

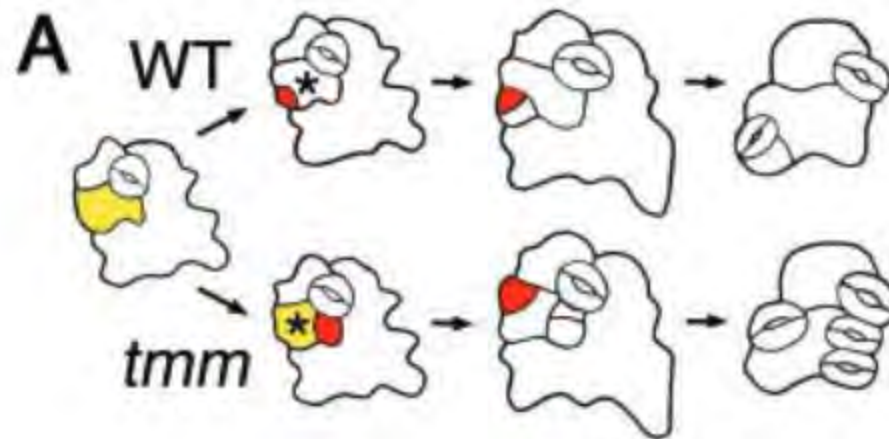
Identification of stomatal related, T-DNA mutants in *Arabidopsis thaliana*

Jennifer Le

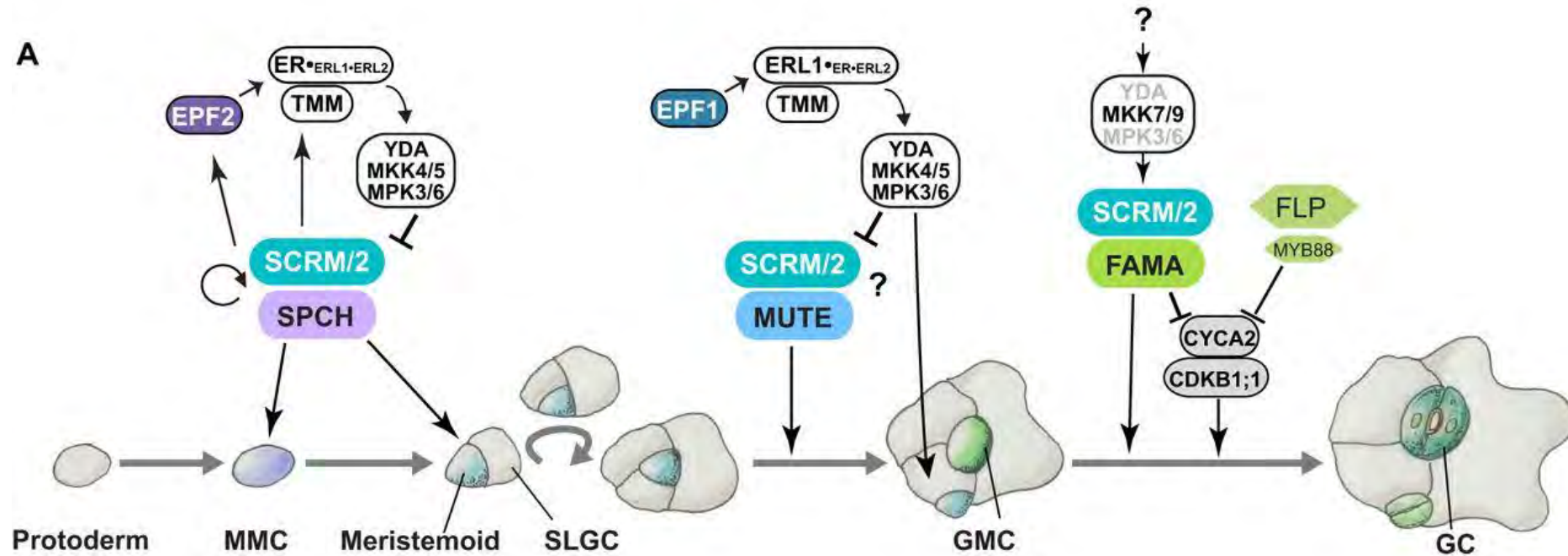
Advisor: Dr. Xingyun Qi

What are stomata?

- Regulate gas exchange (passage of CO₂, O₂, and water vapor)
 - Consist of two guard cells and a pore in between
 - Formed via series of symmetric and asymmetric divisions
- Proper patterning of stomata → efficient gas exchange

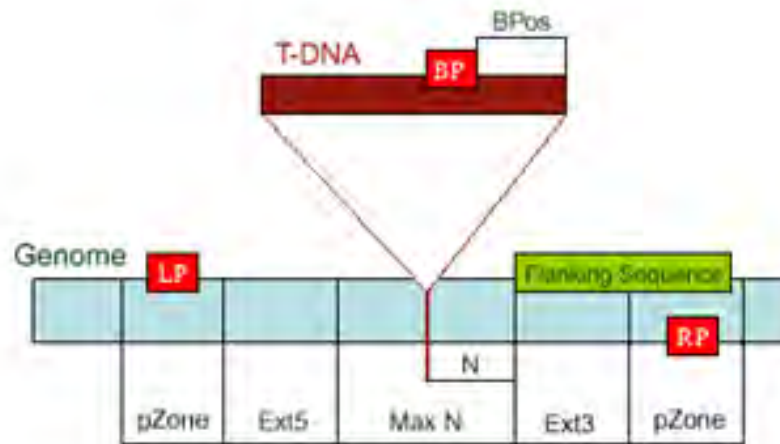


Stomata developmental pathway



What is T-DNA?

