<u>Appendix</u>

Serendipita indica E5'NT modulates extracellular nucleotide levels in the plant apoplast and affects fungal colonization

Authors

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Appendix Figure S1



Appendix Figure S1: Schematic workflow describing the barley inoculation and apoplastic fluid (APF) collection. To ensure that no apoplastic leakage of the *S. indica* GoGFP strain expressing cytosolic GFP used for the inoculation of the barley seedling occurred during APF collection the samples dedicated for mass spectrometric analysis were examined by an anti-GFP western blot.

S. indica protein IDs from apoplastic fluid of barley roots



Appendix Figure S2: *S. indica* putative apoplastic proteins involved in metabolism shown within their respective cellular pathways. Asterisks indicate orthologues identified in the APF of rice leaves infected with the rice-blast pathogen *Magnaporthe oryzae* [43]. The metabolic pathways were modified from [77].

Appendix Figure S3



Appendix Figure S3: Gene Ontology Enrichment Analyses of apoplastic and culture filtrate proteins visualized using REVIGO. Detailed GOeast results are listed in Table EV3.

Appendix Figure S4

Peptides for E5'NT identified by LC-MS/MS from APF of barley roots:

HQLGIIGVITPDTK GTSSGAGPGTEFSDPVQAVQK QLLDIFEGVVSK MNIAFDASGK

PIIN_01005

MQLSRSFALLAIYTTLVLAKVDSLVSERALQKRFVDANGNYNVTIVHTNDVHAHLDQWR AGRGTDCTPGSECISGYARIKQKVSELRQSIQDPIFLNAGDEFQGTLFFTYYGGEKISYA INEVGYDVFTLGNHEFDRGQGELAAFLKNLTFPVVCANFKTNDTAMNALNIQPYTIIEK<mark>H</mark> QLGIIGVITPDTKGTSSGAGPGTEFSDPVQAVQK</mark>AVDELQAKNITRIIALTHIGYDKDIELA QKTKGVDLIVGGHSHTLLGNFTNAMGSYPTTAKNLDGEEVFIVTSYRWGEILGKMNIAF DASGKIVSYEGEPLRLTNTTQQDPKLQAEVNEWRQPFDAMAQVVVGTSSVVLDQSIC QFSECTLGNVITDAMYEYRKNAGGNVDFALINSGGIRASISEGTVTQGDILTSFPFMNG VVDLTWTGKQLLDIFEGVVSKYSTLSHHATTSFIQVSKQVKFSWNPNNVNQTRLITLEIG GQQVDVNKNYTMVTLDFLASNGDYLWGTRTDFAALDTLDVVLASYFKAHSPVGASIEG RITNSTSTTQQLPGKGSSGGSGGASALIVSGLSAGMMLLAGMMLV

Peptide searches against barley proteome retrieved no perfect matches:

HQLGIIGVITPDTK

Best blastP hit vs Hordeum vulgare (taxid:4513)_ Sequence ID: CAX51385.1: Query 4 GIIGVITPD 12 G++GVITPD Sbjct 223 GVVGVITPD 231

GTSSGAGPGTEFSDPVQAVQK

Best blastP hit vs Hordeum vulgare (taxid:4513)_ Sequence ID: BAJ89828.1: Query 9 GTEFSDPVQAV 19 GT+F DPV AV Sbjct 205 GTDFPDPVAAV 215

QLLDIFEGVVSK

Best blastP hit vs Hordeum vulgare (taxid:4513)_ Sequence ID: BAJ94588.1: Query 1 QLLDIFEGVV-SK 12 QLLD FE +V SK Sbjct 673 QLLDSFEEIVASK 685

<u>MNIAFDASGK</u>

Best blastP hit vs Hordeum vulgare (taxid:4513)_ Sequence ID: BAK02710.1: Query 2 NIAFDASG 9 NI FDA G Sbjct 169 NISFDANG 176

Appendix Figure S4: Peptides identified by LC-MS/MS for E5'NT found in the APF of barley. BLAST searches against barley databank retrieved no perfect matches, indicating that these peptides originated from *S. indica* E5'NT.



Appendix Figure S5: Characterization of the *dorn1-3* **mutant line.** ATP and ADP but not AMP nor adenosine induce intracellular calcium release in *Arabidopsis thaliana* expressing aequorin lines (Col-0^{aq}). The ATP receptor deletion mutant *dorn1-3* does not respond to ATP and ADP. After 5 min of background measurements 50 µl of 100 µM adenylates were added, subsequently luminescence was monitored for 30 min. A, ATP; **B**, ADP; **C**, AMP; **D**, adenosine. Data points represent means of six independent biological replicates ±SE.

