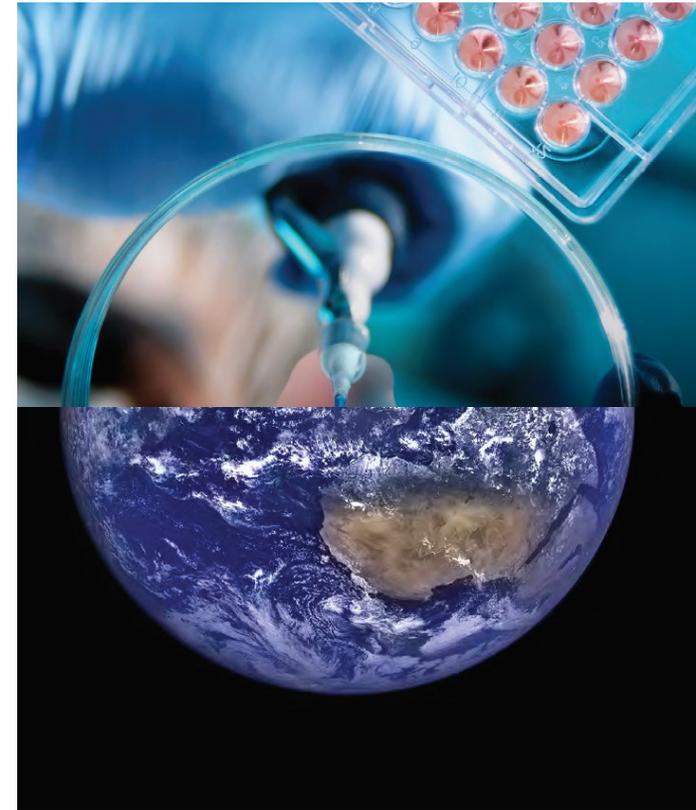
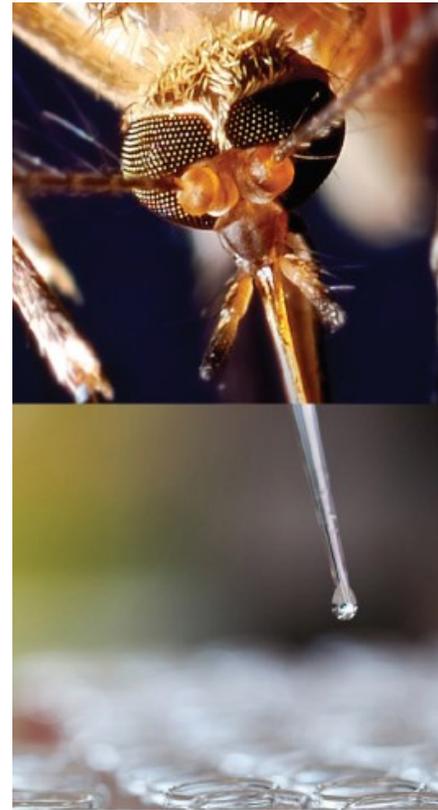
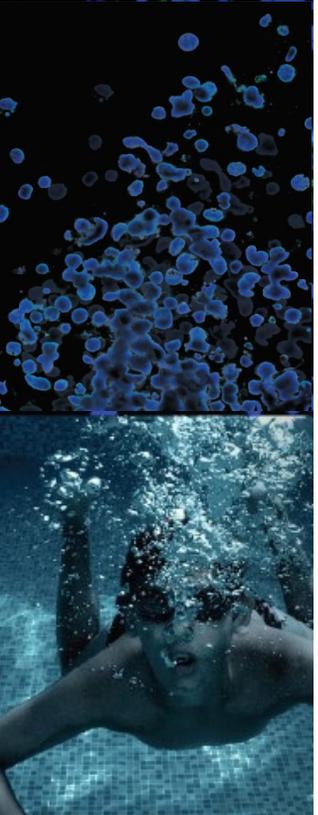




# Novel Epithelial-to-Mesenchymal Transition Reporter Cell Lines Created by CRISPR Technology

Weiguo Shu, Ph.D.  
Senior Scientist, ATCC

Credible Leads to Incredible™



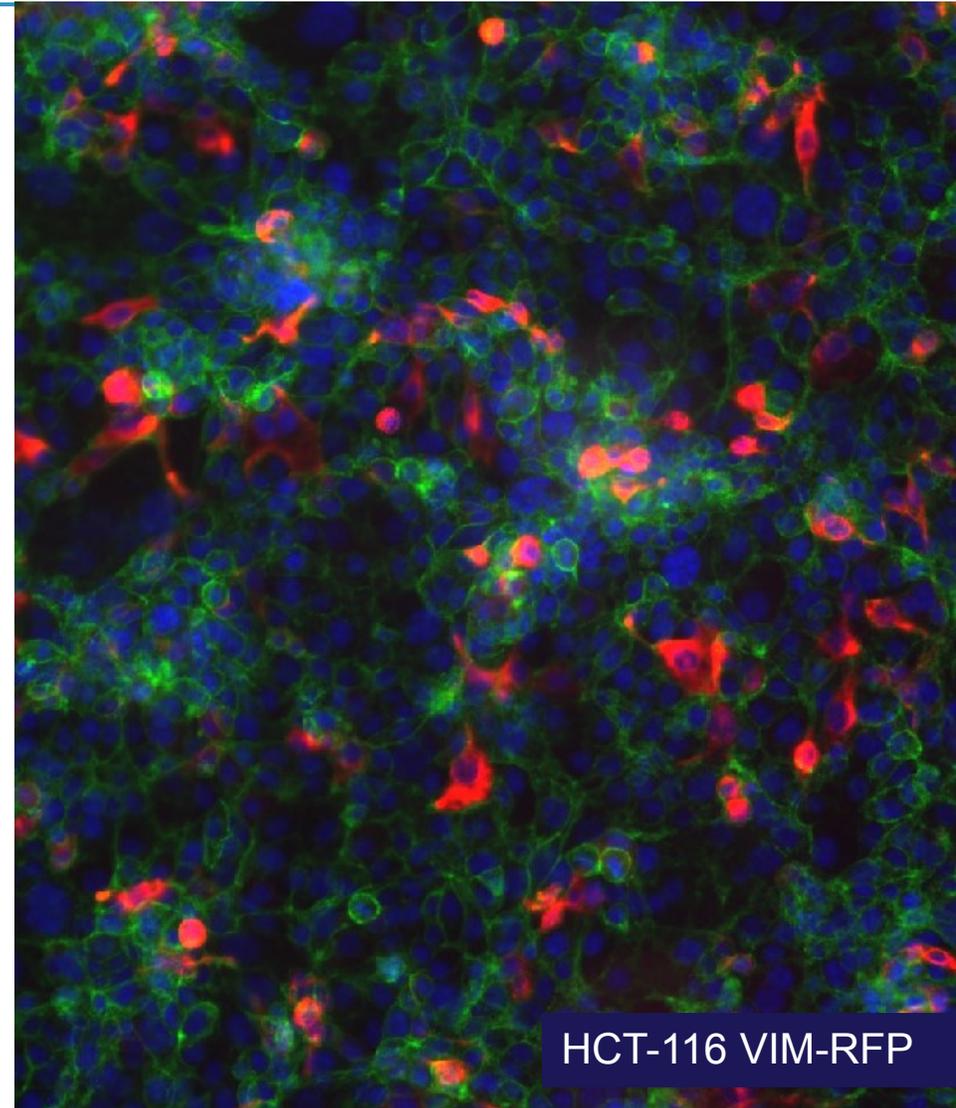
# About ATCC

- 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 premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 15 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees



# Agenda

- EMT background
- Current EMT reporter cell lines
- Generation and validation of CRC HCT-116 VIM-RFP (ATCC® CCL-247EMT™) reporter line
- Generation and validation of NSCLC A549 VIM-RFP (ATCC® CCL-185EMT™) reporter line
- Summary



# Epithelial-to-mesenchymal transition (EMT) key characteristics

Reversible biological process; allows for the trans-differentiation of epithelial cells

- Adoption of the phenotype of mesenchymal cells

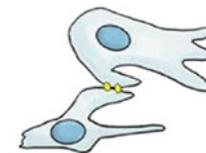
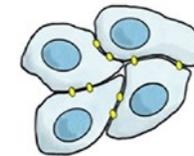
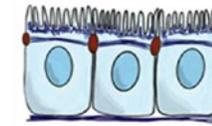
Cancer epithelial cells undergoing EMT:

- Display an array of dynamic states  
“partial EMT”

EMT is involved in pathological processes

- Metastasis
- Chemo-resistance

EMT is a clinically relevant target for the treatment of cancer and overcoming drug resistance



## Epithelial features

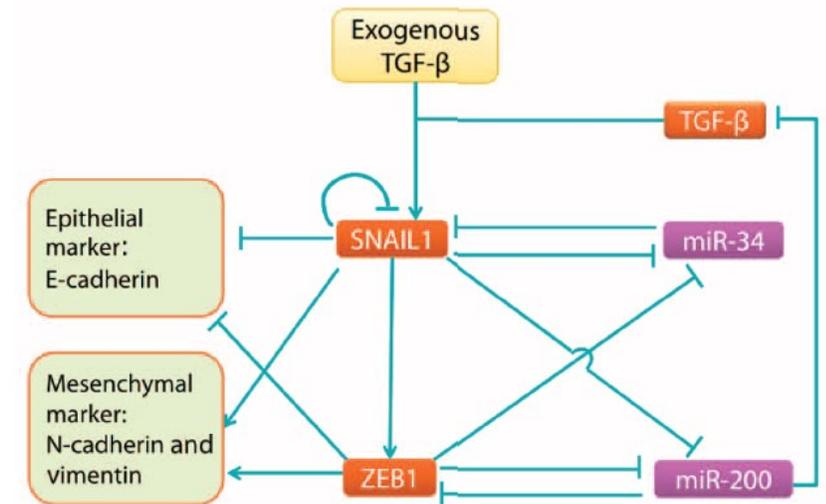
- Regular columnar morphology
- High degree of cell adhesion
- Cell relatively static

## Mesenchymal features

- Irregular rounded or elongate morphology
- Loss of apico-basal polarity
- Cells highly motile

# EMT complex regulatory networks

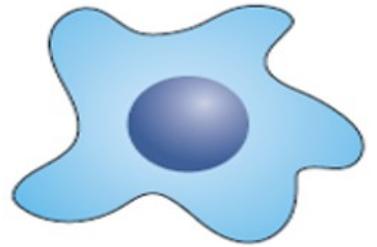
- Signaling pathways/growth factors: TGF $\beta$
- EMT transcription factors: Twist, Snail1/2, Zeb1/2
- Non-coding micro-RNAs: miR-200
- Epigenetic modifiers: Histone demethylase PHF2



Zhang J, et al. *Sci Signal* 7(345):ra91. doi: 10.1126/scisignal.2005304, 2014

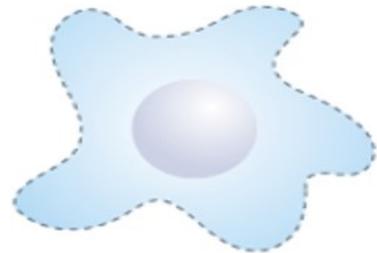
# Strategies for screening compounds targeting EMT

## Targeting mesenchymal tumor cells



Mesenchymal tumor cell

Active compounds

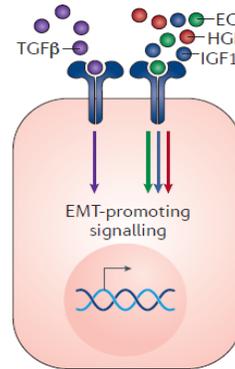


Depletion of functional inhibition

### Readout:

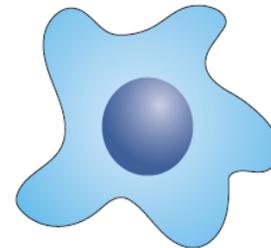
- Induction of apoptosis or cell death
- Inhibition of migration/invasion
- Inhibition of stemness/induction of differentiation
- Reversal of chemo-resistance

## Inhibiting EMT induction



Epithelial tumor cell

Active compounds

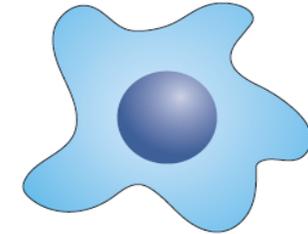


Mesenchymal tumor cell

### Readout:

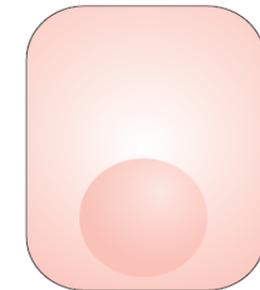
- Preservation of epithelial markers (E-cadherin)
- Inhibition of mesenchymal markers (vimentin, N-cadherin)
- Prevention of chemo-resistance

