A fluorescence microscopy image of an Arabidopsis root. The cytoskeleton is visualized in green, showing a dense network of actin filaments and microtubules. Several bright orange-red spots are visible, likely representing specific organelles or protein localizations. The root is oriented vertically, with the root tip at the bottom.

# A Pair of Trans-Golgi Network/Early Endosome-Localized Proteins Facilitate Cytoskeletal-Mediated Root Skewing in *Arabidopsis thaliana*

J. Alan Sparks<sup>1</sup> and Elison B. Blancaflor<sup>2</sup>

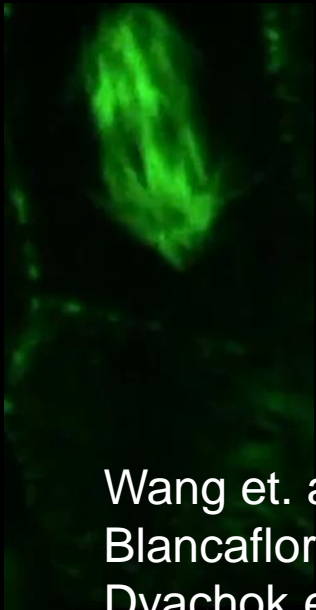
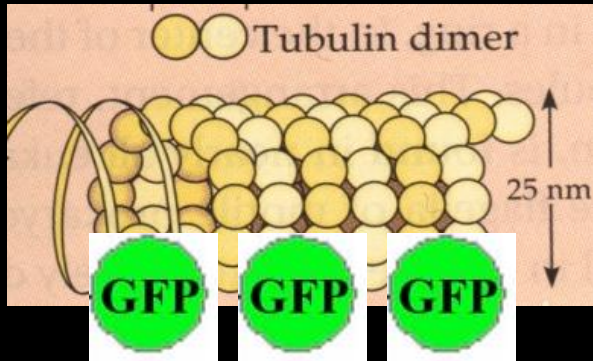
<sup>1</sup>*Noble Research Institute LLC, Ardmore, OK 73401*

<sup>2</sup>*Utilization & Life Sciences Office, Exploration Research & Technology Programs, NASA Kennedy Space Center 32899*



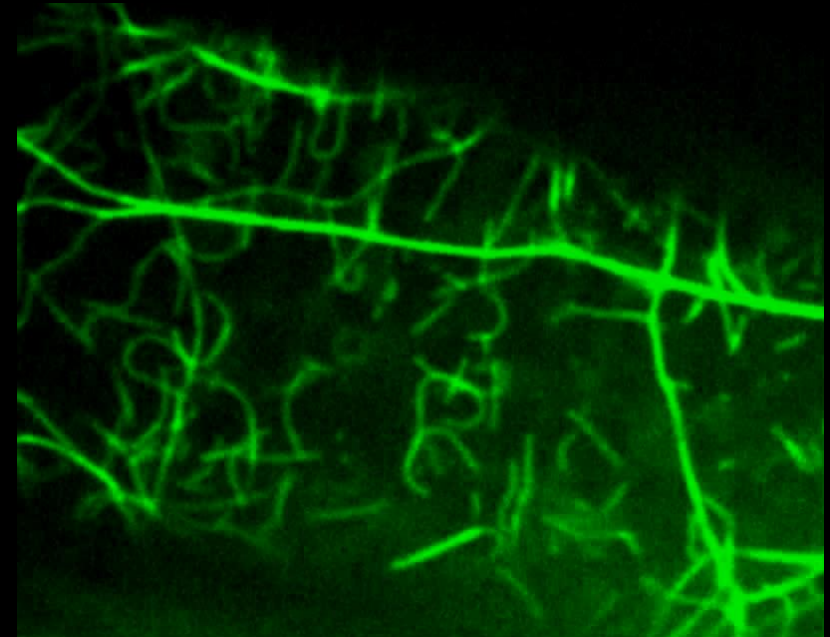
# The cytoskeleton is a network of dynamic filamentous proteins that functions in multiple biological processes

## Microtubules



- Cell division/expansion
- Cell wall remodeling and architecture
- Plant gravity response

## Actin filaments, microfilaments, or Filamentous-actin (F-actin)



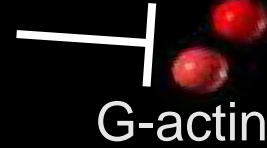
Wang et. al. (2008) *New Phytologist* 177: 525-536  
Blancaflor (2013) *American Journal of Botany* 100:143-152  
Dyachok et. al. (2014) *Cytoskeleton* 71:311-327



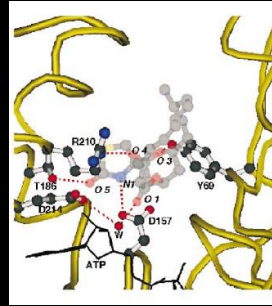
Latrunculins: metabolites from the red sea sponge, *Latrunculia magnifica*, that bind G-actin and prevent polymerization to F-actin



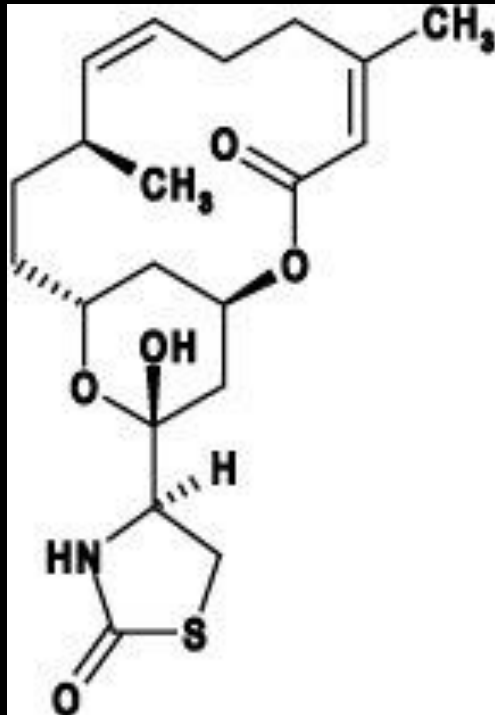
F-actin



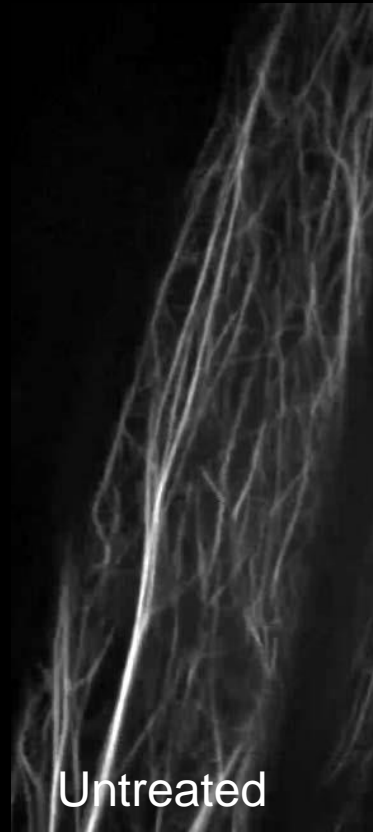
G-actin



Morton et al  
(2000)  
*Nat. Cell Biol.*  
2: 376.



