

This is a repository copy of Stem Cell Regulation by Arabidopsis WOX Genes.

White Rose Research Online URL for this paper: http://eprints.whiterose.ac.uk/98577/

Version: Supplemental Material

Article:

Dolzblasz, A, Nardmann, J, Clerici, E et al. (6 more authors) (2016) Stem Cell Regulation by Arabidopsis WOX Genes. Molecular Plant, 9 (7). pp. 1028-1039. ISSN 1674-2052

https://doi.org/10.1016/j.molp.2016.04.007

© The Author 2016. This is a pre-copyedited, author-produced PDF of an article accepted for publication in Molecular Plant following peer review. The version of record Dolzblasz, A, Nardmann, J, Clerici, E, Causier, B, van der Graaff, E, Chen, J, Davies, BH (orcid.org/0000-0002-9282-3789), Werr, W and Laux, T (2016) Stem cell regulation by Arabidopsis WOX genes. Molecular Plant. ISSN 1674-2052 is available online at: http://dx.doi.org/10.1016/j.molp.2016.04.007.

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



SUPPLEMENTAL FILES:

Figure S1. Related to Figure 2.



Figure S1. Related to Figure 2. Expression analysis of different WOX genes

(A-L) Expression levels of different *WOX* cDNAs visualized by expression of a tandem *GUS* reporter (blue).

(**M**) *WOX* transgene expression levels determined by RT-PCR. *WOX* genes are indicated by numbers. "+" samples prepared with RNA polymerase, "-" without RNA polymerase as negative control, "pos." is transgene DNA as positive control. Lower right: expression of the *PDF2* gene as internal control.

Scale bar: (A-L) 0,5mm

Figure S2. Related to Figure 2.



Figure S2. Related to Figure 2. Complementation of *wus-1* stem cell defects by different *WOX* genes

(A-D) 10-day-old seedling phenotypes.

(E-Q) 40-day-old transgenic plants in comparison to untransformed *wus-1* -/- mutants (L). Scale bars: (A-D) 3mm, (E-Q) 2cm

Figure S3. Related to Figure 2.



Figure S3. Related to Figure 2. Two copies of *pWUS::WOX2* provide an enhanced complementation of *wus-1* defects

(A) Comparison of a 40-day-old *wus-1* mutant plant, where adventitious shoots terminated prematurely in an aerial rosette and a *wus-1* plant expressing homozygously *pWUS::WOX2* which produces an indeterminate shoot with many flowers.

(B) Flower of *wus-1* plant homozygous for *pWUS::WOX2* forming all floral organs.
(C) Comparison of gynoecia of wild-type and *wus-1* plants homozygous for *pWUS::WOX2*, which never elongate to the wild-type level.
Scale bars: (A) 1cm, (B-C) 3mm



Figure S4. Related to Figure 3 and Figure 4

Figure S4. Related to Figure 3 and Figure 4. Schematic representation of variants of WUS used in different experiments

(A) Schematic representation of protein variants used in Figure 3A-C

(**B**) Schematic representation of variants of WUS used as bait in yeast two-hybrid experiments (compare to Figure 3D).

(C) Schematic representation of constructs used for Figure 4.

Boxes represent WOX protein domains: Ac, acidic domain; AD, Gal4 activation domain; C, WOX8 clade C-terminal domain; E, EAR domain; HD, homeodomain (different colors represent HDs from different WOX proteins); N, WOX8-type N-terminal domain; WB, WUS-box; blue strap in WUS-box, "AG" residues; dark red strap in WUS-box, "Q" residue exchange as in WOX5.



Figure S5. Related to Figure 3 and Figure 4

Figure S5. Related to Figure 3 and Figure 4. Expression controls of chimeric *WUS* and *WOX8* constructs

(A) RT-PCR of transgene expression levels. 1, $WUS\Delta EAR$; 2, $WUS\Delta Ac$; 3, $WUS\Delta WB$; 4, WUSmWB; 5, WOX8wusWB; 6, $WOX8wsbx\Delta D$. "WT" is a neg control without constructs, "+" reaction with polymerase, "-" without polymerase, "pos." is the respective cDNA as pos control. Upper left: expression of the *PDF2* gene as internal control.

(**B**) Expression levels of different constructs as indicated, visualized by the tandem *GUS* reporter (blue).