## Promoting MET



Mesenchymal tumor cell

Active compounds



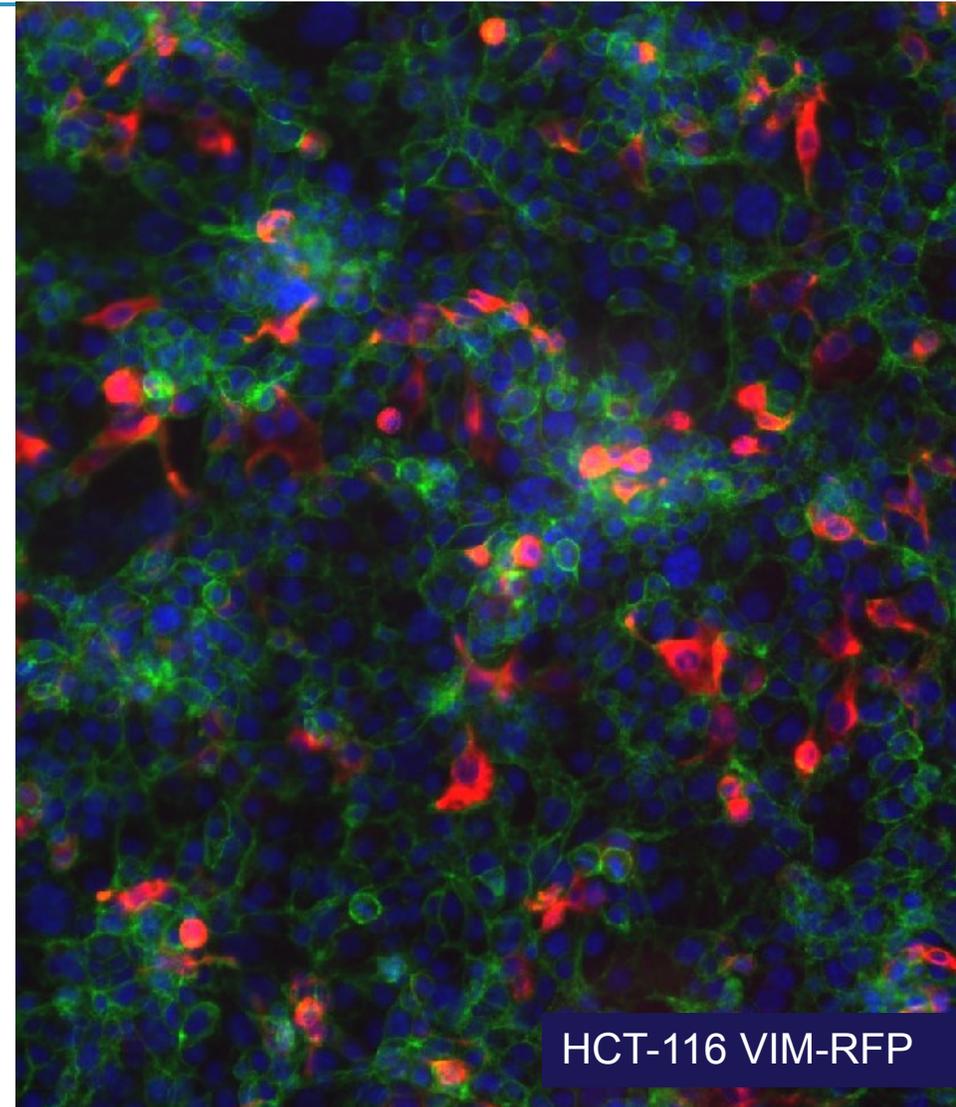
Epithelial tumor cell

### Readout:

- Induction of epithelial markers (E-cadherin)
- Downregulation of mesenchymal markers (vimentin, N-cadherin)
- Reversal of chemo-resistance

# Agenda

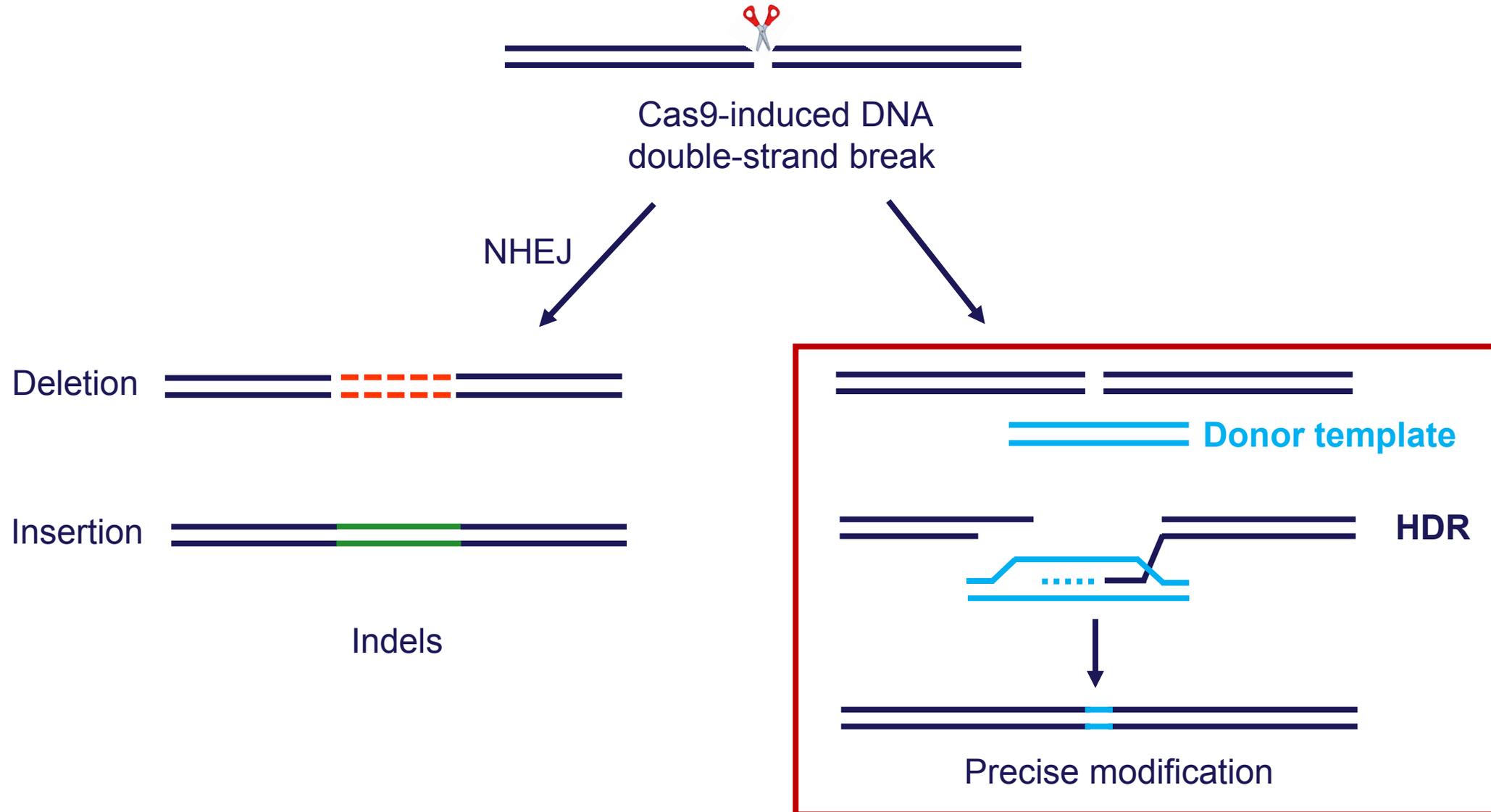
- EMT background
- Current EMT reporter cell lines
- Generation and validation of CRC HCT-116 VIM-RFP (ATCC<sup>®</sup> CCL-243EMT<sup>™</sup>) reporter line
- Generation and validation of NSCLC A549 VIM-RFP (ATCC<sup>®</sup> CCL-185EMT<sup>™</sup>) reporter line
- Summary



# Current EMT reporter cell lines used in high-throughput screening

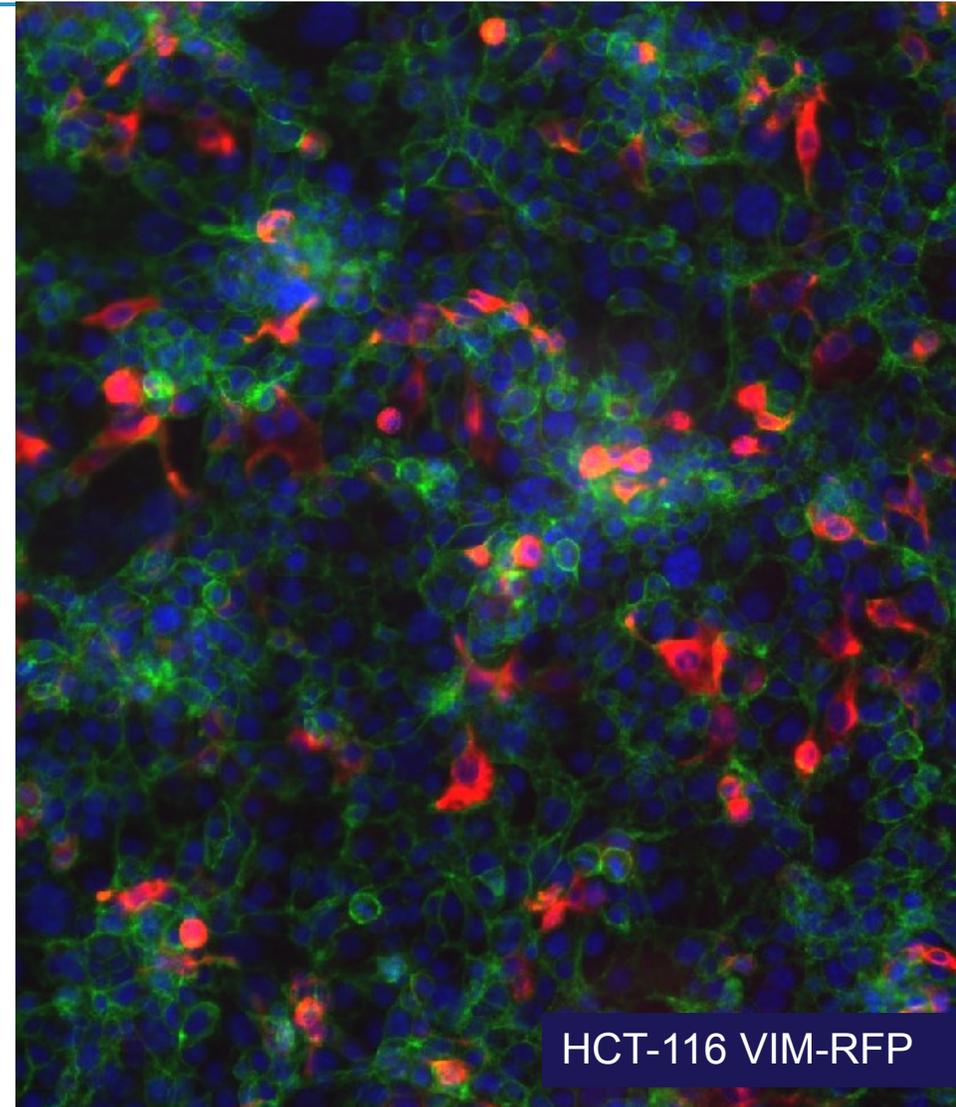
Cell/Cell Line	Phenotype	Cancer/Tissue	Mechanisms	Readout	References
HMLER <sup>shEcad</sup>	Mesenchyme (induced)	Breast (immortalized cells)	Cytotoxicity	Viability	Gupta PB, <i>et al.</i> Cell, 138(4): 645–659, 2009.
NBT-II	Mesenchyme (induced)	Bladder cancer	Inhibiting EMT	Migration	Chua KN, <i>et al.</i> PLoS ONE, 7(3): e33183, 2012.
PANC-1	Mesenchyme	Pancreatic cancer	Promoting MET	ECAD expression	Polireddy K, <i>et al.</i> PLoS ONE 11(10): e0164811, 2016.
MDA-MB-231	Mesenchyme	Breast cancer	Promoting MET	<b>VIM-LUC</b>	Li Q, <i>et al.</i> J Biomol Screen 16(2): 141-54, 2011.
HMLE(N8)	Mesenchyme	Breast (immortalized cells)	Promoting MET	<b>ECAD-LUC</b>	Pattabiraman DR, <i>et al.</i> Science 351 (6277): aad3680, 2016.
SKOV3	Mesenchyme (partial)	Ovarian cancer	Promoting MET	<b>ECAD-LUC</b>	Tang HM, <i>et al.</i> Cell Death Discov 13(2): 16041, 2016.

# Generation of targeted knock-in by CRISPR technology

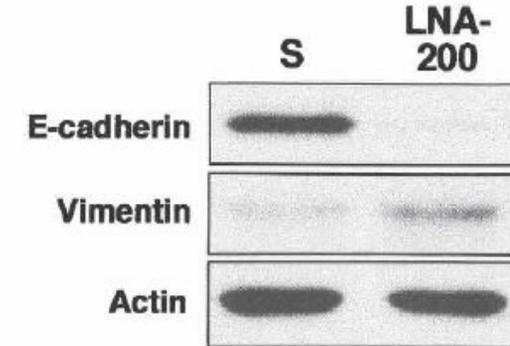
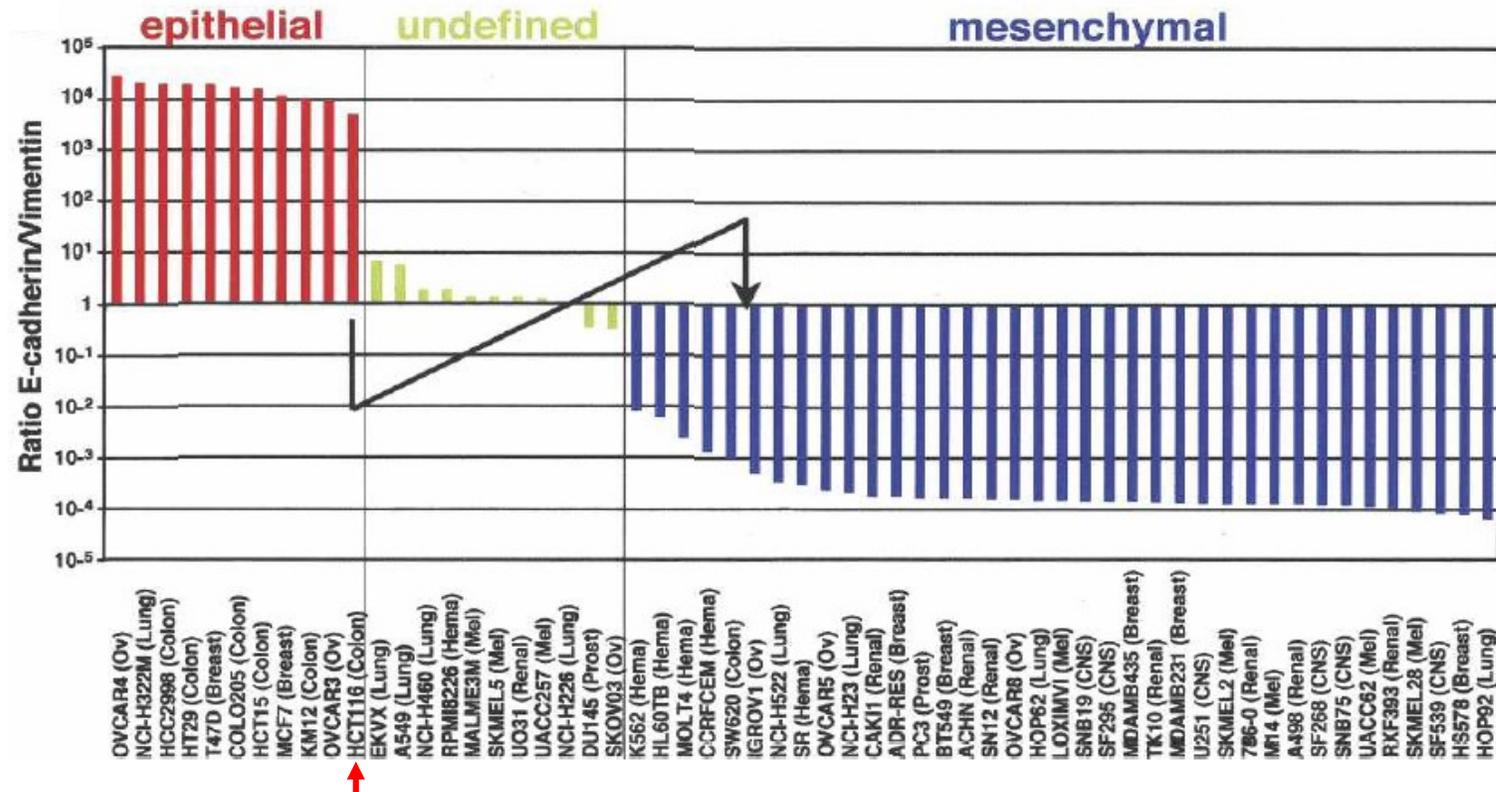


