

Supplemental figure S1. A schematic map of the gene structures of the *GhKOR1* and *AtKOR1* genes. Triangles indicate the location and size of the introns.

Supplemental figure S2. Alignment of eight different plant membrane-bound endo-1,4-b-glucanase protein sequences. Identical amino acids were shaded in black. Stars denote polarized targeting signals. Twenty-two boxed amino acids indicate the predicted transmembrane domains. Arrowheads show the predicted glycosylation sites; Arrows denote the residues essential for catalytic activity. *GhKOR1*, *Gossypium hirsutum*, AAS87601; *AtKOR1*, *Arabidopsis thaliana*, NP_199783(At5g49720); *BnCel16*, *Brassica napus*, CAB51903; *Pa x gKOR*, *Populus alba x grandidentata*, ADB82903; *LeCel3*, *Lycopersicon esculentum*, AAC49704; *OsCel9A*, *Oryza sativa*, BAF37260; *PgKOR*, *Picea glauca*, AEL88496; *PtrKOR1*, *Populus trichocarpa*, AAS45400.

A

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                *           280           *           300           *           3
GhKOR2-cDN : G G A C T T A A C G A T G G - A A A T G A A G - - - - C G A A C T T G - - - - G A T C T T T C C A A : 302
GhKOR1-RNA : G G C C T C C A A G A T G G C A A A T C C G A T C C C T C C G T T T T G A T G A A A G A T C T G G T C G G : 216
                G G C T A G A T G G A A A T C T T T G G A T C T C

                20           *           340           *           360           *
GhKOR2-cDN : G G G G A T G T A T G A T G C T G G A G A T C A C A T G A A A T T T T G G T T T T C C A A T G G C A T T T A : 355
GhKOR1-RNA : C G G A T A T T A C G A T G C T G G A G A T G C T A T C A A G T T T A A C T T T C C T G C A T C T T T T : 269
                G G T A G A T G C T G G A G A T A T A A T T T T T T C C C T T C T T T
  
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B

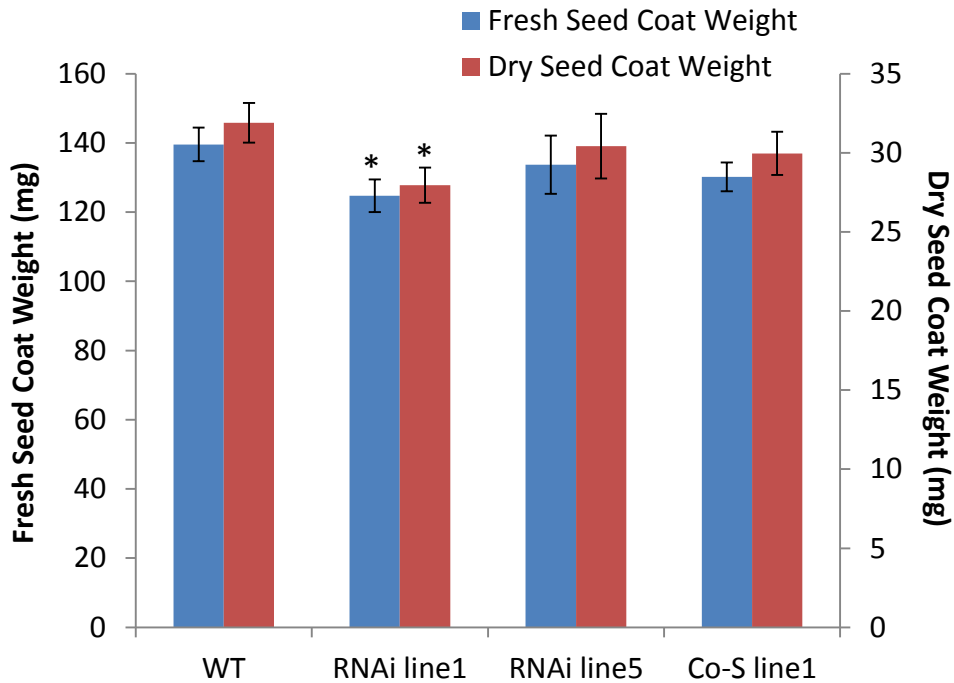
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                520           *           540           *           560
GhGlu3-cDN : G G C C G A G T T A T C A A C C C G C A T T G A A A G G T T T G C T T C A G T T C C A C A A G : 564
GhKOR1-RNA : - - C G G A C T C A T C A - C C C T C A T C G T C A A G A C T G T C C C A - C G T C A T C A C : 43
                C G A T A T C A C C C C A T G A G T G C A C A A

