Supplemental Data. Zhao et al. (2015). Plant Cell 10.1105/tpc.15.00227

The interaction between rice ERF3 and WOX1 1 promotes crown root development by regulating gene expression involved in cy tokinin signaling

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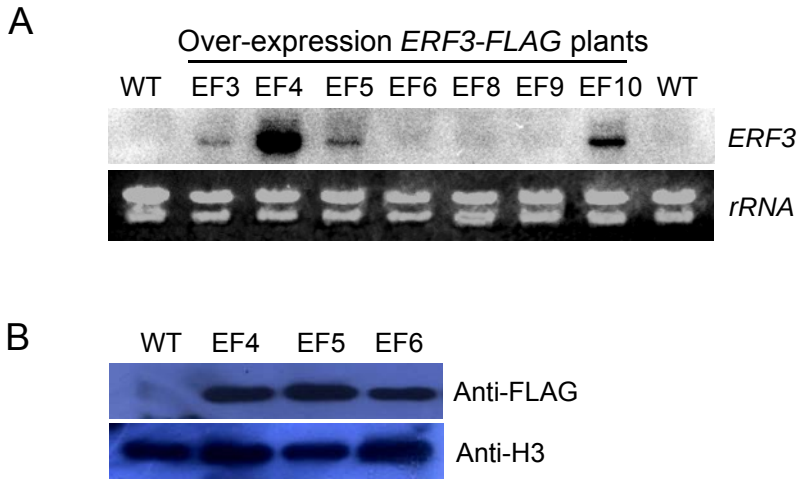
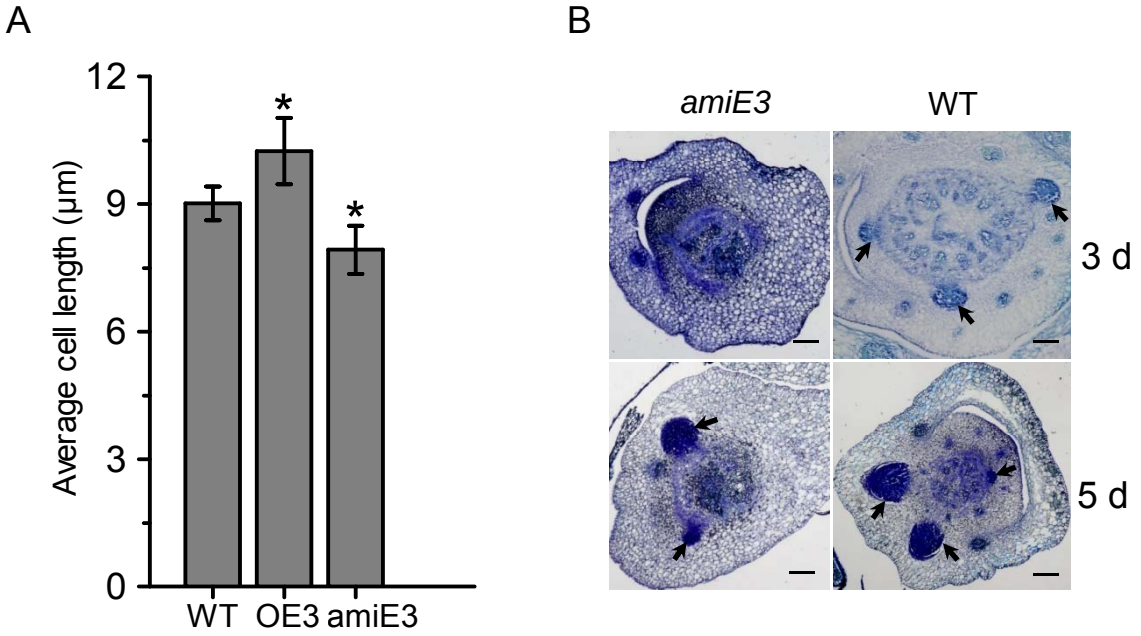
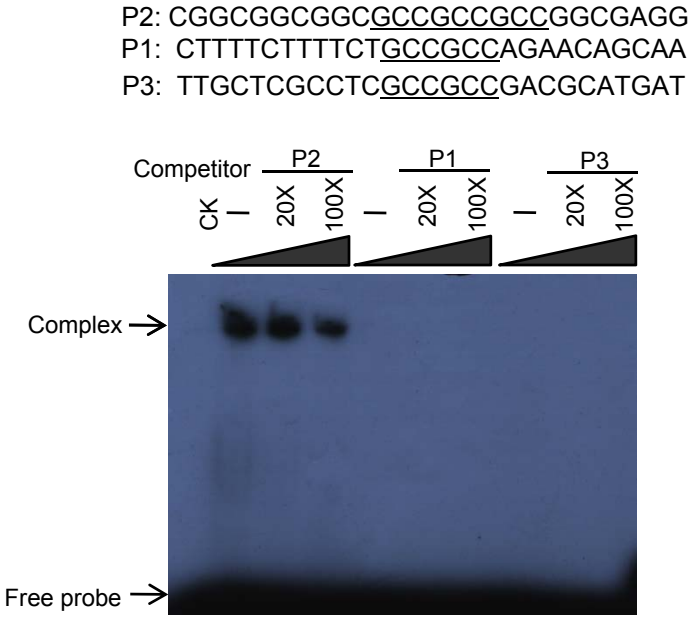
SUPPLEMENTAL DATA