# Agenda

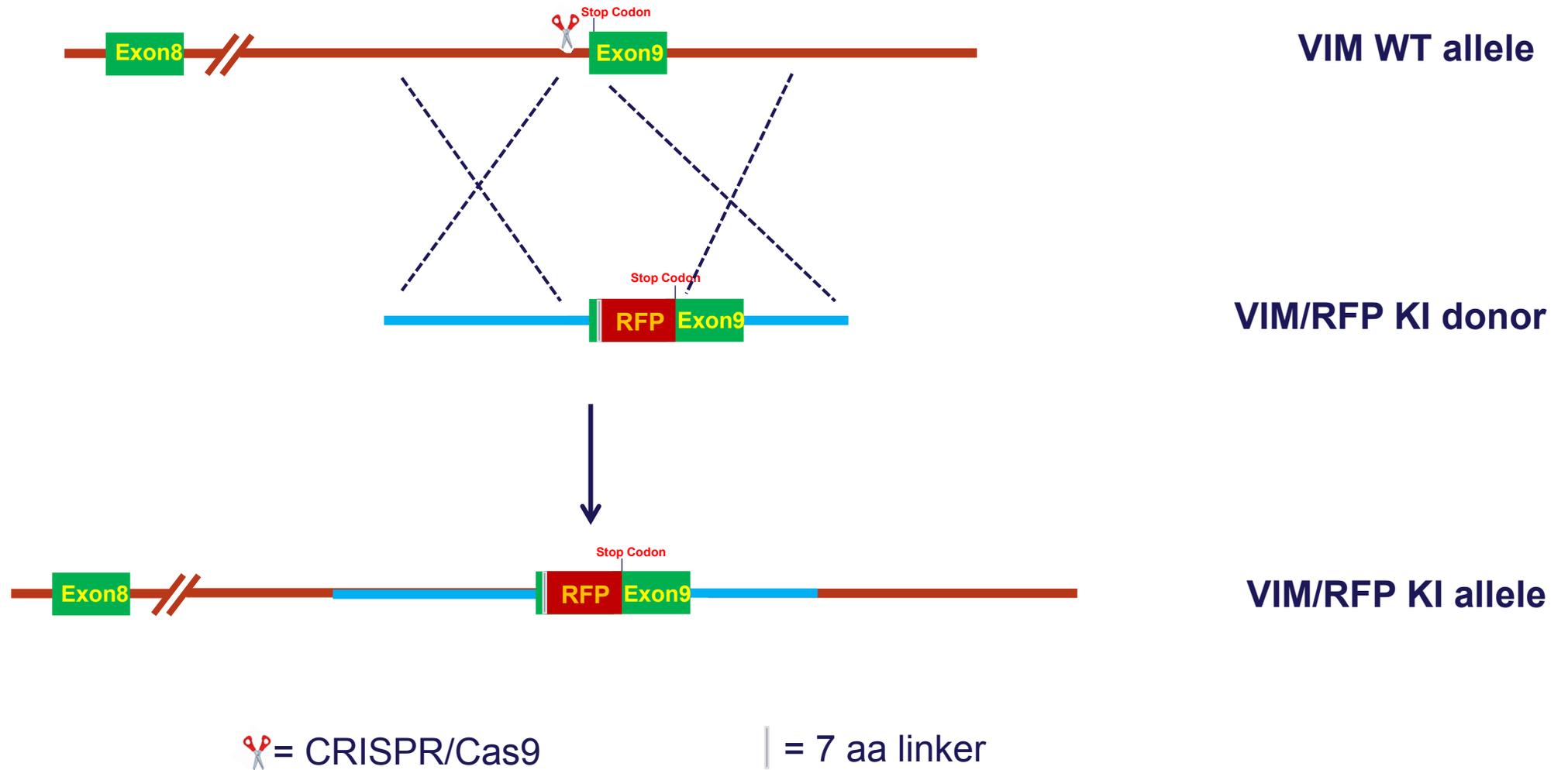
- EMT background
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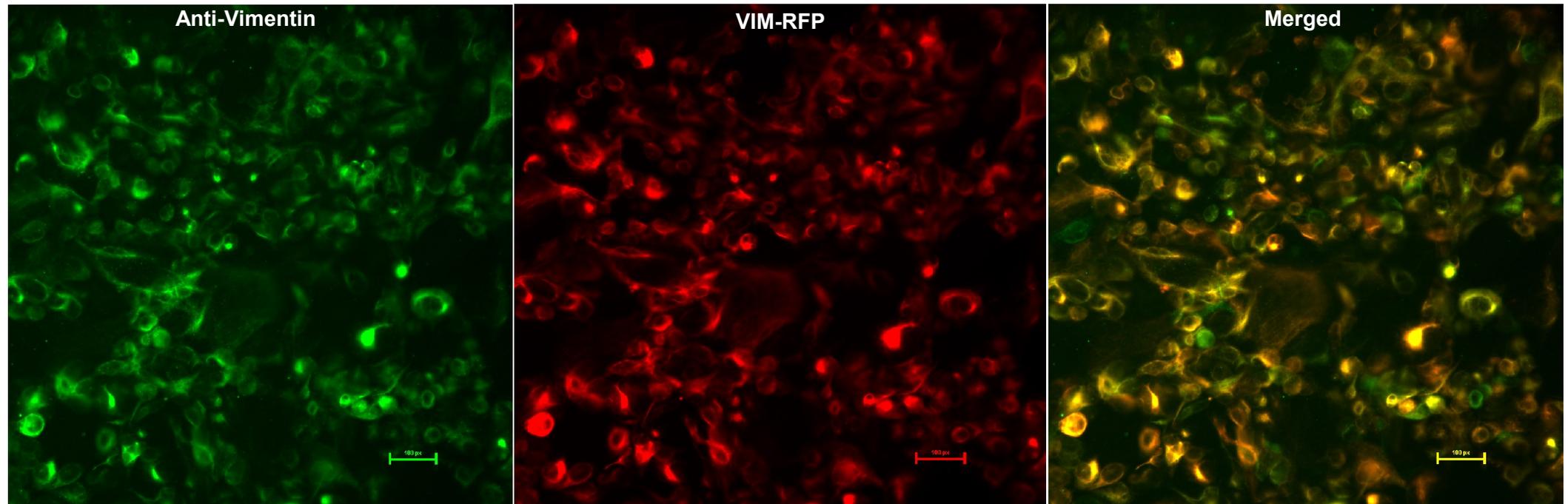
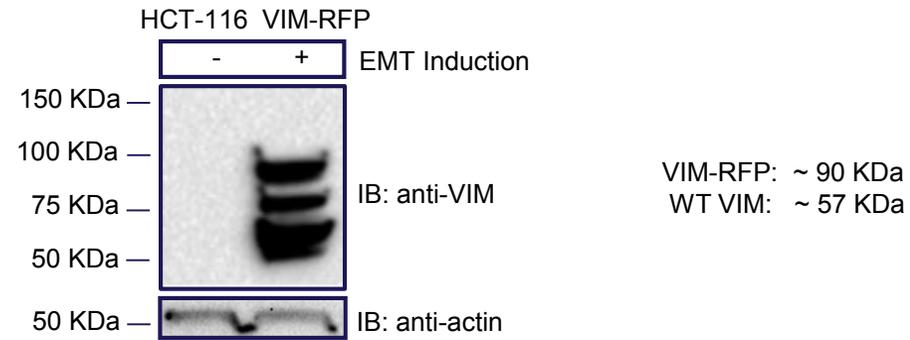
# Colon cancer HCT-116 cells can be induced to undergo EMT



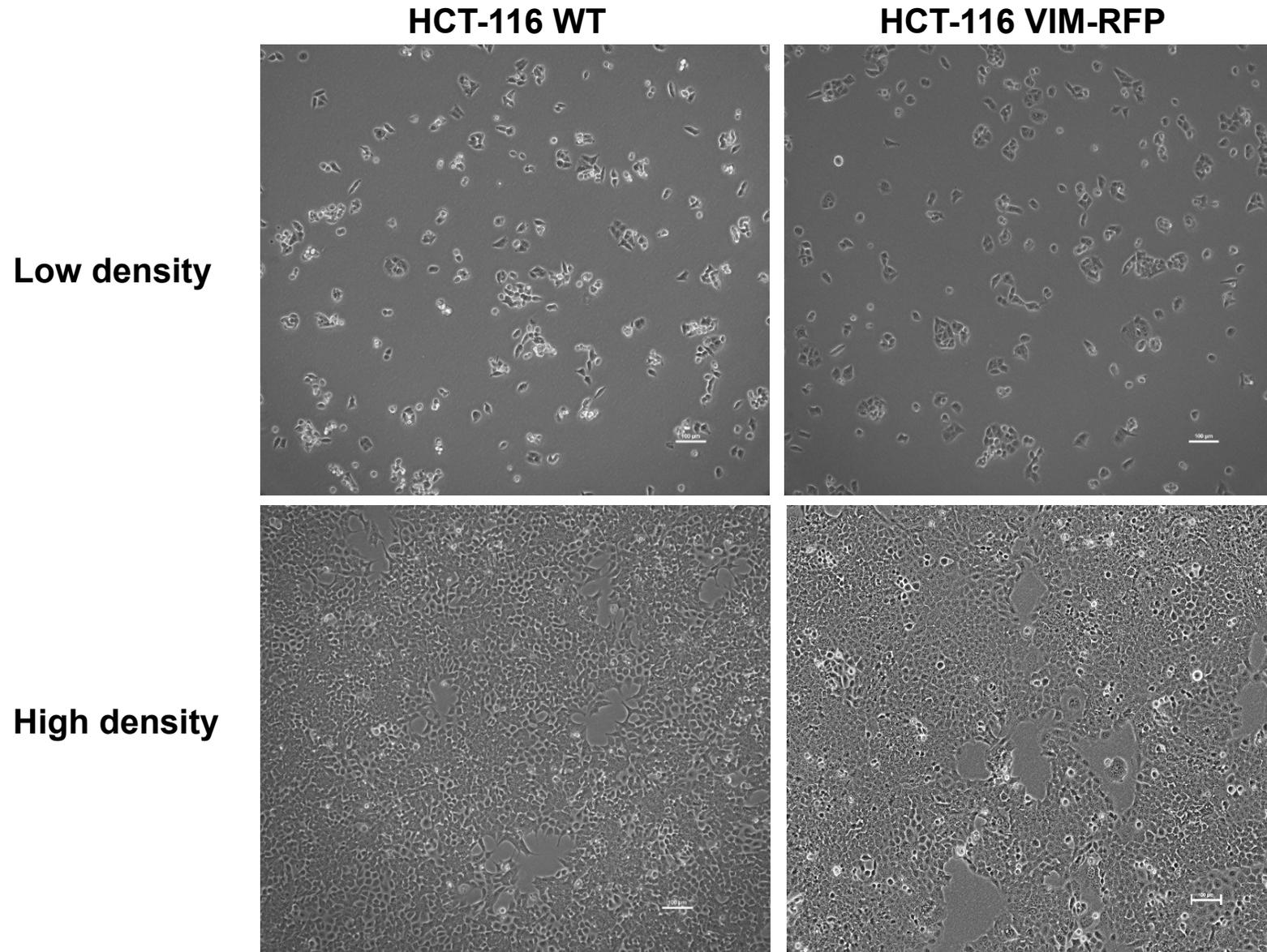
# Precision editing to create vimentin (VIM)-RFP knock-in allele



