

Figure S1. Timeline analysis of RHID markers. Related to Figure 1.

Quantification of protein accumulation at the RHID throughout the early development of root hairs in 18 different marker lines shown in **Figure 1 & Figure 2**. The evenly distributed membrane protein Lti6b is used as reference for protein accumulation. On the left, representative cells are shown from 6 cells before bulging (-6), to 3 cells after bulging (+3). Images are shown in intensity coding false color to highlight intensity differences. On the right, blots of the average polarity index (signal intensity in/outside RHID) for each developmental step. Error bars indicate SEM; y-axis is in log₂-scale; arrowheads indicate first polarity index significantly greater than reference polarity index of GFP-LTI6B (students t-test, $p < 0.05$); Grey bar indicates transition to bulging between cell -1 and +1. Corresponding genes are listed on the very right. Genes were under control of their own promoter, the *UBI10*-Promoter (P15Y), or induced by estradiol (*). Markers are sorted from earliest accumulation (GEF3*, top) to latest accumulation (GEF11, bottom). No accumulation was found for GFP-LTI6B and GFP-SYP132.

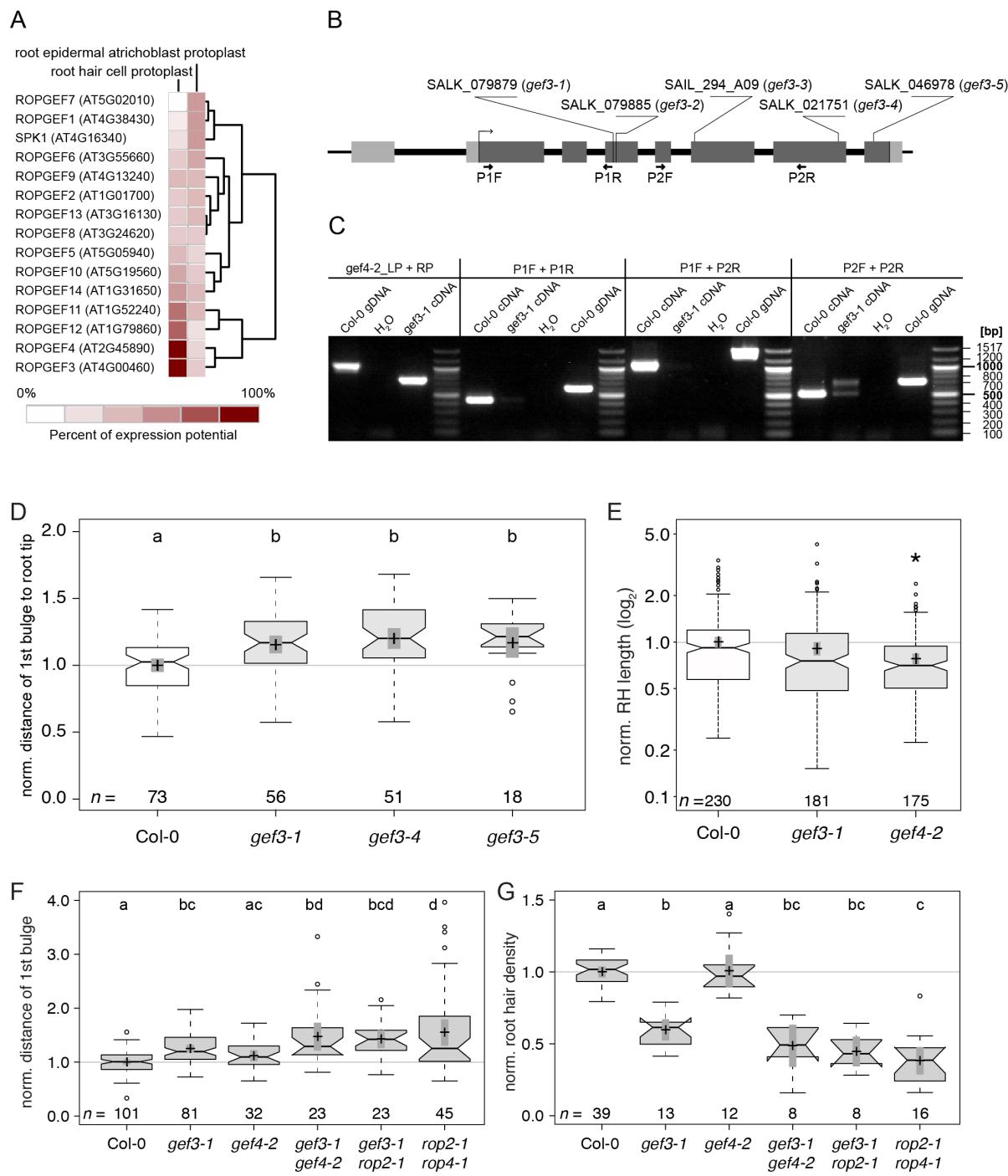


Figure S2. Expression analysis of all *ROPGEFs* and **SPIKE1, gene structure of *GEF3*, and phenotypes of single and double mutants. Related to Figure 2.**

(A) Comparative expression analysis of all *ROPGEFs* and **SPIKE1** in root epidermis cells using hierarchical clustering of published gene expression data in GENEVESTIGATOR. Expression in trichoblasts is compared to atrichoblasts and Genes are sorted from low to high comparative trichoblast expression levels. (B) Gene structure of *GEF3* ([AT4G00460.2](#)) according to [The Arabidopsis Information Resource](#)

