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SCANNER: Single-Cell ANalysis of Arabidopsis immuNE Responses

Julien Lang

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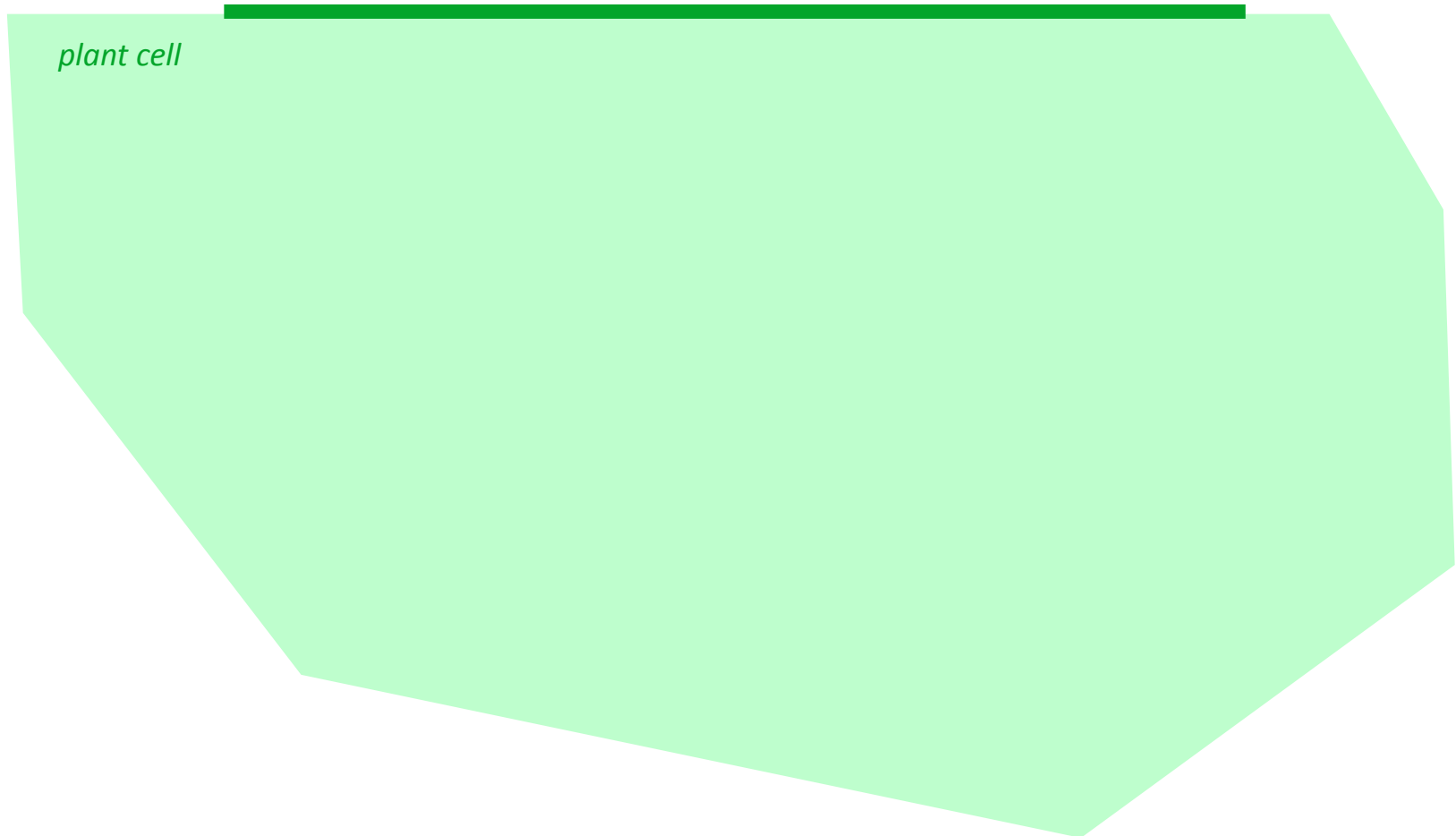
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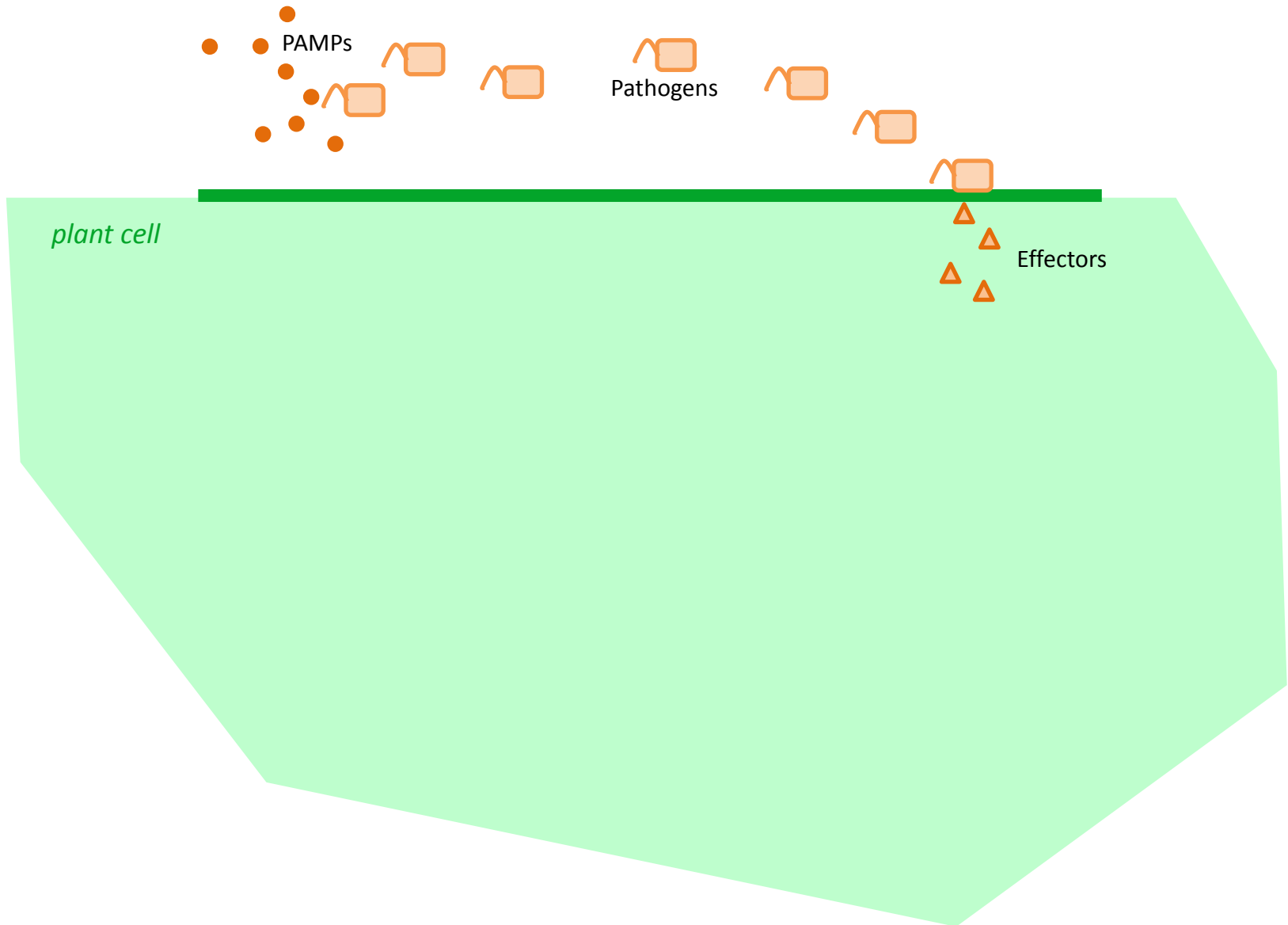
SCANNER: Single-Cell ANalysis of Arabidopsis immuNE Responses

