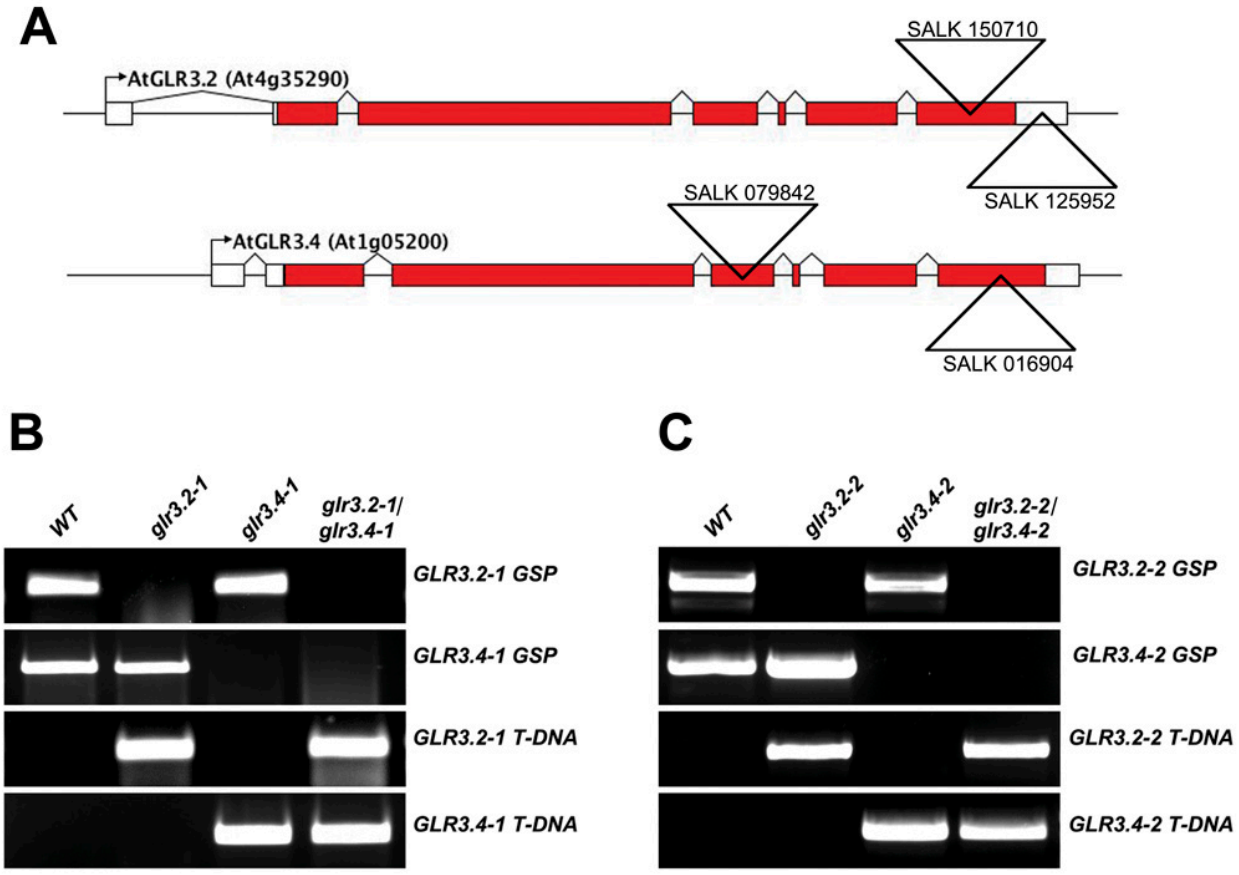
