Regulation and functional differentiation of two actins in *Chlamydomonas*

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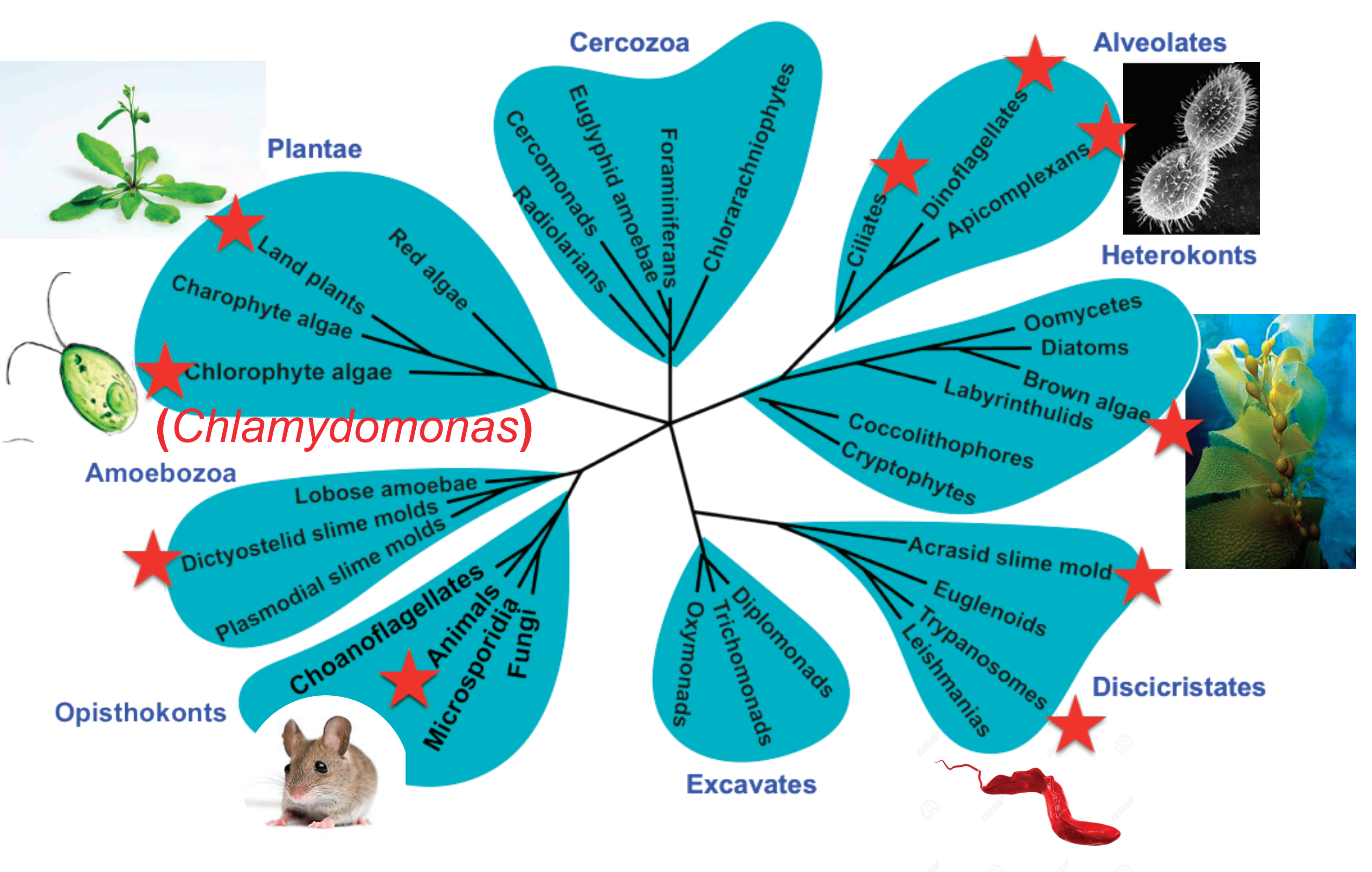
Some of the results have been published:
Onishi, Pringle, & Cross, *Genetics* 2016
Onishi, Pecani, Jones IV, Pringle, & Cross, *PNAS* 2018
Onishi, Umen, Cross, & Pringle, bioRxiv 2019

