Understanding cell signal perception and misperception using optogenetic probes

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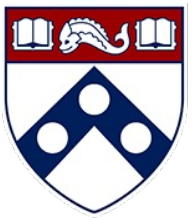
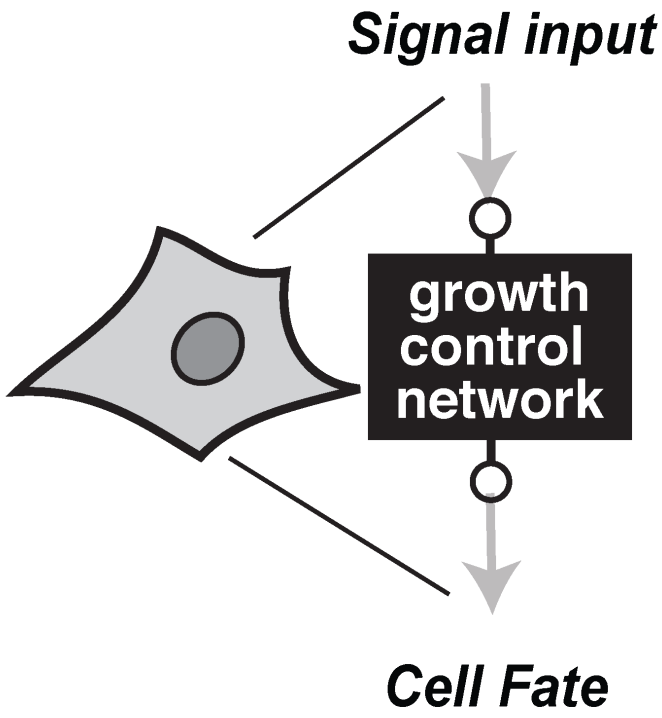
Assistant Professor, Bioengineering
University of Pennsylvania

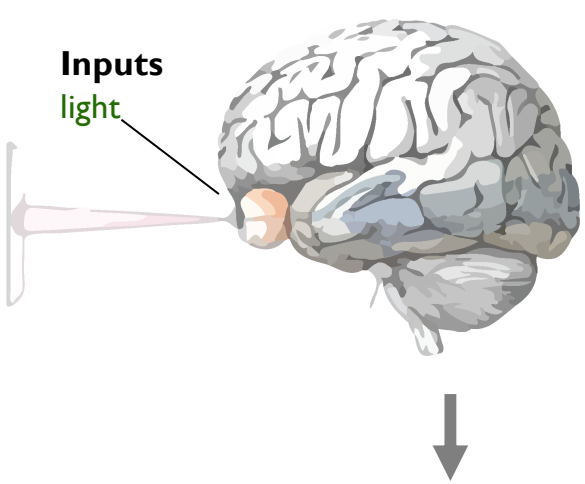


Penn Engineering
University of Pennsylvania

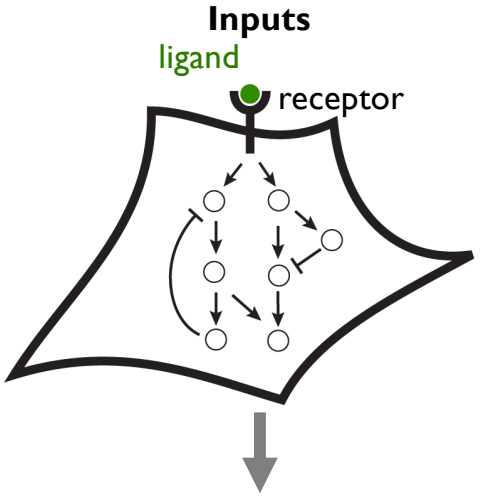
Instytut Podstawowych Problemów Techniki PAN
15.5.2023


Signal input
growth
control
network
Cell Fate

Signaling networks are the brains of the cell

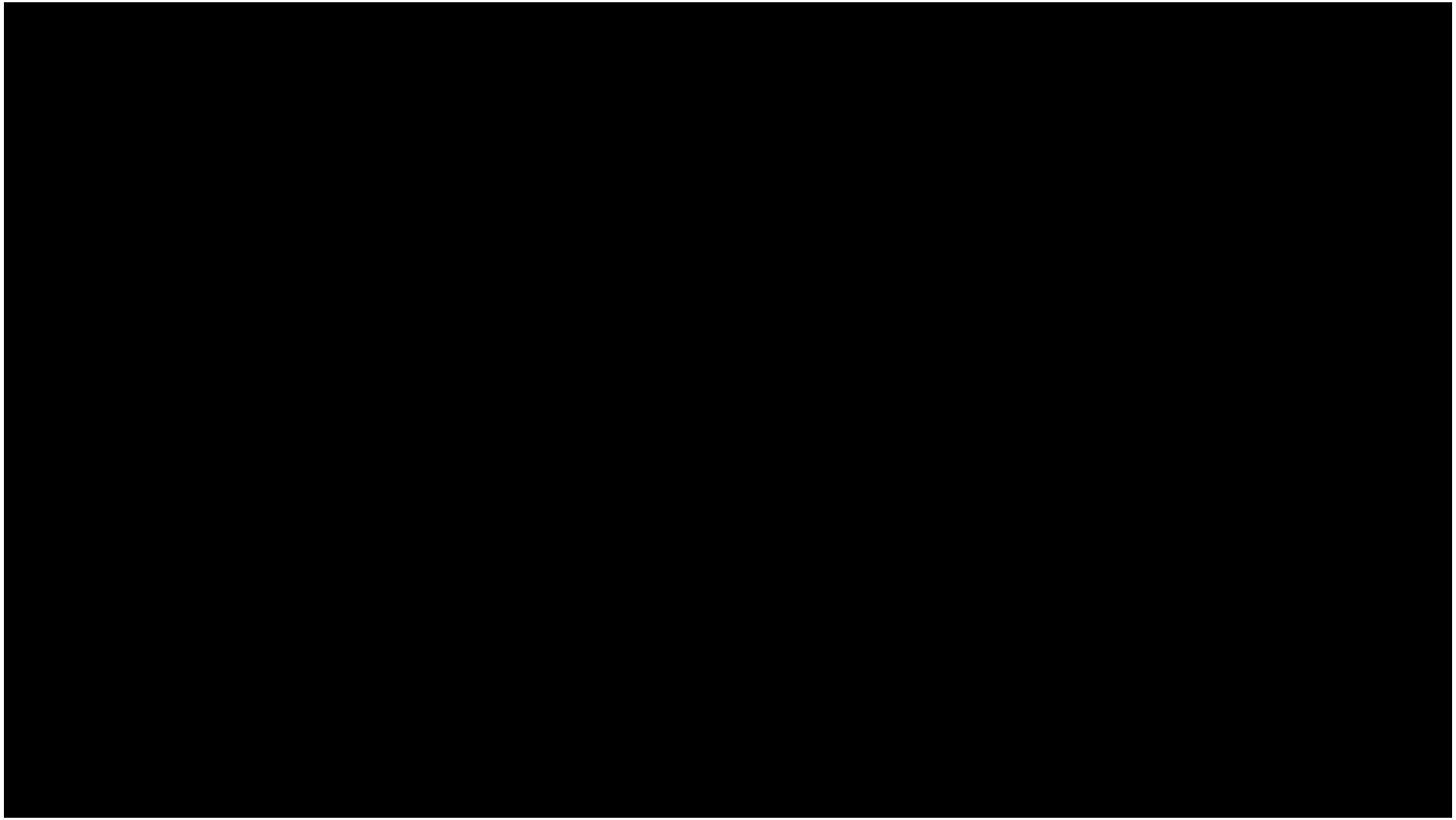


Outputs
(movement, speech, etc)



Outputs
(division, differentiation, death, etc.)

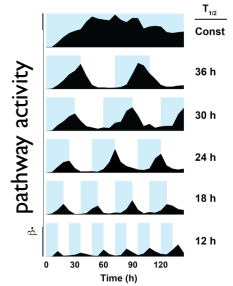
Bugaj Lab:
Precision tools to understand ***signal perception*** and ***misperception***

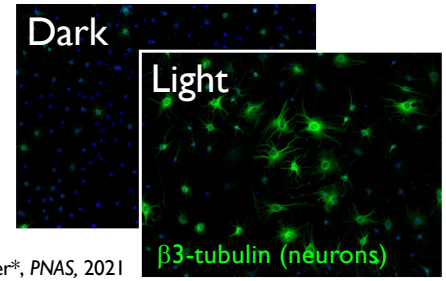


Optogenetic protein clustering and cellular control

Stem cell fate choice (neurogenesis)

β-catenin

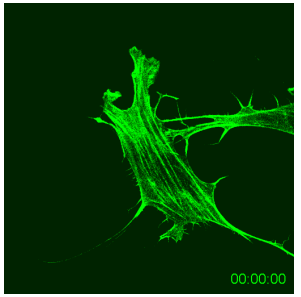




Rosenbloom, …, Bugaj*, Schaffer*, *PNAS*, 2021

receptors
(endogenous)

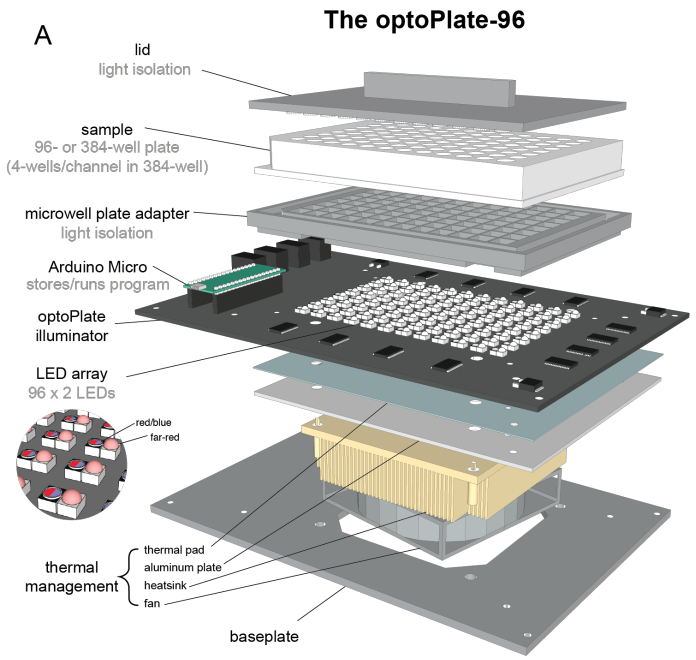
cell movement

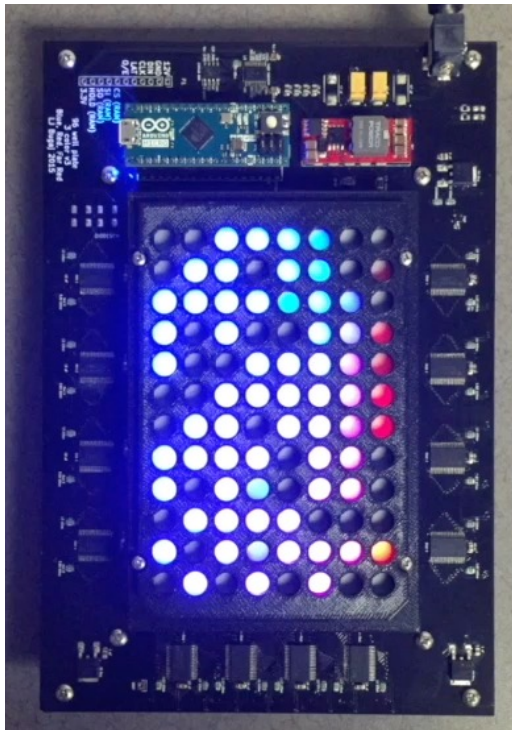


Bugaj et al., *Nature Communications.*, 2015

many more!