(TAIR). Black boxes show introns, grey boxes show exons (light grey: 5' and 3' UTR, dark grey translated regions). Position and ID of used T-DNA insertion lines are indicated. *gef3-1*, *gef3-4* and *gef3-5* were used for phenotyping analysis and further experiments. No homozygous plants were found for *gef3-2* and *gef3-3* in the seeds received from NASC, nor in the following generation. Those lines were not further investigated. Position of primers used for expression analysis of *GEF3* in the *gef3-1* background are indicated by black arrows. (C) Expression analysis of *GEF3* in Col-0 and *gef3-1*. cDNA from seedling roots (10 dag) was used. As a quality control of the *gef3-1* cDNA, *GEF4* was amplified, as it has a similar specific expression pattern compared to *GEF3*. gDNA served as a control for the PCR to exclude gDNA contamination in the samples. H₂O control did not contain any PCR template. Band sizes of ladder are indicated on the right. Three primer sets for *GEF3* were used which amplified fragments in front (P1F+P1R), over (P1F+P2R) and behind (P2F+P2R) the t-DNA insertion site. (D) Normalized distance of first bulge to root tip measured in the indicated mutant (*gef3-4*, *gef3-5*) as compared to Col-0 and *gef3-1*. Letters show results of an ANOVA-Test (significance value 0.01), with same letters indicating no significant differences. (E) Quantification of hair length 3-6 mm away from root tip in *gef3-1* and *gef4-2* mutant lines compared to Col-0. Normalized values are shown in log₂ scale to account for the high variability in individual hairs. Asterisk indicates $p \leq 0.05$ according to two-tailed students t-test. (F,G) Re-plotted data on normalized distance and normalized root hair density for selected mutants for direct comparison of phenotypes. The ANOVA-Test was performed again for this set of mutants; test results are shown as letters. For a detailed explanation of all shown box-plots, see the description given in the legend of Figure 2.

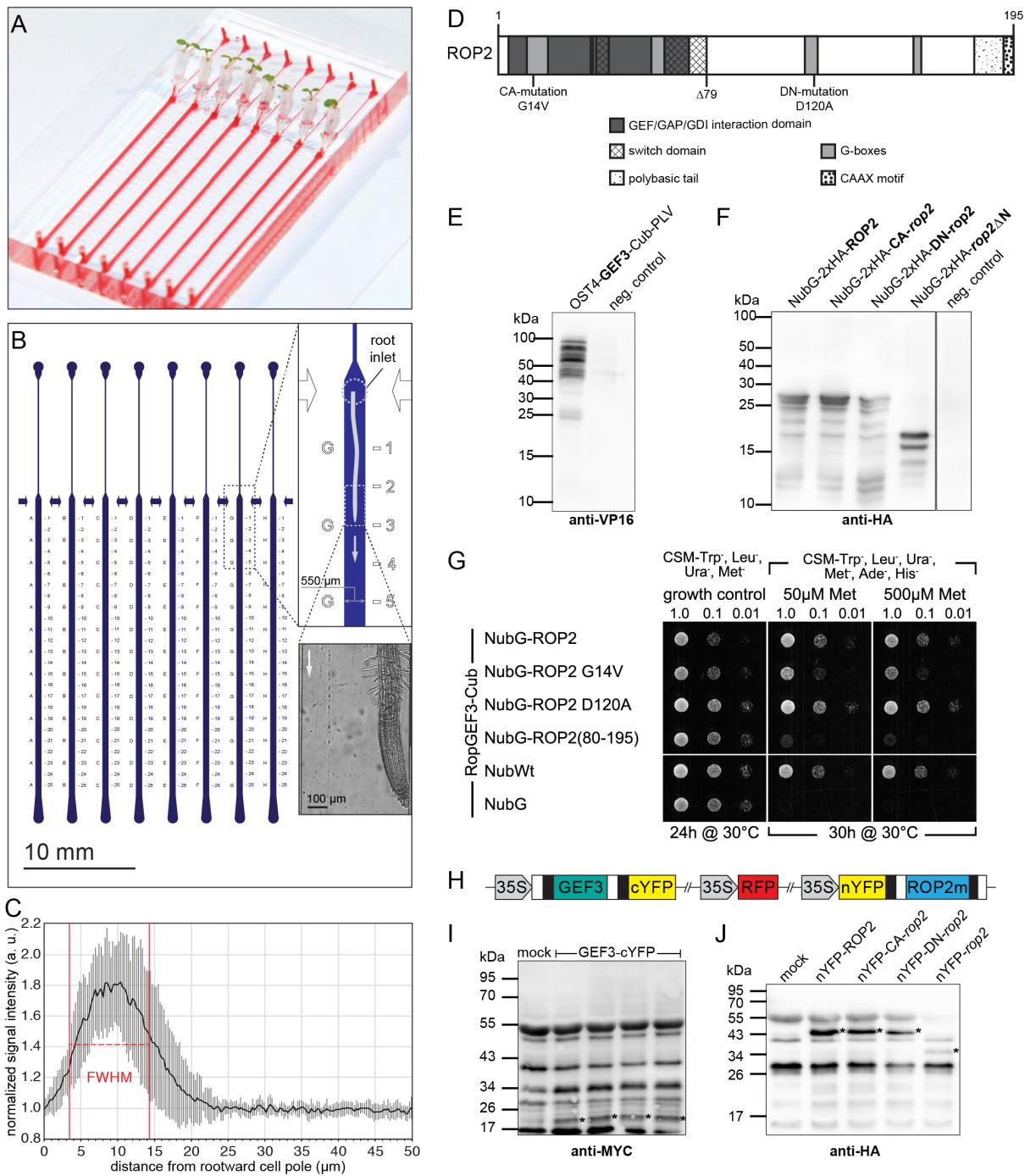


Figure S3. The RootChip-8S, size of the polar GEF3 domain, and physical interaction between GEF3 and ROP2. Related to Figure 3.