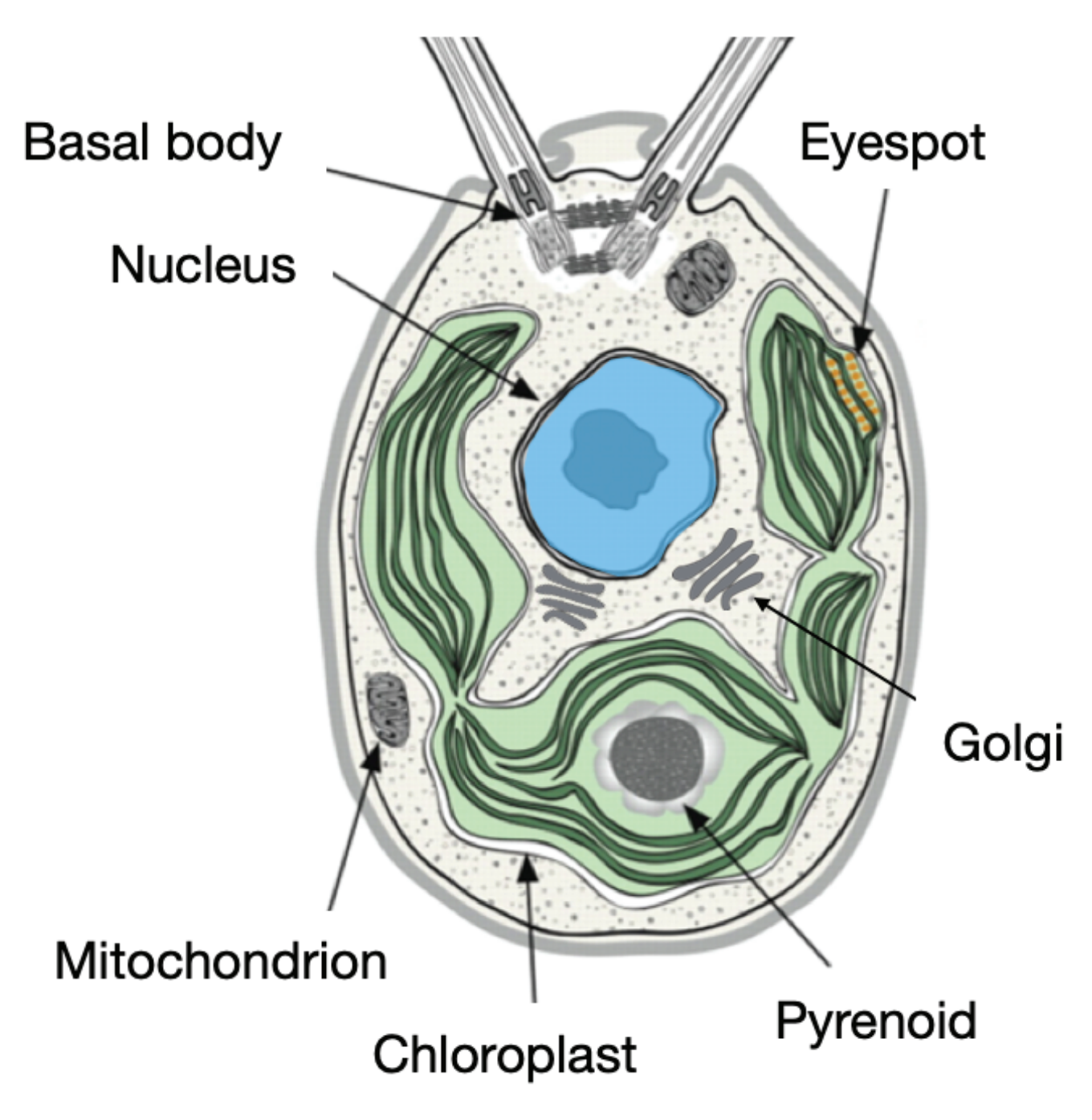
Background

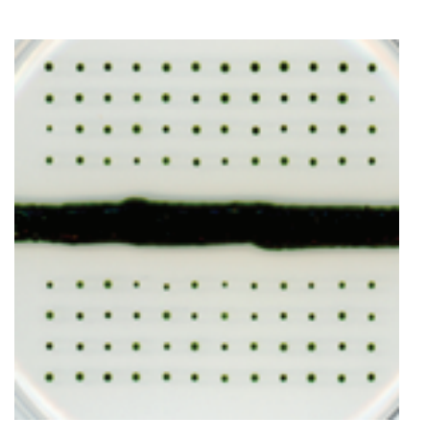
Many eukaryotes have multiple actins (★) that are often divergent, but their functional differences and regulations poorly studied



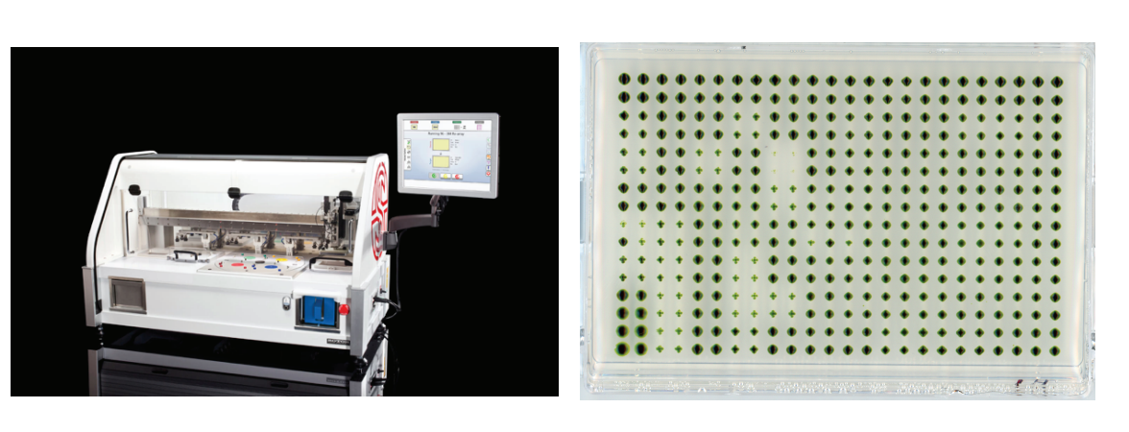
Funcitional differentiation and regulation of thve isoforms?
How do they keep balance of different actin isoforms in a cell?

