

Supplementary Information

**Integrative transcriptome, proteome, phosphoproteome and genetic mapping
reveals new aspects in a fiberless mutant of cotton**

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Supplementary figure and table legends

Supplementary Fig. S1. The experimental workflow in this study. WT -3 DPA and WT 0 DPA: -3-DPA and 0-DPA ovules from WT. *fl* -3 DPA and *fl* 0 DPA: -3-DPA and 0-DPA ovules from *fl* mutant. Bars: 200 μm in WT -3 DPA and *fl* -3 DPA; 20 μm in WT 0 DPA and *fl* 0 DPA.

Supplementary Fig. S2. Alternative splicing events in the common genes between DEGs and DEPs. Yellow bar illustrated the gene sequences same as the cotton genome annotated genes. Blue bar indicated sequences different from the annotated genes in the WT. Red bar indicated sequences different from the annotated genes in the *fl*.

Supplementary Fig. S3. comparison of phosphosites between CotAD_44282 and homologs in other species.

Supplementary Fig. S4. Phosphoraylation motifs between all phosphosites (a) and differential phosphosites (c). (b, d) Top five phosphoraylation motifs.

Supplementary Fig. S5. Western blotting and enzymatic assay validation of selected differentially expressed proteins. (a) Western blotting of actin and PPP1CB expression between WT and *fl* at 0 DPA. (b) Enzymatic assay analysis of the G6PDH enzyme. Three biological replicates were performed. The error bars represent SD (student's t test, *** $p < 0.001$, $p = 5.34\text{E-}05$ at -5 DPA stage, $p = 0.00057$ at 0 DPA stage, $p = 5.87\text{E-}05$ at 3 DPA stage).

Supplementary Fig. S6. Quantification of fatty acids of WT and *fl* ovules at -3 DPA. (student's t test, * $P < 0.05$, ** $P < 0.01$, $p = 0.011$ at C16:0, $p = 0.031$ at C18:0, $p =$

0.0028 at C18:1, $p = 0.0015$ at C18:2, $p = 0.0015$ at C18:3).

Supplementary Fig. S7. Cotton ovule culture in vitro. Scanning electron of unfertilized ovule surface treated with (a) none, (b) 5 μM D - fructose di-*potassium salts* of 6-*phosphorous acid*, (c) 10 μM D-*glucose*, (d) 50 μM dihydroquercetin. Bars = 100 μm .

Supplementary Table S1. The differentially expressed genes (sheet 1-4), proteins (sheet 5-8), and phosphoproteins (sheet 9-12) list. A, Xuzhou 142 WT -3 DPA ovules; B, Xuzhou 142 WT 0 DPA ovules; C, Xuzhou 142 *fl* -3 DPA ovules; D, Xuzhou 142 *fl* 0 DPA ovules.

Supplementary Table S2. A complete list of detected genes (sheet 1), proteins (sheet 2), and phosphoproteins (sheet 3).

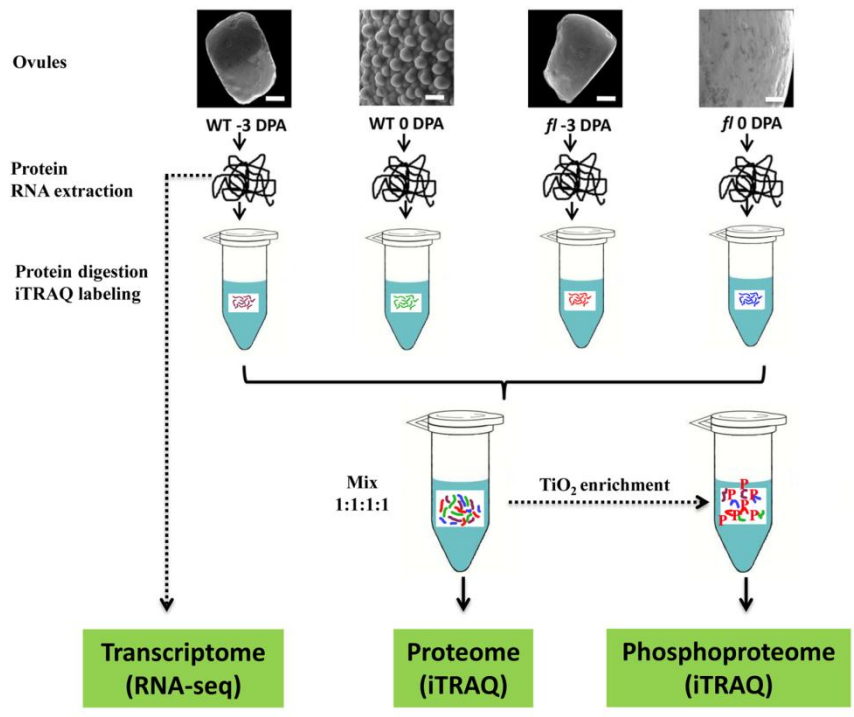
Supplementary Table S3. The previously reported genes, proteins and phosphoproteins.