(C) 40-day-old wus-1 plants expressing chimeric WUS transgenes as indicated.

(**D-F**) Comparison of flowers formed in *wus-1* mutant plants expressing chimeric *WUS* genes as indicated.

Scale bar: (B) 0,5mm; (C) 2cm; (D-F) 1mm

Figure S6. Related to Figure 4.



Figure S6. Related to Figure 4. Complementation of *wus-1* stem cell defects by different homeodomain constructs

(A) Comparison of *wus-1* plants expressing the indicated constructs.

(**B**) Comparison of expression levels of the different transgenes, visualized by tandem *GUS* reporter expression (blue).

(C) Expression levels of transgenes by RT-PCR as indicated. 1, WUS-W8HD; 2, WUS-

W9HD; 3, *WUS-W13HD*. "WT" is a neg control without constructs, "+" reaction with polymerase, "-" without polymerase, "pos." is a transgene DNA as positive control. Lower right: expression of the *PDF2* gene as internal control.

Scale bars: (A - adult plants) 2cm; (A - seedling, flower and siliques) 3mm; (B) 0,5mm

Table S1. Related to Figure 3.Seed production requires the acidic WUS domain

female	mala	carpel lenght (mm)		viable seeds	n
lemale	male	median	m.d.	viable seeds	11
wild-type	wild-type	11,50	0,74	+	10
wild-type	not pollinated	3 ***	0,0	-	11
wus-1-/- $pWUS$: $WUS\Delta Ac$	wus-1-/- $pWUS$: $WUS\Delta Ac$	3 ***	0,74	-	25
wus-1-/- $pWUS$: $WUS\Delta Ac$	not pollinated	3 **	0,37	-	9
wus-1-/- $pWUS:WUS\Delta Ac$	wild-type	3 ***	0,74	-	21
wild-type	wus-1-/- $pWUS:WUS\Delta Ac$	11,75	1,11	+	10
Gynoecia of wild type (wild-type) plants or $wus-1-/-pWUS:WUS\Delta Ac$ transgenic plants were pollinated as					
indicated.					
n, number of gyneocia crossed.					
Plants statistically different to wild-type control (line 1) with P<0,01 ^{***} , <0,001 ^{***} (line 1). Unmarked					
combinations, statistically not significant.					
Probabilities determined using Kruskal-Wallis with Dunn's post-hoc test and corrected for multiple testing					
using the Holm method. m.d., mean absolute deviation.					

genotype	hackground	reference
$wus_l/+$	Lor	$(I_{aux} et al = 1996)$
was^{-1-7+}	Col	(Brauninger at al 2008)
wus 1/1 pWUS · 1 hCA	0	(Breuninger et ut, 2008)
wus-1-/+pw0s.Lh04	Ler	This study
pop.wospop.gos		
wus-1-/+ pw0S:LnG4	Ler	This study
pOp:wOx1 pOp:GUS		-
wus-1-/+ pWUS:LhG4	Ler	This study
pOp:WOX2 pOp:GUS		5
wus-1-/+ pWUS:LhG4	Ler	This study
pOp:WOX3 pOp:GUS	201	1
wus-1-/+ pWUS:LhG4	Ler	This study
pOp:WOX4 pOp:GUS	Lei	This study
wus-1-/+ pWUS:LhG4	Lar	This study
pOp:WOX5 pOp:GUS	Lei	This study
wus-1-/+ pWUS:LhG4	т	This stade
pOp:WOX6 pOp:GUS	Ler	I nis study
wus-1-/+ pWUS:LhG4		
pOp:WOX8 pOp:GUS	Ler	This study
$wus - 1 - /+ pWUS \cdot LhG4$		
pOn:WOX9 pOn:GUS	Ler	This study
pop.word pop.oos		
wus-1-7 + pwos.Lno+	Ler	This study
pop.wox12 pop.003		
wus-1-/+ pwus:LnG4	Ler	This study
wus-1-/+ pWUS:LhG4	Ler	This study
pOp:WUSAWB pOp:GUS		
wus-1-/+ pWUS:LhG4	Ler	This study
pOp:WUS mWB pOp:GUS		
wus-1-/+ pWUS:LhG4	Ler	This study
<i>pOp:WUS∆EAR pOp:GUS</i>		This study
wus-1-/+ pWUS:LhG4	Lar	This study
$pOp:WUS\Delta Ac \ pOp:GUS$	Lei	This study
wus-1-/+ pWUS:LhG4	Lon	This study
pOp:WUS-W8HD pOp:GUS	Lei	This study
wus-1-/+ pWUS:LhG4	т	This stade
pOp:WUS-W9HD pOp:GUS	Ler	I nis study
wus-1-/+ pWUS:LhG4	т	
pOp:WUS-W13HD pOp:GUS	Ler	I his study
$wus - 1 - /+ pWUS \cdot LhG4$		
nOn·WOX8wusWB nOn·GUS	Ler	This study
$wus_l_/+ nWI/S \cdot I hG4$		
nOn:WOY8wshr4D nOn:GUS	Ler	This study
wor8 wor0/		
wurd wurdt +	Ler	This study
p word word p		
	Ler	This study
pw0X9:w0X8-wusWB		-
wox8 wox9/+	Ler	This study
pWOX9:WUS		

