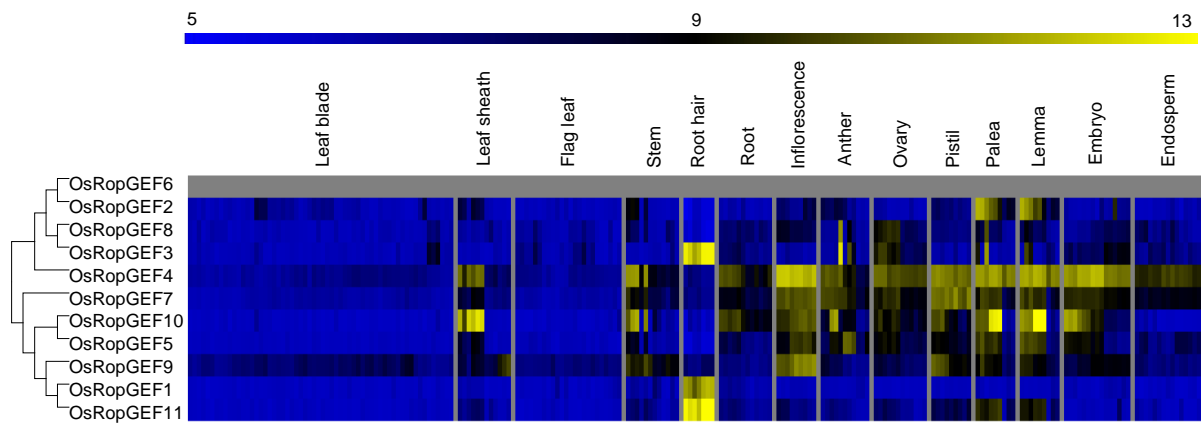
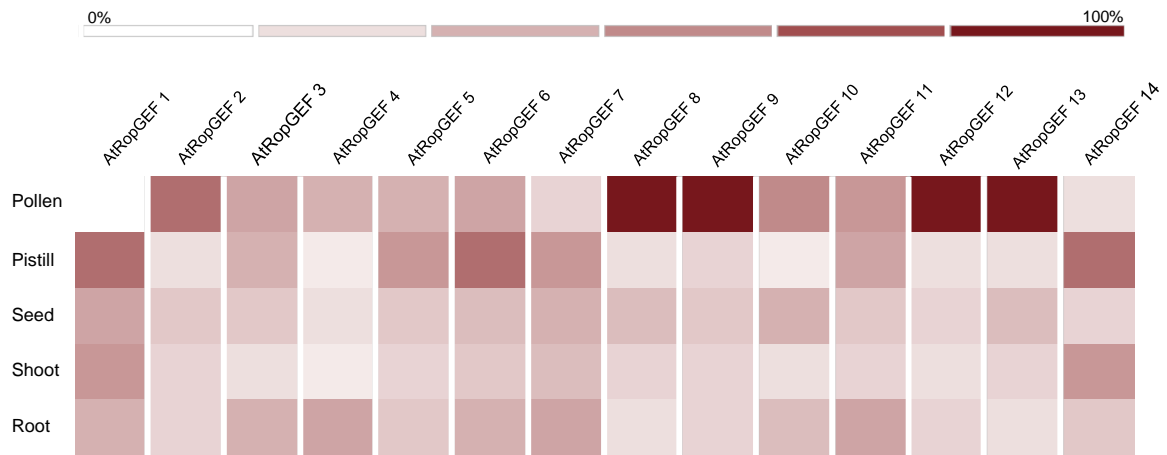


