

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.

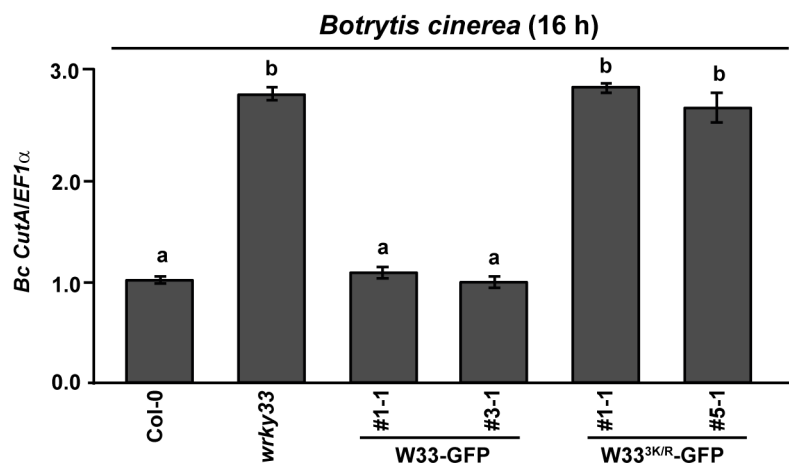


Fig. S3. W33^{3KR}-GFP transgenic lines were more susceptible to *B. cinerea* infection 4-week-old plants of the different genotypes indicated were spray inoculated with 5×10^5 spores/ml and *B. cinerea Cutinase A* expression was analyzed in leaf samples 16 hours post-infection as a measure of susceptibility 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; $P < 0.05$).

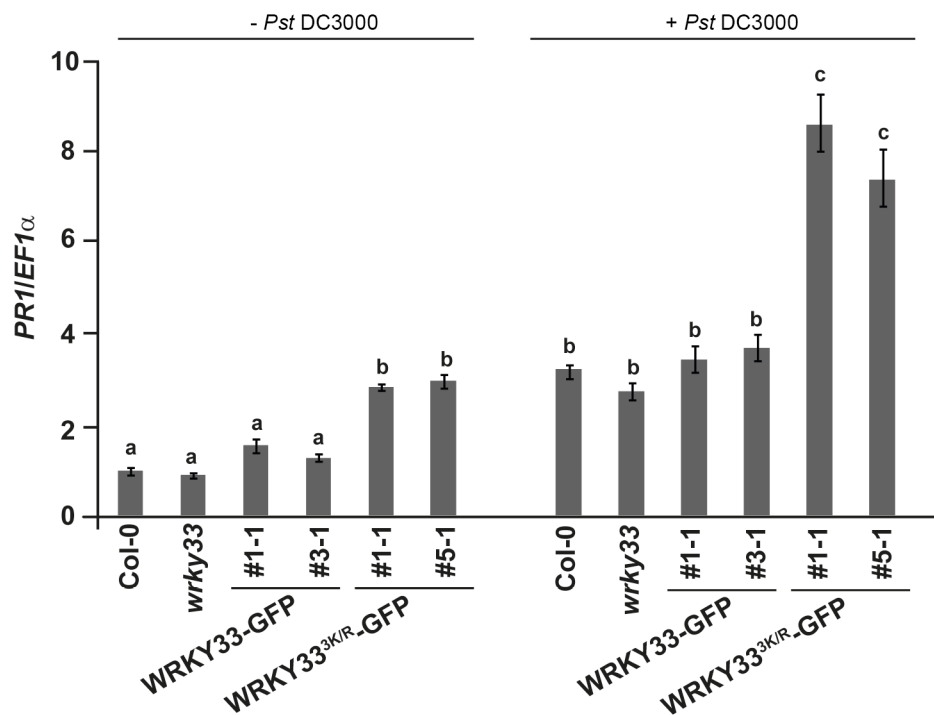


Fig. S4. *PR1* transcript levels are enhanced in *W33*^{3K/R}-GFP transgenic lines upon *Pseudomonas syringae* DC 3000 infection

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. *EF1 α* gene expression was used to normalize the dataset. Bars with different letters were significantly different from others (two-tailed Student's T-test; $P < 0.05$).

