

Supplementary Data

Feedback between PI4P signaling and ER-PM contact sites orchestrates polarized root hair growth

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Supplementary Data 1a. List of primers used in this study.

<i>Plant genotyping</i>	
sac7-3 LP	TTGTTCCAAAGAAACCTCGG
sac7-3 RP	GAGACGGTTGATTCTCGGAAC
syt1-2 LP	AGGTCTCGCGATTTATTAGGG
syt1-2 RP	GCCTCTGACAAGTATAGGGG
syt3 LP	ATCCTCTGGTGGTTTCAAAGG
syt3 RP	GGTTGATCTCAGGTATGTGCC
Salk Lbb1.3	ATTTTGCCGATTTTCGGAAC
Sail Lbb1.3	TAGCATCTGAATTCATAACCAATCTCGATACAC

<i>Cloning of Drosophila SAC1dead constructs</i>		
SAC1dead_part1 FW_C	aaaaGGTCTCaGGCTctATGGATAGCCGTGAGGAGAA	dmSAC1 dead into pGGC000
SAC1dead_part1 RV_C	tctcGGTCTCaTGAAAACGCCTGTTTGCGTTGATA	dmSAC1 dead into pGGC000
SAC1dead_part2 FW_C	tctcGGTCTCaTTCAGGACCAATtctATCGACTGT	dmSAC1 dead into pGGC000
SAC1dead_part2 RV_C	ttttGGTCTCaCTGAACGTCCAGCTCCTCCAG	dmSAC1 dead into pGGC000
UBQ10_GVG_fw_A	tctcGGTCTCaACCTtAGTCTAGCTCAACAGAGCTT	UBQ10prom+GVG into pGGA000
UBQ10_GVG_rv_A	tctcGGTCTCaTGTtctgctttttgtacaactgctc	UBQ10prom+GVG into pGGA000
mCherry_stop_FW_D	tctcGGTCTCaTCAGAAATGGTGAGCAAGGGCGAGgag	mCherry+stop codon into pGGD000
mCherry_stop_RV_D	tctcGGTCTCaGCAGCTACTTGTACAGCTCGTCCATG	mCherry+stop codon into pGGD000

<i>The guide sequences for pi4ka crispr mutant design</i>	
Guide 1 (79 bases de l'ATG)	GTCGTTGTCCACAAACGGAA
Guide 2 (314 bases de l'ATG)	GAGTTATCTTTCTGTGCGG
Guide 3 (629 bases de l'ATG)	TAGTACGAACCGTTATCCCTC
Guide 4 (1493 bases de l'ATG)	GGTACTAGCTGTCTGTGCAC

<i>Cloning of the 35S:GFP-NET3C (Steffen Vanneste, Ghent University)</i>	
NET3C_FW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCCGCCAATGGTTAGAGAAGAGGAGAAATC
NET3C_REV	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAAGGACCTTGTGCCATC

Supplementary Data 1b. The sequence of codon optimized *Drosophila* SAC1¹⁻⁵²¹ gene.