## Additional data

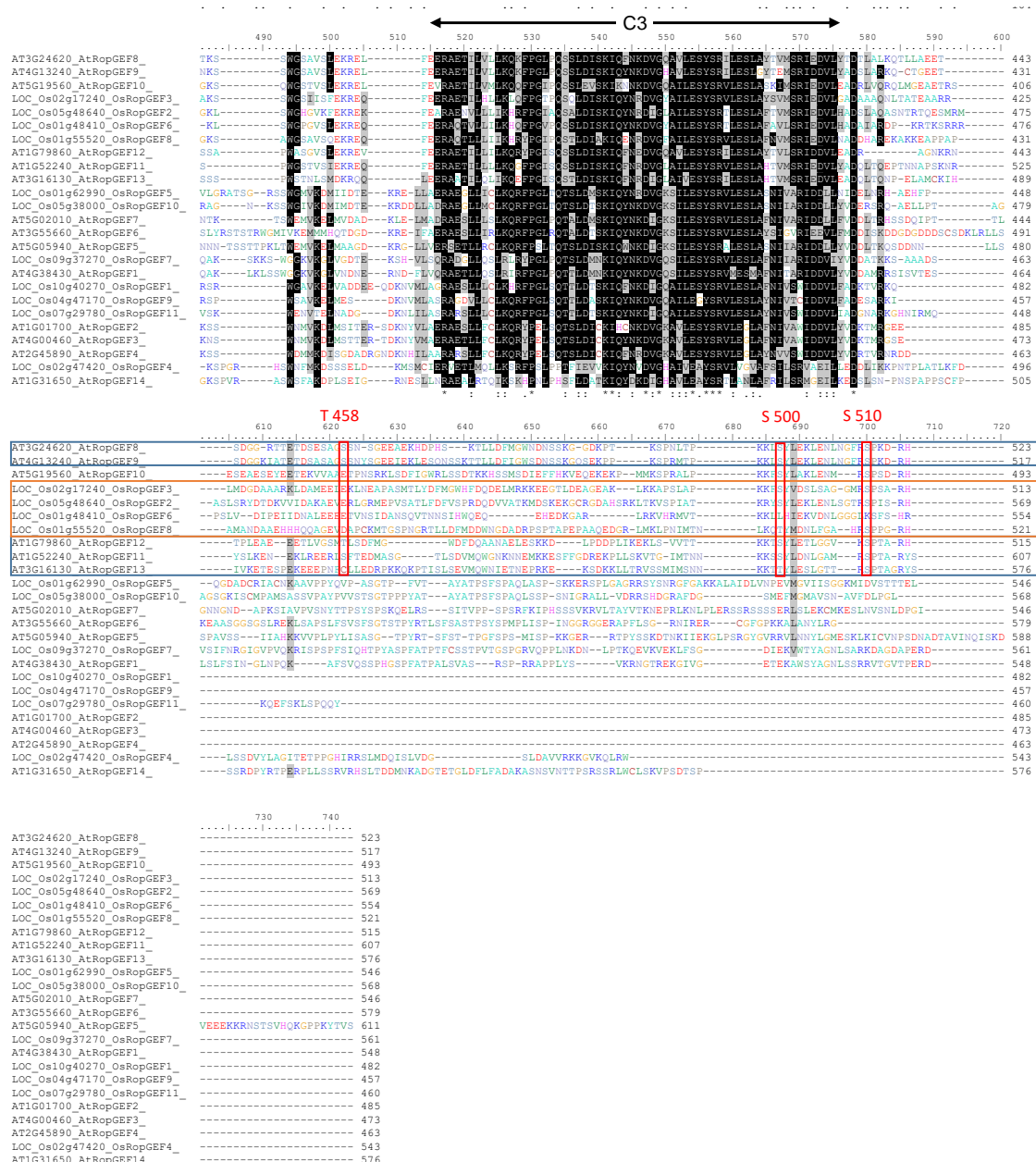


**Additional file 1: Figure S1.** Meta-expression analysis and genome-wide identification of *RopGEF* in rice. The expressions from various rice tissues were examined using Microarray database. Yellow color in the heatmap indicates high level of expression; dark blue, low level of expression. Numeric values indicate the average of the normalized log<sub>2</sub> intensity of microarray data.



