

AtWRKY33	147	FQSQEQQKK--NQSEQWSQETTRP----NNQAVSYNGREQRKGEDGYNWRKYGQKQVK	198
AhWRKY33	141	FQPQEQQRK--NQSEQWNQETTRP----NNQAVSYNGREQRKGEDGYNWRKYGQKQVK	192
AlWRKY33	133	FQPQEQQKN--NQSEQWNQETTRP----NNQAVSYNGREQRKGEDGYNWRKYGQKQVK	184
BnWRKY33	135	-----SVLQSQTDTRPNN--NTHQAVPVNGREQRKGEDGYNWRKYGQKQVK	179
BoWRKY33	135	-----SVLQSQTDTRPNN--NTHQAVPVNGREQRKGEDGYNWRKYGQKQVK	179
BrWRKY33	112	FQAQEGERK--NQSDQWSQTLNNSN---THQAVSYNVNREQRKGEDGYNWRKYGQKQVK	164
StWRKY33	154	FSTAKTGVKSEVAPIQSFSQE-NMPPNNPAPVHYPHQCPSSQYVREQKAEDGYNWRKYGQKQVK	212
SlWRKY33	94	FSTAKTGVKSEVAPIQSFSQE-NMSSNNPAPVHYPHQCPSSQYVREQKAEDGYNWRKYGQKQVK	152
	 : . : . : * . *****	
AtWRKY33		GSENPRSYKCTFPNCPTKKKVERSLEQQITEIVYKGSHNHPKPQSTRRSSS-SSSTFHS	257
AhWRKY33		GSENPRSYKCTFPNCPTKKKVERSLEQQITEIVYKGSHNHPKPQSTRRSSS-SSSTFHS	251
AlWRKY33		GSENPRSYKCTFPNCPTKKKVERSLEQQITEIVYKGSHNHPKPQSTRRSSS-SSSTFHS	243
BnWRKY33		GSENPRSYKCTFPSCPTKKVEMSLDGQITEIVYKGSHNHPKPQSTRRSSS-SSTFHS	238
BoWRKY33		GSENPRSYKCTFPSCPTKKVEMSLDGQITEIVYKGSHNHPKPQSTRRSSS-FSTFHS	238
BrWRKY33		GSENPRSYKCTFPSCPTKKKVERSLEQQITEIVYKGSHNHPKPQSTRRSSSSSTFHS	224
StWRKY33		GSENPRSYKCTFPNCPTKKKVERNLGDGHITEIVYKGSHNHPKPQSTRRSSSQVNLAY	272
SlWRKY33		GSENPRSYKCTFPNCPTKKKVERNLGDGHITEI-----	185
		*****:*****:*****:*****	
AtWRKY33		AVVNASLDHNRQASSDQPNNSNFSHQSDSFGMQQEDNTTSDSVGDDFEQGSSIISRDEE	317
AhWRKY33		AVVNASLDHNRQASSDQPNNSNFSHQSDSFGMQQEDNTTSDSVGDDFEQGSSVVSREEE	311
AlWRKY33		AVVNASLDHNRQASSDQPNNSNFSHHSDSFGMQQEDNTTSDSVGDDFEQGSSIISRREEE	303
BnWRKY33		GG-----DHHGSSDSFAIQQEDNTTSGLGDDEL---SVISRDEE	276
BoWRKY33		GG-----DHHGSSDSFAIQQEDNATSGLGDEL---SVISREEE	276
BrWRKY33		AVFNASLD-----NSFHSDSLAIQQDDNTTSGSVGDDFERGSSVVSRE-E	270
StWRKY33		SNL-----DVTNQPNAFHENGQRDSFAVTD---NSSASFGDEDVDQGPSPIS-KSGE	319
SlWRKY33		-----PNAFLENGQRDSFAVTD---NSSASFGDDDVQGPSPIS-KSGE	224
		*****: : : ** *.*: : : .. *	
AtWRKY33		DCGSEPEAKRWKGDNETNGGNGGGSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	377
AhWRKY33		DCGSEPEAKRWKGDNETNGGNGGGSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	371
AlWRKY33		DCGSEPEAKRWKGENETNGGNGGGSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	363
