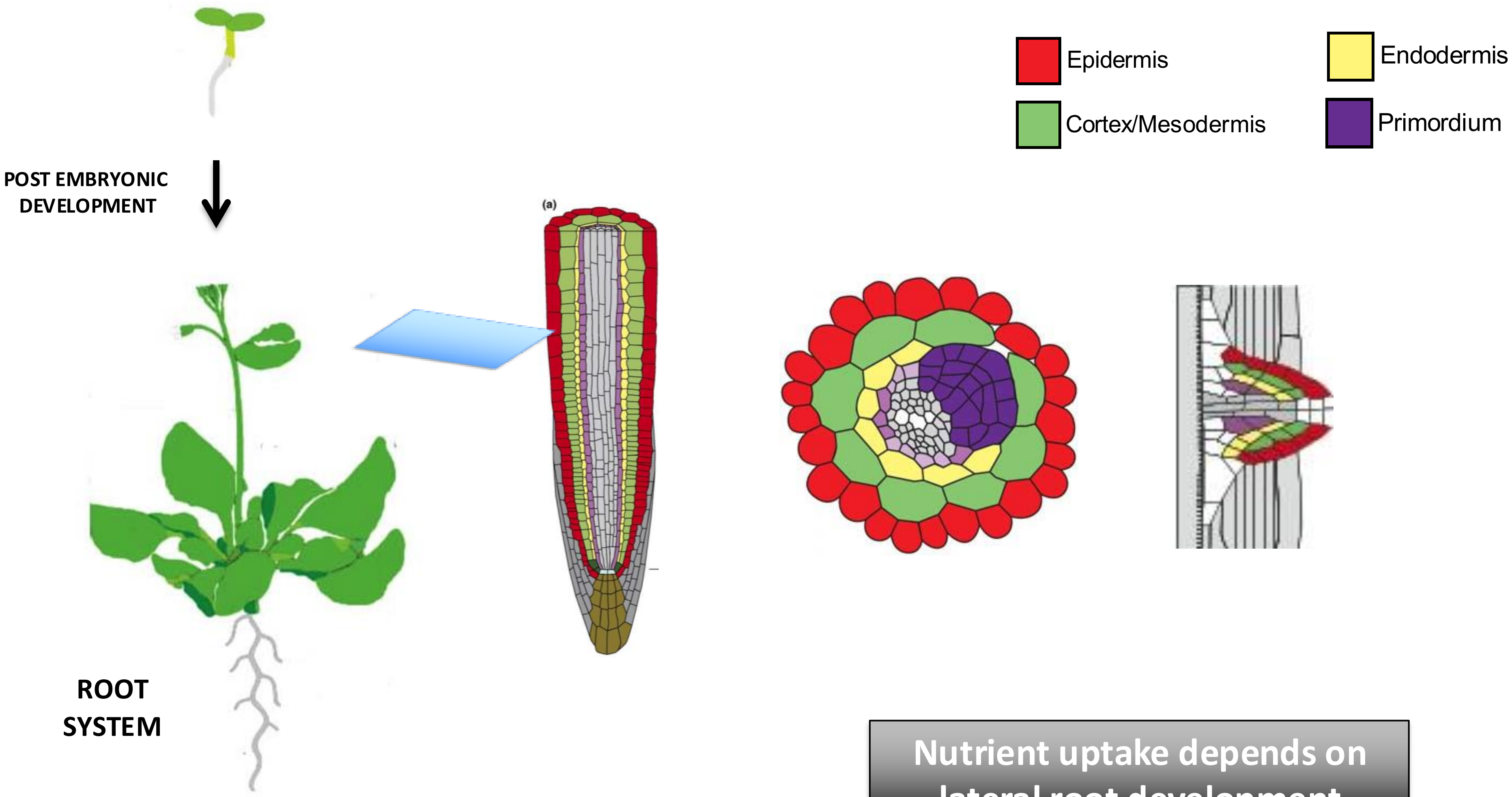
Genome-wide transcript profiling to uncover new genes in the SCF-TIR1/AFBs-independent pathway of lateral root formation in *A. thaliana*.

Patricio Pérez-Henríquez, Boris Parizot, Qian Chen, Tom Beeckman, Lorena Norambuena.

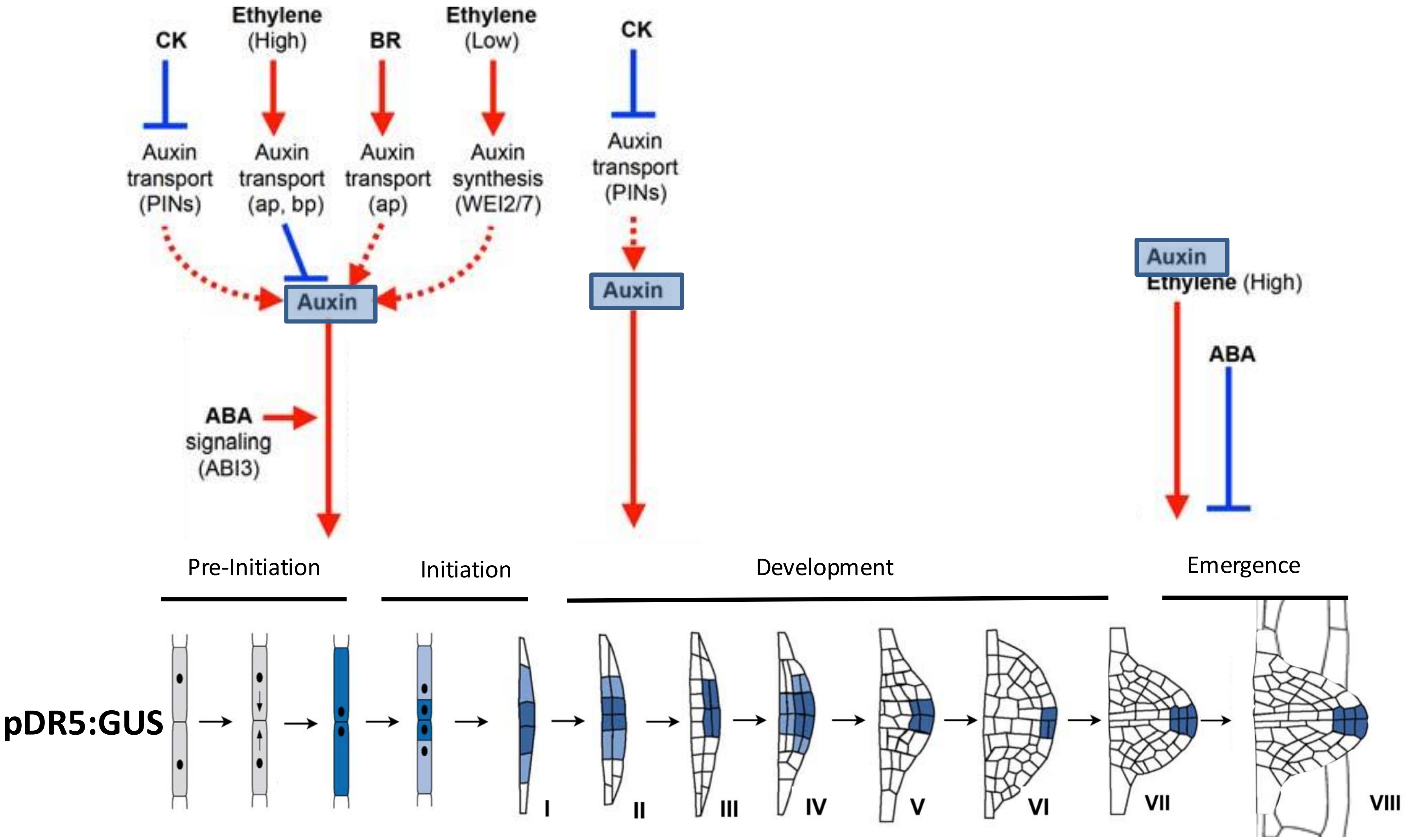
CBMV

Root system architecture relies largely on the continuous process of lateral root initiation



Nutrient uptake depends on lateral root development

Auxin is a key regulator of lateral root development


CK
Auxin transport (PINs)

Ethylene (High)
Auxin transport (ap, bp)

BR
Auxin transport (ap)

Ethylene (Low)
Auxin synthesis (WEI2/7)

Auxin

ABA signaling (ABI3)

CK
Auxin transport (PINs)
Auxin

Auxin
Ethylene (High)
ABA

Pre-Initiation

Initiation

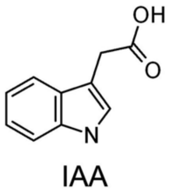
Development

Emergence

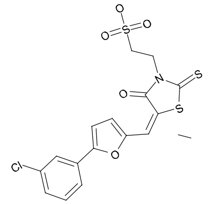
pDR5:GUS

I II III IV V VI VII VIII

Adapted from
Peret *et al*, 2003. *Trends in Plant Biology*
Fukaki *et al, 2004. Plant Phys*.