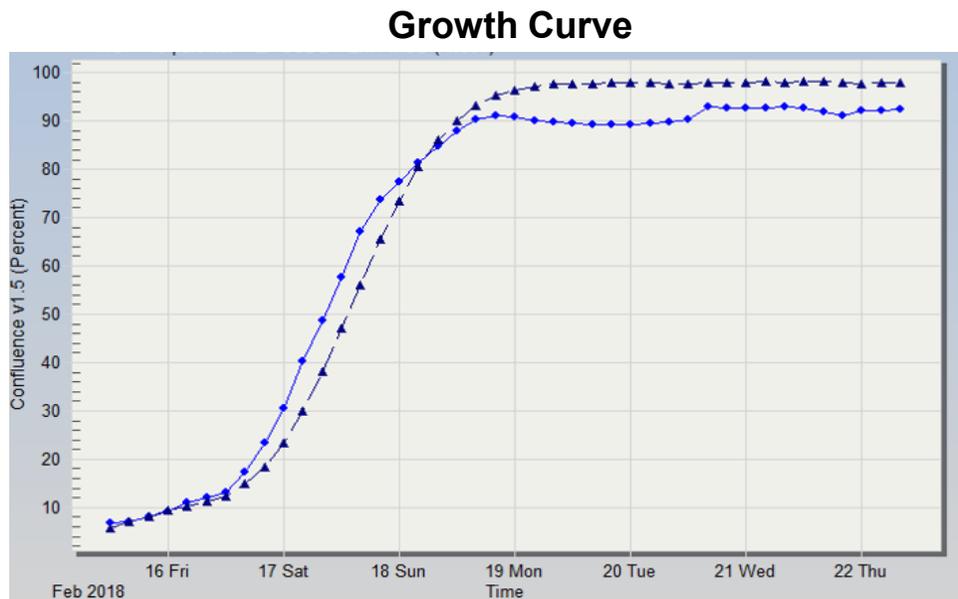
# Detection of VIM-RFP fusion protein in VIM-RFP cells



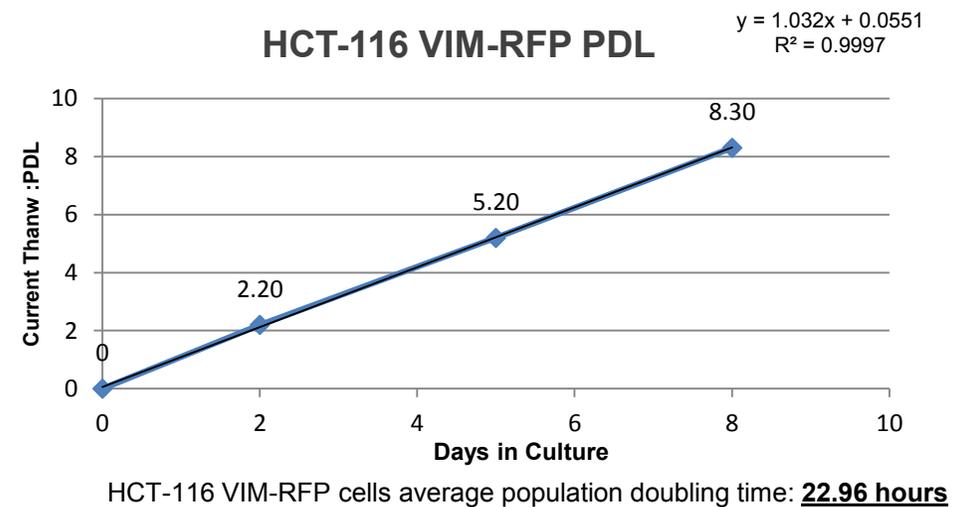
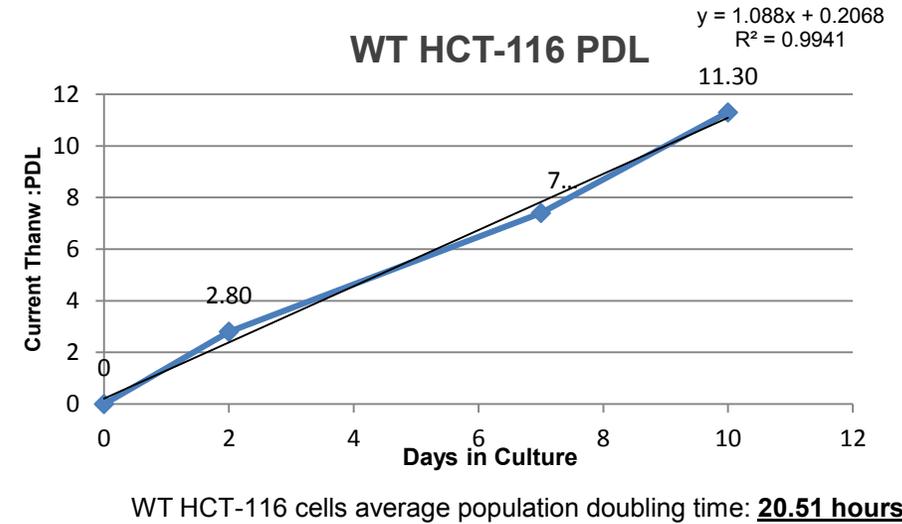
# Morphology of HCT-116 VIM-RFP is similar to the parental line



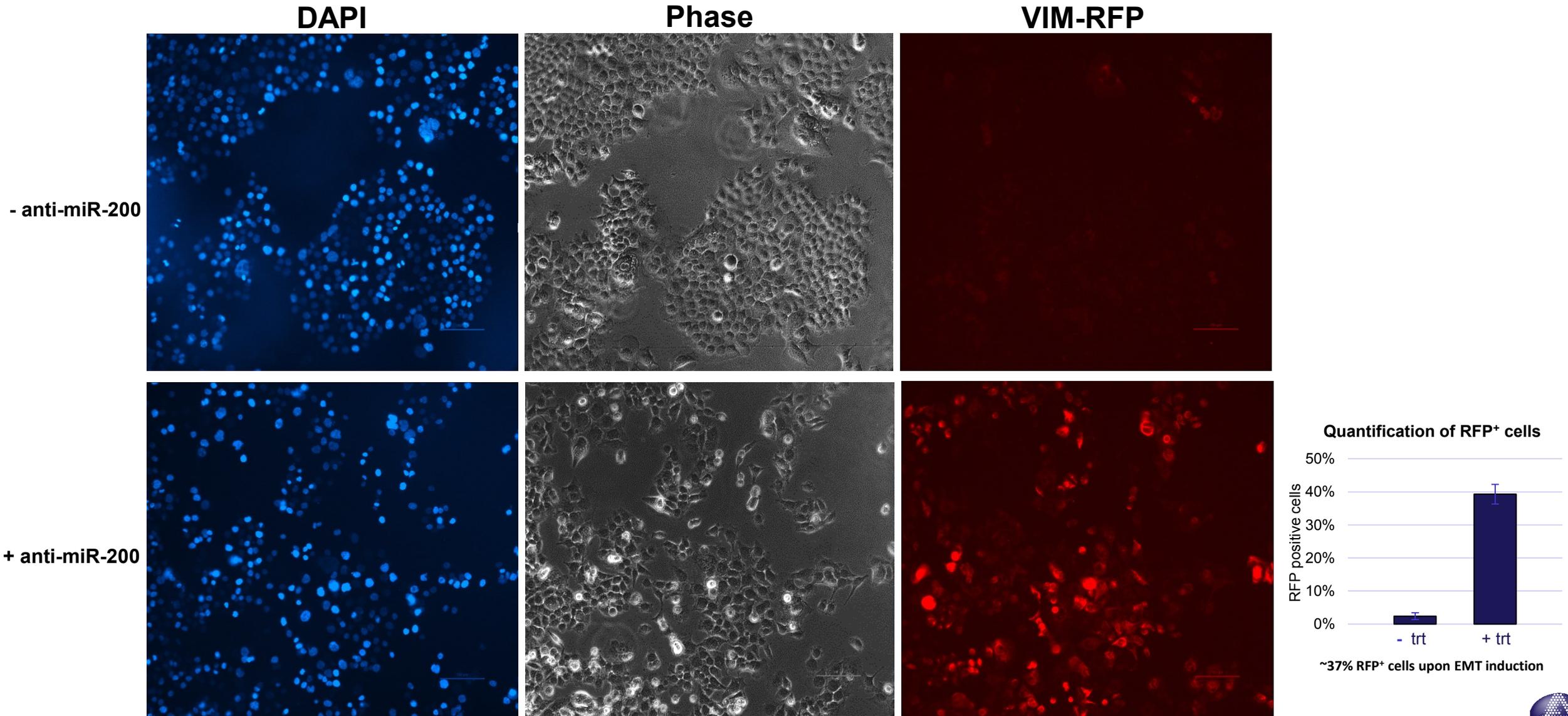
# Growth kinetics of HCT-116 VIM-RFP are similar to WT HCT-116



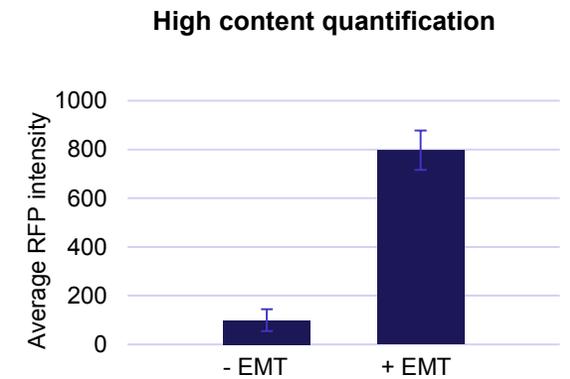
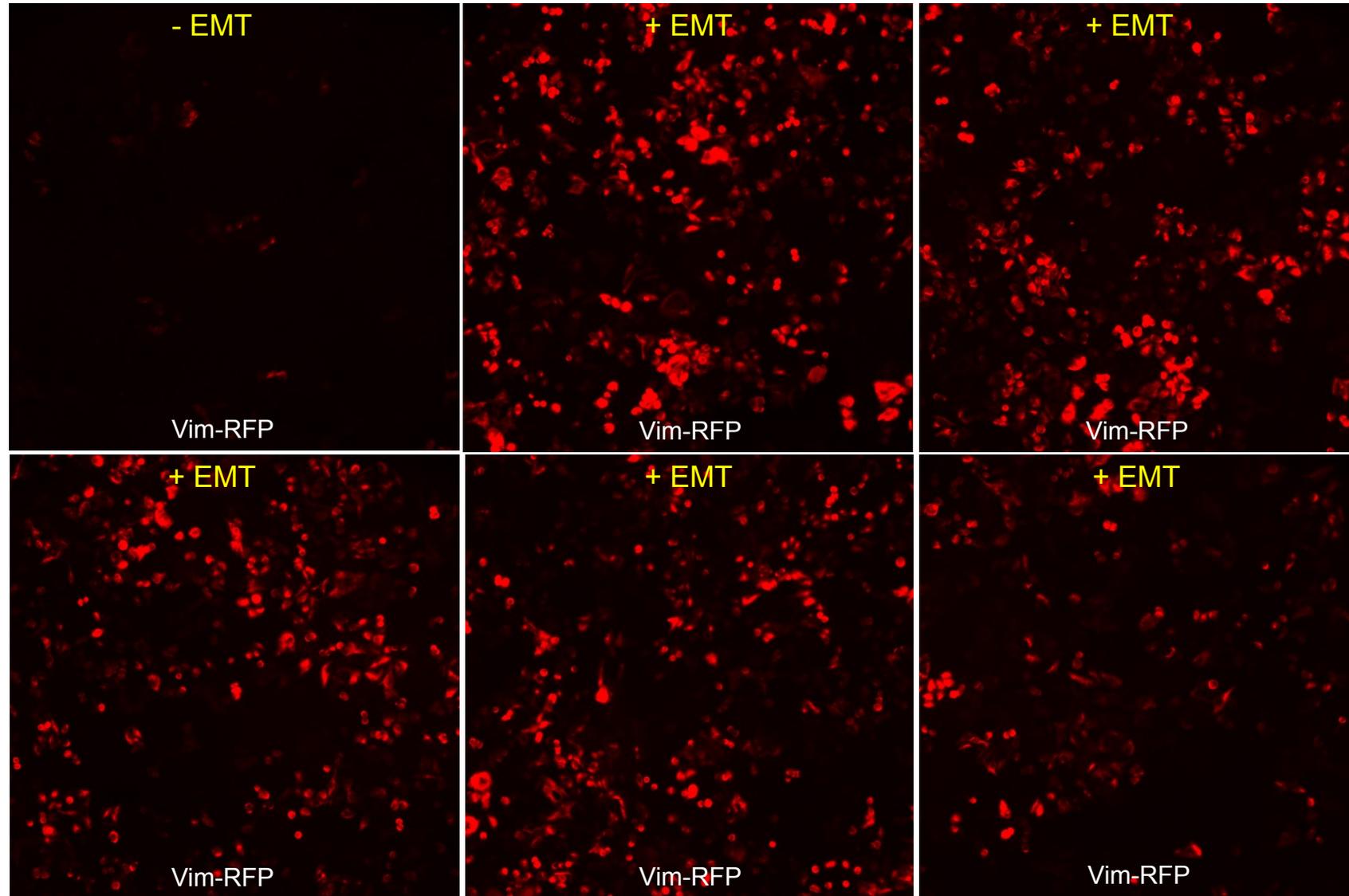
• WT HCT-116    ▲ VIM-RFP HCT-116



