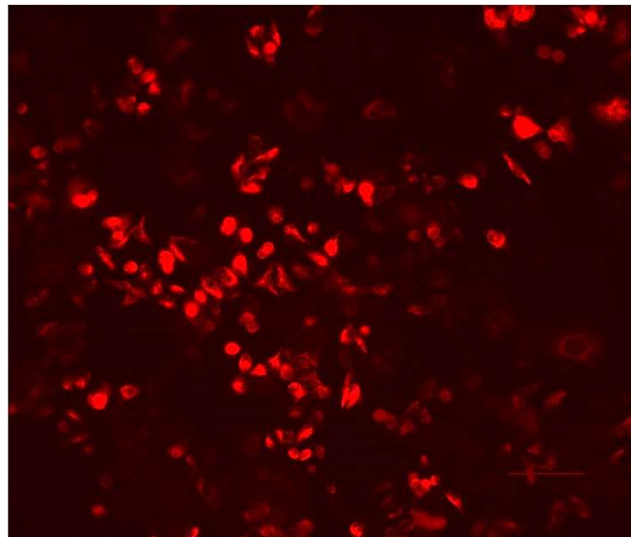# HCT 116 VIM RFP EMT reporter cells display an epithelial-to-mesenchymal morphology change upon induction
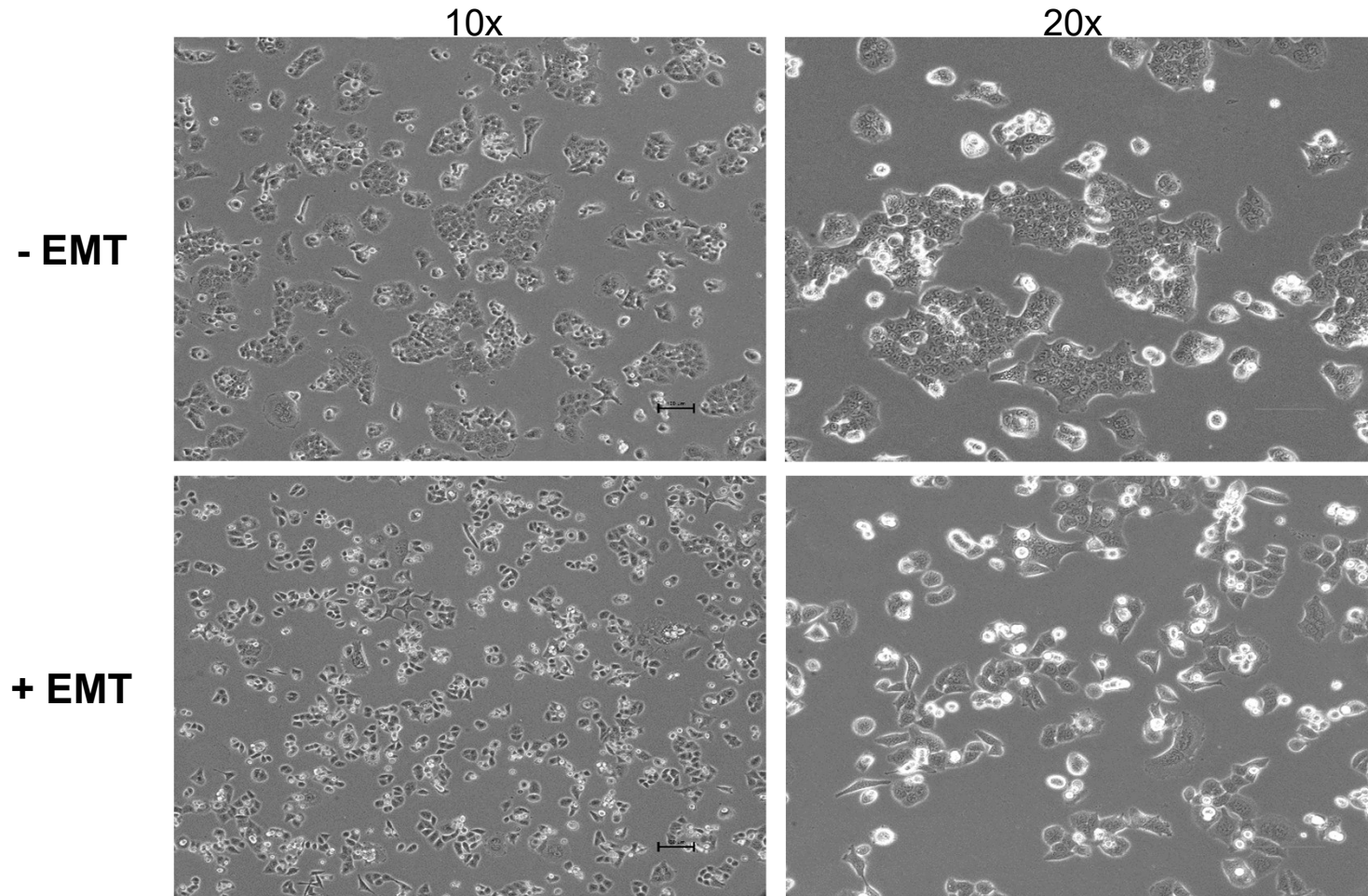
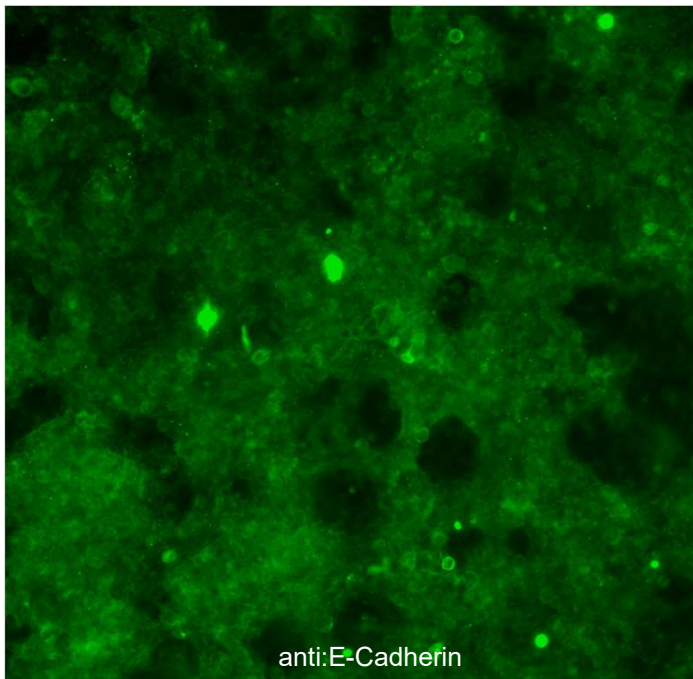# HCT116 VIM RFP reporter cells display an increase in intrinsic vimentin expression
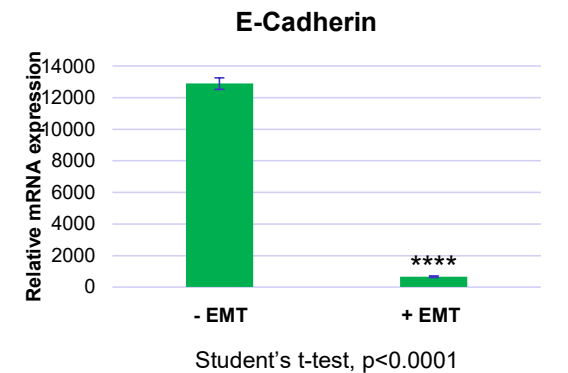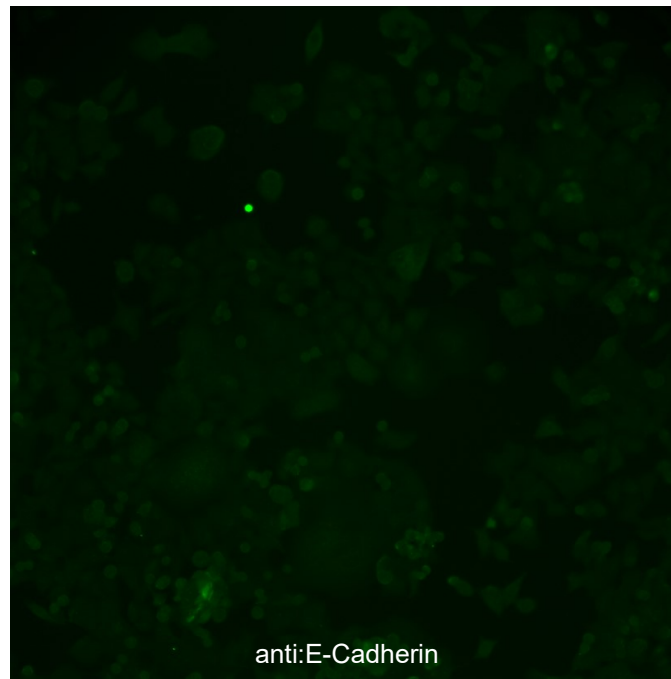
- EMT