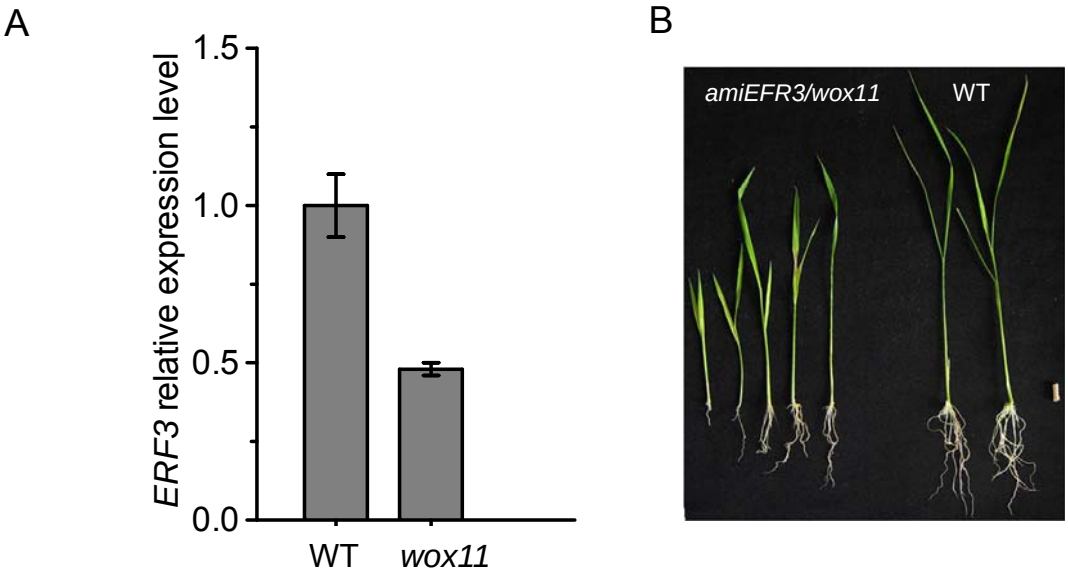
Supplemental Figure 1. Detection of *ERF3* mRNA and protein in *ERF3*-FLAG transgenic plants (EF) and wild type (WT) by RNA gel blot (**A**) and immunoblot (**B**).