Appendix Figure S6



Appendix Figure S6: Expression of *Si*E5'NT in *S. indica* grown in liquid CM supplemented with 100 μ M ATP, ADP, AMP or ATP-imido [adenosine 5'-(β , γ -imido)triphosphate, SIGMA] a non-hydrolysable form of ATP. Mock 0 represents the time point 0 just before addition of the nucleotides and it is set to 1. Y axis shows the relative expression of *Si*E5'NT. An induction of expression was detected at 12 hour post treatment for AMP and ATP-imido. Significances obtained by one way ANOVA testing of each individual time point are indicated. Error bars show the standard error of the mean from 4 biological replicates.



Appendix Figure S7: Sequence alignment of *S. indica,* bacterial and animal 5'NTs. *Si*E5'NT and animal E5'NT contain GPI anchors (predicted by FragAnchor; turquoise) and animal E5'NT a loop involved in dimerization (pink).



Appendix Figure S8: Characterization of *Si*E5'NT. A Cell surface 5'-nucleotidase activity assay of *Ustilago maydis* strains expressing different forms of *Si*E5'NT. To study the ecto-5'-nucleotidase activity of *Si*E5'NT and its substrate preferences, the biotrophic plant pathogen smut fungus *U. maydis* was chosen since an E5'NT orthologue predicted to be secreted is absent in its genome. Ecto-5'-nucleotidase activity of *U. maydis* cells expressing different *Si*E5'NT versions under the control of the constitutive synthetic otef promoter [64] were analyzed. ^{Potef}::mCherry encodes for cytosolic mCherry, ^{Potef}::E5'NT encodes for the full length *S. indica* enzyme, the ^{Potef}::E5'NTwoGPI construct lacks the predicted GPI anchor sequence and ^{Potef}::E5'NTwoSPwoGPI is missing both the predicted signal peptide and the GPI anchor. Cell surface activity of *Si*E5'NT in *U. maydis* washed cell suspensions were analyzed by measuring the amount of phosphate released after 30 min of incubation with 50 µM of either ATP, ADP or AMP. The amount of phosphate is significantly increased in cells transformed with ^{Potef}::E5'NT for all substrates tested. Error bars represent standard deviation of the mean from three biological replicates. Asterisks indicate significance at p< 0.05 (*), 0.01 (**) analyzed by Student's t-test to the mCherry control strains.



Supplementary figure 9: Characterization of *A. thaliana* T3 and T4 transgenic lines in Col-0 background. A *Si*E5'NT and B mCherry expression analysis of different *A. thaliana* T3 transgenic lines by qPCR. Error bars represent standard deviation of the mean from three technical replicates of one biological experiment. C Shoot weight of *A. thaliana* transgenic T3 lines. Error bars represent standard deviation of the mean from in total 12 plants of

two independent biological experiments. Arrows indicate the lines that were selected for further analyses. Black indicates E5'NT expressing lines; Red indicates E5'NT:mCherry expressing lines; Green indicates mCherry expressing lines. **D** Images of seedlings grown on ½ MS with Hygromycin B (15 µg/mL) from the T3 transgenic lines used for detailed analyses. **E** Seed production in the *A. thaliana* transgenic T4 lines used for detailed analyses in Figures S10 and S14. Box plots show the quartile of seed weights of the plant individuals analyzed. The overlaid dot plots shows the respective individual values. Plants were propagated in parallel in greenhouse in soil. Seeds from each plants were collected separately and weighted. Twenty to forty plants for each line were used for one way ANOVA statistical analysis. The activity of *Si*E5'NT might affects seed production in the #303 (1)L3 line by manipulation of eATP levels during propagation. It was reported that optimum concentration of eATP is essential for initiation of pollen germination in *A. thaliana* and in Gymnosperms [78, 79].

Appendix Figure S10



Appendix Figure S10: A Confocal laser scanning microscopy of roots from the lines #304 (6)L8 expressing E5'NT:mCherry and Col-0 WT under the same settings. Plant nuclei were stained with DAPI and visualized with the UV channel. No auto-fluorescence in the UV channel was observed at the laser intensity used. No fluorescence signal was observed in the WT root in the mCherry (red) channel. B Plasmolysis using 0.5 M sorbitol. After plasmolysis a fluorescence signal was detected at the membrane (white arrows) and at the cell wall (yellow arrows), suggesting that E5'NT:mCherry fusion protein is secreted. N indicates a nucleus.