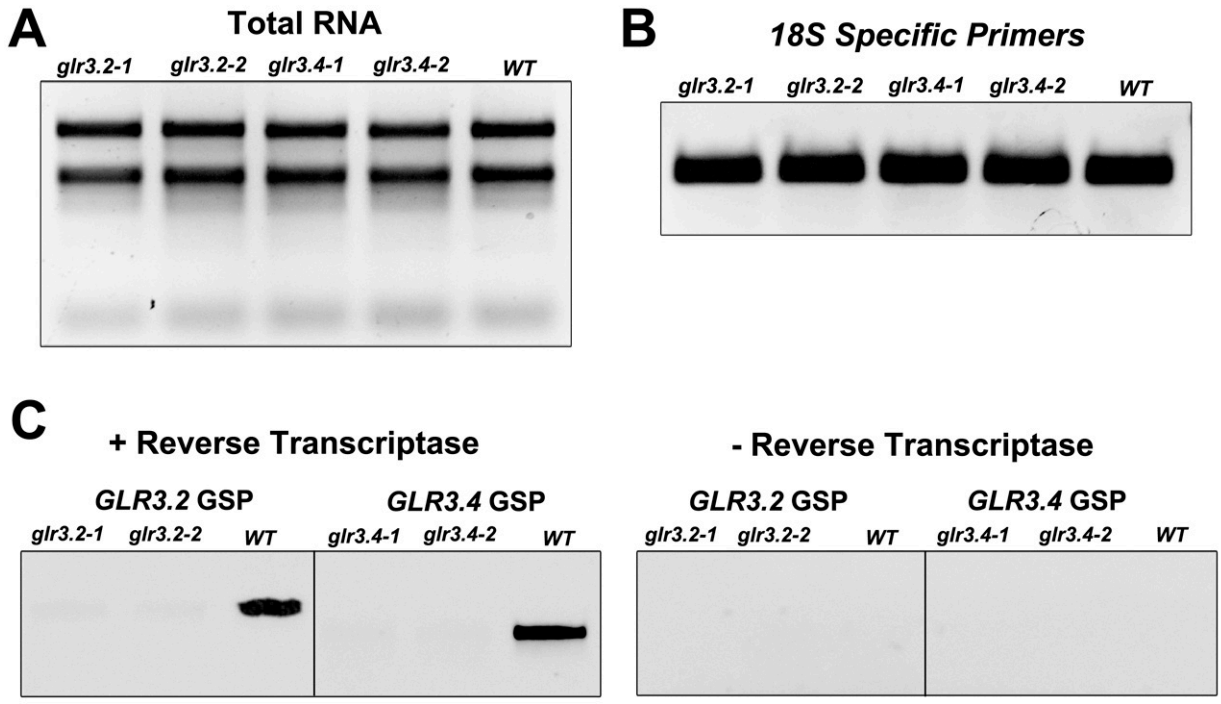