- T-DNA: transfer-DNA from a bacterium into a host plant's genome
 - Genetically and physically stable
 - Discernable effect on gene expression
 - 1.5-2 insertions per line that are randomly distributed



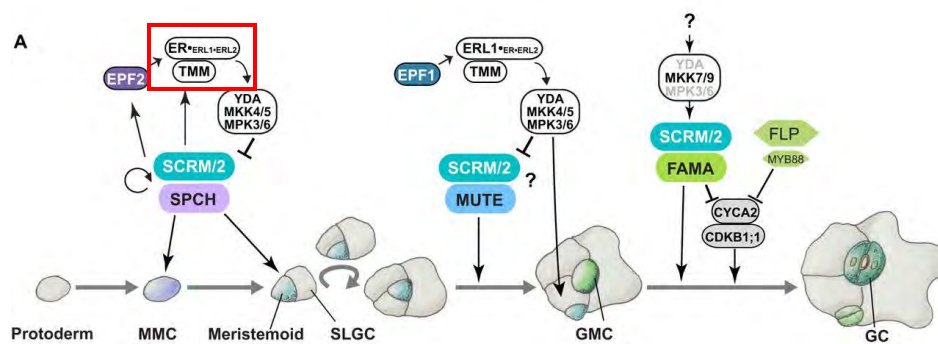
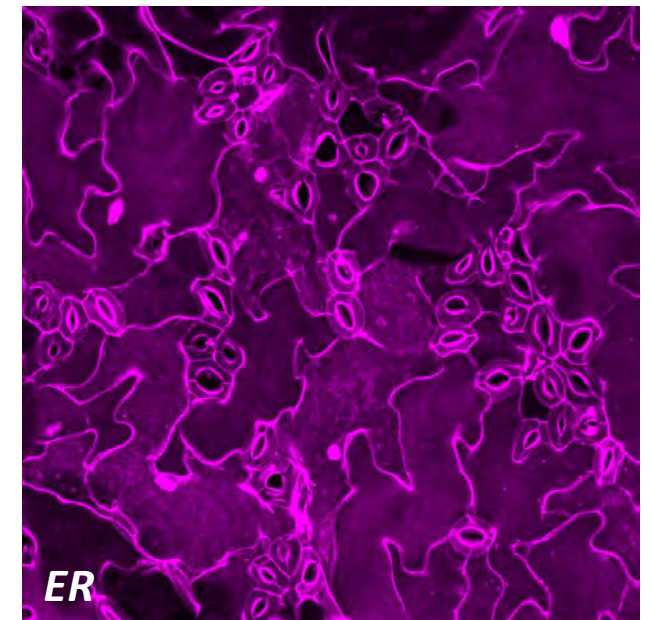
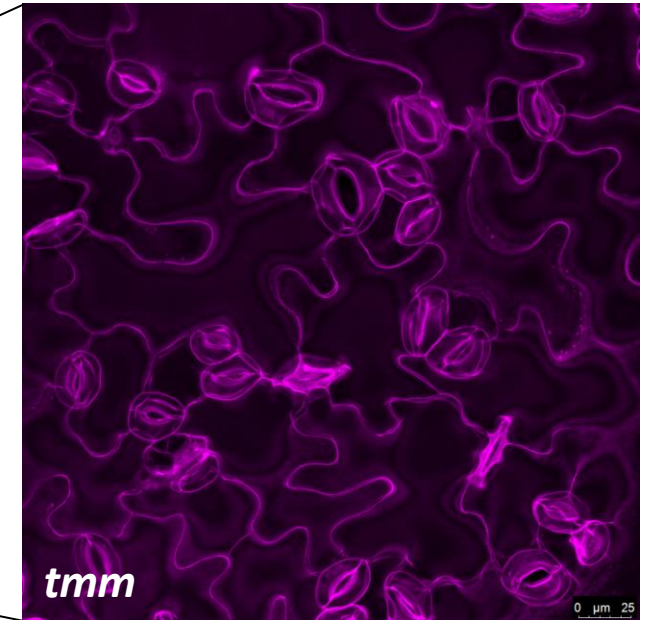
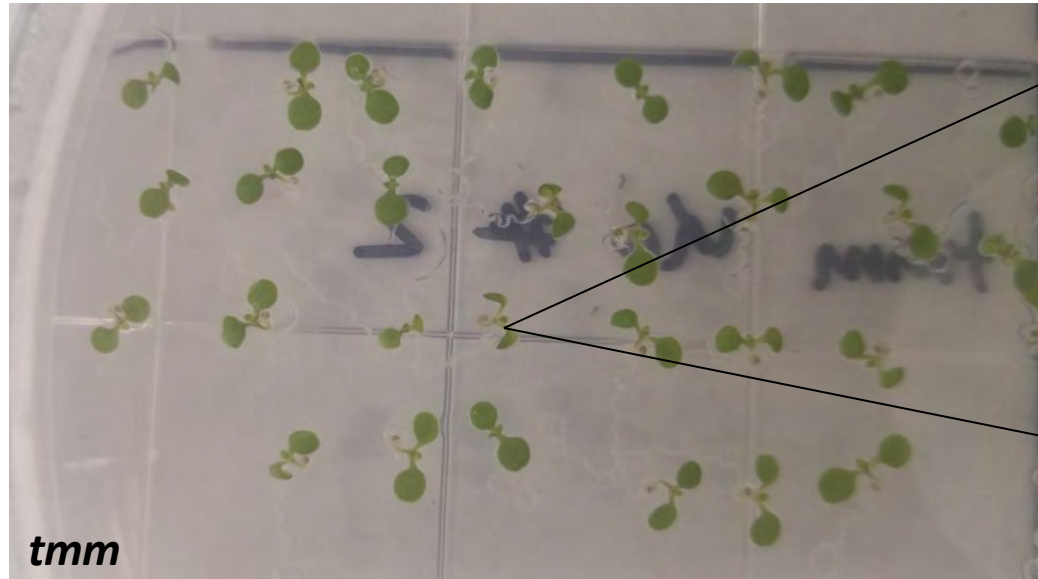
Aims