Julien Lang
16/06/2022

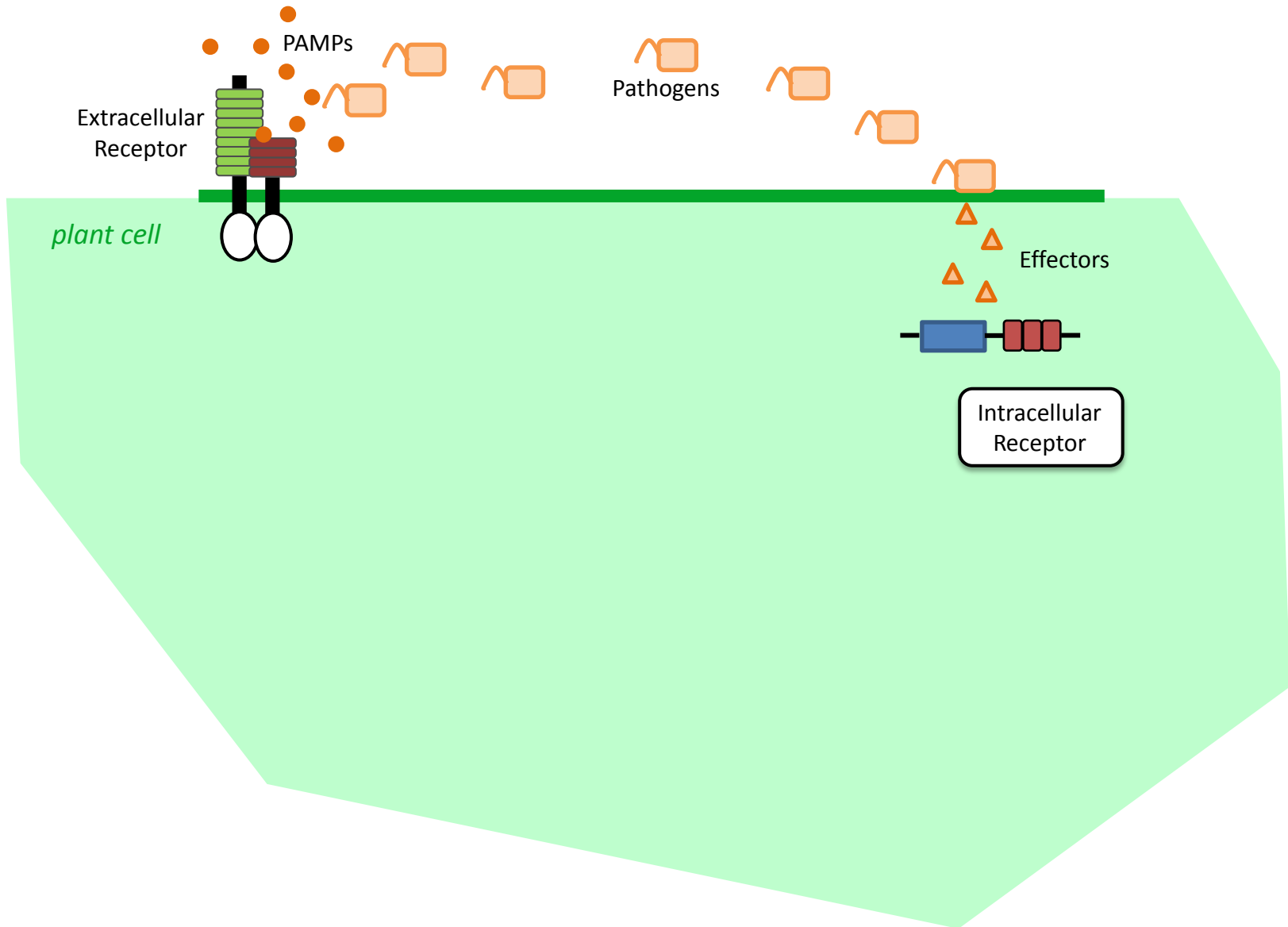
Plant Defense responses



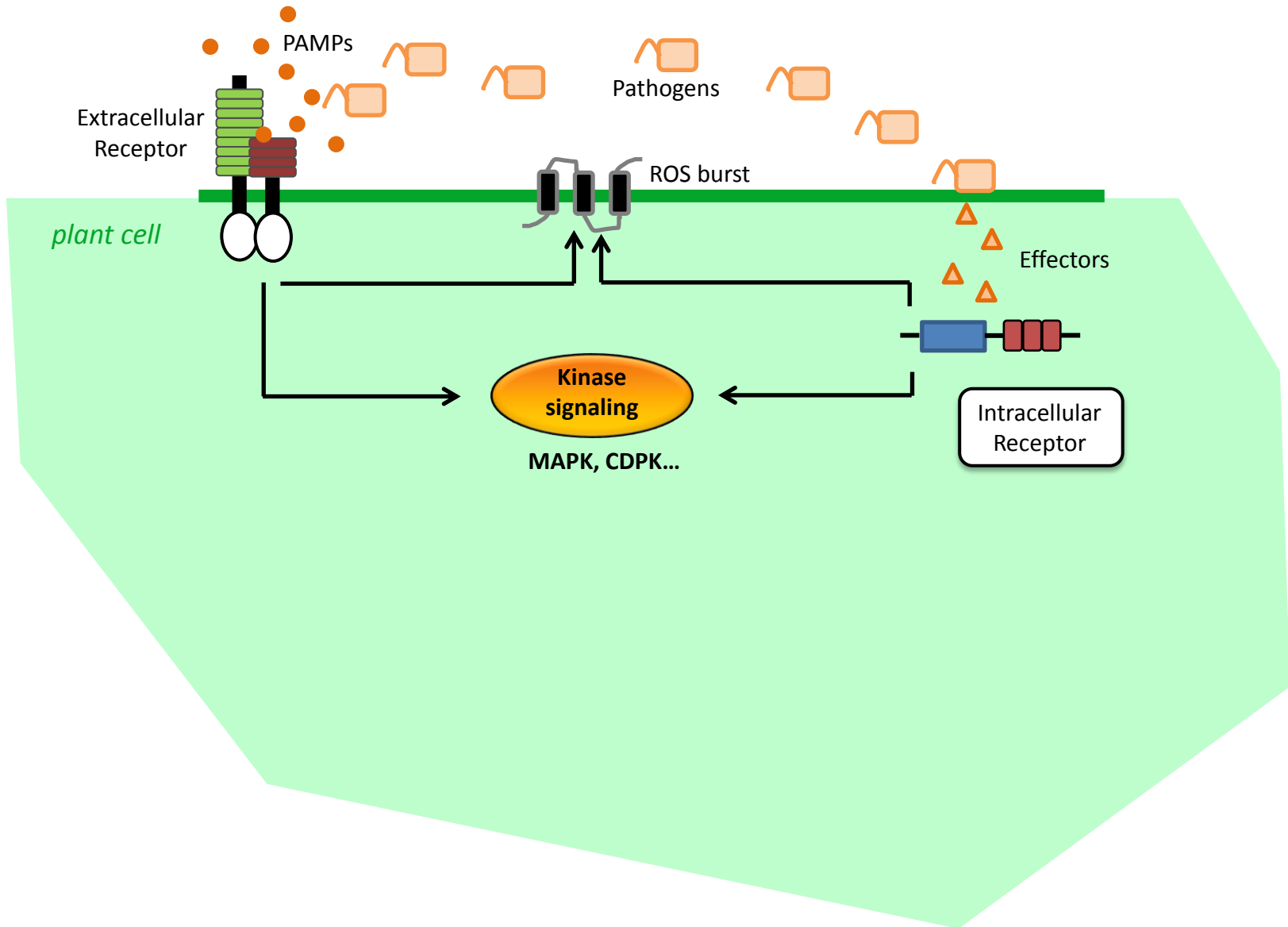
Plant Defense responses



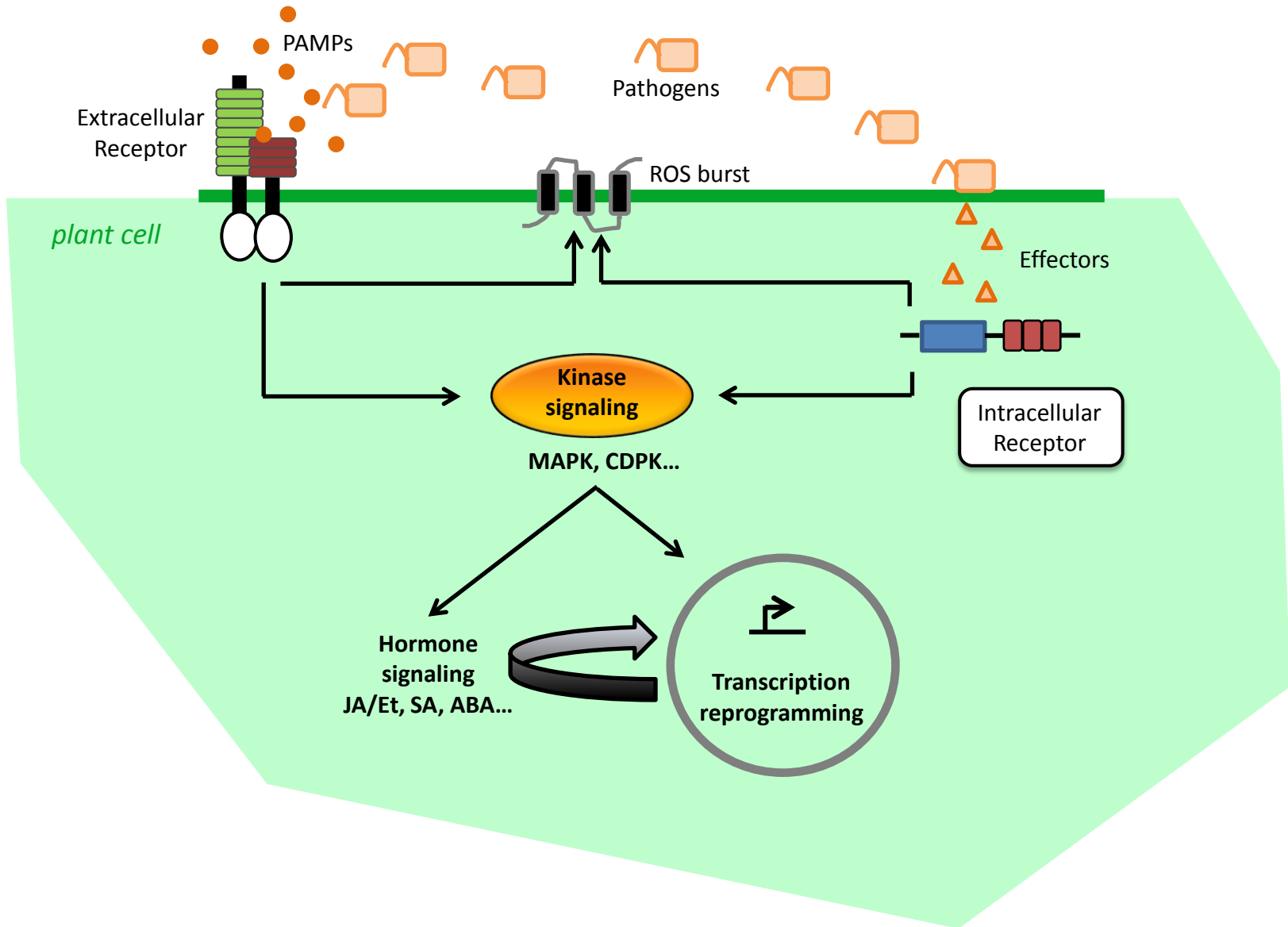
Plant Defense responses



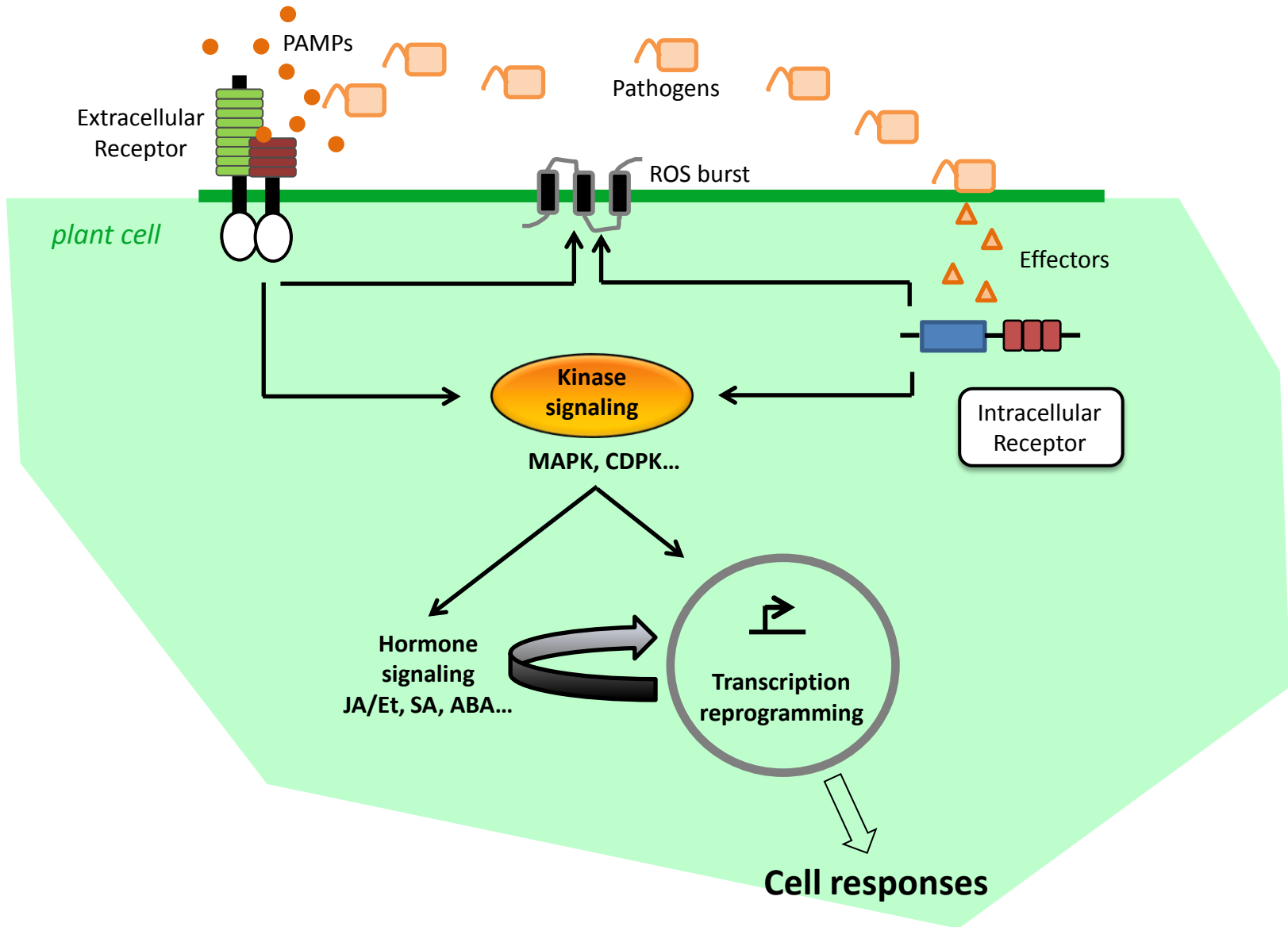
Plant Defense responses



Plant Defense responses

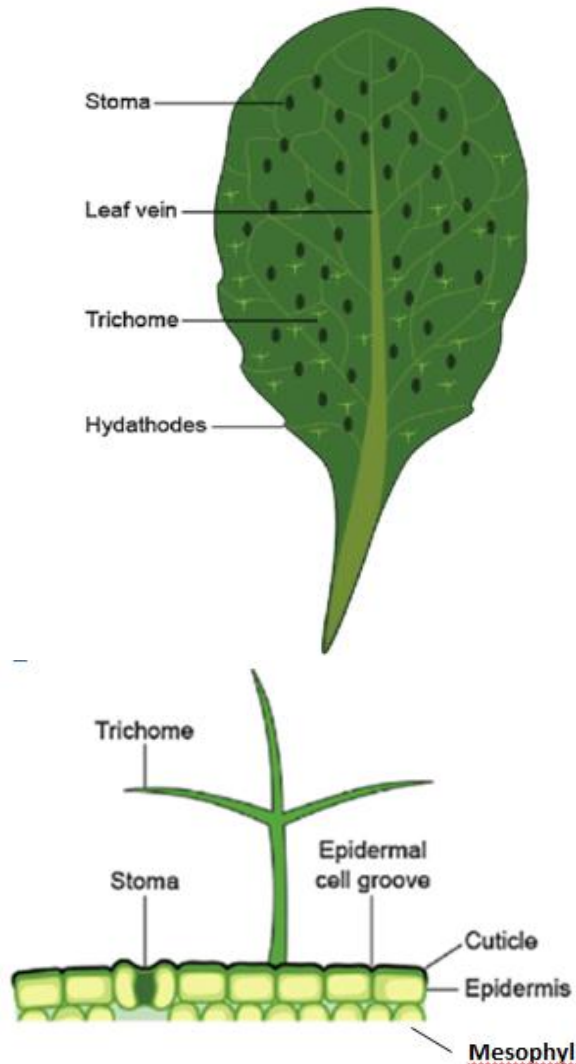


Plant Defense responses



SCANNER: Biological questions

A



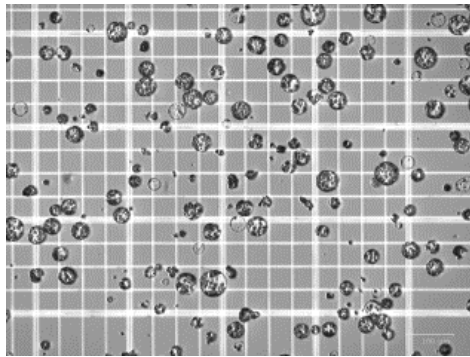
Heterogeneity of transcriptional immune responses in leaves?

- **Cell type immunity**
- **Specialization and coordination of defense responses between different cell populations**

SCANNER: project



- 1,5 month-old leaves Col-0 spray-infected with *P. syringae* DC3000 (OD=0,1)



16 hpi (bulk RT-qPCR, data from literature)

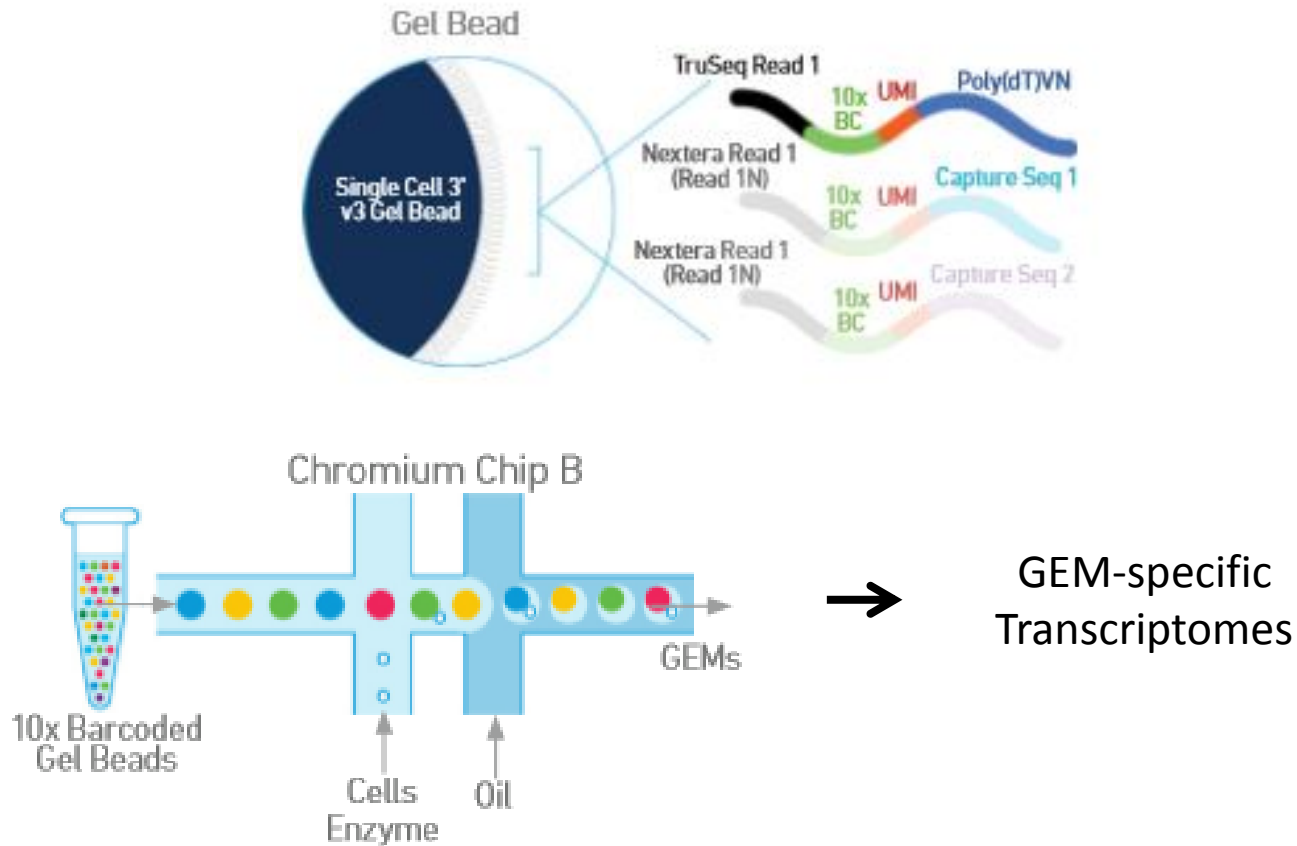
- Cell preparation (Protoplasts)

3 independant replicates

Chromium 10x

SCANNER: the Chromium technology

Gel beads in emulsion (GEMs)



90-99% of droplets contain no cell (but ambient RNA=background) and the remaining contain mostly 1 cell

SCANNER: Cell clustering

