

## Supplementary Information

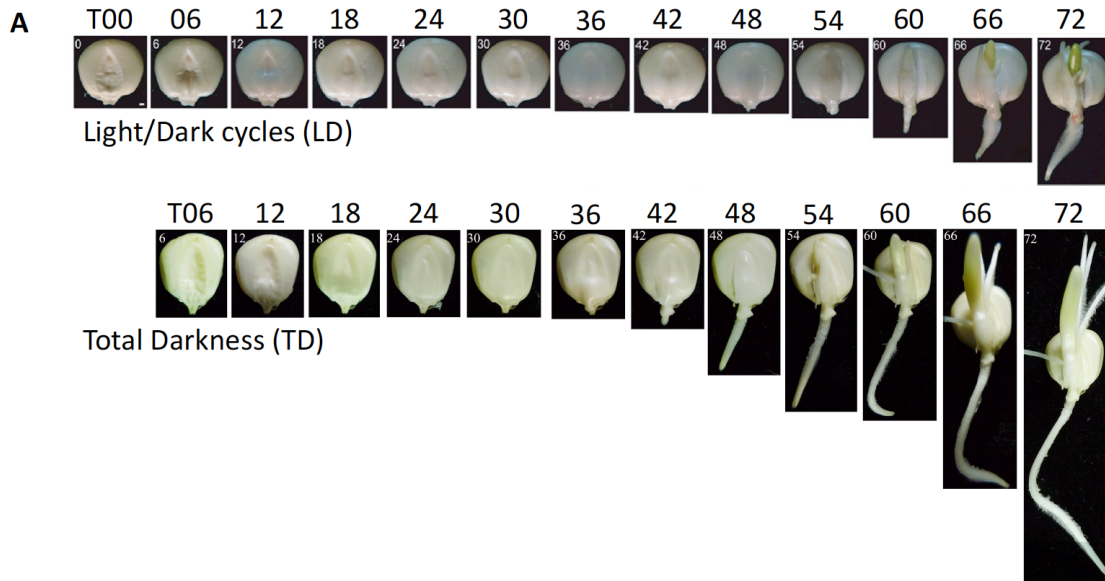
A Comparative Transcriptomics Method to Infer Gene Coexpression Networks and its applications to Maize and Rice Leaf Transcriptomes

Chang et al.