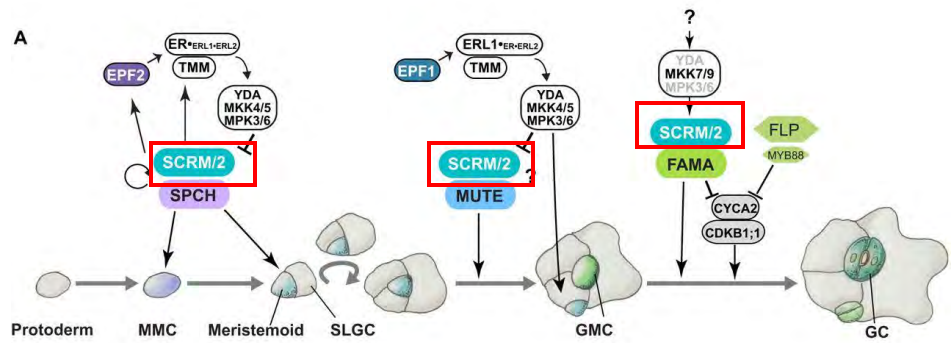
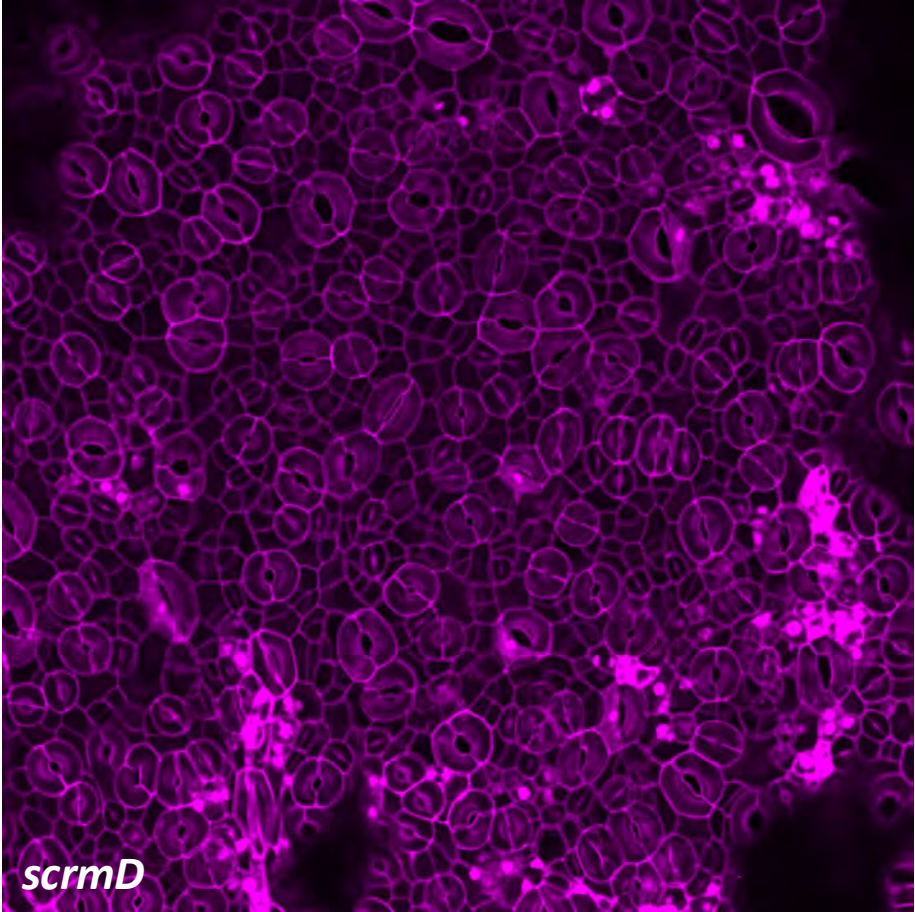
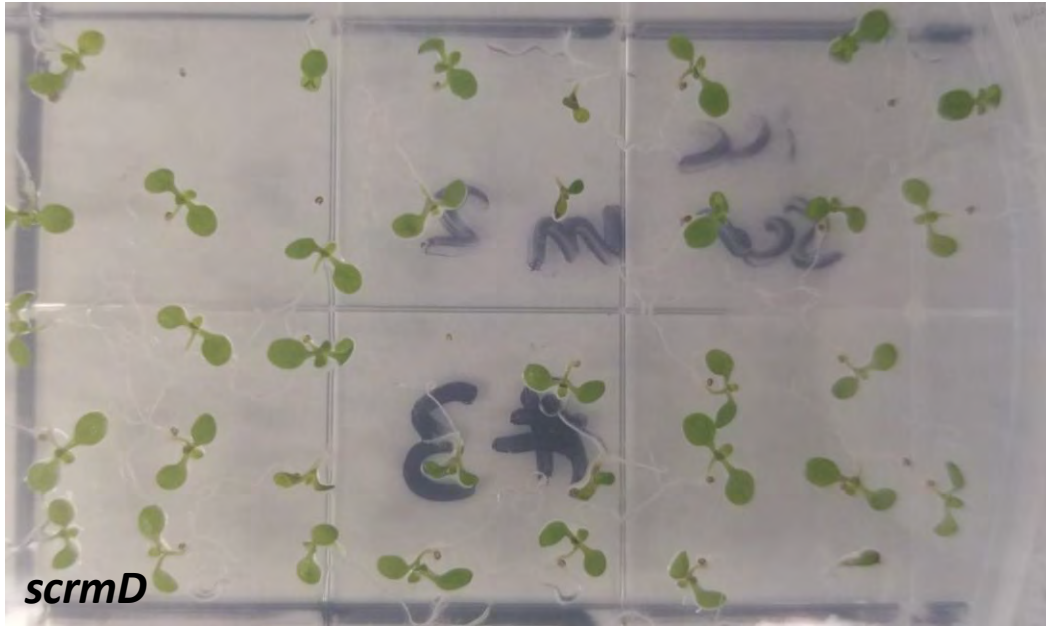
- Genotyping of *EPF1* mutants
- Genetic identification of stomatal-related, T-DNA mutants