(A) Photograph of the RootChip-8S. Channels for sample perfusion are filled with red food color solution to highlight the medium channels. Arabidopsis seedlings grow through medium-filled cones into the perfusion chamber, where the root is imaged from below. (B) Channel design of the RootChip-8S with 8 parallel perfusion chambers and single medium inlets for each perfusion chamber (blue). Seedling inlets are marked by

arrows at the top of the perfusion chambers. Labels next to each chamber help identifying the chamber (letters) and indicate the distance from the root inlet (numbers). The magnified panels show a path of a root (top right, grey sketch; bottom right, brightfield image) grown into the chamber. Grey arrows depict the direction of both medium flow and root growth. Chamber dimensions are 28 x 0.55 x 0.117 (\pm 0.002) mm (LxWxH). **(C)** Dimensions of the mCit-GEF3 domain at the RHID. Average intensity profile (line width 3 px) along the first 50 μ m of the outer cell periphery of 17 trichoblasts just before bulging (stage -1) in GEF3::mCit-GEF3 roots. Starting position was the rootward cell border and values are normalized to the average intensity between 25 μ m and 35 μ m. Full width at half maximum (FWHM) of the GEF3-patch (9.6 μ m \pm 2.5 μ m) is indicated by a dashed line. Error bars indicate standard deviation. **(D)** Schematic protein structure of ROP2. Conserved domains and motives according to [NCBI Conserved Domains](#) are indicated. The GEF/GAP/GDI interaction domain facilitates binding to regulatory proteins. The two switch domains undergo conformational changes upon regulator binding/activity status. The five G-Boxes directly bind GDP/GTP. The polybasic tail helps membrane binding and the CaaX motif is prenylated to facilitate membrane anchorage. The position of the mutations to render ROP2 constitutive active (CA-rop2) or dominant negative (DN-rop2), as well as a deletion at position 79 (rop2 Δ N) to create a mutational construct unable to bind any effectors, are indicated. **(E, F)** Western blots as expression controls for bait **(E)** and prey **(F)** fusion proteins in Split-Ubiquitin-Assay. Asterisks above bands indicate full length constructs. Negative control shows protein extracts of yeast culture transformed with the corresponding ‘empty’ vector. **(G)** Split-ubiquitin-based assay to test for protein-protein interactions of GEF3-Cub with wildtype ROP2, CA-rop2, DN-rop2 and rop2 Δ N (Δ 1-79), respectively. Wildtype NubI (NubWT) served as positive and NubG as negative control. **(H)** Construct used for rBiFC interaction assay. Fusion proteins and the ratiometric marker protein (RFP) are expressed from the same plasmid. GEF3 was C-terminally fused to cYFP and different versions of ROP2 N-terminally tagged with nYFP. **(I, J)** Western blots confirming expression of fusion proteins in rBiFC analysis. Extracts of leaves infiltrated with non-agrobacterium containing medium show cross-reactivity of antibodies with plant proteins (‘Mock’). In **(I)** asterisks indicate degradation product of GEF3-cYFP. The full-length product could not be detected as this is most likely masked by the background band at \sim 55kDa. In **(J)** asterisks indicate full length constructs.

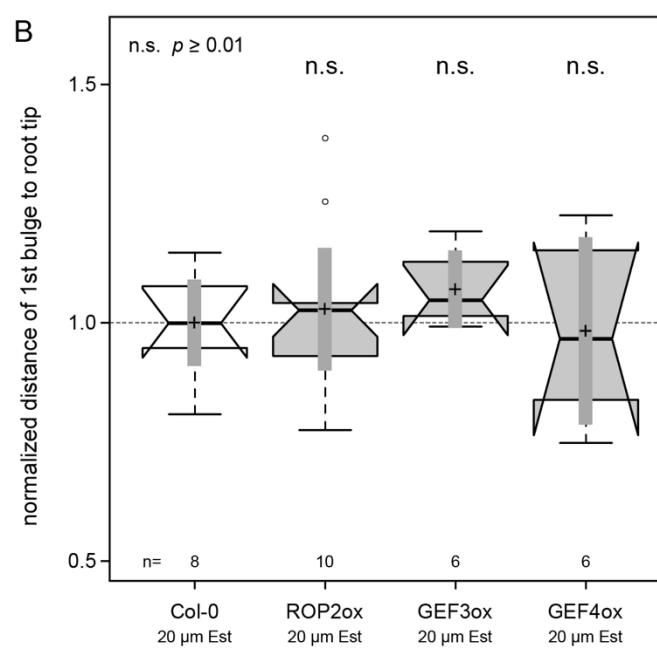
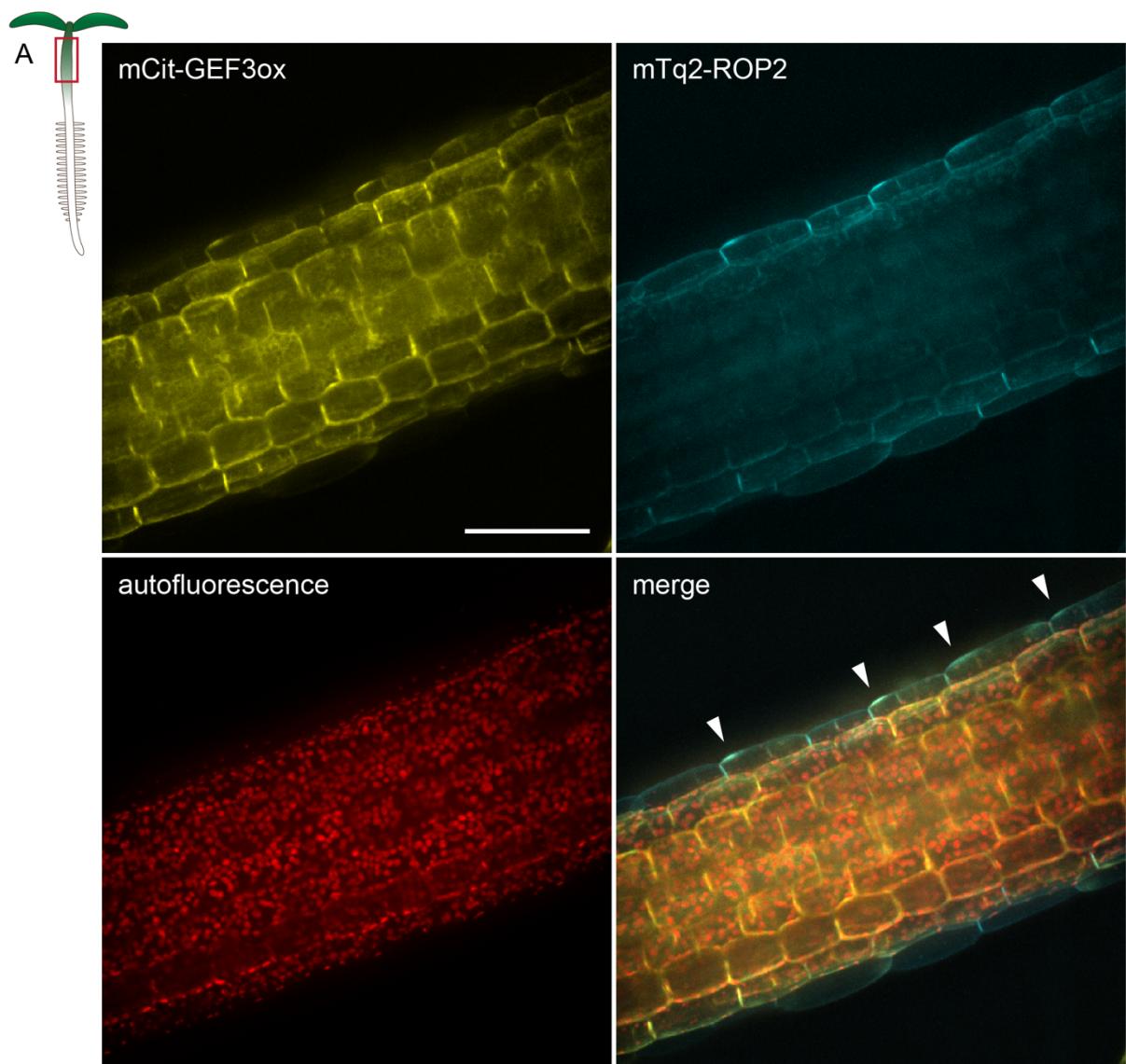