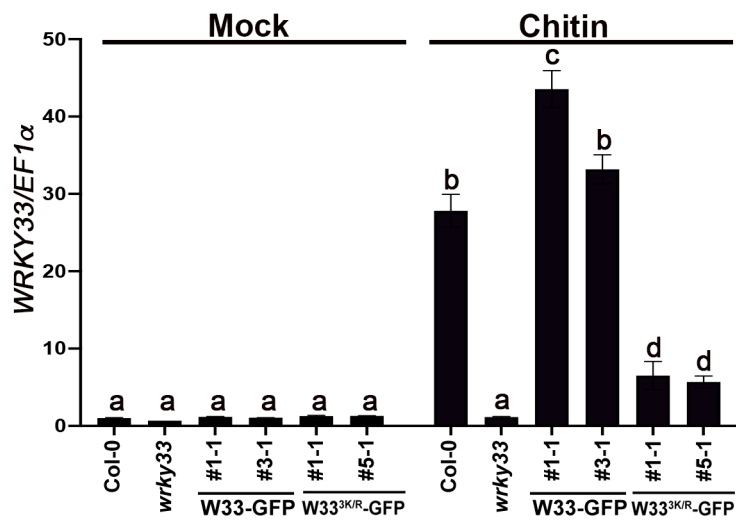


Fig. S5. WRKY33 expression is regulated by chitin in a SUMOylation-dependent manner. 12-day-old seedlings of the different genotypes indicated were treated with 100 μ g/ml chitin for 30 min and WRKY33 expression was analyzed. The mock treatment was done with water and mock-treated seedlings were used as controls. Bars represent means \pm 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; $P < 0.05$).

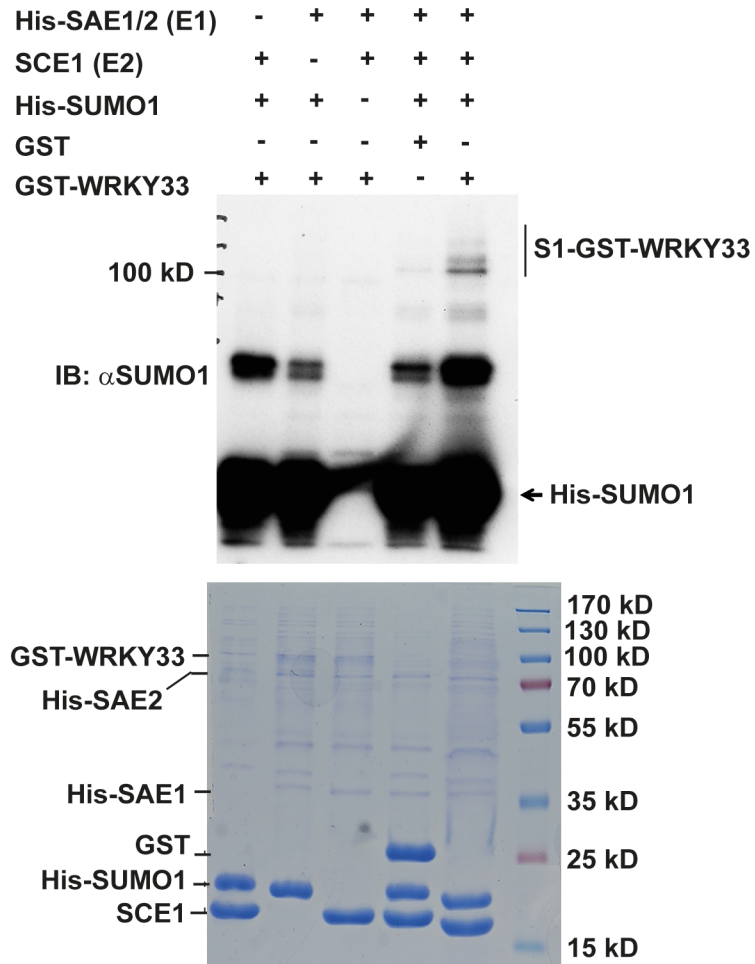


Fig. S6. SPF1 and SPF2 SUMO proteases deSUMOylate WRKY33 (in support of Fig. 3B).

