

Read me:

1. This file displays the phenotypes of **complemented lines for ARENA mutants and their immunoblotting results**. The catalog is on Slide 2.
2. A phenotype image of the original ARENA mutant is shown on each slide and labeled with mutant ID and gene information. See **Data S1** for details.
3. Transformants derived from the mutant background with plasmids carrying wild-type genes were arrayed for phenotyping. Each array includes two colonies of wild-type GV32 (WT) and two of the original mutant as controls.
4. 'M' refers to the original mutant; 'Cx' to phenotypically-rescued lines; 'Nx' to transformants with mutant-like phenotypes.
5. For each gene, over 10% of transformants exhibited rescued phenotypes, explaining why many 'Cx' line neighbors also appeared wild-type.
6. Immunoblotting with an anti-FLAG antibody showed expected fusion protein bands in 'Cx' lines but not in 'Nx' lines for *PMR1*, *CPLD38*, and *CCS5*. Other cases had limited signal-to-background ratios, likely due to transgene expression challenges in *C. reinhardtii*. The high percentage of rescued phenotypes, beyond what random suppressor mutations would suggest, is the basis for our conclusion of successful genetic validation.
7. For each ARENA mutant, two or three lines have been cryopreserved for community sharing to facilitate further research.
8. In the immunoblotting results, the red arrows indicate the bands corresponding to the fusion protein, which are close in size to the expected band. Notably, in the immunoblot of the *hvfn1* complementary strains, we observed two bands.

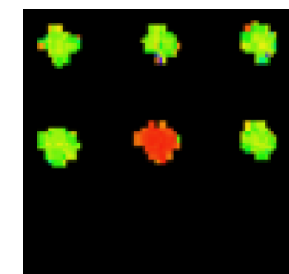
## Catalog

<b>Mutant ID</b>	<b>Mutant name</b>	<b>Slide or figure</b>
<i>arn0638</i>	<i>pmr1</i>	Slide 3
<i>arn0086</i>	<i>rsga</i>	Slide 4
<i>arn0415</i>	<i>not11-1</i>	Slide 5
<i>arn0586</i>	<i>cpld38</i>	Slide 6
<i>arn0095</i>	<i>ccs5</i>	Slide 7
<i>arn0525</i>	<i>hvfn1</i>	Slide 8
<i>arn0068</i>	<i>tsp2</i>	Figure 4