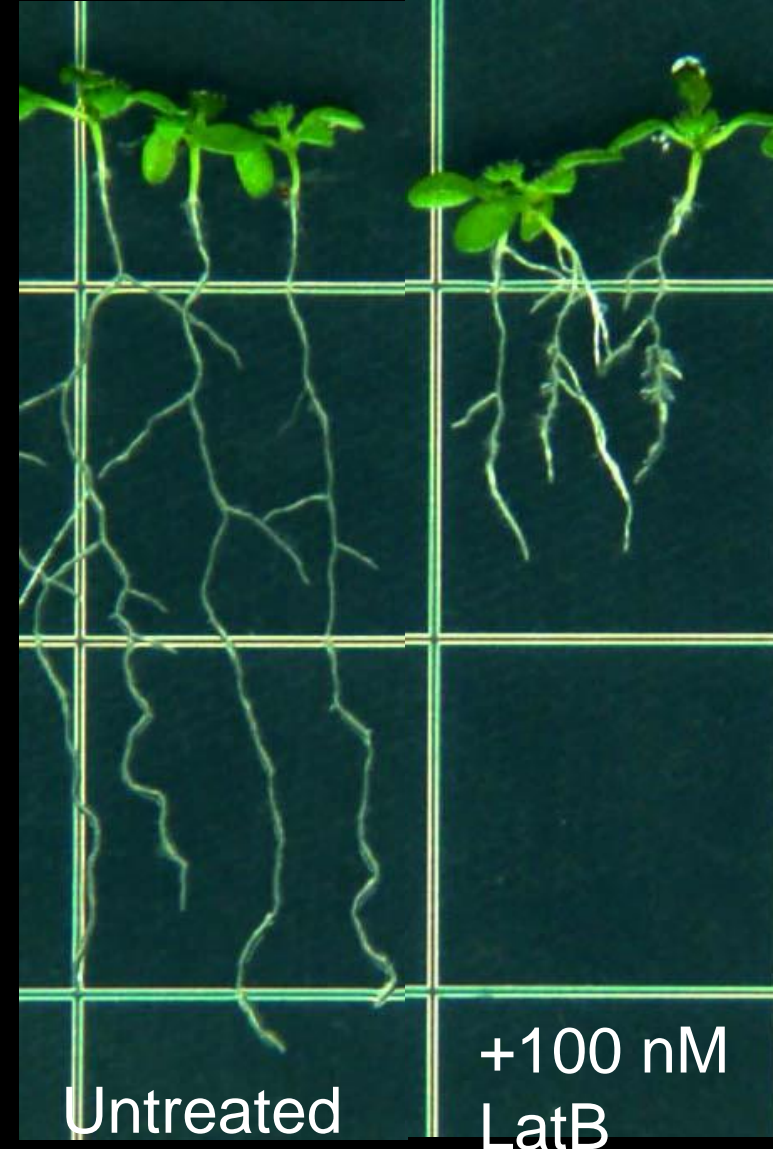
Latrunculin B (LatB)



Untreated



+100 nM  
LatB



Untreated

+100 nM  
LatB



# A forward genetic screen identifies mutants that are hypersensitive to latrunculin B (*hlb*)

Three non-allelic mutants:

- *hlb1* Sparks et. al. (2016) *The Plant Cell* 28:746-769
- *hlb2* Chin et. al. (2021) *The Plant Cell* 33:2131
- *hlb3* Sun et. al. (2019) *Frontiers in Genetics* 10:685



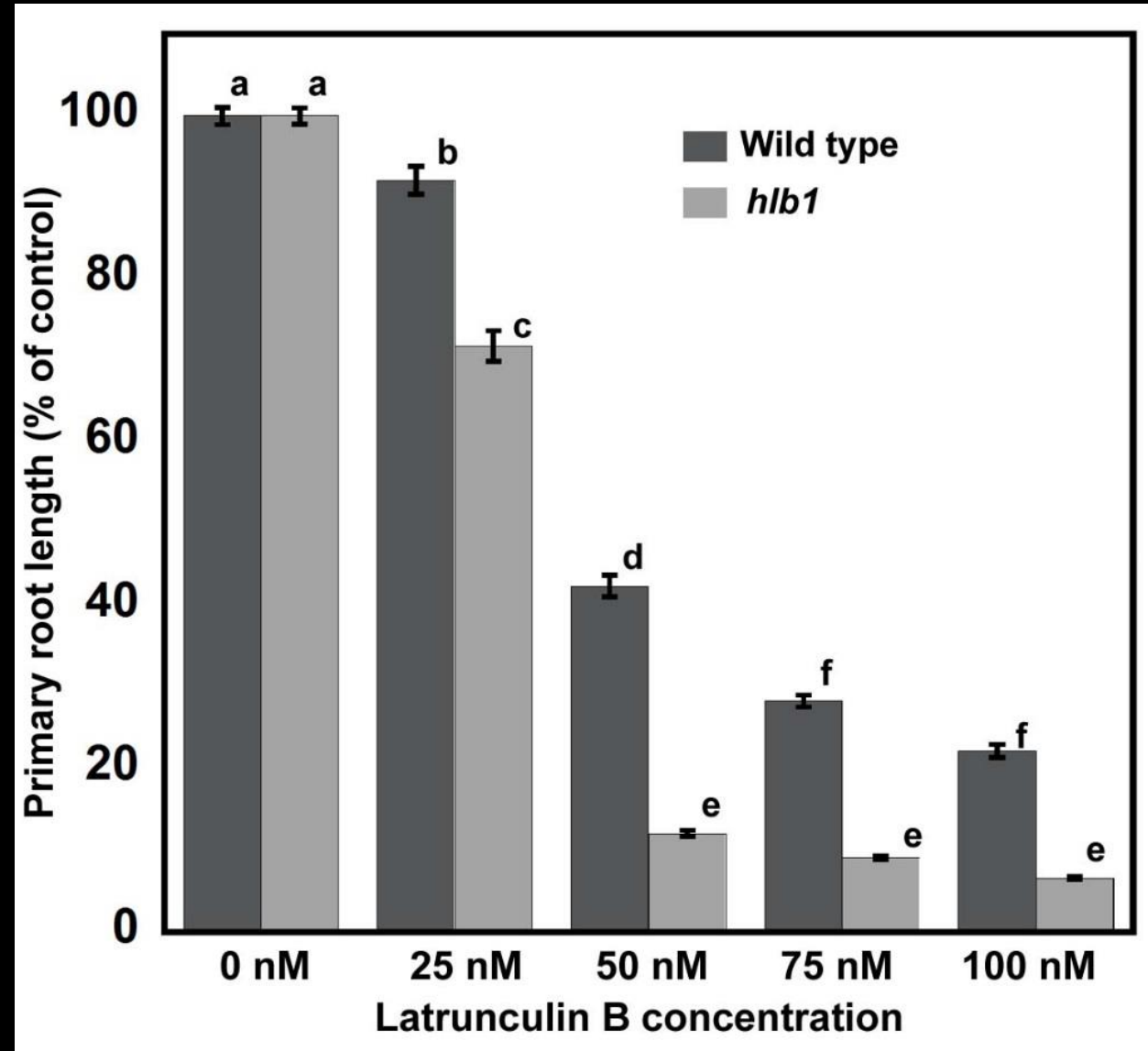
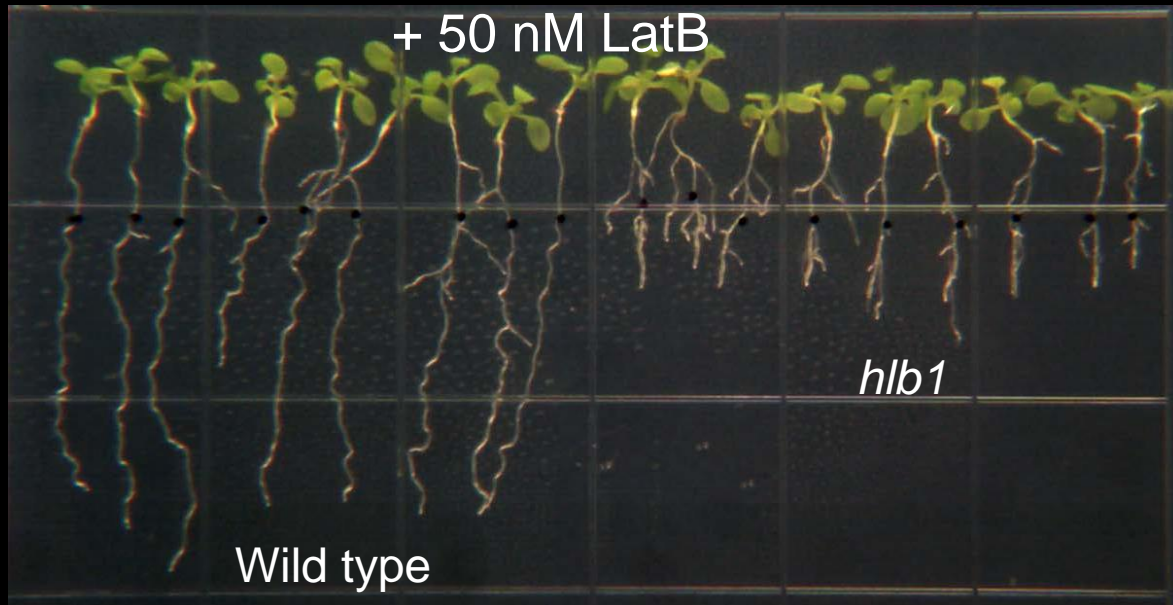
Alan Sparks