Supplementary Table S4. Protein-protein interaction network.

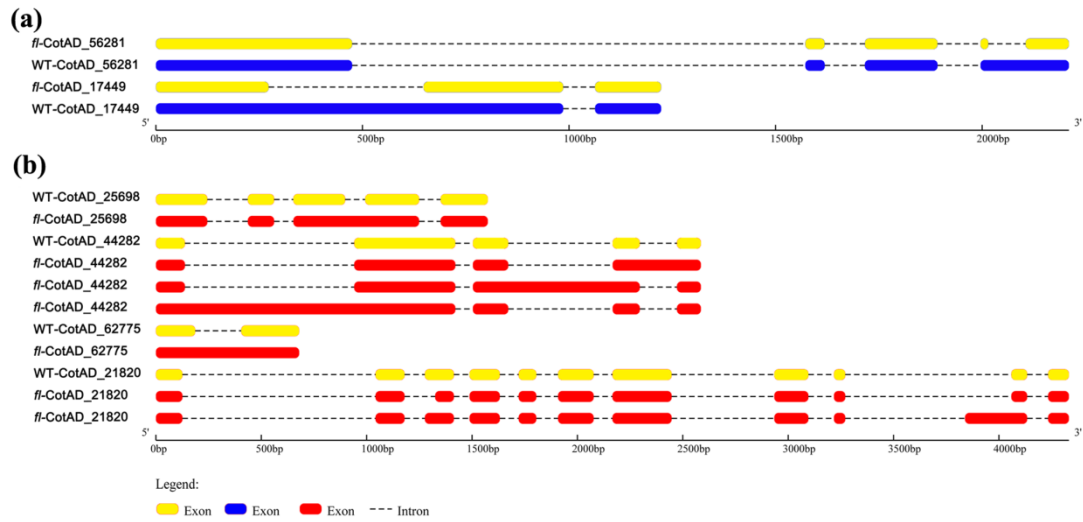
Supplementary Table S5. Phosphorylation motifs distribution between the phosphorylated protein (sheet 1-2) and the differentially phosphorylated protein (sheet 3-4).

Supplementary Table S6. Differentially phosphorylated protein motifs identified in this study.

Supplementary Table S7. The primer sequences list and gene expression profiles following RNA-Seq and qRT-PCR.



Supplementary Fig. S1. The experimental workflow in this study. WT -3 DPA and WT 0 DPA: -3-DPA and 0-DPA ovules from WT. *fl* -3 DPA and *fl* 0 DPA: -3-DPA and 0-DPA ovules from *fl* mutant. Bars: 200 μ m in WT -3 DPA and *fl* -3 DPA; 20 μ m in WT 0 DPA and *fl* 0 DPA.



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CotAD_44282 1 -MLMSRALSRVSKRVGARSLLL---SAPKSFQSPSLSTQFHSLVSESPNKVITNDVCLLQNSNLHWNFRFRFGISSASAP
P3DB-13491 1 -----MEFLSTSMIAA---EAEEYSRSGHNSTHLP-----VQVNFCLCPSINS-----STSKFGFSSASV
P3DB-5227 1 MAAAARLLARISRQGVASAAAARRQAEAAALLGASAGRHLAPPCSSIKALPLLNQPRLYSTS-----TFQRFSGFSSSAP-