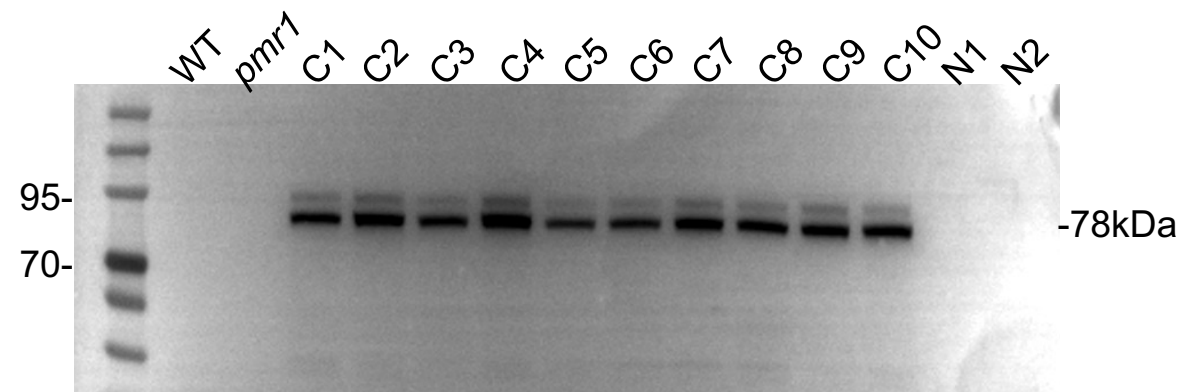
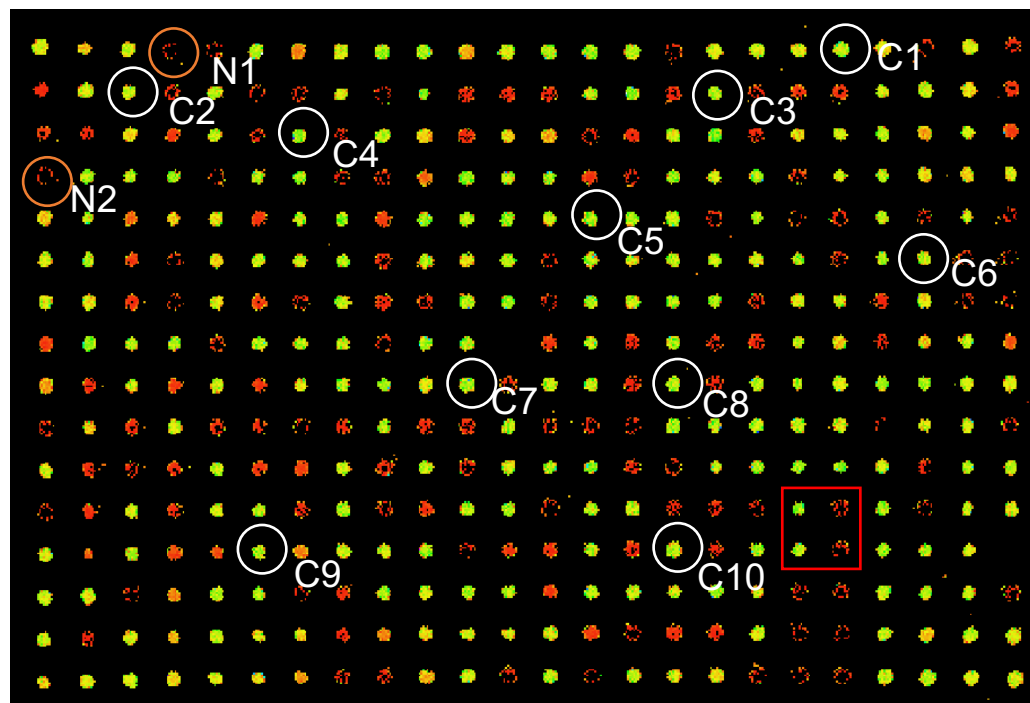
*pmr1, arn0638*

*PMR1*, Cre10.g448950

Nocturnin (CCRN4L)



