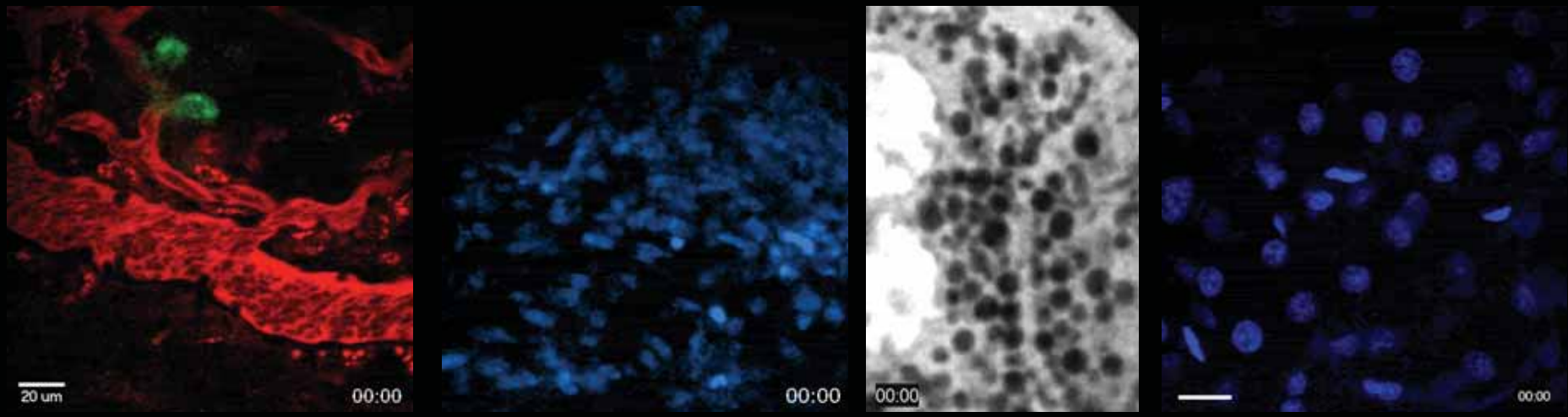
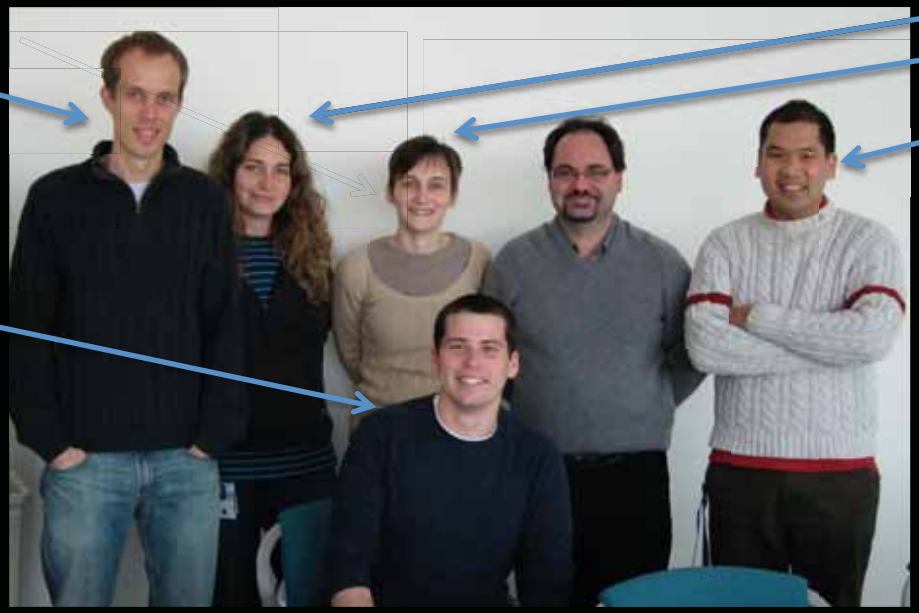
Intravital microscopy: a novel tool to study membrane traffic in physiological conditions and during invasion and metastasis



Roberto Weigert, Ph.D.
Intracellular Membrane Trafficking Unit
Oral and Pharyngeal Cancer Branch
NIDCR-NIH

Intracellular Membrane Trafficking Unit



Andrius Masedunskas

Natalie Porat-Shliom

Monika Sramkova

Panomwat Amornphimoltham

Kamil Rechache

Tim Wigand

PTRU, NIDCR
Thomas H. Bugge
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Kantima Leelahavanichkul
Alfredo Molinolo
VRC, NIDCR
Barton Weick
Rebecca Martinez
NHLBI
Bob Adelstein
Marie Anne Conti
NICHD
Tamas Balla
University of Pittsburgh
Alexander Sorkin
University of South California
Sarah Hamm-Alvarez

Former members

Laura Parente

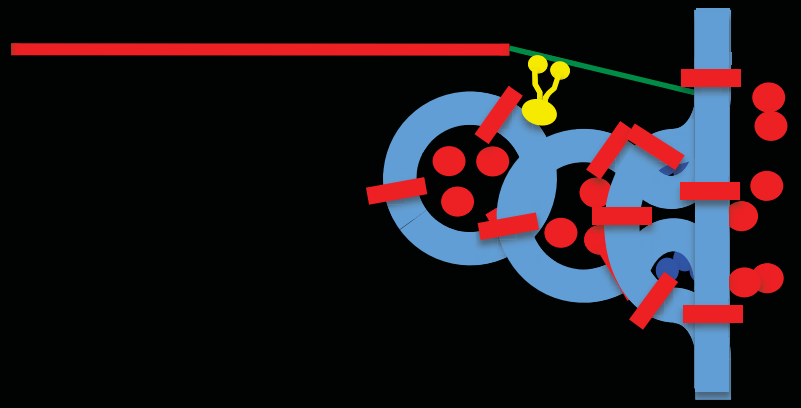
Sonita Bennett

Sara Lamb

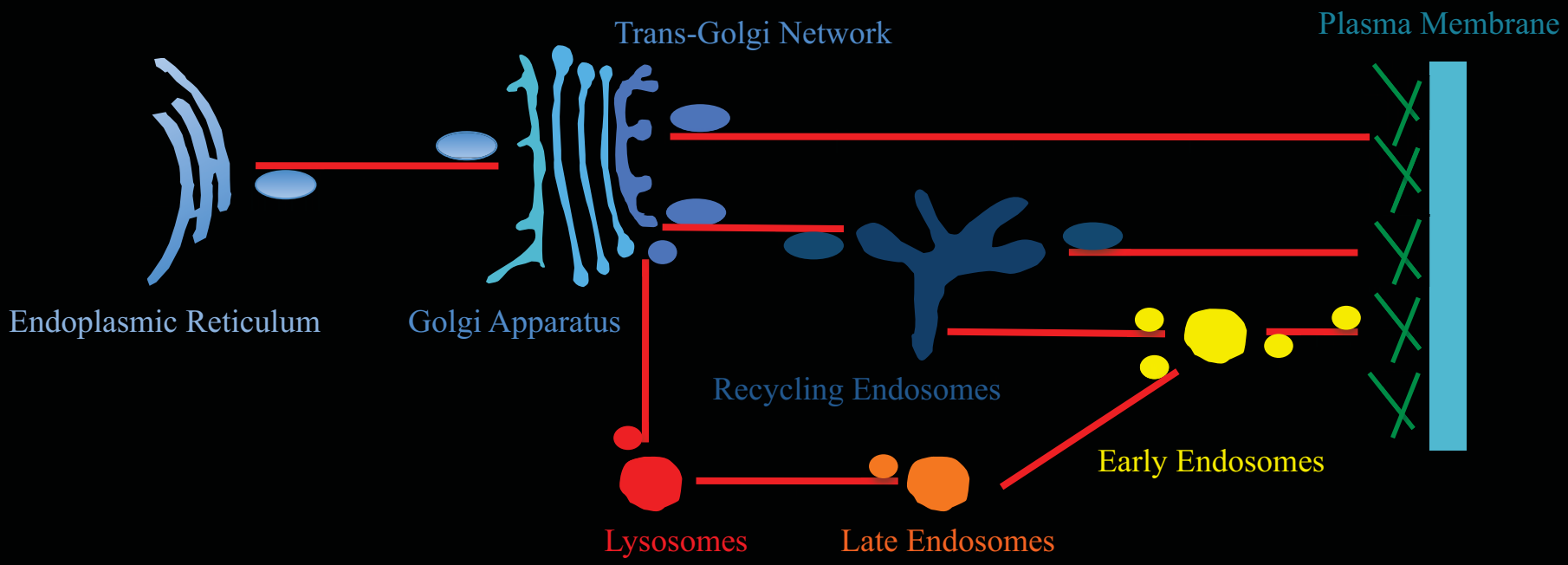
Myo-Pale' Aye

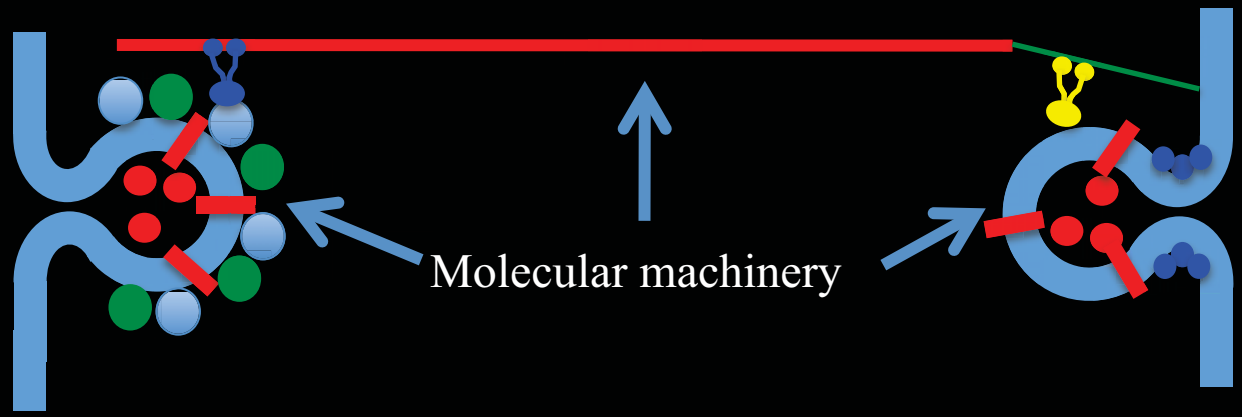
Intracellular Membrane Traffic

Transport intermediates



Aims of the Lab

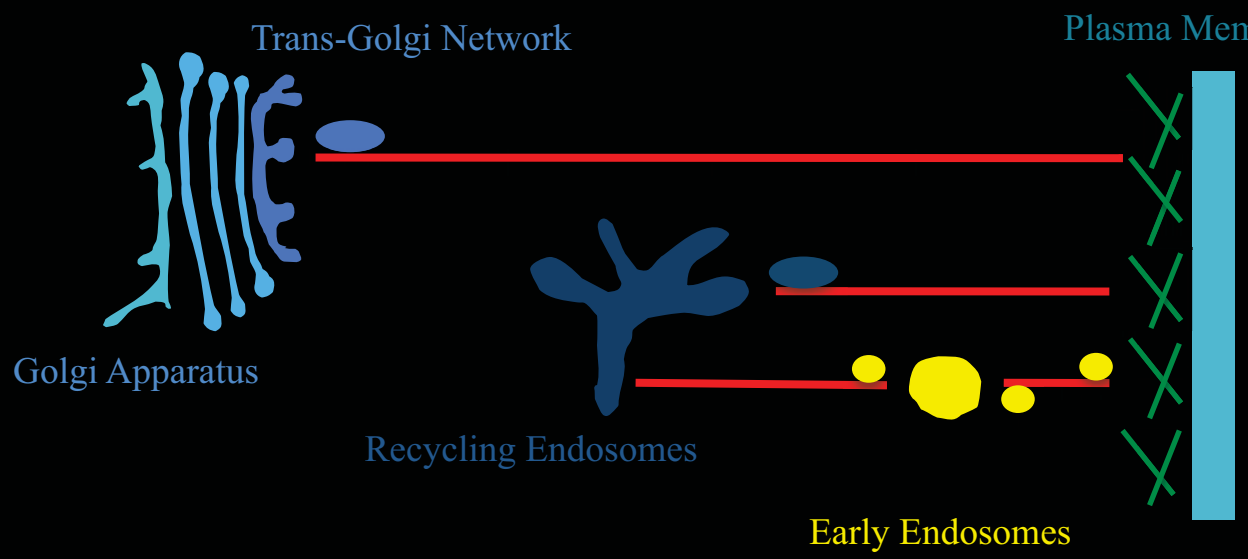




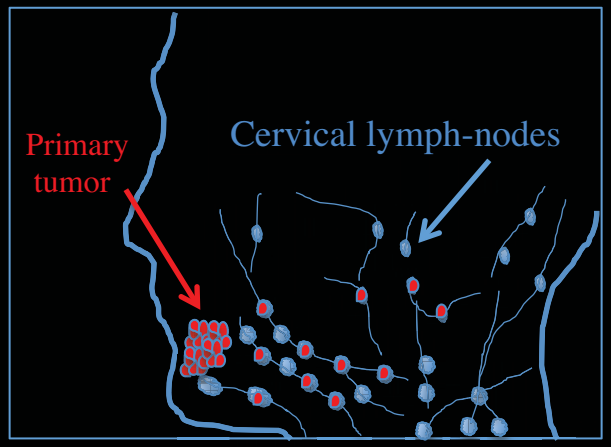
Transport intermediate biogenesis

Transport intermediate targeting/fusion

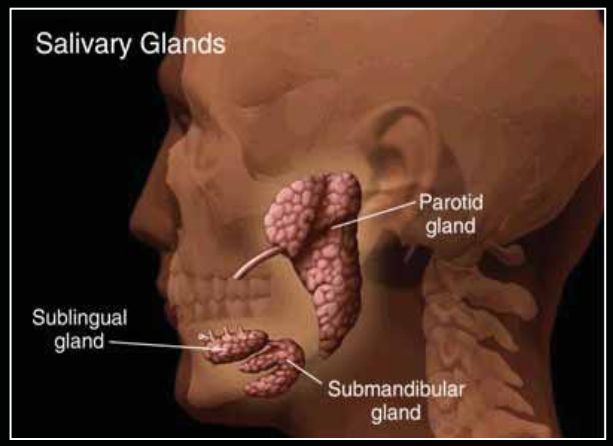
Aims of the Lab



Endosomal recycling

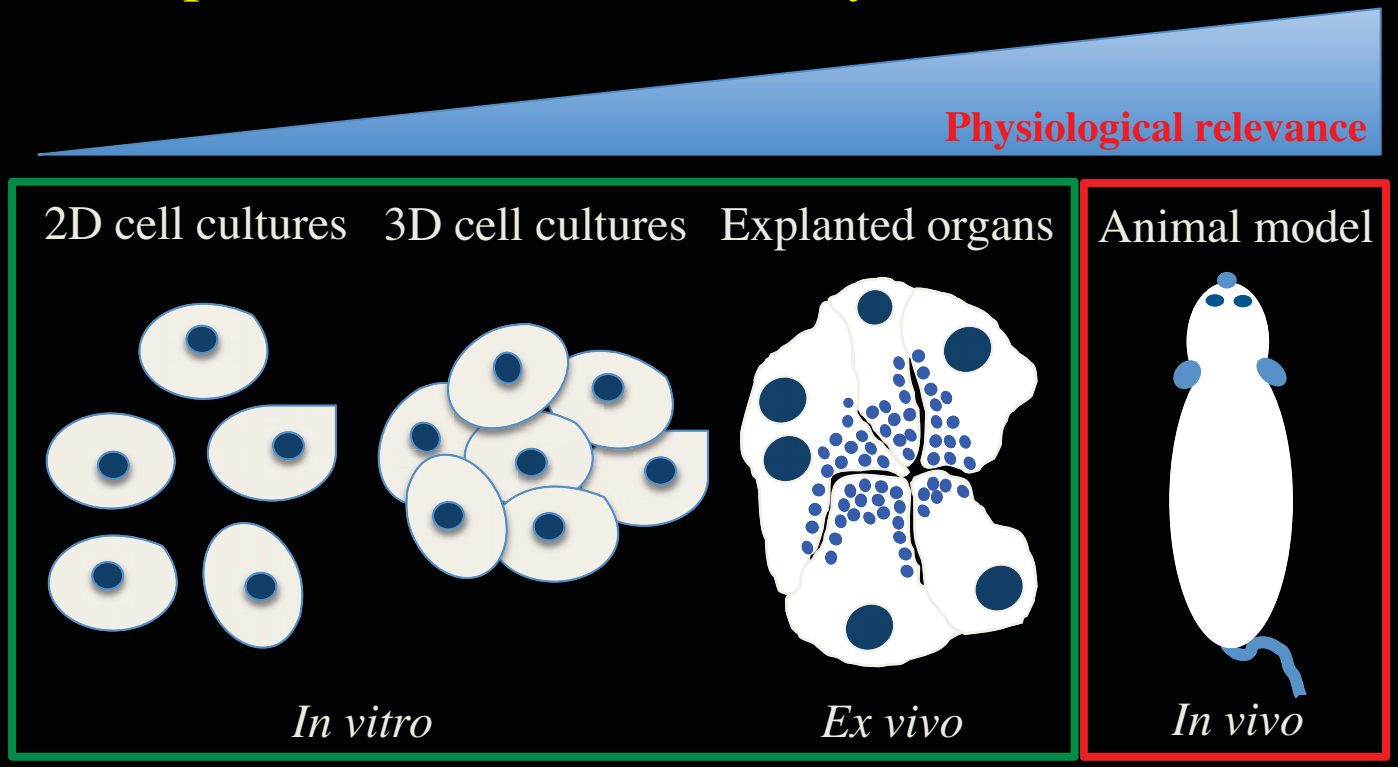


Regulated exocytosis



Physiopathology of the oral cavity

Experimental models to study membrane traffic



1) Biochemical assays — Indirect

2) Imaging of subcellular organelles

-Electron microscopy — Static

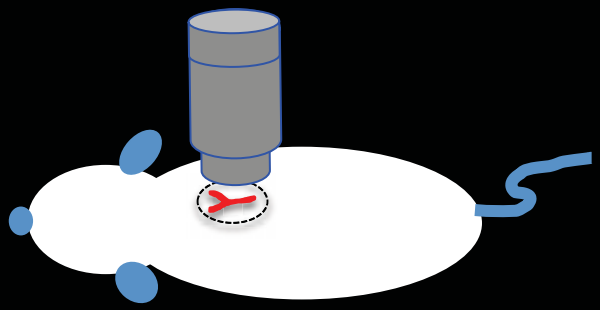
-Light microscopy
Time lapse

→ Intravital Microscopy (IVM)

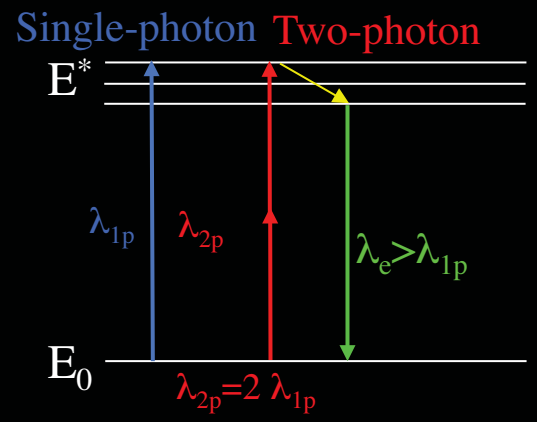
Intravital Microscopy

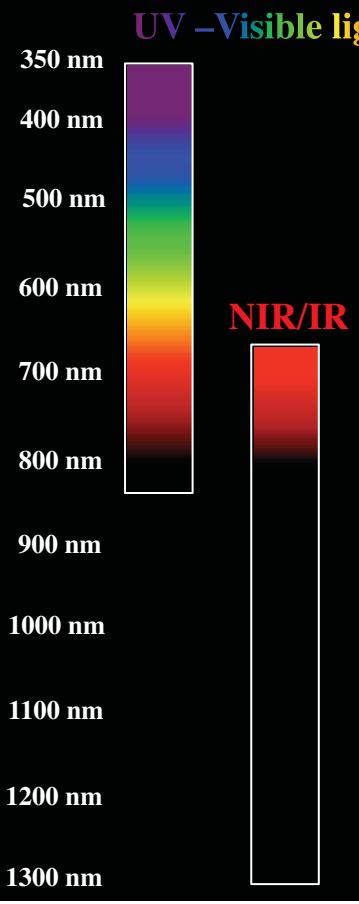
Technical improvements in confocal microscopes and optics

