



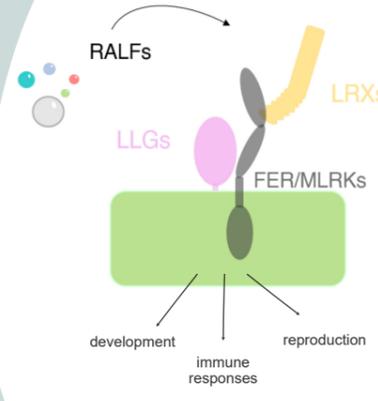
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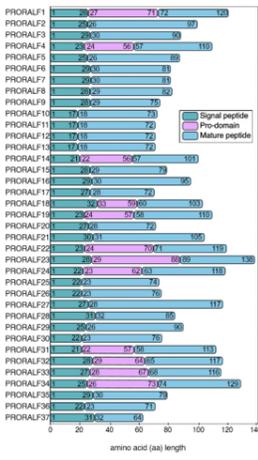
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## Background & motivation

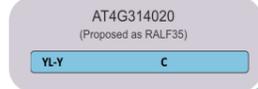
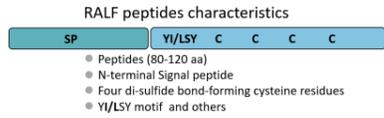
- Rapid alkalization factors (RALFs) are cysteine-rich peptide hormones involved in multiple processes (1). In *A. thaliana*, there are more than 30 predicted RALF peptides and around 1/3 are predicted to be cleaved by the protease S1P. However, there was no consensus regarding the number of members of this family. This discrepancy prompted us to carefully investigate the *A.thaliana* RALF family annotation (5).
- Only a few members of the family have been shown to cause medium alkalization, growth inhibition and recently also to modulate immune responses (2,3,4). Here we tested for the bioactivity of 32 members of the family in *Arabidopsis thaliana* using synthetic peptides.
- RALF23 and its protease S1P have been shown to be negative regulators of immunity. We are interested in deciphering the role other RALF peptides in plant immunity.
- RALFs have been shown to be ligands of *Catharanthus roseus* RLK1-LIKE (CrRLK1L) receptor kinases (1). The CrRLK1L FERONIA (FER) is the best characterized member of the family. Some RALFs are also recognized by heteromeric receptor complexes involving GPI-anchored LRE/LLG1 proteins and leucine-rich repeat extensin proteins (LRXs)(1). Nevertheless, only a few receptor-ligand complexes have been identified. We are interested in identifying more receptor-ligand pairs.



## New proposed *A. thaliana* RALF family annotation

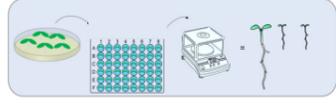
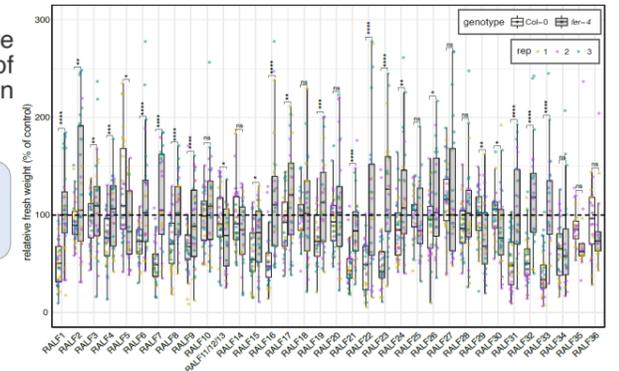


- AT2G32890 and AT4G14020 are not genuine RALF peptides
- We propose a new RALF peptide annotation to keep consistency