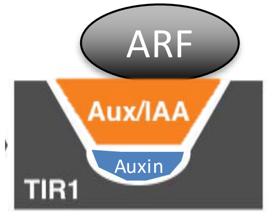


Auxin signaling &
Sortin2, a unique molecule that induces LR

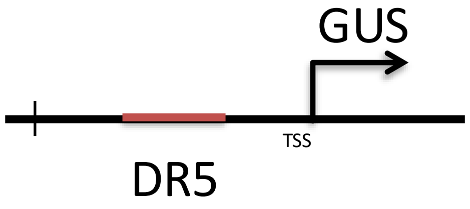


Receptor

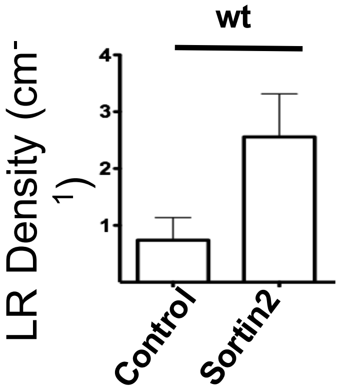
AUX/IAA Degradation

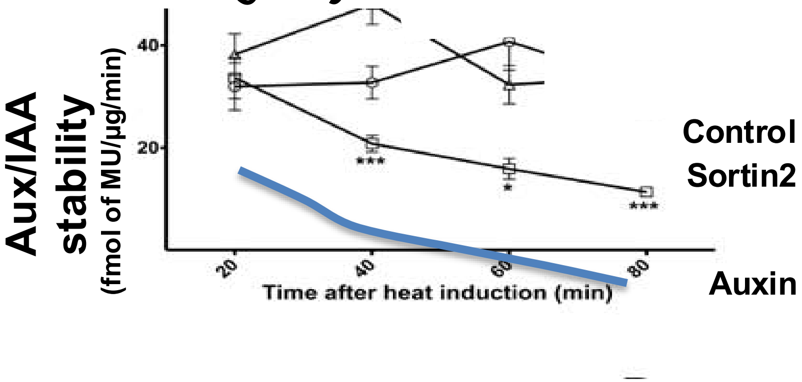


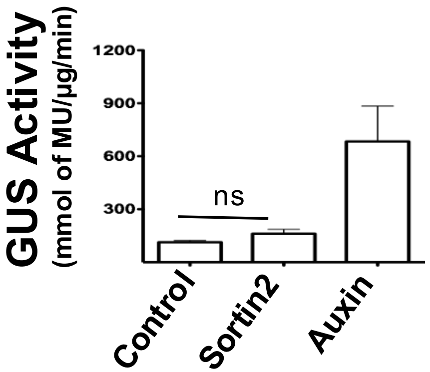
Auxin Response Genes



Adapted from Tan et al . Nature. 2007

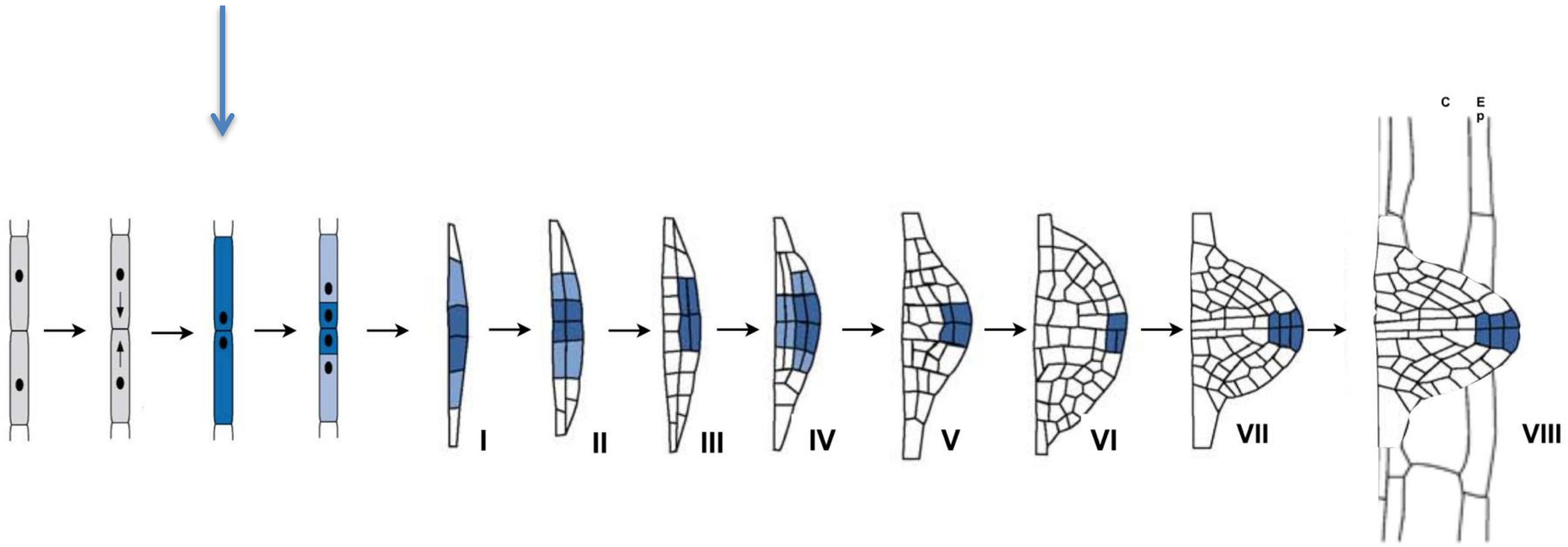






Adapted from
Pérez-Henríquez *et al* .
Molecular Plant. 2012

Sortin2



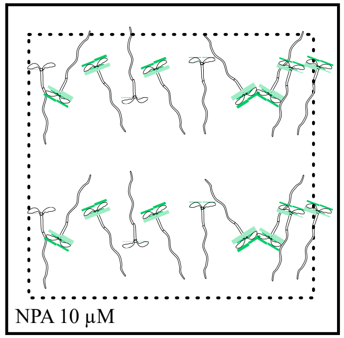
What are the main biological processes targeted specifically by Sortin2?

Compared genome-wide transcript profiling

Understand the specific Sortin2 effect via a compared a genome-wide transcript

1. Sampling methodology Overview for RNA seq

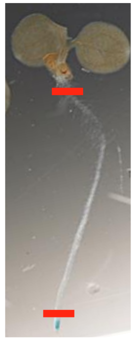
Col-0 grown in NPA



6h

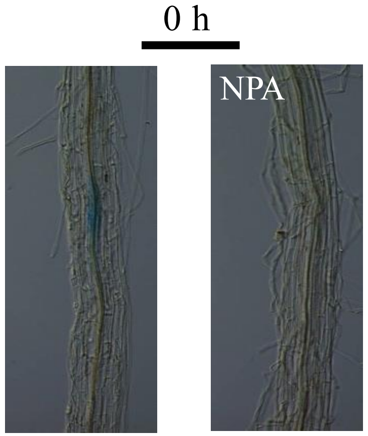
1. **Control**
2. **Sortin2**
3. **Auxin**

remove:
- tip
- aerial part



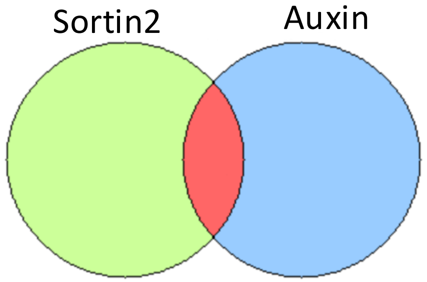
Collect samples

QC

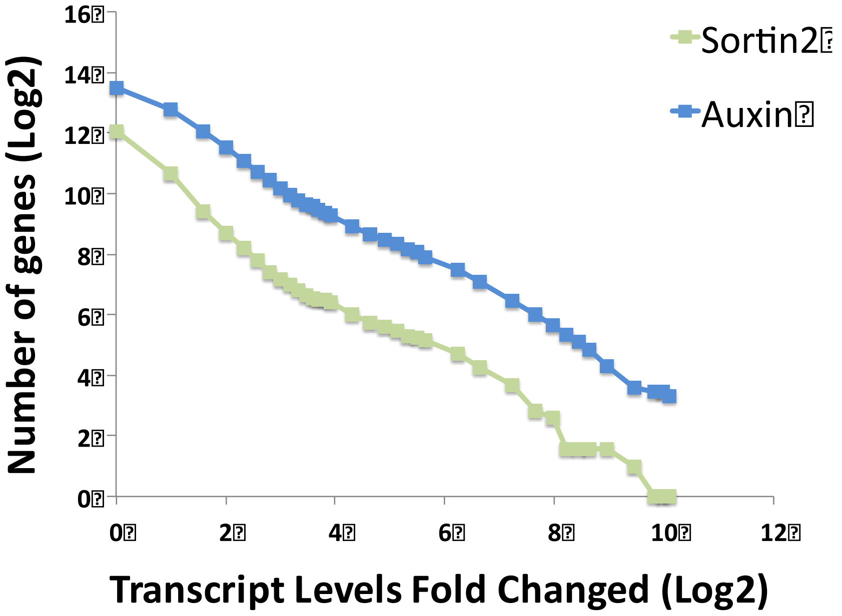


Lateral root inducible system
Himanen *et al*. PNAS. 2004
Vanneste *et al*. Plant Cell. 2005

2. Compare transcript profiles



Compared a genome-wide transcript profiling of Sortin2- and auxin-treated seedlings roots



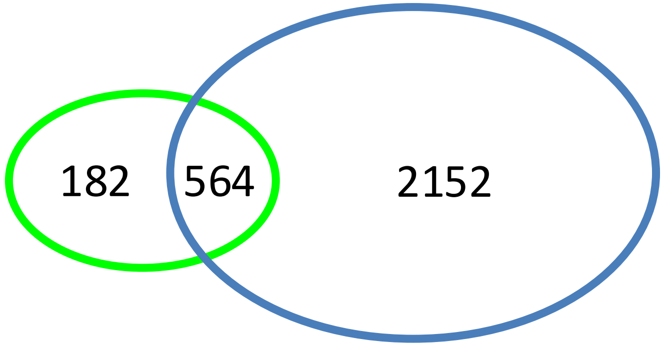
FC = Fold Change

	Auxin	Sortin2	
Total	33800		
5 reads per gene (in at least 2 replicates)	19298		
FDR ≤ 0.05	10899	4018	3x
FC ≥ 2 or FC ≤ 2	6408	1361	5x
FC ≥ 2	3692	615	
FC ≤ 2	2716	746	

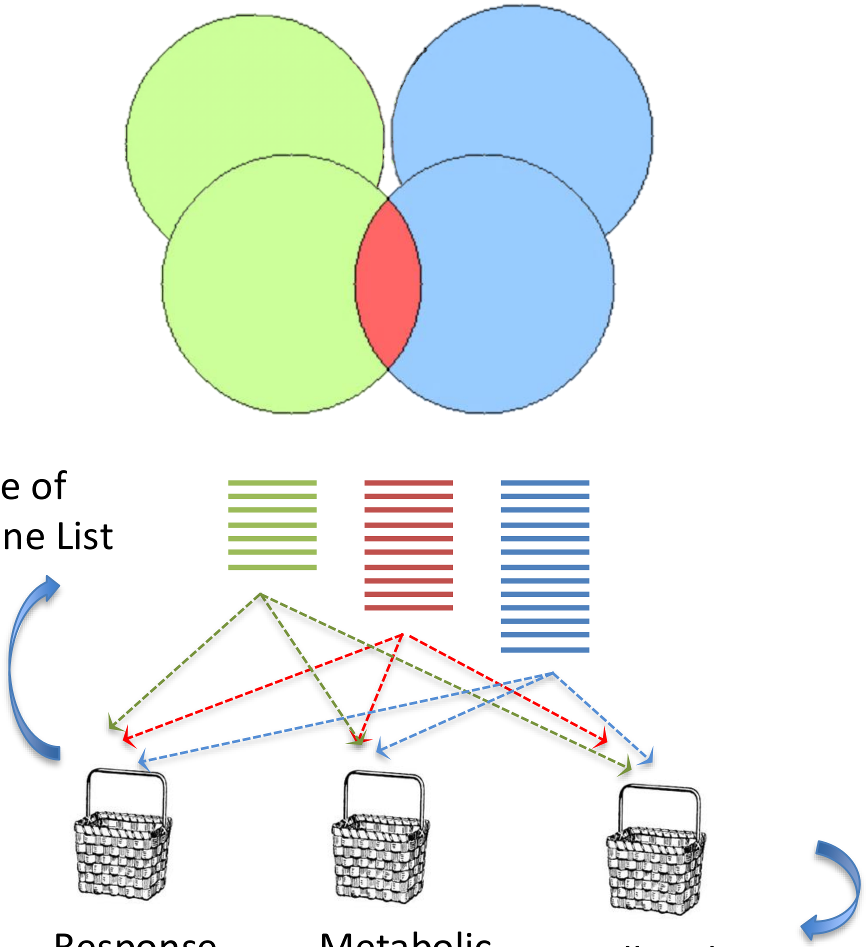
Results suggests that Sortin2 treatment has a narrower effect than auxin

UP REGULATED
FC ≥ 2

DOWN REGULATED
FC ≤ 2



Biological processes Enrichment



Size of Gene List

Response to stress (GO:0006950)

Metabolic process (GO:0008152)

