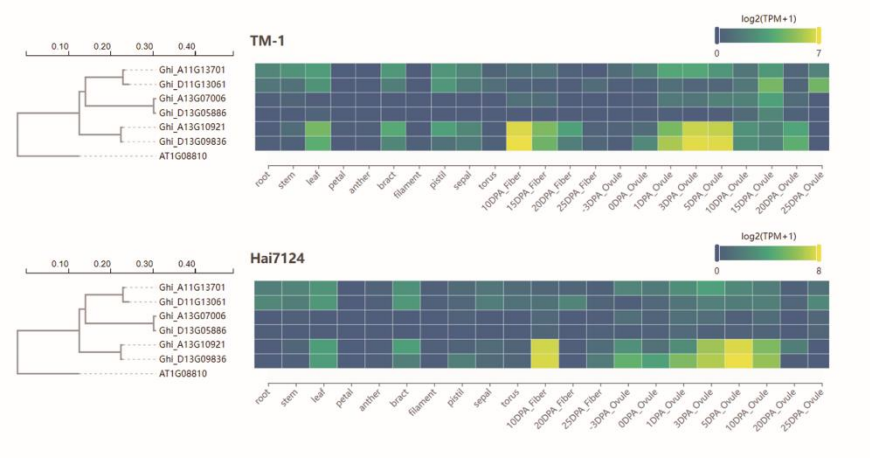
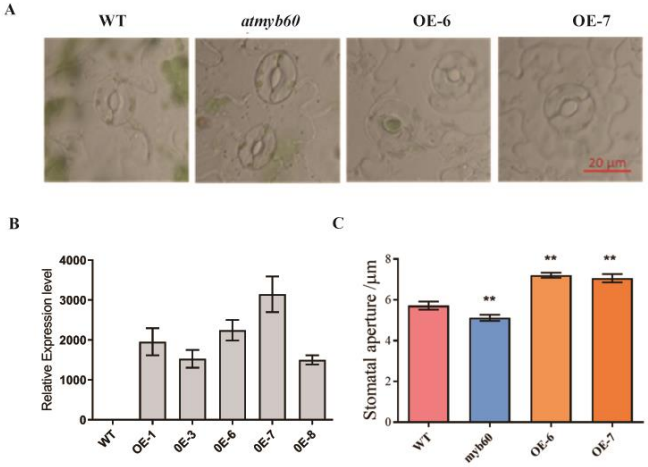
Supplemental Table S1. Transcription factors highly enriched in elongating fibers

Gene_id	Annotation	Average-ef (FPKM)[a]	Average-all (FPKM)[b]	Enrichment Fold[c]	Enrichment Factor[d]
bZIP					
Gh_A01G0419	bZIP	22.57	7.13	3.16	71.41
Gh_D01G0423	bZIP	38.94	10.91	3.57	138.96
Gh_A13G1269	bZIP	49.75	9.09	5.47	272.26
Gh_D13G1573	bZIP	39.59	10.3	3.84	152.23
homeobox					
Gh_A09G0391	HDG	28.7	4.98	5.76	165.29
Gh_Sca007830G01	HDG	46.36	11.24	4.12	191.16
Gh_D10G0270	HOX3	15.76	2.83	5.57	87.83
Gh_D12G2590	HDG11	22.66	6.18	3.67	83.07
bHLH					
Gh_A01G2041		29.51	6.27	4.71	138.96
Gh_A09G1863	bHLH	7.22	0.88	8.23	59.45
Gh_D04G0454	bHLH	12.02	2.09	5.76	69.21
GATA ZF					
Gh_A03G1598	GATA ZF	56.94	10.91	5.22	297.33
Gh_A12G2074	GATA ZF	109.97	25.09	4.38	482.06
Gh_D12G2250	GATA ZF	42.75	10.91	3.92	167.52
Gh_D12G2631	GATA ZF	64.3	14.36	4.48	288.02
Gh_A13G0161	GATA ZF	92.32	20.79	4.44	409.95
Gh_A13G0162	GATA ZF	160.93	44.22	3.64	585.64
MYB					
Gh_A04G1024	MYB 73	23.38	5.74	4.07	95.24
Gh_A05G3123	MYB 66	18.72	4.93	3.8	71.08
Gh_A09G2380	MYB 85	40.94	10.03	4.08	167.12
Gh_A13G1399	MYB 201	108.03	15.95	6.77	731.74
Gh_D13G1712	MYB 201	188.39	27.31	6.9	1299.4
Gh_A13G2154	MYB 106	103.62	29.75	3.48	360.86
Gh_A10G0338	MYB-like	39.04	7.28	5.36	209.41
Gh_D10G0344	MYB-like	36.73	7.31	5.03	184.63
Others					
Gh_A04G0810	GRAS	9.95	1.86	5.35	53.26
Gh_A05G1526	NAC	24.51	7.92	3.09	75.82
Gh_A11G0279	TCP 14	25.1	7.05	3.56	89.32