Appendix Figure S11: A S. indica colonization levels of different independent transgenic T2 and T3 lines. B mCherry transcript levels of the respective untreated plant lines. C SiE5'NT transcript levels of the respective untreated plant lines. Samples were analyzed by qPCR. Error bars show the standard error of the mean of three independent biological replicates. Asterisks indicate significance at p< 0.05 (*) analyzed by Student's t-test to the mCherry control line (#305).

Appendix Figure S12

Dataset: 173 perturbations from data selection: AT AFFY ATH-0 Showing 4 measure(s) of 4 gene(s) on selection: AT O												
Lug²eniu 25 - 20 - 10 - 50 - 00 - 51 - 10 - 20 - 25 Pomregulate U propulsed Domregulate U propulsed												
		173 of 3282 p	perturbations ful	hiled the filter crit	eria							
Fiber values for solected measure(s)												
	140	9		131	0.01							
Arabidopsis thaliana (173)	VRCY VRCY	H Pi-score	Log2-ratio	Fold-Change	p-value							
cycloheximide / mock treated seedlings		16.30	5.52	45.50	0.001	flu / Col-0		4.52	1.89	3.71	0.004	
EF-Tu (elf26) (60min) / untreated whole plant samples (Ler-0) EF-Tu (elf18) (30min) / untreated whole plant samples (fis2-17)		11.16	4.97	30.44 26.83	0.006	HrpZ (1h) / H2O treated leat samples (1h) EE Tu (vit18) study 4 (Col.0) / mode treated coording complex (Col.0)		5.83	1.87	3.70	<0.001	
FLG22 study 6 (penta) / untreated leaf disc samples (penta)		15.18	4.63	22.92	< 0.001	shift low light to dark to high light (mbs1-1) / shift low light to dark (mbs1-1)		3.77	1.81	3.61	0.008	
FLG22 study 6 (Ler) / FLG22 study 8 (1h) chitin / mock treated seedlings		15.45	4,44	20.42 21.36	<0.001	FLG22 (1h) / H2O treated leaf samples (1h)		5.99	1.80	3.51	<0.001	
S. sclerotiorum study 2 (coi1-2) / mock inoculated rosette leaf samples (coi1-2)		17.60	4.40	21.22	<0.001	HR-5 / Ts-1 1		4.01	1.79	3.40	0.002	
FLG22 + GA (1h) / untreated leaf disc samples (Ler) FE-Tu (ell18) (60min) / untreated whole plant samples (fis2-17)		10.99	4.31	16.07	0.003	hst15 / Col-0		4.04	1.78	3.65	0.005	
drought study 9 (355::ABF3-48) / untreated 355::ABF3-48 seedling samples (24h)		16.12	4.14	18.36	<0.001	salicylic acid study 8 (Col-0) / mock treated leaf samples (Col-0)		7.01	1.75	3.37	<0.001	
chitooctaose (erf5-1 erf6-1) / mock treated whole plant samples (erf5-1 erf6-1) chitooctaose (Col.0) / mock treated whole plant samples (Col.0)		16.49	4.12	17.39	<0.001	SALK_074069 ABI3:MTA / Col		6.99	1.75	3.37	<0.001	
FLG22 study 6 (Ler) / untreated leaf disc samples (Ler)		10.09	4.08	13.68	0.003	NFA 10 / To 1 3		3.84	1.74	3.35	0.006	
cycloheximide study 4 (pBeaconRFP_GR-ABI3) / mock treated root protoplast sampl		16.25	4.00	16.63	<0.001	high light study 7 (atwrky40) / untreated leaf samples (atwrky40)		6.00	1.73	3.34	+0.001	
elevated CO2 study 3 (mature leaf 10) / elevated CO2 study 3 (leaf 10 primordia)		15.67	3.92	15.58	+0.001	Knox-18 / Ts-1 0		3.52	1.70	3.19	0.009	
max4 / Col 0 S. eclaration m study 2 (Col-0) / mark incruistad resatta last samplas (Col-0)		15.57	3.89	14.61	~0.001	Bay-0 parent / Fei-0 🚯		6.82	1.70	3.26	<0.001	
Pep2 (ein2-1) / mock treated seedling samples (ein2-1)		14.55	3.64	12.41	<0.001	sali sudy z (early) / uniteated root samples (early) HR-5 / CIBC-17 ()		4.32	1./0	3.18	0.003	
drought study 16 (Col) / mock treated Col whole plant samples Pen2 (Col-0) / mock treated seerling samples (Col-0)		13.90	3.48	11.16	<0.001	abi1-1 / Ler-0 💿		4.50	169	3 18	0.002	
drought study 16 (srk2dei) / mock treated srk2dei whole plant samples		13.38	3.34	10.27	<0.001	bleomycin + INA / untreated seedling samples		3.74	1.67	3.16	0.006	
