



## Supporting Information

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### **A single-nucleotide mutation in a GLUTAMATE RECEPTOR-LIKE gene confers resistance to Fusarium wilt in *Gossypium hirsutum***

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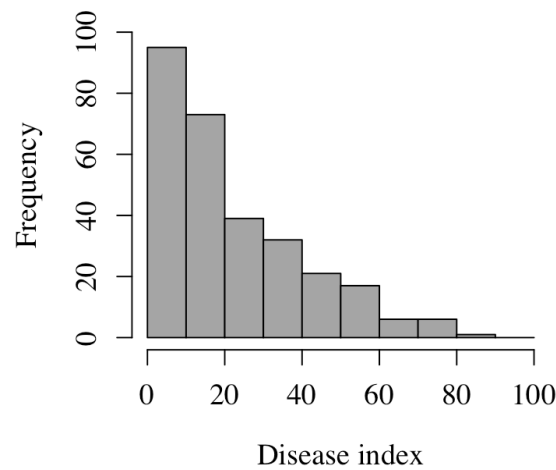
## Supporting Information

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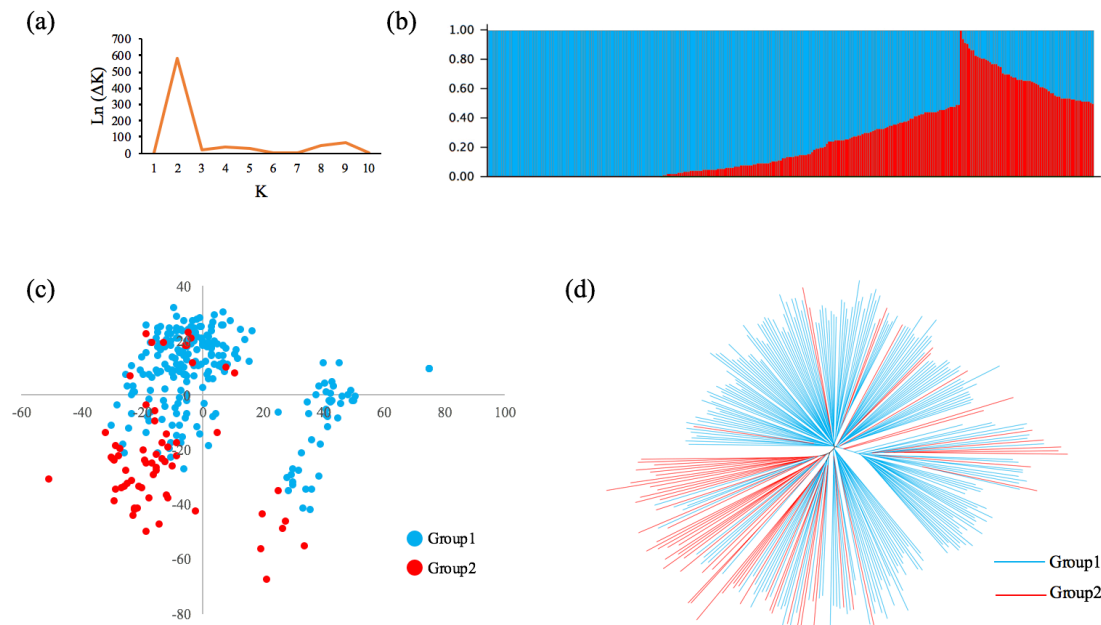
#### Supplementary Figures and Tables

#### Supplementary Figure 1



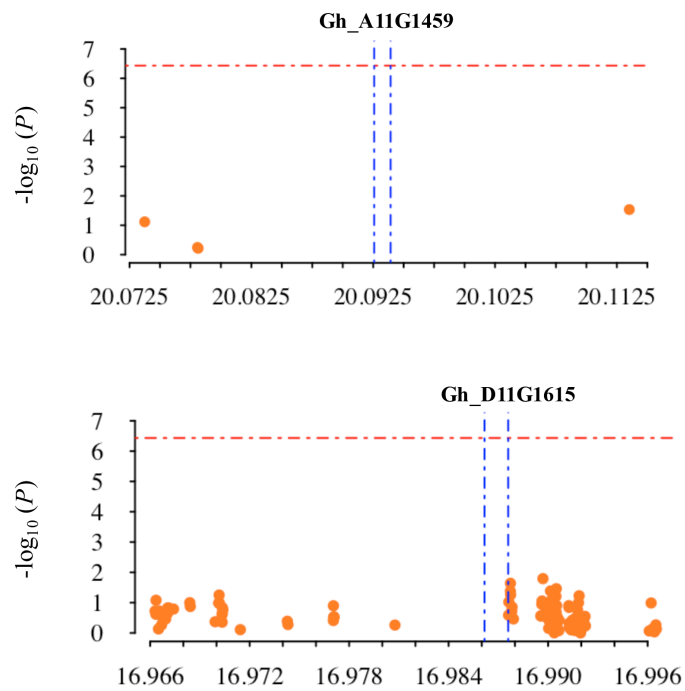
**Supplementary Figure 1.** Frequency distribution histogram of disease index of the 290 Upland cotton varieties.

## Supplementary Figure 2



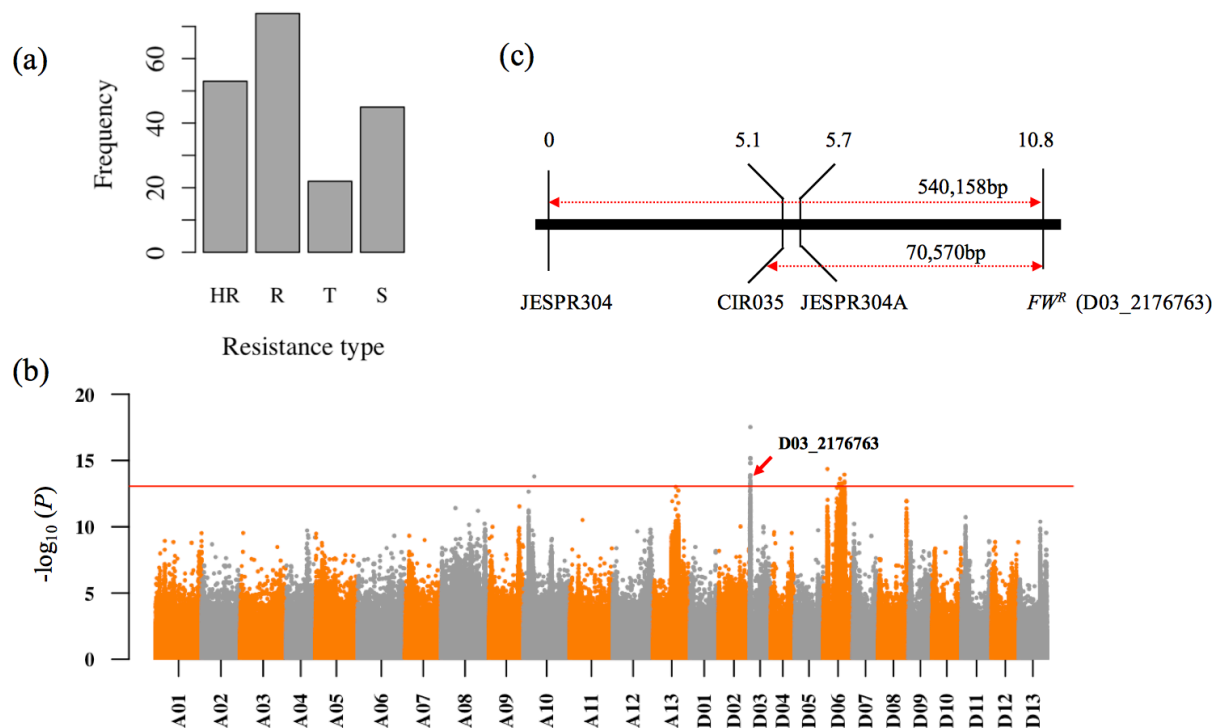
**Supplementary Figure 2.** Population structure of the 290 cotton accessions. **(a)** Corresponding  $\ln(\Delta K)$  values when  $K$  from 1 to 10. **(b)** When  $K=2$ , the population contained 290 cotton accessions was divided into two subpopulations. **(c)** Principal component analysis (PCA) of the population. **(d)** Neighbor-joining tree of the 290 cotton accessions.

### Supplementary Figure 3



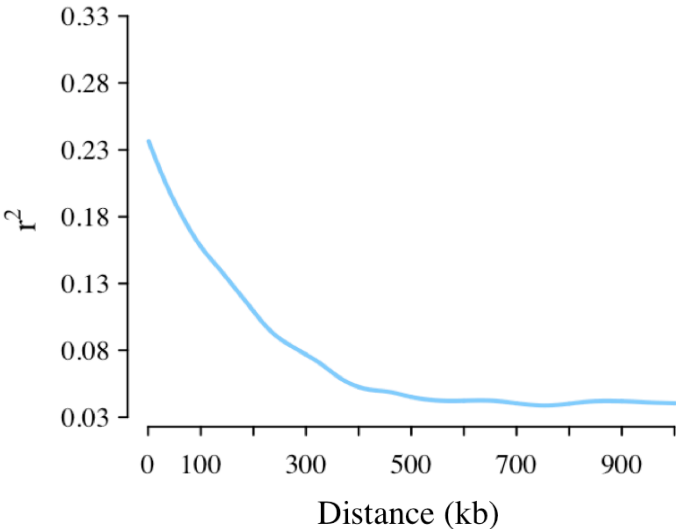
**Supplementary Figure 3.** The nucleotide variations nearby the *GaGSTF9* homologue in *G. hirsutum* are not associated with Fusarium wilt resistance in Upland cotton. *Gh\_A11G1459* and *Gh\_D11G1615* are the homologous genes of *GaGSTF9* in A subgenome and D subgenome, respectively. Blue dashed line represents the genome region of the gene. Red dashed line represents the Bonferroni-adjusted significance threshold ( $-\log_{10}(P) = 6.43$ ).

## Supplementary Figure 4



**Supplementary Figure 4.** GWAS analysis for Fusarium wilt resistance in a population contained 194 Upland cotton cultivars. **(a)** Frequency distribution histogram of disease index of the population. **(b)** Manhattan plot for disease index. The red solid line represents the significance threshold ( $-\log_{10}(P) = 13.05$ ). The significant SNP (D03\_2176763) was marked by arrowhead. **(c)** The distance between  $FW^R$ -linked SSR markers and D03\_2176763 on chromosome D03. Position of  $FW^R$  and SSR markers was determined according to Wang.<sup>[2]</sup> These two SSR markers were mapped to the ‘TM-1’ reference genome recently assembled by PacBio RSII.<sup>[19]</sup> The sequence of JESPR304 matches with a genomic region from 1,707,836 bp to 1,707,629 bp, and CIR035 from 2,177,424 bp to 2,177,088 bp on chromosome D03. The SNP D03\_2176763 is at the 2,247,994 bp-position of on chromosome D03 in the ‘TM-1’ genome.