BnWRKY33		DCGSEPEAKRWKGENETNGGNGDGSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	336
BoWRKY33		DYGSEPEAKRWKGENETNGGSGNGSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	336
BrWRKY33		ECGSEPEAKRWKGESETNGGNNGNSKTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	330
StWRKY33		--NEPEAKRWKGDNENEV-ISSASRTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	375
SlWRKY33		NDENEPEAKRWKGDNENEV-ISSASRTVREPRIVVQTTSIDILDDGYRWRKYGQKVVKG	283
		*****:*. : ****:*****:*****:*****	
AtWRKY33		NPNPRSYYKCTTIGCPVRKHVERASHDMRAVITTYEGKHNHDVPAARGSGYA-TNRPQD	436
AhWRKY33		NPNPRSYYKCTTIGCPVRKHVERASHDMRAVITTYEGKHNHDVPAARGSGYA-TNRPQD	430
AlWRKY33		NPNPRSYYKCTTIGCPVRKHVERASHDMRAVITTYEGKHNHDVPAARGSGYA-TNRPQD	422
BnWRKY33		NPNPRSYYKCTTIGCPVRKHVERASNDMRAVITTYEGKHNHDVPAARGSGYS-TNRLAQD	395
BoWRKY33		NPNPRSYYKCTTIGCPVRKHVERASNDMRAVITTYEGKHNHDVPAARGSGYS-TNRLAQD	395
BrWRKY33		NPNPRSYYKCTTIGCPVRKHVERASQDLRAVITTYEGKHNHDVPAARGSGYA-TNRPVQD	389
StWRKY33		NPNPRLAIPTPYNISCFSHYYILLCKELNDQLA-----	408
SlWRKY33		NPNPRSYYKCTFTGCPVRKHVERASHDLRAVITTYEGKHNHDVPAARGSGSYAMNKPPSG	343
		***** . . : . : . : : :	
AtWRKY33		SS---SVP--IRPAIAIGHSN-----YTTSSQAPYTLQMLHNNN--TNTGPFGYA	479
AhWRKY33		SS---SVP--IRPAIAIGHSN-----YTASSQAPYTLQMLHNNN--TNSGAFGYA	473
AlWRKY33		SS---SVP--IRPAIAIGHSN-----YTTSSQAPYTLQMLHNNN--TNSGAFGYA	465
BnWRKY33		PS---SAP--IRPNAIAGHSH-----YTTSSQAPYTLQMLQHNNNNNTNAGPFGYA	440
BoWRKY33		PS---SAP--IRPNAIAGHSH-----YTTSSQAPYTLQMLQHNN-NTNAGPFGYA	439
BrWRKY33		PS---SAP--IRPAAIAGHTN-----YTTSSRPPYTLQMLQHNNNNNTNAGPYGYA	434
StWRKY33		-----	408
SlWRKY33		SNNNNNSMPVPRPTVLANHSNQGMNFNDTFFNTTQIOPPITLQMLQSSGTSSYSG-FGNS	402
		***** . . : . : . : : :	
AtWRKY33		MNNNNNNNSNLQTQONFVGGSRSRAKEEPNEET--SFFDSFMP	519
AhWRKY33		MNNNNNNNSNLQTQONFVGGSRSRAKEEPNEET--SFFDSFLP	513
AlWRKY33		MNNNNNNNSNLQTQONFVGGSRSRAKEEPNEET--SFFDSFLP	505
BnWRKY33		MNNNNNNNFQ-TQQNNFVGGSRSIAKEEPNEESSSSFFDSFLS	481
BoWRKY33		MNN---NQ-TQQNNFVGGSRSIAKEEPNEESSSSFFDSFLS	477
BrWRKY33		MNNNN--NNLQTQRNDFAGGSRSRAKEEPNDES-SSFLLDSFLS	473
StWRKY33		-----	408
SlWRKY33		SGS-----YMNQMOHTNNSKPISKEEPKD--DLFFSSFLN	435