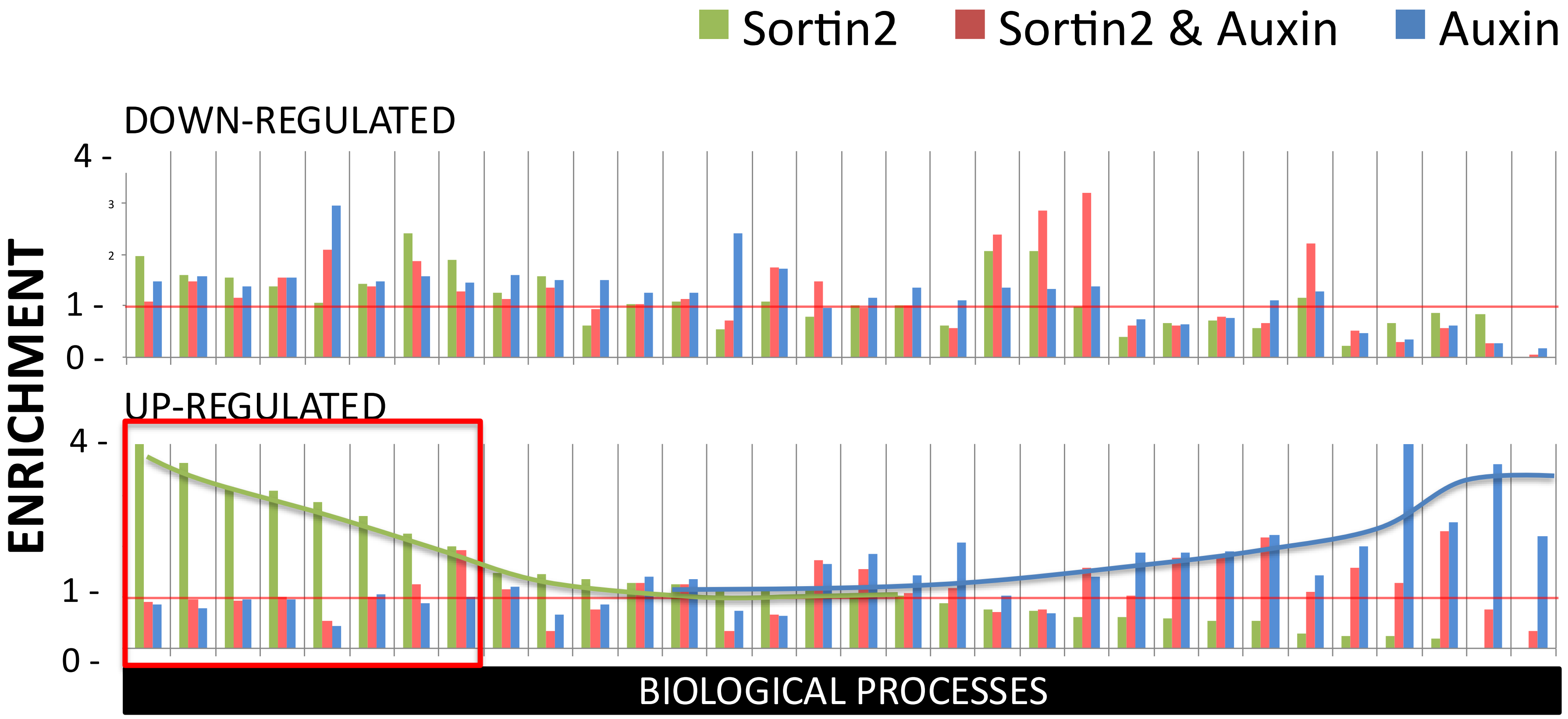
Cell cycle (GO:0007049)

Size of Biological Process

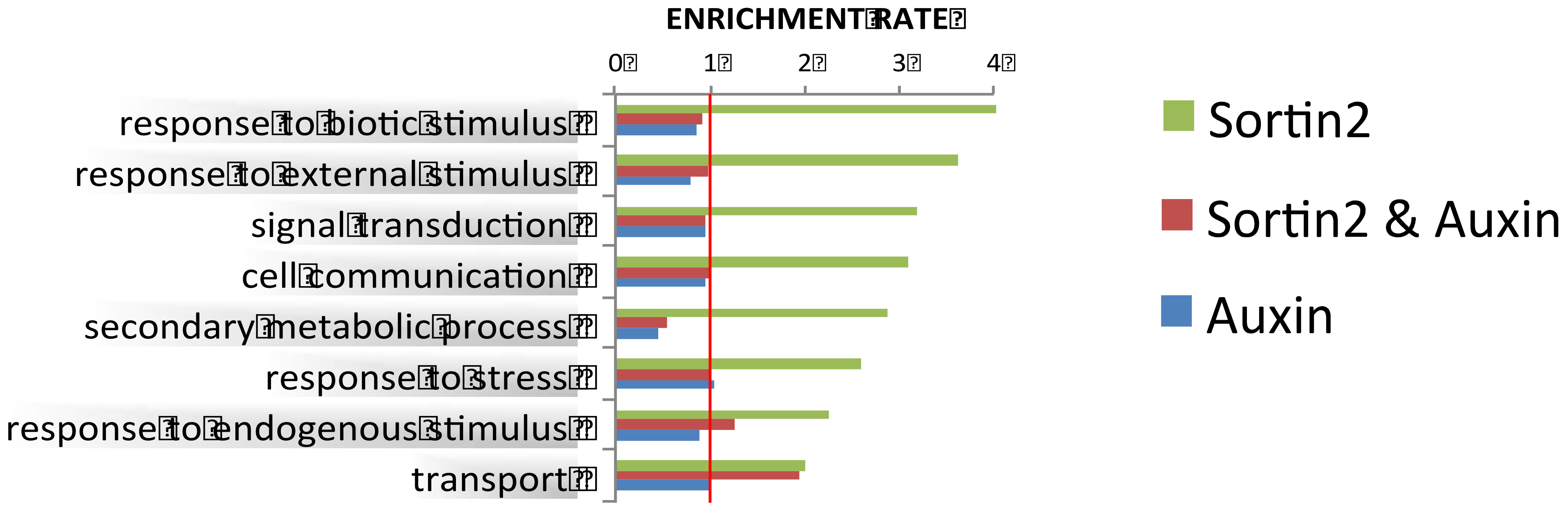
ENRICHEMENT OF EACH BIOLOGICAL PROCESS IN EACH GENE LIST

$$\text{ENRICHMENT} = \frac{\dfrac{\text{N}^\circ \text{ of genes from the list in each GO category}}{\text{N}^\circ \text{ genes in the list}}}{\dfrac{\text{N}^\circ \text{ genes from the genome annotated in each GO category}}{\text{N}^\circ \text{ genes in the genome}}}$$

Enriched biological processes categories discriminates between Sortin2 and Auxin regulated genes

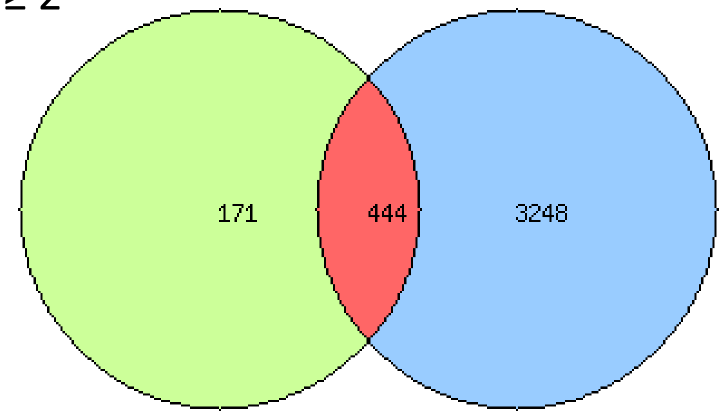


Enriched biological processes categories suggests a unique molecular frame for Sortin2-induced LRI