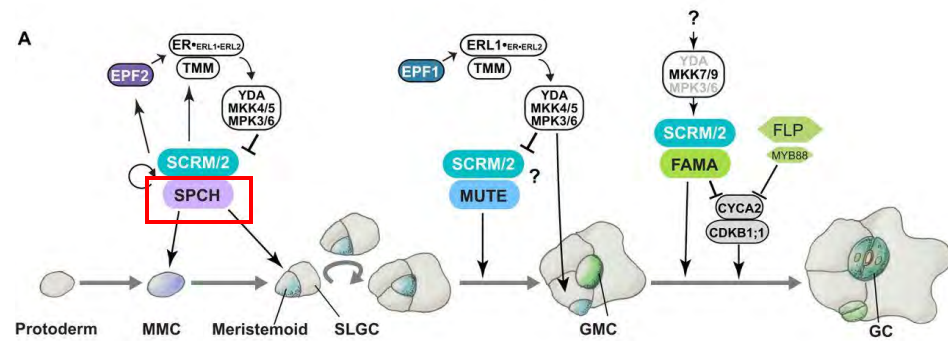
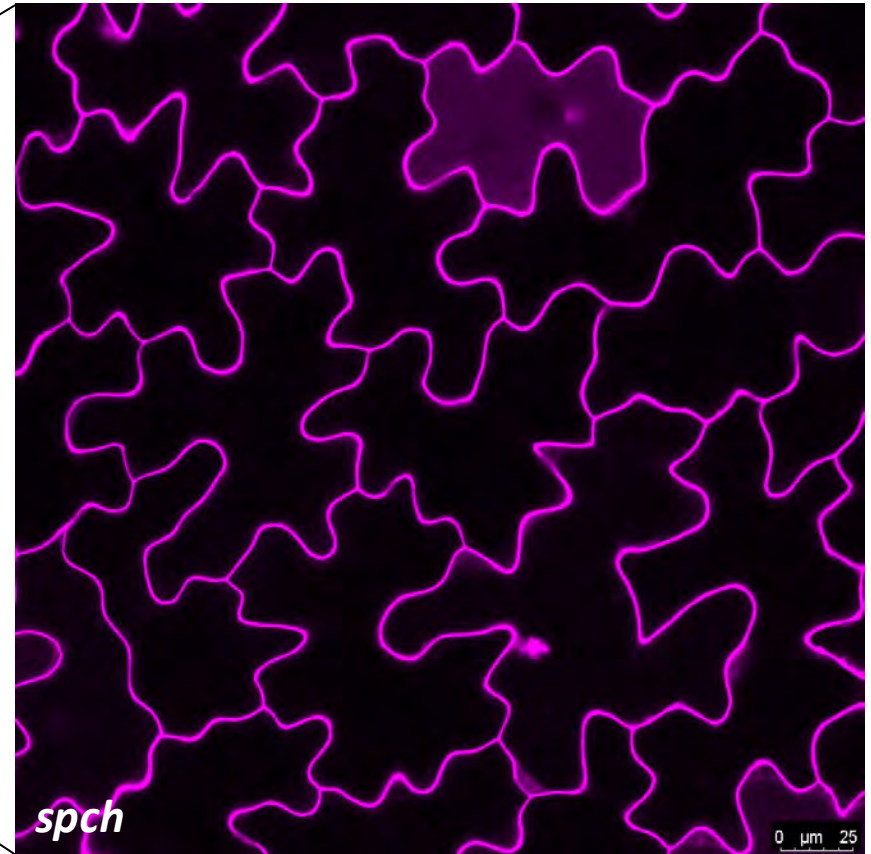
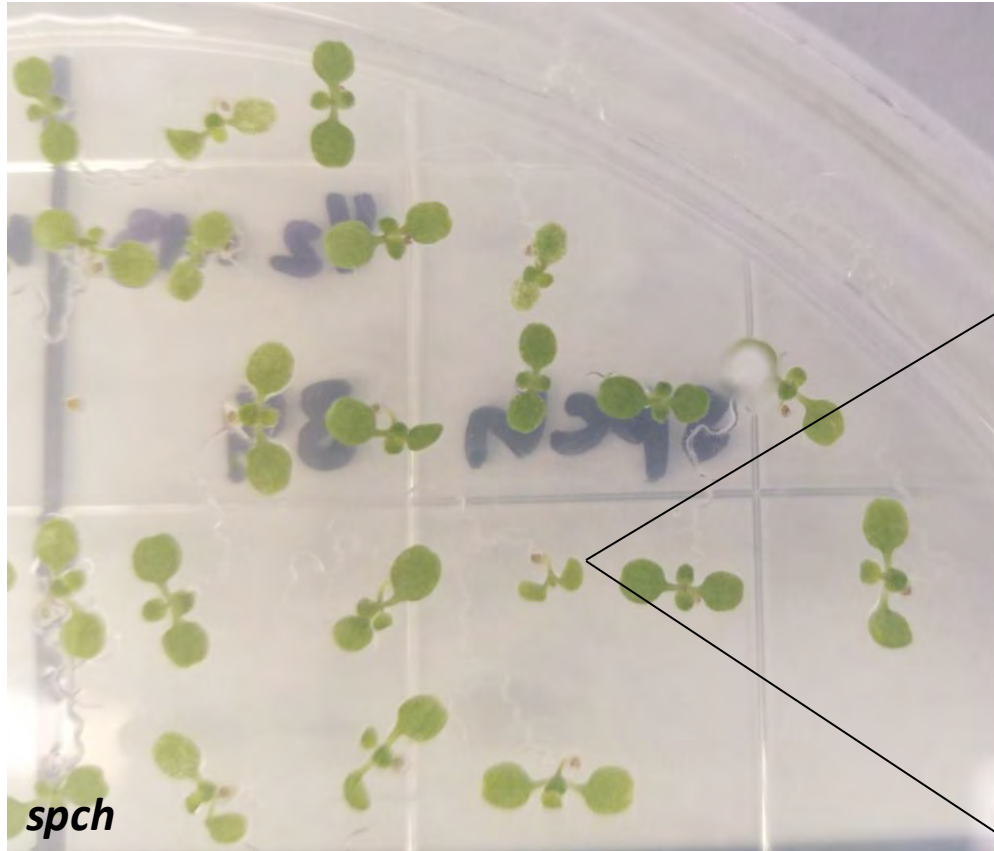
Methods