Figure S4. Ectopic, GEF3-induced polar domains in the hypocotyl epidermis, and analysis of the timing of hair emergence upon mCit-GEF3 overexpression. Related to Figure 4.

(A) Overview representations of ectopic RHID-like domain formation upon induced overexpression of mCit-GEF3ox in the epidermis of the hypocotyl. Polarized mCit signal was occasionally observed (arrow heads), which then frequently coincided with ectopic accumulation of mTq2-ROP2 (expression under the control of the endogenous promoter). In 16 independent seedlings, out of 35 cells with sufficient signal of both mCit-GEF3 and mTq2-ROP2, 30 cells showed a detectable co-accumulation of both markers, five cells showed only accumulation of mCit signal. (B) Normalized distance of first bulge to root tip measured in Col-0 and overexpression lines of mCit-ROP2, mCit-GEF3 and mCit-GEF4 (24h after Estradiol induction). Values were normalized to corresponding Col-0 values for each independent experiment. For a detailed explanation of all shown box-plots, see the description given in the legend of Figure 2. The value of significance (p) was determined by a two tailed students t-test (n. s.= not significantly different).

SUPPLEMENTAL REFERENCE

- S1. Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G., and Yang, Z. (2005). Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* 120, 687–700.

Gene	Gene-ID	Mutant allele	Ecotype	Mutant ID	Mutant NASC ID
ARPC2A	AT1G30825				
FERONIA	AT3G51550				
GEF3	AT4G00460	gef3-1	Col-0	SALK_079879C	N868983
		gef3-2	Col-0	SALK_079885	N579885
		gef3-3	Col-0	SAIL_294_A09	N813628
		gef3-4	Col-0	SALK_021751	N868675
		gef3-5	Col-0	SALK_046978C	N662351
GEF4	AT2G45890	gef4-2	Col-0	SALK_107520	N607520
GEF10	AT5G19560	gef10-1	Col-0	SALK_009456	N653017
GEF11	AT1G52240	gef11-1	Col-0	SALK_126725C	N663923
GEF12	AT1G79860				
GEF14	AT1G31650	gef14-2	Col-0	SALK_046067	N546067
LTI6b	AT3G05890				
PCaP2	AT5G44610				
PIP5K3	AT2G26420				
RHD2	AT5G51060				
ROP2	AT1G20090	rop2-1	Col-0	SALK_055328C	N675195
ROP4	AT1G75840	rop4-1	WS	Wisconsin T-DNA line described by Fu et al. [S1].	
ROP6	AT4G35020				
Sec3a	AT1G47550				
SYP123	AT4G03330				
SYP132	AT5G08080				

Table S1. List of identifiers for genes and single mutant alleles used in this study.

GreenGate Cloning

Double Underlined: Bsal-Sites with standart GreenGate-Overhangs;

Underlined: Bsal-Sites for fusion of multiple PCR-Fragments into one Vector;

