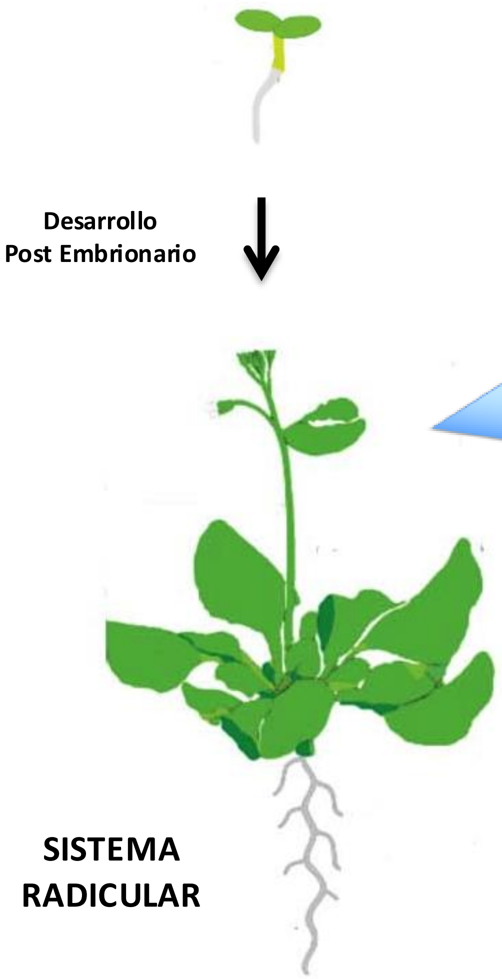
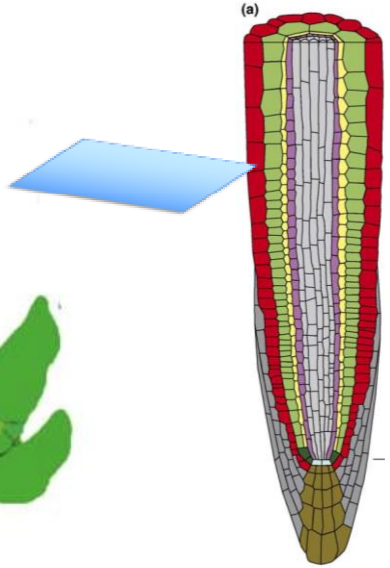
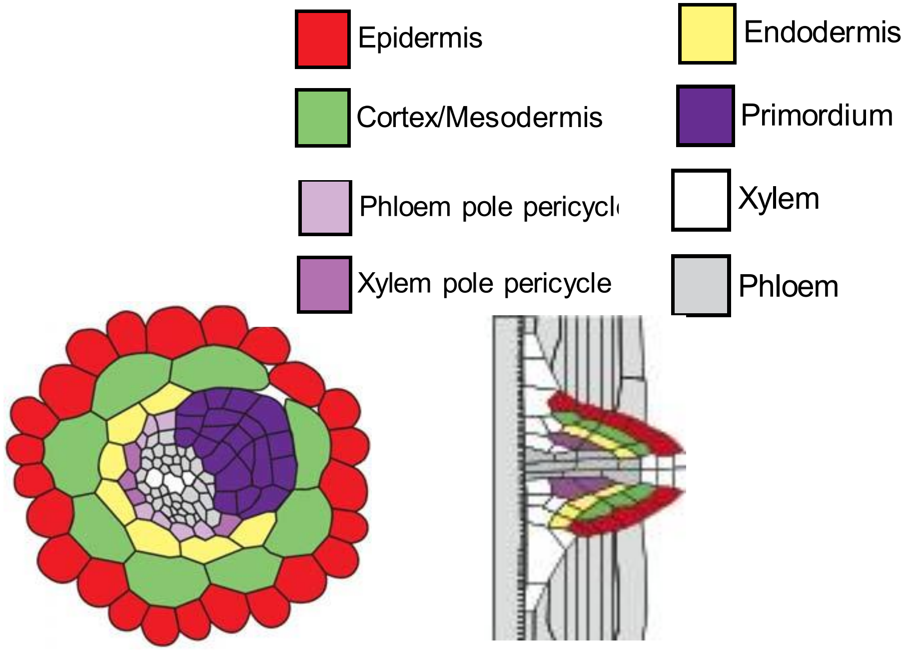
Un nuevo mecanismo molecular que determina la ramificación del sistema radicular

Patricio Pérez-Henríquez
Tutor: Lorena Norambuena
Co-Tutor: Tom Beeckman

Arquitectura del sistema radicular yace en el continuo proceso de iniciar nuevas raíces laterales



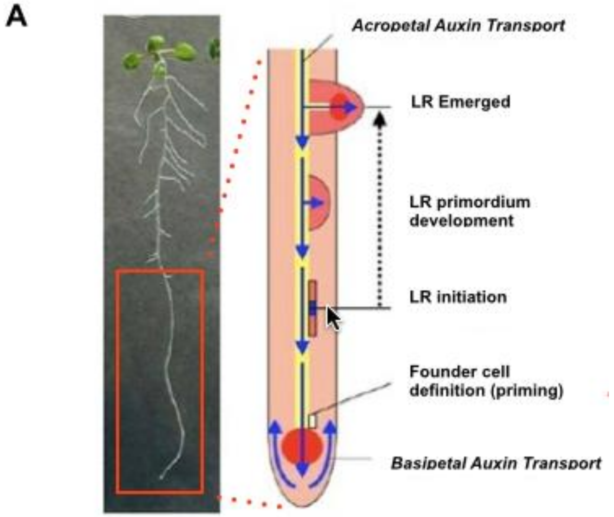


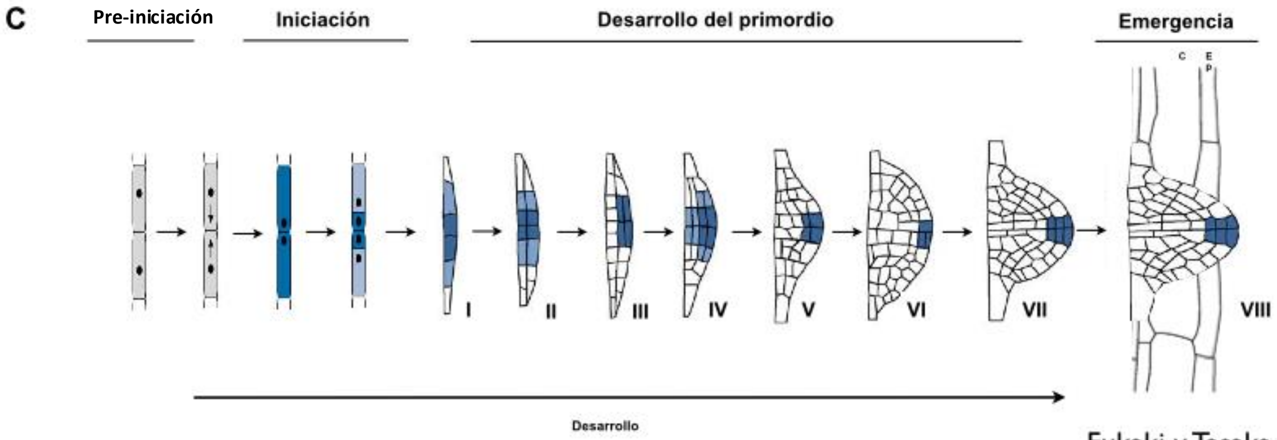


La captación de nutrientes depende de la iniciación de raíces laterales

LR= Lateral Roots

Progresión espacio temporal del desarrollo de raíces laterales

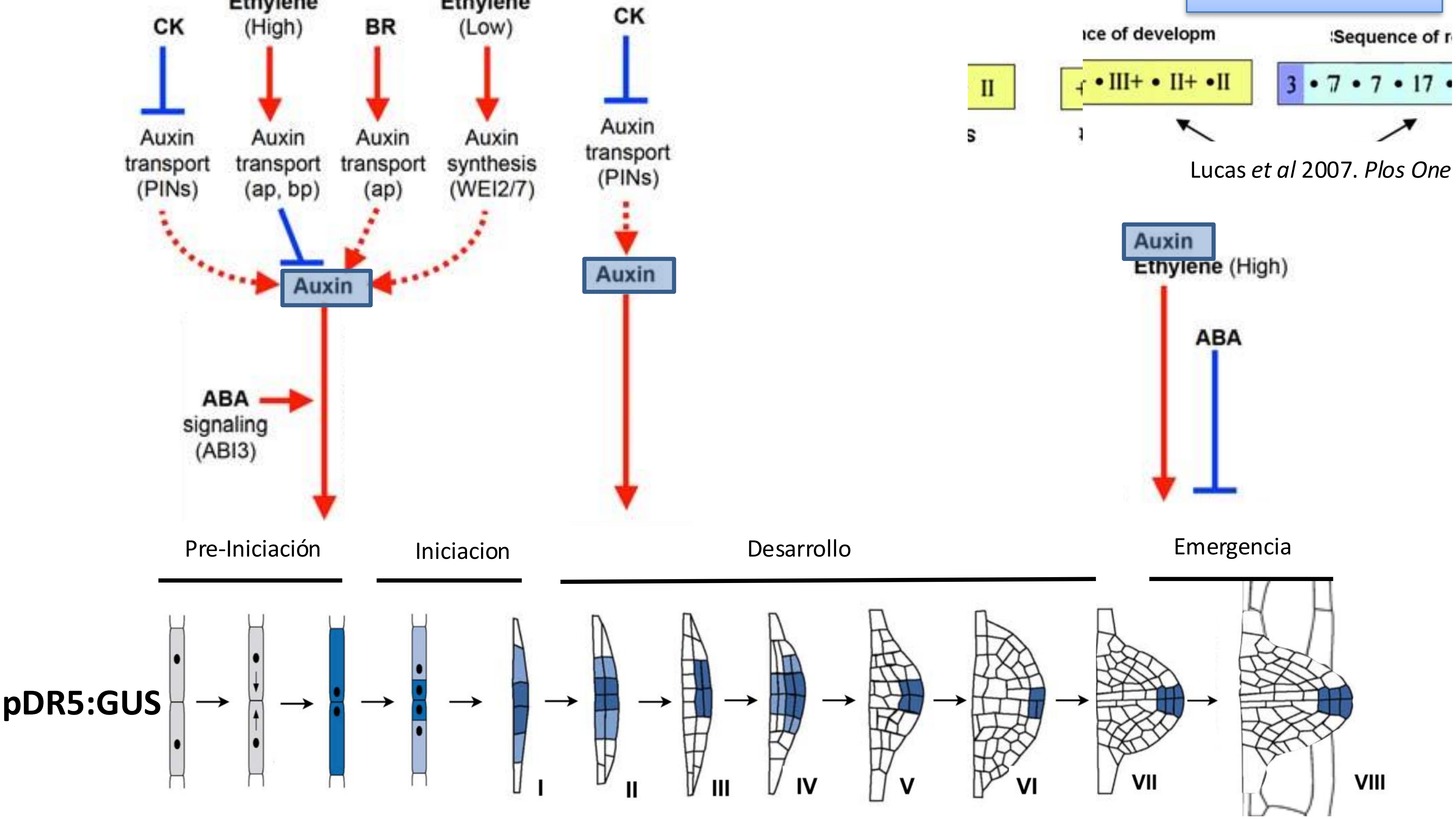




Fukaki y Tasaka. *Plant Mol Biol* (2009)
Péret et al. *Trends in Plant Science* (2009)

Auxina es un regulador clave del desarrollo de raíces laterales

Mitotic activity **pCYCB1;1:GUS**



Lucas *et al* 2007. *Plos One*

Pre-Iniciación

Iniciacion

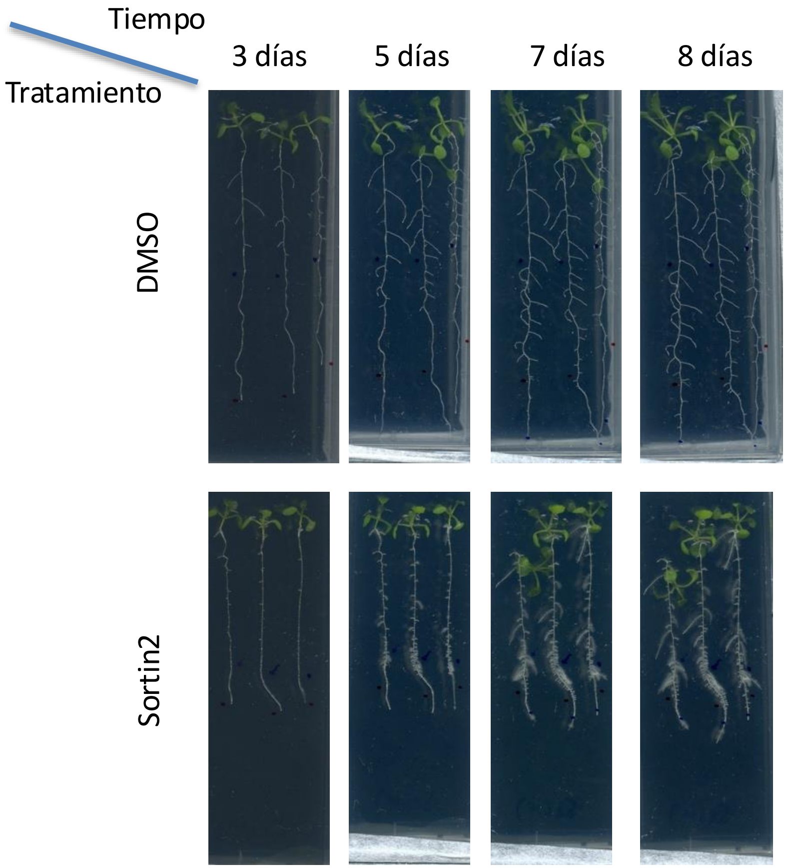
Desarrollo

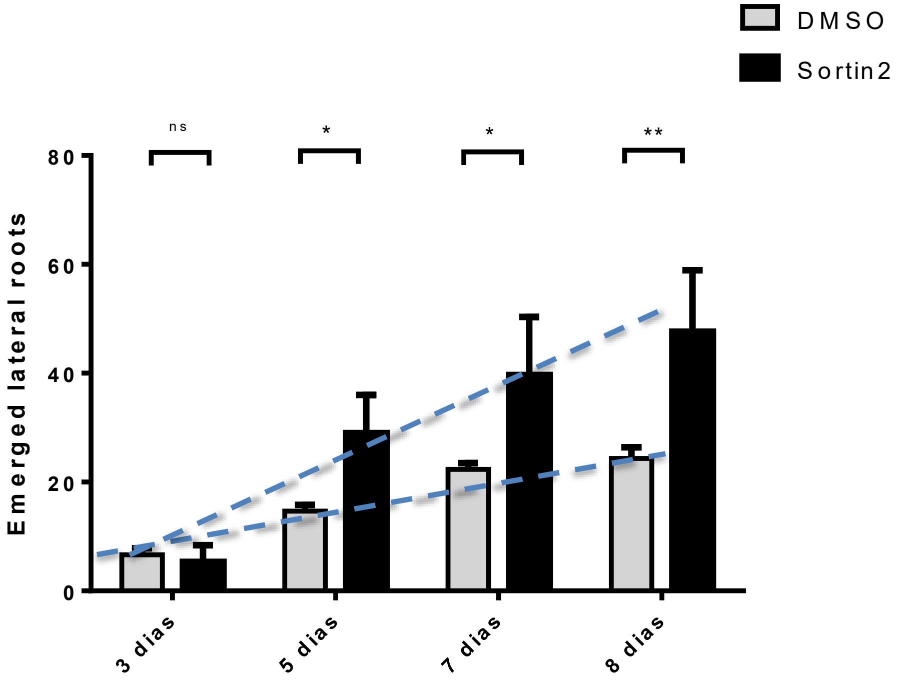
Emergencia

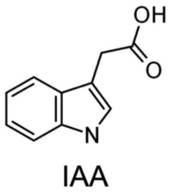
pDR5:GUS

Adaptado desde
Peret *et al*, 2003. *Trends in Plant Biology*
Fukaki *et al, 2004. Plant Phys*.

Sortin2 es una molécula que induce formación de raíces laterales

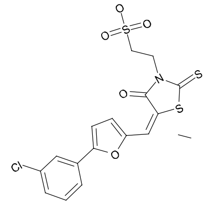


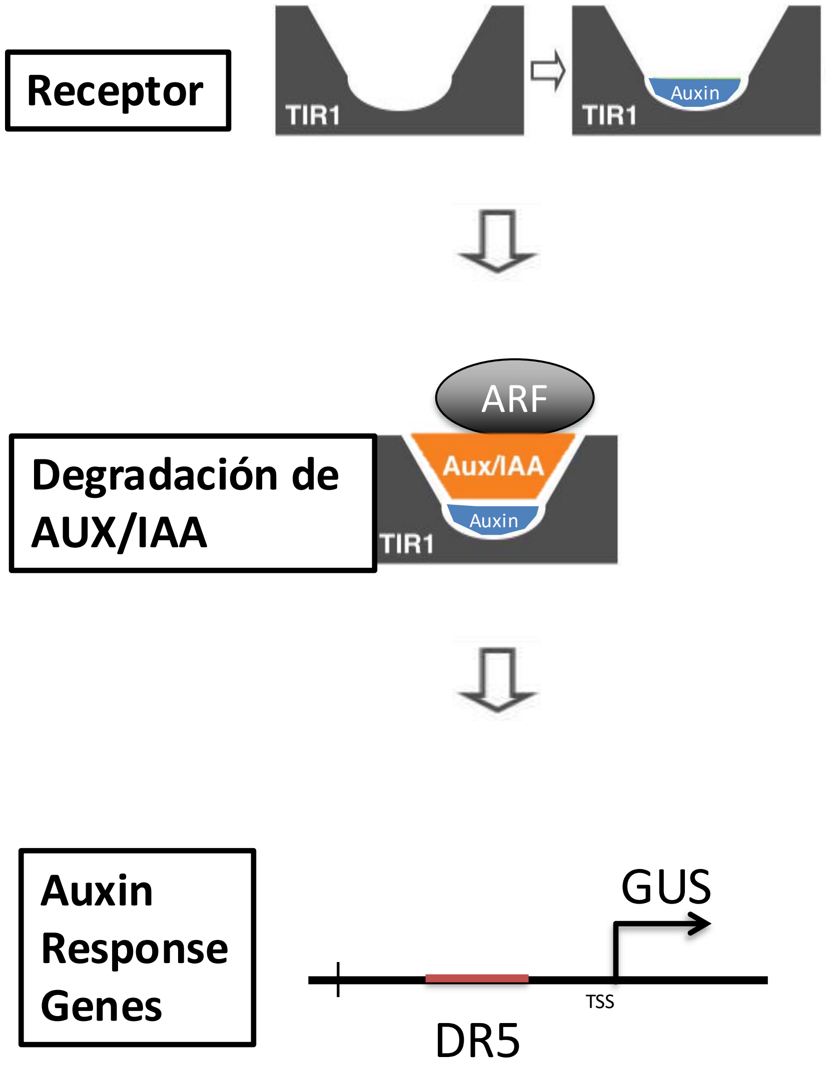




Señalización de auxina

Sortin2 is a [illegible] induces LR



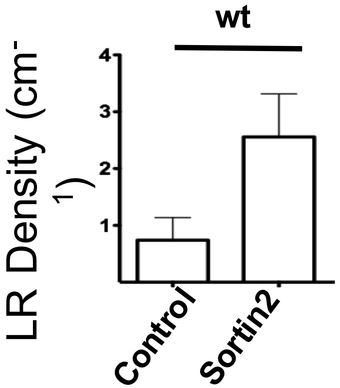


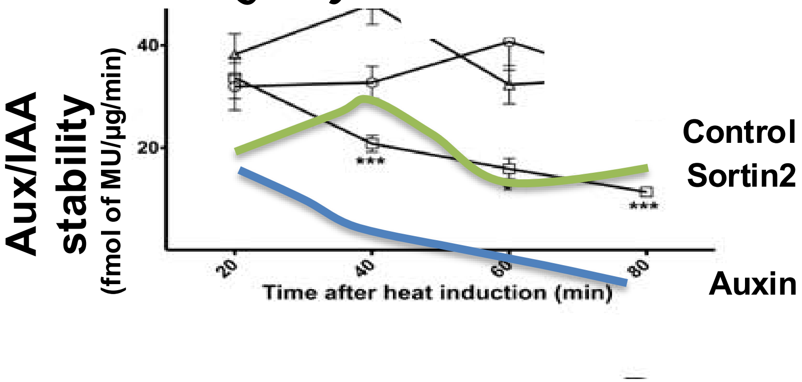
Receptor

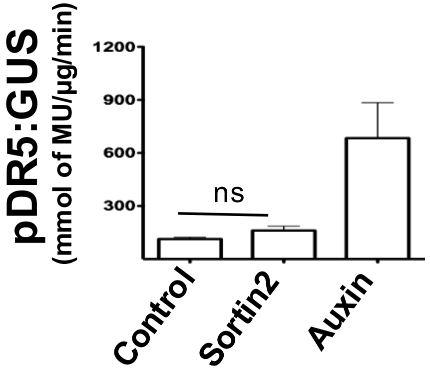
Degradación de AUX/IAA

Auxin Response Genes

Adapted from Tan et al . Nature. 2007







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

HIPOTESIS

Sortin2 estimula la formación de raíces laterales mediante una ruta alternativa a la señalización canónica de auxina.

