

***New Phytologist* Supporting Information Figs S1 & S2 and Table S1**

Article title: The ubiquitin ligase SEVEN IN ABSENTIA (SINA) ubiquitinates a defense-related NAC transcription factor and is involved in defense signaling

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The following Supporting Information is available for this article:

Fig. S1 Alignment of tomato six SINA ubiquitin ligases.

Fig. S2 A weak interaction between NAC1 and SINA3 identified by the LexA-based Y2H assay.

Table S1 List of primers used in this study

Table S1 Primers used in this study

Primer	Sequence	Purpose
NACFE	5'-CTGGTACCATGAACAAAGGAGCAAACGGAAATC-3'	Forward primer for generating pEG202:: NAC ₁₋₂₆₀ construct
NAC260RB	5'- CAGGATCC TTAAAAATCAAGGGTGTTCCTGTGGTCTTC-3'	Reverse primer for generating pEG202:: NAC ₁₋₂₆₀ construct
SINA1FE	5'-CAGAATTCATGGCTCCCGGAGGCAGTAAC -3'	Forward primer for generating pJG4-5:: SINA1 construct
SINA1RSaII	5'- GTCGACTTACAATGGCTCTCGCCAAATACGCCAGC -3'	Reverse primer for generating pJG4-5:: SINA1 construct
SINA2FE	5'- GTGAATTC ATGGAGTTTGATAGCATTGAATG-3'	Forward primer for generating pJG4-5:: SINA2 construct
SINA2R RSaII	5'- CGTGTCGACTTAACTACAGAGATTTGGTATGC -3'	Reverse primer for generating pJG4-5:: SINA2 construct
SINA3FE	5'- GTGAATTCATGGAGTTGGATAGCATTGAATG-3'	Forward primer for generating pJG4-5:: SINA3 and pMAL-c2::SINA3 constructs
SINA3R RSaII	5'- CATGTCGACTTAAGTACAGAGGTTGGGCATAC -3'	Reverse primer for generating pJG4-5:: SINA3 and pMAL-c2::SINA3 constructs
SINA4FE	5'- GATGAATTCATGGAAGAAATGGAAATGGATAG-3'	Forward primer for generating pJG4-5:: SINA4 construct
SINA4R RSaII	5'- GTAGTCGACCTAGCTACCTAGATTTGGTATG-3'	Reverse primer for generating pJG4-5:: SINA4 construct
SINA5FE	5'-TTGAATTCGAAATGGAGAACATTGAGTGTG -3'	Forward primer for generating pJG4-5:: SINA5 construct
SINA5R RSaII	5'-TTGTCGACGCTACACAGATTCGGTATGCA-3'	Reverse primer for generating pJG4-5:: SINA5 construct
SINA6FE	5'- GTAGAATTCATGGAGTCAGATAGCATTGAA -3'	Forward primer for generating pJG4-5:: SINA6 construct

SINA6RSaII	5'-GTAGTCGACTTAACTACATATTGGTATACATGC-3'	Reverse primer for generating pJG4-5::SINA6 construct
NACFE	5'-CTGAATTCATGAACAAAGGAGCAAACGGAAATC-3'	Forward primer for generating pMAL-C2::NAC1-HA construct
NACR_linker	5'-CATAAAAATACGATAGTAACGGGTGAT-3'	Reverse primer for generating pMAL-C2::NAC1-HA construct
SINA1FK	5'-CAGGTACCATGGCTCCCGGAGGCAGTAAC-3'	Forward primer for generating pBTEX::SINA1-HA construct
SINA1RStuI	5'-TTTAGGCCTCAATGGCTCTCGCCAAATACG-3'	Forward primer for generating pBTEX::SINA1-HA construct
SINA2FK	5'-AGTGGTACCATGGAGTTTGATAGCATTGAATG-3'	Forward primer for generating pBTEX::SINA2-HA construct
SINA2RStuI	5'-CGTAGGCCTACTACAGAGATTTGGTATGC-3'	Reverse primer for generating pBTEX::SINA2-HA construct
SINA3FK	5'-GTTGGTACCATGGAGTTGGATAGCATTGAATG-3'	Forward primer for generating pBTEX::SINA3-HA construct or PART27::SINA3-GFP construct
SINA3RPvuII	5'-CATCAGCTGAGTACAGAGGTTGGGCATAC-3'	Reverse primer for generating pBTEX::SINA3-HA construct
SINA3RSaII	5'-CATGTCGACAGTACAGAGGTTGGGCATAC-3'	Reverse primer for generating PART27::SINA3-GFP construct
SINA4FK	5'-GATGGTACCATGGAAGAAATGGAAATGGATAG-3'	Forward primer for generating pBTEX::SINA4-HA construct
SINA4RPvuII	5'-GTACAGCTGGCTACCTAGATTTGGTATG-3'	Reverse primer for generating pBTEX::SINA4-HA construct
SINA5FK	5'-TTGGTACCATGGAAATGGAGAACATTGAGTGTG-3'	Forward primer for generating pBTEX::SINA5-HA construct
SINA5RPvuII	5'-AAACAGCTGGCTACACAGATTCGGTATGCA-3'	Reverse primer for generating pBTEX::SINA5-HA construct
SINA6FK	5'-GTAGGTACCATGGAGTCAGATAGCATT-3'	Forward primer for generating

