
The compound Sortin2 mode of action is unique disrupting *Arabidopsis thaliana* endomembrane system

V Congreso Biología Vegetal



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MSc (c) Biological Science
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Intracellular traffic and the Endomembrane System is required in different physiological roles in plants

Stress response

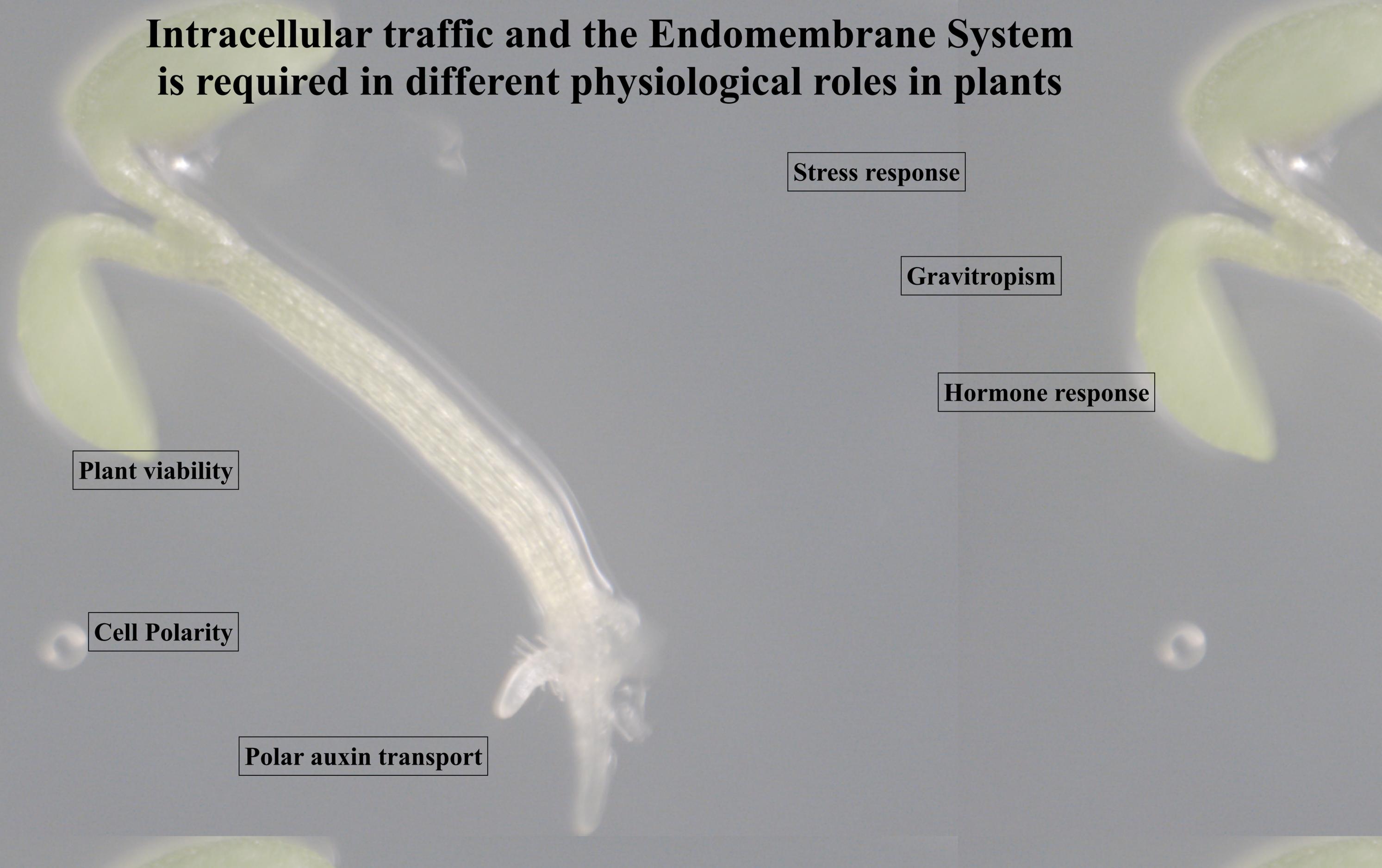
Gravitropism

Hormone response

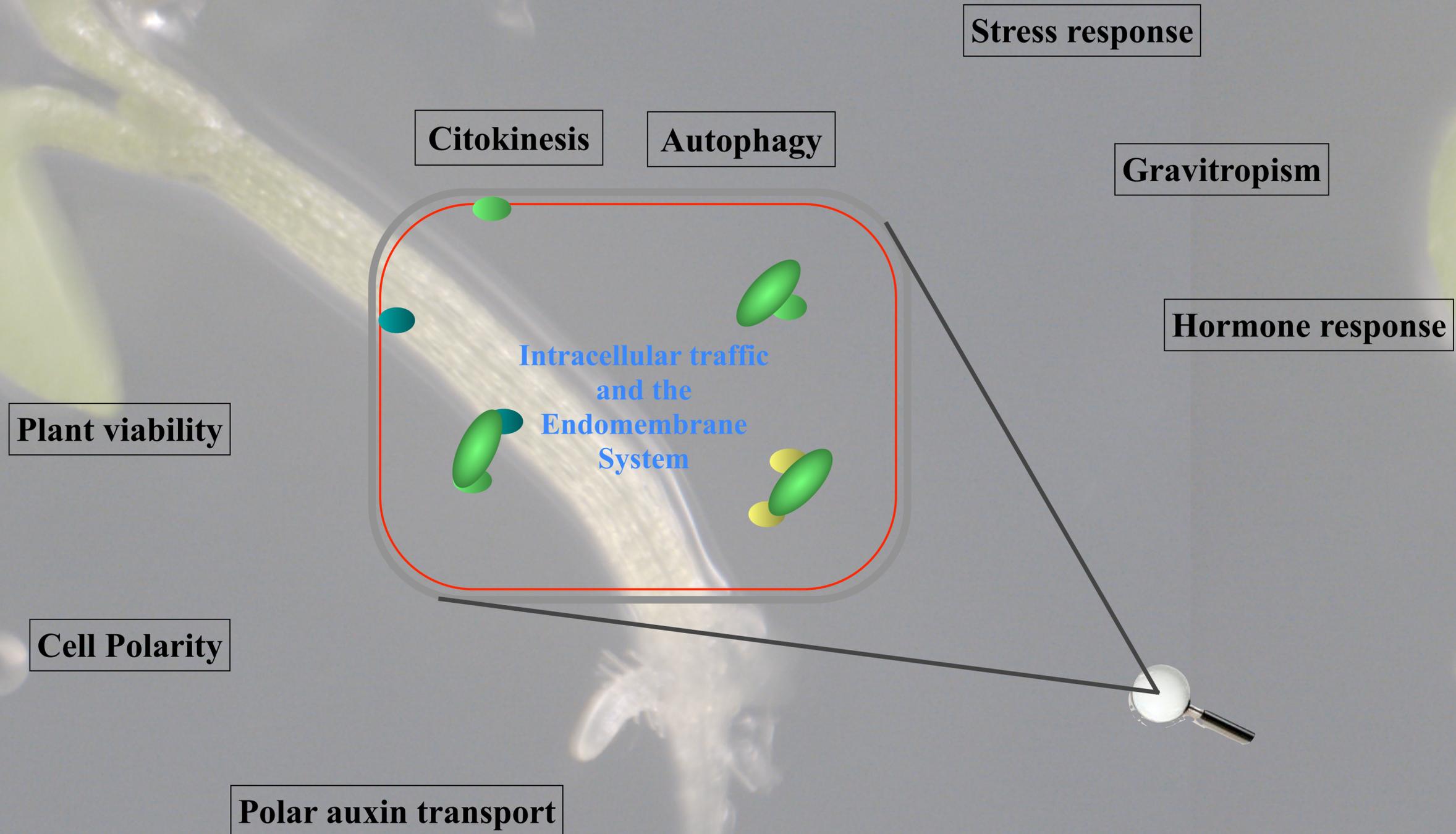
Plant viability

Cell Polarity

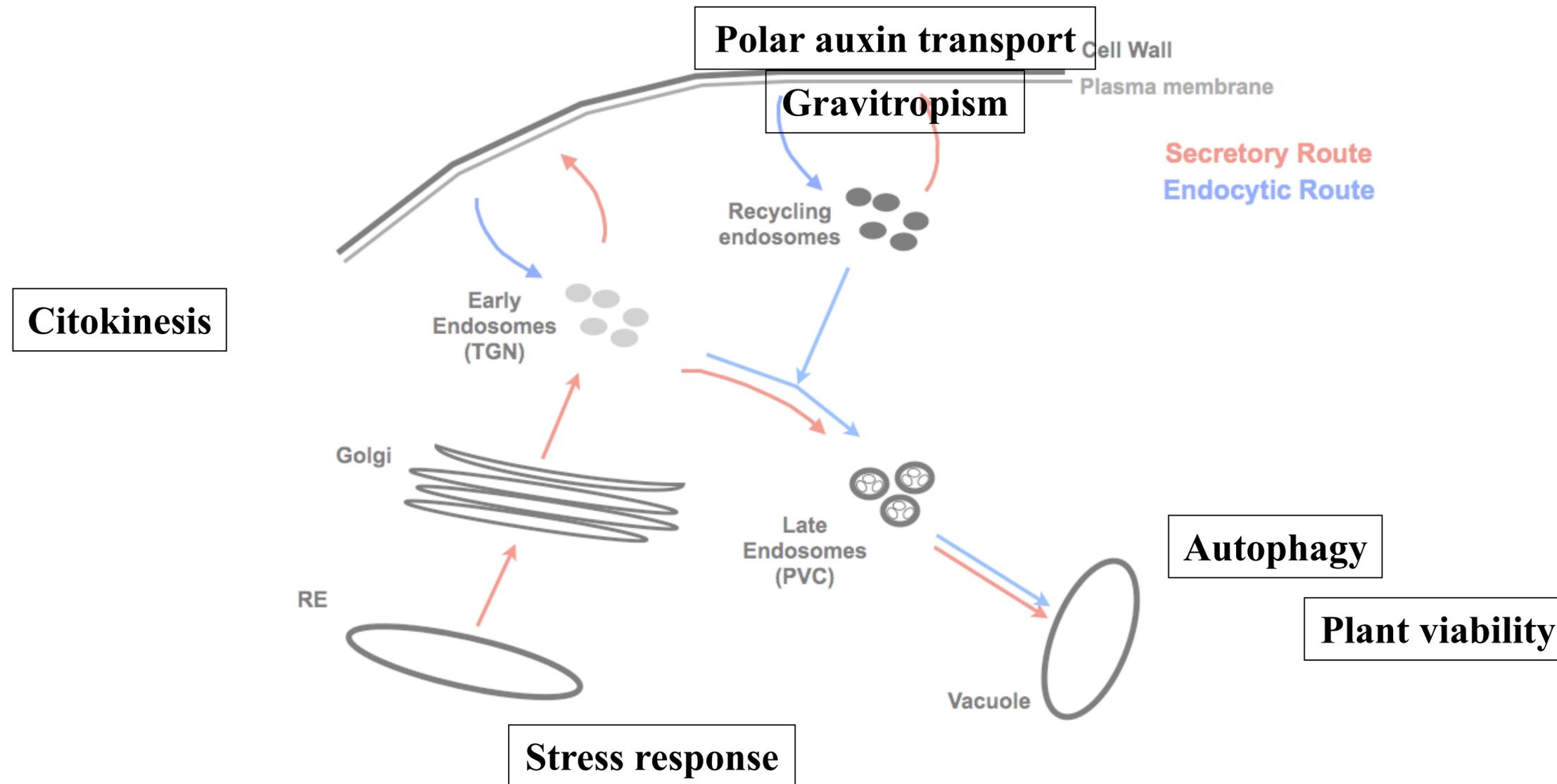
Polar auxin transport



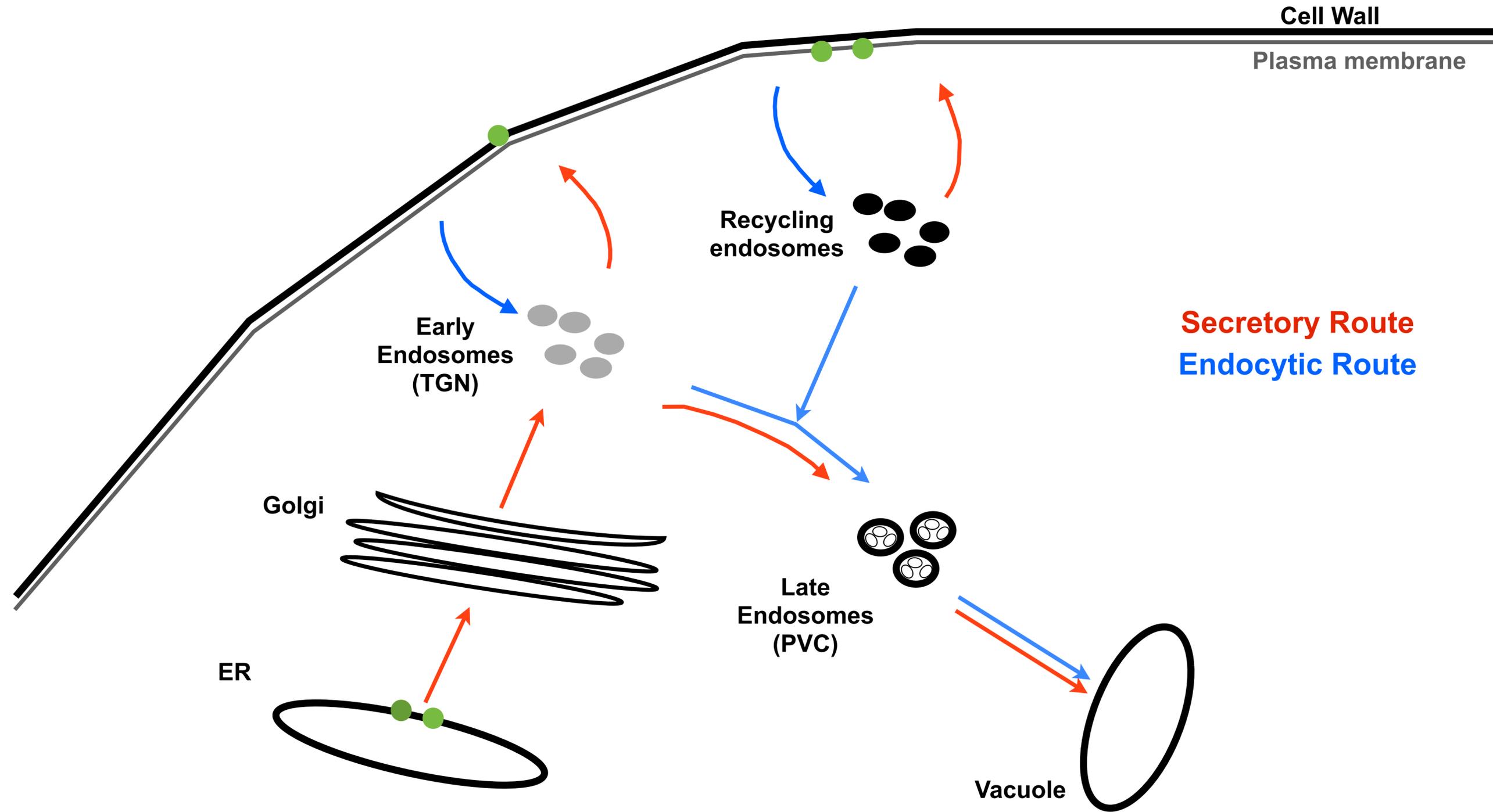
Intracellular traffic and the Endomembrane System is required in different physiological roles in plants



Intracellular traffic and the Endomembrane System is required in different physiological roles in plants



Endomembrane system; essential for eukaryotic functionality



Schematic representation: Some cellular components have been omitted for better understanding

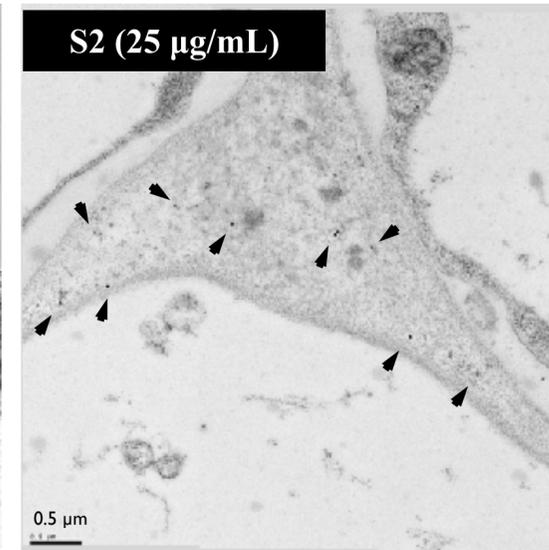
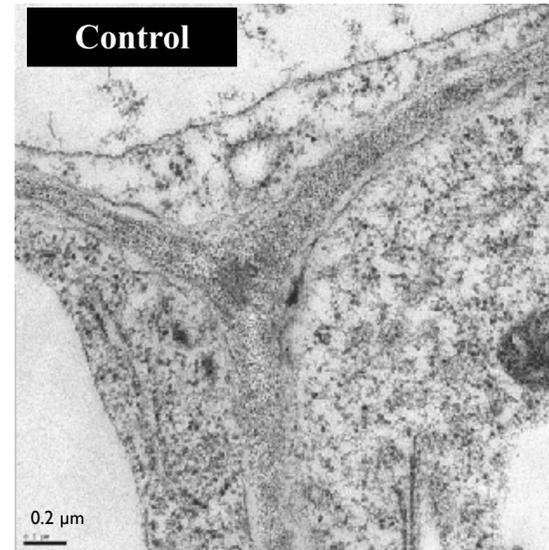
Sortin2 impairs Arabidopsis plant endomembrane system trafficking

Cellular level

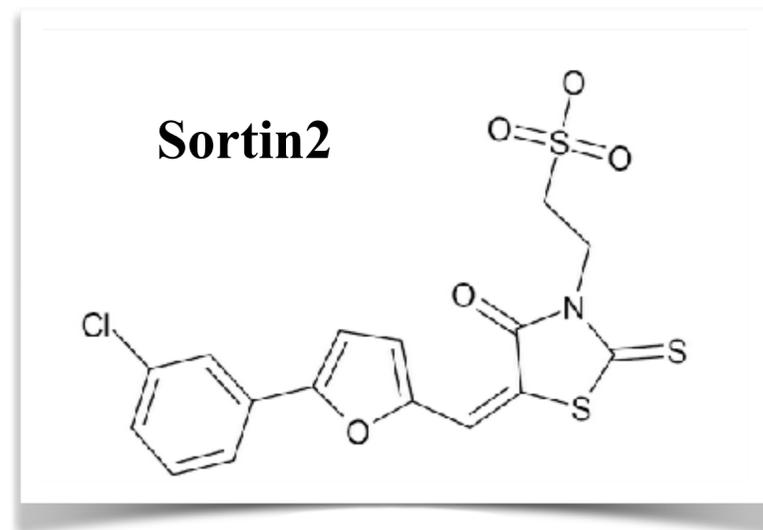


Vacuolar trafficking disruption

Inmunogold assay of AtCPY
in hypocotyls cells



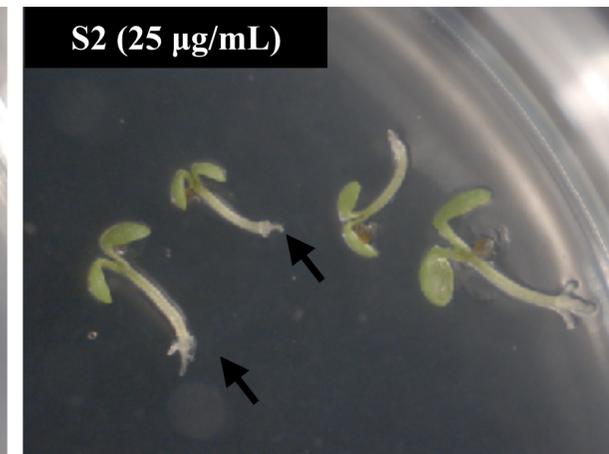
Norambuena & Raikhel, unpublished results



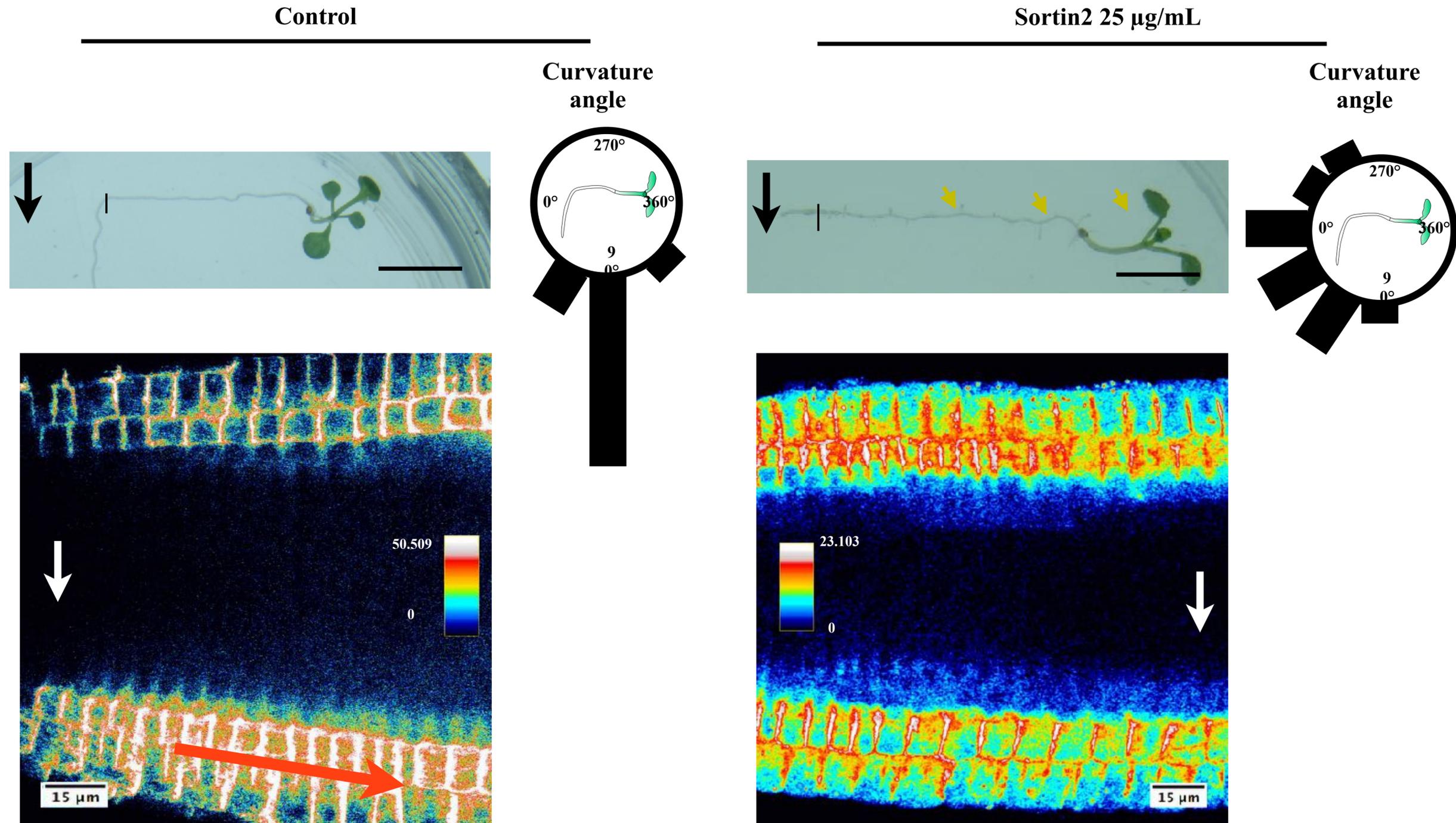
Plant level

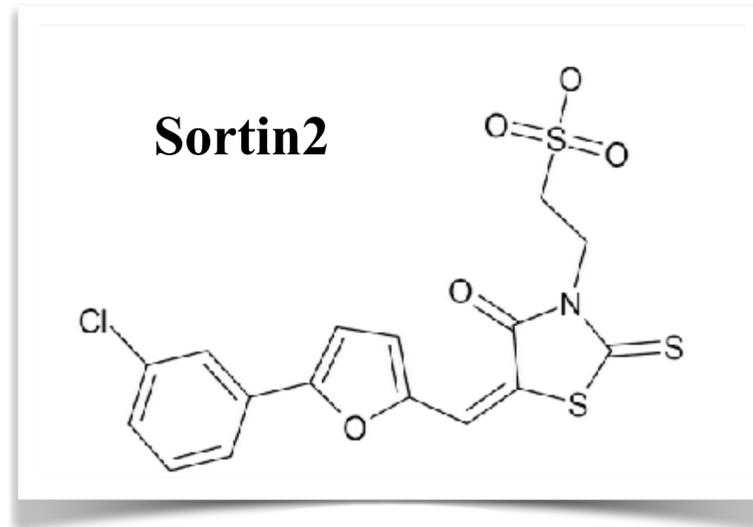


Auxin related phenotypes

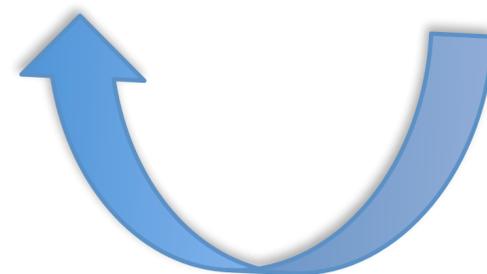
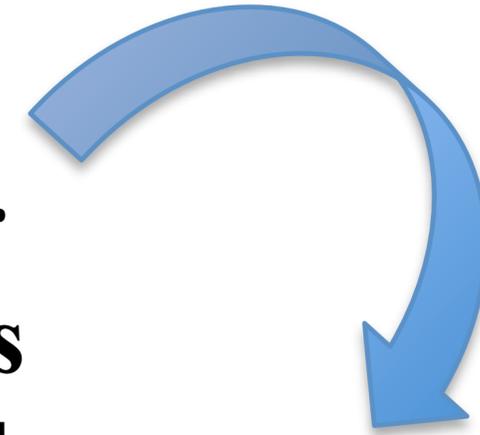
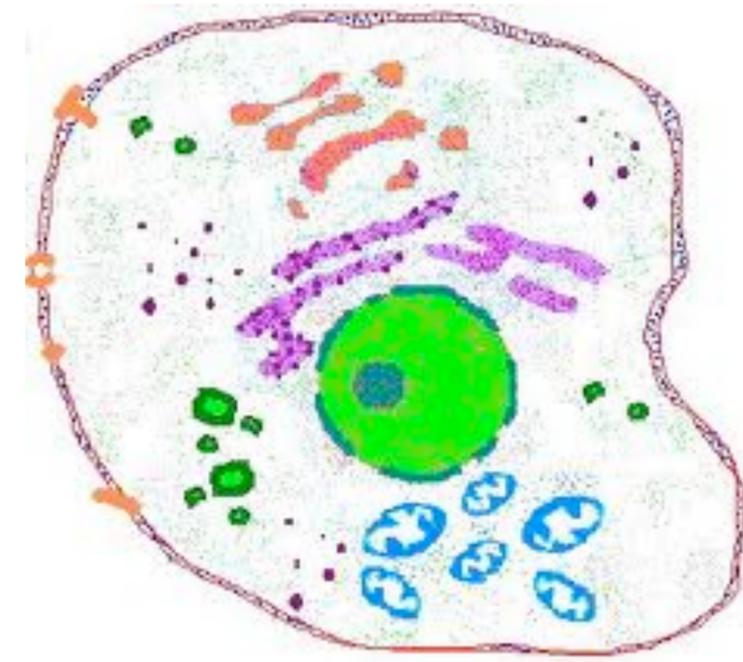
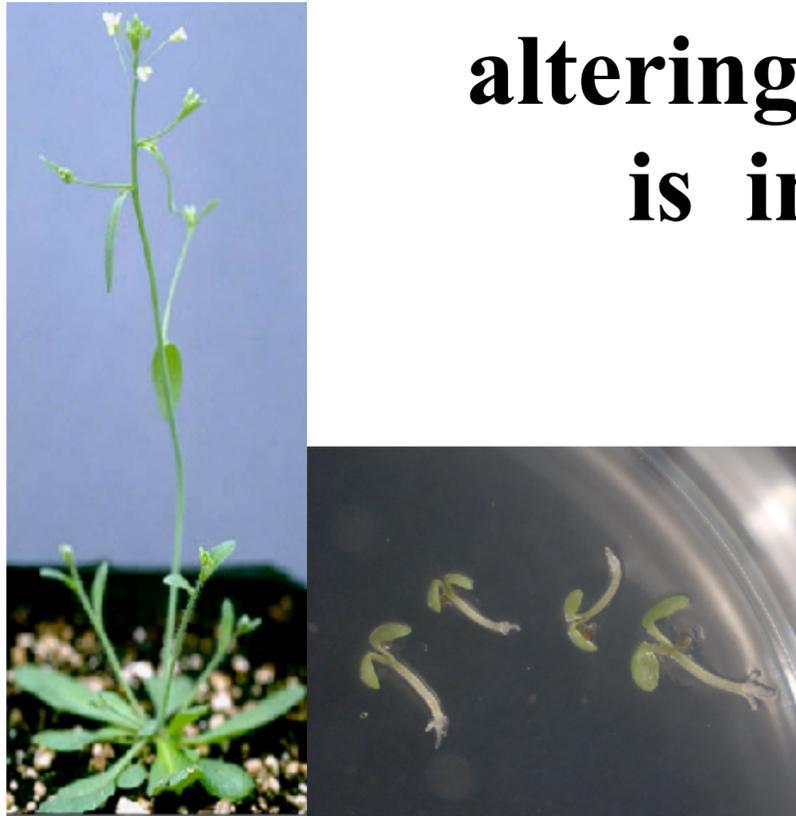


Sortin2 affects gravitropism by altering an auxin transporter differential degradation



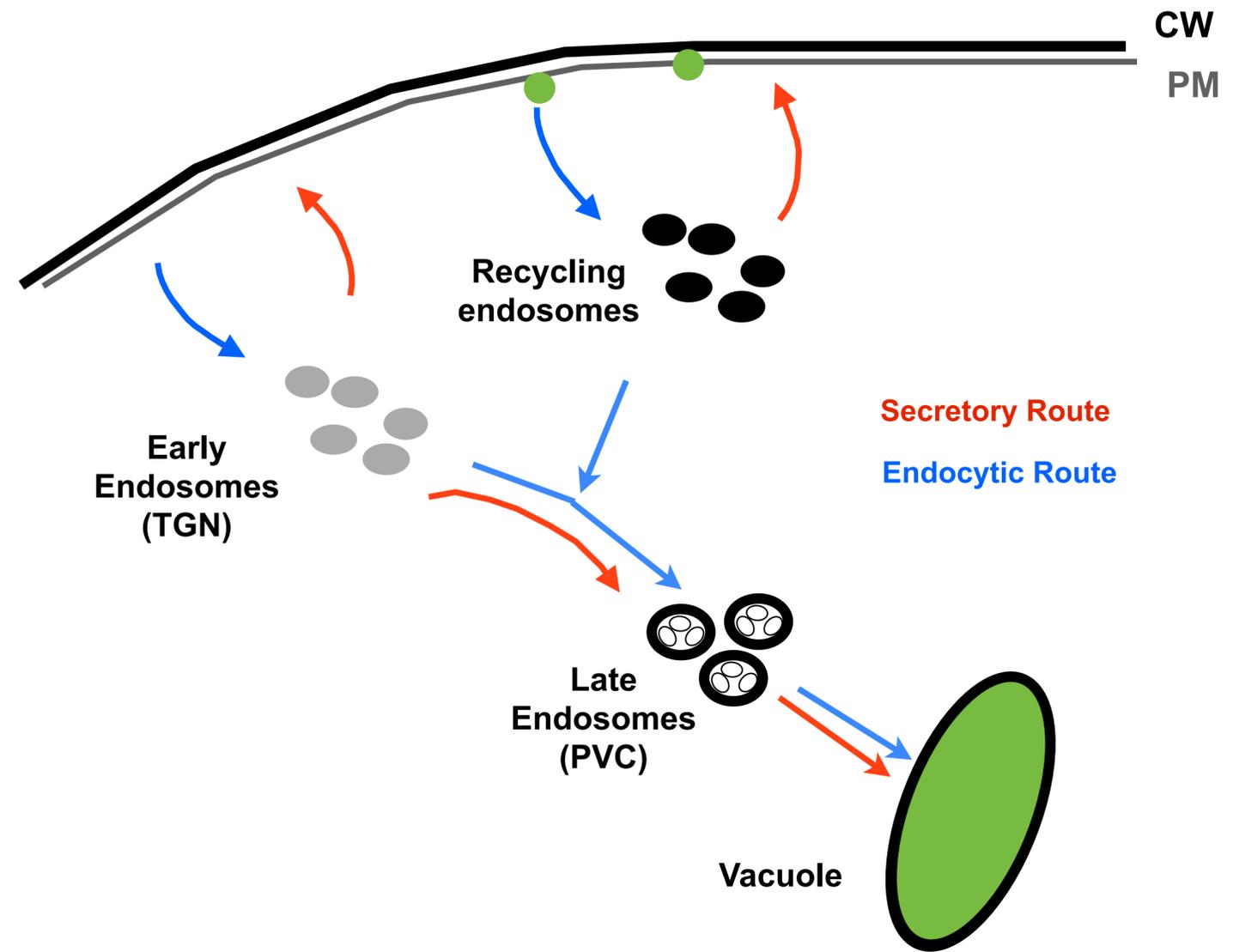
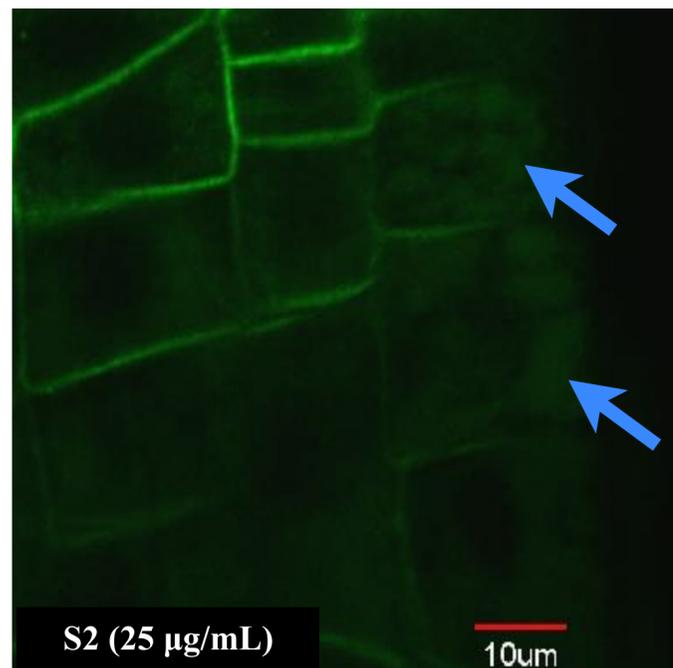
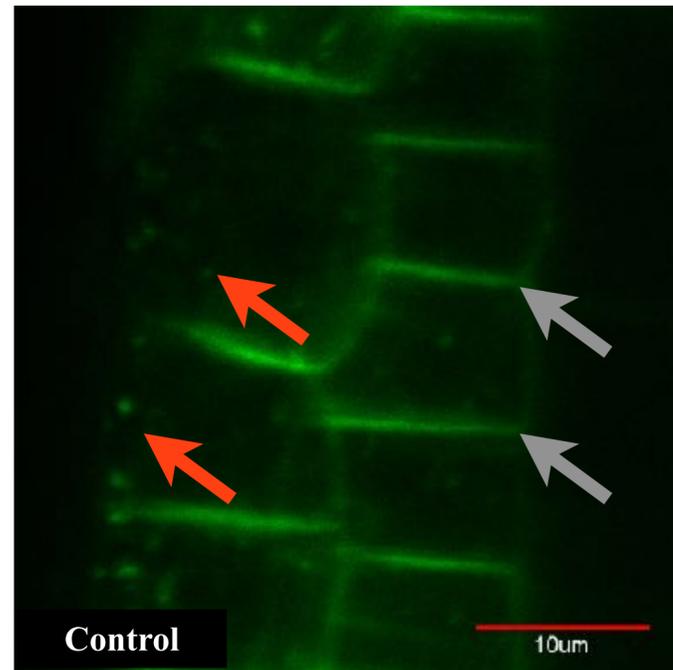


What is the intracellular trafficking that Sortin2 is altering that would explain how it is inhibiting differential degradation?