**Supplementary Figure 5**



**Supplementary Figure 5.** Linkage disequilibrium (LD) decay in D03 chromosome. When  $r^2$  is half of the maximum, the corresponding physical distance is set as the extent of LD (200 kb).



## Supplementary Figure 7

GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7

GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7

GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7



GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7

1 10 20 30 40 50  
 GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7

SNP1

60 70 80 90 100  
 GhGLR4.8  
 GluA2  
 ATGLR1.1  
 ATGLR1.2  
 ATGLR1.3  
 ATGLR1.4  
 ATGLR2.1  
 ATGLR2.2  
 ATGLR2.3  
 ATGLR2.4  
 ATGLR2.5  
 ATGLR2.6  
 ATGLR2.7  
 ATGLR2.8  
 ATGLR2.9  
 ATGLR3.1  
 ATGLR3.2  
 ATGLR3.3  
 ATGLR3.4  
 ATGLR3.5  
 ATGLR3.6  
 ATGLR3.7

	110	120	130
GhGLR4.8	T R R A K A N V L C A L P A I D K A T M G N E S S T S . . . . . Y M S R H M Y E I		
GluA2	S A L T Y D A V Q V M T E A F R N L R K Q R I E I S R R G N . . . . . A G D C L A N P A V P W G Q G V E I E R A		
ATGLR1.1	G L R A H D I A C I L A N A V E K F S V S G K V E A S S N . . . . . V S A D L L D T I		
ATGLR1.2	G V W A H D V A W S L A S A A E V T R M P T V T S . . . . . T L L E A I		
ATGLR1.3	G I W A H D I A F A L A R A A E V I R M P N V T S . . . . . T L L E E I		
ATGLR1.4	S I W A H D I A W S L A R A A E V A K L P G L S V Y . . . . . D L L E A I		
ATGLR2.1	G L W A Y D A T T A L A L A I E E A G . . T S N L T F V K . . . M D A K R N V S E L Q G L G V S Q Y G P K L L Q T L		
ATGLR2.2	G L W A Y D A T T A L A M A I E D A G . . I N N M T F S N . . . V D T G K N V S E L D G L G L S Q F G P K L L Q T V		
ATGLR2.3	G L W A Y D A T T A L A V A I E E A G . . T N N M T F S K V . . V D T G R N V S E L E A L G L S Q F G P K L L Q T L		
ATGLR2.4	G L R A Y D A T T A L A M A V E E A G . . T T N L T F S K . . . M D G . . R N I S D L E A L L S V S E Y G P K L I R S L		
ATGLR2.5	A C W A Y D A T A L A M S V E E I R . . H V N M S F N T T K E D T S R D D I G T D L D E L G V A L S G P K L L D A L		
ATGLR2.6	E C W G Y D T A T A L A M S I E E I S S N V N M S F S Q T K R N T S R D D T G T D L D D L S F A L S G P K L L Q A L		
ATGLR2.7	A R L A Y D I T A L A M A V E K T N . . I K S L R Y D H P . . I A S G N N K T N I G T L G V S R Y G P S L L K A L		
ATGLR2.8	G L W A Y D S T T A L A M A V E K T N . . I S S F P Y N N A . . S G S S N M M T D L G T L H V S R Y G P S L L E A L		
ATGLR2.9	A L W A Y D S I T A L A K A V E K A N . . T K S L W Y D N G . . S T L S K N R T D L G N V G V S R Y G P S L Q K A F		
ATGLR3.1	G L W A Y D T V W I I A R A V K T L L E A G G N L S F S N D A K L G S L K G E A L N L S A L S R F D Q G S Q L D Y I		
ATGLR3.2	G L Y A Y D T V W I I A R A V K R L L D S R A N I S F S S D P K L T S M K G G S L N I G A L S I F D Q G S Q F L D Y I		
ATGLR3.3	G L Y A Y D S V M L L A R G L D K F F K D G G N I S F S N H S M L N T L G K S G N L N L E A M T V F D G G E A L L K D I		
ATGLR3.4	A M Y A Y D S V W L V A R A L D V F F R E N N N I T F S N D P N L H K T N G S T I Q L S A L S V S R Y G E K F M K I I		
ATGLR3.5	A L Y A Y D S V W L V A R A L D V F F S Q G N T V T F S N D P S L R N T N D S G I K L S K L H I F N E G E R F L Q V I		
ATGLR3.6	A L Y A Y D T V W L L A Q A I D D F F K K G G N V S F S K N P I I S E L G G G N L H L D A L K V F D G K I F L E S I		
ATGLR3.7	A L H A Y D T V W M I A H G I E E L L N E G I N I T F S Y S E K L L H A R G T K L H L E K I K F F N S G E L L E K I		

(D03\_2176763) SNP2                      SNP3



	140	150	160	170	180
GhGLR4.8	A R . . E I A S L I G D Y L W L K P G T T V Y E E S D R I S D L D I V T F N L N S R N P T P N T . . . . .				
GluA2	L K Q V Q V E G L S C N I K F D Q N G K R I N Y T I N I M E L K T N G P R K I G Y W S E V D K M V V T . . . . .				
ATGLR1.1	R H S R F K G L S G D I Q I S D N K F I S E T F E I V N I G R E K Q R R I G L W S G G S F S Q R R Q . . . . .				
ATGLR1.2	K E S R F K G L S G F Q L D D M K L L S D K F E I V N M I G S E R R R V G F W N S N G S F S N R R Q L S . . S T H				
ATGLR1.3	T K T R F N G L S G D F Q L N D K K L L S N K F E I V N M I G S S E R R R V G F L N S N G S F S N R R H L S . . S T H				
ATGLR1.4	P E S A K H K G L S G D I K F I D K K F I S D K F E I V N M I G R G E R S V G L W N S G S F I S N R R R R L . . S S T				
ATGLR2.1	S R V R F Q G L A G D F Q F I N G E L Q P S V F E I V N V N Q G G R T I G F W M K E Y G L F K N V D Q K . . P A S				
ATGLR2.2	S T V Q F K G L A G D F H F V S G Q L Q P S V F E I V N M I G T G E R S I G F W T E G N G L V K S L R V D . . . . .				
ATGLR2.3	L T V Q F R G L A G E F R F F R G Q L Q P S V F E I V N I N T G E K S I G F W K E G N G L V K K L D Q E . . A S S				
ATGLR2.4	S Q I Q F K G L S G D Y H F V D G Q L H A S V F E I V N V I D G G G I L V G F W T Q D K G L V K D L S P S . . S G T				
ATGLR2.5	S T V S F K G V A G R F Q L K N G K L E A T T F K I I N I E E S G E R T V G F W K S K V G L V K S L R V D . . . . .				
ATGLR2.6	A T V S F K G V A G R F Q L K N G K L E A T T F K I V N I E E S G E R T V G F W K S K V G L V K S L R V N . . Q T G				
ATGLR2.7	S N V R F N G L A G E F E L I N G Q L E S S V F D V I N I I G S E E R I I G L W R P S N G I V N A K S K N . . T T S				
ATGLR2.8	S E I R F N G L A G R F N L I D R Q L E S P K F E I I N F V G N E E R I V G F W T P S N G L V N V N S N K . . T T S				
ATGLR2.9	S E V R F N G L A G E F K L I D G Q L Q S P K F E I I N F V G N E E R I I G F W T P R D G L M D A T S S N . . K K				
ATGLR3.1	V H T K M S G L T G P V Q F H P D R S M L Q P S Y D I I N I V D D R V H Q I G Y W S N Y S G L S I V P P S . F Y S K				
ATGLR3.2	V N T N M T G V T G Q I Q F L P D R S M I Q P S Y D I I N V D D G F R Q I G Y W S N H S G L S I I P P S . L Y K K				
ATGLR3.3	L G T R M V G L T G Q L Q F T P D R S R T R P A Y D I I N V A G T G V R Q I G Y W S N H S G L S T V L P E L L Y T K E				
ATGLR3.4	L G M N H T G V T G P I Q F D S D R N R V N P A Y E V L N L E G T A P R T V G Y W S N H S G L S V V H P E T . L Y S R				
ATGLR3.5	L E M N Y T G L T G Q I E F N S E K N R I N P A Y D I I N I K S T G P L R V G Y W S N H T G F S V A P P E T . L Y S K				
ATGLR3.6	L Q V D R I G L T G R M K F T S D R N L V N P A F D V L N V I G T G Y T I G Y W F N H S G L S V M P . . . . A D E				
ATGLR3.7	L K V N F T G I A G Q V Q F G S G R N V I G D Y E I I N V N K T D V H T V G F W S R N G G F S V V A P K T R H S Q				

