

## SUPPLEMENTARY DATA

### ***YUCCA*-mediated auxin biogenesis is required for cell fate transition occurring during *de novo* root organogenesis in *Arabidopsis***

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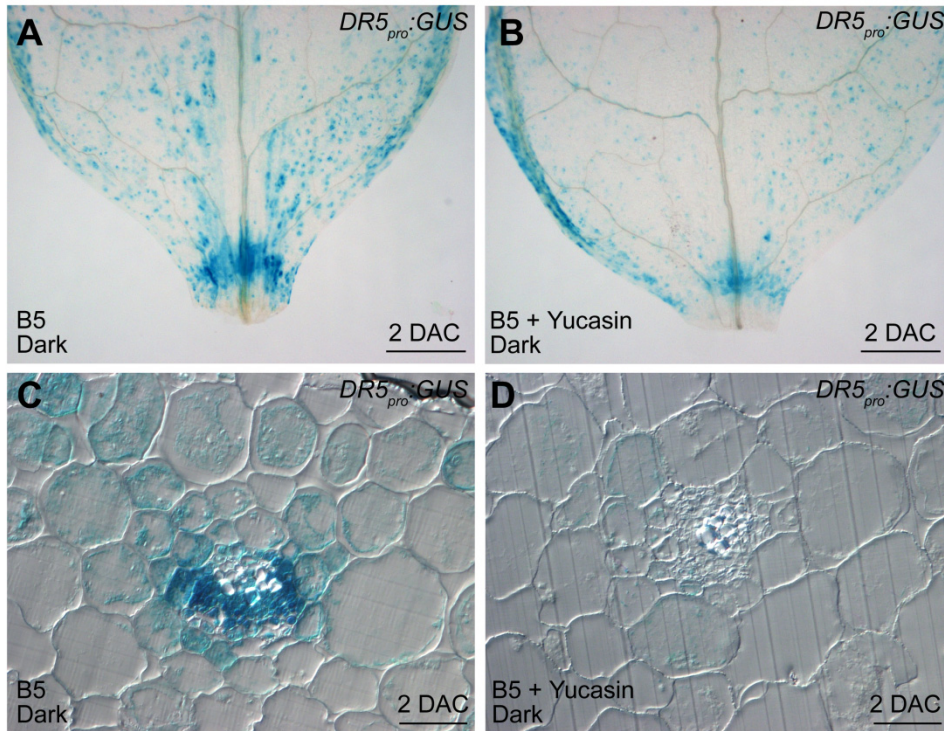
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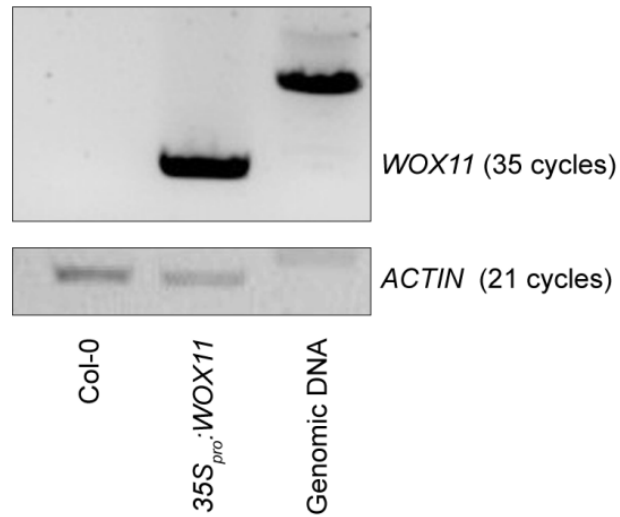
**Fig. S1.** Yucasin inhibits auxin production in regeneration.

(A, B) Observations of the GUS signal in leaf explants from *DR5<sub>pro</sub>:GUS* reporter line at 2 DAC cultured on B5 medium (A) or B5 medium with 200  $\mu$ M yucasin treatment (B).

(C, D) Thin sectioning of 2-DAC leaf explants from *DR5<sub>pro</sub>:GUS* cultured on B5 medium (C) or B5 medium with 200  $\mu$ M yucasin treatment (D) at the leaf base, showing the midrib of vasculature.