Sortin2 affects gravitropism by altering PIN2 cycling

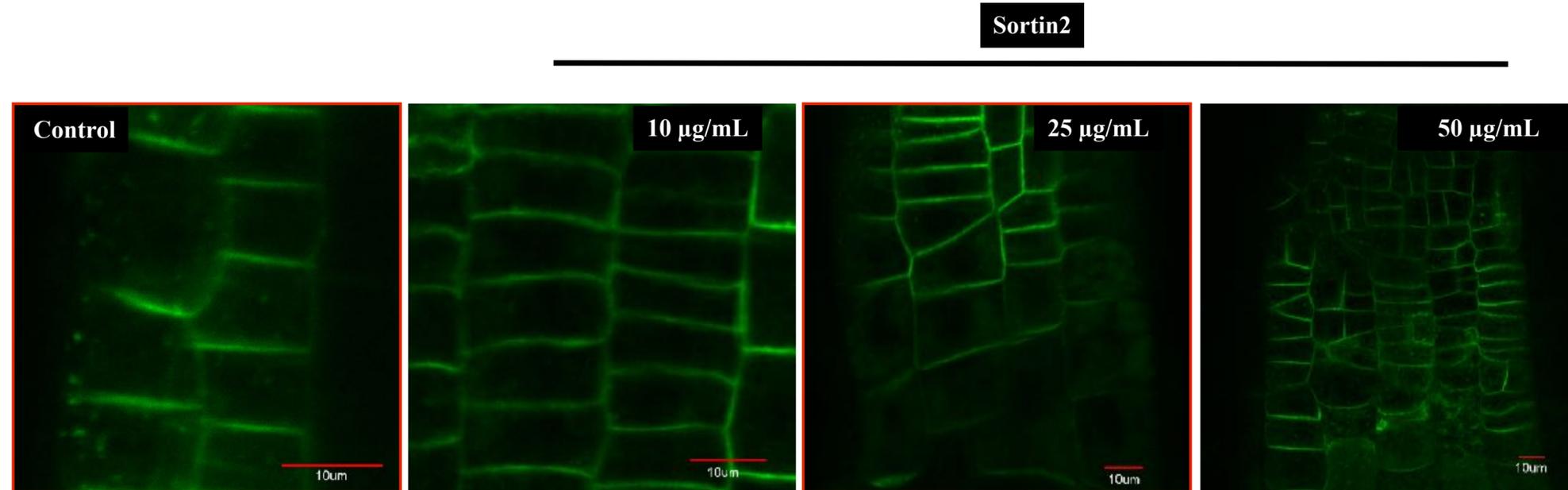
7 day old PIN2:GFP Seedlings grown in the presence of Sortin2



Sortin2 effect is dose-dependent and inducible

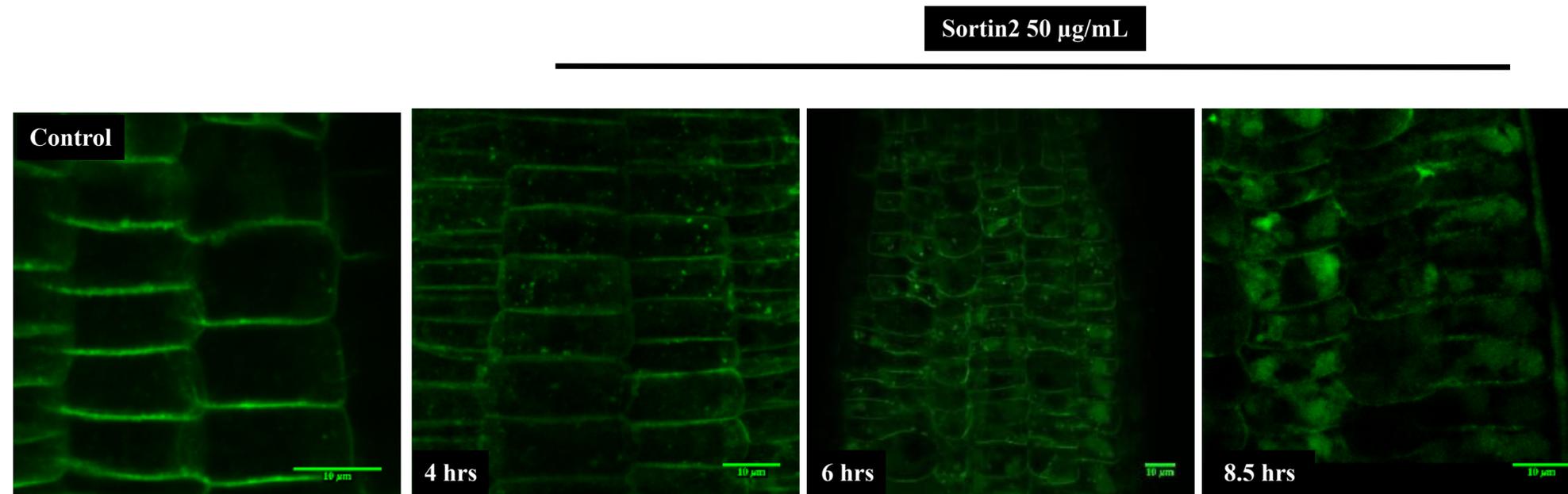
7 day old PIN2:GFP Seedlings grown in the presence of Sortin2

Sortin2
effect is
dose-
dependent

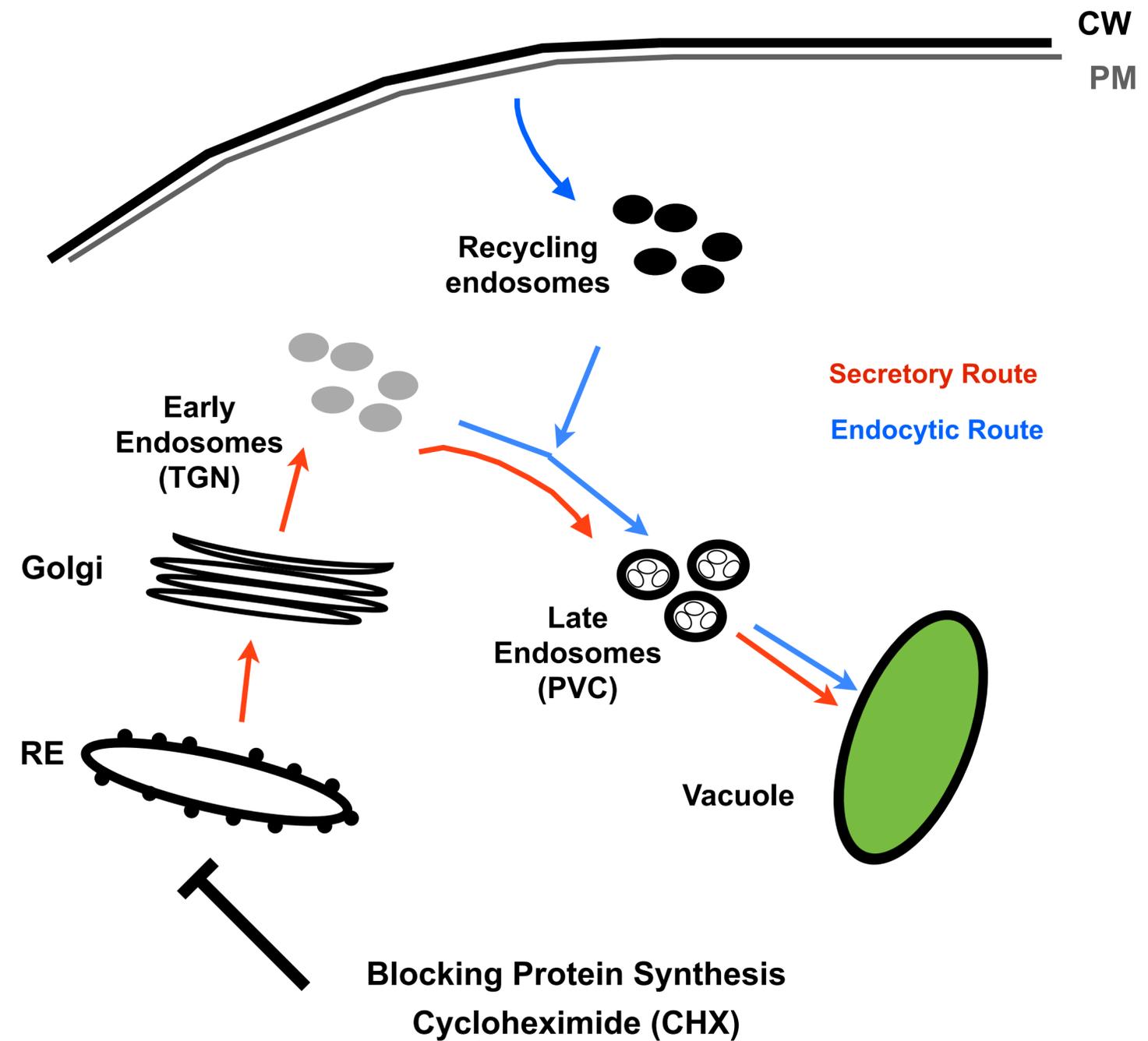
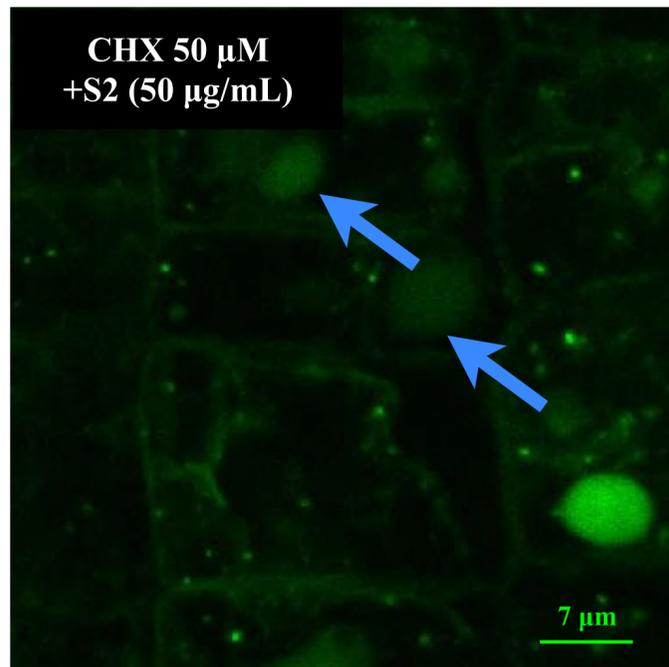
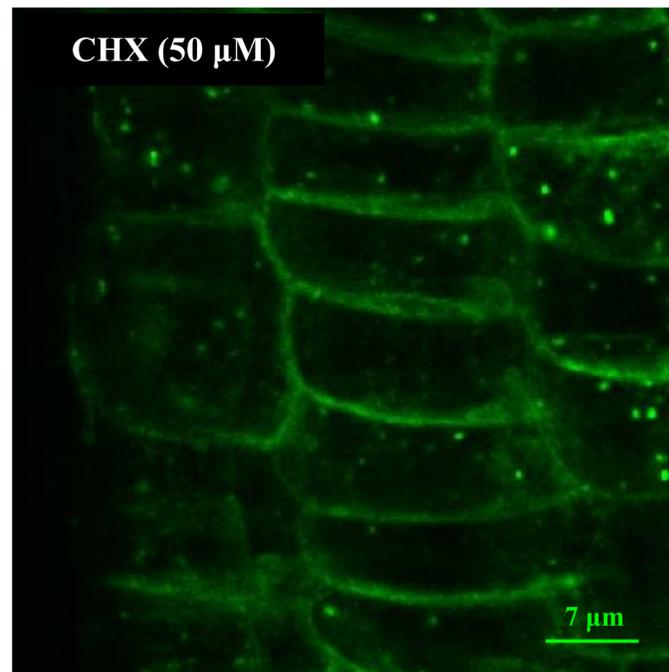


7 day old PIN2:GFP seedlings with hours Sortin2 treatment

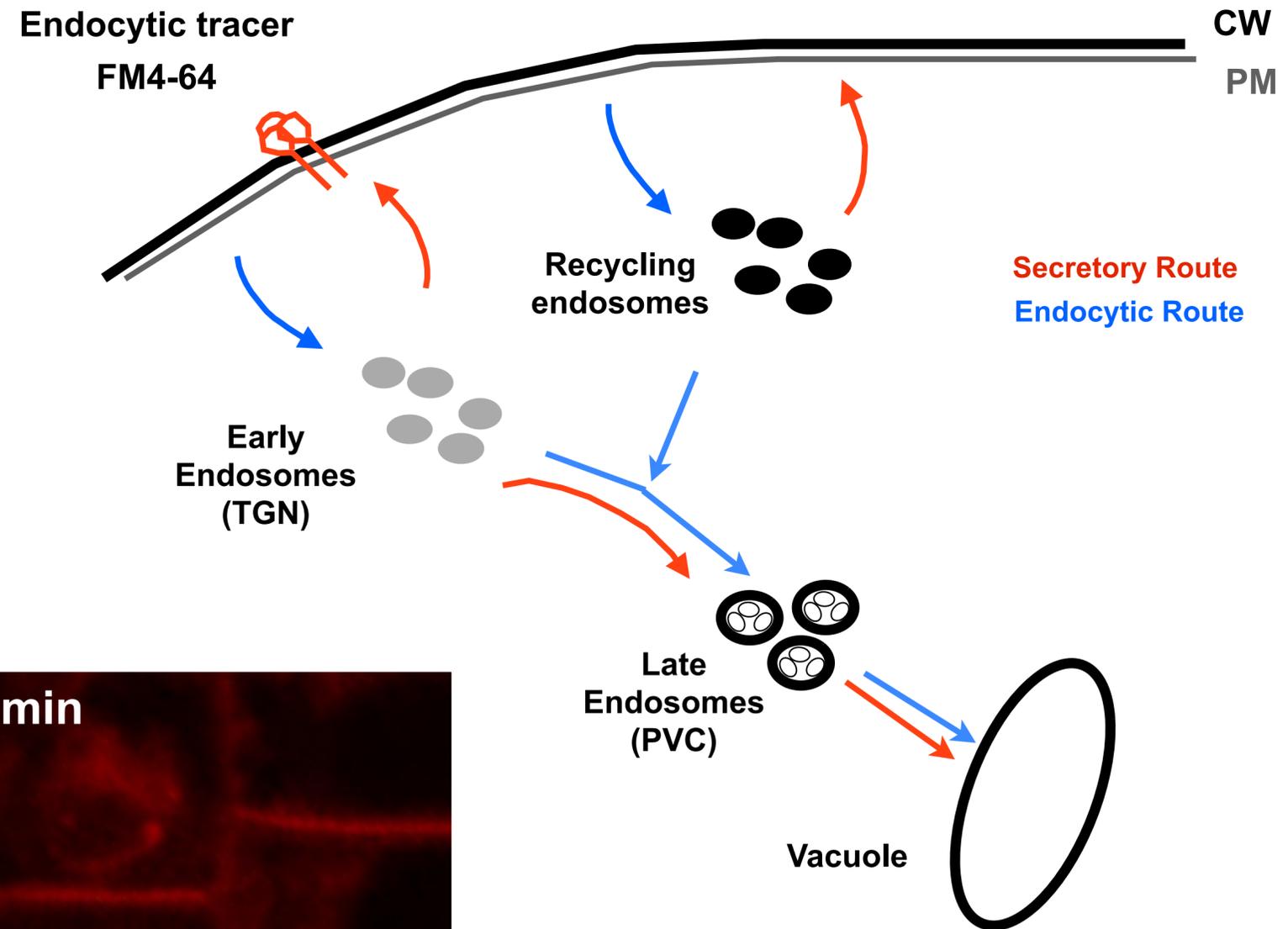
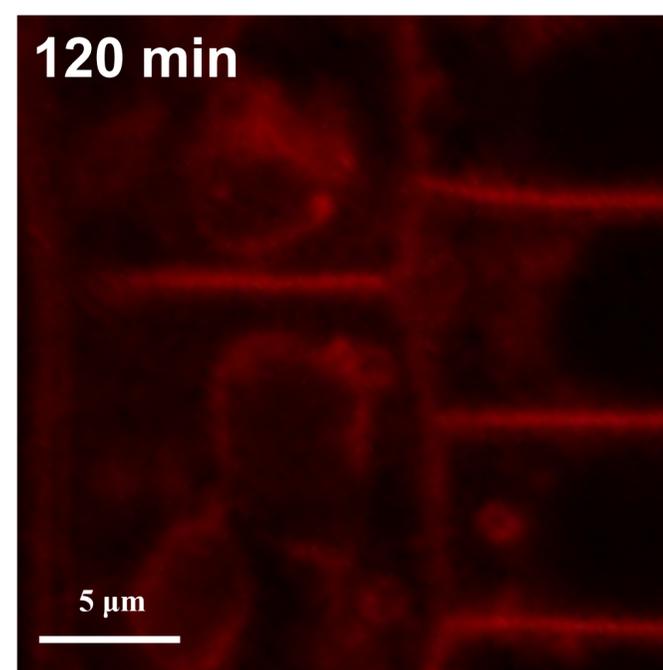
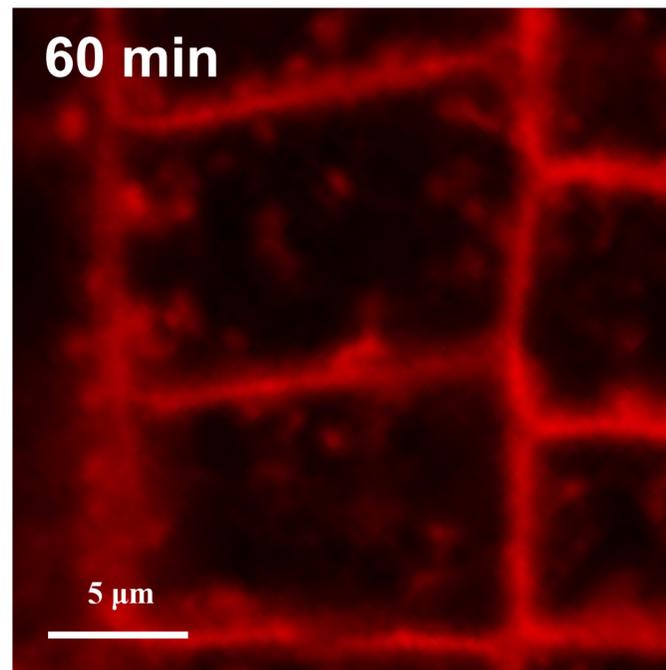
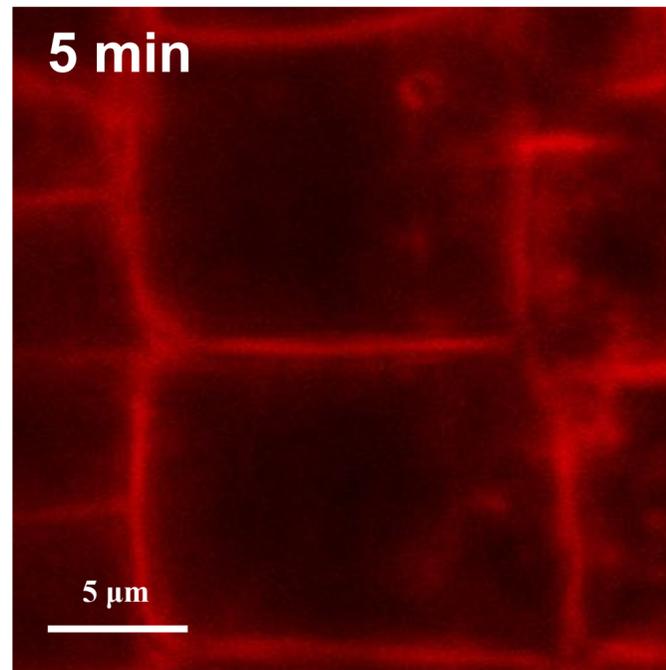
Sortin2
effect is
inducible



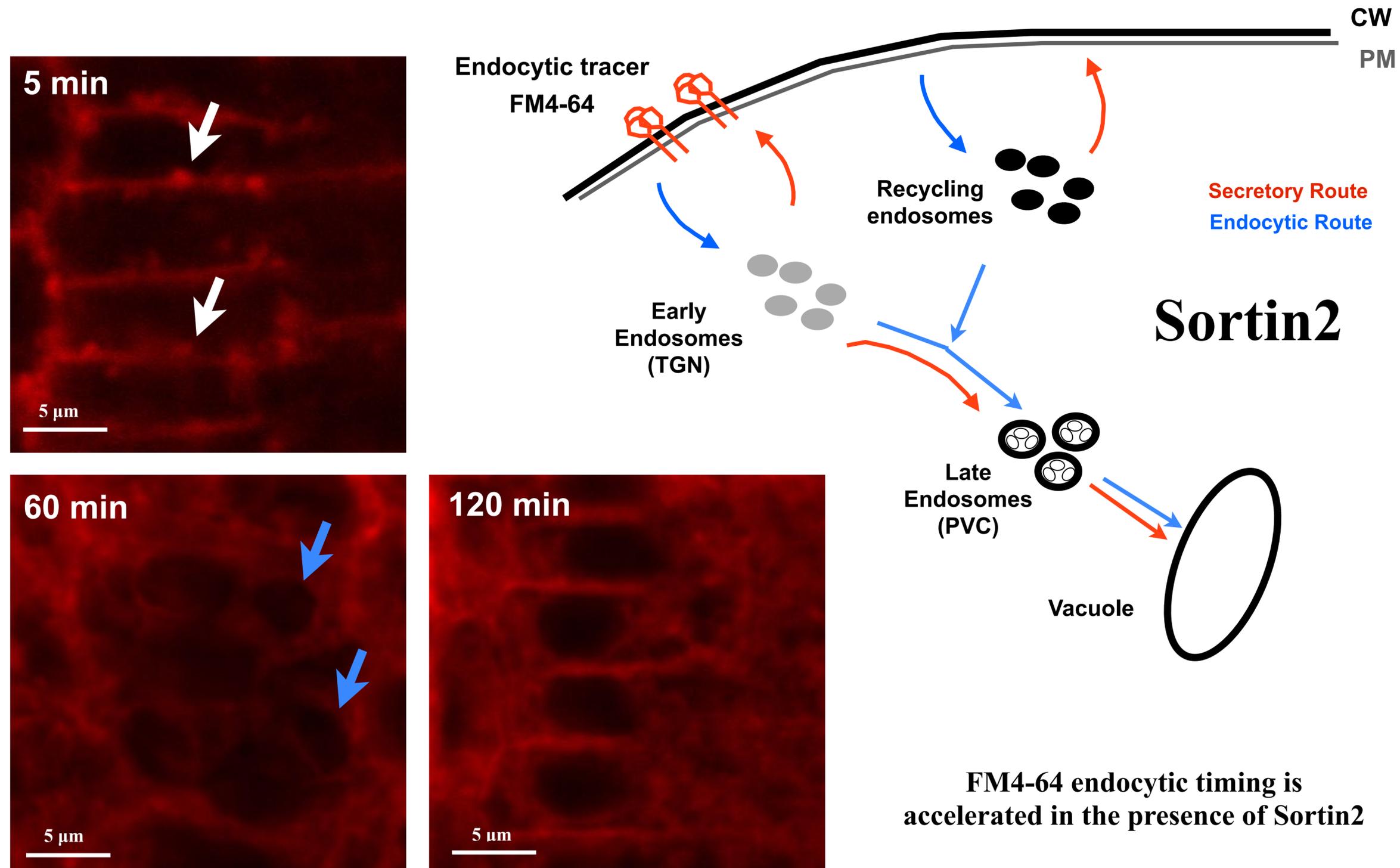
Sortin2 induce PIN2 vacuole accumulation through endocytic route



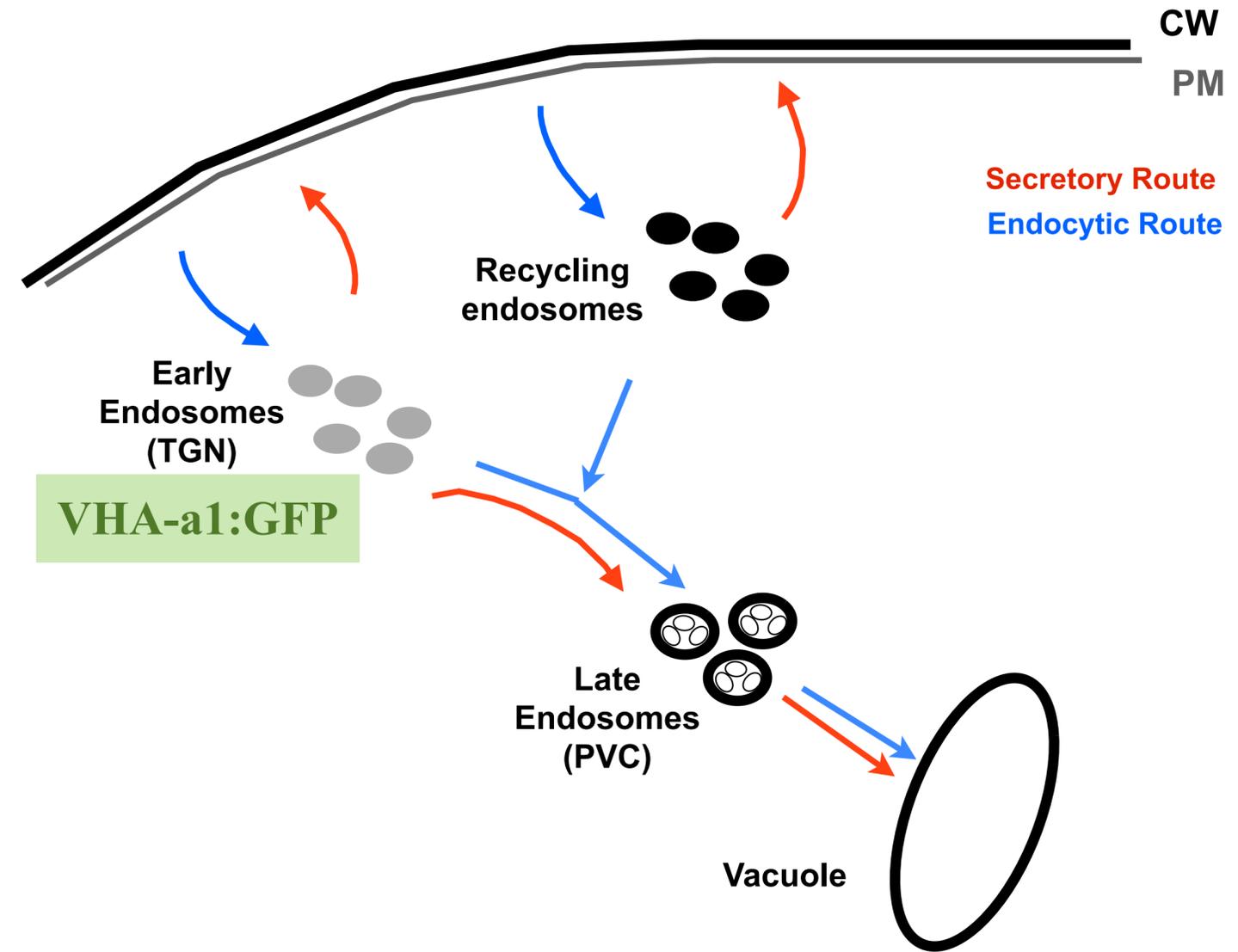
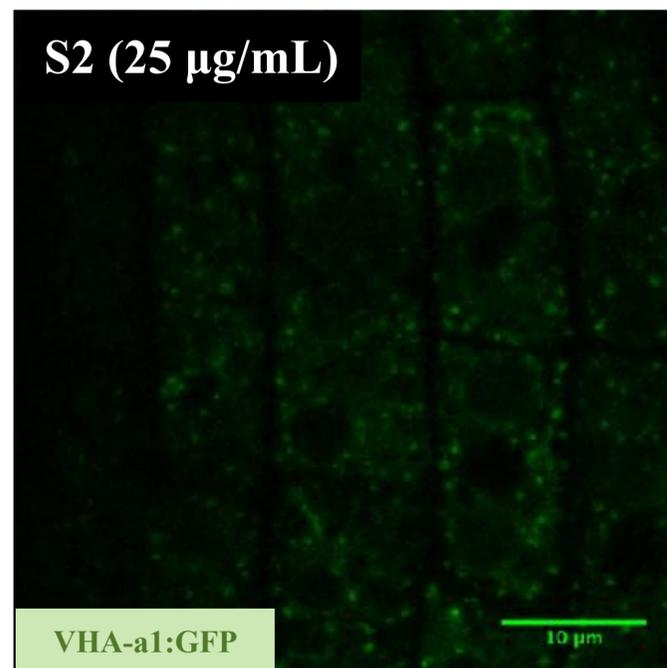
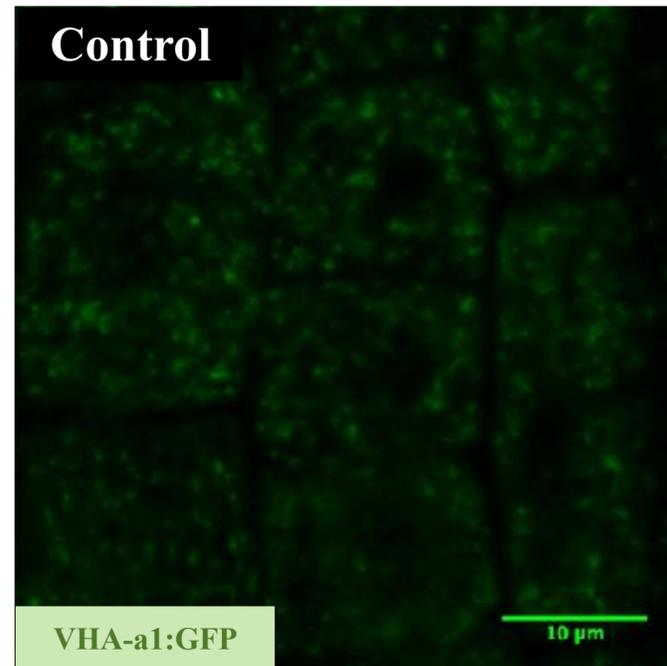
FM4-64 as a general tracer for endocytic trafficking