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 Coomassie 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.

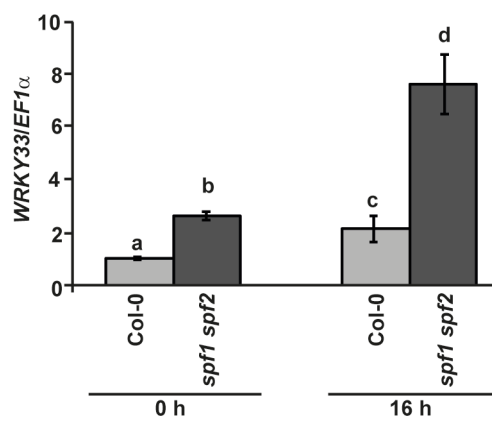


Fig. S7. *WRKY33* transcript levels were higher in *spf1 spf2* mutant after *B. cinerea* infection.

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; $P < 0.05$)

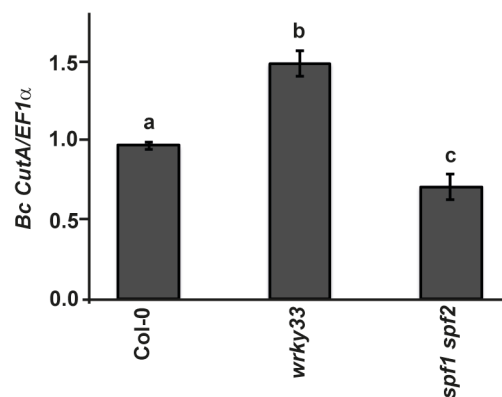


Fig. S8. *spf1 spf2* mutant is more resistant to *B. cinerea* infection than Col-0.

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 α* 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 ($P < 0.05$).

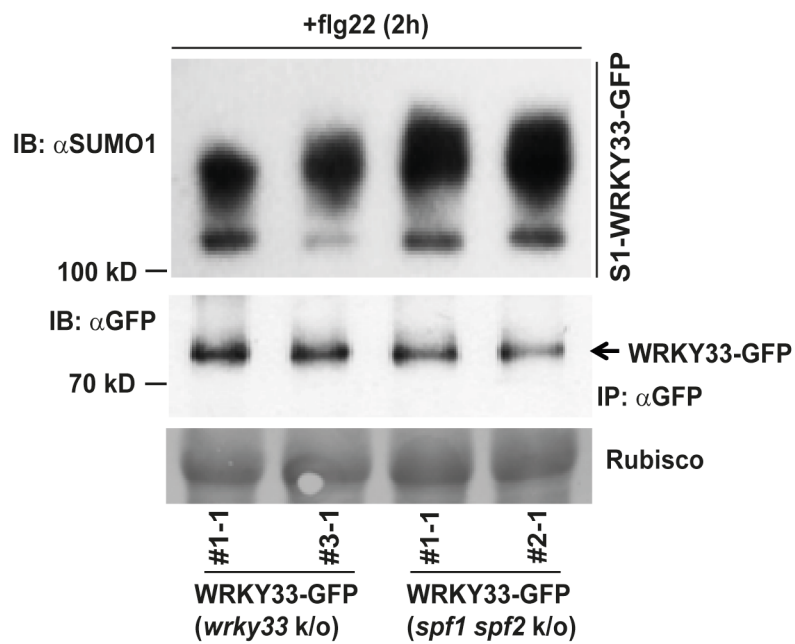


Fig. S9. WRKY33-GFP is more SUMOylated in *spf1 spf2* mutant background than in *wrky33* k/o background. Total protein from 12-day-old seedlings of *ProWRKY33::WRKY33-GFP* (*wrky33* k/o) transgenic lines (#1-1 and #3-1) and *ProWRKY33::WRKY33-GFP* (*spf1 spf2* k/o) transgenic lines (#1-1 and #2-1) treated 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.