Chlamydomonas = Green yeast

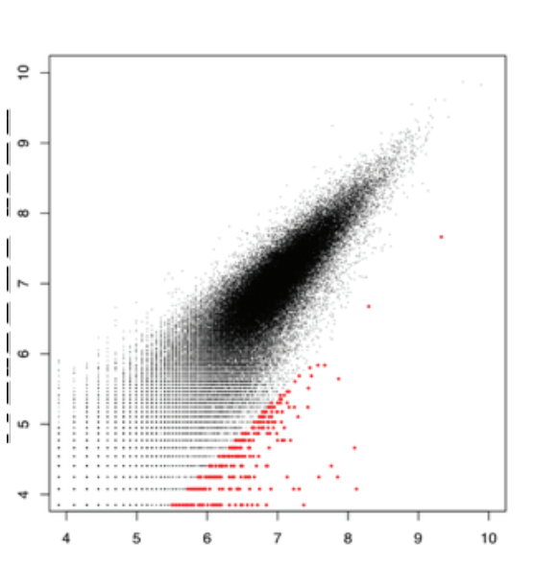




Tetrad dissection



HTP mutant screens

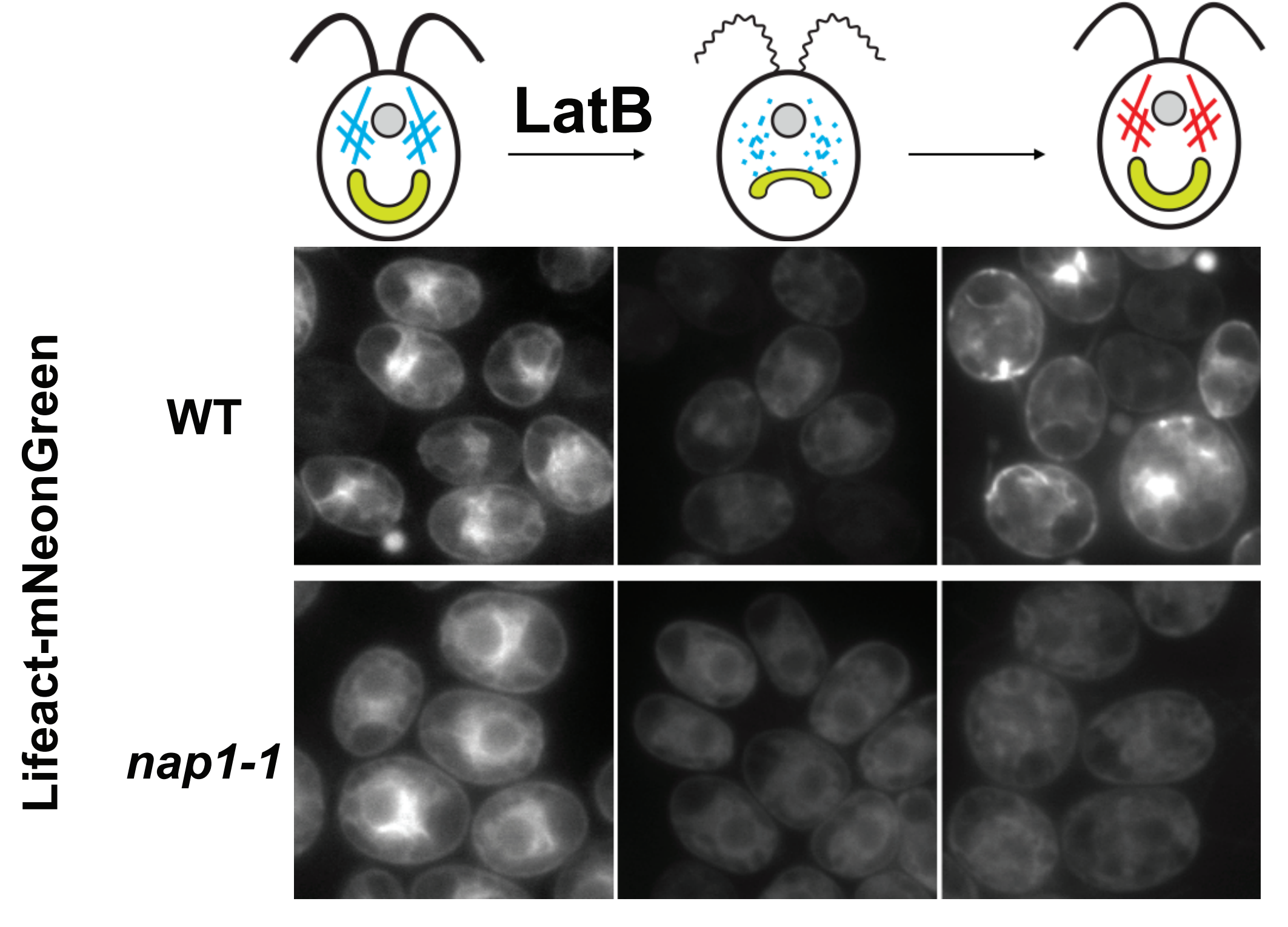


HTP phenotyping

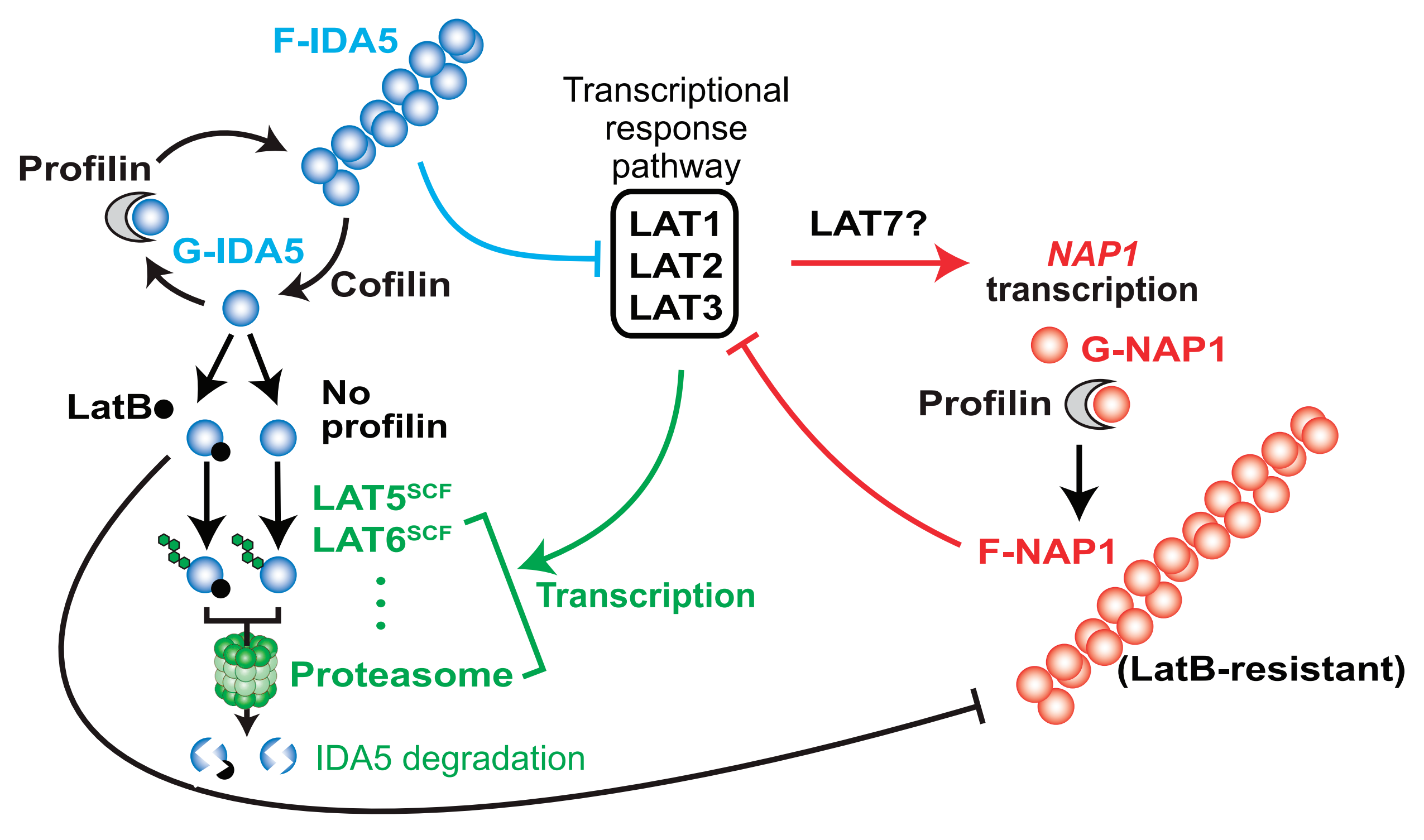
Chlamydomonas forms LatB-resistant filaments

F-IDA5
(canonical actin)

F-NAP1
(divergent actin, LatB-resistant)