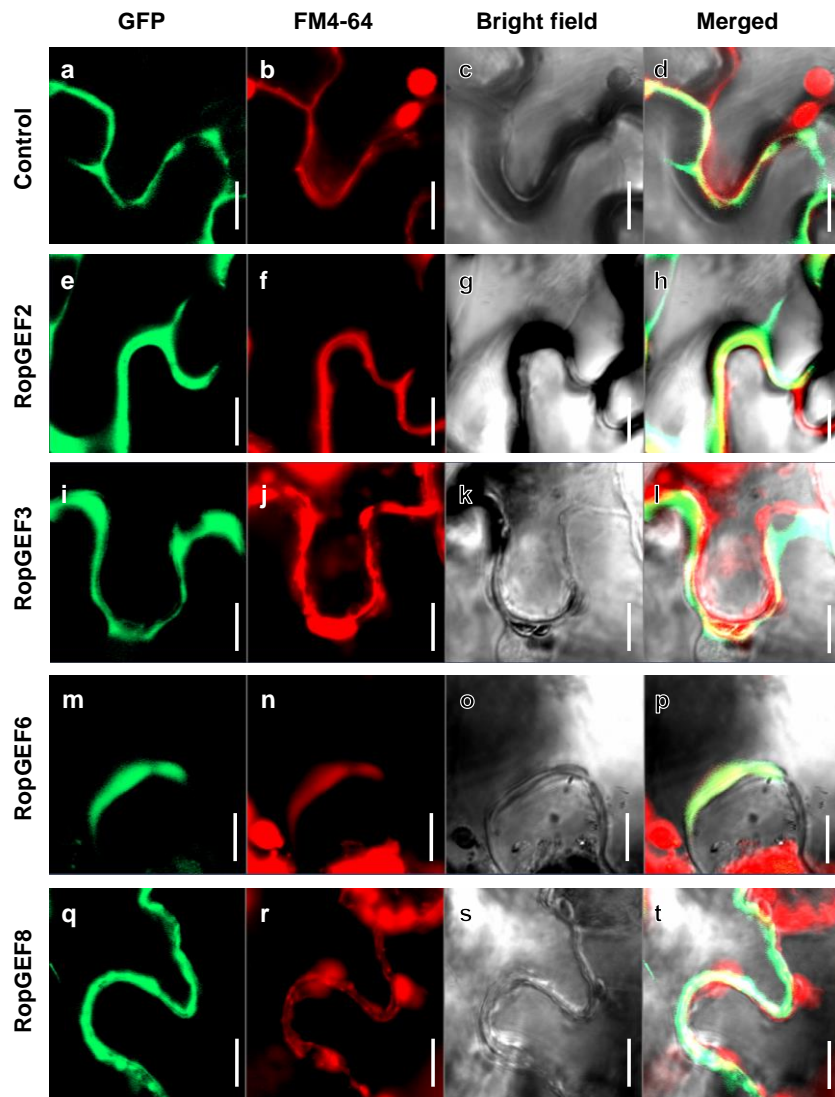
**Additional file 2: Figure S2.** Meta-expression analysis of entire *AtRopGEF* genes. The heatmap was prepared using the Genevestigator. We chose five representative tissues, including pollen. It revealed that five Arabidopsis *RopGEFs* were highly expressed in pollen. The dark red color of the heatmap indicated the highest expression; white color, lowest expression.