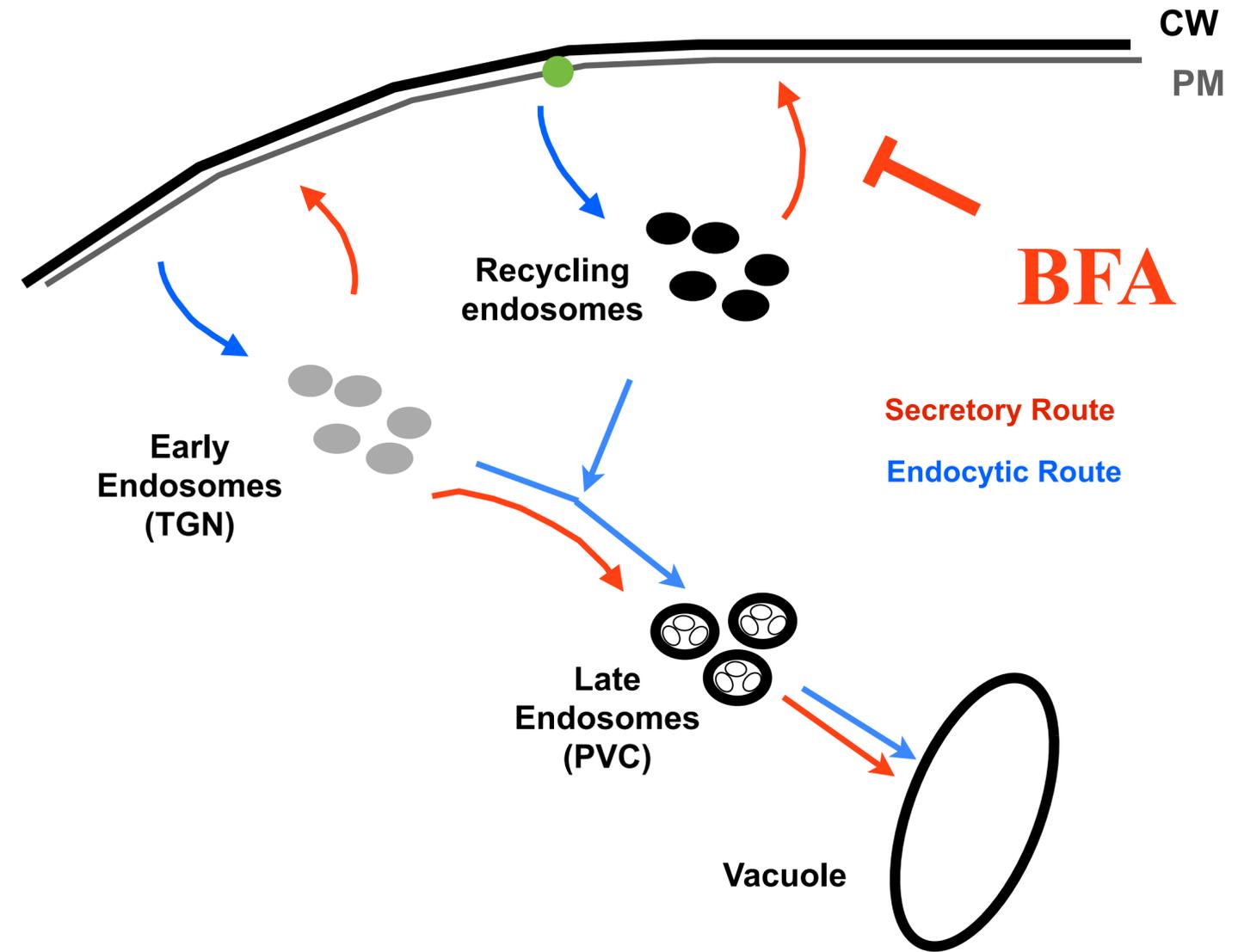
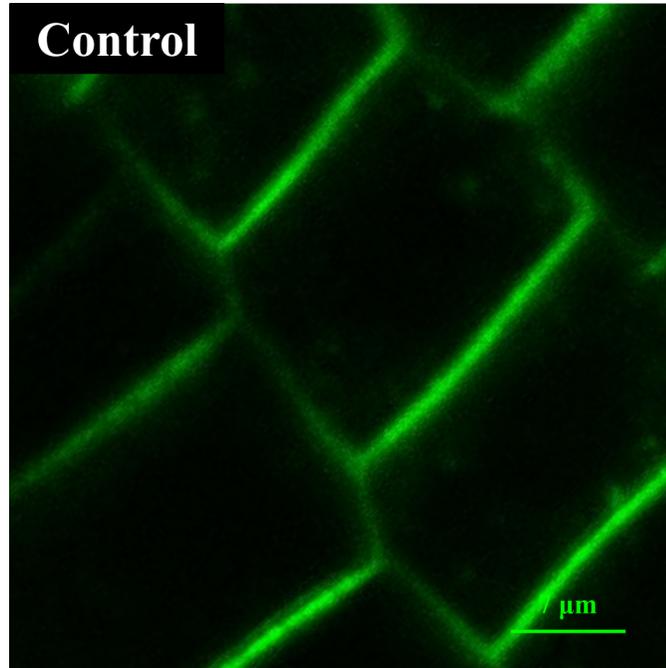
FM4-64 localizes at tonoplast at shorter times with Sortin2 treatments



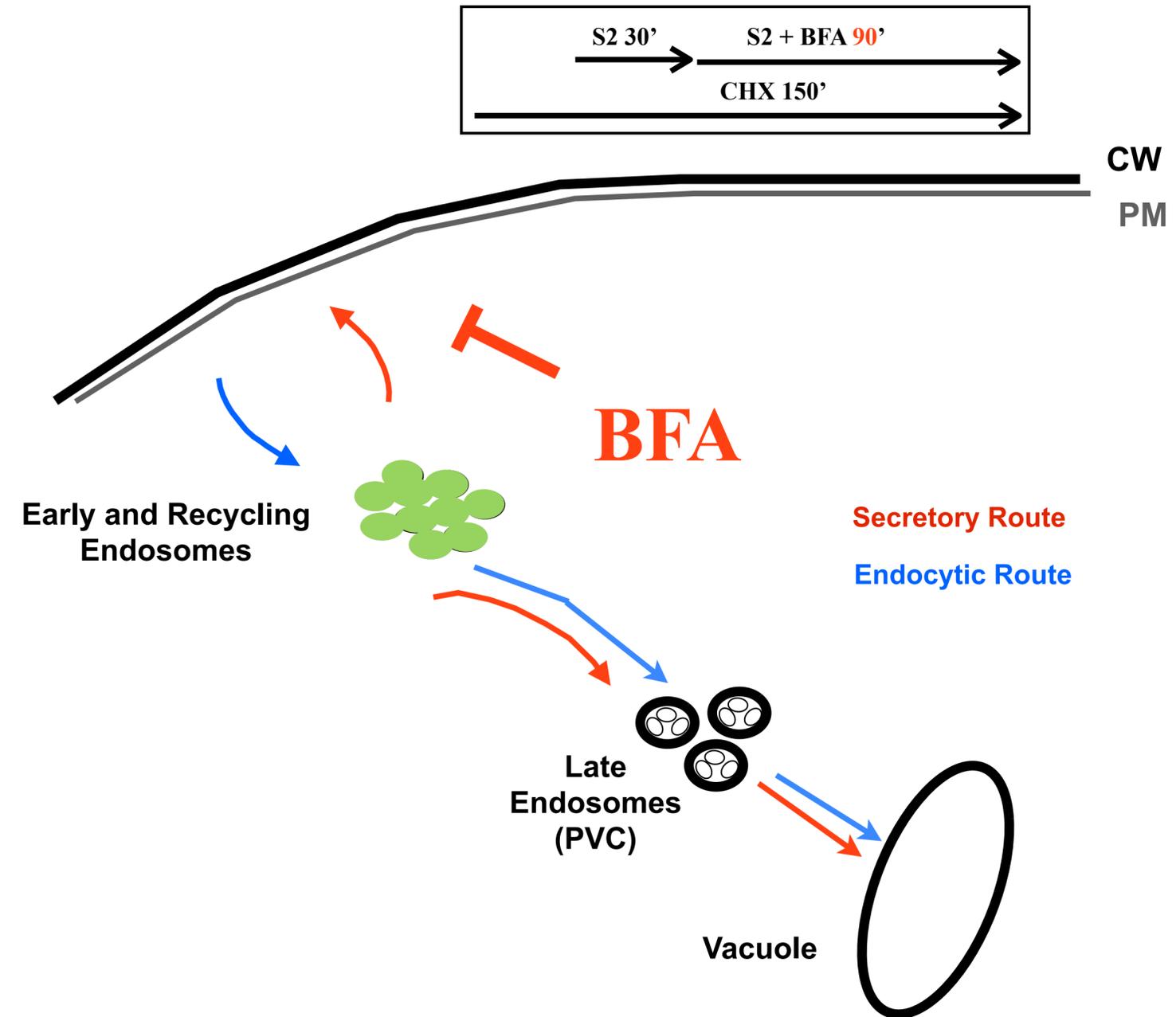
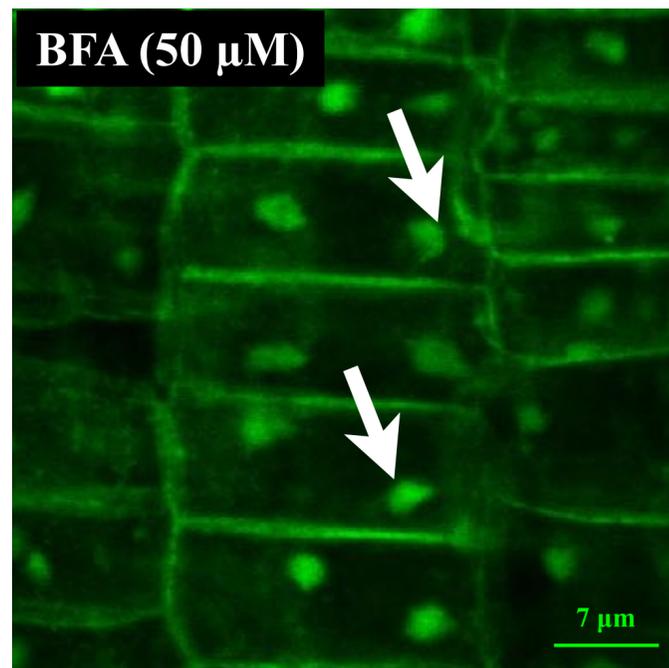
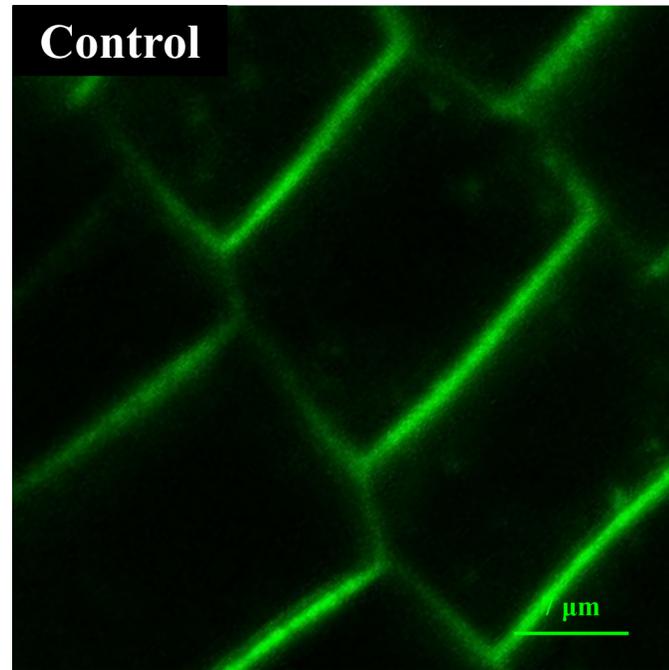
Early Endocytic Route components are not affected by Sortin2



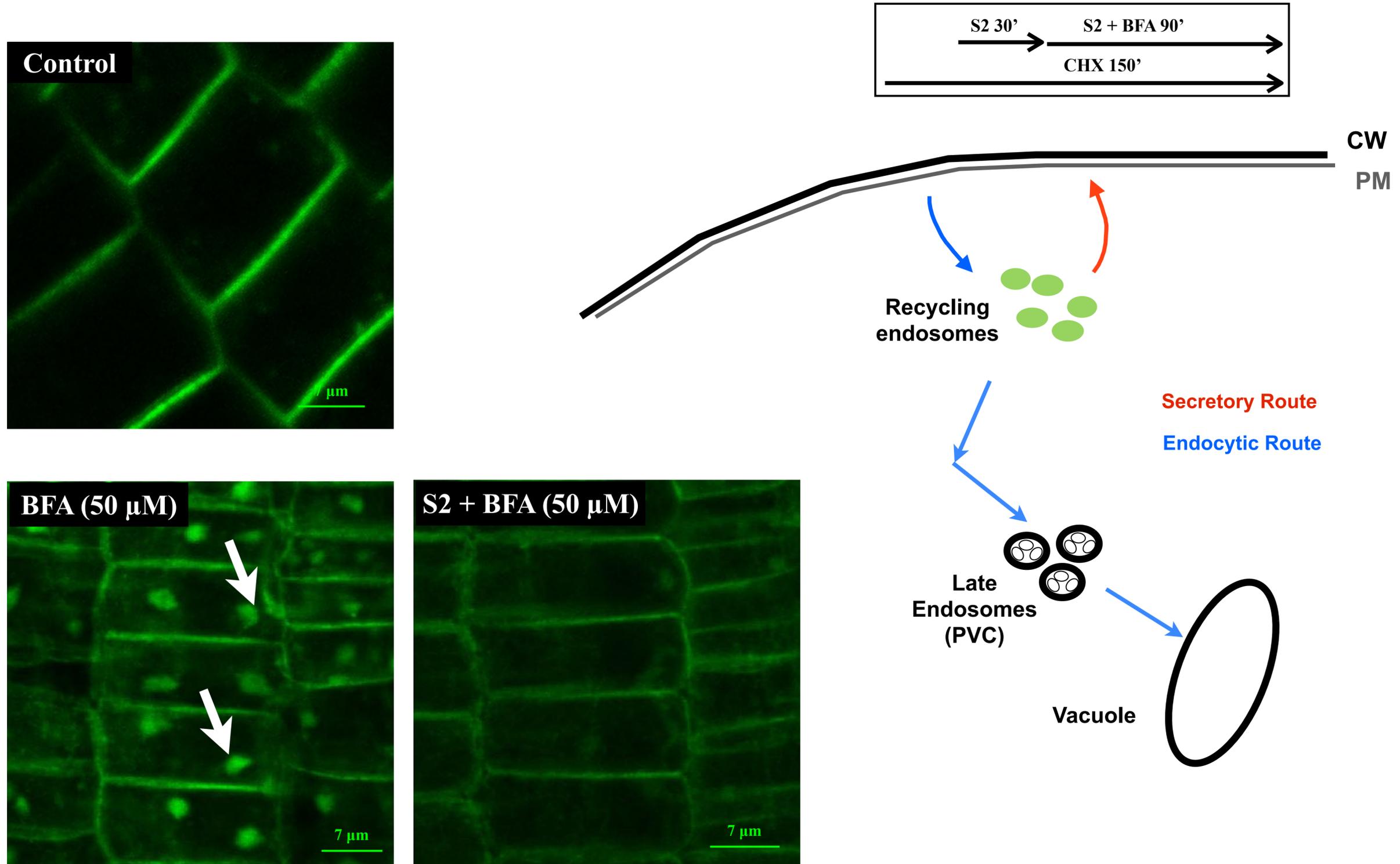
Brefeldin A (BFA) alters PIN2 constitutive cycling



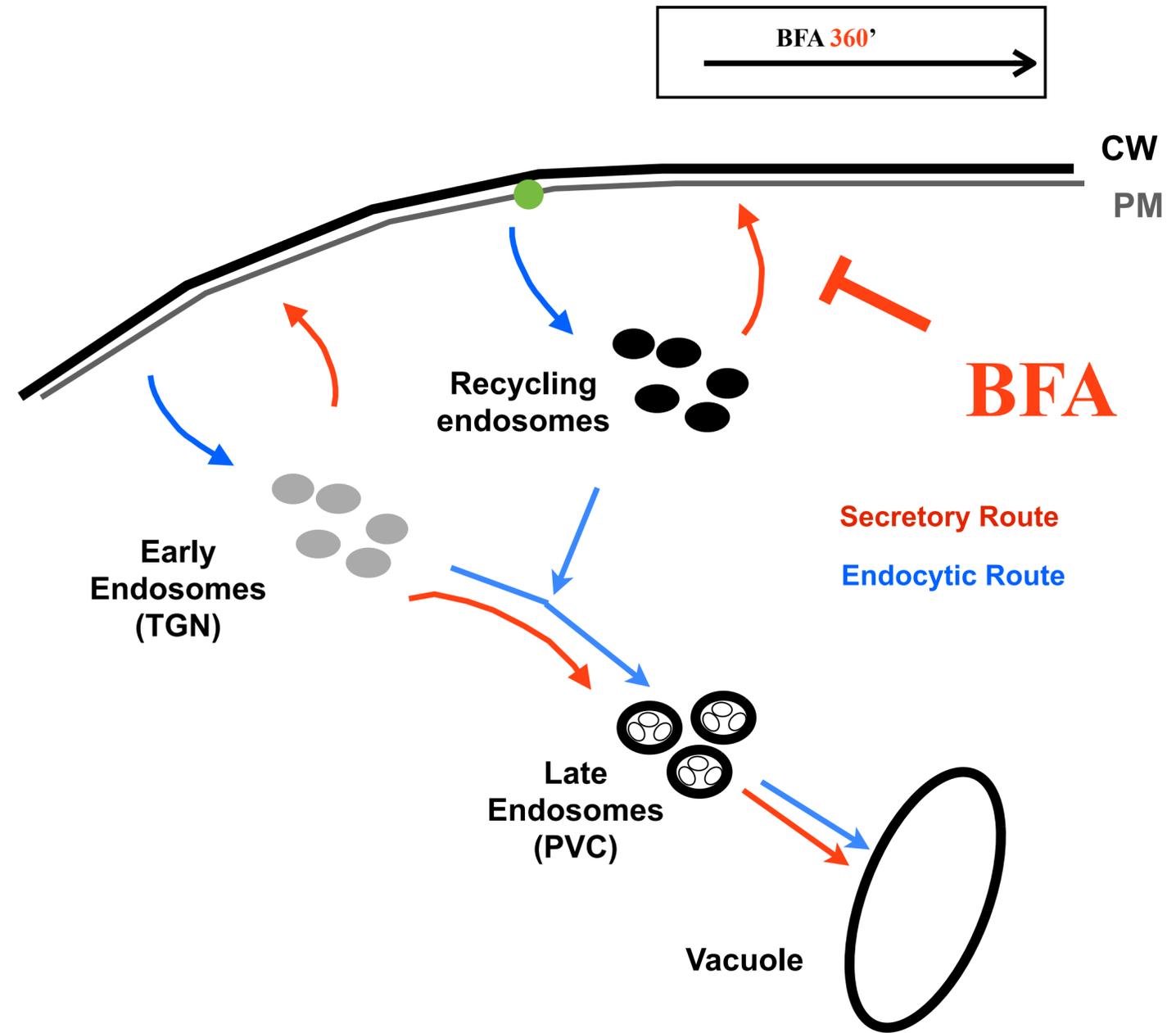
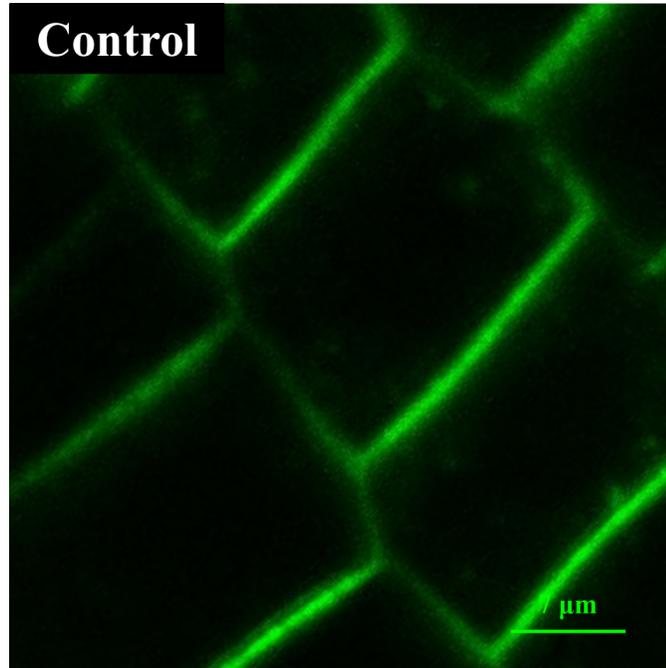
Brefeldin A (BFA); recycling pathway inhibitor accumulates PIN2:GFP in “BFA bodies”



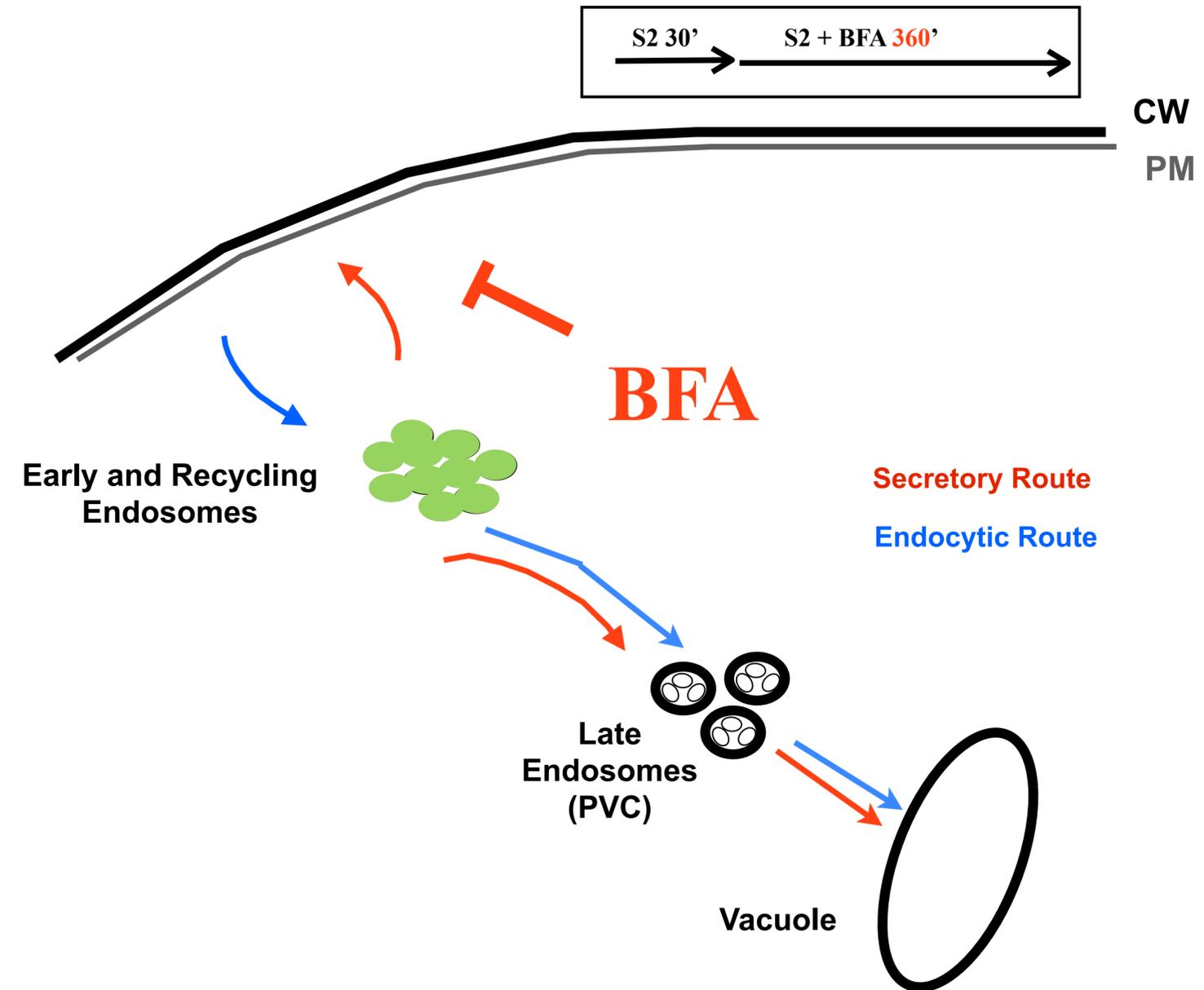
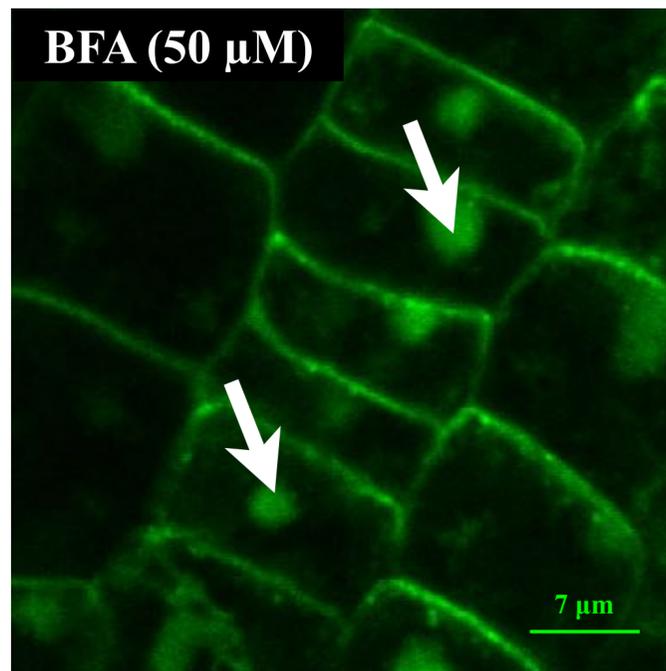
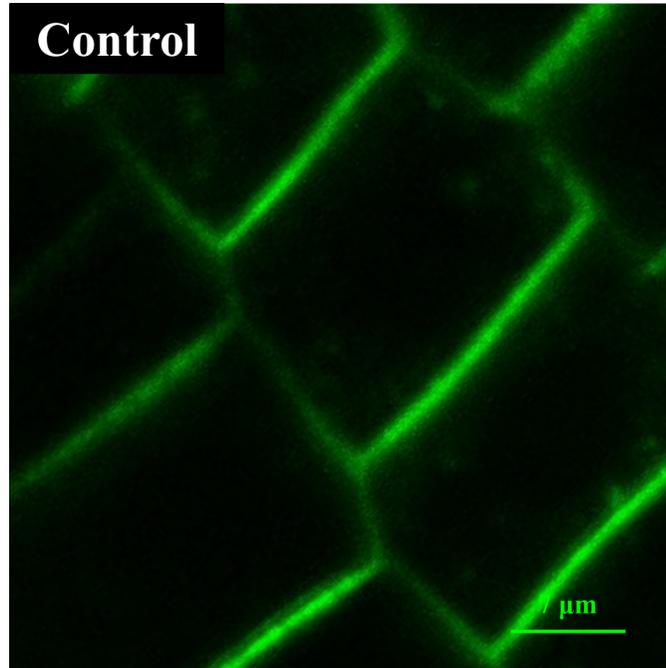
Sortin2 antagonize BFA effect