Two-photon microscopy



Deeper tissue penetration
Limited tissue photo-damage
Limited photo-bleaching



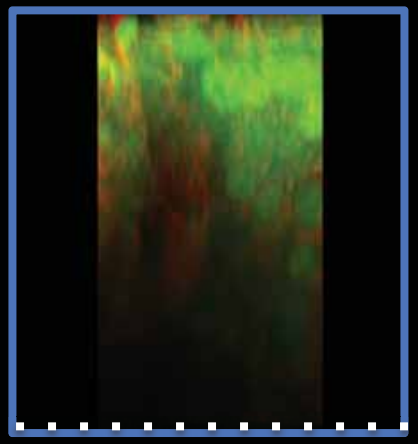


Intravital Microscopy

Homogeneous tissue

Higher spatial resolution

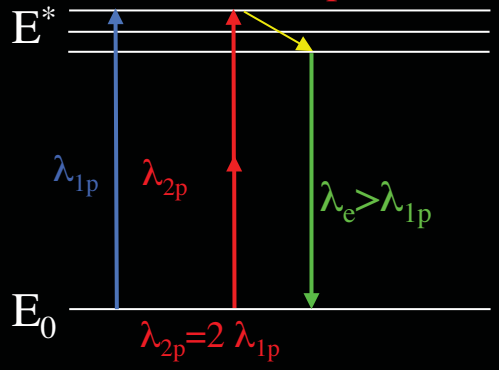
Salivary glands
GFP/mTomato mouse

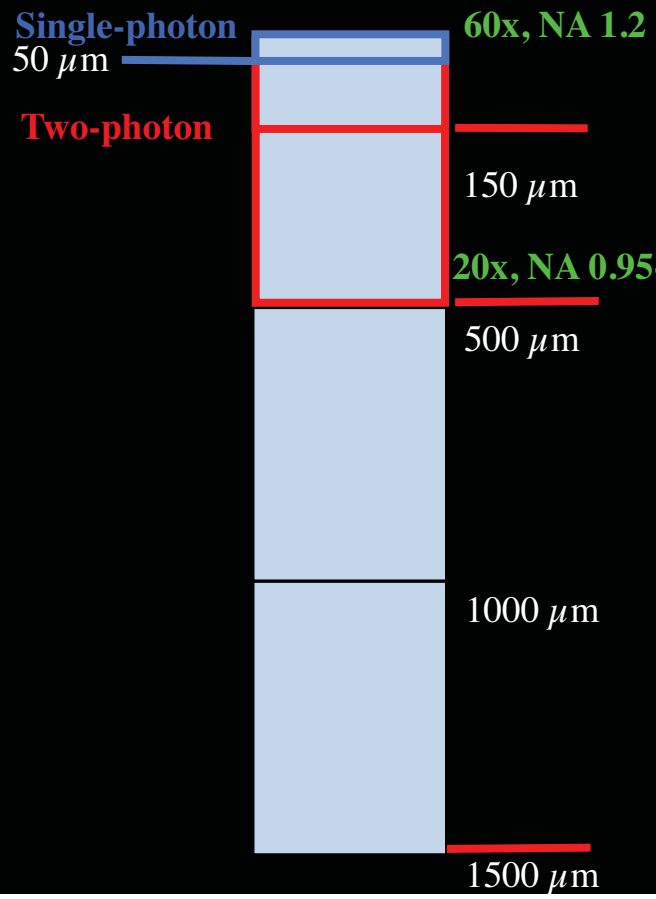


50 μm

Deeper tissue penetration

Single-photon Two-photon

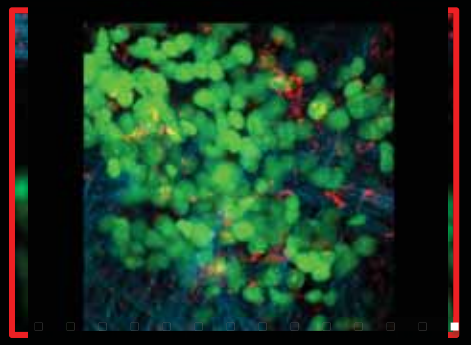




Deep tissue

Long term imaging
Endogenous emission

Tongue Xenograft
GFP-H2B/TX-red dextran/SHG
Excitation 930 nm

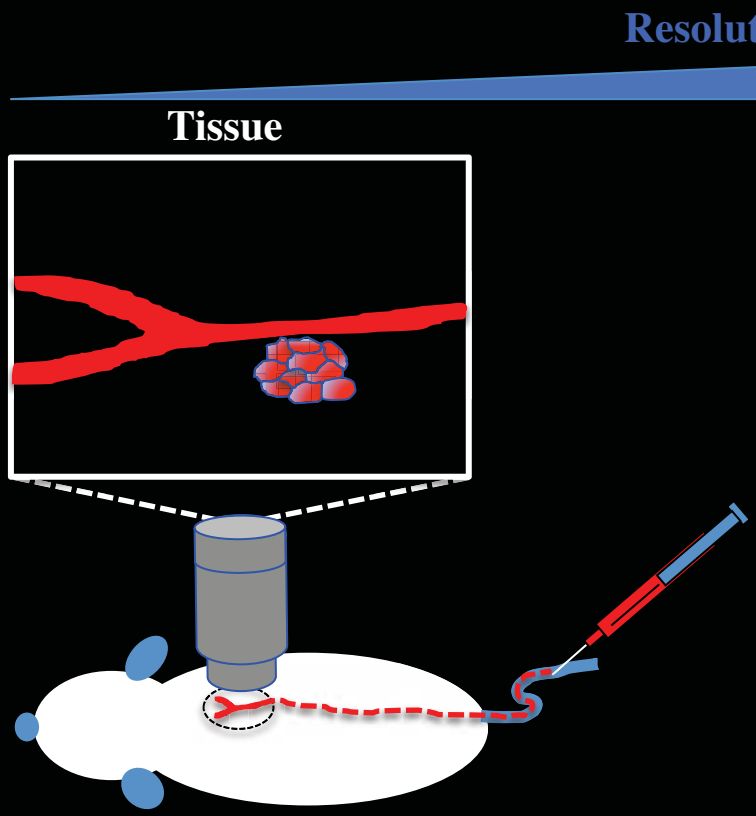


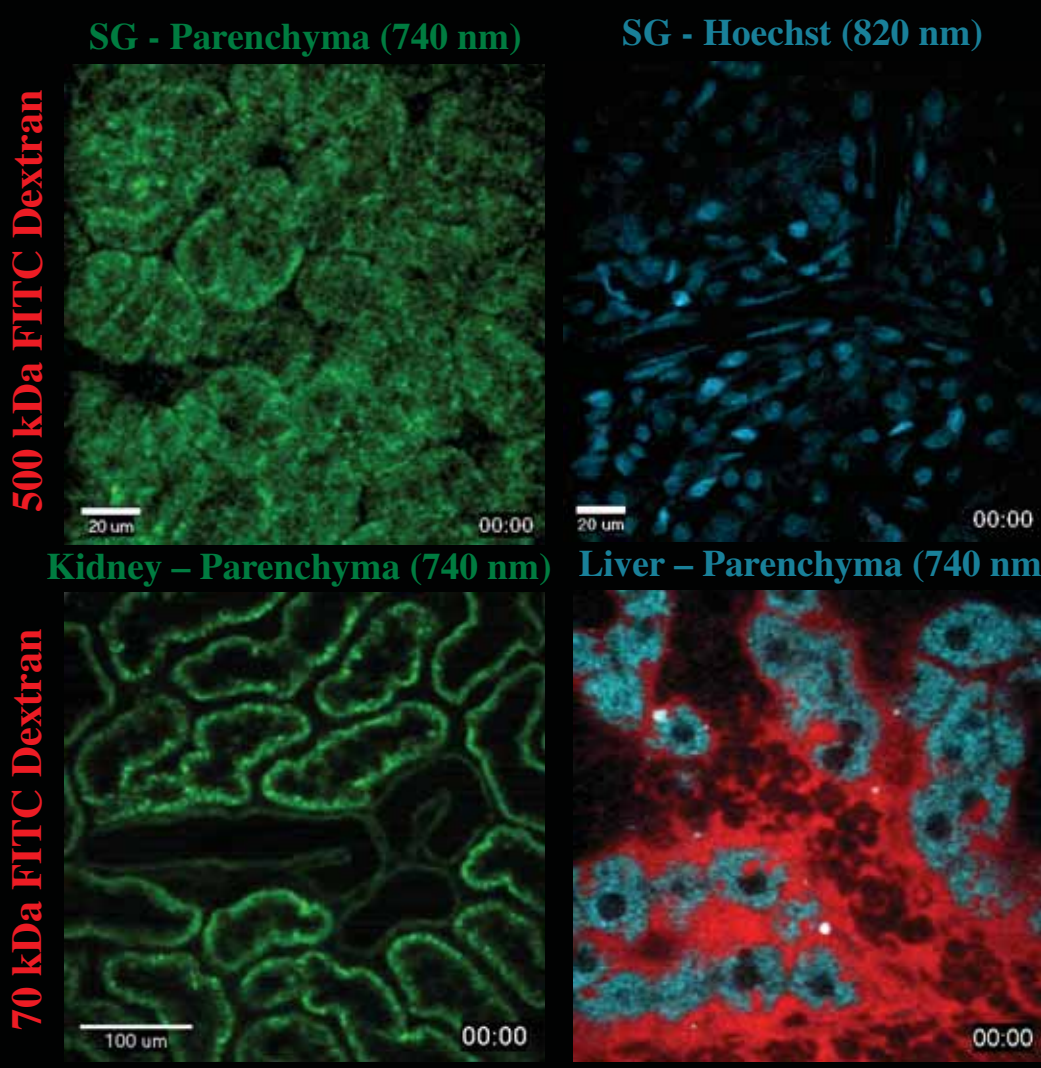
140 μm

Brain
TX-red dextran
Excitation 930 nm

700 μm

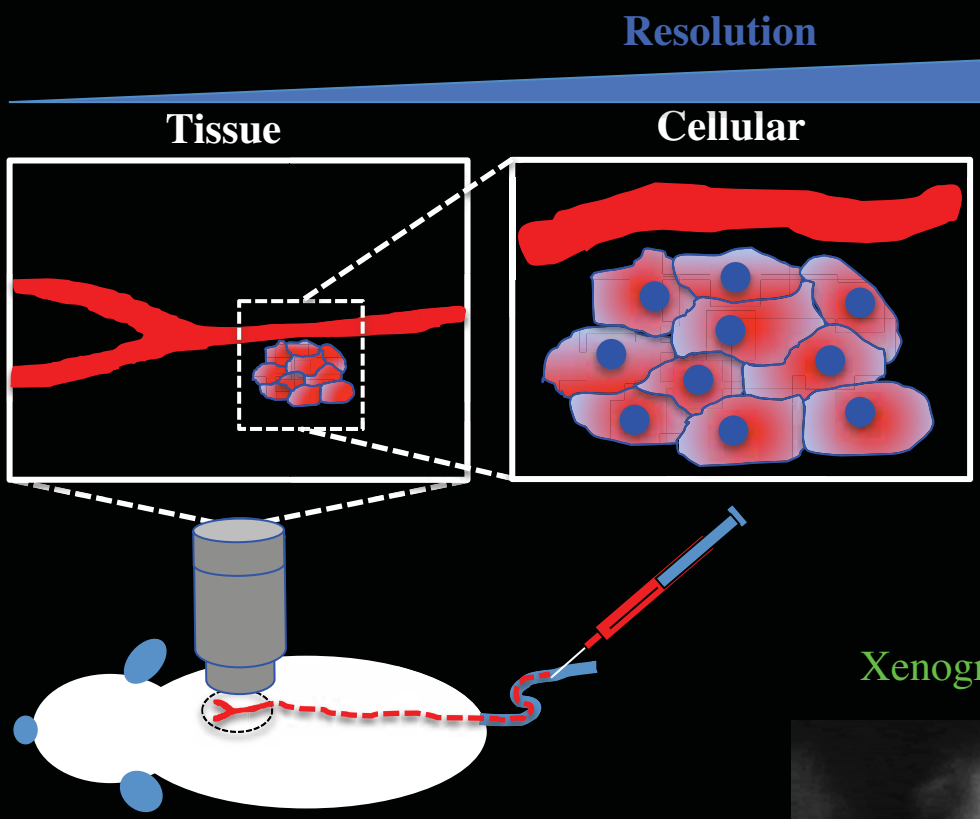
Intravital Microscopy





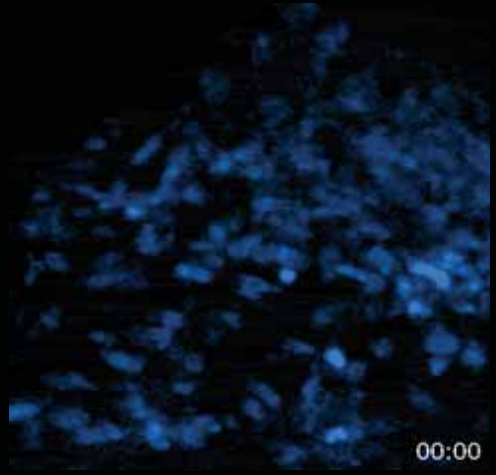
Masedunskas et al., (2008) Traffic, Weigert et al., (2010) Hist. Cell Biol, Amornphimoltham et al., (2010) Adv Drug. Del. Rev

Intravital Microscopy



Xenograft (Back)

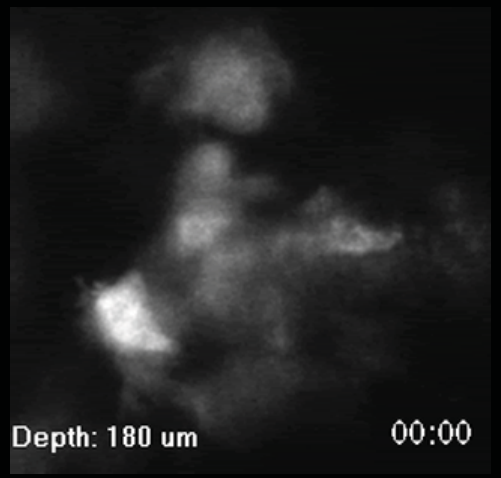
HN12 (Hoechst)

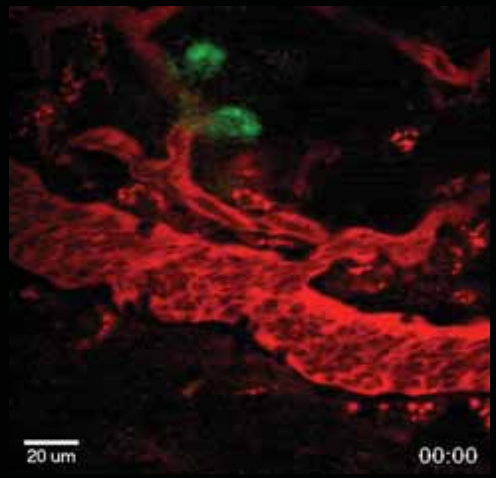


Xenograft (Tongue)

Cervical lymph node

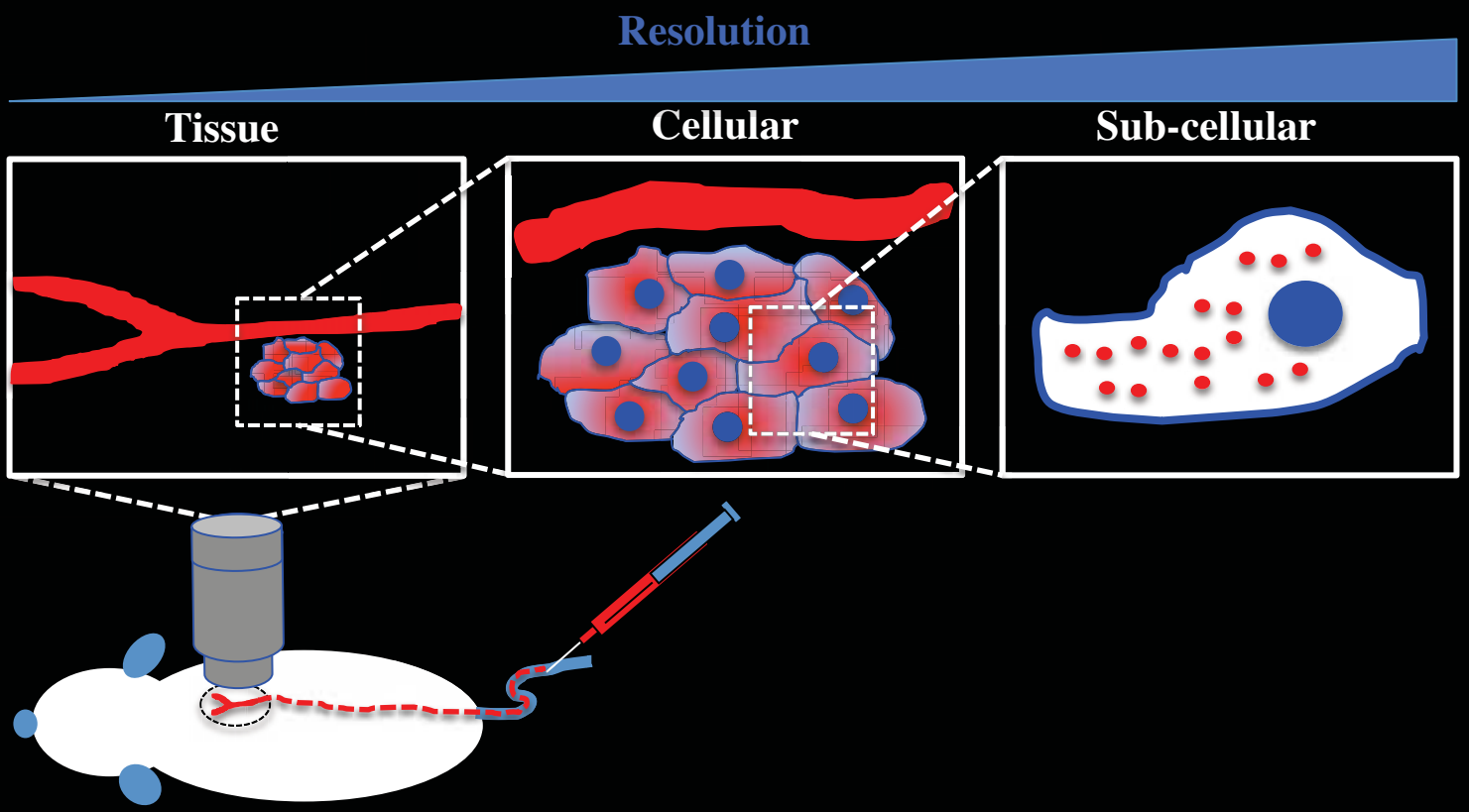
OSCC3-YFP





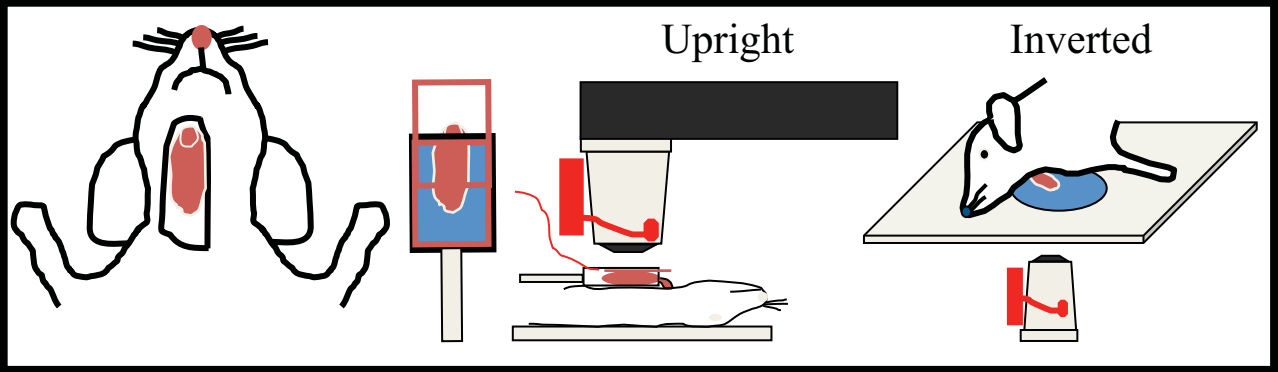
Amornphimoltham et al., (2010) Adv Drug Del. Rev, Bhirde et al., (2009) ACS Nano, Patel et al., (2011) Cancer Research

Intravital Microscopy



Amornphimoltham et al., (2010) Adv. Drug Deliv Rev.

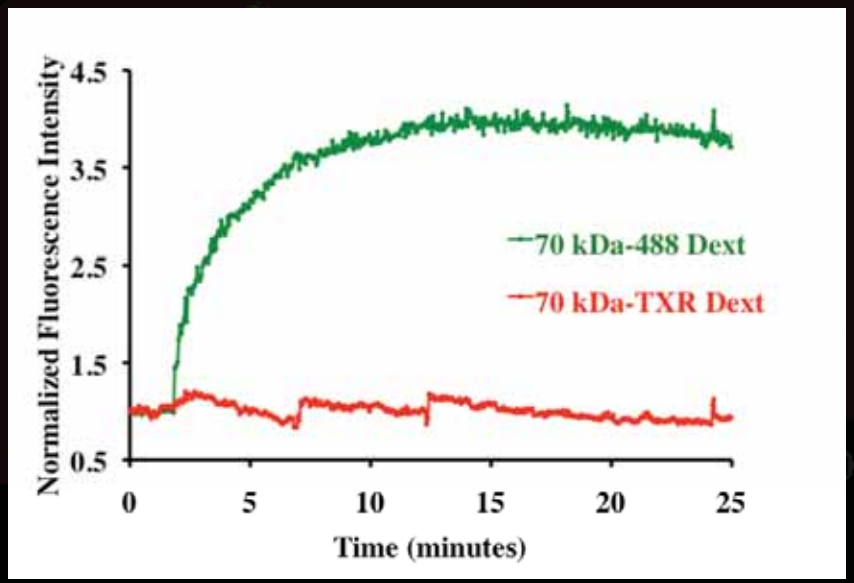
An experimental system to image subcellular organelles in live animals



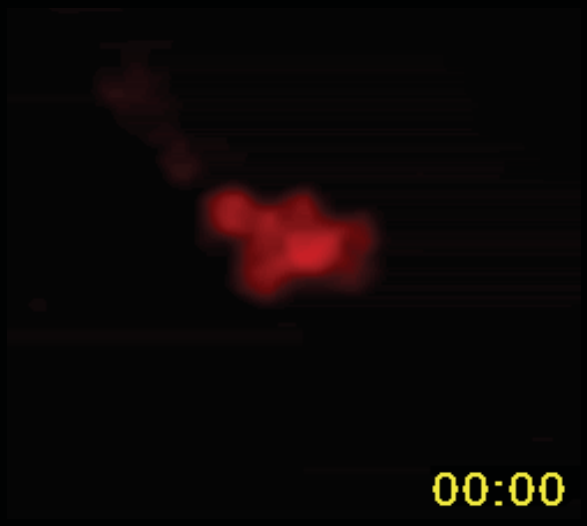
1) Stability
2) Spatial resolution
3) Temporal Resolution
4) Quantitative analysis

Endocytosis of systemically injected fluorescently labeled molecules

500 kDa FITC-Dextran/ 70 kDa TXR-Dextran



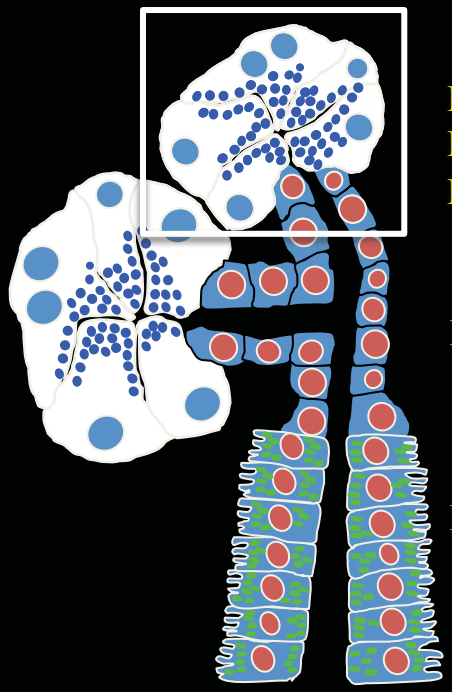
Early endosomes / Lysosomes
70 kDa 488-Dext / 70 kDa TXR-Dext



Masedunskas and Weigert (2008), Traffic

……And amenable to pharmacological and genetic manipulations

1) Delivery of fluorescent molecules
2) Selective delivery of drugs
3) Gene transduction