Sabrina Chin



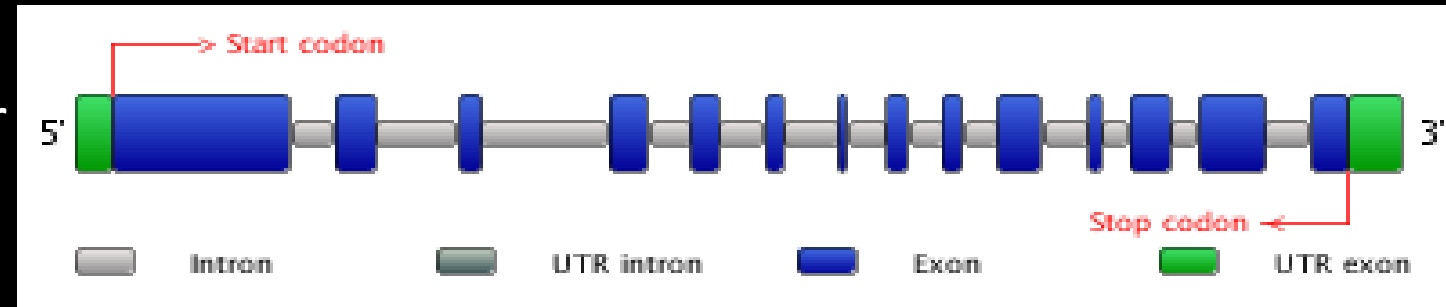
Liang Sun





*HLB1* is a single copy gene in *Arabidopsis* and encodes a tetratricopeptide repeat (TPR) domain-containing protein

*Arabidopsis* locus number  
At5G41950



*HLB1* protein domain organization



Six TPRs – 34 amino acid consensus motif

C-terminus, Pleckstrin Homology (PH)-like domain

<i>A. thaliana</i>	443	NSL	APHSDWK	RTE	FE	LNHER	LLQVLKPEPREM	-GRNLSGKAETMSTNVERKTVKVNITE	IVSVTP	CADLTLPPGAGLCID	521
<i>M. truncatula</i>	399	AIV	APHNDWK	RSEFF	LDHEK	LQQVPRVEQRQA	-SQSLSTRSGDA	-MNGDKKTIKVEIADIVSVSACADLTLPPGAGLCID	476		
<i>P. trichocarpa</i>	386	KPI	APHNDWK	RSEFV	LNHEGL	LQQISKSEKQKV	-RRSLSGRPSDV	-SNSDKKAIKVEVPDIVSVSACADLTLPPGAGLCID	463		
<i>O. sativa</i>	357	SAI	APHKEWERS	QFVLN	HEEL	LQQVNASDQ	-PPSQSPGH	-VDSGRKLFRIVVADIVSVSACADLTLPPGAGLRID	428		
<i>B. distachyon</i>	376	DPI	APHKHWE	RSQFILN	HEEL	LQQANASES	-SPRKSLGH	-VEKSKRFIKVNVVDIVSVSTCSDLTLPPGAGLCIN	447		
<i>P. patens</i>	279	DSL	APHSDWL	RRLWE	VL	DHEALYEVS	-HQHKHLHTSFCPGI	-FAPRLSALRIEMEDILCLAPTADLSLPPGGGFCID	352		

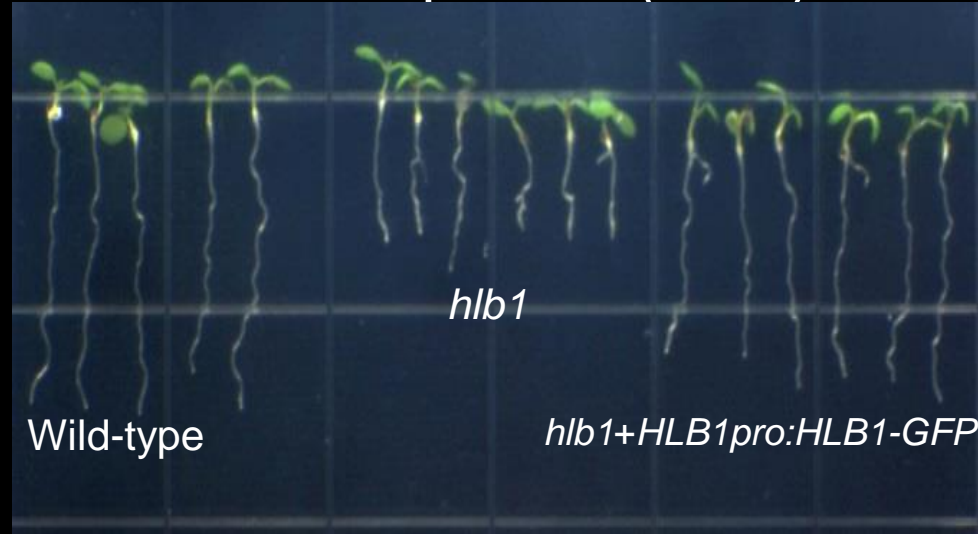
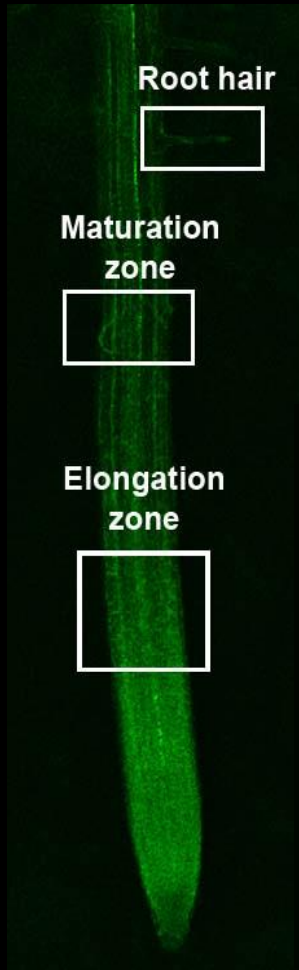
  

<i>A. thaliana</i>	522	TIHG	PVFL	VADSWES	SLDGWL	DAIRLVYT	IYARGKSDVL	AGIITG	565
<i>M. truncatula</i>	477	TVHG	SVYL	VADSWES	SLDGWL	DAIRLVYT	IYVRGKSDVL	AGIITG	520
<i>P. trichocarpa</i>	464	TIHG	PLFL	VADSWES	SLDGWL	DAIRLVYT	IYARGKSEVL	AGIVTS	507
<i>O. sativa</i>	429	TIHG	PRFL	VADNWE	TIDSWL	DAIRLVYT	IFARGRSDVL	AGIITG	472
<i>B. distachyon</i>	448	TIHG	PVYL	VADTWE	SLDGWL	DAIRLVYT	IFARGKSDVL	AGIITG	491
<i>P. patens</i>	353	TVAG	PQYL	IADTWE	SMDAWV	DAIRLVYT	IYAQGKR	DALANVLVV	396