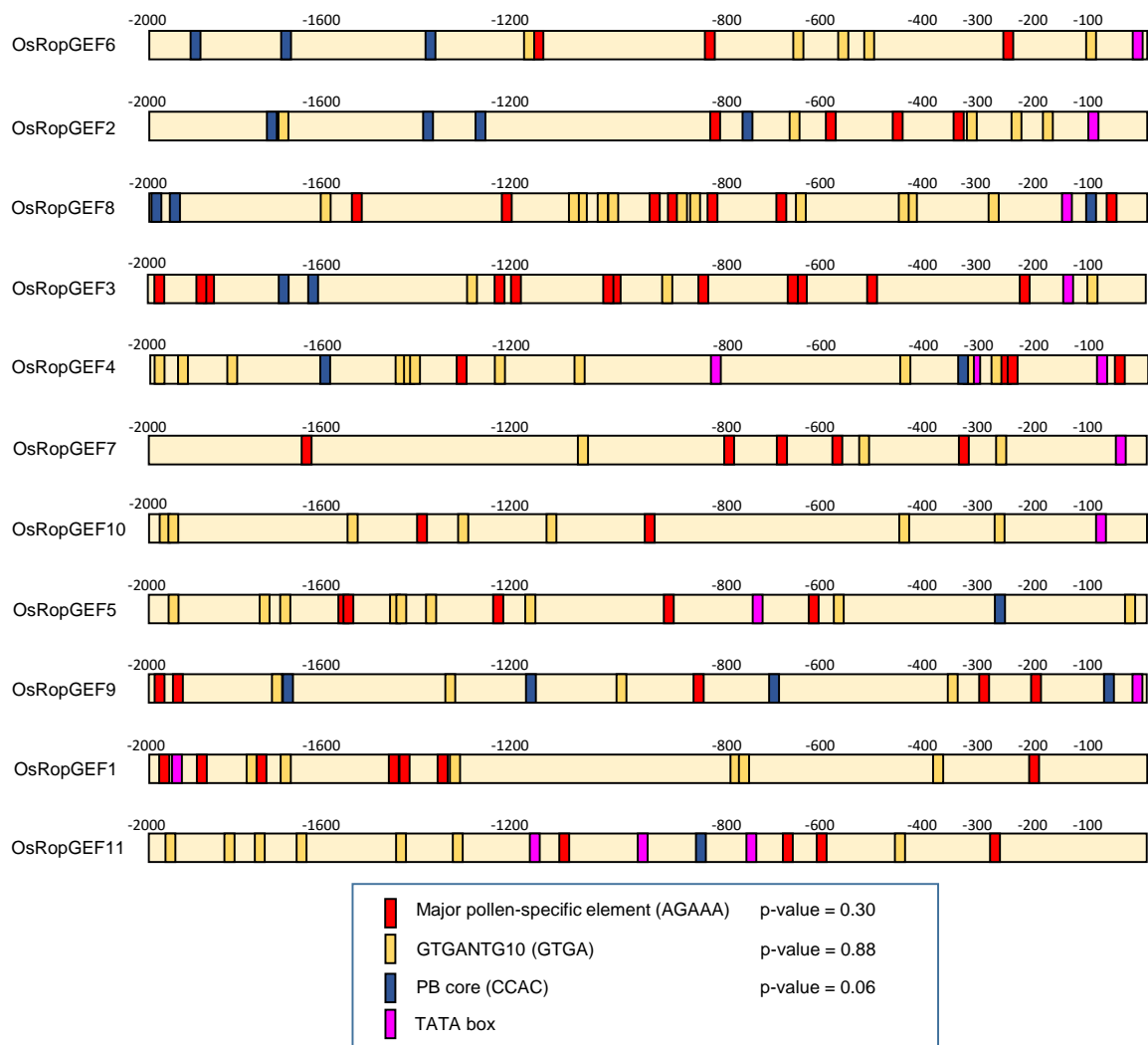


**Additional file 3: Figure S3.** Protein sequence alignment domain analysis and conserved phosphorylated amino acid residues of C-termini of *RopGEF* genes. Every *OsRopGEF* and *AtRopGEF* protein sequence was collected and aligned, followed by the PRONE domain (C1, C2, C3) and the WW-motif. At the end part of the sequence, we found some conserved regions. According to previous studies, S510 in the C-terminus of *AtRopGEF12* is involved in the C-terminal inhibition of GEF activity. As in *AtRopGEF12*, the serine residue is conserved in each