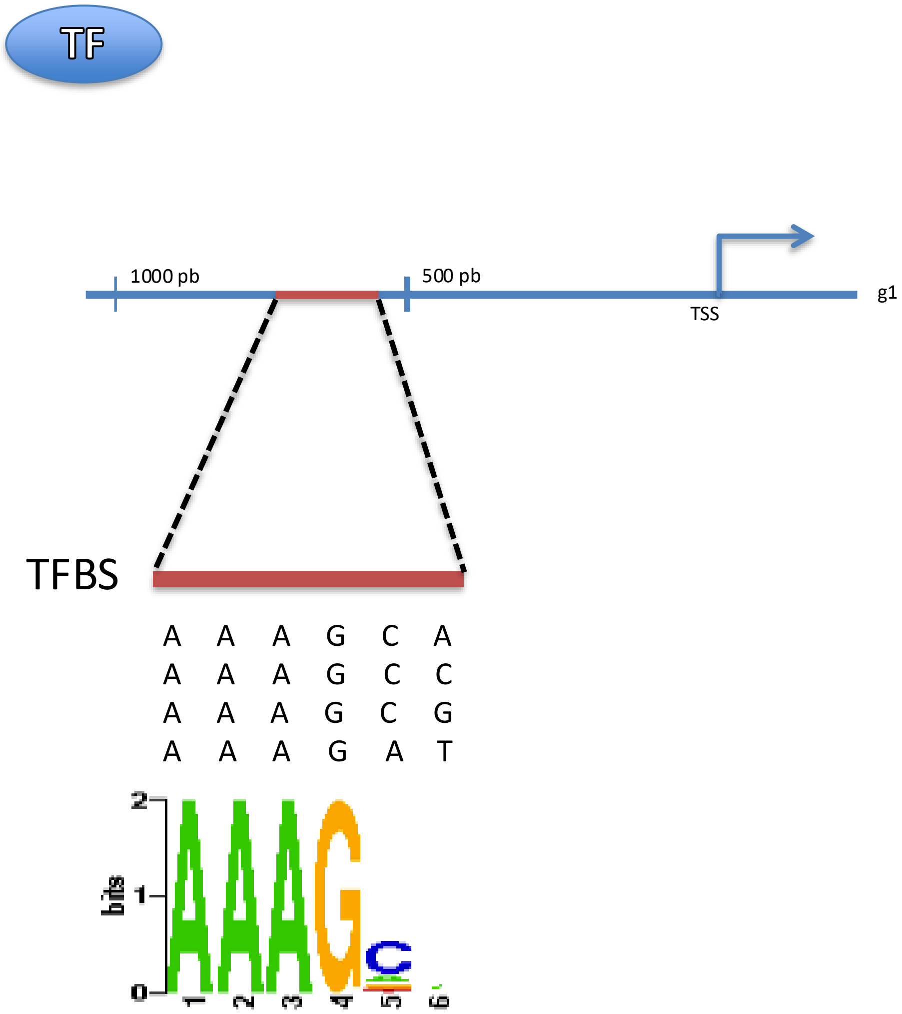


UP REGULATED

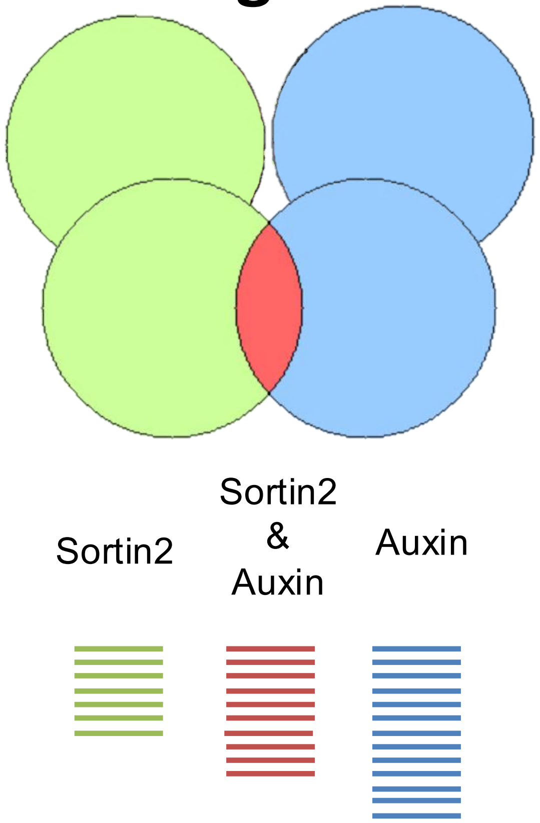
FC ≥ 2



Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes



A A A G C A
A A A G C C
A A A G C G
A A A G A T

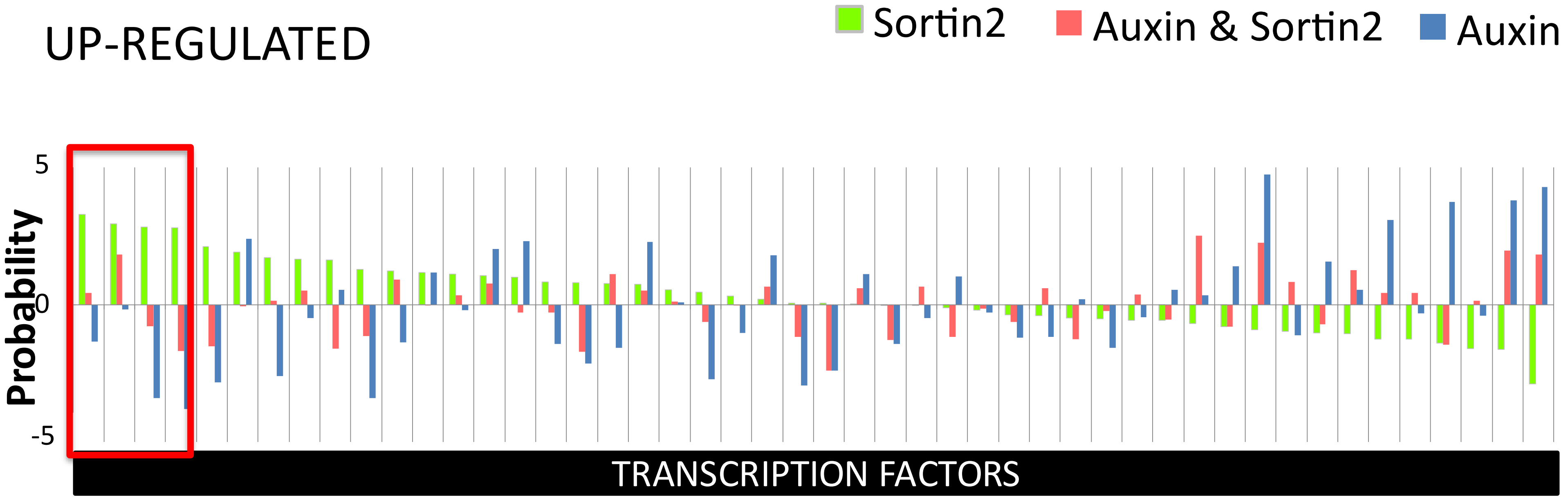


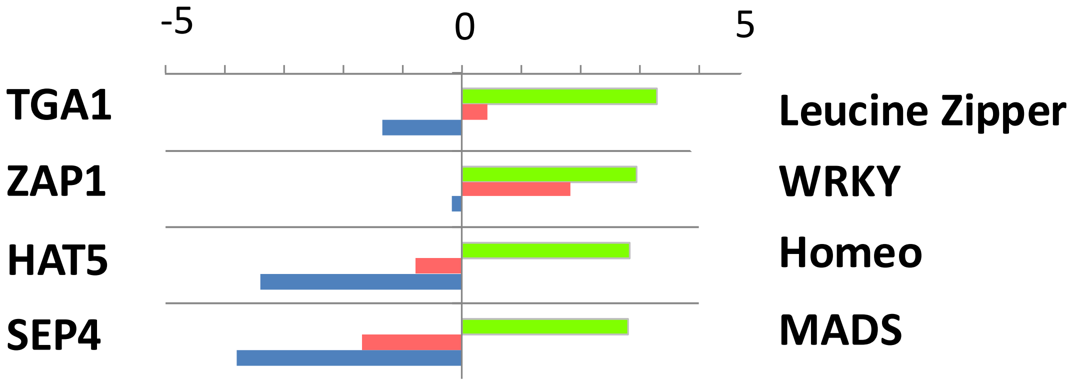
ALGORITHM

Evaluating the probability of regulation by a determined TF

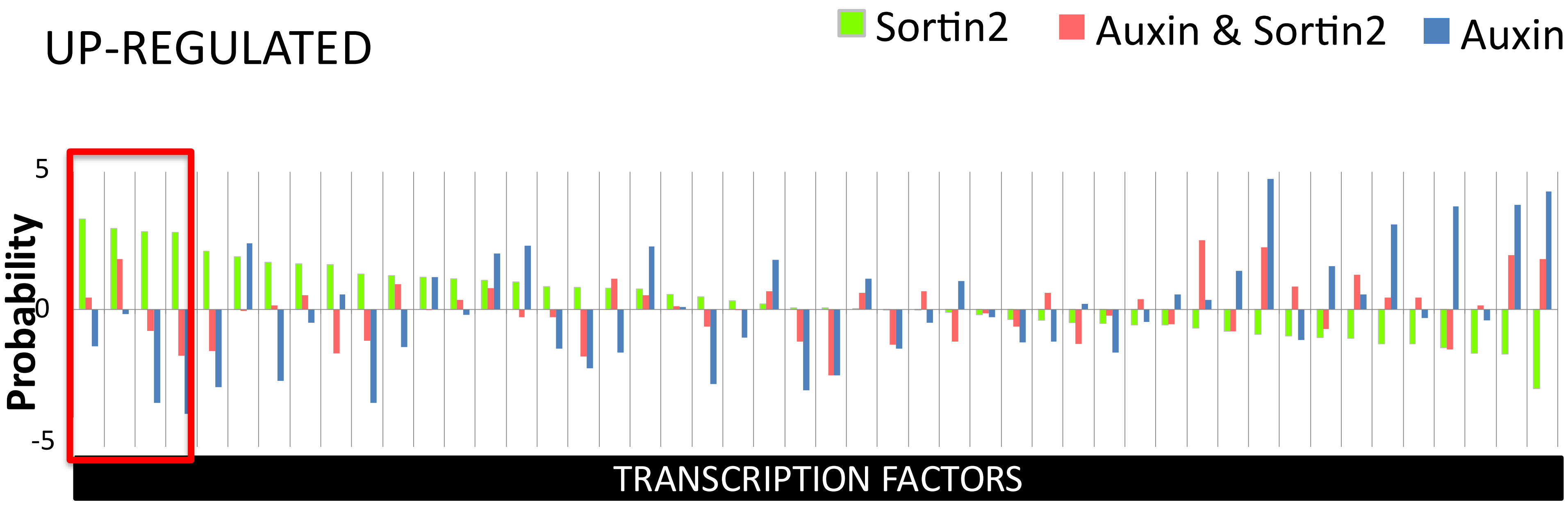
TFBS; Transcription Factor Binding Site

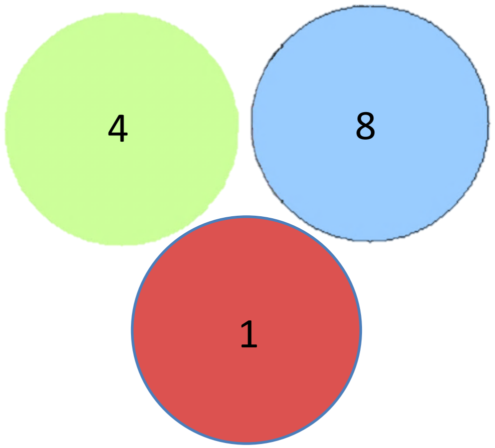
Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes





Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes





🟩 TGA1 , ZAP1 , HAT5 , SEP4

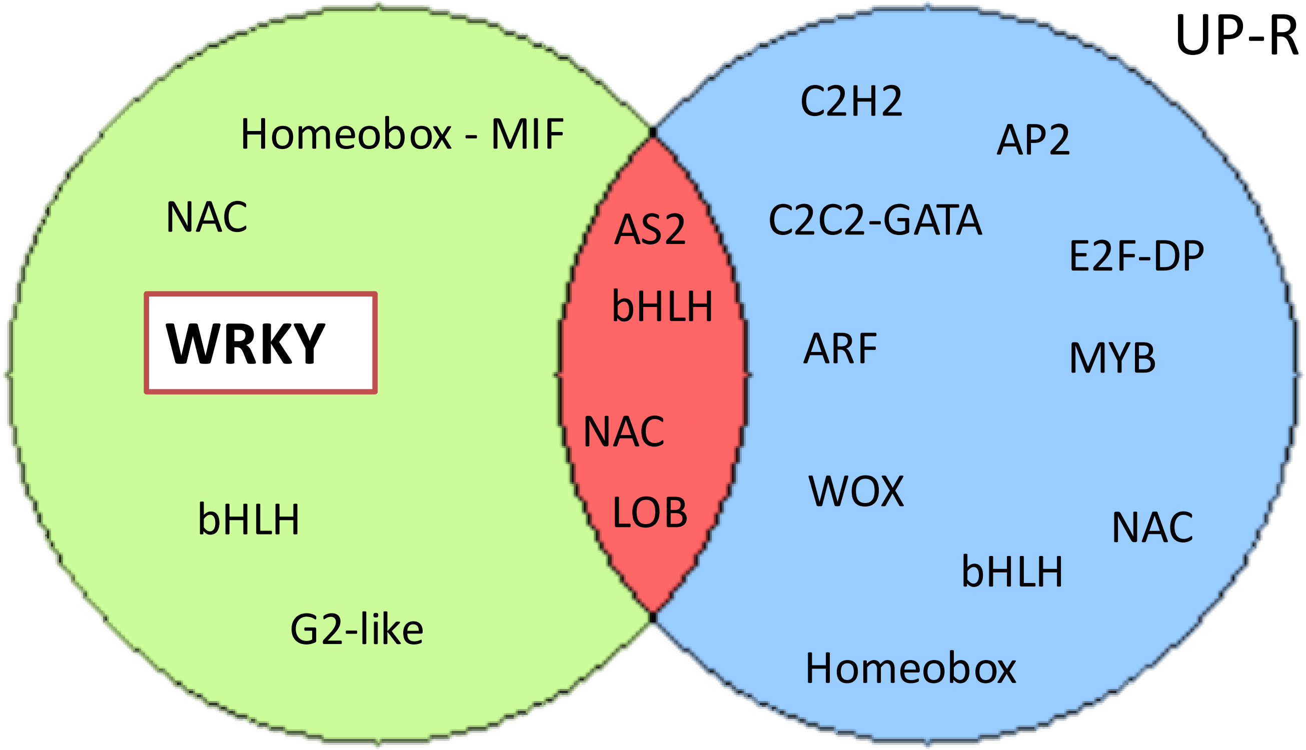
🟦 ERF1 , CDC5 , TCP16 , FHY3 ,
RAV1 , SEP3 , SOC1 , ANT