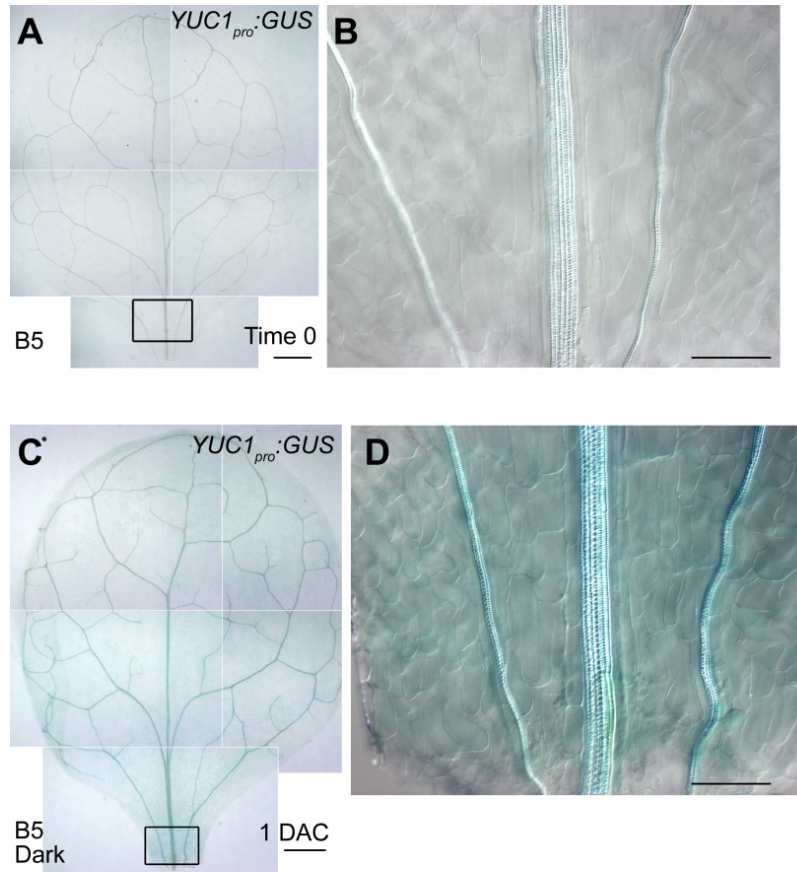
Note that the auxin level was lower in the vasculature of wounded region of leaf explants cultured on B5 medium with 200  $\mu$ M yucasin treatment compared with that in leaf explants cultured on B5 medium. Yucasin treatment could not strictly block all YUC proteins in the whole leaf explant. However, the partially reduced auxin level in the wounding site is sufficient to block rooting.

Scale bars, 500  $\mu$ m in A, B; and 50  $\mu$ m in C, D.



**Fig. S2.** *WOX11* expression in *35S<sub>pro</sub>:WOX11* transgenic plants.

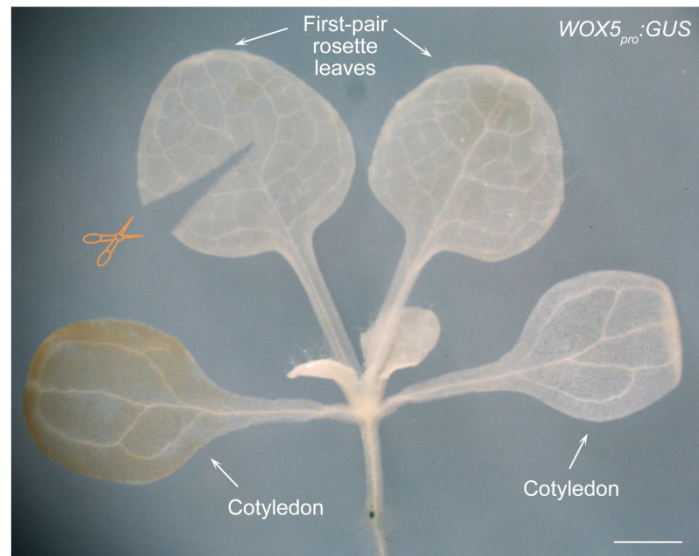
RT-PCR analysis of *WOX11* expression in time-0 leaf explants from Col-0 and *35S<sub>pro</sub>:WOX11* transgenic plants. Expression of *ACTIN* was served as an internal control.