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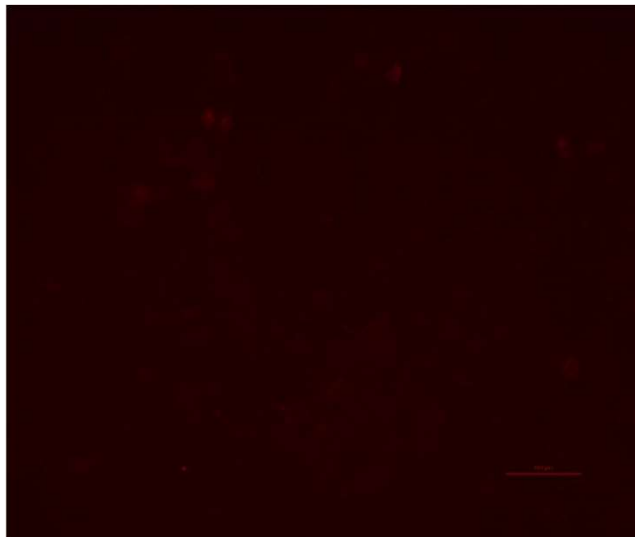
Student's t-test, p<0.001

~ 8.0 fold increase

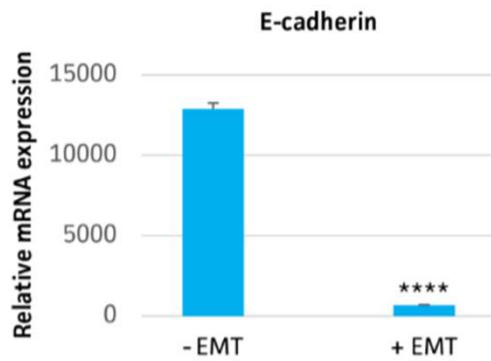# HCT116 VIM RFP reporter cells display a decrease in the epithelial marker E-cadherin

- 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™
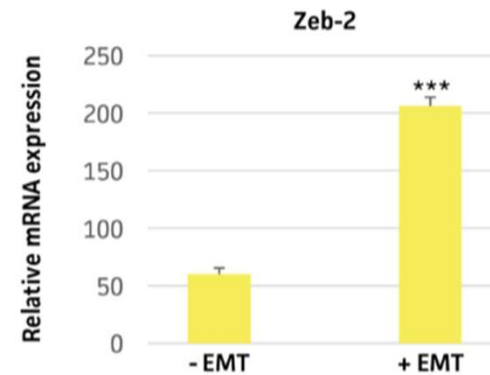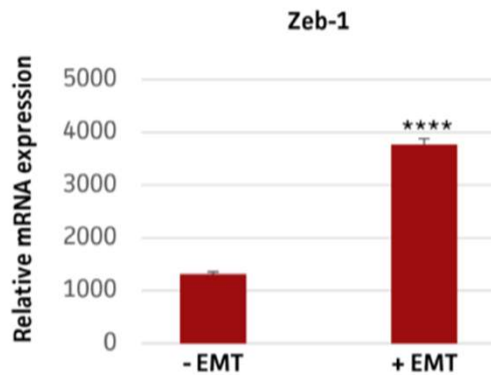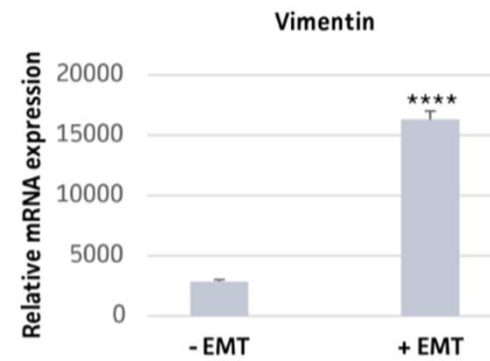
Student's t-test, ***p<0.001, ****p<0.0001