190                      200                      210                      220

GhGLR4.8	. . . . . G G I Q M A A T L S F S T S K P V H Y . . . . . Y T E I S T A V P V R S I P M Q F L N I S Q D E				
GluA2	. . . . . . . . . . . L T E L P S G N D T S G . . . . . L E N K T V V T T I L E S P Y V M M K K N H E				
ATGLR1.1	. . . . . . . . . . . I V W P G R S R K I P R H R V L A E K . . G E K K V L R V L V T A G N K V P H L V S V R P D P				
ATGLR1.2	. . . . . . . . . . . D N L E T I I W P G S A Q S P K G R S L R E . . S G R K K L R V L V T S S N R F P R L M K V E T D P				
ATGLR1.3	. . . . . . . . . . . N K L E T I I W P G S A Q S P K G T S L I D . . S D R K K L R V L V T S S N R F P R L M K V E T D P				
ATGLR1.4	. . . . . . . . . . . K A L E T I I W P G S T R I P K I R S L K E K R H G K K K K L R V L V P A G N I T P Q I L E V K T D F				
ATGLR2.1	K T T F S S W Q D R L R P I I W P G D T T S V P K G W E I P T . . . N G K R L Q I G V P V N N T F Q Q F V K A T R D P				
ATGLR2.2	I G T L S T W P D H L K H I I W P G E A V S V P K G W E I P T . . . N G K K L R I G V P K R I G Y T D L V K V T R D P				
ATGLR2.3	I S A L S T W K D H L K H I V W P G E A D S V P K G W Q I P T . . . K G K K L R I G V P K R T G Y T D L V K V T R D P				
ATGLR2.4	T R I F S S W K N H L N P I L W P G I T L T V P K G W E I P T . . . N G K E L Q I G V P V G T F P Q F V K V T T D P				
ATGLR2.5	. . K V S H S S R R L R P I I W P G D T I F V P K G W E F P T . . . N A K K L R I A V P K K D G F N N F V E V T K D E				
ATGLR2.6	I . K I S H S S H R L R P I I W P G D T I F V P K G W E F P T . . . N A K K L R I A V P K K D G F N N F V E V T K D A				
ATGLR2.7	. . . . . V L G E R L G P V I W P G K S K D V P K G W Q I P T . . . N G K M L R V G I P V K K G F L E F V D A K I D P				
ATGLR2.8	. . . . . F T G E R F G P L I W P G K S T I V P K G W E I P T . . . N G K K I K V G V P V K K G F F N F V E V I T D P				
ATGLR2.9	. . . . . . . . . . . T L G P V I W P G K S K I V P K G W E I P . . . . . G K K L R V G V P M K K G F F D F V K V T I N P				
ATGLR3.1	P P N R S S S N Q H L N S V T W P G G T S V T P R G W I F R N . . . N G R R L R I G V P D R A S F K D F V S R V N G .				
ATGLR3.2	L S N R S S S N Q H L N N V T W P G T S E T P R G W V F P N . . . N G R R L R I G V P D R A S F K E F V S R L D G .				
ATGLR3.3	K P N M S T S P K L K H V I W P G E T F T K P R G W V F S N . . . N G K E L K I G V P L R V S Y K D F V S Q I R G .				
ATGLR3.4	P P N T S T A N Q R L K G I I Y P G E V T K P P R G W V F P N . . . N G K P L R I G V P N R V S Y T D Y V S K D K . .				
ATGLR3.5	P S N T S A K D Q R L N E I I W P G E V I K P P R G W V F P E . . . N G K P L K I G V P N R V S Y K N Y A S K D K . .				
ATGLR3.6	M E N T S F S G Q K L H S V V W P G H S I K I P R G W V F S N . . . N G R H L R I G V P N R Y R F E E V V S V K . . .				
ATGLR3.7	K K T S F V S D E K L G D I T W P G G R E K P R G W V I A D . . . S A D P L K I V V E R R V S F V E F V T E E K N .				

# S1

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230      240      250      260      270
GhGLR4.8 KNHNEAQITGFWTDLFKEAVAVMPINITYKLVPFY..GSD.....DQLFKALVRR...
GluA2      MLEGNERIYEGYCVDLAEIAKHCGFKYKLTIVGDGKYGARDADTKIWNMGVGEGLVYG...
ATGLR1.1  ETGVN.TVSGFCVEVFKTCIAPFNYLELFIPIY.....RGNNDNLAYLSTQR.D
ATGLR1.2  ITHEITIVGFCIEVFQASIAPFNVEVEYIRW.....LN....GTNYTKLAYALHSQK.D
ATGLR1.3  VTNEL.IVSGFCIEVFRAISPFNVEVEYIPW.....LN....GSNDNLAYALHSQK.D
ATGLR1.4  KTGVT.AATGYCIDVFEISLFPFNVEVEYIPWP.GA..IN....YKNYNDLVYTLYSQK.D
ATGLR2.1  ITNST.IFSGFSIDYFEAVIQAIPIYDISYDFIPFQ..DGG.....YDALVYQVYLG...
ATGLR2.2  ITNST.VVKGFCIDVFEAVIQAMPYDVSYEFPF..KPNGEPAGNHNDLVHQVYLG...
ATGLR2.3  ITNST.VVTGFCIDVFEAVIRELPYDVSYEFPF..KPDGKTAGNYNDLVYQVYLG...
ATGLR2.4  LTHET.IVITGFCIDVFEAVIQAMPYDVSYRFPF..DD.....D.GKTN...
ATGLR2.5  NTNVP.TVITGFCIDVNTVMSQMPYAVSYEYIPFD..TPDGKPRGSYDEMVYVFLG...
ATGLR2.6  NTNAP.TITGFCIDVDTAMRQMPYAVPYEYIPF..TPDGKPRGSYDEMVYVFLG...
ATGLR2.7  ISNAM.TPTGYCIEIFEAVLKKLPYSVIPKYIAFL..SPDEN...YDEMVYQVYTG...
ATGLR2.8  ITNIT.TPKGYAIDIFEAAALKKLPYSVIPQYRFE..SPDDD...YDDLVYKVDNG...
ATGLR2.9  ITNKK.TPTGYAIEIFEAAALKELPYLVPIPEYVFE..SPNN...YNNLVYQVYDK...
ATGLR3.1  ...SSNKVQGYCIDVFEAAVKLLSPYVPEHFIFFG..DGLTN..PNYNNLVYKVTGDV...
ATGLR3.2  ...S.NKVQGYAIDVFEAAVKLLISYVPPEHFVLF..DGLKN..PNFNEFVNNVTIGV...
ATGLR3.3  ...TENMFKGFCIDVFEAAVNLNLPYAVPVKFFPYG..NGKEN..PSYTHMVEMITTGN...
ATGLR3.4  ...NPPGVRGYCIDVFEAAIELLPYVVPRTYILYG..DGKRN..PSYDNLVNEVADNSA...
ATGLR3.5  ...NPLGVKGFCDIFFEAAIQLPYVVPRTYILYG..DGKRN..PSYDNLVNEVADNSA...
ATGLR3.6  ...SNGMITGFCVDVFEAAINLNPYVVPFELVAFG..NGHDN..PSNSELRLITTVGV...
ATGLR3.7  ...SSHRITQFCIDVFEAAIKFVPYVVPYIFEPFG..NGHSS..PNYNNLVYKVTGDV...
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280      290      300      310      320      330
GhGLR4.8  TFDAAIGLTVMTRKGSLELFSYPIYFEVGMPLVMKEKPEPN.QVFSFMMPTNEMCTLA
GluA2      KADIAIAPLTIITLVREEVIDFSKPFMSLGISIMIKKPKQSKPGVFSFLDPLAYEIWMCIIV
ATGLR1.1  KYDAAVGDITITSNRSLYVDFTLFPYTDIGIGILT VKKKSQ..GMWTFDPFEKSLWLASG
ATGLR1.2  KYDAAVGDITITSDRSMYVDFTLFPYTEMGLGIVAKERS...MWFVQPLTPNLWITSA
ATGLR1.3  KYDAAVGDITITSNRSTYVDFTLFPYTEMGLGIVAKERS...MWFVQPLTPNLWITSA
ATGLR1.4  KYDAAVGDITITDNRSLYVDFTLFPYTEMGLAVVTAKDKS...MWIFKPLTSLWLITIA
ATGLR2.1  KYDAAVGDITISSNRSMYVDFSLPYPDMSGVGLVVPVKDSVRRSSTIFLMLTLALWLTLSL
ATGLR2.2  QFDAAVGDITILANRSFYVDFTLFPYMSGVGLVPLKDEVKRDKFSFLKPLSIELWLTTL
ATGLR2.3  RYDAAVGDITILVNRSSYVDFTLFPYIKSGVGLVEMTDPVKRDYILFMKPLTSWKLWLTSL
ATGLR2.4  VFDAAVGDITILANRSYVDFTLFPYTTSGVGMVVP LKDNVARS LIFKPLTPGLWGMTL
ATGLR2.5  EFDGAVGDITILANRSHYVDFALPYSETGIVFLVPVKDGKEKGEWVFLKPLTKEWLVTA
ATGLR2.6  EFDGAVGDITILANRSTYVDFALPYSETGIVVVVPVKDEREKKGWVFLKPLTRWLVTA
ATGLR2.7  AYDAAVGDITIVANRSLYVDFTLFPYTESGVMMPVKDN..KNTWVFLRPWSLDLWVTA
ATGLR2.8  TLDAAVGDITITAYRSLYADFTLPYTESGVMMPVVDNENKNTWVFLKPLTWLVTA
ATGLR2.9  TWDAAVGDITITANRSLYADFTLPYTESGVMMPVVDNENKNTWVFLKPLTWLVTA
ATGLR3.1  FDDAAVGDIAIVTKRTRIVDFTPYIESGLVVA PVRTLNE.NPWAFLRPFTLPMWAVTA
ATGLR3.2  FDDAAVGDIAIVTKRTRIVDFTPYIESGLVVA PVRTLND.TPWAFLRPFTLPMWAVTA
ATGLR3.3  FDDAAVGDIAIVTNRKIYVDFTPYIAASGLVVA PFKLLNS.GAWAFLRPFRNRLWAVTG
ATGLR3.4  FDDAAVGDITIVTNRTRYVDFTPYIESGLVVA PVKEAKS.SPWSFLKPFTEIEMWAVTG
ATGLR3.5  FDDAAVGDITIVTNRTKYVDFTPYIESGLVVA PVKGAKS.SPWSFLKPFTEIEMWAVTG
ATGLR3.6  YDAAVGDITITITERTKMADFTQPYVESGLVVA PVRKLG.SAMAFLRPFTPQMWLIAA
ATGLR3.7  YDAAVGDITAVPSRSKLVDFSQPYASTGLVVTIPANDDN..ATWIFLRPFTSRWLVVLI
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340      350      360      370
GhGLR4.8  AMTMFNFAFVIWLV.....ESRTGHESVG.....AIFWFP LATLIFYGGHR
GluA2      FAYIGVSVVLFVLSRFSPYEWHTTEEFEDGRETOSESSTNEFGIFNSLWFSLGAFMQGCD
ATGLR1.1  AFVFLTGIVVWLV.....ERSVNEPFGQSWG.QQLSMMLWFGFSTIVFA.HR
ATGLR1.2  AFVFLTGIVVWLV.....ERAENKEFGQSWP.QQIGVVIWFGFSTLVYA.HR
ATGLR1.3  FVFLTGIVVWLV.....ERAENKEFGQSWP.QQIGVVIWFGFSTLVYA.HR
ATGLR1.4  SFVFLTGIVVWLV.....ERHDNADFQGSFC.QQIGTLLCFGFSTLVYA.HR
ATGLR2.1  LSFFIIGLVVWLV.....EHRVNDPFGPQ.YQLSTIFWFSFSIMVFA.PR
ATGLR2.2  VFVFLVGISVWTL.....EHRVNSDFRGPAN.YQASTIFWFAFSTMVFA.PR
ATGLR2.3  ISFFLVGCTVWV.....EYKRNDPFGPQ.YQASTIFWFAFSTMVFA.PR
ATGLR2.4  GSFVVGFFVWLV.....EHRVNSDFRGPQ.YQISTMFWFAFSTMVFA.PR
ATGLR2.5  ASFFLYIGIMVWIF.....EYQADEFRQMIIDKISVFFWFAFSTLFFA.HR
ATGLR2.6  ASFFLYIGIM.....EYQADEFRQMIIDKISVFFWFAFSTLFFA.HR
ATGLR2.7  CFFVYIGFVWVIL.....EHRVNDPFRGPPH.HQIGTSFVFAFSTMVFA.HR
ATGLR2.8  CFFVYIGFVWVLF.....EHRVNDPFRGPPH.HQIGTSFVFAFSTMVFA.HR
ATGLR2.9  CFFVYIGFVWVLF.....EHRVNDPFRGPPQ.YQIGTSLWFAFSTMVFA.HR
ATGLR3.1  SFVIVGAAIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.2  AFVFLVGSVIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.3  CFFVIVGAVIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.4  GFFVIVGAVIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.5  ALVFLVGAVIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.6  ASVFLVGAVIWVCL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
ATGLR3.7  VSVFLVIAVVIWIIL.....EHRINDPFRGPPR.RQIITILWFAFSTMVFA.HR
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SYTASLTS motif