Fig. S1. Identified SUMO sites are conserved in different WRKY33 homologs.

Sequence alignment of WRKY33 amino acid from *Arabidopsis thaliana* (AtWRKY33) against WRKY33 sequence of *Arabidopsis halleri* (AhWRKY33), *Arabidopsis lyrata* (AlWRKY33), *Brassica napus* (BnWRKY33), *Brassica oleracea* (BoWRKY33), *Brassica rapa* (BrWRKY33), *Solanum tuberosum* (StWRKY33) and *Solanum lycopersicum* (SlWRKY33). The conserved Lysines are highlighted in grey. EnsemblPlants was used to identify WRKY33 homologs in other plant species and the alignment was done using Clustal Omega.

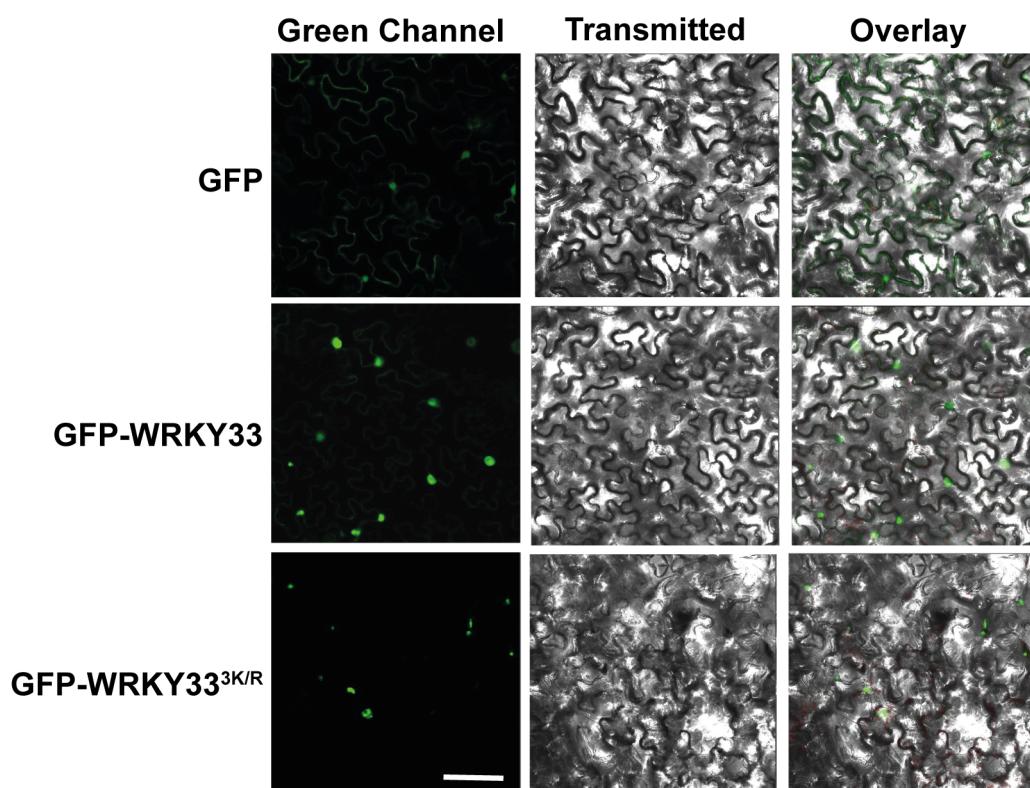


Fig. S2. SUMO site mutations did not affect sub-cellular localization of WRKY33 protein.
Localization of GFP-WRKY33 and GFP-WRKY33^{3K/R} proteins were analyzed in *N. benthamiana* leaves by confocal microscopy. The green channel shows GFP signals, transmitted is bright-field images and overlay is superimposition of the two channels. GFP only construct was used as a control. Scale bar = 10 μ M.

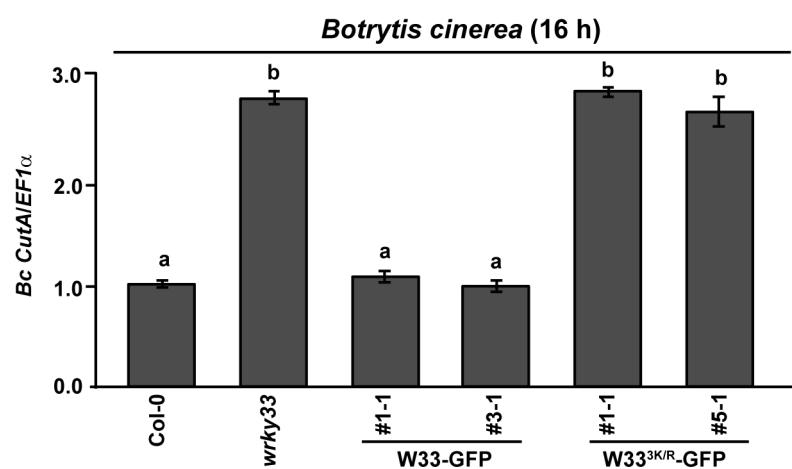


Fig. S3. W33^{3K/R}-GFP transgenic lines were more susceptible to *B. cinerea* infection
 4-week-old plants of the different genotypes indicated were spray inoculated with 5×10^5 spores/ml and *B. cinerea* Cutinase A expression was analyzed in leaf samples 16 hours post-infection as a measure of susceptibility to *B. cinerea*. Data presented are means \pm SD from two independent biological replicates each with 2 independent technical replicates and normalized to *EF1 α* expression. Bars with different letters were significantly different from others (two-tailed Student's t-test; P<0.05).