Open-source devices for optogenetics in cell bio





Bugaj et al., *Science*, 2018

Bugaj et al., *Nature Protocols*, 2019

User interface
Thomas, Hoerner, Weber,
Nature Protocols, 2020

Automated Calibration
Grodem, Sweeney, McClean,
Biotechniques, 2020

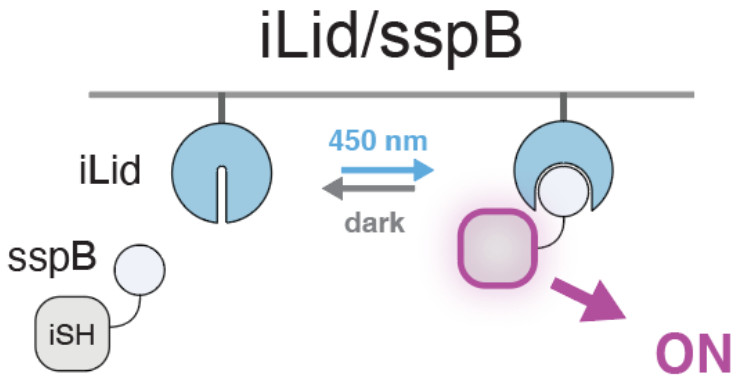
Assembly notes
Mary Dunlop, protocols.io, 2021

all files, protocols, and references:
www.bugajlab.com/optoplate-96

Purchase preassembled:
labMaker.org

But many ways to do optogenetics!

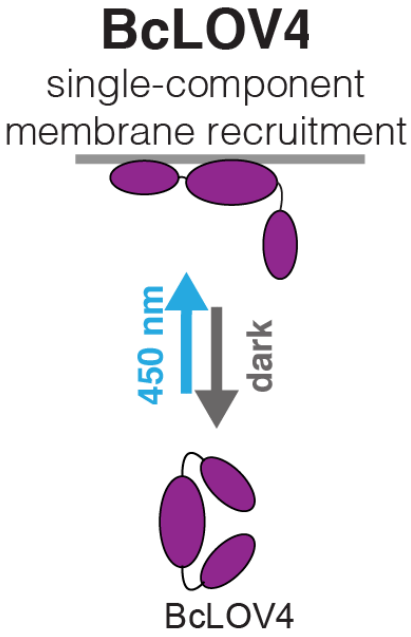
dimerization
membrane translocation

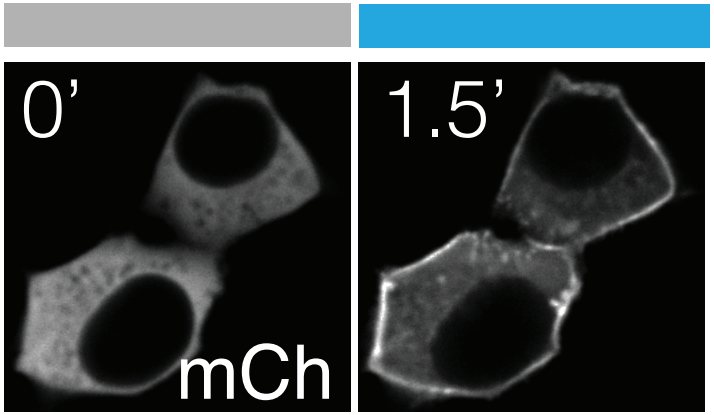


iLid: Guntas et al., *PNAS*, 2015

BcLOV4: single component optogenetic recruitment

Brian Chow, PhD





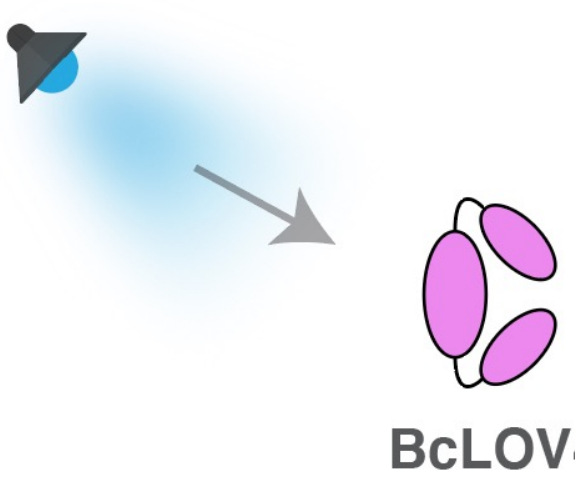
Glantz et al, *PNAS*, 2018

Berlew et al, *Photochem. Photobiol. Sci,* 2020

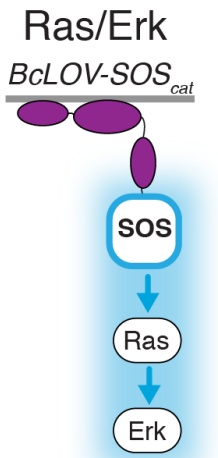
Berlew et al, *bioRxiv,* 2021

INPUT

OUTPUT

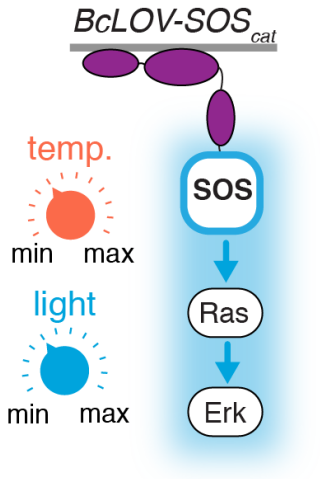


Spontaneous signal decay in mammalian cells



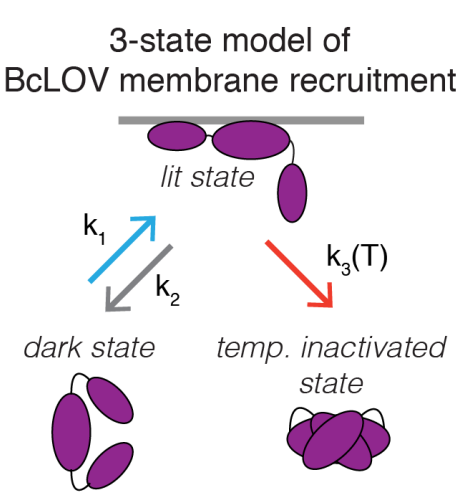
Benman,…, Bugaj, *Nat. Chem. Bio*, 2022

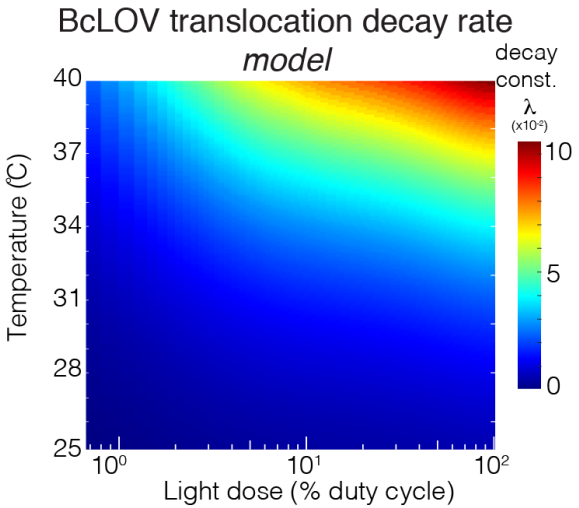
BcLOV decays is a function temperature …and light



Benman,…, Bugaj, *Nat. Chem. Bio*, 2022

Modeling suggests a 3rd state of BcLOV



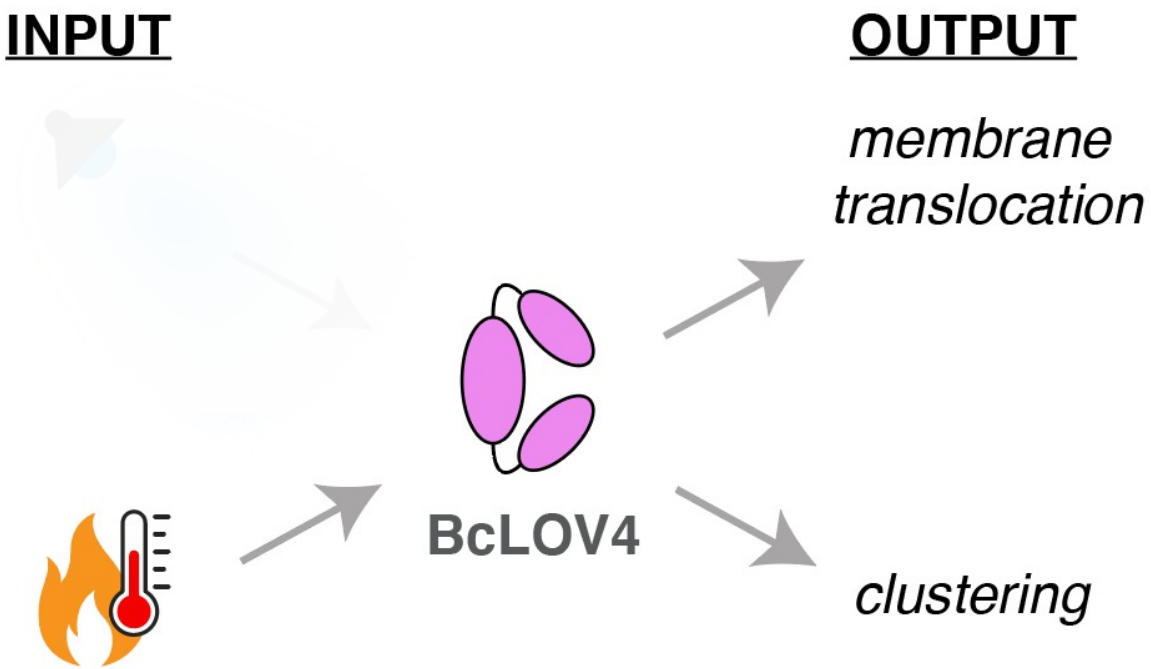


Ongoing:

What is molecular nature of thermosensitivity?

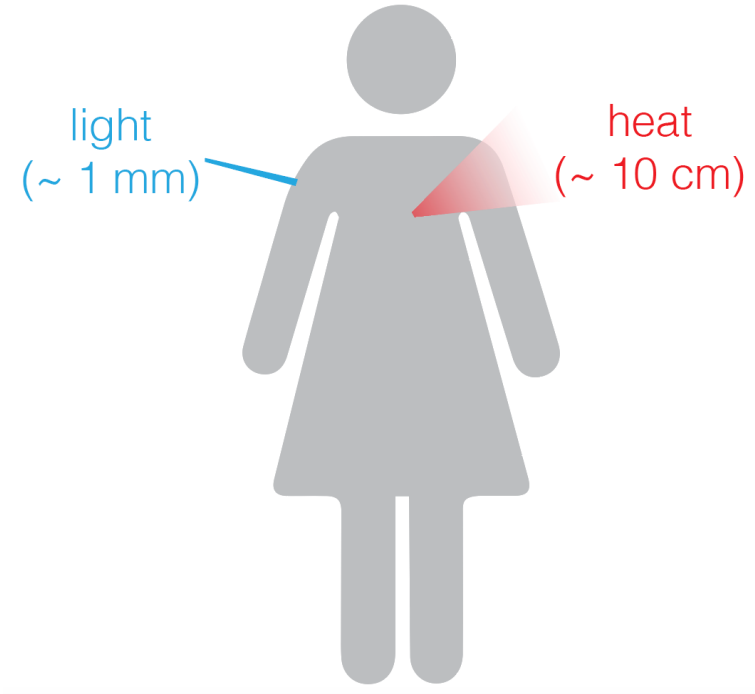
Can we make BcLOV more thermostable?