OBJETIVO GENERAL

Identificar componentes moleculares que participan en el mecanismo de formación de raíces laterales por efecto de Sortin2

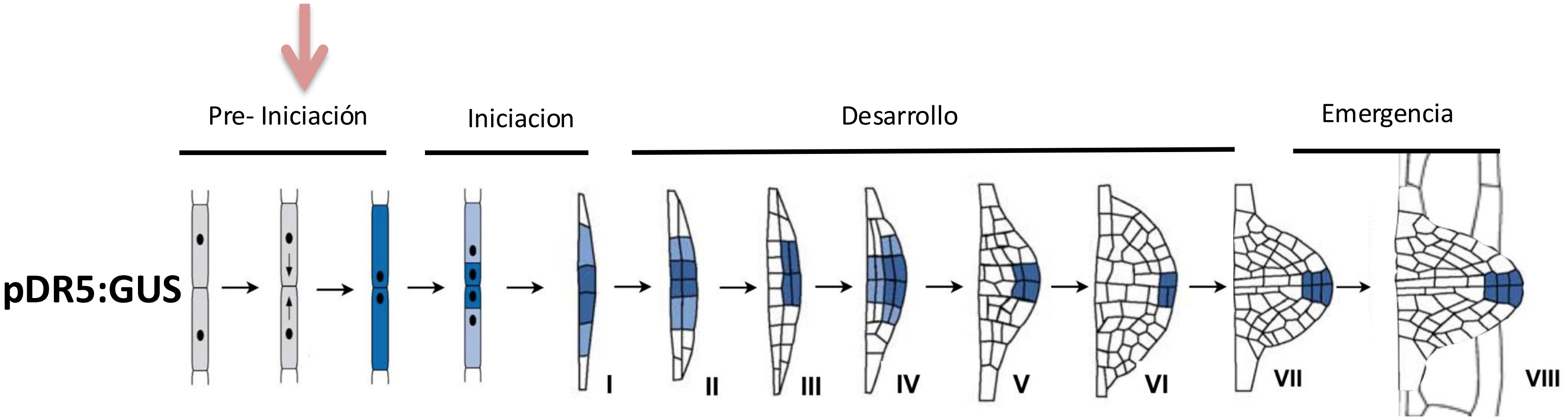
1. **Estudio de la participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2**

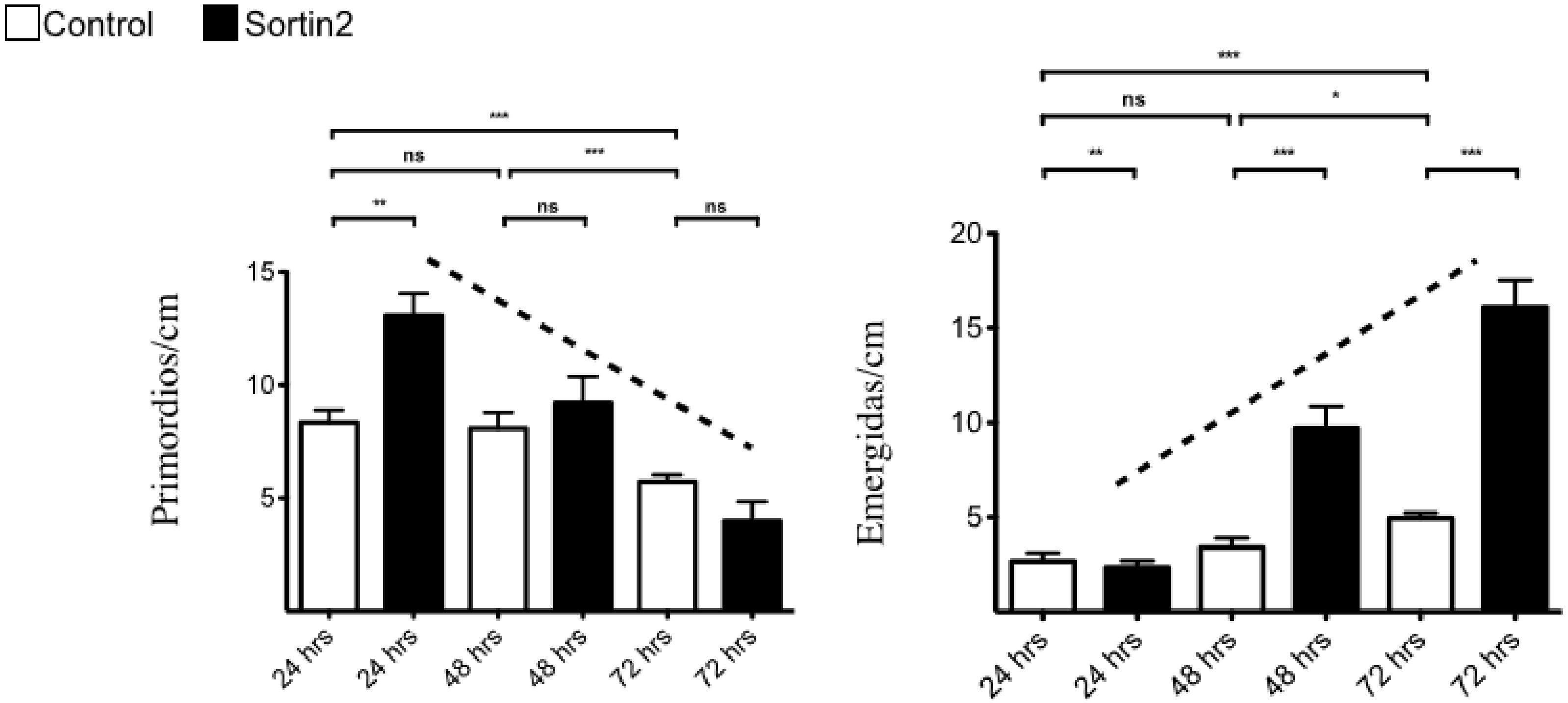
2. Identificación de el(los) grupo(s) de genes que son regulados en tratamientos con Sortin2 mediante análisis global de expresión génica.

3. Identificación de genes importantes para la vía de acción de Sortin2 mediante análisis fenotípico de mutantes insercionales frente a tratamientos con Sortin2.

4. Generación de un modelo que integre la información acerca de los componentes moleculares importantes para la formación de raíces laterales inducidas por Sortin2.

Sortin2 induciría nuevos primordios

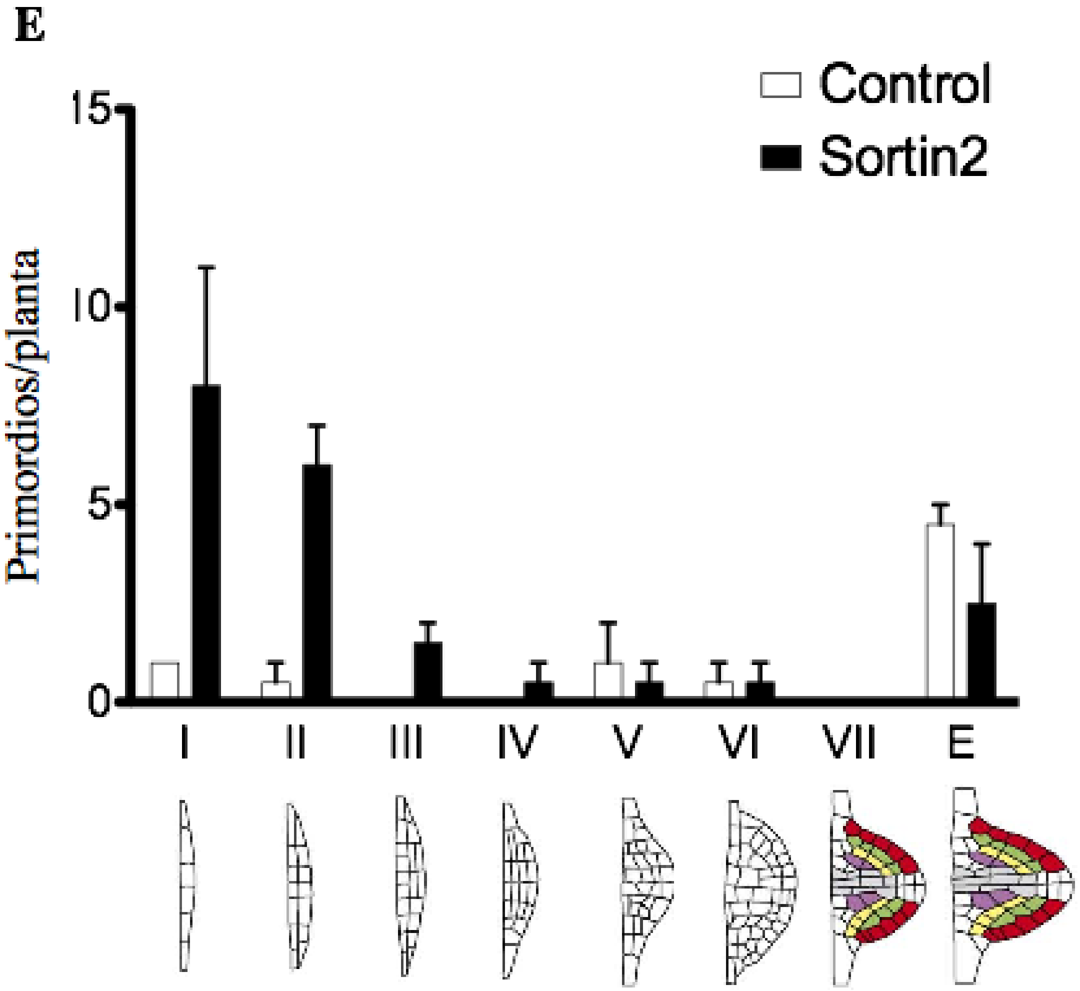




pCYCB1::GUS

Sortin2 induce la iniciación de nuevos primordios

Tratamiento por 24 h



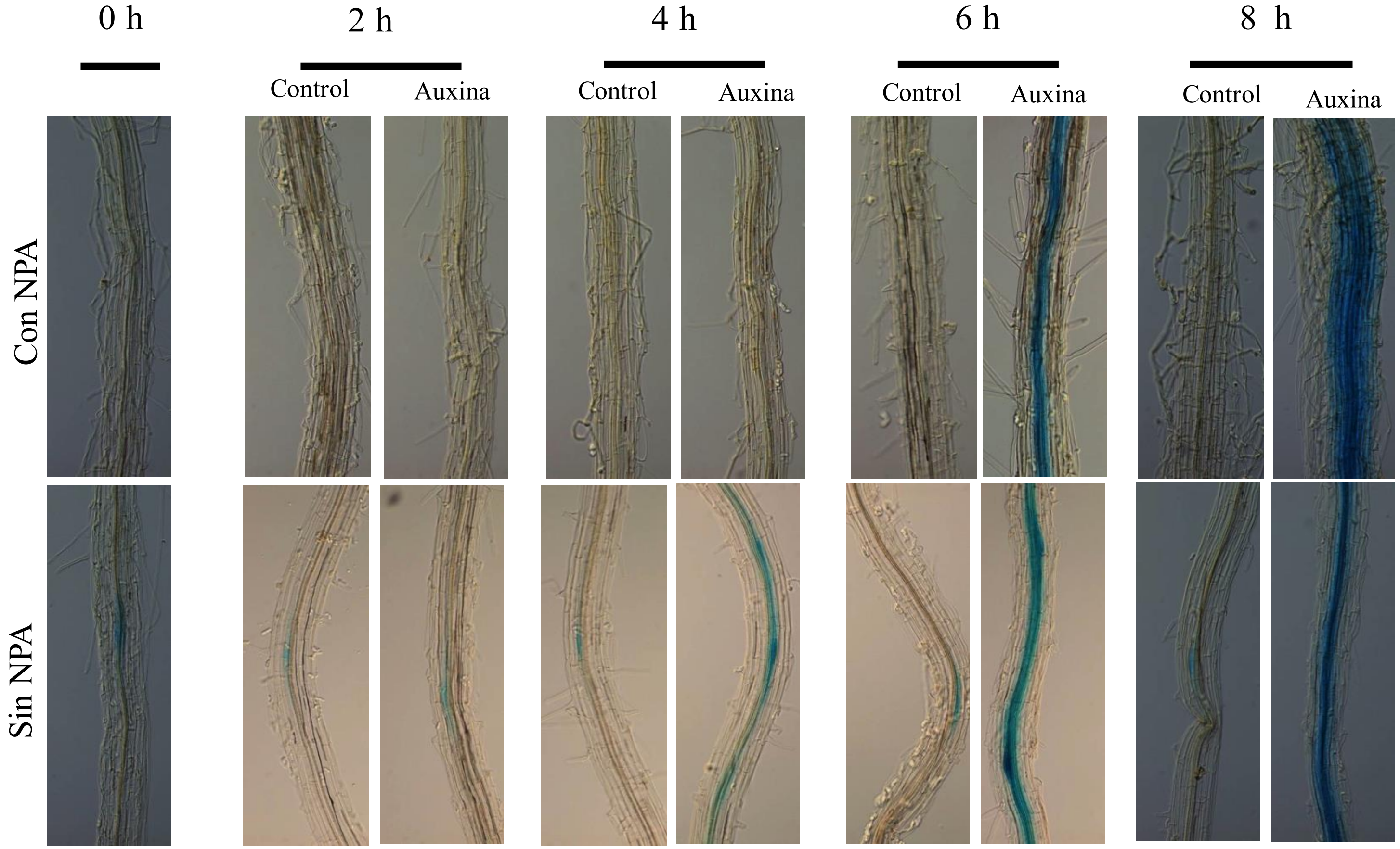
No actuaría sobre una reanudación de primordios detenidos o una aceleración de primordios ya iniciados.

Wild type (Col-0)

De Rybel *et al.* Current Biology. 2010
De Rybel *et al.* Nat Chem Biol. 2012

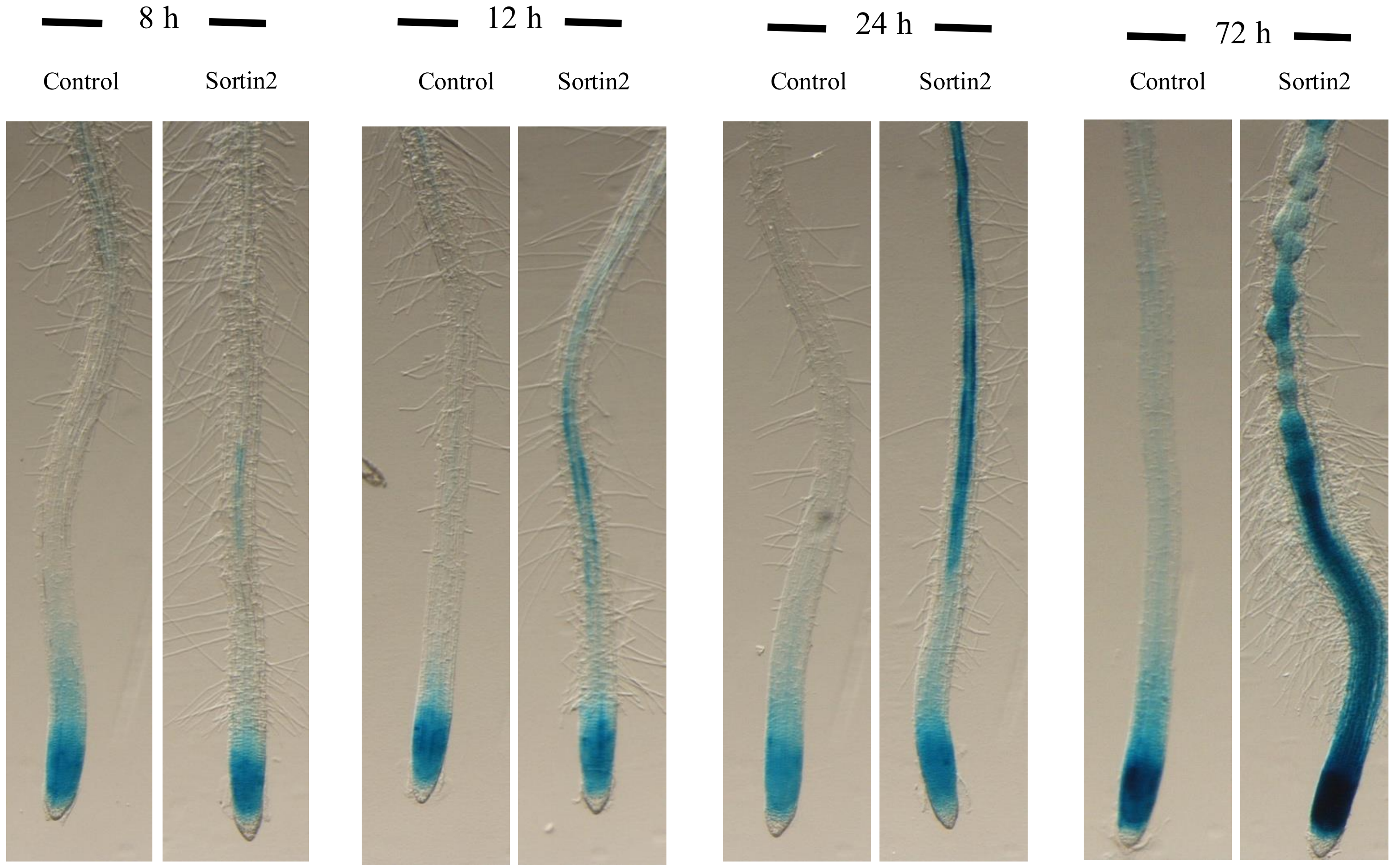
Sistema de inducción de raíces laterales

Himanen et al. PNAS. 2004
Vanneste *et al.* Plant Cell. 2005
De Smet *et al.* Science. 2008
De Smet *et al.* Development. 2007