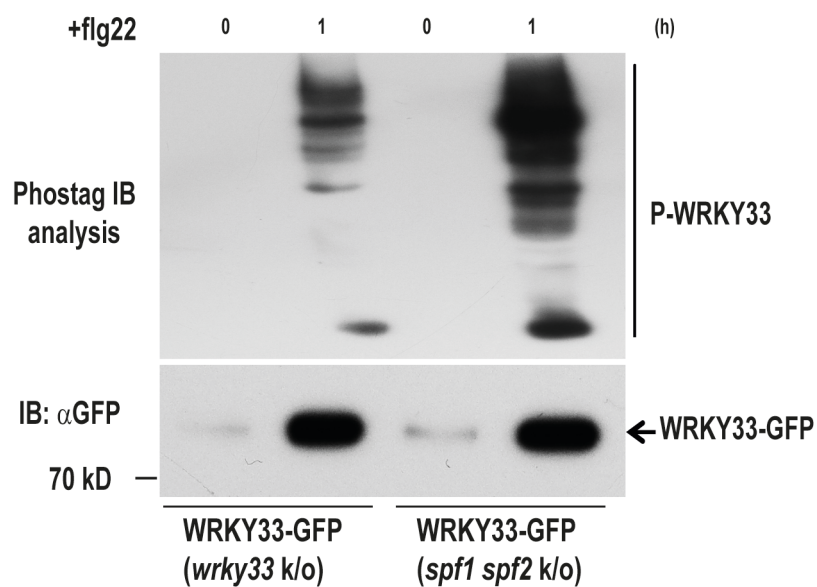


Fig. S10. WRKY33-GFP is more phosphorylated in the *spf1 spf2* mutant than in *wrky33* k/o background. Total protein from 12-day-old seedlings of *ProWRKY33::WRKY33-GFP* (*wrky33* k/o) transgenic line (#1-1) and *ProWRKY33::WRKY33-GFP* (*spf1 spf2* k/o) transgenic line (#1-1) treated with flg22 (1μM)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

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MPK3      MNT-----GGGQYTDFFPAVETHGGQFLSYDIFGSLFEITS 35
MPK4      -----MSAESCFCSSGDQSSSKGVATHGGSYVQYNVYGNLFEVSR 40
MPK6      MDGGSGQPAADTEMTEAPGGFPAAAPSPQMPGIENIPATLSHGGRFLQYNIFGNIFEVTA 60
MPK11     -----MSIEKPF---FGDDSNRQVSIINGGRYVQYNVYGNLFEVSK 37
MPK12     -----MSGESSG--STEHCIVVPTHGGRYVQYNVYQQLFEVSR 38
          .  **  :.:.*:.*:***:

MPK3      36 KYRPPIIPIGRGAYGIVCSVLDTETNELVAMKKIANAFDNHMDAKRTLREIKLLRHLDE 95
MPK4      41 KYVPPLRPIGRGAYGIVCAATNSETGEEVAIKKIGNAFDNIIDAKRTLREIKLLKHMDE 100
MPK6      61 KYKPPIMPIGKAYGIVCSAMNSETNESVAIKKIANAFDNKIDAKRTLREIKLLRHMDE 120
MPK11     38 KYVPPLRPIGRGASGIVCAAWNSETGEEVAIKKIGNAFGNIIDAKRTLREIKLLKHMDE 97
MPK12     39 KYVPPIRPIGRGACGIVCAAVNSVTGEKVAIKKIGNAFDNIIDAKRTLREIKLLRHMDE 98
          **  ** : ** : ** : ** : . : : * . * ** : ** : ** : * : ** : ** : ** : ** : ** : ** :

MPK3      96 NIIAIRDVVPPPLRRQFSDVYISTELMDTDLHQIIRSNQSLSEEHCQYFLYQLLRGLKYI 155
MPK4      101 NVIAVKDIIKPPQRENFDVYIVYELMDTDLHQIIRSNQPLTDDHCRFFLYQLLRGLKYV 160
MPK6      121 NIVAIRDIIPPLRNFNDVYIAYELMDTDLHQIIRSNQALSEEHCQYFLYQLLRGLKYI 180
MPK11     98 NVIAIIDIIIRPPQPDNFNDVHIVYELMDTDLHHIIRSNQPLTDDHRSFFLYQLLRGLKYV 157
MPK12     99 NVITIKDIVRPPQRDIFNDVYIVYELMDTDLQRILRSNQTLSQCRFLVYQLLRGLKYV 158
          * : : : : * : : * *      * . ** : *      * ** : * : ** : * : : : : : : : * : ** : ** :