# miR-200 inhibitors induce VIM-RFP expression in HCT-116 VIM-RFP

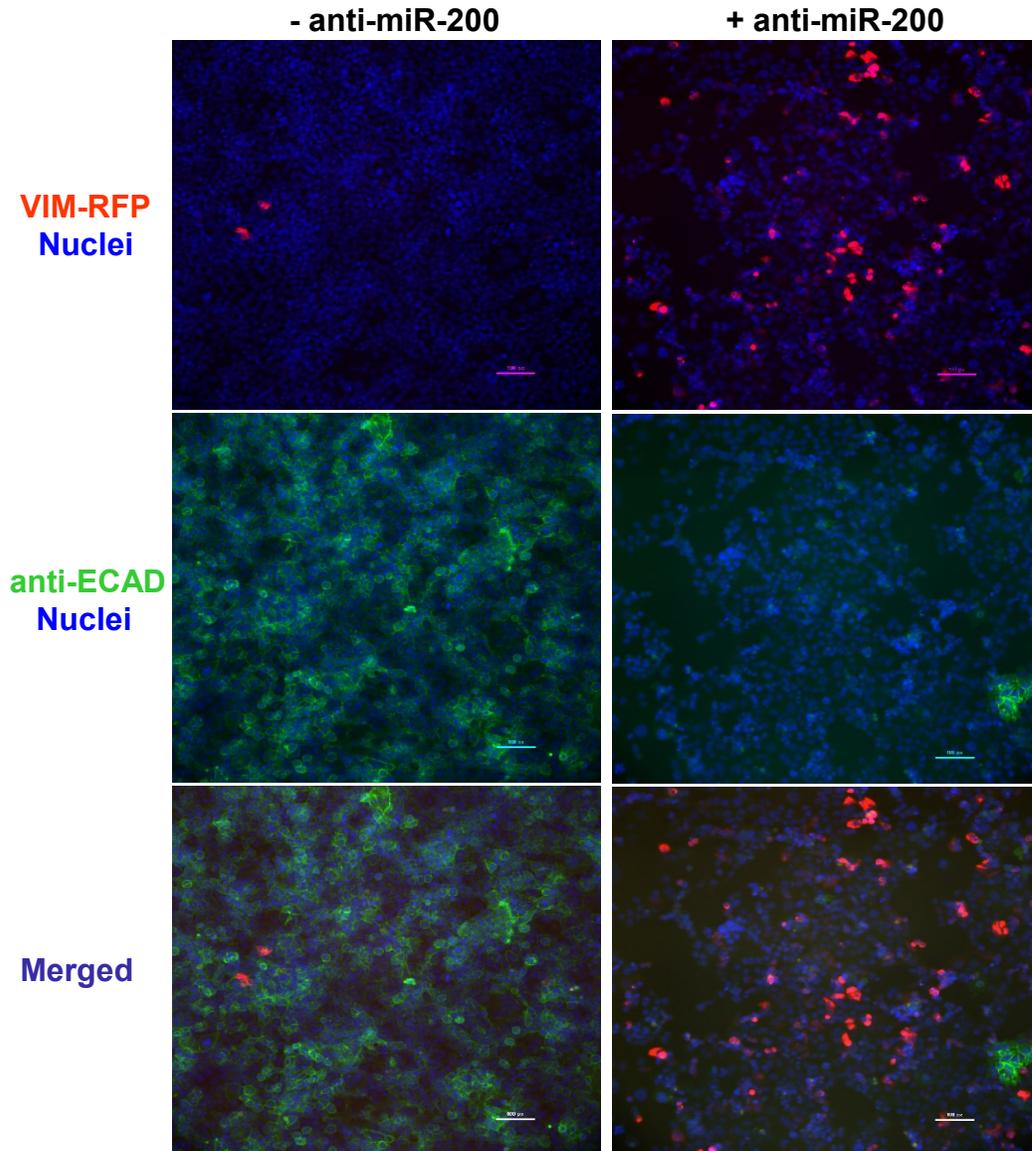


# High-content imaging quantification of VIM-RFP expression

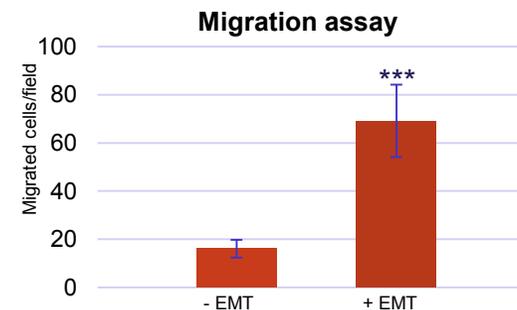
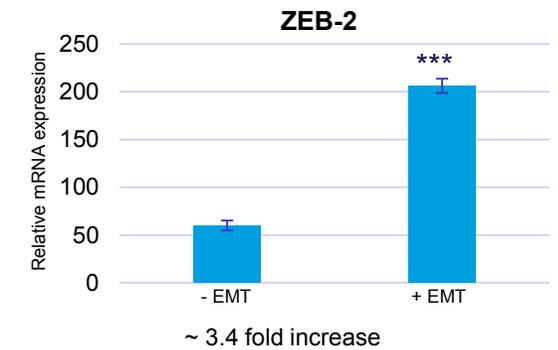
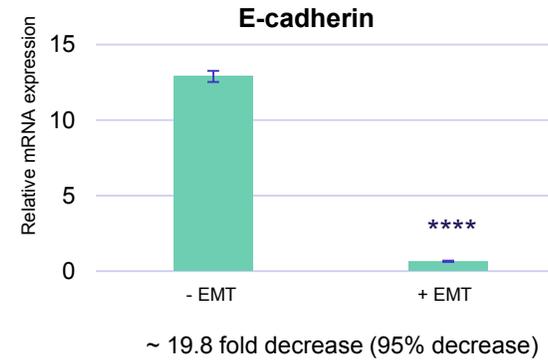
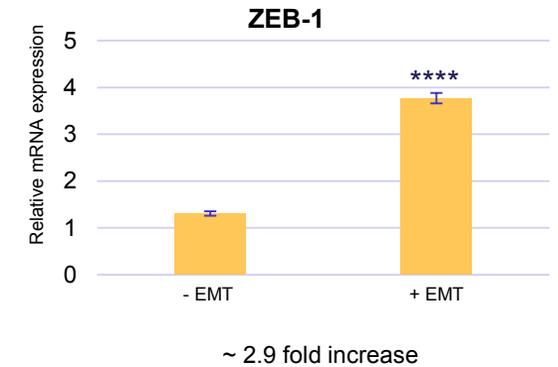
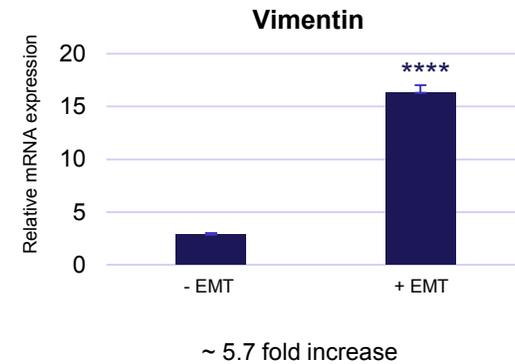


**~ 8.0 fold increase**  
(21 days induction)

# miR-200 inhibitors induce VIM-RFP cells to undergo EMT

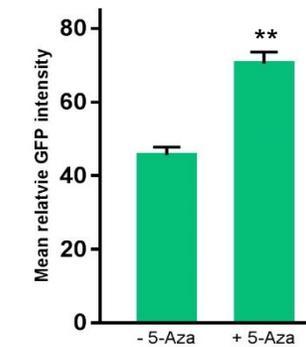
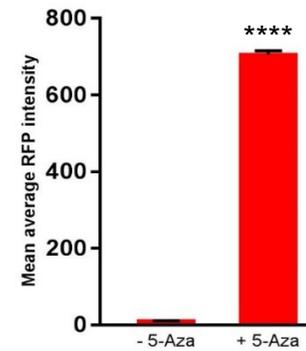
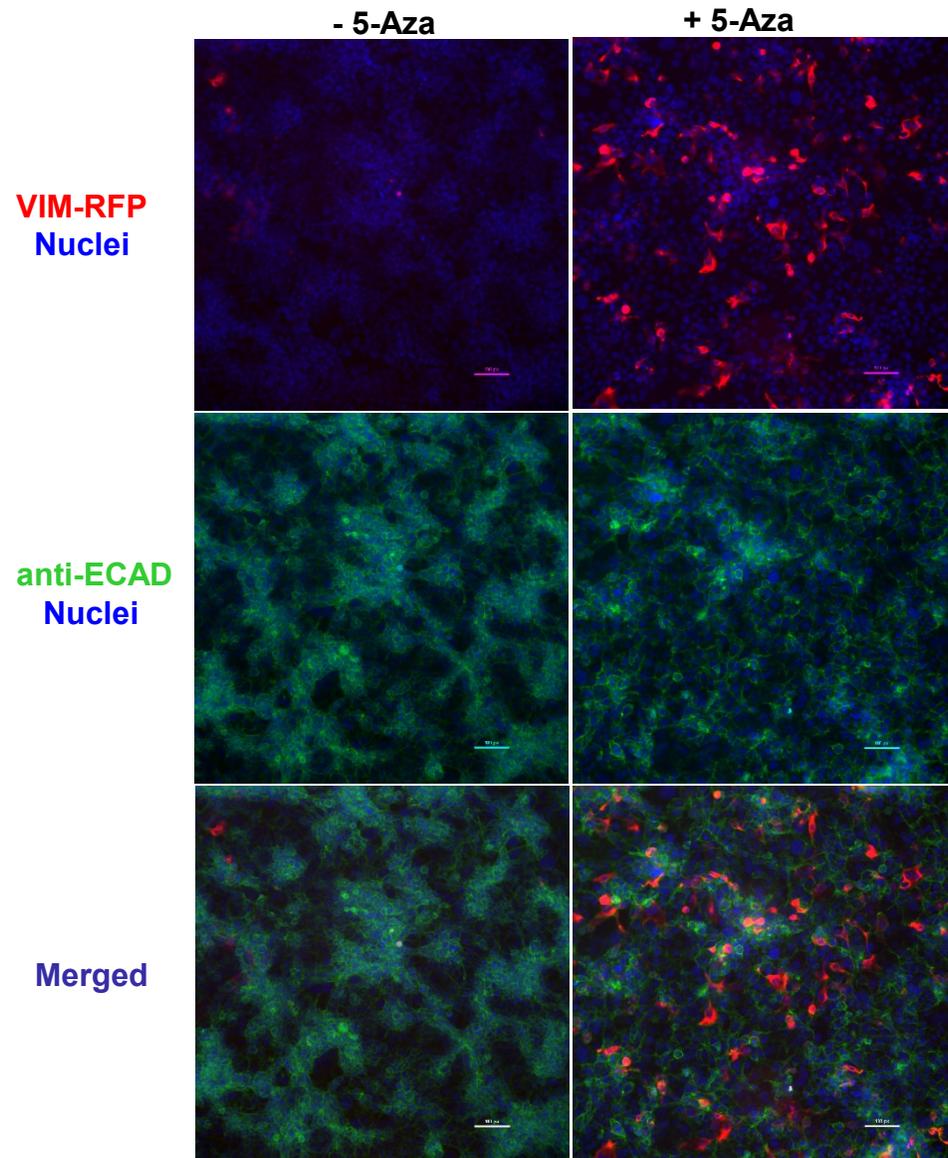


21 days induction

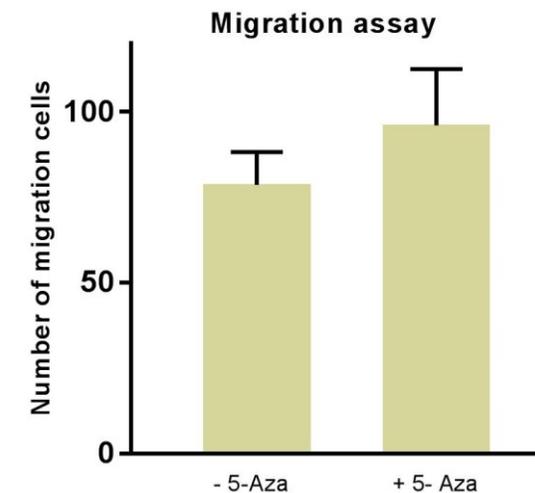
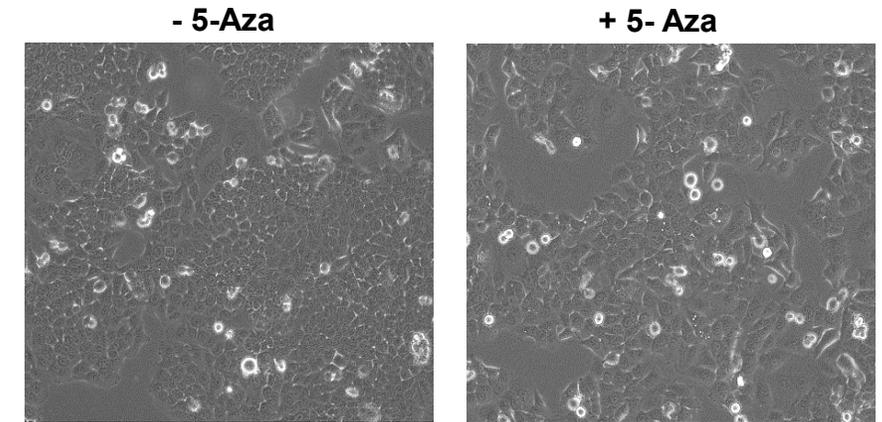


(Student's t-test, \*\*\*p<0.001, \*\*\*\*p<0.0001)