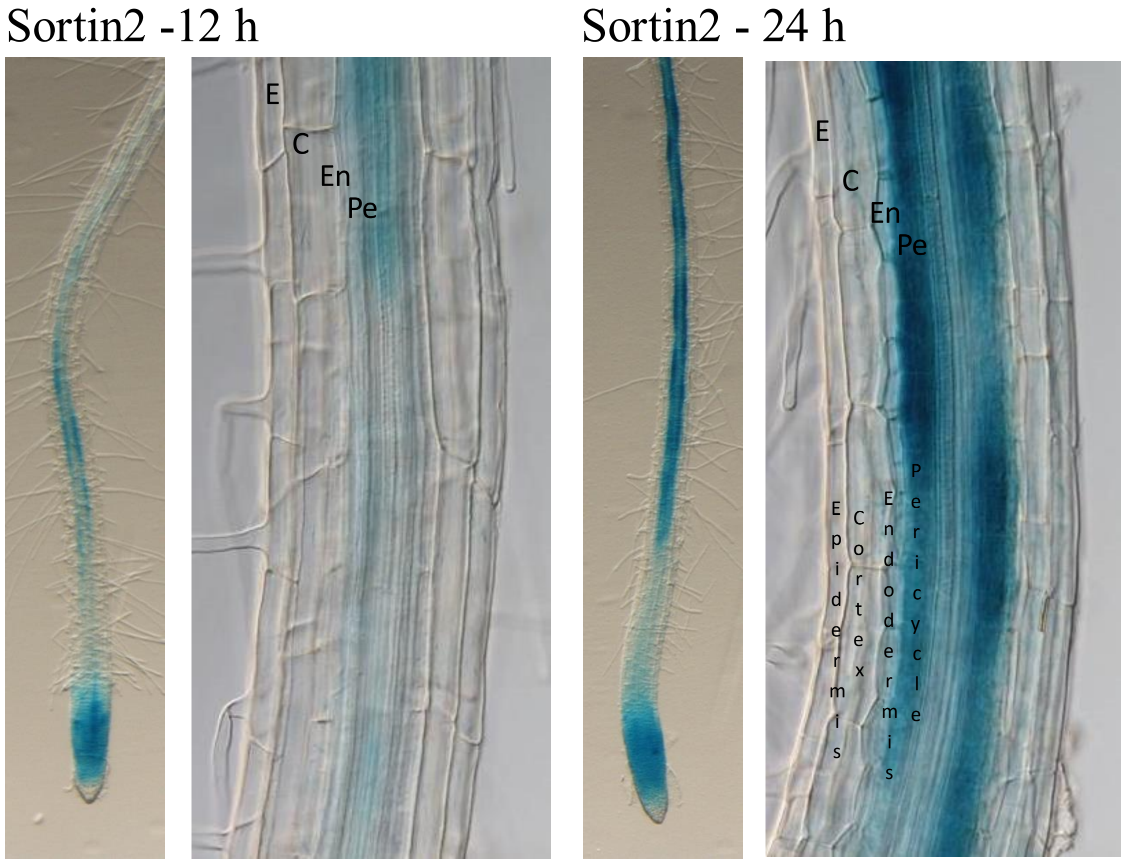
pDR5::GUS

Sortin2 induce actividad mitótica conducente a la formacón de ráices laterales



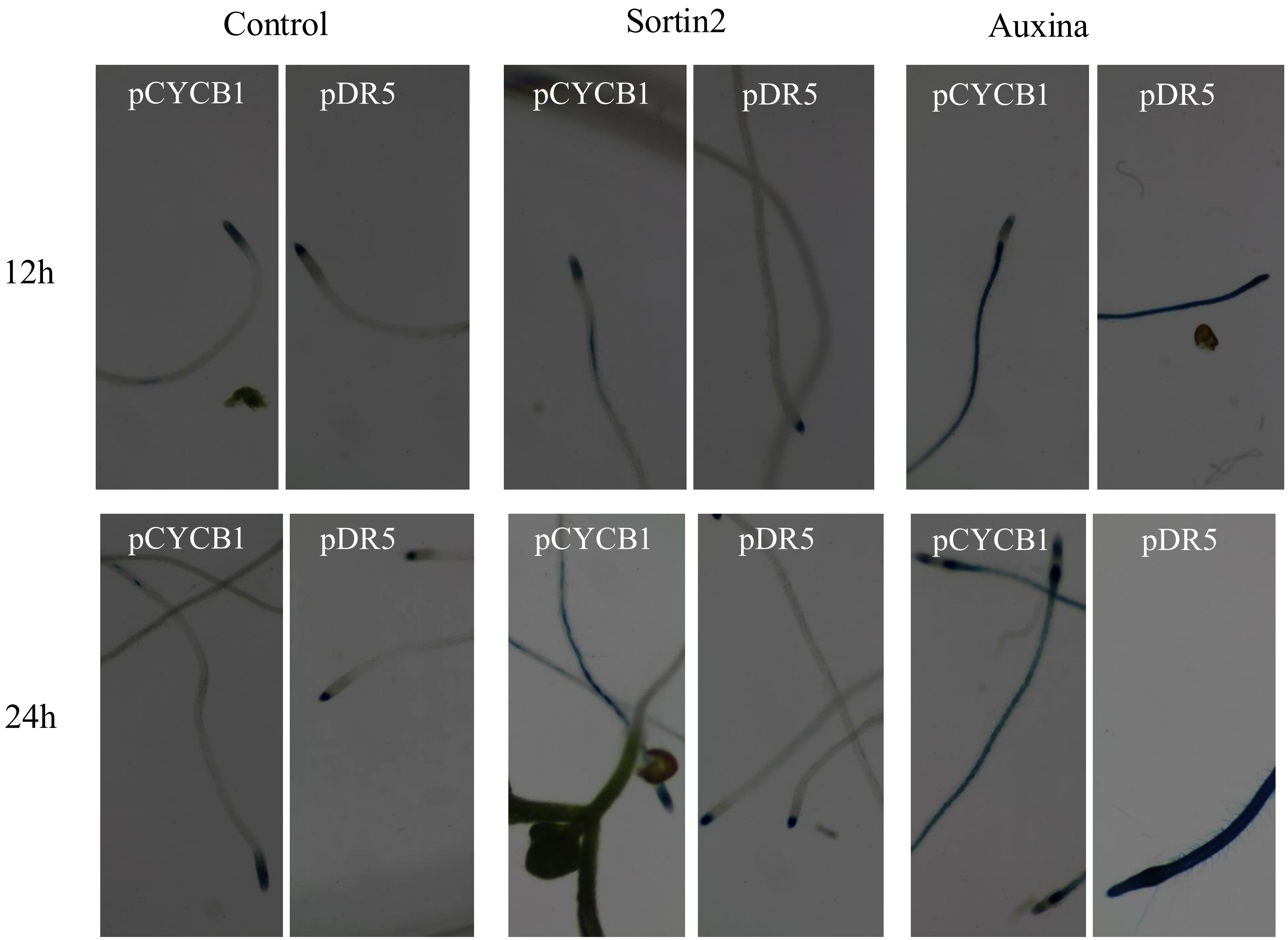
pCYCB1::GUS

Sortin2 induce actividad mitótica específicamente células del periciclo



pCYCB1::GUS

Sortin2 induce LRs por una via pDR5-independiente



La participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2

Niveles hormonales = Sintesis y conjugación de auxina ☑

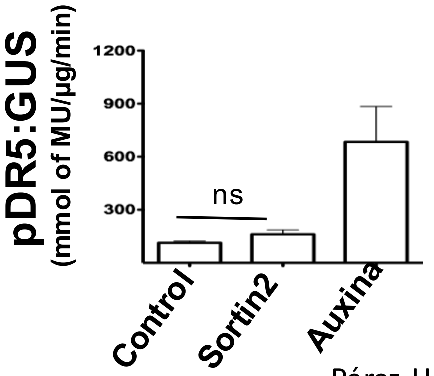
↓

Transporte de auxina ☑

↓

Señalización intracelular de auxina

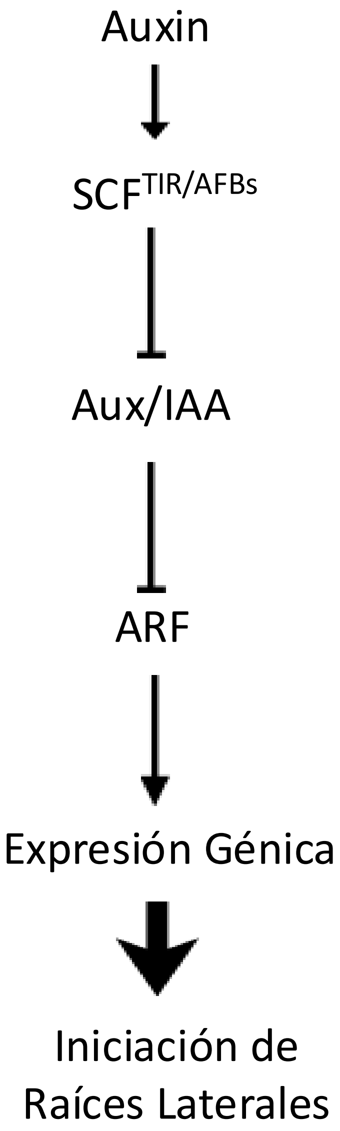
respuesta pDR5
vs niveles hormonales



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

☑ **Identificación de pDR5 a lo largo de la raíz**

La participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2



Auxin

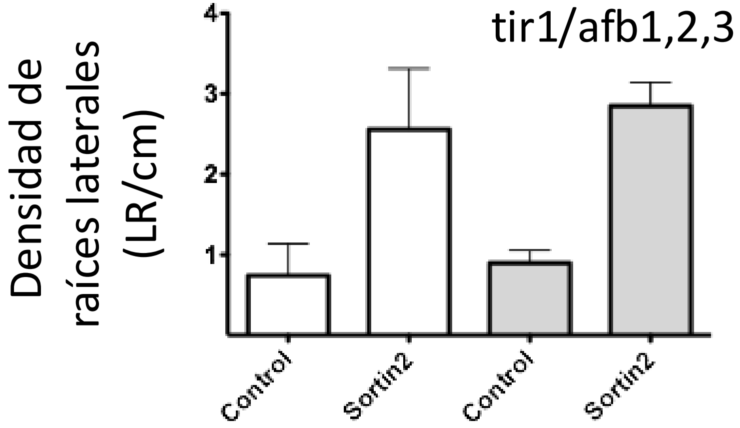
$\text{SCF}^{\text{TIR/AFBs}}$

Aux/IAA

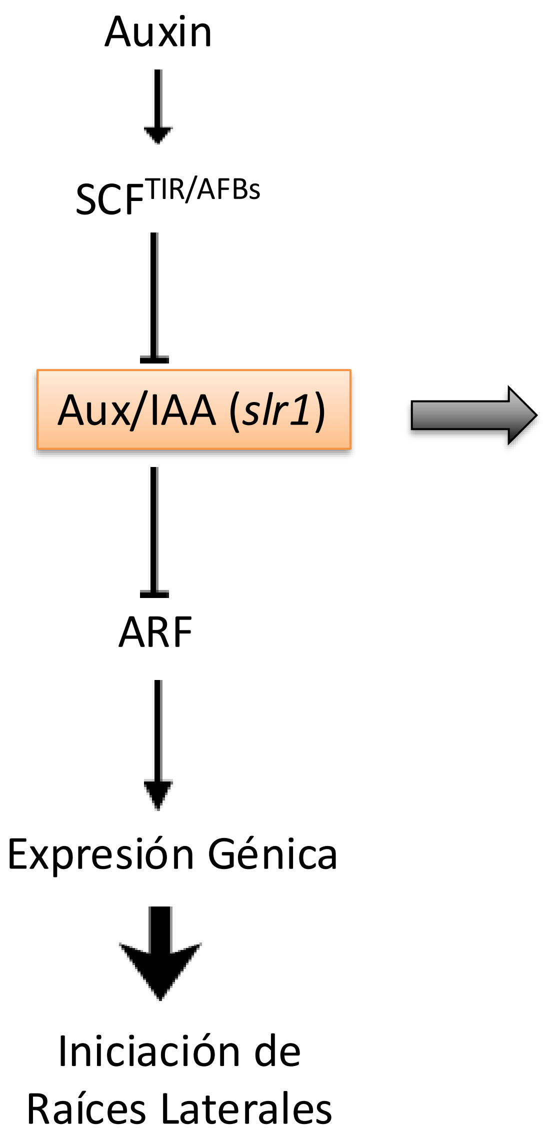
ARF

Expresión Génica

Iniciación de
Raíces Laterales



La participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2



Auxin

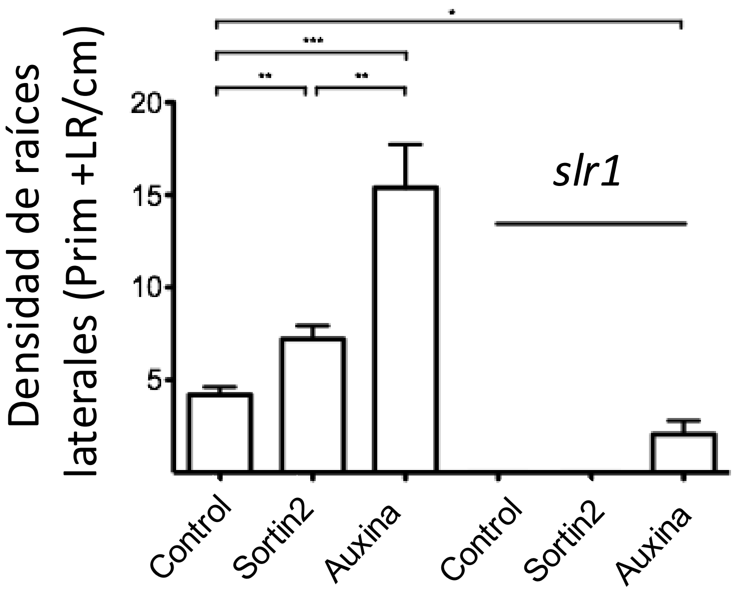
SCF$^{TIR/AFBs}$

Aux/IAA (*slr1*)

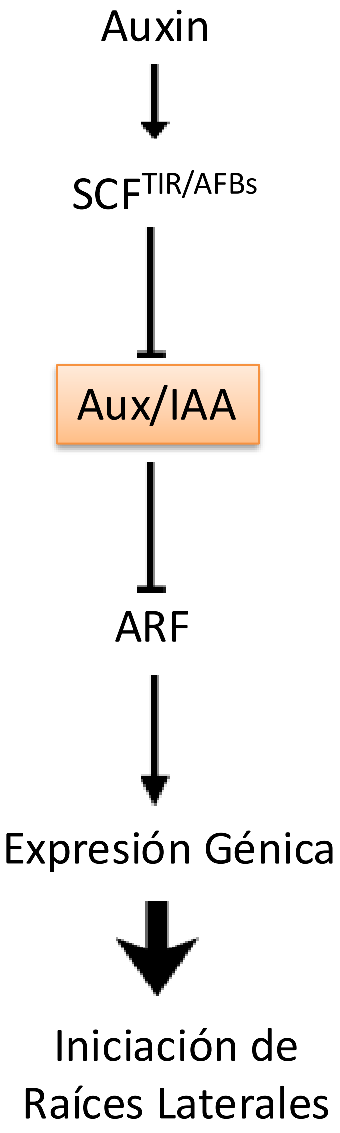
ARF

Expresión Génica

Iniciación de Raíces Laterales



Sortin2 is a unique molecule that induces LR



Auxin

$SCF^{TIR/AFBs}$

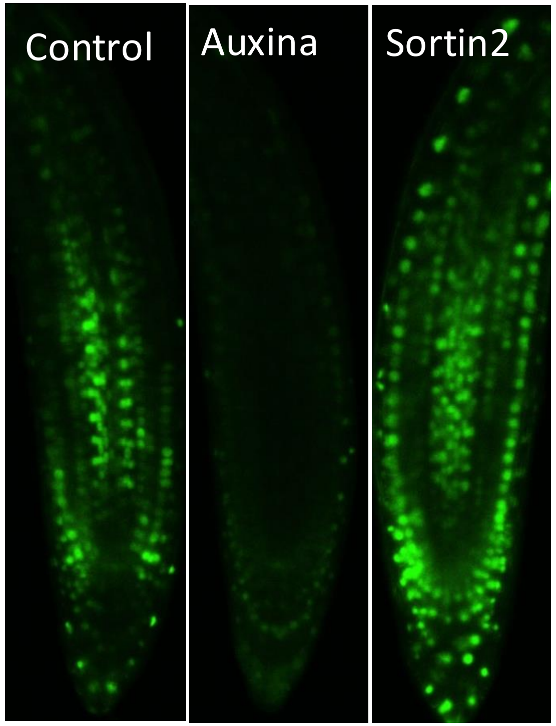
Aux/IAA

ARF

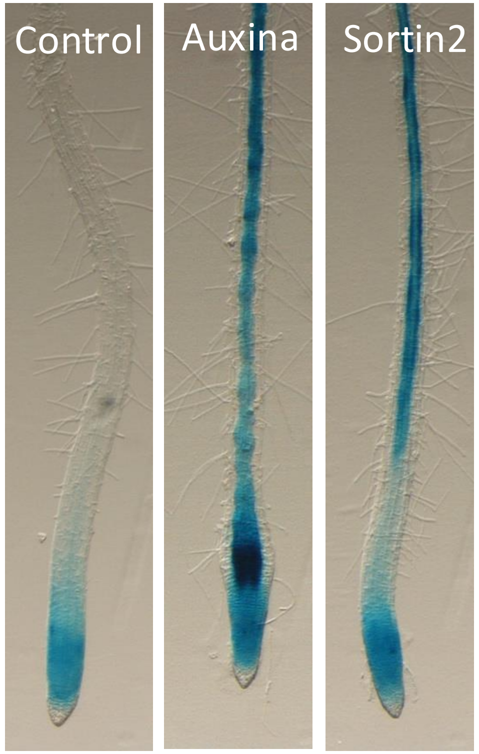
Expresión Génica

Iniciación de
Raíces Laterales

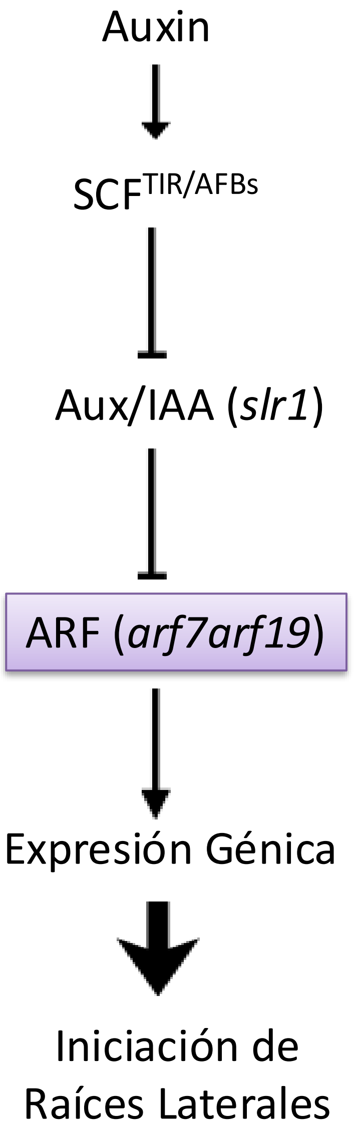
Aux/IAA reporter



pCYCB1;1:GUS



La participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2



Auxin

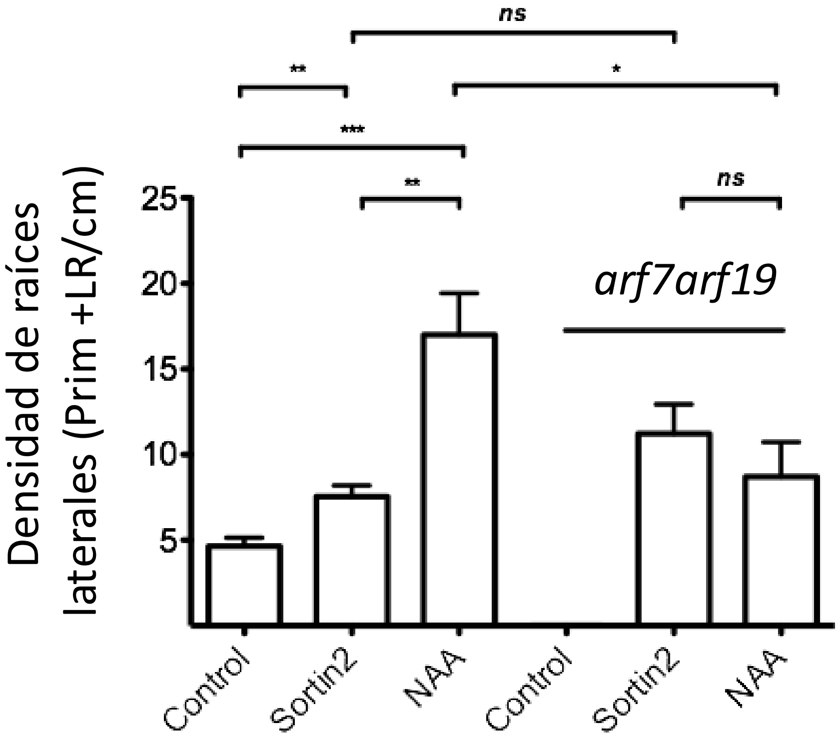
$SCF^{TIR/AFBs}$

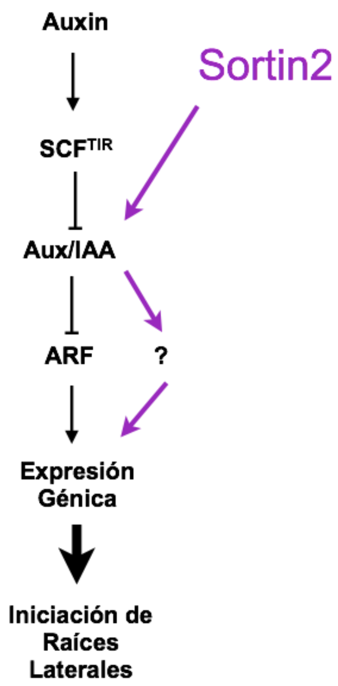
Aux/IAA (*slr1*)

ARF (*arf7arf19*)

Expresión Génica

Iniciación de
Raíces Laterales





Auxin

Sortin2

SCF^{TIR}

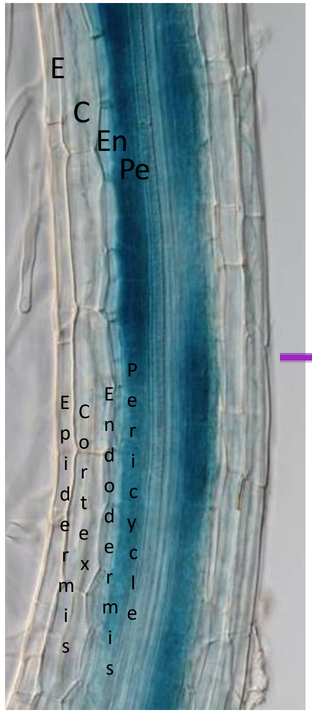
Aux/IAA

ARF

?