GEM-specific transcriptome



Which package? Which version?

Seurat 3.2.2

Replicate pooling

QC filter

Data normalization

PCA, nber of dimensions?

UMAP or tSNE?

Which genes govern the clustering?

Clustering, resolution?

List of marker genes

Cell classification

SCANNER: Cell clustering

GEM-specific transcriptome

Which package? Which version?

Seurat 3.2.2

BASTIEN BATARDIERE, MIA Paris-Saclay

université
PARIS-SACLAY

Tomorrow, 1:30 pm

“Analysis and exploration of single-cell RNA-seq
data using Seurat “



Cell classification

SCANNER: Results

- How to evaluate the biological relevance of the cell clustering
- How to harness it to extend our understanding of the biological question?
 - How to validate it?

SCANNER: Relevance of the clustering?

Metrics:

Nber of high-quality cells: 11206

Median Number of genes / cell : 2386

Median number of reads / cell: 7676

SCANNER: Relevance of the clustering?

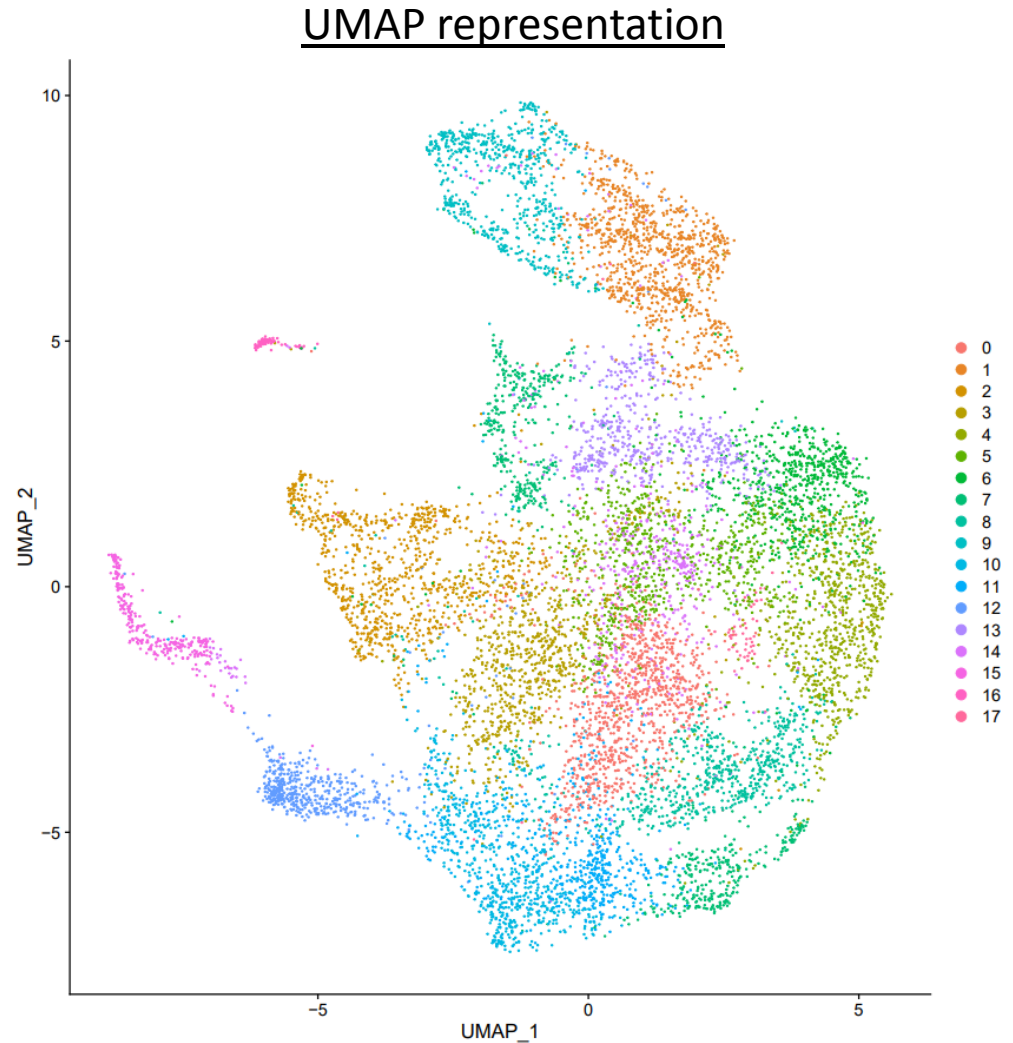
Metrics:

Nber of high-quality cells: 11206

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Number of clusters: 18



SCANNER: Relevance of the clustering?