Bold: Bases different to template for mutation

Cloning of standart modules for GrenGate cloning	Cloning of base entry vector with AD-overhangs	agtgaagct <u>tGGTCTCaACCT</u> ccggat gcgagaatt <u>cGGTCTCaCTG</u> Aggtaccac	oPD0001-fwd oPD0001D-rev	
	Estradiol inducible Promoter module into pGGA	aaca <u>GGTCTCtACCT</u> gcaacaacgaatgttcataactc aaca <u>GGTCTCaCTG</u> Atctggttcgtatggctgttctc aaca <u>GGTCTCaTCAG</u> cgatcgaccaggatccac aaca <u>GGTCTCaTGT</u> tcagcggtccatccaaatgaa	oPD0120-fwd oPD0120-rev oPD0121-fwd oPD0121-rev	
	mCitrine or mTurquoise2 into pGGC, including GAGAGA-Linker	aaaa <u>GGTCTCaTCAGGAGCAGGGCGGG</u> TGCCATGGTGAGCAA GGGCGAG aaaa <u>GGTCTCaGCAGTTACTTG</u> TACAGCTCGTCCA	oPD0002-fwd oPD0002-rev	
	mCitrine or mTurquoise2 into pGGB, including GAGAGA-Linker	aaaa <u>GGTCTCaAACAA</u> TGGTGAGCAAGGGCGAGGA aaaa <u>GGTCTCaAGCCCCCG</u> CTCCTGCTCCCTGTACAGCTCGTC CATGCC	oPD0012-fwd oPD0012-rev	
	HSP18.2 Terminator into pGGE	aaca <u>GGTCTCtCTG</u> Catatgaagatgaagatgaaatattgg aaca <u>GGTCTCaTAGT</u> tttatcttaatcatattccatagtcc	oPD0064-fwd oPD0064-rev	
	GEF3 Promoter with GG-Overhang A GEF3 Promoter with GG-Overhang B	aaca <u>GGTCTCtACCT</u> tcgatcaacccttcacggt aaca <u>GGTCTCaTGT</u> ttttaaaatctaaaacactcctaaaggct	oPD0190-fwd oPD0190-rev	
-terminal tagging only)	GEF3 ORF	Part A with GG-Overhang C	aaca <u>GGTCTCaGGCT</u> ATGGAGATTATCGAATCCAGATGA	oPD0191A-fwd
		Part A for Bsal-Site mutation	aaca <u>GGTCTCCACTACAGAAGCTGTTGGTT</u> CATCTATCTGGTCT GAAAAACCCTGAAGTGGTCTGAAC	oPD0191A-rev
		Part B	aaca <u>GGTCTCGTAGTGAAGCTTCTCCTTGTAACTG</u>	oPD0191B-fwd
		Part B with GG-Overhang D	aaca <u>GGTCTCaCTGATTATTC</u> ACTACCTCTCATGGTTTGT	oPD0191B-rev
	GEF4 Promoter with GG-Overhang A GEF4 Promoter with GG-Overhang B	aaca <u>GGTCTCtACCT</u> tcataatgtgcataagctgctc aaca <u>GGTCTCaTGT</u> tcacgttgtataatgtcaatgttt	oPD0067-fwd oPD0067-rev	
	GEF4 ORF with GG-Overhang C GEF4 ORF with GG-Overhang D	aaca <u>GGTCTCtGGCT</u> ATGGAGAGTTCTCGAATTCCGA aaca <u>GGTCTCtCTGACTAATCATCTGTTCT</u> ACTGTTCT	oPD0068-fwd oPD0068-rev	

GEF10 ORF	GEF10 Promoter with GG-Overhang A	aaca <u>GGTCTCtACCT</u> ggattgtgacattgagatctaccg	oPD0069-fwd
	GEF10 Promoter with GG-Overhang B	aaca <u>GGTCTCaTGT</u> tcttctaaaactaaagatctggcca	oPD0069-rev
	Part A with GG-Overhang C	aaca <u>GGTCTCtGGCT</u> ATGTTGATGGTCGGAACCTCT	oPD0070A-fwd
	Part A	aaca <u>GGTCTCCTG</u> CCCACCGCCTGACAT	oPD0070A-rev
	Part B for Bsal-Site mutation	aaca <u>GGTCTCGGACAGGGGAAACCTCTGCG</u>	oPD0070B-fwd
	Part B	aaca <u>GGTCTCTCGAAAAGCTCCCTTCTCT</u>	oPD0070B-rev
	Part C for Bsal-Site mutation	aaca <u>GGTCTCTTCAAGTCCGAGCTGAACCATTG</u> GG	oPD0070C-fwd
	Part C with GG-Overhang D	aaca <u>GGTCTCtCTGATCAGTGTCAGTAGGGCT</u>	oPD0070C-rev
	GEF11 Promoter with GG-Overhang A	aaca <u>GGTCTCtACCT</u> tttgattttgggttttgtcgcc	oPD0079-fwd
	GEF11 Promoter with GG-Overhang B	aaca <u>GGTCTCaTGT</u> tcttctctatctctctctcaataac	oPD0079-rev
	GEF11 ORF with GG-Overhang C	aaca <u>GGTCTCtGGCT</u> ATGTTGGAAGGCAAAGCAATGG	oPD0080-fwd
	GEF11 ORF with GG-Overhang D	aaca <u>GGTCTCtCTGATCAGGAGTATCTTGCGGTTGG</u>	oPD0080-rev
	GEF12 Promoter with GG-Overhang A	aaca <u>GGTCTCtACCT</u> atccctttctgttttttttatttcttc	oPD0139-fwd
	GEF12 Promoter with GG-Overhang B	aaca <u>GGTCTCaTGT</u> tcctggccctgtatggcaatagag	oPD0139-rev
GEF12 ORF	Part A with GG-Overhang C	aaca <u>GGTCTCtGGCT</u> ATGGTTCGTGCTTCGGAACA	oPD0140A-fwd
	Part A for Bsal-Site mutation	aaca <u>GGTCTCGATCCGGTGGCAGCCTCATCTAACCAAGTCTCGA</u> C	oPD0140A-rev
	Part B	aaca <u>GGTCTCCGGATCCCAGACGCTGAA</u>	oPD0140B-fwd
	Part B	aaca <u>GGTCTCTCTCGCTTCTCCAGACTTACTC</u>	oPD0140B-rev
	Part C for Bsal-Site mutation	aaca <u>GGTCTCGCGAGAGGTCTCGAAGAGCGAGCTGAACCATT</u> TTG	oPD0140C-fwd
	Part C with GG-Overhang D	aaca <u>GGTCTCtCTGATCAATGCCGTGCCGTTGG</u>	oPD0140C-rev
	GEF14 Promoter with GG-Overhang A	aaca <u>GGTCTCtACCT</u> GATGCATTGGTTGCTCACTTCA	oPD0141-fwd
	GEF14 Promoter with GG-Overhang B	aaca <u>GGTCTCaTGT</u> ccttctctctttgaattctttgtga	oPD0141-rev
GEF14 ORF	Part A with GG-Overhang C	aaca <u>GGTCTCtGGCT</u> ATGATGCTGATGAGAAGAAGGTTG	oPD0142A-fwd
	Part A	aaca <u>GGTCTCactataaagaaaaccccaccttagtacat</u>	oPD0142A-rev
	Part B for Bsal-Site mutation	aaca <u>GGTCTCtatagtcaaattctgaacAgtctctaatg</u>	oPD0142B-fwd
	Part B for Bsal-Site mutation	aaca <u>GGTCTCTTACGATCAAAATGTTGAGATCTTCAG</u>	oPD0142B-rev
	Part C	aaca <u>GGTCTCTGCTAAGGAGAAGAAGAAACAAGGC</u>	oPD0142C-fwd
	Part C	aaca <u>GGTCTCGGAGCTAGGGAAAGCAACTCG</u>	oPD0142C-rev
	Part D for Bsal-Site mutation	aaca <u>GGTCTCAGCTCCGTGACCTTATAGGACACCTGAAGACCTC</u>	oPD0142D-fwd
	Part D with GG-Overhang D	aaca <u>GGTCTCtCTGAAGGAGAAGTATCAGAAGGCACTTAC</u>	oPD0142D-rev
PCaP2 ORF	PCaP2 ORF with GG-Overhang C	aaca <u>GGTCTCaGGCT</u> ATGGGTTATTGGAAGTCGAAGGT	oPD0129-fwd
	PCaP2 ORF with GG-Overhang D	aaaa <u>GGTCTCtCTGAAGCCTTTGTGGCGCAGCCG</u>	oPD0003-rev
	Part A with GG-Overhang A	aaca <u>GGTCTCtACCT</u> gcacatctctgtcatctacatcc	oPD0060A-fwd