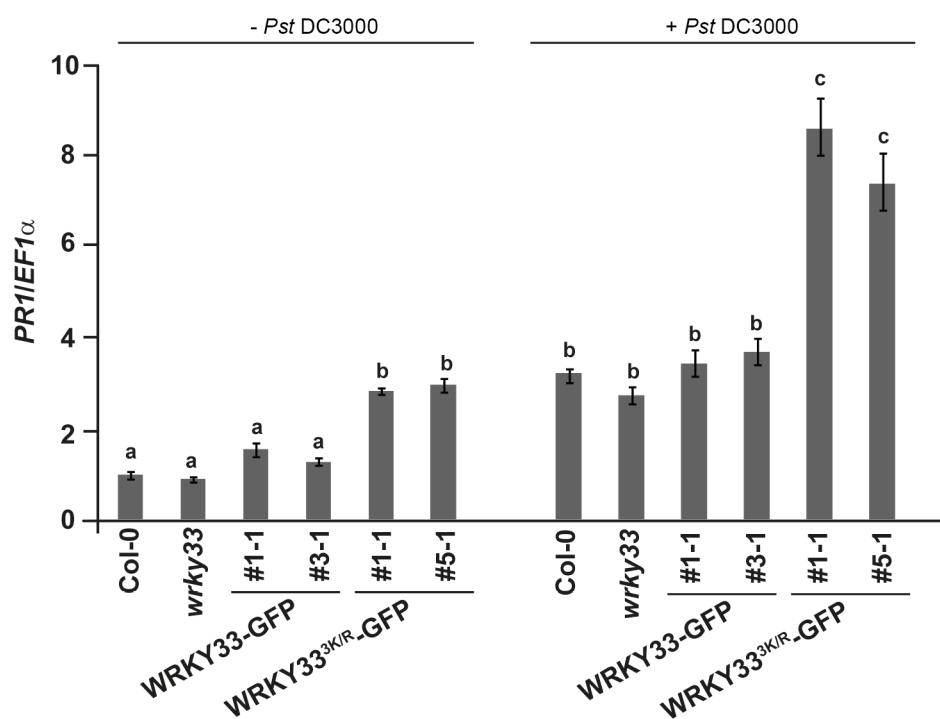


Fig. S4. *PR1* transcript levels are enhanced in *W33^{3KIR}-GFP* transgenic lines upon *Pseudomonas syringae* DC 3000 infection

4 weeks-old-plants of the different genotypes indicated were inoculated with *Pst* DC3000. *PR1* gene expression was analyzed in leaf samples 24 hours post inoculation. Data presented are means \pm SD from 2 independent biological replicates, each with 3 independent technical replicates. *EF1α* gene expression was used to normalize the dataset. Bars with different letters were significantly different from others (two-tailed Student's T-test; P<0.05).

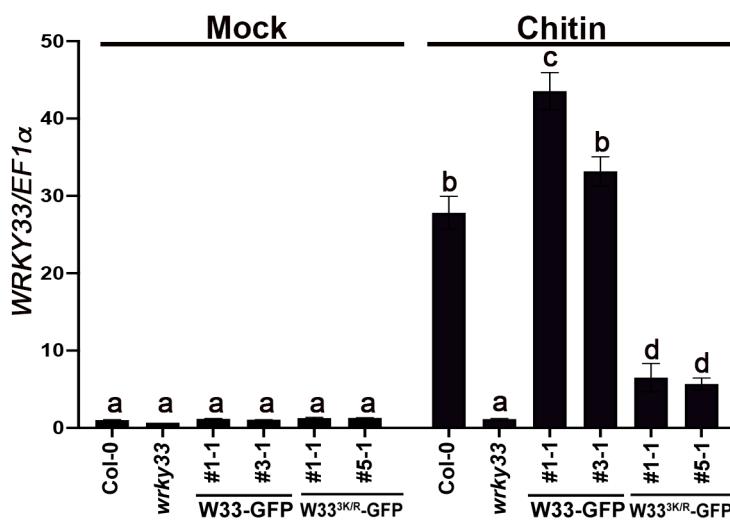


Fig. S5. WRKY33 expression is regulated by chitin in a SUMOylation-dependent manner. 12-day-old seedlings of the different genotypes indicated were treated with 100 µg/ml chitin for 30 min and WRKY33 expression was analyzed. The mock treatment was done with water and mock-treated seedlings were used as controls. Bars represent means ± S.D. from two independent biological replicates. The mock-treated Col-0 was used as reference and normalization was done using *EF1 α* . Genotypes with different letters were significantly different from others (two-tailed Student's t-test; P<0.05).

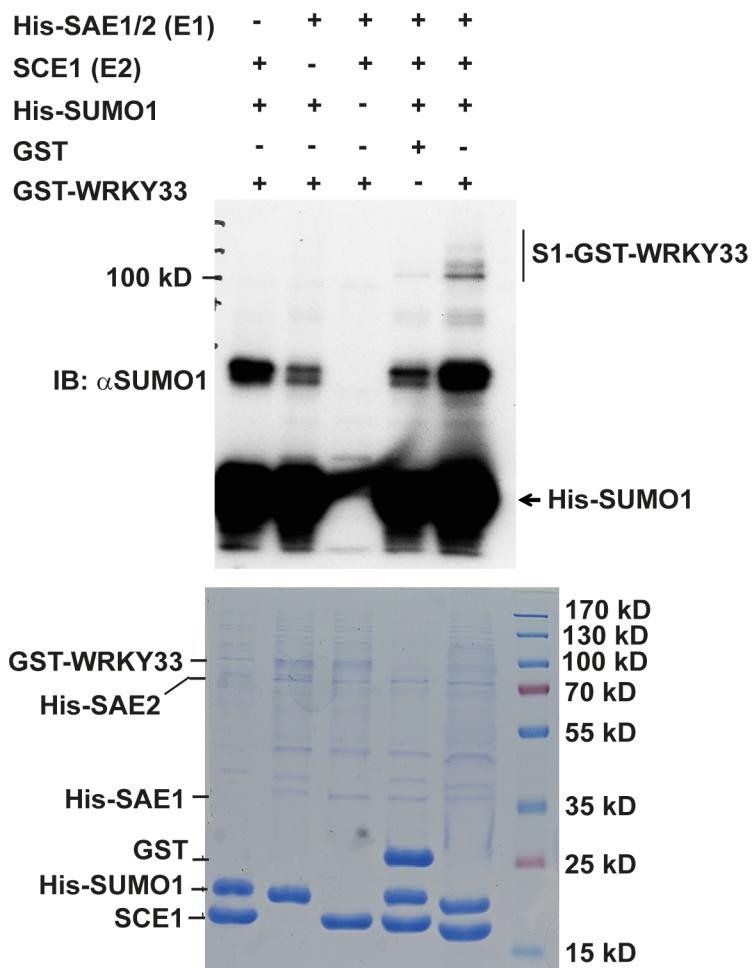


Fig. S6. SPF1 and SPF2 SUMO proteases deSUMOylate WRKY33 (in support of Fig. 3B).