                *           580           *           600           *
GhGlu3-cDN : G A C A A C G G G T C C C C T T T T G C C A T T A A T C C G T A C C C G T T T T T C G C T T A : 611
GhKOR1-RNA : C A C C G C C A C T C T C C G C C C G - - A T A A C T A C A C T C T G G C T C T T C A C - - - : 85
                A C C T C C C G A T A T C C G T T T C C
  
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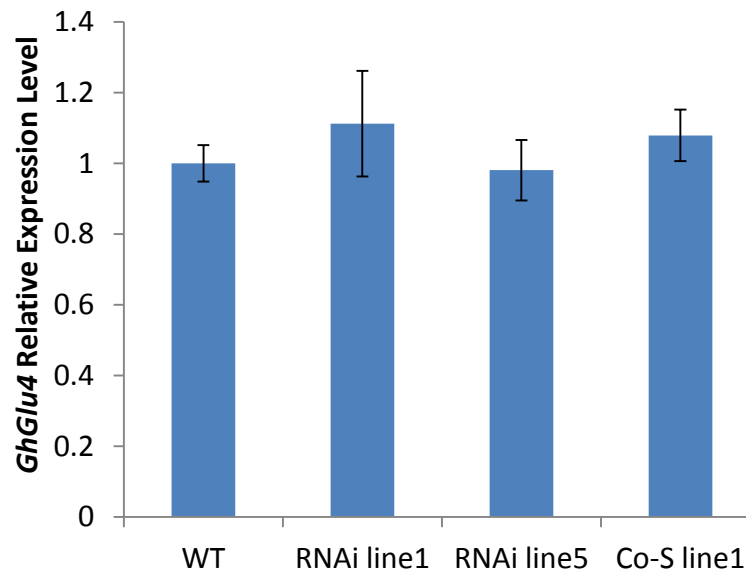
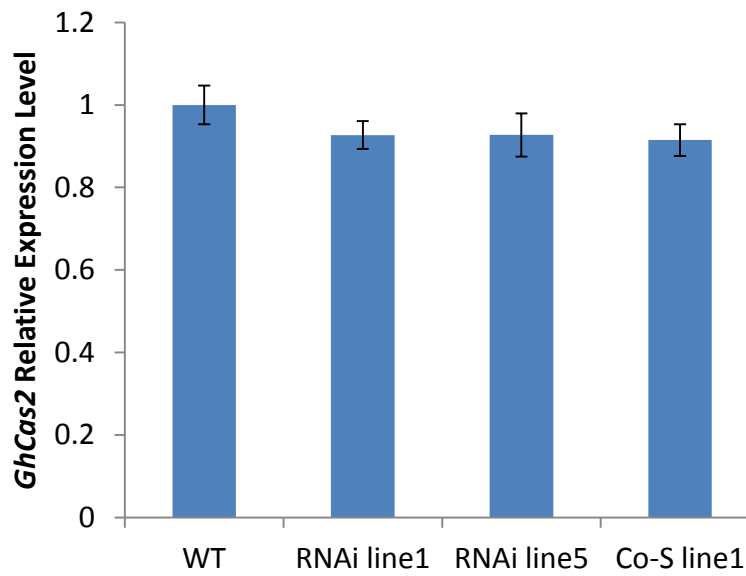
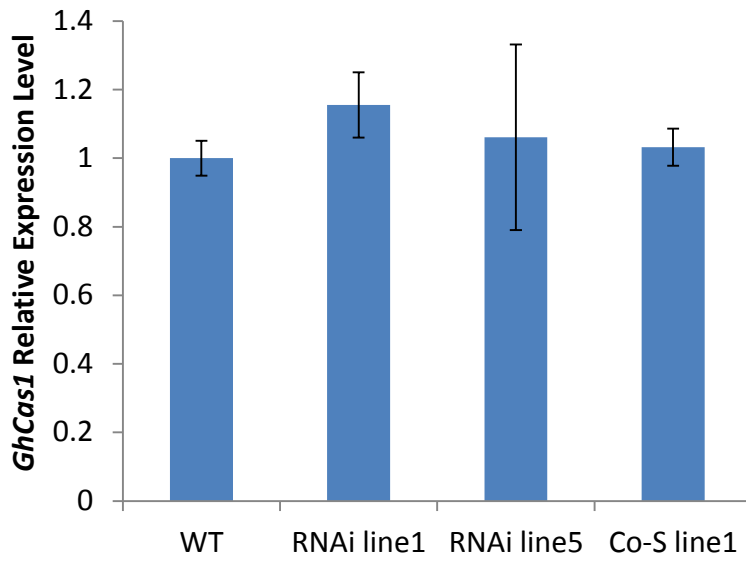
Supplemental Figure S3. The alignment between the RNAi fragment of *GhKOR1* used in the RNAi construct and *GhKOR2* cDNA, *GhGlu3* cDNA sequences. The red boxed sequences indicate regions with high sequence homology which may cause co-suppression.