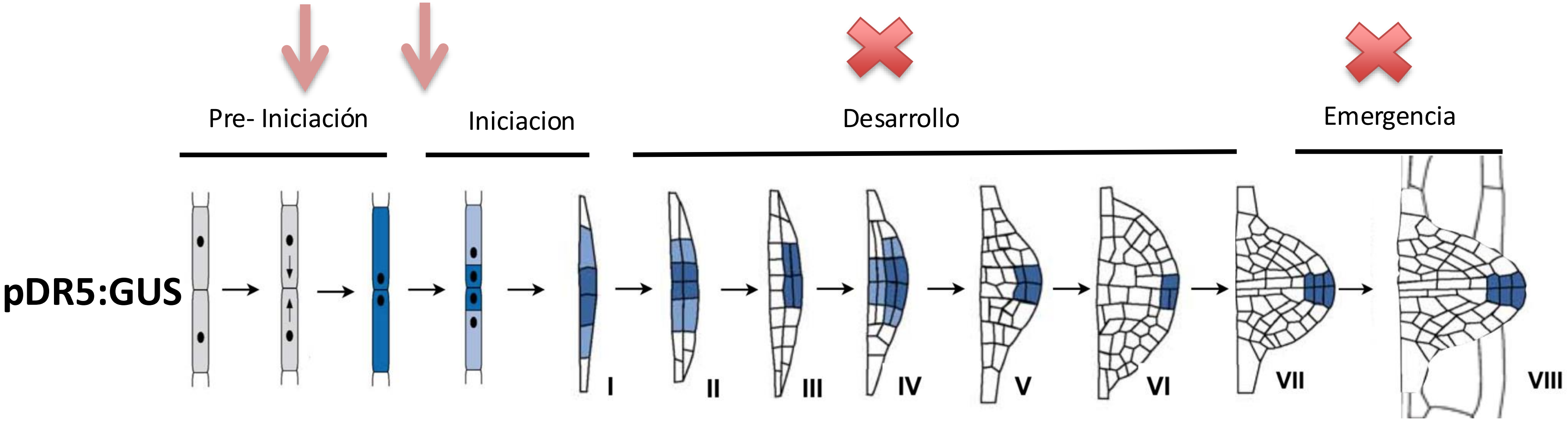
Expresión Génica

Iniciación de Raíces Laterales



Formación de raíces laterales de novo

Redefinicion de células del periciclo hacia células fundadoras



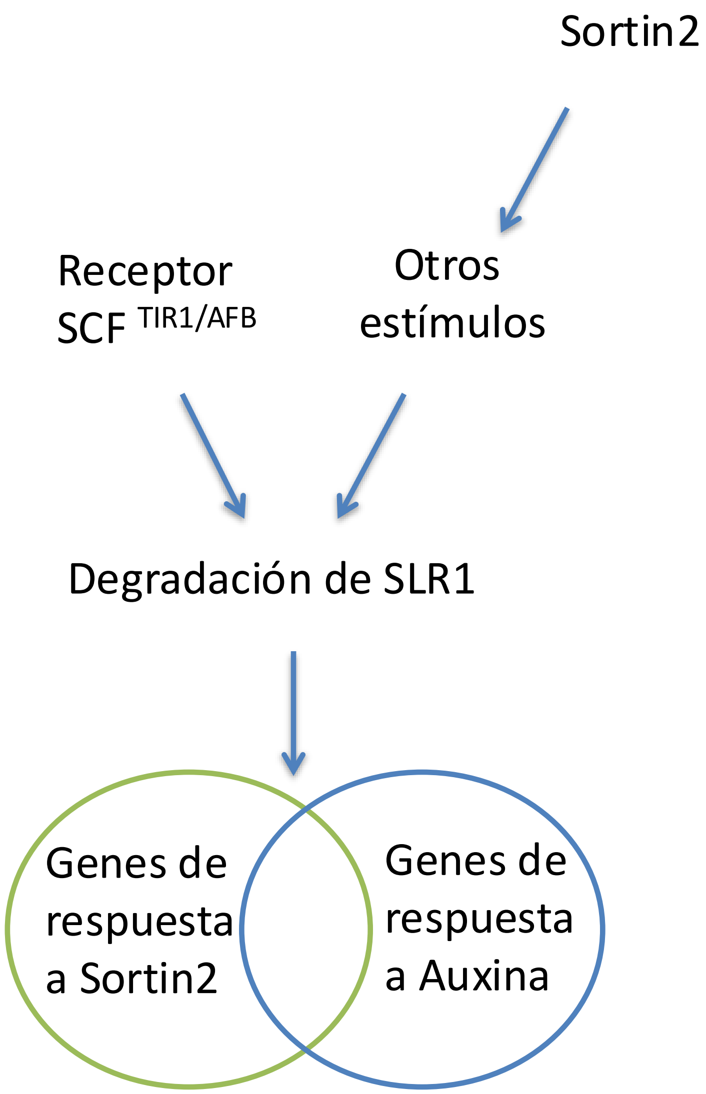
Pre- Iniciación

Iniciacion

Desarrollo

Emergencia

pDR5:GUS



Sortin2

Receptor
SCF $^{TIR1/AFB}$

Otros
estímulos

Degradación de SLR1

Genes de
respuesta
a Sortin2

Genes de
respuesta
a Auxina

1. Estudio de la participación de la vía de señalización canónica de la hormona auxina en la inducción de raíces laterales por Sortin2

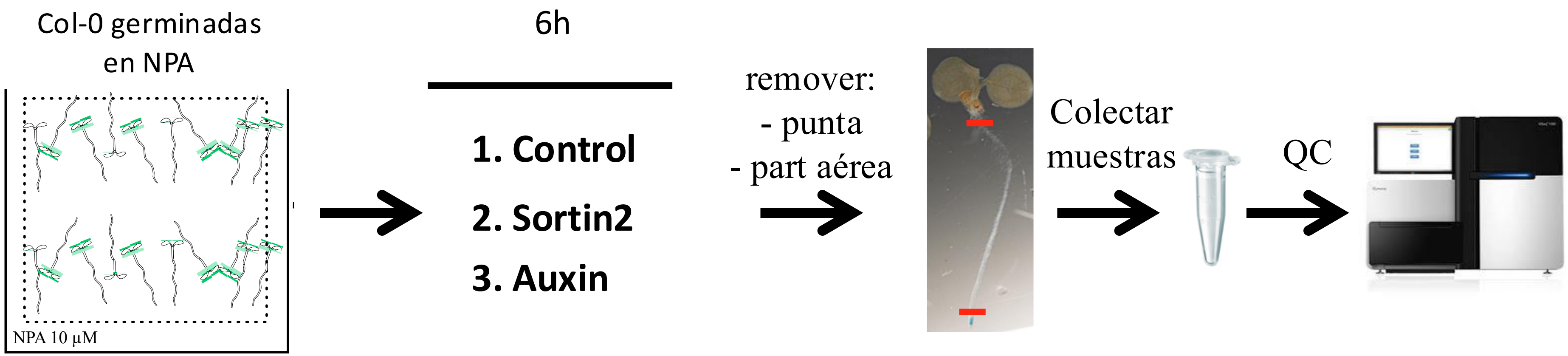
2. **Identificación de el(los) grupo(s) de genes que son regulados en tratamientos con Sortin2 mediante análisis global de expresión génica.**

3. Identificación de genes importantes para la vía de acción de Sortin2 mediante análisis fenotípico de mutantes insercionales frente a tratamientos con Sortin2.

4. Generación de un modelo que integre la información acerca de los componentes moleculares importantes para la formación de raíces laterales inducidas por Sortin2.

Comparación del perfil transcriptómico de raíces tratadas con Sortin2 y auxin

1. Metodología de muestreo



Col-0 germinadas en NPA

6h

1. Control

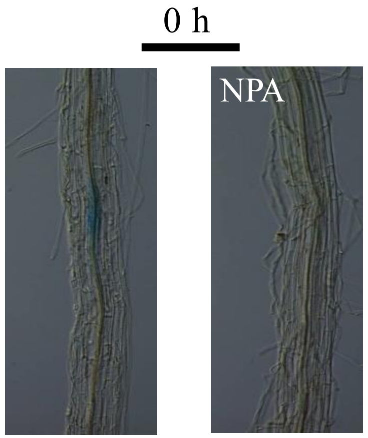
2. Sortin2

3. Auxin

remover:
- punta
- part aérea

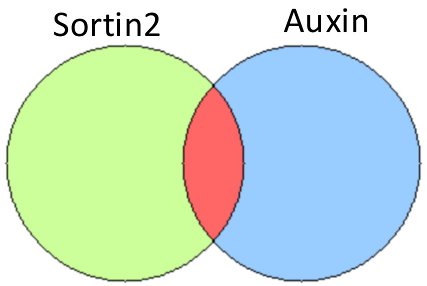
Colectar muestras

QC

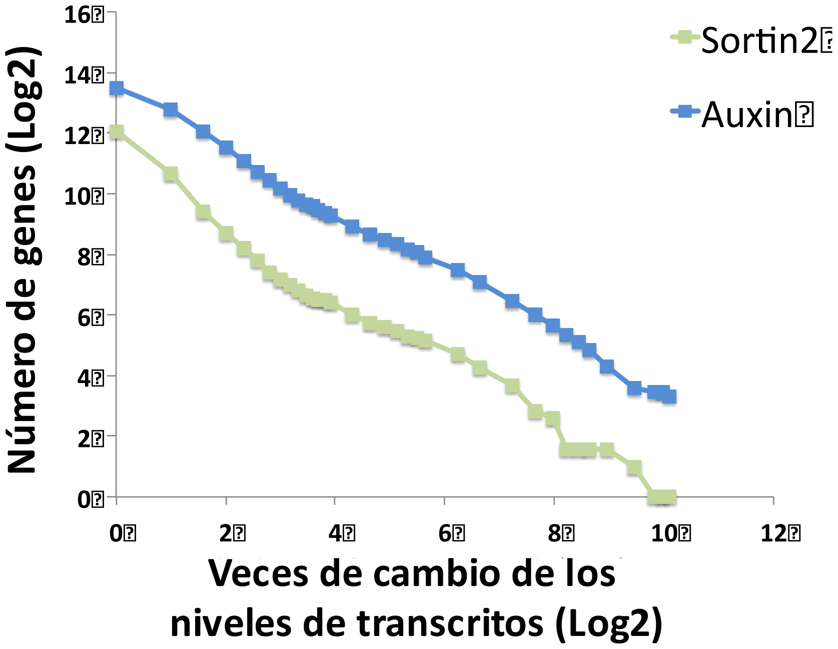


Sistema de inducción de raíces laterales
Himanen *et al*. PNAS. 2004
Vanneste *et al*. Plant Cell. 2005

2. Comparar los perfiles trasncriptómicos



Comparación del perfil transcriptómico de raíces tratadas con Sortin2 y auxin



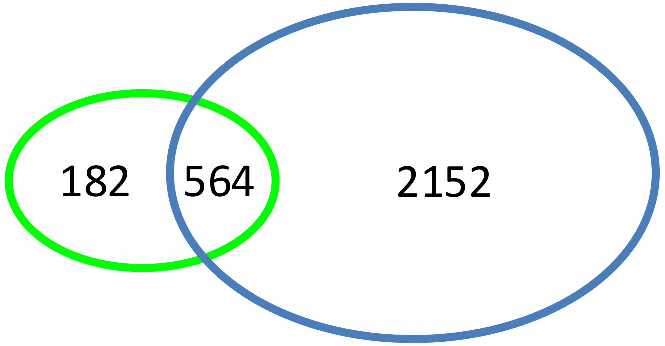
FC = Fold Change

	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	

Resultados sugieren que Sortin2 tiene un efecto más acotadado que auxina

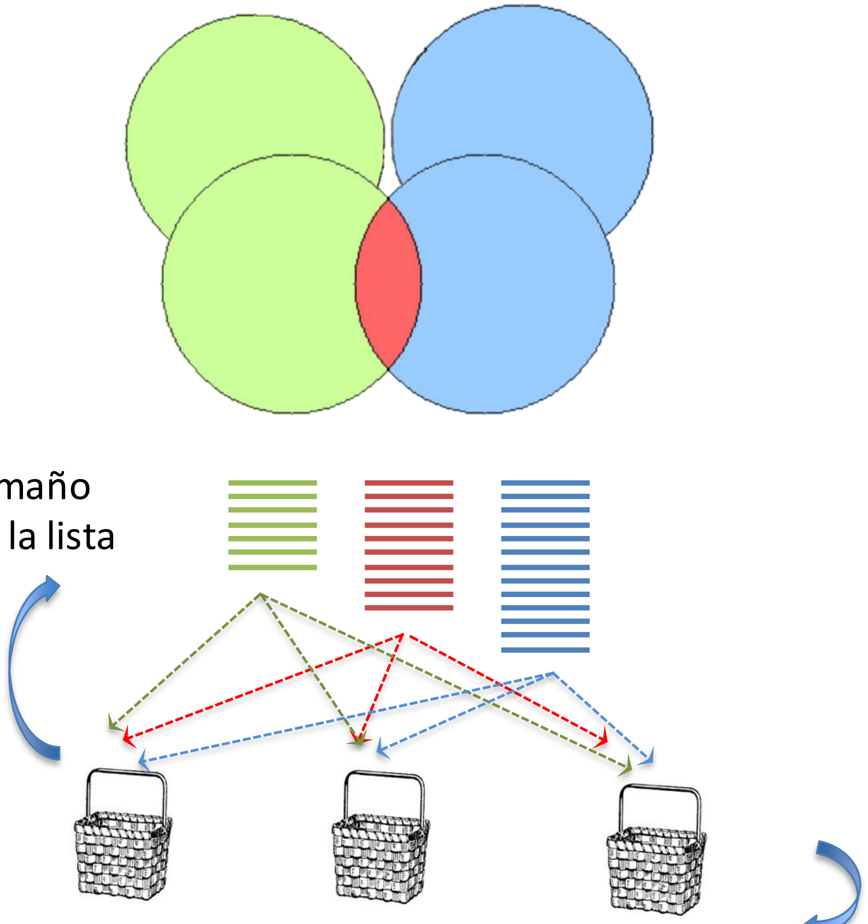
UP REGULATED
FC ≥ 2

DOWN REGULATED
FC ≤ 2



Enriquecimiento de procesos biológicos

ENRIQUECIMIENTO DE CADA PROCESO BIOLÓGICO EN CADA LISTA DE GENES



Tamaño de la lista

Response to stress (GO:0006950)

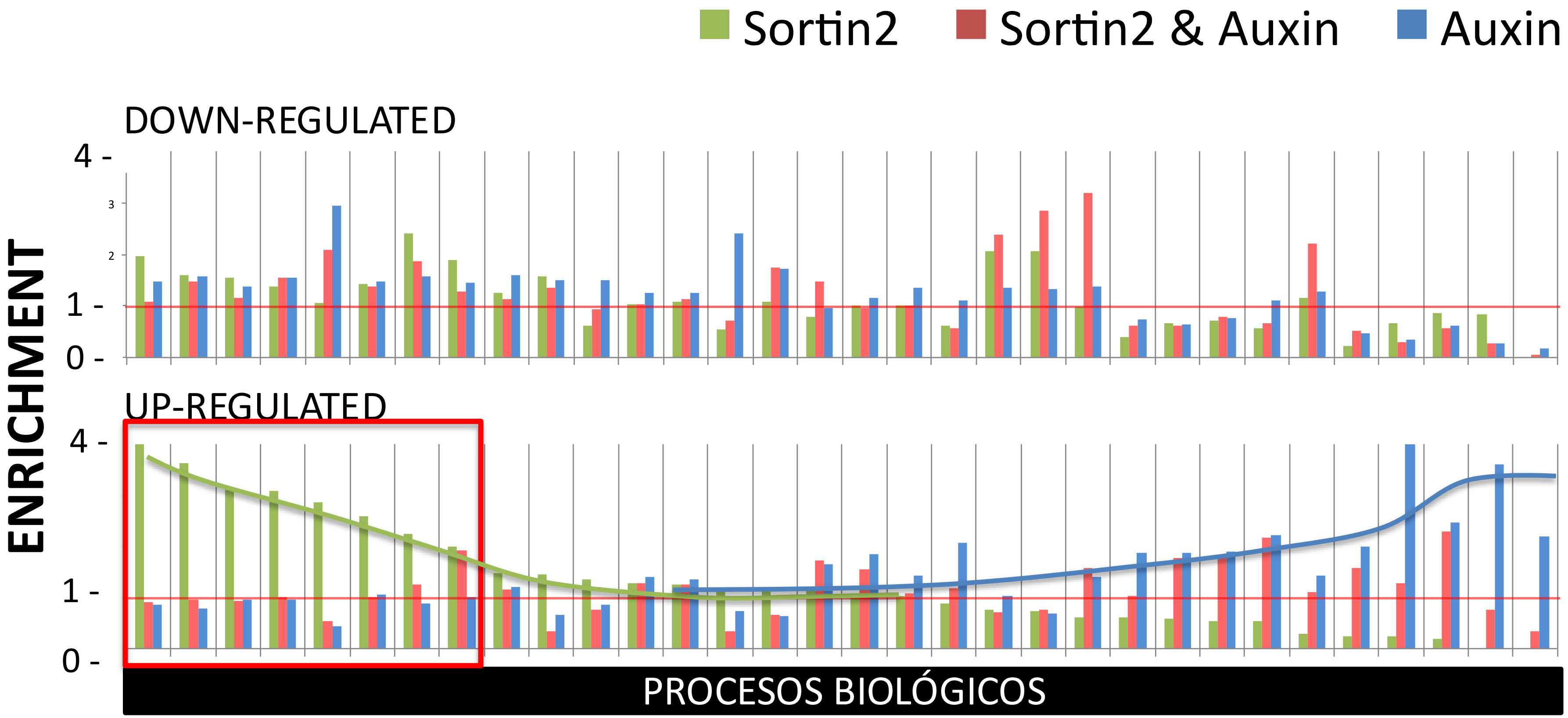
Metabolic process (GO:0008152)

Cell cycle (GO:0007049)

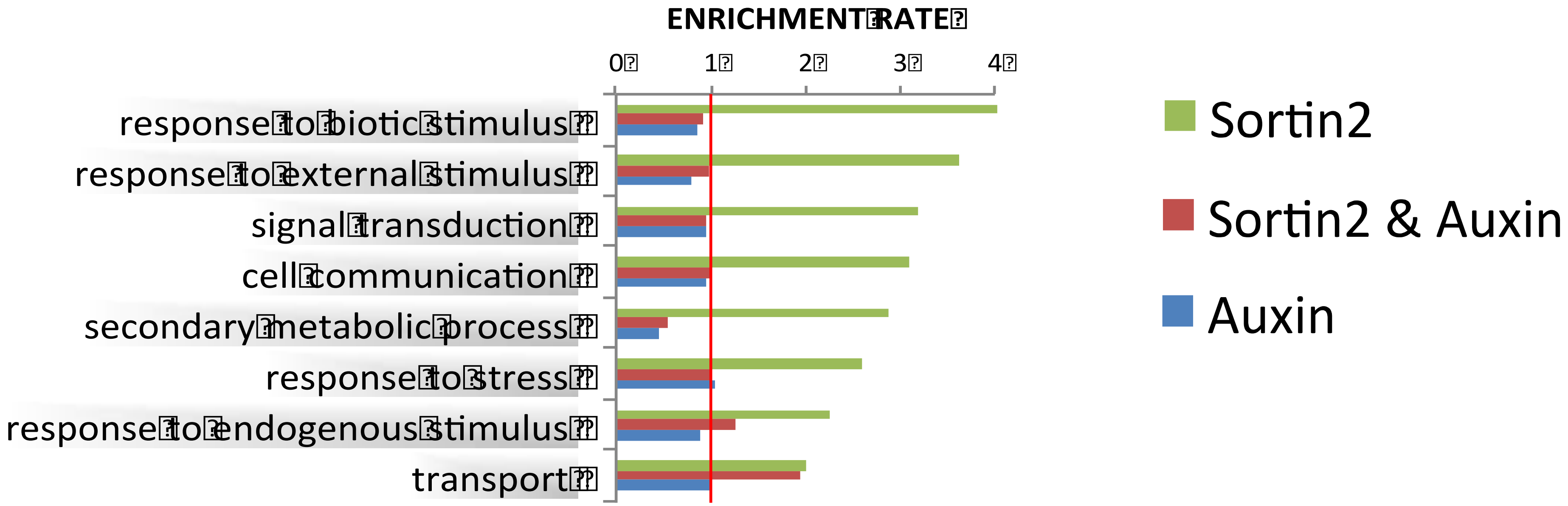
Tamaño de los procesos biológicos

$$\text{ENRICHMENT} = \frac{\dfrac{\text{de la lista en la categoria}}{\text{Total de la lista}}}{\dfrac{\text{del genoma en la categoría}}{\text{Total de la categoría}}}$$