Upper Blot: Full immunoblot of WRKY33 in vitro SUMOylation experiment blotted with anti-SUMO1/2 antibody (IB: α SUMO1) depicting SUMOylated (S1-GST-WRKY33) and free SUMO protein at the bottom (His-SUMO1).

Lower Blot: A commassie gel was run as a control to depict the presence of different proteins in the respective reactions. The proteins were identified and labelled based on their molecular weights and presence in different reactions.

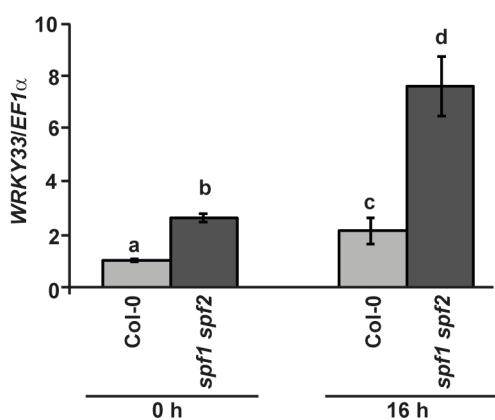


Fig. S7. *WRKY33* transcript levels were higher in *spf1 spf2* mutant after *B. cinerea* infection.

4-week-old plants of Col-0 and *spf1 spf2* mutant were spray inoculated with 5×10^5 spores/ml of *B. cinerea* and *WRKY33* transcripts were analyzed 0 and 16 hours post-infection. Data are means \pm SD from two biological and two technical replicates. Col-0 before infection was taken as the reference and values were normalized to *EF1 α* expression. Bars with different letters were significantly different from others (two-tailed Student's t-test; P<0.05)

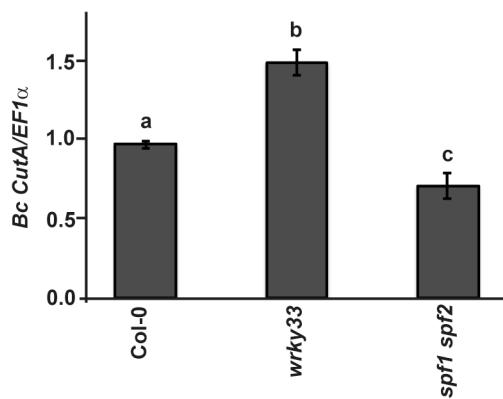


Fig. S8. *spf1 spf2* mutant is more resistant to *B. cinerea* infection than Col-0.

Transcript levels of *B. cinerea* Cutinase A were analyzed in Col-0, wrky33 and *spf1 spf2* mutant 16 h post *B. cinerea* infection. 4-week-old plants of the different genotypes were spray inoculated with 5×10^5 spores/ml and *B. cinerea* Cutinase A expression was analyzed as a measure of susceptibility to *B. cinerea*. Data presented are means \pm SD from two independent biological replicates each with 2 independent technical replicates. *EF1\alpha* was used as an internal control for normalization. A Student's t-test showed that genotypes having different letters on the bars were statistically different in *Bc CutA* expression ($P < 0.05$).