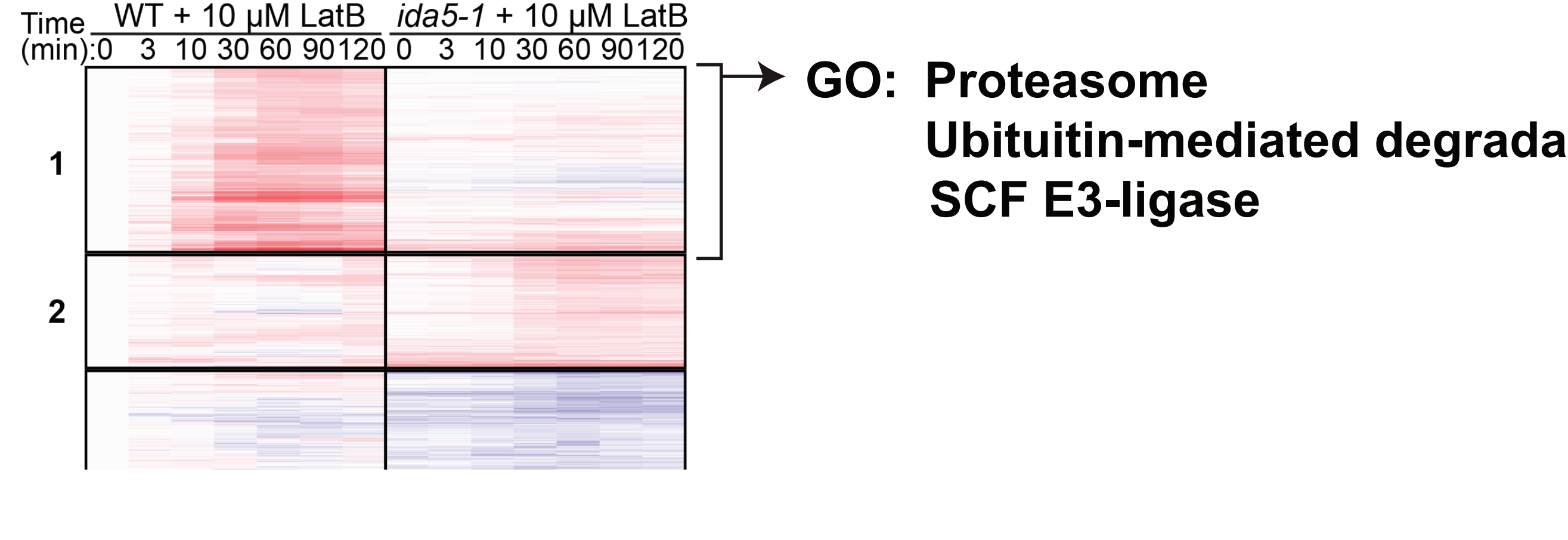


The actin-homeostasis system



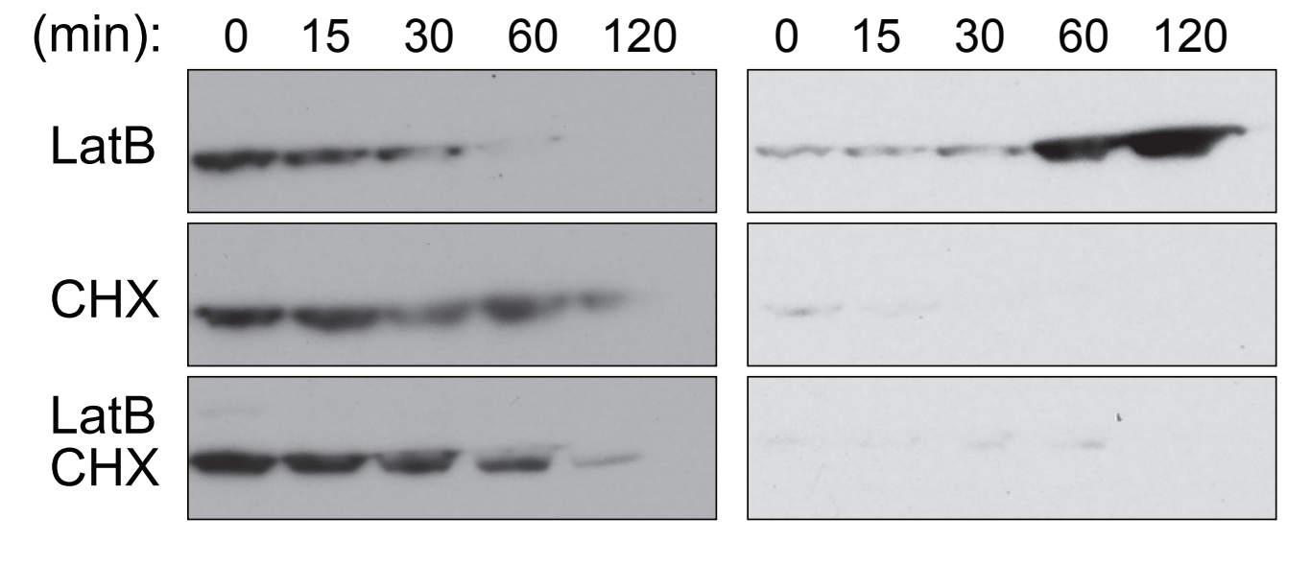
RNA-seq: Proteasome genes are induced by LatB

