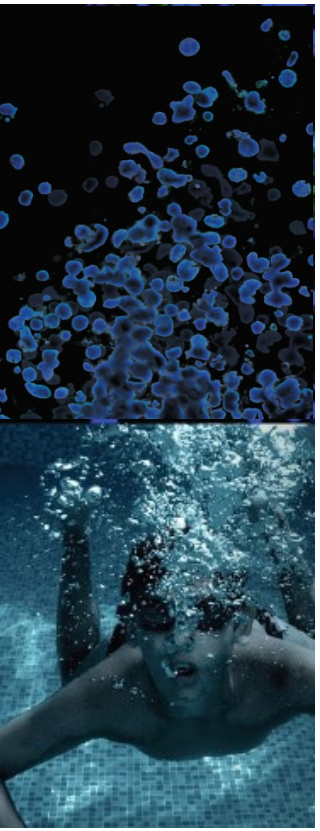
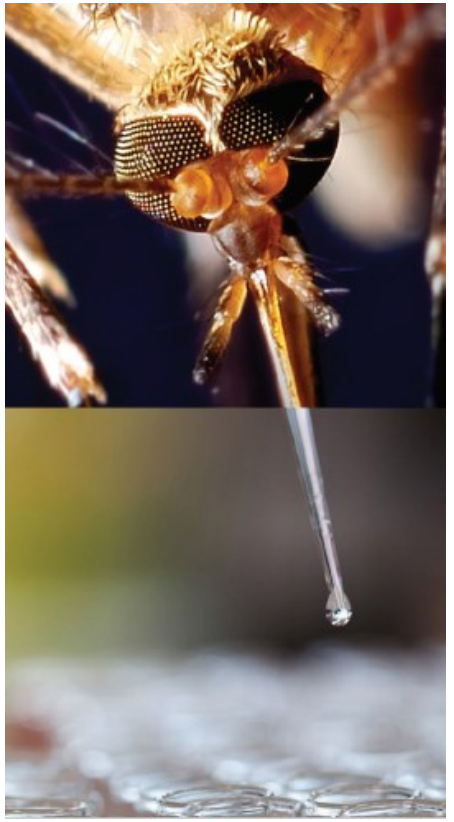
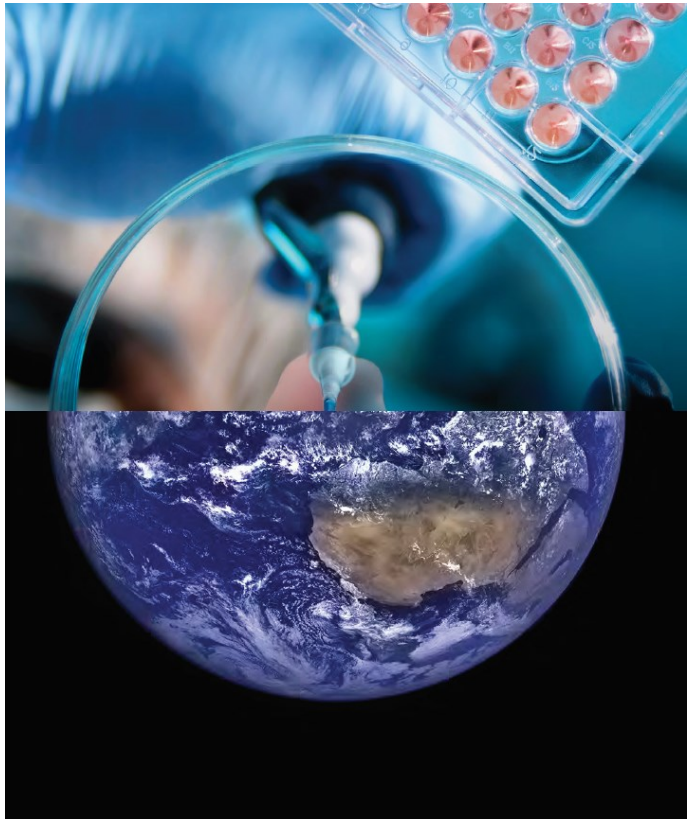
Novel Epithelial-to-Mesenchymal Transition Reporter Cell Lines Created by CRISPR Technology

Weiguo Shu, Ph.D.
Senior Scientist, ATCC

Credible Leads to Incredible™





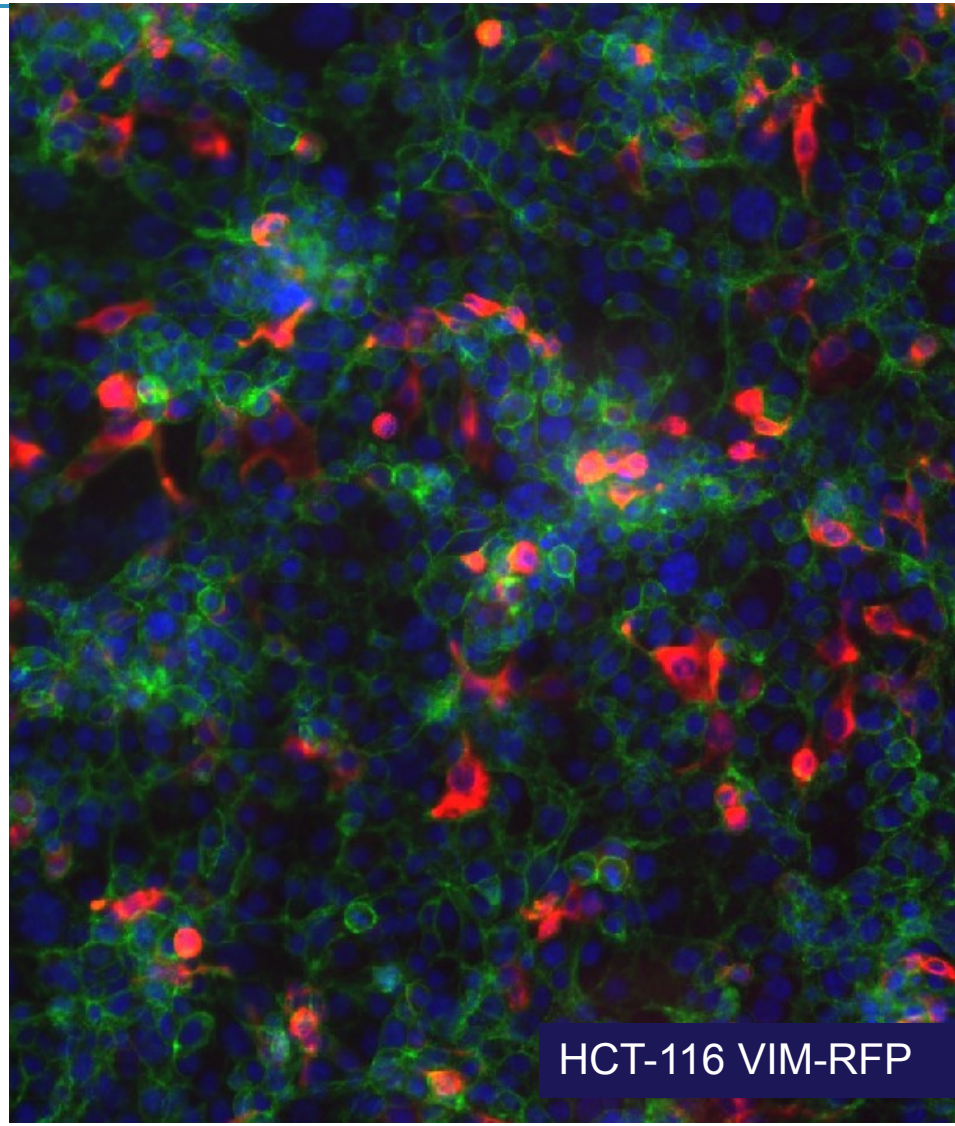


About ATCC

- 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:

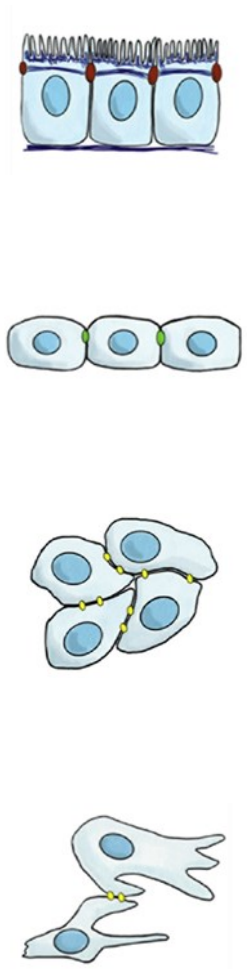
- Display an array of dynamic states

  "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

Modified from: K Campbell, Curr Opin Cell Biol 55:30–35, 2018

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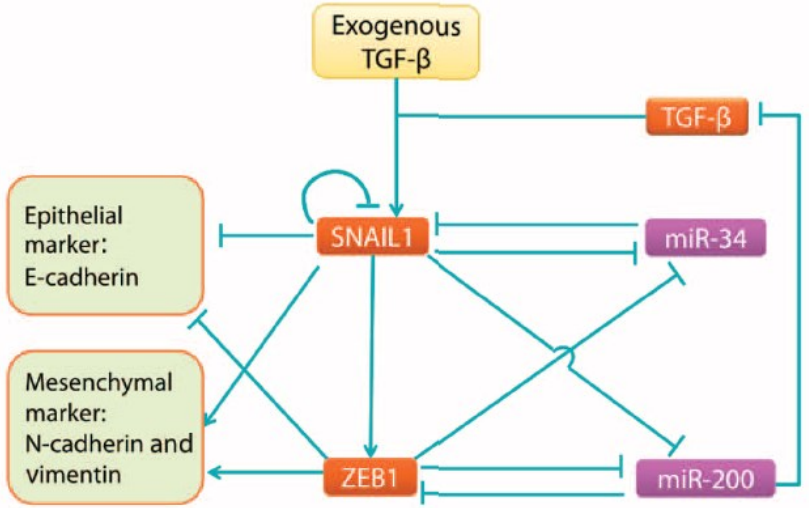
EMT complex regulatory networks

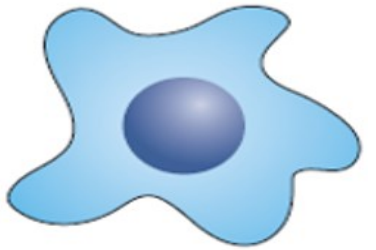
- Signaling pathways/growth factors: TGFβ
- EMT transcription factors: Twist, Snail1/2, Zeb1/2
- Non-coding micro-RNAs: miR-200
- Epigenetic modifiers: Histone demethylase PHF2



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

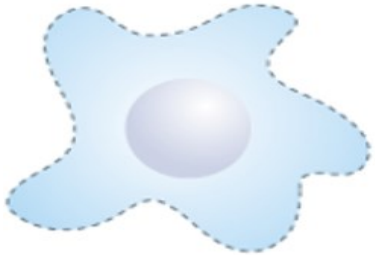
Strategies for screening compounds targeting EMT

Targeting mesenchymal tumor cells



Mesenchymal tumor cell

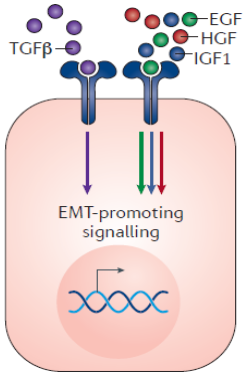
Active compounds



Depletion of functional inhibition

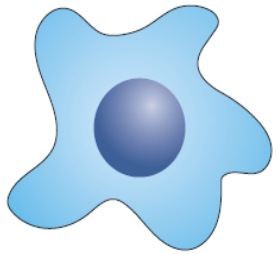
Readout:
- Induction of apoptosis or cell death
- Inhibition of migration/invasion
- Inhibition of stemness/induction of differentiation
- Reversal of chemo-resistance

Inhibiting EMT induction



Epithelial tumor cell

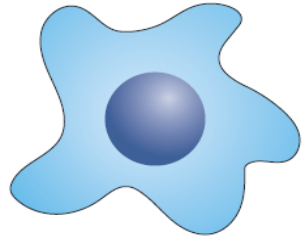
Active compounds



Mesenchymal tumor cell

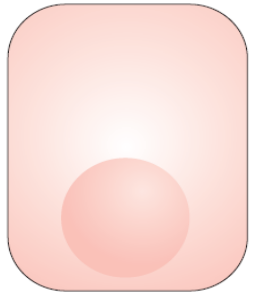
Readout:
- Preservation of epithelial markers (E-cadherin)
- Inhibition of mesenchymal markers (vimentin, N-cadherin)
- Prevention of chemo-resistance

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

Modified from Marcucci et al, Nat Rev Drug Discov 15(5):311-25, 2016.

