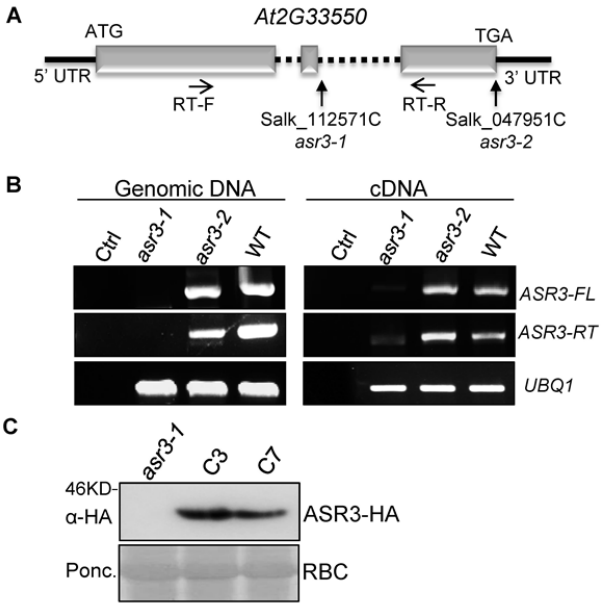
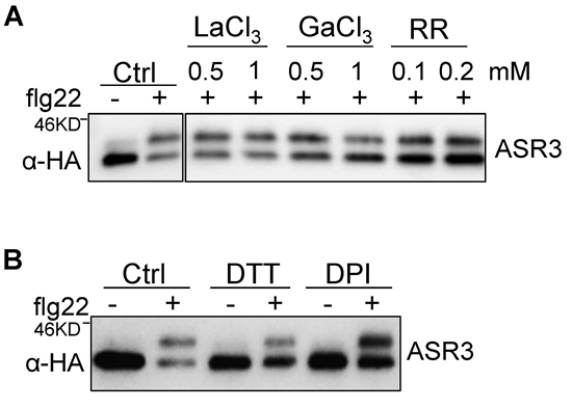
Supplemental Data



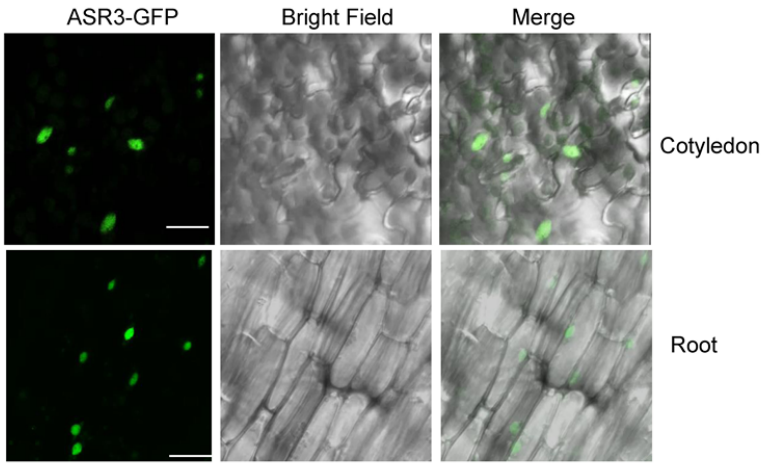
Supplemental Figure 1: Characterization of *asr3* T-DNA knockout lines and complementation transgenic lines.

(A) The scheme of *At2G33550* (*ASR3*) with gray boxes indicating exons and dash lines representing introns. The T-DNA insertions are indicated by arrows for two Salk lines. The positions of RT-PCR primers are labeled with arrows. **(B)** Genotyping and RT-PCR detection of *ASR3* expression in Salk lines. PCR was carried out with genomic DNA or cDNA using either the full-length or RT-PCR primers as indicated above. *UBQ1* was used as an internal control. Ctrl is H_2O. **(C)** Immunoblot analysis of ASR3-HA proteins in two independent transgenic lines (C3 and C7) of *pASR3:ASR3-HA* in the *asr3-1* mutant background. Ponceau staining (Ponc.) of RuBisCo proteins (RBC) serves as a loading control.