MDA-MB-231VIM RFP MET cells display a morphology change upon MET induction

-MET

+MET

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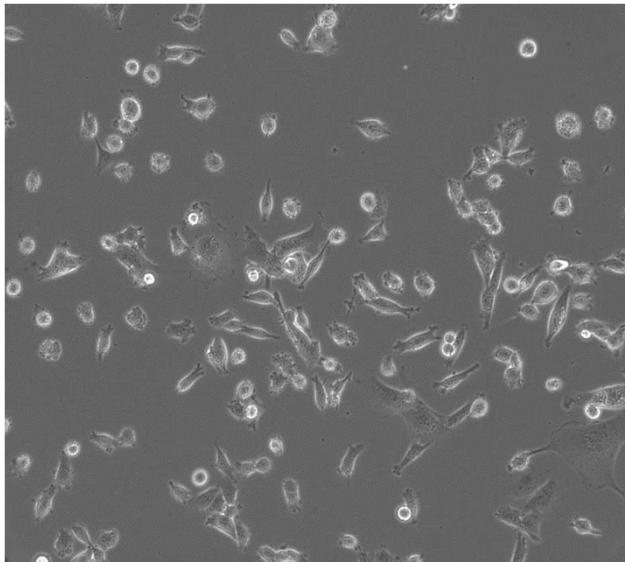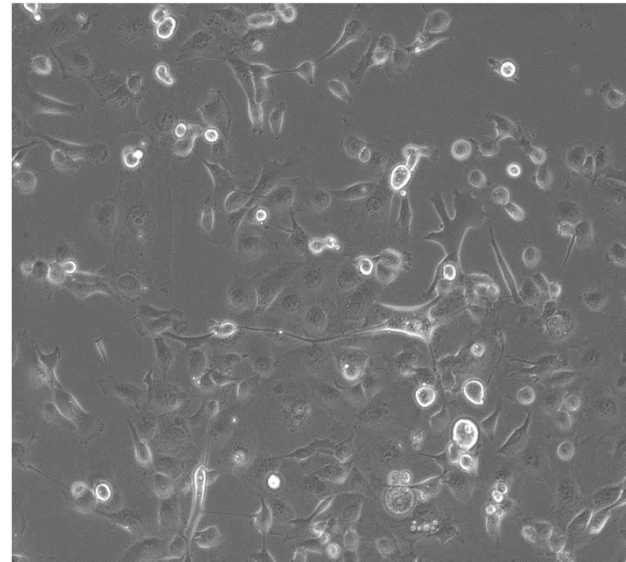
MDA-MB-231 VIM RFP MET cells display a decrease in the intrinsic mesenchymal marker vimentin
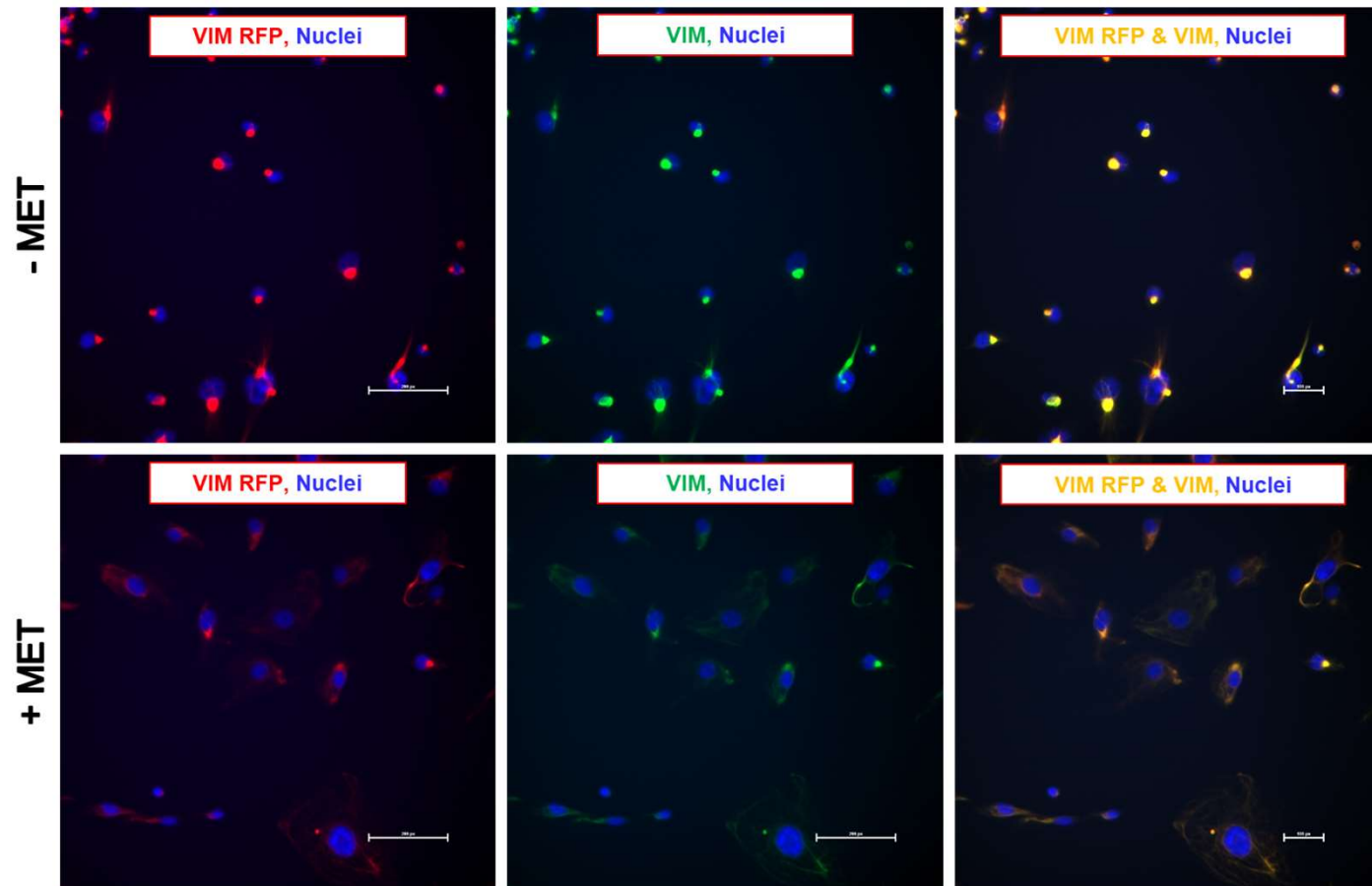
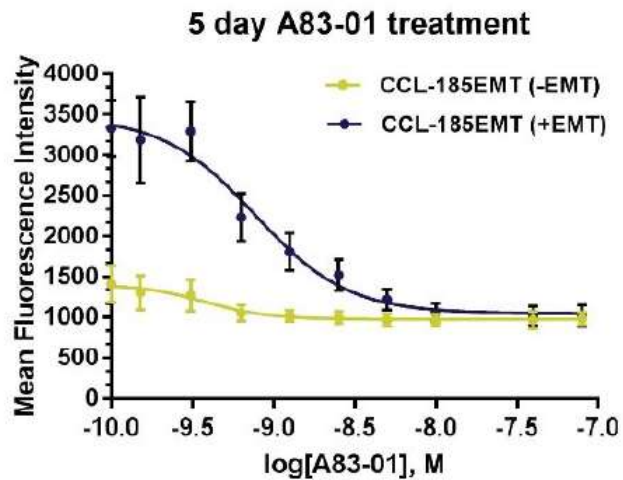# A549 VIM RFP EMT cells display an increase in invasive capacity upon EMT induction

-EMT

+EMT

A549 Invasion
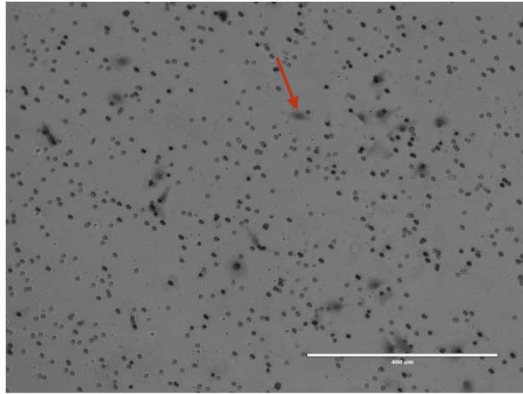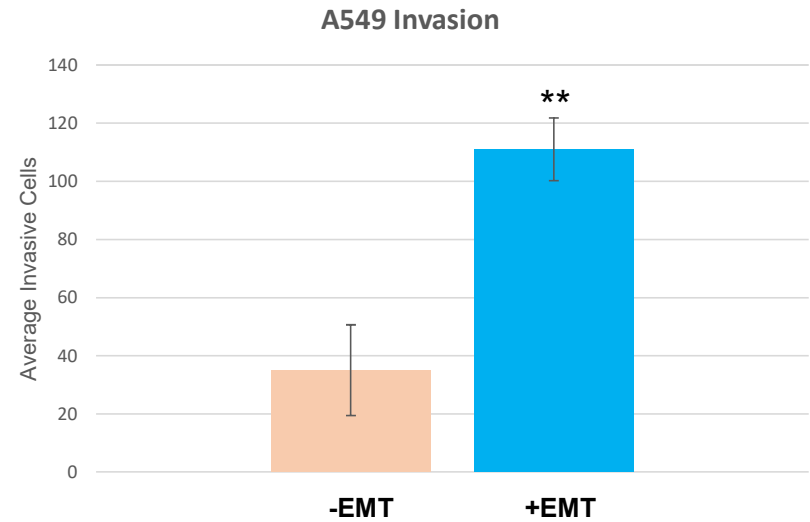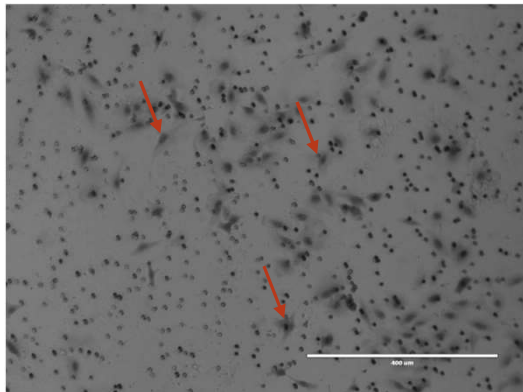
Student's t-test, p<0.01