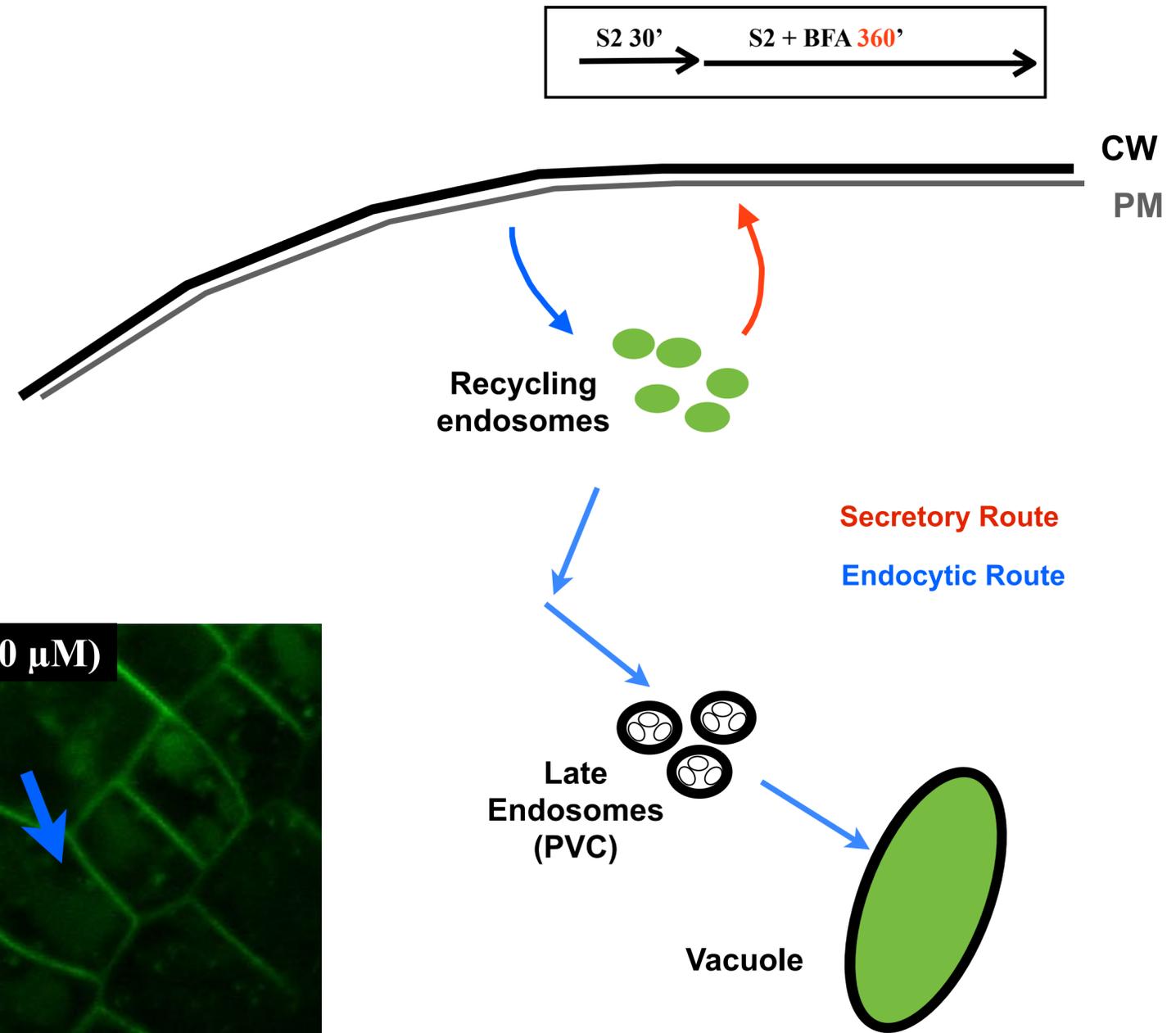
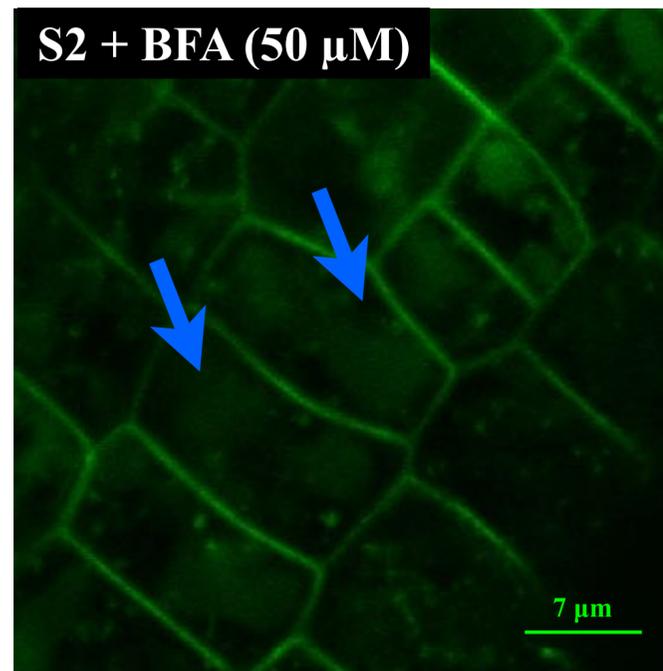
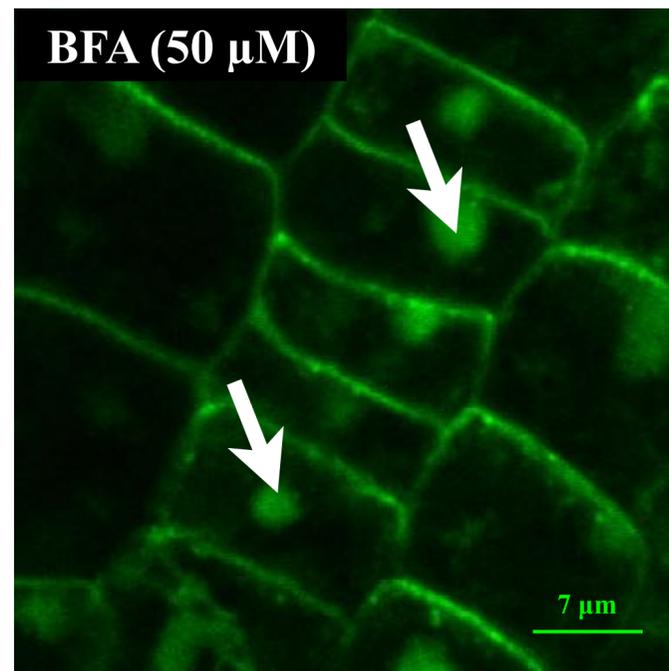
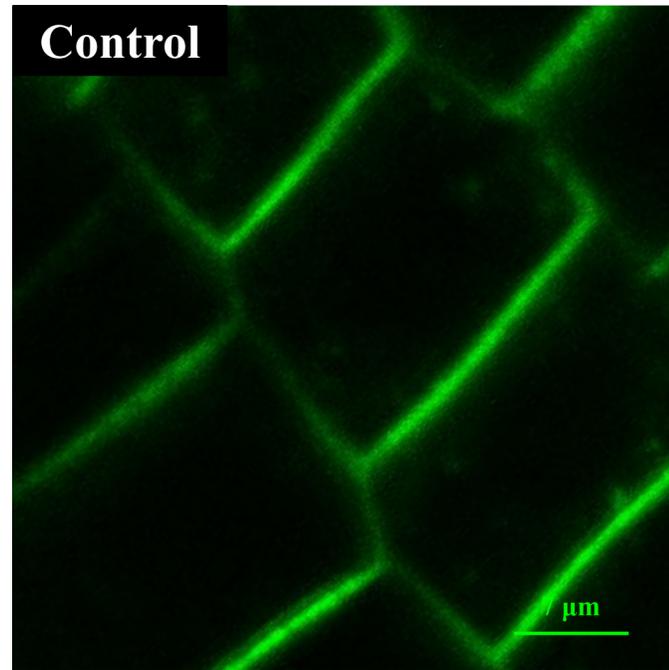
Sortin2 antagonizes BFA inducing PIN2 accumulation in vacuoles



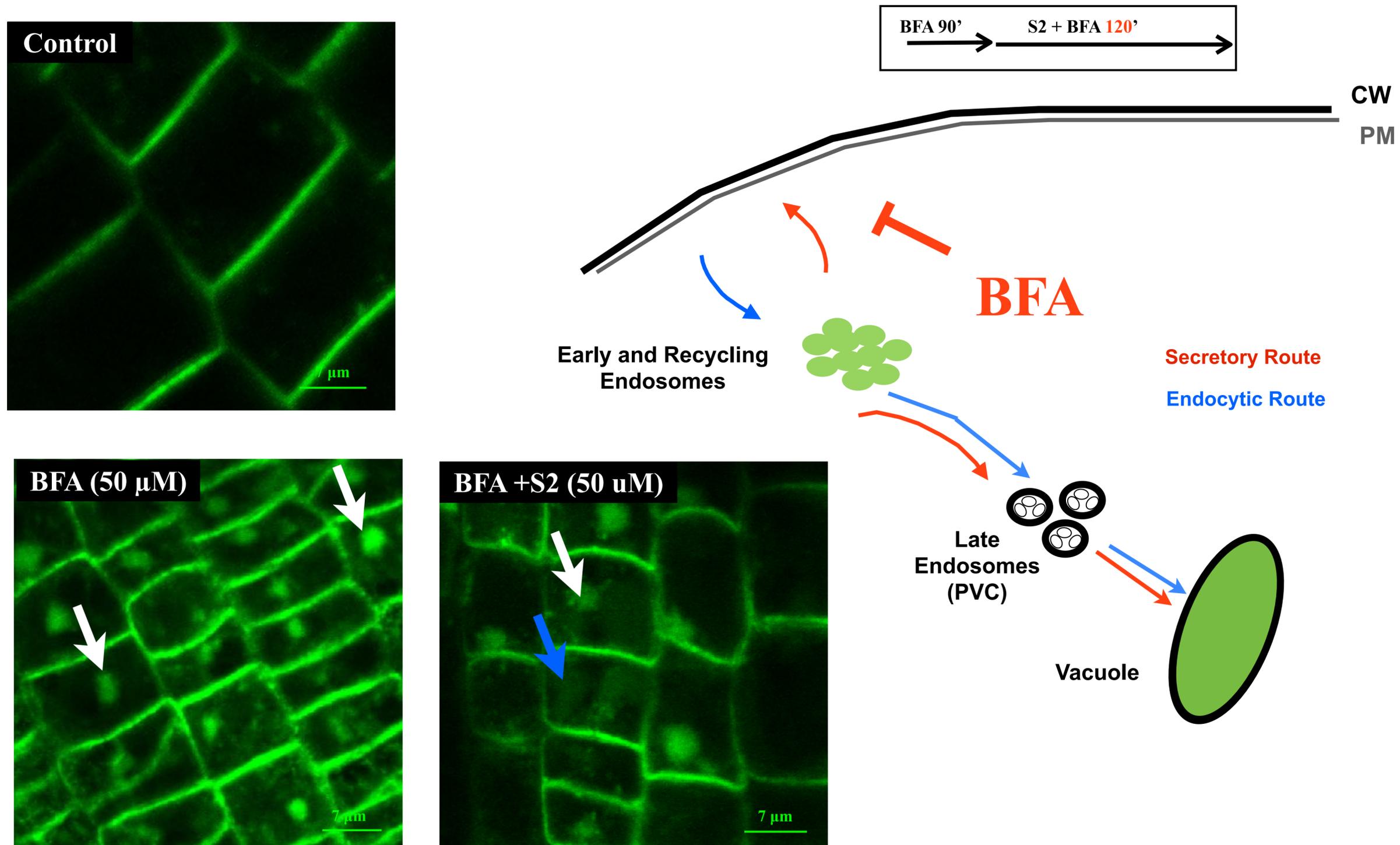
Sortin2 antagonizes BFA inducing PIN2 accumulation in vacuoles



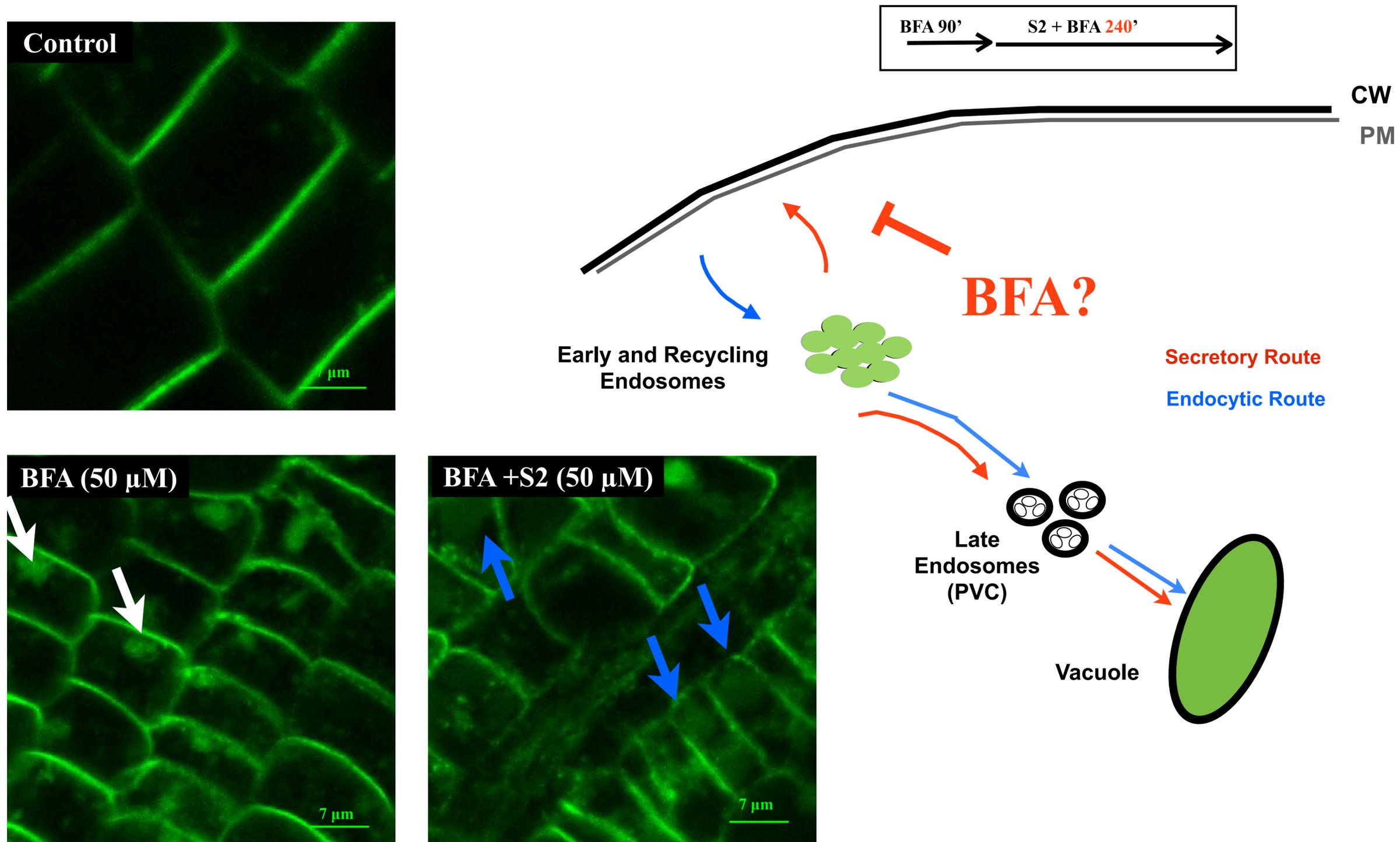
Sortin2 antagonizes BFA inducing PIN2 accumulation in vacuoles



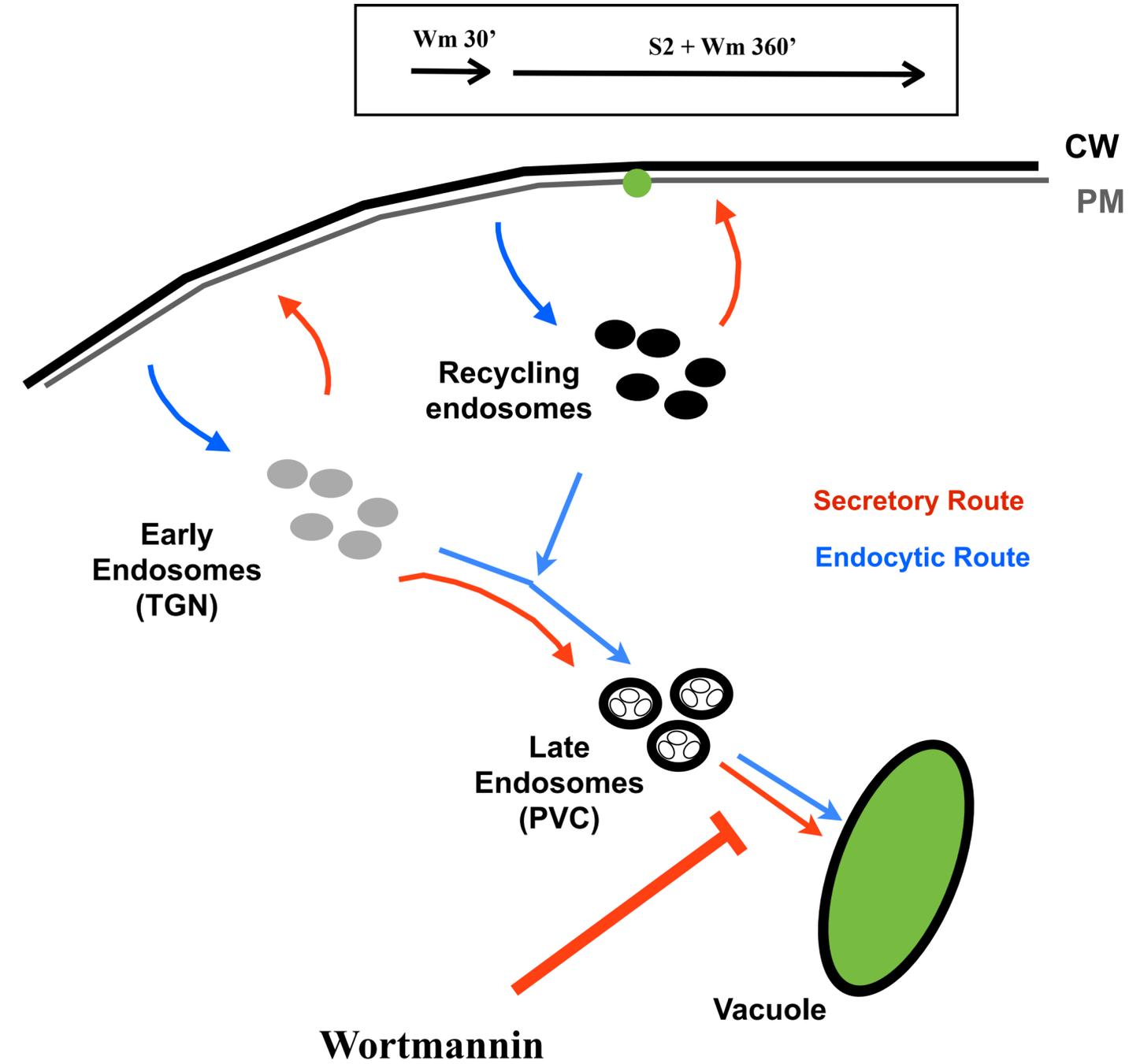
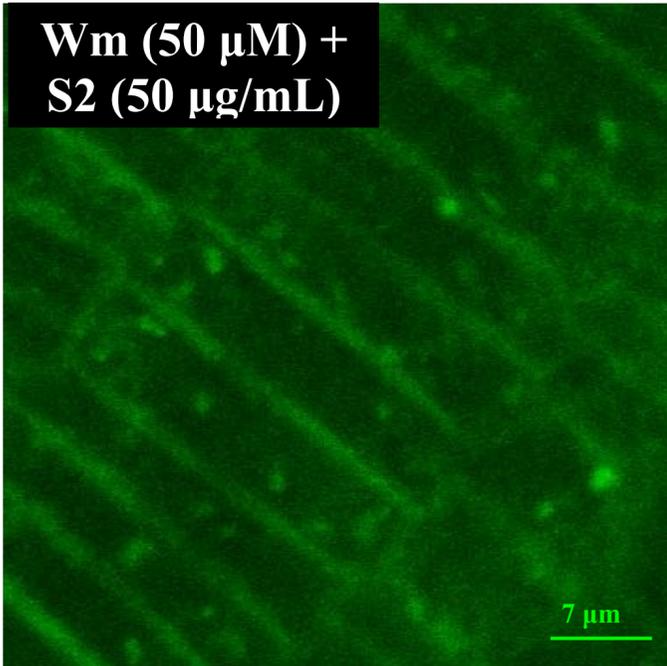
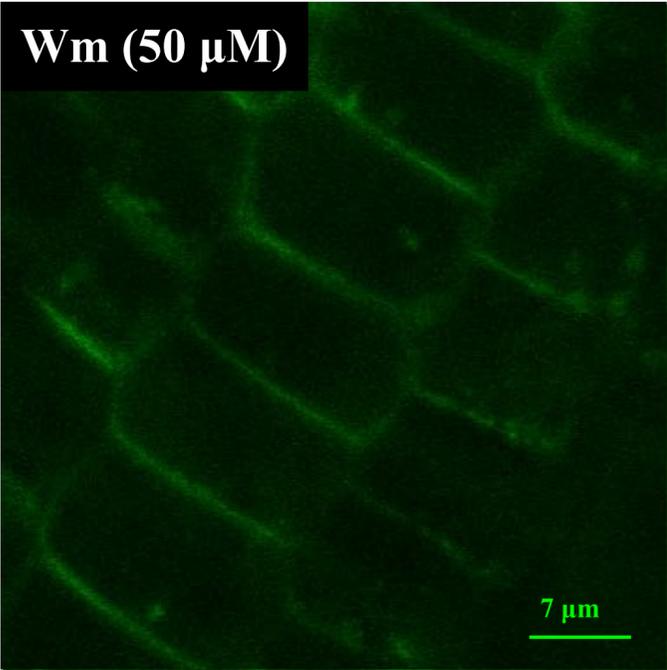
Sortin2 induces PIN2 accumulation in vacuoles from BFA bodies accumulated protein



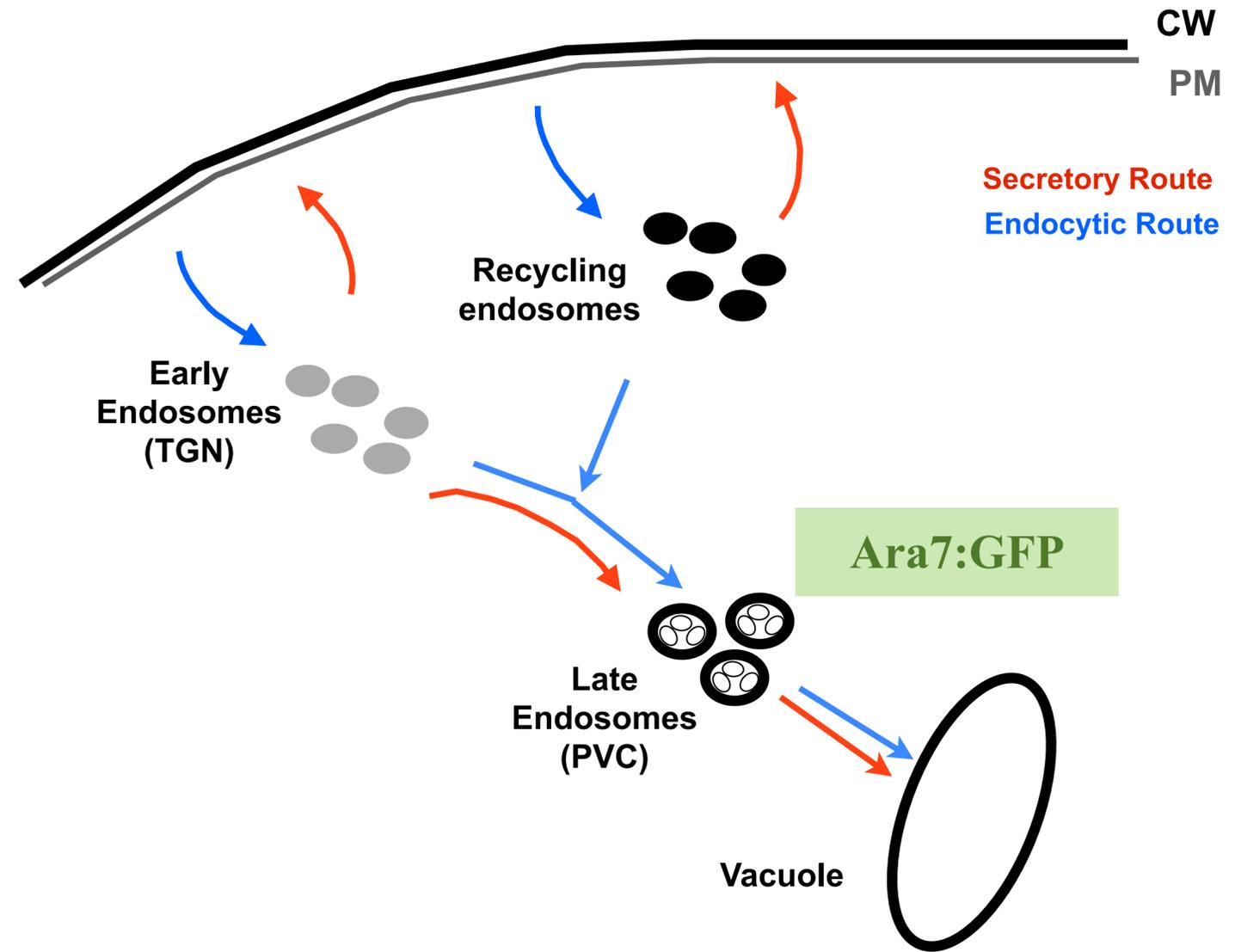
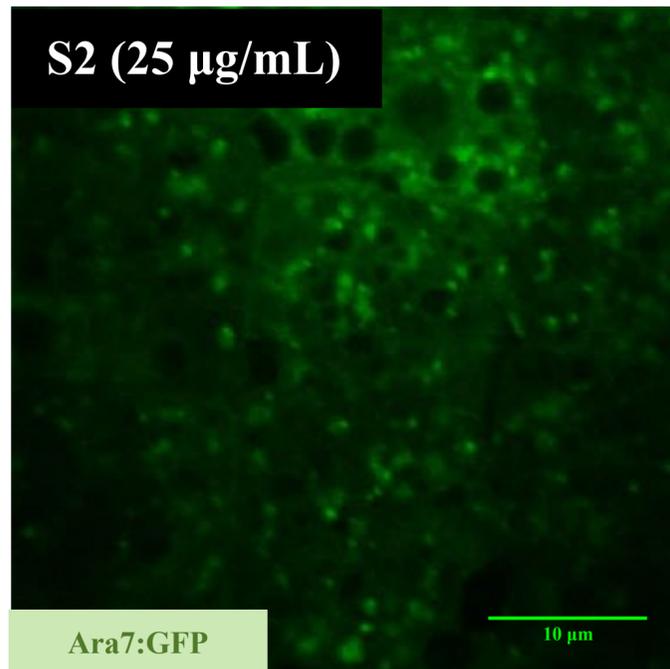
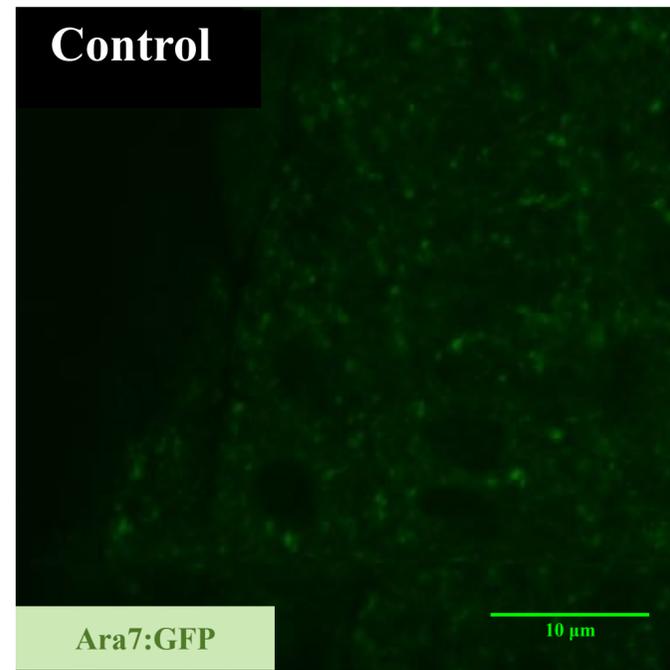
Sortin2 is able to disaggregate PIN2 accumulation within pre-formed BFA bodies



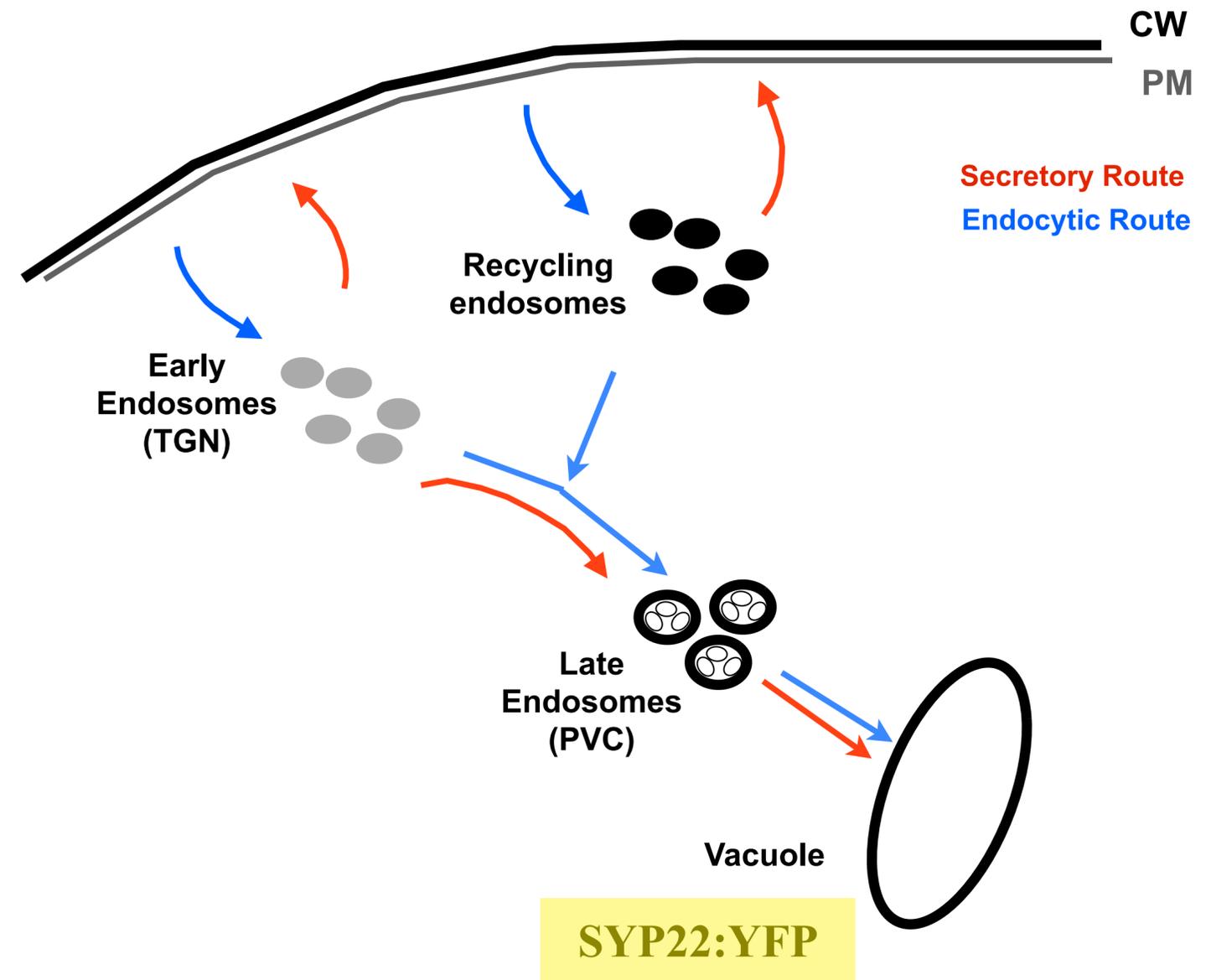
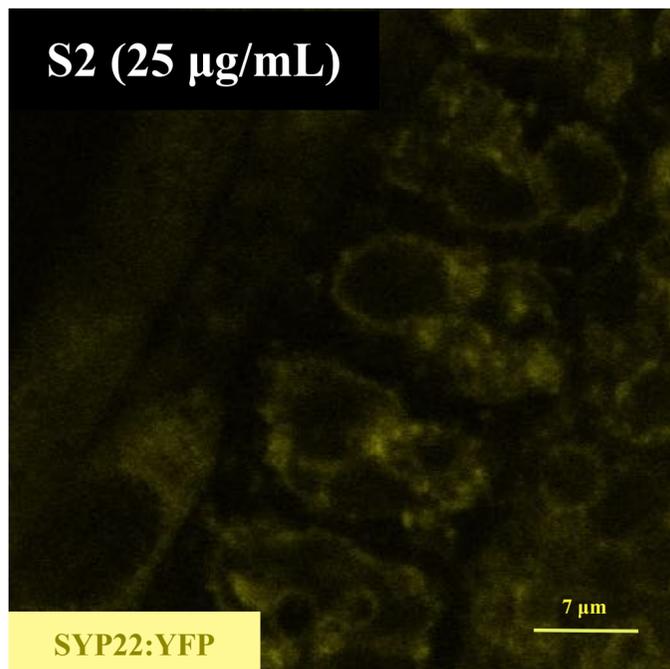
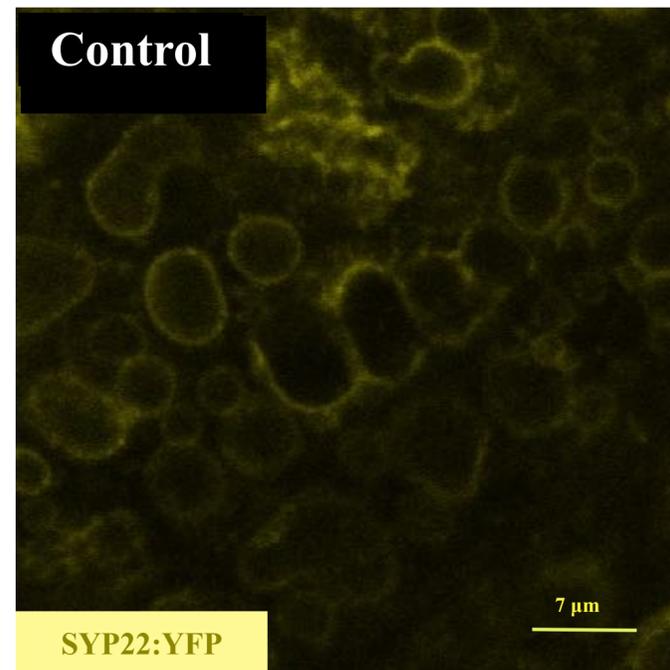
Wortmannin inhibits PIN2 accumulation in vacuole induced by Sortin2