		pBTEX::SINA6-HA construct
SINA6RStuI	5'-GTAAGGCCTACTACATATTGGTATACATGC -3'	Reverse primer for generating pBTEX::SINA6-HA construct
NAC1FKpnI	5'-CTGGTACCATGAACAAAGGAGCAAACGGAAATC-3'	Forward primer for generating pART27::NAC1-GFP construct or pBTEX::NAC1-Flag construct
NAC1RKSaII	5'-CGAGTCGACGTAAGGTTTTTGCATGTATAGGAAC-3'	Reverse primer for generating pART27::NAC1-GFP construct or pBTEX::NAC1-Flag construct
NAC1FXbaI	5'-AAGTTCTAGAATGAACAAAGGAGCAAAC -3'	Forward primer for generating NAC1-NYFP construct
NAC1RXhoI	5'-CGACTCGAGGTAAGGTTTTTGCATGTATAGGAAC-3'	Reverse primer for generating NAC1-NYFP construct
SINA3FXbaI	5'-AAGTTCTAGAATGGAGTTGGATAGCATTG-3'	Forward primer for generating SINA3-CYFP construct
SINA3RXhoI	5'-CACTCGAGTTAAGTACAGAGGTTGGGCATAC-3'	Reverse primer for generating SINA3-CYFP construct
Rpi-blb1 ^{D475V} FKpnI	5'-GAGGTACCATGGCTGAAGCTTTCATTCAAG-3'	Forward primer for generating pBTEX::Rpi-blb1 ^{D475V} -HA construct
Rpi-blb1 ^{D475V} RStuI	5'-GAAAGGCCTAATATATATATATTACATTAGG-3'	Reverse primer for generating pBTEX::Rpi-blb1 ^{D475V} -HA construct
Rx ^{D460V} FKpnI	5'-TTGGTACCATGGCTTATGCTGCTGTTACTT-3'	Forward primer for generating pBTEX::Rx ^{D460V} -HA construct
Rx ^{D460V} RPuvII	5'-GAACAGCTGCTCGACATTATTGCGGCAAGAAG-3'	Reverse primer for generating pBTEX::Rx ^{D460V} -HA construct
NbSINASiIF	5'-AAGAATTCAAATGCGACGTGGATGTTAAC-3'	Forward primer for generating TRV2::NbSINA construct
NbSINASiIR	5'- AATCTAGACCAGAAAAGAAAAGGGCCATG-3'	Reverse primer for generating TRV2::NbSINA construct
SINA3RealF	5'- CTTTCTCCAAGCAGAAGCTTAAA-3'	Forward primer for real-time PCR

		assay of SINA3
SINA3RealR	5'- CACTCTCTGTGTTTCAGATGTGATG-3'	Reverse primer for real-time PCR assay of SINA3
NbSINA3RealF	5'-AATCCTCGAGAAGTTGAAAATGCCA-3'	Forward primer for real-time PCR assay of NbSINA genes
NbSINA3RealR	5'-CCCATAAAACGAAGAAATGCCATGTA-3'	Reverse primer for real-time PCR assay of NbSINA genes
EF1 α F	5'- GGTGGTTTTGAAGCTGGTATCTCC -3'	Forward primer for real-time PCR assay of the EF1- α gene
EF1 α R	5'- CCAGTAGGGCCAAAGGTCACA-3'	Reverse primer for real-time PCR assay of the EF1- α gene

Underlined text represents restriction enzyme sites.