- (A) The alignment between the RNAi fragment of *GhKOR1* and *GhKOR2* cDNA sequences;
- (B) The alignment between the RNAi fragment of *GhKOR1* and *GhGlu3* cDNA sequences



Supplemental Figure S4. Fresh and dry weight of the 20 DPA seed coat in *GhKOR1* down-regulated transgenic cotton in comparison with that of wild-type.

Except for RNAi line1, the fresh and dry weight of 20-d seed coat remained unchanged in the transgenic cotton compared to that in WT. * indicate a significant difference to wild type in *t*-test with *P*-value of 0.05. At least three biological replicates were used for each case.



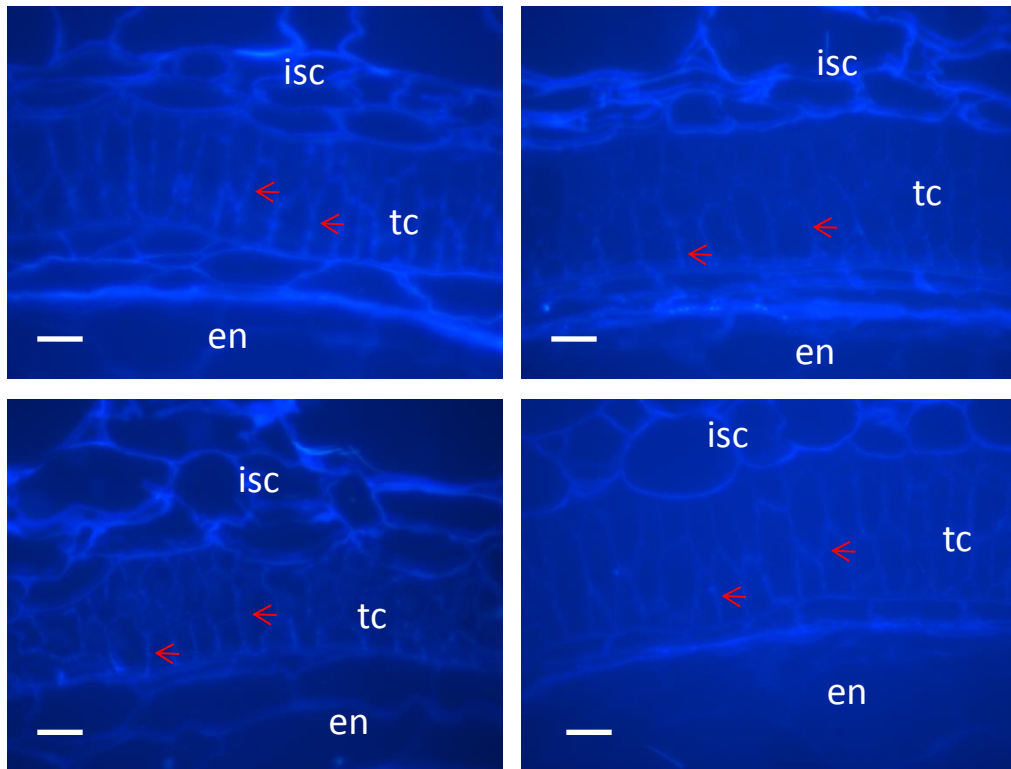
Supplemental Figure S5. Transcript levels of *GhCas1*, *GhCas2* and *GhGlu4* in 15 DPA seed coat of wide type (WT), RNAi and co-suppression (Co-S) transgenic cotton.