Note: a, the average fragments per kilobase per million mapped reads (FPKM) value in fibers of 5 and 10 DPA;
b, the average FPKM value in 20 tissues including fibers of 5, 10, 20 and 25 DPA, ovules of -3, -1, 0, 1, 3, 5, 10, 20, 25 and 35 DPA, roots, stems, leaves, petals, stamens and pistils.
c, Enrichment fold=Average-ef (FPKM)/Average-all (FPKM).
d, Enrichment factor=Enrichment fold × Average-ef (FPKM).

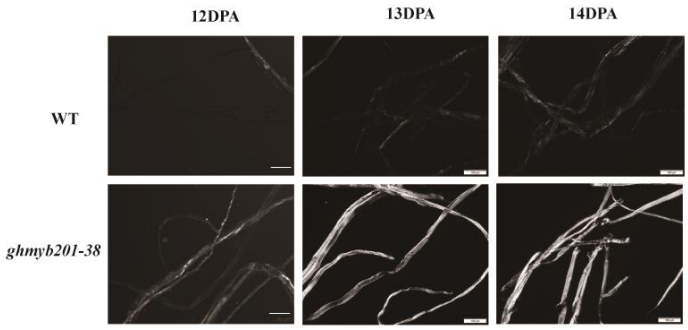


Supplemental Figure S1. Transcript levels of AtMYB60 homologous genes in various cotton tissues
Heatmap indicates fragments per kilobase per million mapped reads (FPKM) values of six AtMYB60 homologous genes (*Ghi_A11G13701/GhMYB105-At, Ghi_D11G13061/GhMYB105-Dt, Ghi_A13G07006/GhMYB192-At, Ghi_D13G05886/GhMYB192-Dt, Ghi_A13G10921/GhMYB201-At,* and *Ghi_D13G09836/GhMYB201-Dt*) in various tissues from *G. hirsutum* acc. TM-1 [1]. Tissues include fibers (Fi) and ovules (Ou) of various DPA, petal (Peta), pistil (Pist), root, stamen (Stamen), stem and leaf.