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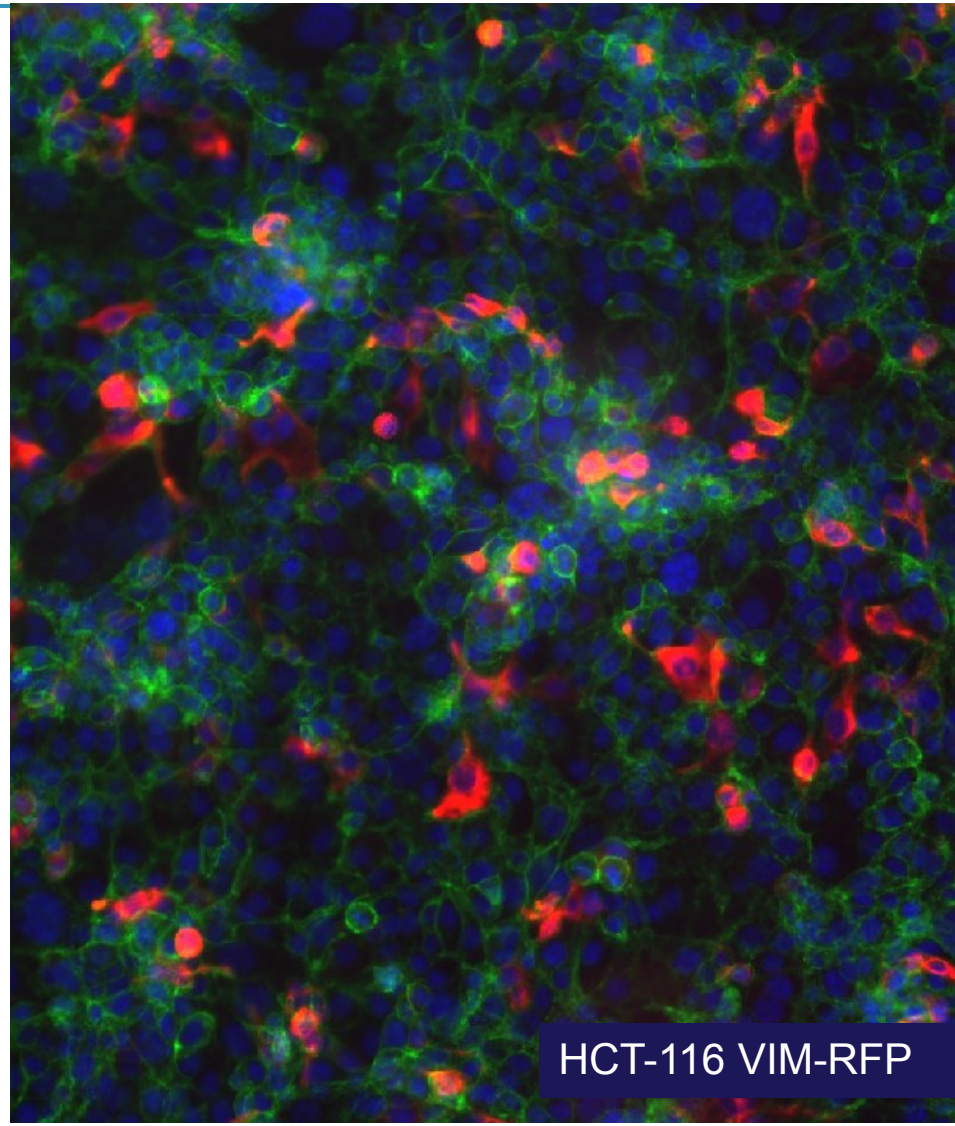

Current EMT reporter cell lines used in high-throughput screening

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

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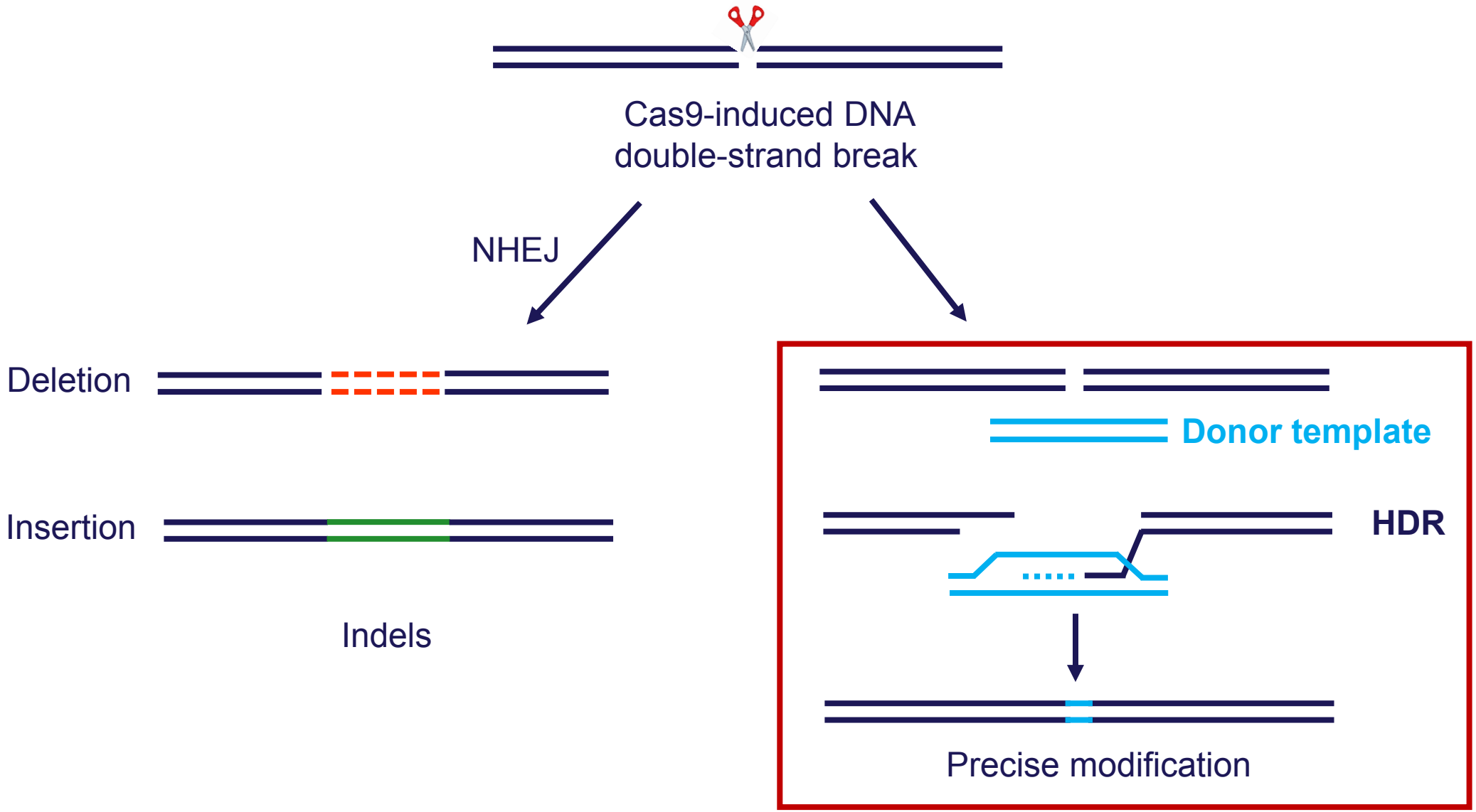
Generation of targeted knock-in by CRISPR technology



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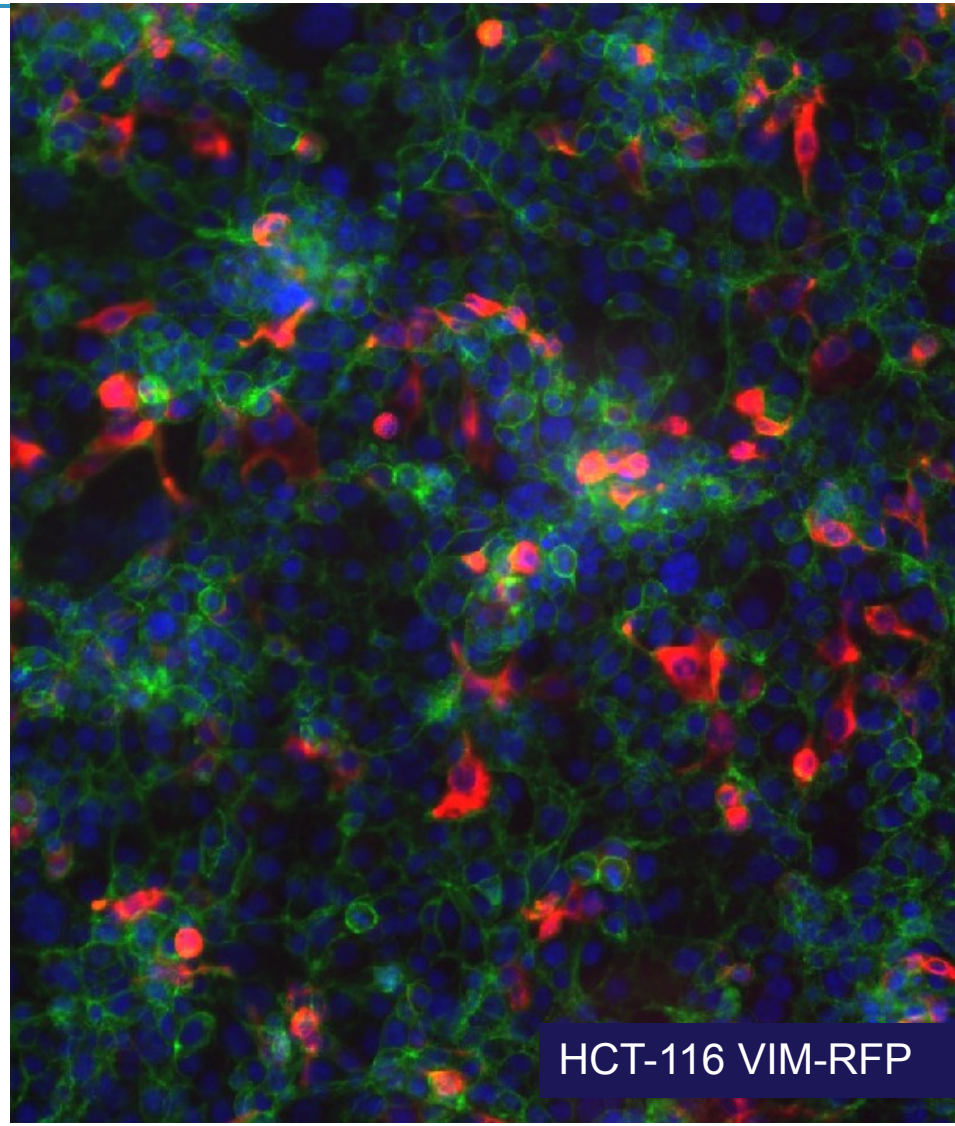
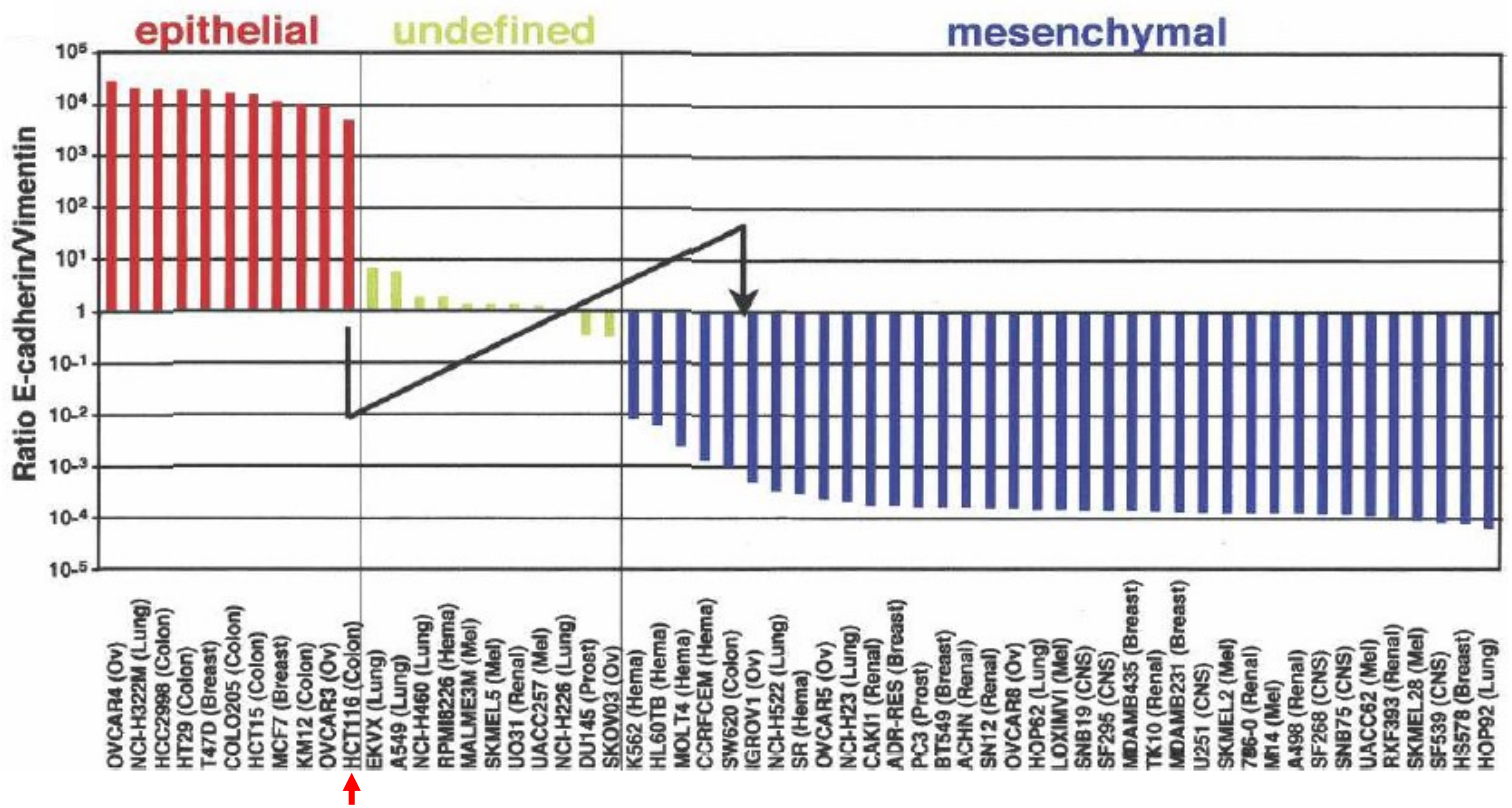
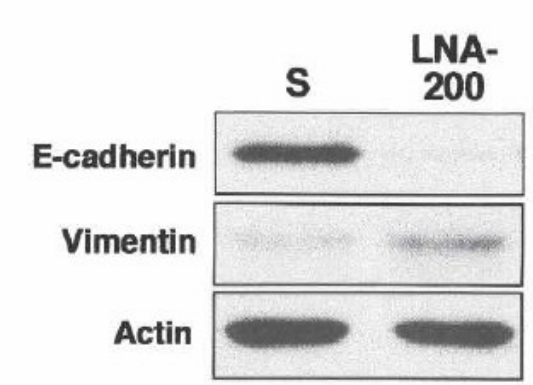