Benman,…, Bugaj, *Nat. Chem. Bio*, 2022



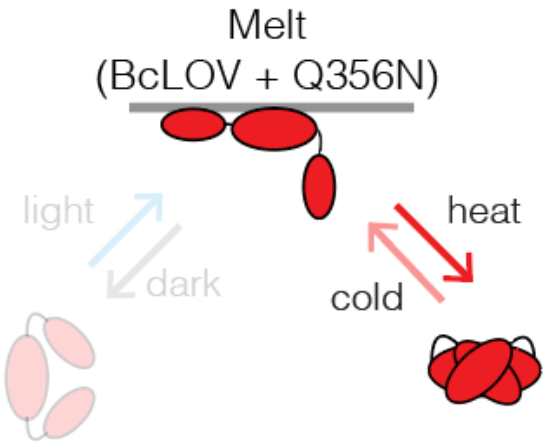
Benman, Huang* et al, 2023 (submitted)*

Temperature is a more penetrant stimulus than light



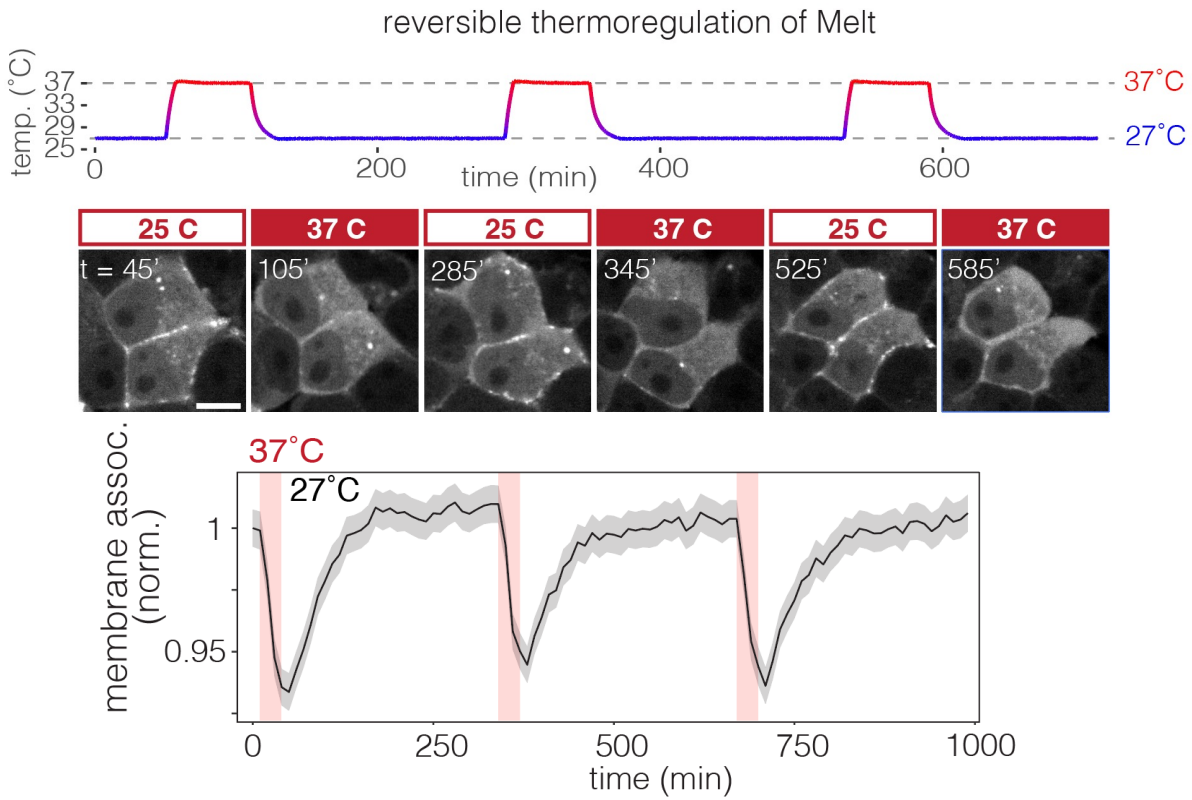
“Melt” senses temperature, but not light

Membrane **l**ocalization using **t**emperature (Melt)



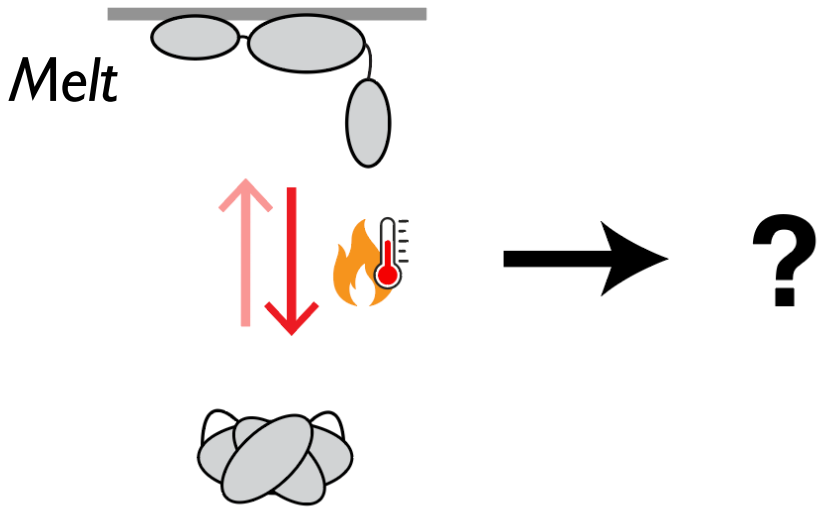
Benman, Huang* et al, 2023 (submitted)*

Reversible, dynamic thermal responses

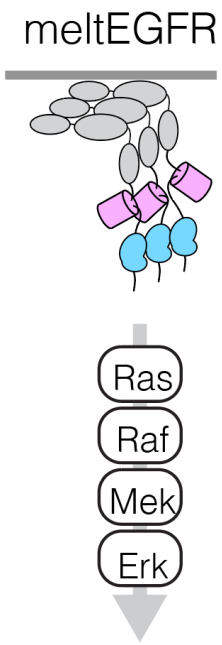


Benman, Huang* et al, 2023 (submitted)*

Can we thermally control cell function?

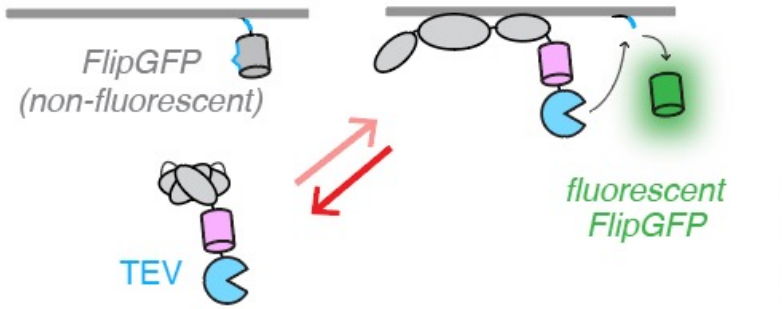


Thermal control of EGFR



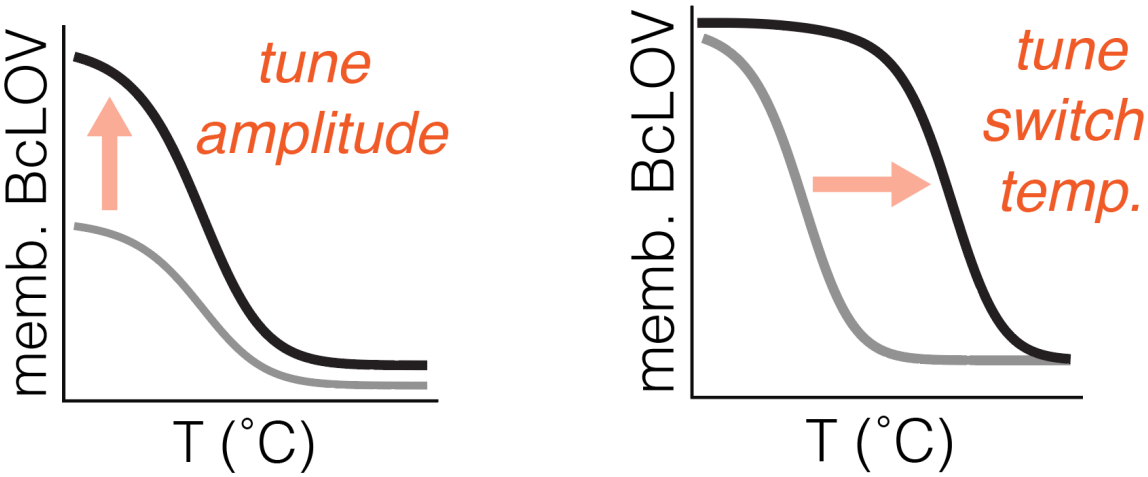
Benman, Huang* et al, 2023 (submitted)*

Thermal control of proteolysis



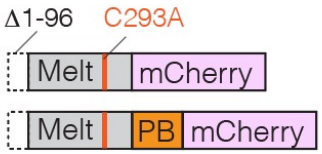
Benman, Huang* et al, 2023 (submitted)*

Shifting the thermal response properties



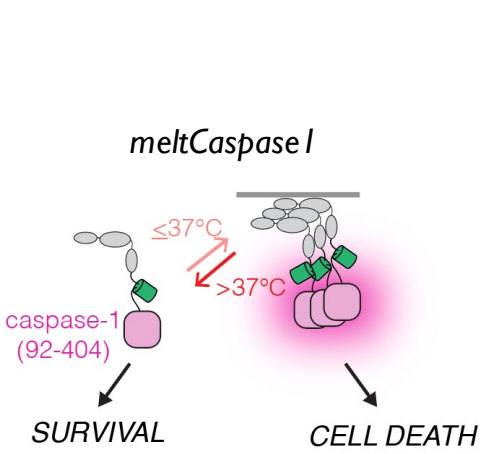
Benman, Huang* et al, 2023 (submitted)*

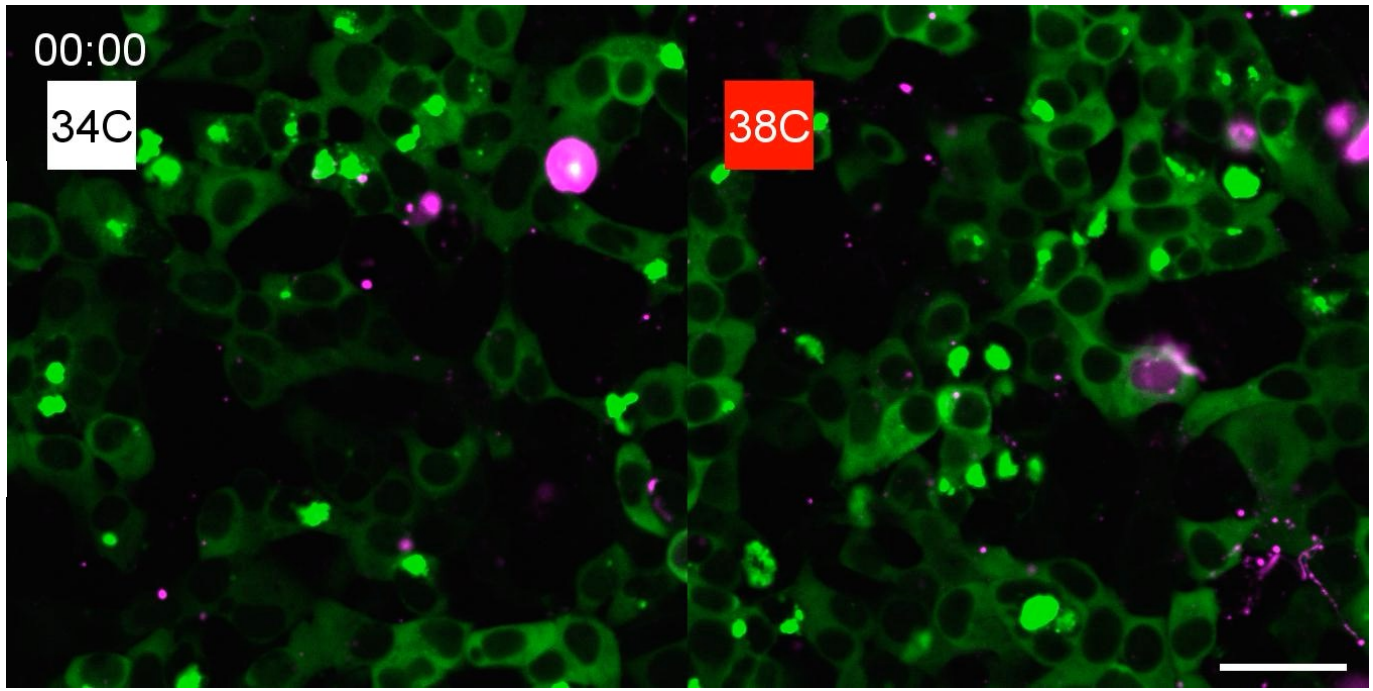
Shifting switch temp intto mammalian range