Los procesos biológicos enriquecidos son distintos en los genes regulados por Sortin2 que por Auxina

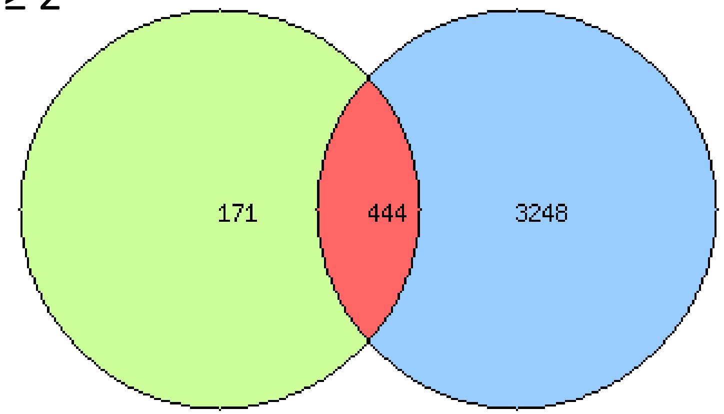


Los procesos biológicos enriquecidos sugieren un contexto biológico particular para Sortin2

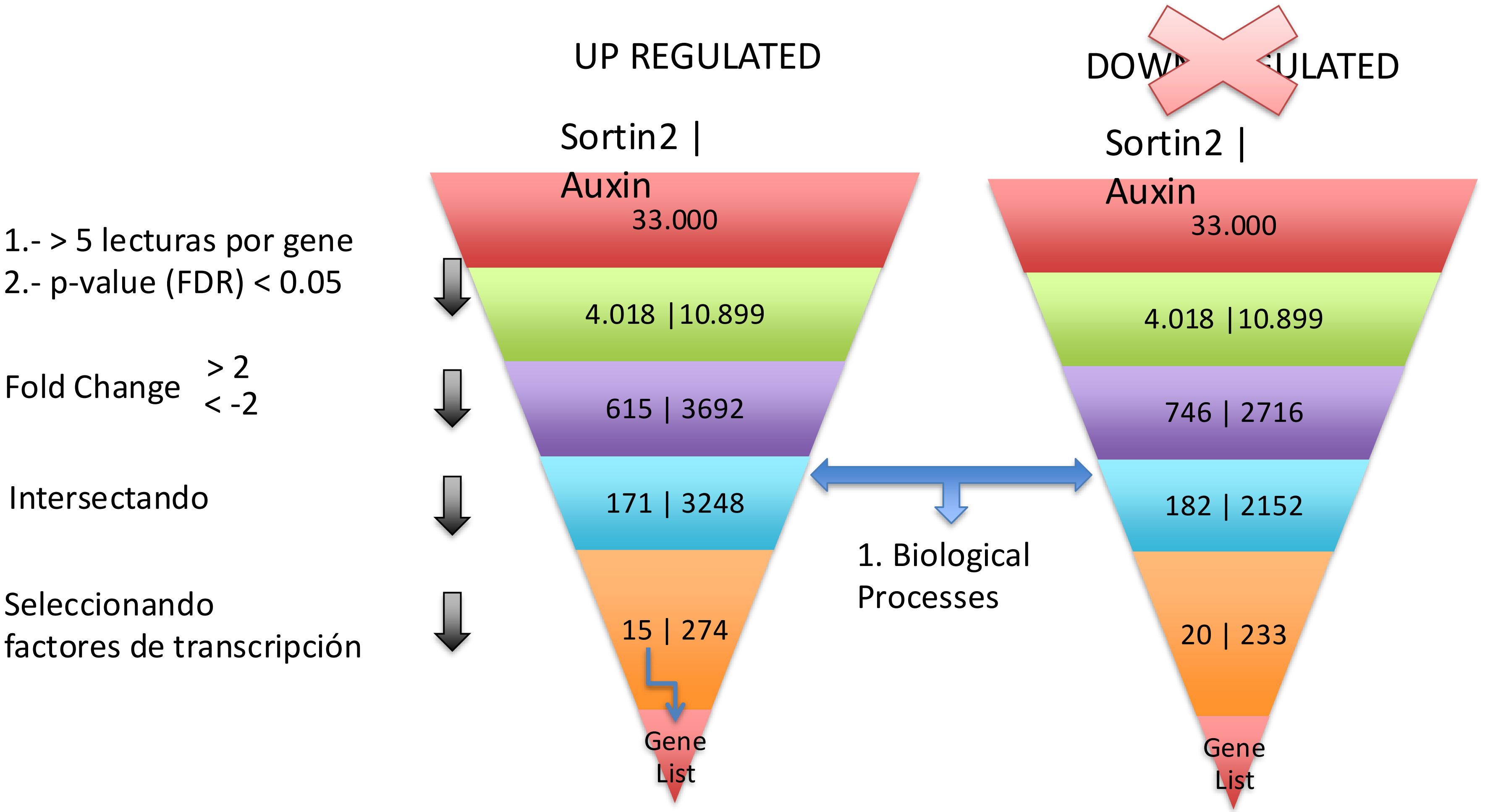


UP REGULATED

FC ≥ 2



Identificando componentes moleculares que participan en el mecanismo de formación de raíces laterales por efecto de Sortin2



1.- > 5 lecturas por gene
2.- p-value (FDR) < 0.05

Fold Change > 2
< -2

Intersectando

Seleccionando
factores de transcripción

UP REGULATED

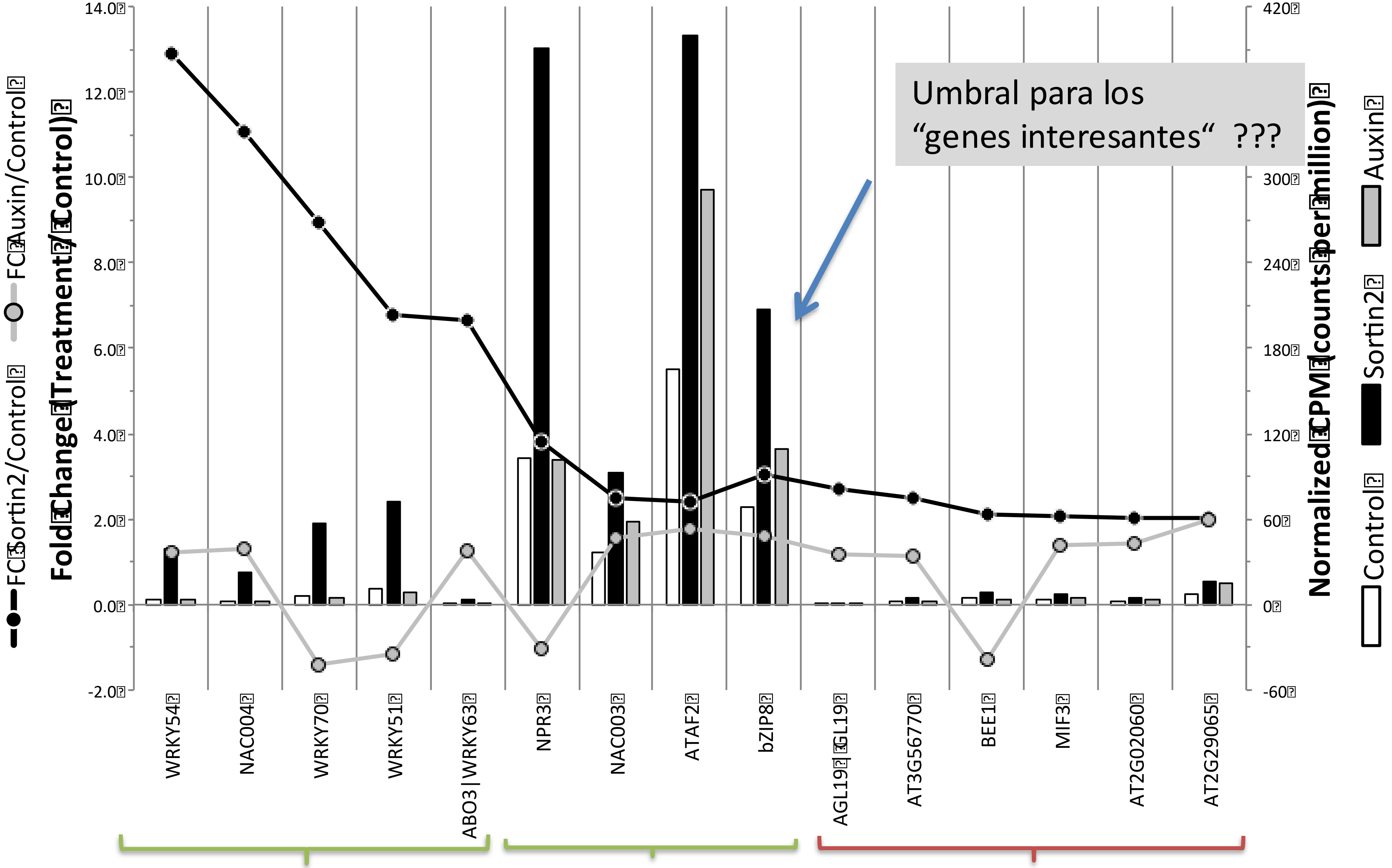
Sortin2 | Auxin
33.000
4.018 |10.899
615 | 3692
171 | 3248
15 | 274
Gene List

DOW ULATED

Sortin2 | Auxin
33.000
4.018 |10.899
746 | 2716
182 | 2152
20 | 233
Gene List

1. Biological Processes

Seleccionando entre los factores de transcripción



Umbral para los
“genes interesantes“ ???

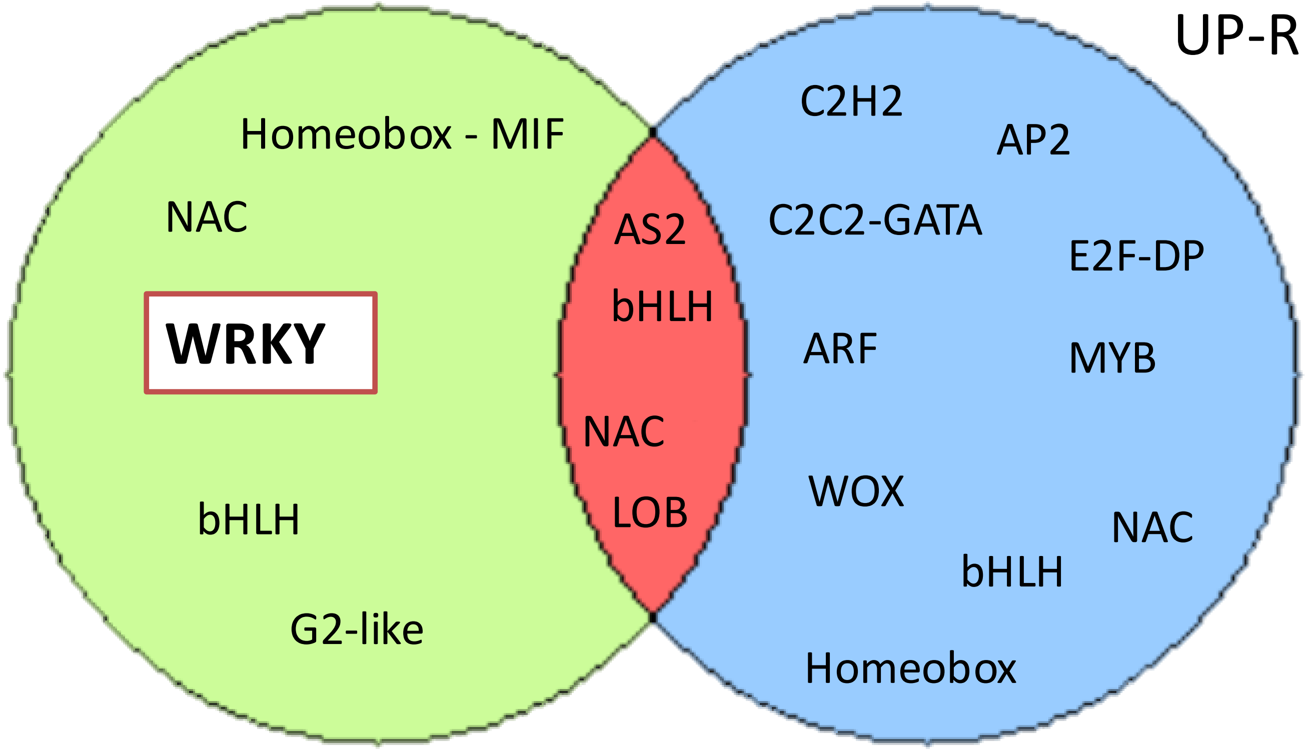
Low| High
Control | Sortin2

High| Higher
Control | Sortin2

Low | Low
Control | Sortin2

Enriquecimiento de familias de factores transcripción

	🟩	🟥	🟦
Size of Gene List	171	444	3248
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

WRKY54

NAC004

WRKY70

WRKY51

✖ ABO3|WRKY63

NPR3

✖ NAC003

ATAF2

✖ bZIP8

✖ AGL19|GL19

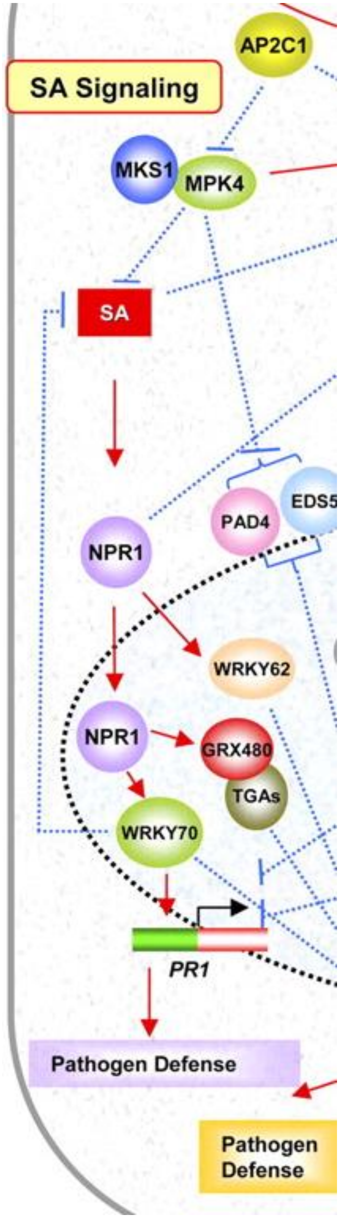
✖ AT3G56770

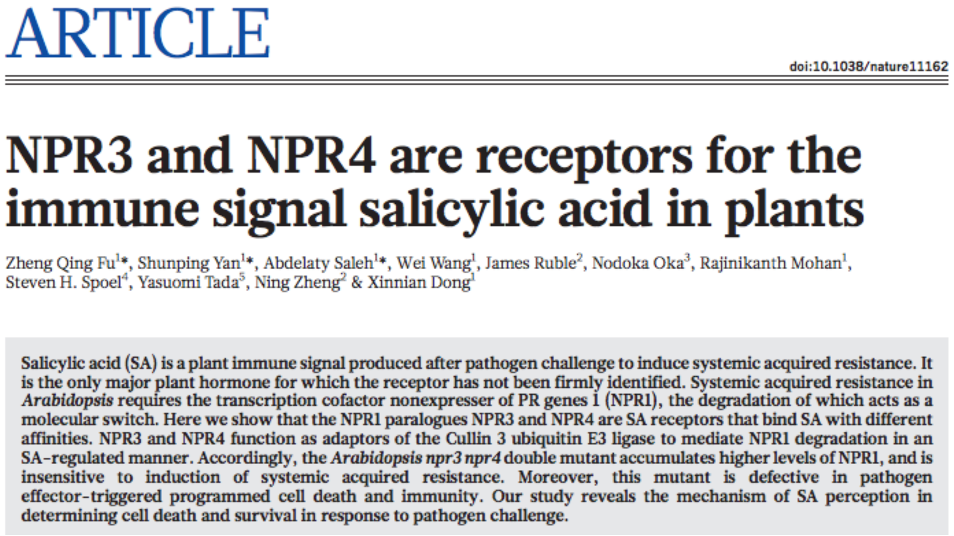
✖ BEE1

✖ MIF3

✖ AT2G02060

✖ AT2G29065





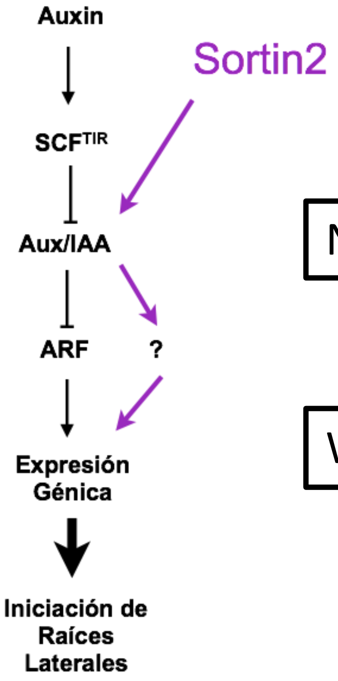
ARTICLE

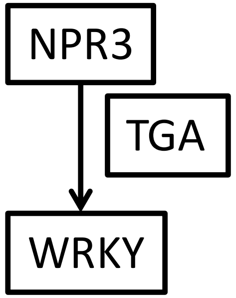
doi:10.1038/nature11162

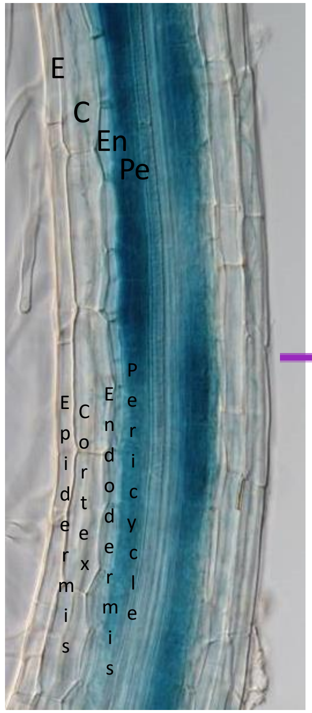
NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants

Zheng Qing Fu[1]*, Shunping Yan[1]*, Abdelaty Saleh[1]*, Wei Wang[1], James Ruble[2], Nodoka Oka[3], Rajinikanth Mohan[1], Steven H. Spoel[4], Yasuomi Tada[5], Ning Zheng[2] & Xinnian Dong[1]

Salicylic acid (SA) is a plant immune signal produced after pathogen challenge to induce systemic acquired resistance. It is the only major plant hormone for which the receptor has not been firmly identified. Systemic acquired resistance in *Arabidopsis* requires the transcription cofactor nonexpresser of PR genes 1 (NPR1), the degradation of which acts as a molecular switch. Here we show that the NPR1 paralogues NPR3 and NPR4 are SA receptors that bind SA with different affinities. NPR3 and NPR4 function as adaptors of the Cullin 3 ubiquitin E3 ligase to mediate NPR1 degradation in an SA-regulated manner. Accordingly, the *Arabidopsis npr3 npr4* double mutant accumulates higher levels of NPR1, and is insensitive to induction of systemic acquired resistance. Moreover, this mutant is defective in pathogen effector-triggered programmed cell death and immunity. Our study reveals the mechanism of SA perception in determining cell death and survival in response to pathogen challenge.