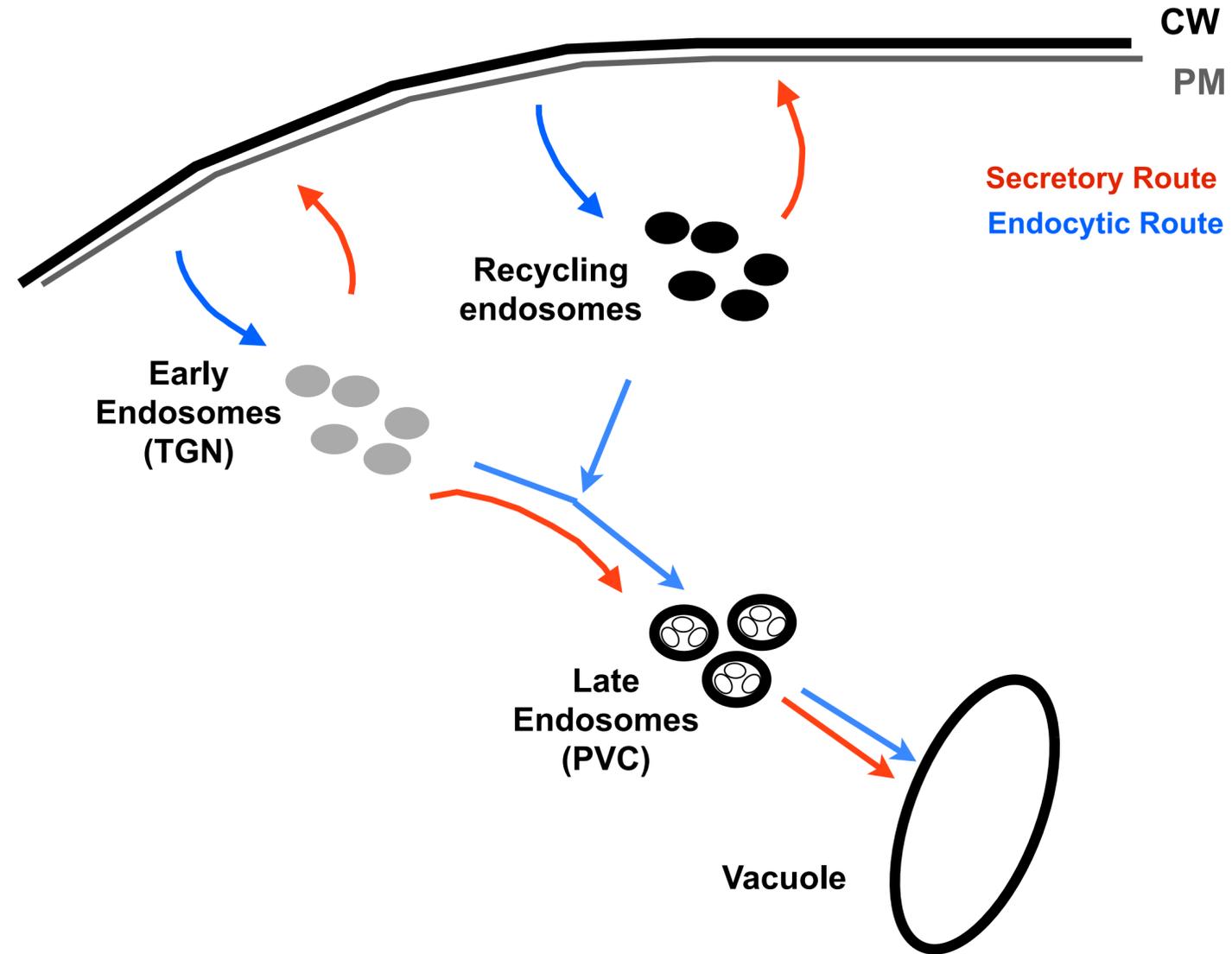
Late Endocytic Route components are affected by Sortin2



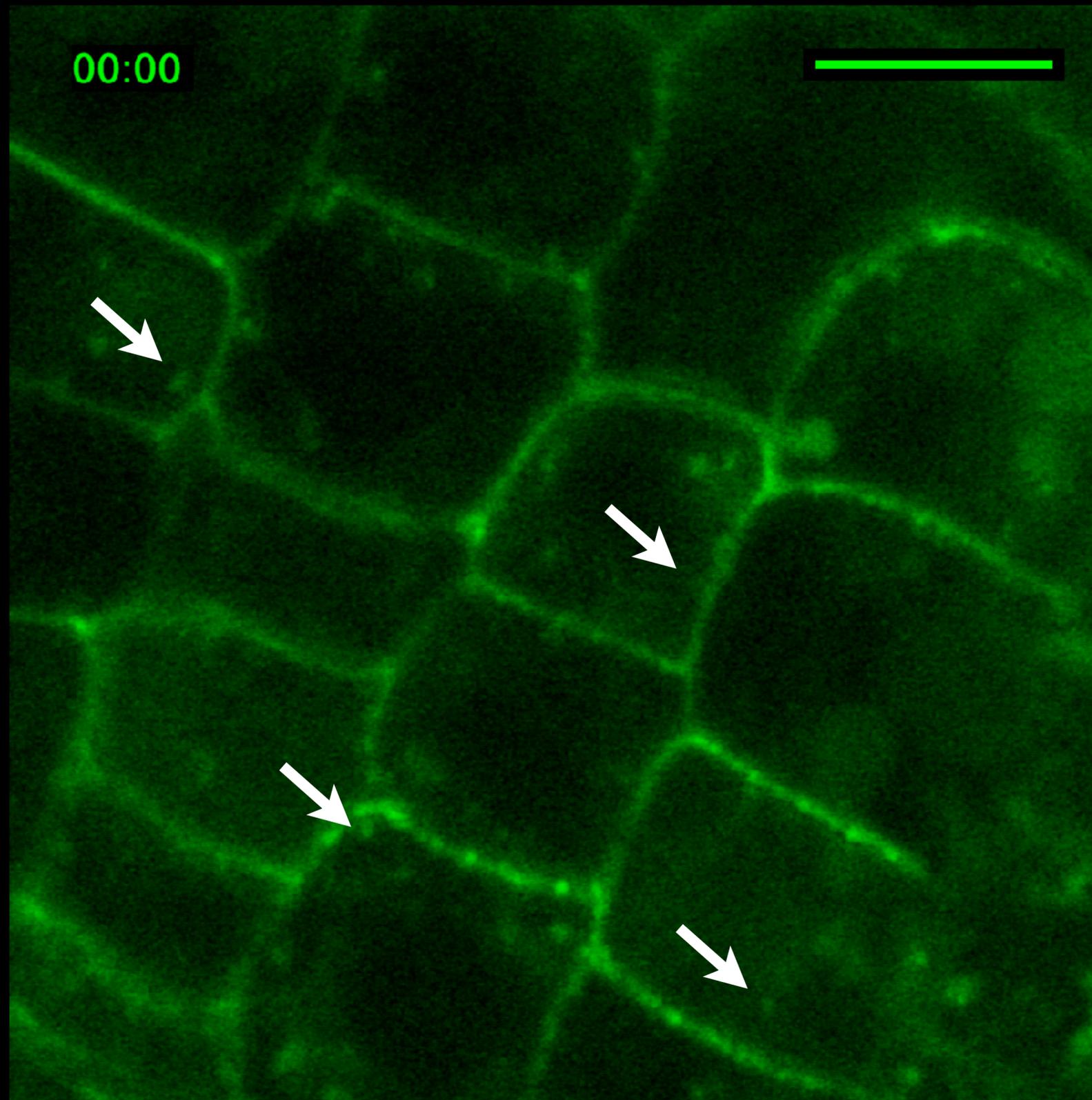
Late Endocytic Route components are affected by Sortin2



Cytoskeleton plays a key role in intracellular trafficking

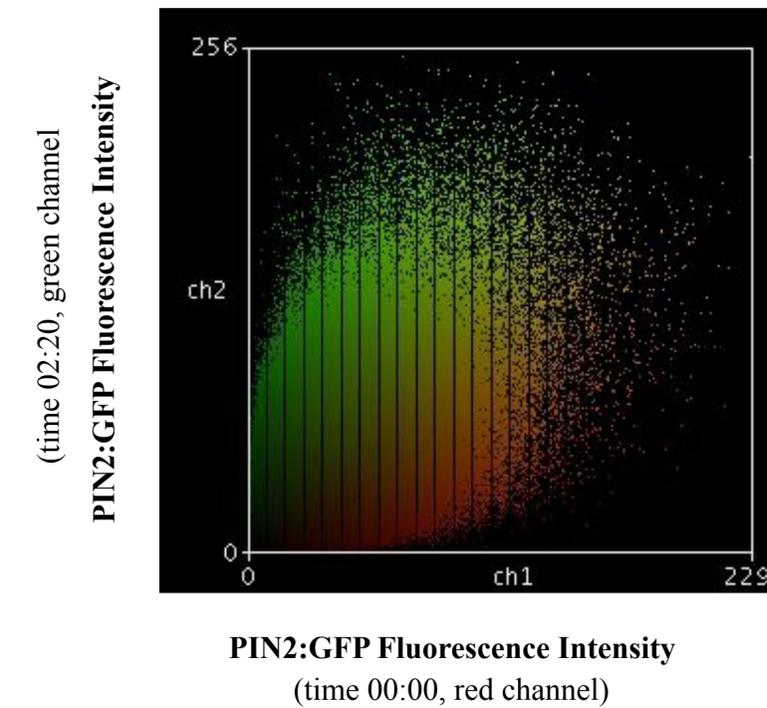
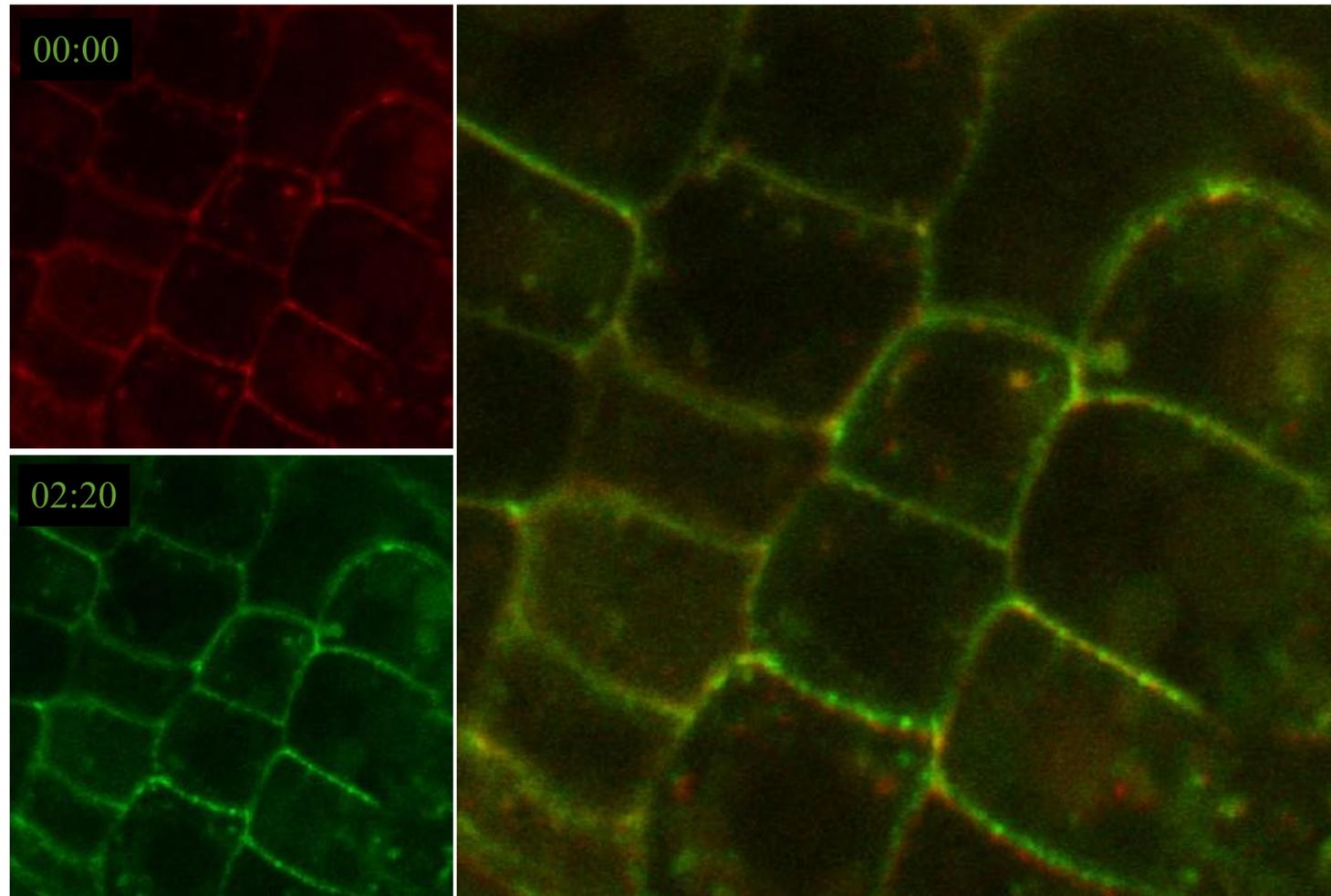


Sortin2 is not affecting endosome movement



Sortin2 is not affecting endosome movement

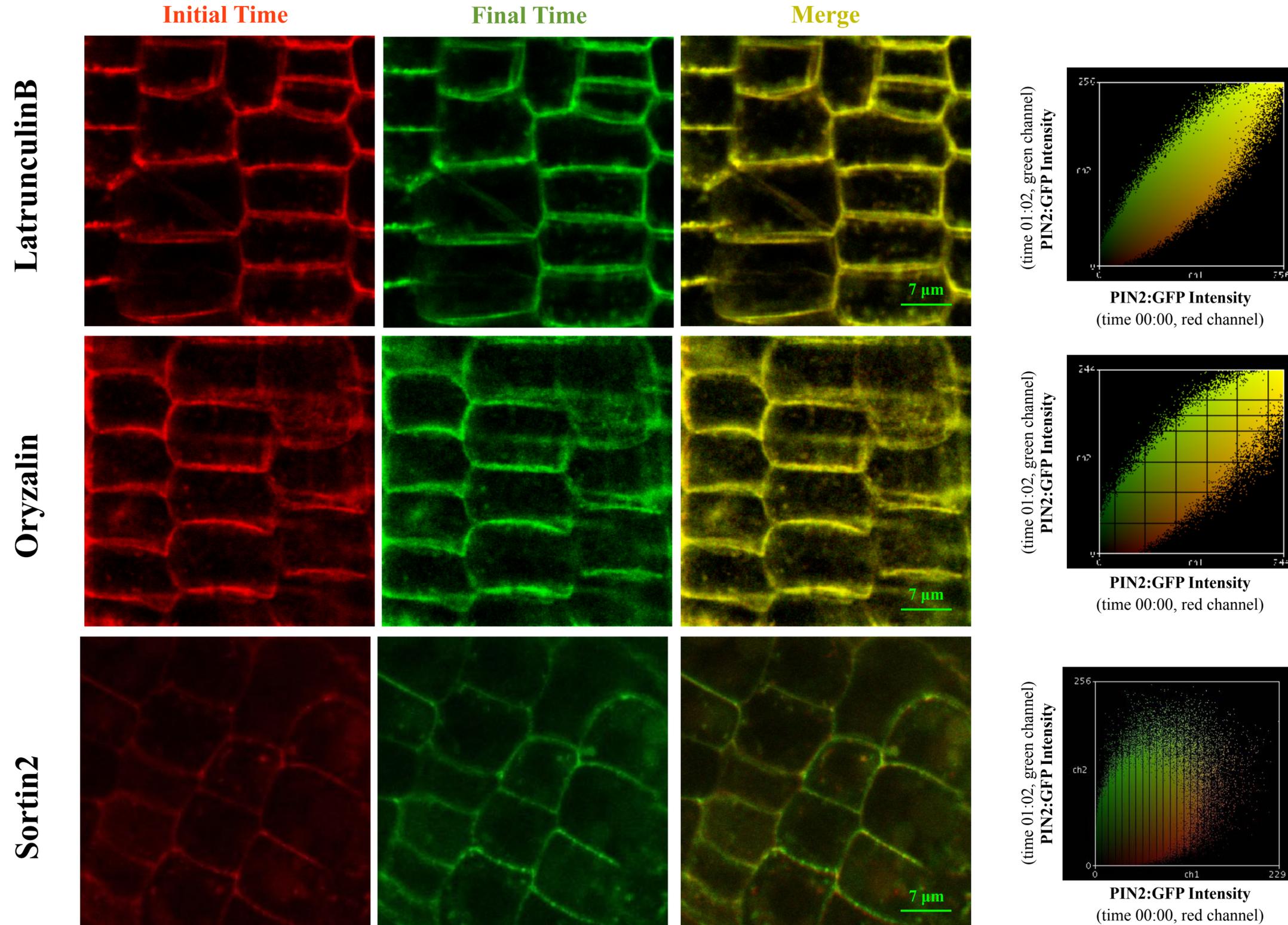
frame 1/frame 6



Latrunculin B
(depolymerizes actin filaments)

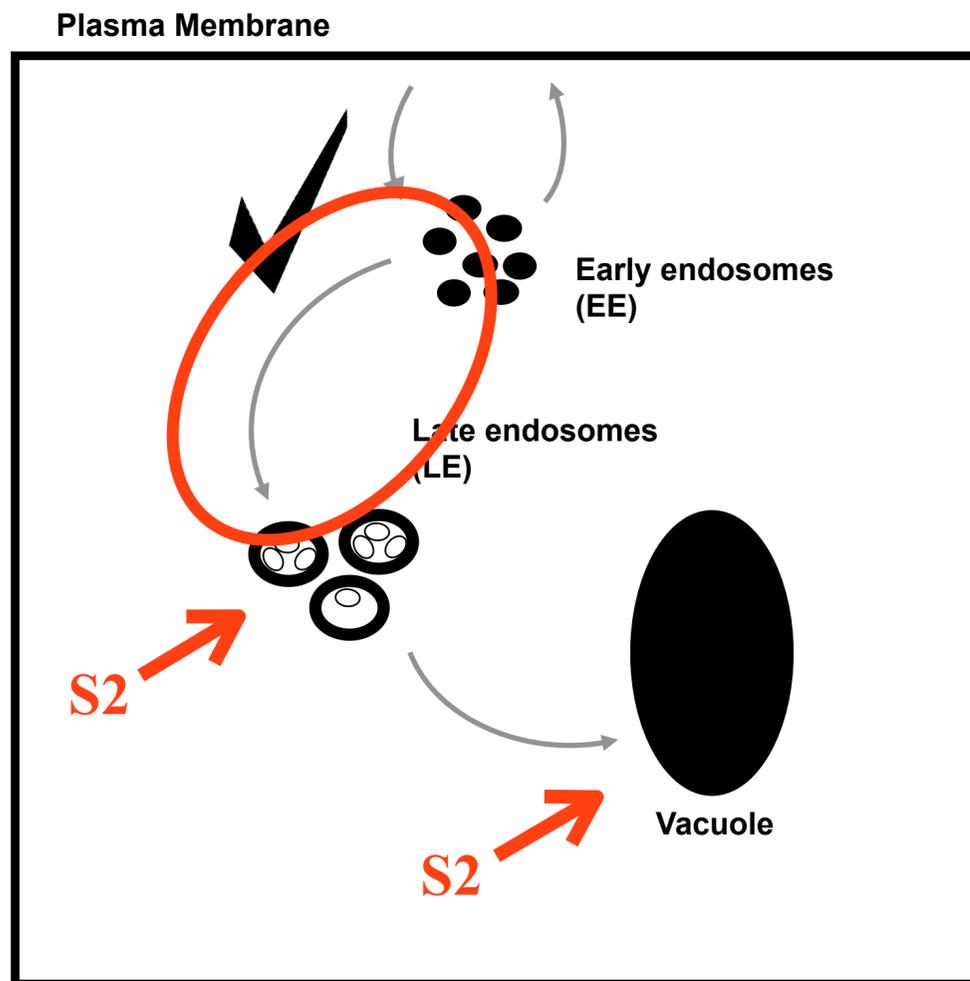
Oryzalin
(depolymerizes microtubule)

Sortin2 is different from Latrunculin B and Oryzalin

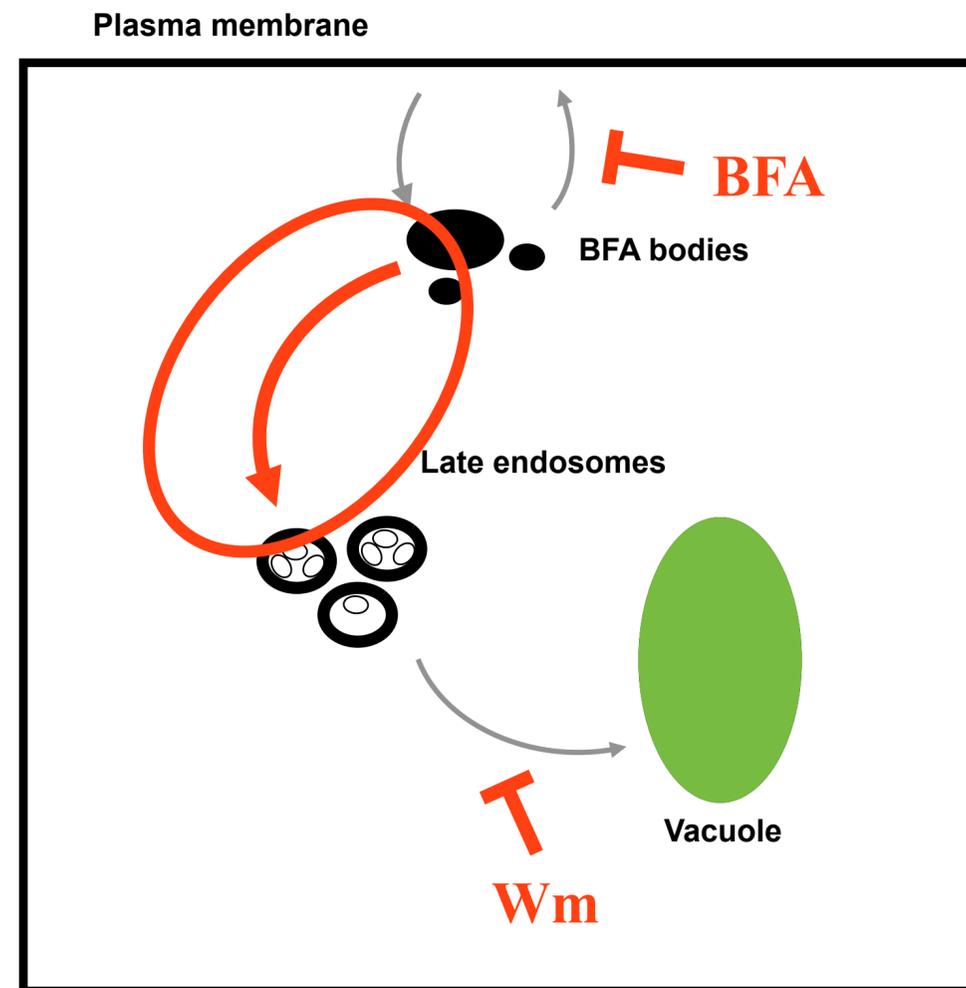


Model for the Sortin2 Action Mode and its Cellular Target

Sortin2 over endomembrane markers



BFA and Sortin2





Laboratorio de Biología Molecular Vegetal
Departamento de Biología, Facultad de Ciencias
Universidad de Chile

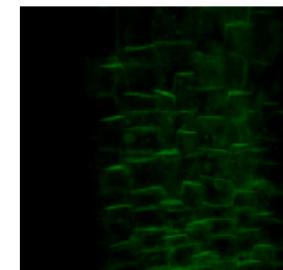
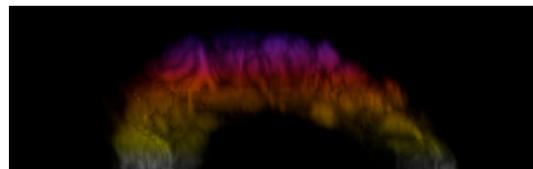


LNM

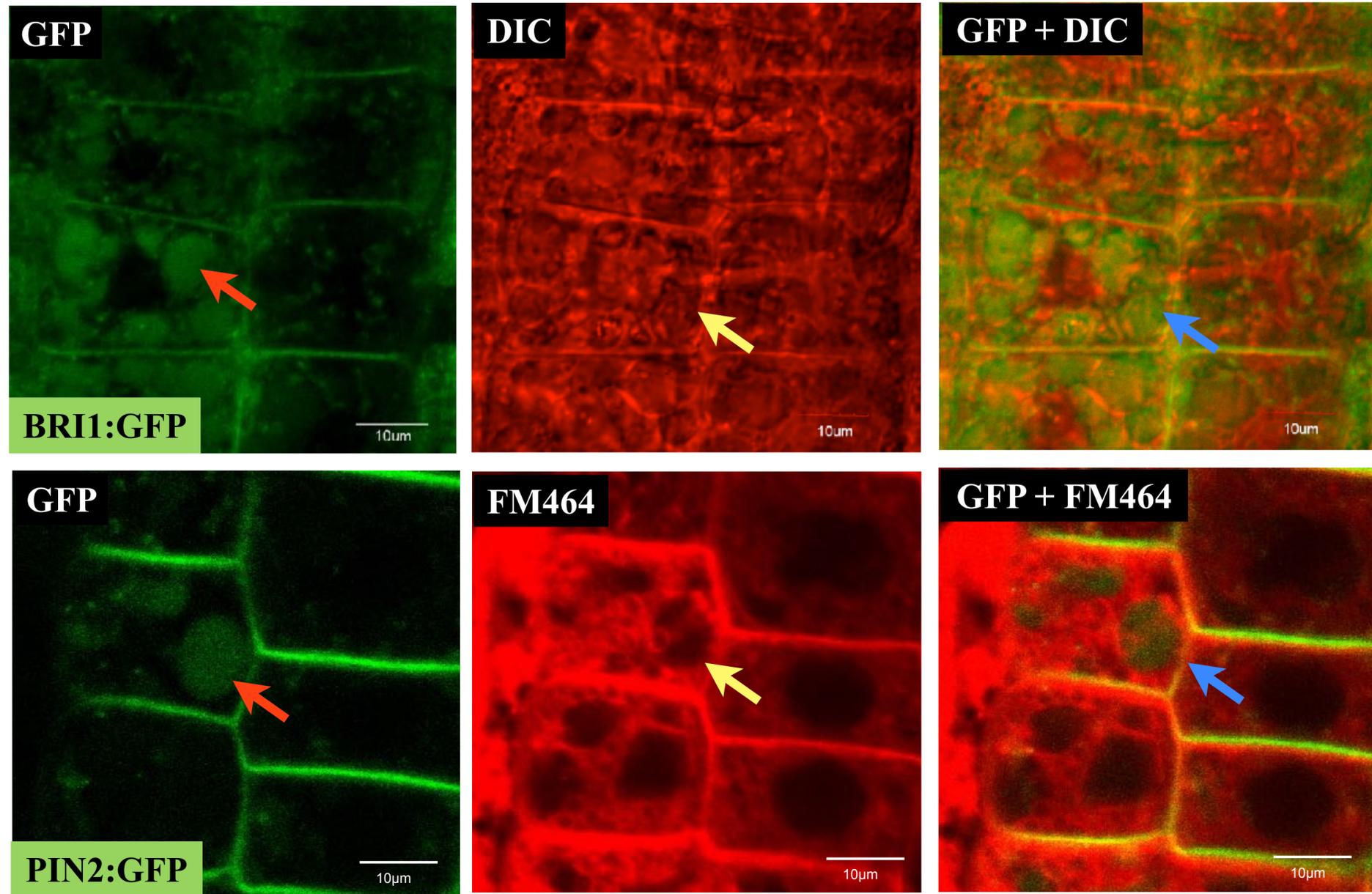
Lorena Pizarro, PhD (c)
Carlos Rubilar, MSc (c)
Nicolás Manríquez, BSc
Felipe Espinoza, BSc

Thanks::GFP

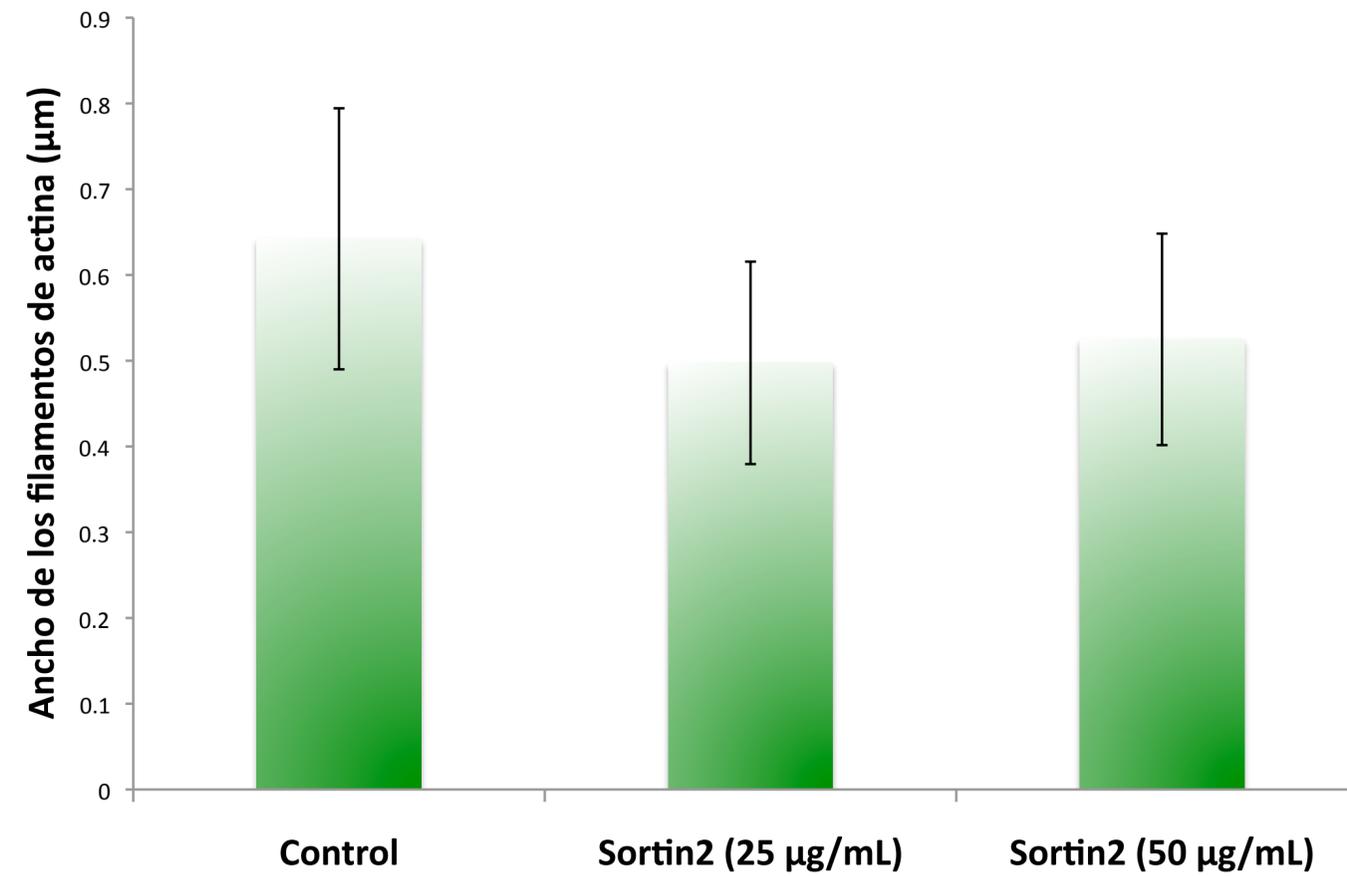
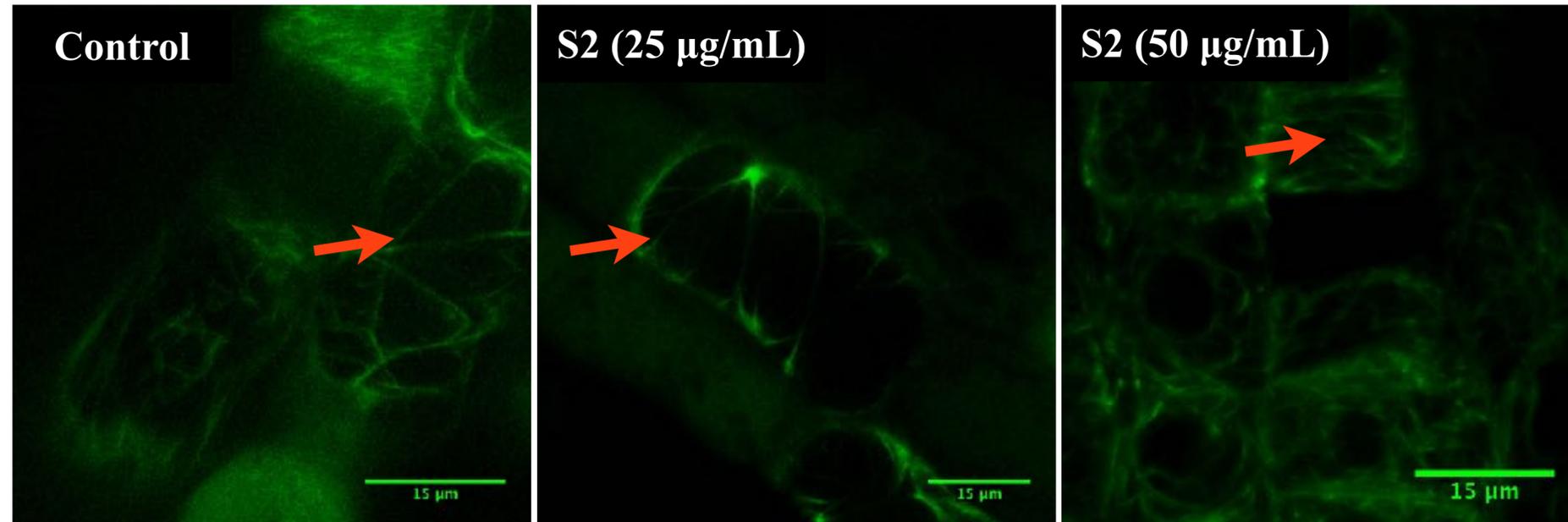
Alejandra Pesce, PhD
Alexander Vergara, PhD
Matias Freire, MSc (c)
Andrés Lagos, BSc