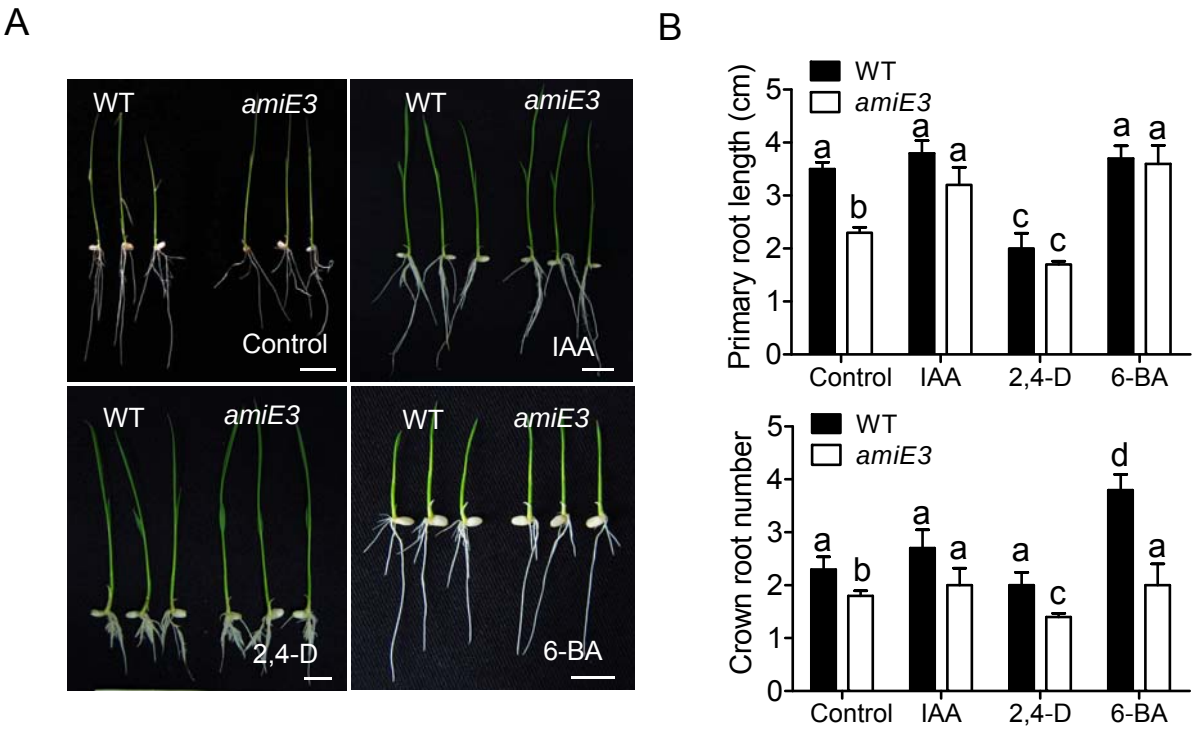
Supplemental Figure 2. Cell longitudinal lengths in root meristem zone and crown root primordium numbers in *ERF3* transgenic plants and wild-type seedlings. **A**. Cell longitudinal lengths in root meristem zone of *ERF3* over-expression (*OE3*, n=20), artificial microRNAs (*amiE3*, n=17) and wild-type (WT, n=16) plants. Error bars represent SD. *, $P < 0.05$, *t*-test. **B**. Crown root primordium numbers in *ERF3* artificial microRNAs (*amiE3*) and wild- type (WT) seedlings at 3 and 5 days after germination as indicated. Arrows indicate emerging crown root initials. Bar=50 μm.