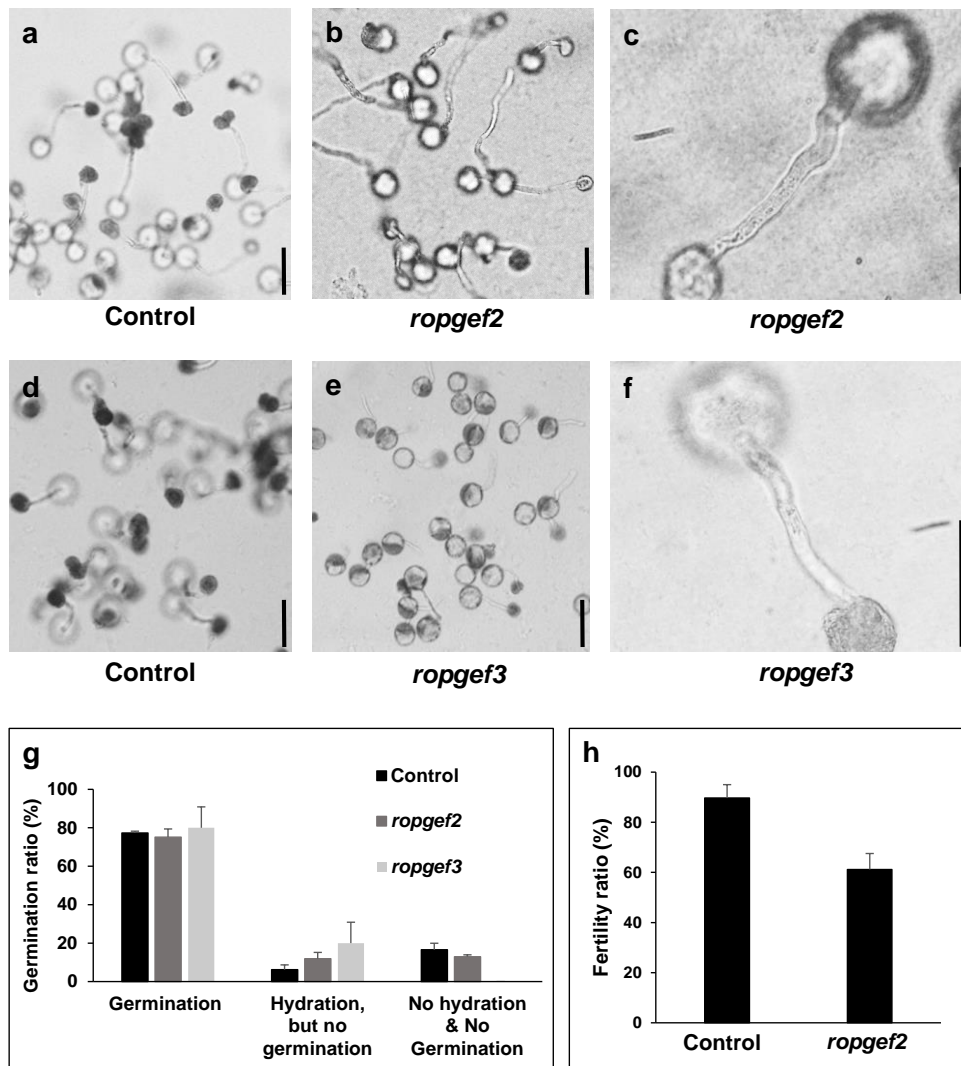
of the *OsRopGEF2*, *OsRopGEF3*, and *OsRopGEF8* genes but not in *OsRopGEF6*. However, K (Lysine) can also be phosphorylated.



**Additional file 4: Figure S4.** Zoom-image of subcellular localization in the tobacco epidermal cells. It shows an enlarged portion of figure 5. The first panels in left side showed control and RopGEF proteins' GFP signal, the second panels showed RFP signal stained with the membrane marker FM4-64, the third panels showed bright image, and the last panels on the right indicate merged images. In addition to the membrane signal, the large spot seen in the RFP channel is due to Chlorophyll's auto fluorescence. a-d, Control (pGREEN-GFP); e-h, RopGEF 2; i-l, RopGEF3; m-p, RopGEF6; q-t, RopGEF8. Bars = 10 um.