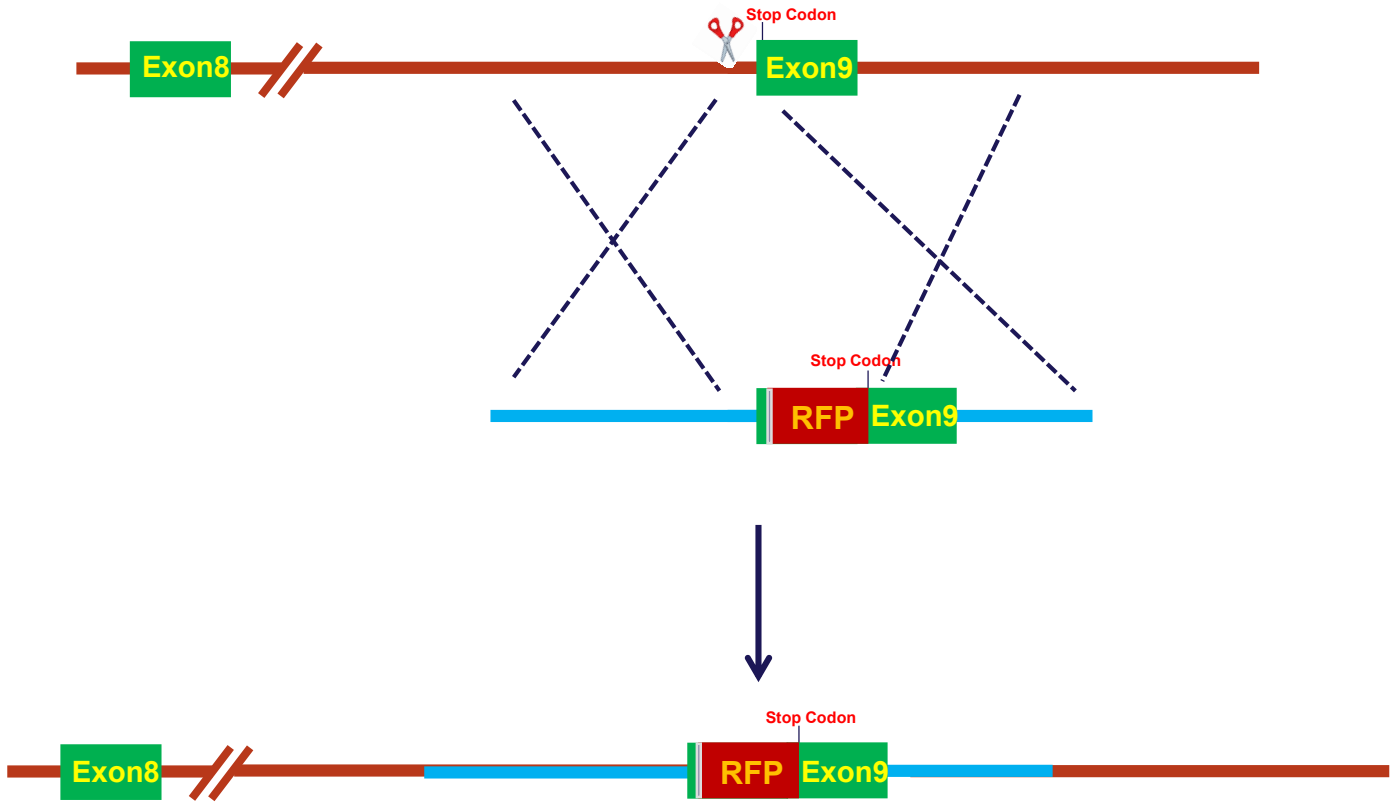
Colon cancer HCT-116 cells can be induced to undergo EMT





Park SM, et al, Genes Dev 22(7):894–907, 2008.

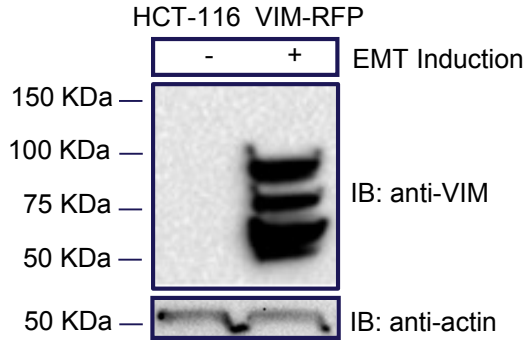
Precision editing to create vimentin (VIM)-RFP knock-in allele



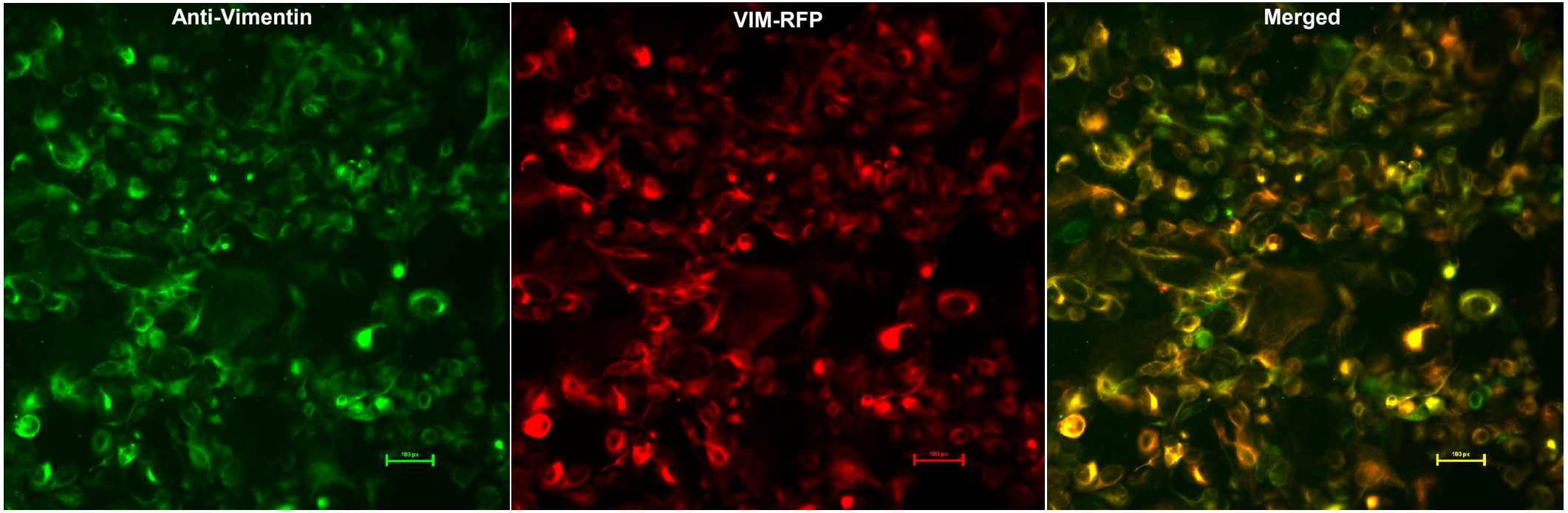
= CRISPR/Cas9

= 7 aa linker

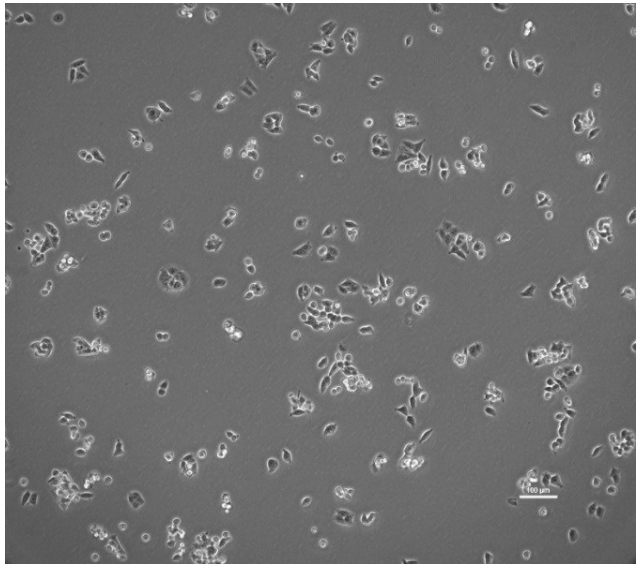
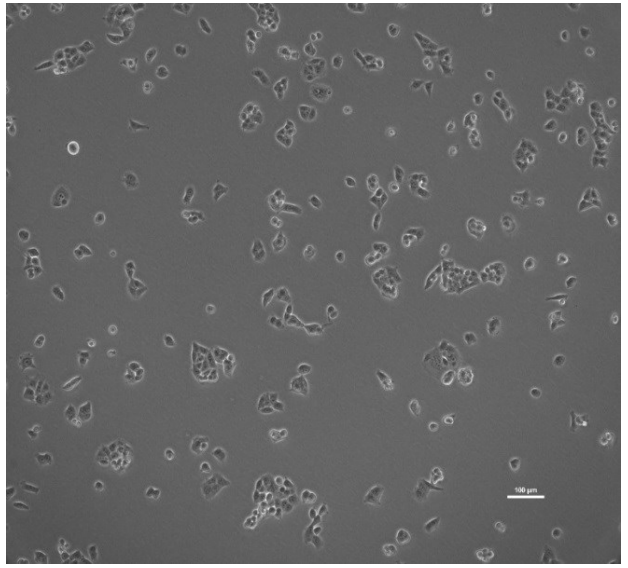
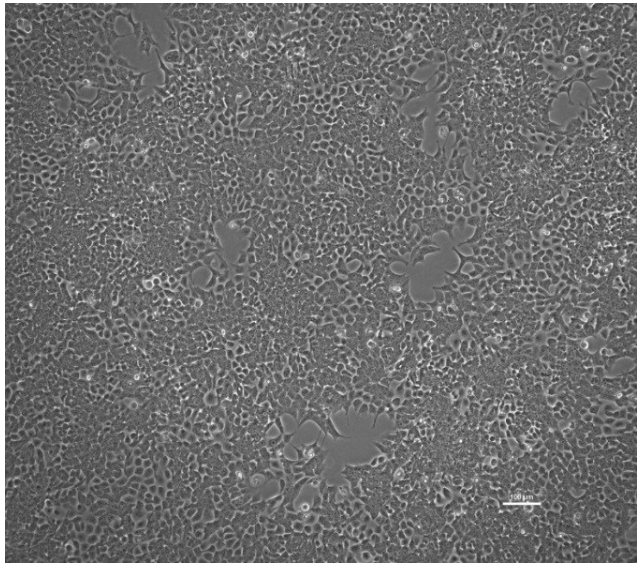
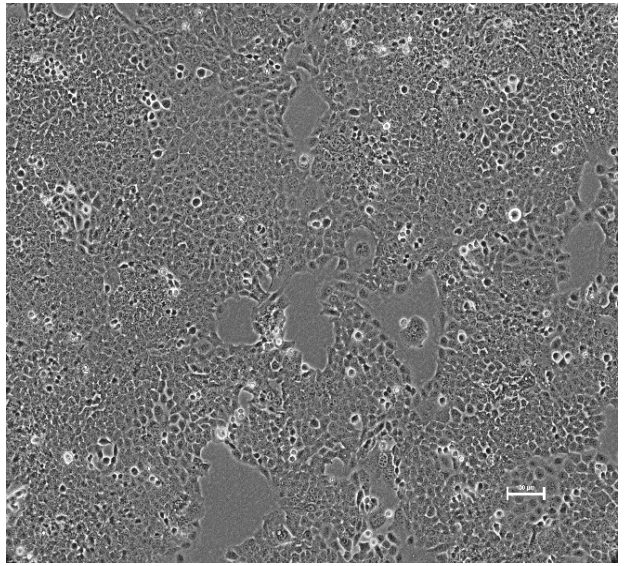
Detection of VIM-RFP fusion protein in VIM-RFP cells