Expression of all genes

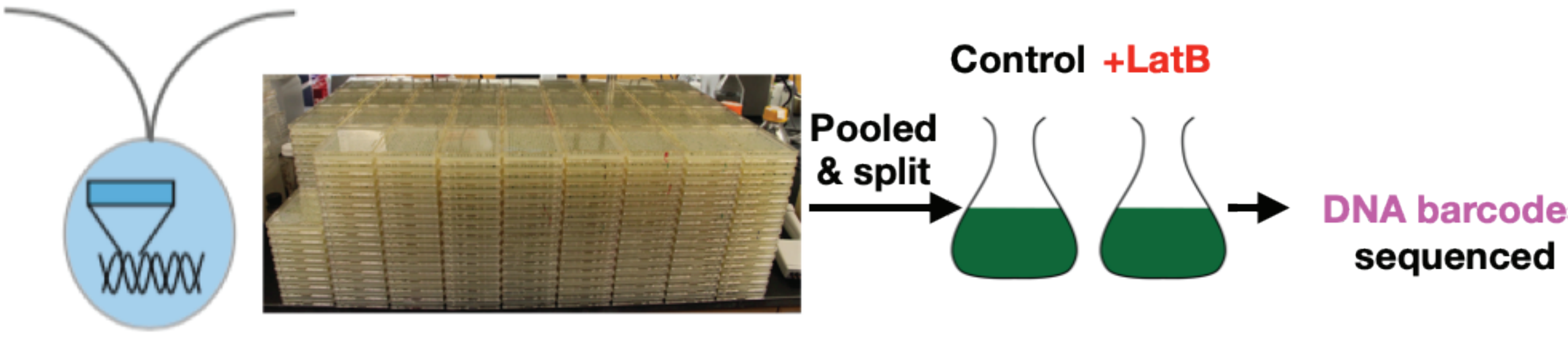


GO: Proteasome
Ubituitin-mediated degradation
SCF E3-ligase

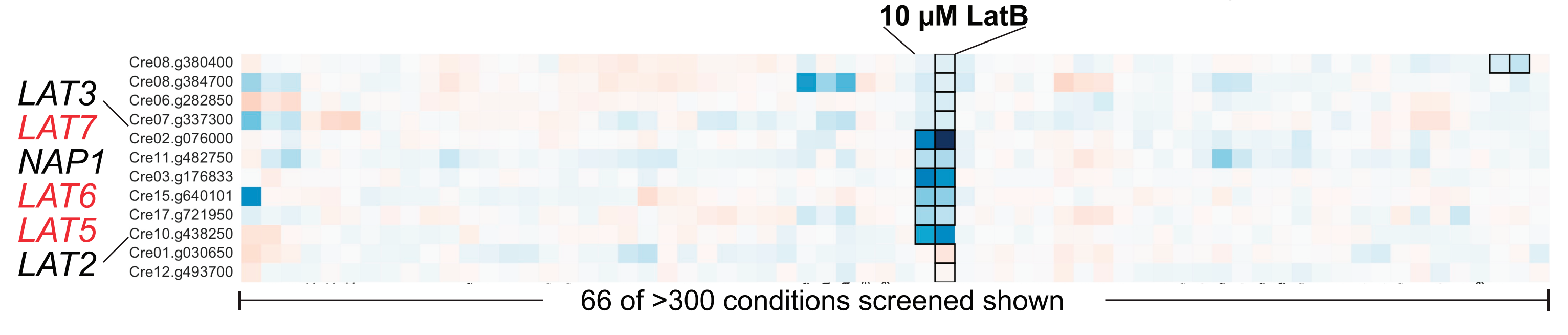
IDA5 is degraded upon LatB addition



SCF E3 ligase identified by genetic screen



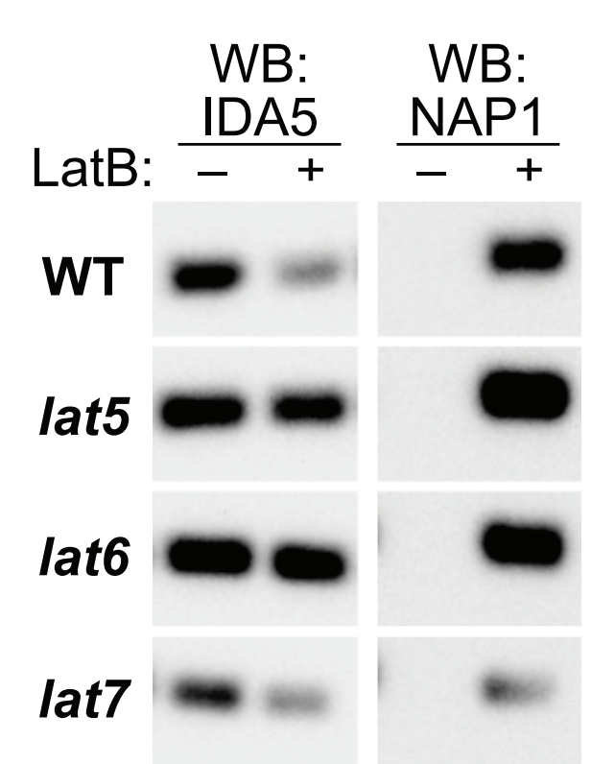