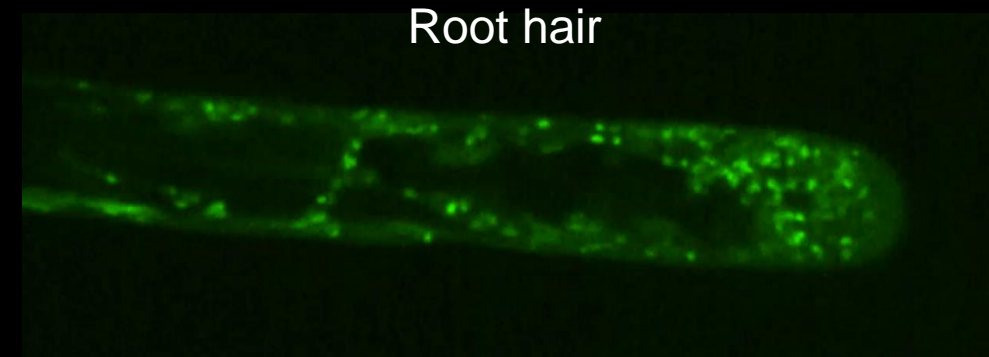
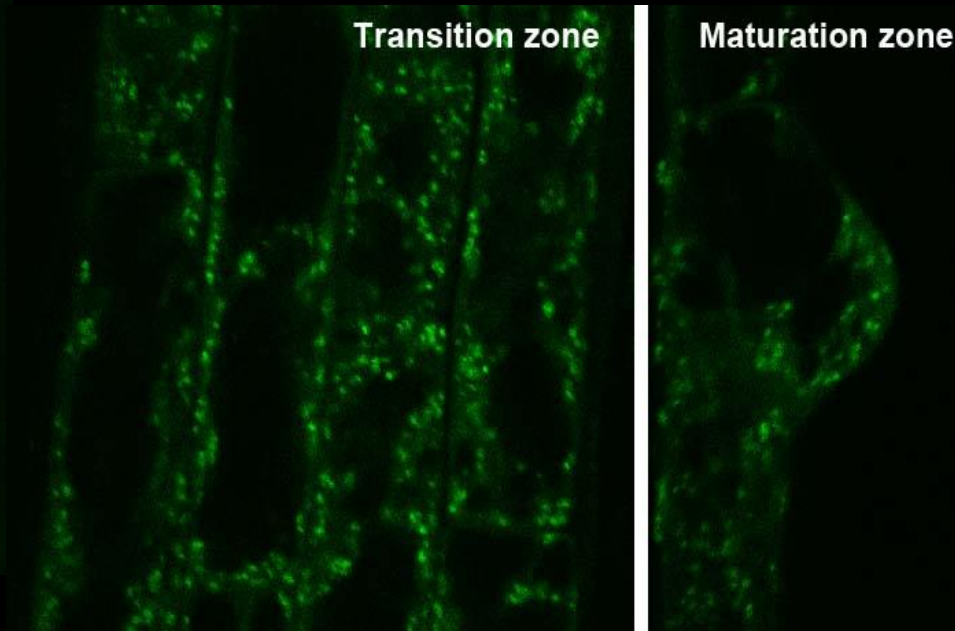
Conserved in land plants:  
moss, monocot, eudicots