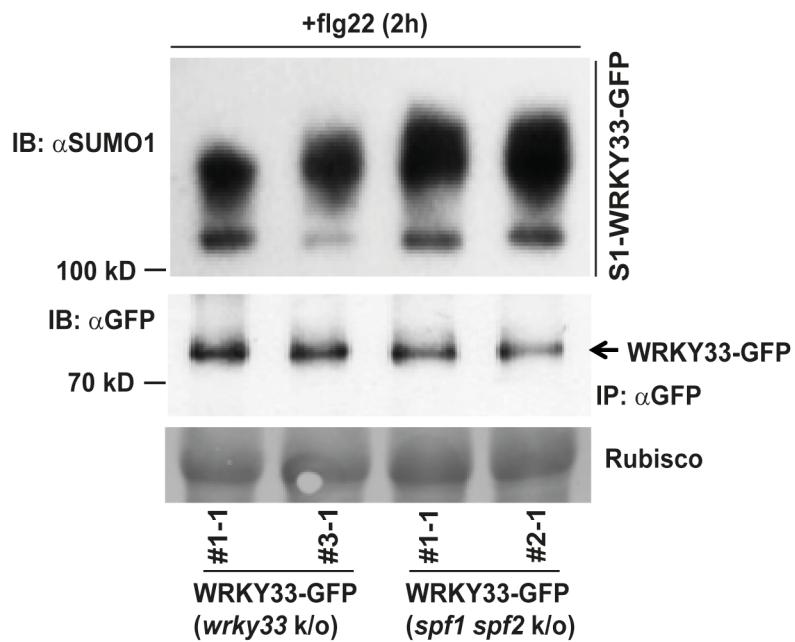


Fig. S9. WRKY33-GFP is more SUMOylated in *spf1 spf2* mutant background than in *wrky33* k/o background. Total protein from 12-day-old seedlings of *ProWRKY33::WRKY33-GFP* (*wrky33* k/o) transgenic lines (#1-1 and #3-1) and *ProWRKY33::WRKY33-GFP* (*spf1 spf2* k/o) transgenic lines (#1-1 and #2-1) treated with flg22 (1 μ M) for 2 h was immunoprecipitated with anti-GFP beads (IP: α GFP) and immunoblotted with anti-GFP (IB: α GFP) for WRKY33-GFP and anti-SUMO1/2 (IB: α SUMO1) for SUMOylated WRKY33-GFP (S1-WRKY33-GFP). Rubisco was used as the loading control.

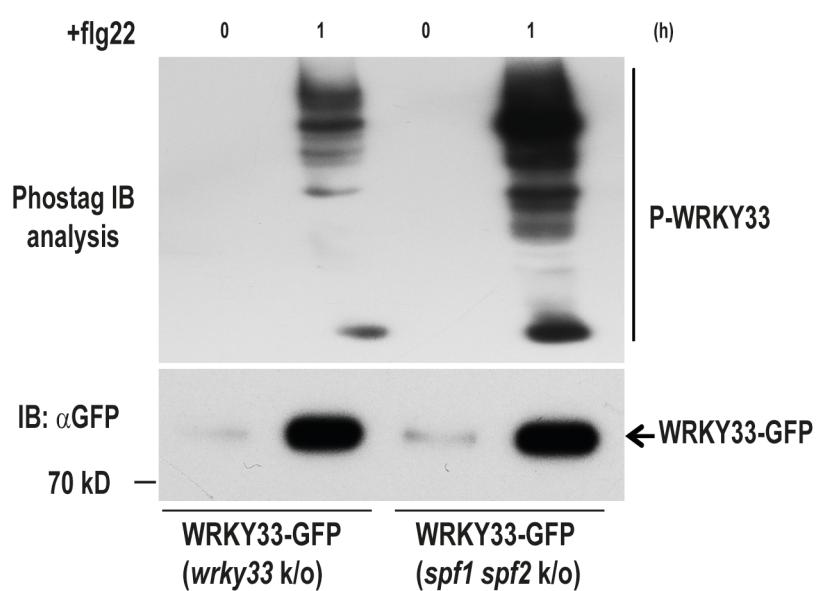


Fig. S10. WRKY33-GFP is more phosphorylated in the *spf1 spf2* mutant than in *wrky33 k/o* background.
 Total protein from 12-day-old seedlings of *ProWRKY33::WRKY33-GFP* (*wrky33 k/o*) transgenic line (#1-1) and *ProWRKY33::WRKY33-GFP* (*spf1 spf2 k/o*) transgenic line (#1-1) treated with flg22 (1 μ M) were immunoprecipitated with anti-GFP (IP: α GFP) beads and subjected to Phos-tag gel analysis to detect WRKY33 phosphorylation status. They were immunoblotted with anti-GFP (IB: α GFP) antibodies (top-panel) and a regular blot was also done to detect total WRKY33 protein (bottom panel).