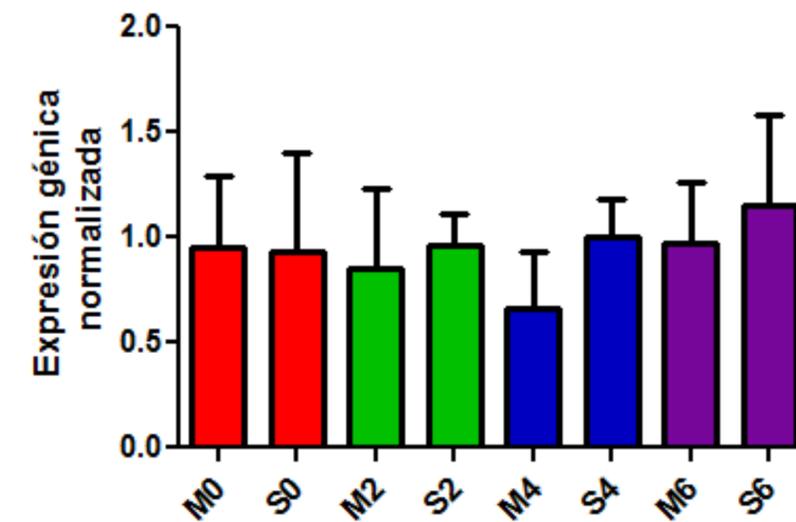
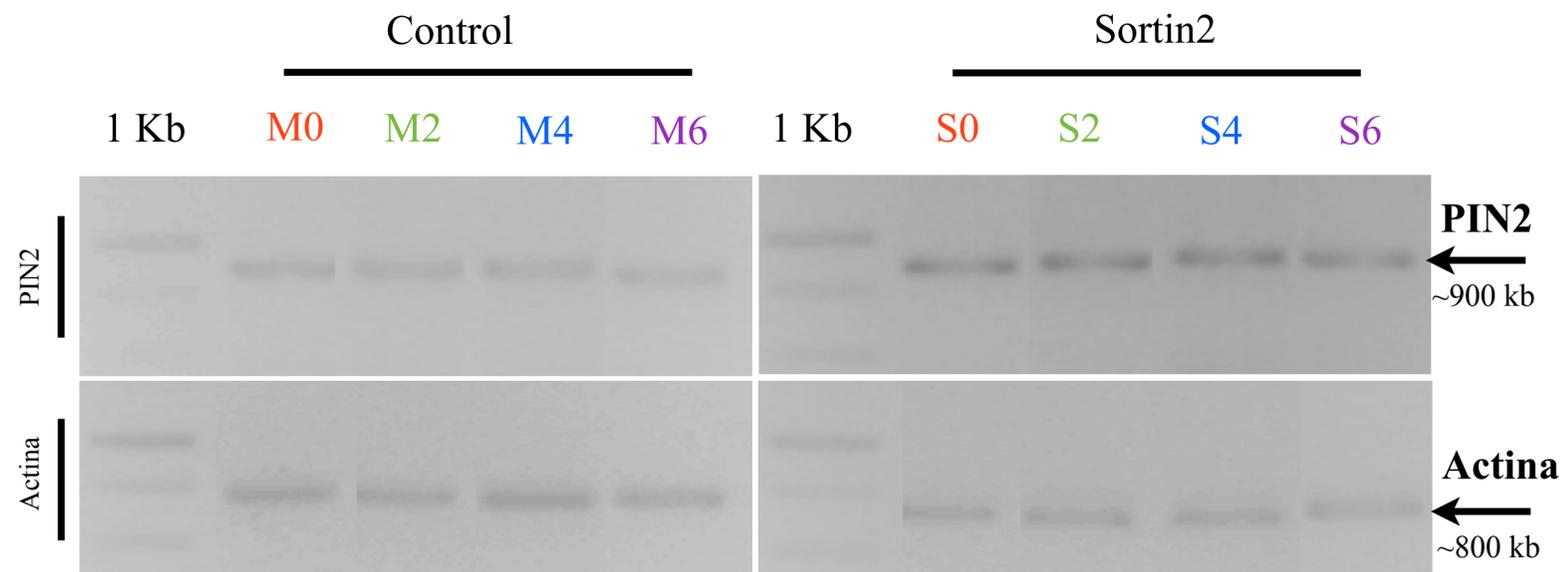
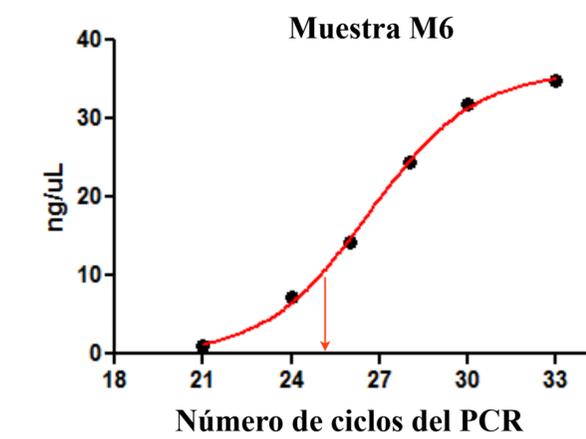
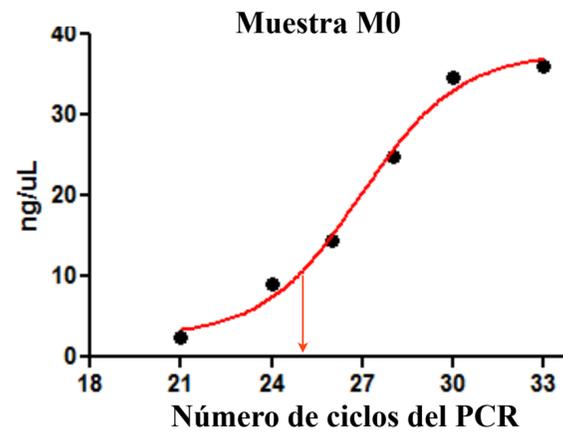
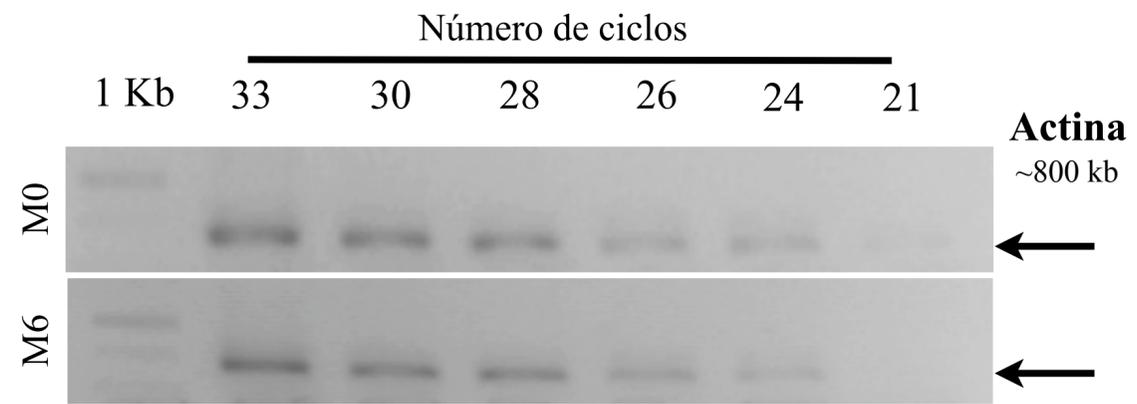
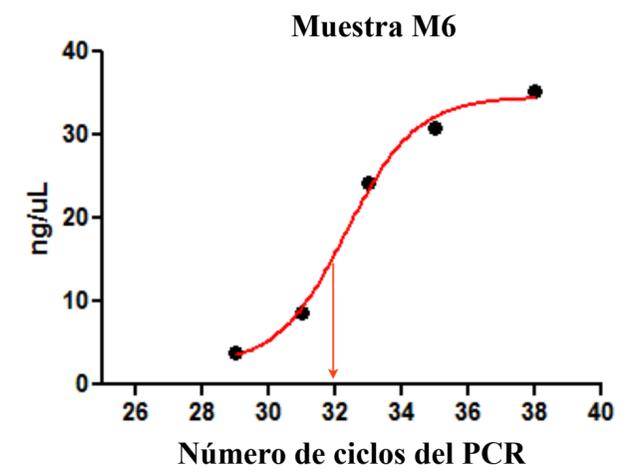
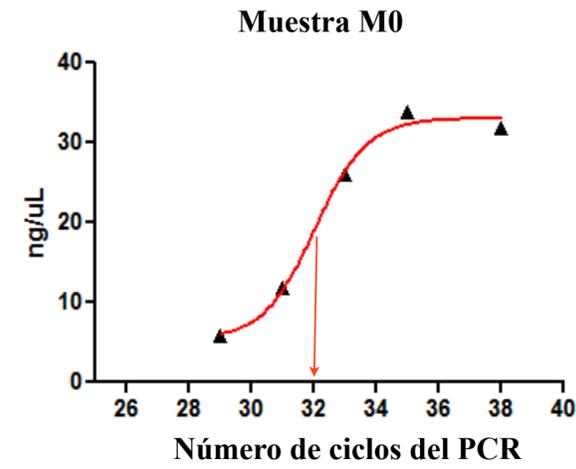
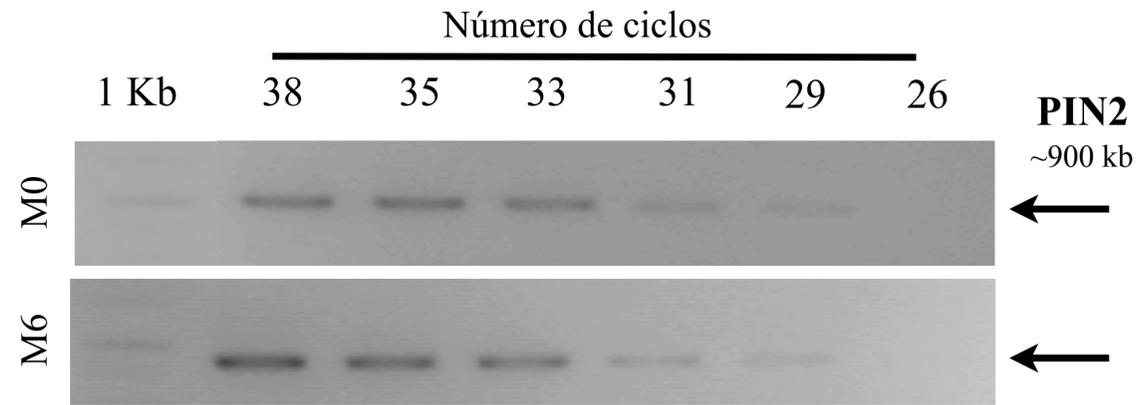


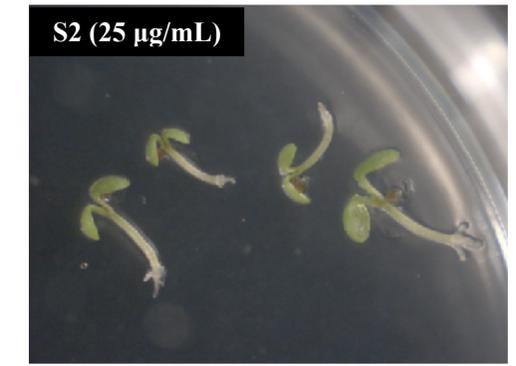
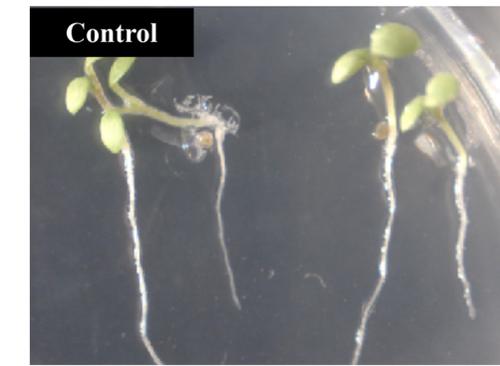
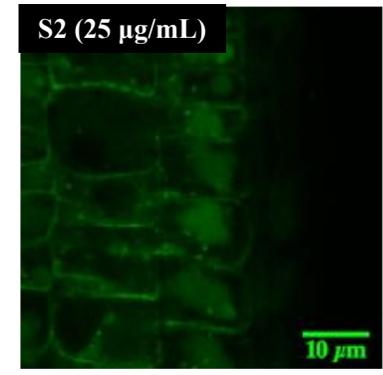
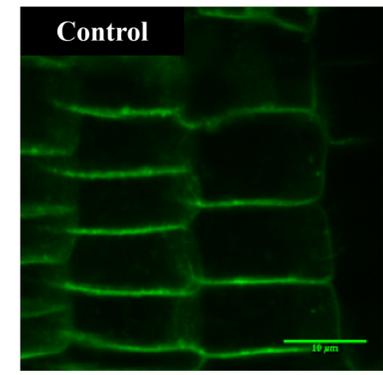
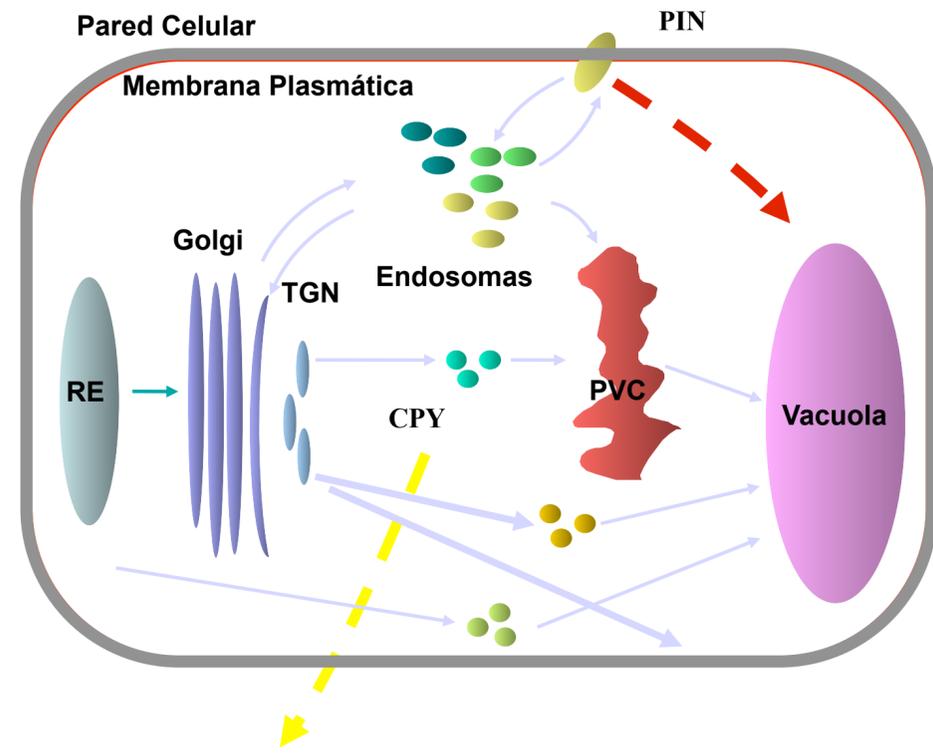
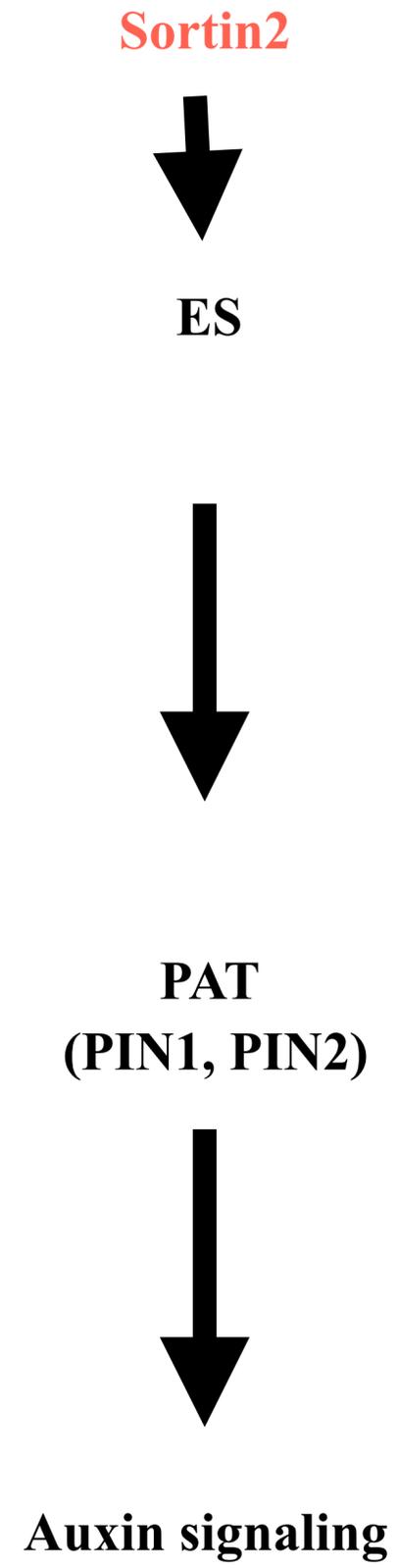
BRI1 and PIN2 are accumulated in vacuoles under Sortin2 treatments

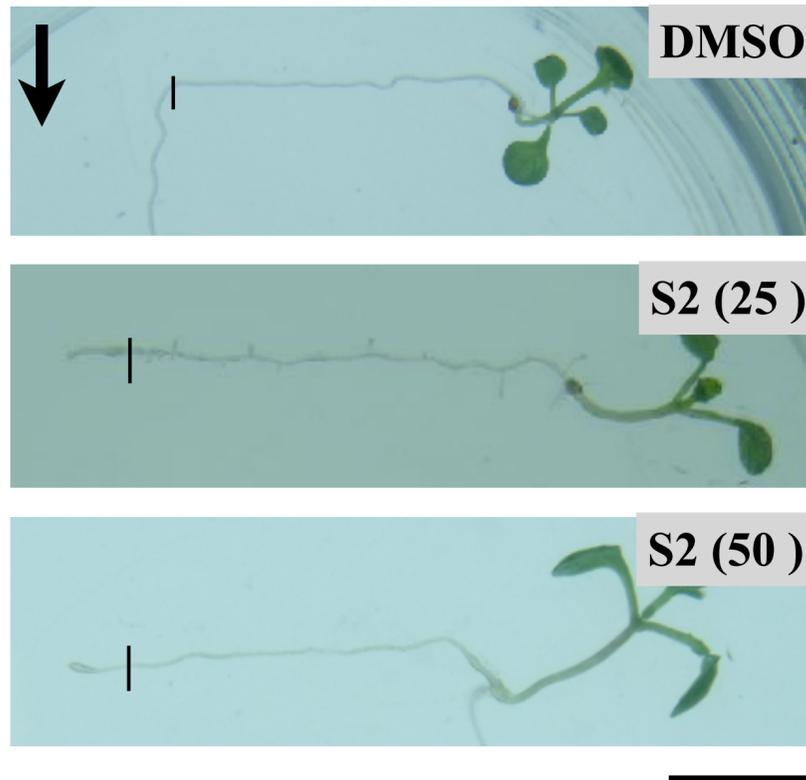
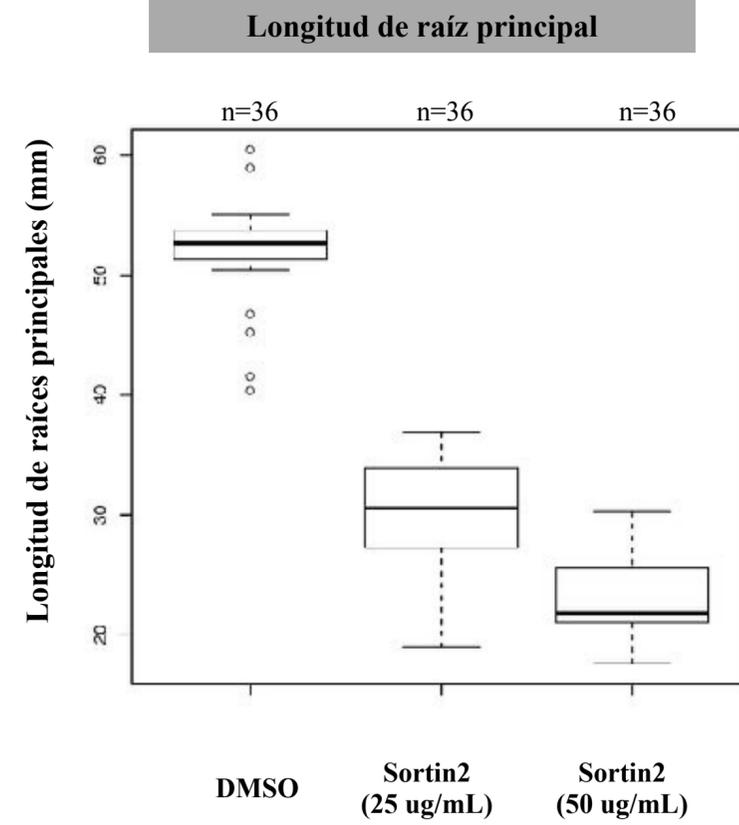
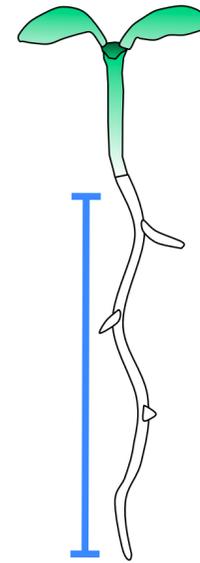
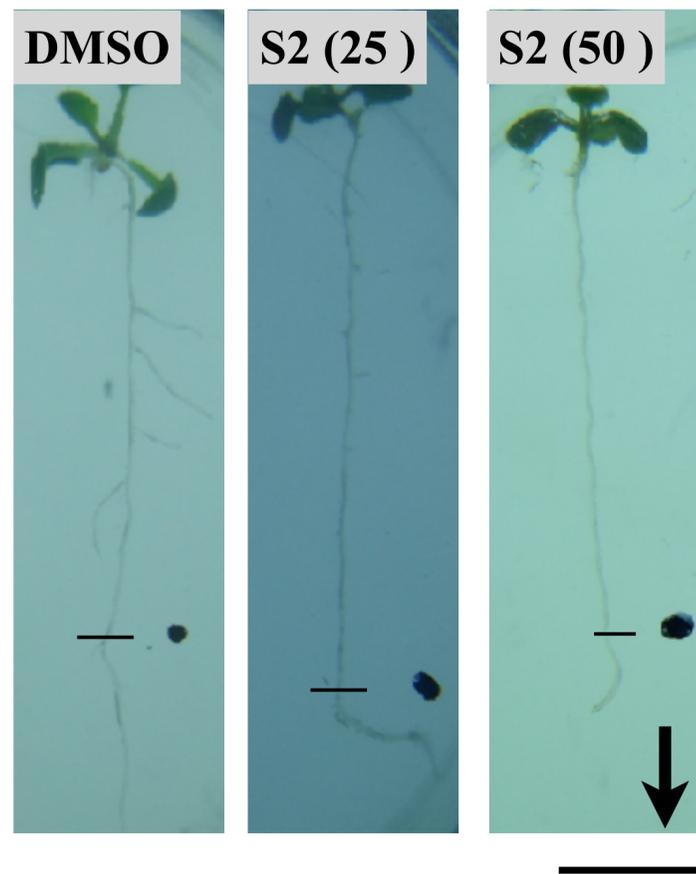


Sortin2 does not alter actin cytoskeleton

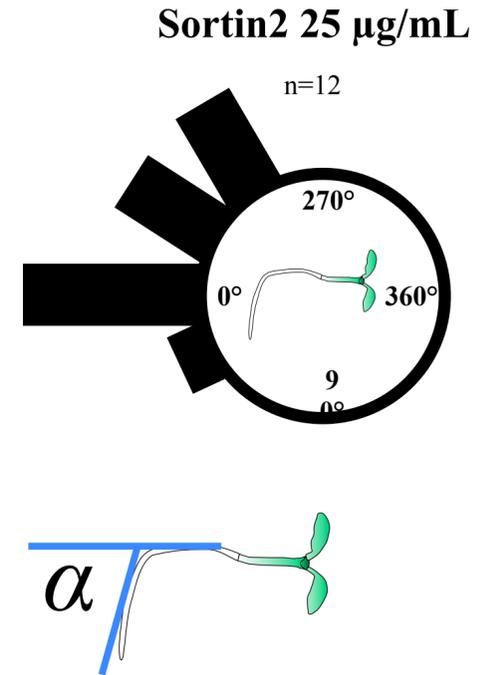
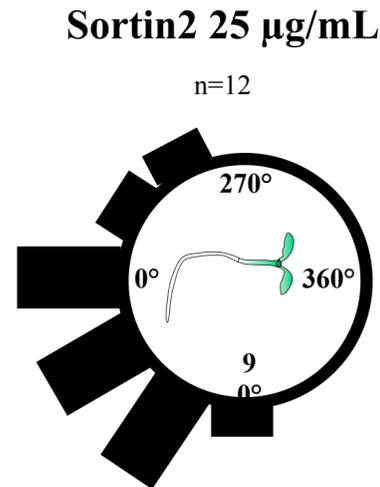
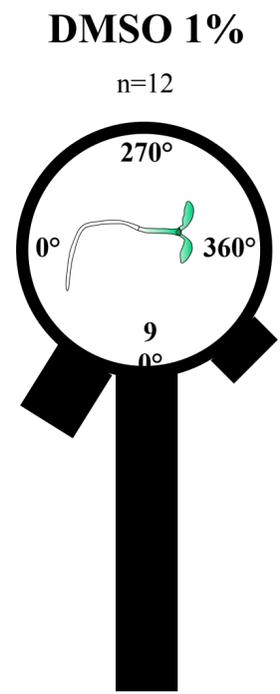




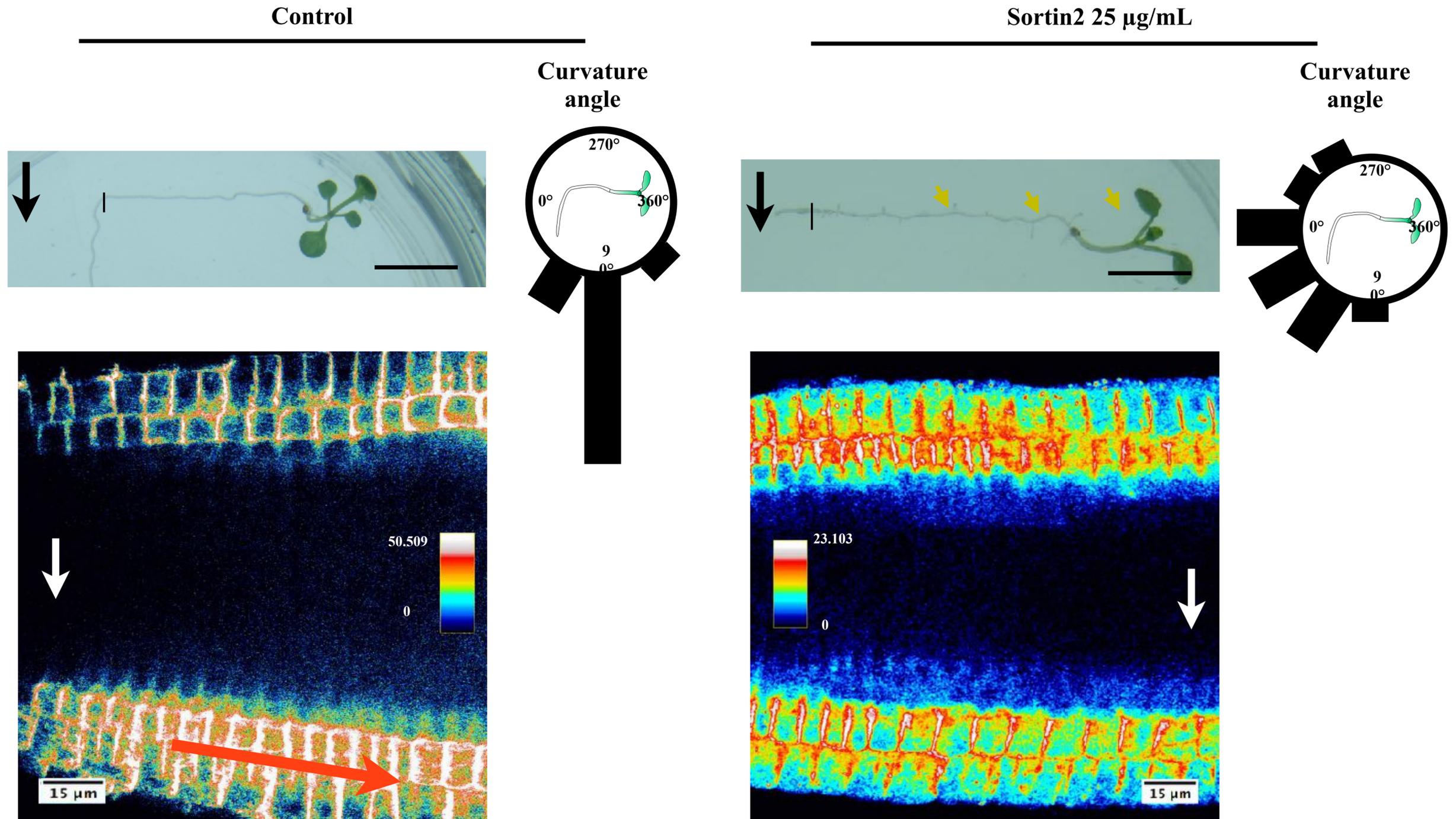




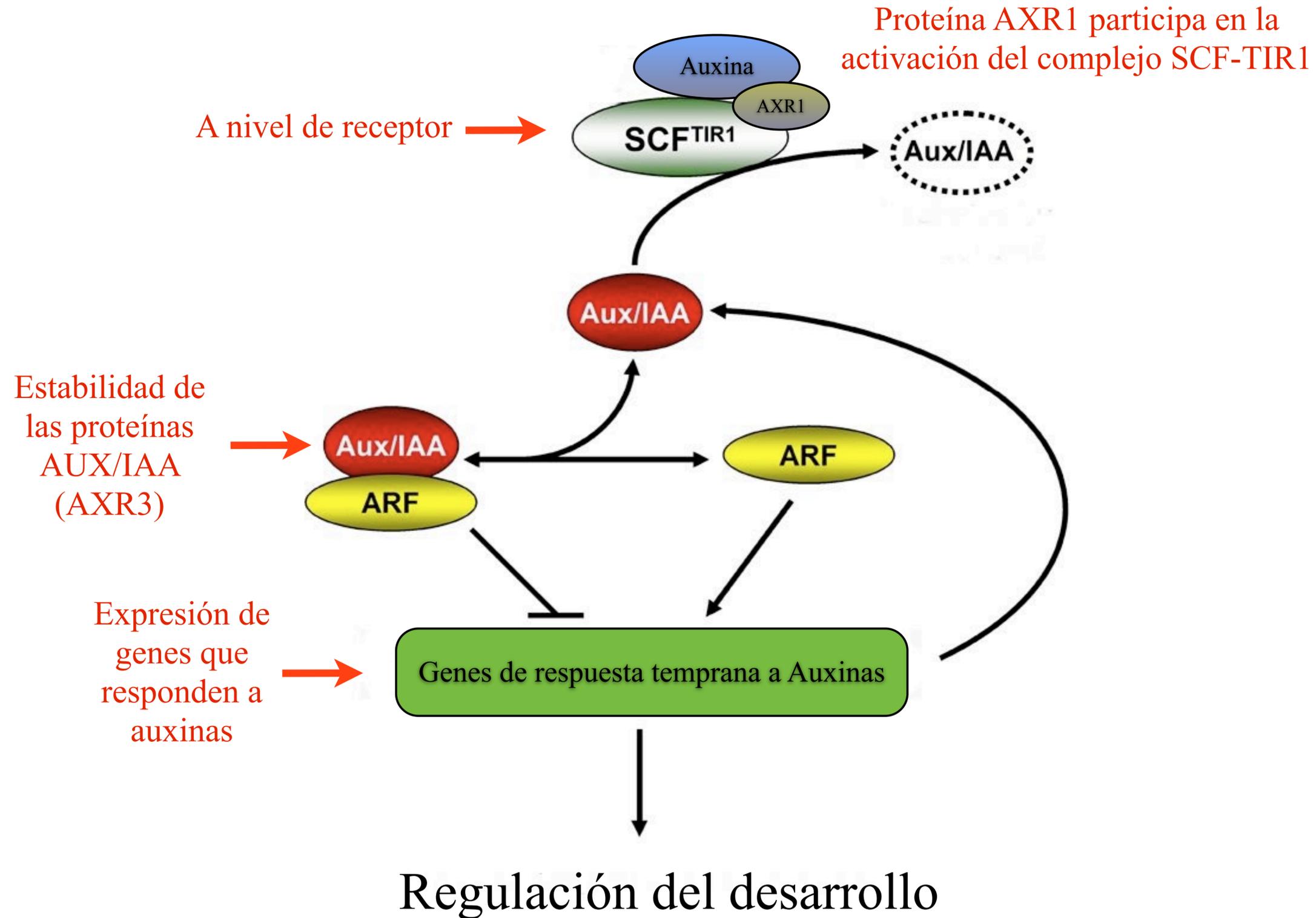
Angulo de curvatura de la raíz



Sortin2 affects gravitropism by altering an auxin transporter differential degradation