~3 fold increase in invasive cells upon EMT induction

ATCC

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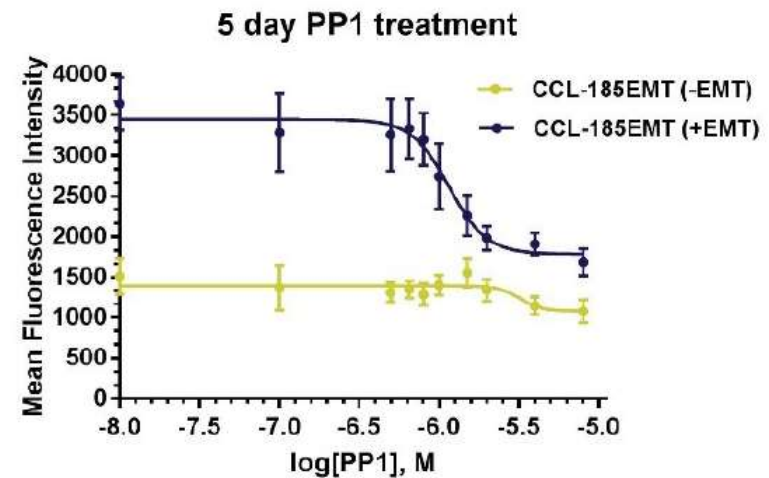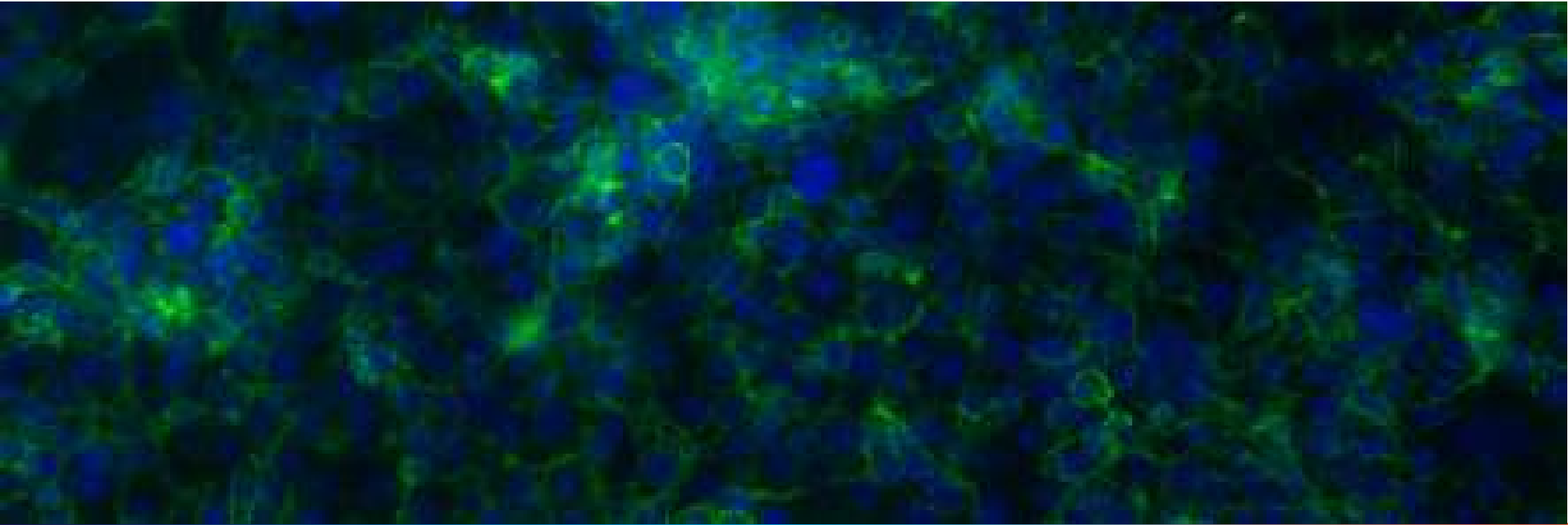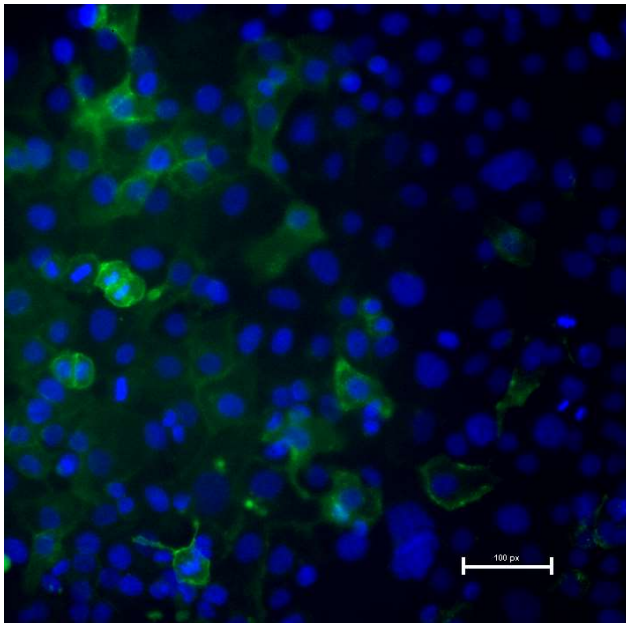
E-cadherin-EmGFP reporter lines