-N<sub>low</sub> NPQ



-PMR1-mCherry-Flag 78kDa

WT	m
WT	m

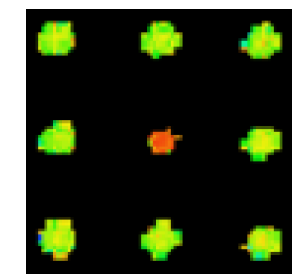


-PMR1-mCherry-Flag 78kDa

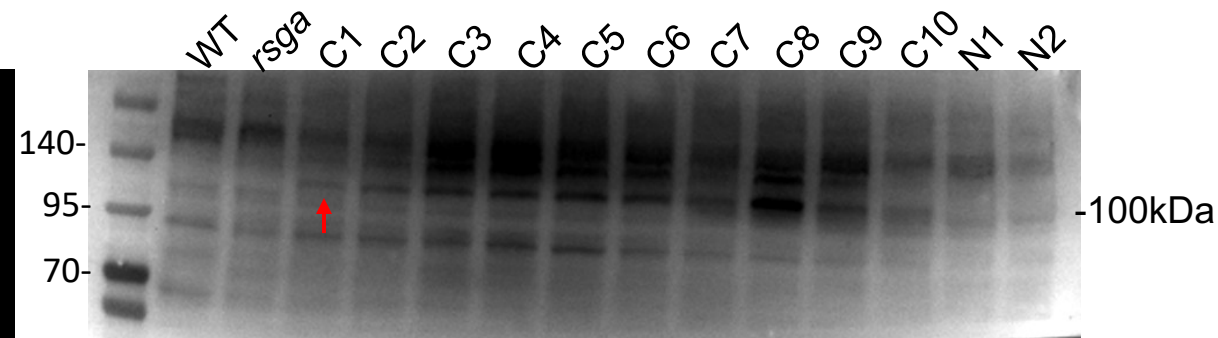
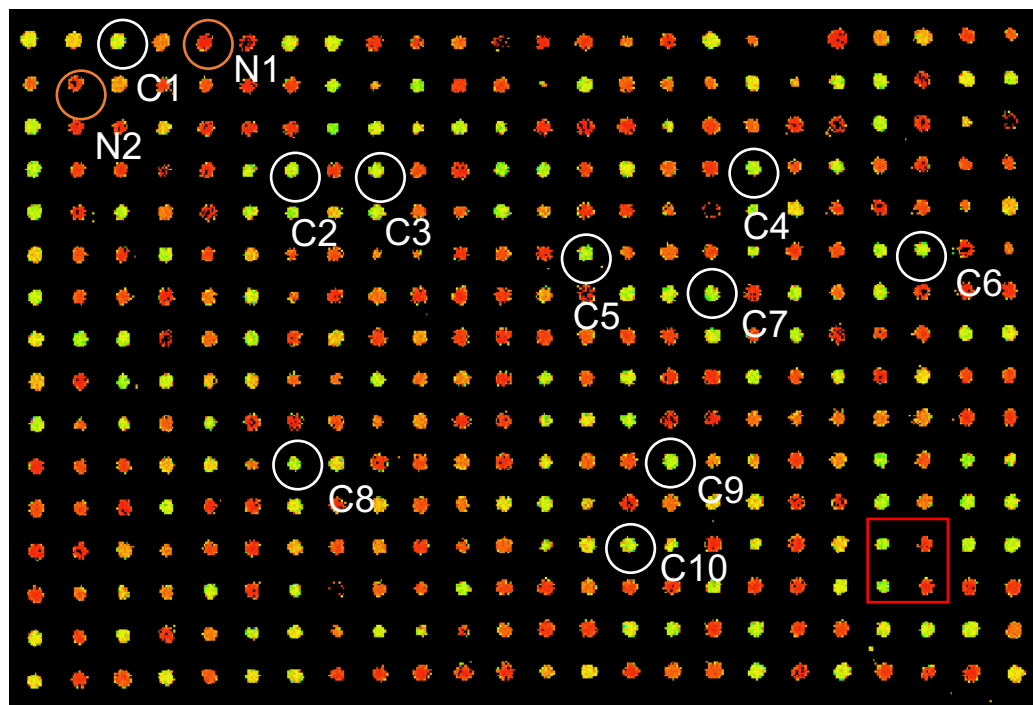
*rsga*, *arn0086*

RSOA, Cre10.g436600

Ribosome biogenesis GTPase (*rsgA*, *engC*)