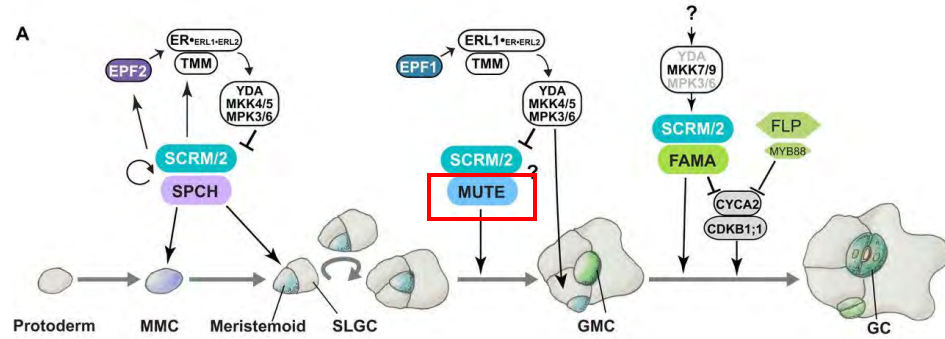
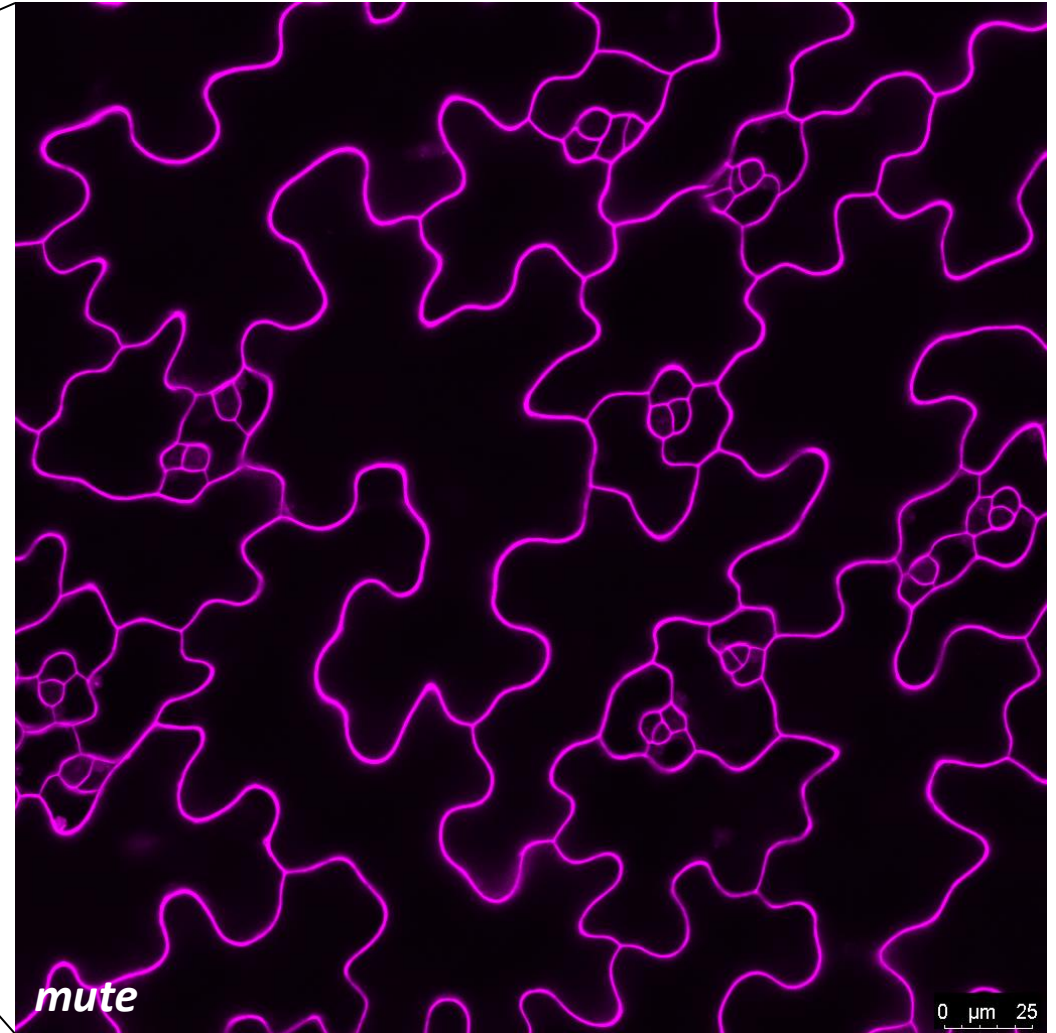
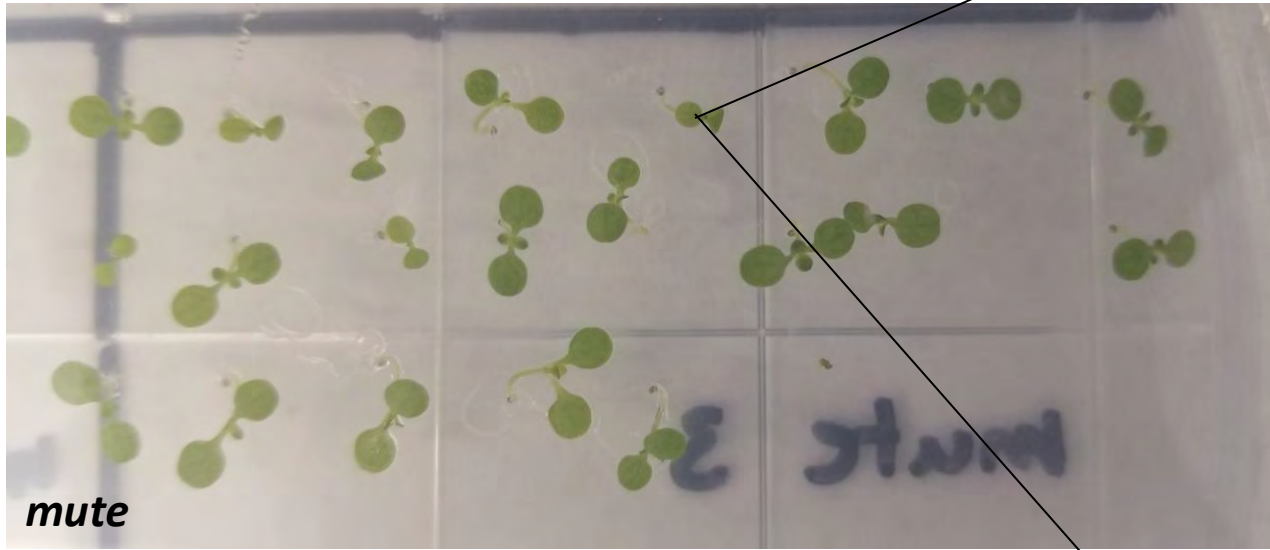
- Approach I: Genetics
 - Mutants with **no** stomata: smaller, yellow leaves
 - Mutants with **excess** stomata
 - Harder to discern from WT plants
 - Will appear greener and healthier than plants with no stomata
 - Have to use microscope to distinguish between these mutants and WT
- Approach II: Molecular
 - Genotypic confirmation of T-DNA insertion through PCR
 - Definite test, if mutants do not show phenotype under confocal