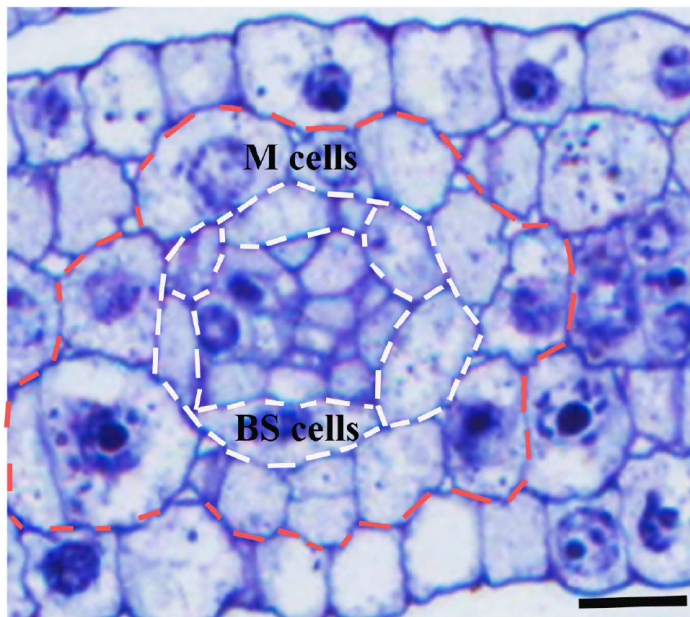
## Supplementary figures and tables

Supplementary Figure S1	Time series of maize seed germination and seedling development
Supplementary Figure S2	Examples of gene expression profiles over the time-series transcriptomes under two conditions
Supplementary Figure S3	The distributions of PCC values between TF genes over the time-series RPKM values under LD and under TD
Supplementary Figure S4	TF-TF gene coexpression networks with LD+TD+ coexpression relationships
Supplementary Figure S5	The predicted TFBSs of four upstream regulators of <i>ZmSHR1</i>
Supplementary Figure S6	EMSA validation of predicted upstream regulators of the C <sub>4</sub> enzyme genes
Supplementary Figure S7	Expression profiles of four TF genes in maize foliar (C <sub>4</sub> ) and husk (C <sub>3</sub> -type) embryonic leaves.
Supplementary Table S1	Read count statistics of the RNA sequencing data for the 12 time points under total darkness (TD). The data were obtained using Illumina HiSeq2000 with the paired-end 2*101nt format
Supplementary Table S2	Numbers of genes and TF-gene pairs in eight expression correlation types under LD and TD growth conditions
Supplementary Table S3	First to fourth order upstream TF candidates of <i>ZmSHR1</i>
Supplementary Table S4	Sequences of gene cloning primers for protoplast transient assay (PTA)
Supplementary Table S5	Statistics of level order changes with different initial seeds
Supplementary Table S6	Sequences of qRT-PCR primers
Supplementary Table S7	EMSA primer sequences for regulator-target pairs in the upstream regulatory pathway of <i>ZmSHR1</i>
Supplementary Table S8	Predicted TFBSs with cross-species conservation
Supplementary Table S9	Sequences of the cloning primers for predicted regulator genes of C <sub>4</sub> enzyme genes
Supplementary Table S10	EMSA primer sequences for TF genes and their target C <sub>4</sub> enzyme genes