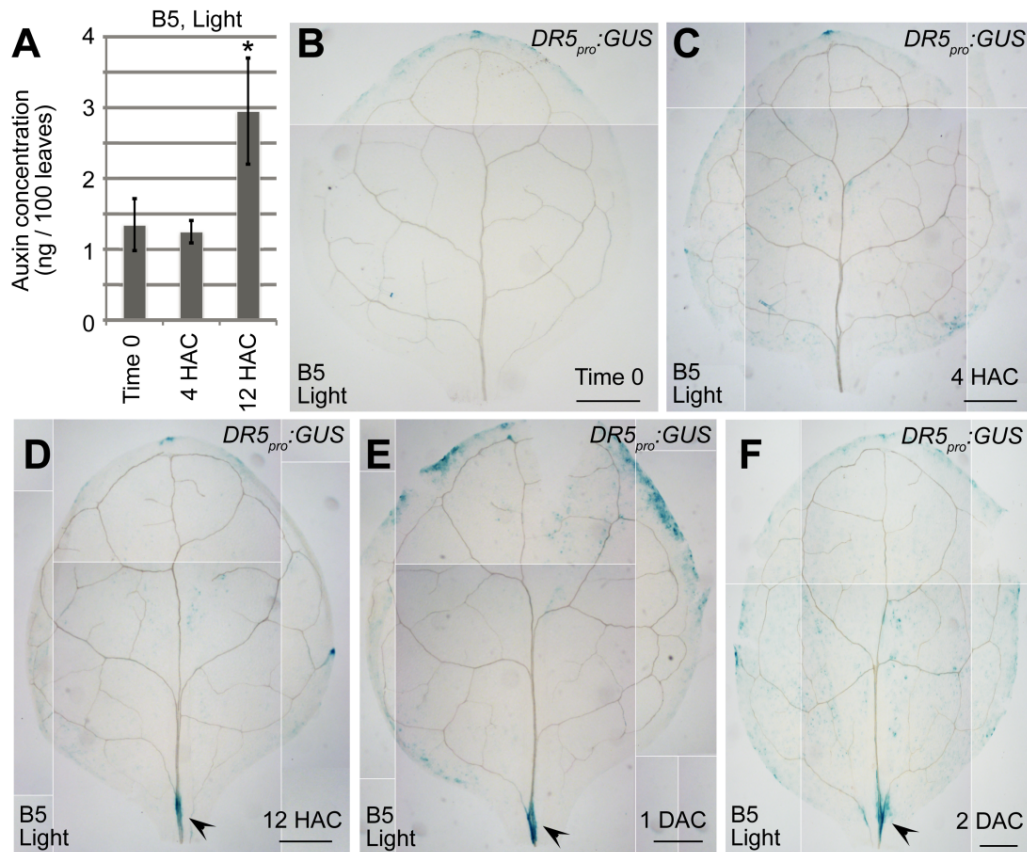
**Fig. S3.** Spatial expression patterns of *YUC1* in regeneration.

(A–D) GUS staining of time-0 (A, B) and 1-DAC (C, D) leaf explants from *YUC1<sub>pro</sub>:GUS* cultured on B5 medium.

The data in A and C were pasted by small pictures of the same leaf explant, because the microscopy is unable to capture the entire leaf explants at a single visual field. B and D are close-ups of the boxed regions in A and C, respectively. Scale bars, 500  $\mu\text{m}$  in A, C; and 50  $\mu\text{m}$  in B, D.



**Fig. S4.** Expression of *WOX5* is not in response to wounding within 4 h. GUS staining of a wounded leaf from *WOX5<sub>pro</sub>:GUS* at 4 hours after wounding, serving as a negative control for non-specific GUS staining in Fig. 6A–C. Note that GUS signal was not observed at the wounded site. Scale bar, 1 mm.

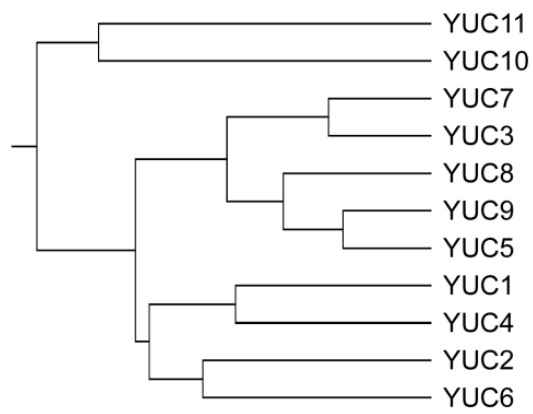


**Fig. S5.** Auxin production in leaf explants in light conditions.

(A) Auxin concentration in leaf explants from time 0 to 12 HAC on B5 medium in light conditions. Bars show SEM with three biological repetitions. Each biological repetition was performed with three technical repetitions. \*  $P < 0.05$  in two-sample  $t$  tests compared with time-0 control.