pGGA000 for promoters, pGGC000 for ORF/CDS and pGGAD000 for Promoter-ORF fusion (C-terminus)

Cloning of Promoters and ORFs and ORF/CDS into GreenGate entry vector	PIP5K3 (Promoter and ORF) for cloning into pGGAD	Part A with GG-Overhang A	aaca <u>GGTCTCCAGCCGTATCACTCACCTCG</u>	oPD0060A-rev
		Part B	aaca <u>GGTCTCCGGCTGCCGAGATTAGAATAGT</u>	oPD0060B-fwd
		Part B for Bsal-Site mutation	aaca <u>GGTCTCGATCCAAGTCTTGAGTGTG</u> T <u>cGTCTCGTCG</u>	oPD0060B-rev
		Part C for Bsal-Site mutation	aaca <u>GGTCTCTGGATCTCAAGTATGTGTTGACTCGA</u> aACCTCA TGG	oPD0060C-fwd
		Part C for Bsal-Site mutation	aaca <u>GGTCTCCTCGTCACGCATGCCAGATTACGGAAATG</u> gAGA CCAATC	oPD0060C-rev
		Part D	aaca <u>GGTCTCGACGACATTCCCTGGGCATC</u>	oPD0060D-fwd
		Part D with GG-Overhang D	aaca <u>GGTCTCtCTGATTGCTTCAATGAATA</u> TTTGT	oPD0060D-rev
	RHD2 ORF	Part A with GG-Overhang C	aaca <u>GGTCTCaGGC</u> ATGTCTAGAGTGAGTTGAAGTGT	oPD0087A-fwd
		Part A	aaca <u>GGTCTCcACAC</u> cgctttattataatgaatttagt	oPD0087A-rev
		Part B for Bsal-Site mutation	aaca <u>GGTCTG</u> ggggagacGacaactaaaa	oPD0087B-fwd
		Part B	aaca <u>GGTCTCCCTCGTACTTCTGAGTCTTGTG</u> C	oPD0087B-rev
		Part C for Bsal-Site mutation	aaca <u>GGTCTCACGGAGGTGGTT</u> ACTAGTTGG <u>t</u> AGGGATTGG	oPD0087C-fwd
		Part C with GG-Overhang D	aaca <u>GGTCTCaCTGATTAGAAATTCTCTTG</u> GGAAAGGA	oPD0087C-rev
	ROP2 Promoter with GG-Overhang A	aaca <u>GGTCTCtACCI</u> gacaaaataattataagaagctaccgtctg	oPD0038-fwd	
	ROP2 Promoter with GG-Overhang B	aaaa <u>GGTCTCtTGTT</u> ctcgccgcaagatcgaaaa	oPD0006-rev	
	ROP2 ORF & CDS with GG-Overhang C	aaaa <u>GGTCTCaGGC</u> ATGGCGTCAAGGTTATAAAGTGTG	oPD0007B-fwd	
	ROP2 ORF & CDS with GG-Overhang D	aaaa <u>GGTCTCtCTGAT</u> CACAAGAACCGCGAACCGGT	oPD0007-rev	
	ROP4 ORF with GG-Overhang C	aaaa <u>GGTCTCaGGC</u> ATGAGTGCTCGAGGTTAT	oPD0009-fwd	
	ROP4 ORF with GG-Overhang D	aaaa <u>GGTCTCtCTGAT</u> CACAAGAACACCGCAGCGGT	oPD0009-rev	
	ROP6 ORF with GG-Overhang C	aaaa <u>GGTCTCaGGC</u> ATGAGTGCTCAAGGTTAT	oPD0011-fwd	
	ROP6 ORF with GG-Overhang D	tttt <u>GGTCTCtCTGAT</u> CAGAGTATAGAACAAACCTT	oPD0011-rev	
Mutagenesis of ROP2	rop2 ^{CA} -mutation (G14V) with oPD0007-rev Primer	aaca <u>GGTCTCtGGC</u> ATGGCGTCAAGGTTATAAAGTGTGACC GTCGGAGATG <u>t</u> GCCGTCGG	oPD0092-fwd	
	rop2 ^{DN} -mutation (D120A) with oPD0007B-fwd Primer	aaca <u>GGTCTCTTG</u> CCCAACAAGGATAATGGGAA	oPD0093A-rev	
	rop2 ^{DN} -mutation (D120A) with oPD0007-rev Primer	aaca <u>GGTCTCGGACAAAC</u> ACTCG <u>c</u> TCTCGAGAT	oPD0093B-fwd	
	rop2 ^{A20} -mutation with oPD0007-rev Primer	aaca <u>GGTCTCtGGC</u> ITGCATGCTCATTTCTACACTAGC	oPD0094-fwd	
	rop2 ^{A43} -mutation with oPD0007-rev Primer	aaca <u>GGTCTCtGGC</u> ATGTGCTAATGTGGTTGATGG	oPD0095-fwd	
	rop2 ^{A80} -mutation with oPD0007-rev Primer	aaca <u>GGTCTCtGGC</u> TTCAATTCTGCTTCTCTTATTAGCA	oPD0096-fwd	
Gef3	Gef3-P1F	Expression Analysis of Gef3	TGAAAACGACGATCATCAATCACC	oPD0231-fwd
	Gef3-P1R	Expression Analysis of Gef3	GGCTCTAACCTCAGATTCTGTCC	oPD0231-rev
	Gef3-P2F	Expression Analysis of Gef3	TGGAGAGTAGACCAAGAGCAGA	oPD0232-fwd
	Gef3-P2R	Expression Analysis of Gef3	TCTCCATGTGTACATCGAAGCT	oPD0232-rev