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

ATCC®

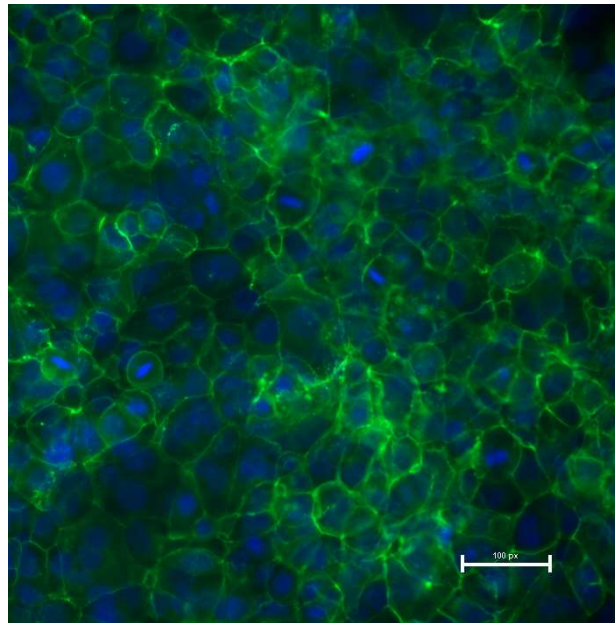# PANC-1 ECAD EmGFP MET reporter cells display a morphology change upon induction
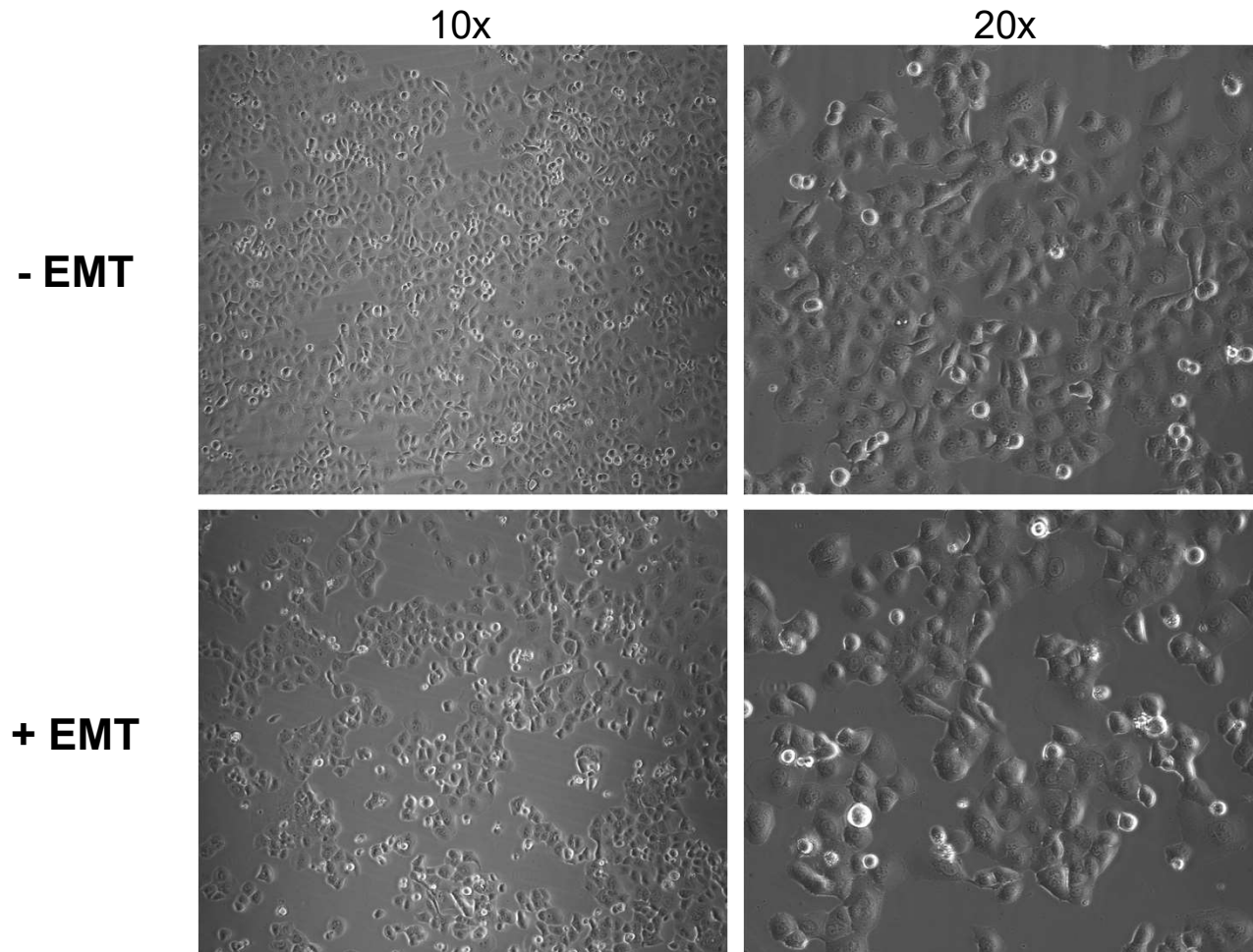
PANC-1 ECAD EmGFP *MET* cells display an increase in intrinsic E-cadherin expression
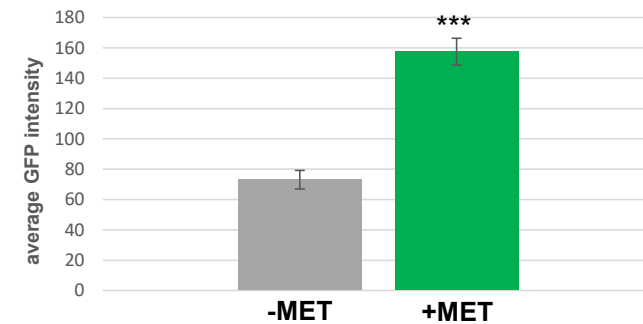
- MET

+ MET (Pre-miR-200)

100 px

100 px

PANC-1 clone B3 ECAD expression

average GFP intensity

180
160
140
120
100
80
60
40
20
0

***

-MET   +MET

Student's t-test, p<0.001

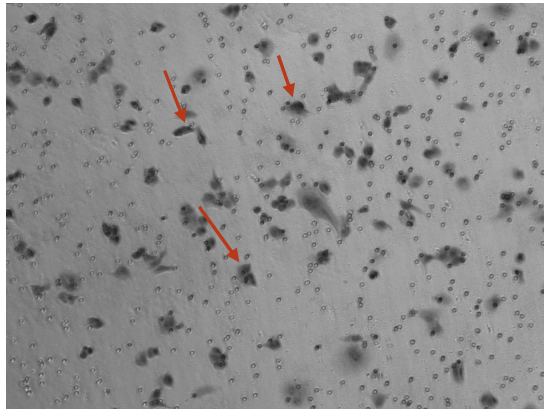**Increase in ECAD expression upon MET induction > 2-fold**

ATCC

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

PANC-1 clone B3 SNAIL expression

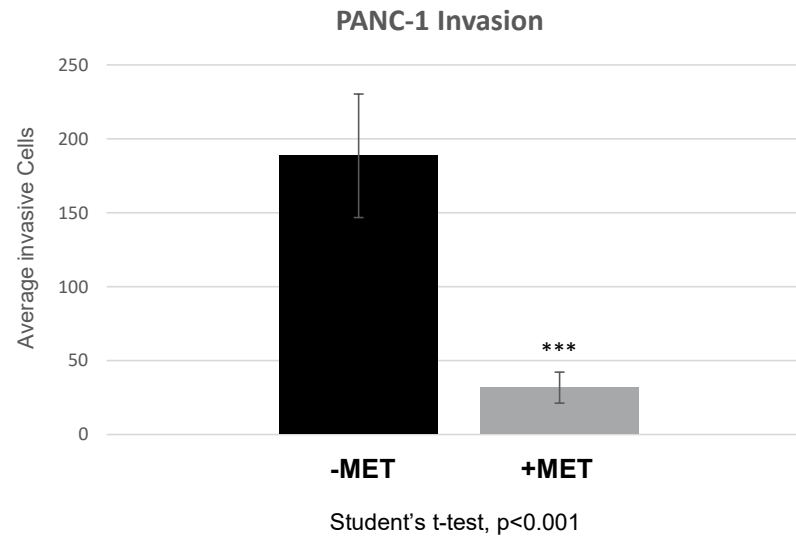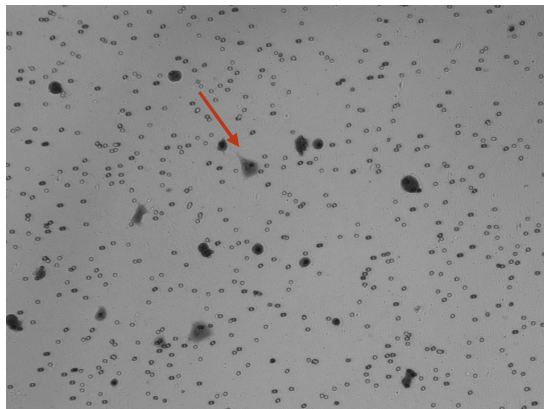Student's t-test, p<0.001