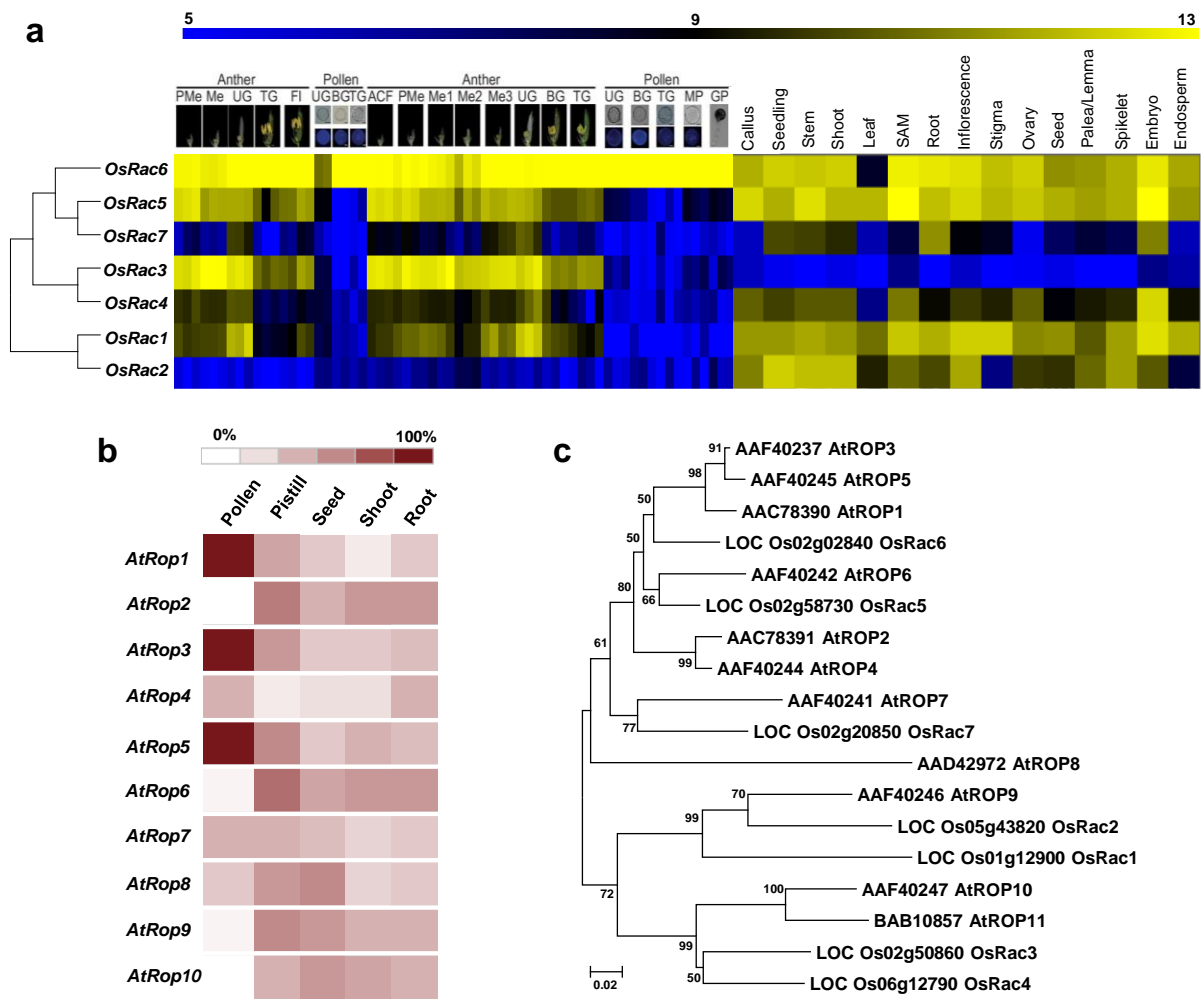


**Additional file 5: Figure S5.** Promoter analysis of each *OsRopGEF* gene. Specific *cis*-acting element (CRE) known to affect expression in pollen were identified using PLACE database, namely three pollen-CREs and the TATA box. The number above the yellow bar shows the upstream position of the promoter base pair when taken at +1 of ATG. We analyzed up to the upstream 2,000-base pair. The p-values indicate how significantly the four genes exhibiting high expression in pollen differed from the other seven genes.