Expression test and Genotyping of t-DNA lines	gef3-1_LP	Genotyping of gef3-1 (SALK_079879C) & gef3-2 (SALK_079885)	TCGAATCCAGATGAAAACGAC TCCTGAATGATCCAGTCGAAG	oPD0197-LP oPD0197-RP
	gef3-1_RP			
	gef3-3_LP	Genotyping of gef3-3 (SAIL_294_A09)	CGCTGTTCAAACAGAAGAGG CAGAACATCTGAGGTTAGAGCCG	oAR012-LP oAR012-RP
	gef3-3_RP			
	gef3-4_LP	Genotyping of gef3-3 (SALK_021751)	AGAAAGGAGCAAAAGCTTGG GATTCATAAAGCTGCAATGGC	oAR013-LP oAR013-RP
	gef3-4_RP			
	gef3-5_LP	Genotyping of gef3-5 (SALK_046978C)	TCGAAGATGGGACAAGTCAG TGCAGTGTGGTAAAAGCAGTG	oAR014-LP oAR014-RP
	gef3-5_RP			
	gef4-2_LP	Genotyping of gef4-2 (SALK_107520)	AACCTTCAGCAGGAACACATG AGAGTTCTCGAATTCCGACC	oPD0181-LP oPD0181-RP
	gef4-2_RP			
	gef10-1_LP	Genotyping of gef10-1 (SALK_009456)	TTGACCGAAATAAGAAGTCCTC TTTGAAACGATGTGGTGTGG	oPD0110-LP oPD0110-RP
	gef10-1_RP			
	gef11-1_LP	Genotyping of gef11-1 (SALK_126725C)	TCAGAGAGAGGTCAAAATTGAGG TACCTGCGAGATTGGTAATGG	oPD0168-LP oPD0168-RP
	gef11-1_RP			
	gef14-2_LP	Genotyping of gef14-2 (SALK_046067)	TGGTAAGACACCGAAACTTGC TGCTGATGAGAAGAAGGTTG	oPD0176-LP oPD0176-RP
	gef14-2_RP			
	rop2-1_LP	Genotyping of rop2-1 (SALK_055328C)	TCGAATTGGGTGATTCTCAG TGTGGACTCGAAAGATTCAACC	oPD0104-LP oPD0104-RP
	rop2-1_RP			
	rop4-1_LP	Genotyping of rop4-1 (Wisconsin t-DNA line, Fu et al. 2005, Cell)	aaaaGGTCTCaGGCTATGAGTGCTCGAGGTTAT aaaaGGTCTtCTGATCACAAGAACACGCAGCGGT	oPD0009-fwd oPD0009-rev
	rop4-1_RP			
	LB1.3x_Salk	internal Primer for Salk t-DNA lines	GGATTGGCCGATTCGGAACCACC	
	LB3_Sail	internal Primer for Sail t-DNA lines	TAGCATCTGAATTCATAACCAATCTCGATACAC	
	JL-202	internal Primer for Wisconsin t-DNA lines	CATTTTATAATAACGCTGCGGACATCTAC	

Gateway cloning

Small Letters: attB Site

Red: Additional bases for correct reading frame

Cloning of PCR fragments for Gateway cloning	
DM-GEF3 -attB1	ggggacaagttgtacaaaaaaaggcaggct CT ATGGAGAATTATCGAATCCAGATG
DM-GEF3 -attB2-ST	ggggaccacttgcataagaaaaggctgggt TT ATTCACTACCTCTCATGGTTTG
DM-GEF3 -attB2-wo	ggggaccacttgcataagaaaaggctgggt TT CACTACCTCTCATGGTTTG
DM-GEF3 -attB4-ST	ggggacaacttgcataaaaaaggctgggt TT ATTCACTACCTCTCATGGTTTG
DM-GEF3 -attB4-wo	ggggacaacttgcataaaaaaggctgggt TT CACTACCTCTCATGGTTTG
DM-ROP2 -attB1	ggggacaagttgtacaaaaaaaggcaggct CT ATGGCGTCAAGGTTATAAAGTGTG
DM-ROP2 -attB2-ST	ggggaccacttgcataagaaaaggctgggt TC ACAAGAACGCGAACGG
DM-ROP2 -attB3	ggggacaacttgcataataaaaagg CT ATGGCGTCAAGGTTATAAAGTGTG
DM-rop2ΔN -attB1	ggggacaagttgtacaaaaaaaggcaggct CT TTCATTCTGCTTCTCTTATTAGC
DM-rop2ΔN -attB3	ggggacaacttgcataataaaaagg CT TTCATTCTGCTTCTCTTATTAGC

Table S2. List of PCR primers used for GreenGate and Gateway cloning.