Metrics:

Nber of high-quality cells: 11206

Median Number of genes / cell : 2386

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Number of clusters: 18

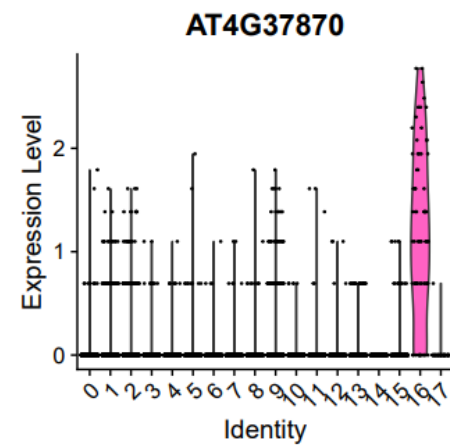
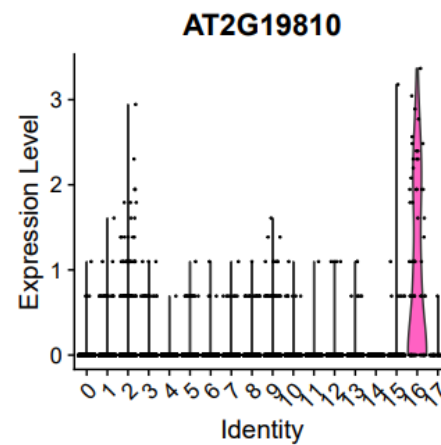
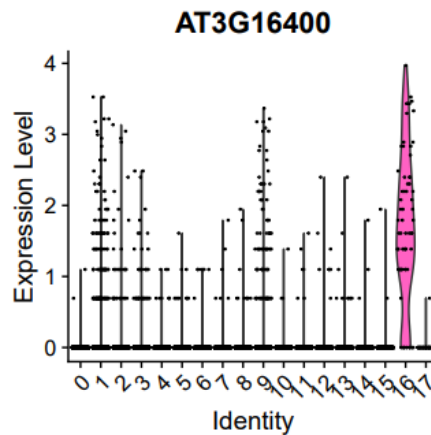
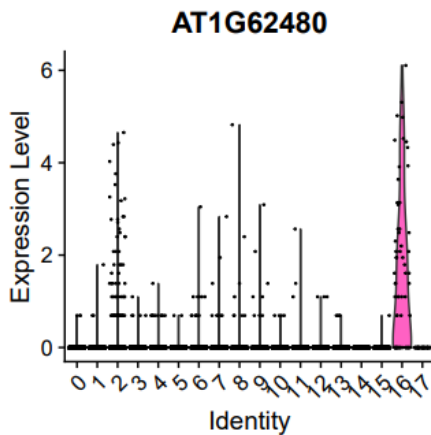
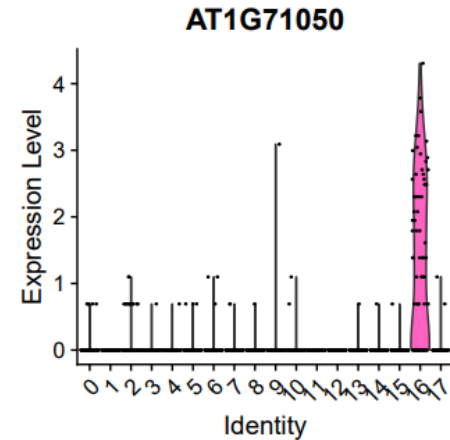
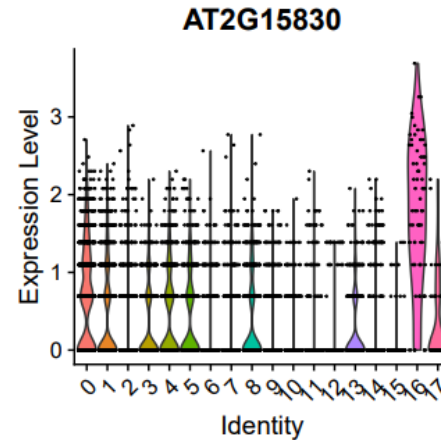
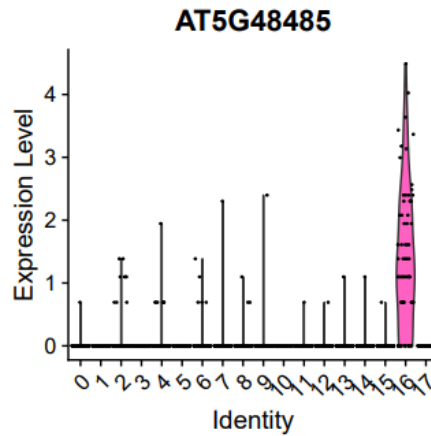
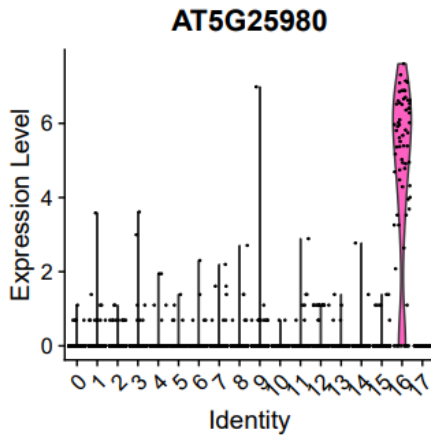
Number of cells / cluster

Number of marker genes

Clusters	Nber cells	Nber markers
C0	1003	306
C4	788	506
C10	648	695
C11	621	225
C7	693	498
C8	690	541
C12	534	130
C1	976	840
C3	833	298
C5	729	693
C6	728	293
C13	499	692
C2	908	445
C9	656	653
C16	71	316
C15	289	344
C14	479	43
C17	61	152

SCANNER: Relevance of the clustering?

Retrieve cell types (Guard cell markers)



SCANNER: Relevance of the clustering?

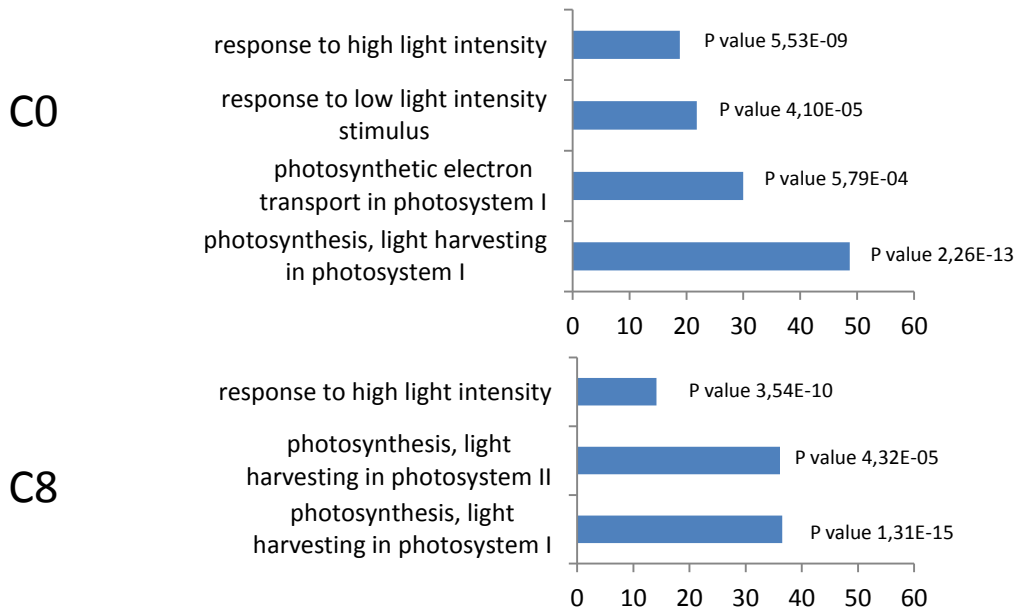
Retrieve cell types

Clusters	Cell Types
C0	Mesophyl cells
C4	
C10	
C11	
C7	
C8	
C12	
C1	
C3	
C5	
C6	
C13	
C2	
C9	Epiderm cells
C16	Guard cells
C15	Companion cells
C14	
C17	

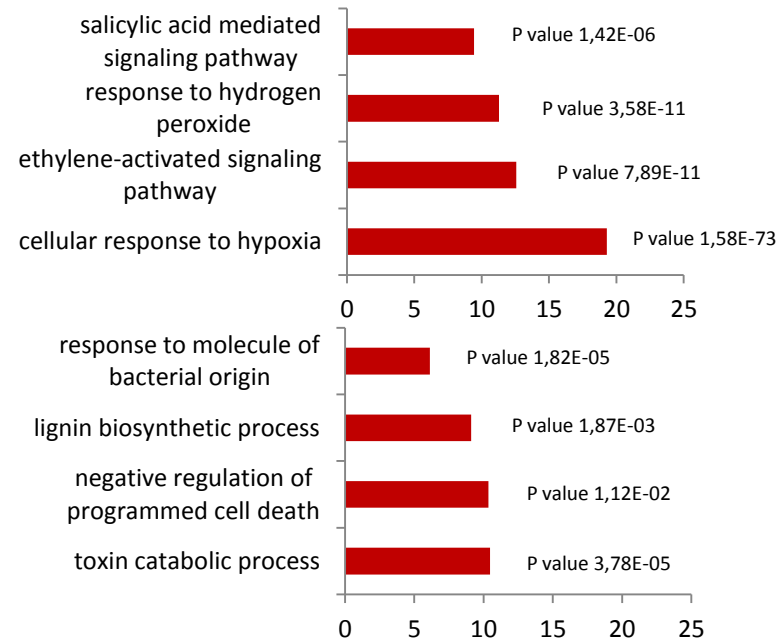
SCANNER: Relevance of the clustering?

