

## Appendix for

### **E3 ligases SNIPER1 and SNIPER2 broadly regulate the homeostasis of plant sensor NLR immune receptors**

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**Running title:** Global control of NLRs by ubiquitination

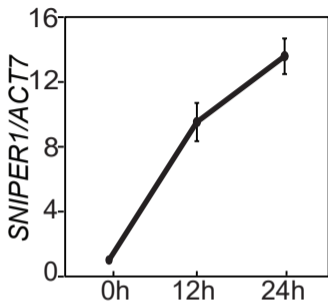
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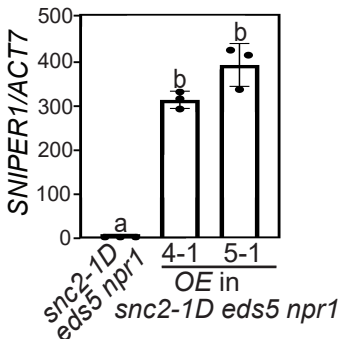
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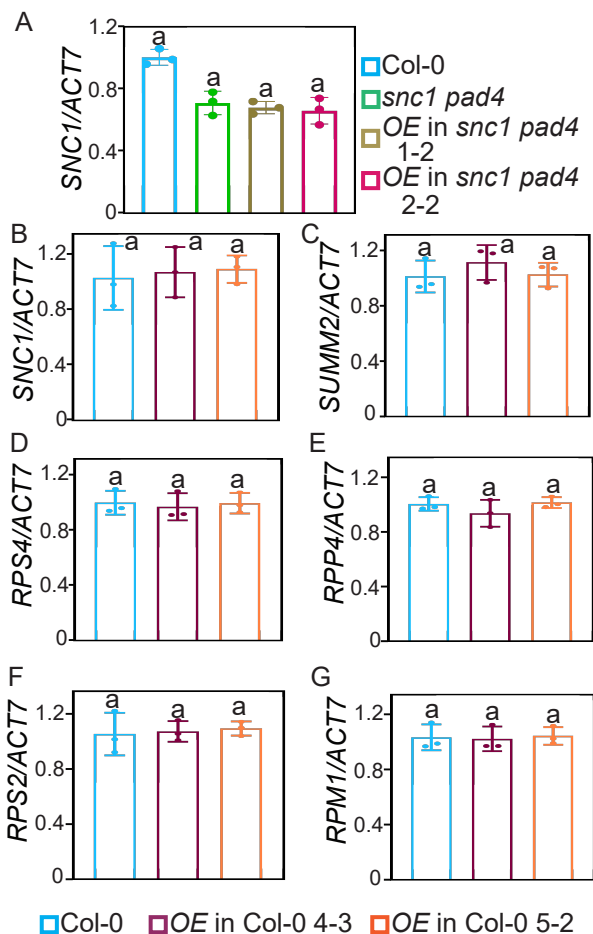
**Appendix Figure S1. *SNIPER1* expression is induced by pathogen.**

RT-PCR analysis of the expression of *SNIPER1* after *P.s.m.* ES4326 infection. Samples were collected at 0, 12 h and 24 h after *P.s.m.* ES4326 infection. Values were normalized to the level of *ACTIN7*. Error bars represent means  $\pm$  SD (n = 3).

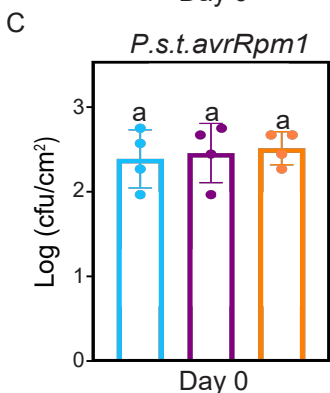
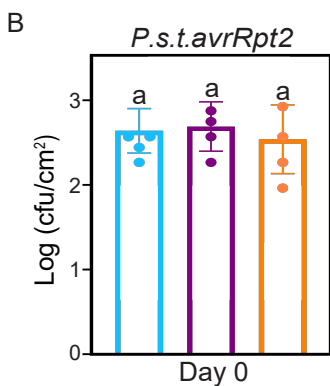
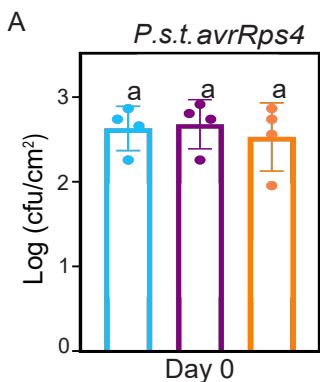


**Appendix Figure S2. *SNIPER1* gene expression in the *snc2-1D eds5 npr1* *SNIPER1* overexpression lines. In the triple mutant, *eds5 npr1* is used to overcome the sterility of *snc2-1D*.**

*SNIPER1* gene expression in the indicated plants as determined by RT-PCR. Statistical significance is indicated by different letters ( $p < 0.01$ ). Error bars represent means  $\pm$  SD ( $n = 3$ ). Two independent experiments were carried out with similar results.