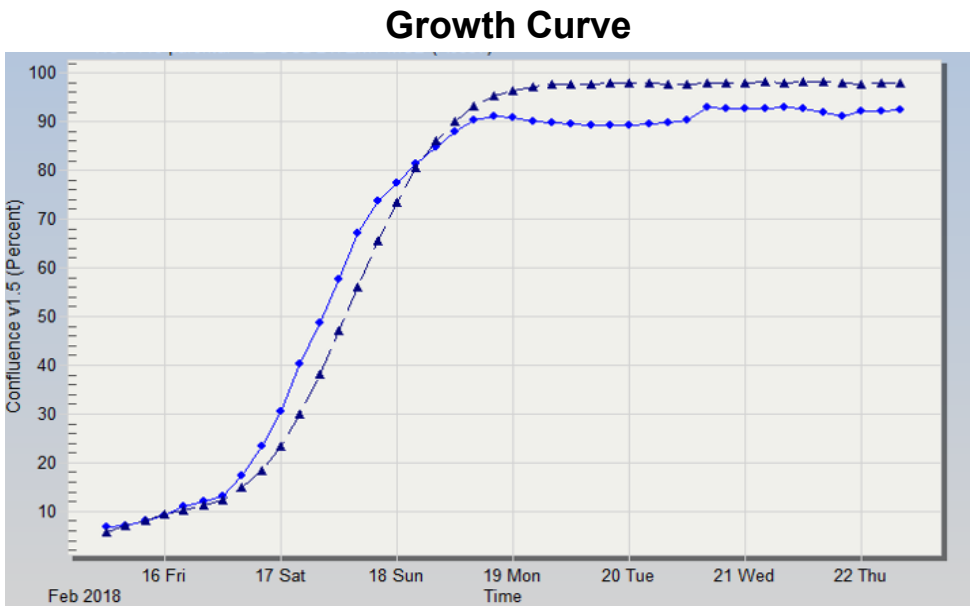
VIM-RFP: ~ 90 KDa
WT VIM: ~ 57 KDa



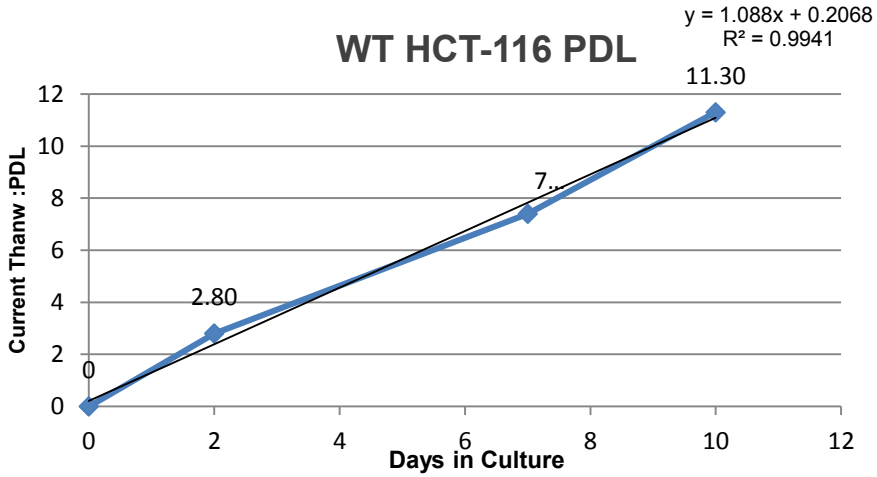
Morphology of HCT-116 VIM-RFP is similar to the parental line

	HCT-116 WT	HCT-116 VIM-RFP
Low density		
High density		

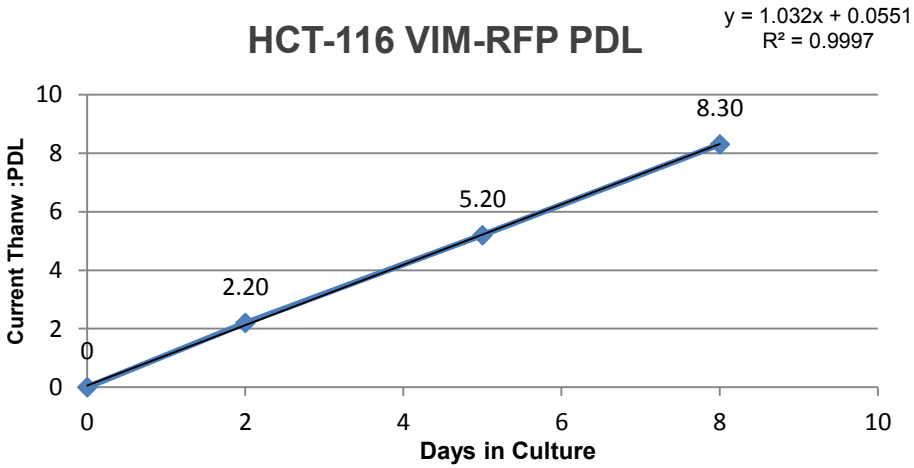
Growth kinetics of HCT-116 VIM-RFP are similar to WT HCT-116



• WT HCT-116 ▲ VIM-RFP HCT-116

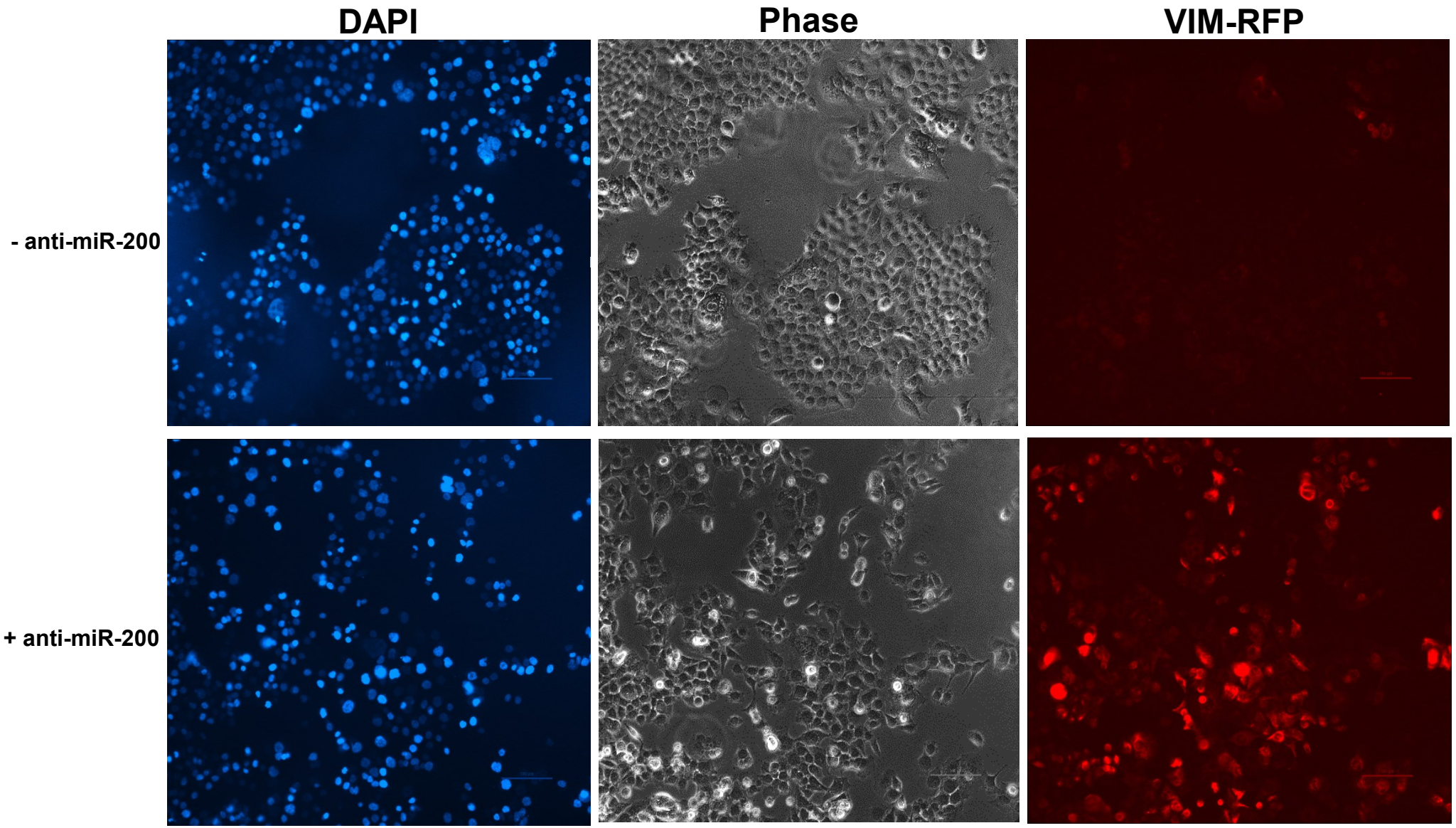


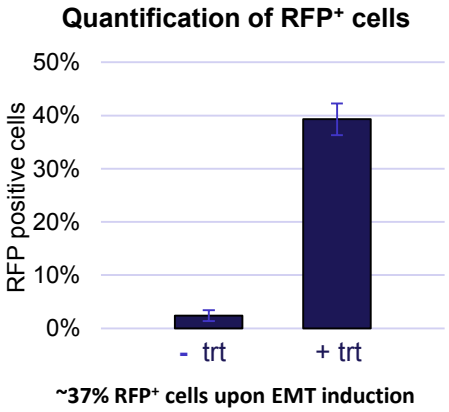
WT HCT-116 cells average population doubling time: **20.51 hours**



HCT-116 VIM-RFP cells average population doubling time: **22.96 hours**

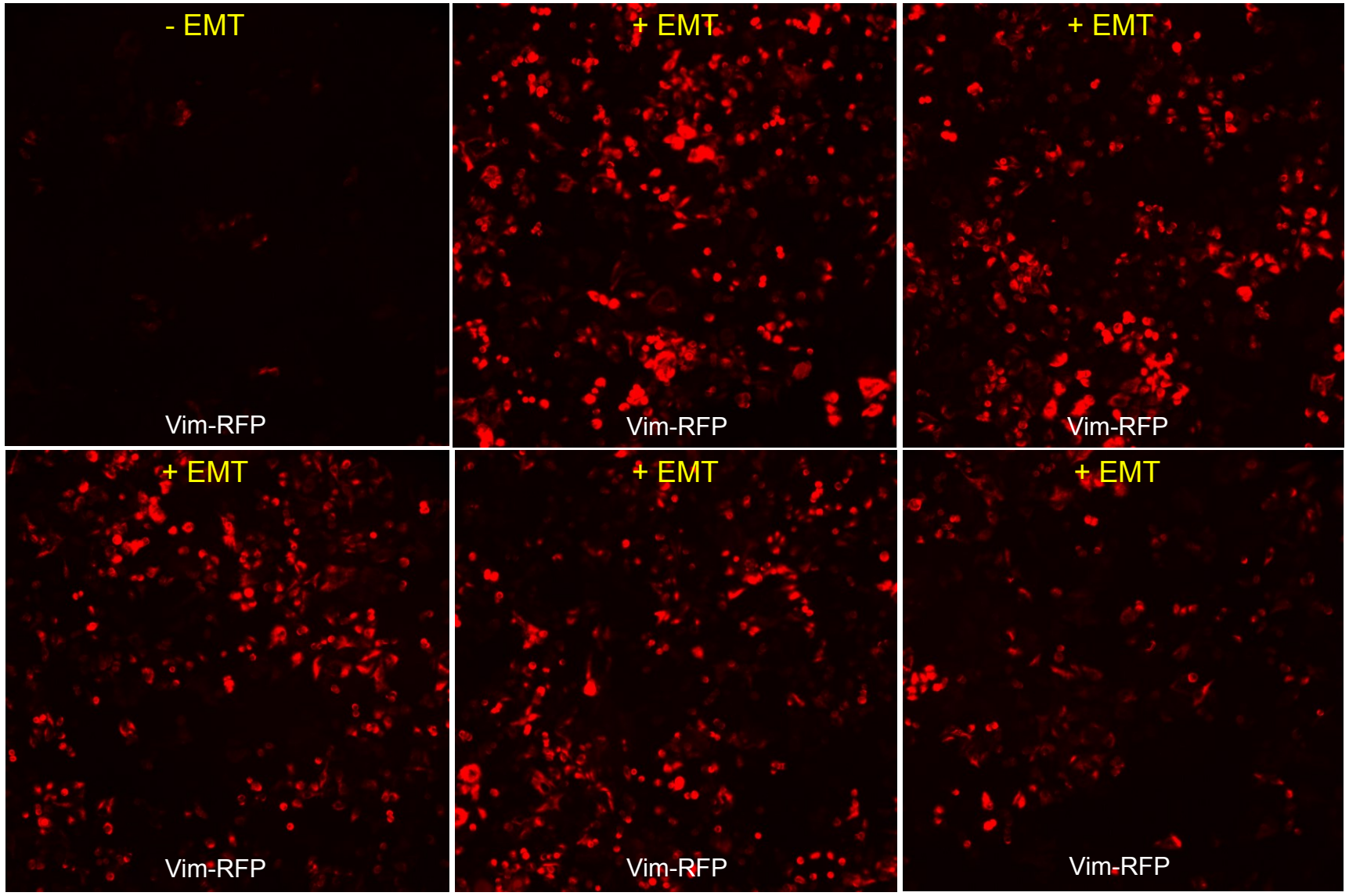
miR-200 inhibitors induce VIM-RFP expression in HCT-116 VIM-RFP