# Demethylating agent azacitidine induces VIM-RFP expression



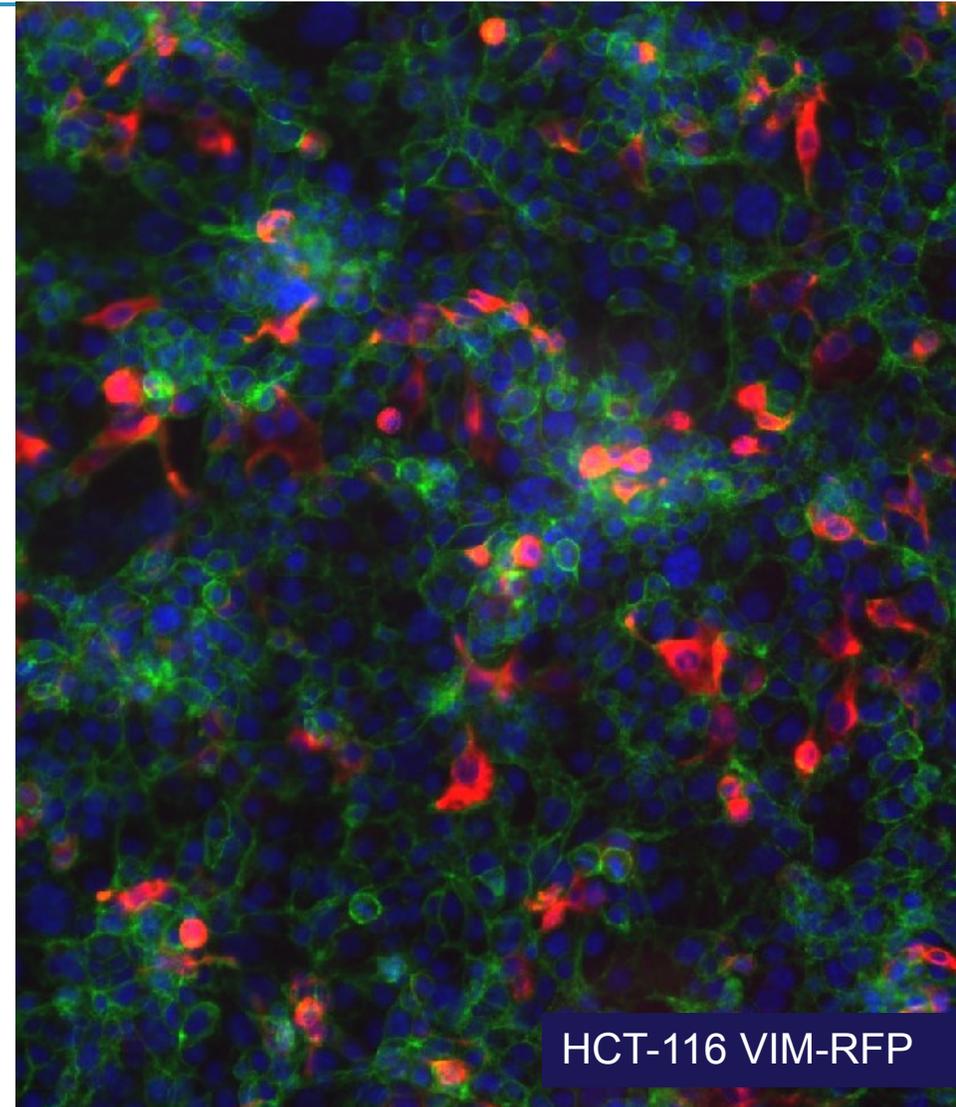
(Student's t-test, \*\*p<0.01, \*\*\*\*p<0.0001)



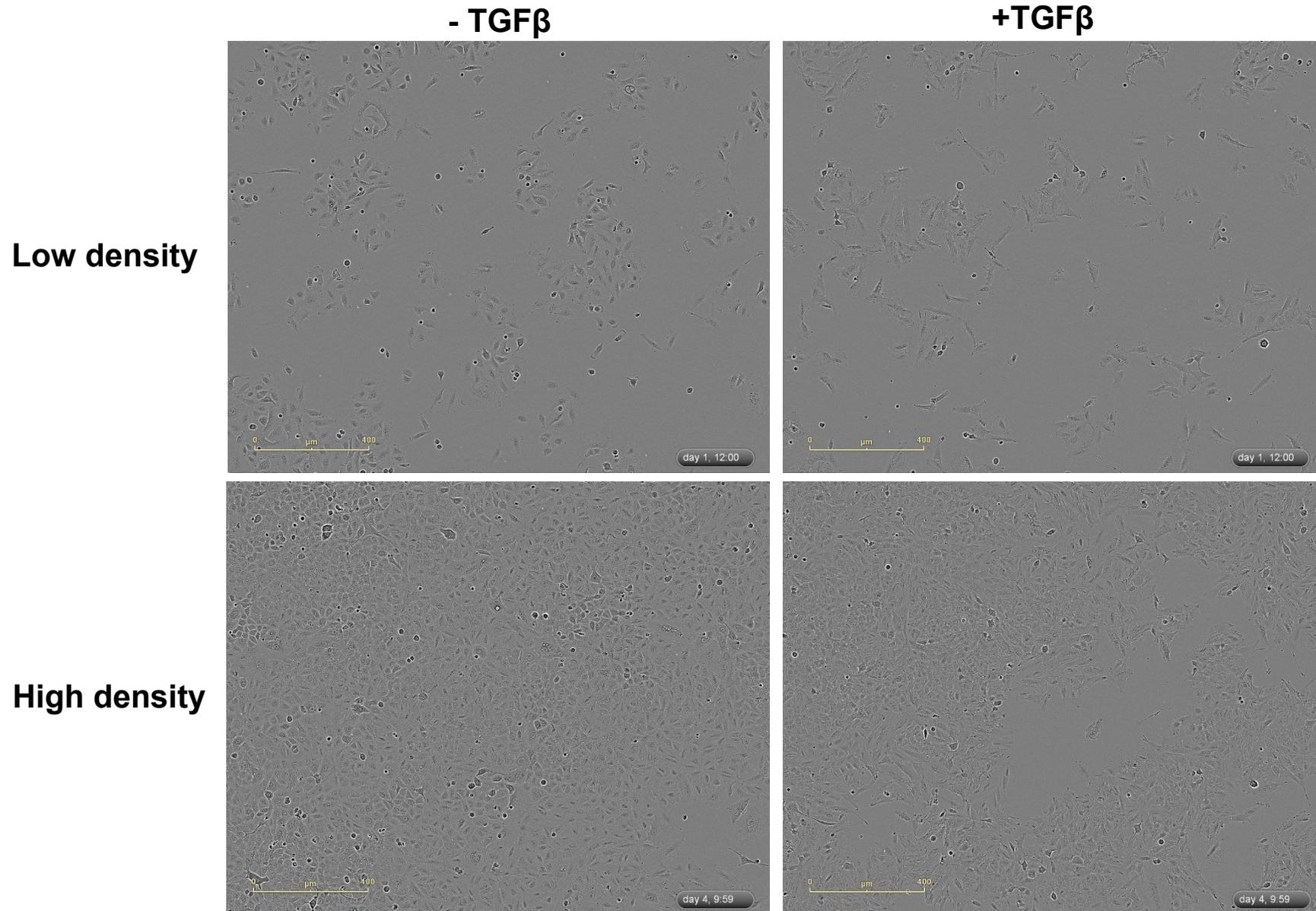
2 days induction

# Agenda

- EMT background
- Current EMT reporter cell lines
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- Summary

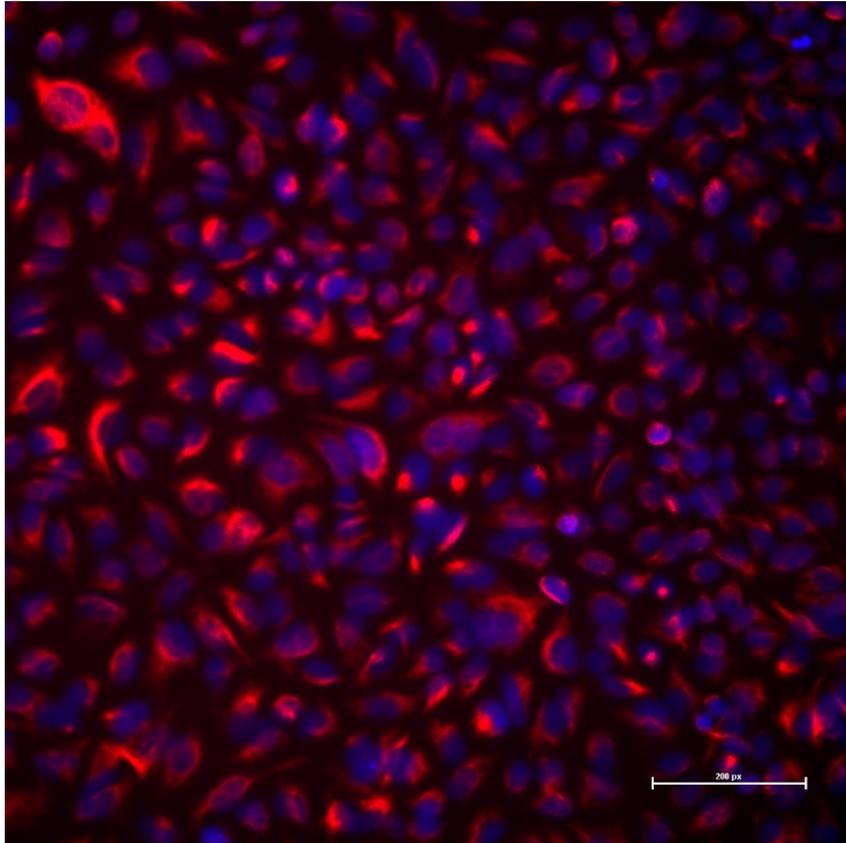


# TGF- $\beta$ treatment induces morphological changes in A549 VIM-RFP



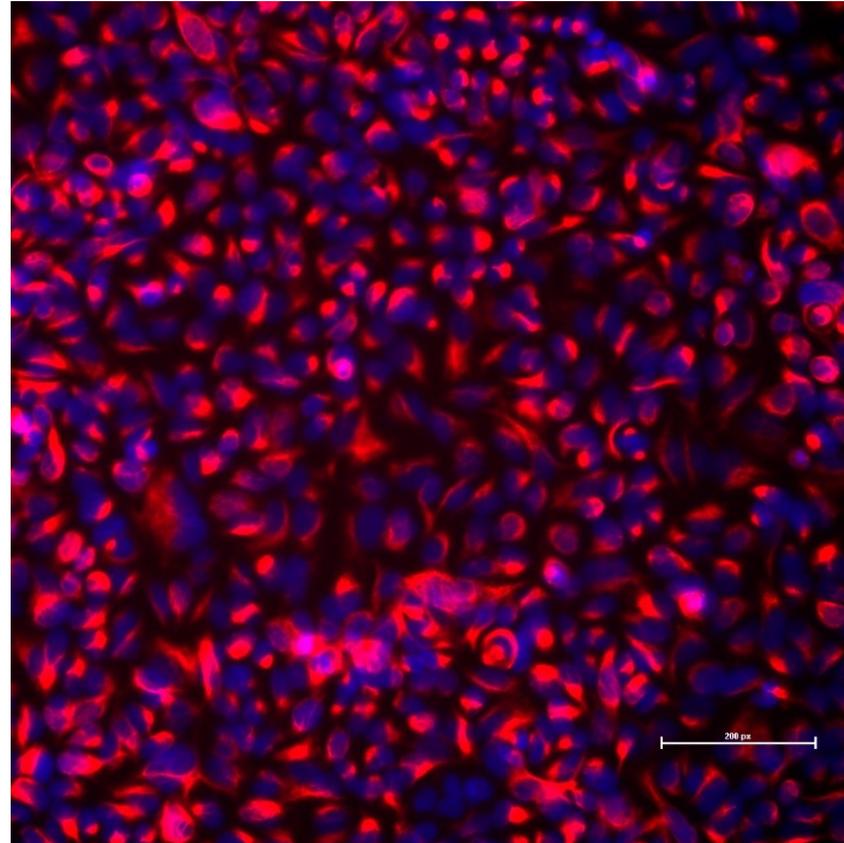
# VIM-RFP expression is increased upon TGF- $\beta$ EMT induction