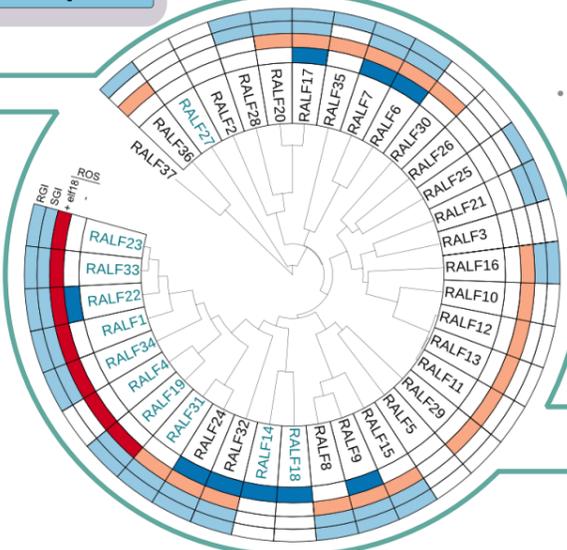
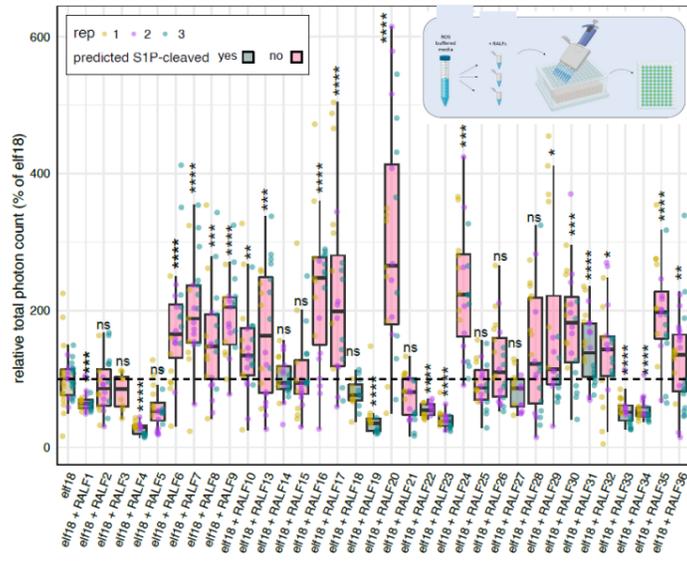
## Most RALFs induce FER-dependent seedling and root growth inhibition

- We compared seedling and root growth inhibition data of Col-0 with the mutant line *fer-4* to test for bioactivity of RALFs and their FER-dependency.
- *fer-4* mutant seedlings were insensitive to the majority of RALFs that are active in Col-0.



## Multiple RALFs modulate PAMP-induced ROS production

- Which RALFs modulate ROS responses?

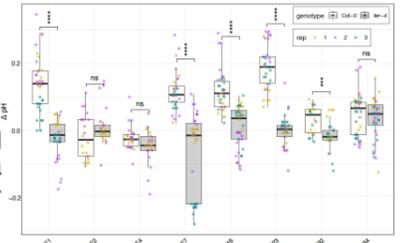
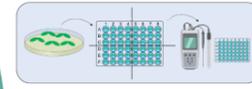


- Predicted S1P-cleaved RALFs inhibit elf18-induced ROS production.
- Non S1P-cleaved RALFs increase elf18-induced ROS production.

- Which RALFs are positive regulators of immunity?

- Based on expression data, ROS burst modulation and predicted LLG1 perception, we selected candidates for generation of high order CRISPR mutants

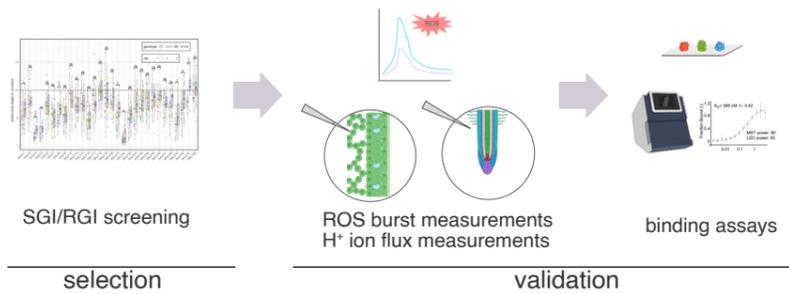
- Is the alkalization activity of RALFs related with the growth inhibition?



- Growth inhibition and alkalization data correlate in a FER-dependent manner

## In search of CrRLK1L-RALF pairs

- Only a few CrRLK1L-RALF receptor ligand pairs have been identified so far



- These assays with different *CrRLK1L* mutants provide the basis for the future identification of additional receptor-ligands pairs.

## Conclusions & future directions

- AT2G32890 and AT4G14020 are not genuine RALF peptides.
- The majority of RALF peptides inhibit growth and alkalize the media in a FER-dependent manner.
- The majority of predicted S1P-cleaved RALF peptides inhibit elf18-induced ROS burst.
- The majority of non S1P-cleaved RALF peptides increase elf18-induced ROS burst.

- Test SGI and RGI in different *CrRLK1L* mutants to identify additional receptor-ligand pairs.
- Generate CRISPR-Cas9 mutant of different RALFs, and test their implication in pattern-triggered immunity.

## References

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## Funding



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