	380	390	400	410	420	430
GhGLR4.8	E S P R S N L T Y F V L A P W L V L I L V V S S T Y T Q S F T S M I T S S D T E S S S C L D I E D L K K T N A I V G C D					
GluA2	I S P R S L S G R I V G G V W F F T L I I S S Y T A N L A A F L T V E R M V S P I E S A E D L S K Q T E I A Y G T L					
ATGLR1.1	E K L Q K M S S R F L V I V W V F V V L I L T S S Y S A N L T S T K T I S R M Q L N . . . . . H Q M V F G G S					
ATGLR1.2	E K L Q H N L S R F V V T V W V F A V L I L V T S Y T A T L T S M M T V Q Q I R F N A . . . . . N E D Y V G H L					
ATGLR1.3	E K L K H N L S R F V V T V W V F A V L I L T A S Y T A T L T S M M T V Q Q I R F N S . . . . . N E D Y V G H L					
ATGLR1.4	E R L Q H N M S R F V V I V W I F A V L I L T S N Y T A T L T S V M T V Q Q I R G L K . . . . . S N E N I G F F					
ATGLR2.1	E R V L S F W A R V V V I I W Y F V L V L V T Q S Y T A S L A S L L T T Q H L H P T V T N . I N S L L A K G E S V G Y Q					
ATGLR2.2	E R V L S F G A R S L V V T W Y F V L L V L T Q S Y T A S L A S L L T S Q Q L N P T I T S . M S S L L H R G E T V G Y Q					
ATGLR2.3	E R V F S F W A R A L V I A W Y F L V L V L T Q S Y T A S L A S L L T S Q K L N P T I T S . M S S L L E K G E T V G Y Q					
ATGLR2.4	E R V M S F T A R V V V I T W Y F I V L V L T Q S Y T A S L S S L L T T Q Q L N P T E T S . I K N V L A K G G P V A Y Q					
ATGLR2.5	R P S E S F F T R V L V V V W C F V L L I L T Q S Y T A T L T S M L T V Q E L R P T V R H . M D D L R K S G V N I G Y Q					
ATGLR2.6	. . . . . S Y T A T L T S M L T V Q E L R P T V R H . M D D L R N S G V N I G Y Q					
ATGLR2.7	E K V V S N L A R F V V L V W C F V V L V L I Q S Y T A N L T S F F T V K L L Q P T V T N . W K D L I K F N K N I G Y Q					
ATGLR2.8	E K V V S N L A R F V V V V W C F V V L V L T Q S Y T A N L T S F L T V Q R F O P A A I N . V K D L I K N G D Y V G Y Q					
ATGLR2.9	E N V V S N L A R F V V V V W C F V V L V L T Q S Y T A S L T S F L T V Q S L Q P T V T N . V N D L I K N R D C V G Y Q					
ATGLR3.1	E T T V S T L G R M V L L I W L F V V L I I T S S Y T A S L T S I L T V Q Q L N S P I K G . V D T L I S S T G R I G Y Q					
ATGLR3.2	E N T V S T L G R A V L L I W L F V V L I I T S S Y T A S L T S I L T V Q Q L N S P I R G . V D T L I S S S G R V G F Q					
ATGLR3.3	E N T V S T L G R L V L I I W L F V V L I I N S S Y T A S L T S I L T V Q Q L S S P I K G . I E S L R R E R D D P I G Y Q					
ATGLR3.4	E N T V S S L G R F V L I I W L F V V L I I N S S Y T A S L T S I L T I R Q L T S R I E G . I D S L V T S N E P I G V Q					
ATGLR3.5	E N T V S T L G R F V L L V W L F V V L I I N S S Y T A S L T S I L T V Q Q L T S R I E G . M D T L I A S N E P I G V Q					
ATGLR3.6	E T T T S N L G R I V L I I W L F V V L I I N S S Y T A S L T S I L T V H Q L S S P I K G . I E T L Q T N H D P I G Y P					
ATGLR3.7	E D T I S N L A R L V M I V W L F L L M V L T A S Y T A N L T S I L T V Q Q L P S A I T G . I D S L R A S E V P I G Y Q					

S2

	440	450	460	470	480
GhGLR4.8	M E D S I M L Q H L V E Y I G F Q R K N I K H I A Q S S I D D Y A K A L S T G . . . . . K T K A A F F W A P Y S G				
GluA2	D S G S T K E F F R R S K I A V F D K M W T Y M R S A E P S V F V R T T A E G V A R V R K S K G K Y A Y L L E S T M N E				
ATGLR1.1	T T . S M T A K L G . . . . . S I N A V E A Y A Q L L R D G . . . . . T L N H V I N E I P Y L S				
ATGLR1.2	S G . S L I A N A A L T N S S L R A M . . R L L G L N T S E D Y A Q A L M N K . . . . . S V S Y I V S E L P Y L K				
ATGLR1.3	S G . S L I A N V A L T S S S L R A M . . R S L G L N S A A D Y A Q A L N K . . . . . T V S F V V D E L P Y L K				
ATGLR1.4	S A . S I A A N V V N D N P T F Q G P . R Y K G L K T A D D F T N A L R N G . . . . . T I S F I V D E V P Y V K				
ATGLR2.1	S . . S F I L G R L R . D S G F S E A . S L V S Y G S P E H C D A L L S K G Q A . . . . . E G G V S A A F M E V P Y V R				
ATGLR2.2	R T . S F I L G K L N . E T G F P Q S . S L V P F D T A E E C D E L L K K G P K . . . . . N G G V A A A F L G T P Y V R				
ATGLR2.3	R T . S F I L G K L K . E R G F P Q S . S L V P F D T A E E C D E L L S K G P K . . . . . K G G V S G A F L E I P Y L R				
ATGLR2.4	R D . S F V L G R L R . E S G F P E S . R L V P F T S P E K C E E L L N K G P S . . . . . K G G V S A A F M E V P Y V R				
ATGLR2.5	T G . S F T F E R L K . Q M R F D E S . R L K T Y N S P E E M R E L F L H K S S . . . . . N G G I D A A F D E V A Y I K				
ATGLR2.6	T G . S F T F E R L K . Q M G Y K E S . R L K T Y D T P Q E M H E L F L K K S S . . . . . N G G I D A A F D E V A Y I K				
ATGLR2.7	R G . T F V R E L L K . S Q G F D E S . Q L K P F G S A V E C D E L F S N G . . . . . T I T A S F D E V A Y I K				
ATGLR2.8	H G . A F V K D F L I . K E G F N V S . K L K P F G S S E E C H A L L S N G . . . . . S I S A A F D E V A Y L R				
ATGLR2.9	G G . A F V K D I L L . G L G F H E D . Q L K P F D S A K D A D D L L S K G K S . . . . . K . G I A A A F D E V A Y L K				
ATGLR3.1	V G . S F A E N Y M T D E L N I A S S . R L V P L A S P E E Y A N A L Q N G . . . . . T V A A I V D E R P Y I D				
ATGLR3.2	V G . S Y A E N Y M I D E L N I A R S . R L V P L G S P K E Y A A A L Q N G . . . . . T V A A I V D E R P Y V D				
ATGLR3.3	V G . S F A E S Y L R N E L N I S E S . R L V P L G T P E A Y A K A L K D G P S . . . . . K G G V A A I V D E R P Y V D				
ATGLR3.4	D G . T F A R N Y L I N E L N I L P S . R I V P L K D E E Q Y L S A L Q R G P N . . . . . A G G V A A I V D E L P Y I E				
ATGLR3.5	D G . T F A W K F L V N E L N I A P S . R I V P L K D E E E Y L S A L Q R G P R . . . . . G G G V A A I V D E L P Y I K				
ATGLR3.6	Q G . S F V R D Y L I H E L N I H V S . R L V P L R S P E E Y D K A L R D G P G . . . . . K G G V A A V D E R A Y I E				
ATGLR3.7	A G . T F T L E Y L T Y S L G M A R S . R L V P L D S T E E Y E K A L K L G P T . . . . . N W G G V A A I V D E L P Y I E				