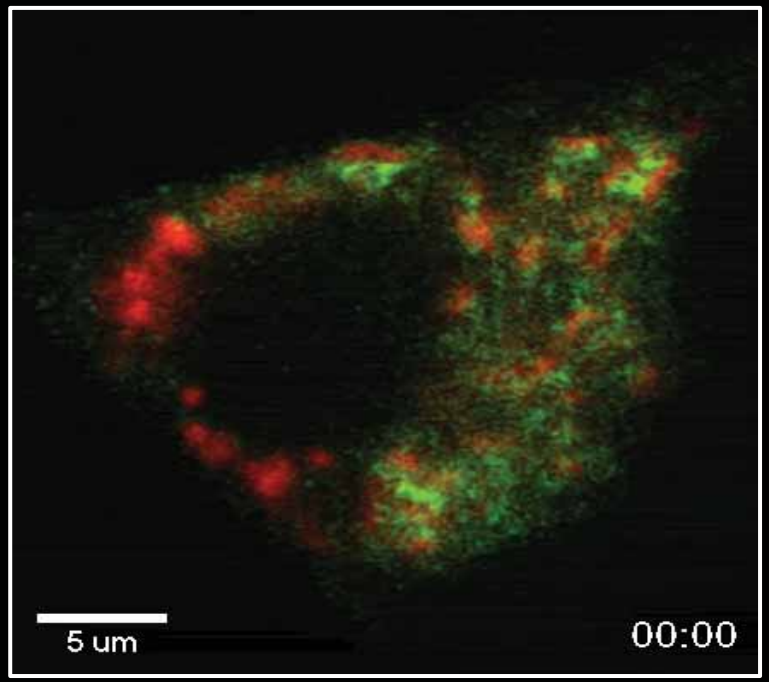


Plasmid DNA/Ad5
Plasmid DNA/PEI
Plasmid DNA/Isoproterenol

Plasmid DNA

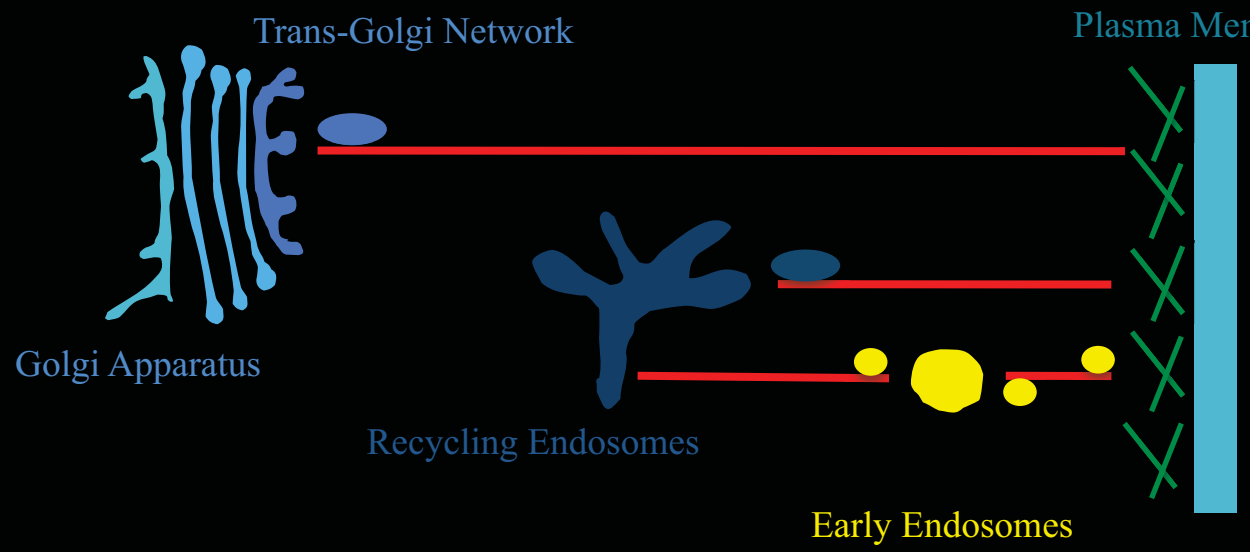
Plasmid DNA/Ad5

GFP-Clathrin / TGN38-mcherry

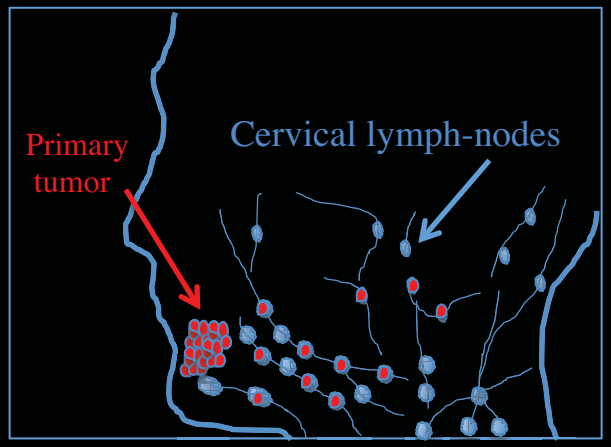


Masedunskas and Weigert (2008) Traffic - Sramkova et al. (2009), Am. J. Phys - Weigert et al. (2010) Hist. and Cell Bio

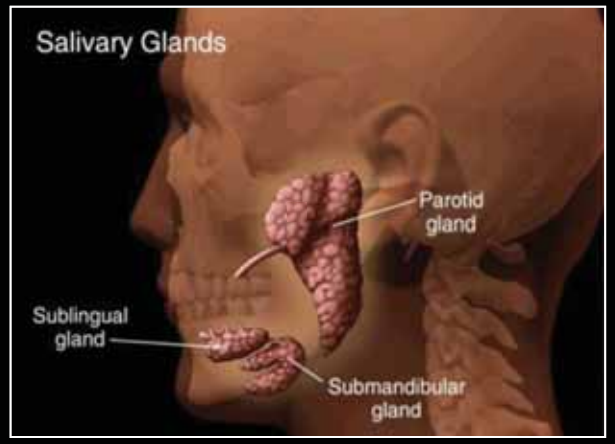
Aims of the Lab



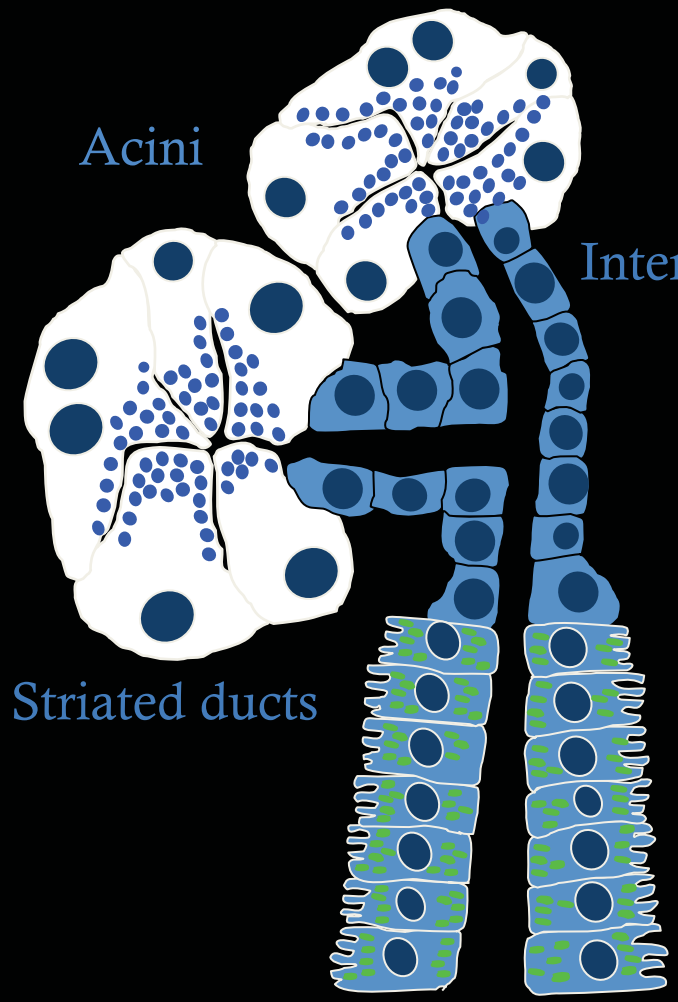
Endosomal recycling

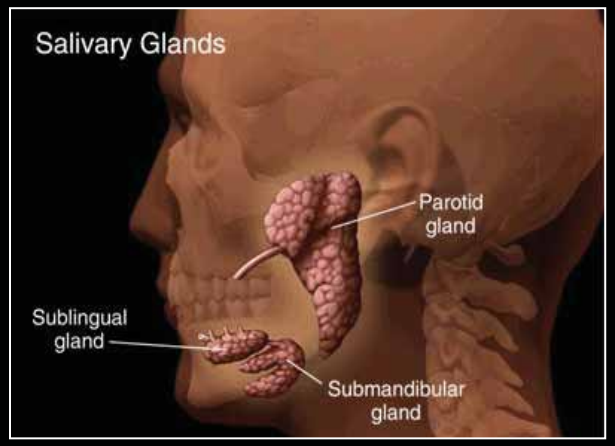


Regulated exocytosis

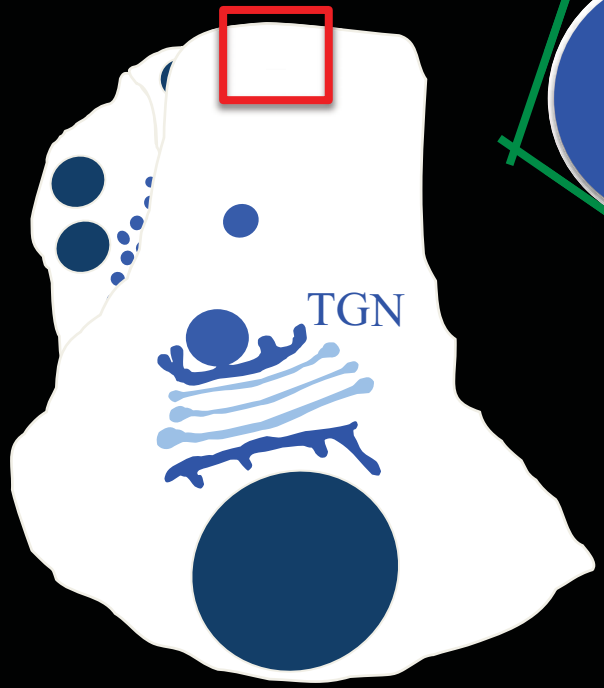


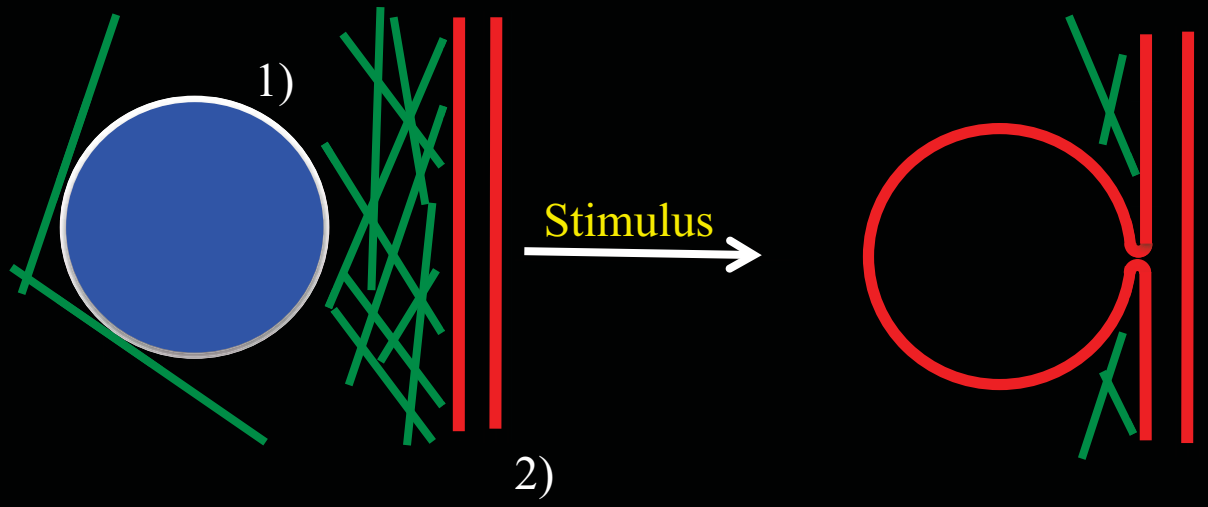
Salivary glands





Regulated exocytosis in salivary glands



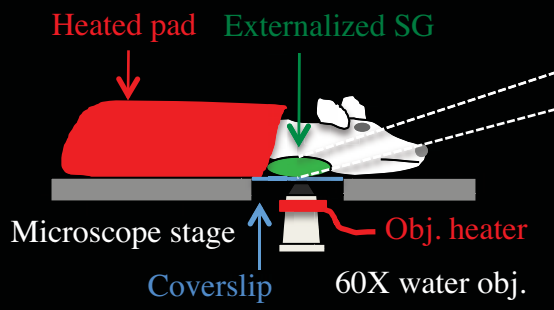


How is the remodeling of the apical membrane, the actin cytoskeleton and the surface of the granule regulated by a stimulus originated from the basolateral pole?

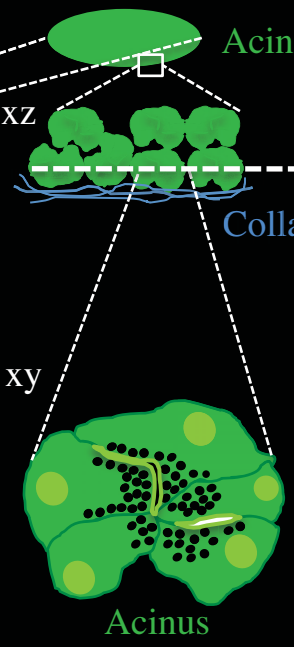
Which molecules are activated during secretion…

1) on the surface of the granule?
2) at the apical plasma membrane?

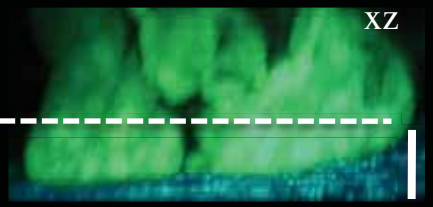
A model to study exocytosis in salivary glands: the GFP mouse



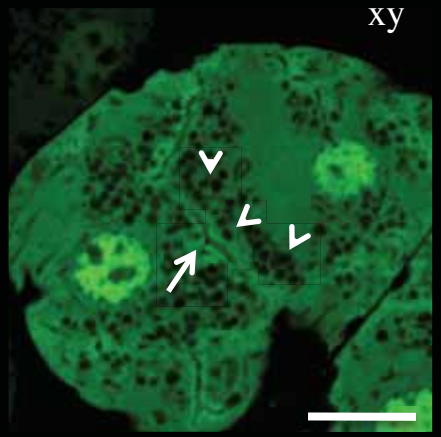
GFP-FVB

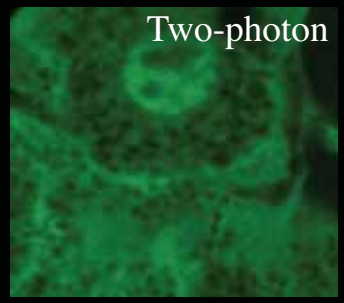


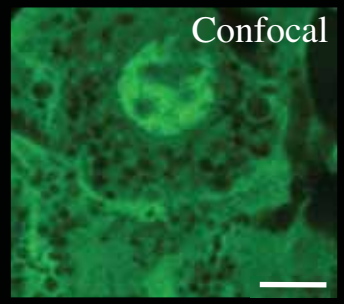
Collagen / GFP



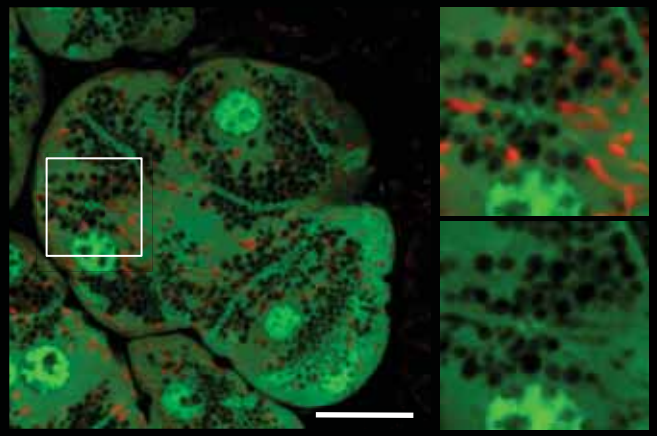
GFP



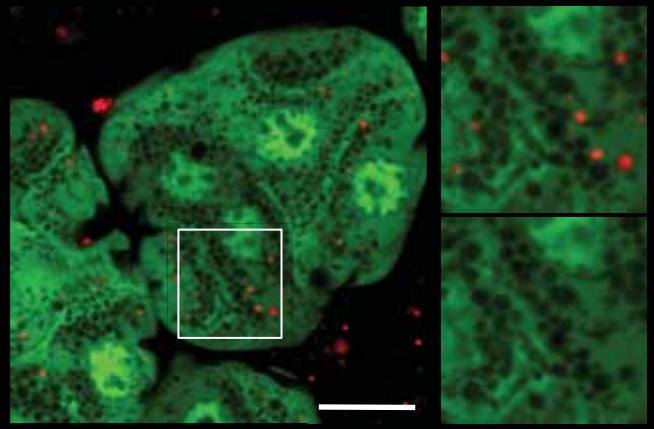




Mitotracker



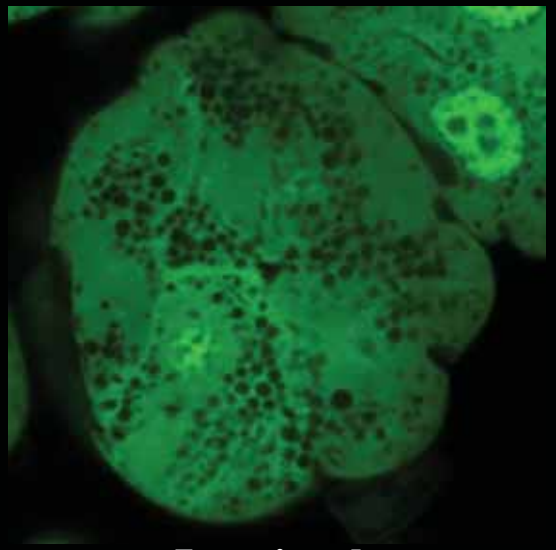
Lysotracker



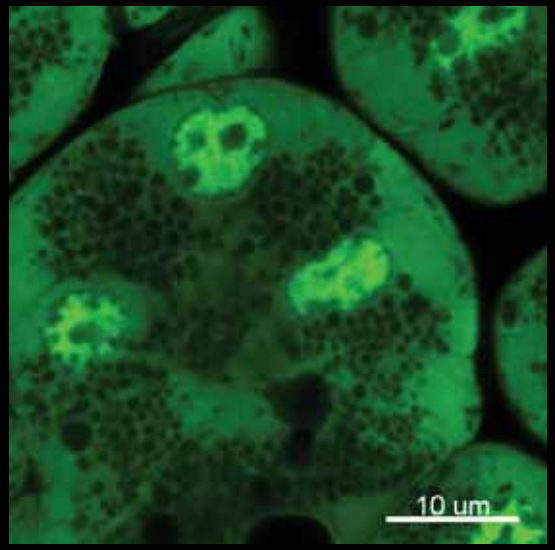
Masedunskas et al., submitted

Exocrine glands in the GFP mouse

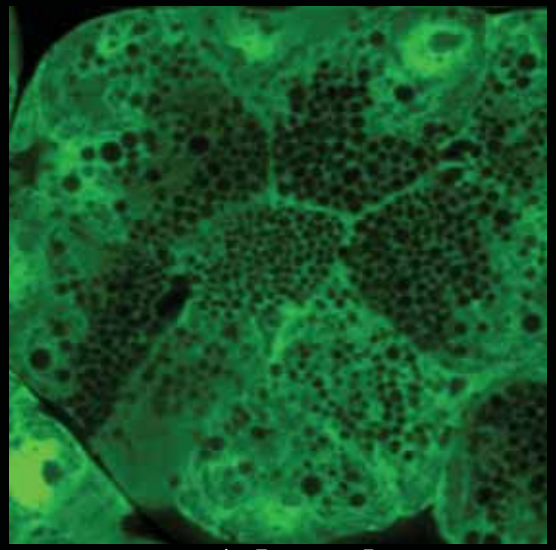
Submandibular



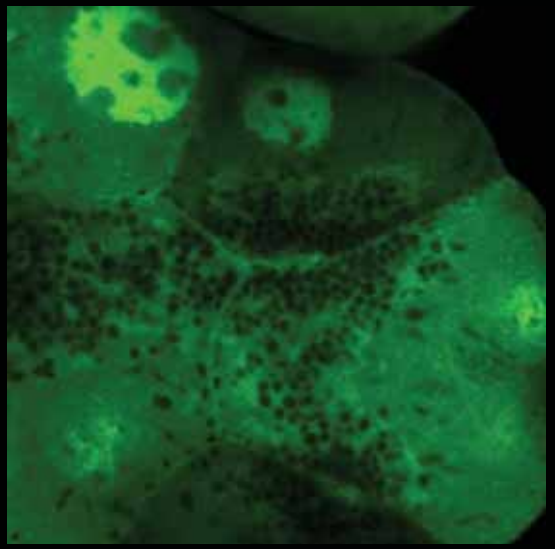
Parotid



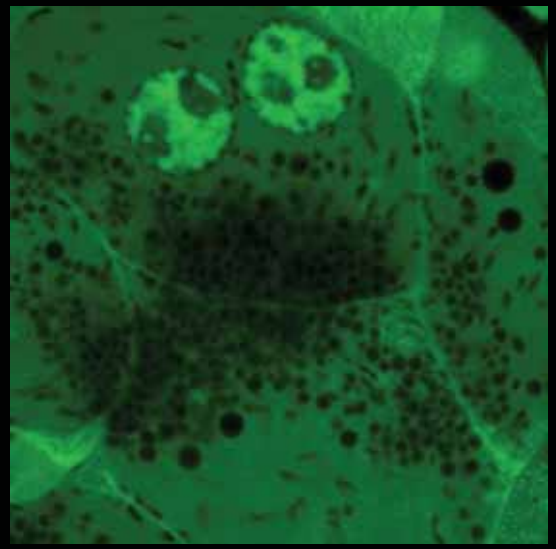
Sublingual



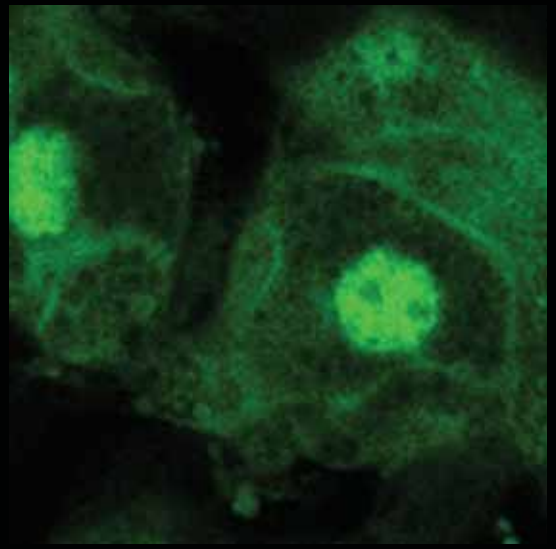
Lacrimal



Pancreas



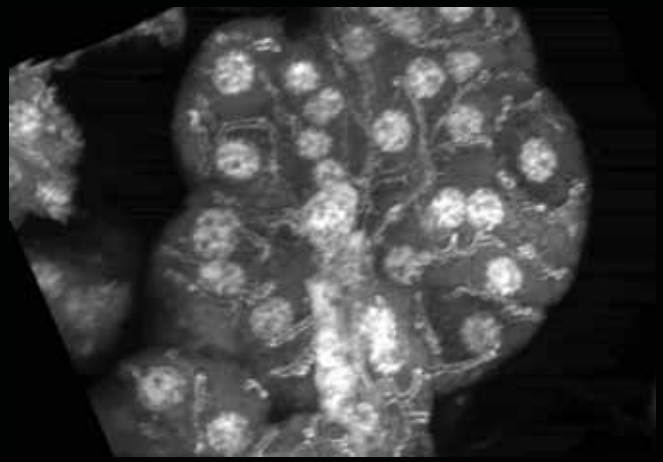
Adrenal



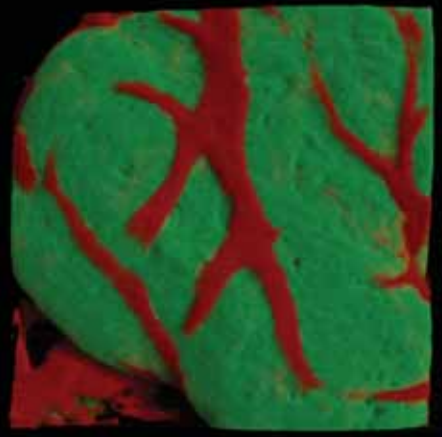
Masedunskas et al., submitted

The architecture of the acini

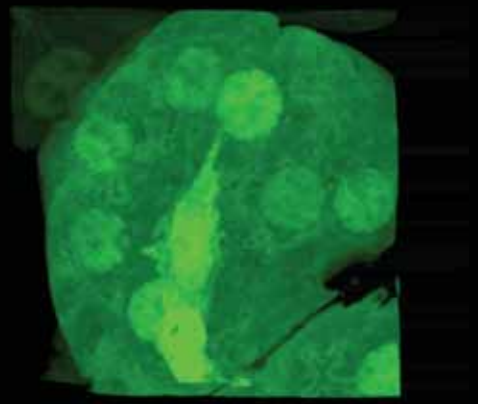
GFP



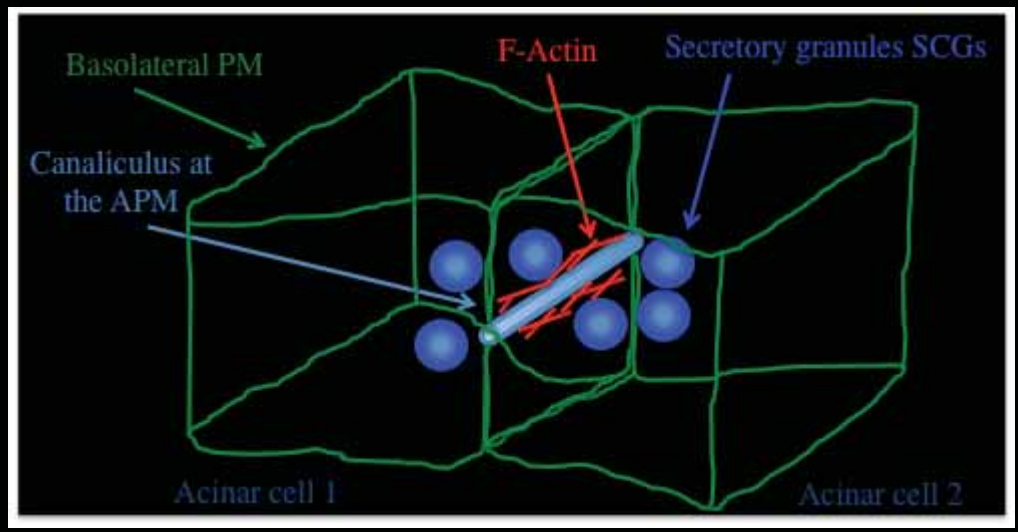
GFP/Phalloidin



GFP/Secretory granules



Secretory granules per acinus	2300-3100
Cells per acinus	9-10
Diameter of the granules (μm)	1.5 – 2.0
Diameter of the canaliculi (μm)	0.3-0.4