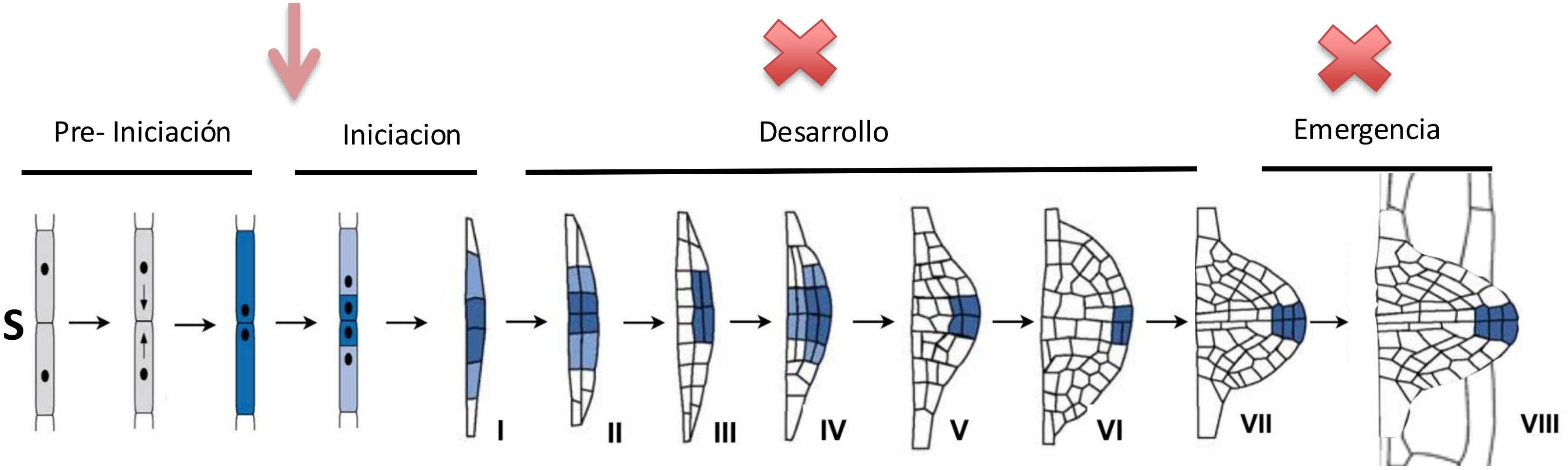




Formación de raíces laterales de novo

Redefinicion de células del periciclo hacia células fundadoras

pDR5:GUS



CONCLUSION

1. Sortin2 induce formación de raíces laterales estimulando etapas tempranas

1. Sortin2 requiere pero no induce la degradación de proteínas Aux/IAA (slr1)

2. Existen grupos de genes específicos que responden a Sortin2 y que se excluyen del marco regulatorio de la hormona auxina

3. Respuesta a estimulos bióticos, transducción de señal y comunicación celular son procesos exclusivamente enriquecidos entre los genes regulados por Sortin2

4. El efecto de Sortin2 podría estar enmarcado en un vía identificable que involucra NPR3, TGA y factores WRKY

5. Mutantes disponibles en los genes identificados (cadidatos) serán evaluados


Norambuenas' Lab


Root Development Group
Tom Beeckman

T H A N K S !

CBMV

UNIVERSITEIT GENT

VIB

Funding

CONICYT
Ministerio de Educación
Gobierno de Chile

1120289

FONDECYT
Fondo Nacional de Desarrollo Científico y Tecnológico

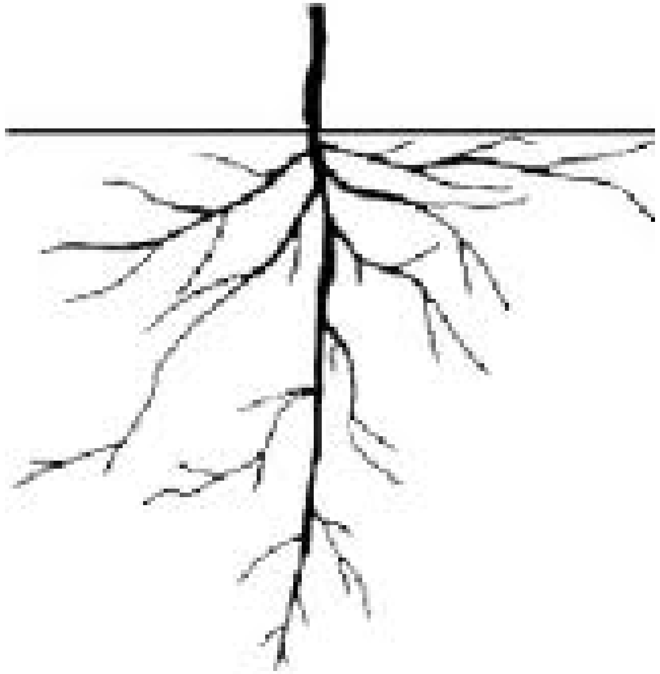
CONICYT
Ministerio de Educación
Gobierno de Chile

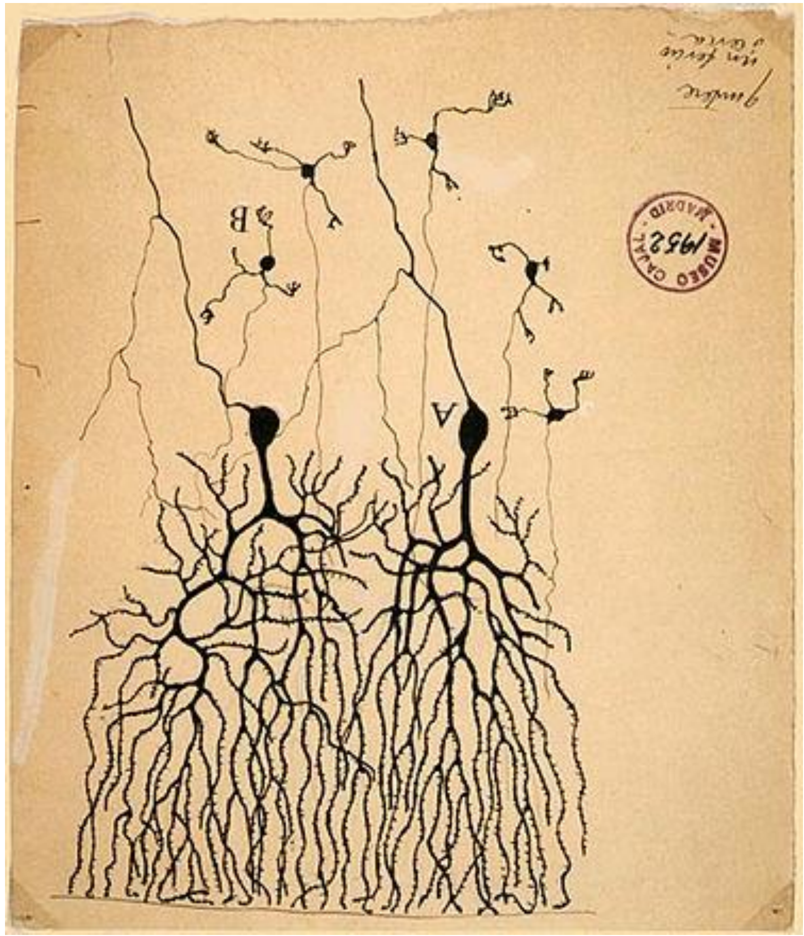
GO 21110627

BECAS CONICYT
Programa de Formación de Capital Humano Avanzado

Vicerrectoría de Asuntos Académicos
UNIVERSIDAD DE CHILE
FUNDADA EN 1842

La ramificación





T-DNA availability of interesting gene list

	Loci ID	Name	Description	Allele 1	Allele 2
	AT2G40750	WRKY54	WRKY DNA-binding protein 54	SALK_111964C (05/06)	SALK_017254C (05/06)
	AT3G56400	WRKY70	WRKY DNA-binding protein 70	SALK_025198C (09/07)	GK-324D11.07 02/09
	AT5G64810	WRKY51	WRKY DNA-binding protein 51	SALK_022198C (11/08)	SALK_076577(Targeted)
	AT1G66600	ABO3	ABA overly sensitive mutant 3	SALK_068280C (08/07)	SALK_075986C (06/08)
	AT1G02230	NAC004	NAC domain containing protein 4	SALK_054446C (06/06)	SALK_035023c (01/11)
	AT5G45110	NPR3	NPR1-like protein 3	WiscDsLox5G05 (04/10)	GK-684H02(Confirmed)
	AT1G68880	bZIP	basic leucine-zipper 8		
x	AT4G22950	AGL19	AGAMOUS-like 19	SALK_000234C (01/06)	SALK_016657C (01/06)
	AT1G02220	NAC003	NAC domain containing protein 3		
x	AT3G56770		basic helix-loop-helix (bHLH) DNA-binding superfamily protein	WiscDsLox461-464N24 (06/10)	
	AT5G08790	ATAF2	NAC (No Apical Meristem) domain transcriptional regulator superfamily protein	SALK_136355C (05/07)	SALK_015750C (05/07)
x	AT1G18400	BEE1	BR enhanced expression 1	WiscDsLoxHs102_04G (07/10)	
x	AT1G18835	MIF3	mini zinc finger	SALK_009428C (03/08)	SALK_014097C (10/09)
x	AT2G02060		Homeodomain-like superfamily protein	SALK_099488C (10/08)	SAIL_2_E03 (03/09)
x	AT2G29065		GRAS family transcription factor		

with an x the ones with relative low increase upon Sortin2

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

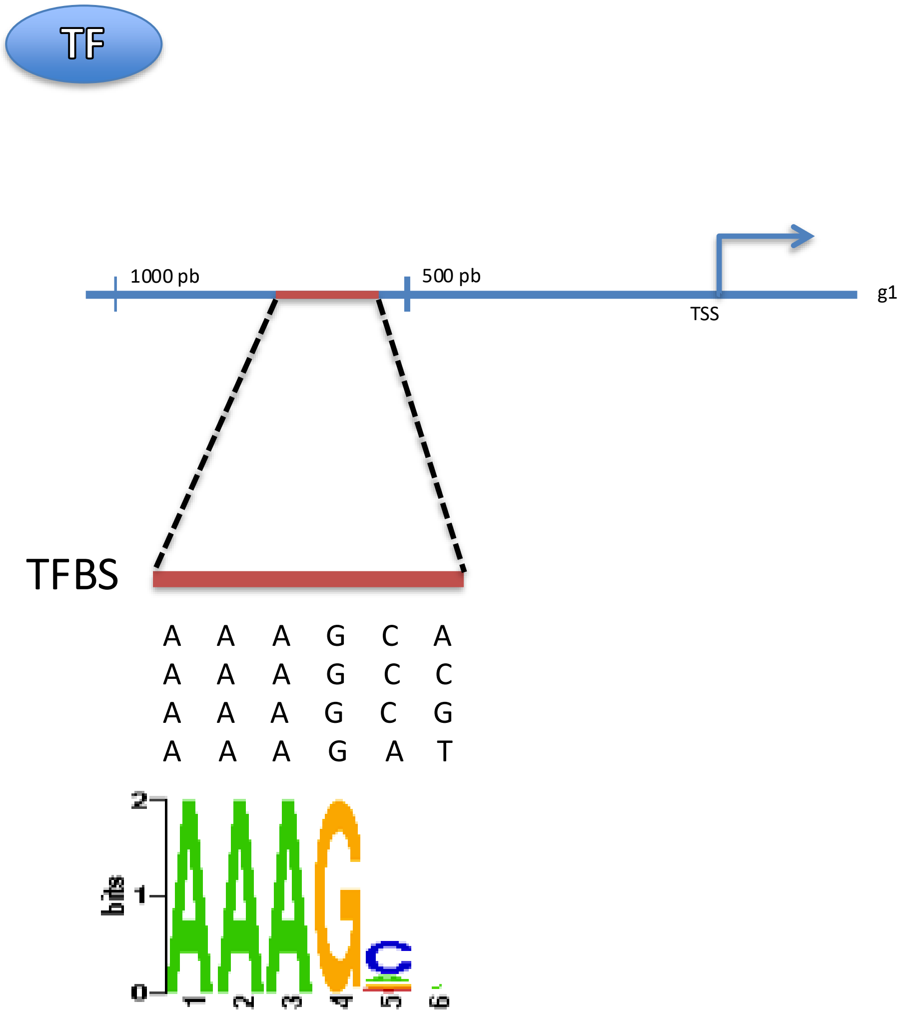
JA response and Synthesis

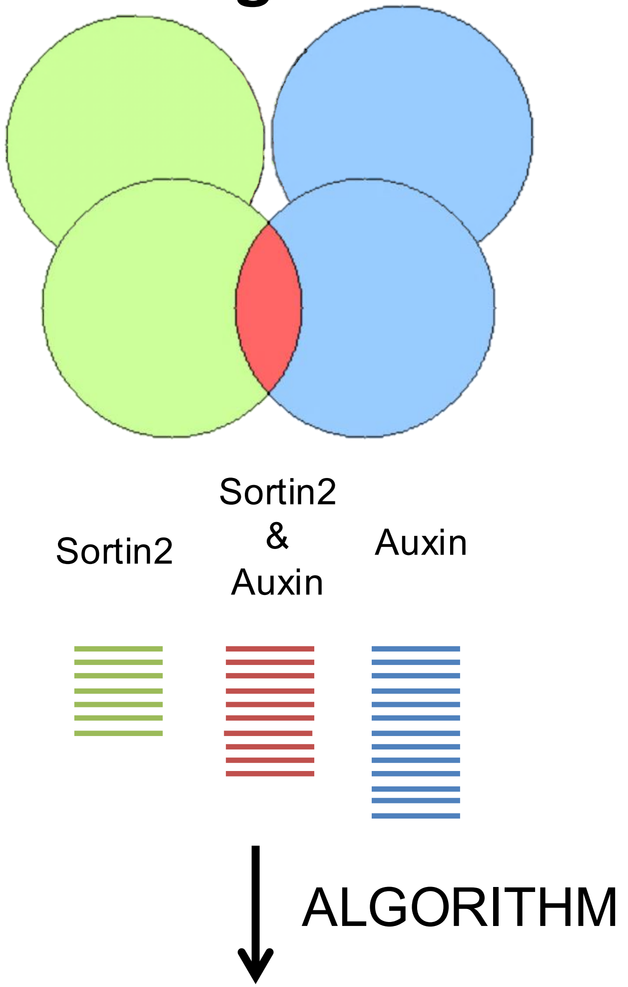
According to Méndez-Bravo et al. Plos One 2011

	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

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

Transcription factors binding sites (TFBS)



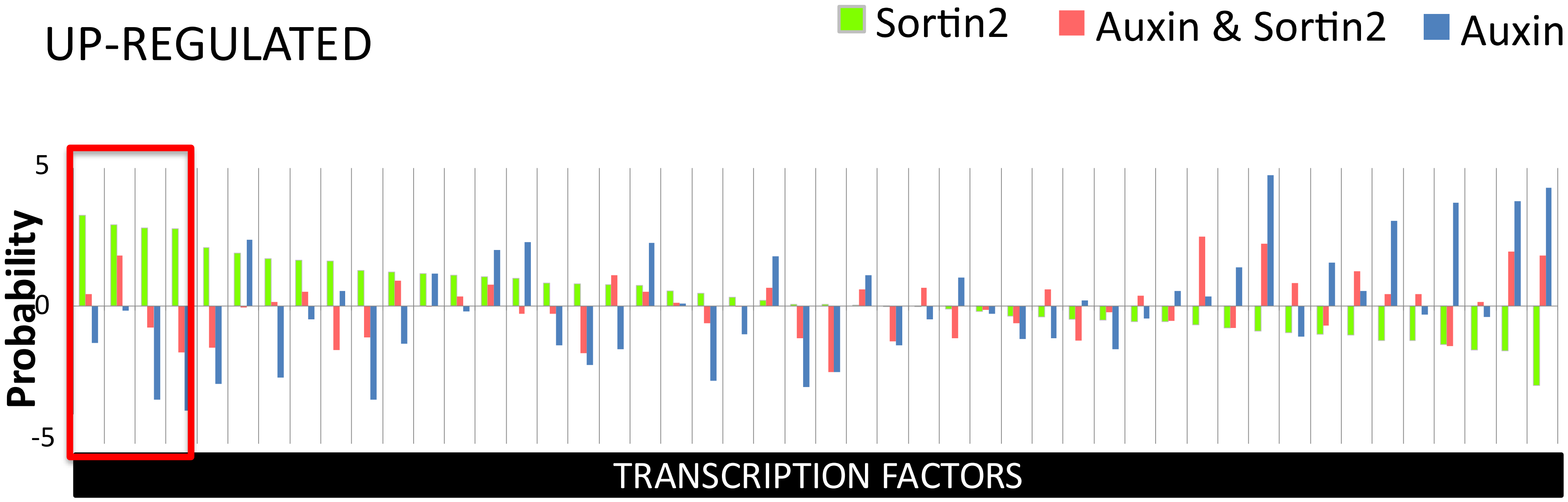


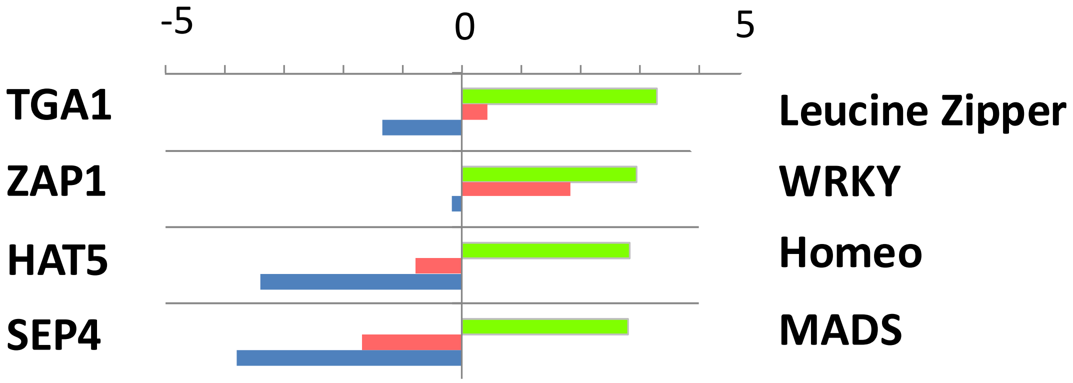
Evaluating the probability of regulation by a determined TF

TFBS; Transcription Factor Binding Site

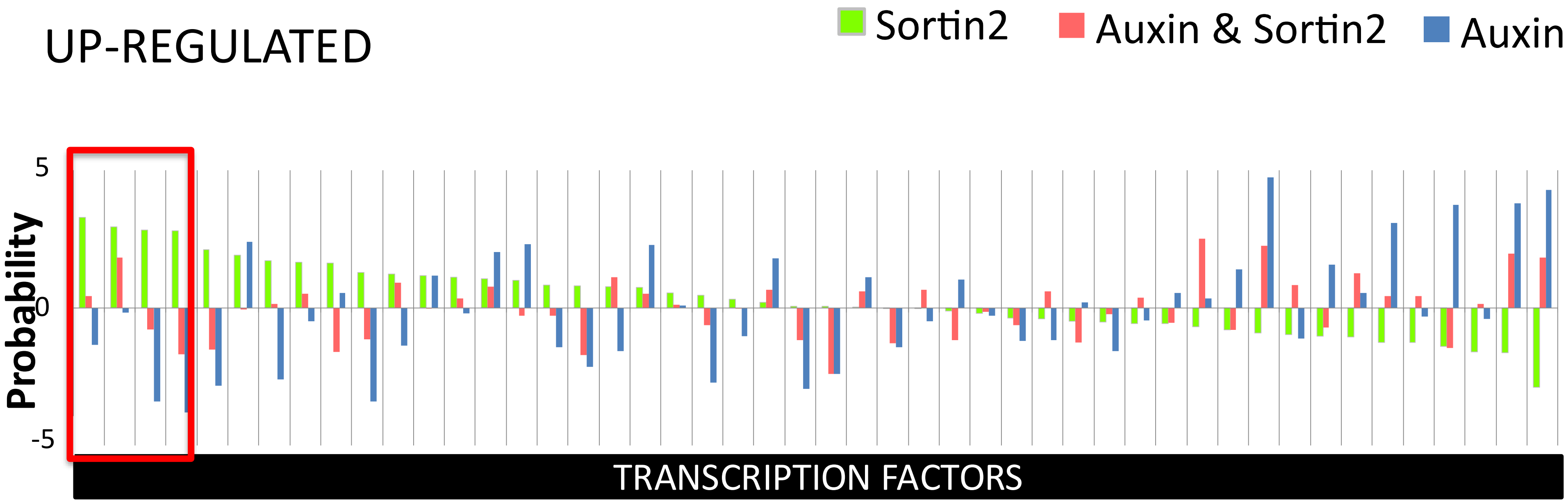
Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes

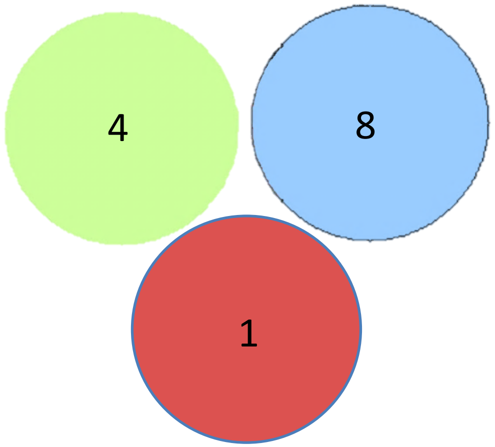
UP-REGULATED





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



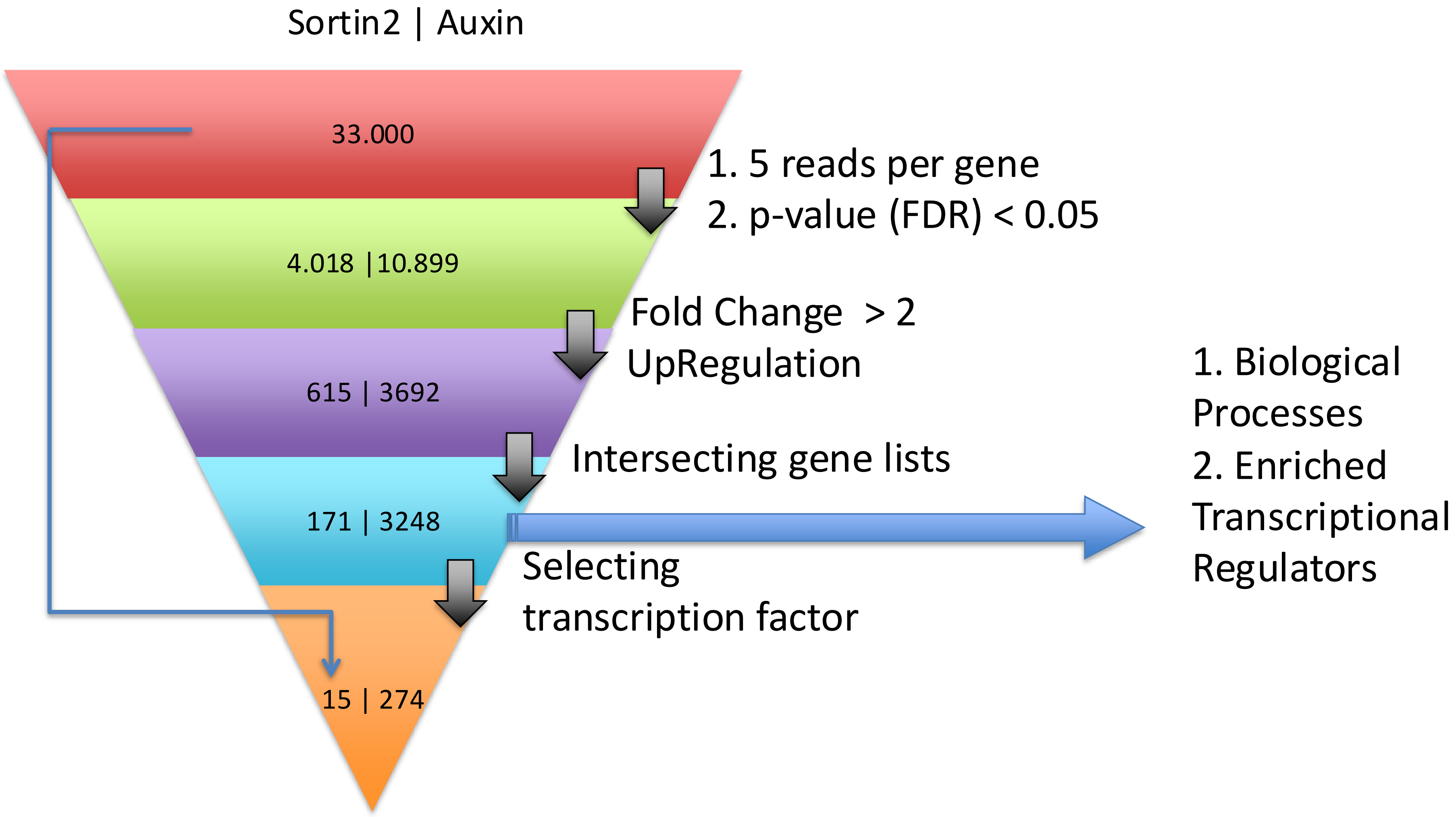


🟩 TGA1 , ZAP1 , HAT5 , SEP4

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

🟥 LFY

Filtering process



Sortin2 | Auxin

33.000

4.018 | 10.899

615 | 3692

171 | 3248

15 | 274

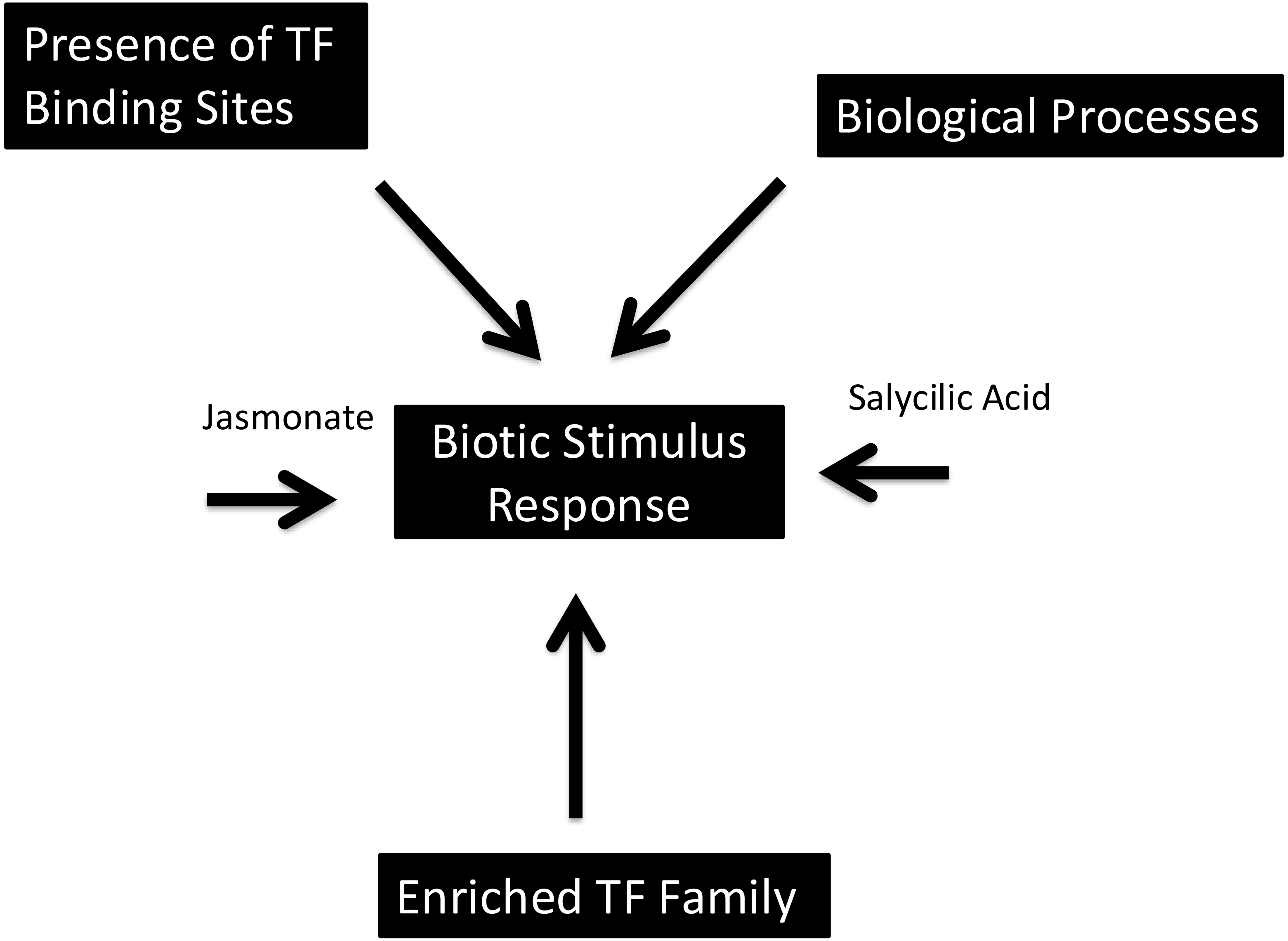
1. 5 reads per gene
2. p-value (FDR) < 0.05

Fold Change > 2
UpRegulation

Intersecting gene lists

Selecting
transcription factor

1. Biological Processes
2. Enriched Transcriptional Regulators



Presence of TF Binding Sites

Biological Processes

Jasmonate

Biotic Stimulus Response

Salycilic Acid

Enriched TF Family

PROJECTIONS

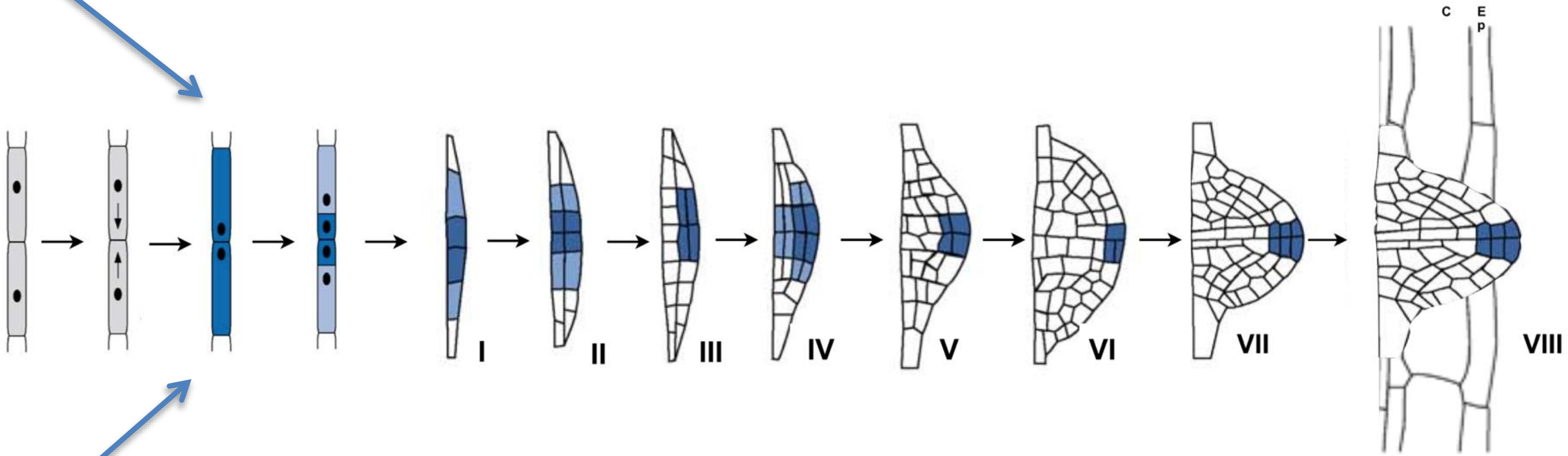
Genome-wide transcript profiling suggested a collection of genes that are not identified as part of the canonical pathway for LRI but that might be needed for for a SCF-TIR1/AFBs-independent LRI pathway.

SCF-TIR/AFBs - independent

Sortin2

SA response genes?
WRKY TF?

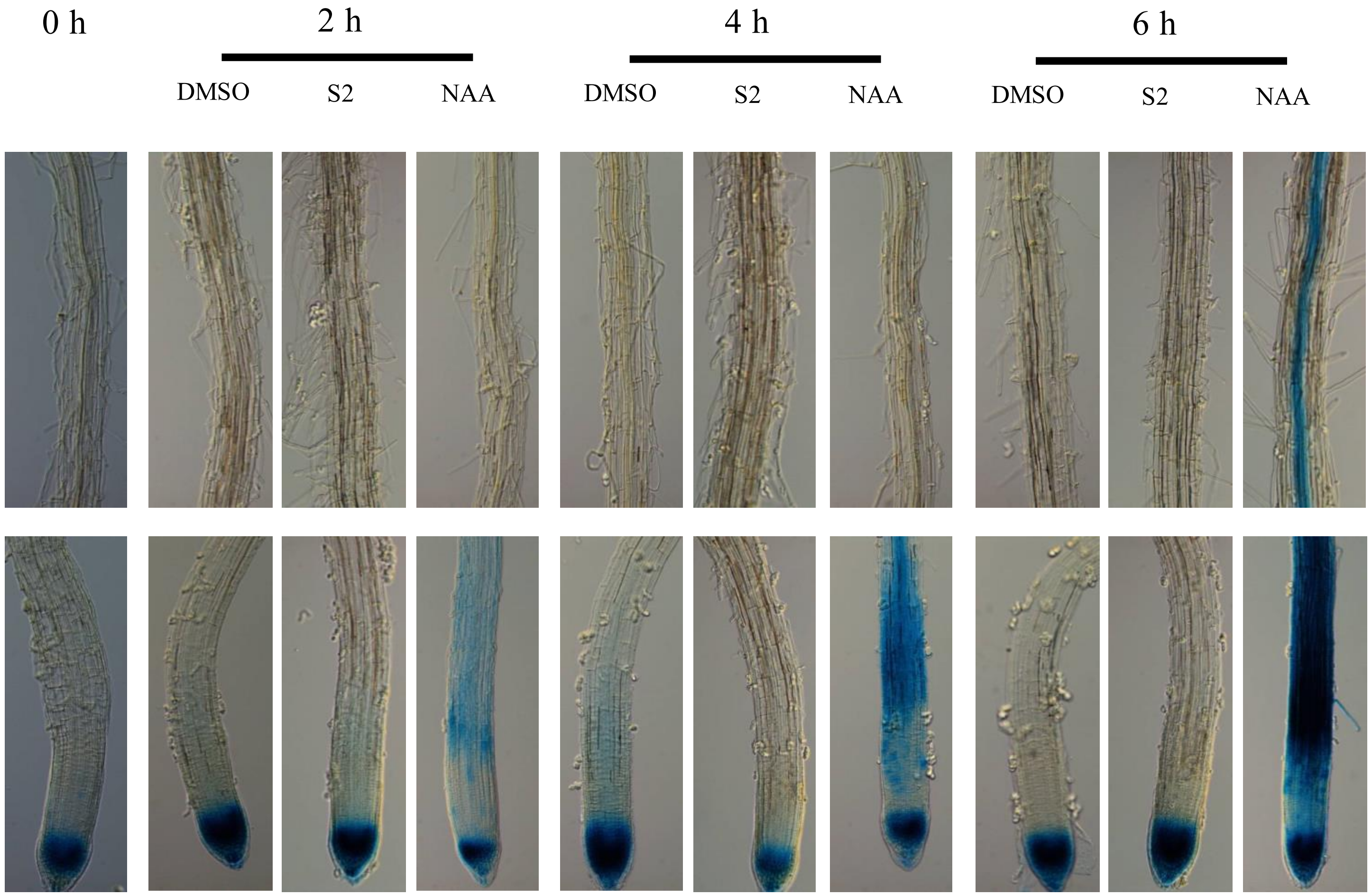
Stefanía
Morales



Auxin

SCF-TIR/AFB - dependent

Sortin2 induce LRs por una via pDR5-independiente



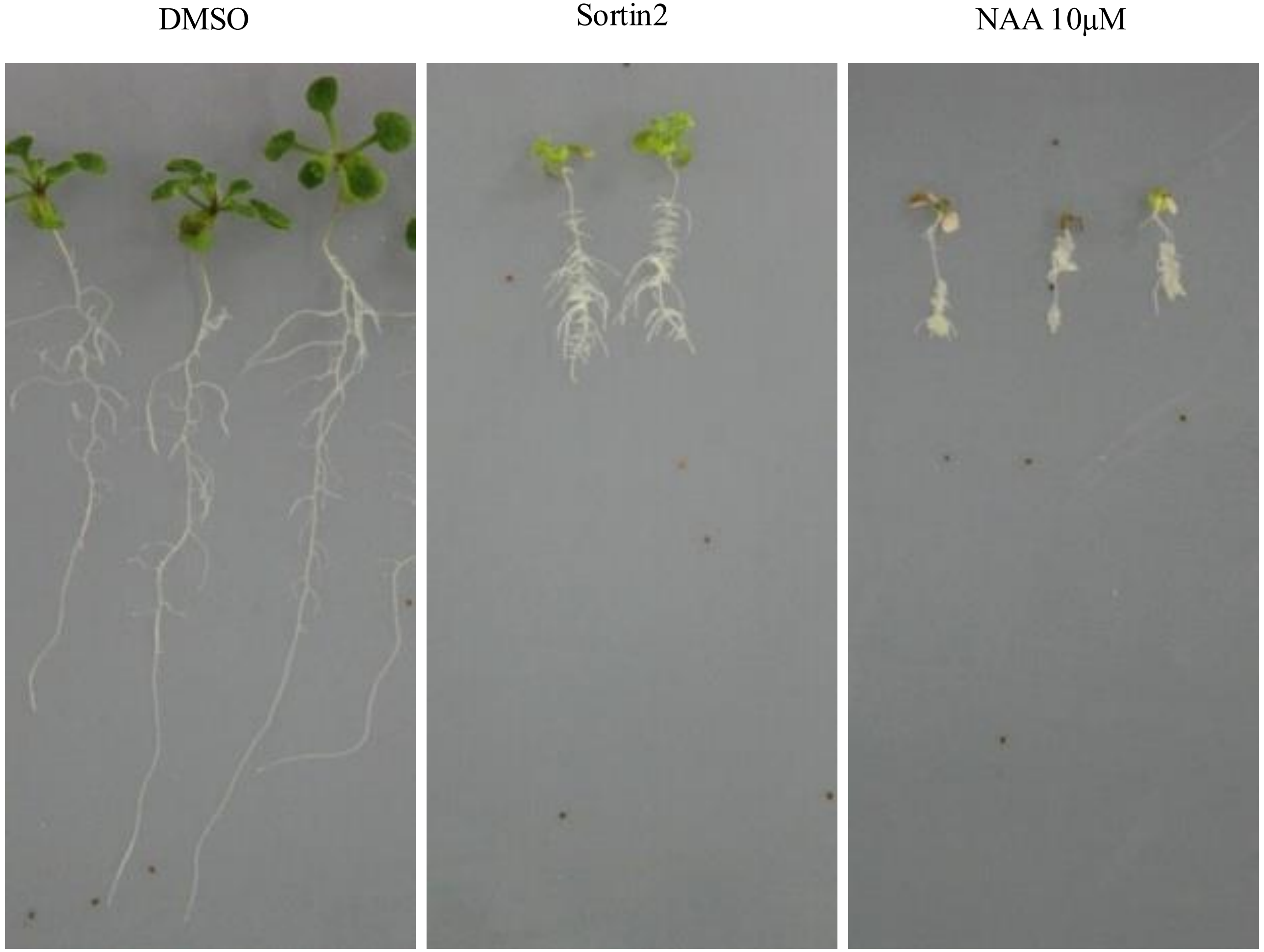
germinadas
en
NPA

transferencia →

Control
Sortin2
Auxin

pDR5::GUS

La malla no interfiere en la captura ni en la manifestación del efecto de Sortin2



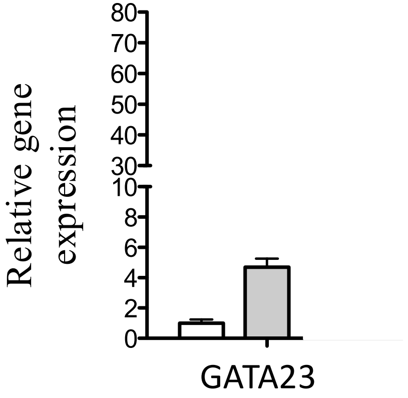
11 días de tratamiento

Sortin2 involves transcript levels regulatory activity

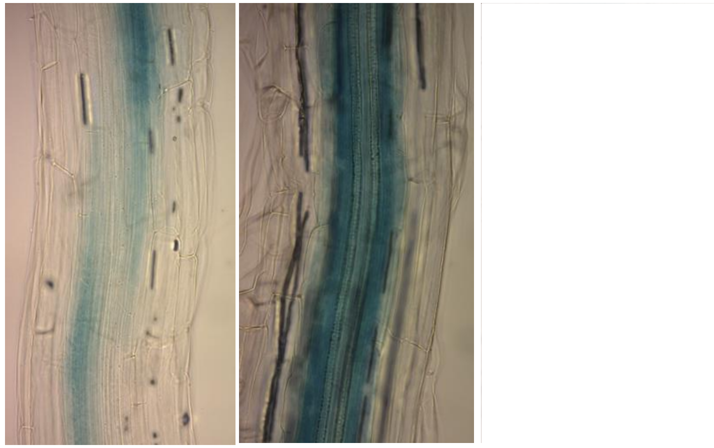
GATA23 expression
↓
founder cell specification