🟥 LFY

Transcription Family Enrichment

	🟩	🟥	🟦
Size of Gene List	171	444	3133
TF	15	33	274
TF %	8.7%	7.4%	8.7%

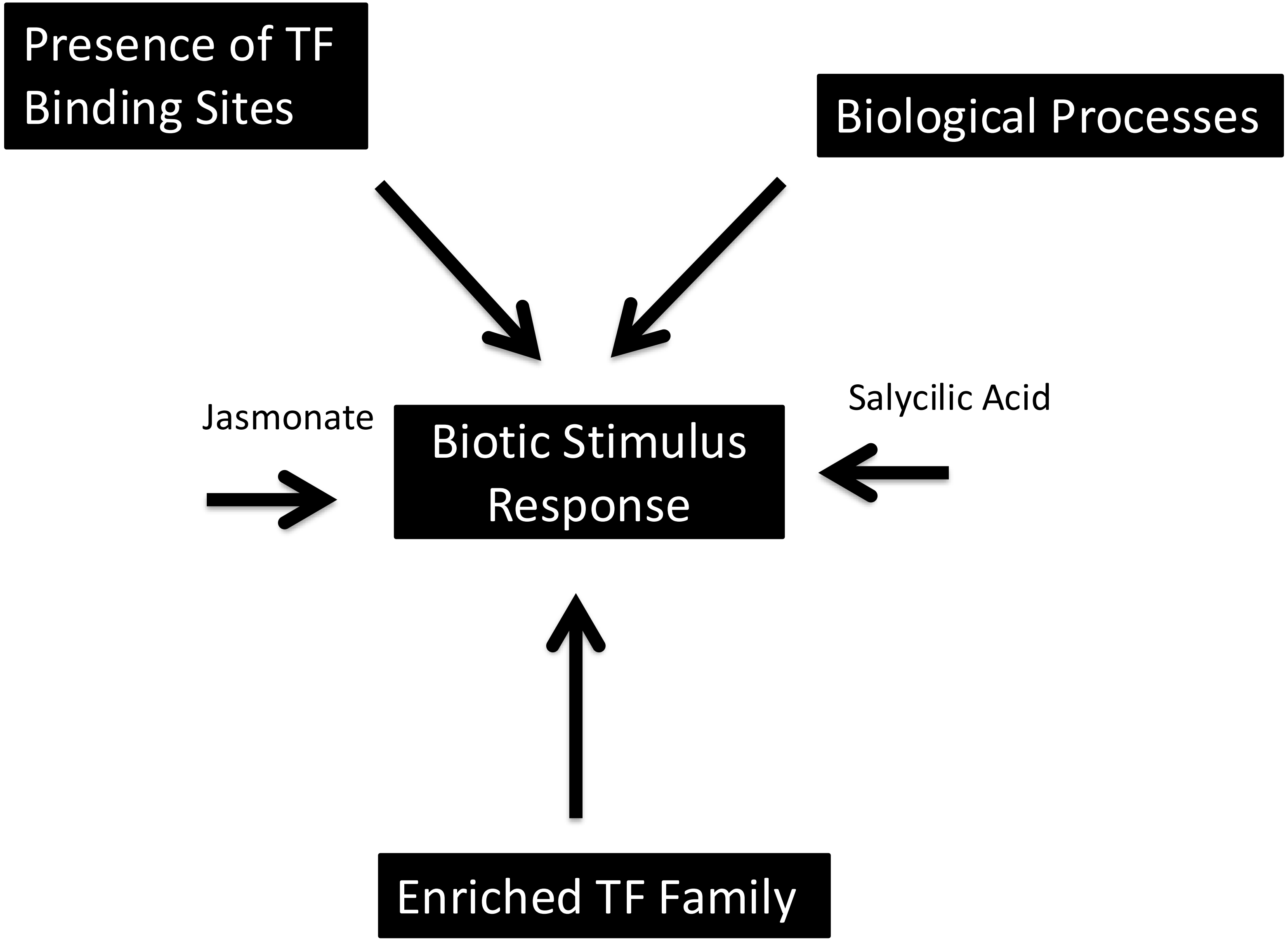
UP-REGULATED



Homeobox - MIF
NAC
WRKY
bHLH
G2-like

AS2
bHLH
NAC
LOB

C2H2
AP2
C2C2-GATA
E2F-DP
ARF
MYB
WOX
NAC
bHLH
Homeobox



Presence of TF Binding Sites

Biological Processes

Jasmonate

Biotic Stimulus Response

Salycilic Acid

Enriched TF Family

Salicylic acid response is specifically associated with Sortin2 response

min max

SA response genes

According to Krinke et al. Plant Phys 2007

	NIMIN-1	ATWRKY66	AT5G03350	ATWRKY38	AT1G19960	LHT7	CXC750	ATWRKY54	AtTN10	GDPDL1	AT3G29240	NIMIN-2	UGT76B1	ATSAGT1	DMR6	AT1G49750	GRX480	ATGSTF5	ATGRXS13	PGDH	AT4G34630	ATSAR1	ARFA1D	SVB	AT2G44370
Auxin	1	-3	-1	-4	-7	-2	-125	1	2	-21	-6	-2	1	2	-1	-6	-3	1	-2	-2	-2	-2	-1	-1	-1
Sortin2	142	103	71	49	17	14	14	13	8	7	6	6	5	5	5	4	3	2	1	1	1	1	1	1	-1

JA response and Synthesis

According to Méndez-Bravo et al. Plos One 2011

	SAG13	ATMBP	ATCLH1	anac081	LTP3	ATLOX6	ATERF-2	AOC4	ATOPR1	cdf3	ATOPR2		CYP71B15	ATHCHIB	PDF1.2c	LCR77	atnudt24	CORI3	ATLOX2	PDF1.2b	AtOPR3	LCR67	ATERF-4	AOC3		AOS	ATLOX1	ATLOX5	AOC2	TAT	ATLOX3	AOC1	JAZ8	ATLOX4	ATVSP2	FAMT
Auxin	2	5	-2	2	3	1	1	2	-3	1	-1	6	-3	1	-1	-1	-1	-1	-1	-1	-2	1	1	-2	11	-2	-2	-5	-37	-6	-2	-25	-3	-2	-8	-13
Sortin2	7	4	3	2	2	2	2	2	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-2	-2	-2	-2	-3	-3	-3	-3	-5	-10

CONCLUSION

1. Sortin2 induces much lower transcript level changes than auxin suggesting a narrower effect of Sortin2

2. We could identified 'gene sets' that are specifically up or down regulated by Sortin2 and that are excluded from the auxin regulatory activity

3. Response to stimulus either endogenous, external or biotic, and signal transduction processes were enriched among Sortin2- specific up-regulated genes.

4. Sortin2 activity is framed in a identifiable and unique regulatory context

5. Salicylic acid response genes are targeted in Sortin2 treatments

PROJECTIONS

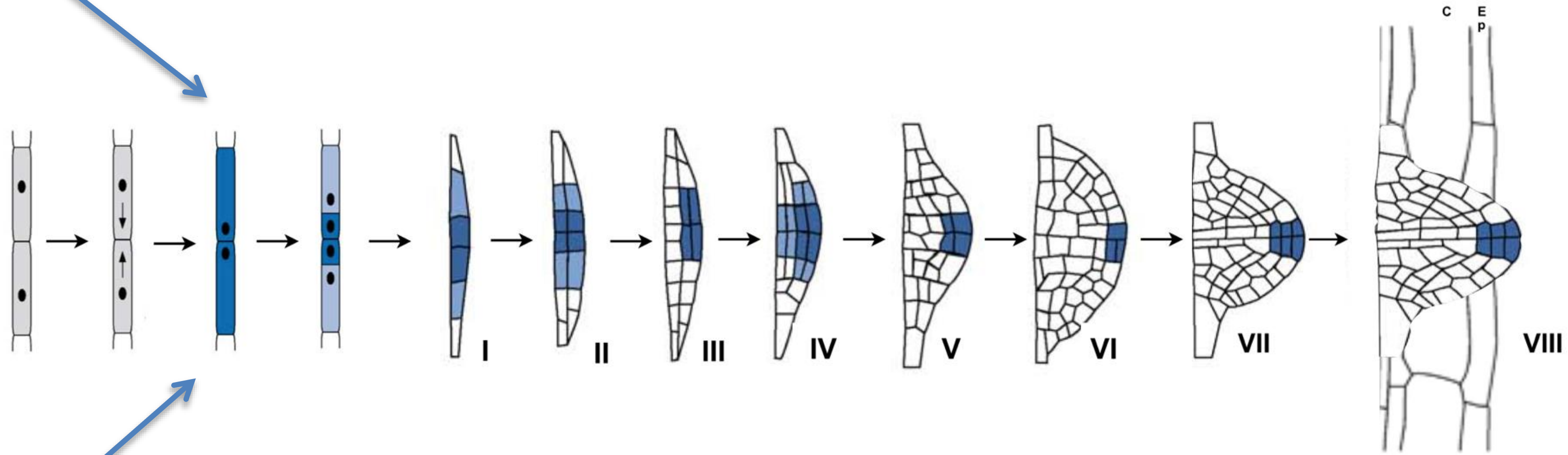
Genome-wide transcript profiling suggested a collection of genes that are not identified as part of the canonical pathway for LRI but that might be needed for for a SCF-TIR1/AFBs-independent LRI pathway.

SCF-TIR/AFBs - independent

Sortin2

SA response genes?
WRKY TF?

Stefanía
Morales



Auxin

SCF-TIR/AFB - dependent

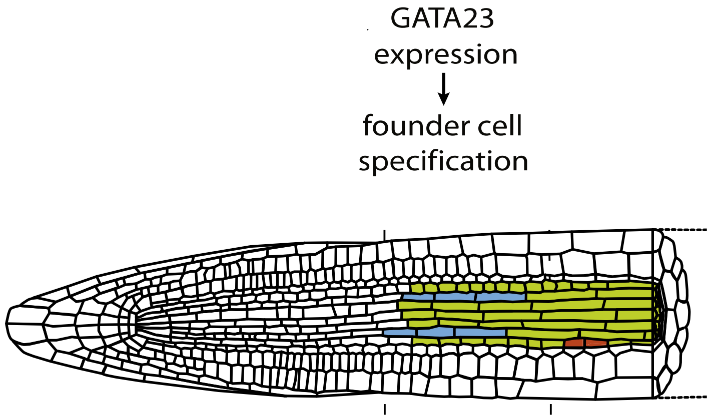
THANK YOU
FOR YOUR TIME!