dmSAC1¹⁻⁵²¹

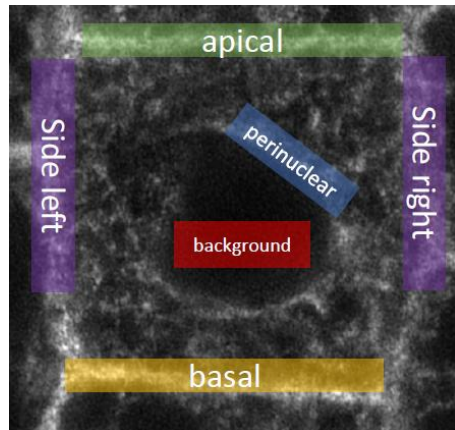
ATGGATAGCCGTGAGGAGAACGCGGTTTATGACGACATGAACTTGTACATCGCTCCACAGAGTTTCATAATCGAGCCCAATGGTGCGCAGAGCT
CCTCGTTATAGGAAGGCATGATAAGGTAACCAGAGTACAGCCGGCGAGCGGGGCCCTTGTTGCTAACCTAAGGCCAACACGTCGAATCTGTGGC
GTTCTCGGAACCATTCACCTTGTATCATGCGATTATCTGTTAGTAGCCACTCACCGTTTATTTGTAGGTGTGCTAAATGGGGCCGTTGTCTGGCGTC
TCGCGGGATACGACATTATACCTTACATTCCCAATTCCTTCCAGCGTAAGGAGAACGAGAACTACCTAAGGCTTCTACGACAGACTCTCGACACCA
AATTCCTTCTATTTAGTTACAGATATGATCTTACCAACTCTTTACAACGTCAGAGGGAAGTTGCACAGTCCAGACCCGAAGTATCAGGCCTTCTTCAG
AGGGCCGAACAACGTTTTGTATGGAATGGCTATGTCTTAGGCAGTTTAACTGTGATAAGATGGAGAAGTTTCAGCTTCCGCTGGTTCTAGGATTT
GTTAGCATCAATCAGGTTTCAGATAAAATGGCCAGACCTTTTTTTGGAGTATCATAACGCGTCAAGCGTACAGAGGGCTGGAACCCGCTCTGTTCTGC
CGTGGAAGTGATGAGCAAGGGCATGTCGCAAACTTTGTAGAaaCCGAGCAAATTGTGAATTCAACGGGCAACTTACGGGATTCTGTACAAACACGT
GGTAGCATGCCTTTTCACTGGCATCAACTCCCTAATTTGCGTTACAAACCAAGGCCGGTGTTAGTTCCGGGAAAGGATCACCTGGCAGCTTGTGga
CTTCATTTCAAAGAGCAGATTAGATTATATGGAACAACGTTGCGGTCAACCTCGTTGATCACAAGGCGCTGAAGGCGAGCTTGAAGCCACGTAT
GCGGCTCTCGTTTCGAGAAATGGGCAATCCACAGGTGCGATATGAGTCATTTCGATTTTTCACAGCGAGTGCAGAAAGATGCGTTGGGACAGACTTAA
CATTTTGATAGACCGATTAGCCACGAACAAGATCAATTTGGGGTTTATCATGTCTTTGACGACGGCAAGCTGGTATCAACGCAACAGGCGTTTTTC
AGGACCAAT **TGCATCGACTGTCTTGACAGAACA**AATGTCGTACAGTCCATGTTAGCGAGACGAAGTCTCAGAGCTGTCTTCAGAAATTAGGGGTC
CTCCATGTCGGGCAAAAGGTAGAGCATGCAAGCGATATCTTCAAGCATCTTTAAAGGCGTTTGGGCTGACAATGCCGACTTAGTGAGCTTGCAA
TACTCCGGAACATGTGCTTTGAAAACCGATTTACCCGTAAGGAGGACAAAGAGTGCGCCATGCAAGATGGCAAGAACTCCCTTATGAGA
TACTACCTAAACAACCTTCGCCGATGGACAGAGACAGGACTCAATTGATCTGTTCTTGAAAAATACTTGGTAAATGACAATGAAGGCGGTGCAGTC
CCCAGCCCGCTAGAATCTAAACACGGGG **GGAGGTACTGCTCGTGGAGCTGCTGCTGGAGCTGGAGGAGCTGGACGT**

Red letters represent the catalytic motif and the sequence encoding for the catalytic cysteine is highlighted in yellow. For designing the dmSAC1^{dead}, at the place of catalytic cysteine, serine was introduced by changing TGC sequence to TCT sequence. Blue letters indicate the linker sequence.

Supplementary Data 1c. Website links to Fiji macros used in this study.

Fiji macro for contact site density quantification	https://github.com/RDP-vbayle/SiCE_FIJI_Macro/blob/main/misc/MacroPatternERcs%20Markovic%20et%20al.%202026.ijm
Fiji macro for quantification of the root hair length and density	https://github.com/RDP-vbayle/SiCE_FIJI_Macro/blob/main/misc/MacroRootHair%20Markovic%20et%20al.%202026.ijm
Fiji macro for contact site segmentation of SYT1-GFP-mCherry-SAC7 time-lapses	https://github.com/RDP-vbayle/SiCE_FIJI_Macro/blob/main/misc/MacroSAC7SYT1Kymo%20Markovic%20et%20al.%202026.ijm
Fiji macro for colocalization analysis of SYT1-GFP and mCherry-SAC7	https://github.com/RDP-vbayle/SiCE_FIJI_Macro/blob/main/misc/MacroSAC7SYT1coloc%20Markovic%20et%20al.%202026.ijm

Supplementary Data 1d. Quantification of the cortical index.



Root meristematic cell

Cortical index:

$$\frac{(\text{Apical Fluo} - \text{Background}) + (\text{Side right} - \text{Background})}{2 \times (\text{Perinuclear Fluo} - \text{Background})}$$