limiting NH4NO3 / elevated CO2 (midnight) / limiting NH4NO3 / elevated CO2 (midd antimycin & (AOX1a:LLC) / mock treated short samples (AOX1a:LLC)		10.06	3.28	9.21 9.45	<0.001	salicylic acid study 7 (npr1-1 sni1) / solvent treated whole plant samples (npr1-1 sni1)		5.07	1.67	3.10	<0.001	
Pep2 (bak1-3) / mock treated seedling samples (bak1-3)		13.04	3.26	9.54	<0.001	ozone study 4 (abi1ld) / untreated rosette leaf samples (abi1td) cold (late) / untreated green tissue samples (late)		4.21	1.66	3.21	<0.003	
P. parasitica (10.5h) / non-infected root samples (Col-0) antimusia A study 2 (append 7.1) (mark transfer depart samples (append 7.1)		6.61	3.21	9.43	0.009	gamma irradiation (cmt3-11) / untreated cmt3-11 rosette leat samples (1d)		3.40	1.66	3.18	0.009	
antimycin A (rao1-1) / mock treated shoot samples (rao1-1)		12.72	3.18	9.06	<0.001	LBD37OX / Col-0 HR-5 / Ts-1 0		5.58	1.65	3.15	<0.001	
FLG22 study 4 (Col-0) / untreated leaf disc samples (Col-0)		6.94	3.17	9.20	0.006	MeJa (late) / opr3 stamen samples (0h)		6.02	1.65	3.16	<0.001	
NAA + FLG22 (1h) / untreated leaf disc samples (Col-0)		7.84	3.09	8.52	0.003	NFA-10 / CIBC-17 👽 mvb61 / Col-0 🚯		3.41 4.48	1.65	3.14	0.009	
axr1-12 / Col-0		12.10	3.08	8.37	<0.001	low light + DBMIB (0.5h) / low light study 4 (6h)		4.34	1.61	3.08	0.002	
drought study 7 (srk2cf) / untreated plant samples (srk2cf)		10.14	3.01	7.77	<0.001	shift low light to dark to high light (35S:MBS1) / shift low light to dark (35S:MBS1) IAA study 10 (8h) / mock treated root samples (8h)		3.99	1.60	3.07	0.003	
X. campestris pv. campestris study 2 (AtMYB30-ox-20A) / untreated leaf samples (At X. campestris pv. campestris study 2 (AtMYB30-ox-20A) / untreated leaf samples (At		6.19	2.99	7.78	0.008	sulfometuron methyl (24h) / mock treated leaf samples (24h)		3.64	1.55	3.01	0.004	
antimycin A study 2 (rao2-1) / mock treated shoot samples (rao2-1)		11.73	2.93	7.53	<0.001	hypoxia study 9 (AtERF73/HRE1-RNAi20) / untreated root samples (AtERF73/HRE1 pai/L pr 6)		4.08	-1.56	-3.08	0.007	
ABA study 8 (Col-0) / solvent treated leaf samples (Col-0) El C22 study 4 (2EC:AED1) / untreated leaf dire samples (2EC:AED1)		8.27	2.92	7.31	0.001	Bay-0 parent / Col-0 💿		4.45	-1.69	-3.04	0.002	
high light study 7 (AtWRKY63 OE1) / untreated leaf samples (AtWRKY63 OE1)		11.62	2.91	7.42	~0.001	BI-1 / Bur-0 👽 C24 / Bur-0 🚯		6.18	-1.60	-0.05	<0.001	
B. graminis (ataf1-1) / non-infected rosette leaf samples Bur.0 / Eei.0 6		5.67	2.83	6.47	0.010 ¢0.001	BI-1 / Bur-0 1		4.45	-1.72	-3.31	0.002	
drought study 6 (srk2cf) / untreated plant samples (srk2cf)		8.78	2.78	6.69	<0.001	OE/a-1 / Col-0 9 CIBC-17 / Bur-0 🚯		5.14	-1.74	-3.34	0.001	
salt study 2 (late) / untreated root samples (late) ambient CO2 (mature leaf 10) / ambient CO2 (leaf 10 primordia)		6.49	2.78	7.78	<0.001	syringolin study 2 / solvent treated leaf samples (syl_404_bc2)		3.58	-1 76	-3.37	0.009	
5-AC / solvent treated seedling samples (Ws)		6.74	2.75	7.36	0.004	C24 / Bur 0 👀 Ta-1 / Bur 0 🚯		5.21	-1.76	-3.45	0.001 <0.001	
ample NH4NO3 / elevated CO2 (midnight) / ample NH4NO3 / elevated CO2 (midday) ELG22 study 7 (penta) / untreated leaf disc samples (penta)		7.32	2.73	6.30 5.98	0.002	Sha / Col-0 📵		5.51	-1.79	-3.51	<0.001	
drought study 6 (Col-0) / untreated plant samples (Col-0)		9.25	2.64	6.13	<0.001	M. incognita study 2 (Pico) / non-infested root cell samples (Pico) BI-1 / CoI-0 0		4.00	-1.01	-3.70	<0.003	
X. campestris pv. campestris (Ws-4) / untreated leaf samples (Ws-4) aba1-1 / Ler-0 0		10.54	2.63	6.21	<0.001	npr1-1 sni1 brca2a / npr1-1 sni1 0		6.62	-1.85	-3.60	<0.001	