CotAD_44282 77 EPSEKEHGSVDN---NGQDPVKPSED---TIPGEAEATKESG-FNSESQNAMPQSDKRRRKAARKRTAFSDSDESDDG
P3DB-13491 54 ETEGKE-GNNVHNGNFTSADPAKTSEEAEEIDQPGQAKSADQTEESGSIADSHSQTVKRRRRSIKRTAFSDSDESSED
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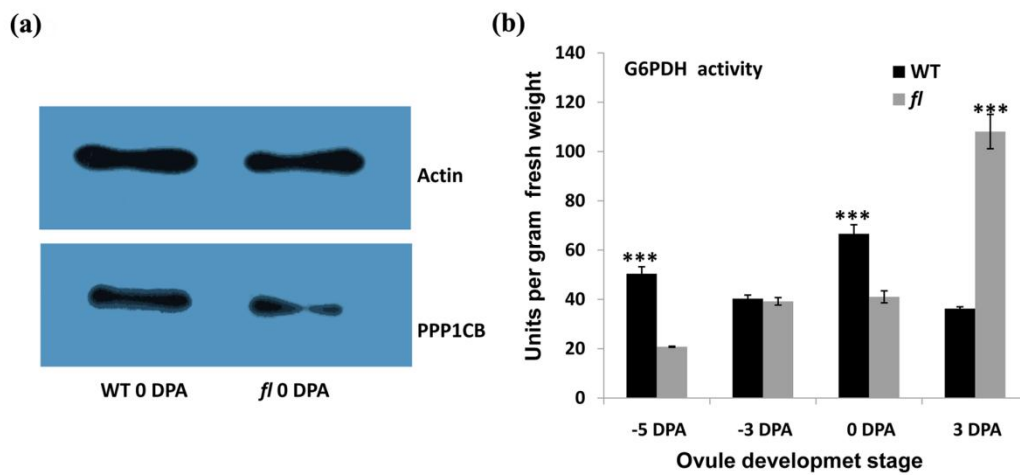
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P3DB-13491 133 LSRDDLKLVAEKEELLKLLKKEIEKMQDKVLRTYAEMENVMDRTRREAENSRKFAIQNFAKSLLDVADNLGRASSVVK
P3DB-5227 144 LSKEDLTKLVLEKEELLKSKDEEVKMDKVLRSYAEMENVIARTKRESNKKYAVQGFSSKSLLDVADNLSRASSVVK

CotAD_44282 229 SFSKIDESND-TAGAVPLLKTLLEGVEMTEKQLGEVFRKFGVEKFDPTNEPFDPHRHNAVFQVPDNSKPPGTVAHVVKAG
P3DB-13491 213 SFSKIDTSSDSSAEAVKLLKTLLEGVEMTEKQLSEALKKFGVEKFDPTNEPFDPHRHNAVFQVPDGSKPPGTVAAVLKAG
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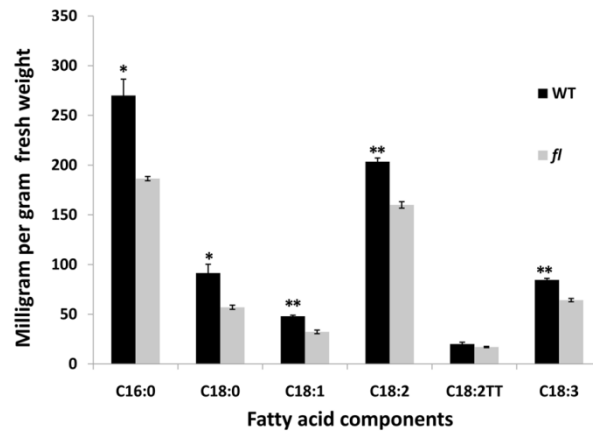
CotAD_44282 308 YMLYDRVIRPAEVEGVTQATDDGATENNTGDKVSDA
P3DB-13491 293 YLLHDRVIRPAEVEGVTQAKENGNSAE-----
P3DB-5227 303 YMLHDRVLRPAEVEGVTTEGGPIEEPEEKSDKSD--

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Supplementary Fig. S3. comparison of phosphosites between CotAD_44282 and homologs in other species.