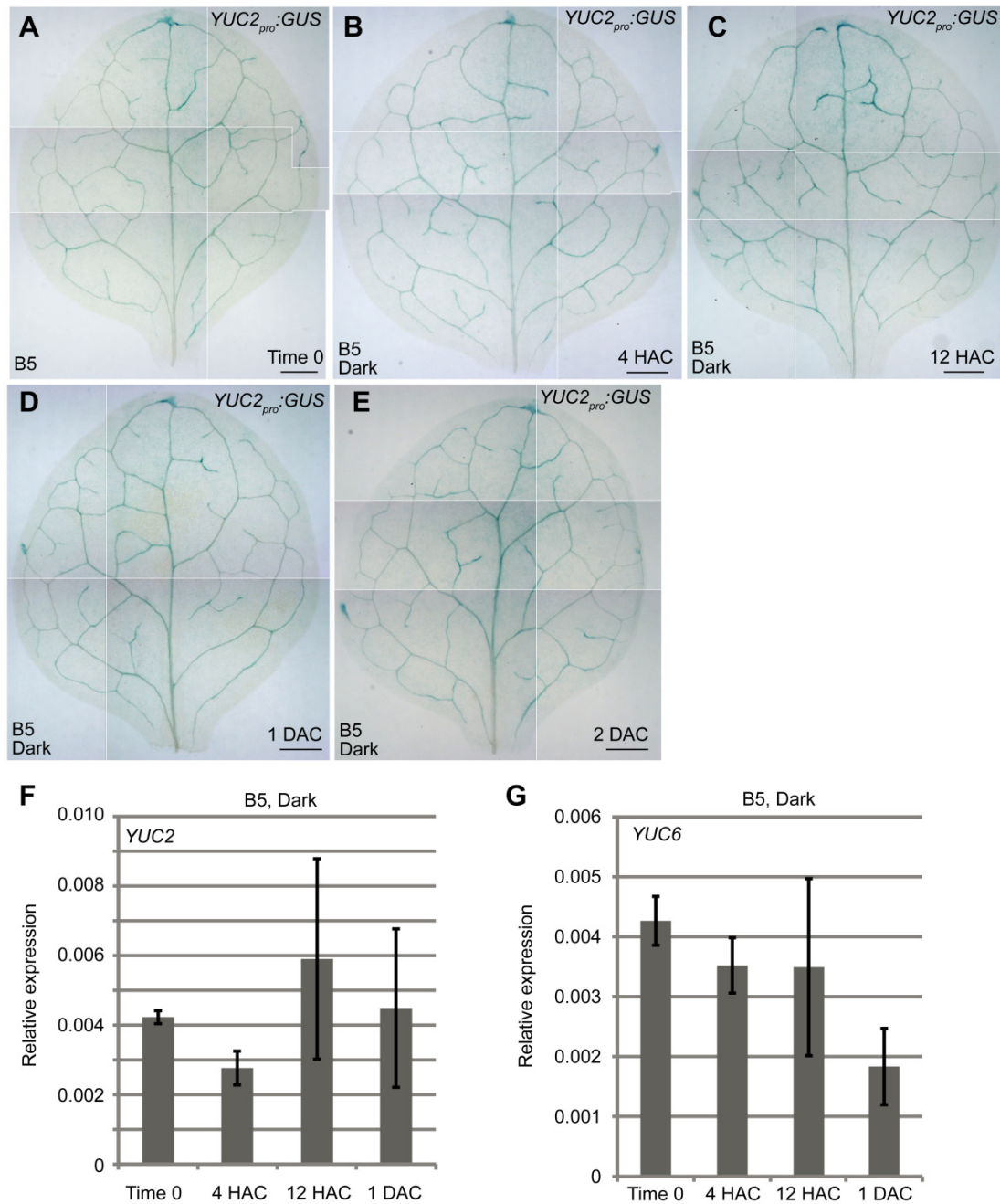
(B–F) Observations of the GUS signal in leaf explants from *DR5<sub>pro</sub>:GUS* reporter line at time 0 (B), 4 HAC (C), 12 HAC (D), 1 DAC (E) and 2 DAC (F) cultured in light conditions. Arrowheads in D–F indicate the GUS signal in vasculature near the wound.