Table S2. Mutant and transgenic lines used in the study

Table S3.	Sequence of	oligonucleotides	used for	RT-PCH
		a		

construct	Forward Primer	Reverse Primer
PDF2	CGTTACTGCCAGCCATTGTAGAA	CCGCAGGTAAGAGTTTGGAACAT
pOp:WUS pOp:GUS	CTAGCTTATCGATACCGTCG	CATACTTCCAGATGGCACCA
pOp:WOX1 pOp:GUS	CTAGCTTATCGATACCGTCG	TTGGAGCAATCTTCATGTTGTTCTTGATC
pOp:WOX2 pOp:GUS	CTAGCTTATCGATACCGTCG	TGGAGGAGGCGATTGAAGTAAGCC
pOp:WOX3 pOp:GUS	CTAGCTTATCGATACCGTCG	GGTGATGATGATAAGGATTGTG
pOp:WOX4 pOp:GUS	CTAGCTTATCGATACCGTCG	CCTATCTGTTCTTGAGTCGG
pOp:WOX5 pOp:GUS	CTAGCTTATCGATACCGTCG	CGTCGTGGTGGTCTCTCGAAT
pOp:WOX6 pOp:GUS	CTAGCTTATCGATACCGTCG	CCTGATGATGAATCCTTGACGTCT
pOp:WOX8 pOp:GUS	CTAGCTTATCGATACCGTCG	TAGGATCTGGTATCCGATCTGAGG
pOp:WOX9 pOp:GUS	CTAGCTTATCGATACCGTCG	AATCCTACATCTCGTTTTTCTTGAG
pOp:WOX12 pOp:GUS	CTAGCTTATCGATACCGTCG	CCATTGTTGTTCTCACACCC
pOp:WOX13 pOp:GUS	CTAGCTTATCGATACCGTCG	CCAGAAGAACCTCCACCATGC
$pOp: WUS\Delta WB$ pOp:GUS	CTAGCTTATCGATACCGTCG	CGTACGCTTCCTCTCTCCC
pOp: WUS mWB pOp:GUS	CTAGCTTATCGATACCGTCG	CGTACGCTTCCTCTCTCCC
<i>pOp: WUS∆EAR</i> <i>pOp:GUS</i>	CTAGCTTATCGATACCGTCG	TCTGGGAACTACTCACAC
$pOp: WUS\Delta Ac$ pOp:GUS	CTAGCTTATCGATACCGTCG	CGTACGCTTCCTCTCTCCC
pOp: WUS-W8HD pOp:GUS	GGCTTCTTGAGATCCTTCA	CATACTTCCAGATGGCACCA
pOp: WUS-W9HD pOp:GUS	CTAGCTTATCGATACCGTCG	CATACTTCCAGATGGCACCA
pOp: WUS-W13HD pOp:GUS	CTAGCTTATCGATACCGTCG	CATACTTCCAGATGGCACCA
pOp:WOX8wusWB pOp:GUS	CTAGCTTATCGATACCGTCG	CGTACGCTTCCTCTCTCCC
pOp:WOX8wsbx∆D pOp:GUS	CTAGCTTATCGATACCGTCG	CGTACGCTTCCTCTCTCCC