~60% decrease in SNAIL upon induction
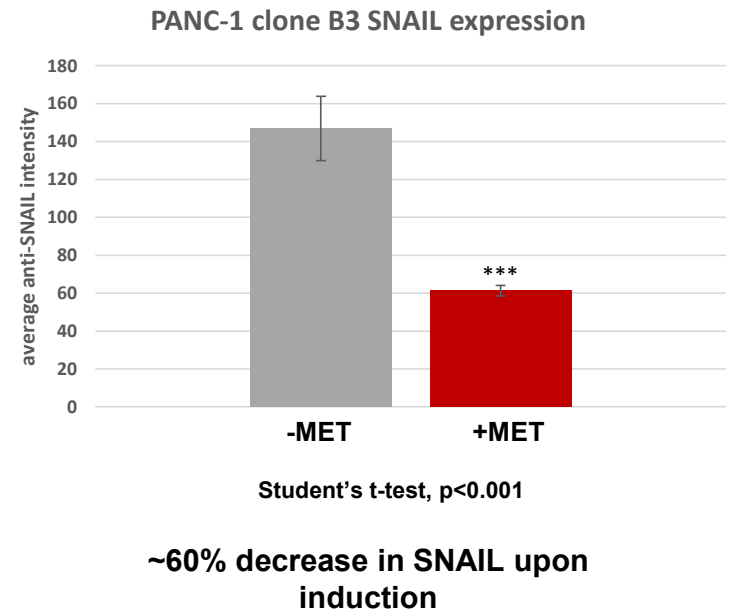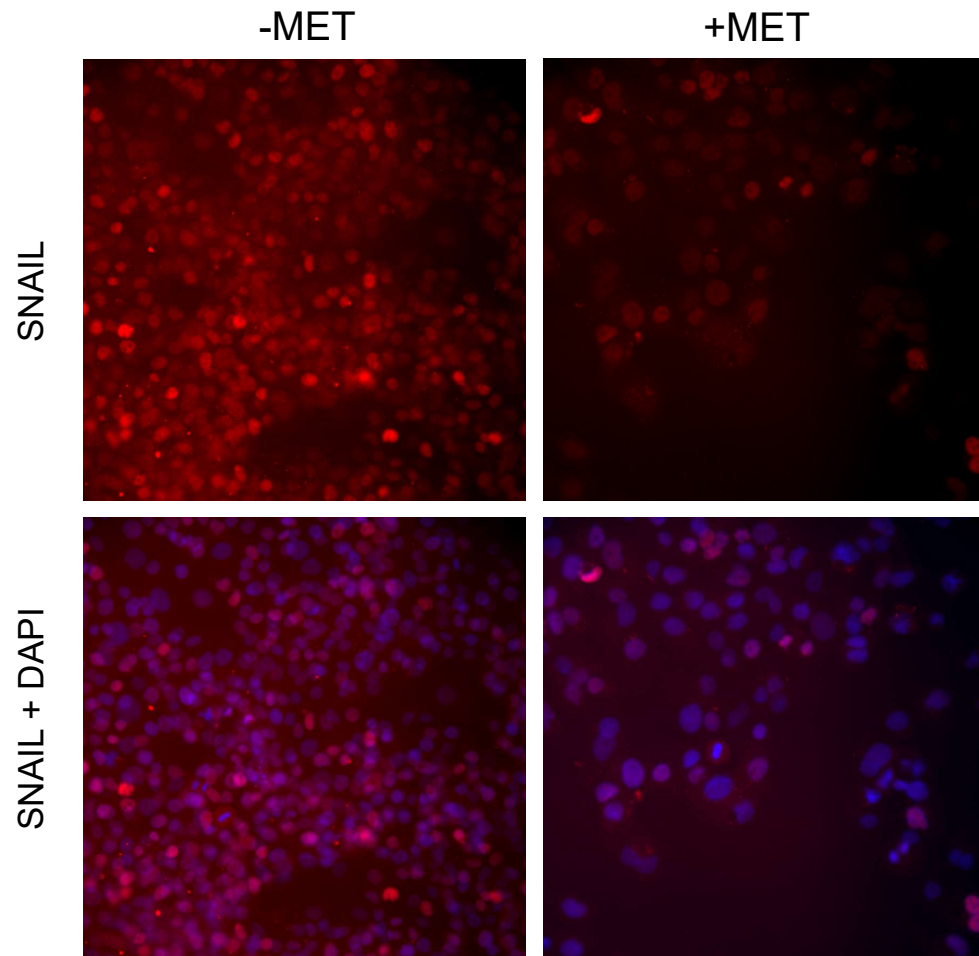
PANC-1 ECAD EmGFP cells show a decreased invasive capacity after EMT

-MET
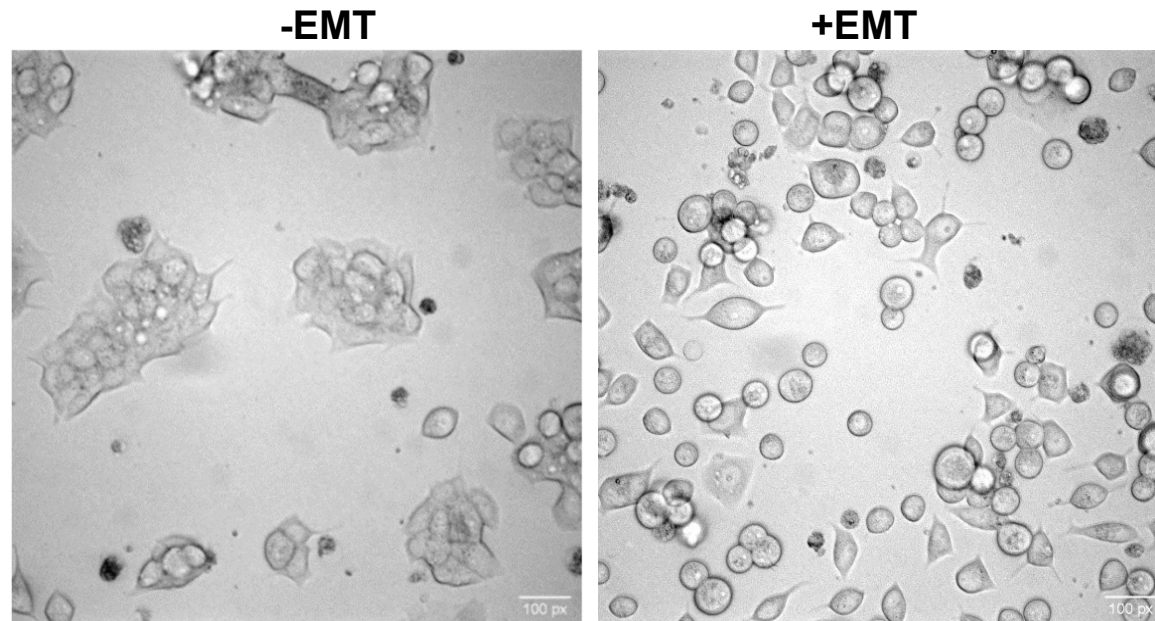
+MET
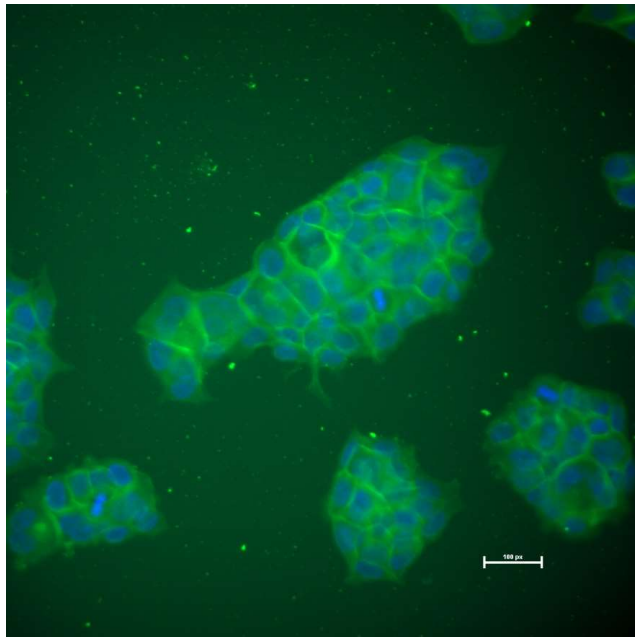
PANC-1 Invasion