The data in B–F were pasted by small pictures of the same leaf explant, because the microscopy is unable to capture the entire leaf explants at a single visual field. Scale bars, 500 μm in B–F.



**Fig. S6.** YUC family in Arabidopsis.

Phylogenetic analysis of *Arabidopsis* YUC protein sequences was conducted using MEGA3.0 (Kumar *et al.*, 2004).



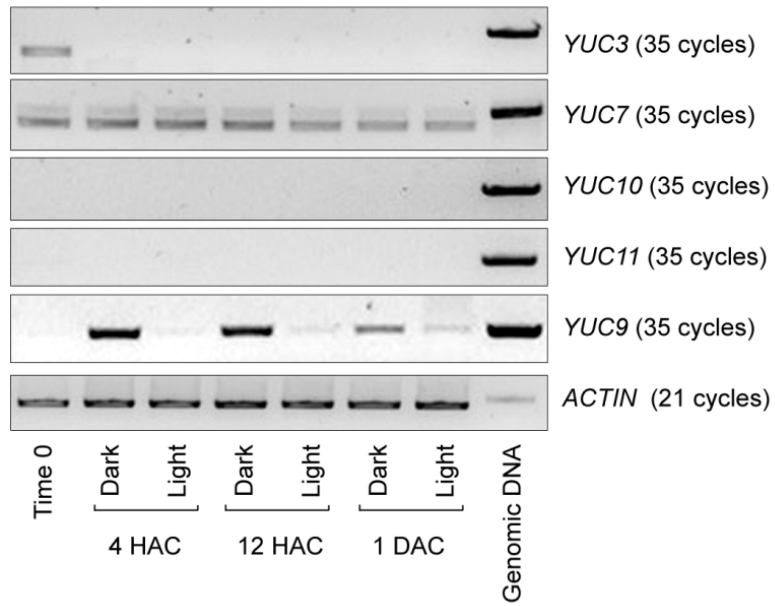
**Fig. S7.** Expression of *YUC2* and *YUC6* genes in regeneration.

(A–E) GUS staining of time-0 (A), 4-HAC (B), 12-HAC (C), 1-DAC (D) and 2-DAC (E) leaf explants from *YUC2<sub>pro</sub>:GUS* cultured on B5 medium. The data in A–E were pasted by small pictures of the same leaf explant, because the microscopy is unable to capture the entire leaf explants at a single visual field.

(F, G) qRT-PCR analysis of *YUC2* (F) and *YUC6* (G) during rooting from leaf explants on B5 medium. Bars show SEM from three biological repetitions. Each biological repetition was performed with three technical repetitions. Note that the



expression levels of the two genes were not significantly upregulated within 1 DAC. We also constructed *YUC6<sub>pro</sub>:GUS* construct; however, this construct did not show GUS signal in leaf explants in our conditions. Scale bars, 500  $\mu\text{m}$  in A–E.



**Fig. S8.** *YUC3*, *YUC7*, *YUC10*, *YUC11*, and *YUC9* expression during rooting from leaf explants.

RT-PCR analysis of *YUC* gene expression in time-0, 4-HAC, 12-HAC and 1-DAC leaf explants from Col-0 in dark or light conditions. *YUC9* was served as a positive control. Expression of *ACTIN* was served as an internal control.

