

1 **Supplementary Data**

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3 ***OsWRKY42* represses *OsMT1d* and induces reactive oxygen**
4 **species and leaf senescence in rice**

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11 **Supplementary Data Contents**

12 Supplementary Table 1

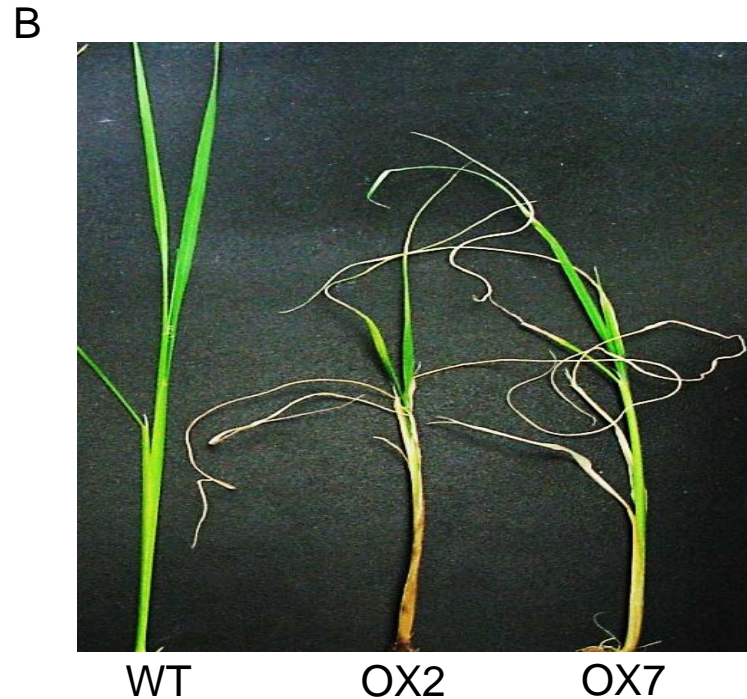
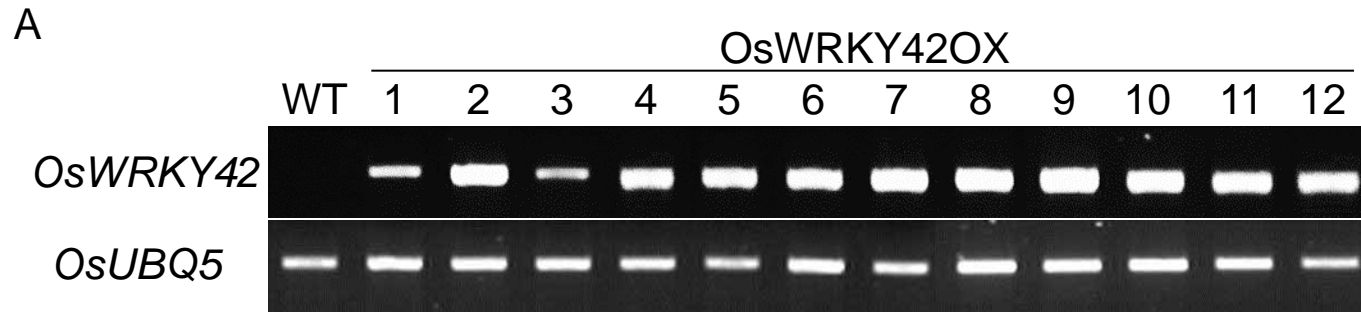
13 Supplementary Figures 1-4