MPK3	MNT-----	GGGQYTDFPAVETHGGQFISYDIFGSLFEITS	35
MPK4	-----MSAESCFCGSSGDQSSSKGVATHGGSYVQYNVYGNLFEVSR	40	
MPK6	MDGGSGQPAADTEMTEAPGGFPAAAPSPQMPGIENIPATLSHGRFIQYNIFGNIFEVTA	60	
MPK11	-----MSIEKPF---FGDDSNRGVSINGGRYVQYNVYGNLFEVSK	37	
MPK12	-----MSGESSSG--STEHCIKVVPTHGGRYVQYNVYGNLFEVSR	38	
	. : * : * : * : * : * :		
MPK3	36 KYRPPIIPIRGAYGIVCSVLDTETNELVAMKKIANAFDNHMDAKRTLREIKLLRHMDHE	95	
MPK4	41 KYVPPRLPIRGAYGIVCAATNSETGEEVAIKKIGNAFDNIIDAKRTLREIKLLKHMDHE	100	
MPK6	61 KYKPPIMPIKGAYGIVCSAMNSETNESVAIKKIANAFDNKIDAKRTLREIKLLRHMDHE	120	
MPK11	38 KYVPPRLPIRGAGSAGIVCAAWNSETGEEVAIKKIGNAFGNIIDAKRTLREIKLLKHMDHD	97	
MPK12	39 KYVPPIRPIRGAGCAGIVCAAVNSVTGEKVAIKKIGNAFDNIIDAKRTLREIKLLRHMDHE	98	
	* * : * * : * * : * : * : * : * : * : * : * : * : * : * : * :		
MPK3	96 NIAIRDVVPPPLRRQFSVDVYISTELMDTDLHQIIRSNQLSEEHQCQYFLYQLLRGLKYI	155	
MPK4	101 NVIAVKDIKPKPQRENFDNDVYIVYELMDTDLHQIIRSNQPLTDDHCRFFLYQLLRGLKYV	160	
MPK6	121 NIVAIRDIIIPPLRNRNAFDNDVYIAYELMDTDLHQIIRSNQALSEEHQCQYFLYQILRGLKYI	180	
MPK11	98 NVIAIIDIIIRPPQPDNFNDVHVIVYELMDTDLHHIIRSNQPLTDDHSRFFLYQLLRGLKYI	157	
MPK12	99 NVITIKDIVRPPQRDIFNDNDVYIVYELMDTDLQRILRSNQTLTSQCRFLVYQLLRGLKYV	158	
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :		
MPK3	156 HSANIIHRDLKPSNLLNNANCNCDLKICDFGLARPTSENDFMTEYVVTRWYRAPELLLNSSD	215	
MPK4	161 HSANVLHRDLKPSNLLNNANCNCDLKLGFGLARTKSETDFMTEYVVTRWYRAPELLNCSE	220	
MPK6	181 HSANVLHRDLKPSNLLNNANCNCDLKICDFGLARVTSESDFMTEYVVTRWYRAPELLNNSSD	240	
MPK11	158 HSANVLHRDLKPSNLLNNANCNCDLKIGDFGLARTKSETDFMTEYVVTRWYRAPELLNCSE	217	
MPK12	159 HSANILHRDLKPSNLLNNSKNELKIGDFGLARTTSDTDFMTEYVVTRWYRAPELLNCSE	218	
	* * : * * : * * : * : * : * : * : * : * : * : * : * : * : * : * :		
MPK3	216 YTAIAIDVWSVGCIFMELMNRKPLFPKGDKDHVHQMRLLTELLGPTESDLGFTHNEDAKRYI	275	
MPK4	221 YTAIAIDIWSVGCILGETMTREPLFPKGDKDYVHQLRLITELGSPDDSSLGFLRSNDARRYV	280	
MPK6	241 YTAIAIDVWSVGCIFMELMDRKPLFPGRDGHVHQLRLMELIGTPSEELEFL-NENAKRYI	300	
MPK11	218 YTAIAIDIWSVGCILGEIMTREPLFPGRDGYVQQLRLITELGSPDDSSLGFLRSNDARRYV	277	
MPK12	219 YTAIAIDIWSVGCILGEIMTGQPLFPKGDKDYVHQLRLITELVGSPDNSSLGFLRSNDARRYV	278	
	***** : ***** : * * : * : * : * : * : * : * : * : * : * : * : * :		
MPK3	276 RQLPNFPRQPLAKLFSHVNPMIALDVLDRMLTFDPNRRITVEQALNHQYLAKLHDPEPI	335	
MPK4	281 RQLPQYPRQNFQAARFPNMSAGAVDLLEKMLVFDPSSRRITVDEALCHPYLAPLHDINEEPV	340	
MPK6	301 RQLPPYPRQSTSITDKFPTVHPLAIDLIEKMLTFDPSSRRITVLDALAHPYLNSLHDISDEPE	360	
MPK11	278 RQLPQYPRQNFQAARFPNMSVNAV DLLQKMLVFDPNRRITVDEALCHPYLAPLHEYNEEPV	337	
MPK12	279 RQLPRYPKQQFAARFPKMPTTAIDLLERMLVFDPNRRISVDEALGHAYLSPHHDVKEPV	338	
	* * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : . **		
MPK3	336 CQKPFSFEEQQPLDEEQIKEMIYQEAIALNPETY-	370	
MPK4	341 CVRPFNFDFEQPTLTEENIKELIYRETVKFNPQDSV	376	
MPK6	361 CTIPFNFDFENHALSEEQMKEIYREALAFNPEYQQ	396	
MPK11	338 CVRPFHDFDFEQPSLTEENIKELIYRESVKFNP---	368	
MPK12	339 CSTPFSDFDFEHPSCTEEHIKELIYKESVKFNPDH--	371	
	* * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : . **		

Fig. S11. Identified SIM site is conserved in different MPK homologs.

Sequence alignment of MPK3 with other MPKs from Arabidopsis. The identified SIM site in MPK3, Ile22, is conserved (replaced by Val in MPK4, MPK11 and MPK12) in other MPKs and is highlighted in grey.