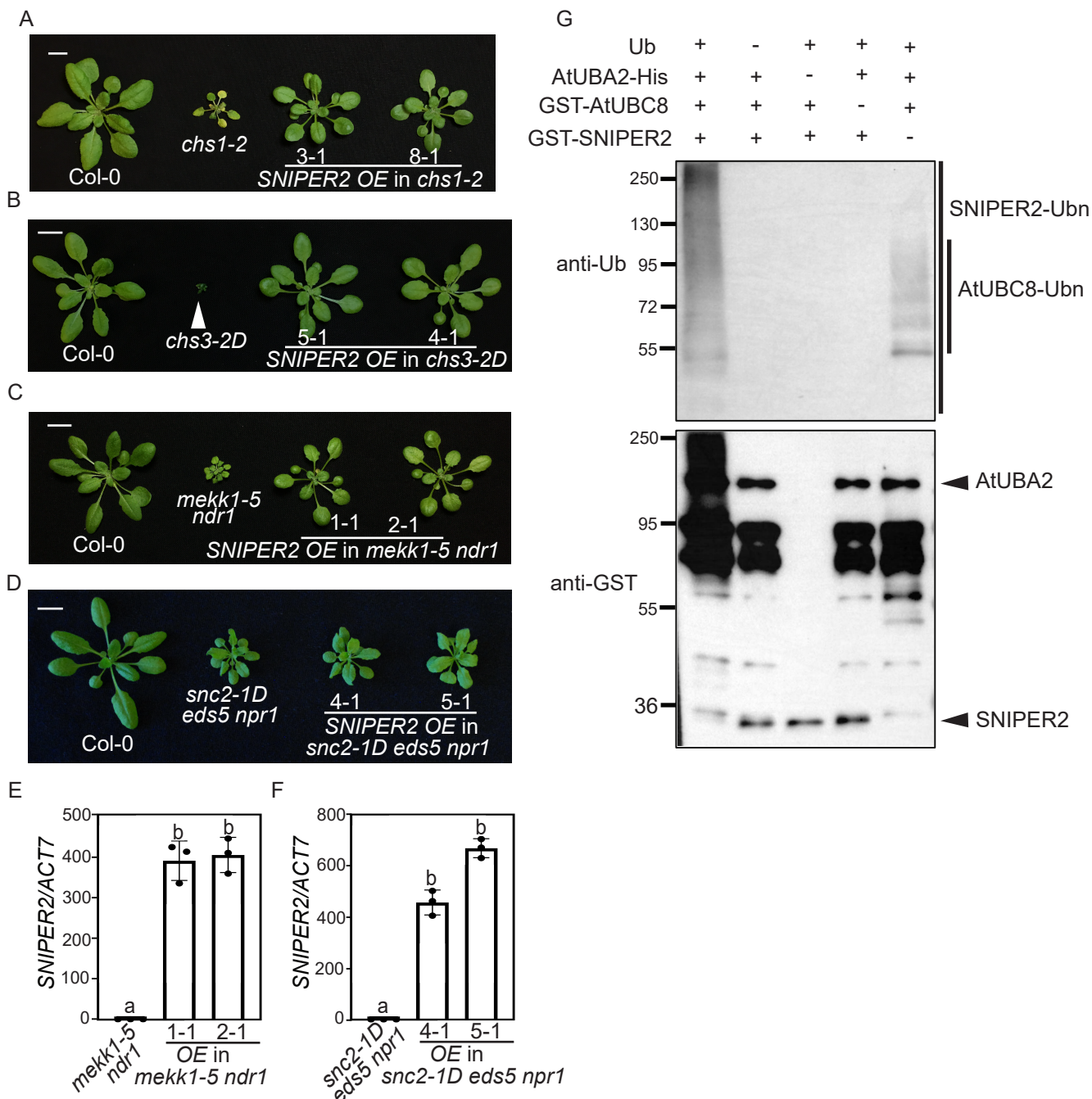


**Appendix Figure S3. Overexpression of *SNIPER1* does not affect sNLRs transcript levels.** RT-PCR analysis of the expression of *SNC1* (A-B), *SUMM2* (C), *RPS4* (D), *RPP4* (E), *RPS2* (F) and *RPM1* (G) in the indicated genotypes. Statistical significance is indicated by different letters ( $p < 0.01$ ). Error bars represent means  $\pm$  SD ( $n=3$ ). At least two independent experiments were carried out with similar results. Color squares shown on the bottom are for B-G.