# A functional HLB1-green fluorescent protein (GFP) fusion localizes to dynamic bodies

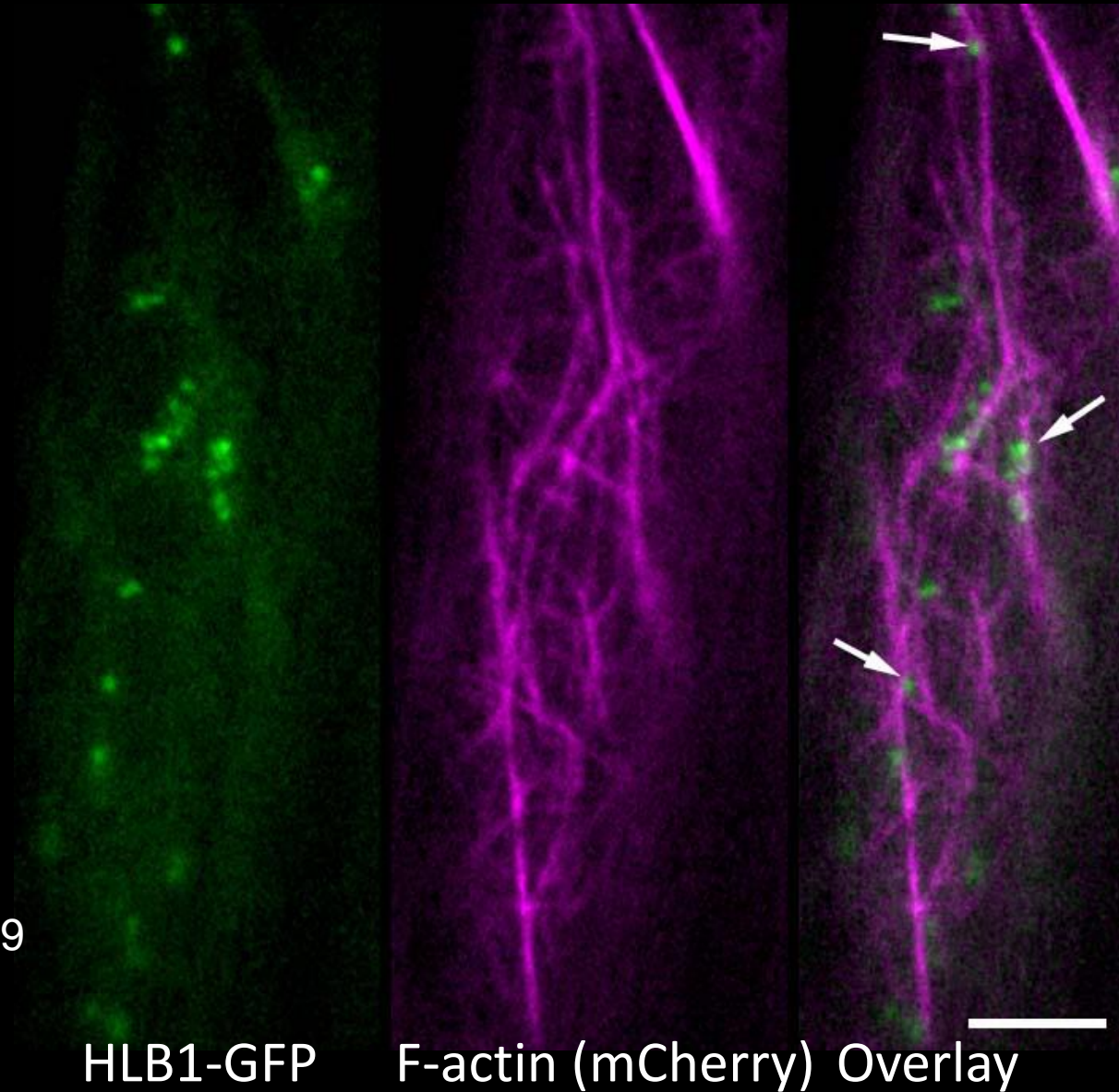


+50 nM Latrunculin B





# HLB1 bodies localize to F-actin



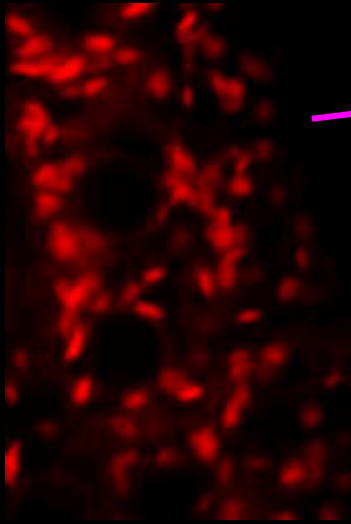
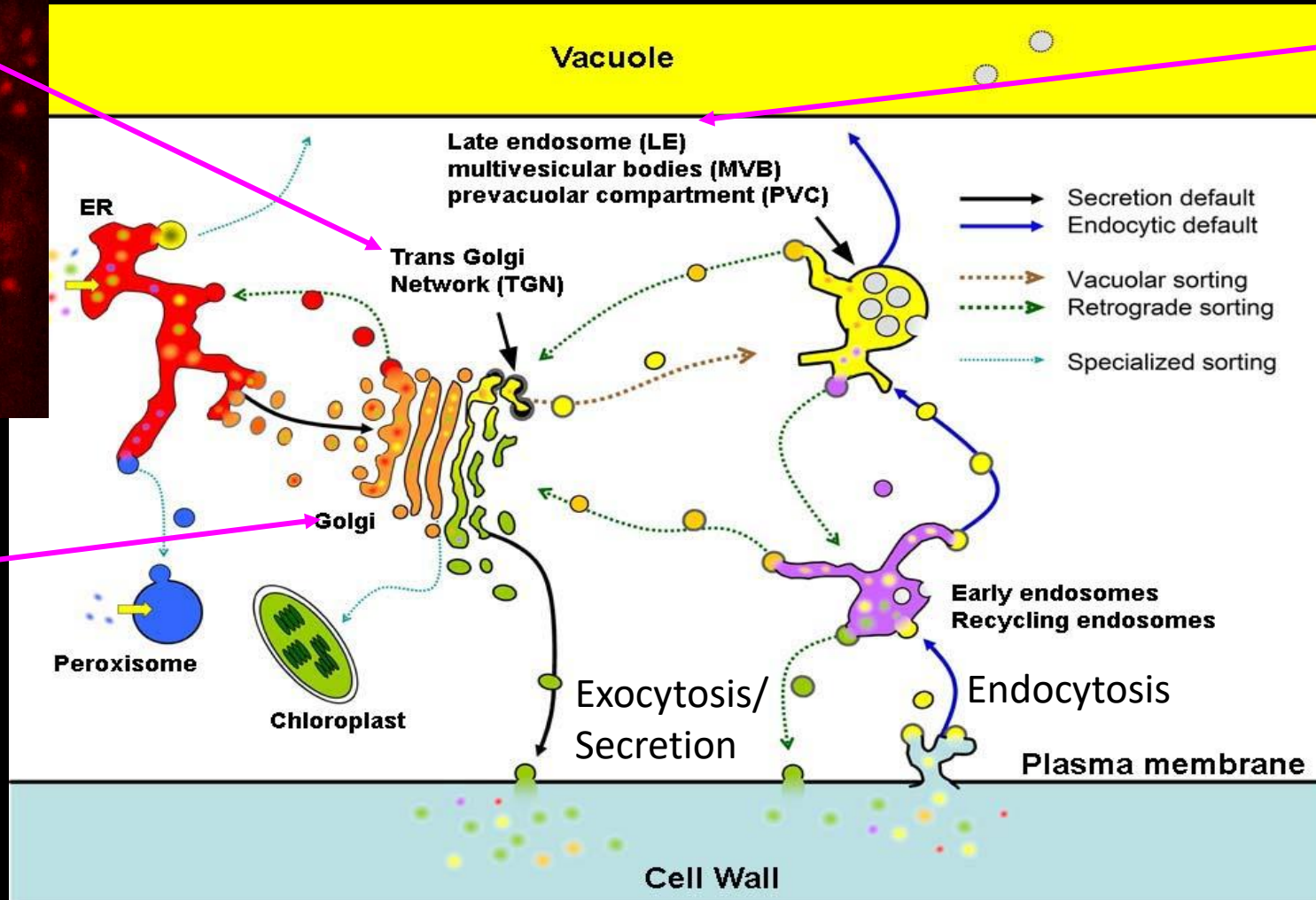
Sparks et. al. (2016)  
*The Plant Cell* 28:746-769



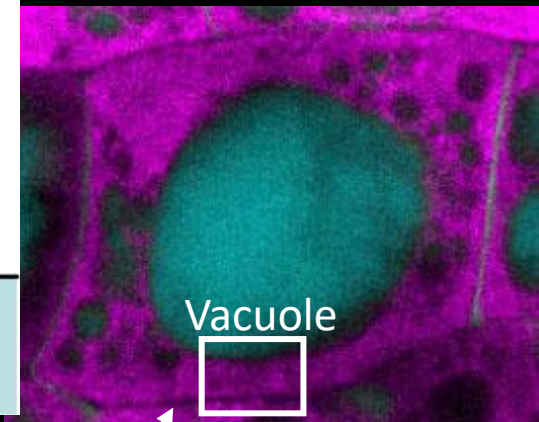
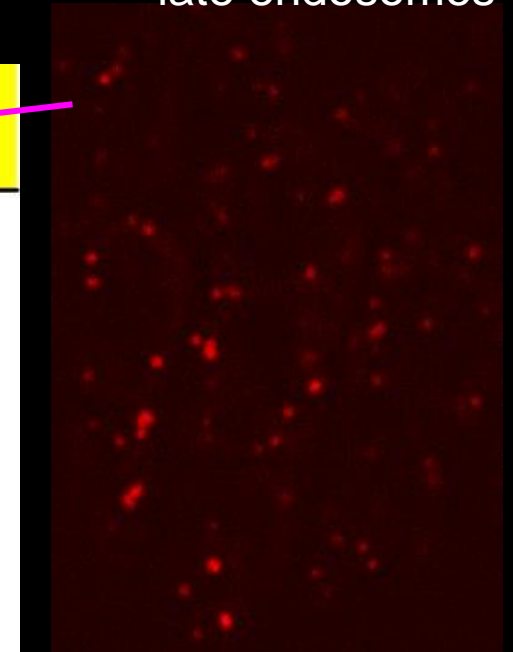
# The secretory/endomembrane system of Arabidopsis (from Bassham et al., 2008- The Arabidopsis Book)

mCherry-*trans*-Golgi-  
Network (TGN)- Early  
Endosomes (EE)