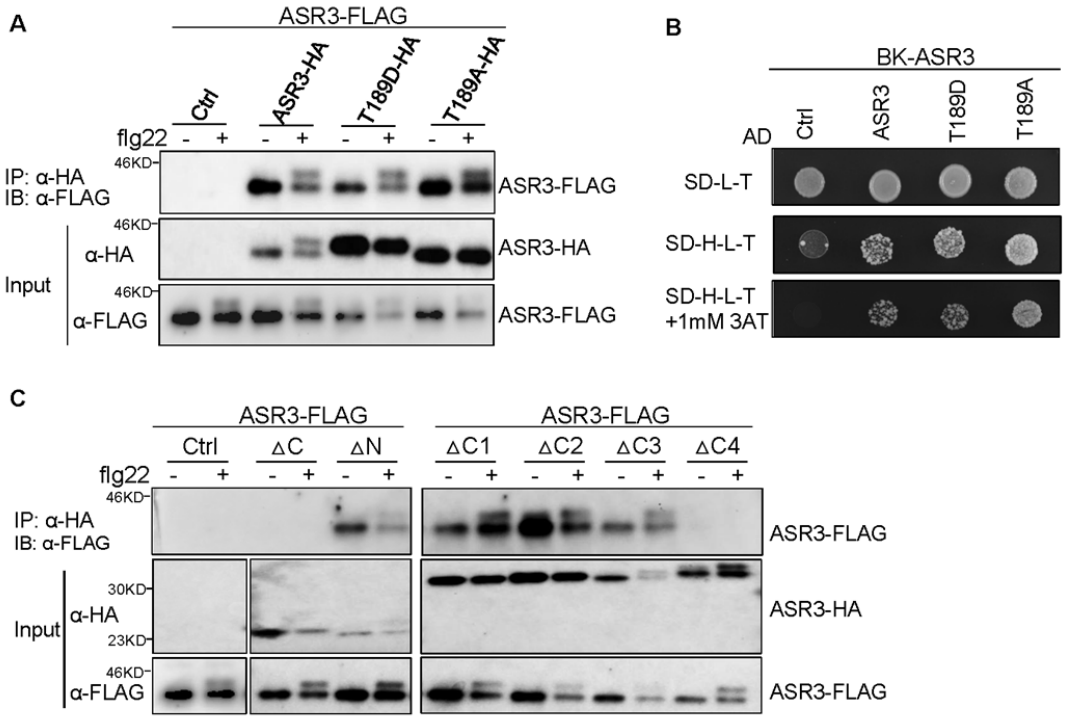
Supplemental Figure 2. The flg22-induced ASR3 phosphorylation is not affected by Ca^{2+} channel inhibitor or NADPH oxidase inhibitor treatment.

(A) flg22-induced ASR3 mobility shift is not affected by Ca^{2+} channel inhibitor treatment. Inhibitors $LaCl_3$, $GaCl_3$ and ruthenium red (RR) were added to protoplasts expressing ASR3-HA 30 min before flg22 treatment. **(B)** flg22-induced ASR3 mobility shift is not affected by NADPH oxidase inhibitor diphenylene iodonium (DPI) or redox reagent Dithiothreitol (DTT). Similar results were obtained from two independent biological repeats.



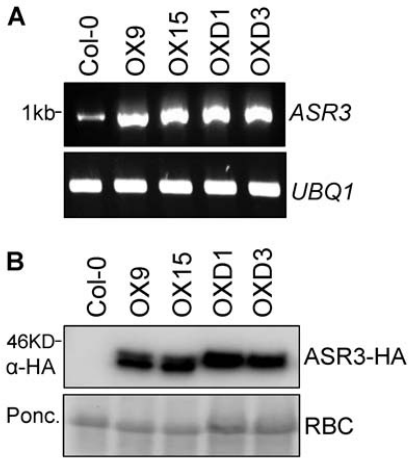
Supplemental Figure 3. ASR3 localizes to nucleus in *35S:ASR3-GFP* transgenic plants.

Four-day-old seedlings were subjected to confocal microscopic examination. Scale bar is equivalent to 20 μm.



Supplemental Figure 4. The coiled-coil domain of ASR3 is required for ASR3 homo-dimerization.

(A) Dimerization of ASR3 with WT ASR3, phospho-inactive ASR3^{T189A} or phospho-mimetic ASR3^{T189D} by co-IP assay. Protoplasts co-transfected with ASR3-FLAG and Ctrl or HA-tagged ASR3 and phosphorylation variants were incubated for 10 h and treated with or without 100 nM flg22 for 30 min. **(B)** Interaction of ASR3 with itself or the phosphorylation variants in yeast two-hybrid assay. Yeast colonies carrying pGBKT7-ASR3 and pGADT7 empty vector (Ctrl), pGADT7-ASR3, pGADT7-ASR3^{T189A} or pGADT7-ASR3^{T189D} were grown on the indicated SD medium. **(C)** Identification of ASR3 dimerization domain. Different truncation or deletion mutants tagged with HA were co-expressed with ASR3-FLAG in protoplasts for 12 h and followed by 100 nM flg22 treatment for 15 min.