Student's t-test, p<0.001

~6 fold decrease in invasive cells upon MET induction

ATCC®

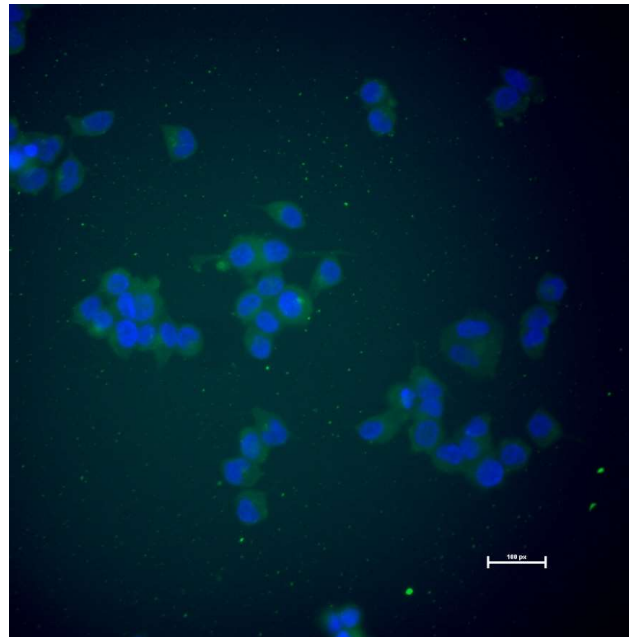# BT-474 ECAD EmGFP EMT cells display a morphology change upon EMT induction

-EMT

+EMT

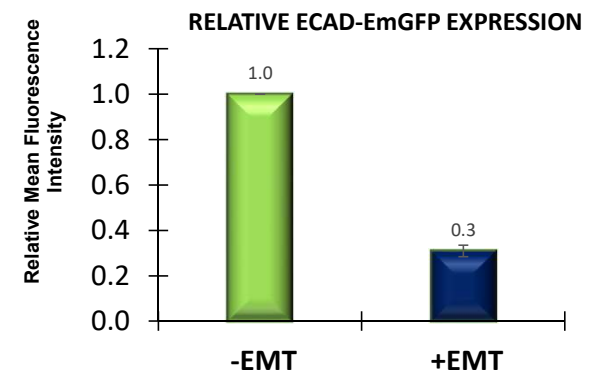# BT-474 ECAD EmGFP EMT display a decrease in endogenous E-cadherin expression
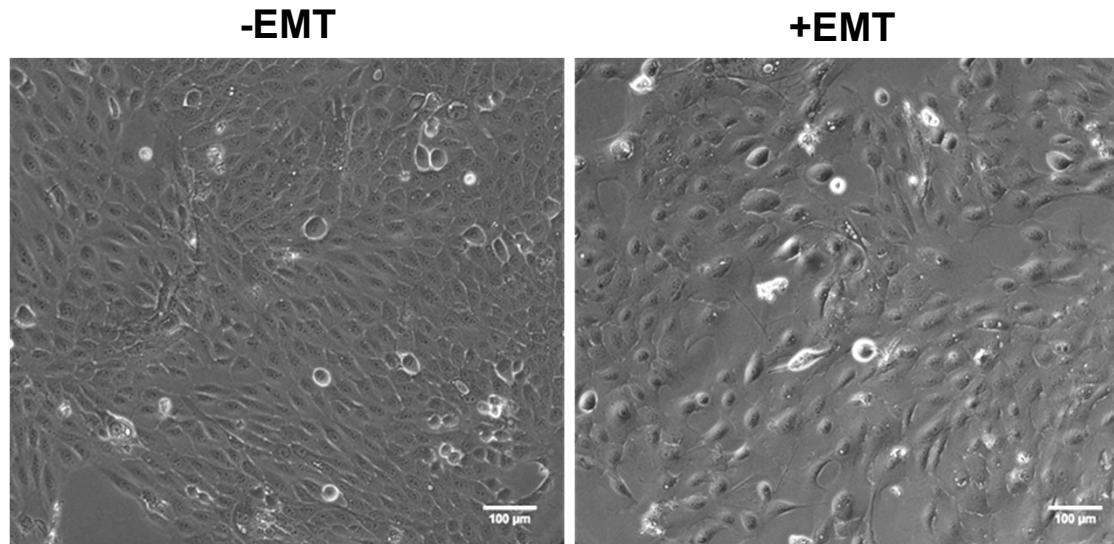
-EMT
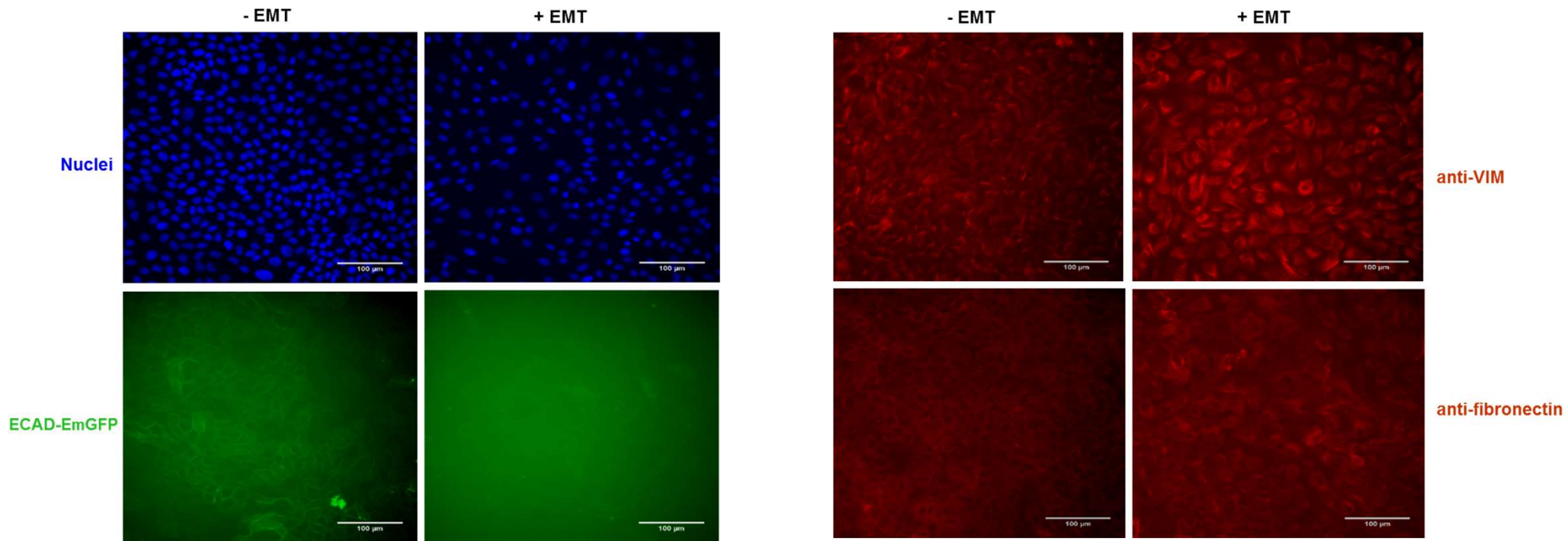
+EMT