Norambuena'sLab

CONICYT
BECA DOCTORADO
BECA ASISTENCIA A CONGRESO

CBMV

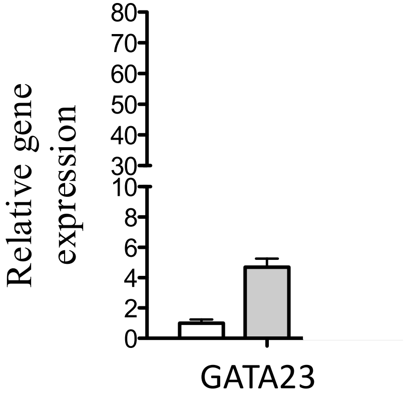
Sortin2 involves transcript levels regulatory activity



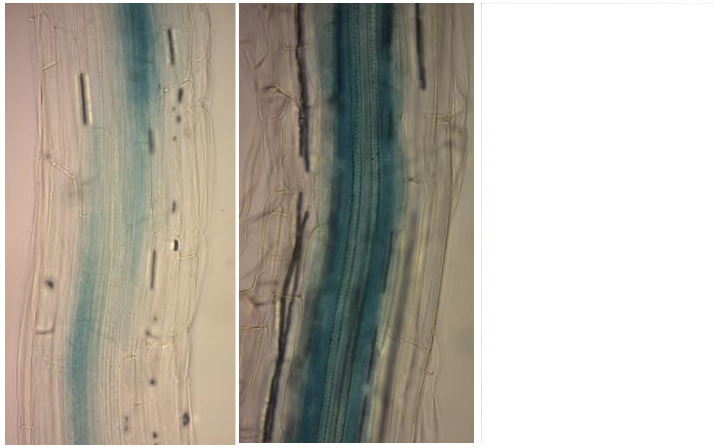
GATA23
expression
↓
founder cell
specification

De Rybel *et al*. Current Biology. 2010

GATA23 expression
(qRT in wild type plants)

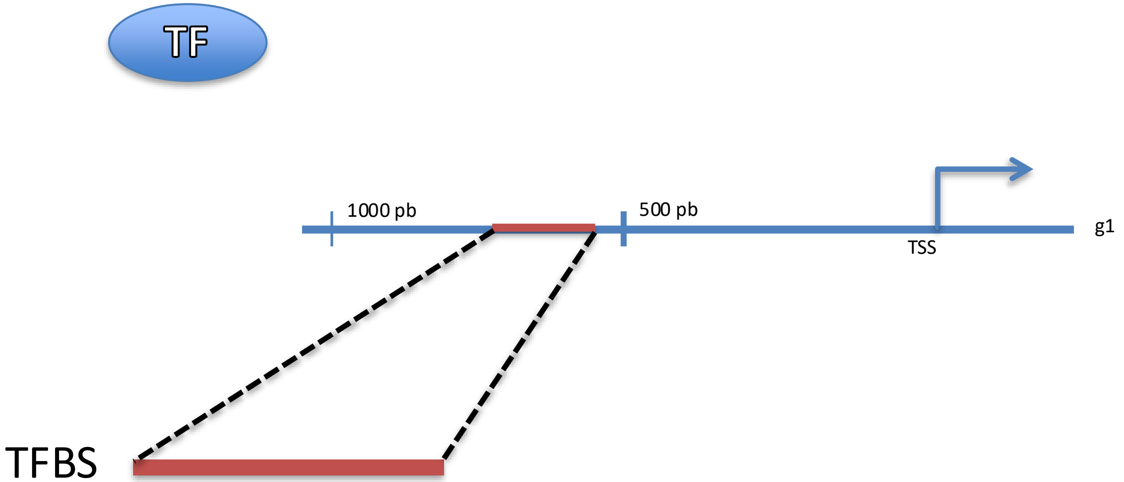


GATA23 expression
(pGATA23:GUS lines)



Control Sortin2 Auxin

Transcription factors binding sites (TF



A A A G C A
A A A G C C
A A A G C G
A A A G A T

Profile - Position specific frequency matrix

MA0020.1

	1	2	3	4	5	6
A	21	21	21	0	3	7
C	0	0	0	0	14	6
G	0	0	0	21	2	3
T	0	0	0	0	2	5

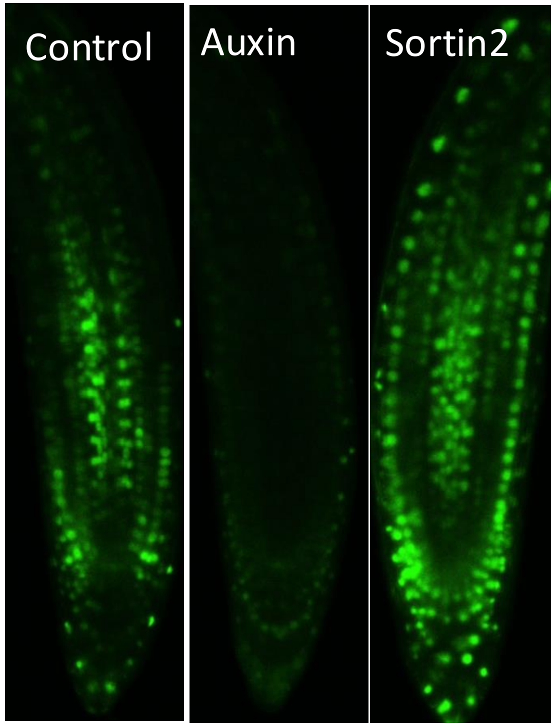
Nitrate response

	AO	ACH1	AtbZIP	AT5G09800	AT4G32950	ATHNIR	CYP707A3	ATRFNR1	AT5G41670	G6PD2	AHB1	ATRFNR2	AT3G47980	MAPKKK14	AtLEA5	AT3G60750	AT1G32920	PGM	AT2G48080	G6PD3	AT4G25835	LBD38	ASL39	AT1G25550	AT5G62720
Auxin	3	27	2	13	2	-1	1	-1	-1	-1	2	-1	-2	2	1	-1	-1	1	2	-1	-2	-2	-2	-1	-2
Sortin2	7	5	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1

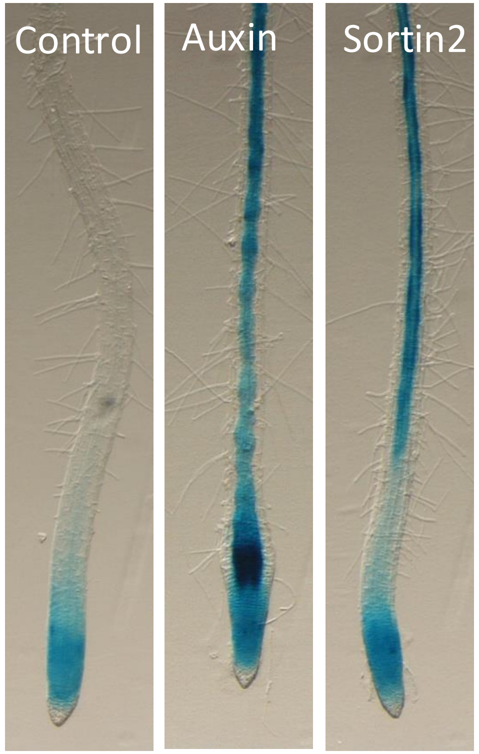
	AT4G18340	UPM1	LBD39	AT1G25550	ATNAS1	AT4G18340	AT5G19970	GNR1	AT1G68670	AtbZIP3	CIPK3	ATGSTF14	AT1G80380	AT3G62930	AT1G16170	AT3G07350	BT5	AT3G25790	HRS1	AT5G10210	AT5G01740	ATBT2	ATTPPB	SKOR	A
Auxin	-1	-2	1	-3	-1	-1	-4	1	-2	-7	-2	2	1	-3	-2	-3	-7	-7	-7	-4	-23	2	-36	-29	-7
Sortin2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-2	-2	-2	-2	-3	-3	-3	-3	-3	-4	-4	-4	-5

Sortin2 is a unique molecule that induces LRI

Aux/IAA reporter

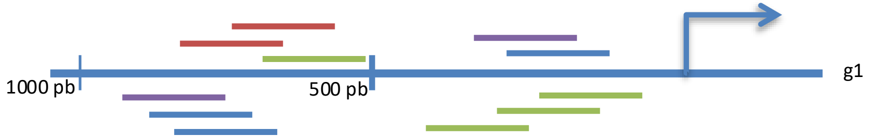


pCYCB1;1:GUS



How to asses TFBS in the regulatory region of co-expressed genes

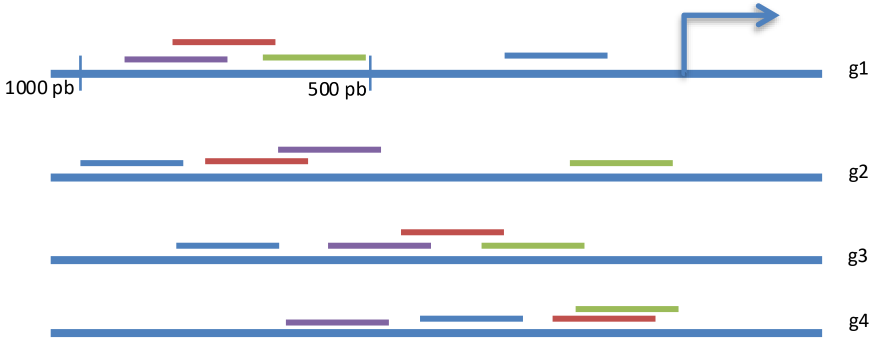
CLOVER



TFBS profile	Hit1	Hit2	Hit3	Hit4	Average matching value
Verde	**4**	**5**	**4**	**2**	**3.75**
Azul	4	4	3		3.67
Rojo	3	2			2.50
Lila	3	4			3.50

PSCAN

PSCAN; In this algorithm, instead of computing a count of predicted sites, we rather compute for each input sequence a raw matching value, representing the likelihood for the TF to bind the promoter.



TFBS profile	g1	g2	…	gN	Average matching value
Verde	4	5	4	2	3.75
Azul	4	4	3	2	3.25
Rojo	3	2	4	3	3.00
Lila	**5**	**5**	**3**	**3**	**4.00**

⋮

Average for matching value within group of genes

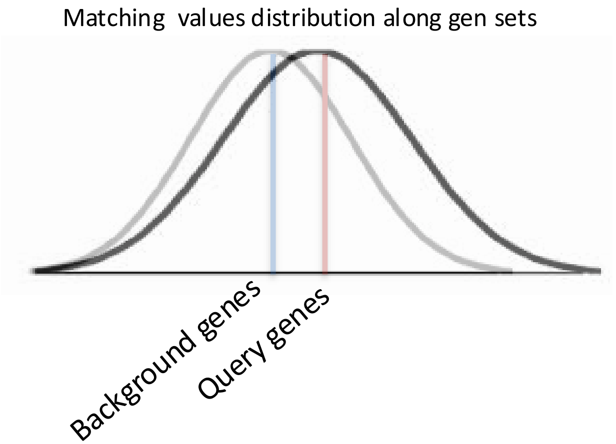
→ Sample (Query)

→ Background (Whole genome)