Supplemental Figure 5. Characterization of *35S:ASR3-HA* and *35S:ASR3^{T189D}-HA* transgenic lines.

(A) RT-PCR detection of *ASR3* gene expression in Col-0 WT and *ASR3* transgenic plants. OX9 and OX15 are two representative lines for *35S:ASR3-HA*, and OXD1 and OXD3 are two representative lines for *35S:ASR3^{T189D}-HA*. *UBQ1* was used as an internal control. **(B)** ASR3 protein expression in transgenic lines detected by immunoblotting with α-HA antibody. Ponceau staining (Ponc.) of RBC serves as a loading control.

Supplemental Table 1. LC-MS/MS analysis of in vivo phosphorylation of ASR3 upon flg22 treatment.

Peptide[a]	Identified residue	Mr (expt)[b]	Mr (calc)[c]	Expect[d]	Ion[e] Score	Freq.[f]
1-M.ALEQLGLGVpSAVDGGE-17	S11	2994.3148	2994.3092	0.0027	42	1
180-R.EpSPEKLNSTPVAK.S-194	S182	1478.7034	1478.7018	0.36	23	1
180-R.ESPEKLNpSTPVAK.S-194	S188	1478.7032	1478.7018	0.25	18	2
185-K.LNSpTPVAK.S-194	T189	908.4194	908.4368	0.03	50	7
180-R.ESPEKLNSpTPVAK.S-194	T189	1478.709	1478.7018	0.0014	50	6
217-K.QPEAANVEGGSTpSQEE.R-234	S230	1867.765	1867.7585	4.70E-05	58	3

[a] The number before and after the peptide indicates the position of detected peptide in the ASR3 protein sequence. Phosphorylated residues are denoted as pS and pT.
[b] Experimental m/z transformed to a relative molecular mass.
[c] Relative molecular mass calculated from the matched peptide sequence.
[d] Frequency this match would occur by chance, confident matches typically have Expect values <0.05.
[e] Individual ions scores > 45 indicate identity or extensive homology ($p<0.05$).
[f] The number of peptide matches to the same peptide sequence with the same modifications and charge.

Supplemental Table 2. Primers used in this study.

Cloning and mutation primers

Gene	Forward primer	Reverse primer
ASR3	CGGGATCC*ATG*GCTCTGGAACAGTTAGG	GAAGGCCTCATCTTATCCGCGATTTTTG
ProASR3	CCGCTCGAGGAGCTCCAATCCTTATATGATTTGTTTCTCC	CGGGATCCTCAAACGGAATATTCCTTCC
ASR3⊿C	CGGGATCC*ATG*GCTCTGGAACAGTTAGG	GAAGGCCTAGGAATCACACCACCGTCAAC
ASR3⊿N	CGGGATCC*ATG*CCGGCGGTTCCGGTTC	GAAGGCCTCATCTTATCCGCGATTTTTG
ASR3⊿C1	GACGGTGGTGTGATTC\CTACTCCGGTGGCTAAATCA	TGATTTAGCCACCGGAGTAG\GAATCACACCACCGTC
ASR3⊿C2	CCTGAGAAGTTGAATTCT\GCAAACGTGGAAGGTGGA	TCCACCTTCCACGTTTGC\AGAATTCAACTTCTCAGG
ASR3⊿C3	GAGAAACAGCCAGAAGCAAAGA\TGCAGAATCAGTTG	CAACTGATTCTGCA\TCTTTGCTTCTGGCTGTTTCTC
ASR3⊿C4	GAAGAAGGAGAAACAA\AGCAAAGAAAAGATCACGGT	ACCGTGATCTTTTCTTTGCT\TTGTTTCTCCTTCTTC
ASR3^{S169A}	GGATTGGCTCCGGCGGCAGACGAGGGATTGTTG	CAACAATCCCTCGTCTGCCGCCGGAGCCAATCC
ASR3^{S175A}	GACGAGGGATTGTTGGCTGATTTAGATCGGAGA	TCTCCGATCTAAATCAGCCAACAATCCCTCGTC
ASR3^{S182A}	TTAGATCGGAGAGAAGCTCCTGAGAAGTTGAAT	ATTCAACTTCTCAGGAGCTTCTCTCCGATCTAA
ASR3^{T189A}	GAGAAGTTGAATTCTGCTCCGGTGGCTAAATCA	TGATTTAGCCACCGGAGCAGAATTCAACTTCTC
ASR3^{T189D}	GAGAAGTTGAATTCTGATCCGGTGGCTAAATCA	TGATTTAGCCACCGGATCAGAATTCAACTTCTC
ASR3^{T196A}	GTGGCTAAATCAGTTGCTGATGTTATAGACAAA	TTTGTCTATAACATCAGCAACTGATTTAGCCAC
ASR3^{S230A}	GAAGGTGGATCGACAGCACAAGAAGAGAGGAAG	CTTCCTCTCTTCTTGTGCTGTCGATCCACCTTC
ASR3-ear-A	CGGCGGTTCCGGTTGCTTCGGCTGGAGCGGCTCCGGCGTCAGAC	GTCTGACGCCGGAGCCGCTCCAGCCGAAGCAACCGGAACCGCCGG
ASR3-ear-B	CTTGAGGTTCAGAATGCAAACGCAAAAGCAGACAGAGAGCAAAGA	TCTTTGCTCTCTGTCTGCTTTTGCGTTTGCATTCTGAACCTCAAG