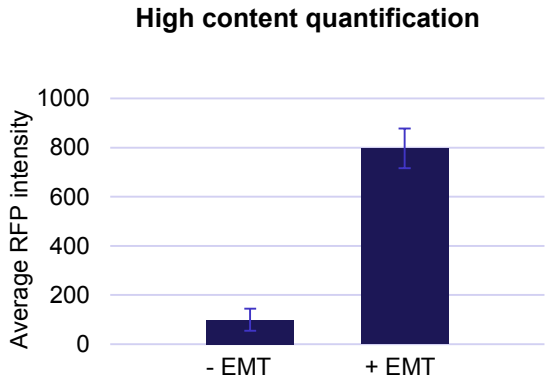




~37% RFP+ cells upon EMT induction

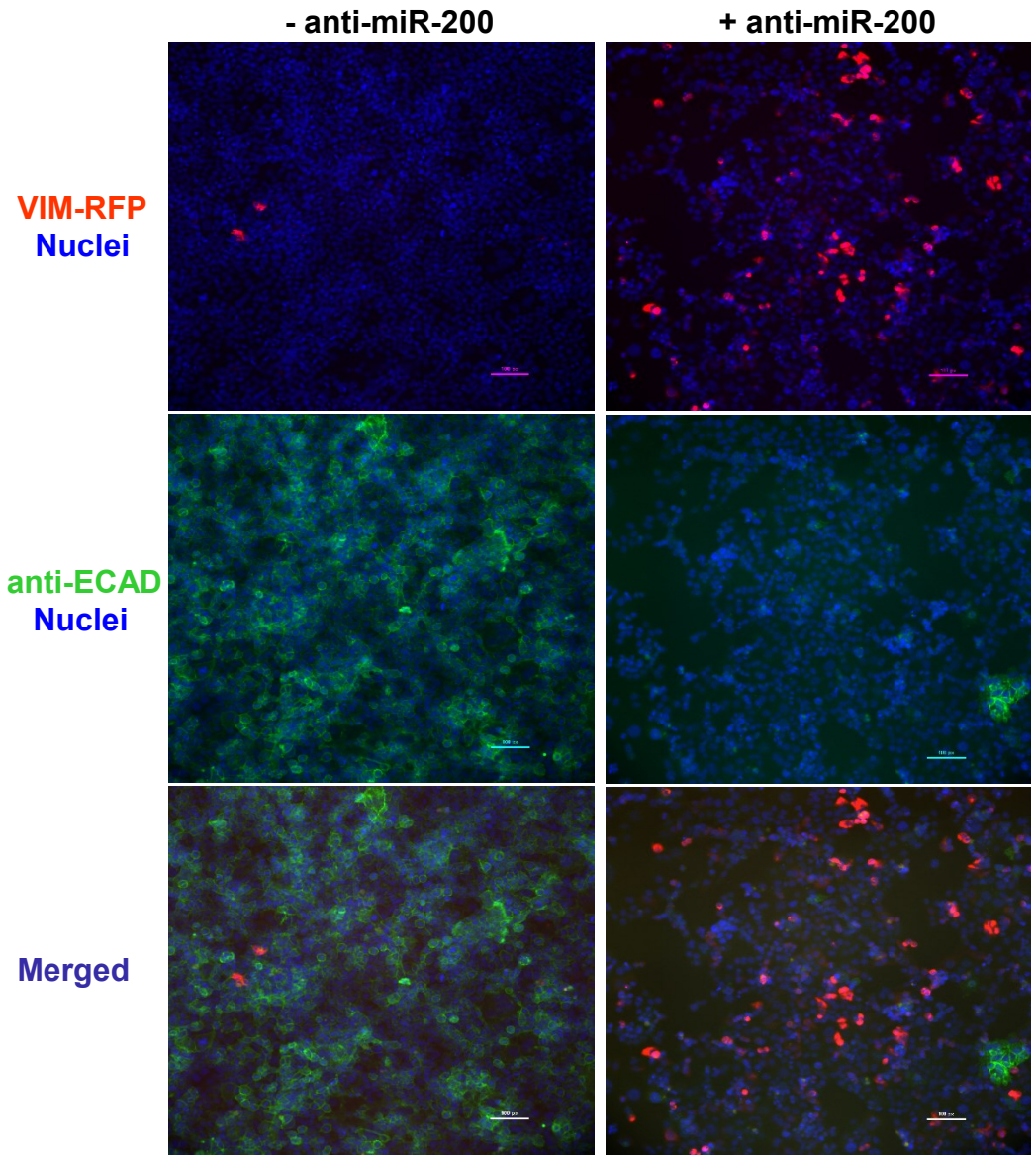
High-content imaging quantification of VIM-RFP expression

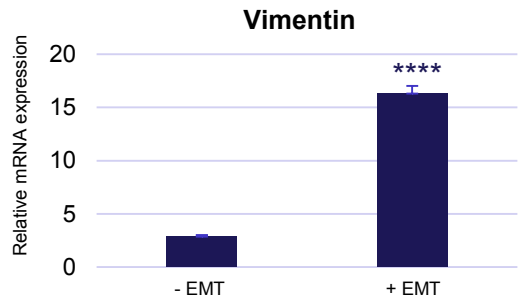




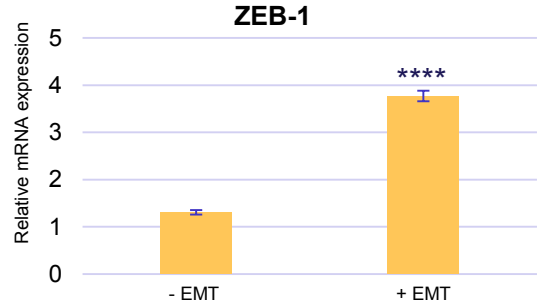
~ 8.0 fold increase
(21 days induction)

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

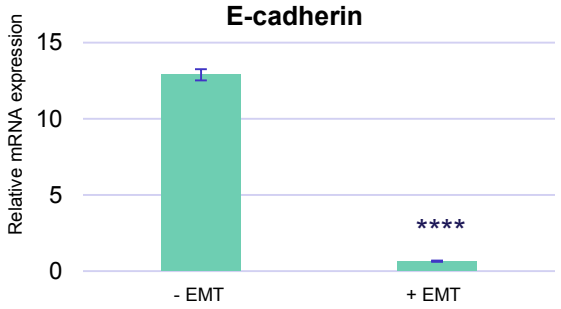




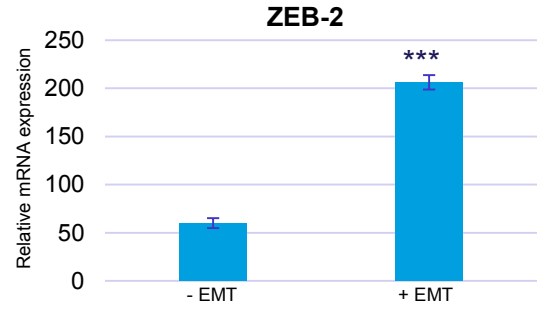
~ 5.7 fold increase



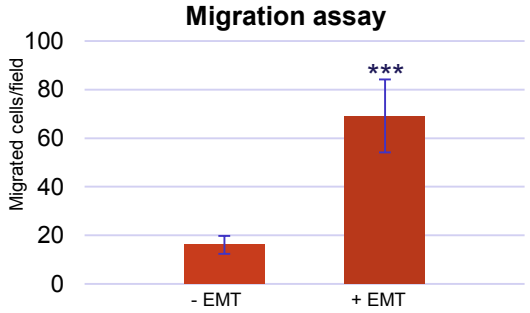
~ 2.9 fold increase



~ 19.8 fold decrease (95% decrease)

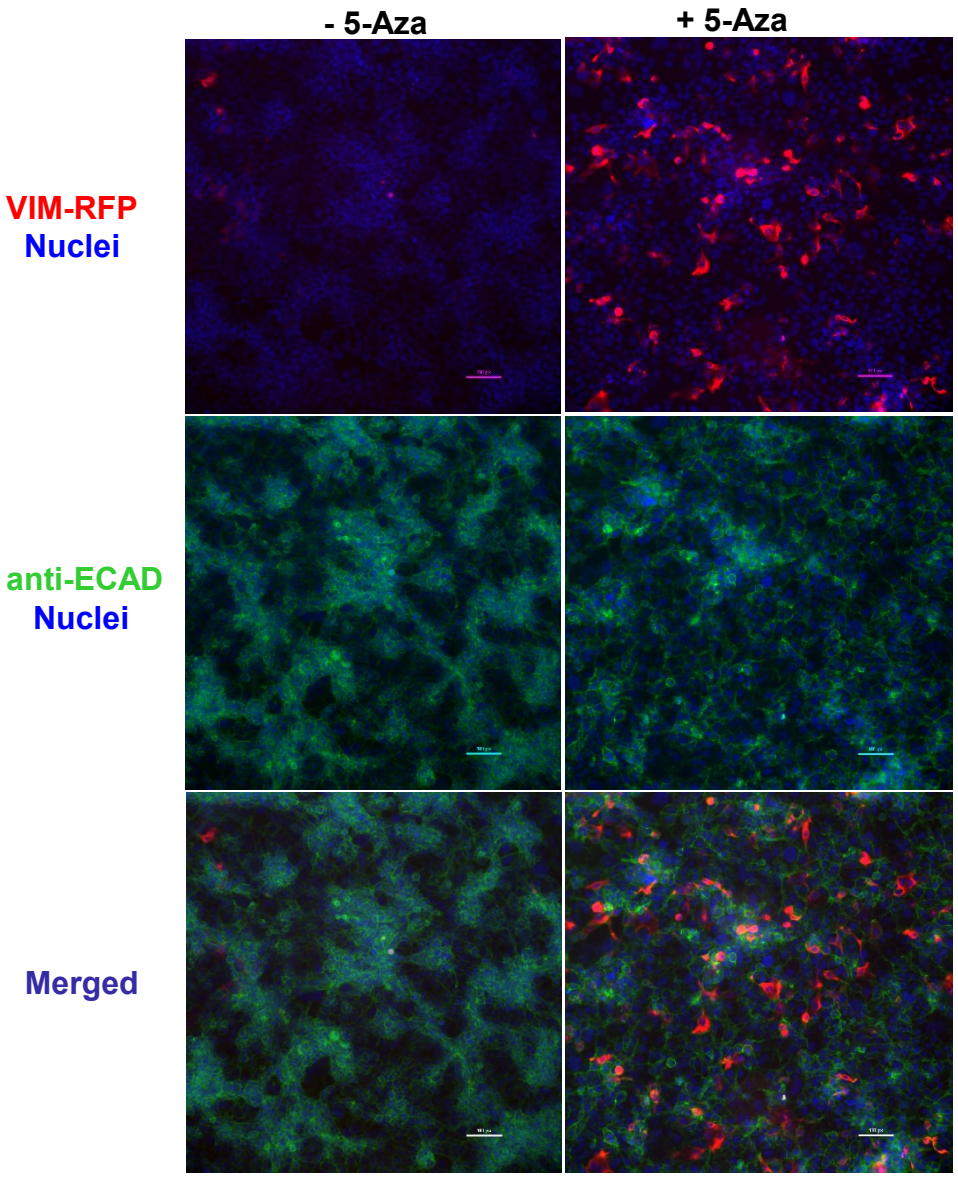


~ 3.4 fold increase

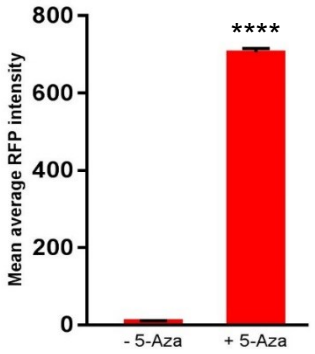


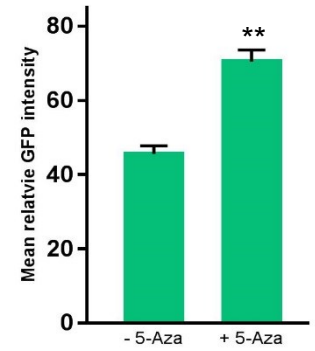
(Student's t-test, ***p<0.001, ****p<0.0001)