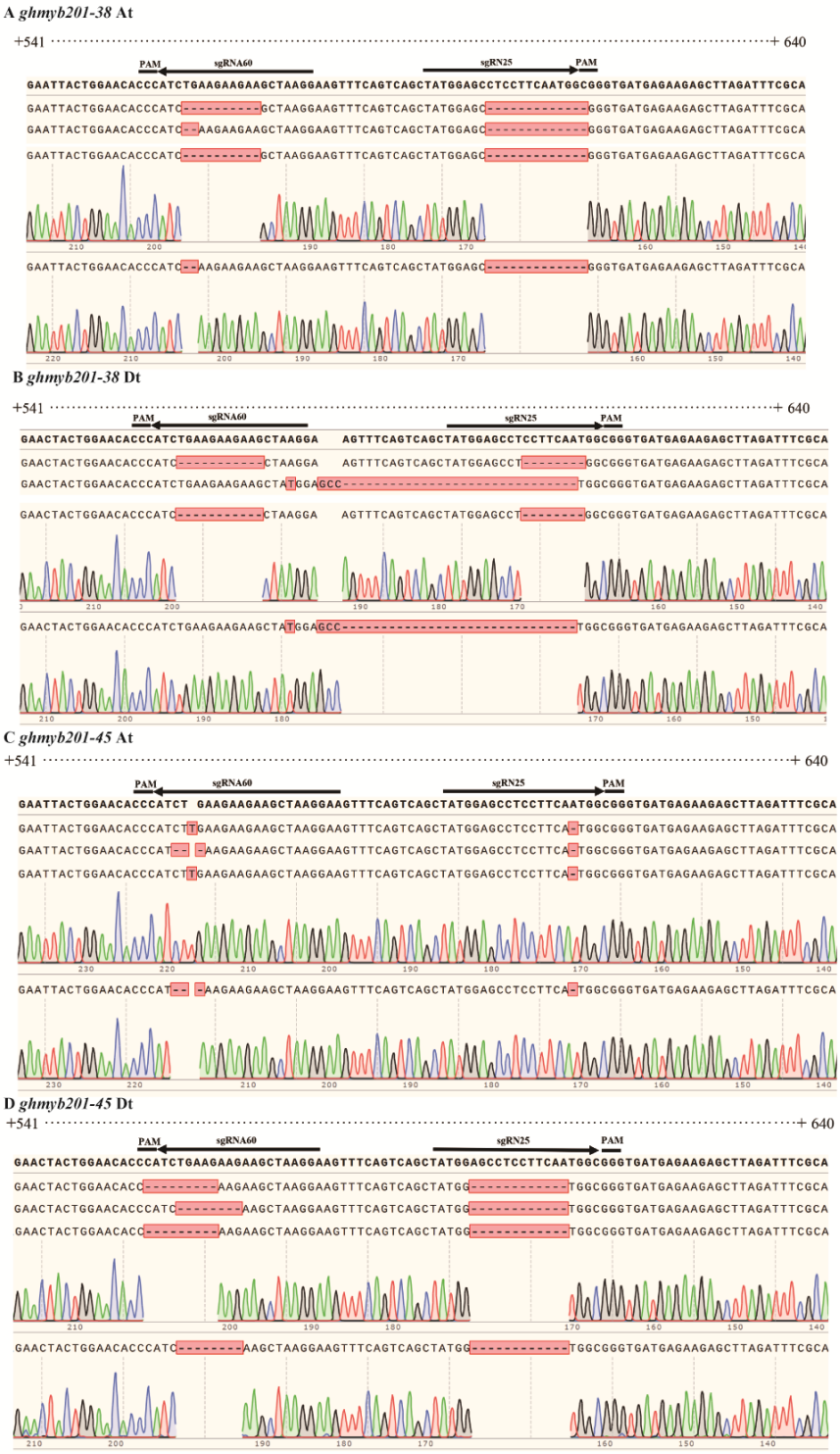


Supplemental Figure S2. *GhMYB201* rescues the regulation of stomatal aperture in Arabidopsis *atmyb60* mutant

A, The stomatal aperture of wild type, *atmyb60* mutant (SALK_013126C) and *GhMYB201* over-expressing Arabidopsis lines (OE). Bar = 20 μm. B, RT-qPCR analysis of *GhMYB201* transcript levels in transgenic Arabidopsis lines. Mean values and standard errors were calculated from three biological replicates. C, Statistical analysis of stomatal aperture in A. Data are means ±SEM, n > 100 stomata.