(A) Transcript levels of *GhCas1* in 15 DPA seed coat.

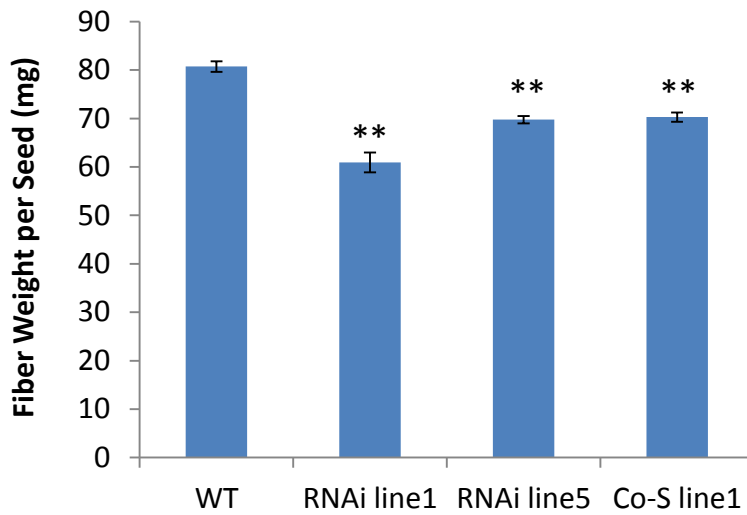
(B) Transcript levels of *GhCas2* in 15 DPA seed coat.

(C) Transcript levels of *GhGlu4* in 15 DPA seed coat.

No significant difference was detected in the transcript level of these three callose metabolism genes in 15 DPA seed coat.