SNP4

	490	500	510	520	530	540
GhGLR4.8	L F L A K Y C K G F R S W G P N H N L R G S S V I F P R D S P F A P Y M S E A M V R L C G S G K F K R M K D L Q S F P					
GluA2	Y I E Q R K P C D T M K V G G N L D S K G Y G I A T P K G S S L R T P V N L A V L K L S E Q G V L D K L K N K W Y D K					
ATGLR1.1	I L I G N Y P N D F V M T D R V T N T N G F G F M F Q K G S D L V P K V S R E I A K L R S L G M I K D M E K K W F Q K L					
ATGLR1.2	I L L G E N P G H F L M V K T Q S T T N G F G F M F Q K G S E L A P N V S R E I A K L R T S E R L N E M E R R W F D K Q					
ATGLR1.3	V V L G E N P I H F F M V K T Q S T T N G F G F M F Q K G F E L V P N V S R E I S K L R T S E K L N E M E K R W F D N Q					
ATGLR1.4	L F V A K H P S E F V I V E T E S V T N G F G F A F Q K G S P L V Q K V S R E I E K L R R T E K L K A I E N W W F Q R Q					
ATGLR2.1	I F L G Q Y C N K Y K M V Q T P F K V D G L G F V F P I G S P L V A D I S R A I L K V E E S N K A N Q L E N A W F K P I					
ATGLR2.2	L F L G Q Y C N T Y K M V E E P F N V D G F G F V F P I G S P L V A D V S R A I L K V A E S P K A M E L E H A W F K K K					
ATGLR2.3	L F L G Q F C N T Y K M V E E P F N V D G F G F V F P I G S P L V A D V S R A I L K V A E S P K A M E L E R A W F K K K					
ATGLR2.4	V F L G Q Y C K Y K M V E V P F D V D G F G F V F P I G S P L V A D V S R A I L K V A E S N K A T Q L E T A W F K N I					
ATGLR2.5	L F M A K Y C S E Y S I I E P T F K A D G F G F A F P L G S P L V S D I S R Q I L N I T E G D A M K A I E N K W F L G .					
ATGLR2.6	L F M A K Y C S K Y T I I E P T F K A D G F G F A F P L G S P L V P D L S R Q I L N I T E G E T M K A I E N K W L L G .					
ATGLR2.7	V I L S Q N S S K Y T M V E P T F K T A G F G F V F P K K S P L T D D V S R A I L N V T Q G E E M Q H I E N K W F K K .					
ATGLR2.8	A I L S Q Y C S K Y A I V E P T F K T A G F G F A F P R N S P L T G D V S K A I L N V T Q G E M Q H I E N K W F M K .					
ATGLR2.9	A I L S Q S C S K Y V M V E P T F K T G F G F A F P K N S P L T G E F S R A I L N L T Q N N V T Q Q I E D R W F P K .					
ATGLR3.1	L F L S . D Y C K F A I R G Q E F T R C G W G F A F P R D S P L A V D M S T A I L G L S E T G E L Q K I H D R W L S . K					
ATGLR3.2	L F L S . E F C G F A I R G Q E F T R S G W G F A F P R D S P L A I D M S T A I L G L S E T G Q L Q K I H D K W L S . R					
ATGLR3.3	L F L S . S N C A Y R I V G Q E F T K S G W G F A F P R D S P L A I D L S T A I L L A E N G D L Q R I H D K W L M . K					
ATGLR3.4	V L L T N S N C K F R T V G Q E F T R T G W G F A F Q R D S P L A V D M S T A I L Q L S E E G E L E K I H R K W L N Y K					
ATGLR3.5	A L L S N S N C K F R T V G Q E F T R T G W G F A F Q R D S P L A V D M S T A I L Q L A E E G K L E K I R K W L T Y D					
ATGLR3.6	L F L S . N R C E F G I V G Q E F T K N G W G F A F P R N S P L A V D V S A A I L Q L S E N G D M Q R I R D K W L L . R					
ATGLR3.7	L F L A . E R T G E K I V G E P F M H R G W G F A F K R D S P L A I D M S T A I L K L S E T R K L Q E I R K K W L C . K					

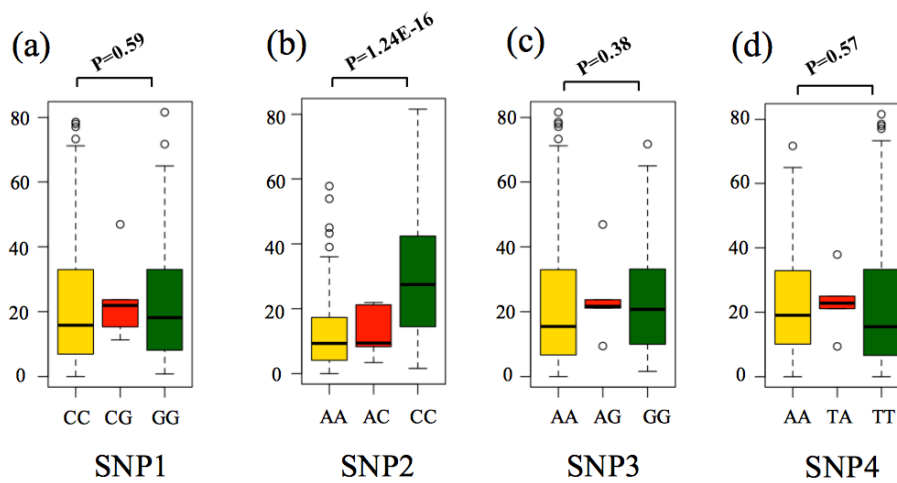
	550	560	570	580	590	
GhGLR4.8	ECSSST.....	IDVTMKR.....	GIGPGPFS	GLFII	SGTASAVAILI	TVIRPMR
GluA2	GECGAKD.....	SGSKEKTS.....	ALSLSNVA	GVFYI	LVGGGLG	LAMLV
ATGLR1.1	DLSLVHSNTEEVASTND	DDEASK.....	RFTFRELR	GLFII	AGAAHV	LVLAL
ATGLR1.2	LP.....	YTTDDTSN.....	PIITLYRFR	GLFMI	TGVSFAF	FALAV
ATGLR1.3	LP.....	YTTDDTSN.....	PIITLYRFR	GLFII	TGVSFAF	FALAV
ATGLR1.4	TTS.....	ATSEDTFH.....	PLTVYTFR	GLFMI	TGVSFAF	FALIV
ATGLR2.1	DESCPD.....	PLTNPDPNPSVSFRQ	LGFDSF	WVLF	LVAAIV	CTMAL
ATGLR2.2	EQSCPD.....	PVTNPDSNPTVTAIQ	LGVGSF	WVLF	VVVF	CVLAL
ATGLR2.3	EQSCPD.....	PIITNPDPNPSFTSRO	LDIDSF	FLFV	GVLV	CVMAL
ATGLR2.4	DKTCPD.....	PMNPNPDPNPTVSFRK	LSLDSF	LLFV	AAAV	CTLAL
ATGLR2.5	EKHCLD.....	STITSDSP.....	IQLDHH	SFEA	LFLI	VFVVS
ATGLR2.6	EKHCLD.....	STITSDSP.....	IRLDHH	SFEA	LFTI	VFVVS
ATGLR2.7	PNNCPD.....	LNITSLSSN.....	HLSLS	SF	WGLF	LIAGIAS
ATGLR2.8	QNDCPD.....	PKTALSSN.....	RLSLR	SF	WGLF	LIAGIAS
ATGLR2.9	KNDCPD.....	PMTALSSN.....	RNLNS	SF	WGLF	LIAGIAS
ATGLR3.1	SNCSSPHG.....	SQSSG.DSE.....	QLNVH	SF	WGMFL	VGIA
ATGLR3.2	SNCSSNLNG.....	SVSDEDESE.....	QLKLR	SF	WGLF	LVCGIS
ATGLR3.3	NACTLEN.....	PMAELESD.....	RHLK	SF	WGLF	LVCGVA
ATGLR3.4	HECSMQI.....	SNSSEDS.....	QLSLK	SF	WGLF	LVCGIT
ATGLR3.5	HECTMQI.....	SSTENY.....	QISVQ	SF	WGLF	LVCGV
ATGLR3.6	KACSLQG.....	AEIEVD.....	RLLEK	SF	WGLF	VVCGVA
ATGLR3.7	TNCAGKSN.....	WNPEPN.....	QLHLK	SF	WGLY	LVCAITV

	600
GhGLR4.8	RRWER.....LVQGLMG.....RGLWV
GluA2	KSRAEAKR.....MKVAKNAQN.....INPSS
ATGLR1.1	EVSRLCTK.....LQSFYK.....
ATGLR1.2	ERWEILVNS.....VNIYFSQRLRHFRILFTR.....TIHPS
ATGLR1.3	DKWEILVD.....NLDLSQRLRHFRHFVR.....SIHTS
ATGLR1.4	EQRQVVLK.....HFHRYVSHRFAR.....EIRPS
ATGLR2.1	EN.....PNQRNLRVLWEKFN.....EPDQKS
ATGLR2.2	KT.....KGDLDWKEFL.....KRDTDS
ATGLR2.3	K.....DQVS
ATGLR2.4	QNRILND.....EFYRGKRMKEMWLKFM.....ESDGES
ATGLR2.5	QERQHN.....ASPMLNDQ.....ANAAQE
ATGLR2.6	ROESKSGEINANNSPDTGNMRAPPNQPTDDNMRAPTSPPIDQVLEPPGALNEADDQDQ
ATGLR2.7	EKHHTLFD.....DSENSFRGKLLKFLVRNFDEKDIKSHMPKENAVHNVSSPITQSSSP
ATGLR2.8	ENRHTLCD.....DSEDSIWRKLTSLFRNFDEKDIKSHMPKSSAVHHVSSPMTQYIPSPS
ATGLR2.9	EHRHTLGD.....DSEDSLWRKLLKFLKIFDEKDMNSHTFKNSAIHNISLPMAVYCTWLMIR
ATGLR3.1	DFCKDTP.....EUVVEEAIPS...PK.....SSRLTK
ATGLR3.2	DFFRHG.....KYDEEATVPS...PE.....SSRSKS
ATGLR3.3	QLYKKPT.....DDAIARDQQNHDS.....SMRSTR
ATGLR3.4	QYQRLLP.....ESADEERAGEVSEPSRSG.....RGSRAP
ATGLR3.5	QYQRLRP.....EESDEVQARSEAGSSRG.....KSLRAVS
ATGLR3.6	QFGQQCP.....EEAEGSIRRR.....SSPSAR
ATGLR3.7	QFVRYRR.....MERTSSMPRASWSASP.....TLRLREL

	610	620	630	640
GhGLR4.8	WLTTLFSRDQRGNQLQVQLARISFTSQTQLTSS.....			
GluA2	SQNSQNFATYKEGYNVYGIESVKI.....			
ATGLR1.1	.....			
ATGLR1.2	PLGLDNPIGENAVQMAQRNRR.....			
ATGLR1.3	PL..DDPIGETAVQMAQQNRQ.....			
ATGLR1.4	PT..TPNRQNEANSVI.....			
ATGLR2.1	YIKDVTKQCQSSGQMPKNGQEGAN.....AVNNGN.....			
ATGLR2.2	YINDIEKCLCSQEMPENSNAKATNQTYGMELVRNIVQVNTDPDCL.....			
ATGLR2.3	YLDKVMSPCQSSSQMPVKKRKTQLN.....MSQVHDQDSL.....			
ATGLR2.4	YIS...RVRSTCPQVLIQPREEDID.....PING.....			
ATGLR2.5	EVNEEGNVG...DHIVEVDTALVRRK..KLTSNTIPIRRVAPLSRLKSA.			
ATGLR2.6	LLNDEVNVGDRNEVDIIVEVDPTLVHRRN.LITSKTIPTRRALFSRIKSA.			
ATGLR2.7	TDQSTPLPRSPEQYRELELRVSSISSGELFTTQ.SEQVEDEESAIQCEGE			
ATGLR2.8	TLQIAPRPHSPSQDRAFELRRVSFTPNNEERLTTQ.TIHFEDEESDIECVVEQ			
ATGLR2.9	TVQITPWPQSPSQNREFELRRVSFSPSEERFTTQPIIHHEDEESDIECRVEQ			
ATGLR3.1	LQTFLLAFVDEKEEETKRRLLKRRRNNHDSMNANSIISRTASRRPI.....			
ATGLR3.2	LQTFLLAFVDEKEEESKRRMRRRNNDDLSLKPS.....RPI.....			
ATGLR3.3	LQRFLSLMDEKEESKHESSKRRKIDGSMNDTSGSTRSRGDFRERSFNVPDLD			
ATGLR3.4	FKELIKVVDKREAEIKEILKQKSSKLLKSTQSAAGTSQSQHGEIT.....			
ATGLR3.5	FKDLIKVVDKREAEIKEMLKEKSSKLLKDGQSSAENSQSKDHETPQ.....			
ATGLR3.6	IHDFLSFVKEKEEDAKARSSRERQLEDISANGSSRCN.....			
ATGLR3.7	VDFDFVEFVDEKEEAIKRMFRSDDSNPNP SHVGEVQADTEVPRN.....			