mCherry-  
late endosomes



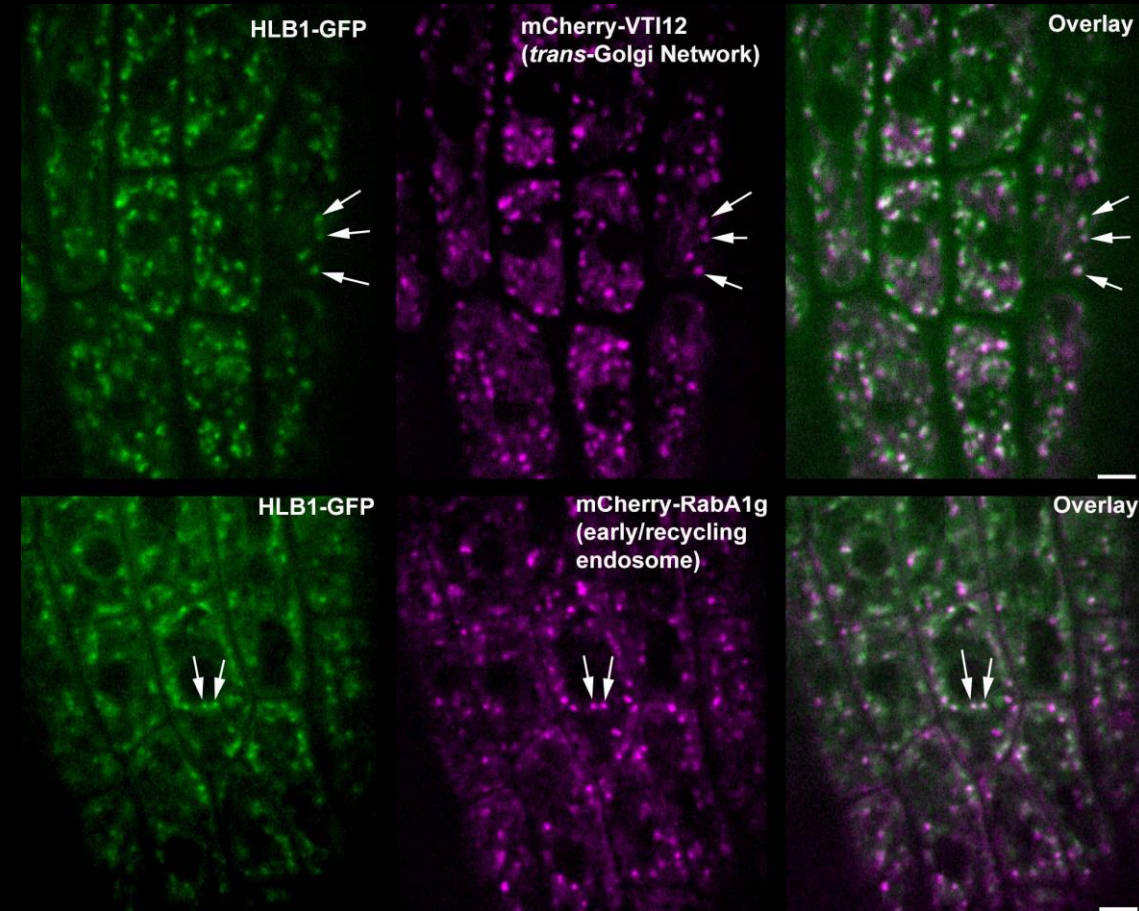
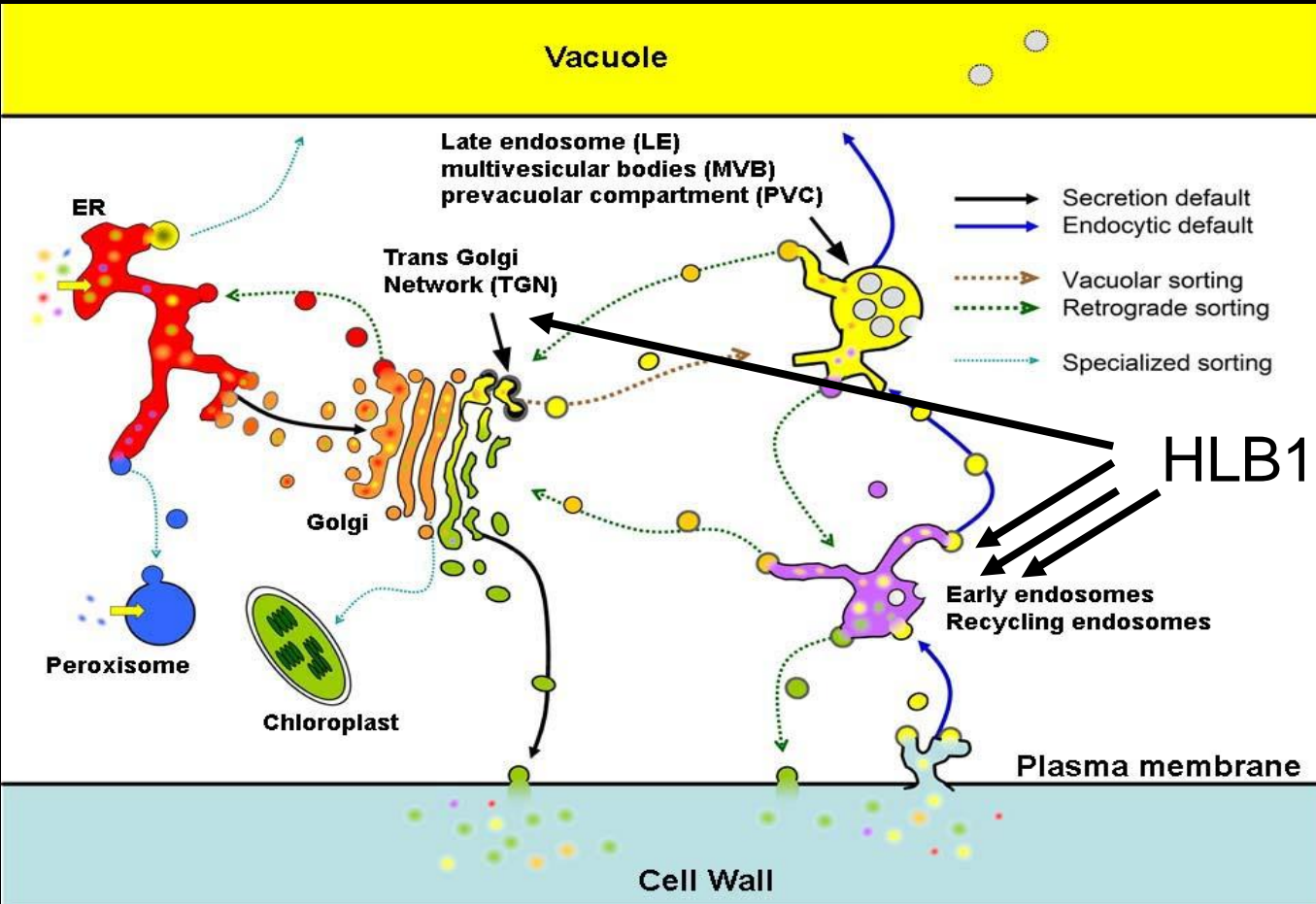
mCherry-Golgi