- TGF- $\beta$

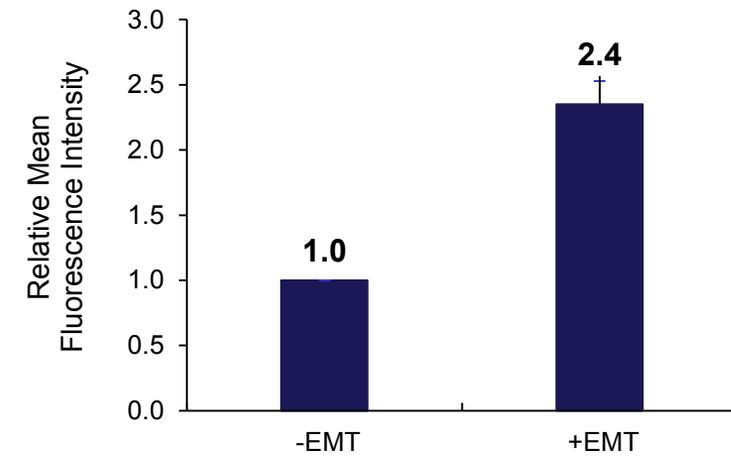


VIM-RFP, Nuclei

+ TGF- $\beta$

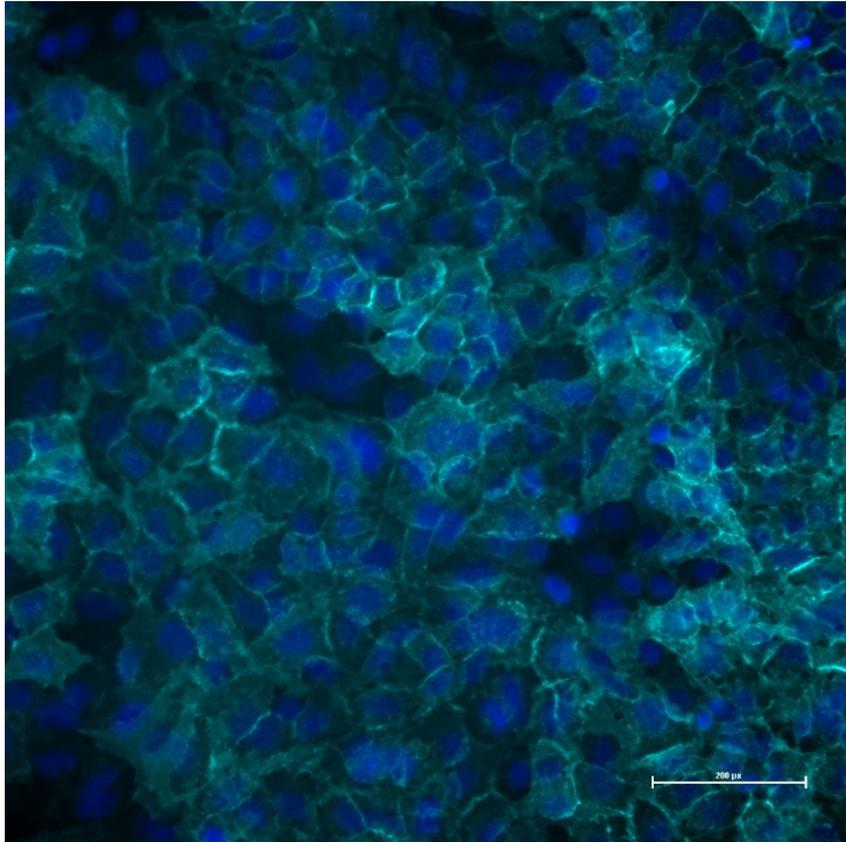


VIM-RFP, Nuclei



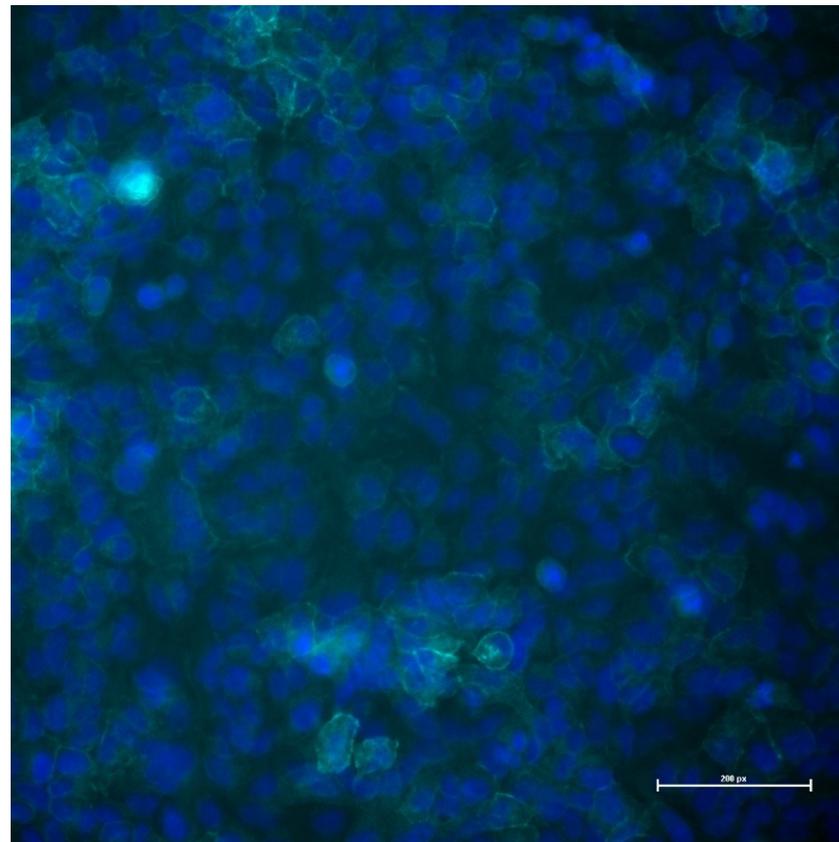
# E-cadherin expression is decreased upon TGF- $\beta$ induction

- TGF- $\beta$

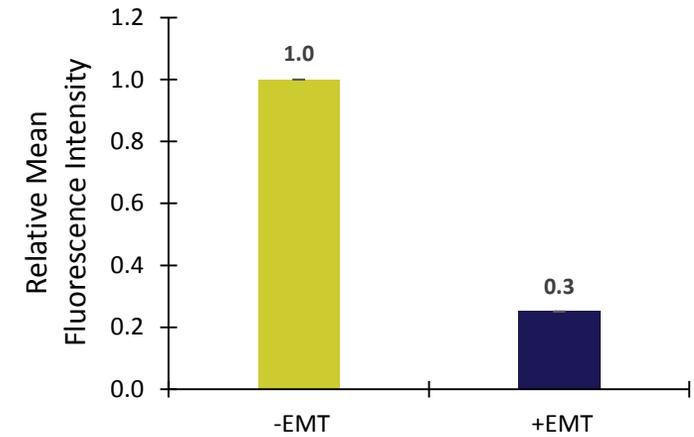


E-cadherin, Nuclei

+ TGF- $\beta$

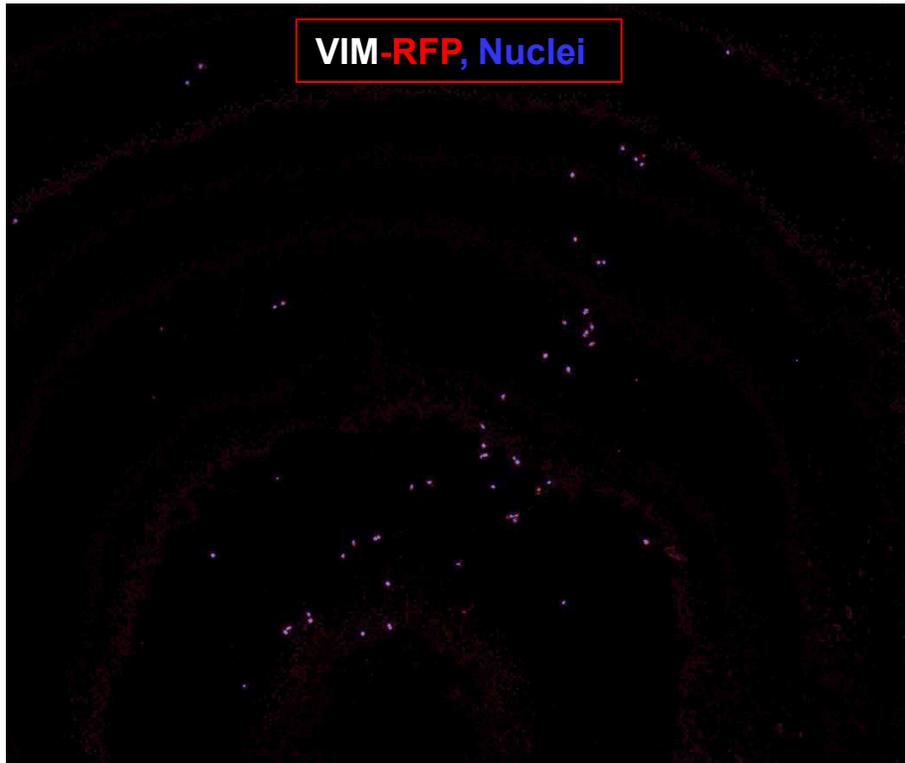


E-cadherin, Nuclei

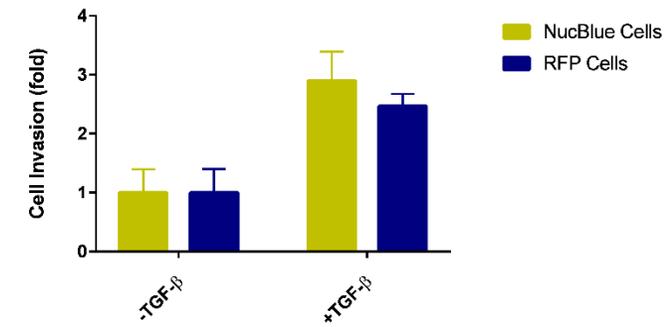
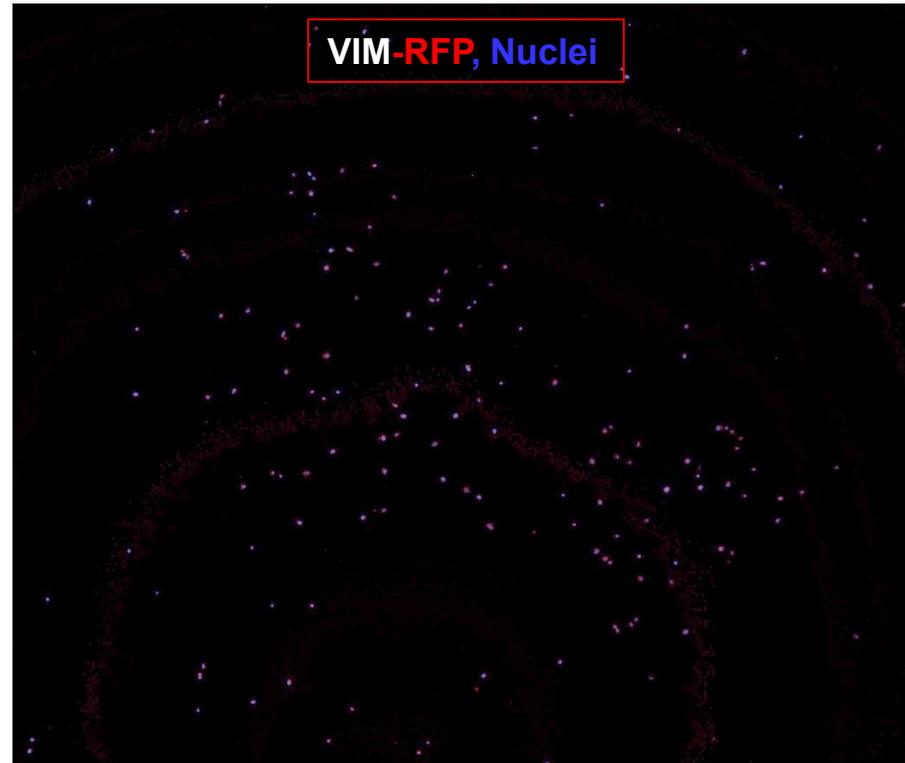