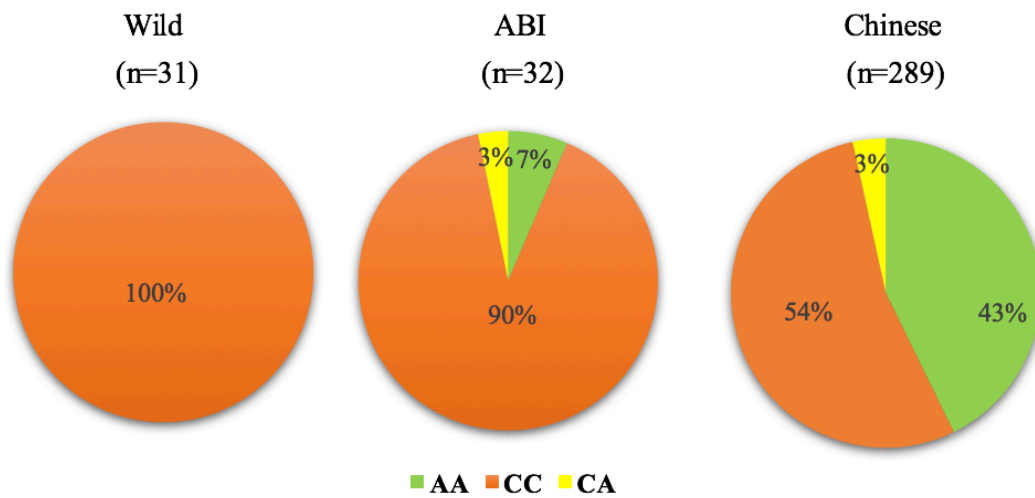
**Supplementary Figure 7.** Amino acid sequence alignment of GhGLR4.8 with AtGLRs and GluA2. SNP1-SNP4 are marked by red arrow, S1 segment of LBD is indicated by red line, S2 segment of LBD is indicated by green line, the conserved 'SYTASLTS' motif of M3 is indicated by orange line, and the number indicates the amino acid length of GhGLR4.8. The amino acid sequence from top to bottom is GhGLR4.8, GluA2 and *Arabidopsis* 20 AtGLRs.

## Supplementary Figure 8



**Supplementary Figure 8.** Association analysis between FW resistance and four nucleotide variations in the *GhGLR4.8* coding sequence. **(a), (c) and (d)** Other three polymorphisms in *GhGLR4.8* are not associated with disease index. In box plots, center line indicates median, box limits denote upper and lower quartiles, and points indicate outliers. Statistical significance was determined by a two-sided *t*-test. **(b)** The disease index shows significant difference between allele 'AA' and allele 'CC' of the strongest association SNP (D03\_2176763).

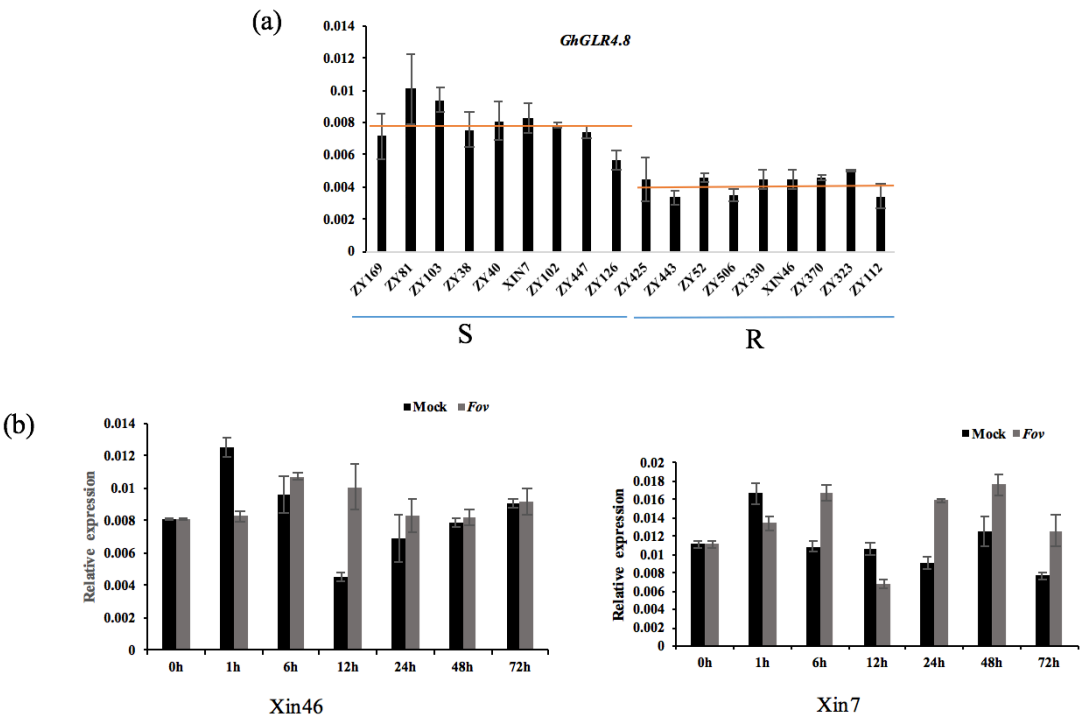
### Supplementary Figure 9



**Supplementary Figure 9.** Genotype frequency distribution of SNP D03\_2176763. The SNP information of 352 resequenced accessions is derived from our previous study.<sup>[12]</sup> Wild group, wild cottons; ABI group, cottons from America, Brazil and India; Chinese group, cottons from China.

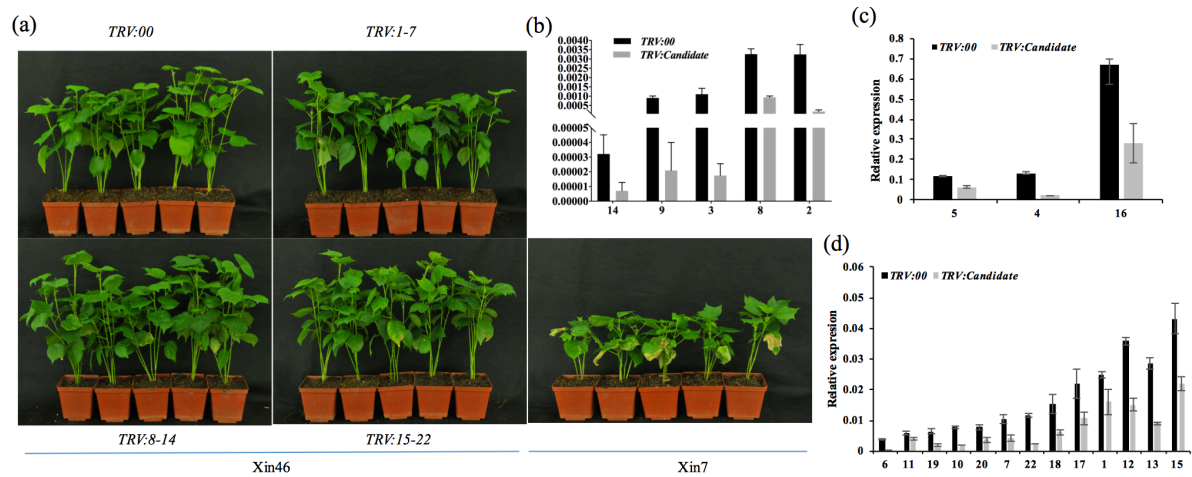


**Supplementary Figure 10**



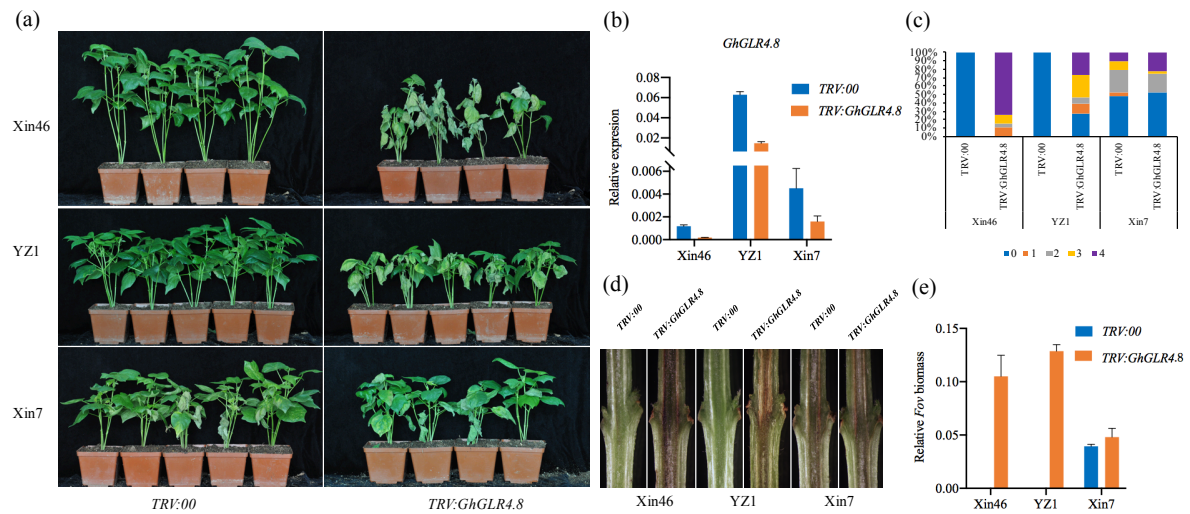
**Supplementary Figure 10.** The expression pattern of *GhGLR4.8* gene. **(a)** qRT-PCR analysis of the expression levels of *GhGLR4.8* in resistance (R) and susceptible (S) cotton varieties **(b)** qRT-PCR analysis of the expression levels of *GhGLR4.8* in resistant and susceptible cotton varieties at different time points after inoculation. The expression level of genes was normalized by the expression of *G. hirsutum* *UB7*, data are presented as mean  $\pm$  SD from three technical replicates.