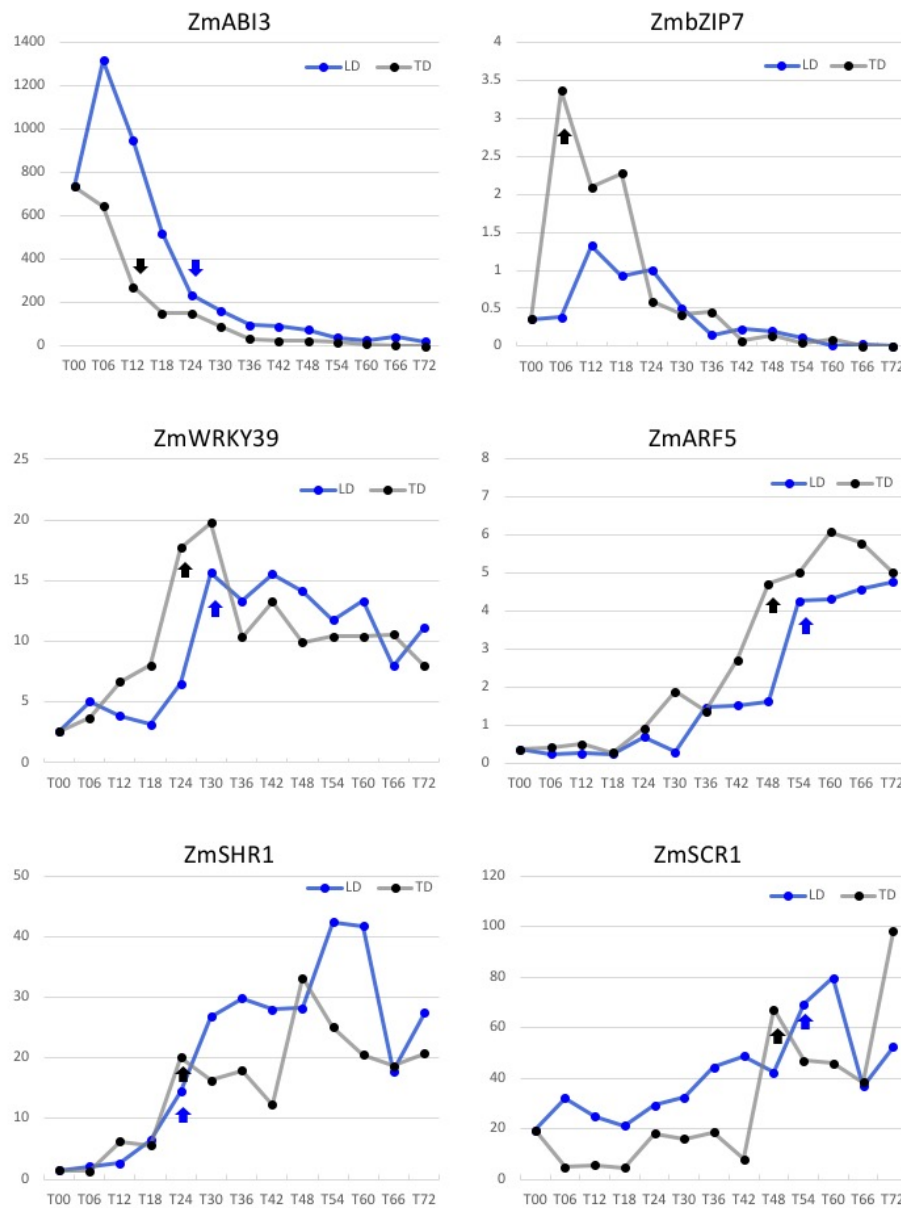
## Supplementary figures



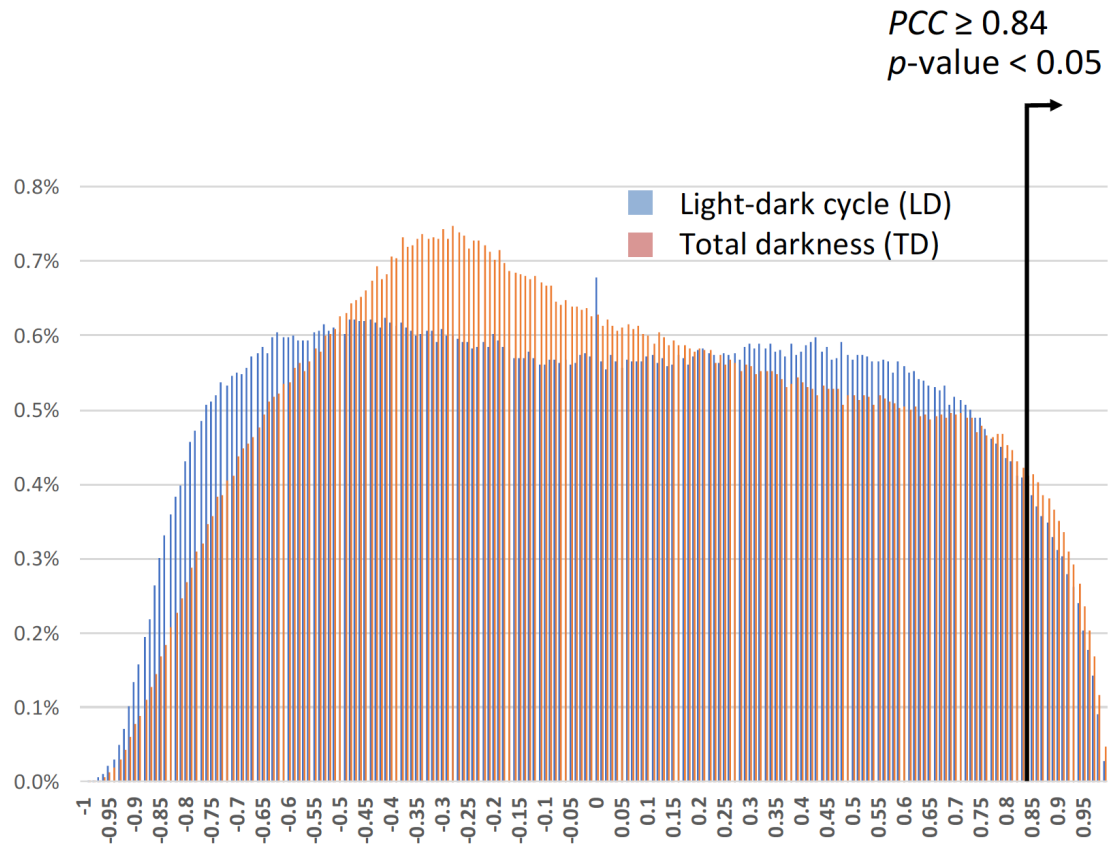
**B**



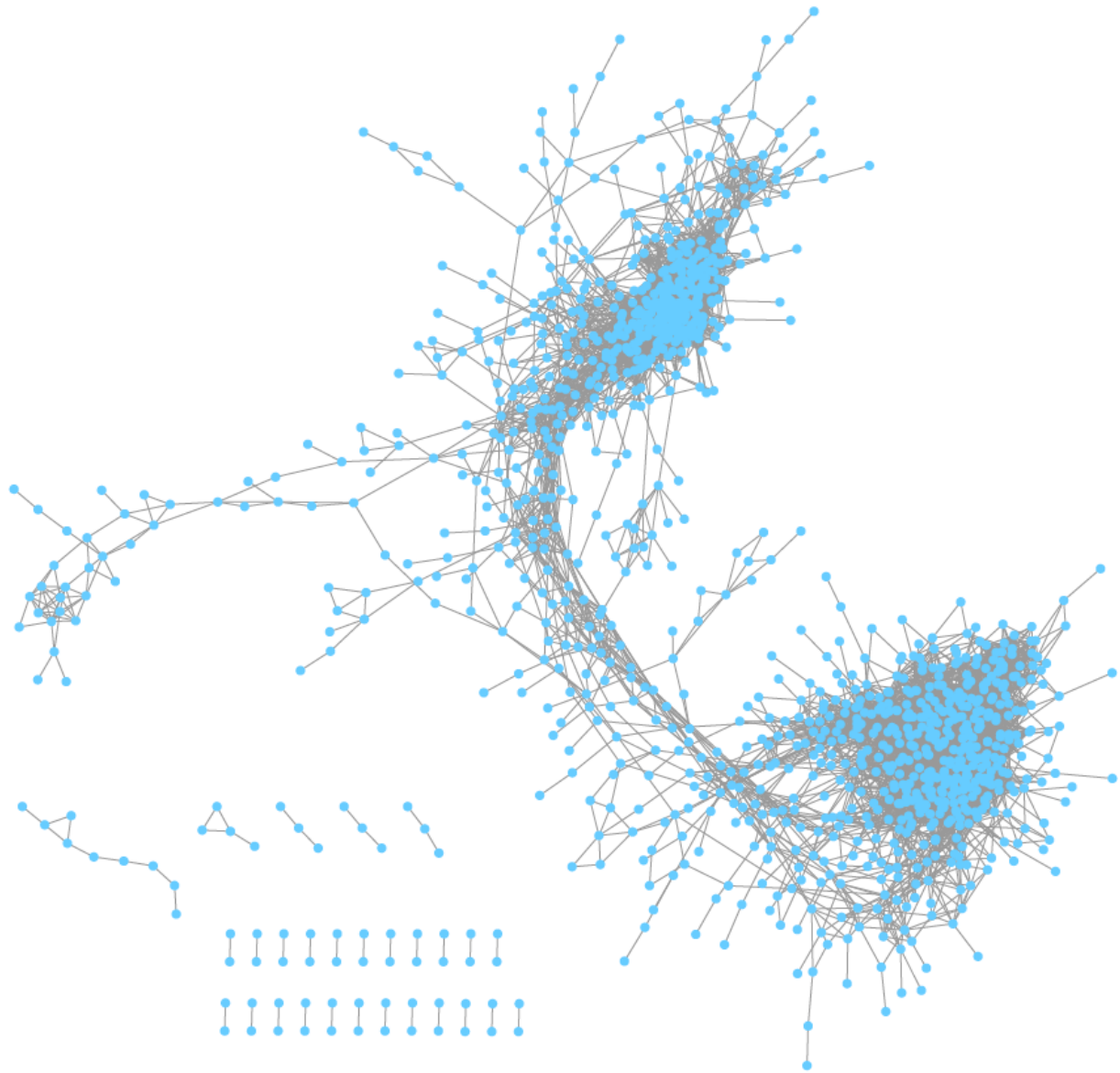
**Supplementary Figure S1.** Time series of maize seed germination and seedling development. (A) Morphologies of seed and germinating seedling from 0 h (T00, dry seed) to 72 h (T72) post imbibition under the natural light/dark (LD) cycle or under total darkness (TD). Scale bars: 1 mm. (B) Cross section of a second order vein under total darkness at 72 h (T72). The Kranz anatomy is shown with one layer of bundle sheath (BS) cells and one layer of mesophyll (M) cells. Scale bar: 10  $\mu$ m.