Benman, Huang* et al, 2023 (submitted)*

Thermal control of cell death





Benman, Huang* et al, 2023 (submitted)*

Bugaj Lab:
Precision tools to understand ***signal perception*** and ***misperception***

Control

Light – "optogenetics"

Bugaj, *Nat Methods.*, 2013 Bugaj, *Nat Protocol.*, 2018

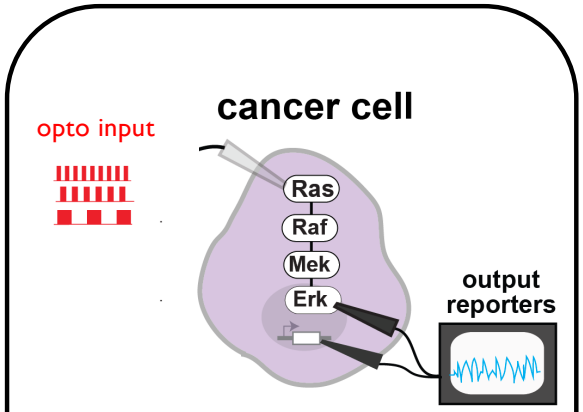
Temperature – "thermogenetics"

Benman,…,Bugaj*. *Nat Chem Bio*, 2022 *unpublished*

Visualization

Mumford, …, Bugaj*, *bioRxiv.*, 2022

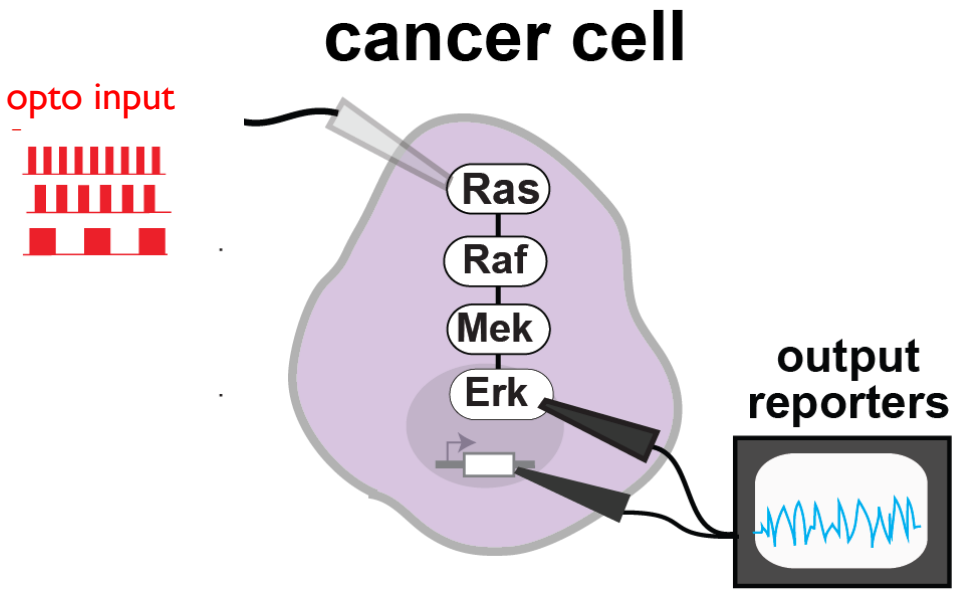
Cancer signal misperception



Bugaj et al., *Science*, 2018

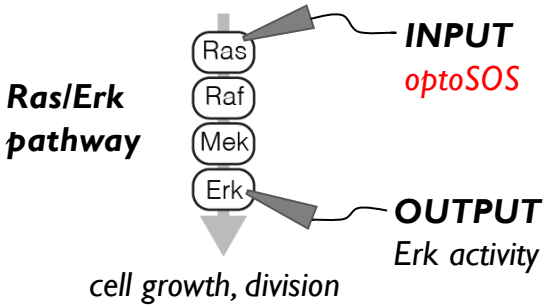
Gonzalez-Martinez, Roth, …,Bugaj*, *bioRxiv*, 2022

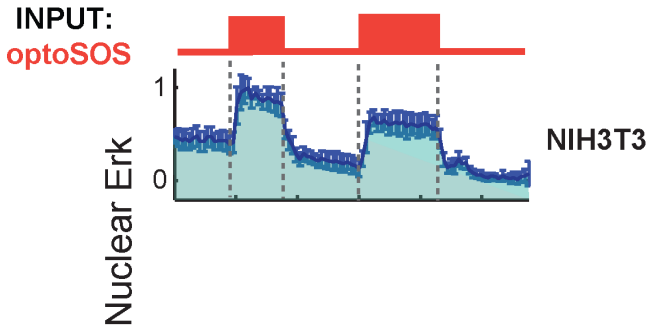
Misperception: signal corruption in cancer

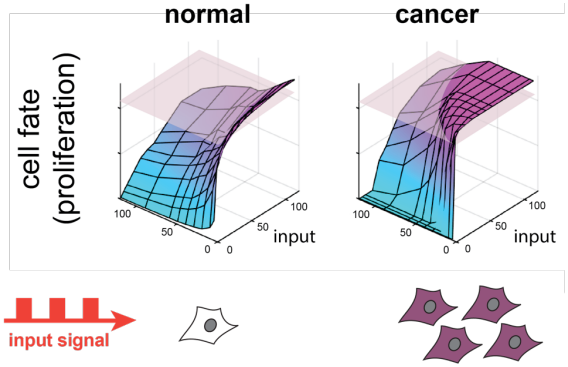


Bugaj et al., *Science*, 2018

Corruption in growth signal transmission

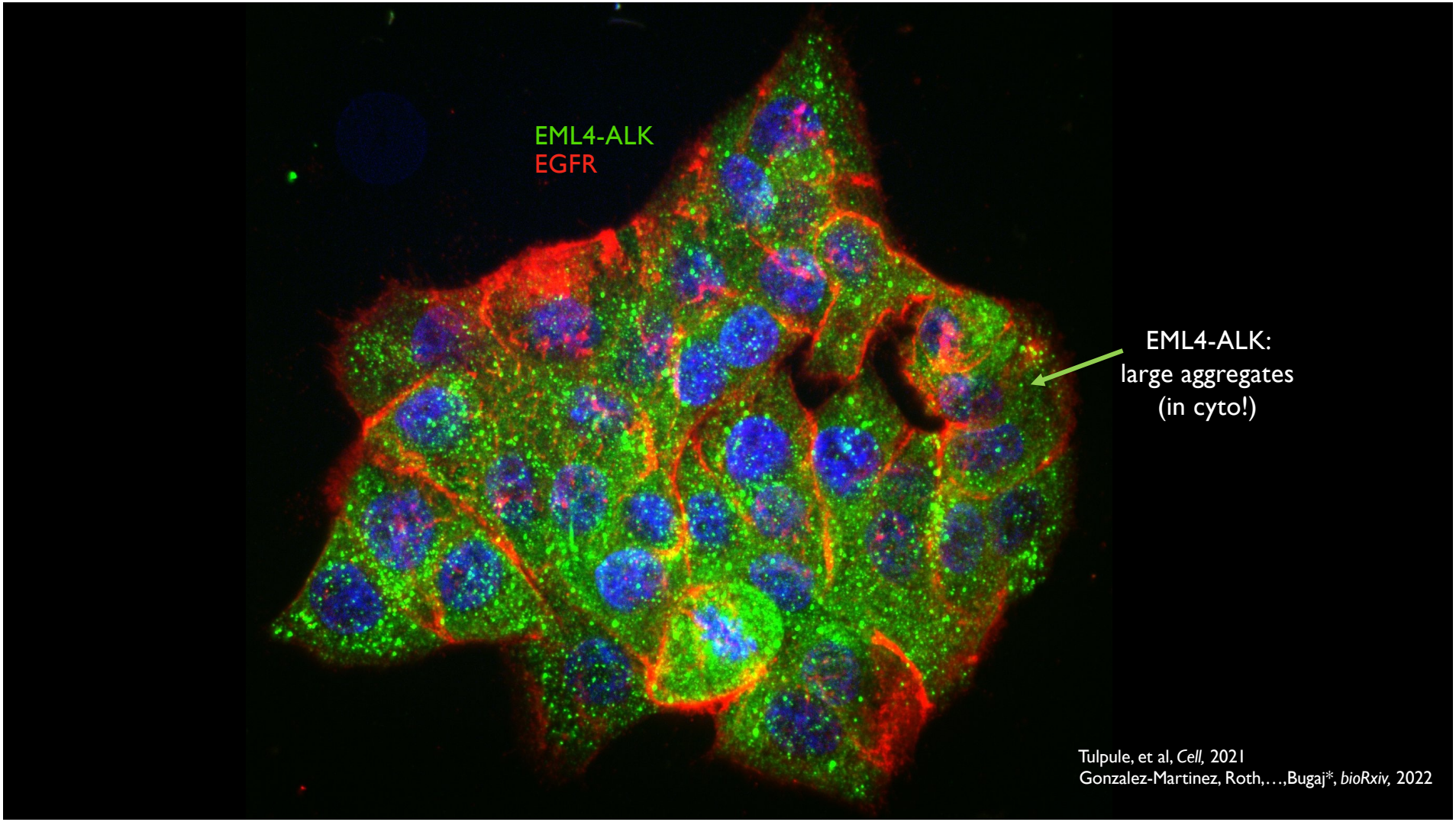






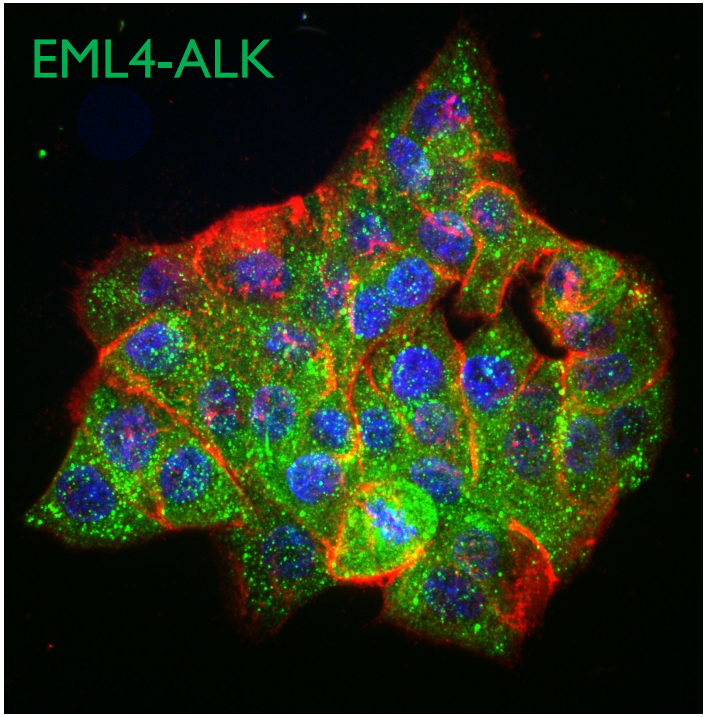
Misperception of Ras dynamics
➡ *inappropriate proliferation*

Bugaj et al., *Science*, 2018


EML4-ALK
EGFR
EML4-ALK:
large aggregates
(in cyto!)
Tulpule, et al, *Cell,* 2021
Gonzalez-Martinez, Roth,…,Bugaj*, *bioRxiv,* 2022