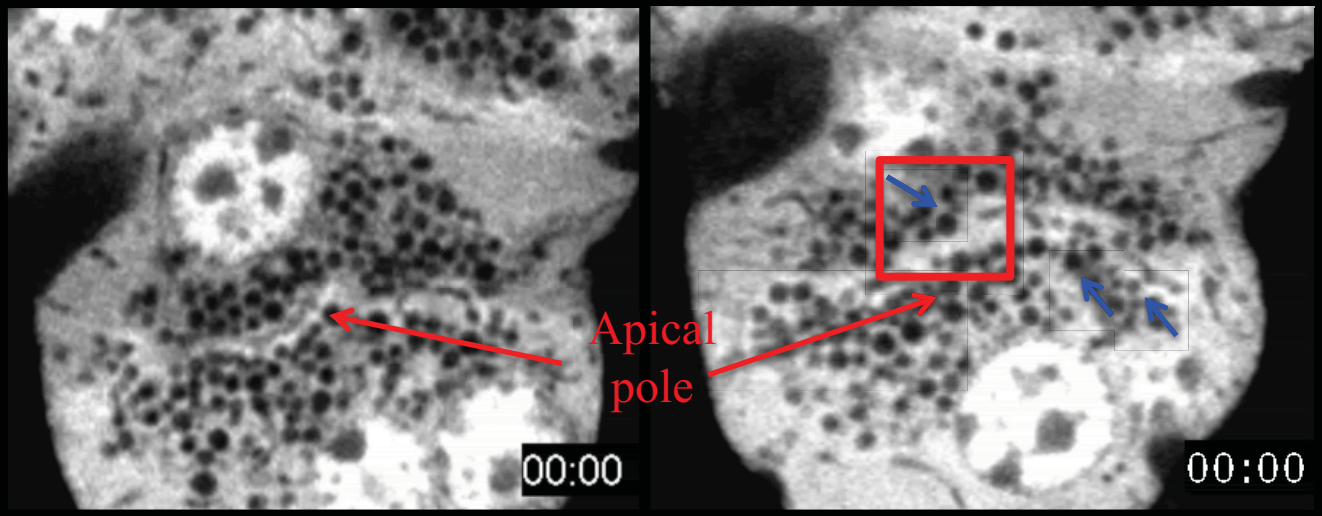
Masedunskas et al., submitted

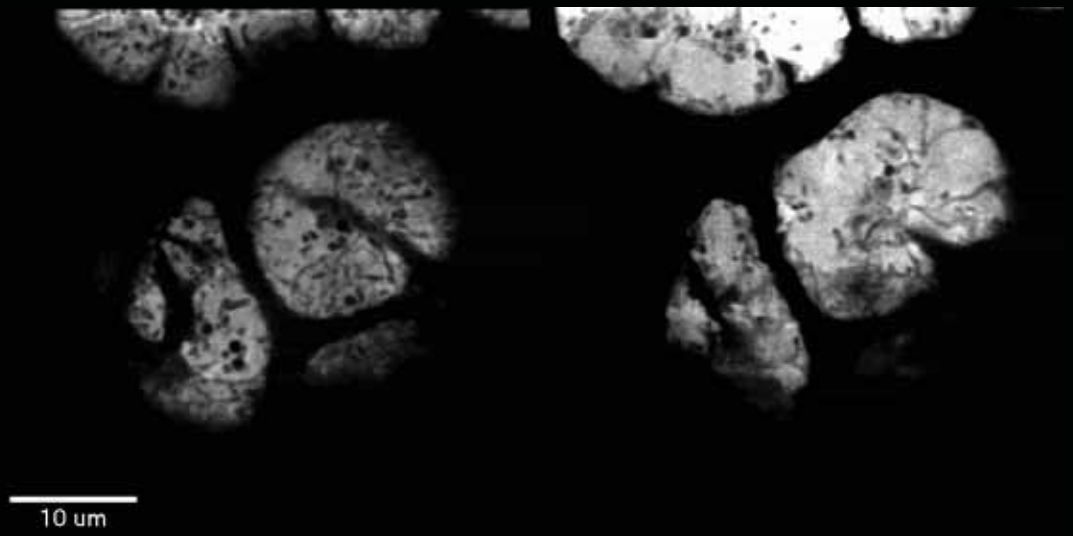
Dynamics of the secretory granules during regulated exocytosis *in vivo*

Resting

Stimulated – Iso/Carb

Submandibular gland



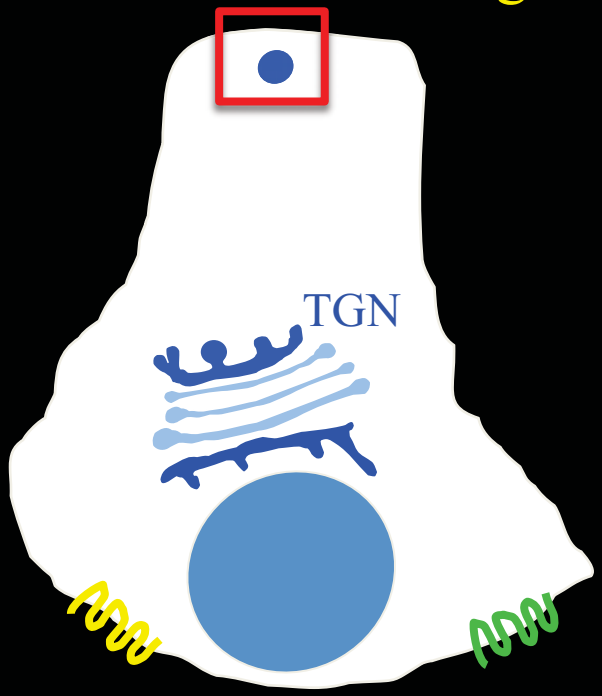


t=0

t=30

Masedunskas et al., submitted

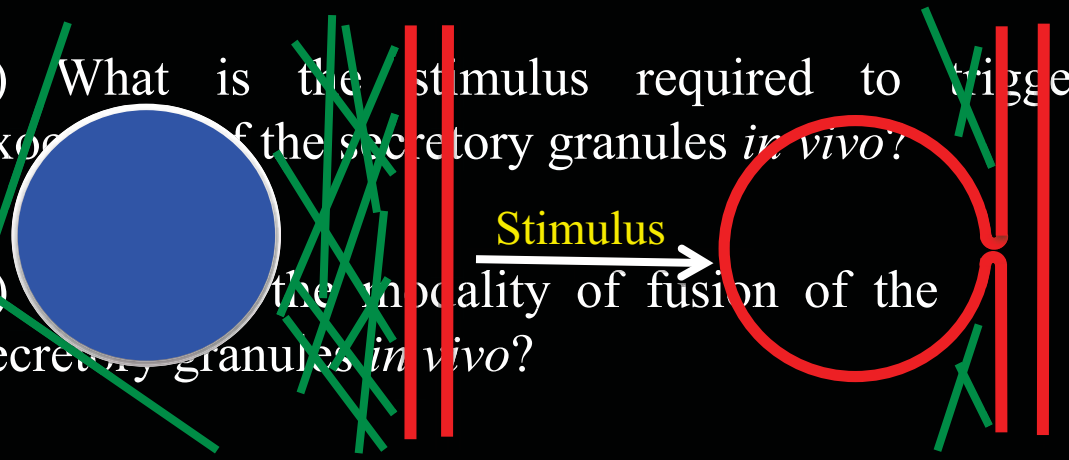
Regulated exocytosis in salivary glands



In vivo
β-adrenergic receptors

Ex-vivo
M1/M3 muscarinic receptor
β-adrenergic receptors

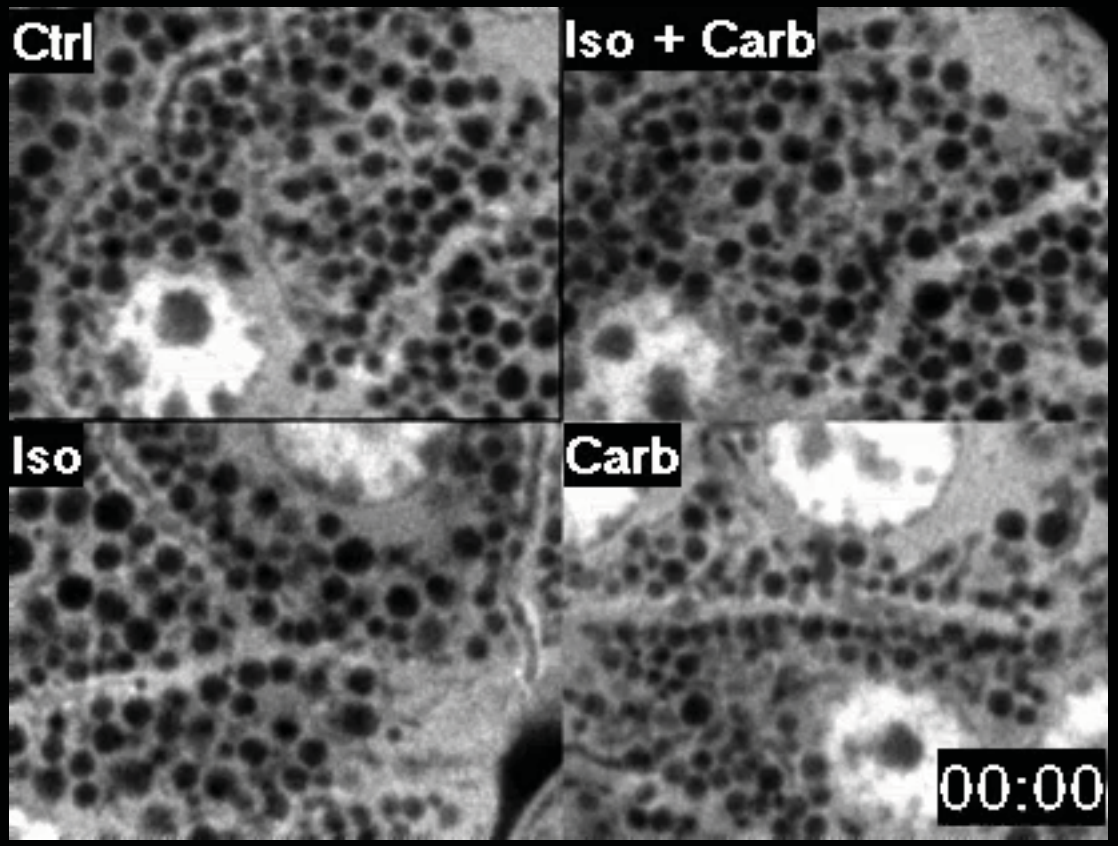
1) What is the stimulus required to trigger exocytosis of the secretory granules *in vivo*?

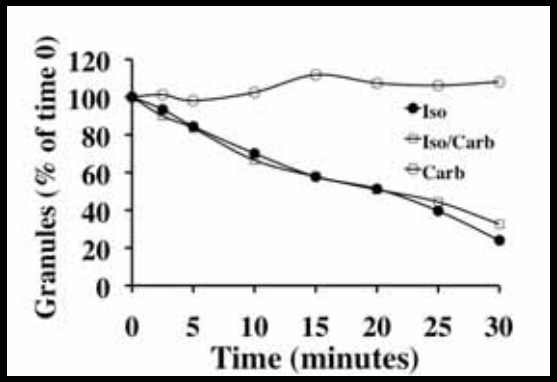


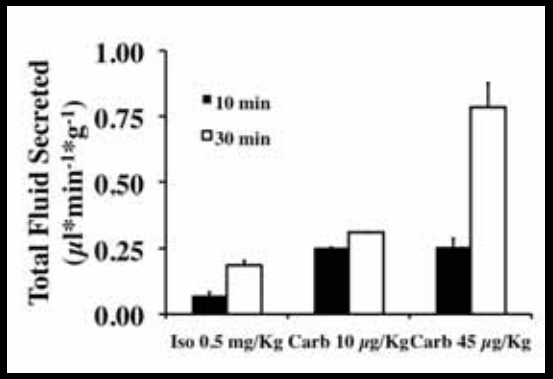
2) [illegible] the modality of fusion of the secretory granules *in vivo*?

3) How is fusion regulated?

Signaling through β-adrenergic and not muscarinic receptors stimulate the exocytosis of the secretory granules in the salivary glands *in vivo*

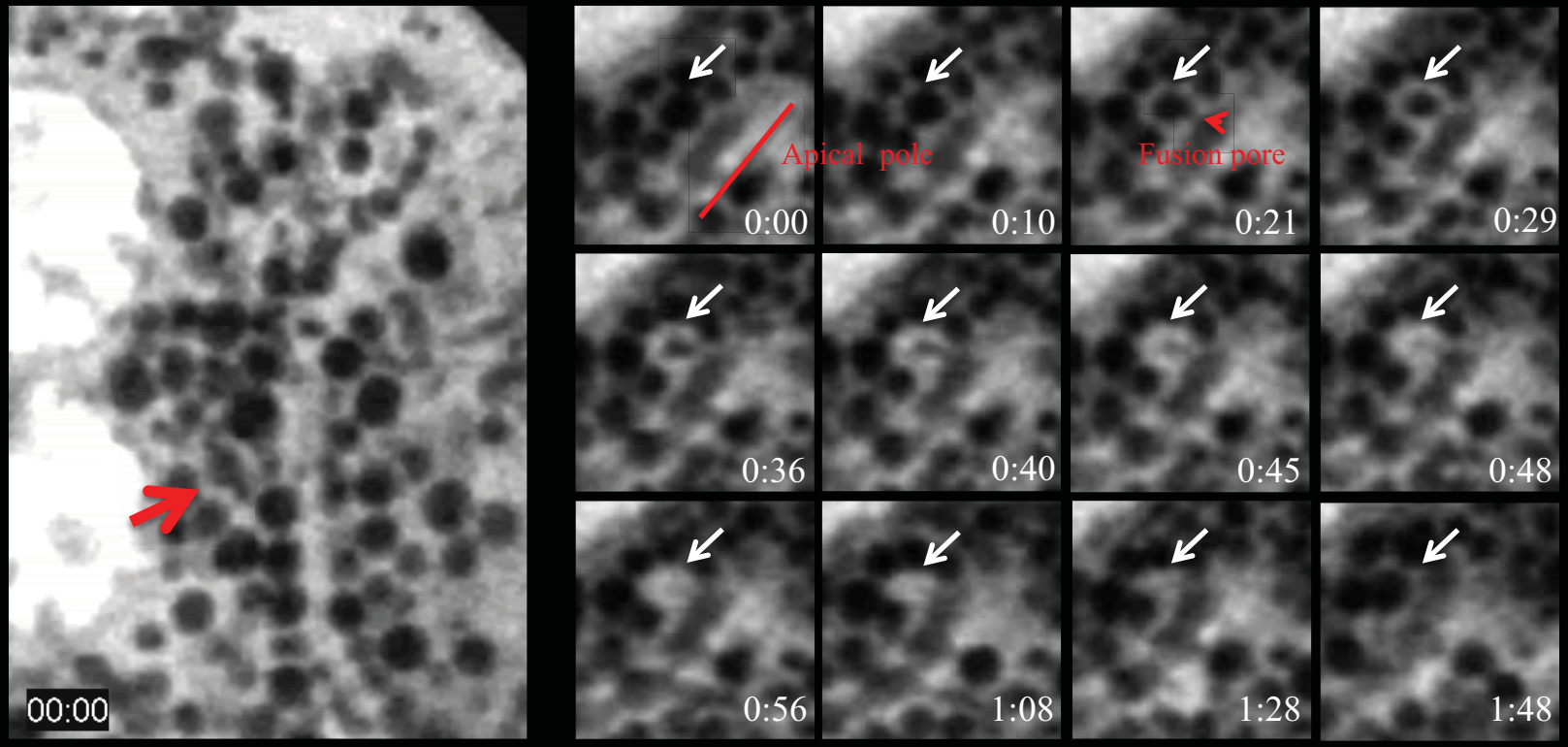






Masedunskas et al., submitted

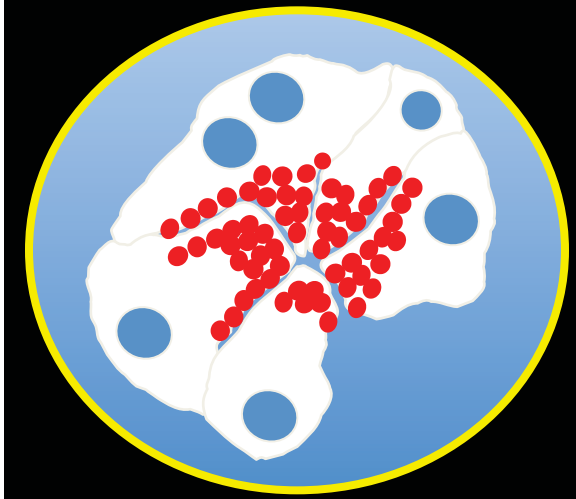
Secretory granules exocytosis *in vivo* occurs through single fusion events

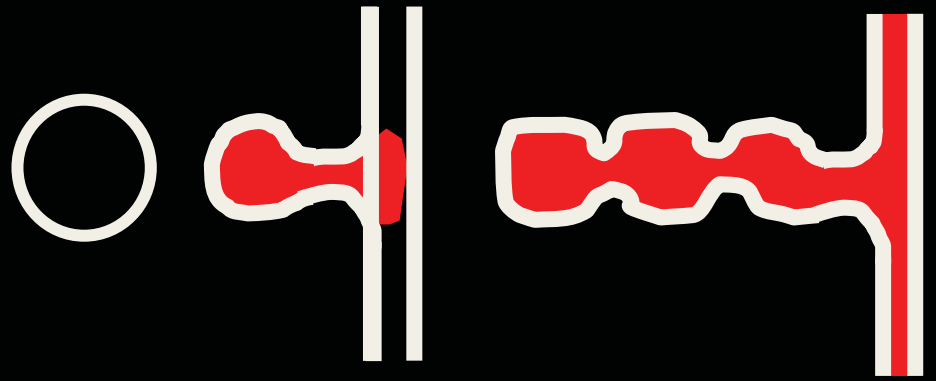


Sec Granule Plasma membrane

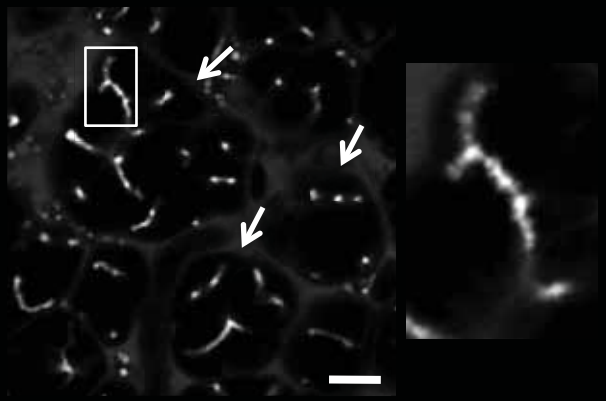
Masedunskas et al., submitted

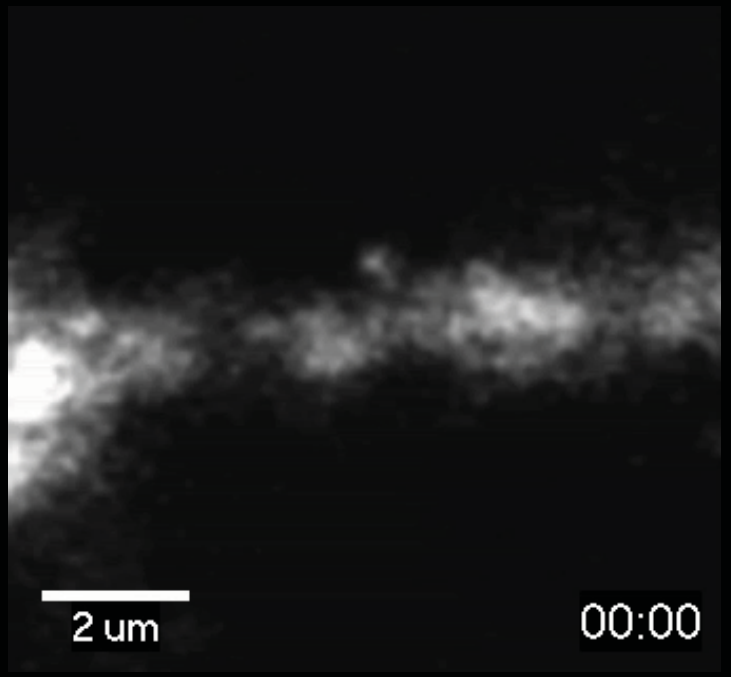
Secretory granules exocytosis *in vivo* occurs through single fusion events