□ Col-0    □ OE in Col-0 4-3    □ OE in Col-0 5-2

**Appendix Figure S4. Growth of *P.s.t. avrRps4*, *avrRpt2* and *avrRpm1* on four-week-old leaves of the indicated genotypes at 0 dpi with bacterial inoculum of OD600 = 0.0001 for Figure 5. Statistical significance is indicated by different letters ( $p < 0.01$ ).**



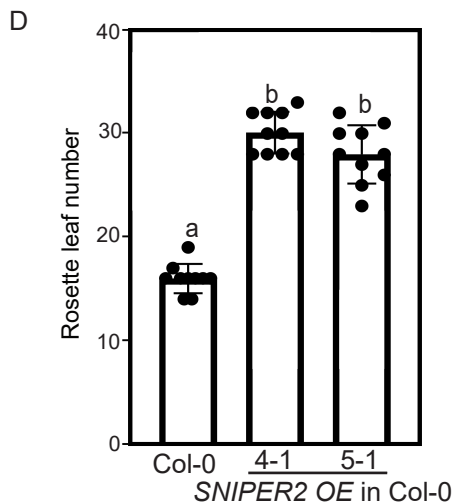
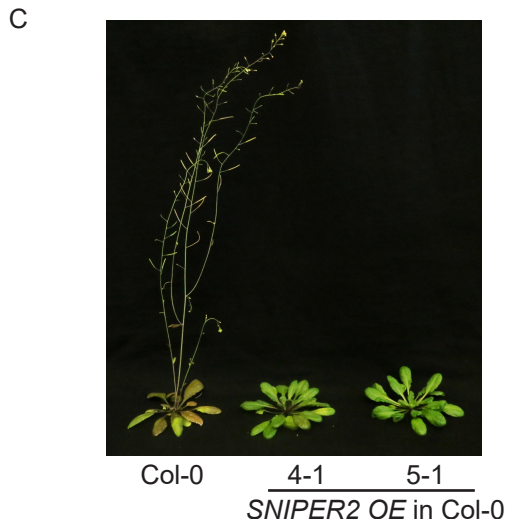
**Appendix Figure S5. SNIPER2 exhibits E3 ligase activity and overexpression of SNIPER2 fully suppresses both TNL- and CNL-mediated autoimmunity.**

A. Morphology of four-week-old soil-grown plants of Col-0, *chs1-2* and two independent transgenic lines of *SNIPER2* OE into *chs1-2* background. Plants were grown at 16 °C under long day conditions. Bar = 1cm.

B-D. Morphology of four-week-old soil-grown plants of Col-0, *chs3-2D* (B), *mekk1-5 ndr1* (C), *snc2-1D eds5 npr1* (D) and two independent transgenic lines of *SNIPER2* OE into *chs3-2D* (B), *mekk1-5 ndr1* (C), and *snc2-1D eds5 npr1* (D) backgrounds. Bar = 1cm

E-F. *SNIPER2* gene expression in the indicated plants as determined by RT-PCR. Statistical significance is indicated by different letters ( $p < 0.01$ ). Error bars represent means  $\pm$  SD ( $n = 3$ ). Two independent experiments were carried out with similar results.

G. *In vitro* ubiquitination assay using *E. coli*-expressed *SNIPER2* (E3), AtUBC8 (E2), AtUBA2 (E1) and/or Ubiquitin. The molecular mass markers are indicated on the left (kDa). Ubiquitination was detected by immunoblotting with an anti-ubiquitin antibody. AtUBA2 and *SNIPER2* were detected by immunoblotting with anti-GST antibody.