	Promoter	N-terminal Tag	ORF / CDS	C-terminal Tag	Terminator	Plant selection marker	Plant Expression Vector	Bacterial selection marker	Plasmid ID	
	Module A	Module B	Module C	Module D	Module E	Module F	Module Z			
GreenGate expression vectors	GEF3-mCitrine	GEF3-Promoter	mCitrine w/ Linker	GEF3-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0332
	GEF3-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF3-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0333
	GEF3-mTurquoise2 (inducible)	Ubi-XVE_oLexA-35S	mTurquoise2 w/ Linker	GEF3-ORF	Decoy	HSP18.2	Kanamycin	pGGZ003	Spec/Strep	pPD0334
	GEF4-mCitrine	GEF4-Promoter	mCitrine w/ Linker	GEF4-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0301
	GEF4-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF4-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0202
	GEF10-mCitrine	GEF10-Promoter	mCitrine w/ Linker	GEF10-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0302
	GEF10-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF10-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0203
	GEF11-mCitrine	GEF11-Promoter	mCitrine w/ Linker	GEF11-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0303
	GEF11-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF11-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0204
	GEF12-mCitrine	GEF12-Promoter	mCitrine w/ Linker	GEF12-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0304
	GEF12-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF12-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0205
	GEF14-mCitrine	GEF14-Promoter	mCitrine w/ Linker	GEF14-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0305
	GEF14-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	GEF14-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0206
	PCaP2-mCitrine (inducible)	Ubi-XVE_oLexA-35S	Decoy	PCaP2-ORF	mCitrine w/ Linker	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0215
	PIP5K3-mCitrine		PI5K3-Promoter and ORF		mCitrine w/ Linker	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0241
	RHD2-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	RHD2-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0209
	ROP2-mTurquoise2	ROP2-Promoter	mTurquoise2 w/ Linker	ROP2-ORF	Decoy	RubisCO	Kanamycin	pGGZ003	Spec/Strep	pPD0042
	ROP2-mCitrine	ROP2-Promoter	mCitrine w/ Linker	ROP2-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0240
	ROP2-ORF-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	ROP2-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0199
	ROP2-CDS-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	ROP2-CDS	Decoy	HSP18.3	Basta	pGGZ003	Spec/Strep	pPD0184
	rop2ΔN-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	rop2ΔN-CDS	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0195
	ROP4-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	ROP4-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0200
	ROP6-mCitrine (inducible)	Ubi-XVE_oLexA-35S	mCitrine w/ Linker	ROP6-ORF	Decoy	HSP18.2	Basta	pGGZ003	Spec/Strep	pPD0201
Gateway expression vectors										
	NubG-ROP2	ADH	NubG w/ 2xHA	ROP2-CDS	-	-	Yeast: TRP	-	Amp	D1288
	NubG-CA-ROP2	ADH	NubG w/ 2xHA	CA-ROP2-CDS	-	-	Yeast: TRP	-	Amp	D1289
	NubG-DN-ROP2	ADH	NubG w/ 2xHA	DN-ROP2-CDS	-	-	Yeast: TRP	-	Amp	D1290
	NubG-rop2ΔN	ADH	NubG w/ 2xHA	rop2ΔN-CDS	-	-	Yeast: TRP	-	Amp	D1291
	Ost4-GEF3-Cub	met25	mOst4	GEF3-CDS	Cub	-	Yeast: Leu	-	Amp	D1295
	GEF3-cYFP--nYFP-ROP2	3x35S	nYFP-HA	GEF3-CDS/RFP/ROP2-CDS	MYC-cYFP	T35S	-	-	Spec	D1189
	GEF3-cYFP--nYFP-CA-ROP2	3x35S	nYFP-HA	GEF3-CDS/RFP/CA-ROP2-CDS	MYC-cYFP	T35S	-	-	Spec	D1190
	GEF3-cYFP--nYFP-DN-ROP2	3x35S	nYFP-HA	GEF3-CDS/RFP/DN-ROP2-CDS	MYC-cYFP	T35S	-	-	Spec	D1191
	GEF3-cYFP--nYFP-rop2ΔN	3x35S	nYFP-HA	GEF3-CDS/RFP/rop2ΔN-CDS	MYC-cYFP	T35S	-	-	Spec	D1192
	FER-GFP	FER	-	FER	mGFP6	NOS	Hygromycin	pMDC111	Kanamycin	pMDC111

Table S3. List of used expression vectors.

```

> # This script has been modified from the script #84 TUKEY TEST, deposited on R-graph-gallery
  (https://www.r-graph-gallery.com/84-tukey-test/) by Yan Holtz, Queensland Brain Institute,
  University of Queensland.

> # script modifications by Guido Grossmann, Centre for Organismal Studies, Heidelberg
University
> # library
> library(multcompView)
>
> # Import root hair distance data
> data <- read.csv("~/distance.csv",header=TRUE)
>
> # What is the effect of the line on the value?
> model=lm( data$value ~ data$line )
> ANOVA=aov(model)
>
> # Tukey test to study each mutant line pair:
> TUKEY <- TukeyHSD(x=ANOVA, 'data$line', conf.level=0.99)
>
> # Tukey test representation :
> plot(TUKEY , las=1 , col="brown" )
>
> # Grouping the mutant lines that are not different to each other.
> generate_label_df <- function(TUKEY, variable){
+ # Extract labels and factor levels from Tukey post-hoc
+ Tukey.levels <- TUKEY[[variable]][,4]
+ Tukey.labels <- data.frame(multcomLetters(Tukey.levels)['Letters'])
+
+ #Ordering the labels as in the boxplot :
+ Tukey.labels$line=rownames(Tukey.labels)
+ Tukey.labels=Tukey.labels[order(Tukey.labels$line) , ]
+ return(Tukey.labels)
+ }
> # Apply the function on the dataset
> LABELS=generate_label_df(TUKEY , "data$line")
>
> # A panel of colors to draw each group with the same color :
> my_colors=c( rgb(143,199,74,maxColorValue = 255),rgb(242,104,34,maxColorValue = 255),
  rgb(111,145,202,maxColorValue = 255),rgb(254,188,18,maxColorValue = 255) ,
  rgb(74,132,54,maxColorValue = 255),rgb(236,33,39,maxColorValue =
255),rgb(165,103,40,maxColorValue = 255))
>
> # Draw the basic boxplot
> a=boxplot(data$value ~ data$line , ylim=c(min(data$value) , 1.1*max(data$value)) ,
  col=my_colors[as.numeric(LABELS[,1])], ylab="value" , main="")
>
> # Adding a letter over each box. Over is how high I want to write it.
> over=0.1*max( a$stats[nrow(a$stats),] )
>
> #Adding the labels
> text( c(1:nlevels(data$line)) , a$stats[nrow(a$stats),]+over , LABELS[,1] ,
  col=my_colors[as.numeric(LABELS[,1])] )

```