ECAD, Nuclei

ECAD, Nuclei

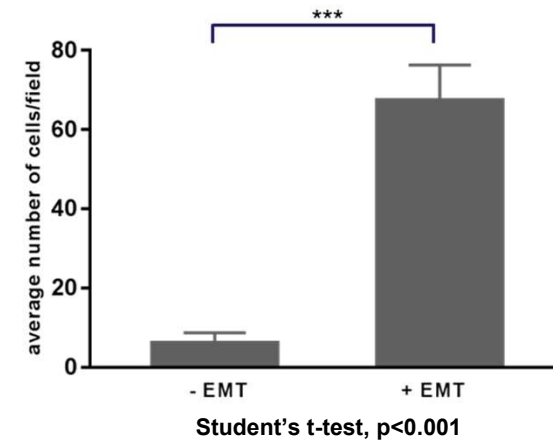**RELATIVE ECAD-EmGFP EXPRESSION**
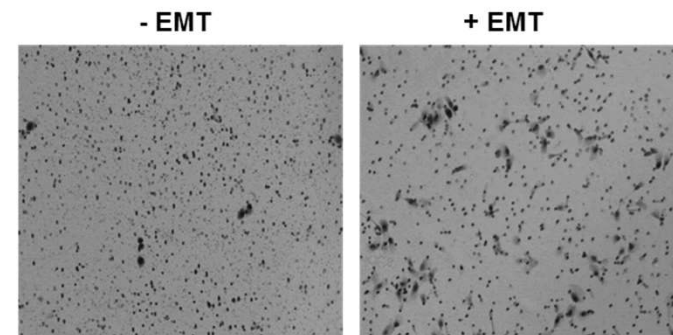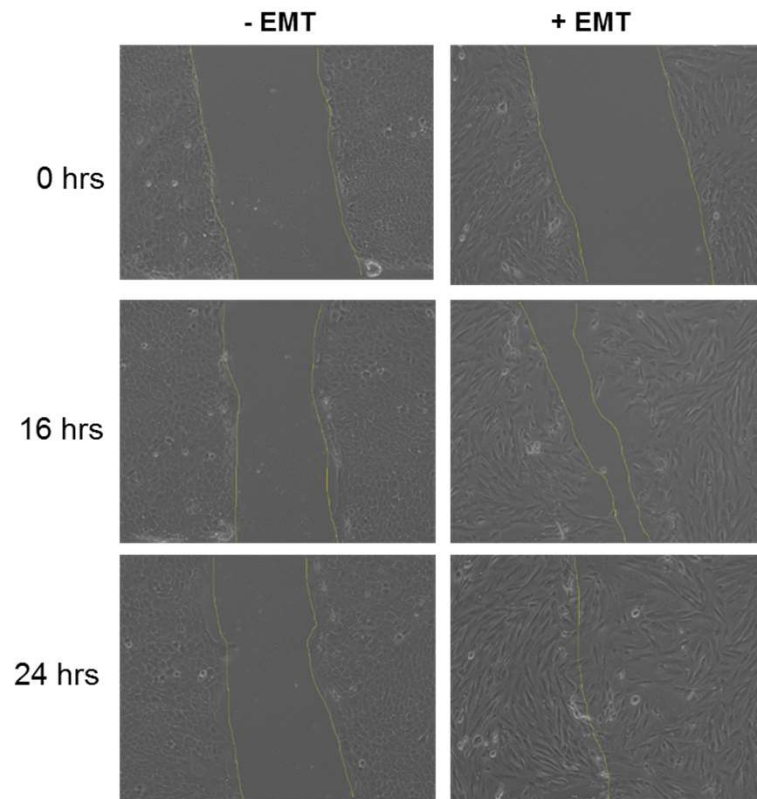
ATCC

MCF10A ECAD EmGFP EMT cells display a morphology change upon EMT induction

-EMT   +EMT

MCF10A ECAD EmGFP cells display both epithelial and mesenchymal marker changes

MCF10A ECAD EmGFP EMT cells show a significant increase in motility in a wound healing assay

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
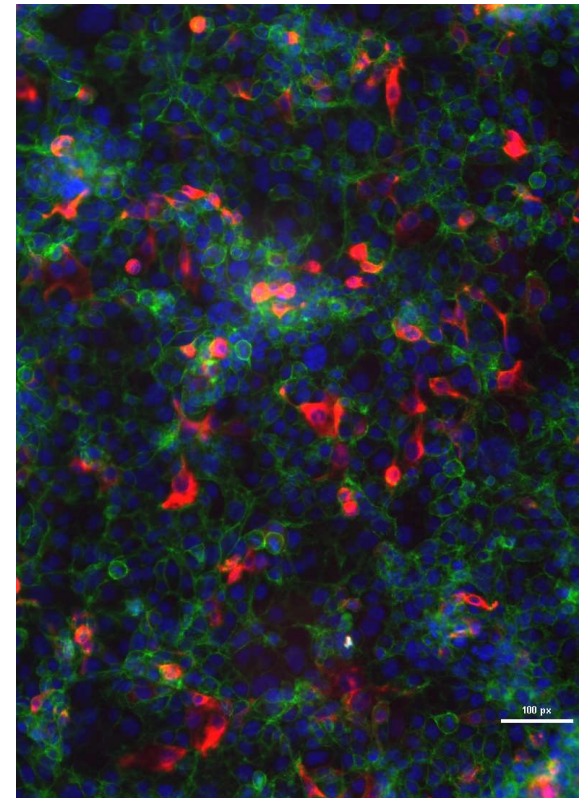
Cultivating collaboration to support global health

Go to www.atcc.org/EMT for more information

Upcoming webinars:

- **On the edge of the bubble: Use of exosomes as reference materials in biomedical research**
  October 31, 12:00 ET

- **Prevent Analysis Variability by Using Reference-quality Microbial Genomes — Shift from Consensus to Discernible**
  November 14, 12:00 ET

www.atcc.org/webinars