## Supplementary Figure 11



**Supplementary Figure 11.** Knockdown of candidate genes through VIGS except for *GhGLR4.8* has no effect on FW resistance. (a) Phenotype of *TRV:00* plants and genes-silenced plants at 20 days after inoculation with *Fov*. VIGS assay was conducted in a resistant cultivar Xinluzao 46 (Xin46) background. Susceptible cultivar Xinluzao 7 (Xin7) was inoculated with *Fov* simultaneously. (b-d) Detection of genes silencing efficiency through qRT-PCR. Data in b, c and d are presented as mean  $\pm$  SD from three technical replicates.

## Supplementary Figure 12



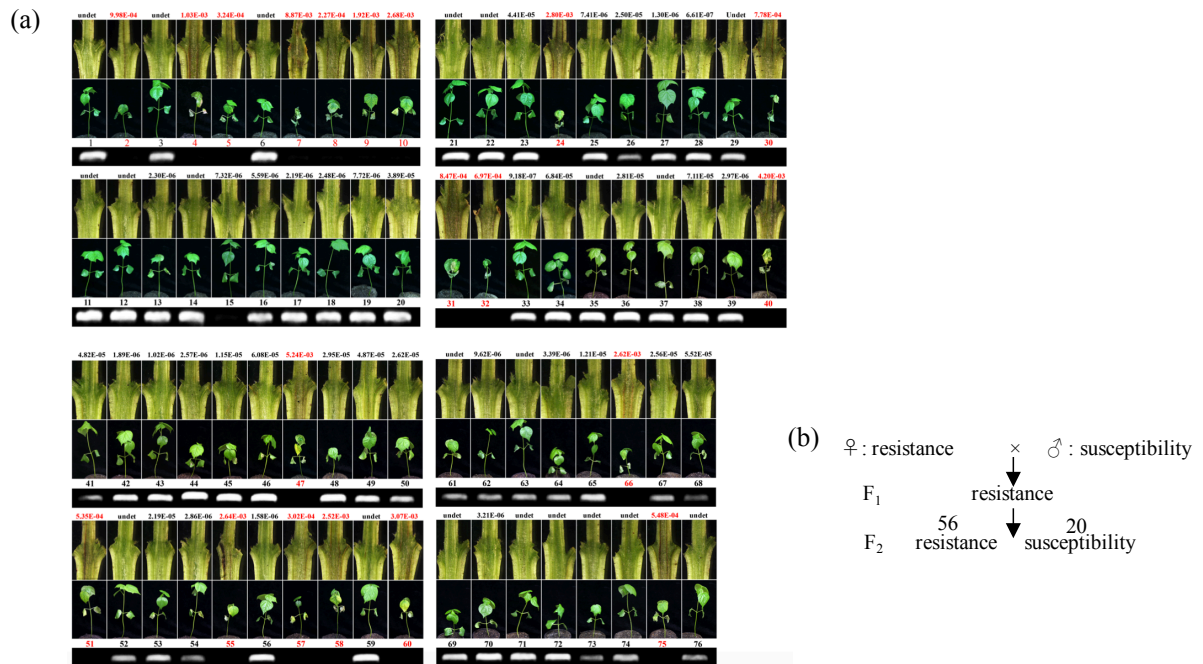
**Supplementary Figure 12.** Knockdown of *GhGLR4.8* strongly suppressed resistance to *Fov* in Upland cotton. **(a)** Disease symptoms of *TRV:00* and *TRV:GhGLR4.8* plants at 20 days after inoculation with *Fov*. Two highly resistant cultivars (Xin46 and YZ1) and one susceptible cultivars (Xin7) were selected. **(b)** Expression levels of *GhGLR4.8* in *TRV:00* and *TRV:GhGLR4.8* plants was determined by qRT-PCR. **(c)** Distribution of disease grade of *TRV:00* and *TRV:GhGLR4.8* plants. **(d)** Vascular bundles coloration in longitudinal-sections of inoculated *TRV:00* and *TRV:GhGLR4.8* plants. **(e)** Relative content of *Fov* DNA in inoculated stem of *TRV:00* and *TRV:GhGLR4.8* plants. Data in b and e are presented as mean  $\pm$  SD from three technical replicates.

## Supplementary Figure 13



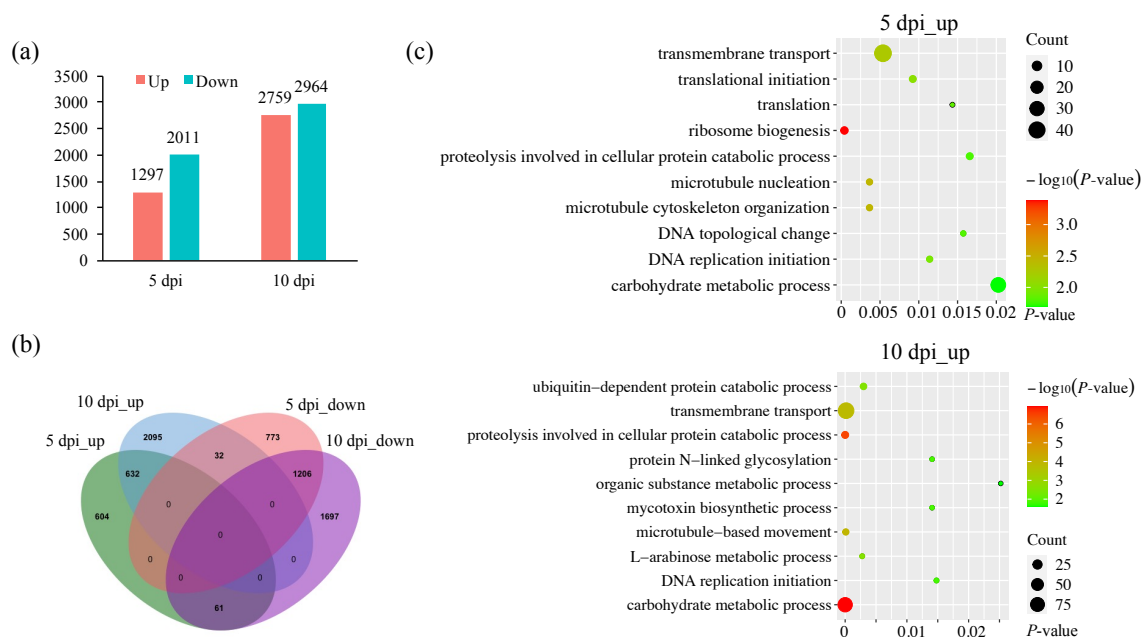
**Supplementary Figure 13.** Knockdown of *GhGLR4.8* through VIGS has no effect on Verticillium wilt resistance. **(a)** Disease symptoms of *TRV:00* and *TRV:GhGLR4.8* plants at 20 days after inoculation with *V. dahliae*. **(b)** Expression levels of *GhGLR4.8* in *TRV:00* and *TRV:GhGLR4.8* plants were determined by qRT-PCR. Data in b are presented as mean  $\pm$  SD from three technical replicates.

## Supplementary Figure 14



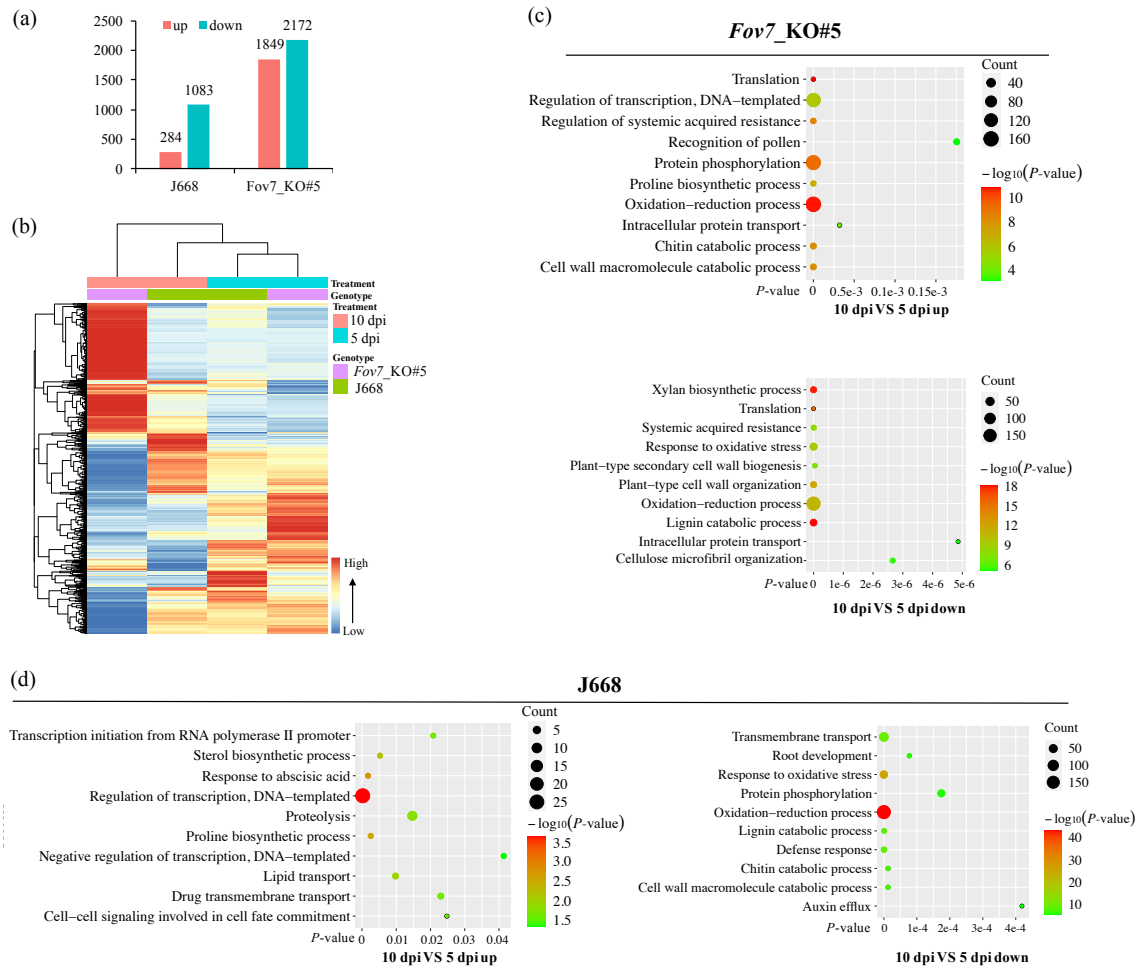
Supplementary Figure 14. Repeat of co-segregation analysis between *GhGLR4.8*<sup>SNP(A/C)</sup> genetic marker and *Fov* resistance. The detailed description refers to Figure 3.