Demethylating agent azacitidine induces VIM-RFP expression

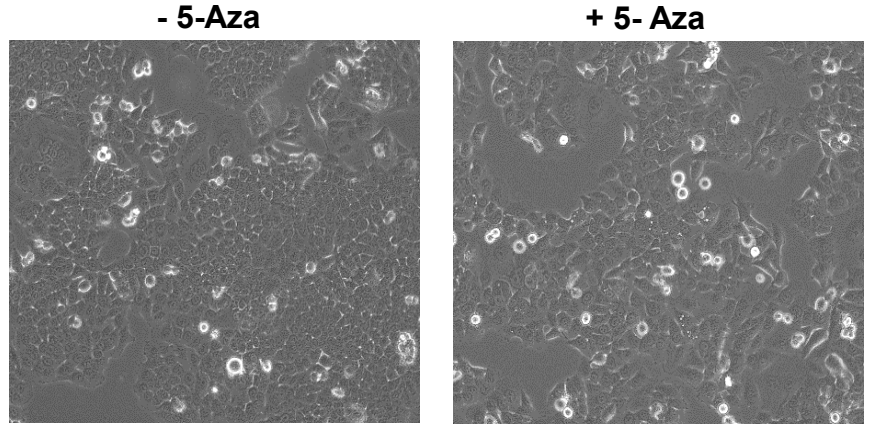


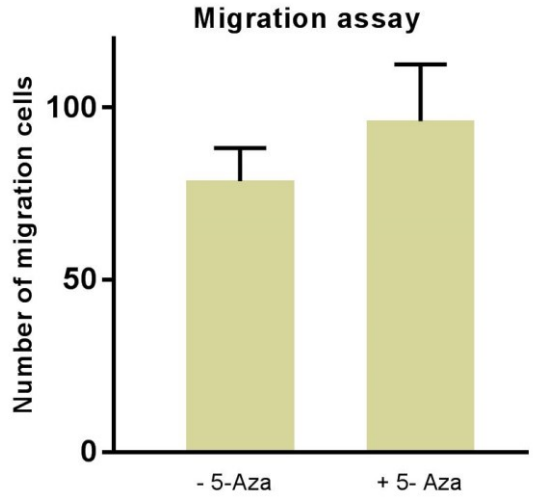
2 days induction





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





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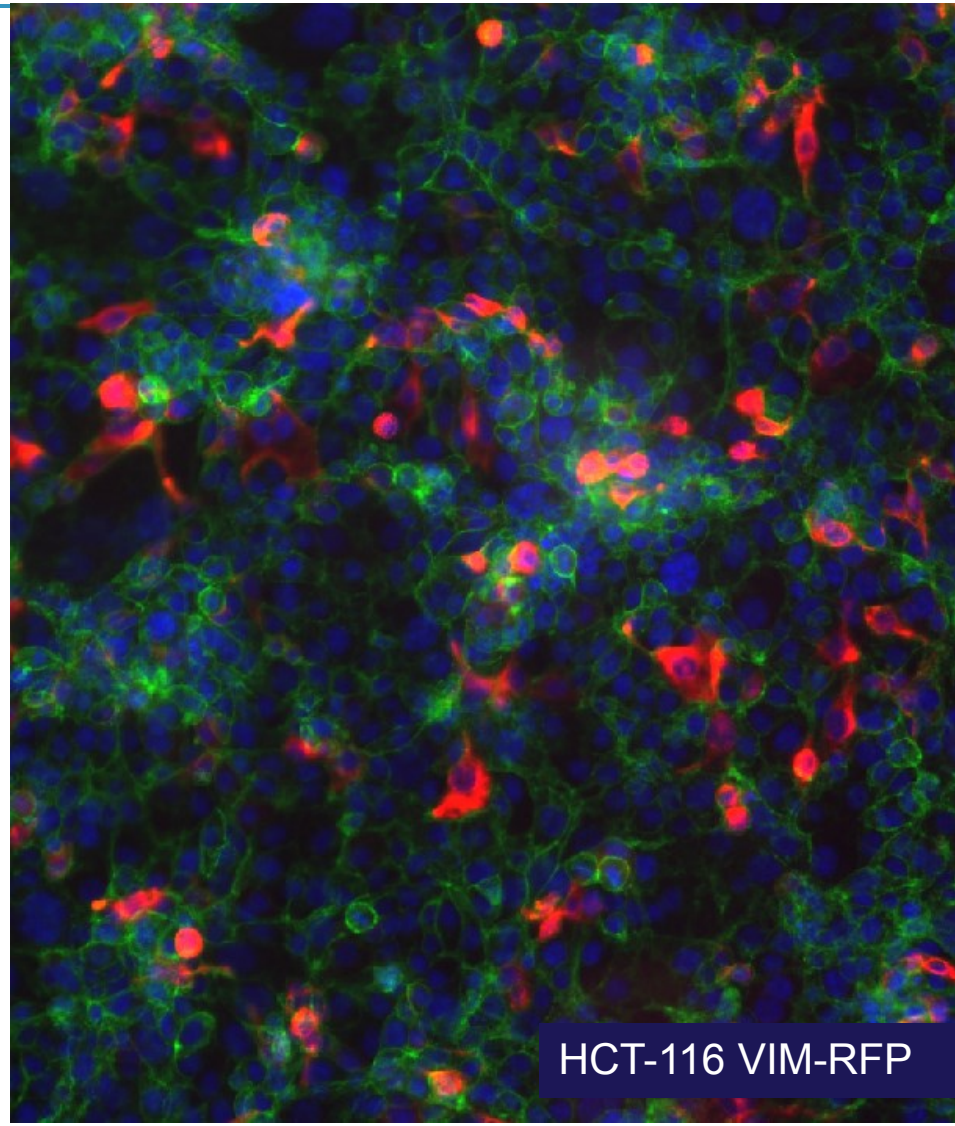
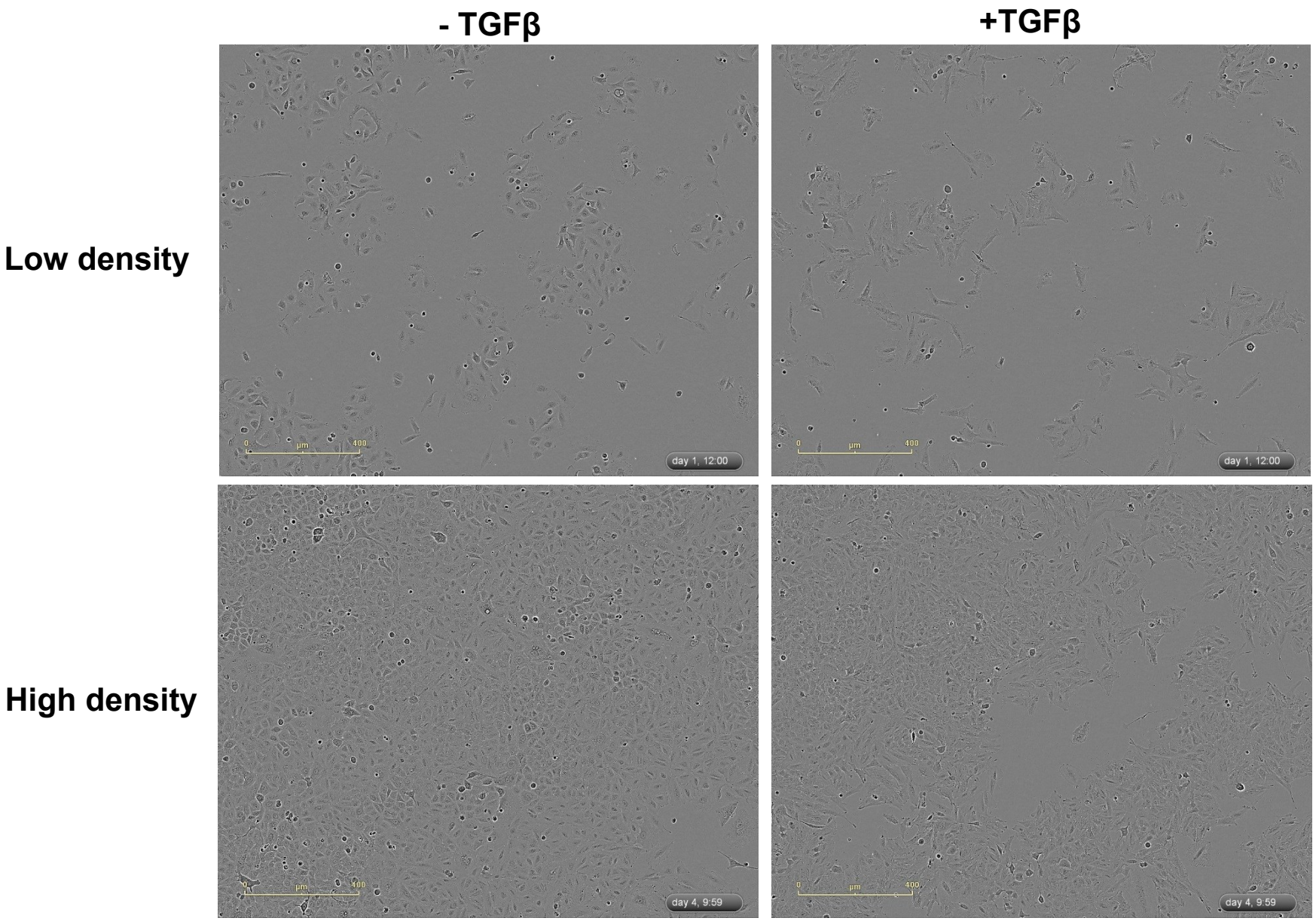
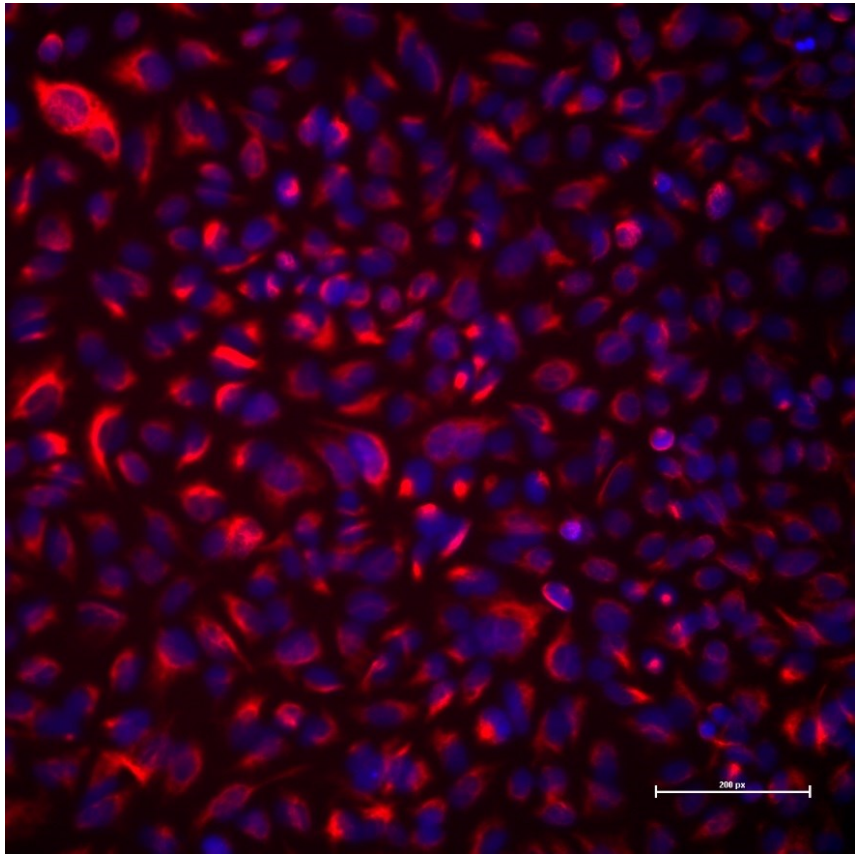


TGF-β treatment induces morphological changes in A549 VIM-RFP



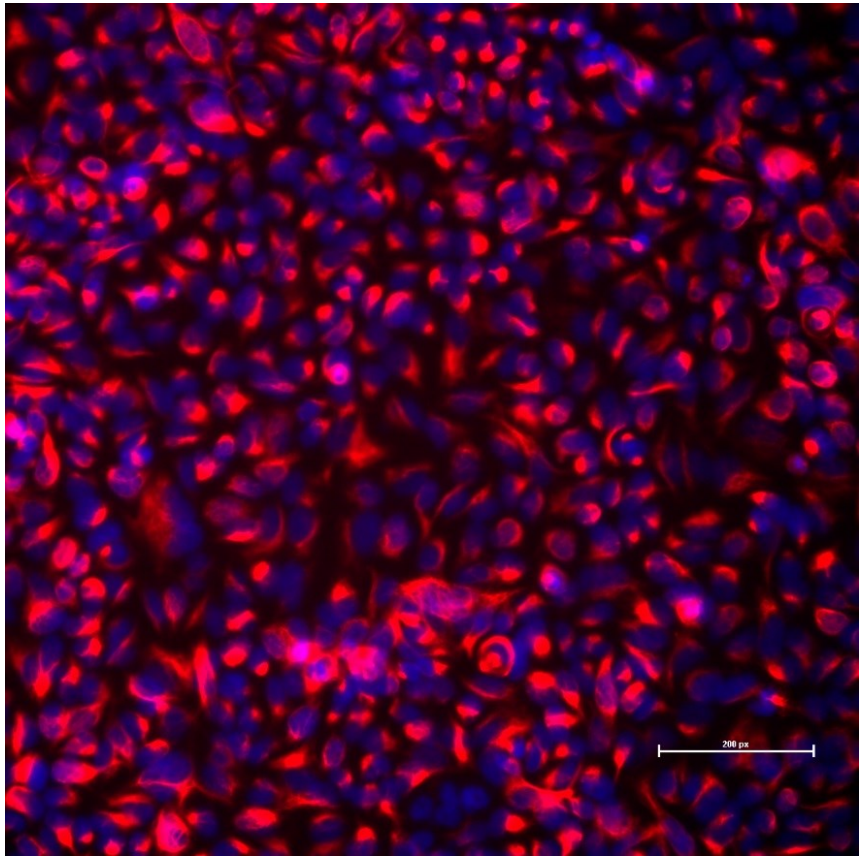
VIM-RFP expression is increased upon TGF-β EMT induction