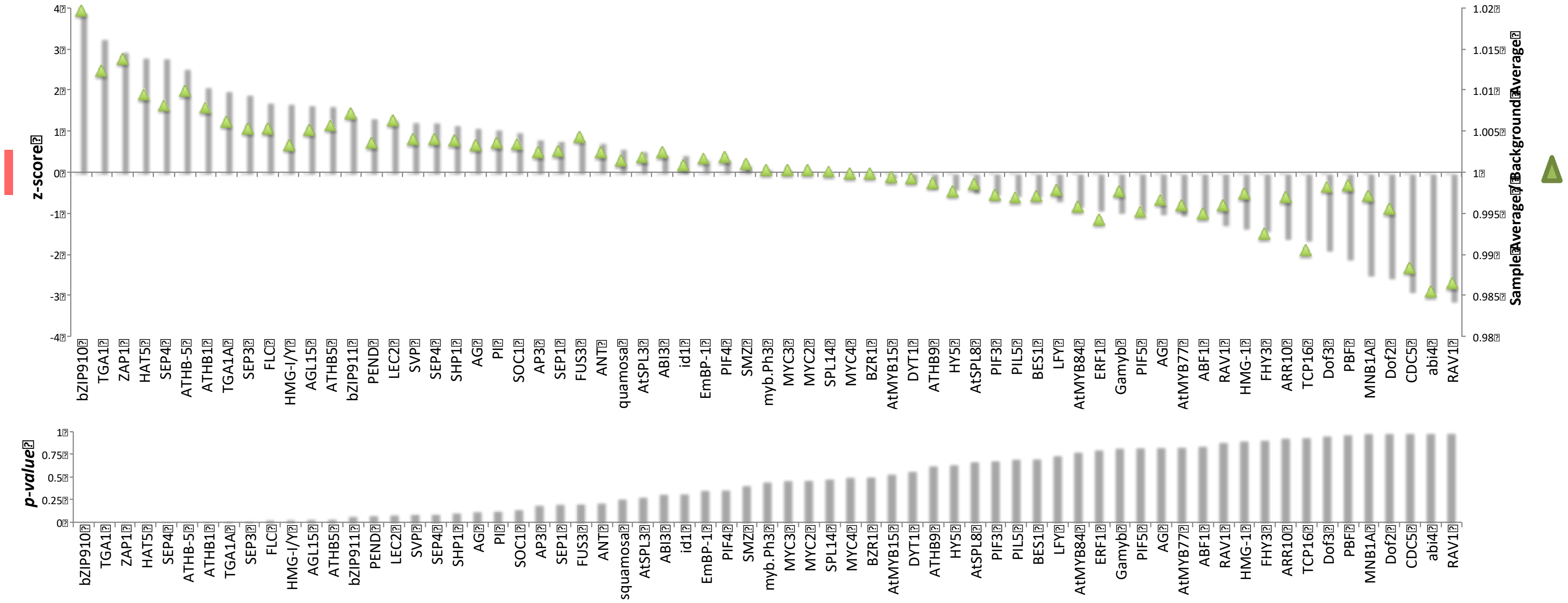
→ Random Subset

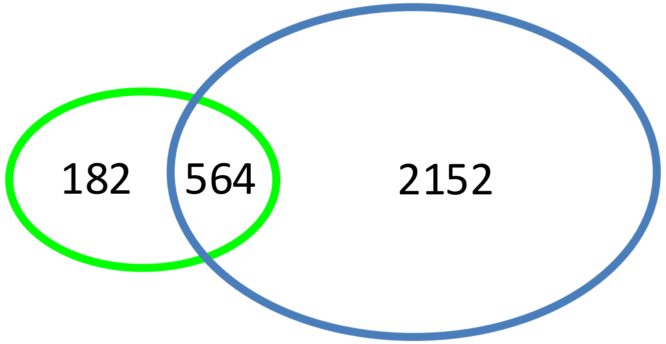
JASPAR database
69 FTBS FOR PLANTS

Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes



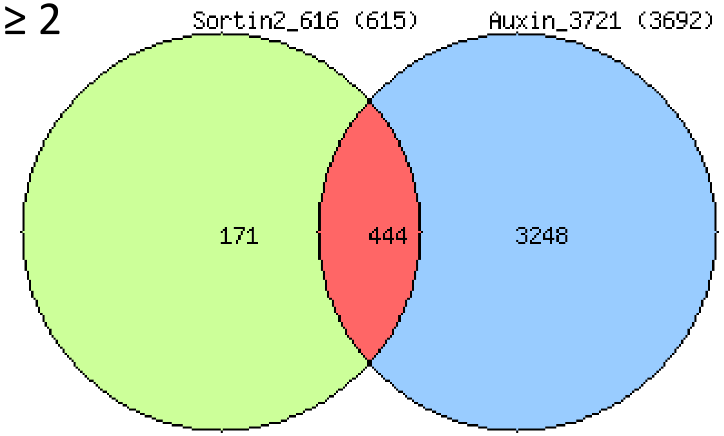
$$\text{FC Rate} = \frac{\text{Matching value Avg}_{\text{Query genes}}}{\text{Matching value Avg}_{\text{Background genes}}}$$