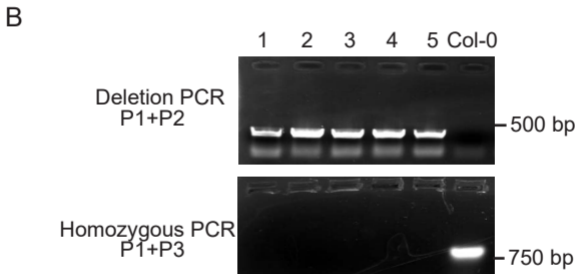
**Appendix Figure S6. *SNIPER2* OE lines are late flowering and exhibit pleiotropic alterations in leaf development.**

A. Morphology of four-week-old soil-grown plants of Col-0 and two independent transgenic lines of *SNIPER2* OE into Col-0 background. Bar = 1cm.

B. Rosette leaves of Col-0 and *SNIPER2* OE plants grown under LD conditions. All leaves, including cotyledons, are shown in order of production from the first true leaf.

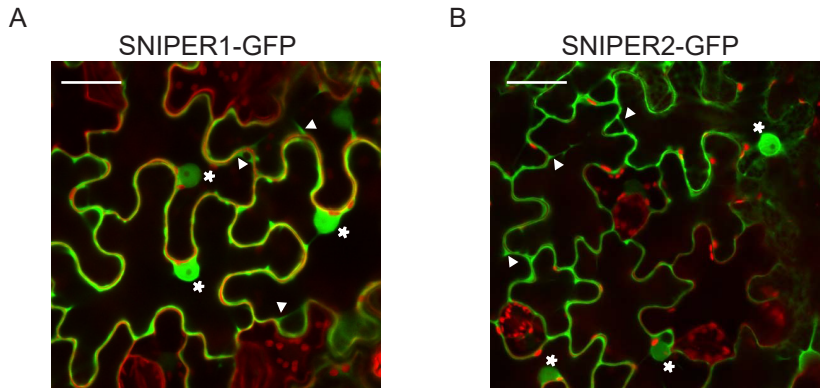
C. Morphology of eight-week-old soil-grown plants of Col-0 and two independent transgenic lines of *SNIPER2* OE into Col-0 background grown under long day conditions.

D. Quantification of flowering time under long day conditions by counting the number of leaves produced at bolting. Error bars represent means  $\pm$  SD (n = 10). Three independent experiments were carried out with similar results.



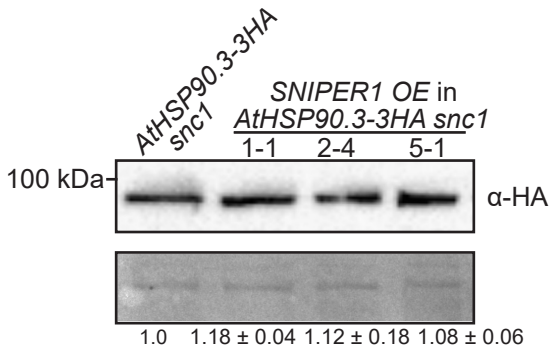
**Appendix Figure S7. Schematic description of CRISPR/Cas9 construct design (A) and PCR genotyping (B) of mutants.**





**Appendix Figure S8. Subcellular localization of SNIPER1-GFP and SNIPER2-GFP.**

Confocal images of SNIPER1-GFP (A) and SNIPER2-GFP (B) fluorescence in *N. benthamiana* leaves. The nuclear areas are indicated by white arrowheads. white asterisks indicate location of cytosol. Bar = 25  $\mu\text{m}$ .



**Appendix Figure S9. AtHSP90.3 protein level is not altered by *SNIPER1* overexpression.**

Western blot analysis of AtHSP90.3-3HA protein level in stable transgenic *Arabidopsis* lines overexpressing *SNIPER1* in *AtHSP90.3-3HA snc1* background. Equal loading is shown by Ponceau S staining of a non-specific band. The numbers below represent normalized band intensity  $\pm$  SD (n = 2). Molecular mass marker in kilo Daltons is indicated on the left. Two independent experiments were carried out with similar results.