-N\_low NPQ



WT	m
WT	m

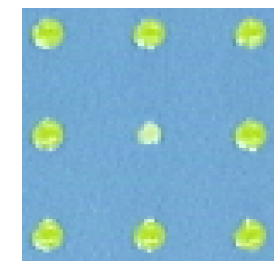


-RSGA-mCherry-Flag 100kDa

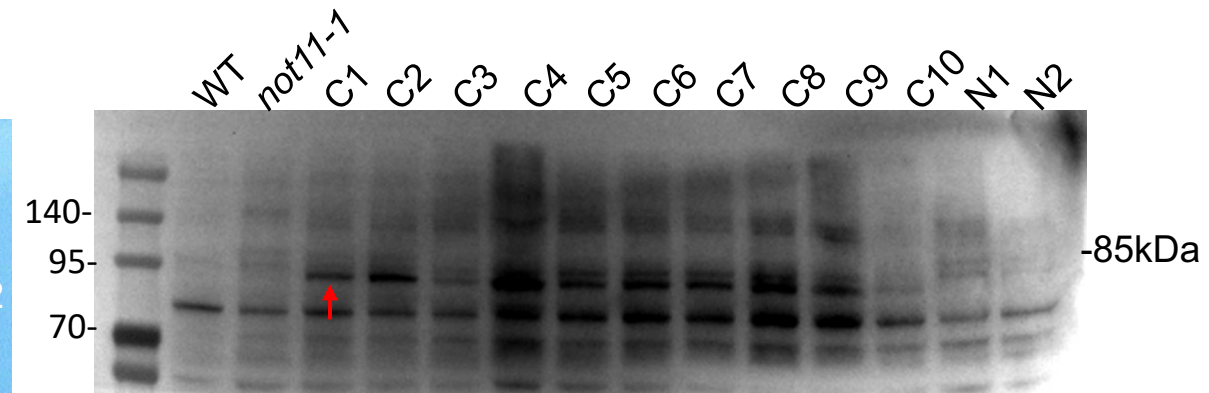
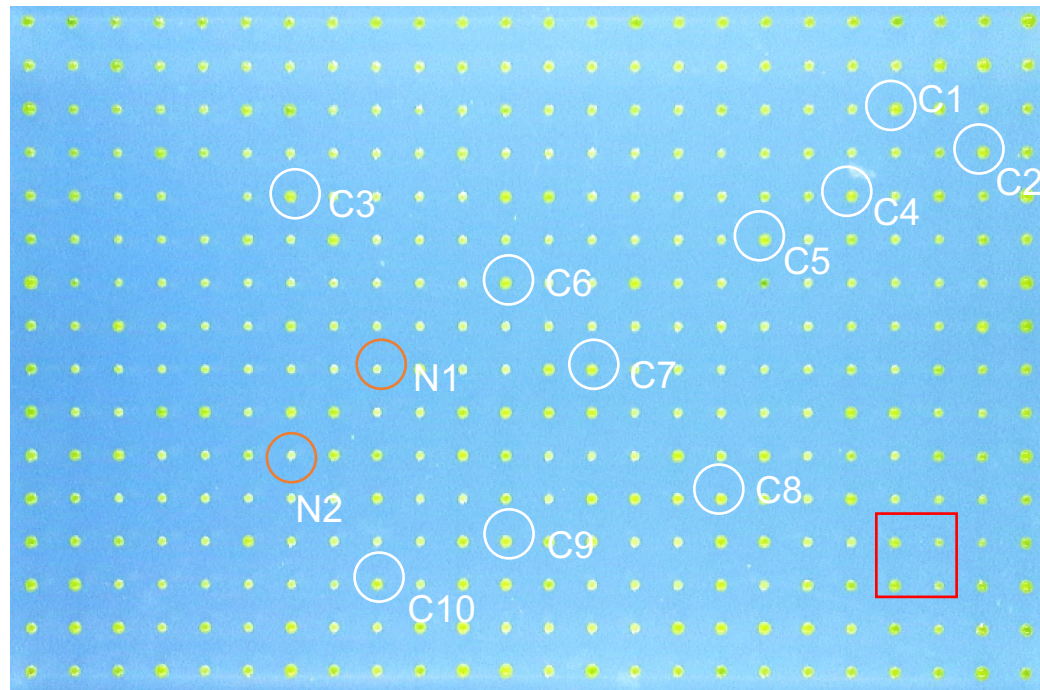
*not11-1, arn0415*

*NOT11*, Cre17.g726800

Uncharacterized conserved protein (DUF2363)