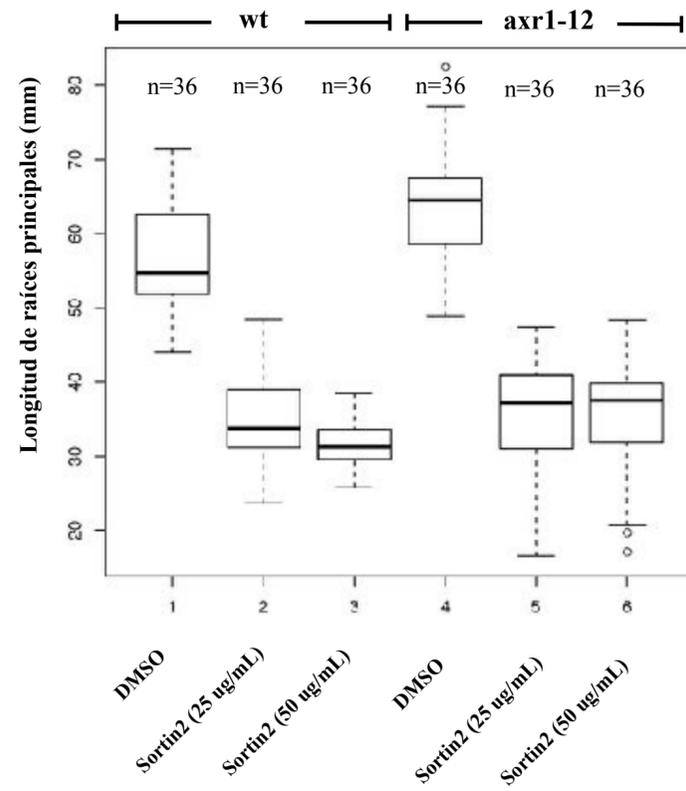
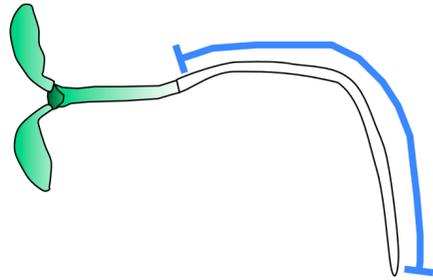


Auxin signaling

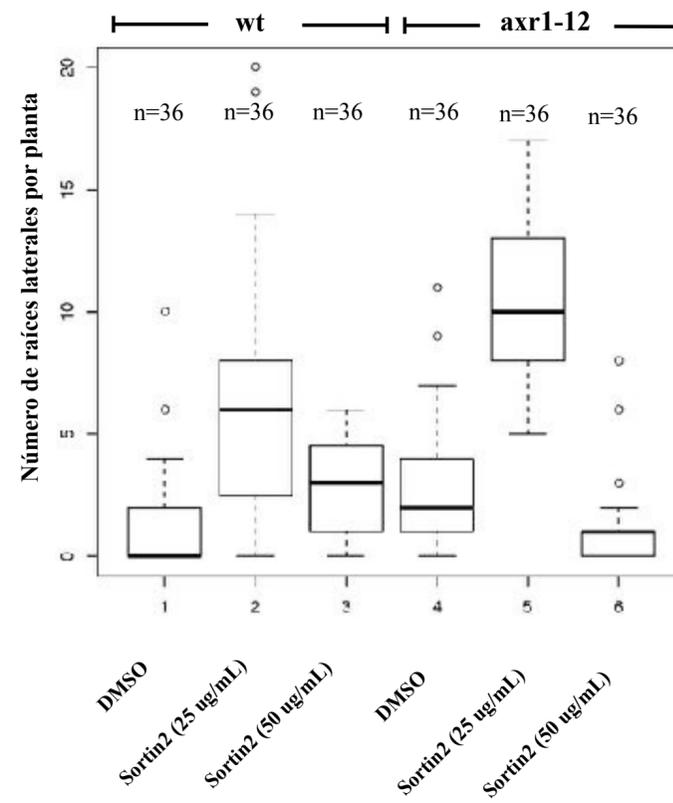
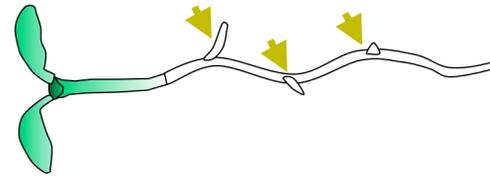


axr1 and wt behave the same under Sortin2 treatment

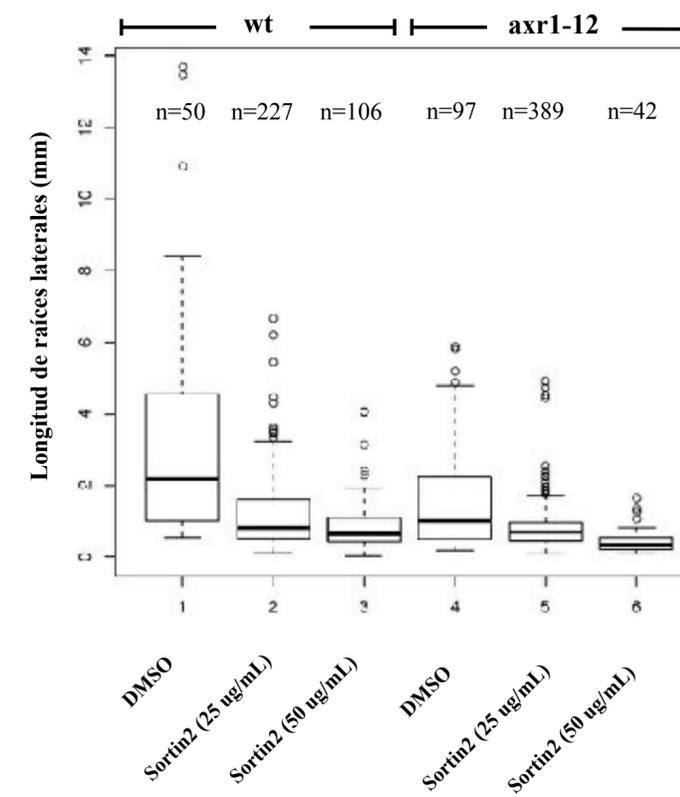
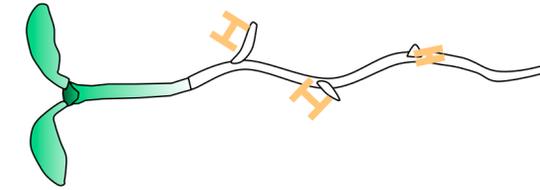
Longitud de raíz principal



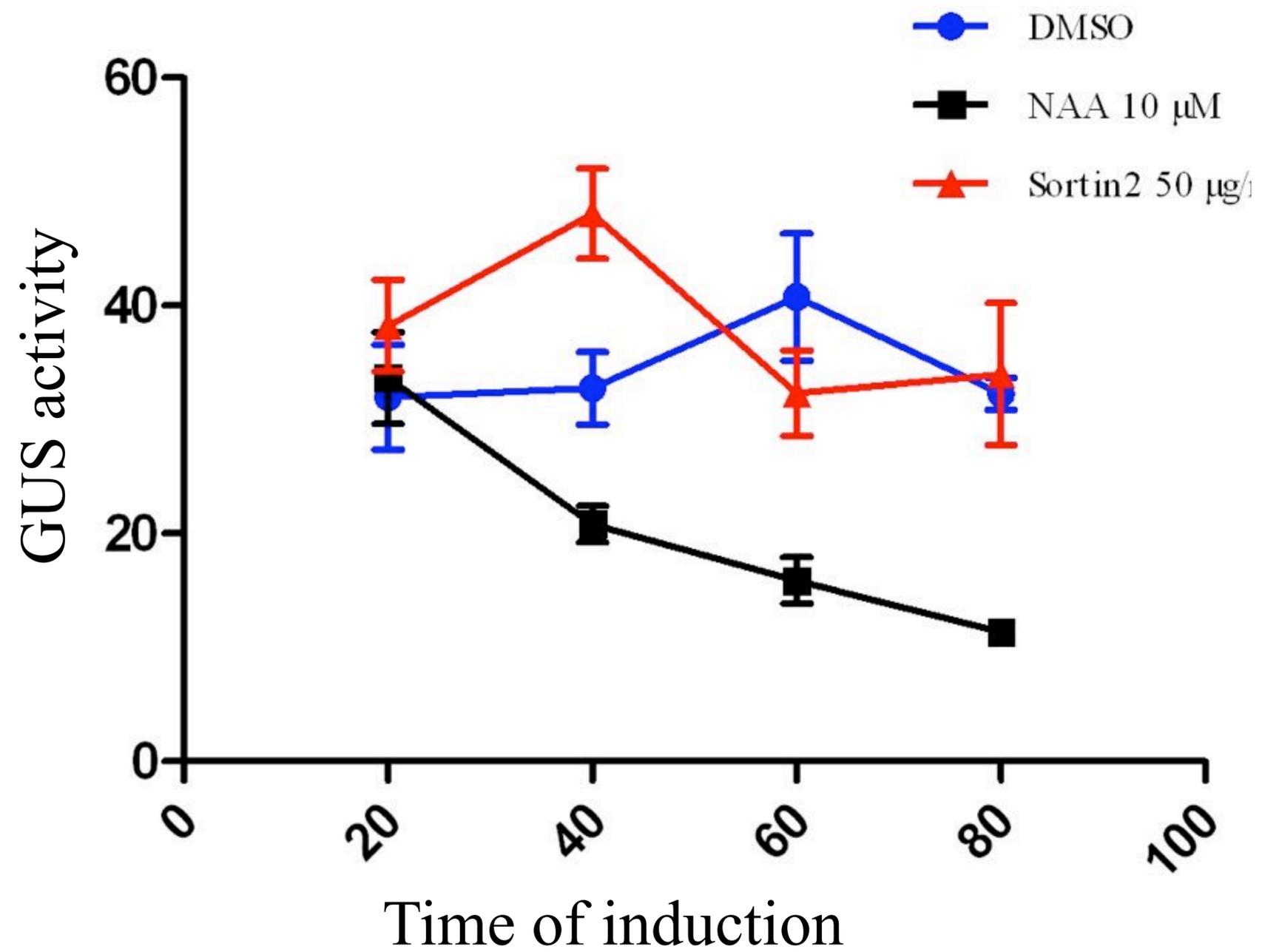
Número de raíces laterales por planta

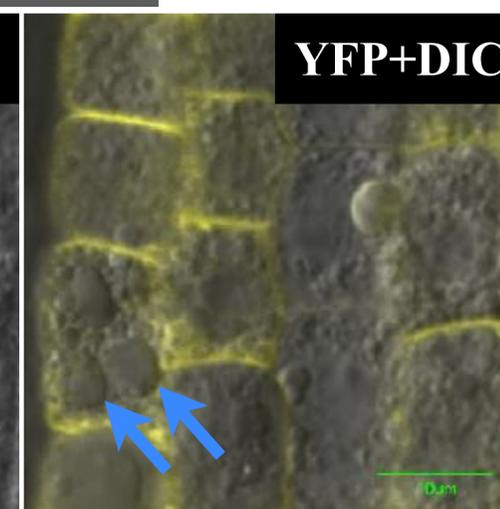
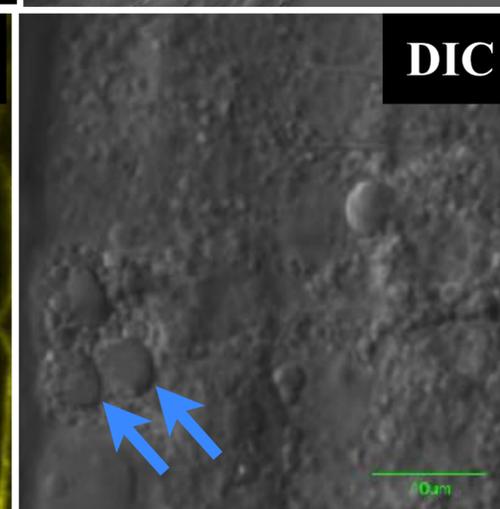
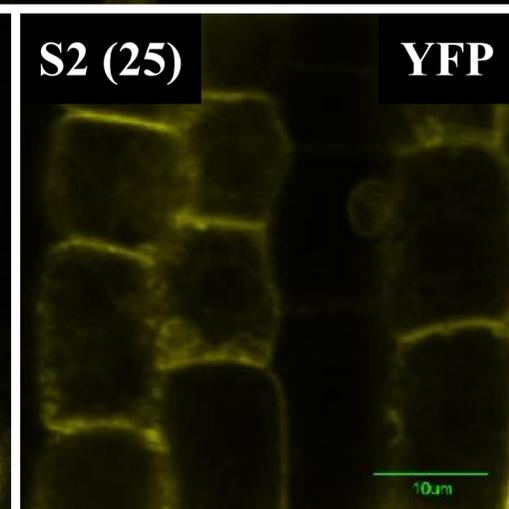
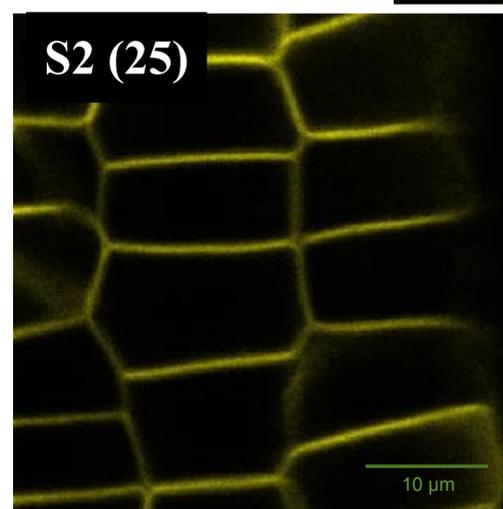
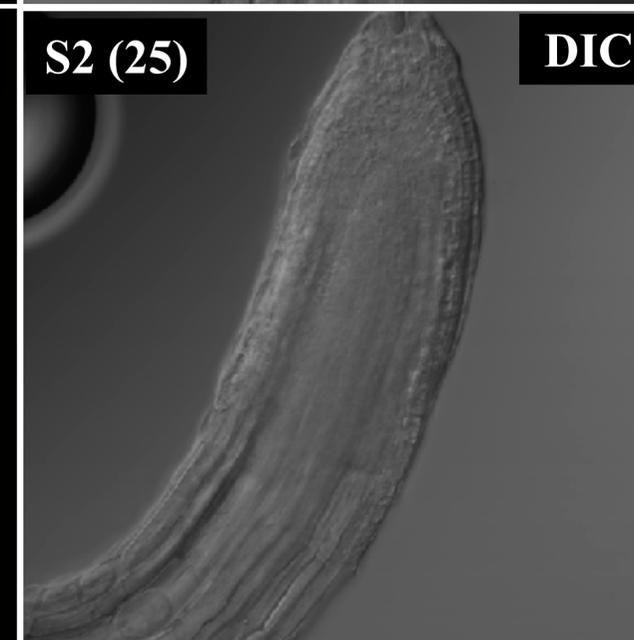
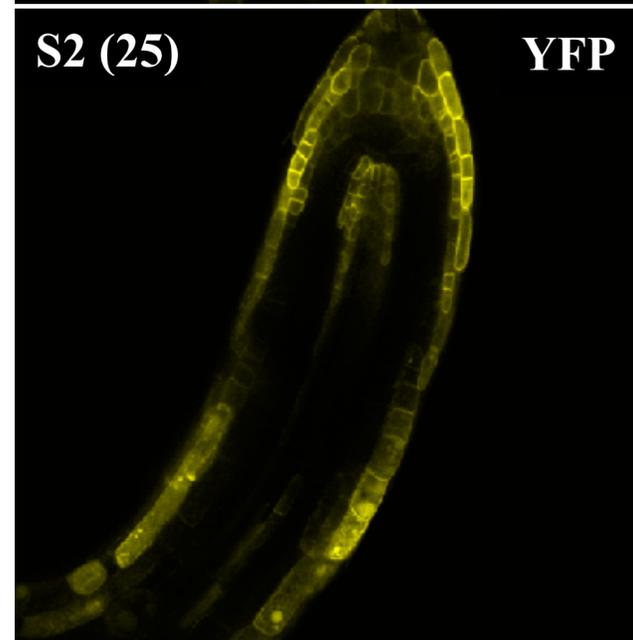
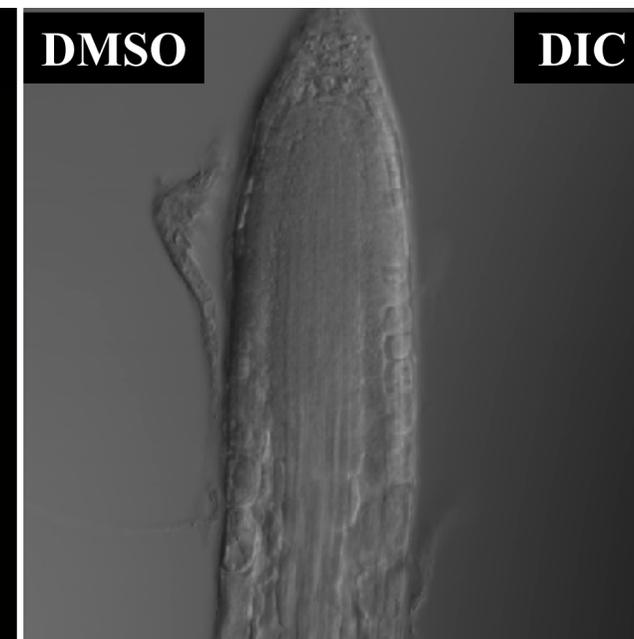
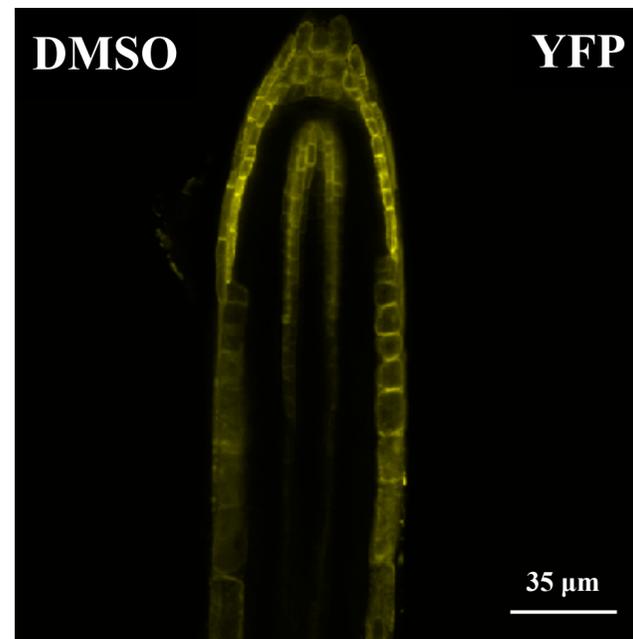


Longitud de raíz lateral



Soritin2 does not affect





Sortin2

It *Does* dislocalize PIN2 and PIN1 proteins.

Also receptor BRI1

**It *Does Not* dislocalize AUX1 and PIN7
auxin carriers.**

The Plant Cell, Vol. 18, 3171–3181, November 2006, www.plantcell.org © 2006 American Society of Plant Biologists

Subcellular Trafficking of the *Arabidopsis* Auxin Influx Carrier AUX1 Uses a Novel Pathway Distinct from PIN1 ^W

Jürgen Kleine-Vehn,^a Pankaj Dhonukshe,^a Ranjan Swarup,^b Malcolm Bennett,^b and Jiří Friml^{a,1}

^aCenter for Molecular Biology of Plants, University of Tübingen, D-72076 Tübingen, Germany

^bPlant Sciences Division, School of Biosciences, University of Nottingham, Loughborough LE12 5RD, United Kingdom

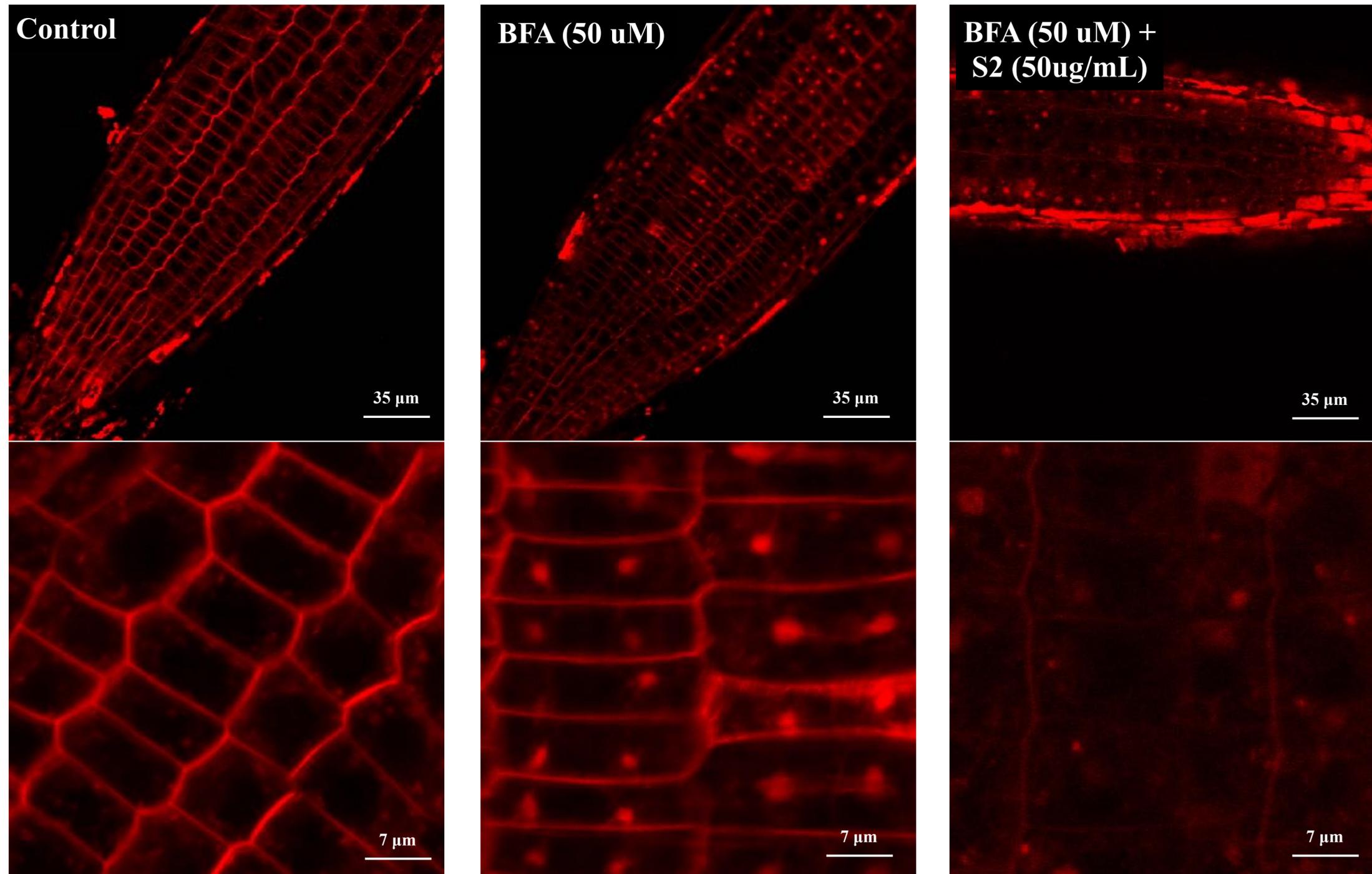
Endosidin1 defines a compartment involved in endocytosis of the brassinosteroid receptor BRI1 and the auxin transporters PIN2 and AUX1

Stéphanie Robert, S. Narasimha Chary, Georgia Drakakaki, Shundai Li*, Zhenbiao Yang, Natasha V. Raikhel,
and Glenn R. Hicks[†]

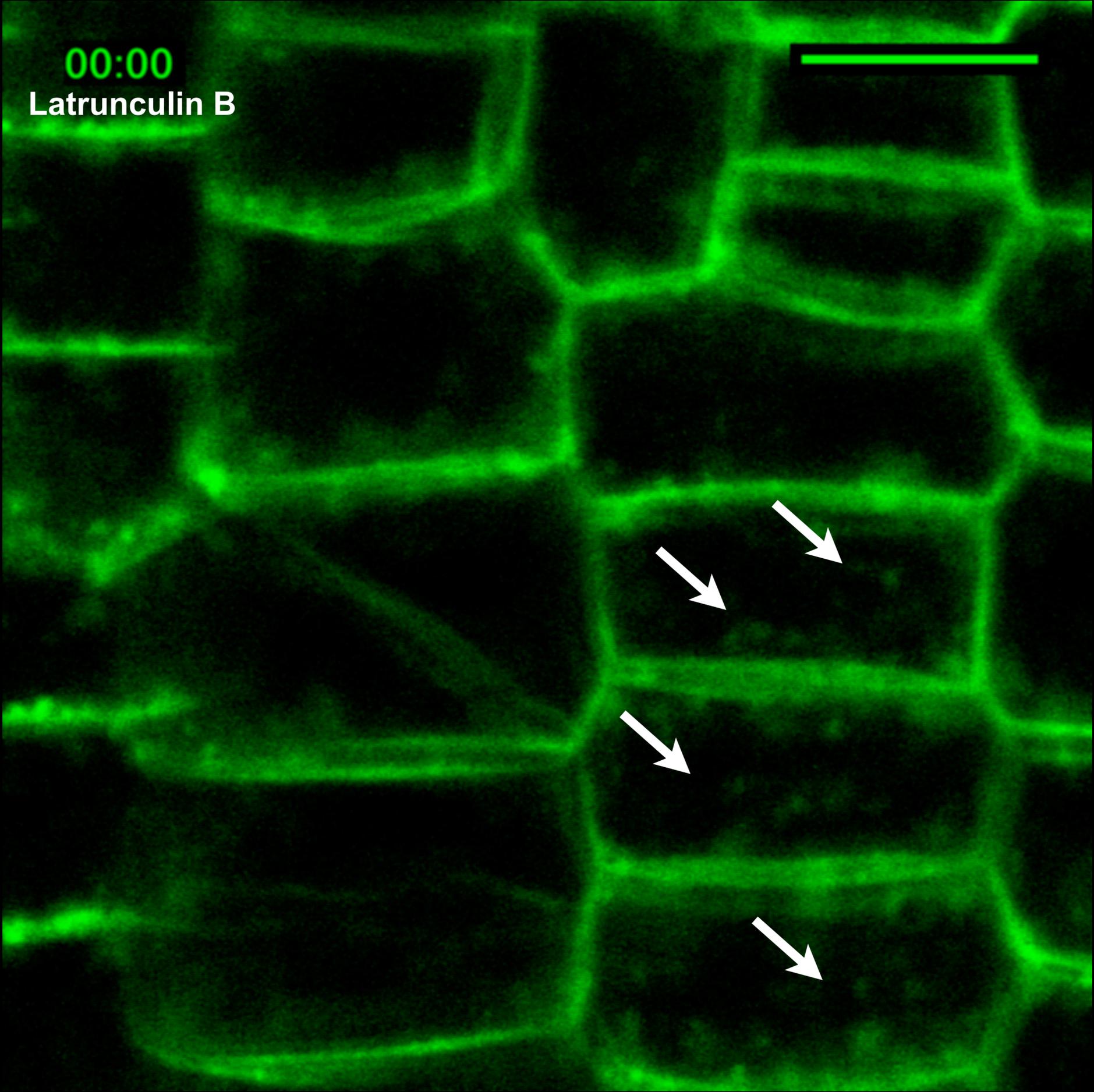
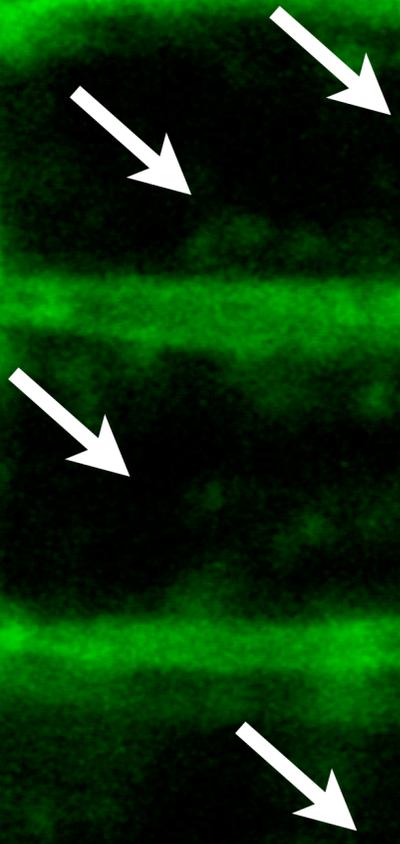
Center for Plant Cell Biology, Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, University of California,
Riverside, CA 92521

Edited by Joanne Chory, Salk Institute for Biological Studies, La Jolla, CA, and approved April 1, 2008 (received for review December 10, 2007)