10 kDa Texas-Red Dextran

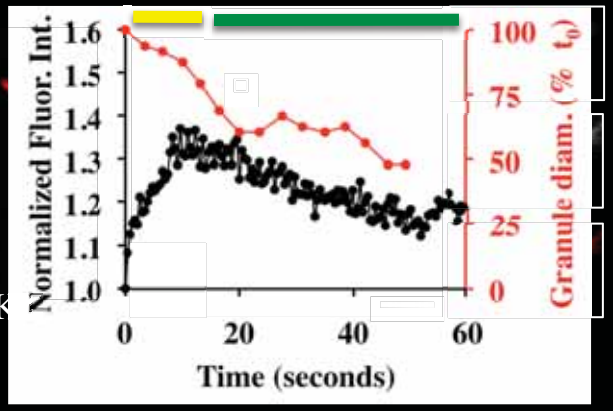




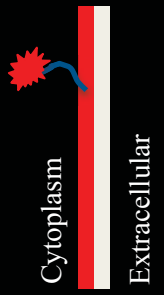
Masedunskas et al., submitted

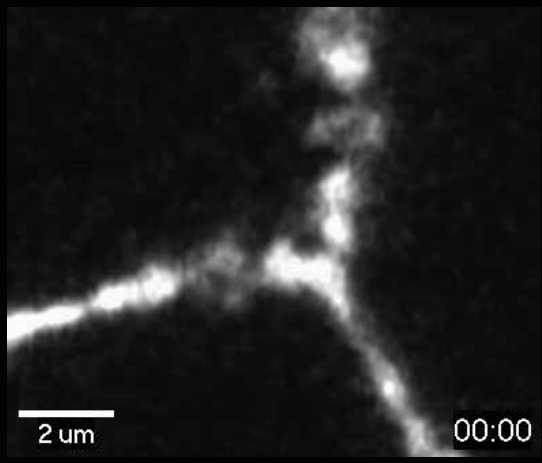
Secretory granules exocytosis *in vivo* occurs through single fusion events

Tomato- MGCCFSK



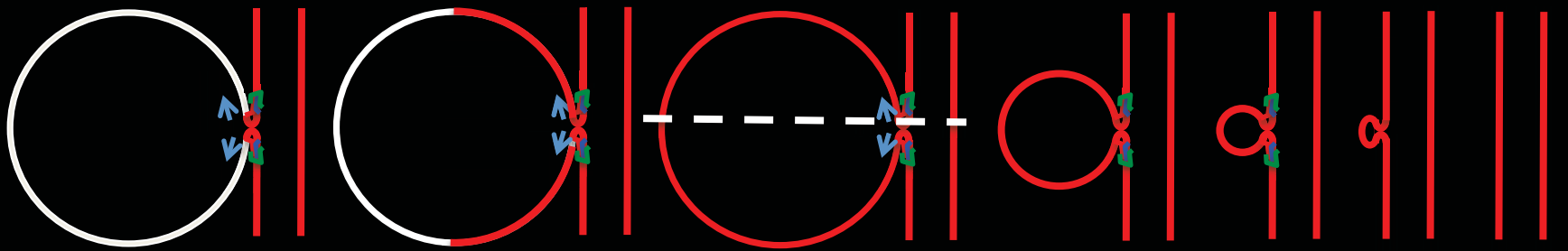
PM bilayer





Membrane diffusion

Collapse



Sec Granule Plasma membrane

Φ=1.5 μm Φ=0.3 μm

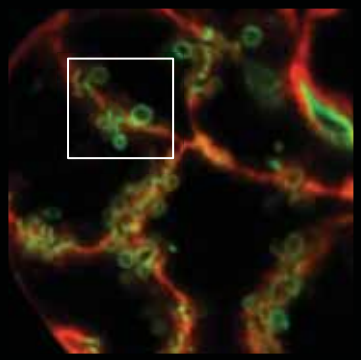
t=0

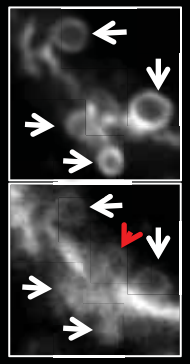
t=5-10 sec

t=40-90 sec

Masedunskas et al., submitted

Actin is recruited onto the secretory granules after fusion with the APM

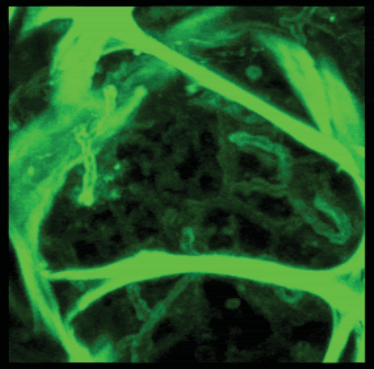




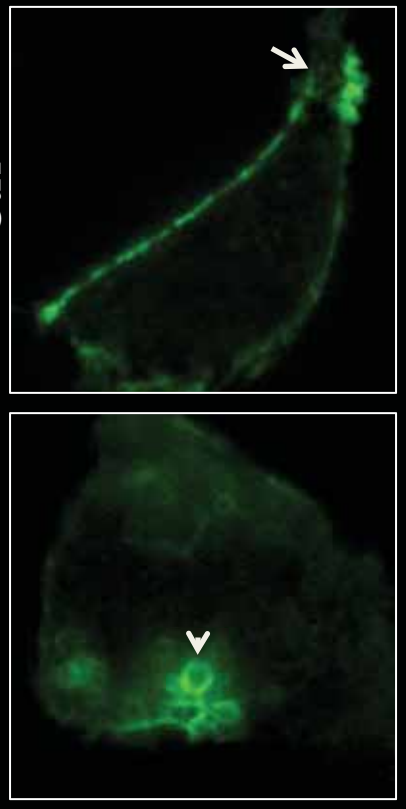
GFP-Lifeact (F-actin)

Riedl et al., (2008) Nat. Methods
Courtesy of Tamas Balla (NICHD)

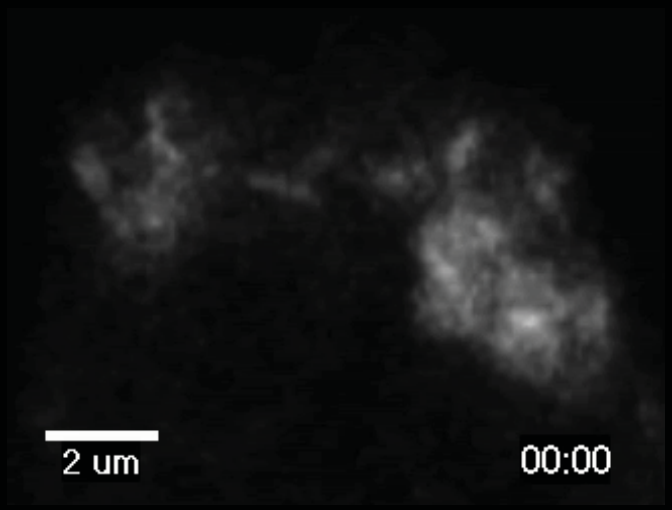
GFP-Lifeact mouse

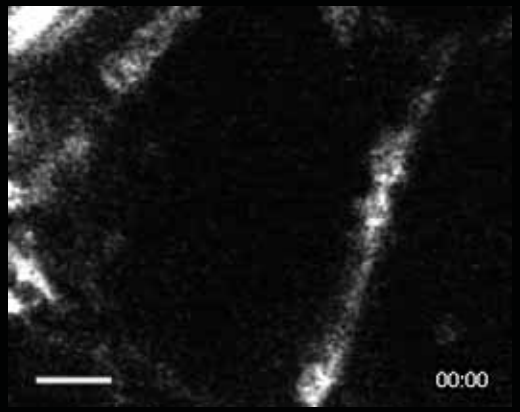


GFP-Lifeact



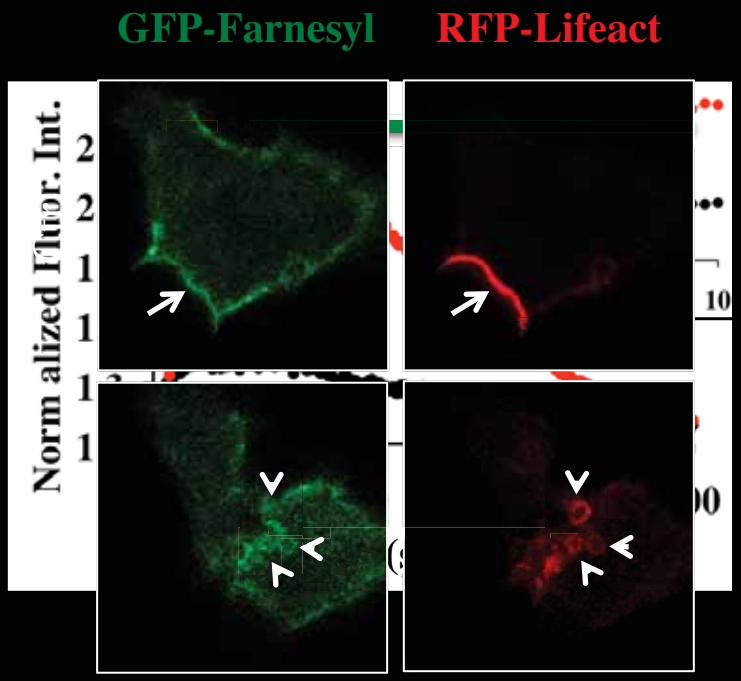
Iso

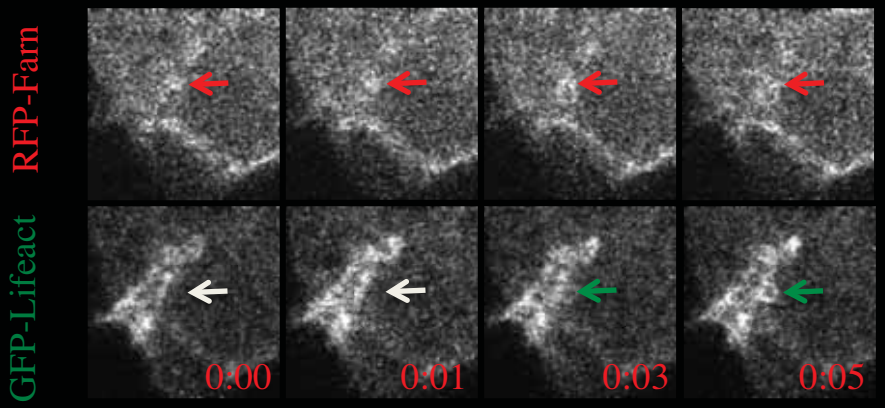


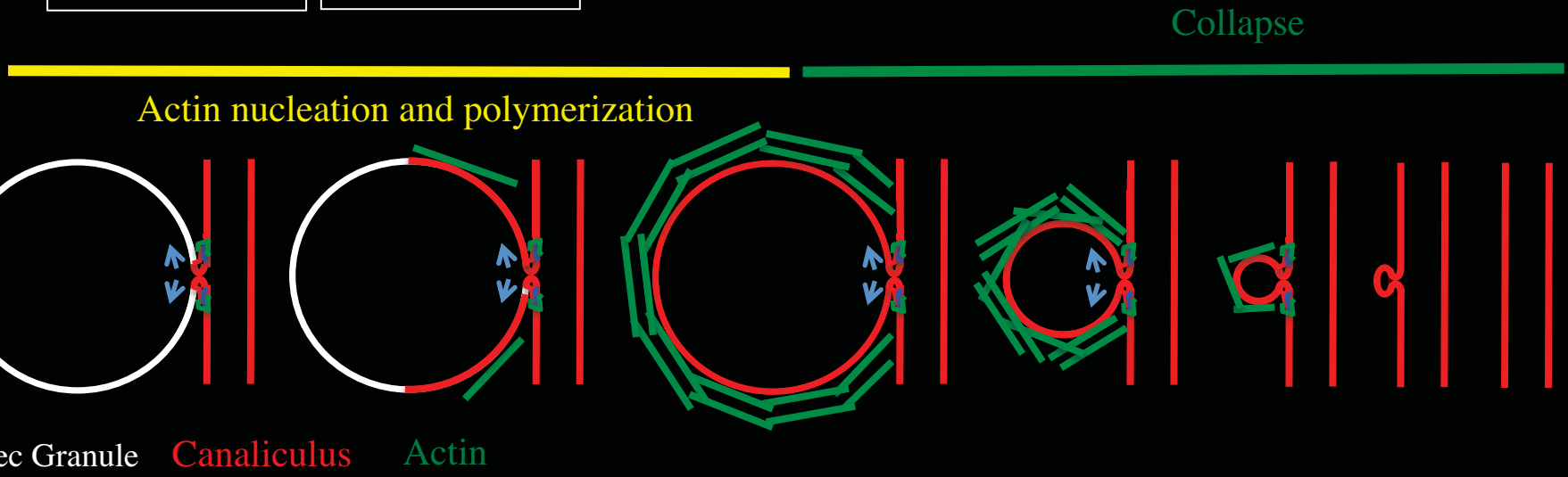


Masedunskas et al., submitted

Actin is recruited onto the secretory granules after fusion with the APM







Masedunskas et al., submitted

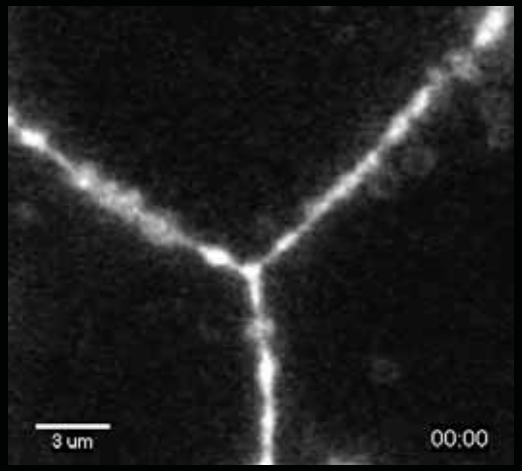
Actin is required to complete the collapse of fused the secretory granules

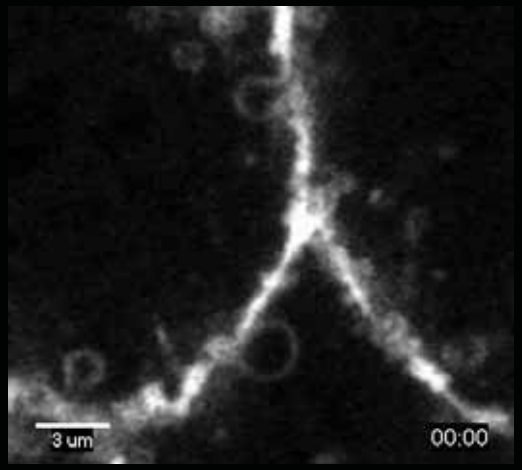
Cytochalasin D

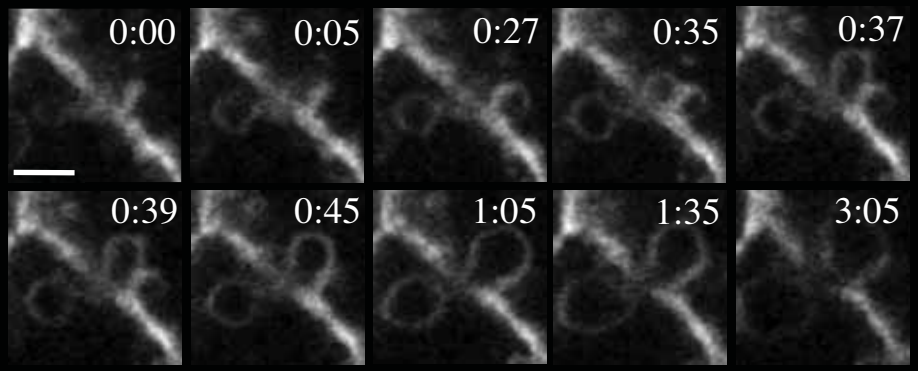
Cytochalasin D

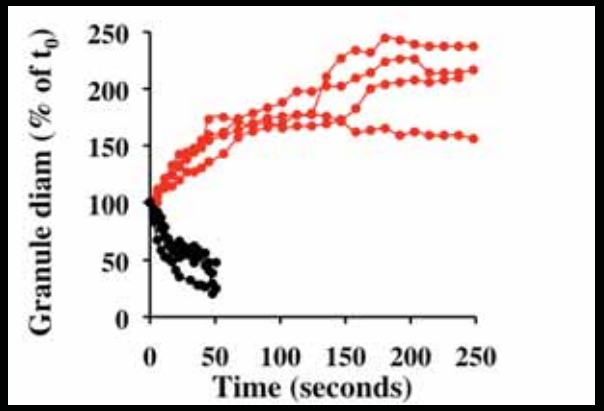
Control

Isoproterenol



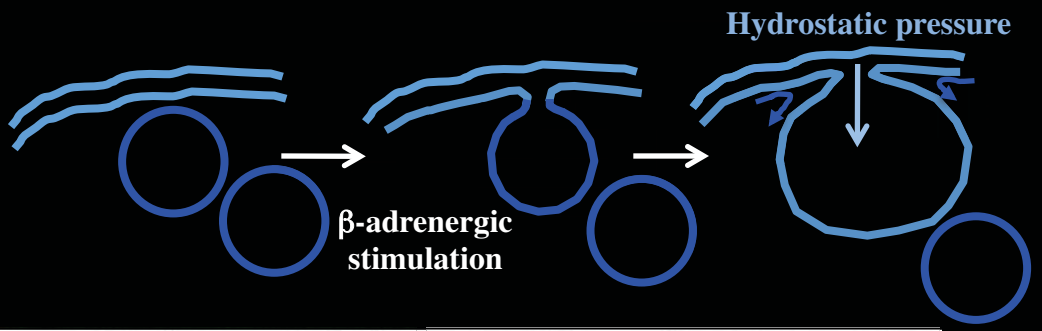


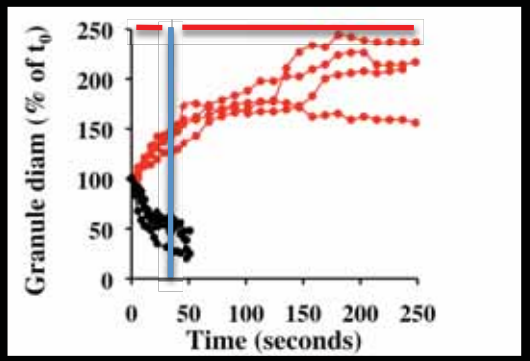


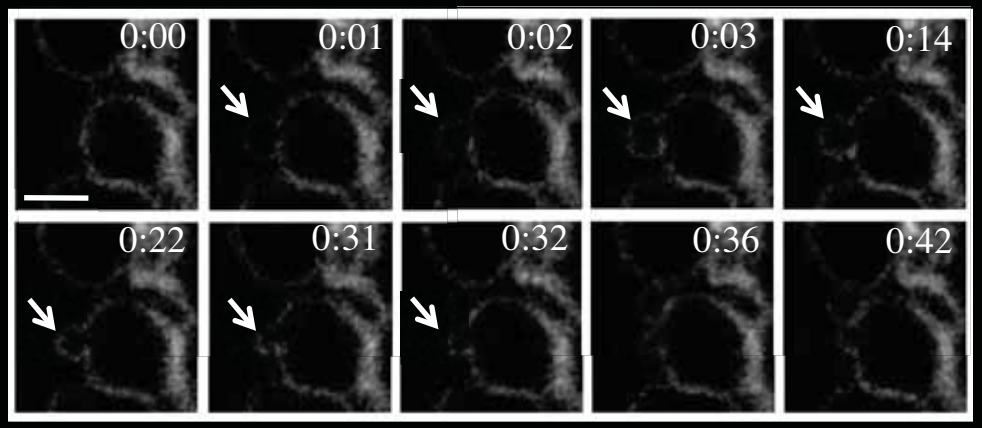


Masedunskas et al., submitted

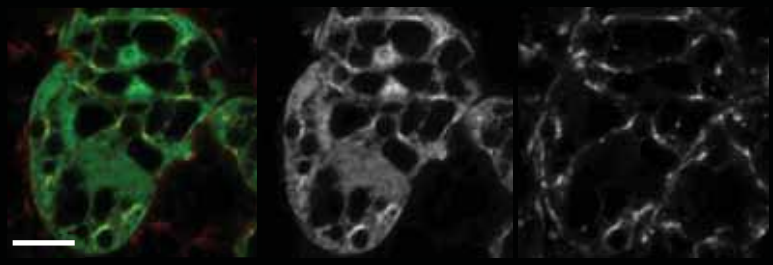
Actin is required to complete the collapse of fused the secretory granules

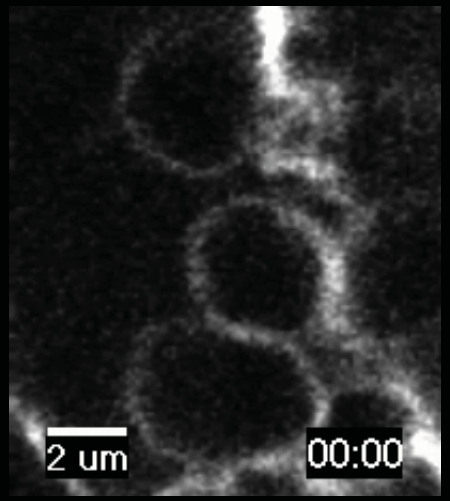


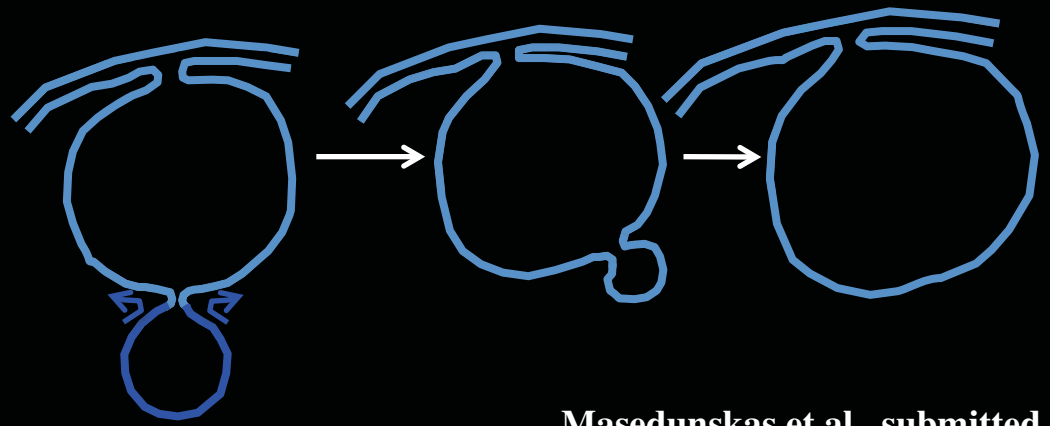




GFP Phalloidin





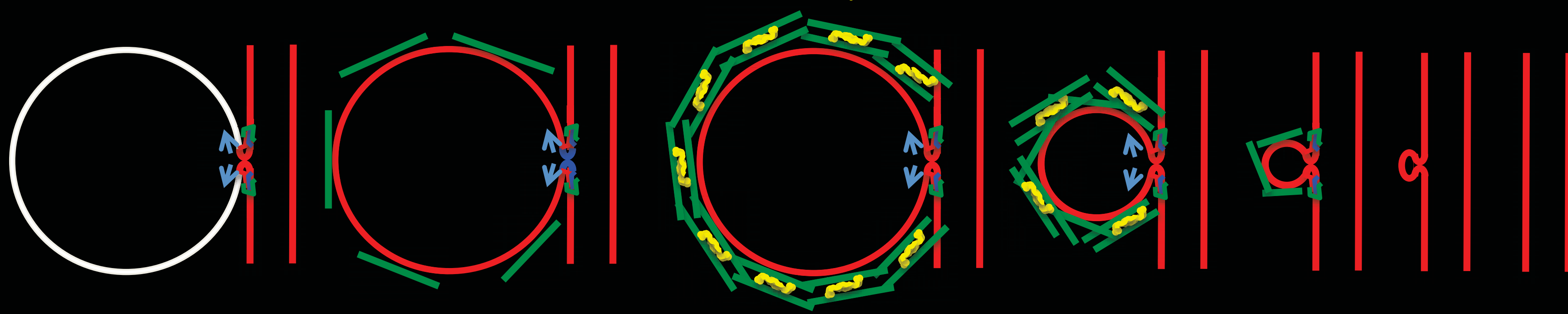


Masedunskas et al., submitted

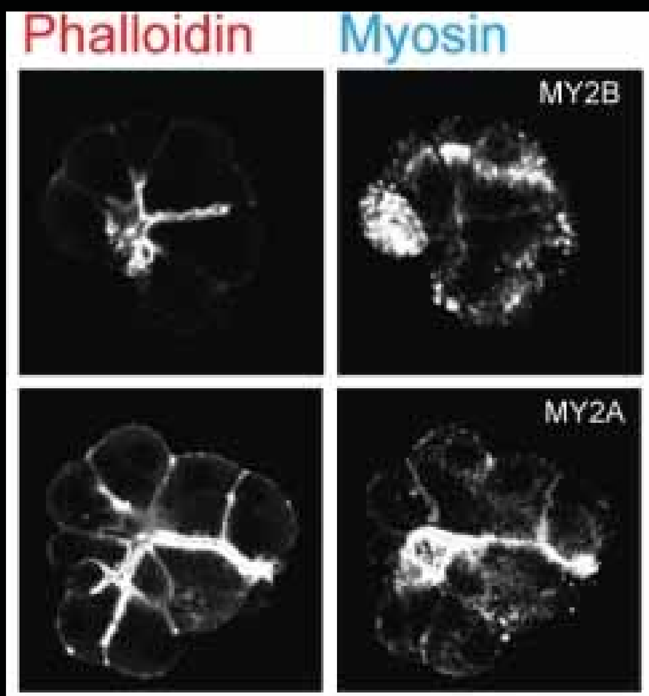
Actin is required to complete the collapse of the fused secretory granules