-N\_fast  
chlorosis



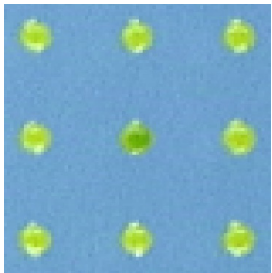
WT	m
WT	m



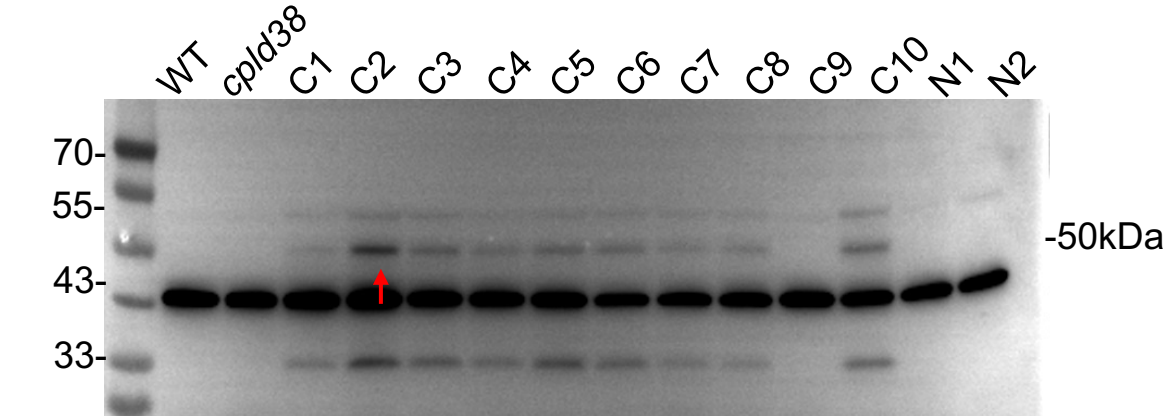
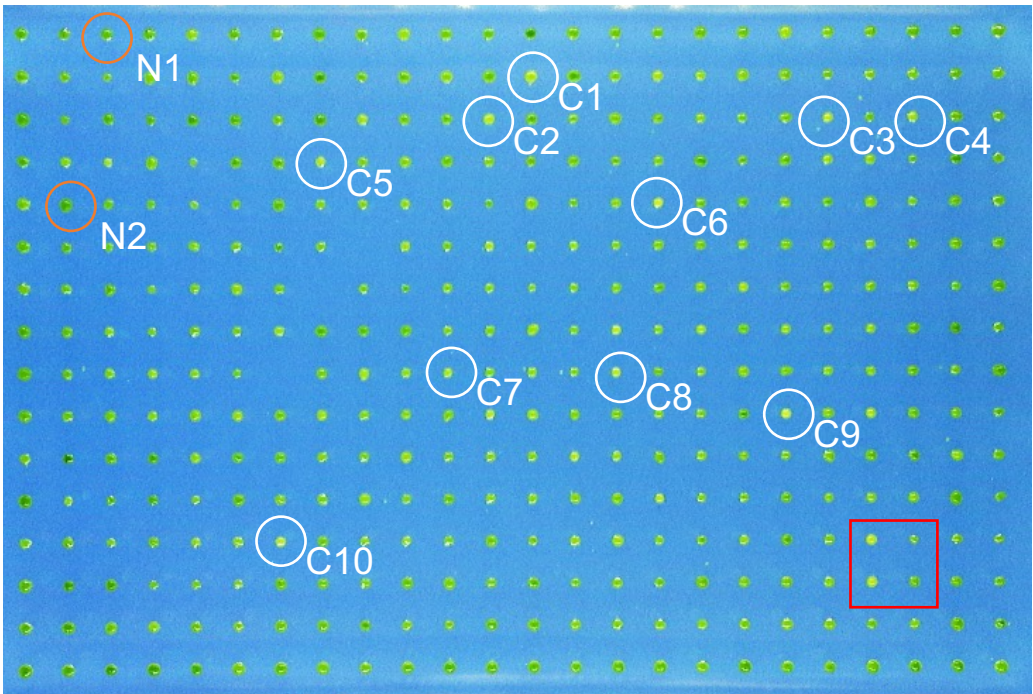
-NOT11-mCherry-Flag 85kDa