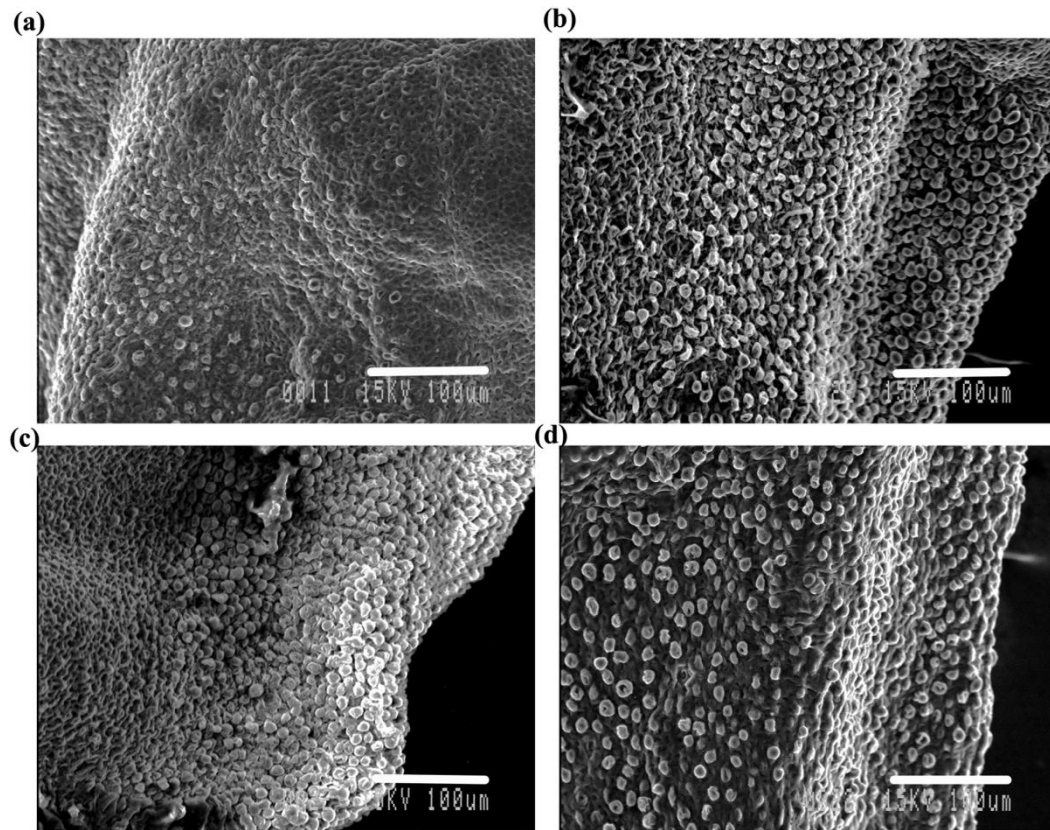


Supplementary Fig. S5. Western blotting and enzymatic assay validation of selected differentially expressed proteins. (a) Western blotting of actin and PPP1CB expression between WT and *fl* at 0 DPA. (b) Enzymatic assay analysis of the G6PDH enzyme. Three biological replicates were performed. The error bars represent SD (student's t test, *** $p < 0.001$, $p = 5.34E-05$ at -5 DPA stage, $p = 0.00057$ at 0 DPA stage, $p = 5.87E-05$ at 3 DPA stage).



Supplementary Fig. S6. Quantification of fatty acids of WT and *fl* ovules at -3 DPA.

(student's t test, *P < 0.05, **P < 0.01, p = 0.011 at C16:0, p = 0.031 at C18:0, p = 0.0028 at C18:1, p = 0.0015 at C18:2, p = 0.0015 at C18:3).



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Supplementary Table S3. The previously reported genes, proteins and phosphoproteins.

Gene list	Type
CotAD_00199	The previously reported gene
CotAD_00236	The previously reported gene
CotAD_00696	The previously reported gene
CotAD_00940	The previously reported gene
CotAD_01748	The previously reported gene
CotAD_02255	The previously reported gene
CotAD_02378	The previously reported gene
CotAD_02433	The previously reported gene
CotAD_02553	The previously reported gene
CotAD_02961	The previously reported gene
CotAD_03497	The previously reported gene
CotAD_04193	The previously reported gene
CotAD_04477	The previously reported gene
CotAD_04628	The previously reported gene
CotAD_04637	The previously reported gene
CotAD_05386	The previously reported gene
CotAD_05977	The previously reported gene
CotAD_06350	The previously reported gene
CotAD_06406	The previously reported gene
CotAD_06986	The previously reported gene
CotAD_07026	The previously reported gene
CotAD_07129	The previously reported gene
CotAD_07491	The previously reported gene
CotAD_07567	The previously reported gene
CotAD_09649	The previously reported gene
CotAD_10182	The previously reported gene
CotAD_10228	The previously reported gene
CotAD_10611	The previously reported gene
CotAD_10936	The previously reported gene
CotAD_11005	The previously reported gene
CotAD_11036	The previously reported gene
CotAD_12715	The previously reported gene
CotAD_12781	The previously reported gene
CotAD_12926	The previously reported gene
CotAD_13206	The previously reported gene
CotAD_13625	The previously reported gene
CotAD_13946	The previously reported gene
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CotAD_14663	The previously reported gene