- TGF-β

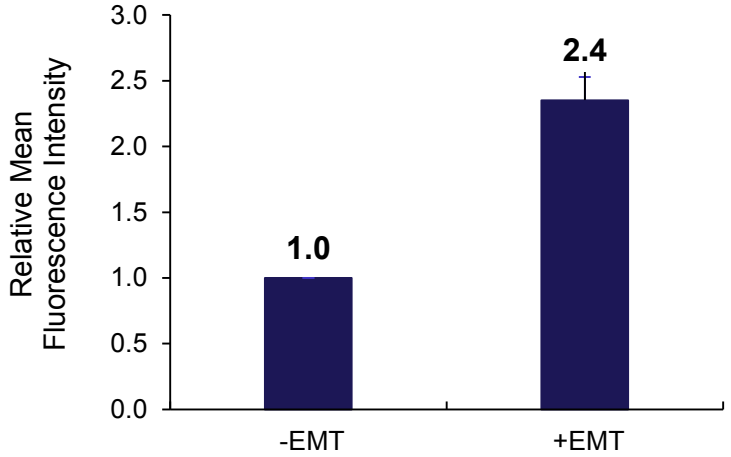


VIM-RFP, Nuclei

+ TGF-β

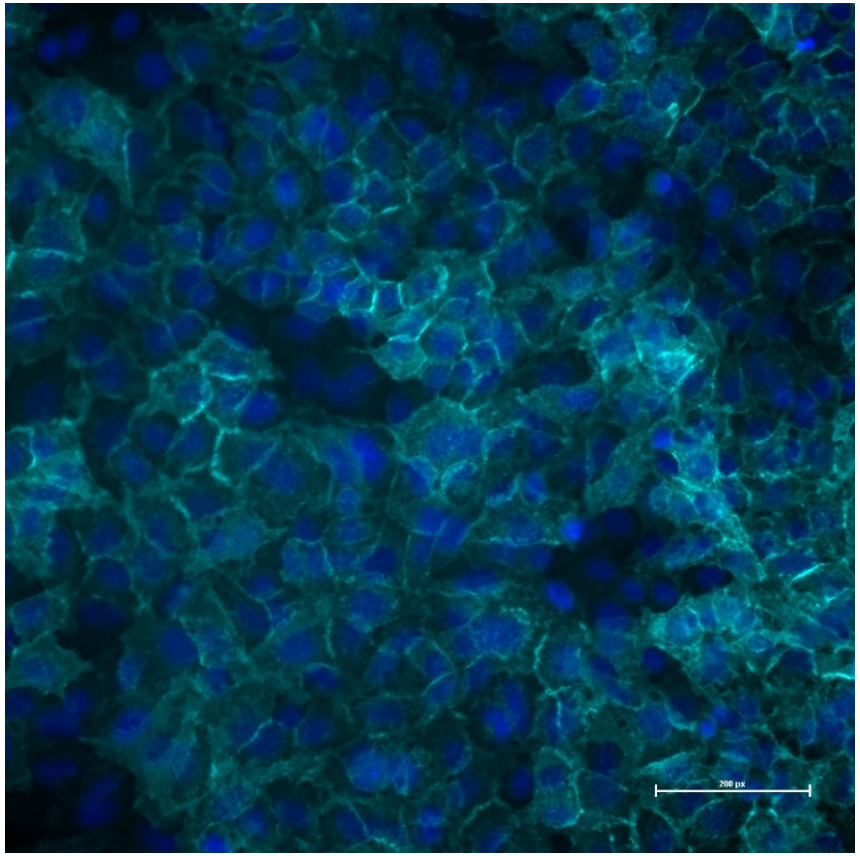


VIM-RFP, Nuclei



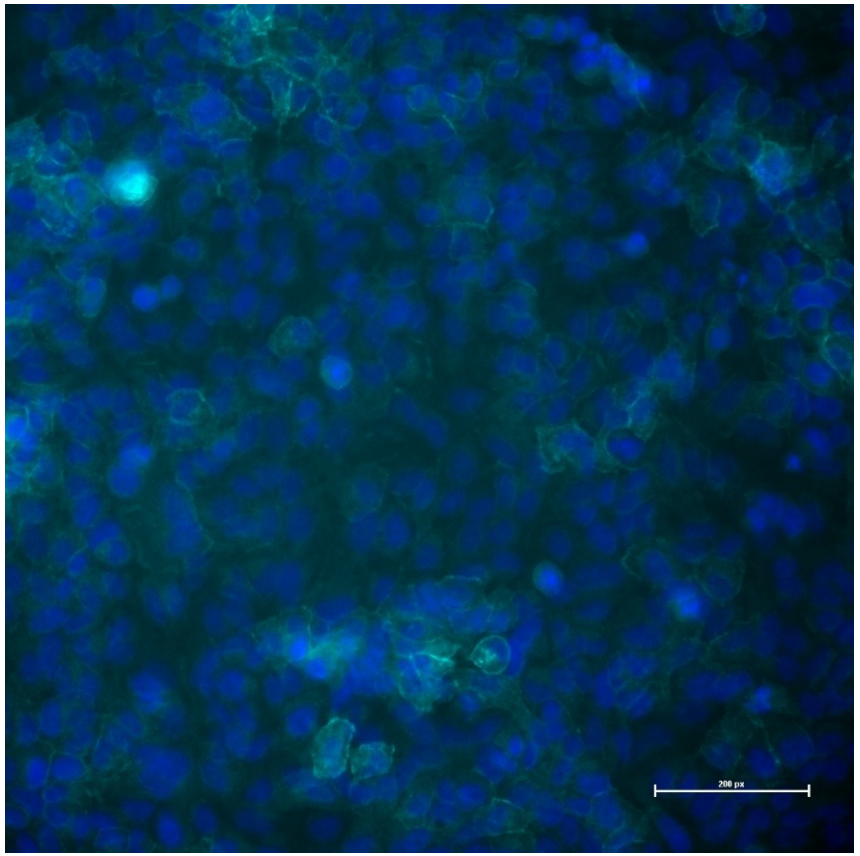
E-cadherin expression is decreased upon TGF-β induction

- TGF-β

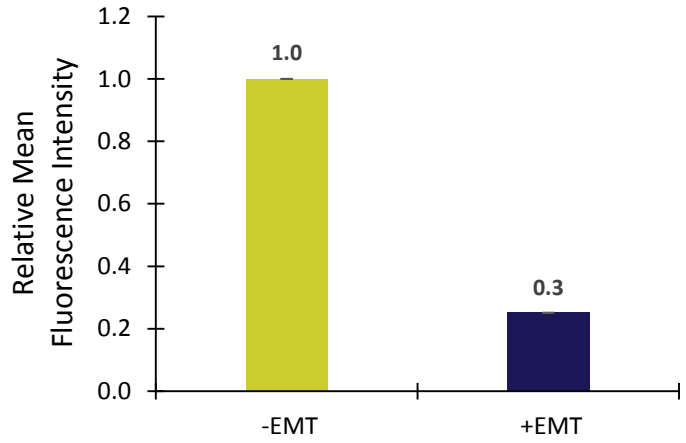


E-cadherin, Nuclei

+ TGF-β

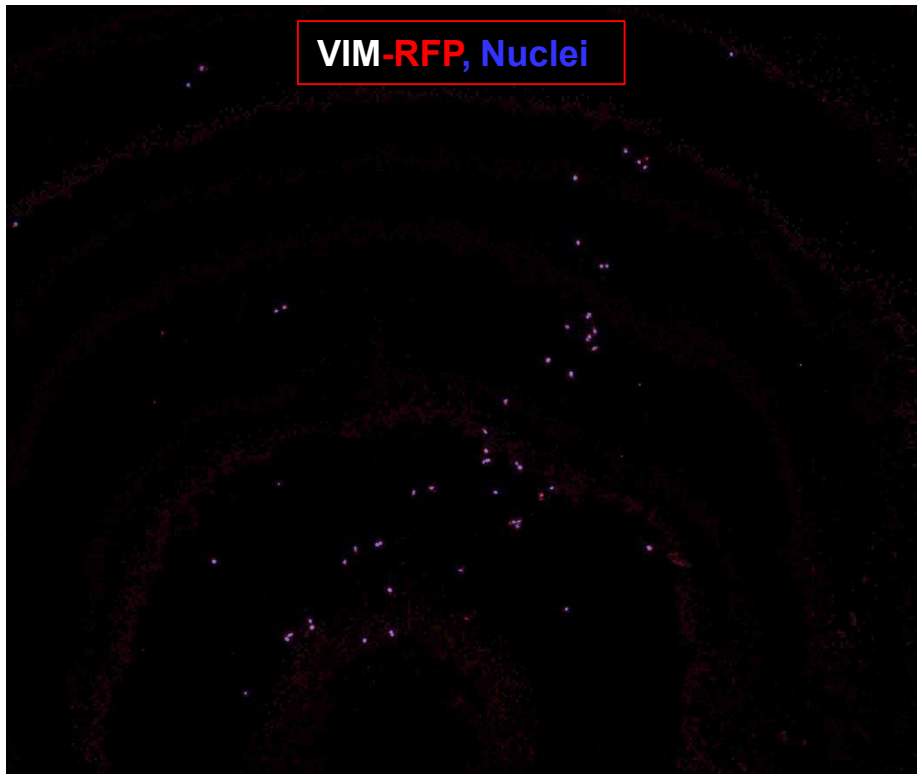


E-cadherin, Nuclei

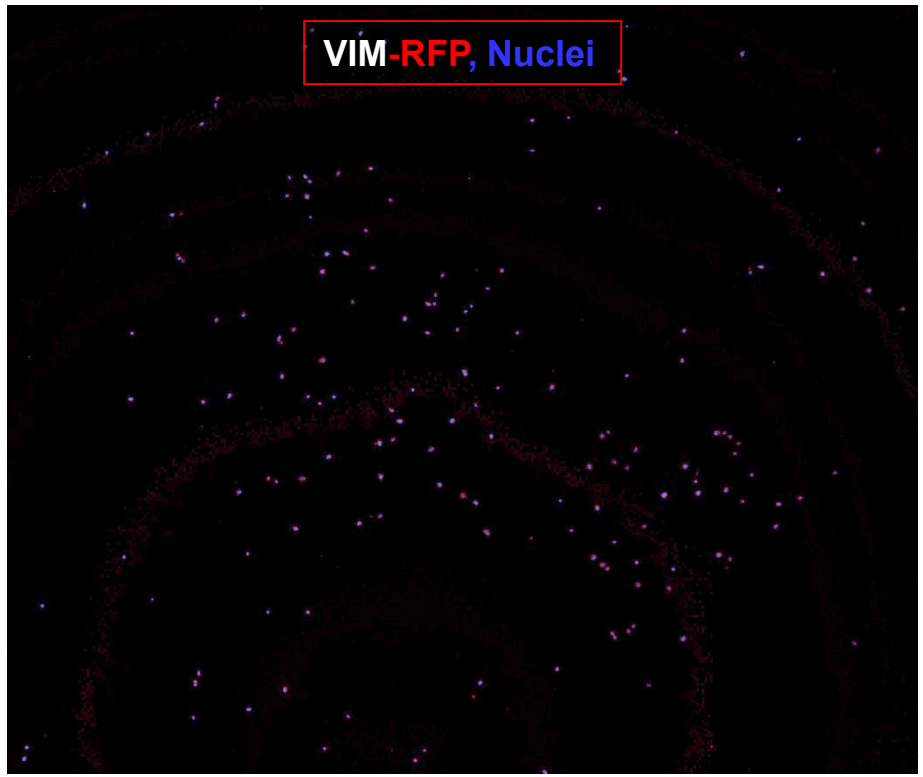


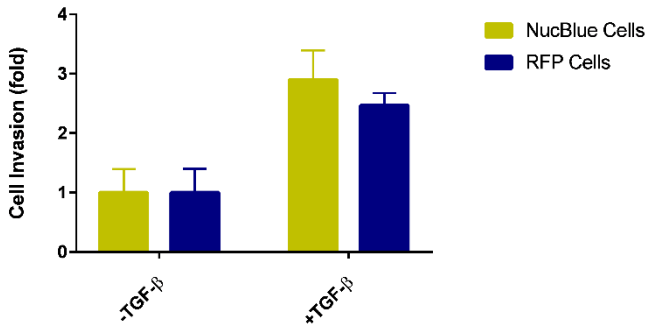
TGF-β Induced A549 VIM-RFP Cells Display Increased Invasiveness