BFA bodies are not disgregating by Sortin2

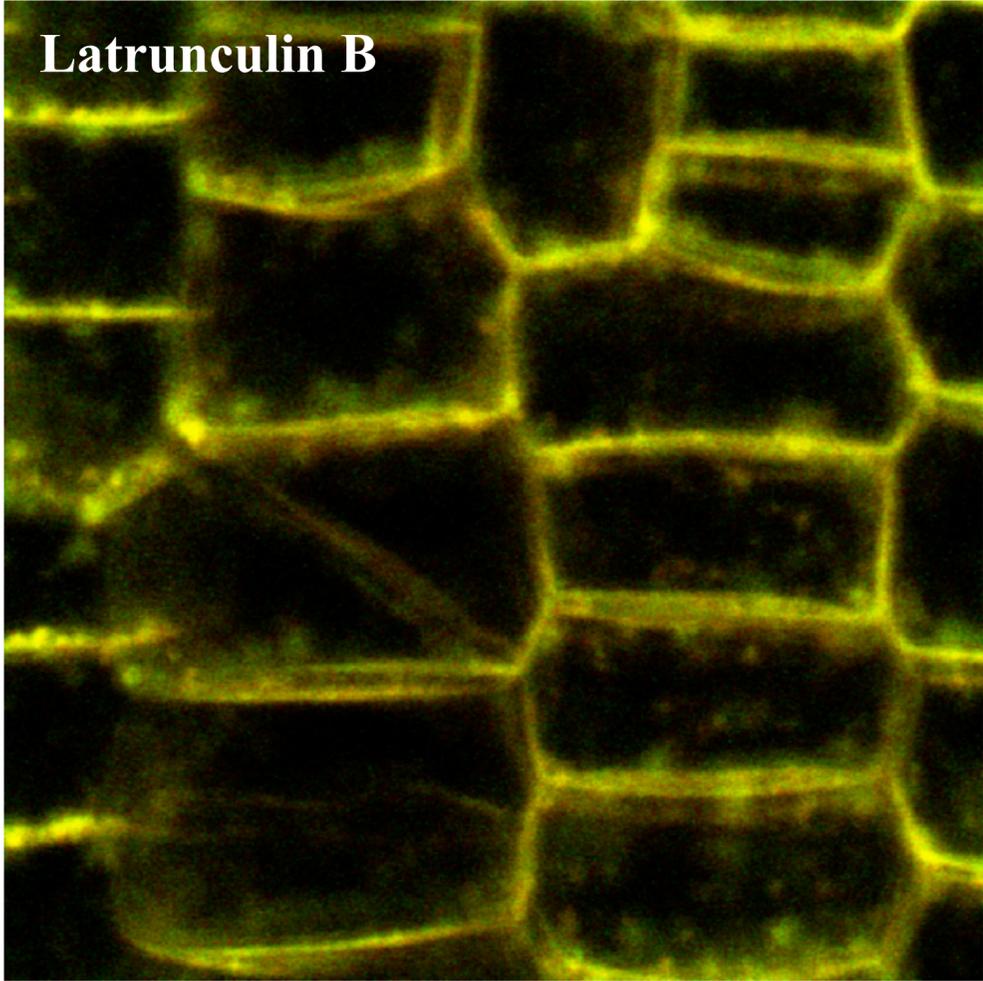


00:00
Latrunculin B

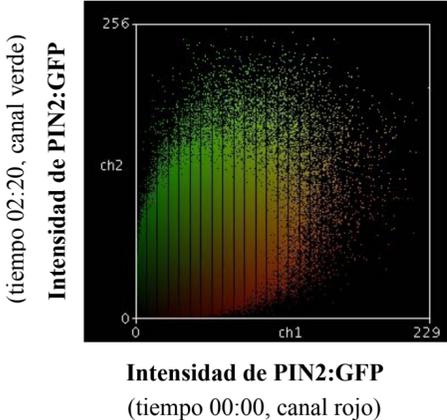
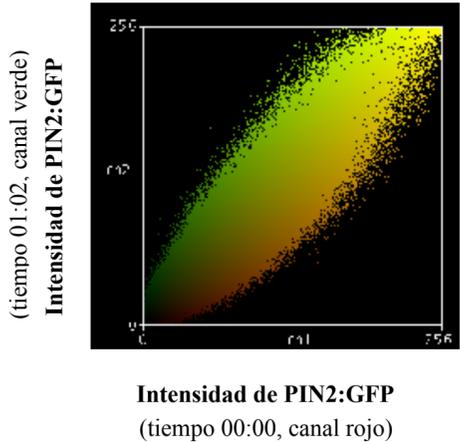


Sortin does not alter actin cytoskeleton

frame 1 (00:00)/frame 6 (01:02)

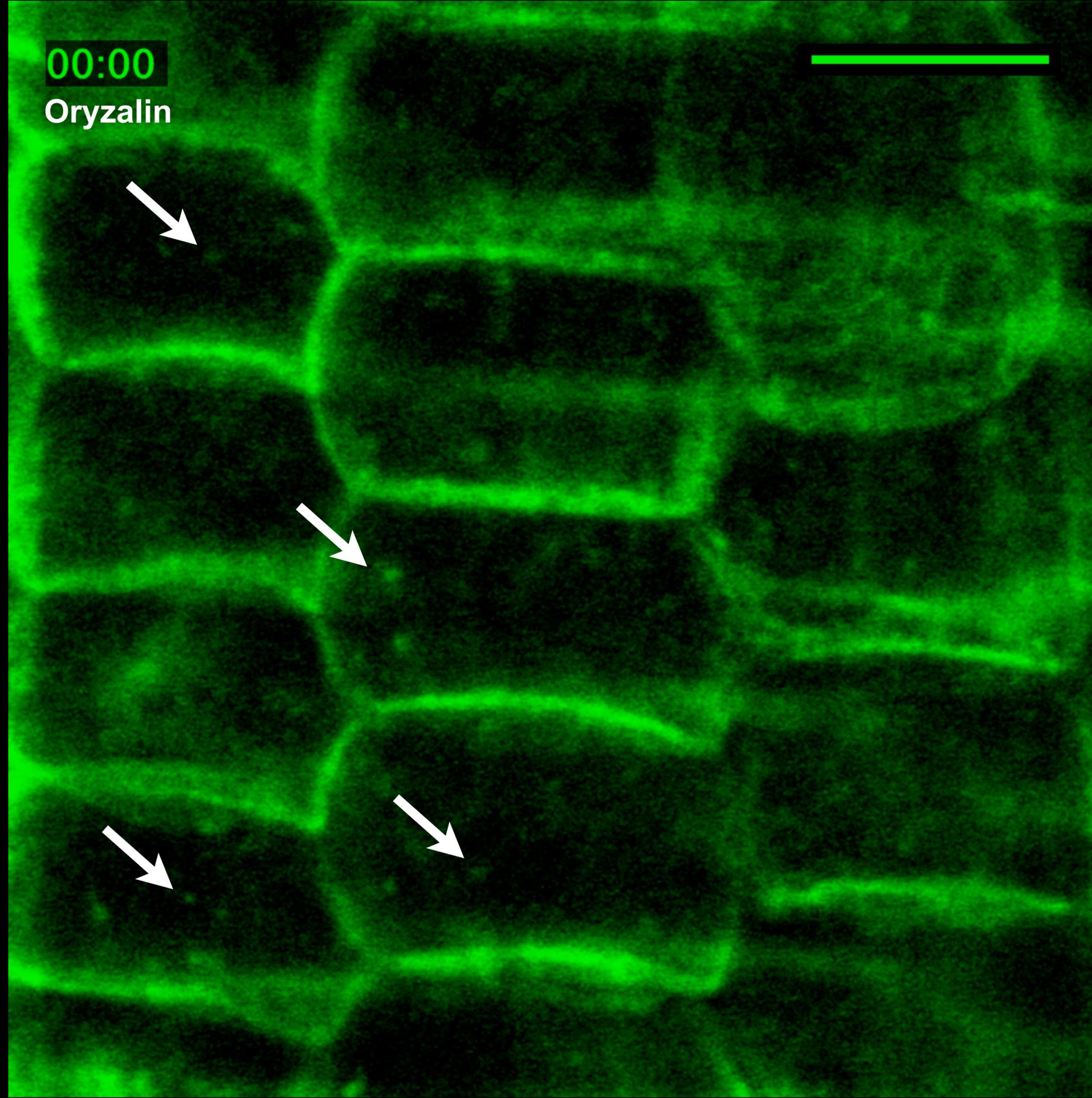


frame 1 (00:00)/frame 6 (02:20)



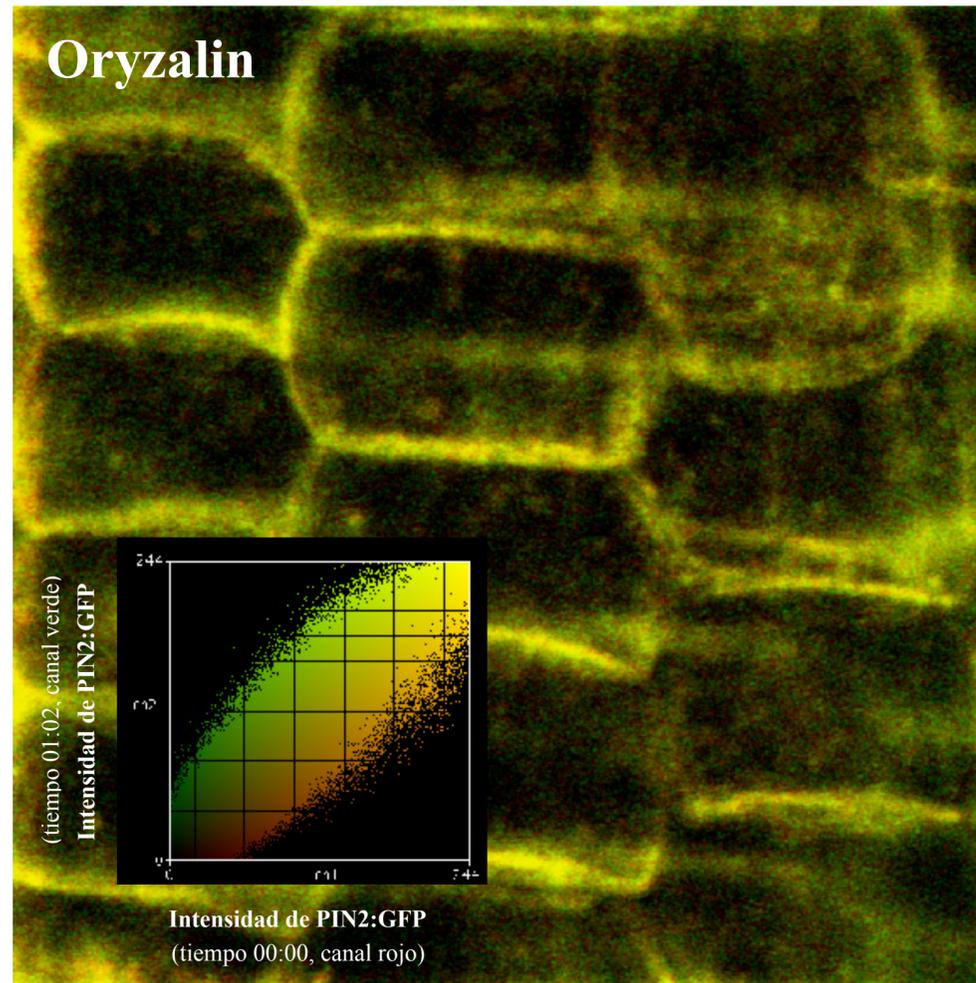
00:00

Oryzalin

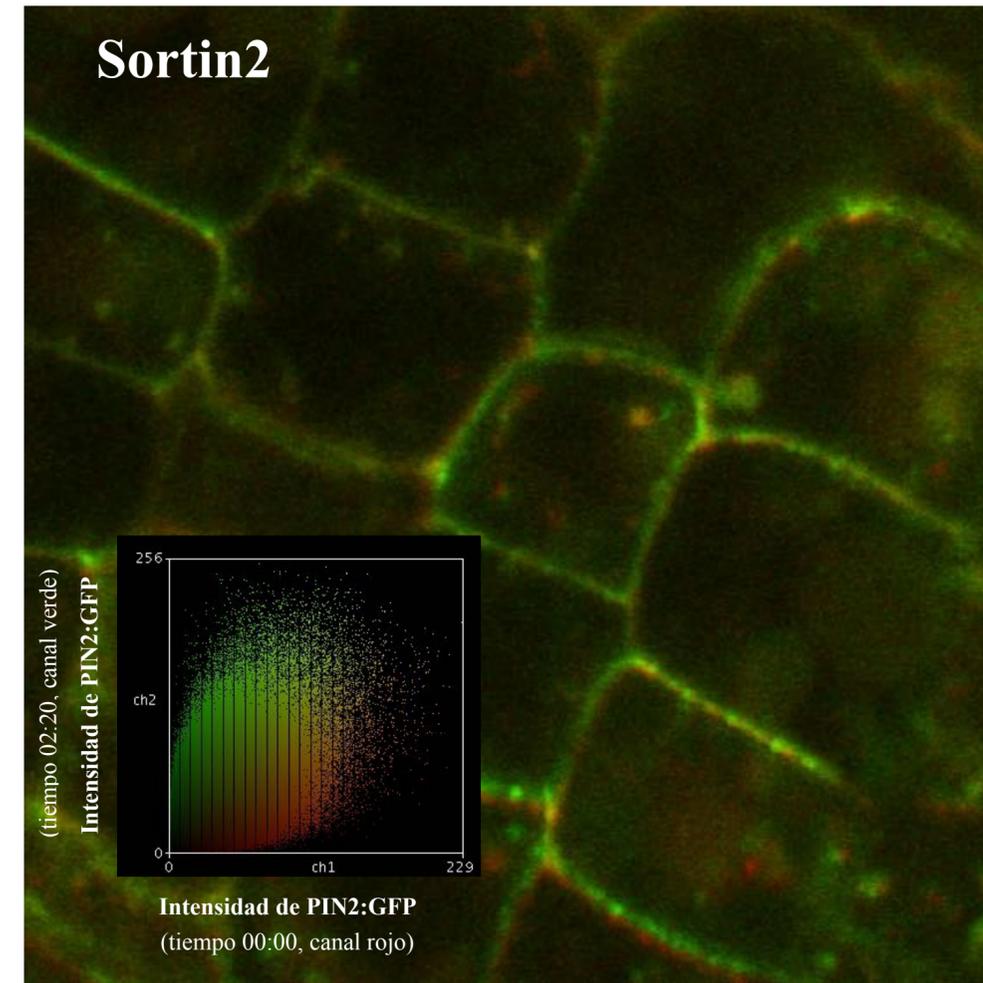


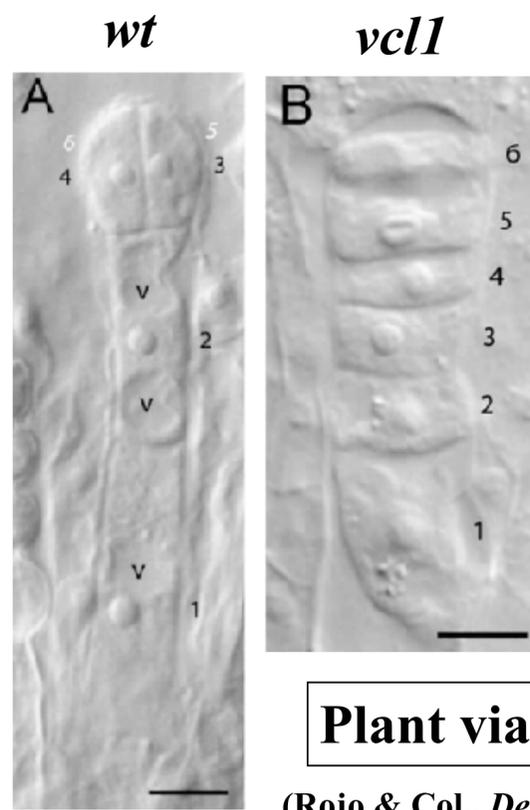
Sortin2 does not alter microtubule cytoskeleton as oryzalin

frame 1 (00:00)/frame 6 (01:02)

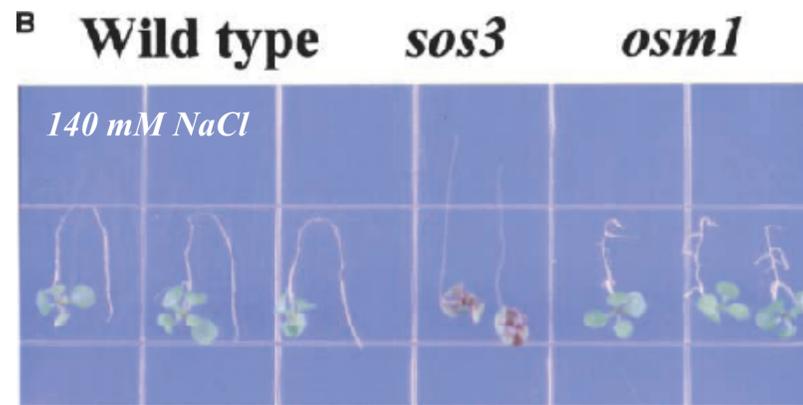


frame 1 (00:00)/frame 6 (02:20)





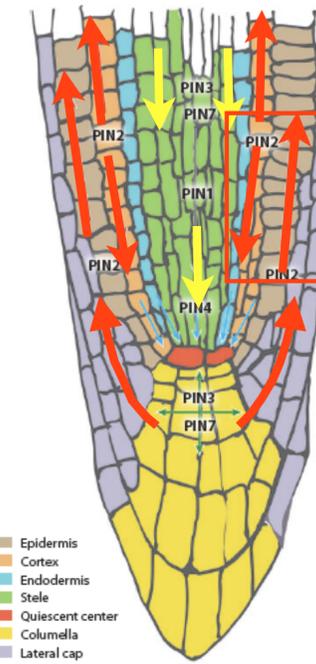
Plant viability
(Rojo & Col., *Dev Cell*, 2001)



Stress response

(Zhu *et al*, *Plant Cell*, 2002)

Intracellular traffic and the Endomembrane System

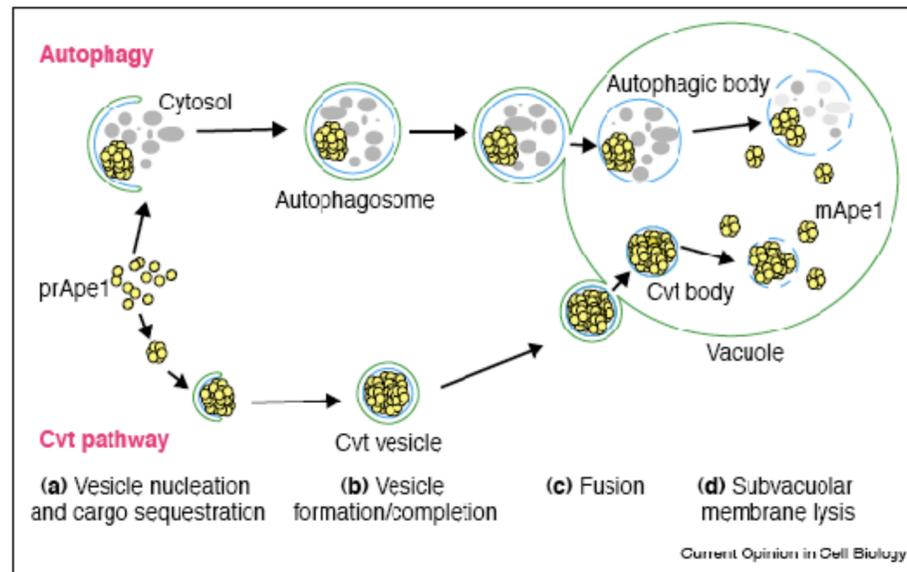


Polar auxin transport

(Kleine-Vhen & Friml, *Ann Rev Cell Dev*, 2008)

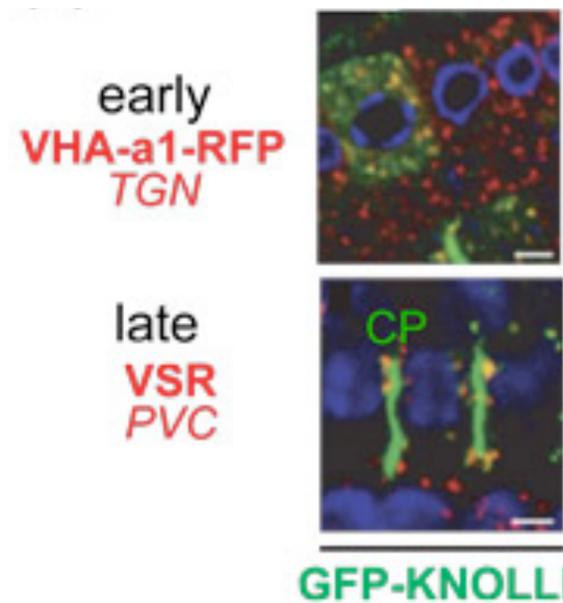
Autophagy

(Khlafan & Klinsky, *Curr Opin Cell Biol*, 2002)



Citokinesis

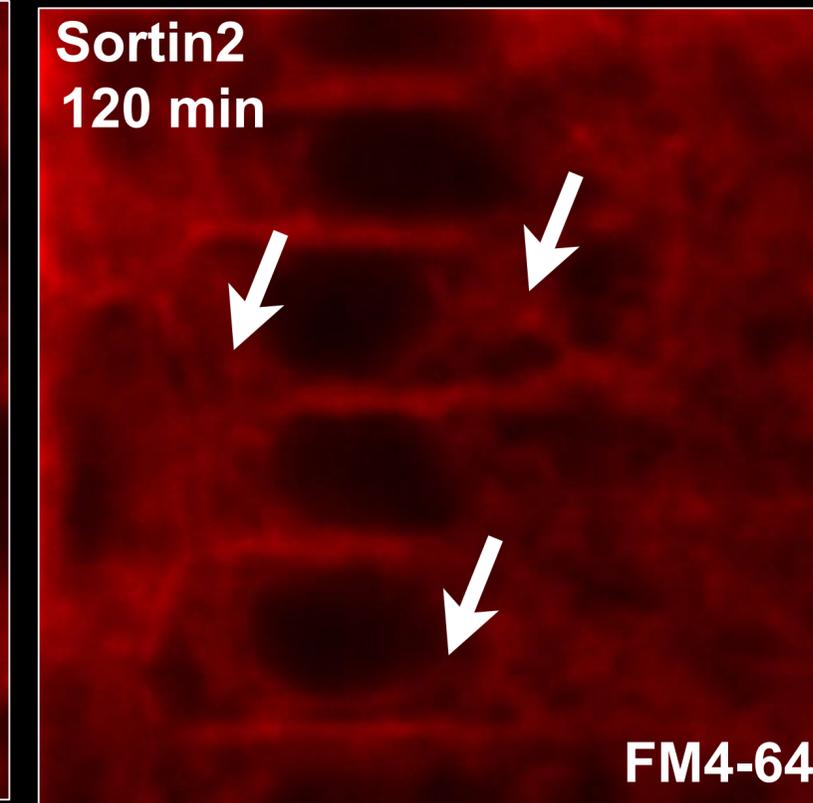
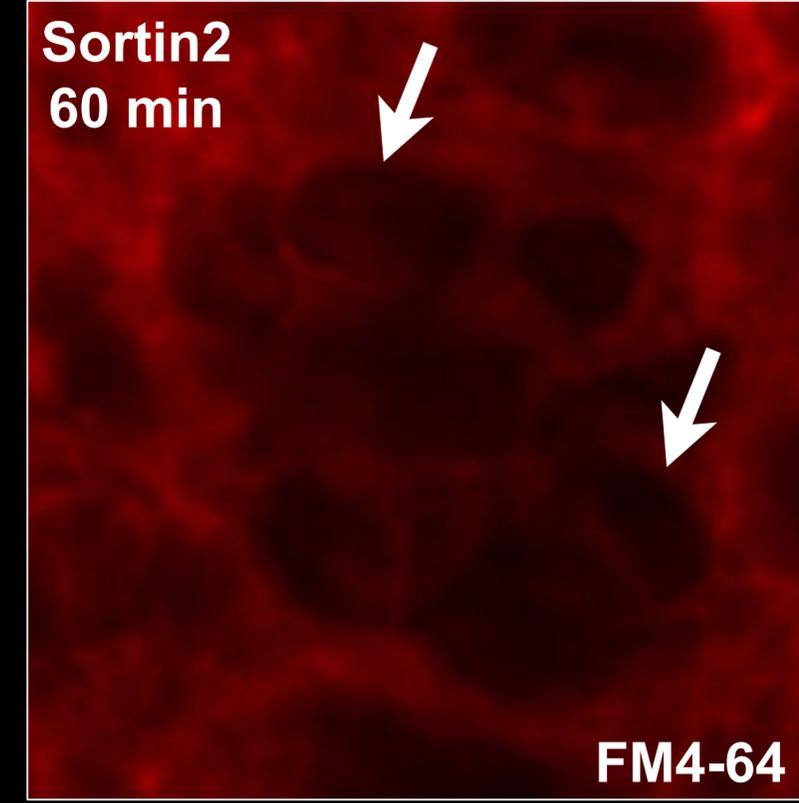
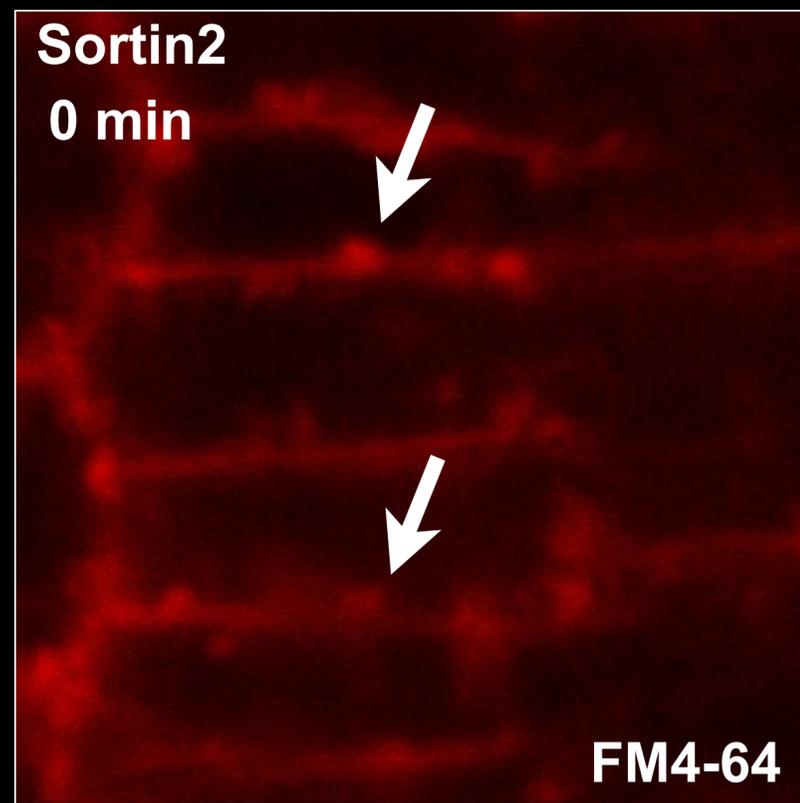
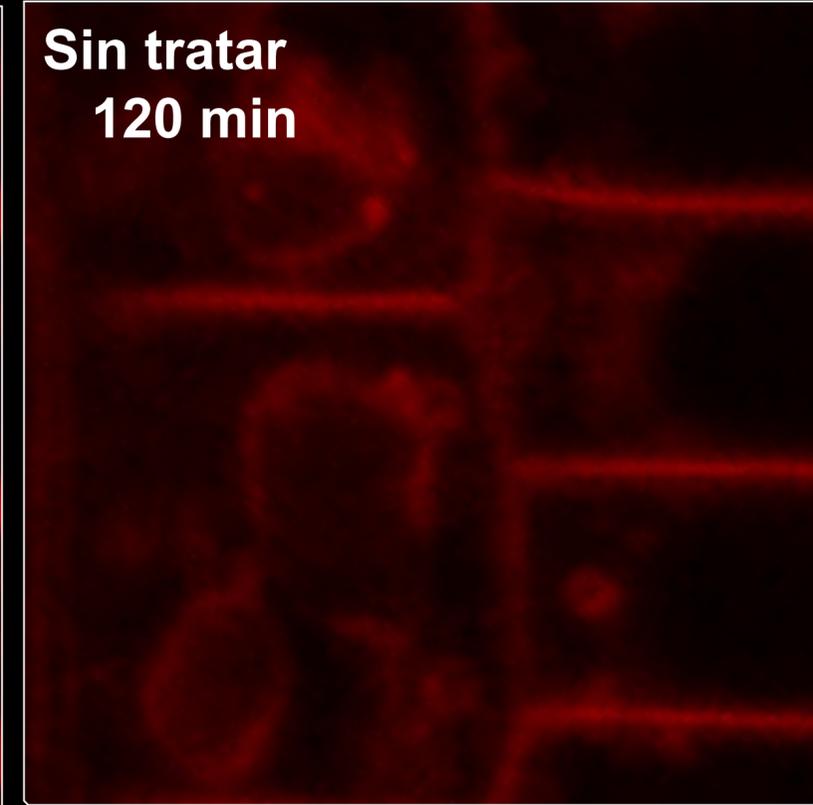
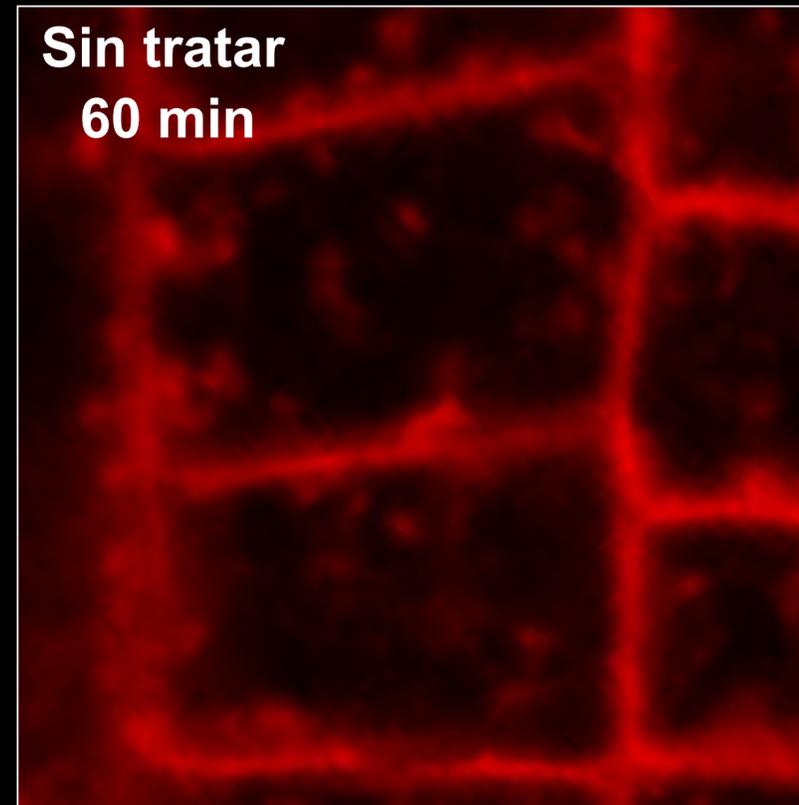
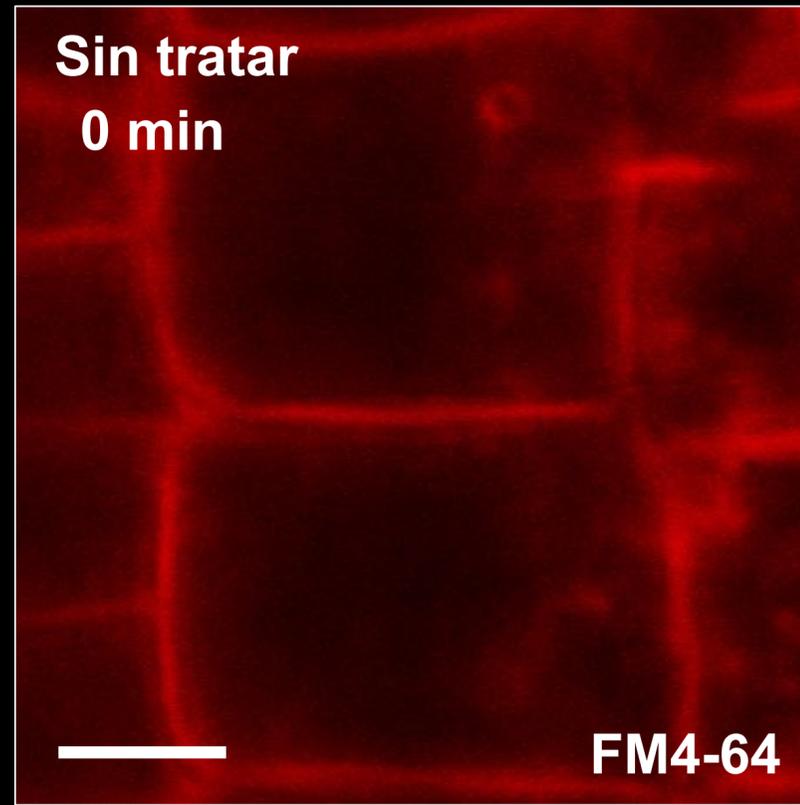
(Reichardt *et al*, *Curr Biol*, 2007)



Gravitropism

(Venneste & Friml, *Cell*, 2010)





Sortin2 altera degradación diferencial de PIN2 bajo estímulo gravitrópico