Myosin IIa and IIb are recruited onto the secretory granules

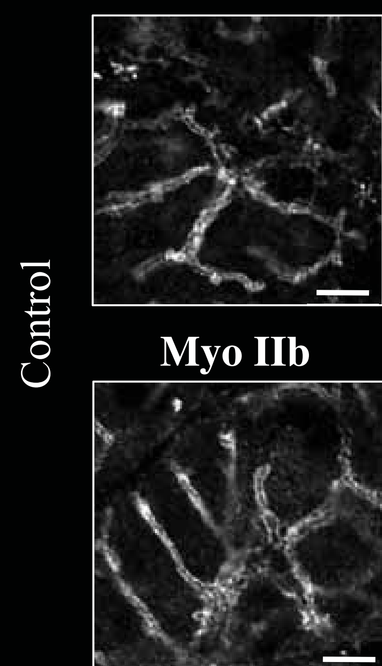
Myosin motor

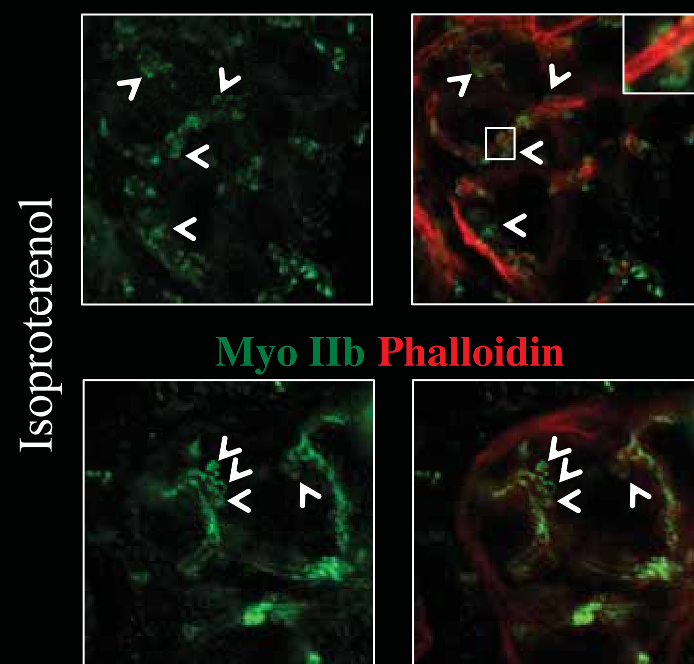


Pancreatic acini



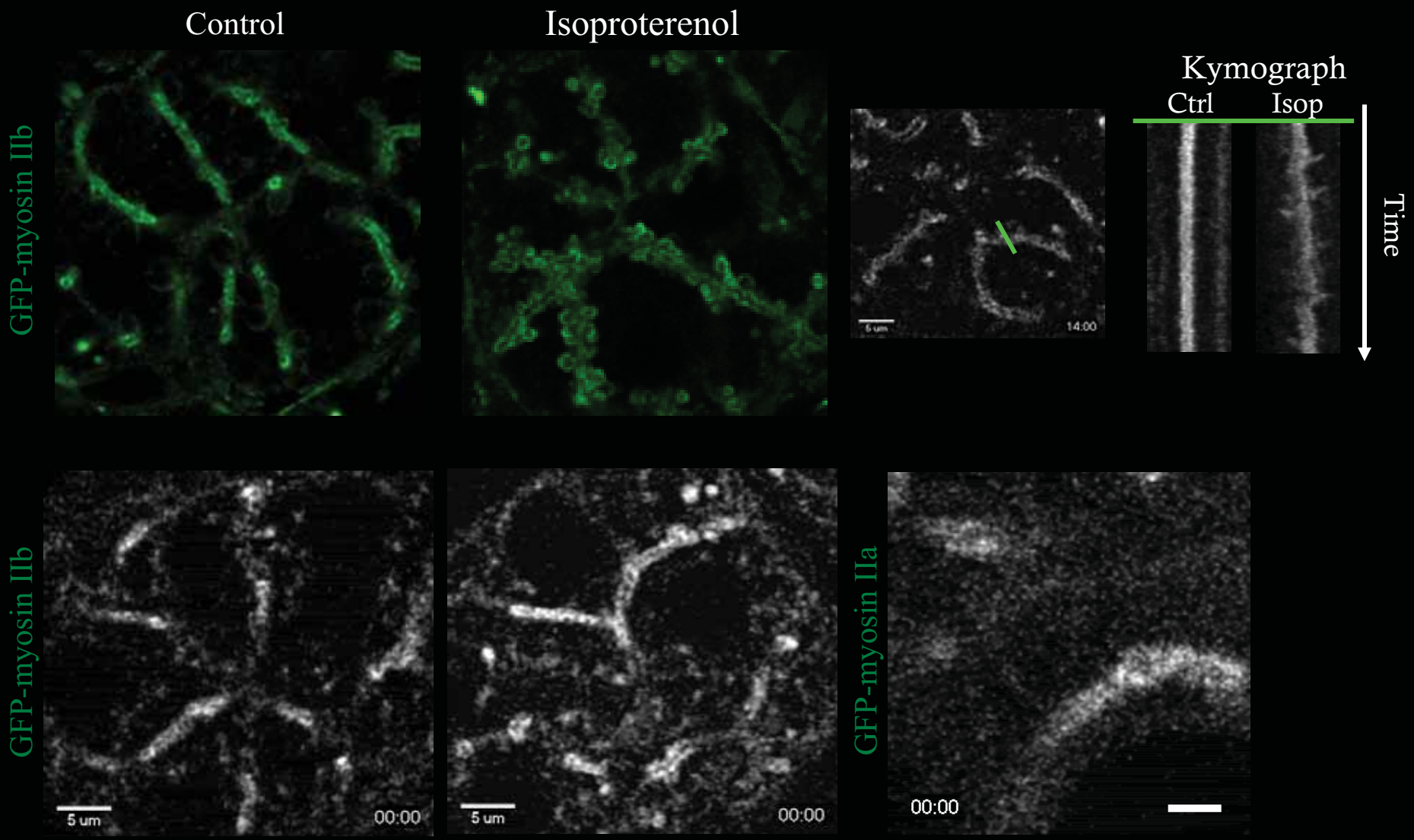
Bhat and Thorn, (2009) MBC





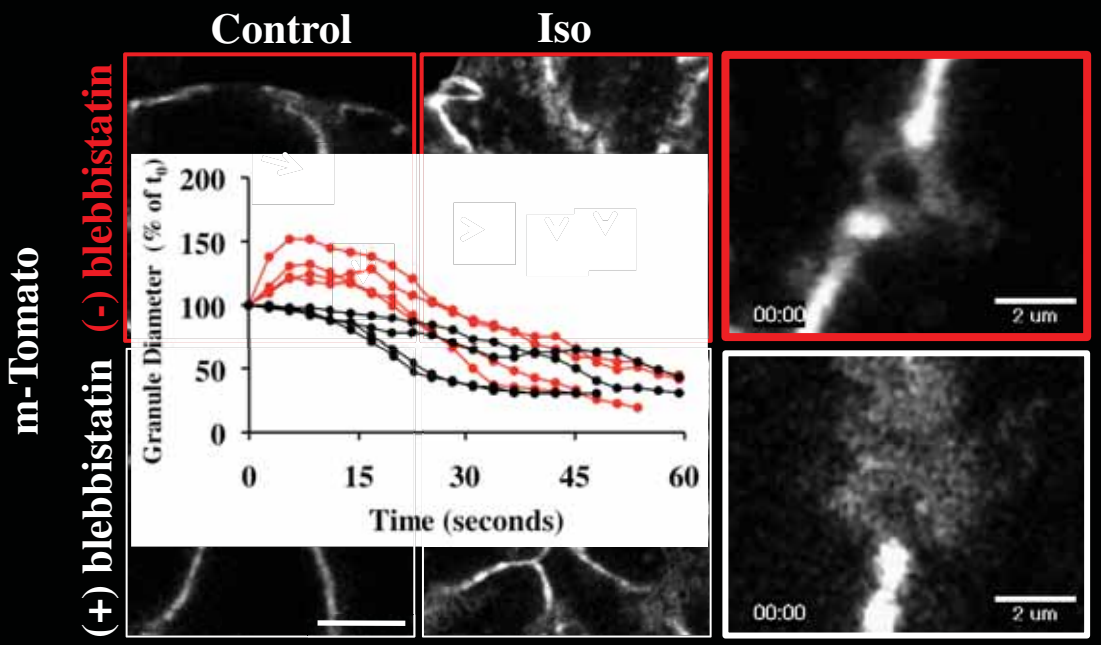
Masedunskas et al., submitted

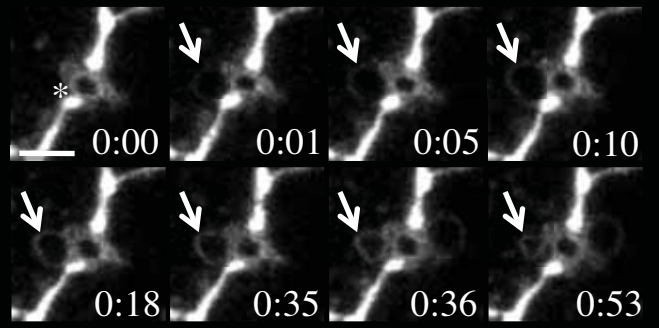
Myosin IIa and IIb are recruited onto the secretory granules

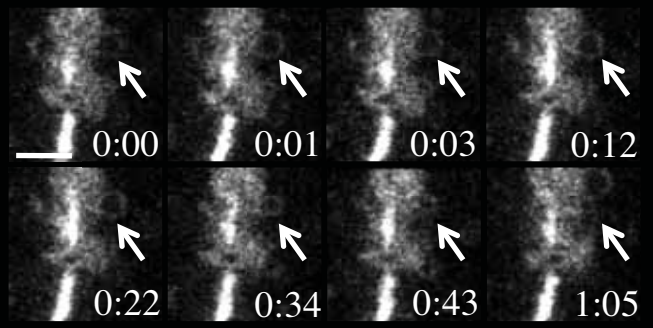


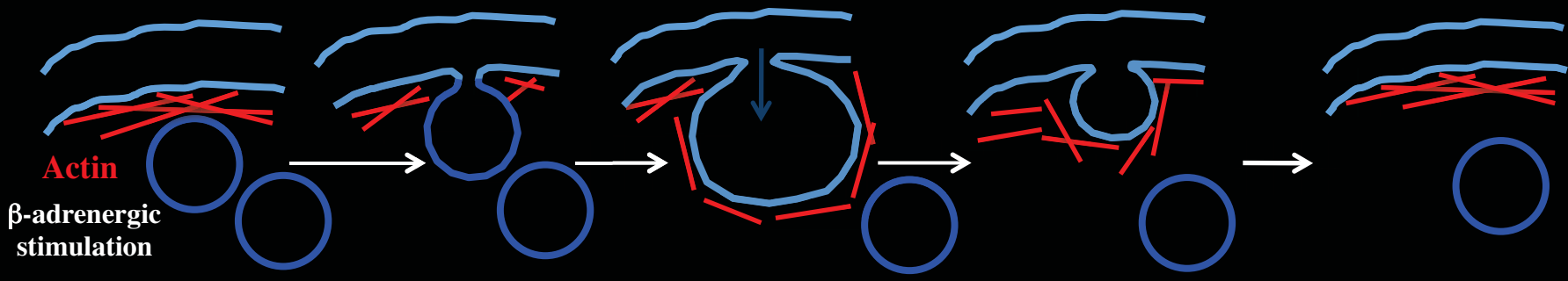
Andrius Masedunskas, Bob Adelstein, Marie Anne Conti

The impairment of the motor activity of myosin II affects the collapse of the SCGs



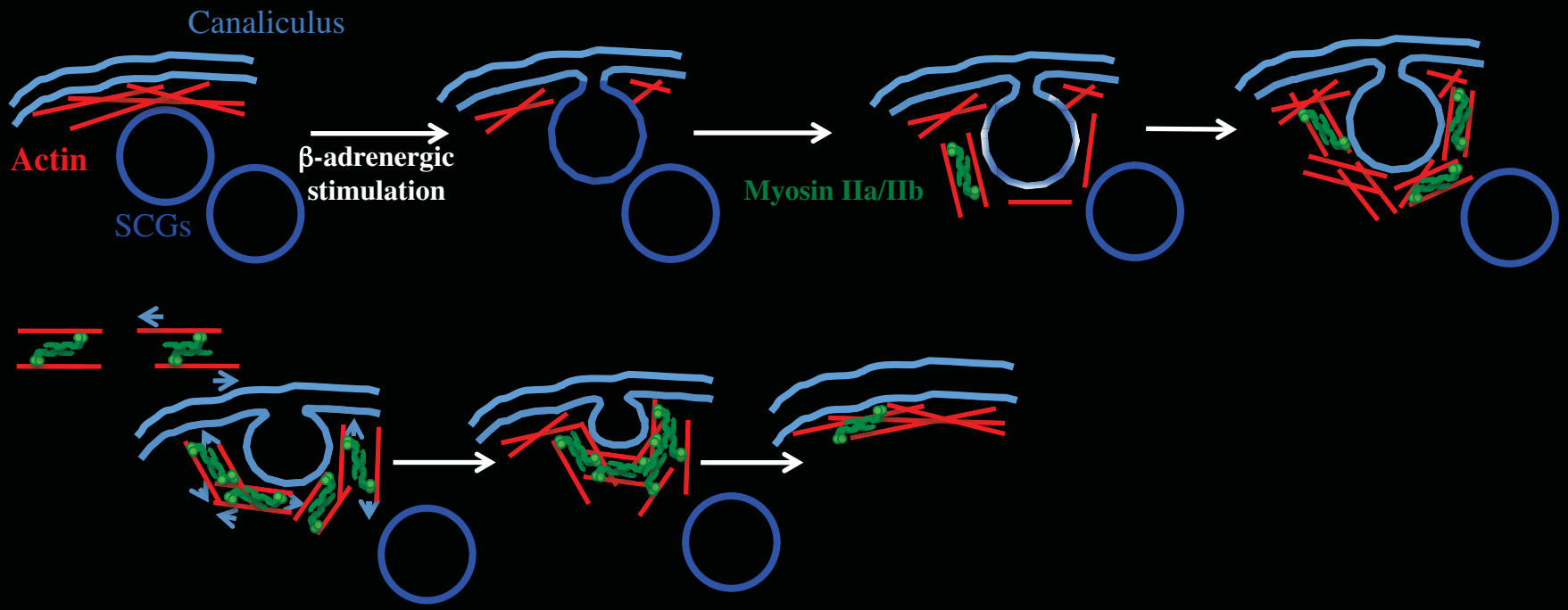


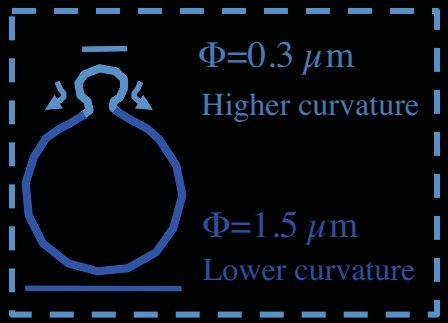


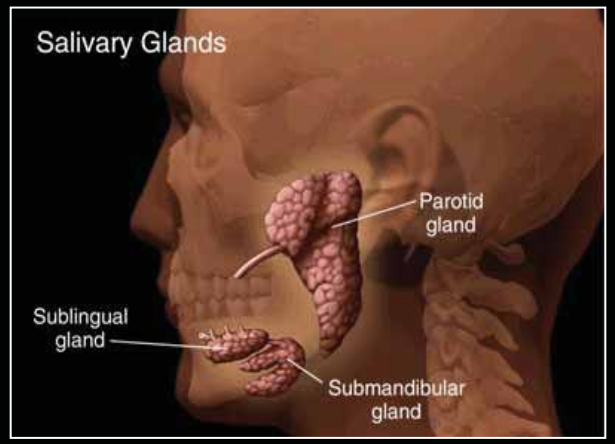


Masedunskas et al., submitted

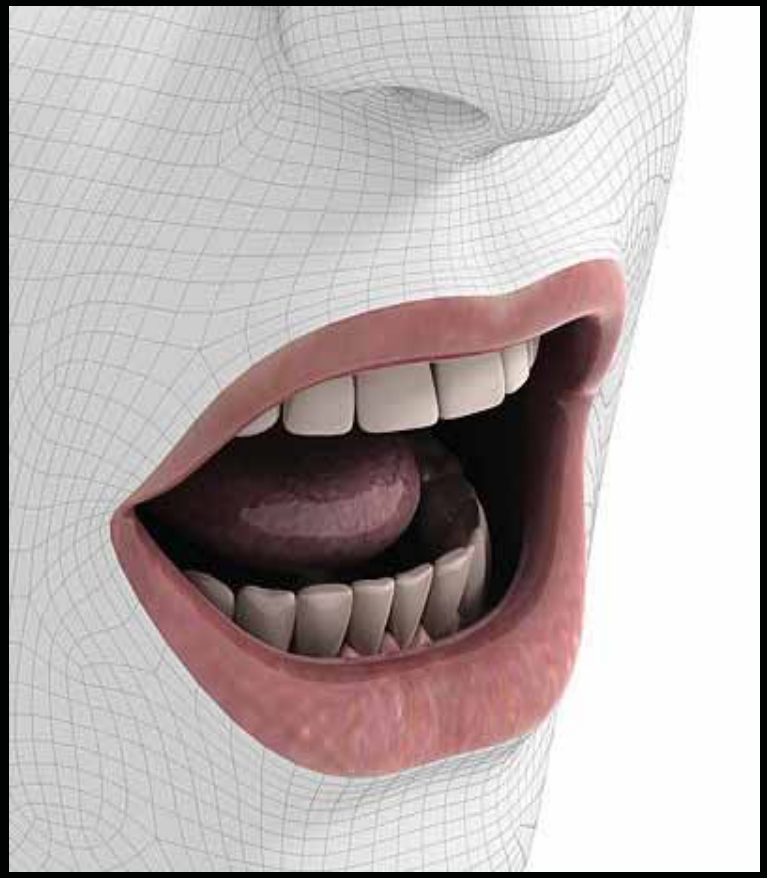
Model


Canaliculus
Actin
SCGs
β-adrenergic stimulation
Myosin IIa/IIb

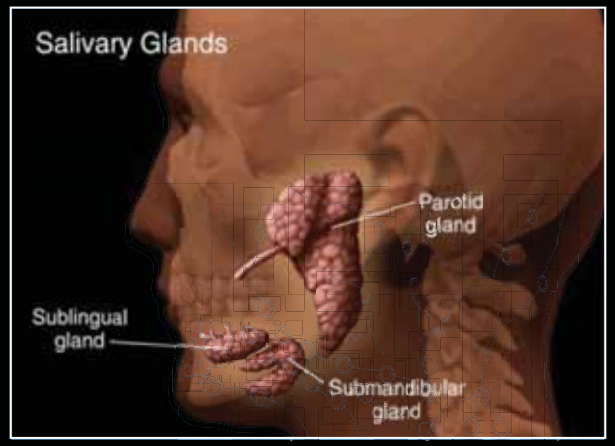

Φ=0.3 μm
Higher curvature
Φ=1.5 μm
Lower curvature


Salivary Glands
Parotid gland
Sublingual gland
Submandibular gland

Head and Neck Cancer

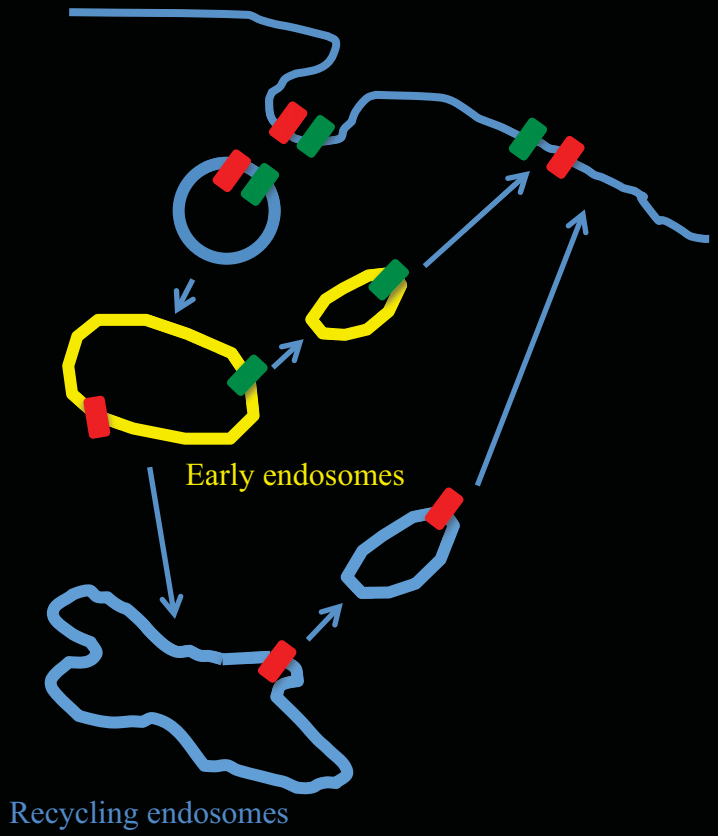


- Sixth most common cancer in the developed world (500,000 new cases; 250,000 deaths/year)
- 37,000 new cases of head and neck cancer/year (8,000 deaths/year) in U.S. (Cancer satistics, 2010)
- The incidence of oral cancer varies greatly worldwide
- 90-95% are squamous cell carcinoma
- 30-40% are originated from dorsal and lateral tongue
- Survival rate less than 50%



What is the role of membrane trafficking in invasion and metastasis?