Quantitative Pooled Screen of >100k mutants identified mutants with moderate LatB sensitivity



66 of >300 conditions screened shown

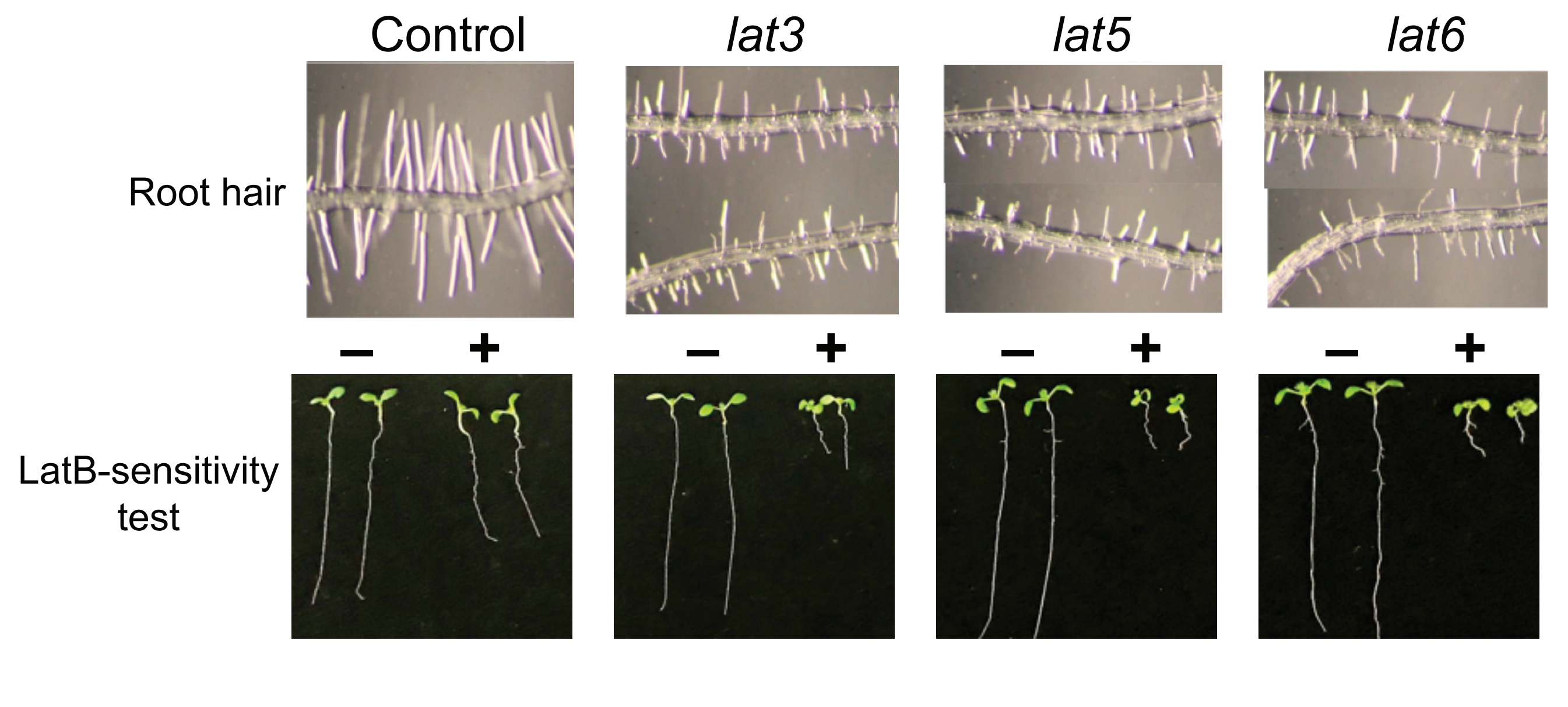
E3-ligase subunits LAT5 (Cullin) and LAT6 (F-box) are required for IDA5 degradation