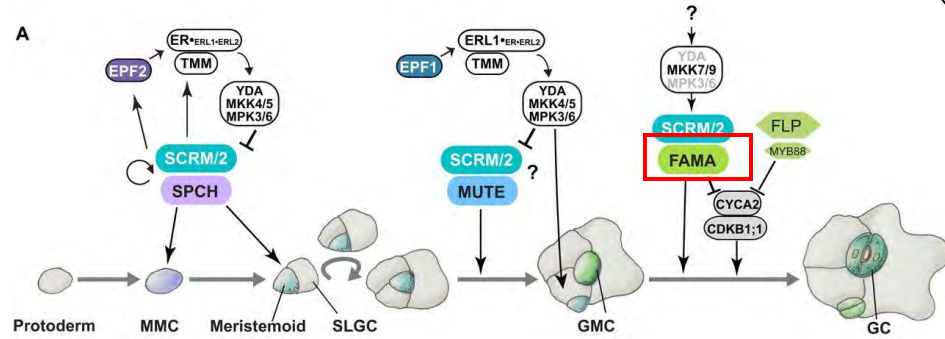
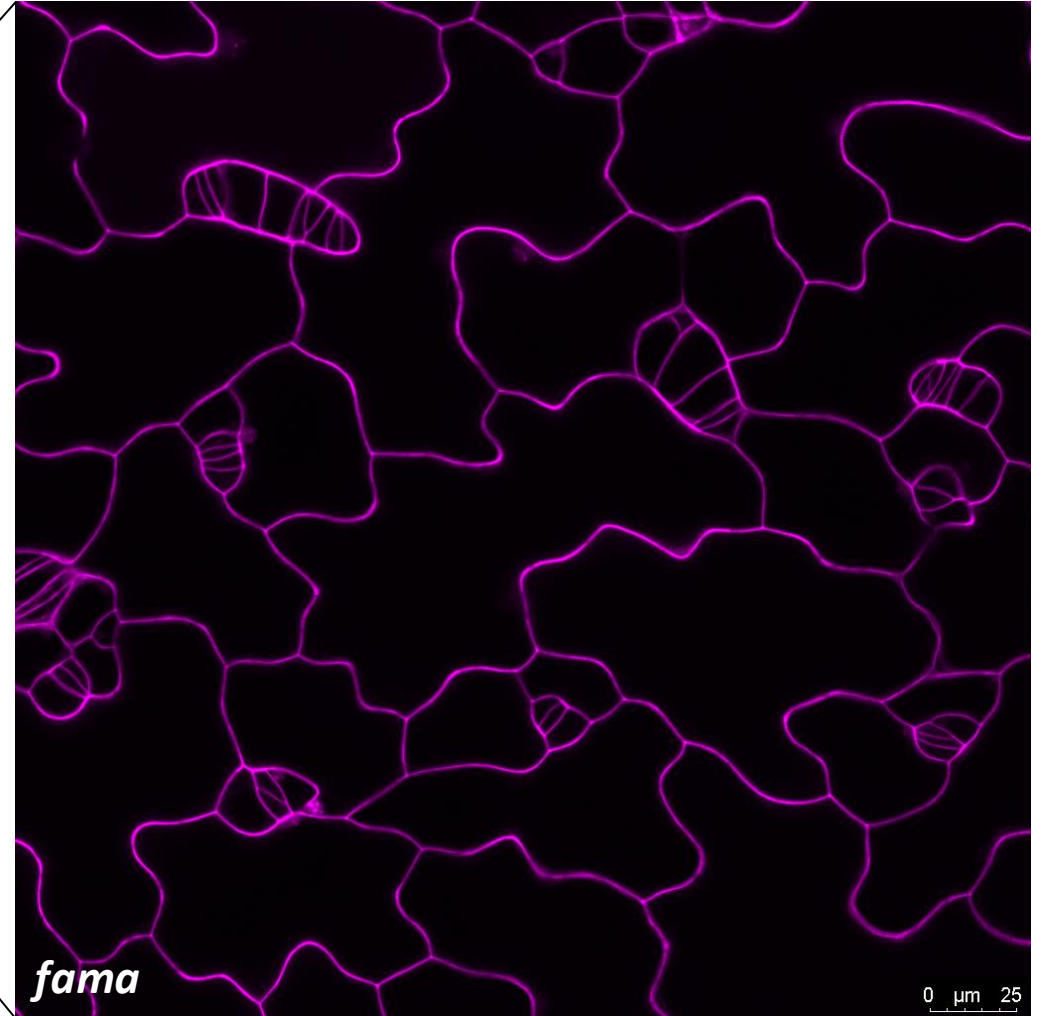
Results