14 Supplementary Table 1. Primers used for cloning and RT-PCR analysis in this
 15 study

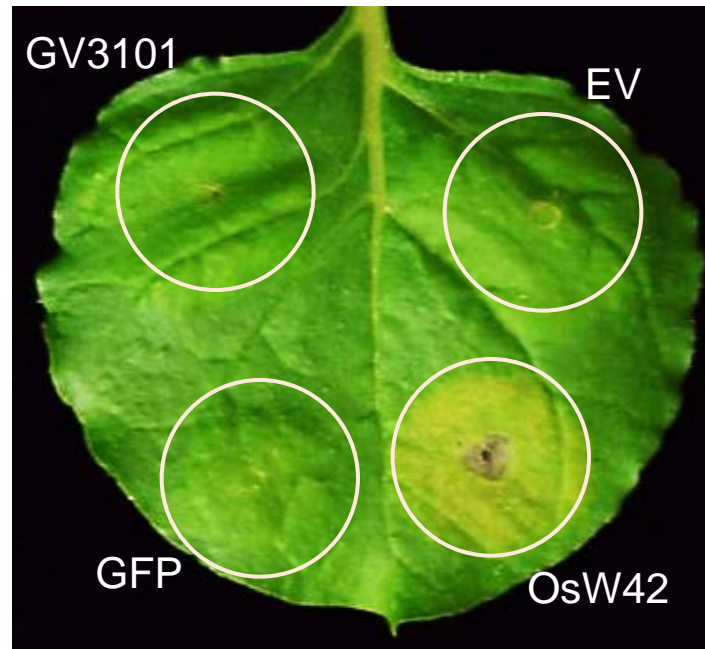
Genes	Forward primer (F)	Reverse primer (R)	Size (bp)
For cloning*			
cDNA	<u>CTCTAGA</u> ATGGCGGATCCGTTT	CCTCGAGCTAGAGCAATCTTCCA	776
GFP	<u>CTCTAGA</u> ATGGCGGATCCGTTT	CCTCGAGAGAGCAATCTTCCAGTAGT	774
FL	<u>CCCCGGG</u> ATGGCGGATCCGTTT	<u>CGTCGAC</u> CTAGAGCAATCTTCCA	776
<i>OsMT1a</i> pro	<u>CGGATCCTGCAGGAATTT</u> CACAGT TTCT	<u>CGTCGAC</u> GTCAGCAATTGCTGAAAGC AT	1496
<i>OsMT1d</i> pro	<u>CGGATCCGCTCTCTAATCC</u> ATTGC CTCGT	<u>CGTCGAC</u> CTTGATCGTCTGGGTCTCT TGT	1427
For ChIP			
P1	GCTCTCTAATCCATTGCCTCGT	TGAAATACGTTGCCTTTAATCA	286
P2	TGATTAAAGGCAACGTATTTCA	TCATACGCCAAGAATTCAAATC	256
P3	GATTTGAATCTTGCGGTATGA	GATGCATTAGTGCACAGCTTGA	305
P4	TCAACTAGGAATGACACAATTG	CTTGATCGTCTGGGTCTCTTGT	292
P5	ATGTCGTGCGGTGGAAGTTG	GTGATCTTCTCAGCCAGGTCA	332
For RT-PCR			
<i>OsWRKY42</i>	CAGTCTGCTTCAGATTATGCTT	TCTCCAGTAGTTTGGTCAGTC	411
<i>OsW42VP16</i>	CAGTCTGCTTCAGATTATGCTT	TCACCCACCGTACTCGTCAAT	654
<i>OsMT1a</i>	ATGCATTGCACTCATCTCAAG	TTAGCAGTTGCAGGGGTTGCA	324
<i>OsMT1b</i>	ATGTCTTGCACTGTGGATC	AGCAGTTGCAAGGGTTGCAC	217
<i>OsMT1c</i>	ATGTCGTGCGGTGGAAGTTG	TGCCAACCATCACCTCAAAGT	163
<i>OsMT1d</i>	ATGTCGTGCGGTGGAAGTTG	TCAGTCATAGATCGCCTTG	240
<i>OsMT1f</i>	ATGTCGTGCGGTGGAAGTTG	CTTTGCCAACTCCCTCAAATT	163
<i>OsMT1g</i>	TTGCAACTGCGGTTTAAGCTG	TTAGCAGTTGCAGGGGTTGCA	214
ROS scavenging			
Os01g22352	CTCGTCGACGGCTTCAAGCT	GTCGATCTCCGTCGTGATC	216
Os04g59150	CATCGTGAGGTGGCACGTCA	CAGAGGCACGTGAAGTAG	310
<i>FeSOD1</i>	GATCCTATGATGGCATGAT	ACATAGTCAGATCTCCGATC	268
<i>FeSOD2</i>	GTATGGAACCATCACTTCT	TCATGCAACTGGGATATTTG	423
<i>MnSOD1</i>	CATGTCAATCATTGATCT	CTGGTTAGCAGTTGTTTCCA	228
<i>Cu-ZnSOD2</i>	TGACACCACTAATGGCTGC	CTAACCCTGGAGTCCGATGA	310
<i>Cu-ZnSOD4</i>	GATACTACGAATGGGTGCA	ACTGAGACTAAGTCTCATG	255
<i>OsCat2</i>	GTGGTGATTGCCAAGGAGA	ACATGCTCGGCTTCGCGCTG	220