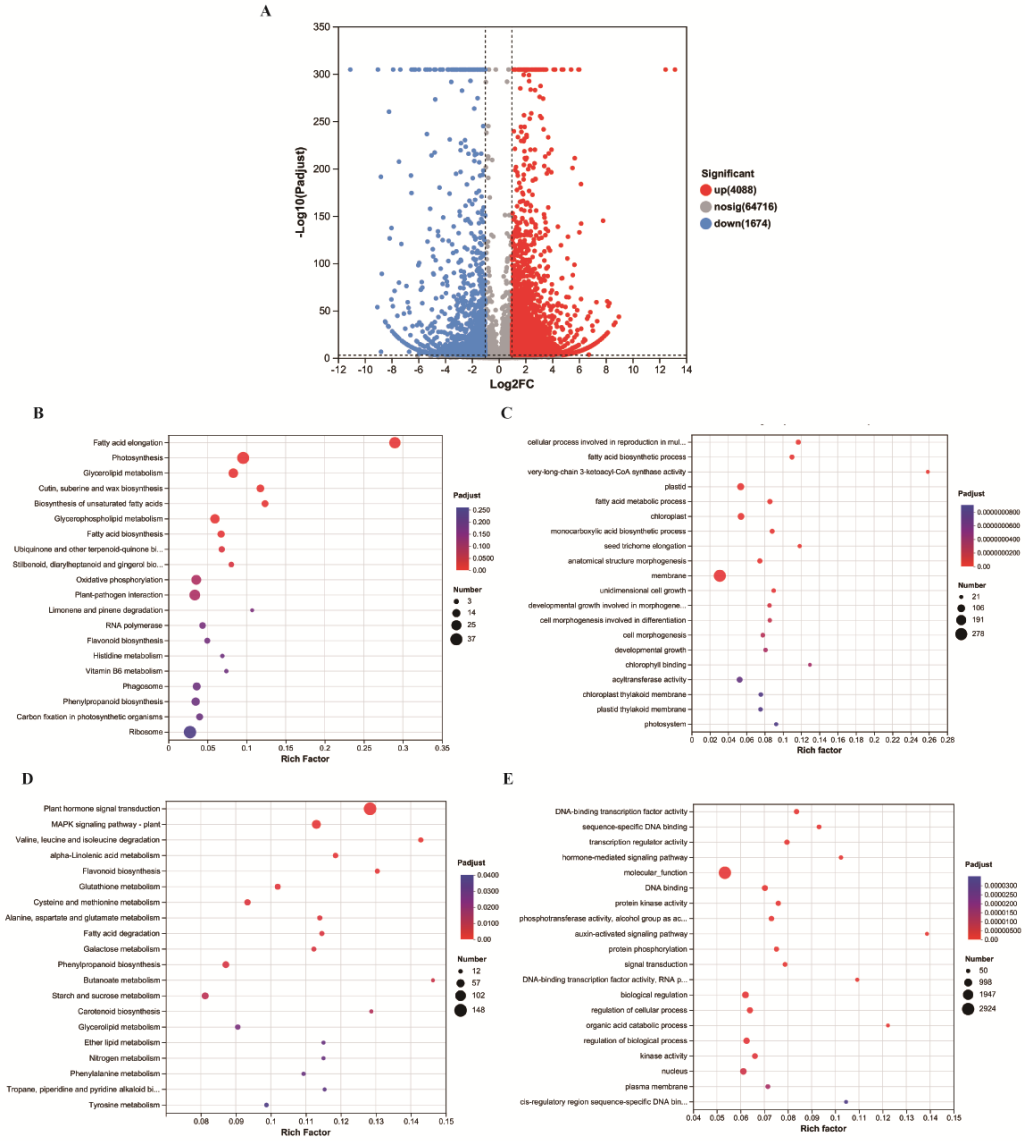


Supplemental Figure S3. Birefringence microscopy of Cellulose deposition in developing fibers
WT and *ghmyb201-38* fibers were observed at 12, 13 and 14 DPA fibers. Scale bar = 100 μm.



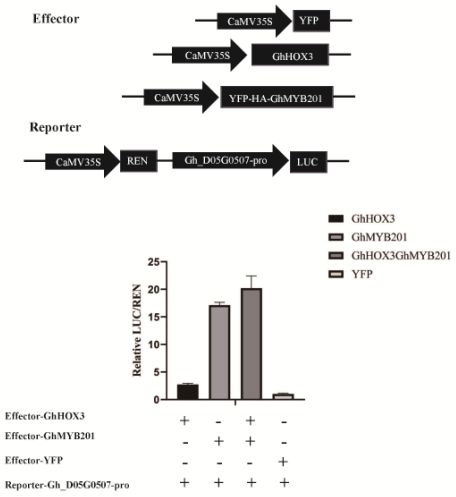
Supplemental Figure S4 Characterization of GhMYB201 plants generated by CRISPR/Cas9 gene editing

A-B *ghmyb201-38* genomic sequences of target sites from T1 plants, obtained by Sanger sequencing. The mutation type of *ghmyb201-38* knockout line with 2 sgRNAs. C-D *ghmyb201-45* genomic sequences of target sites from T1 plants, obtained by Sanger sequencing. The mutation type of *ghmyb201-45* knockout line with 2 sgRNAs.



Supplemental Figure S5. RNA-Seq analysis of 7-DPA fibers of *GhMYB201* knockout line (*ghmyb201-38*) and wild type

(A) Volcano map of the differentially expressed genes (DEGs). Blue and red dots represent the down- and up-regulated genes in *GhMYB201* knockout fibers, compared with the wild type, respectively. (B) Enriched KEGG pathways in downregulated DEGs. (C) Enriched GO pathways in downregulated DEGs. (D) Enriched KEGG pathways in upregulated DEGs. (E) Enriched GO pathways in upregulated DEGs.



Supplemental Figure S6. Transcriptional regulation of target gene by GhMYB201 and the effect of GhHOX3

1. Zhang, T.; Hu, Y.; Jiang, W.; Fang, L.; Guan, X.; Chen, J.; Zhang, J.; Saski, C.A.; Scheffler, B.E.; Stelly, D.M.; et al. Sequencing of allotetraploid cotton (Gossypium hirsutum L. acc. TM-1) provides a resource for fiber improvement. *Nat Biotechnol* **2015**, *33*, 531-537, doi:10.1038/nbt.3207.