Receptor Tyrosine Kinase (RTK) fusion oncogenes

lung cancer cells (STE-1)



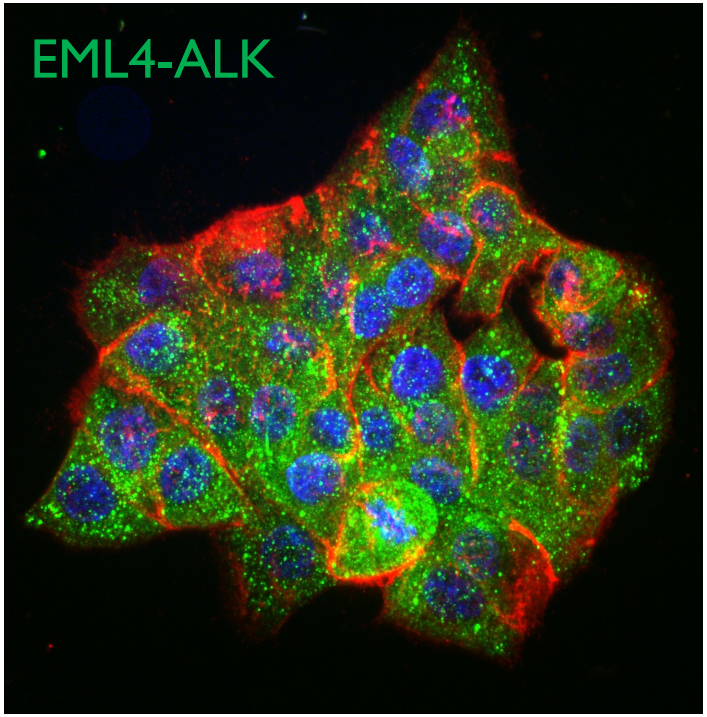
partner	RTK (kinase)

(often oligomeric)

> 50 RTK fusions described, across cancer types

Receptor Tyrosine Kinase (RTK) fusion oncogenes

lung cancer cells (STE-1)



EML4 | ALK

(often oligomeric)

> 50 RTK fusions described, across cancer types

EML4-ALK

- ~3-7% of non-small-cell lung cancer
- Oncogenic RTK signaling (primarily through Ras/Erk)
- ALK inhibitors work (3 gen.)

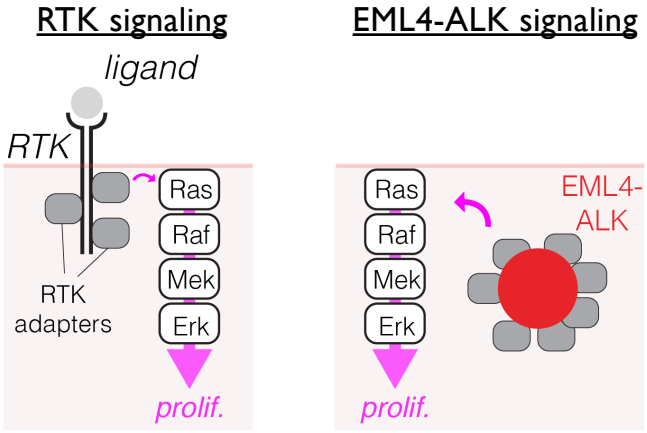
…*but*, drug resistance emerges

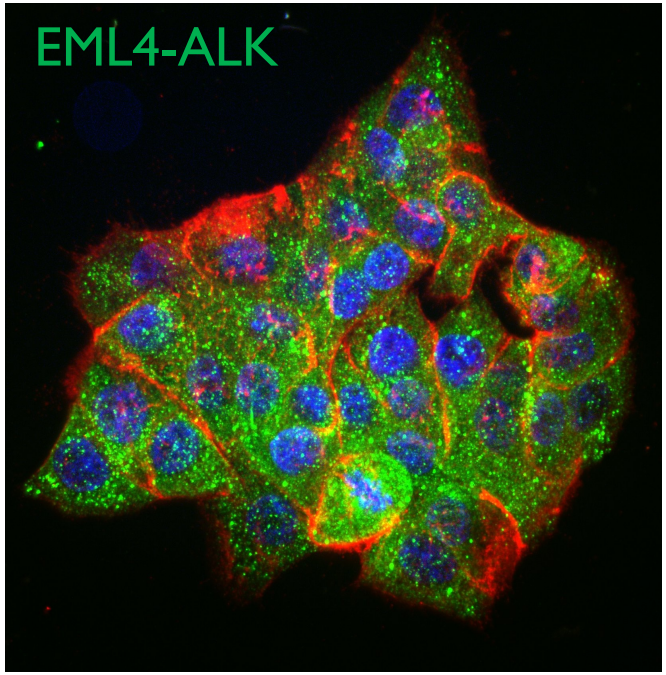
EML4-ALK signals as cytoplasmic aggregates

Article

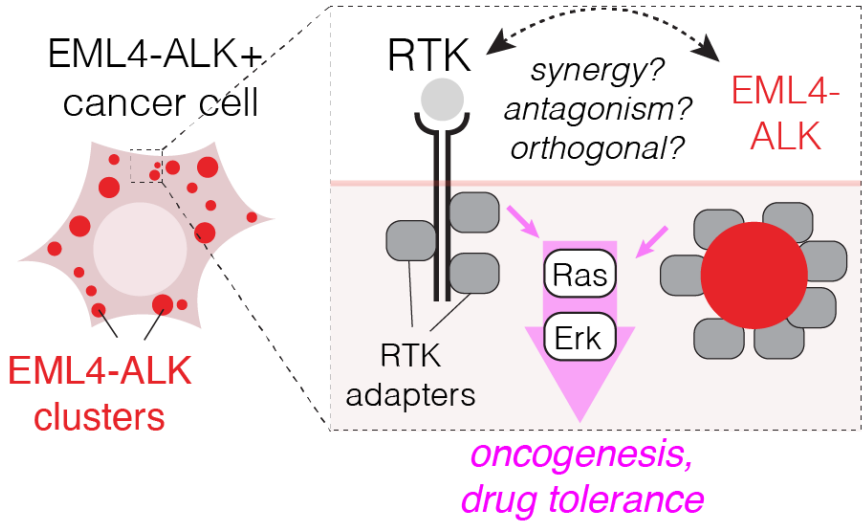
Kinase-mediated RAS signaling via membraneless cytoplasmic protein granules

Asmin Tulpule,[1,9] Juan Guan,[2,3,9] Dana S. Neel,[4,9] Hannah R. Allegakoen,[1] Yone Phar Lin,[1] David Brown,[2] Yu-Ting Chou,[4] Ann Heslin,[1] Nilanjana Chatterjee,[4] Shriya Perati,[1] Shruti Menon,[1] Tan A. Nguyen,[5] Jayanta Debnath,[5] Alejandro D. Ramirez,[2] Xiaoyu Shi,[2] Bin Yang,[2] Siyu Feng,[6] Suraj Makhija,[5] Bo Huang,[2,7,8,*] and Trever G. Bivona[4,10,*]

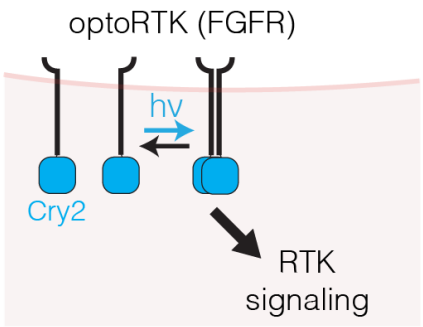




Can EML4-ALK condensates corrupt signaling through transmembrane receptors (RTK)?

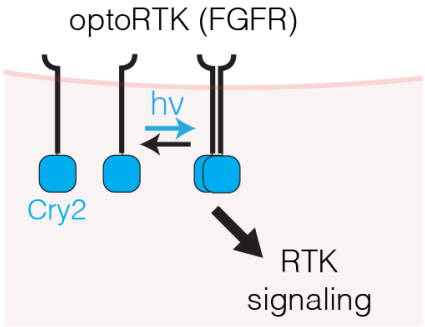


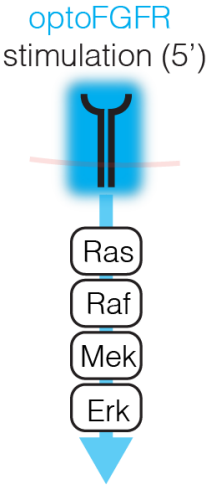
EML4-ALK+ cells do not respond to optoRTK

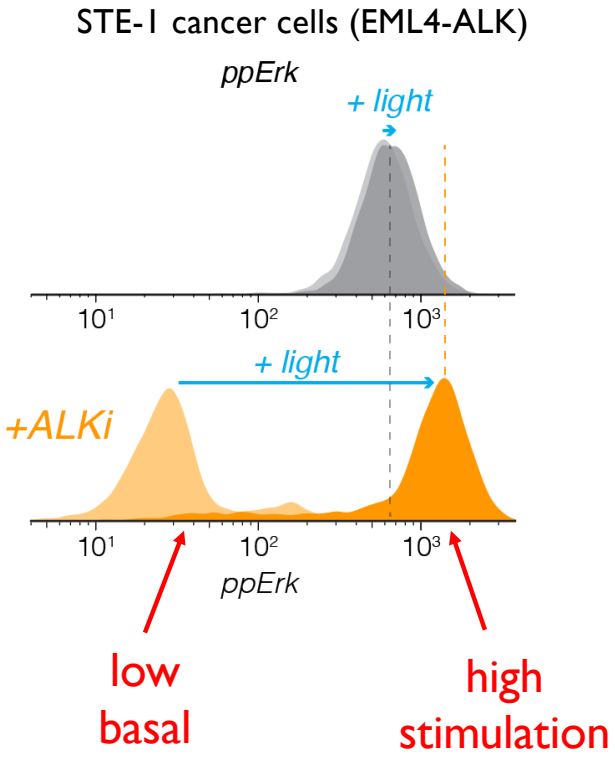


optoFGFR: Kim et al, ***Chem Biol,*** 2014

…but ALK inhibition *hypersensitizes* RTK stim.



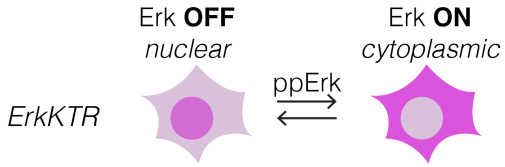




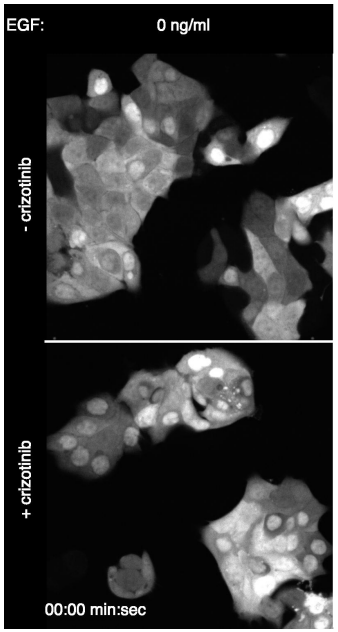
EML4-ALK suppresses RTK sensitivity

optoFGFR: Kim et al, ***Chem Biol,*** 2014

ALKi allows perception of EGF stimulus (re-sensitization)