<i>OsCatA</i>	TCATGCACCGGGACGAGGAG	TCACATGCTTGGCTTCACG	332
<i>OsCatB</i>	CTCGTGTTCTAACAGGCTGT	CTACATGTTTGGTTTCAGGT	251
ROS producing			
<i>Os04g57560</i>	GAAGTACTGCAGTGGTGCA	AGCTTGTCCCATCTTCAAC	244
<i>OsRbohA</i>	CACTTATGACGATTACCTCA	GACTGGAGCTCCACAGTAGA	976
<i>OsRbohE</i>	TGGTCTTGGGAATTGGTGCTAC	CTTGTGGAAGTGGGAATCTAGT	526
<i>OsRbohF</i>	AGAGATCTGCTGAACAACATC	TCAGAAGTACTCCTTGTGGA	546
Other marker genes			
<i>SGR</i>	CTACCAAACCGAGCCAAAAT	ACCAAACGACTCTTGACAGC	170
<i>RbcS</i>	AGTACTCGAGCAAAGAAGGAGAG	GTGGTTCTCACGGTAGACAAATC	341
<i>OsUBQ5</i>	GACTACAACATCCAGAAGGAGTC	TCATCTAATAACCAGTTCGATTC	365

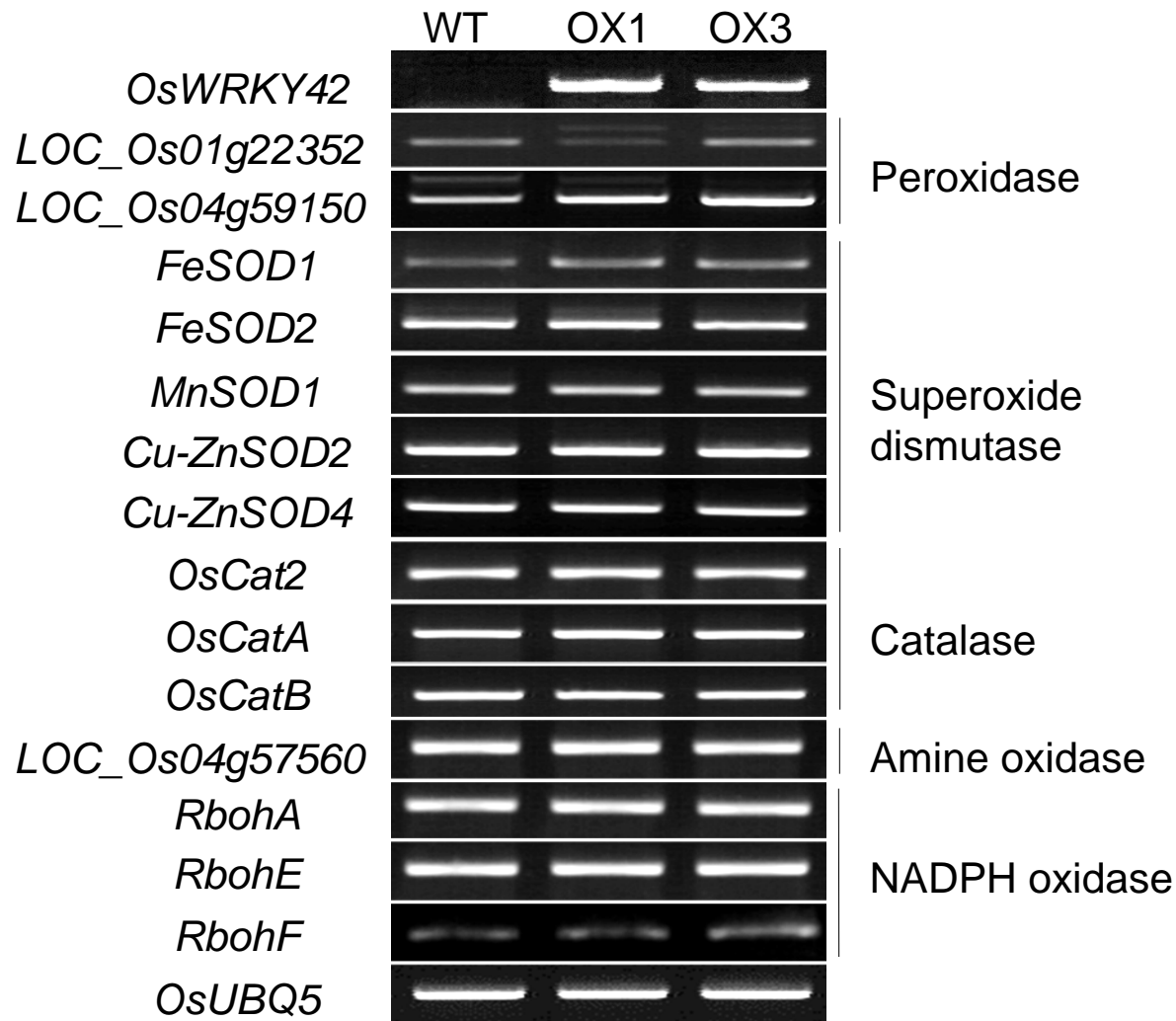
16 *Underlined sequences indicate restriction enzyme digestion sites.