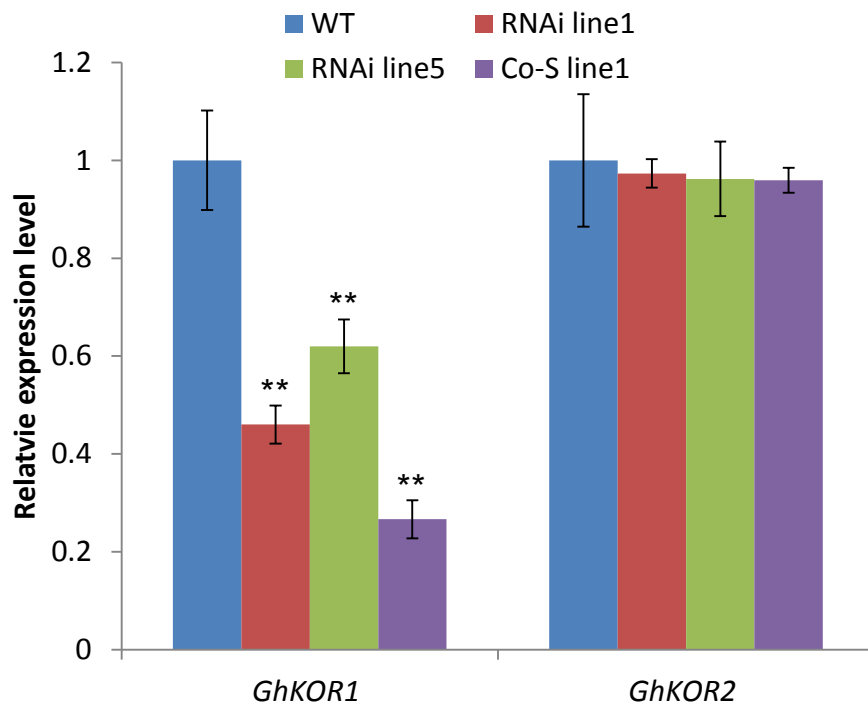
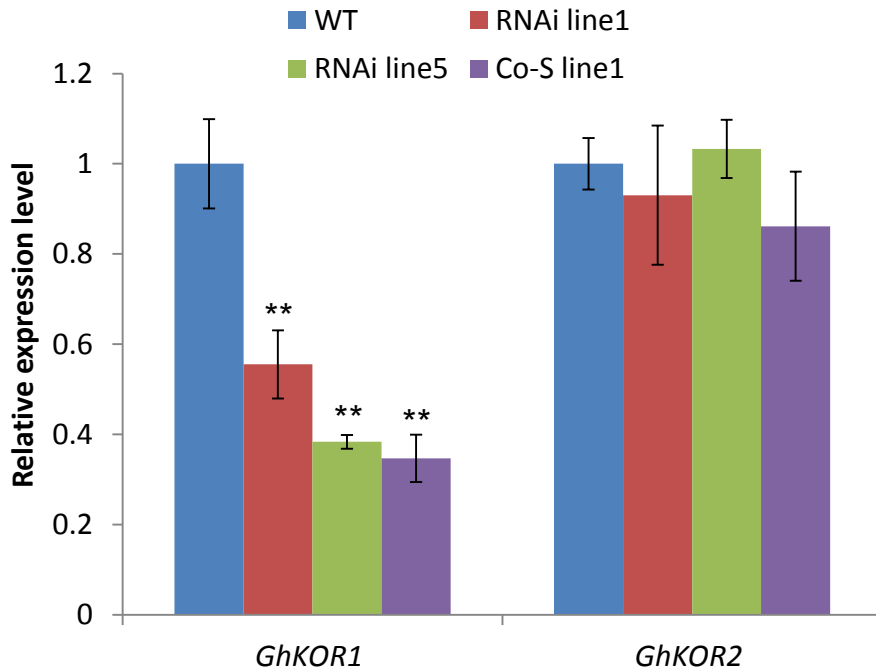


Supplemental Figure S6. Comparison of aniline blue – labelled cellulose signals in 15DPA seed transfer cells (TCs) between WT and *GhKOR1* down-regulated transgenic cotton. Images are micrographs of 2 μ m cross sections of 15DPA resin embedded seeds stained with Calcofluor White. (A) Image of the WT 15DPA cotton seed section; (B to D) Images of the RNAi line1, RNAi line5 and Co-S line1 15DPA cotton seed section, respectively. No significant difference of the cellulose signals could be detected in the TCs between the transgenic lines and the WT, which showed similar flange morphology of the TCs (red arrows). Three biological replicates were observed for each line with similar results obtained. Representative images were provided. en, endosperm; isc, inner seed coat; tc, transfer cell. Bars = 50 μ m in A to D.



Supplemental Figure S7. Reduced fiber weight in *GhKOR1* down-regulated transgenic cotton. Mature fiber weight per seed of transgenic cotton is significantly reduced as compared to that in the wild type.

** indicate a significant difference to wild type in *t*-test with *P*-value of 0.01.



Supplemental Figure S8. Transcript levels of *GhKOR1* and *GhKOR2* in root and above ground tissue of wide type (WT), RNAi and Co-Suppression (Co-S) transgenic cotton.

(A) and (B) qRT-PCR results showing that *GhKOR1* gene was down-regulated in the root (A) and above ground tissue (B) of the three transgenic lines. While *GhKOR2* mRNA level did not appear to exhibit major changes.

** indicate a significant difference to wild type in *t*-test with *P*-value of 0.01. Samples were collected on 30 days after germination (DAG).