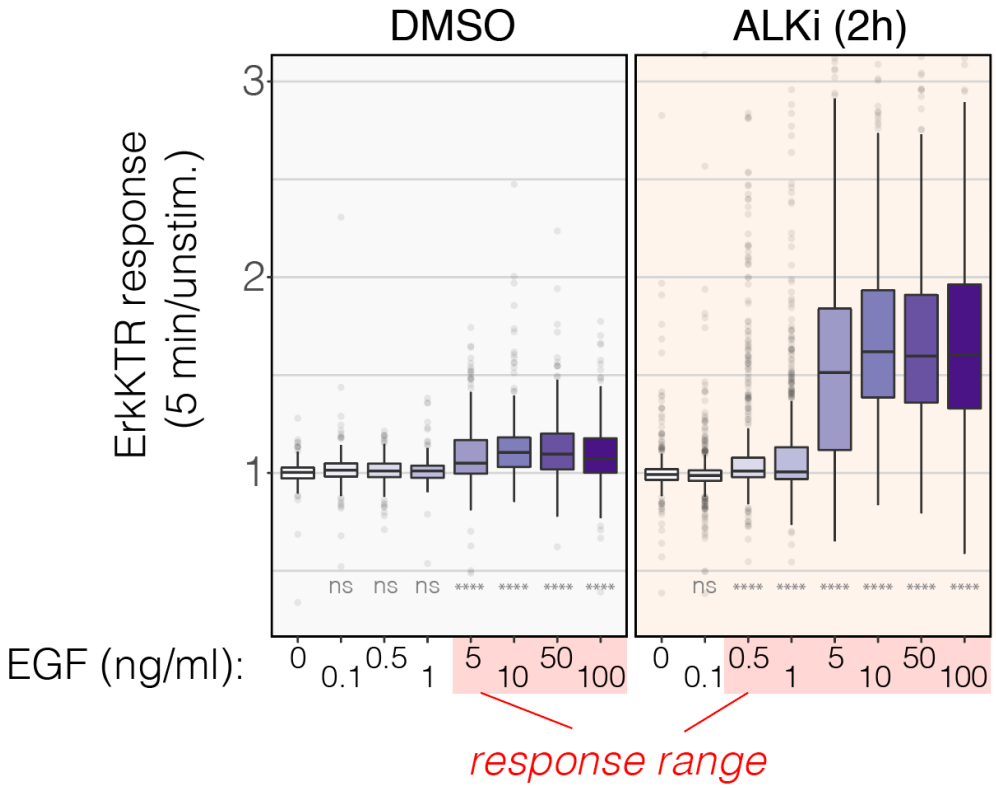
STE-1 cancer cells



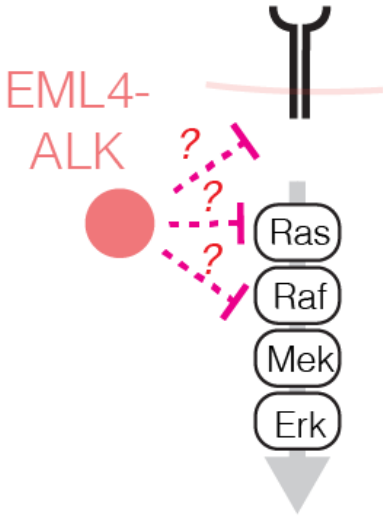
-

+ALKi

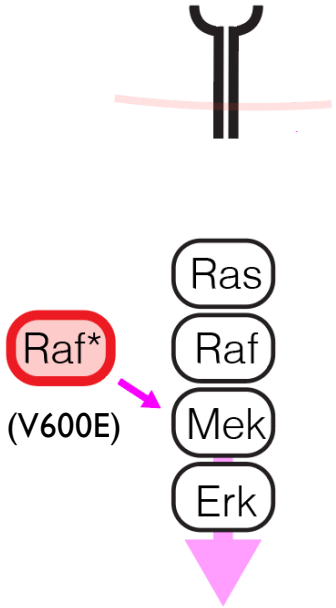
ALKi allows perception of EGF stimulus (re-sensitization)



Optogenetics pinpoints RTK suppression



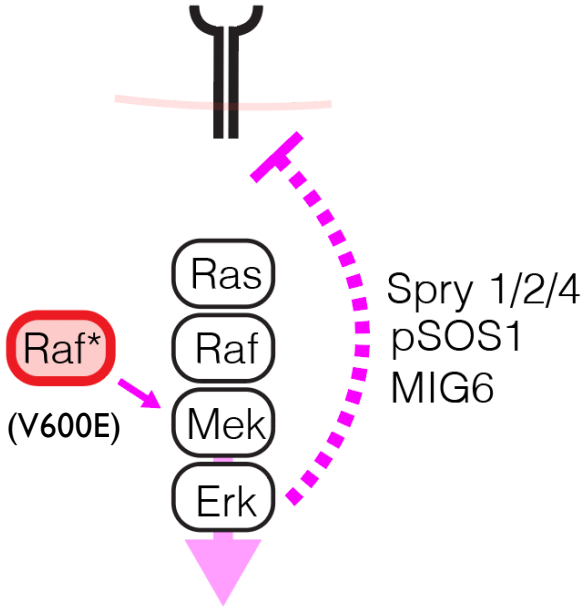
RTK suppression through Erk-dep. feedback?



BRAF V600E+ melanoma, colon cancer

Lito et al, ***Cancer Cell***, 2012
Prahallad, et al, ***Nature***, 2012
Corcoran et al, ***Cancer Disc***, 2012
Gerosa et al, ***Cell Systems***, 2012

RTK suppression through Erk-dep. feedback?

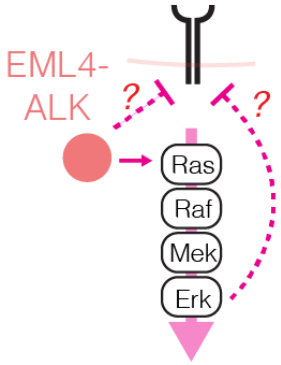


BRAF V600E+ melanoma, colon cancer

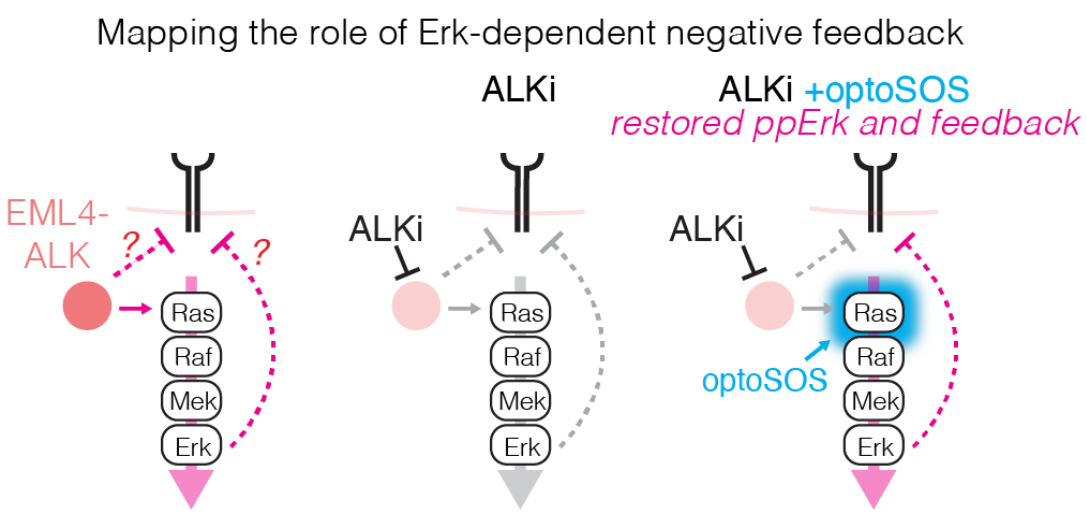
Lito et al, ***Cancer Cell***, 2012
Prahallad, et al, ***Nature***, 2012
Corcoran et al, ***Cancer Disc***, 2012
Gerosa et al, ***Cell Systems***, 2012

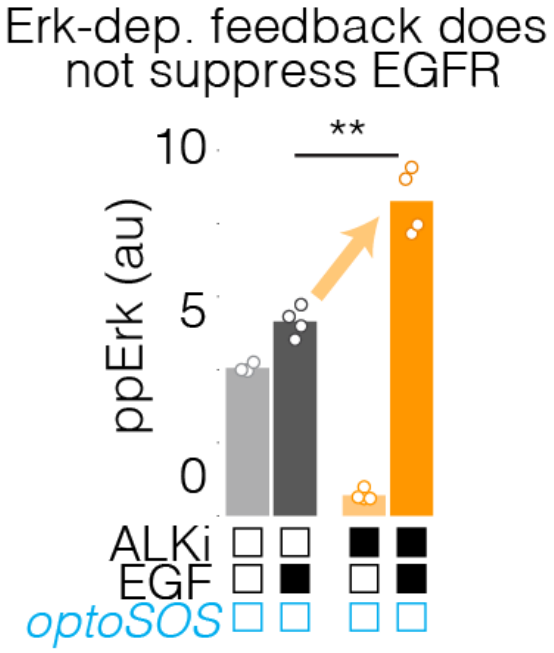
EGFR suppression is not Erk-feedback dependent

Mapping the role of Erk-dependent negative feedback

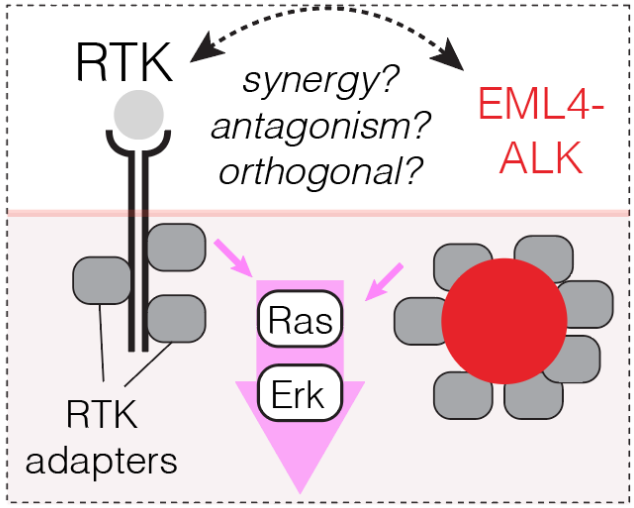


EGFR suppression is not Erk-feedback dependent

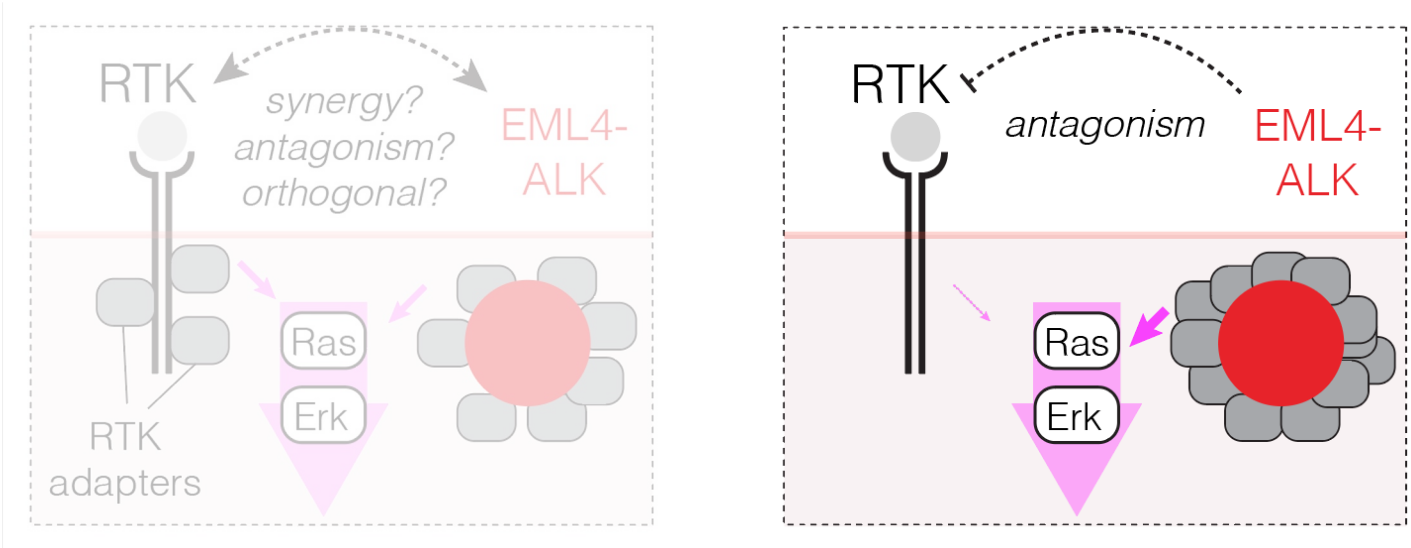




Does EML4-ALK condensation play a role?