GO Analysis (+ KEGG)

Cell identity GO enrichment



Stress-related GO enrichment



C13

SCANNER: Relevance of the clustering?

Clusters	Cell Types	Biological processes	
C0	Mesophyl cells	Healthy	
C4			
C10			
C11			
C7	Mesophyl cells	Hormone signaling (JA/Et/SA) ROS stress Hypoxia...	
C8			UPR, Kinase signaling...
C12			
C1	(???)	Lignin synthesis JA signaling Toxin catabolism Cell death regulation	
C3			
C5			
C6			
C13			
C2	S cells	H sulfide, glucosinolates synthesis, callose deposition...	
C9	Epiderm cells	Wax, cuticle, cellulose, glucosinolate synthesis....	
C16	Guard cells	ABA signaling (stomata closure)	
C15	Companion cells	Polyamine synthesis	
C14	rubbish		
C17	Dividing cells?		

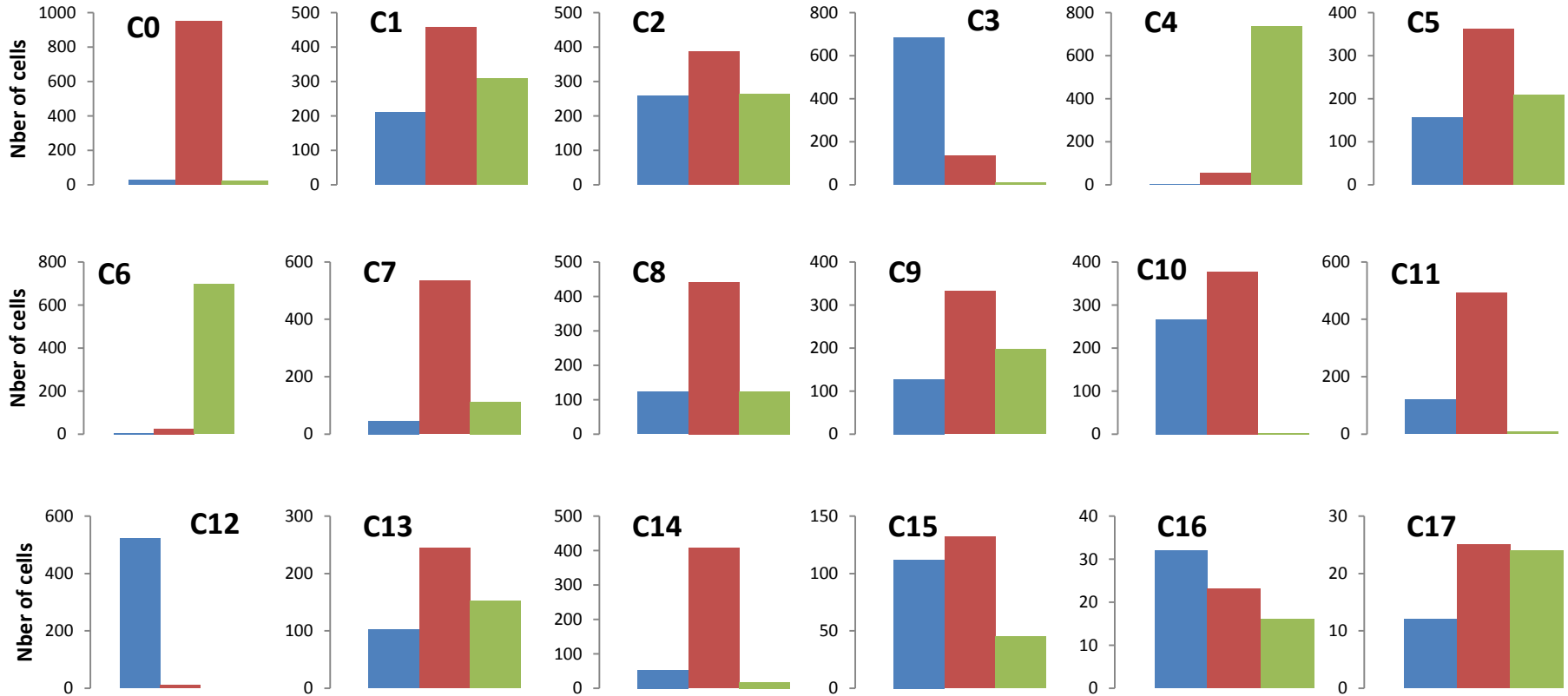
SCANNER: Relevance of the clustering?

Batch effects



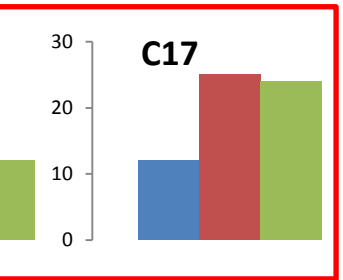
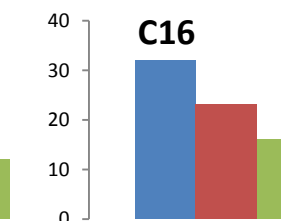
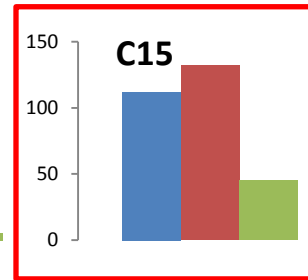
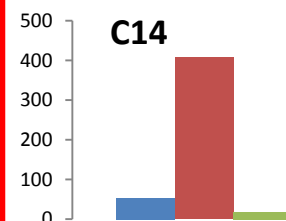
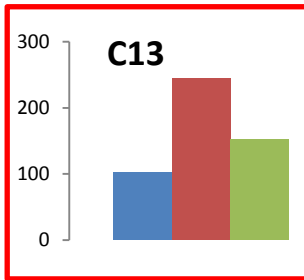
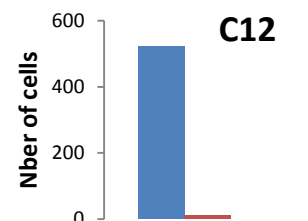
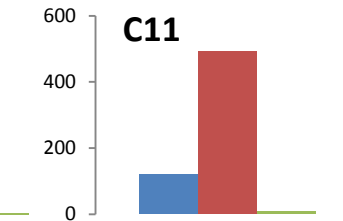
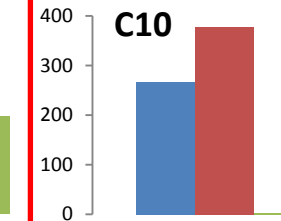
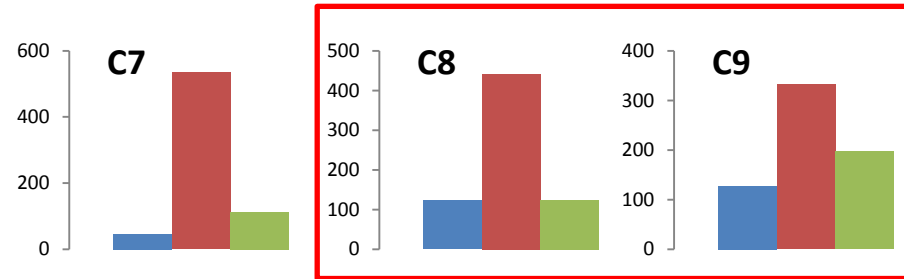
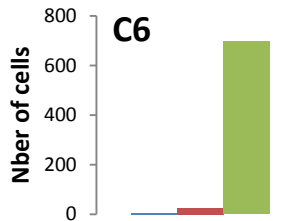
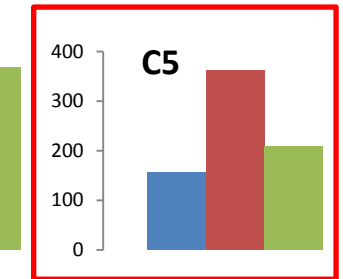
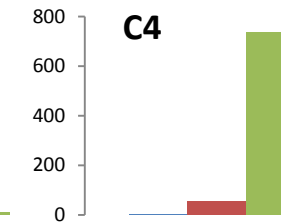
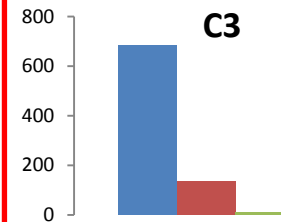
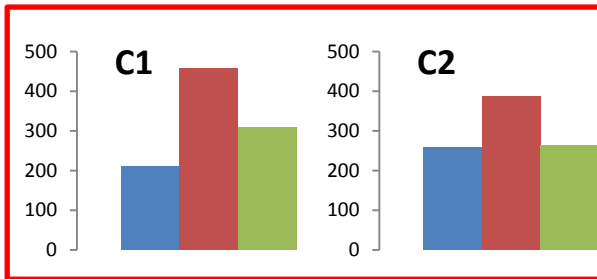
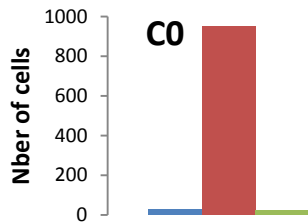
SCANNER: Relevance of the clustering?

Batch effects



SCANNER: Relevance of the clustering?

Batch effects



SCANNER: Relevance of the clustering?

Clusters	Cell Types	Biological processes	
C0	Mesophyl cells	Healthy	
C4			
C10			
C11			
C7	Mesophyl cells	Hormone signaling (JA/Et/SA) ROS stress ...	
C8			UPR, Kinase signaling...
C12			
C1	(???)		Lignin synthesis JA signaling Toxin catabolism Cell death regulation
C3			
C5			
C6			
C13			
C2	S cells	H sulfide, glucosinolates synthesis, callose deposition...	
C9	Epiderm cells	Wax, cuticle, cellulose, glucosinolate synthesis....	
C16	Guard cells	ABA signaling (stomata closure)	
C15	Companion cells	Polyamine synthesis	
C14	rubbish		
C17	Dividing cells?		

SCANNER: Harnessing the clustering

Transcriptional dynamics within and between clusters?