CotAD_14769	The previously reported gene
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CotAD_15105	The previously reported gene
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CotAD_17026	The previously reported gene
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CotAD_76868	The previously reported gene
CotAD_49525	The previously reported protein
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CotAD_07844	The previously reported protein
CotAD_24342	The previously reported protein
TC251823	The previously reported protein
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CotAD_42608	The previously reported protein
CotAD_51639	The previously reported protein
Cotton_D_gene_10017826	The previously reported protein
CotAD_21698	The previously reported protein
Cotton_A_11375	The previously reported protein
CotAD_09528	The previously reported protein
Cotton_D_gene_10008766	The previously reported protein
EV482881	The previously reported protein
CotAD_05159	The previously reported phosphoprotein
CotAD_07377	The previously reported phosphoprotein
CotAD_07810	The previously reported phosphoprotein
CotAD_09667	The previously reported phosphoprotein
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Cotton_A_19569	The previously reported phosphoprotein
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Cotton_A_29925	The previously reported phosphoprotein
Cotton_A_38233	The previously reported phosphoprotein
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Cotton_D_gene_10014636	The previously reported phosphoprotein
Cotton_D_gene_10018388	The previously reported phosphoprotein
Cotton_D_gene_10026593	The previously reported phosphoprotein
Cotton_D_gene_10027878	The previously reported phosphoprotein
Cotton_D_gene_10028753	The previously reported phosphoprotein
Cotton_D_gene_10035684	The previously reported phosphoprotein
Cotton_D_gene_10040894	The previously reported phosphoprotein
TC229918	The previously reported phosphoprotein
Cotton_A_19478	The previously reported phosphoprotein
Cotton_A_03590	The previously reported phosphoprotein
CotAD_12923	The previously reported phosphoprotein
TC247099	The previously reported phosphoprotein
CotAD_44734	The previously reported phosphoprotein
TC271223	The previously reported phosphoprotein
CotAD_52648	The previously reported phosphoprotein
CotAD_74126	The previously reported phosphoprotein
Cotton_A_12625	The previously reported phosphoprotein
CotAD_38392	The previously reported phosphoprotein
CotAD_51219	The previously reported phosphoprotein
CotAD_18320	The previously reported phosphoprotein
CotAD_40997	The previously reported phosphoprotein
TC233322	The previously reported phosphoprotein
CotAD_50655	The previously reported phosphoprotein

Supplementary Table S6. Differentially phosphorylated protein motifs identified in this study.

NO	Motif	Special for DEPs or not	Found in other species	Motif pattern	Motif category
1SP.....	No	Yes	sP	Pro-directed
2DSD.....	No	Yes	[D/E]s	Acidic
3SD.E...	No	Yes	sXX[D/E]	Acidic
4S.SD...	Special	Novel	sXX[D/E]	Acidic
5DS.....	Special	Yes	[D/E]s	Acidic
6S.D....	No	Yes	sX[D/E]	Acidic
7SD.....	No	Novel	s[D/E]	Acidic
8	...R..S.....	No	Yes	RXXs	Basic
9SE.....	No	Yes	s[D/E]	Acidic
10TP...P.	No	Novel	Unclear	Unclear
11TP.....	No	Yes	Unclear	Unclear
12TD.....	No	Novel	Unclear	Unclear
13TSD....	No	Yes	Unclear	Unclear
14	...S..T.....	Special	Yes	Unclear	Unclear

Supplementary Table S7. The primer sequences list and gene expression profiles following RNA-Seq and qRT-PCR.

Gene list	Forward	Reverse	RNA-Seq(Expression)		qRT-PCR (Expression)	
			-3_WT:-3_fl	0_WT:0_fl	-3_WT:-3_fl	0_WT:0_fl
CotAD_35784	AGACTCGCCATTGCTGCTC	ATAATCCGGCCACAAACCA	3.05	4.2	2.5	9.65
CotAD_60970	ACTTGCCTTACGGGATTGA	CCAGTTGCTGTCCGGTCTC	8.3	9.3	3.5	6.87
CotAD_68165	CATCAGTGGAGCAAAGGGTC	GGTAGGAGCAATGGCAGGA	2.01	2.3	2.6	6.72
CotAD_13206	GGTATTCGGACACGTTAATG	AAGACAGAAGAACCAGATGG		4.17		5.5
CotAD_52285	ATAGCGAAACCGACTACCC	GCTGAGCAACGGCACATAA		2.38		4.26
CotAD_07889	GACCCAACAGTTCCCAACC	GGACACCTTCCCATCTTTC	1.37	1.22	1.67	4
CotAD_42040	TAACCCGAGTTTACGAGGAT	AGGCGAGTGAAGTTCTACCAT	-3.1	-2.69	0.25	0.429
CotAD_12714	TTGCTGCTCAATTCTTGGA	AGGACTTGCTTCACTAGGA	-2.6		0.12	
CotAD_22057	GCAGGATGTTGAAGATGG	CCCAATTAAGAGAAAGAGGA	-1.59		0.58	