Supplemental Figure 1. Identification of homozygous *glr3.2*, *glr3.4*, and *glr3.2 glr3.4* plants by PCR-based genotyping. A. Outline of gene structure for *AtGLR3.2* and *AtGLR3.4*. Untranslated regions are shown as white boxes, exons as red boxes, and introns as intervening thin black lines. Two separate T-DNA insertion alleles were identified in each gene and mapped to the insertion points, indicated with triangles. Primers for PCR-based genotyping were designed using the T-DNA Express program of the Salk Institute (http://www.salk.org). B, C. Photograph of ethidium bromide agarose gel of amplified gene products from genomic DNA isolated from the single and double T-DNA insertion lines using the gene-specific primers and T-DNA-specific border primer (see Table S2 for exact sequences).



Supplemental Figure 2. Analysis of *GLR3.2* and *GLR3.4* expression in respective mutant alleles. (A) RNA integrity was assessed by resolving the preparation on an ethidium bromide agarose gel. (B) PCR amplification of the 18S ribosomal RNA demonstrated the effectiveness of the reverse transcriptase step. (C) *GLR*-specific primers (GSP) amplified the expected product in wild-type samples but not in *glr* T-DNA insertion alleles. In preparations not subjected to reverse transcriptase, no amplification products were detected.

Plant and mammalian expression vectors used in this study

cDNA in pENTR-D Entry Vector	Destination Vector	Promoter	Fluorescent Tag	Resulting Plasmid After Recombination
Plant Expression				
AtGLR3.2	pEARLEYGATE 102*	AtGLR3.2 native promoter (2,006 bp)†	EGFP	pGATE_proGLR3.2-GLR3.2-EGFP
AtGLR3.2	pEARLEYGATE 101	Cauliflower mosaic virus promoter (35S)	YFP	pGATE_proGLR3.2-GLR3.2-EGFP
AtGLR3.3	pEARLEYGATE 102*	AtGLR3.3 native promoter (3,500 bp)†	EGFP	pGATE_proGLR3.3-GLR3.3-EGFP
AtGLR3.4	pEARLEYGATE 102*	AtGLR3.4 native promoter (1,902 bp)†	EGFP	pGATE_proGLR3.4-GLR3.4-EGFP
AtGLR3.4	pEARLEYGATE 102	Cauliflower mosaic virus promoter (35S)	CFP	pGATE_proGLR3.4-GLR3.4-EGFP
Mammalian Expression				
AtGLR3.2	pDS_EF1-XB-CFP	Human elongation factor-1 (EF1)	CFP	pDS_EF1-AtGLR3.2-CFP
AtGLR3.2	pDS_EF1-XB-YFP	Human elongation factor-1 (EF1)	YFP	pDS_EF1-AtGLR3.2-YFP

Supplemental Table 1

AtGLR3.3	pDS_EF1-XB-CFP	Human elongation factor-1 (EF1)	CFP	pDS_EF1-AtGLR3.3-CFP
AtGLR3.3	pDS_EF1-XB-YFP	Human elongation factor-1 (EF1)	YFP	pDS_EF1-AtGLR3.3-YFP
AtGLR3.4	pDS_EF1-XB-CFP	Human elongation factor-1 (EF1)	CFP	pDS_EF1-AtGLR3.4-CFP
AtGLR3.4	pDS_EF1-XB-YFP	Human elongation factor-1 (EF1)	YFP	pDS_EF1-AtGLR3.4-YFP

*modified (see **materials and methods**) †sequence upstream from start codon

Supplemental Table 2

Primers used for genotyping and expression analysis

Primers used for Genotyping

GLR3.2-1 GSP RP: 5′-TTT TGC GGT TTT GTT TGT AGG-3′	GLR3.2-2 GSP RP: 5′-AGA TAG TCC GCG ACT TCT TCC-3′
GLR3.2-1 GSP LP: 5′-TTT TGG ATC CAG CAT TAG TCG-3′	GLR3.2-2 GSP LP: 5′-GTC TCG GTT GTT AGC GAT TCC-3′
GLR3.4-1 GSP RP: 5′-GAA GTG AGA CTG GCC GTG TAG-3′	GLR3.4-2 GSP RP: 5′-TGC AAA TTC CGT ACA GTA GGG-3′
GLR3.4-1 GSP LP: 5′-GGG TTA ATC CGG CTT ATG AAG-3′	GLR3.4-2 GSP LP: 5′-CAG AGC CAT TCA AAT ACC TCG-3′
T-DNA Specific LBP: 5′-TGG TTC ACG TAG TGG GCC ATC G-3′	

Primers used for RT-PCR

GLR3.2 GSP Forward: 5′-CGA TGA GCT CGA AGG AAG-3′	GLR3.4 GSP Forward: 5′-GCA CTA AGT GTT TTC AAT GAA GGA-3′
GLR3.2 GSP Reverse: 5′-TAC CCT ATC TGC CTA AAC CC-3′	GLR3.4 GSP Reverse: 5′-GGA TTT CTC TTC CCG TCT CC-3′
18S Ribosome Forward: 5′-TAT GGT CGC AAG GCT GAA ACT T-3′	
18S Ribosome Reverse: 5′-TTT CCC CGT GTT GAG TCA AAT T-3′	

GSP = gene-specific primer

RP = right primer

LP = left primer