Appendix Table S1. The list of primers used in this study

Name	Sequence	Purpose
U4h-EcoRI-F	ccggaattcATGTCTTCTGAGAATGATT	pHAN SNIPER1 OE construct
U4h-BamHI-R	cgcgatccTTAGTTTCTTCTGTGCGCCGGC	
U4h H129YF	GAGATGCCTTGTAATAT AAGTTTCACTCAA	DN form of SNIPER1
U4h H129YR	TTGAGTGAAACTTATTTTTACAAGGCATCTC	
U4h-KpnI-F	gcgcggtaccATGTCTTCTGAGAATGATT	Cluc/HATurobI D vector
U4h-SpeI(no stop)-R	gactagtcGTTTCTTCTGTGCGCCGGCGTT	
U4h-SfiI-F	CCCGGCCGTCAAGGCCa ATGTCTTCTGAGAATG ATT	pGST1FLAG- SNIPER1
U4h-SfiI-R	GCCGGCCCATGAGGCCTTAGTTTCTTCTGTGCG CGG	
U4hZZ-BamHI-F	cgcgatccATGTCTTCTGAGAATGATT	pACYCDuet vector
U4HstuI (ns)-R	GAAGGCCTGTTTCTTCTGTGCGCCGGCGTT	
SALKseq_28010_U4h_ LP	TCCAAGGTTCTGTGATGATCG	<i>sniper1-1</i> genotyping PCR
SALKseq_28010_U4h_ RP	cccattgtgcatcttgagg	
SUMM2-DraIII-F	cggcggCACTCAGTGATGGGAGCTTGTTAACT	Nluc/FLAG vector
SUMM2-DraIII(ns)-R	cggcggCACTCAGTGCCGCACATAACTAATTGC	
SUMM2(NB)-EcoRI-F	CGGAATTCATGGAAACAATGCTCGAAAGG	Nluc/FLAG/GS T-HA vector
SUMM2(NB)-BamHI- NF	cgcgatccATGGAAACAATGCTCGAAAGG	
SUMM2(NB)-StuI-R	GAAGGCCTATTTTCATCTATGAATCCCT	
RPP4-KpnI-F	gcgcggtaccATGGCTTCTTCTTCTTCTTC	Nluc/FLAG vector
RPP4-SalI (ns)-R	CGCGTCGACTATCAACATCAGCATCATCA	
RPP4(NB)-EcoRI-NF	CGGAATTCATGGGTGACTTCGTCGGAATTG	Nluc/FLAG/GS T-HA vector
RPP4(NB)-KpnI-F	gcgcggtaccATGGGTGACTTCGTCGGAATTG	
RPP4(NB)-StuI-R	GAAGGCCTGACGTTACTGACTTTAAAACC	
<u>CHS1(NB)-EcoRI-F</u>	CGGAATTCATGCGTCACATGAAAGCATTGTA	Nluc/FLAG/GS T-HA vector
CHS1(NB)-BamHi-NF	cgcgatccATGCGTCACATGAAAGCATTGTA	

CHS1(NB)-StuI-R	GAAGGCCTCATAATCTTCCAAACTTCCT	
RPP4-RT-F	AACTCAGCCACTTGGAAG	RT PCR
RPP4-RT-R	CAAGCGACTGGATGC	
Summ2-RT-F	GGTTCTGGACTCGGAAC	
Summ2-RT-R	CCATAAGGGATGAAGAACTTG	
U4H-CR-DT1-BsF	ATATATGGTCTCGATTGaagtcttaagcgtcgtcaaGTT	SNIPER1 CRISPR construct
U4H-CR-DT1-F0	TGaagtcttaagcgtcgtcaaGTTTTAGAGCTAGAAATAG C	
U4H-CR-DT2-R0	AACcaagatgcaacatggggctAATCTCTTAGTCGACTCT AC	
U4H-CR-DT2-BsR	ATTATTGGTCTCGAAACcaagatgcaacatggggctC	
1g14200-del-NF	AACGAGTTCCGAGAATGTCAG	SNIPER1 deletion PCR
1g14200-del-R	CTCAAGGTAGCCACAACCA	
SALKseq_63073-U4-LP	GGCGACAGAACAAGAAGCTG	<i>sniper2-1</i> genotyping PCR
FLAG_311D05-U4-R	ACCATCACTGTTTCCACCG	
U4-SfiI-F	CCCGGCCGTCAAGGCCaATGGCGACAGAACAAG AAG	pGST1HA- SNIPER2 OE constuct
U4-SfiI-R	GCCGGCCCATGAGGCCCTAATTCTCGGACTCAAC AC	
U4-BamHI-F	cgcgatccATGGCGACAGAACAAGAAG	GST-SNIPER2
U4-SalI-R	cgcgctgacCTAATTCTCGGACTCAACAC	
eds1-2 geno F	ACAAGCCAAAGTGTC AAGCC	<i>eds1-2</i> genotyping PCR
eds1-2 geno R	CAAGCATCCCTTCTAATGTC	