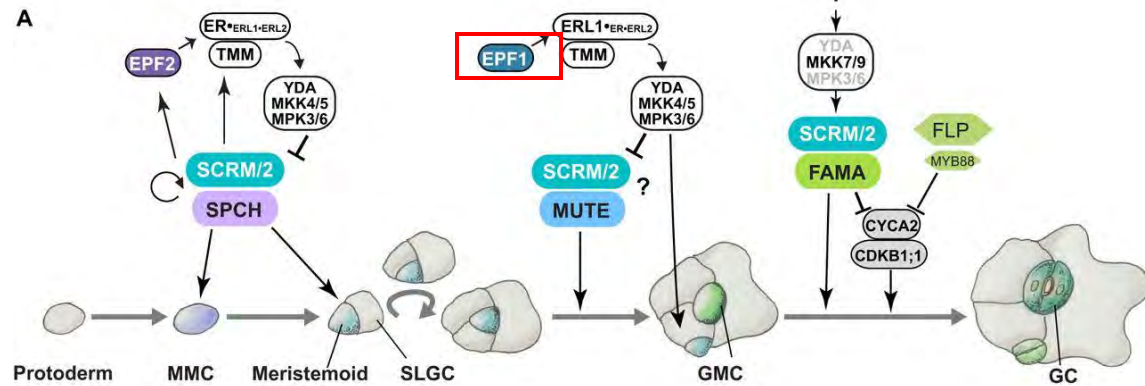




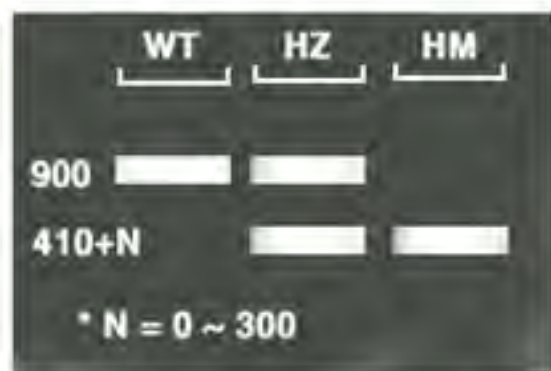
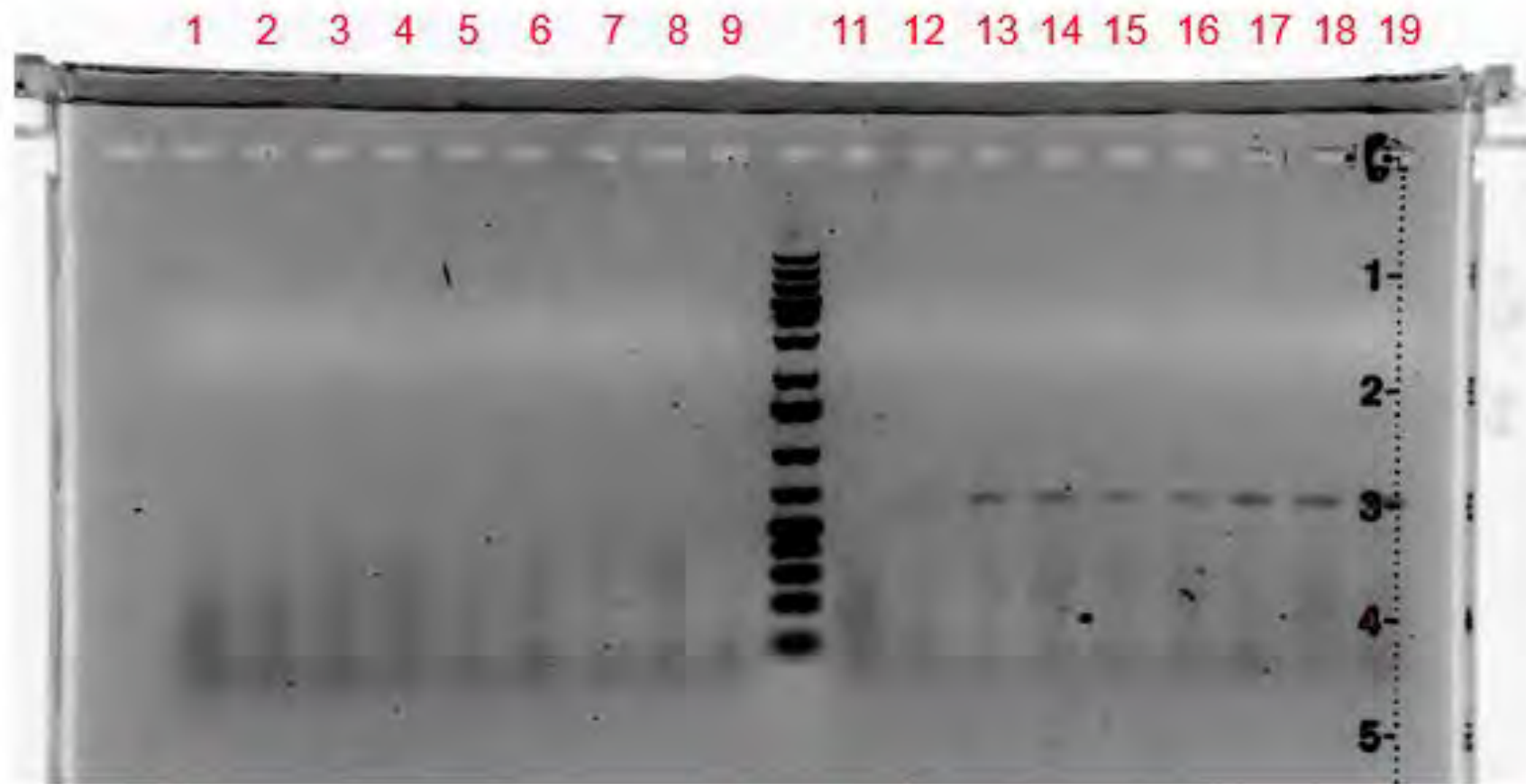








Mutant	Insertion Site
SAIL_532_B07	Exon
SALK_063396	5'UTR
SALK_204587	5'UTR
SALK_112714	3'UTR
SALK_111174	3'UTR
SALK_137549	Intron
SAIL_1054_A10	Promoter
SALK_091319	Promoter
SALK_035606	-
SALK_016986	-
SALK_035511	-
SAIL_367_D11	-
SALK_144857	-
SALK_091323	Promoter
SALK_016920	-
SALK_023569	Promoter
SALK_016911	-



WT
Primers
 SALK 035606 LP and RP

Mutant
Primers
 LBb1.3 and SALK 035606 RP

Future Directions

- Assess mRNA expression of SALK T-DNA mutants using RT-PCR
- Determine phenotype of those mutants using the confocal microscope