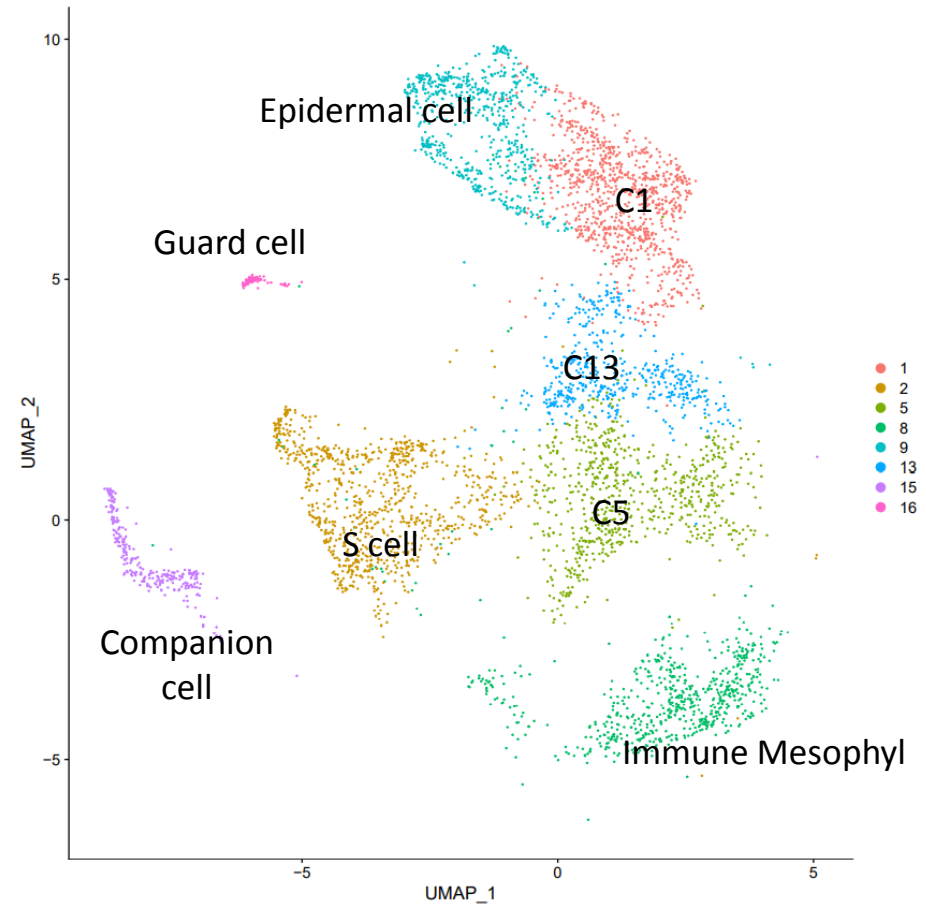
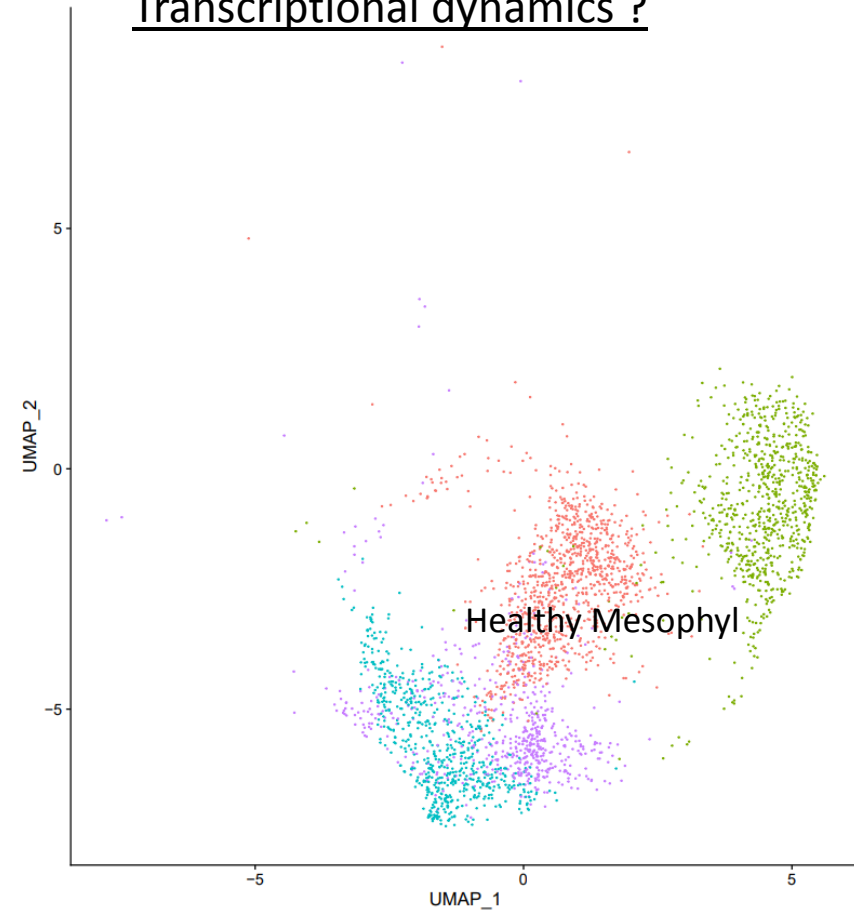
- Is there a trajectory between healthy and stressed cells in each cluster and which genes govern this trajectory?
- Do some clusters evolve towards others? Convergence/Divergence between different clusters?

SCANNER: Harnessing the clustering

Clusters	Cell Types	Biological processes	
C0	Mesophyl cells	Healthy	
C4			
C10			
C11			
C7	Mesophyl cells	Hormone signaling (JA/Et/SA) ROS stress ...	
C8			UPR, Kinase signaling...
C12			
C1	(???)	Lignin synthesis JA signaling Toxin catabolism Cell death regulation	
C3			
C5			
C6			
C13			
C2	S cells	H sulfide, glucosinolates synthesis, callose deposition...	
C9	Epiderm cells	Wax, cuticle, cellulose, glucosinolate synthesis....	
C16	Guard cells	ABA signaling (stomata closure)	
C15	Companion cells	Polyamine synthesis	
C14	rubbish		
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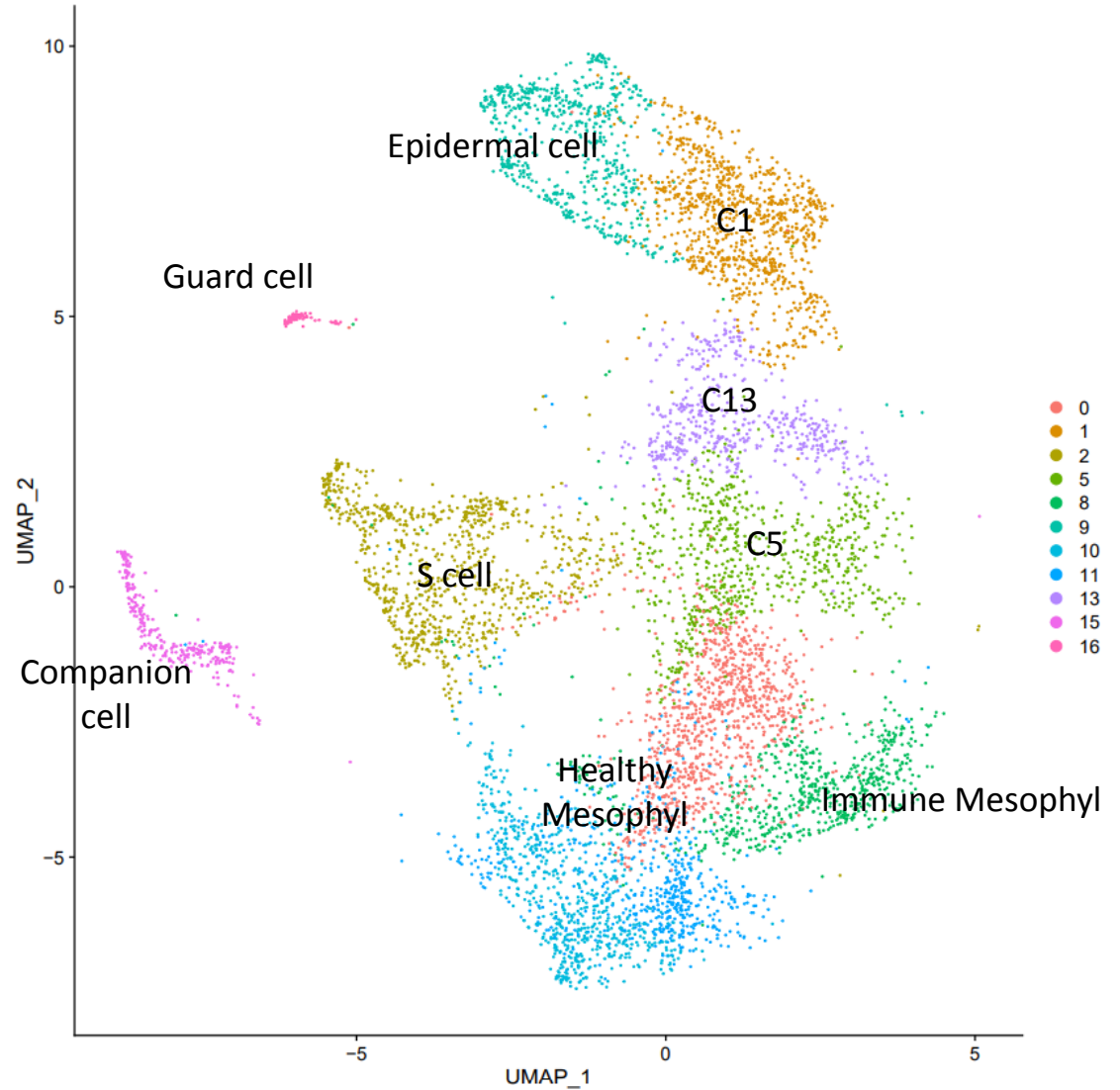
SCANNER: Harnessing the clustering

Transcriptional dynamics ?



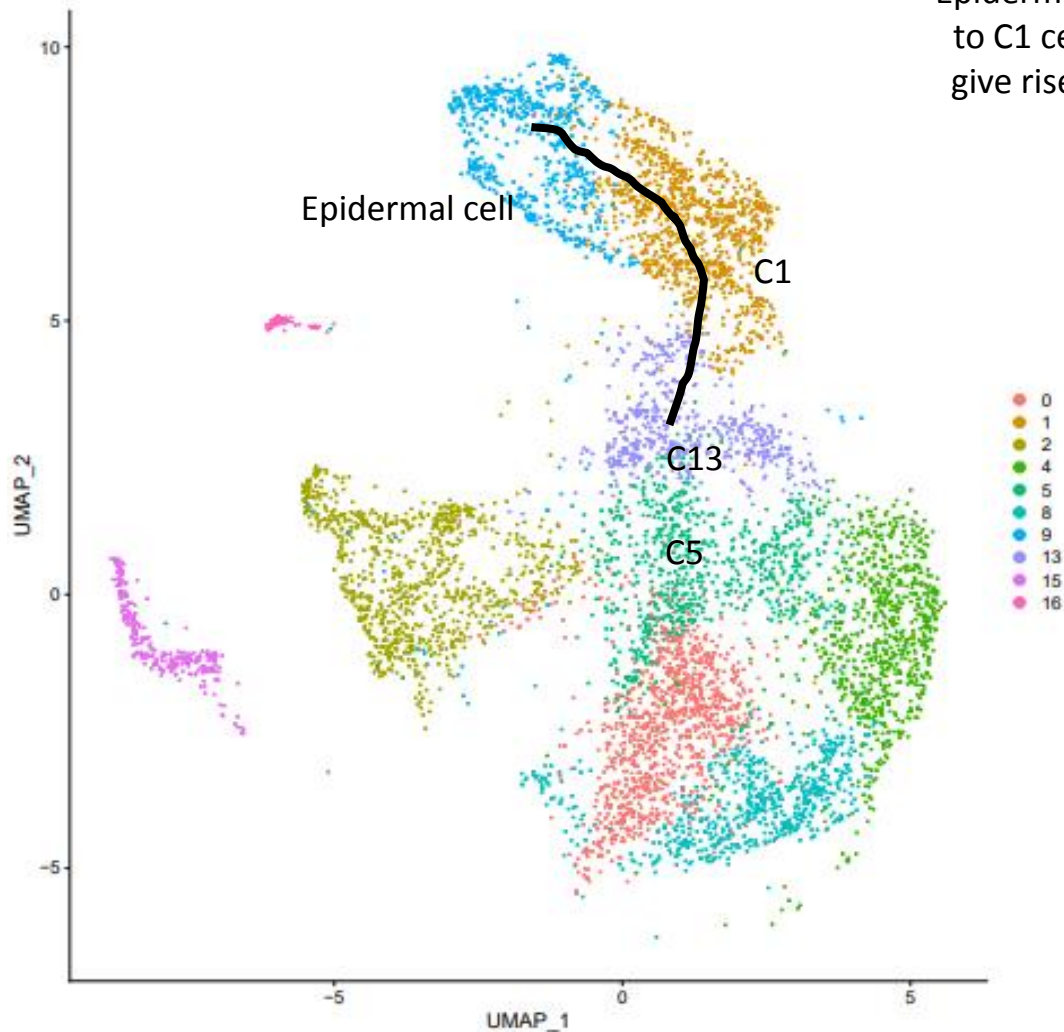
SCANNER: Harnessing the clustering

Transcriptional dynamics ?