SINA1	1	...MAPGGSNYQDIGDSRSAYSIDYG...IAPESAEFKNSPFRKAAAVIGG.....	44
SINA2	1	...MEFDSEICVSSSDG.IEEDIPLQLHPHIIRSQFSSSKTTTHKNNNGSSI LCEGN	53
SINA3	1	...WELDSIECVSSSDGMIDDEIPLHHPHIHSQYSSSKTSNNNNINNS...SSNN	51
SINA4	1	MEEMEMDSVECVSLMDG.VEDEEQS...SIASHQYPLGK..PNNILSAG.....	44
SINA5	1	...MEMENIECVSTTDG.IEDEEQ...LSHNQFSSAK..PHNILFPG.....	39
SINA6	1	...WESDSIECIPSLDG.IDVN.....EIVHSQFSSSKTPHNNNNKN.....N	40
RING			
SINA1	45	..KNGVGSNSAVHELLECPVCMNPMYPPIH.QCPNGHTLCVKCKSKVH.VCPICRHE	97
SINA2	54	NGGIAIHPATSVHELLECPVCMNPMYPPIH.QCHNGHTICSTCKARVHNRCPTCRQE	109
SINA3	52	DGIAAIHSTTSVHELLECPVCMNPMYPPIH.QCHNGHTICSTCKARAHNRCPTCRQE	107
SINA4	45NVPTSVHELLECPVCMNPMYPPIH.QCHNGHTICSTCKTRVHNRCPTCRQE	94
SINA5	40LAPTSVHELLECPVCMNPMYPPIH.QCHNGHTICSTCKTRVHNRCPTCRQE	89
SINA6	41	N...SIRPATSVHELLECPVCMNPMYPPIH.QCHNGHTICSTCKERVHNRCPTCRQE	94
Zn Finger			
SINA1	98	LGNIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.QMCRFRPYMCPYAGSEC	154
SINA2	110	LGDIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.AACNFRPYMCPYAGSEC	166
SINA3	108	LGDIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.SVCNFRPYMCPYAGSEC	164
SINA4	95	LGDIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.TLCNFRPYMCPYAGSEC	151
SINA5	90	LGDIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.ALCNFRPYMCPYAGSEC	146
SINA6	95	LGDIRCLALEKVAESLELPCRYQIYGCQDIFTYHTRLRHE.AMCMFRPYMCPYAGSEC	151
Zn Finger SBD			
SINA1	155	AVTGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLT.VFNCFGHQF	210
SINA2	167	AAVGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLT.VFNCFGQYF	222
SINA3	165	SVVGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLT.VFNCFGQCF	220
SINA4	152	SVTGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLT.VFNCFGQYF	207
SINA5	147	SVTGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLT.VFNCFGQYF	202
SINA6	152	SIVGDIPYLVVHLEKDDHKVDMHSGCTFMHRYVKSNPQEVENATWMLTVFNCFGQSF	208
SBD			
SINA1	211	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	267
SINA2	223	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	279
SINA3	221	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	277
SINA4	208	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	264
SINA5	203	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	259
SINA6	209	CLHFEAFQLGVAIPVYMAFLRFMGDEEARNYSYSLEVGGNGRKLIMEGTPRSIRD SH	265
SBD			
SINA1	268	KIVRDSLDGLI IQRMMALFFSGGDRKELKLRVTGRIWREQL.....	308
SINA2	280	RKVRDSHDGLV IQRMMALFFSGGDRKELKLRVTGRIWREQQNPDEGACIPNLC S	333
SINA3	278	RKVRDSHDGLV IQRMMALFFSGGDRKELKLRVTGRIWREQQNLDGGACMPNLC T	331
SINA4	263	RKVRDSHDGLI IQRMMALFFSGGDRKELKLRVTGRIWREQQNQDGGVCIPNLG S	318
SINA5	260	RKVRDSHDGLI IQRMMALFFSGGDRKELKLRVTGRIWREQQNPEGGVCIPNL C S	313
SINA6	266	RKVRDSHDGLI IQRMMALFFSGGDRKELKLRVTGRIWREKRNPDGGACIP. I C S	318

Fig. S1 Alignment of tomato six SINA ubiquitin ligases. The conserved RING, Zn-Finger and SB domains are indicated.

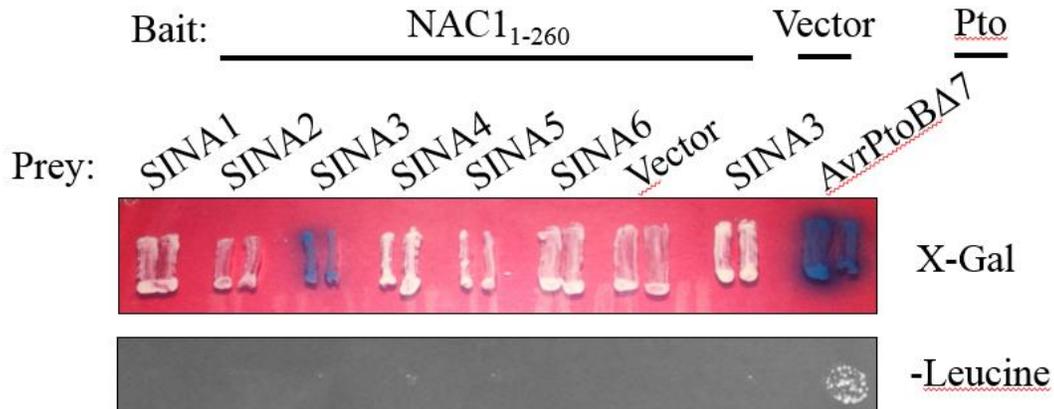


Fig. S2 A weak interaction between NAC1 and SINA3 identified by the LexA-based Y2H assay. The N-terminal 260 amino acids (NAC1₁₋₂₆₀), which did not exhibit self-activation, was expressed as bait and SINA1-6 were expressed as prey. AvrPtoBΔ7 and Pto were expressed as prey and bait, respectively, serving as a positive control (Abramovitch *et al.*, 2003). Upper panel, blue yeast colonies grown on the X-Gal-containing medium indicate the interaction between NAC1 and SINA3. Lower panel, yeast cells collected from clones on the top panel were diluted and loaded on medium lacking leucine. Only cells containing AvrPtoBΔ7 and Pto were able to grow, suggesting the interaction between NAC1 and SINA3 is weak.

Abramovitch RB, Kim Y-J, Chen S, Dickman MB, Martin GB. 2003. *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO Journal* **22**: 60–69.