Gene	Protein	Growth +/- LatB by Pooled Screen		Expression +/- LatB	IDA5 degradation	NAP1 expression
LAT1	Kinase	NA	NA	-0.18	No	No
LAT2	Volvocale-specific protein	-2.7	-3.3	0.30	No	No
LAT3	Kinase	-5.0	-5.2	0.32	No	No
NAP1	Divergent actin	-6.1	-6.1	6.2	Yes	No
LAT5	Cullin-RING	-2.3	-3.5	0.25	No	Yes
LAT6	F-box	-3.0	-3.5	4.0	No	Yes
LAT7	Importin-like	-1.3	-1.3	1.2	Yes	Weak

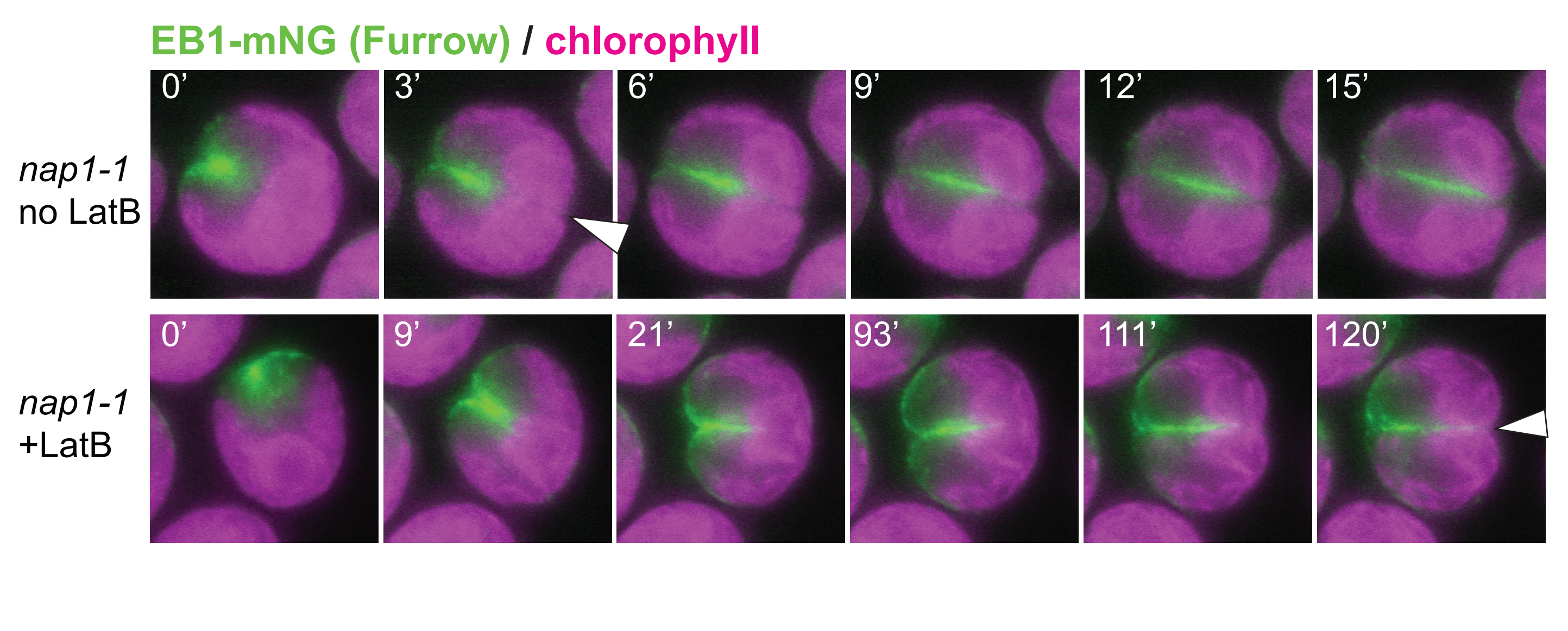


Conserved in plants (and other organisms)?