salt / FACS study 3 (8h) / root endodermis and quiescent center protoplast samples		10.50	2.63	6.26	< 0.001	BL/H3BU3 (100) / untreated cell culture samples Bav-0 parent / Col-0 🚯		3.95	-1.91	-3.76	<0.008	
cold study 2 (late) / untreated root samples (late) Bur-0 / Fei-0 1		9.03	2.59	5.78	<0.001	BL/H3BO3 (6d) / untreated cell culture samples		4.03	-2.00	-4.00	0.010	
high light study 7 (atwrky63) / untreated leat samples (atwrky63)		6.74	2.49	5.36	0.002	efr-1 / Col-0 😏 CS57053 / Bay-0 parent		6.99	2.01	-4.04 4.64	0.001	
salt / FACS study 2 (48h) / root cortex protoplast samples of mock treated pCOR315		9.65	2.46	5.50	<0.001	BI-1 / Col-0 😗		0.15	-2.04	-4.12	<0.001	
drought study 7 (Col-0) / untreated plant samples (Col-0)		8.50	2.44	5.30	-0.001	CS5/696 / Bay-U parent BL/H3BO3 (8d) / untreated cell culture samples		4.57	-2.05	-4.21	0.005	
Imiting NH4NO3 / ambient CO2 (midnight) / limiting NH4NO3 / ambient CO2 (midday) RALF (30min) / mock treated seedling samples (30min)		9.65	2.43	5.00	+0.001	psad1-1/stn7-1 / Col-0		4.60	-2.09	-4.60	0.006	
antimycin A study 2 (AOX1a:LUC) / mock treated shoot samples (AOX1a:LUC)		6.98	2.41	5.10	0.001	CS57865 / Bay-0 parent BL/H3BO3 (4d) / untroated cell culture samples		4.60	-2.23	-4.69	0.009	
cc26 / Col-0 0		7.70	2.40	5.34	<0.001	anac017-2 / AOX1a:LUC 🚯		6.53	-2.26	-4.63	0.001	
CS57803 / Sha parent		6.45	2.39	4.62	0.002	C24 / Bur-0 19 C24 / Col-0 19		6.01	-2.28	-5.01	0.002	
shift 28°C to 19°C study 3 (355°HPS4-HS frs1-11) / 28°C (355°HPS4-HS frs1-11) stratification (12h) / seed desiccation		8.61	2.36	5.22	<0.001	MeJa study 5 (gal) / untreated leaf disc samples (gal)		7.22	-2.47	-5.65	0.001	
cold / cordycepin (24h+1h) / cordycepin (1h)		9.36	2.34	5.04	<0.001	HBH depletion (HNA); 24h) / untreated leat samples (Un) gai / penta 🟮		5.99	-2.52	-5.65	0.002	
ample NH4NO3 / ambient CO2 (midnight) / ample NH4NO3 / ambient CO2 (midday)		4.85	2.32	6.01	0.008	BI-1 / Col-0 0		9.97	-2.63	-6.13	<0.001	
shift cold to freezing (Rsch-0) / cold study 23 (Rsch-0)		4.70	2.27	4.59	0.009	C24 / Col-0 0 RBR depletion (RNAi: 12h) / untreated leaf samples (0h)		10.70	-2.67	-6.39	0.001	
elevated CO2 study 3 (expanding leaf 10) / elevated CO2 study 3 (leaf 10 primordia)		5.51	2.23	4.79 5.30	0.003	C24 / Col·0 💿		10.21	2.71	6.40	<0.001	
salt / FACS study 3 (20h) / root endodermis and quiescent center protoplast sample		8.05	2.20	4.63	<0.001	carus romation (12h) / untreated root samples long day (Col-0) / short day study 2 (Col-0)		9.28	-2.95	-7.40	<0.001	
EF-Tu (elf18) study 3 (ein2-1) / mock treated seedling samples (ein2-1)		7.32	2.11	4.18	<0.001	Fei-0 / Col-0 0		9.47	-3.15	-9.04	<0.001	
Sha / Fei-0 0		6.09	2.07	4.29	0.001	Fel-0 / Col-0 👽 ssi2-1 act1-1 / ssi2-1		12.99	-3.25	-9.54	<0.001	
drought study 8 (control-48) / untreated control-48 seedling samples (2h)		5.65	2.06	4.20	0.002	Foi 0 / Col 0 🚯		13.67	-3.42	-10.71	-0.001	
Sha / Fel-0 😨		5.76	2.04	4.22	0.001	Se 0 / Mir-0/Se 0 callus formation (96h) / untreated root samples		14.21	-3.60	-11.76	<0.001 ∢0.001	
benzothiadiazole study 3 (mkk2) / untreated (mkk2) plant samples		5.56	2.02	4.02	0.000	pepr1-1 pepr2-3 / Col-0 🔞		15.19	-0.00	-13.57	~0.001	
rose bengal / untreated cell culture samples		6.16	2.01	4.03	<0.001	Mir-0 / Mir-0/Se-0 callus formation (24h) / untreated root samples		15.84	-3.96	-15.66	<0.001	
Sha / Fei 0 🚯		5.91	1.98	4.05	0.001	callus formation (48h) / untreated root samples		15.90	-3.97	-15.81	<0.001	
benzothiadiazole (mil4) / mock treated rosette tissue samples (mil4) CS57565 / Sha parent		6.47 4.05	1.96	3.88	∠0.001 0.008	1152-177 Lef-0 😈		10.94	-4.3U	-30.57 with GENEV/	STIGATOR	