Cell wall/Plasma membrane

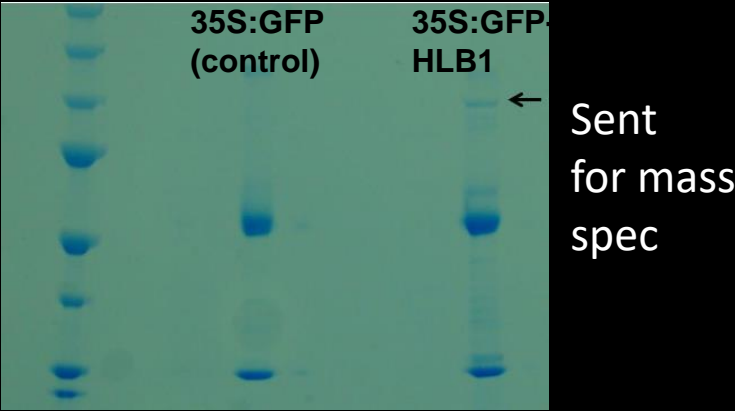


# HLB1 localizes to the *trans*-Golgi Network (TGN)/Early Endosomes (EE)/Recycling Endosomes in Arabidopsis Roots

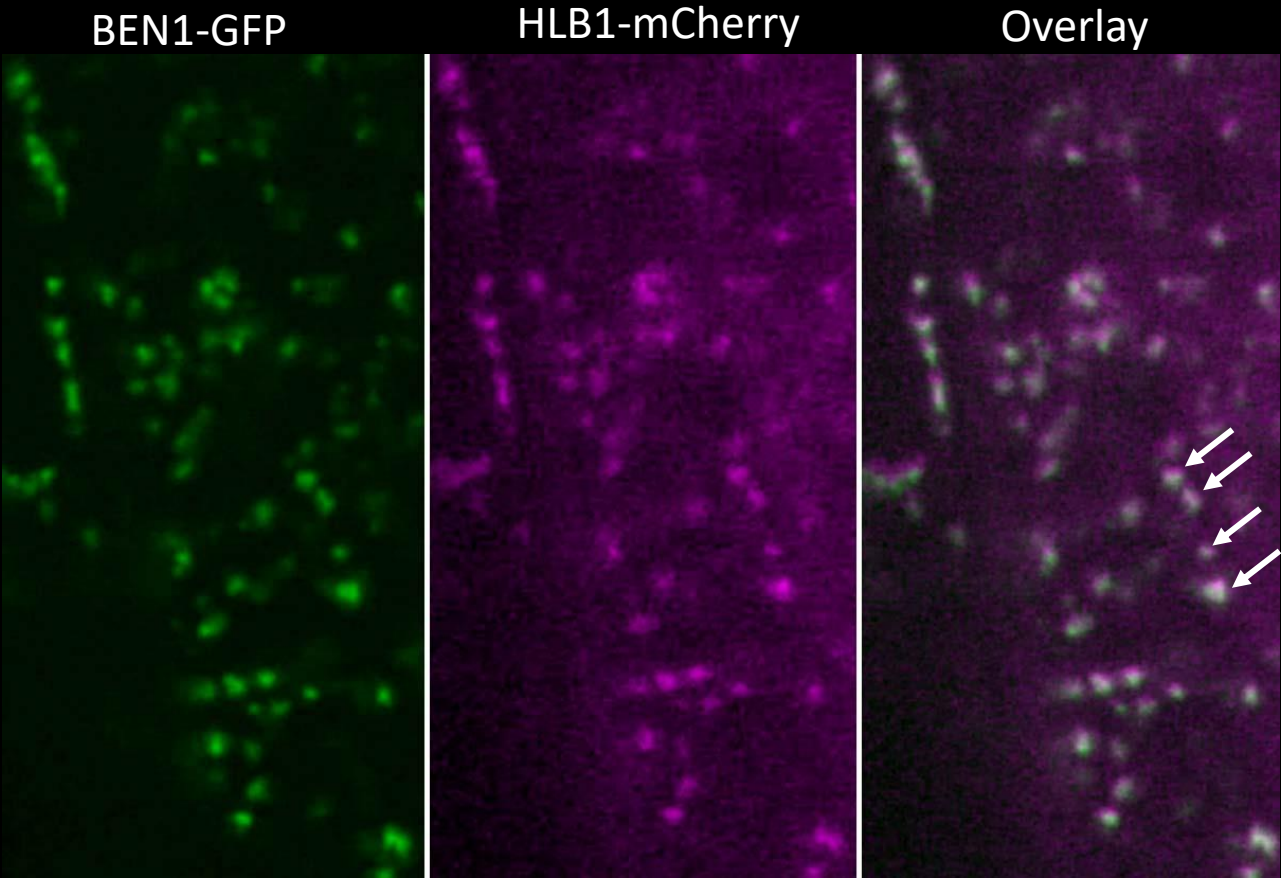




# Co-immunoprecipitation identifies BEN1 as an HLB1 interactor



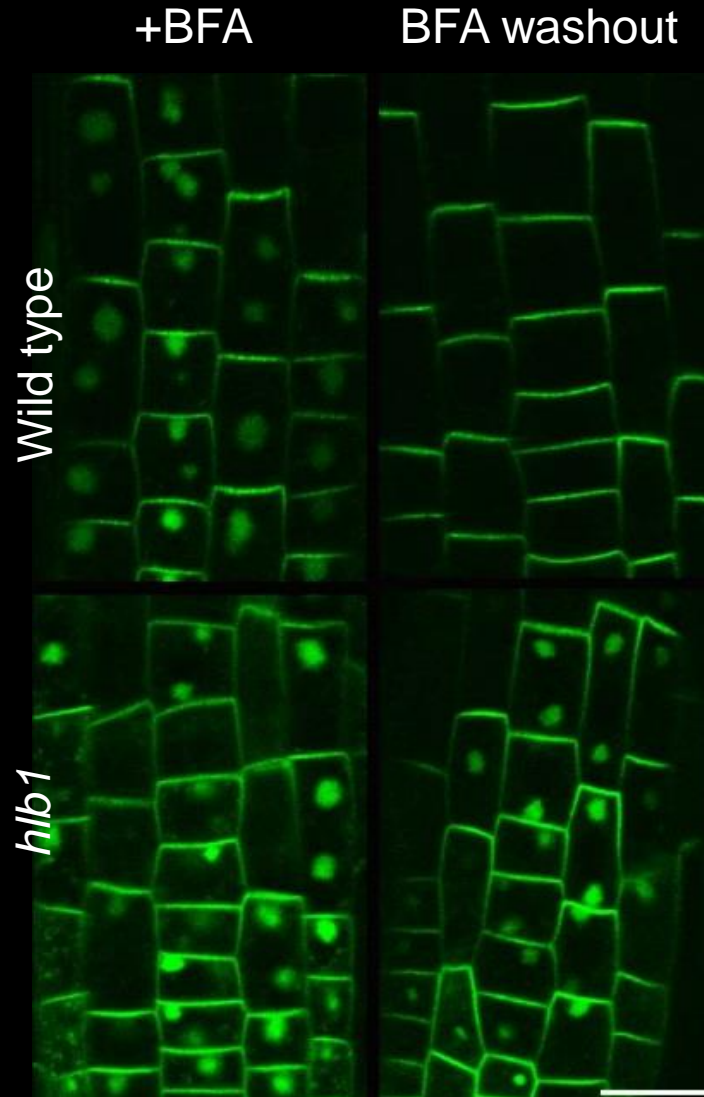
<b>At1G49240</b>	<b>ACTIN8</b>
<b>At5g41950</b>	<b>HLB1</b>
At5g39570	Uncharacterized
At5g09590	Heat Shock
At5g18800	NADH dehydrogenase 1 alpha subcomplex subunit 8-B
At3g02090	Mitochondrial-processing peptidase subunit beta
At3G06330	RING/U-box superfamily protein
At5G19990	26S protease regulatory subunit 8 homolog A
At1G15210	ABC transporter G family member 35
At1G77250	RING/FYVE/PHD-type zinc finger family protein
At4G23930	Late embryogenesis abundant hydroxyproline-rich glycoprotein
At5G20010	GTP-binding nuclear protein Ran-1
At5G20020	GTP-binding nuclear protein Ran-2
At1G11580	Pectinesterase inhibitor 18
At5g43060	Cysteine protease component of protease-inhibitor complex
At5G55190	GTP-binding nuclear protein Ran-3
At2G43910	Thiocyanate methyltransferase 1
<b>At3G43300</b>	<b>BEN1</b>