**Additional file 6: Figure S6.** Single mutant assay using CRISPR-Cas9 system. (a-b, d-e) *In-vitro* pollen germination test of wild-type (a, d), *ropgef2* (b, c), and *ropgef3* (e-f) single mutants on solid pollen media, Bar = 50  $\mu$ m; (c,f) enlarged pollen pictures, Bar = 20  $\mu$ m. Each control and mutant pollen in-vitro assays were performed in the same environment on the same day. (g) The ratio of pollen germination. Black bar indicates wild type; grey bar indicates *ropgef2*; white bar indicates *ropgef3* mutant pollen. Error bars are standard deviation of three technical repeats. (h) Fertility ratio comparison between wild-type and *ropgef2* single mutant plants. Error bars are standard deviation of more than three panicles in the plants.





**Additional file 7: Figure S7.** Meta-expression analysis and genome-wide identification of the seven *OsRac* and ten *AtRop* genes. (a) Heatmap expression analysis of *OsRac* gene. (b) Heatmap of *AtRop* using Genevestigator. (c) Phylogenetic tree constructs including every *OsRac* and *AtRop*.

**Additional file 8: Table S1.** Gene identification of RopGEF genes and RAC/ROP genes in rice and Arabidopsis.

<b>Gene</b>	<b>Locus ID</b>	<b>NCBI</b>	<b>Gene</b>	<b>Locus ID</b>	<b>NCBI</b>	<b>Gene</b>	<b>Locus ID</b>	<b>NCBI</b>
		<b>Gene ID</b>			<b>Gene ID</b>			<b>Gene ID</b>
<i>OsRopGEF1</i>	Loc_Os10g40270	None	<i>AtRopGEF1</i>	AT4G38430	830000	<i>AtROP1</i>	AT3G51300	824293
<i>OsRopGEF2</i>	Loc_Os05g48640	None	<i>AtRopGEF2</i>	AT1G01700	839250	<i>AtROP2</i>	AT1G20090	838598
<i>OsRopGEF3</i>	Loc_Os02g17240	None	<i>AtRopGEF3</i>	AT4G00460	827967	<i>AtROP3</i>	AT2G17800	816290
<i>OsRopGEF4</i>	Loc_Os02g47420	None	<i>AtRopGEF4</i>	AT2G45890	819197	<i>AtROP4</i>	AT5G45970	834637
<i>OsRopGEF5</i>	Loc_Os01g62990	None	<i>AtRopGEF5</i>	AT5G05940	831858	<i>AtROP5</i>	AT4G35950	829750
<i>OsRopGEF6</i>	Loc_Os01g48410	None	<i>AtRopGEF6</i>	AT3G55660	824732	<i>AtROP6</i>	AT4G35020	829654
<i>OsRopGEF7</i>	Loc_Os09g37270	None	<i>AtRopGEF7</i>	AT5G02010	830000	<i>AtROP7</i>	AT5G45970	834637
<i>OsRopGEF8</i>	Loc_Os01g55520	None	<i>AtRopGEF8</i>	AT3G24620	822058	<i>AtROP8</i>	AT2G44690	819077
<i>OsRopGEF9</i>	Loc_Os04g47170	None	<i>AtRopGEF9</i>	AT4G13240	826941	<i>AtROP9</i>	AT4G28950	829016