MPK3      156 HSANIHRDLKPSNLLNANCDLKICDFGLARPTSEDFMTEYVTRWYRAPELLNSSD 215
MPK4      161 HSANVLRDLKPSNLLNANCDLKLGFGLARTKSETDFMTEYVTRWYRAPELLNCSE 220
MPK6      181 HSANVLRDLKPSNLLNANCDLKICDFGLARVTSEDFMTEYVTRWYRAPELLNSSD 240
MPK11     158 HSANVLRDLKPSNLLNANCDLKLGFGLARTKSETDFMTEYVTRWYRAPELLNCSE 217
MPK12     159 HSANILHRDLRPSNVLLNSKNELKIGDFGLARTTSDTFMTEYVTRWYRAPELLNCSE 218
          * ** : * ** : * ** : * ** : : * * : * ** * . * : * ** : ** : ** : ** : ** :

MPK3      216 YTAADIVWSVGCIFMELMNRKPLFPGKDHYVHQLRLITELIGSPDDSSLGFLRSDNARYV 275
MPK4      221 YTAADIDWSVGCILGETMTREPLFPGKDHYVHQLRLITELIGSPDDSSLGFLRSDNARYV 280
MPK6      241 YTAADIVWSVGCIFMELMDRKLPLFGRDHYVHQLRLIMELIGTPSEEELEFL-NENAKRYI 300
MPK11     218 YTAADIDWSVGCILGEIMTREPFLFPGRDYVQQLRLITELIGSPDDSSLGFLRSDNARYV 277
MPK12     219 YTAADIDWSVGCILGEIMTGPFLFPGKDHYVHQLRLITELVGPDDSSLGFLRSDNARYV 278
          * ** : * ** : * ** : * * : * ** : * : * : * : * : * : * : * : * : * : * : * : * :

MPK3      276 RQLPNFPRQPLAKLFSHVNPMAIDLVDRLTFDPNRRITVEQALNHQYLAKLHDPNDEPI 335
MPK4      281 RQLPQYPRQNFAARFPNMSAGAVDLEKMLVDFPSRRITVDEALCHPYLAPLHDINEEPV 340
MPK6      301 RQLPPYPRQSITDKFPTVHPLAIDLIEKMLTFDPRRRITVLDALAHPYLNSLHDISDEPE 360
MPK11     278 RQLPQYPRQNFAARFPNMSVNAVDDLQKMLVDFPNRRITVDEALCHPYLAPLHEYNEEPV 337
MPK12     279 RQLPRYPKQQFAARFPKMPPTAIDLLERMLVDFPNRRISVDEALGHAYLSPHHDVAKEPV 338
          * ** : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :

MPK3      336 CQKPFSEFEQQLDEEQIKEMIYQEAIALNPTYG- 370
MPK4      341 CVRPFNFDFEQPTLTEENIKELIYRETVKFNPDQSV 376
MPK6      361 CTIPFNDFENHALSEEQMKELIYREALAFNPEYQQ 396
MPK11     338 CVRPFHDFEQPSLTEENIKELIYRESVKFNP--- 368
MPK12     339 CSTPFSDFEHPSCTEEHKELIYKESVKFNPDH-- 371
          *  * * * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :

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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.