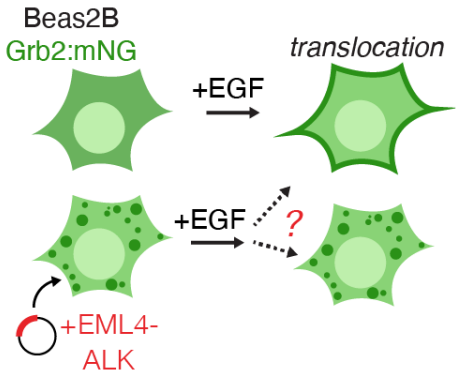


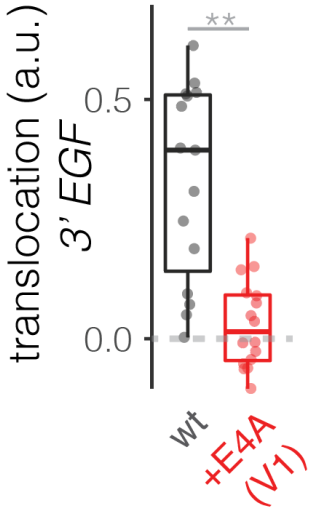
Does EML4-ALK condensation play a role?



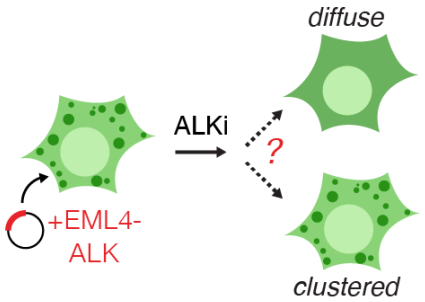
Hypothesis: EML4-ALK aggregates sequester adapters

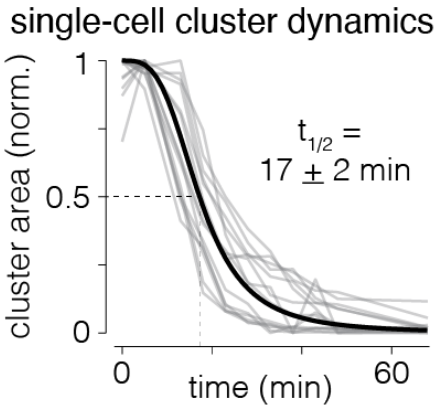
Grb2 sequestration prevents membrane translocation



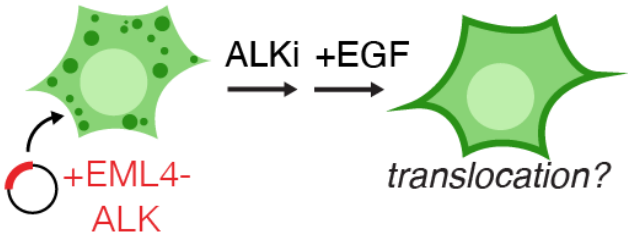


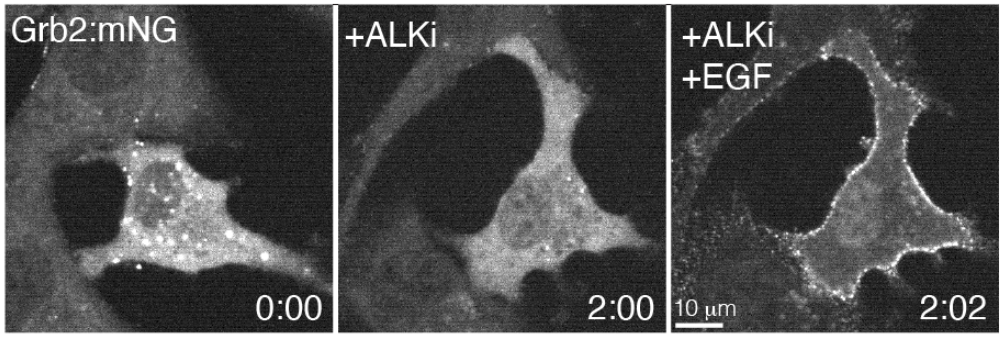
ALKi dissolves Grb2 clusters

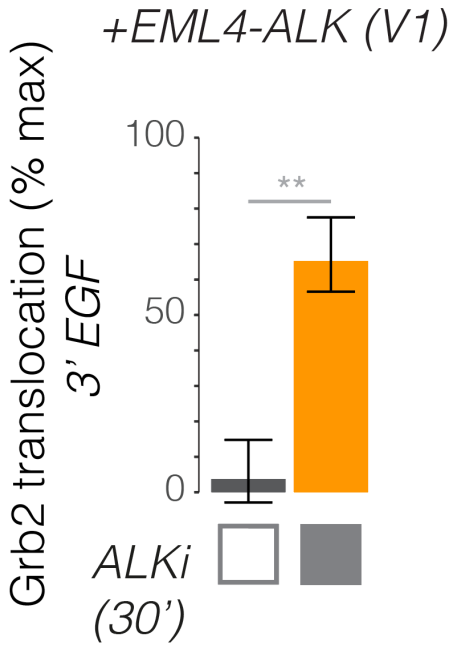




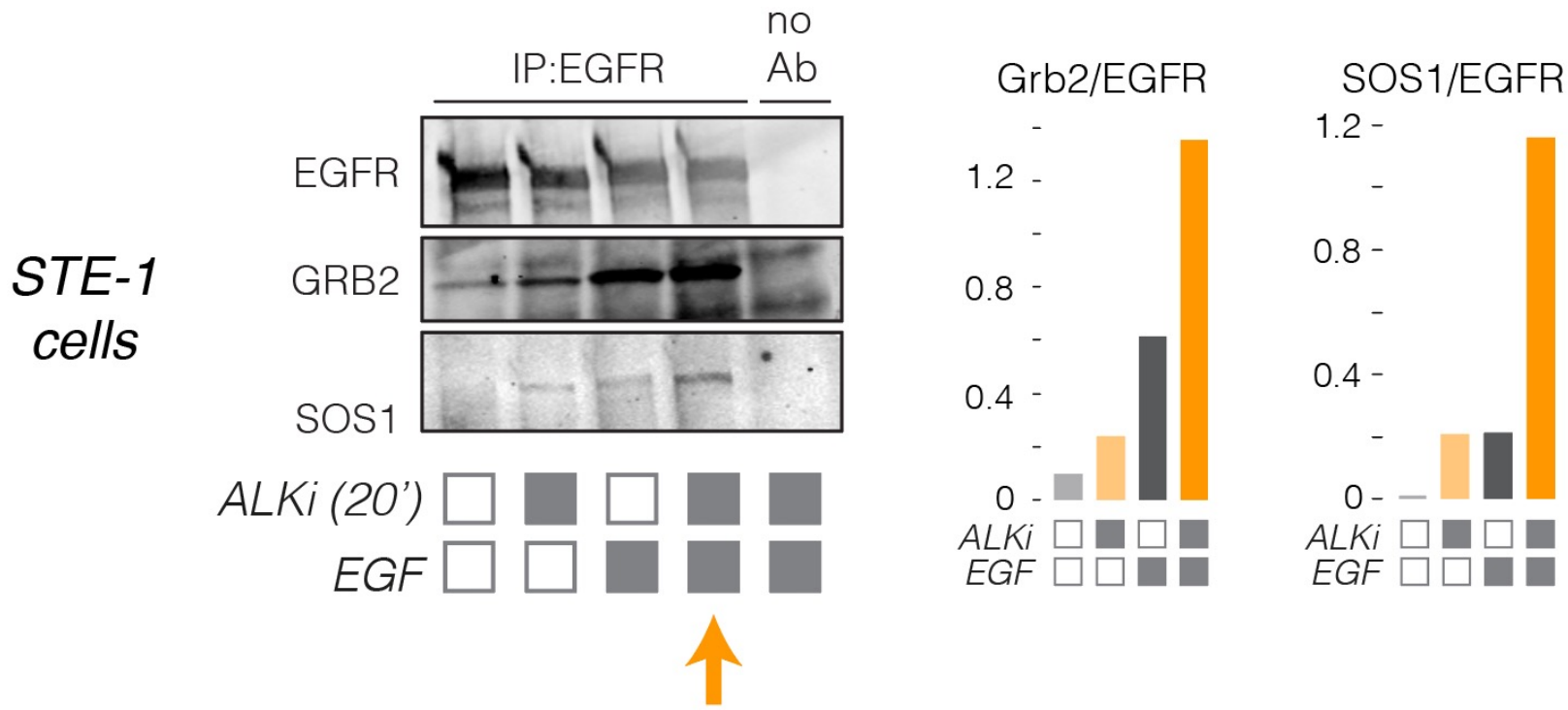
ALKi permits Grb2 translocation in EML4-ALK+ cells



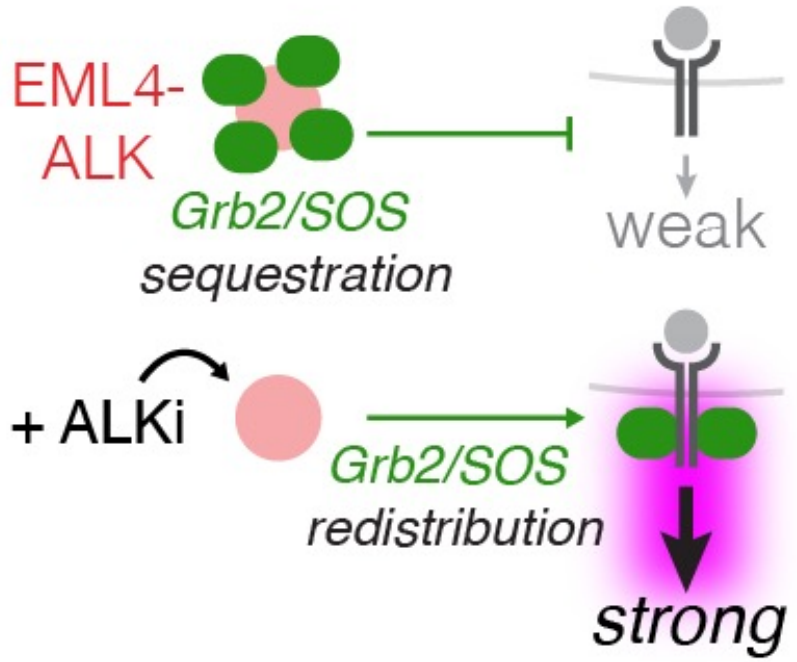




Suppression of adapters in cancer cells

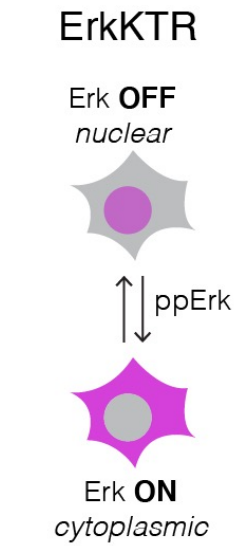


EML4-ALK suppresses RTKs
by competition for adapter proteins



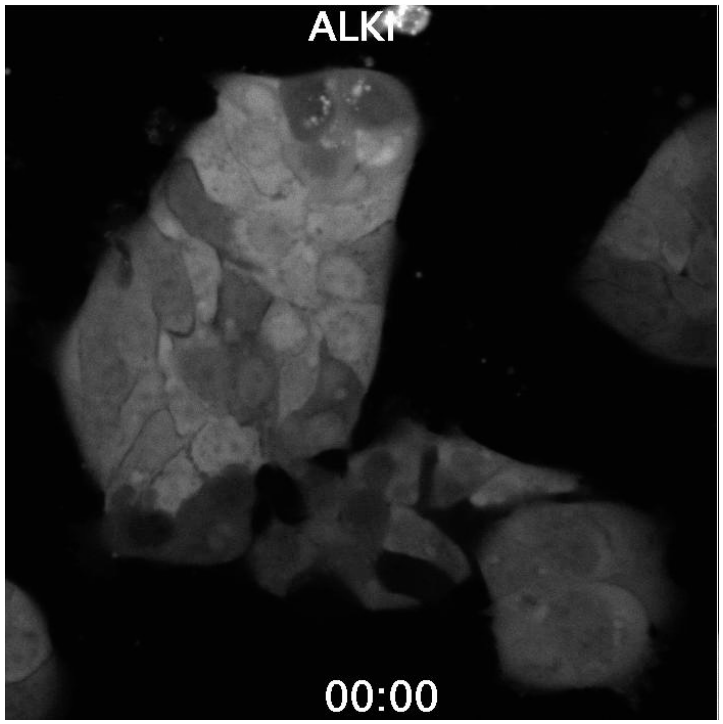
Does RTK re-sensitization matter?