<i>OsRopGEF10</i>	Loc_Os05g38000	None	<i>AtRopGEF10</i>	AT5G19560	832076	<i>AtROP10</i>	AT3G48040	823959
<i>OsRopGEF11</i>	Loc_Os07g29780	None	<i>AtRopGEF11</i>	AT1G52240	841654	<i>AtROP11</i>	AT5G62880	836408
<i>OsRac1</i>	Loc_Os01g12900	4325879	<i>AtRopGEF12</i>	AT1G79860	844325			
<i>OsRac2</i>	Loc_Os02g02840	4328116	<i>AtRopGEF13</i>	AT3G16130	820858			
<i>OsRac3</i>	Loc_Os02g20850	4329132	<i>AtRopGEF14</i>	AT1G31650	840052			
<i>OsROP4</i>	Loc_Os02g50860	4330693						
<i>OsROP5</i>	Loc_Os02g58730	4331272						
<i>OsRacB</i>	Loc_Os05g43820	4339304						
<i>OsRacD</i>	Loc_Os06g12790	4340590						

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**Additional file 9: Table S2.** *OsRopGEF* isogene-specific primers for qPCR and cloning analyses.

<b>Genes</b>	<b>Sense Primers</b>	<b>Antisense Primers</b>
<b>qPCR</b>		
<i>OsRopGEF2</i>	5'-CATTGCTCAGTCAGCTCTTG-3'	5'-TGAAGAACATCTTCGATTTCG-3'
<i>OsRopGEF3</i>	5'-TGACGAGGAAGTGGCTGCAG-3'	5'-AGCGACTCGATGTACACCTC-3'
<i>OsRopGEF6</i>	5'-TTCGGTATTCGGGGAGCAGC-3'	5'-ATGCTGGTTCCATCCGTGGA-3'
<i>OsRopGEF8</i>	5'-CAAGTCTTGGACAACGGCGT-3'	5'-TTGAAGTTGTCCAGGTAATC-3'
<b>pGREEN cloning</b>		
<i>OsRopGEF2</i>	5'-TCGACGGTATCGATAAGCTT ATGGCGCGGCCGCTGCTGA-3'	5'-CAGGAATTCGATATCAAGCTT GTGTCTAGATATTGGGCT-3'
<i>OsRopGEF3</i>	5'-TCGACGGTATCGATAAGCTT ATGGTGCGGTTCCCTCCGG-3'	5'-CAGGAATTCGATATCAAGCTT GTGGCGCGCGGAGGGGCT-3'
<i>OsRopGEF6</i>	5'-TCGACGGTATCGATAAGCTT ATGGTGCGGCGGCACCTG-3'	5'-CAGGAATTCGATATCAAGCTTC CTATGAGAAAAGCTTTTC-3'
<i>OsRopGEF8</i>	5'-TCGACGGTATCGATAAGCTT ATGGCAGCGAGCGGCGGT-3'	5'-CAGGAATTCGATATCAAGCTT ATGGCGACCTGGTGGGCTT-3'
<b>Single CRISPR-Cas cloning</b>		

*OsRopGEF3* 5'-GGCAGTCGAATGCGATCACGAA 5'-AAACGGTTCGTGATCGCATTCCG  
(Target 1) CC-3' AC-3'

*OsRopGEF3* 5'-GGCAAGCGAGTTCTGGTACGAG 5'-AAACTTCTCGTACCAGAACTC  
(Target 2) AA-3' GCT-3'

**Multiple CRISPR-Cas cloning**

*OsRopGEF2* 5'- TAGGTCTCCTCTCCGGGTCCA 5'-CGGGTCTCACATTGAGCTCAA  
GTTTTAGAGCTAGAA-3' TGCACCAGCCGGG-3'

*OsRopGEF6* 5'-TAGGTCTCCAATGTCGCGCGC 5'-CGGGTCTCATCGCCATGATCG  
GTTTTAGAGCTAGAA-3' TGCACCAGCCGGG-3'

*OsRopGEF8* 5'-TAGGTCTCCGCGACGACTTAC 5'-CGGGTCTCAGAGATGCCGCC  
GTTTTAGAGCTAGAA-3' TGCACCAGCCGGG-3'

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