Endosomal recycling



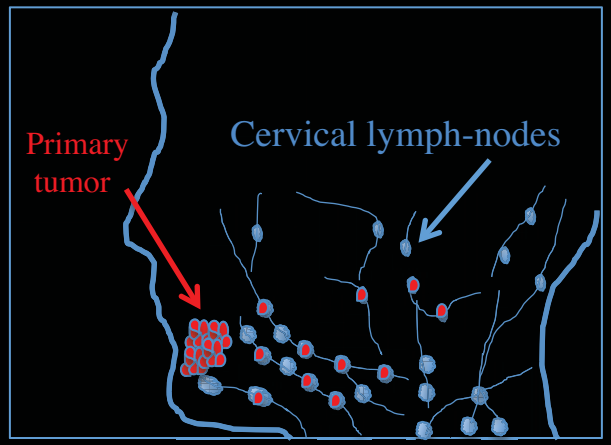
Cell motility ⟶ Invasion and metastasis

Directing molecules to specific locations of the PM
Adhesion
Signaling
Matrix degradation

Moving membranes in the direction of migration

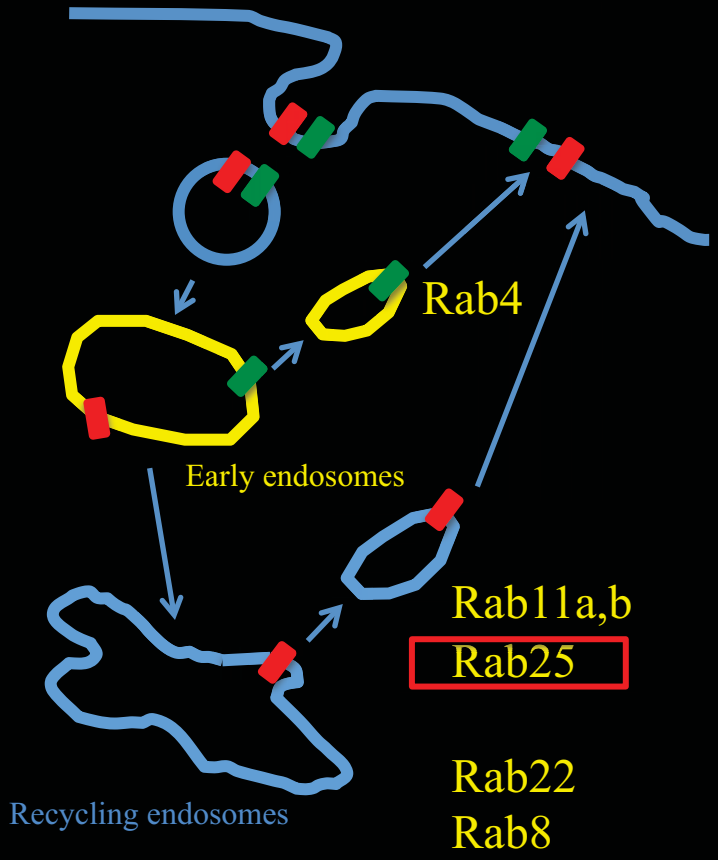
1) Which recycling pathway controls the invasion process ?

2) How is invasion regulated by endosomal recycling ?



What is the role of membrane trafficking in invasion and metastasis?

Endosomal recycling



Rab GTPase

Close homologue of Rab11a and Rab11b

Implicated in recycling in epithelial polarized cells

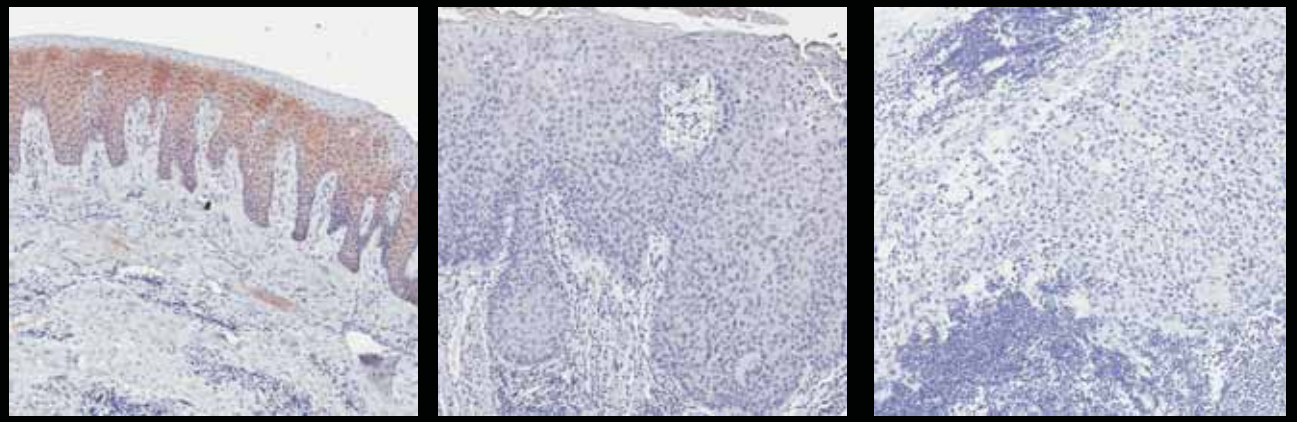
Interacts with integrin α5β1
(Caswell PT et al., 2007)

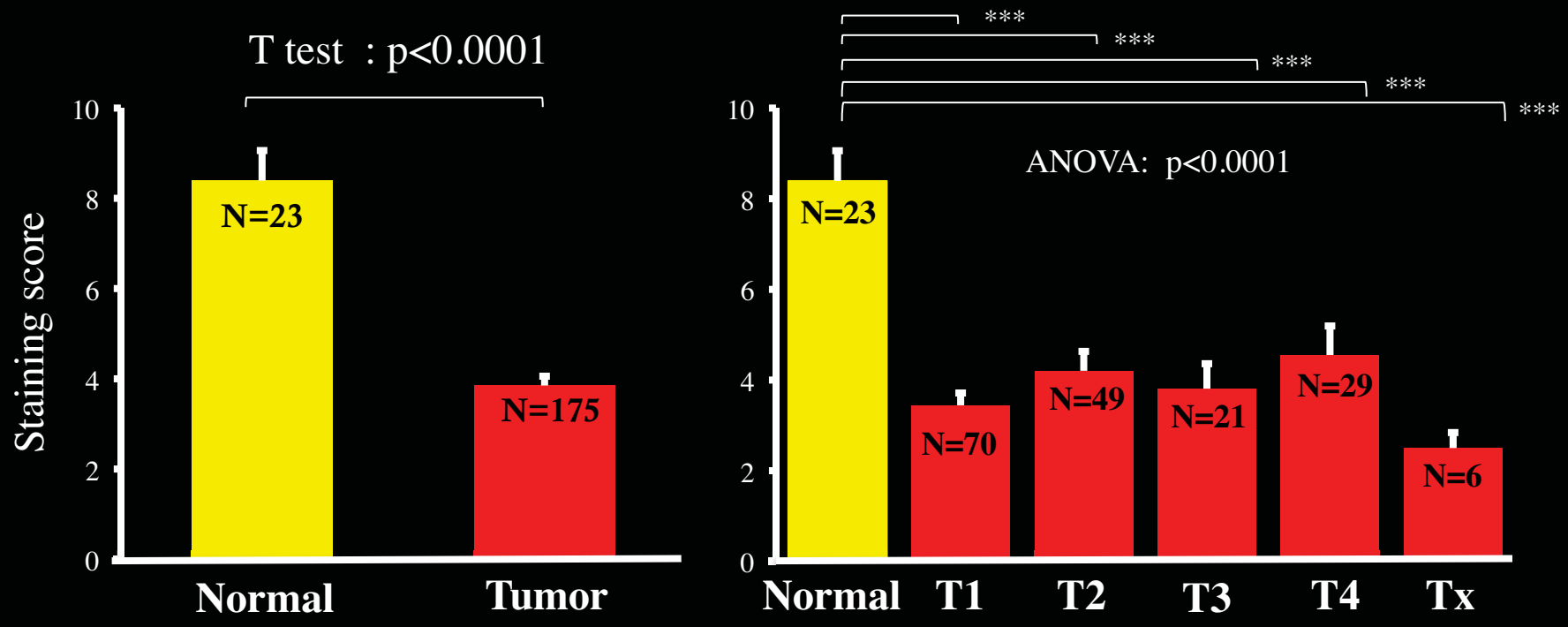
Overexpressed in breast and ovarian cancer
(Cheng JM et al., 2004)

Downregulated in breast cancer
(Cheng KW et al., 2006)

Downregulated in colon cancer
(Nam KT et al., 2010)

The small GTPase Rab25 is down regulated in human HNSCC tissues

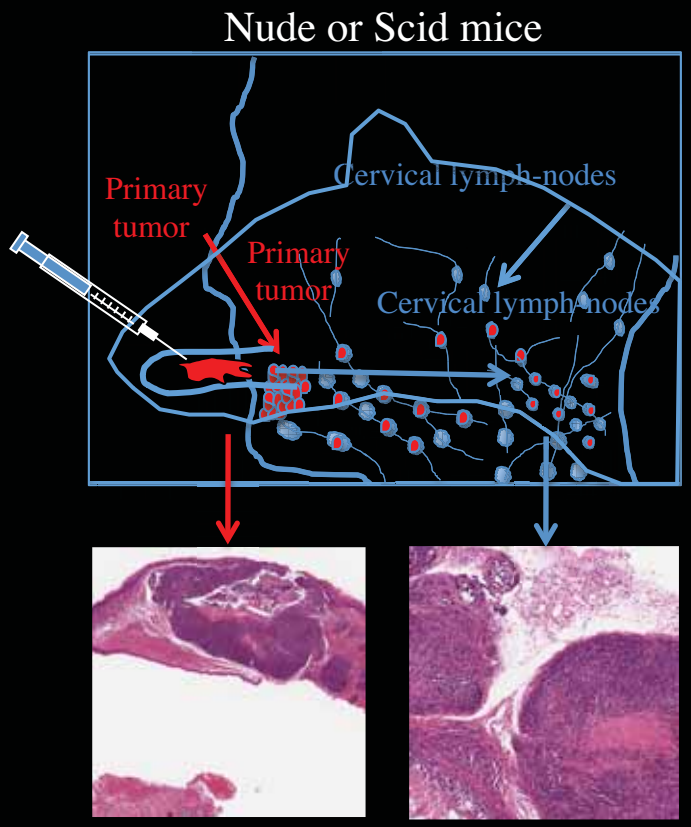


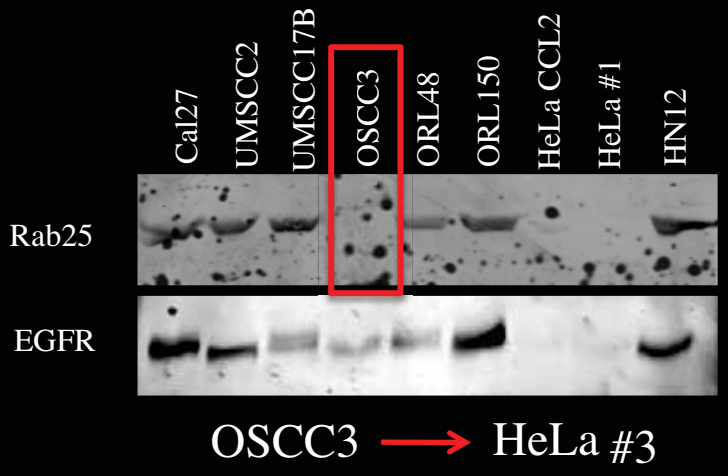


Amornphimoltham et al., man in prep

Experimental model

Xenograft in the mouse tongue



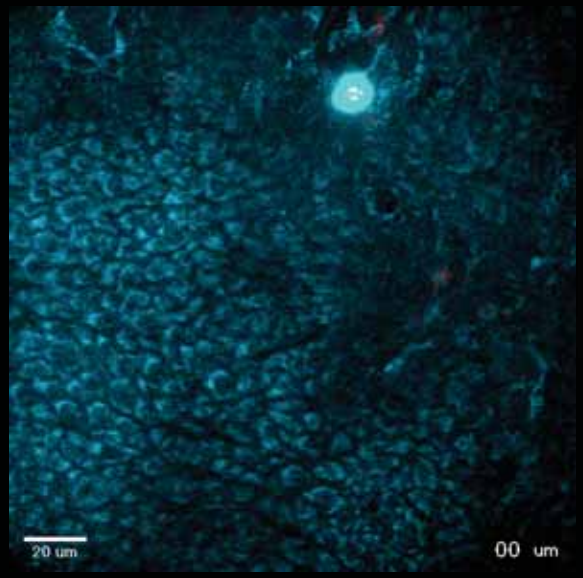


Patel et al., (2011) Cancer Research, Amornphimoltham et al., man in prep

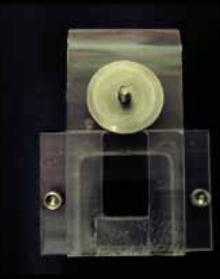
Experimental model

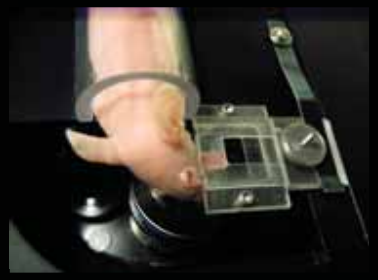
Xenograft in the mouse tongue

H2B Endog fluo Stromal cells



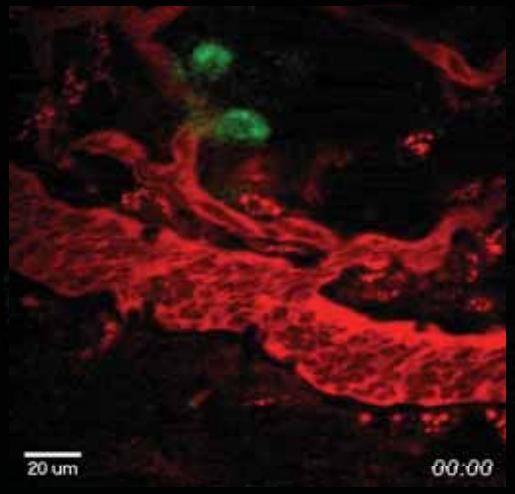
Excitation 740/930 nm

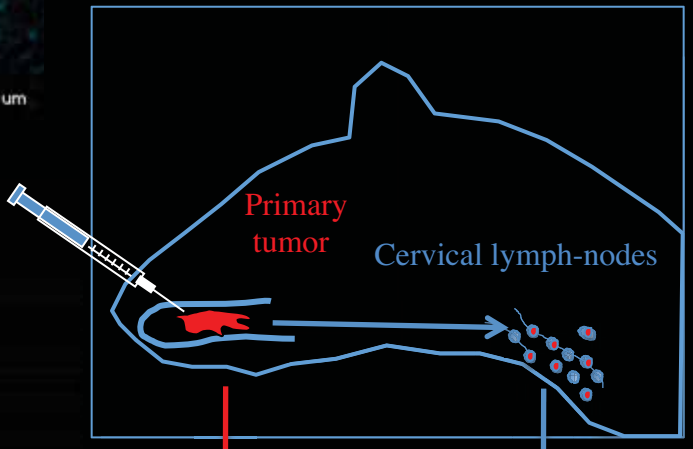


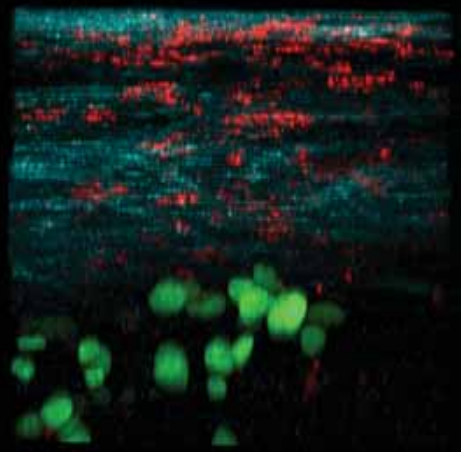


Nude or Scid mice

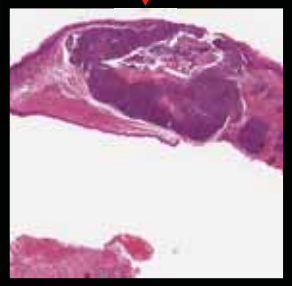
H2B H2B Collagen Lymphatic

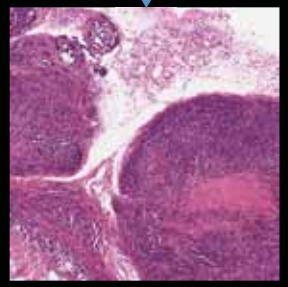






Excitation 930 nm

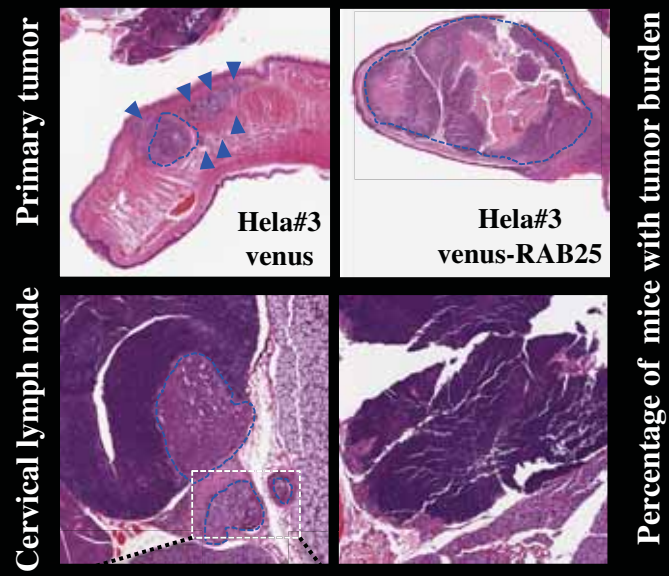


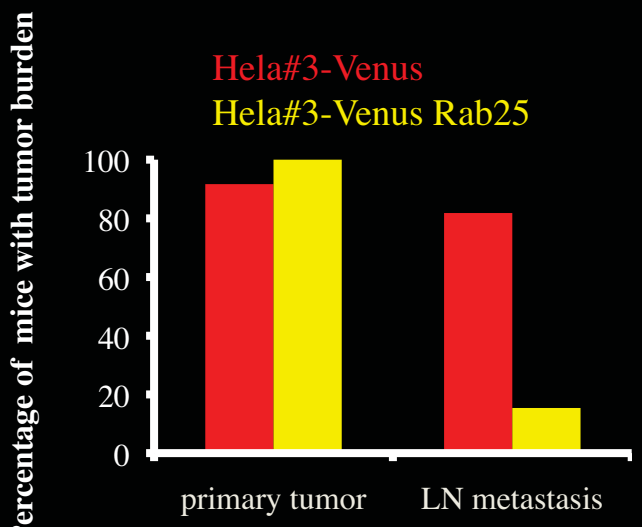


Excitation 930 nm

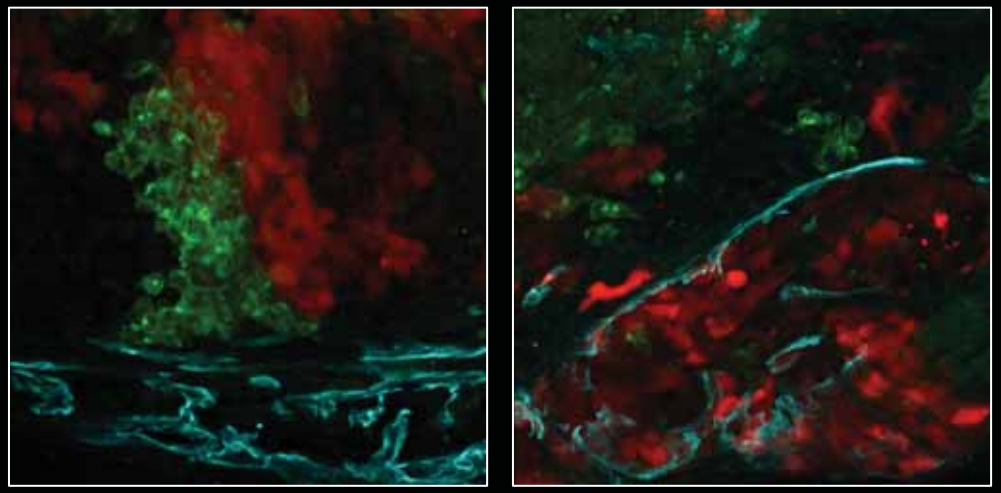
Patel et al., (2011) Cancer Research, Amornphimoltham et al., man in prep

Rab25 re-expression blocks invasion and metastasis *in vivo*





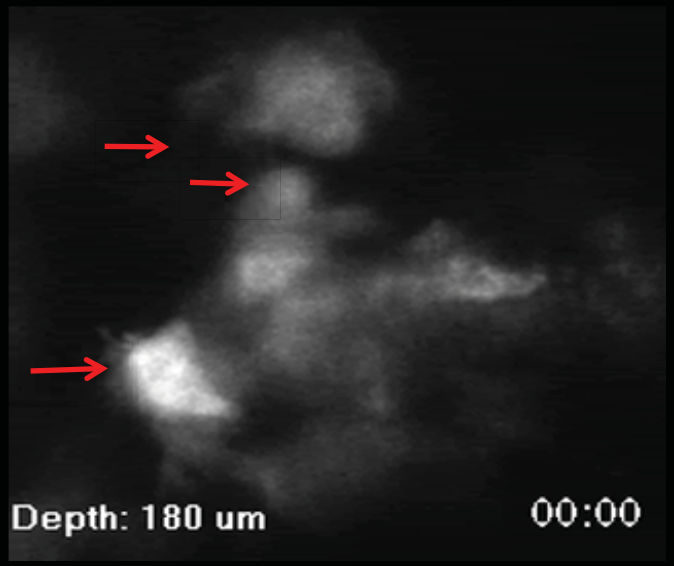
Hela#3-Venus Rab25/ Hela#3-mCherry/ Lyve1



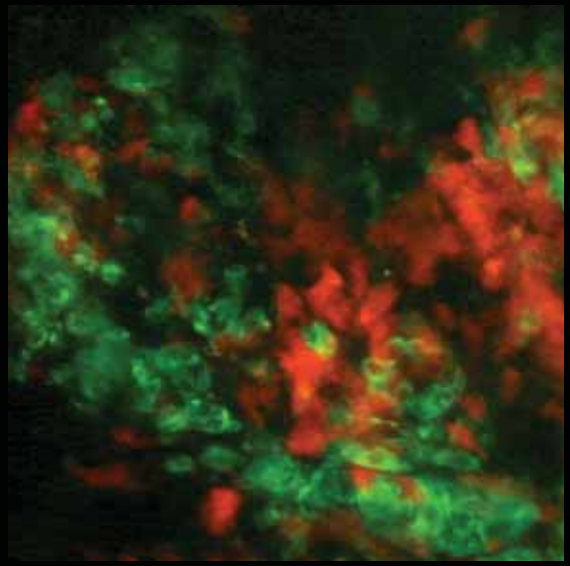
Amornphimoltham et al., (Man. in prep.)