SCANNER: Harnessing the clustering

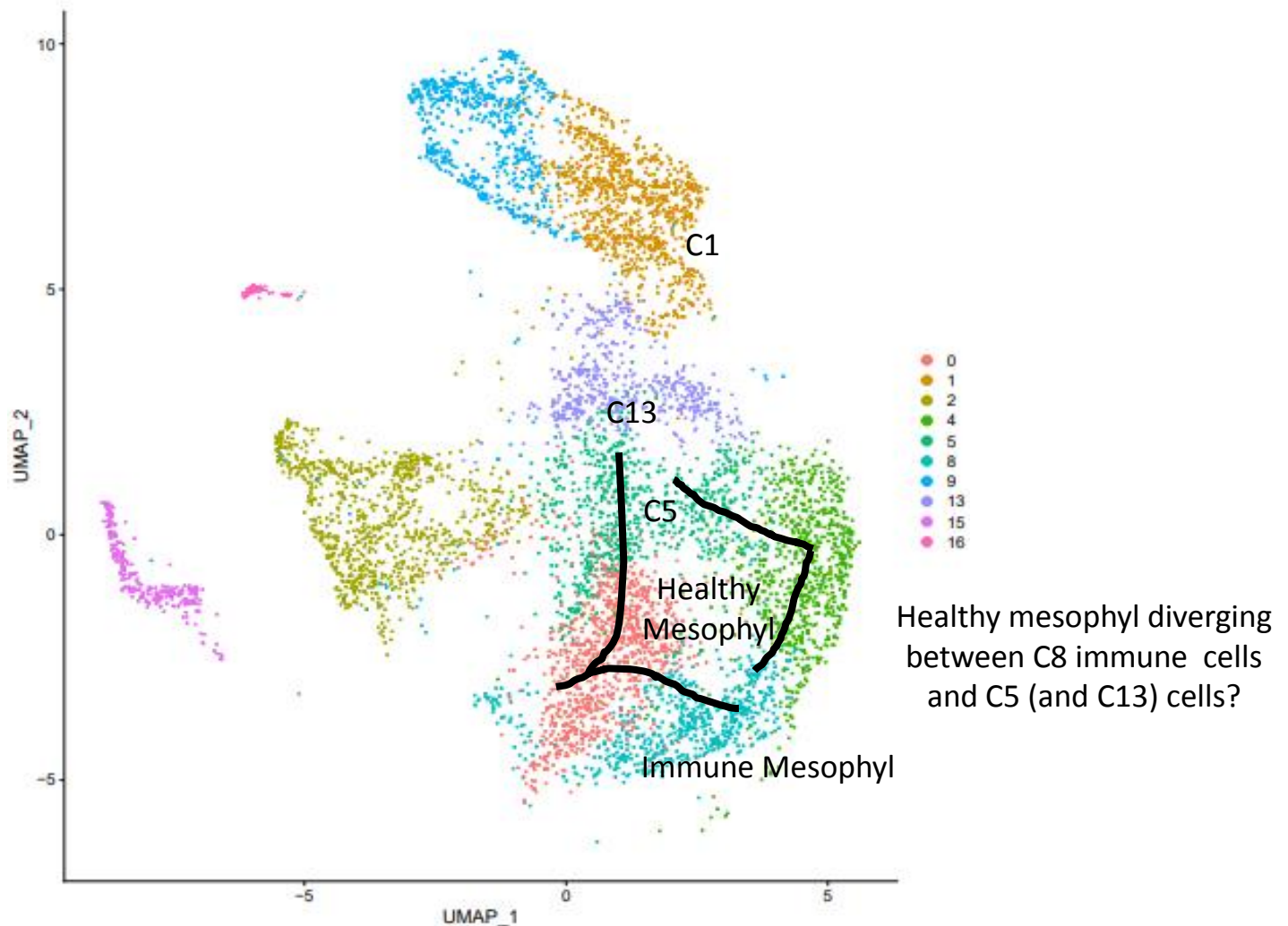
Transcriptional dynamics ?



Epidermal cells giving rise to C1 cells which in turn give rise to C13 (and C5) cells?

SCANNER: Harnessing the clustering

Transcriptional dynamics ?



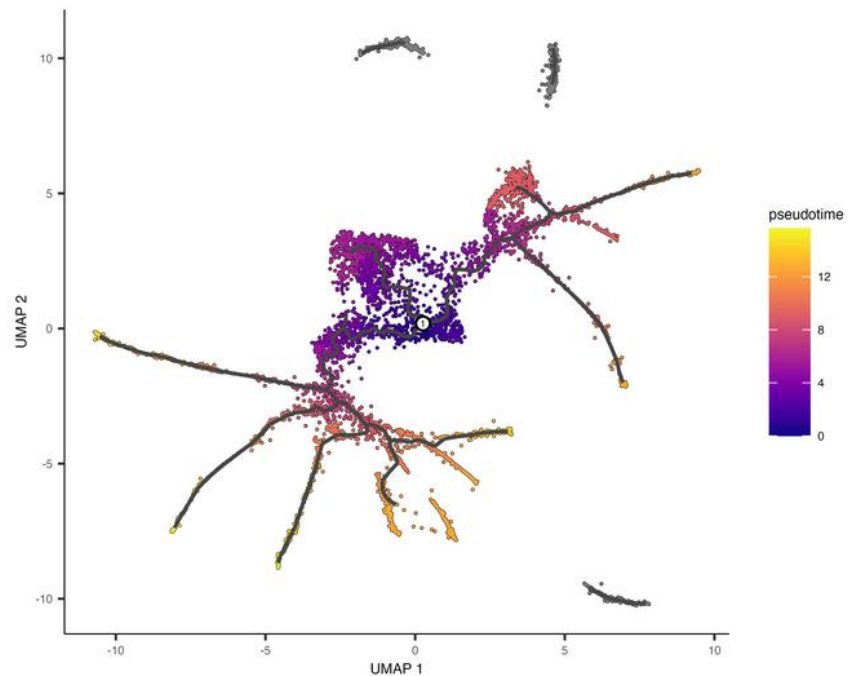
SCANNER: Harnessing the clustering

Transcriptional dynamics within and between clusters?

MONOCLE package ongoing analysis....

<https://cole-trapnell-lab.github.io/monocle3/>

- Cell ordering
- Pseudotime
- Genes governing trajectories

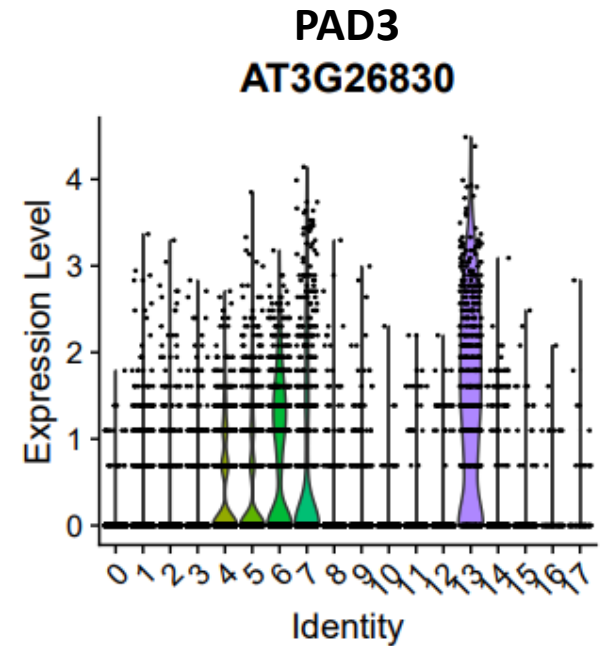
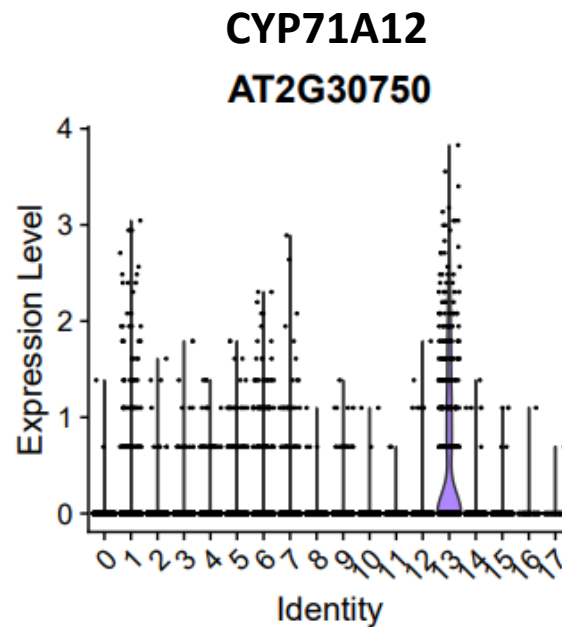
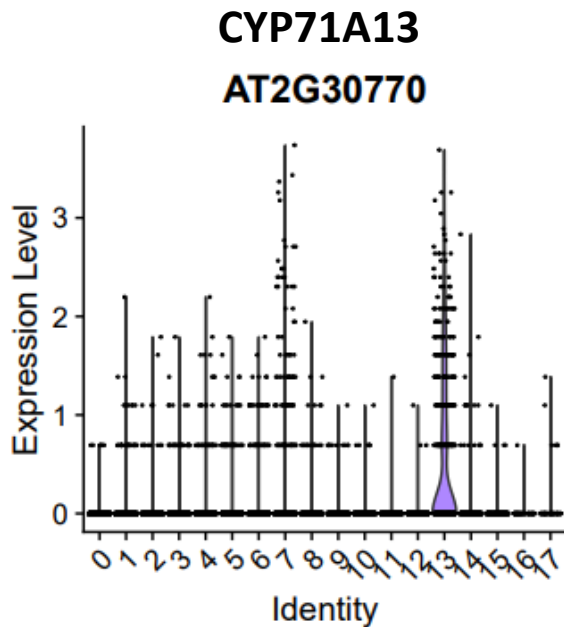