*cpId38, arn0586*  
*CPLD38*, Cre01.g000850  
Predicted protein, CPLD38



-N\_fast  
chlorosis



WT	m
WT	m

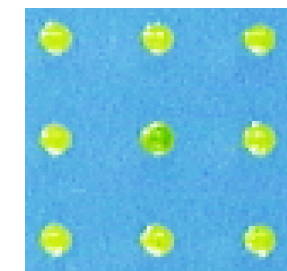


-CPLD38-mCherry-Flag 50kDa

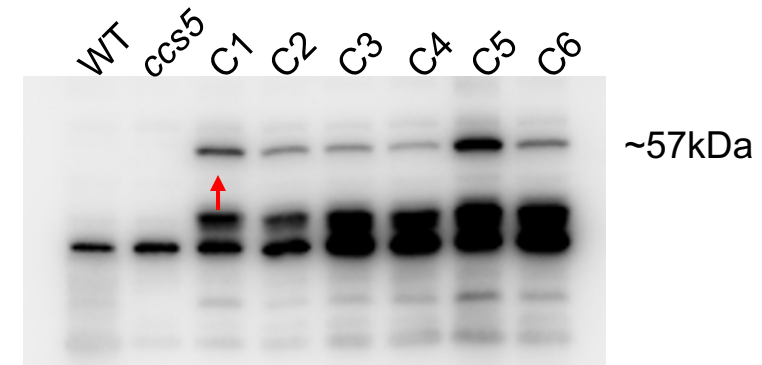
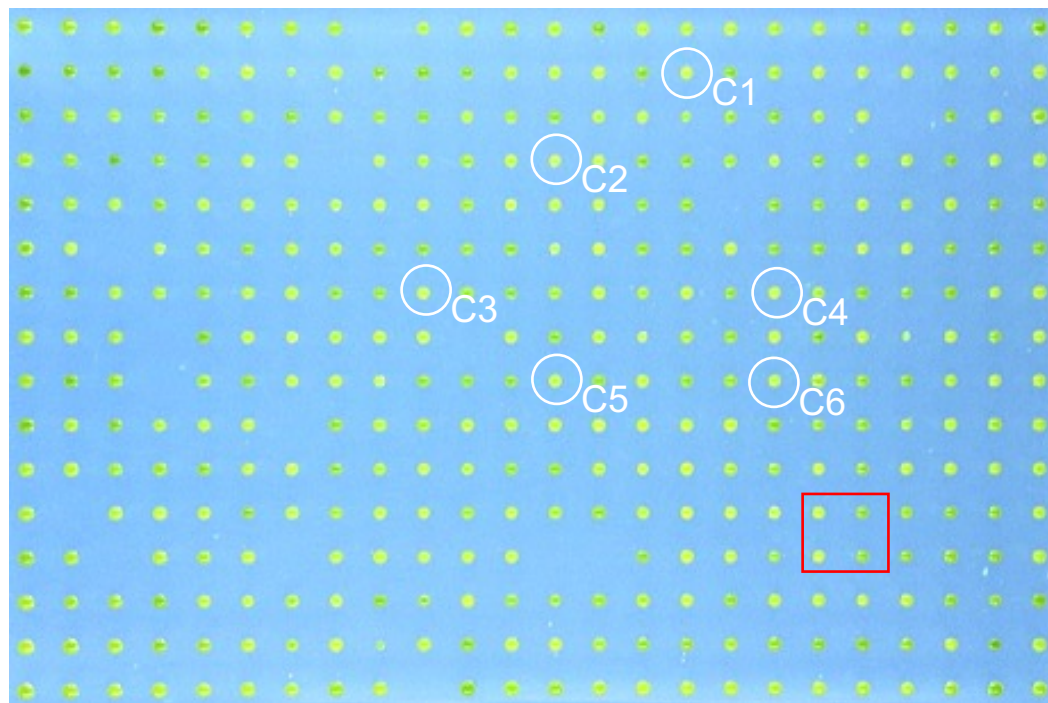
*ccs5, arn0095*