## Supplementary Figure 15



**Supplementary Figure 15.** Transcriptome profile of fungal genes in hypocotyls of *Fov*-infected *Fov7\_KO#5* versus *in vitro*-grown *Fov*. **(a)** The number of differentially expressed fungal genes (DEFGs) ( $P < 0.05$ ,  $|\log_2(\text{FC})| > 1$ ). Fold change was calculated by  $(\text{in planta } Fov)/(\text{in vitro-grown } Fov)$ . **(b)** The venn diagram of DEFGs. **(c)** GO enrichment of host-induced genes of *Fov*. TOP10 significantly enriched biological process GO terms are show. Three biological replicates were included for each treatment.

## Supplementary Figure 16



**Supplementary Figure 16.** Transcriptome profile of plant genes in hypocotyls of *Fov*-infected *Fov7\_KO#5* and J668 at 10 dpi versus at 5 dpi. (a) Number of differentially expressed plant genes (DEPGs) ( $P < 0.05$ ,  $|\log_2(\text{FC})| > 2$ ) in *Fov*-infected hypocotyls of *Fov7\_KO#5* and J668 at 10 dpi versus 5 dpi. Fold change is calculated by 10 dpi/5 dpi, dpi, days post inoculation. (b) Heat map of DEPGs in hypocotyls of *Fov7\_KO#5* and J668 at 10 dpi versus 5 dpi. (c) and (d) Gene ontology (GO) enrichment analysis of DEPGs in hypocotyls of *Fov7\_KO#5* (c) or J668 (d) at 10 dpi versus 5 dpi. Three biological replicates were included for each treatment.

# Supplementary Figure 17



**Supplementary Figure 17.** Sequence comparison of *Fov7* in *G. hirsutum* (TM-1),<sup>[19]</sup> *G. barbadense* (3-79)<sup>[19]</sup> and *G. arboretum* (Shixiyal).<sup>[10]</sup> *Ghir\_A02G017230.1* is the *Fov7* homologue in A subgenome of TM-1, *Ga02G0002.1* is the *Fov7* homologue in Shixiyal, *Gbar\_A02G016930.1* and *Gbar\_D03G002290.1* are the *Fov7* homologues in A and D subgenome of 3-79, respectively.



## Supplementary Table

Supplementary Table 1, 3, 7-10 were offered in a separate file.

**Supplementary Table 1.** The disease index of 290 cotton accessions for GWAS.

**Supplementary Table 2.** The significant SNPs associated with Fusarium wilt.

SNP	Chromosome	Allele	Position	<i>P</i> value	$-\log_{10}(P)$	MarkerR <sup>2</sup>
D03_2176763	D03	G/T	2176763	7.84E-11	10.106	0.17535
A01_30018512	A01	A/T	30018512	6.47E-09	8.189	0.12425
D03_2135880	D03	C/T	2135880	9.11E-09	8.040	0.13716
D03_2146908	D03	G/A	2146908	1.81E-08	7.742	0.13175
D03_2115717	D03	G/A	2115717	2.34E-08	7.631	0.12975
D03_2148731	D03	G/A	2148731	6.76E-08	7.170	0.12145
D03_2135218	D03	A/T	2135218	7.34E-08	7.134	0.1208
D03_2115674	D03	C/T	2115674	9.72E-08	7.012	0.11862
D13_41766280	D13	T/G	41766280	1.35E-07	6.869	0.11606
D03_2125319	D03	A/G	2125319	1.54E-07	6.813	0.11506
D03_2076487	D03	A/C	2076487	1.78E-07	6.749	0.11393
D03_2380413	D03	T/G	2380413	2.04E-07	6.691	0.11288
D03_2145709	D03	G/A	2145709	2.32E-07	6.634	0.11187
D03_2147303	D03	C/T	2147303	2.79E-07	6.554	0.11045
D03_2138339	D03	A/G	2138339	2.99E-07	6.524	0.10991
D03_2073788	D03	G/A	2073788	3.40E-07	6.468	0.10893
D03_2075791	D03	G/A	2075791	3.52E-07	6.453	0.10866

**Supplementary Table 3.** The FW resistance of 194 cotton accessions collected from previous study.

**Supplementary Table 4.** Genes located in candidate region of *Fov7* locus and its annotation.

Gene ID	Strand	Start	Stop	Annotation
Gh_D03G0203	-	2027606	2029122	heat shock factor 4
Gh_D03G0204	+	2045424	2045591	unknown
Gh_D03G0205	+	2094618	2094950	chloride channel B
Gh_D03G0206	-	2125102	2144777	cytochrome P450, family 83
Gh_D03G0207	-	2166718	2167364	Protein of unknown function (DUF1685)
Gh_D03G0208	-	2169915	2173932	unknown
Gh_D03G0209	-	2175025	2177322	glutamate receptor 3.3
Gh_D03G0210	+	2179082	2182850	unknown
Gh_D03G0211	+	2193477	2194673	unknown
Gh_D03G0212	-	2203285	2203968	bHLH DNA-binding superfamily protein
Gh_D03G0213	+	2218541	2219557	mitogen-activated protein kinase kinase kinase 19
Gh_D03G0214	+	2226884	2228258	F-box/RNI-like superfamily protein
Gh_D03G0215	-	2229735	2231887	PLC-like phosphodiesterases superfamily protein
Gh_D03G0216	-	2232858	2235938	Plant protein of unknown function (DUF869)
Gh_D03G0217	-	2238291	2240938	protein serine/threonine kinases
Gh_D03G0218	-	2255453	2257785	Integrase-type DNA-binding superfamily protein
Gh_D03G0219	+	2293920	2297367	Cystathionine beta-synthase (CBS) family protein
Gh_D03G0220	-	2304123	2304617	DREB and EAR motif protein 3
Gh_D03G0221	-	2314748	2317694	Dentin sialophosphoprotein-related
Gh_D03G0222	+	2338597	2347155	P-loop containing nucleoside triphosphate hydrolases
Gh_D03G0223	-	2348736	2351761	Leucine-rich receptor-like protein kinase family protein
Gh_D03G0224	+	2368534	2373145	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase
Gh_D03G0225	-	2373295	2374910	FMN-linked oxidoreductases superfamily protein

**Supplementary Table 5.** Features of the *GLR* genes in Upland cotton

Gene ID	Gene Name	Chr	Length (aa)	Genomic Region
Ghir_A07G021120	GhGLR1.1	A07	627	91819388-91821699
Ghir_A07G021130	GhGLR1.2	A07	847	91850132-91854091
Ghir_D07G009840	GhGLR1.3	D07	792	11852666-11858516
Ghir_D07G021310	GhGLR1.4	D07	862	53661343-53664759
Ghir_D07G021320	GhGLR1.5	D07	776	53666922-53669913
Ghir_A07G003890	GhGLR2.1	A07	979	4330442-4336180
Ghir_D04G008970	GhGLR2.2	D04	851	18628789-18638112
Ghir_D04G015310	GhGLR2.3	D04	914	49556864-49564141
Ghir_D07G003910	GhGLR2.4	D07	915	4037638-4043561
Ghir_A02G019470	GhGLR3.1	A02	947	107934254-107939151
Ghir_A05G018230	GhGLR3.2	A05	851	17246944-17253044
Ghir_A12G001330	GhGLR3.3	A12	885	1683238-1688933
Ghir_A12G016770	GhGLR3.4	A12	813	91878901-91883964
Ghir_A13G006510	GhGLR3.5	A13	905	14068841-14073278
Ghir_A13G009800	GhGLR3.6	A13	821	52745568-52750576
Ghir_A13G009810	GhGLR3.7	A13	921	52799328-52804364
Ghir_D03G000100	GhGLR3.8	D03	310	98496-99510
Ghir_D05G018240	GhGLR3.9	D05	909	15825979-15832082
Ghir_D12G001360	GhGLR3.10	D12	810	1630516-1636164
Ghir_D12G017030	GhGLR3.11	D12	813	49647233-49652268
Ghir_D13G005830	GhGLR3.12	D13	810	7699695-7704665
Ghir_D13G009280	GhGLR3.13	D13	821	19596508-19601650
Ghir_D13G009290	GhGLR3.14	D13	921	19625438-19630396
Ghir_A02G017230	GhGLR4.1	A02	481	105680541-105683016
Ghir_A02G017250	GhGLR4.2	A02	566	105697917-105714265

Ghir_A05G016210	GhGLR4.3	A05	900	15273260-15279087
Ghir_A05G020820	GhGLR4.4	A05	405	20134953-20137186
Ghir_A10G002660	GhGLR4.5	A10	904	2350198-2354896
Ghir_A10G002670	GhGLR4.6	A10	972	2354914-2358484
Ghir_A10G002680	GhGLR4.7	A10	889	2361358-2365538
Ghir_D03G002390	GhGLR4.8	D03	641	2246112-2248669
Ghir_D05G016050	GhGLR4.9	D05	818	13966076-13971543
Ghir_D05G020760	GhGLR4.10	D05	740	18081772-18085238
Ghir_D10G003440	GhGLR4.11	D10	904	2872905-2876333
Ghir_D10G003450	GhGLR4.12	D10	932	2877535-2881153
Ghir_D10G003460	GhGLR4.13	D10	897	2882280-2888831

**Supplementary Table 6.** Percentage and number of unique RNA-seq reads aligned to *Fov* race 4.

		Days Post Inoculation			
		5	10	5	10
		Number of			
Biological		Percentage of <i>Fov</i>		Uniquely Aligned	
Replicate	Plant	Reads		Reads	
1	<i>Fov7_KO#5</i>	0.10%	0.70%	17429	315378
2	<i>Fov7_KO#5</i>	0.09%	0.69%	31558	201704
3	<i>Fov7_KO#5</i>	0.06%	0.66%	13410	193339
Average		0.08%	0.68%	20799	236807

**Supplementary Table 7.** Transcriptome profiles of fungal genes in hypocotyls of *Fov*-infected *Fov7\_KO#5* versus *in vitro*-grown *Fov*, related to Figure S15.

**Supplementary table 8.** Transcriptome profiles of plant genes in hypocotyls of *Fov*-infected *Fov7\_KO#5* versus J668 at 5 dpi and 10 dpi, related to Figure 5.

**Supplementary table 9.** Transcriptome profiles of plant genes in hypocotyls of *Fov*-infected *Fov7\_KO#5* and J668 at 10 dpi versus 5 dpi, related to Figure S16.

**Supplementary Table 10.** Primers used in this study.