Supplementary Figure 1. Characterization of *OsWRKY42*-overexpressing (*OsWRKY42OX*) independent primary transgenic rice plants. (A) RT-PCR analysis of the *OsWRKY42* expression in 12 independent transgenic lines. *OsUBQ5* was amplified as RT-PCR control. (B) A representative image of *OsWRKY42OX2* (OX2) and *OsWRKY42OX2* (OX7) plants with highly expressing transgenes compared with wild type (WT) rice plants transformed with empty vector.



Supplementary Figure 2. Expression of *OsWRKY42* in tobacco leaves by *Agrobacterium*-mediation. The photo was taken 10 days after inoculation. GV3101, *Agrobacterium* background strain; EV, empty vector; GFP, 35S:*GFP*; OsW42, 35S:*OsWRKY42-cMyc*.



Supplementary Figure 3. RT-PCR analysis of representative ROS producing and scavenging genes in the leaves of *OsWRKY42OX* lines (OX1 and OX2) and wild type (WT) plants. Locus numbers of the ROS producing and scavenging genes are as follows: *LOC_Os01g22352* and *LOC_Os04g59150* (peroxidases), *LOC_Os06g02500* (*FeSOD1*), *LOC_Os06g05110* (*FeSOD2*), *LOC_Os05g25850* (*MnSOD1*), *LOC_Os07g46990* (*Cu-ZnSOD2*), *LOC_Os08g44770* (*Cu-ZnSOD4*), *LOC_Os03g03910* (*OsCat2*), *LOC_Os02g02400* (*OsCatA*), *LOC_Os06g51150* (*OsCatB*), *LOC_Os04g57560* (Amine oxidase), *LOC_Os01g53294* (*OsRbohA*), *LOC_Os01g61880* (*OsRbohE*), *LOC_Os08g35210* (*OsRbohF*). *OsUBQ5* was used RT-PCR control.

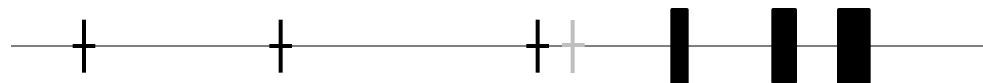
LOC_Os12g38300
(*OsMT1d*)



LOC_Os12g38010
(*OsMT1f*)



LOC_Os12g38290
(*OsMT1g*)



0.5 kb

Supplementary Figure 4. Promoter sequence analysis of *OsMT1d*, *OsMT1f*, and *OsMT1g* genes. †, W-boxes with the consensus sequence TTGACT present within about 1.5 kb upstream regulatory regions. ‡, potential W-boxes with a consensus sequence variant A/CTGACC.