Spontaneous and rapid Erk reactivation upon Alk inhibition

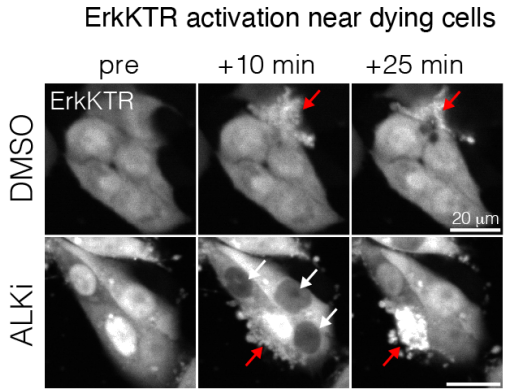


Regot et al, *Cell,* 2013

STE-1 cancer cells (EML4-ALK)



Erk reactivation is driven by paracrine signaling from dying cells



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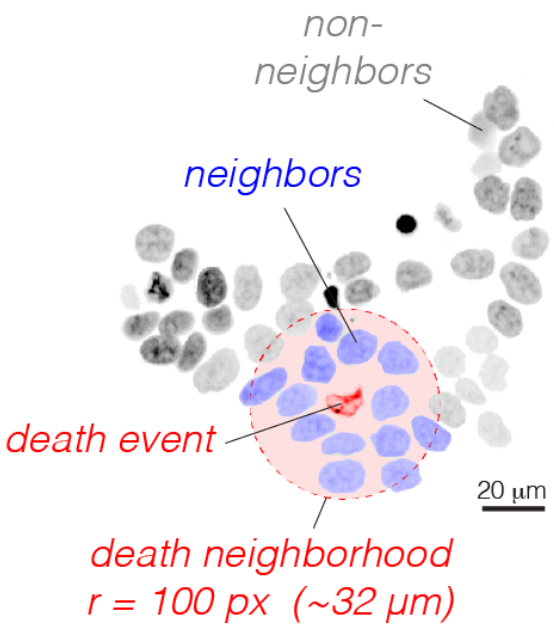
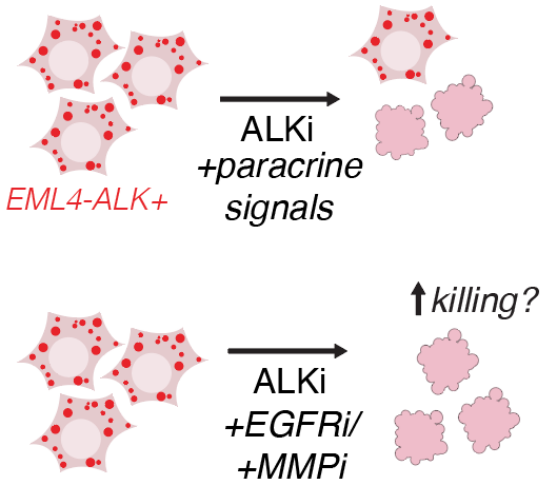


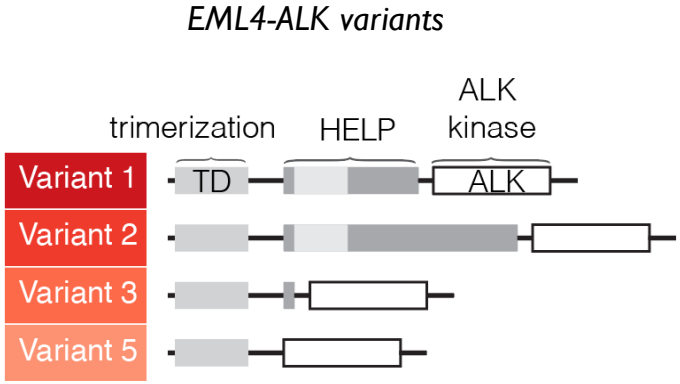
Erk pulses are associated with cell survival

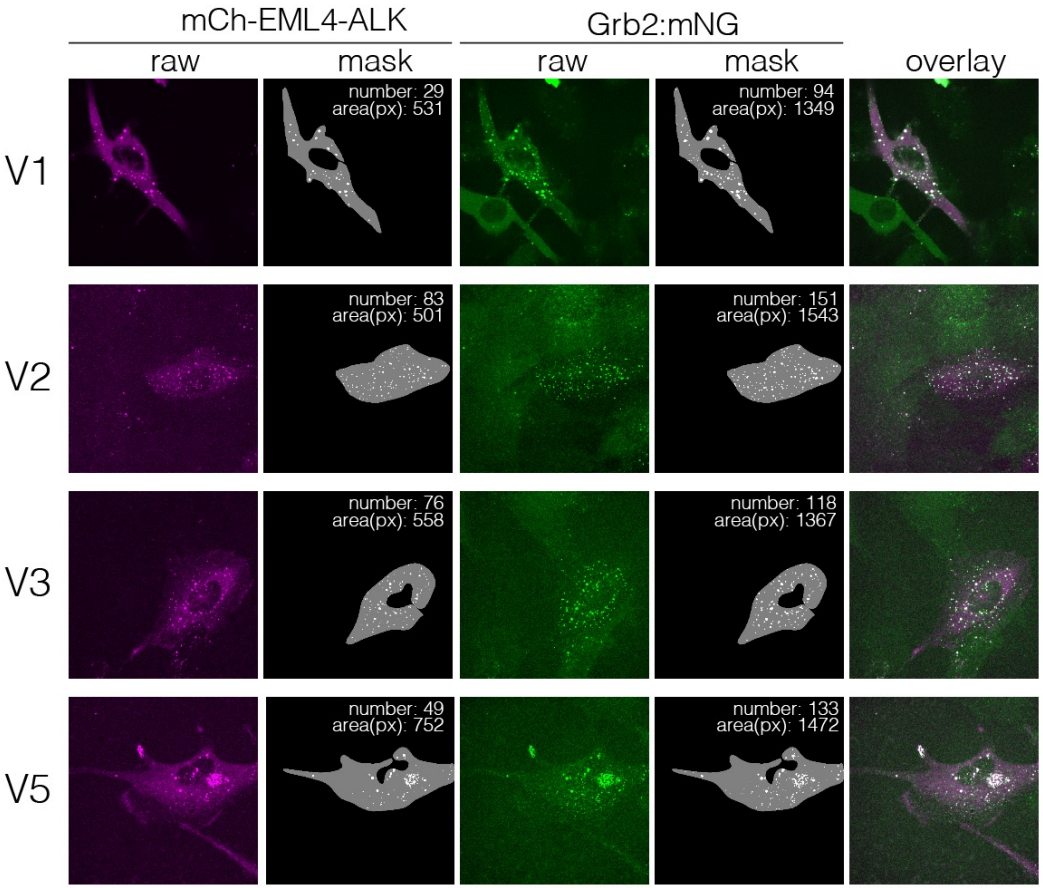


Contributions

1. EML4-ALK condensates desensitize RTKs through adapter sequestration

Condensation and colocalization are common among EML4-ALK variants

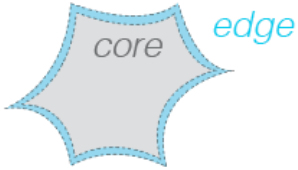




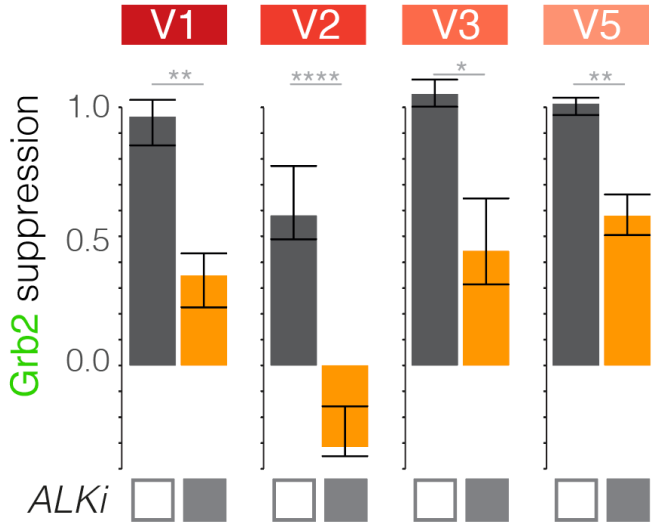
Adapter sequestration is common among EML4-ALK variants

$$\text{transloc. suppression} = \frac{\text{transloc.}_{wt} - \text{transloc.}_{EML4\text{-}ALK}}{\text{transloc.}_{wt}}$$

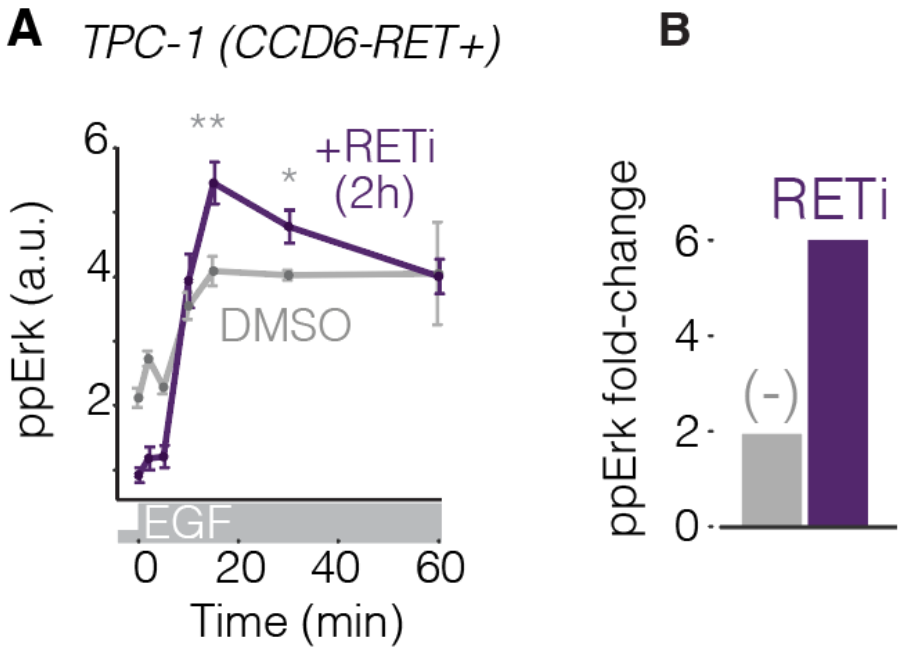
quantifying adapter translocation



$$\text{translocation} = \left(\frac{edge}{core}\right)_{post\text{-}EGF} - \left(\frac{edge}{core}\right)_{pre\text{-}EGF}$$



Other RTK fusion cancer cells have suppressed RTK signaling



Future work

- Generality of:
  - RTK fusion condensation
  - Adapter sequestration/suppression
  - Drug-induced sensitization
  - Drug-induced paracrine survival signaling

- Novel drug combinations, *in vivo*

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