**Table S1.** List of primers used in this study.

Experiments	Primers	Sequence (5' →3')
<b>Molecular cloning</b>		
<i>YUC4<sub>pro</sub>:</i> <i>GUS</i>	YUC4 <sub>pro</sub> -F1	cgcggatccGAAGCCGTTGATTCTTACATGGTG
	YUC4 <sub>pro</sub> -R1	tccccgggGTCGACTAATAAAAGCGAAAG
<i>YUC1<sub>pro</sub>:</i> <i>GUS</i>	YUC1 <sub>pro</sub> -F1	cttgcgatcctgcaggtcgacCACGTTTTTGGGTGGACCAC
	YUC1 <sub>pro</sub> -R1	cggggatcctctagagtcgacTCTTGATGGATGATGGAAAA
<i>YUC2<sub>pro</sub>:</i> <i>GUS</i>	YUC2 <sub>pro</sub> -F1	acgcgtcgacGCATAATCAAATTTTAGTTAC
	YUC2 <sub>pro</sub> -R1	cgggatccACAATGTTGAGGACGAGCCAATGG
<i>YUC9<sub>pro</sub>:</i> <i>GUS</i>	YUC9 <sub>pro</sub> -F1	cttgcgatcctgcaggtcgacCCAATTGAAAAAAGTGTTAAAC
	YUC9 <sub>pro</sub> -R1	cggggatcctctagagtcgacTTTCTTGAGTGAGTTTTTGAATG
<b>qRT-PCR</b>		
<i>YUC1</i>	YUC1-F	CGATGTCGGAGCTATGTCTC
	YUC1-R	CTGTACAAGTTTATTACTTCG
<i>YUC2</i>	YUC2-F	CGGTTAGGGTTAGTTCGACC
	YUC2-R	GAACCTCAATATCCTCAGCG
<i>YUC4</i>	YUC4-F	CCGTTCTTGATGTCGGTGCC
	YUC4-R	AAGGATTTATTGAAATGAAGATG
<i>YUC6</i>	YUC6-F	GGTAGTTAAGCACACGTGTC
	YUC6-R	GGCTAGCGTGCCAACGTCGAG
<i>YUC5</i>	YUC5-F	CCATGATGTTGATGAAGTGG
	YUC5-R	CCAATATCTTGAGCGATG
<i>YUC8</i>	YUC8-F	TGTATGCGGTTGGGTTTACGAGGA
	YUC8-R	CCTTGAGCGTTTCGTGGGTTGTTT
<i>YUC9</i>	YUC9-F	GCTCGGTTTCATGTTTTACCG
	YUC9-R	CTTGGCTTCAGGAAAGTGAG
<i>WOX11</i>	WOX11-F	CGCAACCACCAACACTTGTGACC
	WOX11-R	AAGACATCTGTTGCATCACC
<i>WOX5</i>	WOX5-F	GTGAAAGGTCGAAGCTTACG
	WOX5-R	GTA CTGGTTATTGCCTCTAGC

<i>ACTIN</i>	ACTIN-F	TGGCATCA(T/C)ACTTTCTACAA
	ACTIN-R	CCACCACT(G/A/T)AGCACAATGTT
<b>RT-PCR</b>		
<i>YUC3</i>	YUC3-F	CGTTTGAATTAGGAGTTACG
	YUC3-R	GGTATCCCATCATCGGAG
<i>YUC7</i>	YUC7-F	GGATGTGGAAACTCAGGC
	YUC7-R	GTGGCAAGAATCACTGAATC
<i>YUC10</i>	YUC10-F	GGTCGACACATTGGTGACG
	YUC10-R	CCATCCTTCTTCATCACG
<i>YUC11</i>	YUC11-F	GATCTATCTAAGTGCAACGC
	YUC11-R	GAACAGATCTCCATCATCG
<i>YUC9</i>	YUC9-F	GCTCGGTTTCATGTTTTACCG
	YUC9-R	CTTGGCTTCAGGAAAGTGAG
<i>ACTIN</i>	ACTIN-F	TGGCATCA(T/C)ACTTTCTACAA
	ACTIN-R	CCACCACT(G/A/T)AGCACAATGTT
<b>ChIP</b>		
<i>YUC1</i>	YUC1-I-F	GTCGTATCTCGGTATGCTATAG
	YUC1-I-R	CGTGAACTTCTGCCGACAG
	YUC1-II-F	GAGTAACTTTACCACTAATCTAC
	YUC1-II-R	CATTGTGCATTGTGTGAGCC
<i>YUC4</i>	YUC4-I-F	CATGAGAGGAGTCGAGTTGC
	YUC4-I-R	CTTGCAAGGCCATGTTAAATGTG
	YUC4-II-F	GCTATTGAATAAGTATCTTTTCG
	YUC4-II-R	CTGCATGCATGTATCTCTAGC
<i>AG</i>	AG-F	GGCTTTGGAGCAGCAATCAC
	AG-R	GCAAACCATTCTACGTTTGC

Note that lower case letters represent additional nucleotides to introduce restriction sites.

## **SUPPLEMENTARY REFERENCE**

**Kumar S, Tamura K, Nei M.** 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform.* **5**, 150-163.