CCS5, Cre17.g702150

Thioredoxin-like protein hcf164, chloroplastic HCF164, TRX20, CCS5



-N\_fast  
chlorosis



WT	m
WT	m

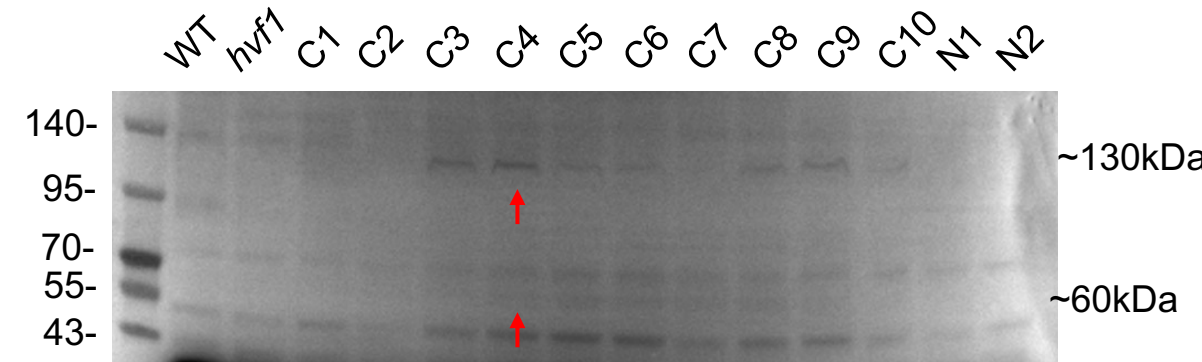
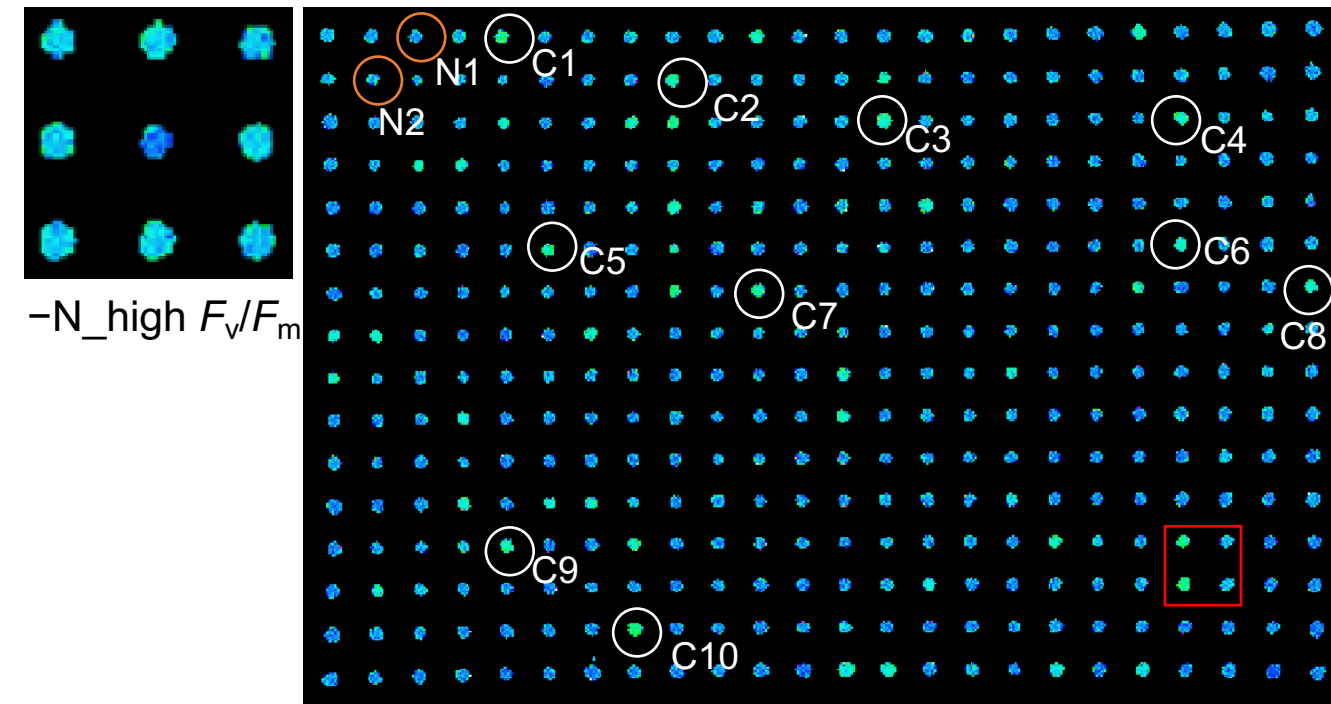


-CCS5-mCherry-Flag 57kDa

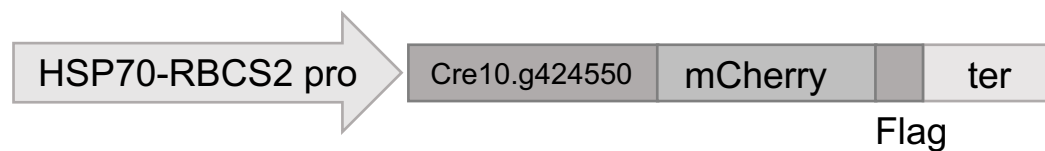
*hvf1*, *arn0525*

*HVFN1*, Cre10.g424550

Coexpressed with genes in phototrophic condition specific coexpression subnetwork



WT	m
WT	m



-HVFN1-mCherry-Flag 74kDa