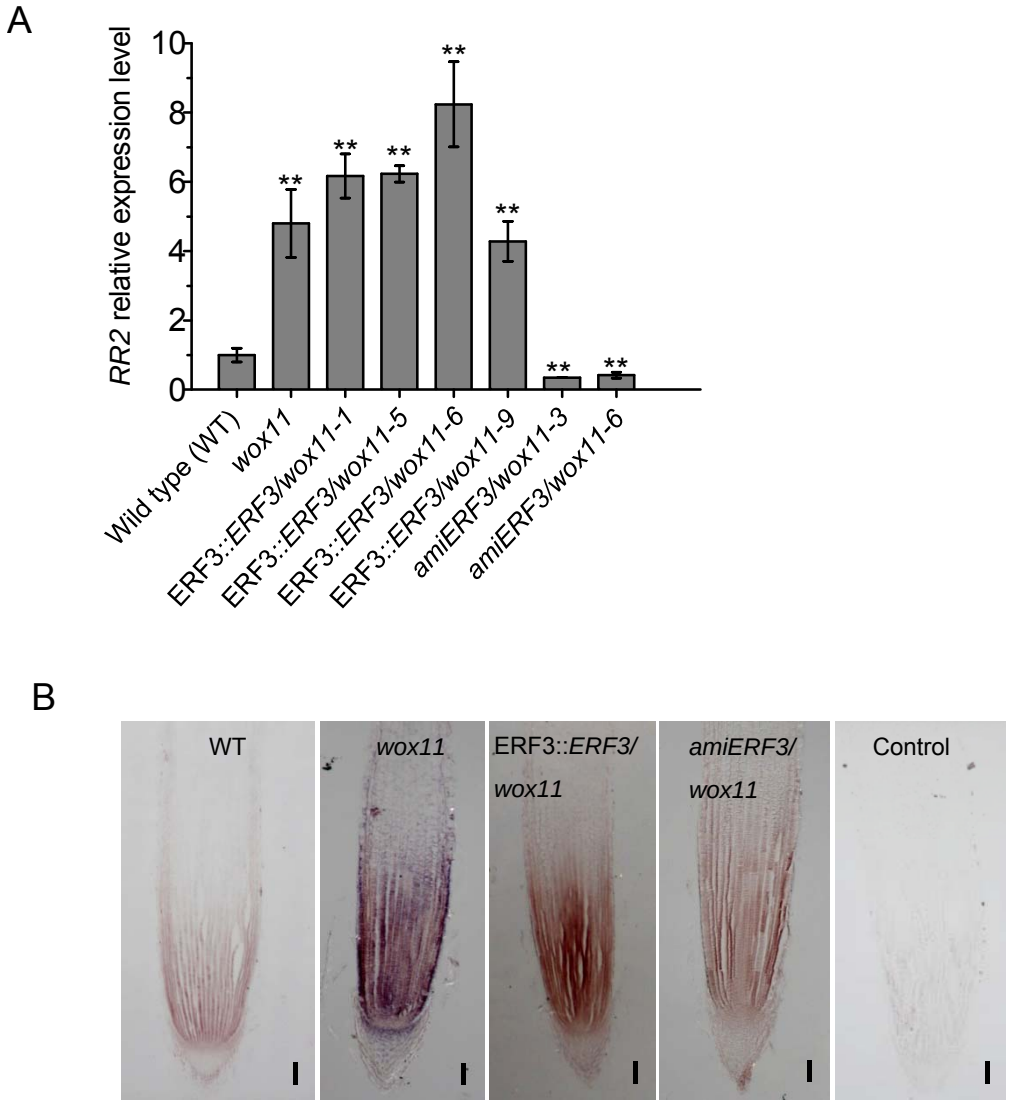
Supplemental Figure 3. Gel shift assay of ERF3 protein binding to P1, P2 and P3 regions of *RR2* containing the ERF binding sites (underlined). *E. coli*-produced ERF3 protein was incubated with ^{32}P-labeled P1, P2 and P3 and analyzed by eletrophoresis. The shifted band is indicated by the arrow.



Supplemental Figure 4. Detection of expression level of *ERF3* in wild type (WT) and *wox11* (**A**) and phenotype of crown root *of amiERF3/wox11* transgenic plants at rooting stage (**B**). Bar=1 cm.



Supplemental Figure 5. Auxin (2,4-D and IAA) and cytokinin (6-BA) partially rescued the root phenotype of *ERF3* artifical microRNAs (*amiE3*) transgenic plants. Different letters in B represent significant differences at $P<0.05$, *t*-test. Bar=1 cm.



Supplemental Figure 6. RT-qPCR (**A**) and *in situ* hybridization (**B**) detection of *RR2* expression in *wox11,* ERF3::*ERF3/wox11, amiERF3/wox11* plants and wild-type (WT) roots. Error bars in (A) represent SD. *, *P*<0.05; **, *P*<0.01. Bar =75 μm in (B).

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Supplemental Table 1. Primers Used in This Study