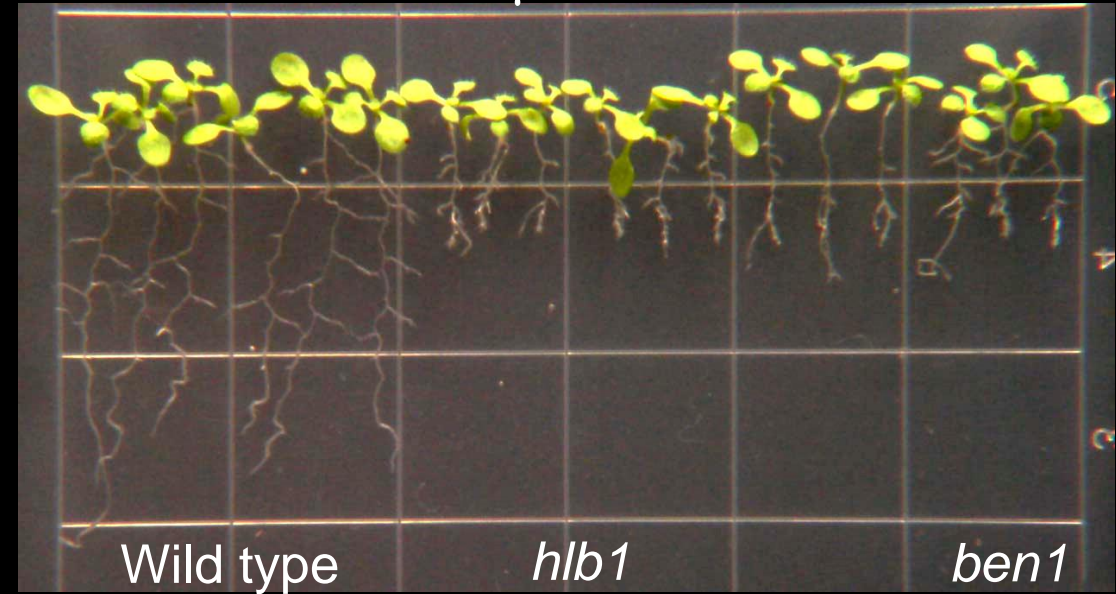


# *hlb1* and *ben1* mutants are defective in protein recycling and phenocopy each other

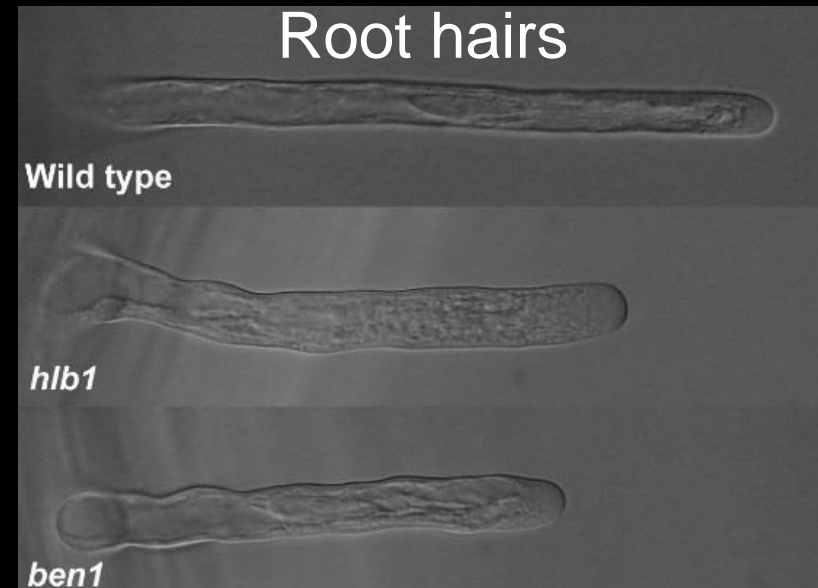
GFP plasma membrane marker



+50  $\mu$ M LatB

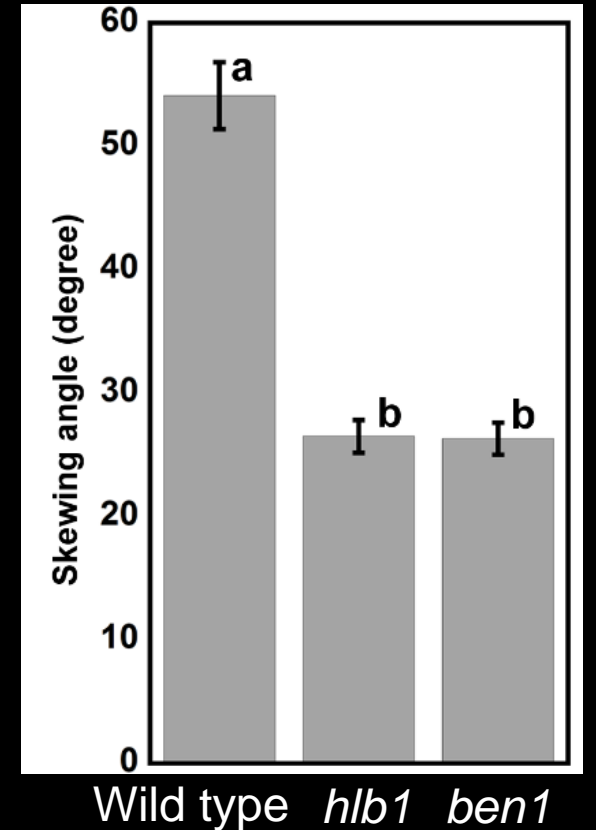
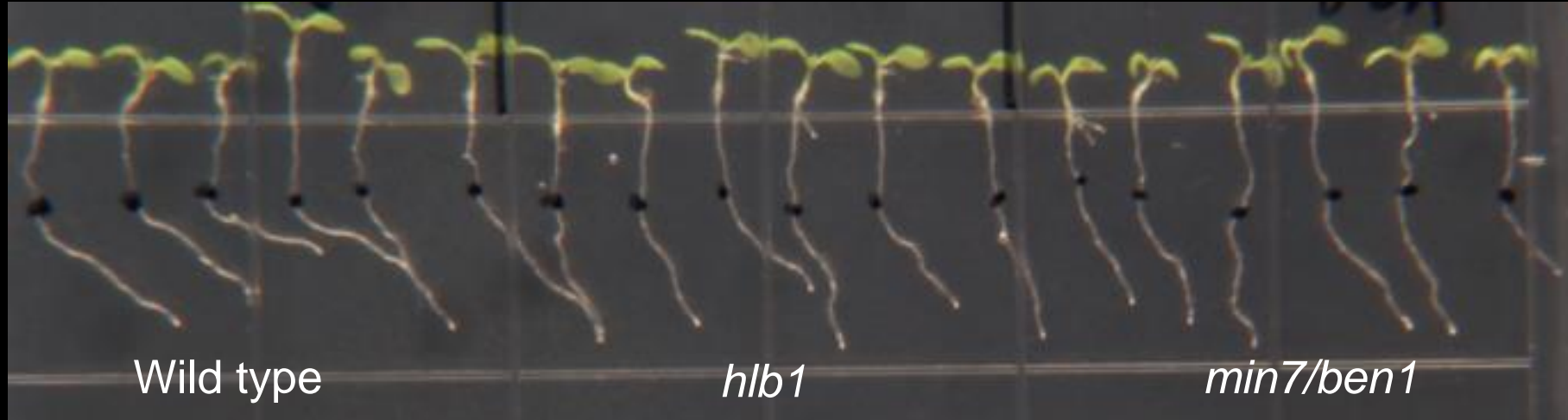


Root hairs





# Root skewing of *hlb1* and *ben1* mutants is dampened



## What is BEN1?

BreFeldin A (BFA) visualized ENdocytic trafficking defective 1 (BEN1) is an Adenosine DiPhosphate (ADP) Ribosylation Factor(ARF)-Guanine Nucleotide Exchange Factor (GEF).





# SUMMARY

- A forward genetic screen on latrunculin B led to the identification of new proteins involved in actin function
- HLB1 and BEN1 protect F-actin against Latrunculin B disruption
- HLB1 and BEN1 form a complex with actin and the Trans Golgi Network/Early Endosomes to facilitate root skewing and root hair tip growth



# Acknowledgments

NASA Biological and Physical Sciences (BPS) grants  
80NSSC22K0029 and 80NSSC22K0024

Colleagues at the Utilization and Life Science  
Office, Exploration Research and Technology  
Programs, Kennedy Space Center and  
Noble Research Institute

Happy Holidays