# TGF- $\beta$ Induced A549 VIM-RFP Cells Display Increased Invasiveness

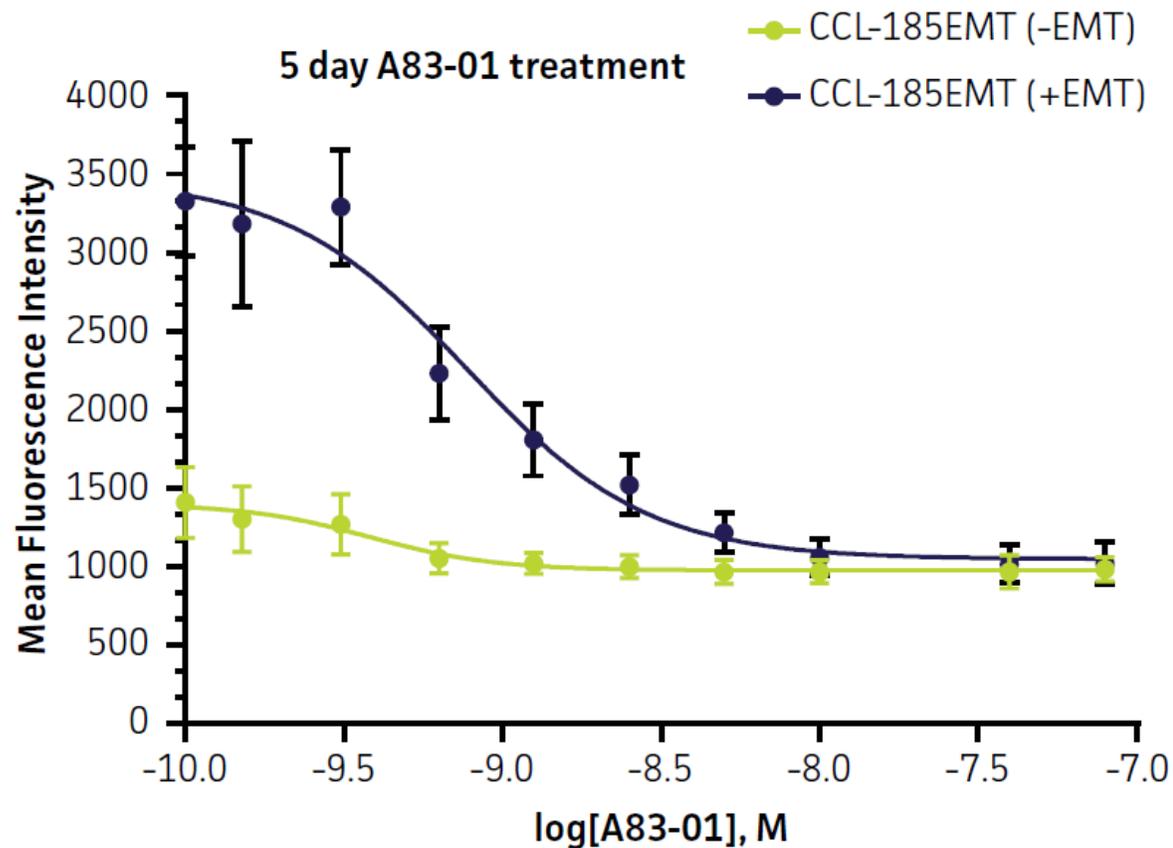
- TGF- $\beta$



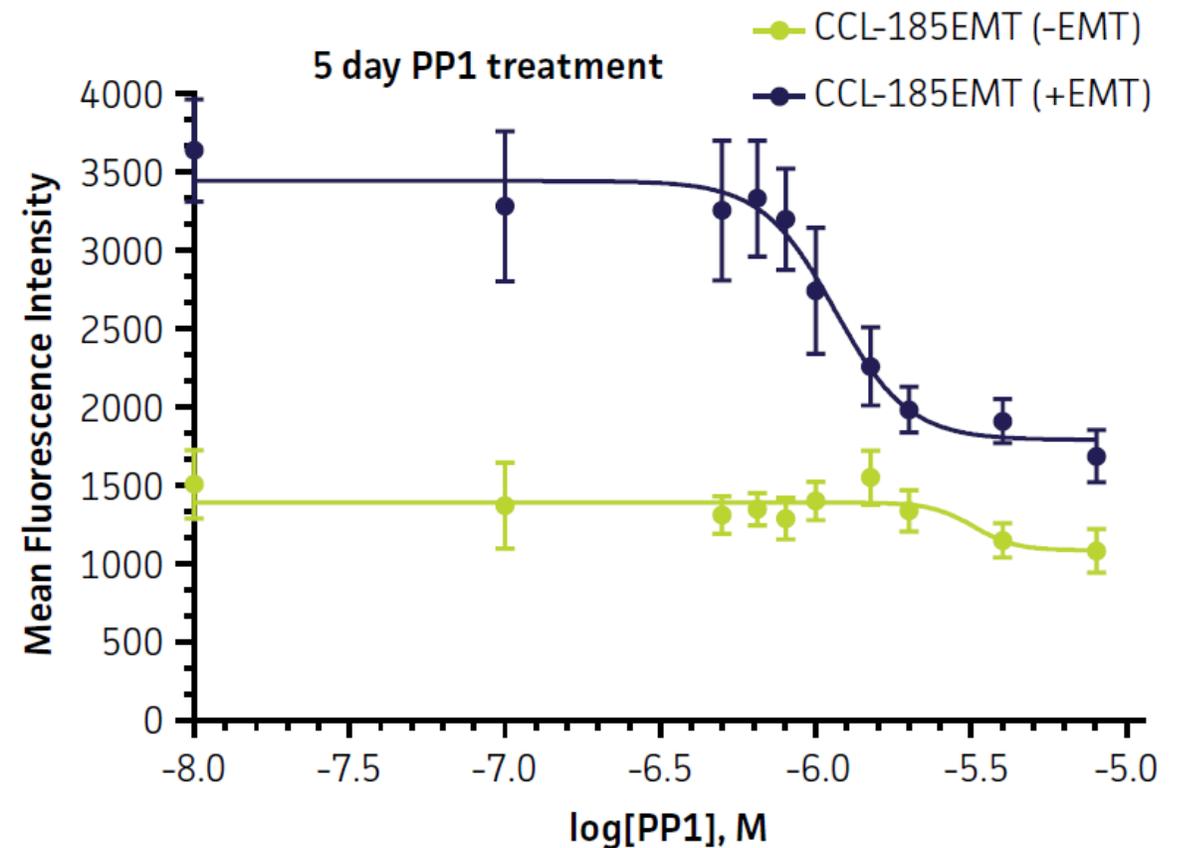
+ TGF- $\beta$



# Small molecule EMT inhibitors block transition in A549 VIM-RFP



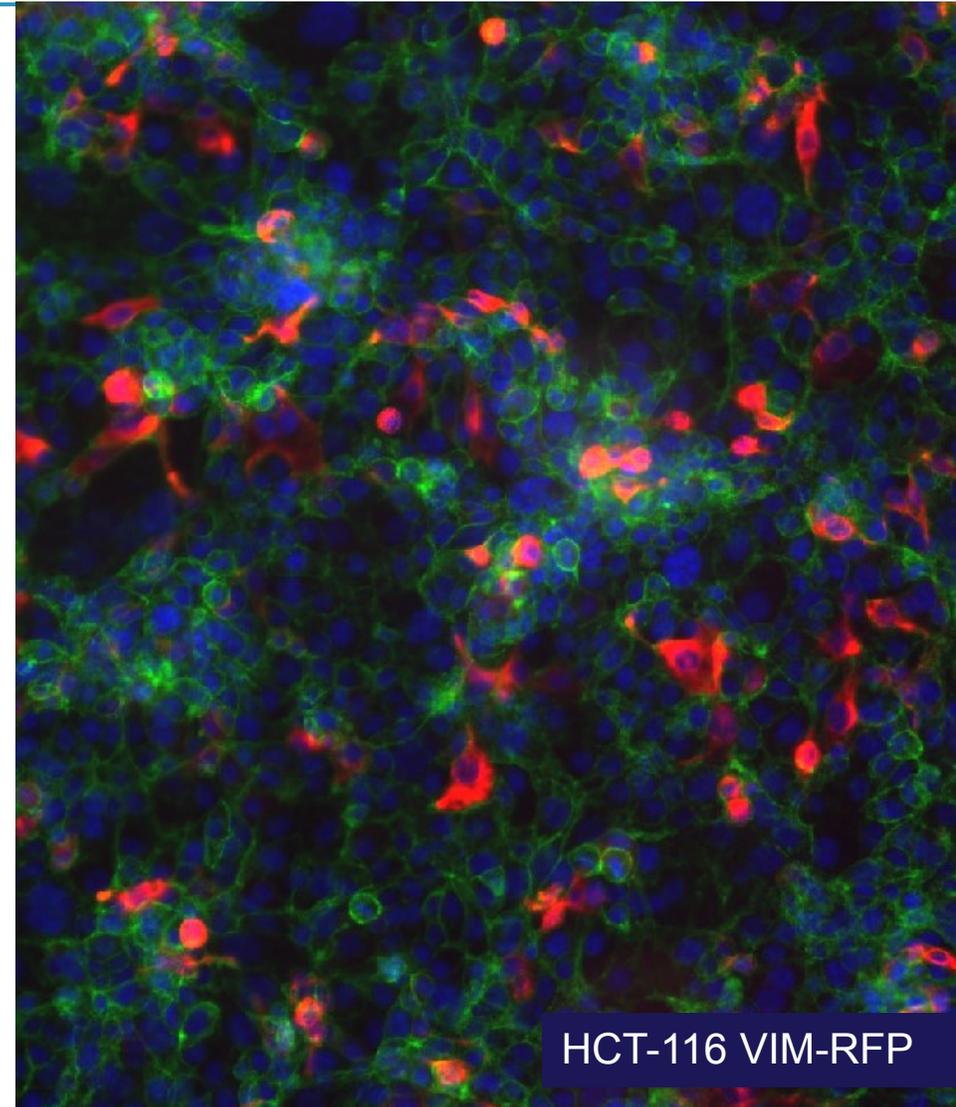
	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
IC50	3.822e-010	7.733e-010



	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
IC50	3.22e-006	1.154e-006

# Agenda

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# Summary

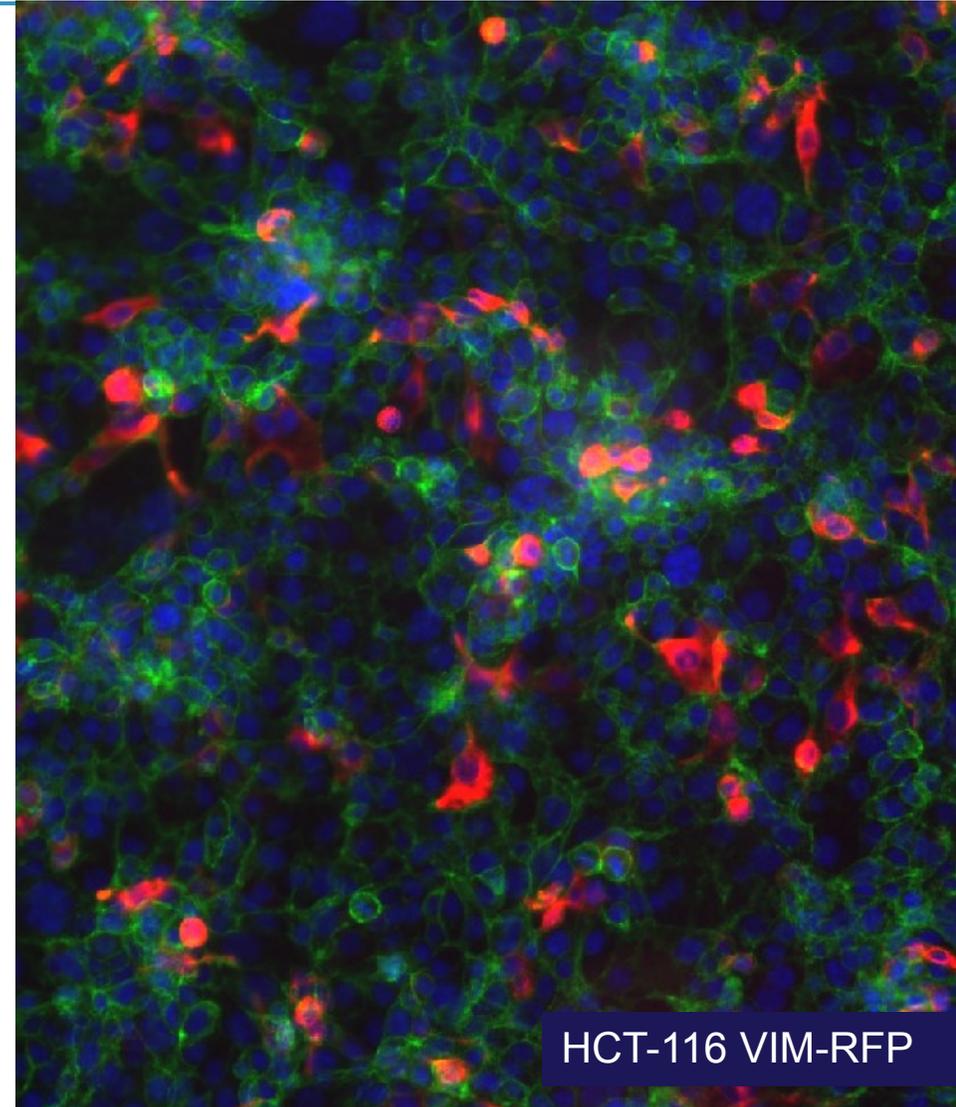
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- We have successfully generated VIM-RFP fusion EMT reporter cell lines via CRISPR/Cas9 gene-editing technology.
- VIM-RFP reporter cells undergo EMT upon induction, enabling real-time monitoring of EMT intermediate states in live cells.
- VIM-RFP EMT reporter cell lines are suitable and sensitive models for studying the molecular mechanisms underlying EMT and for development of novel anticancer drugs that target EMT.

Thank you!

# Disclaimer

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# For more information

- Website: [www.atcc.org/EMT](http://www.atcc.org/EMT)
- Flyer: Epithelial-mesenchymal Transition Reporter Cell Line
- Email: [wshu@atcc.org](mailto:wshu@atcc.org)

Thank you!

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## EPITHELIAL-MESENCHYMAL TRANSITION REPORTER CELL LINE

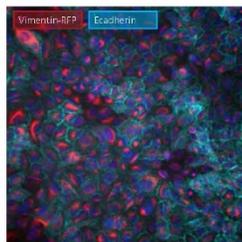
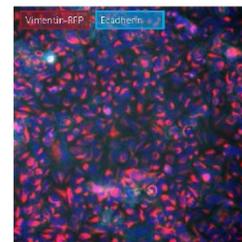
Epithelial-mesenchymal transition (EMT) and its reverse, mesenchymal-epithelial transition (MET) are developmental processes which have been shown to play critical roles in promoting metastasis and invasion in carcinoma. Recent studies have shown that EMT of cancer cells not only causes tumor metastasis but also contributes to drug resistance. To help researchers investigating this phenomenon, ATCC has employed CRISPR/Cas9 gene editing to develop A549 Vim-RFP (ATCC® CCL-185EMT™).

This reporter line is designed to enable the real-time monitoring of the changing status of cells from epithelial to mesenchymal via the expression of red fluorescent protein (RFP)-tagged vimentin. This cell line is not only an aid in dissecting the EMT/MET pathway in the research field, but also a robust platform for new cancer drug development.

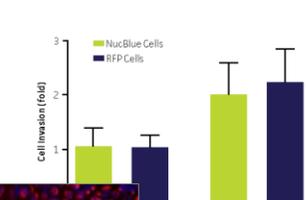
- CRISPR/Cas9 gene-edited vimentin-RFP fusion protein
- Strong RFP signal due to upregulated vimentin upon EMT induction
- Physiological E-cadherin expression in the absence of EMT
- Similar growth kinetics as the parental A549
- TGF-β1 responsive
- Increased invasive capacity following EMT
- EMT sensitive to A83-01 and PP1 inhibition

ATCC No.	Designation	Volume	Cells/Vial
CCL-185EMT™	A549 Vim-RFP	1 mL	1 x 10 <sup>6</sup>

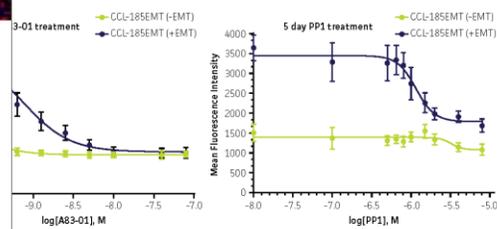
**VALIDATION DATA**

**Figure 1.** A549 Vim-RFP shows increased mesenchymal and decreased epithelial marker protein expression after EMT. Treatment of A549 Vim-RFP with the EMT induction agent TGF-β1 results in increased vimentin-RFP expression (red) and decreased E-cadherin expression (cyan). The cells in both panels were counterstained with NucBlue fixed cell ReadyProbes reagent (blue).



**Figure 2.** After a 5-day incubation with (+EMT) or without (-EMT) TGF-β1, A549 Vim-RFP cells were monitored over a 24 hr period for invasion through an 8 μm pore filter of the basement membrane of the BD 24 well fluoroblock cell invasion system. EMT induced A549 Vim-RFP cells show increased invasive capacity. The similar number of RFP positive and NucBlue nuclear counterstained cells depict the utility of RFP expression to monitor invaded cells.



**3-01 treatment**

log[A83-01], M	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
-9.0	~150	~3500
-8.5	~150	~3000
-8.0	~150	~2500
-7.5	~150	~2000
-7.0	~150	~1500

**5 day PP1 treatment**

log[PP1], M	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
-8.0	~150	~3500
-7.5	~150	~3000
-7.0	~150	~2500
-6.5	~150	~2000
-6.0	~150	~1500
-5.5	~150	~1000
-5.0	~150	~500

**IC50 values:**

IC50	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
322±10	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
773±10	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
322±906	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
1154±906	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)

EMT inhibitors block transition in A549 Vim-RFP cells. Two pathways associated with EMT were targeted: TGF-β and I, respectively. In both cases, TGF-β1-induced EMT was inhibited by the compound.

**3/EMT for more information.**

**PHONE**  
800.638.6597  
703.365.2700

**EMAIL**  
SalesRep@atcc.org

**WEB**  
www.atcc.org

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10801 University Blvd.  
Manassas, VA 20110



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