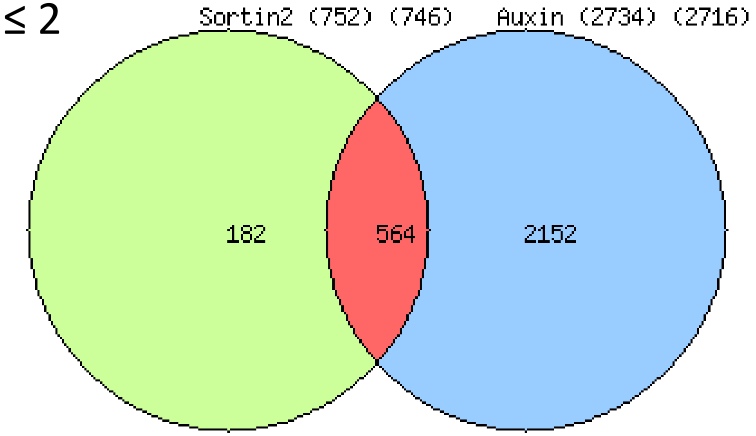
UP REGULATED

FC ≥ 2

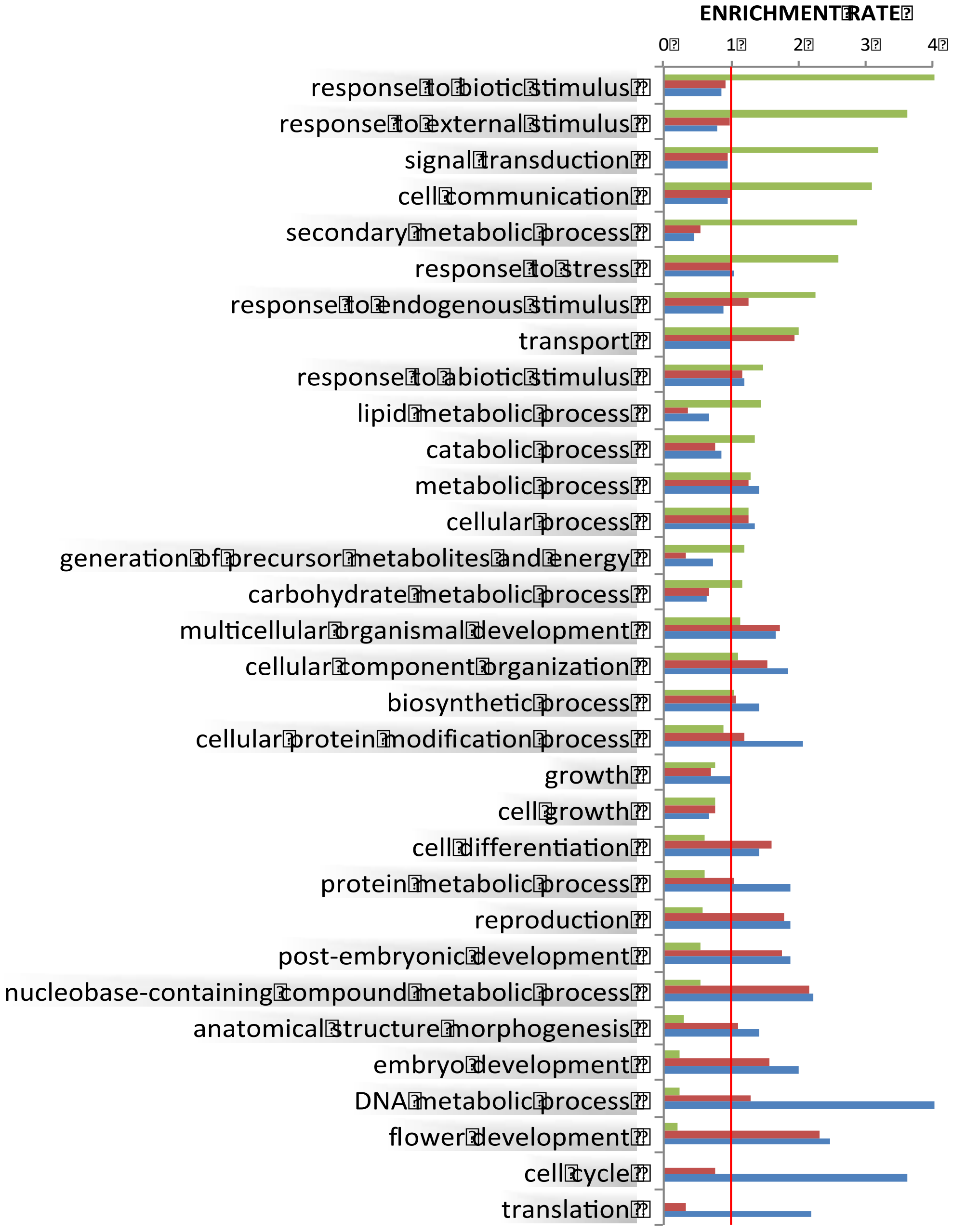


DOWN REGULATED

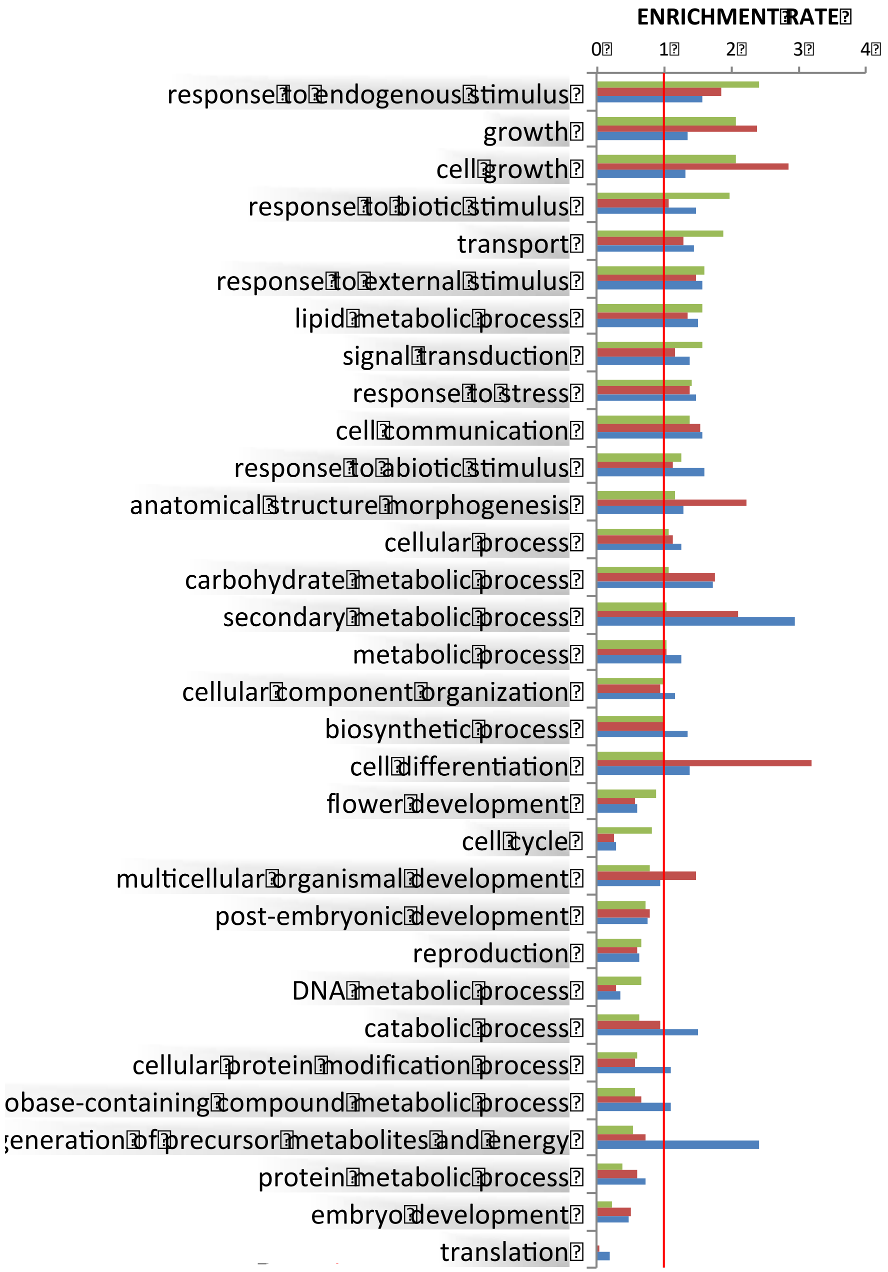
FC ≤ 2



UPREGULATED

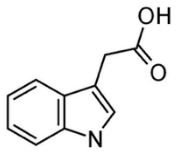


DOWNREGULATED



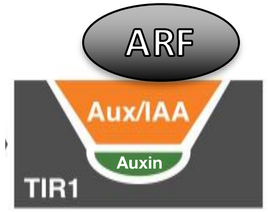
🟩 Sortin2 (171) 🟥 Sortin2 & Auxin (444) 🟦 Auxin (3248)

Auxin signaling requires Aux/IAA degradation

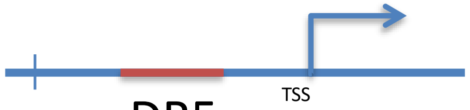

IAA

Receptor

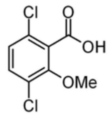
AUX/IAA Degradation

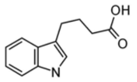


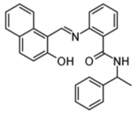
Auxin Response Genes

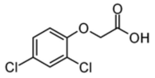


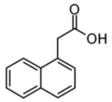
DR5 promoter

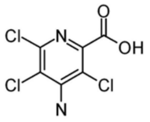

Dicamba


IBA



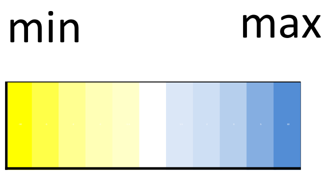

2,4-D


1-NAA


Picloram

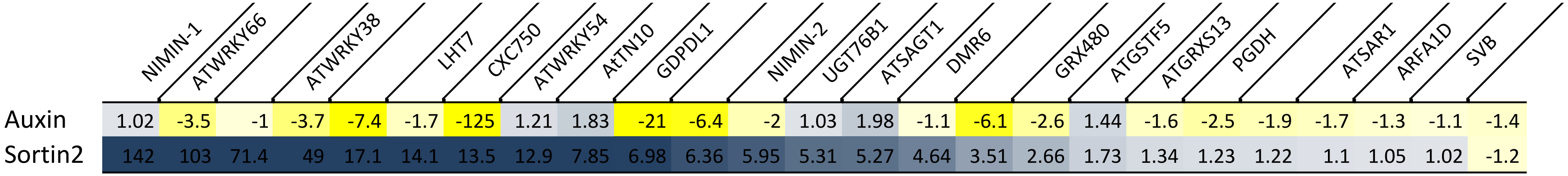
Adapted from
Tan et al . Nature. 2004

Salicylic acid response is specifically associated with Sortin2 response



SA response

According to Krinke et al. Plant Phys 2007

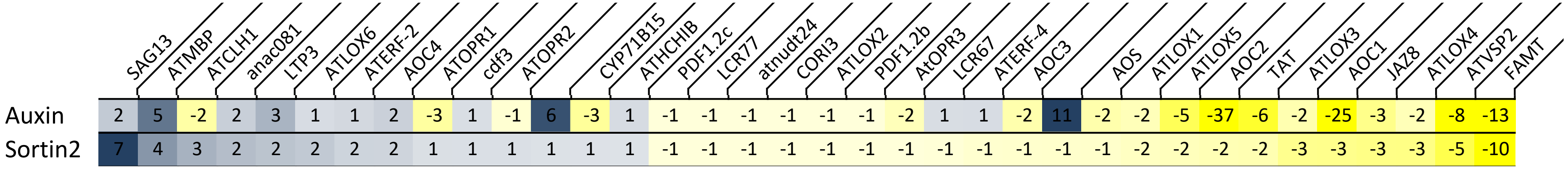


	NIMIN-1	ATWRKY66		ATWRKY38		LHT7	CXC750	ATWRKY54	AtTN10	GDPDL1		NIMIN-2	UGT76B1
Auxin	1.02	-3.5	-1	-3.7	-7.4	-1.7	-125	1.21	1.83	-21	-6.4	-2	1.03
Sortin2	142	103	71.4	49	17.1	14.1	13.5	12.9	7.85	6.98	6.36	5.95	5.31

	ATSAGT1	DMR6		GRX480	ATGSTF5	ATGRXS13	PGDH		ATSAR1	ARFA1D	SVB	
Auxin	1.98	-1.1	-6.1	-2.6	1.44	-1.6	-2.5	-1.9	-1.7	-1.3	-1.1	-1.4
Sortin2	5.27	4.64	3.51	2.66	1.73	1.34	1.23	1.22	1.1	1.05	1.02	-1.2

JA response and Synthesis

According to Méndez-Bravo et al. Plos One 2011



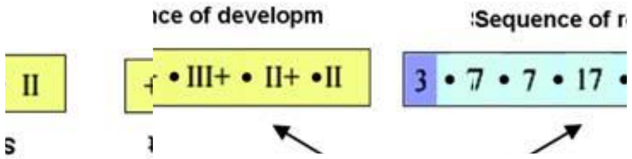
	SAG13	ATMBP	ATCLH1	anac081	LTP3	ATLOX6	ATERF-2	AOC4	ATOPR1	cdf3	ATOPR2	
Auxin	2	5	-2	2	3	1	1	2	-3	1	-1	6
Sortin2	7	4	3	2	2	2	2	2	1	1	1	1

	CYP71B15	ATHCHIB	PDF1.2c	LCR77	atnudt24	CORI3	ATLOX2	PDF1.2b	AtOPR3	LCR67	ATERF-4	AOC3
Auxin	-3	1	-1	-1	-1	-1	-1	-1	-2	1	1	-2
Sortin2	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

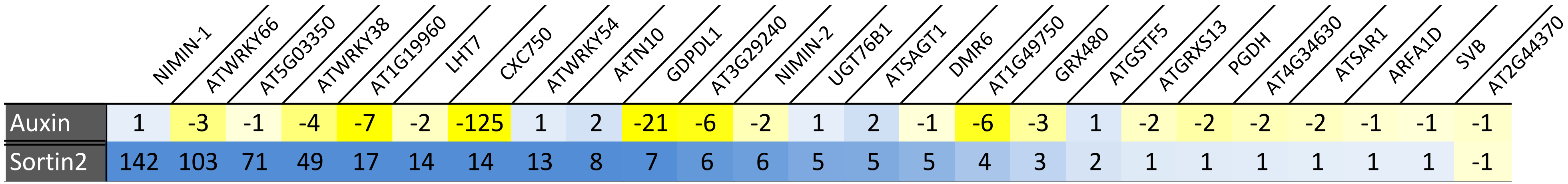
		AOS	ATLOX1	ATLOX5	AOC2	TAT	ATLOX3	AOC1	JAZ8	ATLOX4	ATVSP2	FAMT
Auxin	11	-2	-2	-5	-37	-6	-2	-25	-3	-2	-8	-13
Sortin2	-1	-1	-2	-2	-2	-2	-3	-3	-3	-3	-5	-10

Mitotic activity

pCYCB1;1:GUS



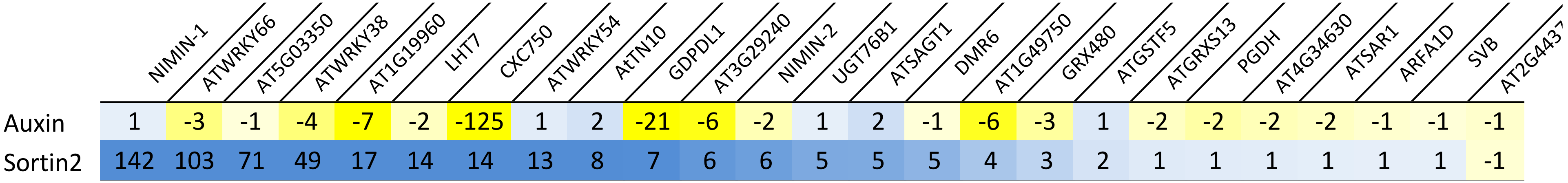
Lucas *et al* 2007. *Plos One*



	NIMIN-1	ATWRKY66	AT5G03350	ATWRKY38	AT1G19960	LHT7	CXC750	ATWRKY54	AtTN10	GDPDL1	AT3G29240	NIMIN-2	UGT76B1	ATSAGT1	DMR6	AT1G49750	GRX480	ATGSTF5	ATGRXS13	PGDH	AT4G34630	ATSAR1	ARFA1D	SVB	AT2G44370
Auxin	1	-3	-1	-4	-7	-2	-125	1	2	-21	-6	-2	1	2	-1	-6	-3	1	-2	-2	-2	-2	-1	-1	-1
Sortin2	142	103	71	49	17	14	14	13	8	7	6	6	5	5	5	4	3	2	1	1	1	1	1	1	-1

AUXIN

SORTIN2



	NIMIN-1	ATWRKY66	AT5G03350	ATWRKY38	AT1G19960	LHT7	CXC750	ATWRKY54	AtTN10	GDPDL1	AT3G29240	NIMIN-2	UGT76B1	ATSAGT1	DMR6	AT1G49750	GRX480	ATGSTF5	ATGRXS13	PGDH	AT4G34630	ATSAR1	ARFA1D	SVB	AT2G4437
Auxin	1	-3	-1	-4	-7	-2	-125	1	2	-21	-6	-2	1	2	-1	-6	-3	1	-2	-2	-2	-2	-1	-1	-1
Sortin2	142	103	71	49	17	14	14	13	8	7	6	6	5	5	5	4	3	2	1	1	1	1	1	1	-1