**Supporting Data**

**Figure S1** Genotyping of polymorphic marker for *Gh\_A03G0489* in genetic populations.

P1: TM-1, I4005, CSIL028; P2, *im*. 9-44 showed the amplification products of *Gh\_A03G0489* in 36 randomly selected individuals with fluffy fiber phenotype (mature fiber; 21-44) or not (Immature fiber; 9-20). The genetic populations included (CSIL028×*im*) F2 with 342 individuals (Wang *et al*. 2013), (TM-1×*im*) F2 with 737 individuals and (I4005×*im*) F2 with 1837 individuals.



**Figure S2** Subgenome-specific primers for *GhImA* and *GhImD* genes.

A. The SNP locus for designing subgenome-specific primers for qRT-PCR (arrows).

B. Detection of subgenome-specific primers. *G.a* and *G.r* represented diploid cotton *G. arboreum* (AA) and *G. raimondii* (DD) respectively. 1. Marker; 2-5. Specific-primers for *GhImA*; 6-9. Specific-primers for *GhImD*.



**Figure S3** Motif analysis of *GhImA* (A) and *GhImD* (B) in TM-1.

Different colors showed the different motifs of PPR proteins. SP, P and S showed the N-terminal signal peptide, PPR motif and short motif in PPR proteins.



**Figure S4 Phylogenetic relationships of GhIm and its homologs from different species**

The full-length protein sequences of GhIm and its homologous representative protein sequences from other species, including St: *Solanum tuberosum*, Sl: *Solanum lycopersicum*, Dz: *Durio zibethinus*, Tc: *Theobroma cacao*, Hu: *Herrania umbratica*, Cc: *Corchorus capsularis*, Qs: *Quercus suber*, Md: *Malus domestica*, Hb: *Hevea brasiliensis*, Me: *Manihot esculenta*, Jc: *Jatropha curcas*, Rc: *Ricinus communis*, Pt: *Populus trichocarpa*, Cu: *Citrus unshiu*, Vv: *Vitis vinifera*, Al: *Arabidopsis lyrata*, At: *Arabidopsis thaliana* and EMP10, EMP11, EMP12, EMP16, EMP602 and DEK2 from *Zea mays*, respectively, were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) to construct phylogenetic tree using MEGA-X software. Scale bar, 0.2.



**Figure S5 Investigation on mature transcripts and the splicing efficiency of *nad7* in fibers of TM-1 and *im* mutant**

A. RT-PCR analysis of 36 mitochondrion-encoded transcripts in fibers of TM-1 and *im* mutant. RNA was from 22 DPA fibers of TM-1 and the *im* mutant. Mitochondrial *18S rRNA* (GenBank ID: 24679539) as a reference.

B. Splicing efficiency of *nad7* introns. The ratio of mature transcripts to unspliced fragments was used for measuring differences in splicing efficiency, showing that the intron2 splicing efficiency of *nad7* was potentially affected in *im* mutant.



**Figure S6** qRT-PCR analysis of mature transcripts and unspliced fragments. Primers spanning adjacent exons or spanning adjacent exons and introns were used for measuring differences in each spliced fragment, respectively.



**Figure S7** BN-PAGE (left) and In-gel Complex 1 activity (right) detection of mitochondrial complexes. The positions of Complex I were indicated respectively. Each lane was loaded with equal quantitative mitochondrial protein for TM-1 and *im*. M represents the protein molecular weight marker. In-gel NADH dehydrogenase activity assay of the mitochondrial protein Complex I was shown.



**Figure S8** Plant phenotypes after silencing *GhIm* gene in the *im* mutant.

**A.** Phenotypes of control (CK), empty vector (TRV:00), positive control (TRV:*CLA1*) and *GhIm*-silenced (TRV:*GhIm*) plants after two weeks post-agroinﬁltration. Scale bar, 35 mm.

**B.** Transcript level of *GhImA* and *GhImD* in leaves of CK, TRV:00 and TRV: *GhIm* plants. The relative expression level was calculated using the 2-△CT method with three biological replicates. Data are represented as mean ± SE. Statistical significance was determined by Student’s *t*-test. \*\*P < 0.01.

**C.** Plant height comparison of CK, TRV:00 and TRV: *GhIm* plants after *GhIm* gene was silenced. Data are represented as mean ± SE with 20 biological replicates for each treatment. Statistical significance was determined by Student’s *t*-test. \*\*P < 0.01).

**D.** Phenotypes of CK, TRV:00, TRV:*CLA1* and TRV:*GhIm* plants at five weeks post-agroinﬁltration. Scale bar, 35mm.



**Figure S9** Correlations of fold change between RNA-seq data and qRT-PCR.

Expression data were from 20 genes (File S1) on 13 DPA, 16 DPA, 19 DPA, 22 DPA and 25 DPA fibers in TM-1 and *im* mutant. The RNA-seq log2 (expression ratio) values were plotted against the log2 (expression ratio) obtained by qRT-PCR.



**Figure S10** Detection of H2O2 content in TM-1 and *im* fibers.

Data are represented as mean ± SE and the significant differences in H2O2 content between TM-1 and the *im* mutant at the same stage of fiber development are shown. Statistical significance was determined by Student’s *t*-test. \*P < 0.05 and \*\*P < 0.01.