SCANNER: Harnessing the clustering

Screening for defense actors and defense processes

SCANNER: Harnessing the clustering

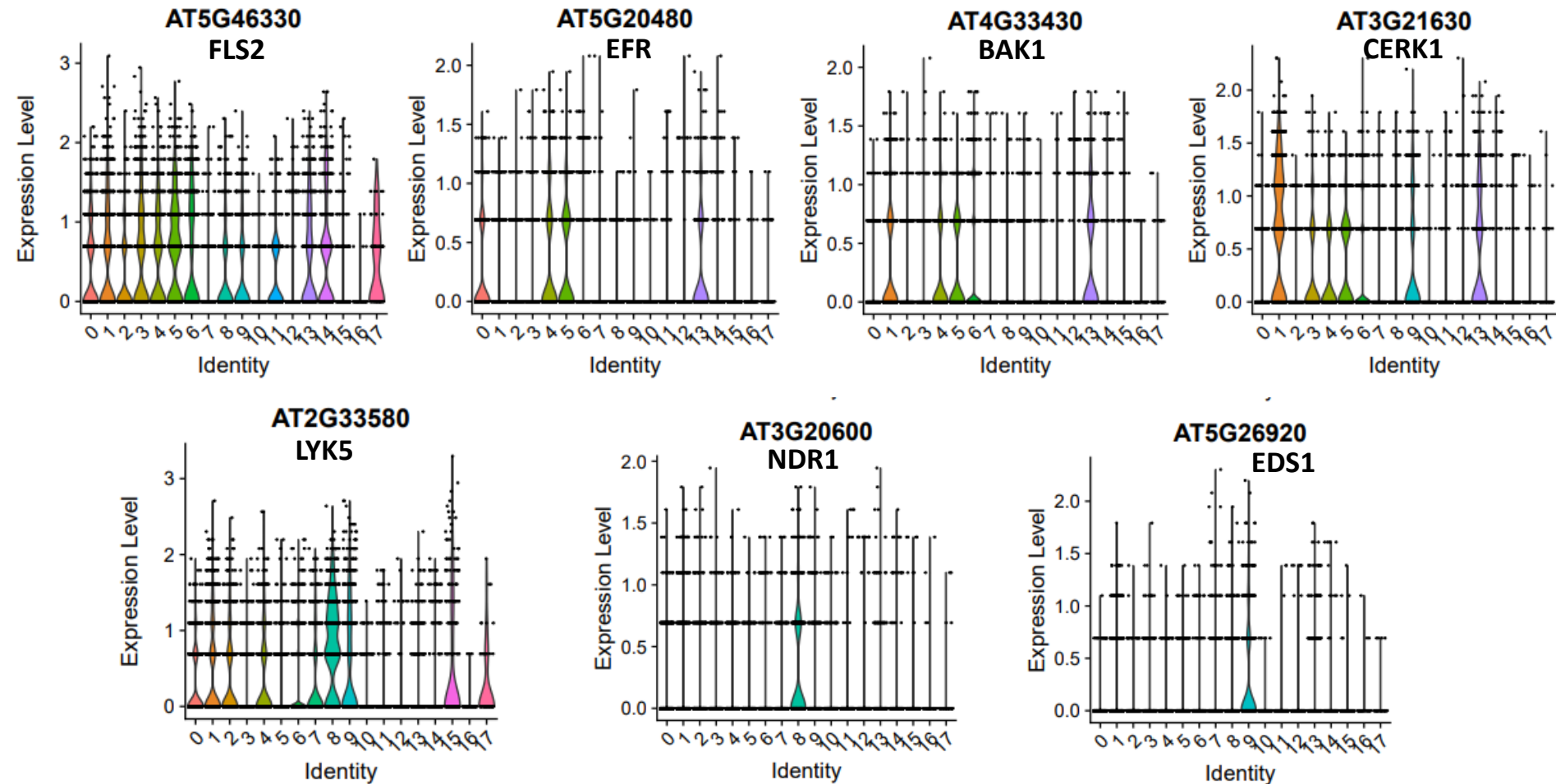
Screening for defense actors and defense processes



Camalexin synthesis is specific of Cluster 13

SCANNER: Harnessing the clustering

Screening for defense actors and defense processes



Inequalities in sensing / fighting pathogens between cell types (?)

SCANNER: Harnessing the clustering

Identify important genes for stress responses in specific cell populations

SCANNER: Harnessing the clustering

Identify important genes for stress responses in specific cell populations

C8	Unfolded Protein	HSP17.6A/B HSP18 HSP17.6II HSP17.4A/B
C13	Lignification	CASPL1D1 CASPL4D2
C16 Guard cell	Mustard oil bomb	TGG1 TGG2

SCANNER: Harnessing the clustering

Identify cell-type specific Regulators of defense reprogramming

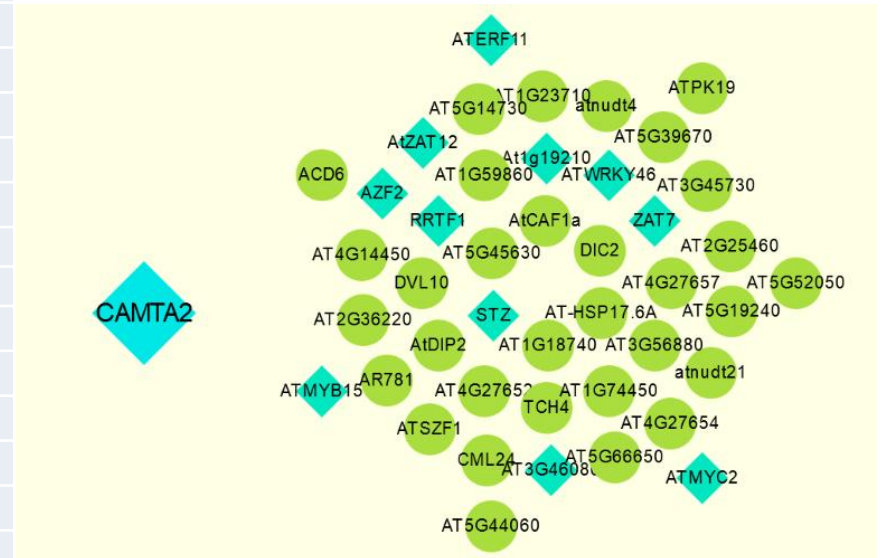
SCANNER: Harnessing the clustering

Identify cell-type specific Regulators of defense reprogramming

TF2Network <http://bioinformatics.psb.ugent.be/webtools/TF2Network/>

C8

Regulator	Symbol	rank	q-value
AT5G64220	CAMTA2	1	2,11E-10
AT5G64220	CAMTA2	2	2,11E-10
AT2G22300	CAMTA3	3	1,71E-09
AT2G22300	CAMTA3	4	5,97E-09
AT5G09410	CAMTA1	5	5,97E-09
AT3G02990	ATHSFA1E	6	4,6418E-06
AT1G23810	AT1G23810	7	4,6418E-06
AT5G43840	AT-HSFA6A	8	1,2608E-05
AT3G22830	AT-HSFA6B	9	1,2608E-05
AT2G22300	CAMTA3	10	3,4993E-05
AT5G16820	ATHSF3	11	3,4993E-05
AT4G11660	AT-HSFB2B	12	3,4993E-05
AT2G41690	AT-HSFB3	13	3,4993E-05
AT2G15660	AGL95	14	3,4993E-05
AT4G34000	ABF3	15	3,4993E-05
AT4G18880	AT-HSFA4A	16	7,2654E-05
AT1G46264	AT-HSFB4	17	7,2654E-05
AT2G01818	AT2G01818	18	7,2654E-05

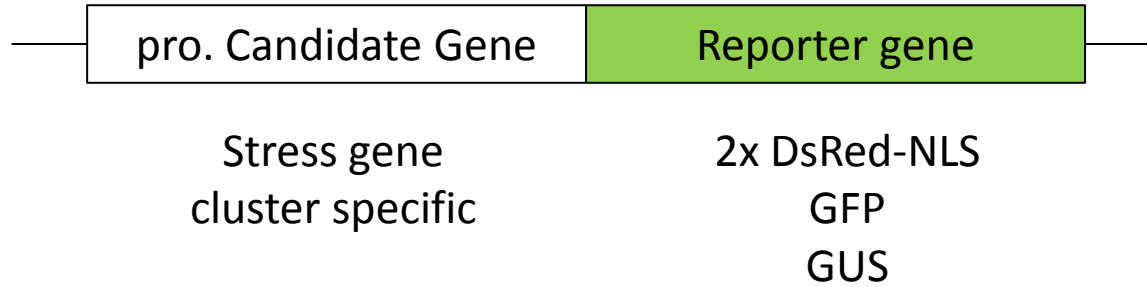


SCANNER: Harnessing the clustering

Clusters	Cell Types	Stress Responses		Regulators
C0	Mesophyl cells	Healthy		/
C4				
C10				
C11				
C7	Mesophyl cells	Hormone signaling (JA/Et/SA) ROS stress ...		
C8			UPR, Kinase signaling, camalexin...	CAMTA1/2/3, HSFs..
C12				
C1	(???)	Lignin synthesis JA signaling Toxin catabolism Cell death regulation		WRKYs...
C3				WRKYs... ANACs....
C5				
C6				
C13				
C2	S cells	H sulfide, glucosinolates synthesis, callose deposition...		
C9	Epiderm cells	Wax, cuticle, cellulose, glucosinolate synthesis....		
C16	Guard cells	Myrosination, ABA signaling (stomata closure)		ABFs...
C15	Companion cells	Polyamine synthesis		
C14	rubbish			
C17	Dividing cells?			

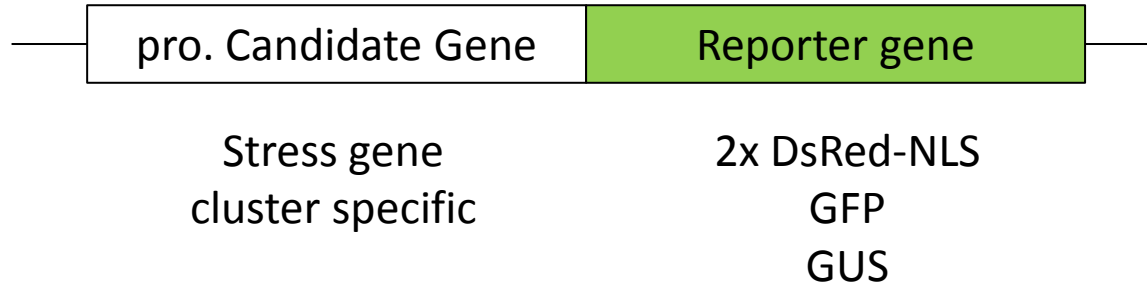
SCANNER: Validation

Create reporter lines



SCANNER: Validation

Create reporter lines



Test Resistance Phenotypes of selected mutants



Pseudomonas
DC3000



Mutant
No Stress Gene
Upregulated in specific Cluster(s)
Not upregulated in bulk RNAseq



Thank you !

Julien Lang
16/06/2022