- TGF-β

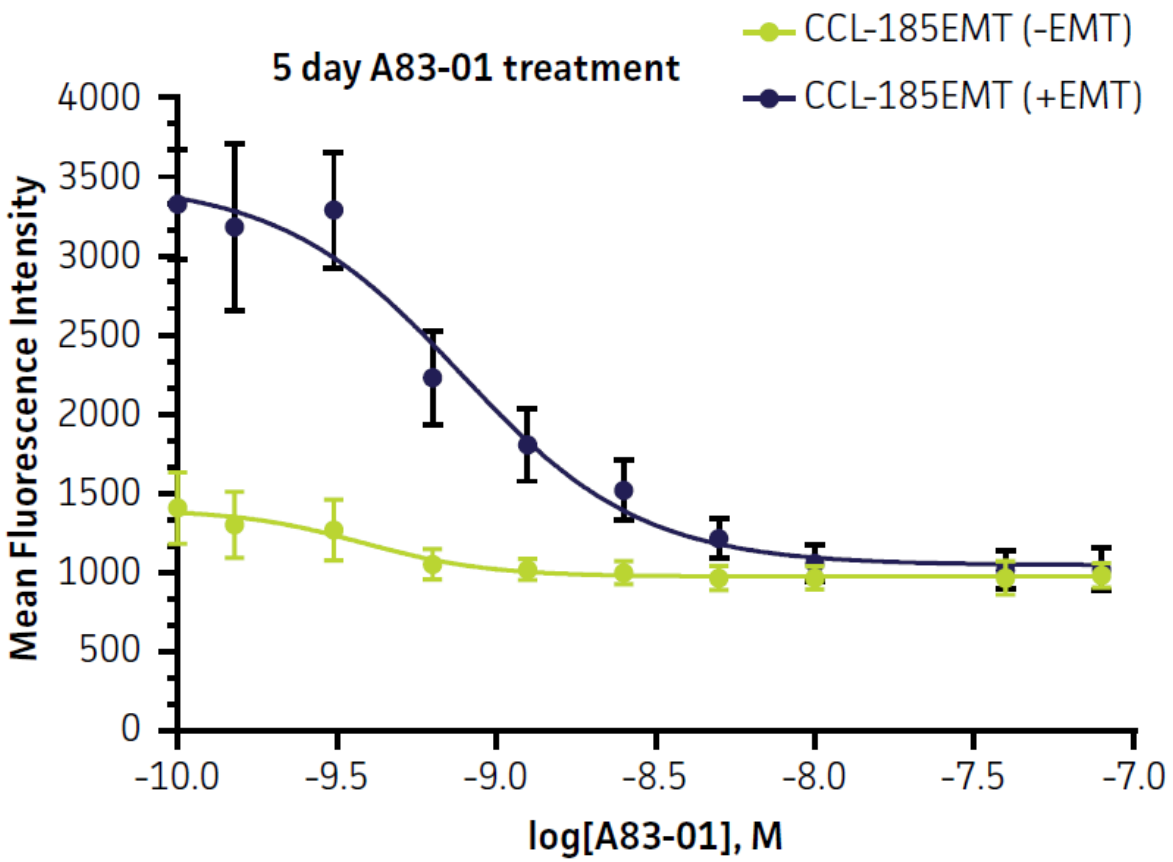


+ TGF-β

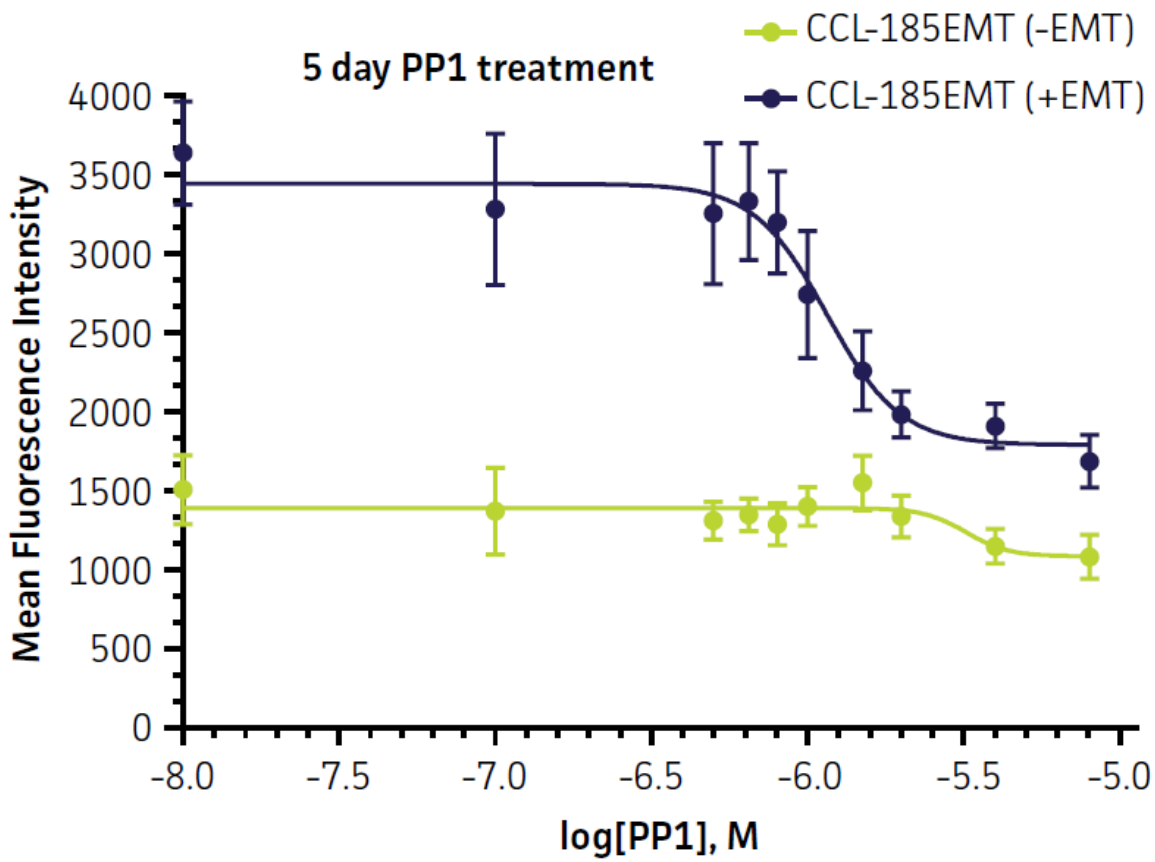




Small molecule EMT inhibitors block transition in A549 VIM-RFP



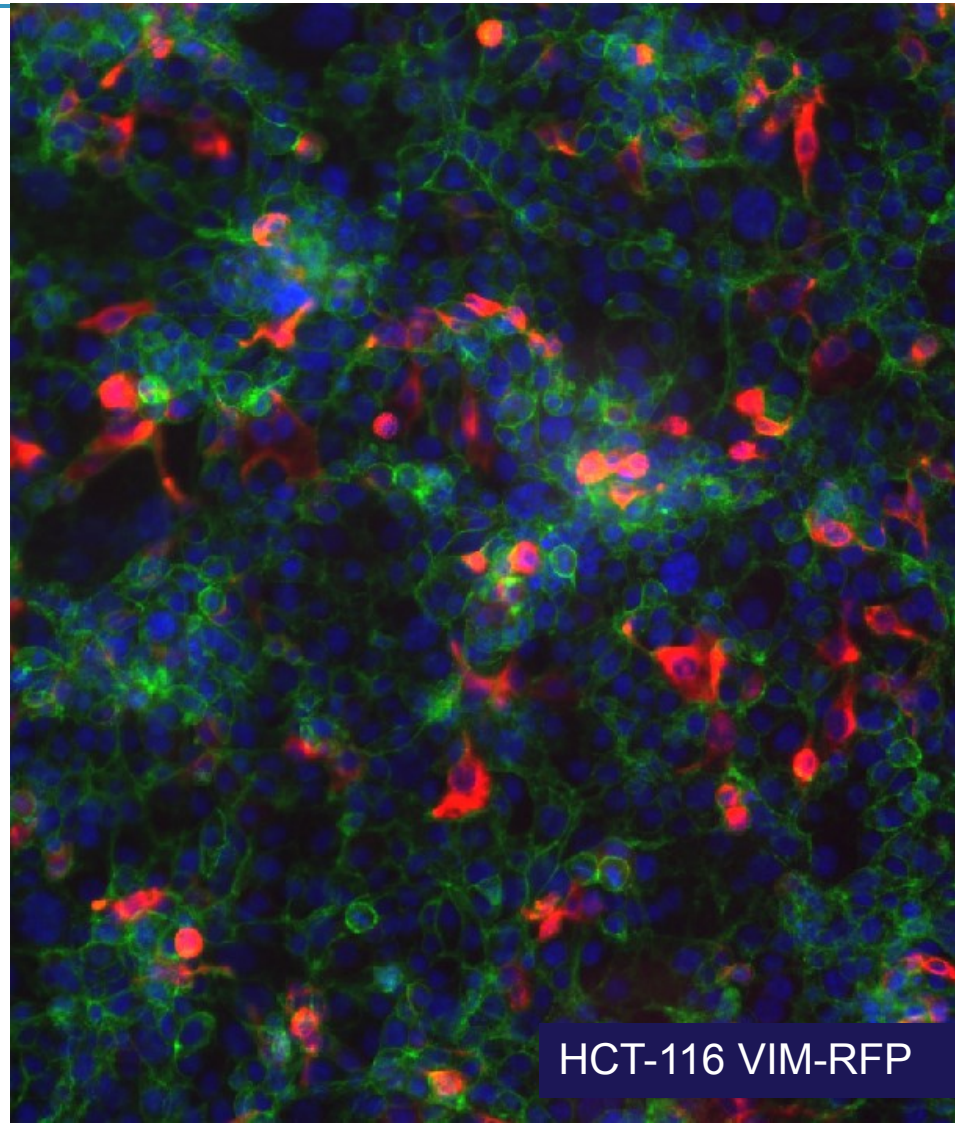
	CCL-185EMT (-EMT)	CCL-185EMT (+EMT)
IC50	3.822e-010	7.733e-010



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

Agenda

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



Summary

- 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.

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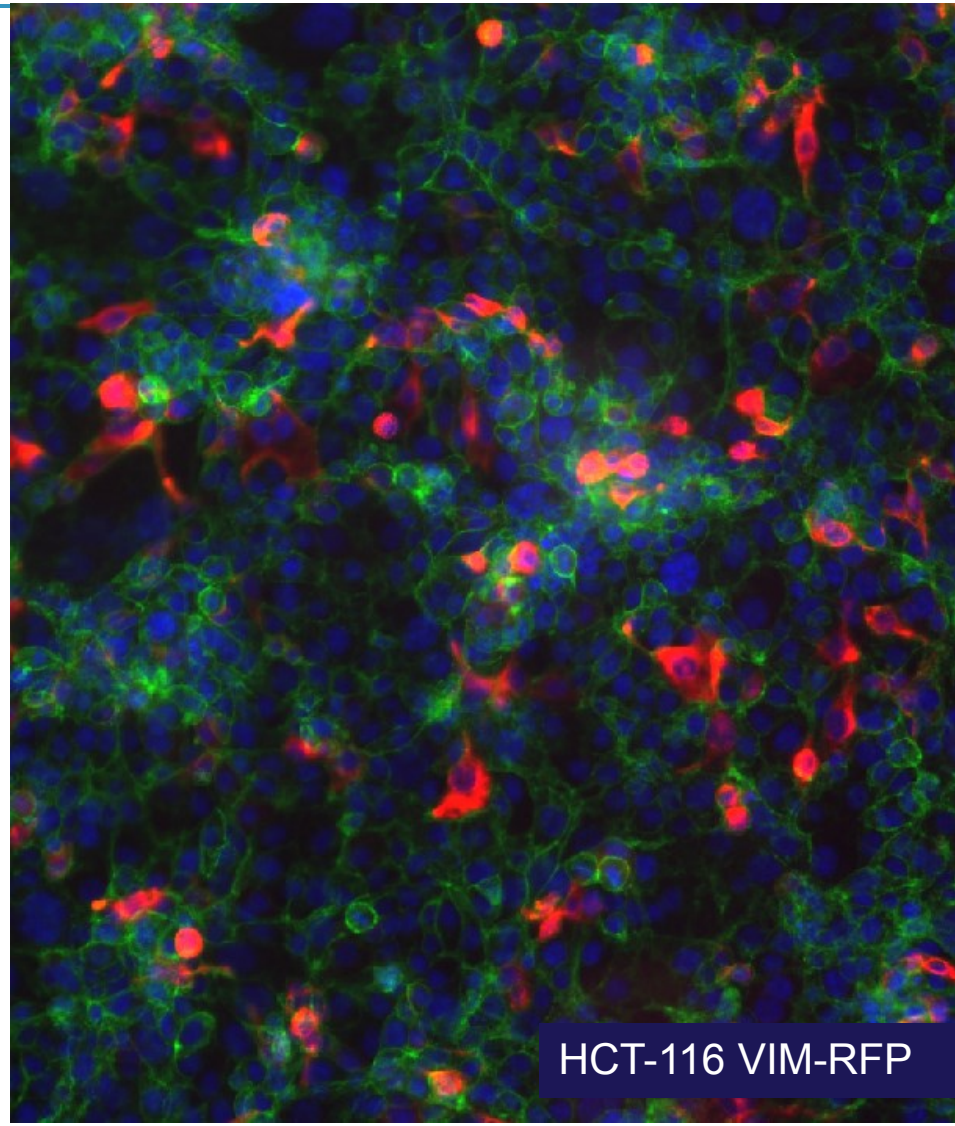
Thank you!

Disclaimer



For more information

- Website: www.atcc.org/EMT
- Flyer: Epithelial-mesenchymal Transition Reporter Cell Line
- Email: wshu@atcc.org

Thank you!