Rab25 re-expression blocks invasion and metastasis *in vivo*

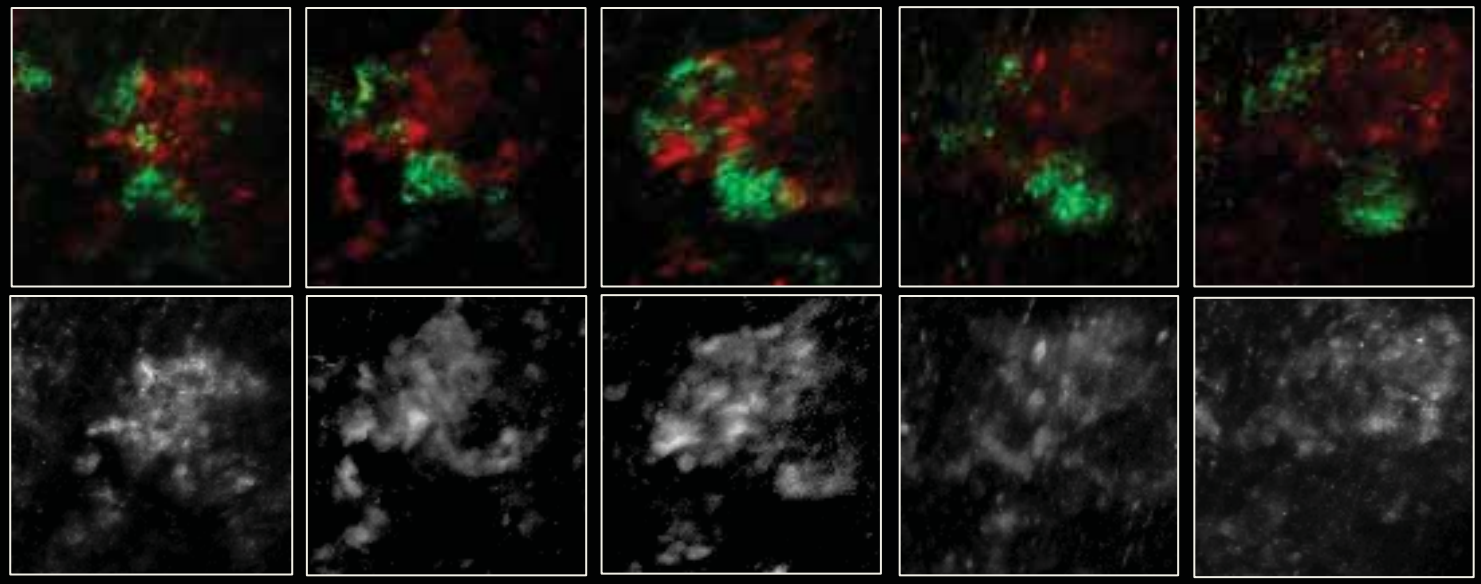
Hela#3-Venus



Hela#3-Venus Rab25/ Hela#3-mCherry

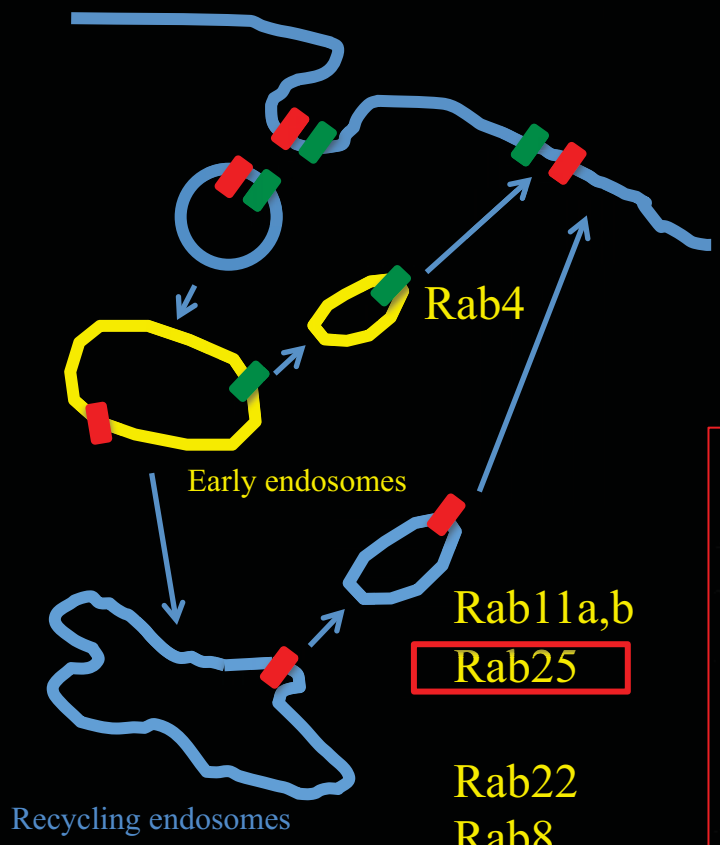


Day 23 24 25 26 27



Amornphimoltham et al., (Man. in prep.)

Rab25 plays an important role in preventing invasion and metastasis

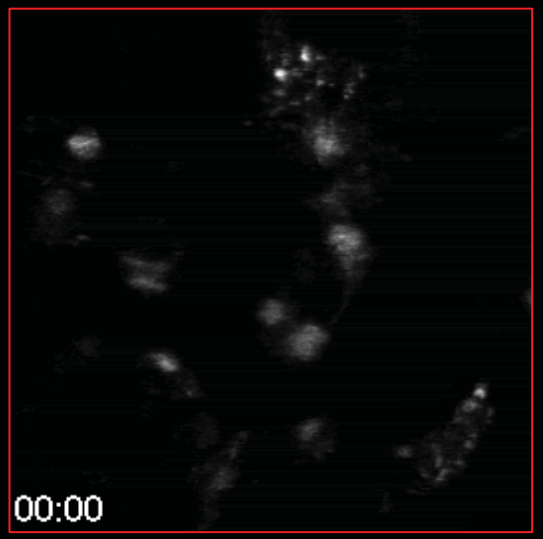


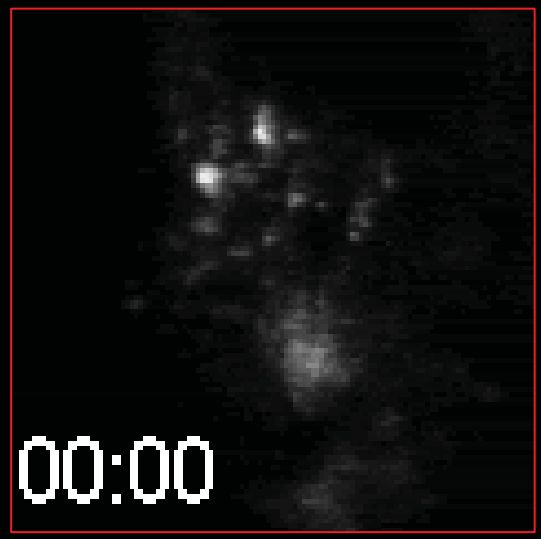
What is the mechanism?

1) ~~Pro apoptotic~~
2) ~~Prevent angiogenesis~~
3) ~~Regulate cell cycle~~
4) Cell motility
5) Adhesion

Integrin trafficking?

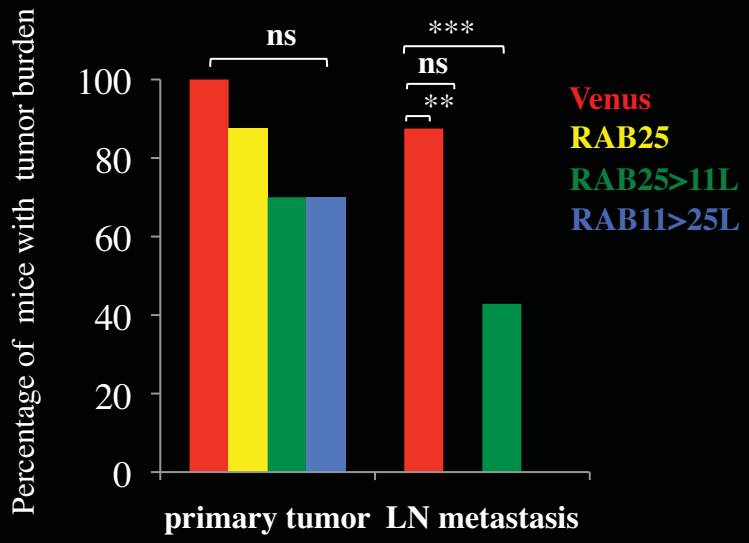
Venus Rab25





The C-terminus of Rab25 is necessary and sufficient to block cell invasion *in vitro*

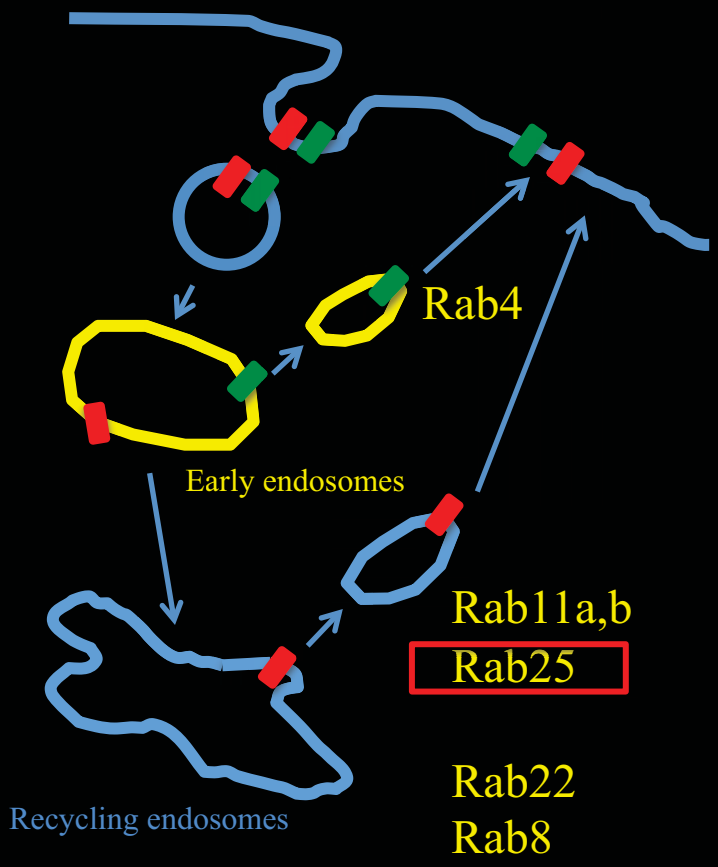
Caswell PT et al., 2007



No effect of Rab25 on integrin localization or trafficking

Amornphimoltham et al., (Man. in prep.)

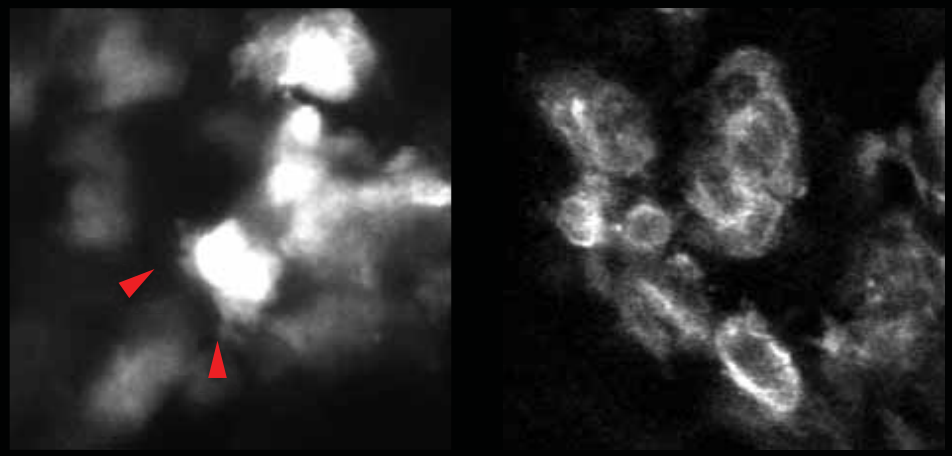
Rab25 plays an important role in preventing invasion and metastasis



What is the mechanism?

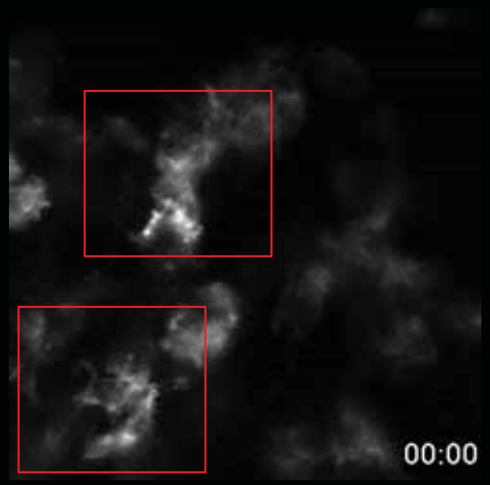
1) ~~Pro apoptotic~~
2) ~~Prevent angiogenesis~~
3) ~~Regulate cell cycle~~
4) Cell motility
5) Adhesion

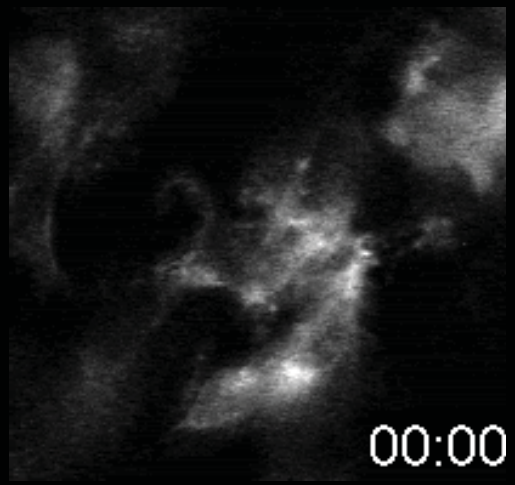
Cytoskeleton

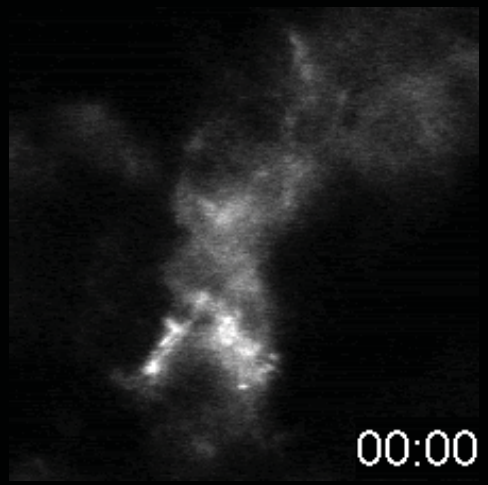


Rab25 re-expression reduces actin-rich structures at the PM

Hela#3-GFP-lifeact



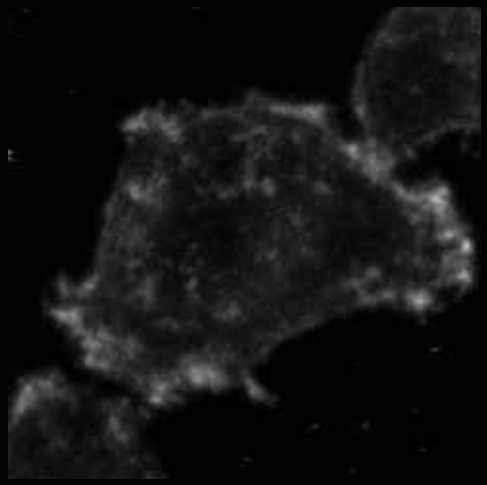




Cells migrating in 3D

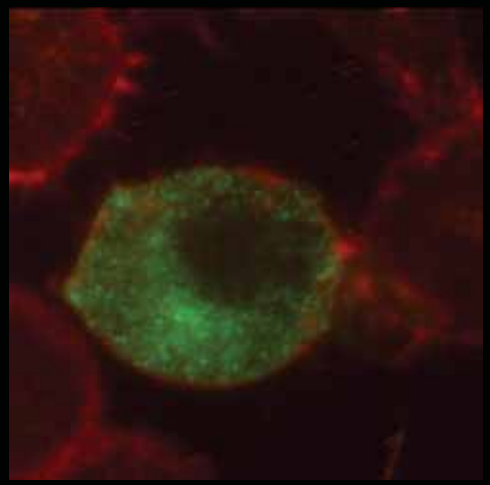
Phalloidin

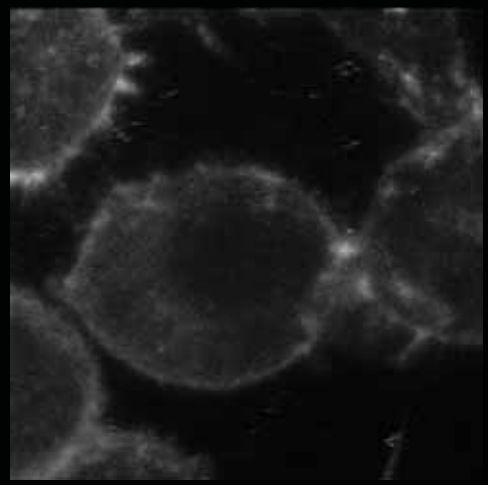
Hela#3-Venus



Venus Rab25/ Phalloidin

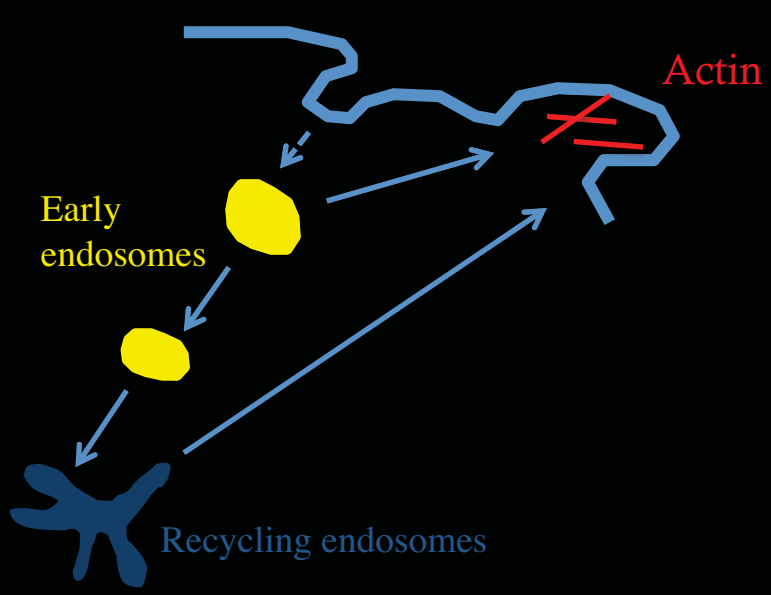
Hela#3-Venus Rab25

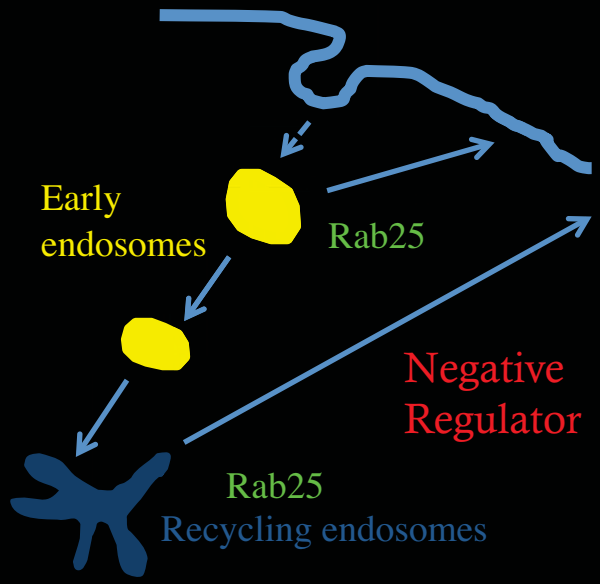




Amornphimoltham et al., (Man. in prep.)

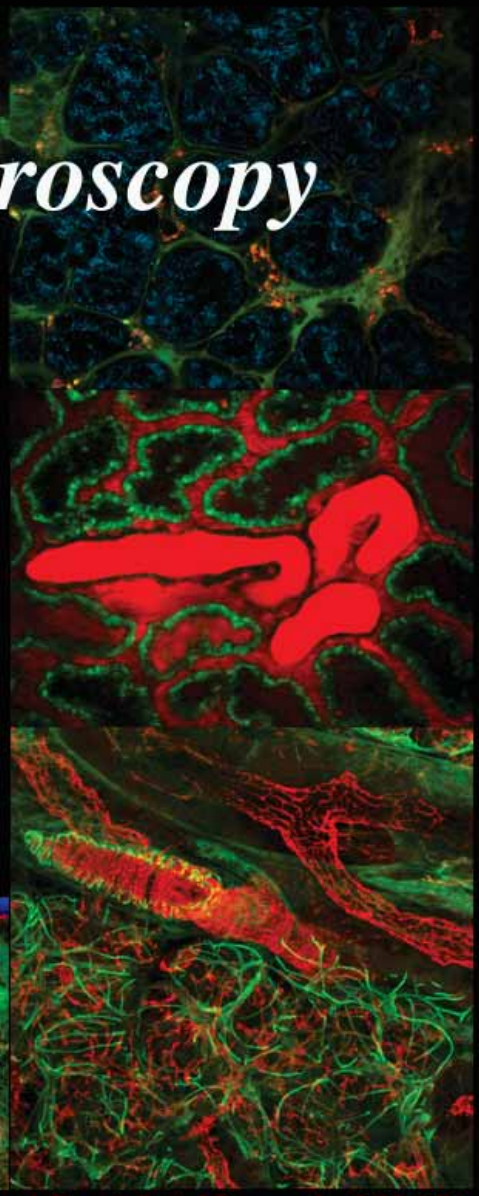
Rab25 re-expression reduces actin-rich structures at the PM





MAY 18-19, 2011

Frontiers in Intravital Microscopy

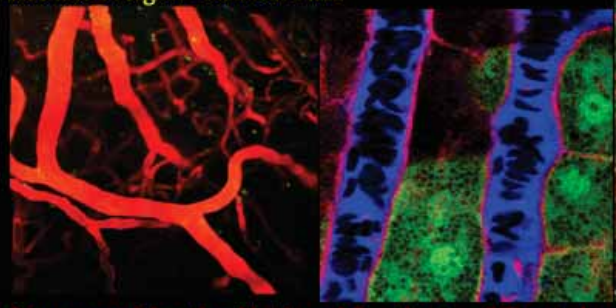


Symposium on Intravital Microscopy

May 18-19, 2011
Natcher building (Main auditorium)
National Institute of Health
Bethesda, MD

Speakers

Robert S. Balaban - NHLBI-NIH
Micheal D. Cahalan - University of California, Irvine
John J. Condeelis - Einstein College of Medicine
Kenneth H. Dunn - Indiana University
Peter Friedl - NCMLS - Nejmegen (NL)
Ron Germain - NIAID-NIH
Bradley T. Hyman - Mass General Hospital
Rakesh K. Jain - Mass General Hospital
Michael J. Levene - Yale School of engineering
John J. Lemasters - University South Carolina
Xinde Li - Johns Hopkins University
Marshall H. Montrose - University of Cincinnati
Mark Schnitzer - Stanford University
Ulrich H. Von Andrian - Harvard Medical School
Roberto Weigert - NIDCR-NIH



Sponsored by the National Institute of Dental and Craniofacial Research (NIDCR)

For more information please contact
Roberto Weigert IMTU/OPCB/NIDCR
(weigertr@mail.nih.gov)

Event will be videocast LIVE at http://videocast.nih.gov/