De Rybel *et al*. Current Biology. 2010

GATA23 expression
(qRT in wild type plants)



GATA23 expression
(pGATA23:GUS lines)

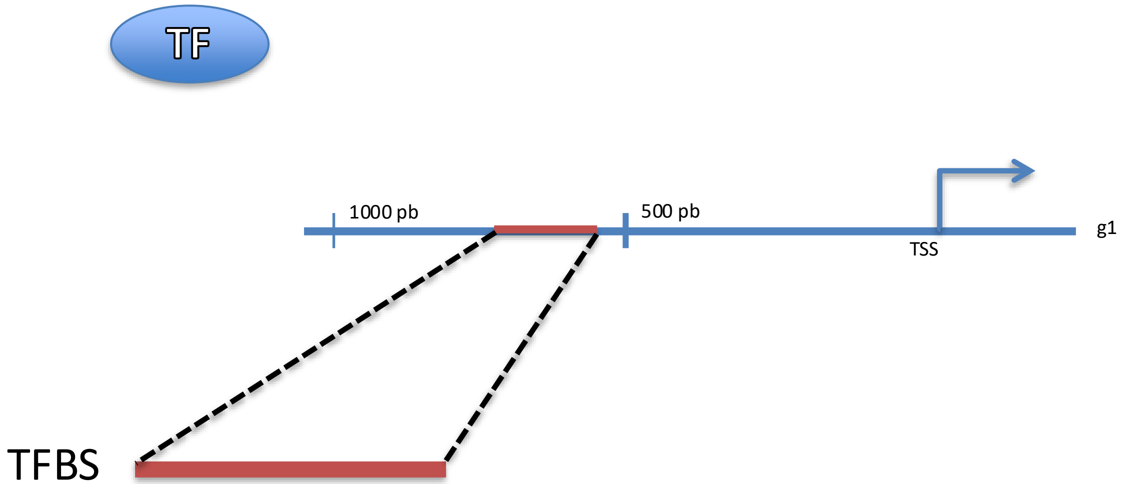


Control

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

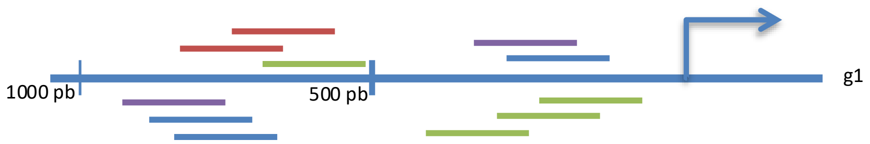
	AO	ACH1	AtbZIP	AT5G09800	AT4G32950	ATHNIR	CYP707A3	ATRFNR1	AT5G41670	G6PD2	AHB1	ATRFNR2	AT3G47980	MAPKKK14	AtLEA5	AT3G60750	AT1G32920
Auxin	3	27	2	13	2	-1	1	-1	-1	-1	2	-1	-2	2	1	-1	-1
Sortin2	7	5	3	2	2	1	1	1	1	1	1	1	1	1	1	1	1

	PGM	AT2G48080	G6PD3	AT4G25835	LBD38	ASL39	AT1G25550	AT5G62720	AT4G18340	UPM1	LBD39	AT1G25550	ATNAS1	AT4G18340	AT5G19970	GNR1	AT1G68670
Auxin	1	2	-1	-2	-2	-2	-1	-2	-1	-2	1	-3	-1	-1	-4	1	-2
Sortin2	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

	AtbZIP3	CIPK3	ATGSTF14	AT1G80380	AT3G62930	AT1G16170	AT3G07350	BT5	AT3G25790	HRS1	AT5G10210	AT5G01740	ATBT2	ATTPPB	SKOR	A
Auxin	-7	-2	2	1	-3	-2	-3	-7	-7	-7	-4	-23	2	-36	-29	-7
Sortin2	-1	-1	-1	-2	-2	-2	-2	-3	-3	-3	-3	-3	-4	-4	-4	-5

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

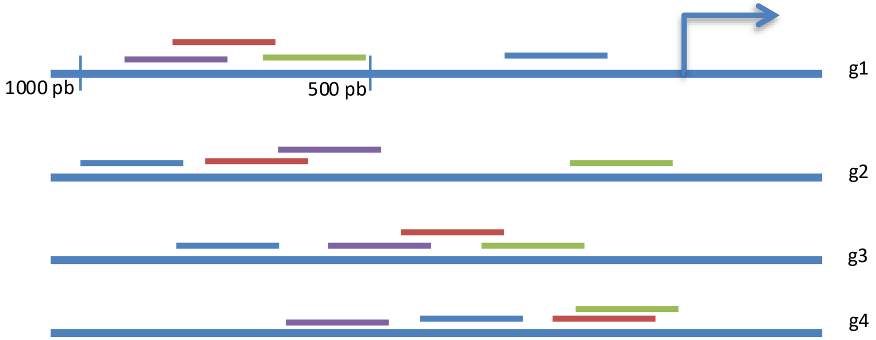
CLOVER



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)

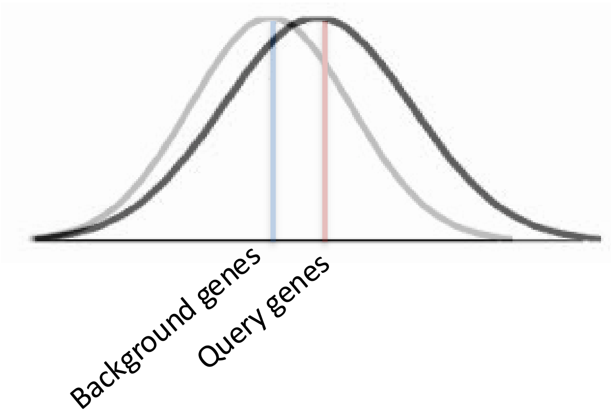
→ Background (Whole genome)

→ Random Subset

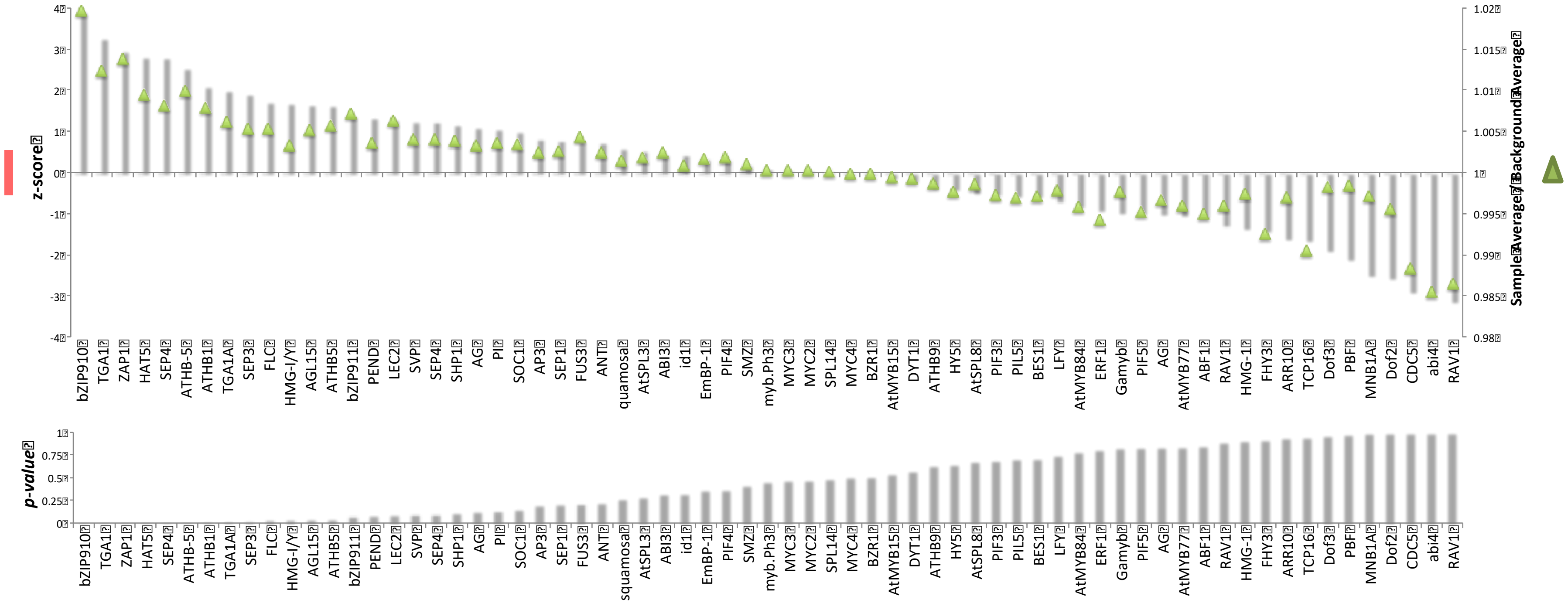
JASPAR database
69 FTBS FOR PLANTS

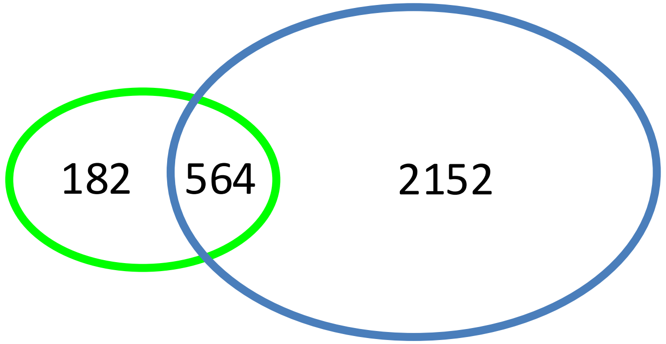
Finding overrepresented TFBS motifs in regulatory sequences from co-expressed genes

Matching values distribution along gen sets



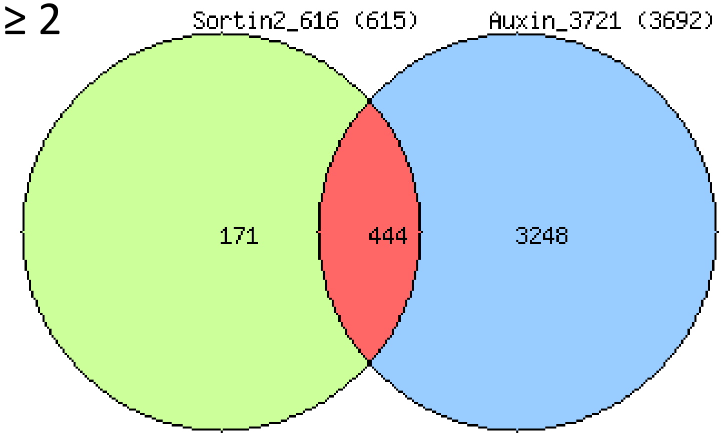
$$\text{FC Rate} = \frac{\text{Matching value Avg}_{\text{Query genes}}}{\text{Matching value Avg}_{\text{Background genes}}}$$





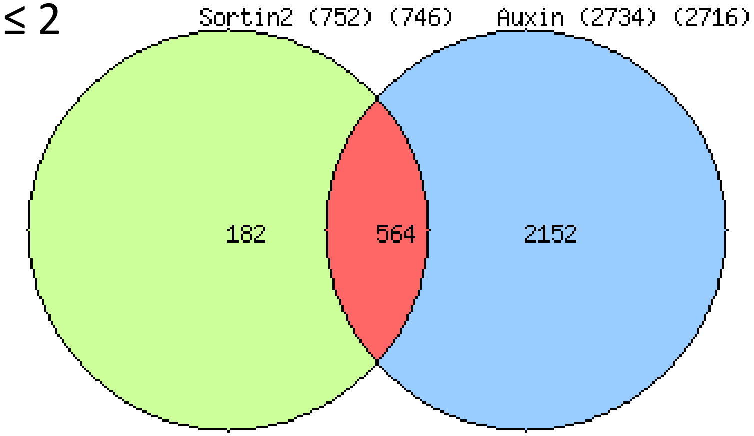
UP REGULATED

FC ≥ 2



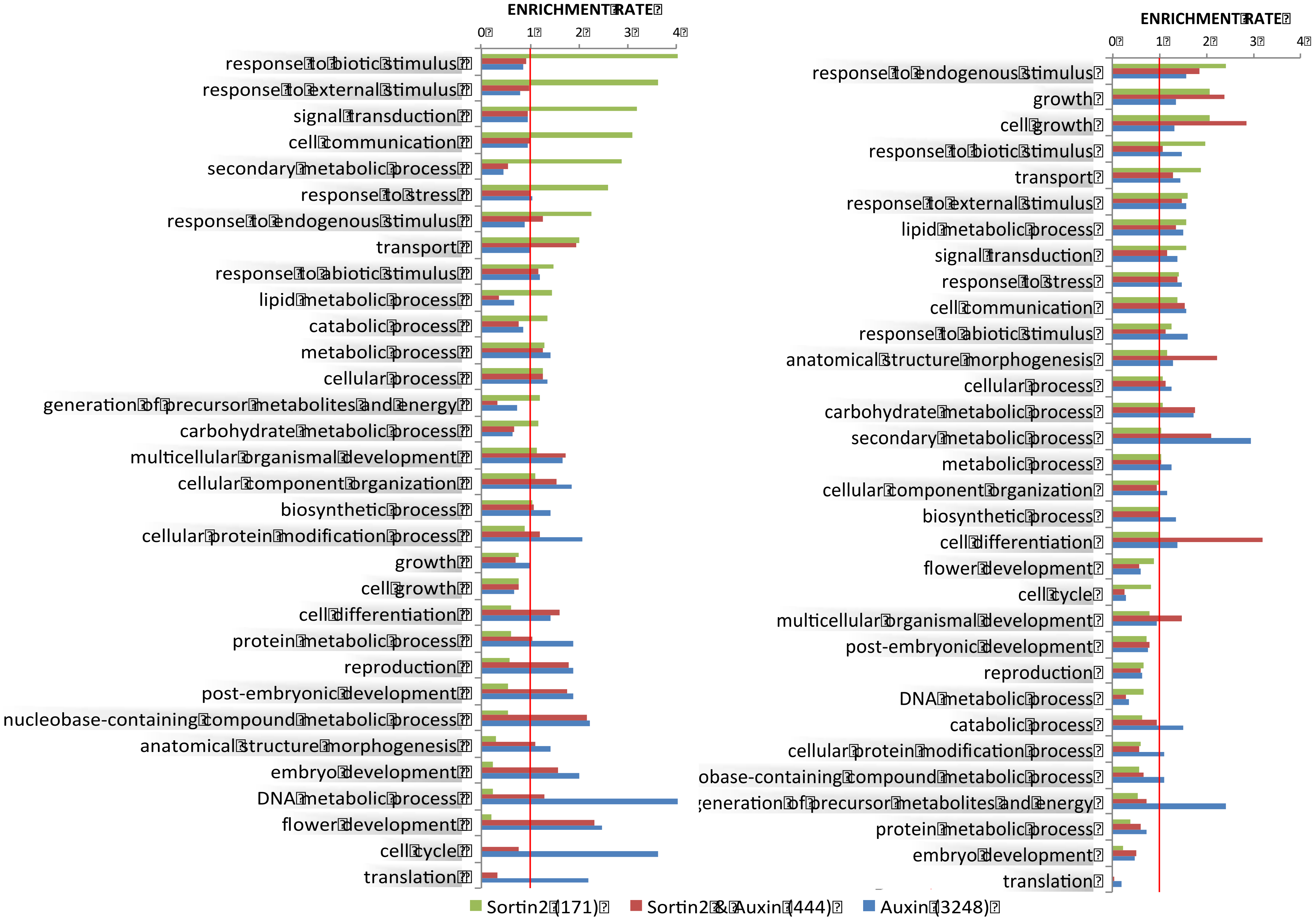
DOWN REGULATED

FC ≤ 2

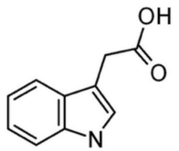


UPREGULATED

DOWNREGULATED



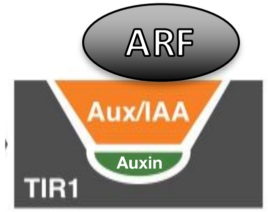
Auxin signaling requires Aux/IAA degradation



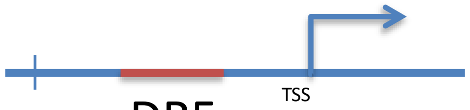
IAA

Receptor

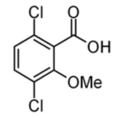
AUX/IAA Degradation



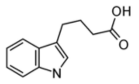
Auxin Response Genes



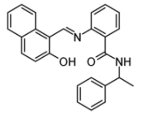
DR5 promoter

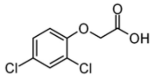


Dicamba

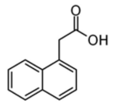


IBA

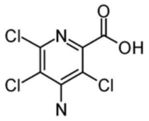




2,4-D



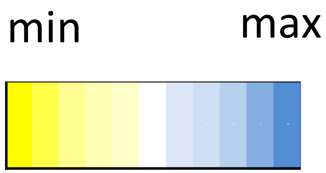
1-NAA



Picloram

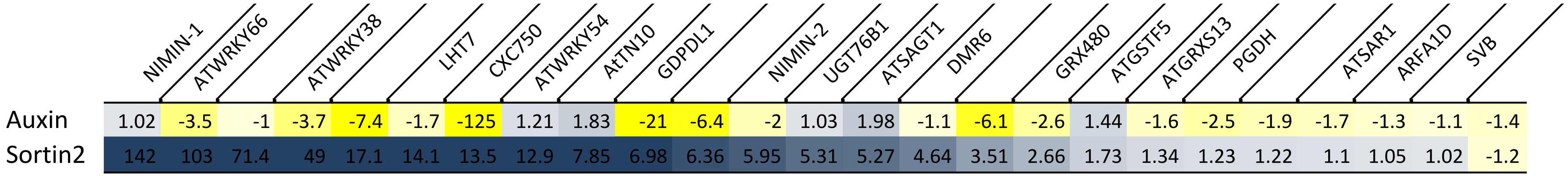
Adapted from
Tan et al . Nature. 2004

Salicylic acid response is specifically associated with Sortin2 response



SA response

According to Krinke et al. Plant Phys 2007

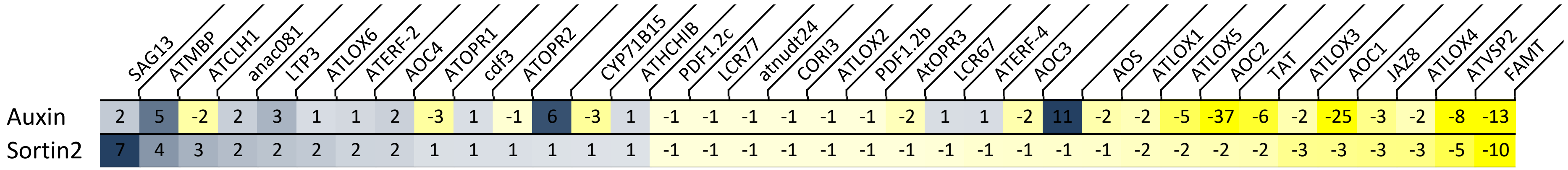


	NIMIN-1	ATWRKY66		ATWRKY38		LHT7	CXC750	ATWRKY54	AtTN10	GDPDL1		NIMIN-2	UGT76B1
Auxin	1.02	-3.5	-1	-3.7	-7.4	-1.7	-125	1.21	1.83	-21	-6.4	-2	1.03
Sortin2	142	103	71.4	49	17.1	14.1	13.5	12.9	7.85	6.98	6.36	5.95	5.31

	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



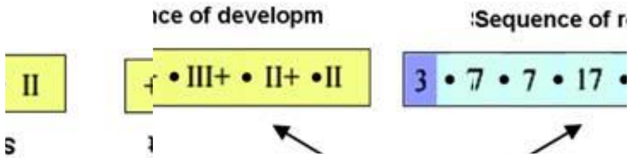
	SAG13	ATMBP	ATCLH1	anac081	LTP3	ATLOX6	ATERF-2	AOC4	ATOPR1	cdf3	ATOPR2	
Auxin	2	5	-2	2	3	1	1	2	-3	1	-1	6
Sortin2	7	4	3	2	2	2	2	2	1	1	1	1

	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