Expriments	Name	Primer Sequence
Vector constructs	WOX11(2YH)-F	ATAGTCGACACAAGGCAGCTAGCTAGTG
	WOX11(2YH)-R	ATAACTAGTATACGTACGTTGCCATCGA
	ERF3Protein-F	AAGAAGAAGGGATCCATGGCGCCCAGAGCAGCTAC
	ERF3Protein-R	AAGAAGAAGGTCGACCTAGTTCTCTACCGGCGGCGG
	ERF3BiFC-F	GGGGGATCCACTTGTAAACCTGCTGCACCC
	ERF3BiFC-R	GTCGTCGACCATCCTGAGCTAGTTCTCTACC
	ERF3ox-F	GGGGGTACCACTTGTAAACCTGCTGCACC
	ERF3ox-R	CGCGGATCCCTAGTTCTCTACCGGCGGCGG
	ERF3miR-s	AGTAAAGTAAGGATGCGTTGCTTCAGGAGATTCAGTTTGA
	ERF3miR-a	TGAAGCAACGCATCCTTACTTTACTGCTGCTGCTACAGCC
	ERF3miR*s	CTAAGCATCGCTTCCTTACTTTATTCCTGCTGCTAGGCTG
	ERF3miR*a	AATAAAGTAAGGAAGCGATGCTTAGAGAGGCAAAAGTGAA
	ERF3flag-F	GGGGGTACCACTTGTAAACCTGCTGCACC
	ERF3flag-R	GGGGGATCCCCGGTTCAGATCCAGATCG
	ERF3pgus-F	CGCGGATCCGCTAGCAGCGCCTGGTACAT
	ERF3pgus-R	ACGCGTCGACGTGTTTTGGGAGGTTGGGTT
	RiRR2-F(in situ)	GGGACTAGTGGTACCGTCGTCGGAGAATGAGCC
	RiRR2-R(in situ)	GGGGAGCTCGGATCCGACCATCTGTGCAGGAGC
	OxRR2-F	CGGGGTACCTGTGTGGGTGAGACCTGAGA
	OxRR2-R	CGCGGATCCGAGGAGGCTGCTGCCATT
In situ hybridization	ERF3situ-F	GGGACTAGTGGTACCCAGCAGCAATAGCACGGTAG
	ERF3situ-R	GGGGAGCTCGGATCCTTCAGATCCAGATCGAAAGCT
Gel shift assays	EMSAp1-F	TTTCTTTTCTGCCGCCAGAACAGCAA
	EMSAp1-R	TTGCTGTTCTGGCGGCAGAAAAGAAA
	EMSAp2-F	CGGCGGCGGCGCCGCCGCCGGCGAGG
	EMSAp2-R	CCTCGCCGGCGGCGGCGCCGCCGCCG
	EMSAp2d-F	CGGCGGCGGCGCCGGCGAGG
	EMSAp2d-R	CCTCGCCCGGCGCCGCCGCCG
	EMSAp3-F	TGCTCGCCTCGCCGCCGACGCATG
	EMSAp3-R	CATGCGTCGGCGGCGAGGCGAGCA

ChIP assays	ERF3ChIPs4-F	TGTGGGTGAGACCTGAGAGGA
	ERF3 ChIPs4-R	GAGAAGCAGCTCCACGAC
	ERF3ChIPs5-F	GAGGGTGCTGGTGGTGGACGACTC
	ERF3ChIPs5-R	ACGGTAGCGCTCACCGTGGAAC
	ERF3ChIPs6-F	CCACGGTGAGCGCTACCGTCTC
	ERF3ChIPs6-R	GGTGACTGGAGGATCGAGGA
Expression analysis	ERF3qPCR-F	TGCACGTCCAGCAACGCATC
	ERF3qPCR-R	TGCCGCCTTGTTCGCCGTA
	RR1qPCR-F	AGGATCAGCAGATGCATGAATG
	RR1qPCR-R	GAGACGCTGTACGTCCTTGCTT
	RR2qPCR-F	ACGATCTTCTCAAAGCCATCAAG
	RR2qPCR-R	TGAGAGGCTTAAGGATGAAATCCT
	RR3qPCR-F	AGGGTTCGATCTCCTCAAGAG
	RR3qPCR-R	GAATTCTCCGACGACATTAGC
	RR4qPCR-F	GCGATTTGCTGTGGAGATTC
	RR4qPCR-R	GTGTGGCTGGCTTGGCTA
	CRL1qPCR-F	ATGACGGGATTTGGATCGC
	CRL1qPCR-R	CTTGCTCGTGGCAGAAGTAT
	CRL4qPCR-F	GGATTGGGAATGCTACTTCG
	CRL4qPCR-R	CCTTCTTTGGGTCTCTGTTG
	CRL5qPCR-F	CCCTTCCACACACATCAACT
	CRL5qPCR-R	CTCCTTAAGTGAGCCACATACTC
	IAA5qPCR-F	GAAATTGAAAATCATGAGAGGATCTG
	IAA5qPCR-R	TGCTCTGCCCTGACTGCTCTA
	IAA11qPCR-F	CGACGTCGCCATGTACAAGA
	IAA11qPCR-R	TGGTGAAGGAGGTGAACATGTT
	IAA23qPCR-F	TGCCCACCTACGAGGACAAG
	IAA23qPCR-R	TTGCAGGACTCGACGAACATC
	IAA31qPCR-F	CGACGTCCCATTCGAGATGT
	IAA31qPCR-R	TTGCTCCTAGGCCTCTTGCTT
	WOX11qPCR-F	CCAGATGGGCGAGAGCTACT
	WOX11qPCR-R	CGTTGCCATCGATCAATCAA
	ACTIN1qPCR -F	TGTATGCCAGTGGTCGTACCA
	ACTIN1qPCR -R	CCAGCAAGGTCGAGACGAA