Note: The restriction enzyme sites are underlined and start codon is italicized. For deletion primers, the deletion fragment is indicated by back slash. For point mutation primers, the mutation sites are underlined

RT-PCR primers

Gene	Forward primer	Reverse primer
ASR3	TGTCCGTTTACGCTTCCTCT	CCGGAAAAGATGGAGCAAT
UBQ1	ACCGGCAAGACCATCACTCT	AGGCCTCAACTGGTTGCTGT

qRT-PCR primers

Gene	Forward primer	Reverse primer
FRK1	ATCTTCGCTTGGAGCTTCTC	TGCAGCGCAAGGACTAGAG
WRKY30	GCAGCTTGAGAGCAAGAATG	AGCCAAATTTCCAAGAGGAT

At2G17740	TGCTCCATCTCTCTTTGTGC	ATGCGTTGCTGAAGAAGAGG
At1G07160	CGTGTTGGGGATTGATTCG	AGAGCTCGGGCGGTTATG
At4G25110	GCTCATCTTAGGCGCTTCTC	ACAGCAAATGCCTCGTTAGC
At2G40180	GATTGAAGCATTGGGTGGTT	GGTTTCCGGTTCAGCTATCA
At1G02360	ATTTGACACCGTTTCTGGATTC	CAACTATCGTGGAACTACAATTACG
UBQ10	AGATCCAGGACAAGGAAGGTATTC	CGCAGGACCAAGTGAAGAGTAG

ChIP-PCR Primers

Name	Forward primer	Reverse primer
P1	TTATGCATATAGTTTCATTCGC	GTCAACGCTTACTCTCATTAAC
P2	TCACCGTAACACATTGATATTCAAC	TGCTATAACACCACATCACAAATG
P3	TGACTGTATTCGCCATGTGA	TTCCAACCTGCAATCACTAACC
P4	TCCTCACTTCCTCTTCCTCG	ATTTGGAGGAGGACTTGACG

Supplemental Table 3. Elicitors and chemical inhibitors used in this study.

Name	Company	Cat#	Concentration
Flagellin peptide flg22	Genscript	Custom Peptide synthesis	100 nM
EF-Tu peptide elf18	Genscript	Custom Peptide synthesis	100 nM
Chitin	Sigma	C9752	50 µg/mL
Lipopolysaccharide (LPS)	Sigma	L-2012	50 µg/mL
K-252a	A.G. Scientific	K-1006	1 µM
Diphenylene Iodonium (DPI)	A.G. Scientific	D-1011	5 µM
U0126	A.G. Scientific	U-1026	5 µM
Lanthanum Chloride ($LaCl_3$)	Sigma	L4131	0.5-1 mM
Gallium Chloride ($GaCl_3$)	Sigma	413089	0.5-1 mM
Ruthenium Red (RR)	Sigma	R2751	0.1-0.2 mM
Calf intestinal alkaline phosphatase (CIP)	New England BioLabs	M0290S	0.5 U/µl
Lamda protein phosphatase (λPP)	New England BioLabs	P0753S	2 U/µl