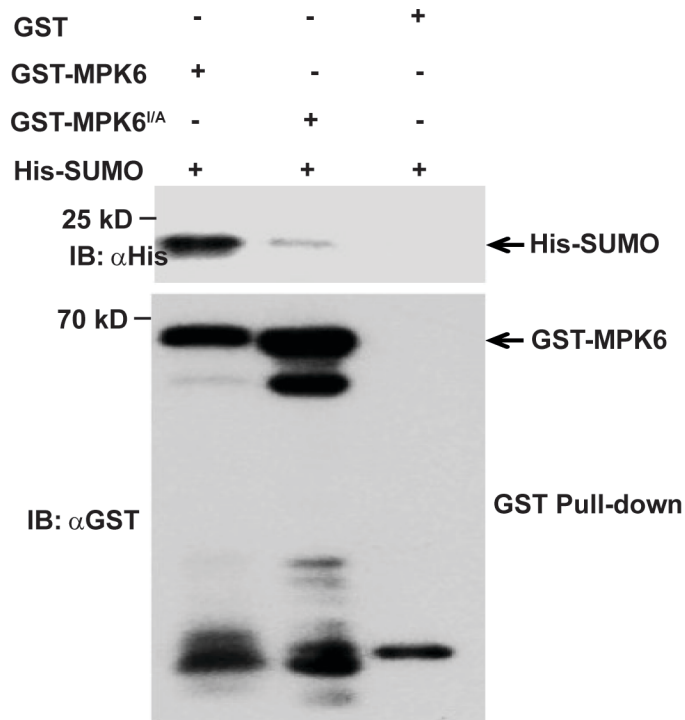


Fig. S12. MPK6 SIM (MPK6^{I47A}) site is crucial for interaction with SUMO.

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.

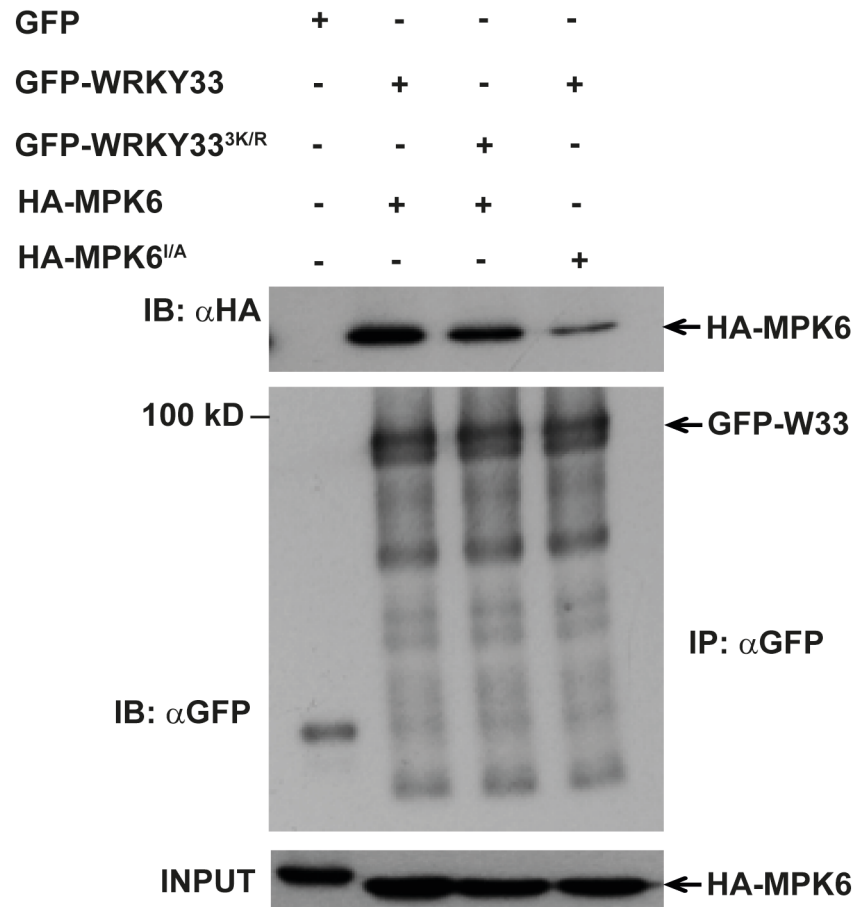


Fig. S13. SUMO-SIM module is required for WRKY33-MPK6 interaction.

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: αGFP) and immunoblotted with anti-HA (IB: αHA) for HA-fusion proteins and anti-GFP (IB: αGFP) 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.