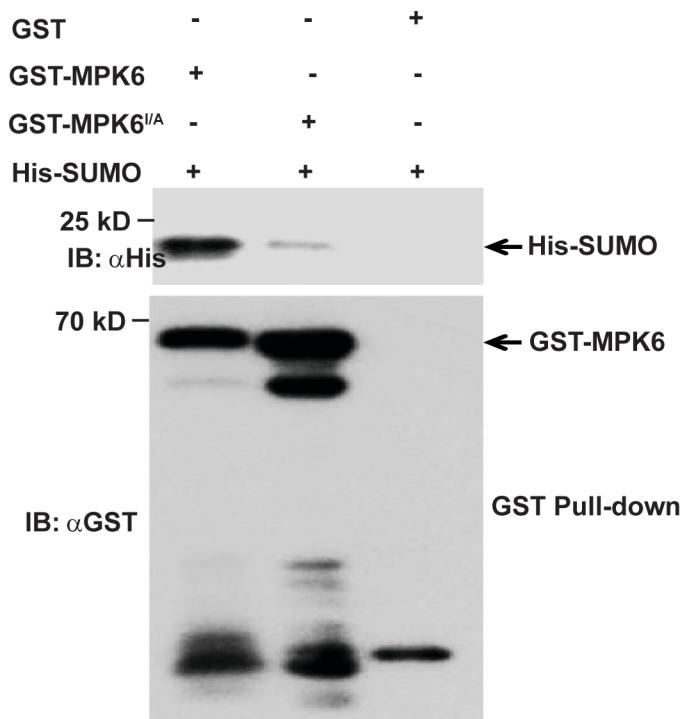


Fig. S12. MPK6 SIM (MPK6^{I47A}) site is crucial for interaction with SUMO.

Recombinant GST-MPK6 and GST-MPK6^{I47A} were immobilized on GST resin and then incubated with His-SUMO for 1 h. Reactions were thoroughly washed to remove any unbound proteins and elution was done using 4x SDS loading buffer. Samples were subjected to SDS-PAGE followed by immunoblotting with anti-GST (IB: αGST) and anti-His (IB: αHis) antibodies. GST only protein was used as a negative control. The experiment was conducted twice with consistent results.

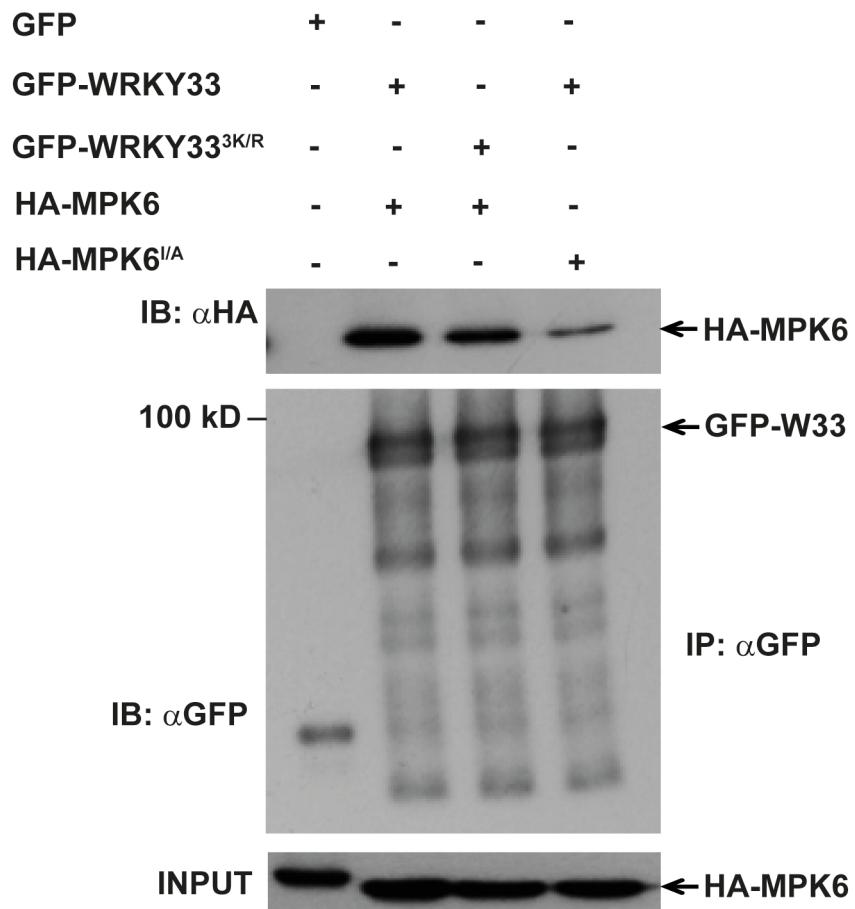


Fig. S13. SUMO-SIM module is required for WRKY33-MPK6 interaction.

Total protein extracted from *N. benthamiana* leaves transiently expressing GFP-WRKY33 or GFP-WRKY33^{3K/R} with either HA-MPK6 or with HA-MPK6^{I47A} was subjected to immunoprecipitation with anti-GFP beads (IP: α GFP) and immunoblotted with anti-HA (IB: α HA) for HA-fusion proteins and anti-GFP (IB: α GFP) for GFP/GFP-fusion proteins. Total protein extracts were probed with anti-HA antibody (HA-MPK6/HA-MPK6^{I47A} input) for equal HA-fusion proteins.