Appendix Figure S12: Expression patterns of At1g58420, *At*WRKY40, *At*RBOHD and *At*CPK28 under different perturbations, available in the GENEVESTIGATOR database. The filter for the generation of the dataset was set to a cutoff of fold change >3. These genes are eATP responsive only at low level (below cutoff) and strongly responsive to MAMPs and biotic stresses.



Appendix Figure S13: AtRBOHD and AtCPK28 expression levels upon S. indica colonization (addition to Figure 4E/F). Expression levels of the eATP and wounding responsive genes AtRBOHD and AtCPK28 [8] in transgenic lines colonized by S. indica at 5 dpi. For visualization data were normalized by setting the values from the mock treated samples to 1. Error bars represent ±SE of the mean from three independent biological replicates.



Appendix Figure S14: Colonization assay of transgenic lines by the phytopathogenic fungus *Colletotrichum incanum*. Left: Characterization of the transgenic lines after propagation in the T4 generation. Plants were propagated in parallel in greenhouse in soil. Lines #303 (2)L3, #304 (6)L8 and #305 (2)L1 were chosen for colonization analysis with *C. incanum* based on the expression strength for the respective heterologous gene. *Si*E5'NT transcript levels were measured by qPCR. **Right:** Root colonization of transgenic lines by *C. incanum* at 5 dpi. Error bars show the standard error of the mean of three independent biological replicates. Asterisks indicate significance at p< 0.05 (*) and p< 0.01 (**) analyzed by one way ANOVA.