Arabidopsis lat mutants show root-hair growth defects and LatB hypersensitivity

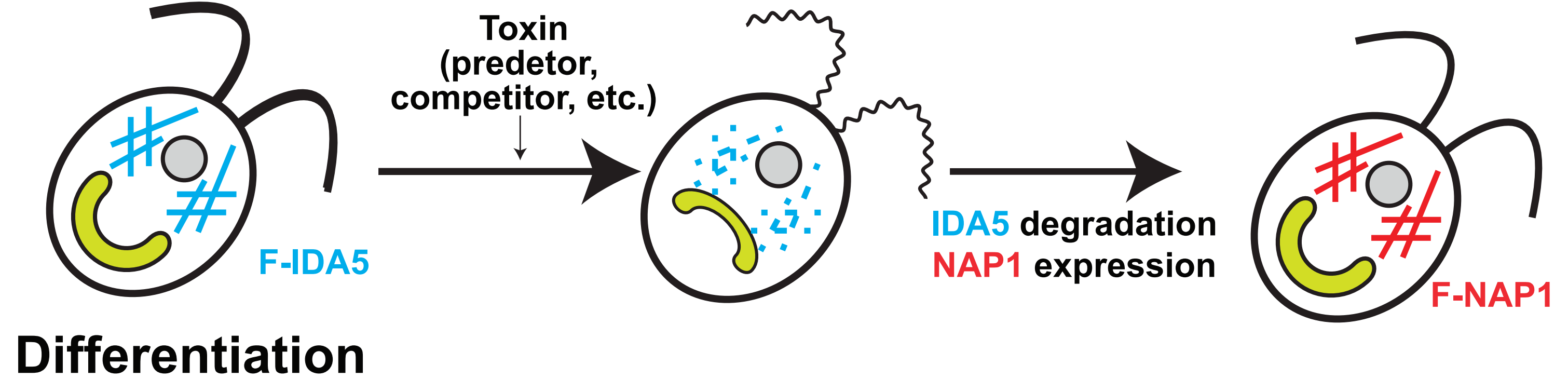


Cytokinesis without actin

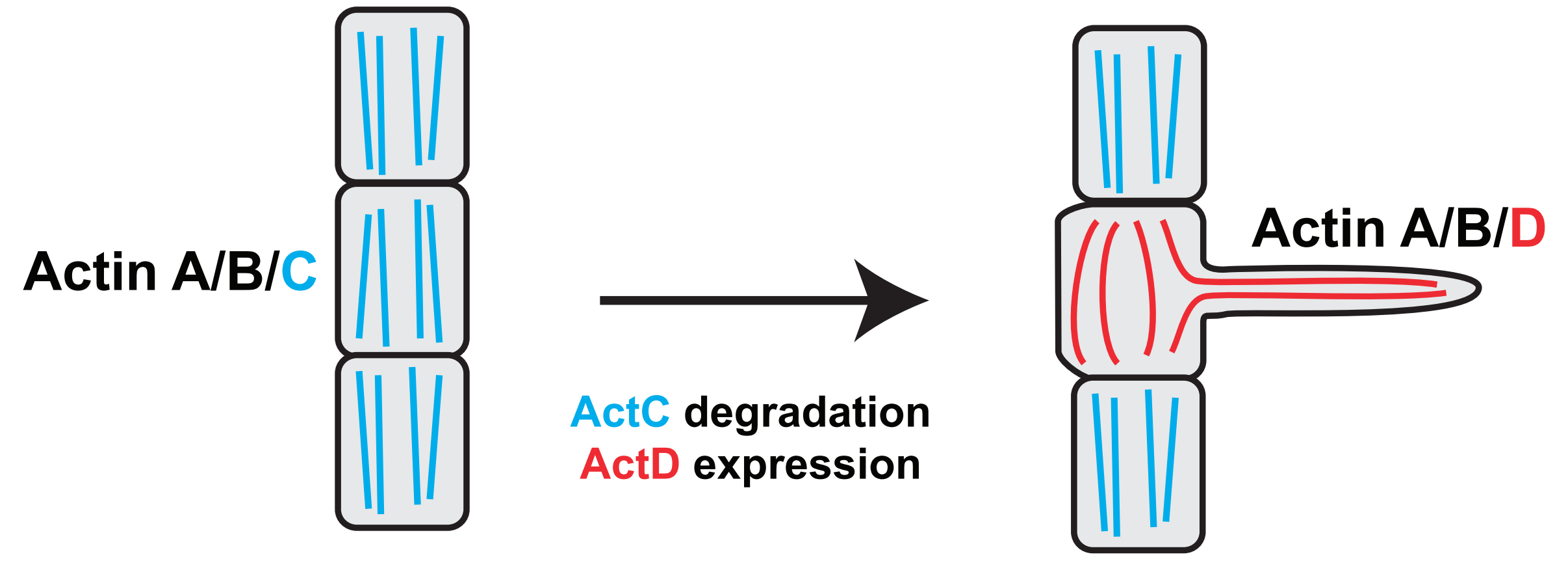


Significance of regulating actin isoforms

- Defense agaist actin-targeting toxins



- Differentiation



Funding:
NSF MCB EAGER 1548533 (2015-2018), MCB 1818383 (2018~)