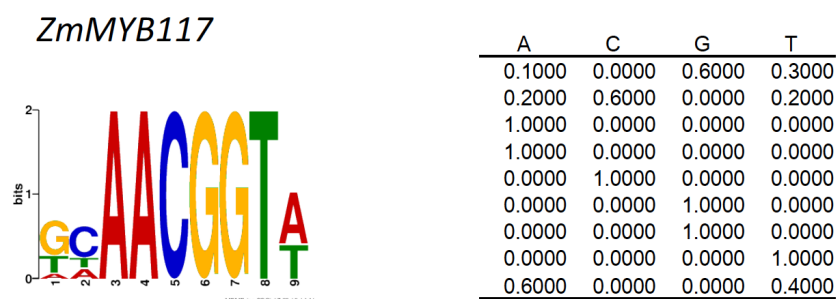
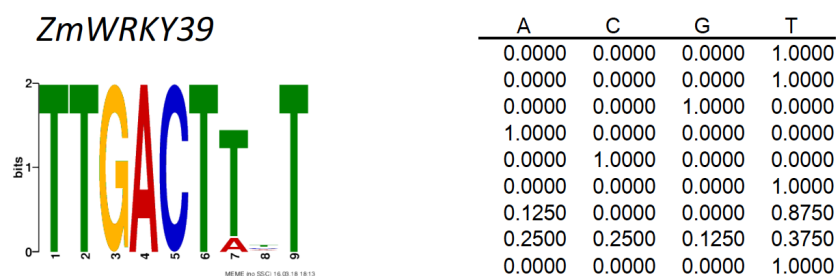
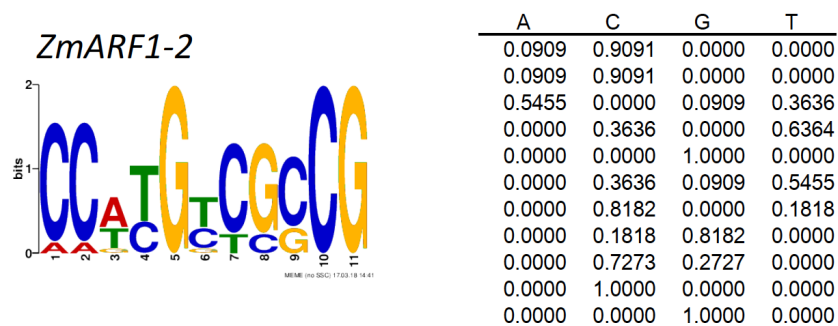
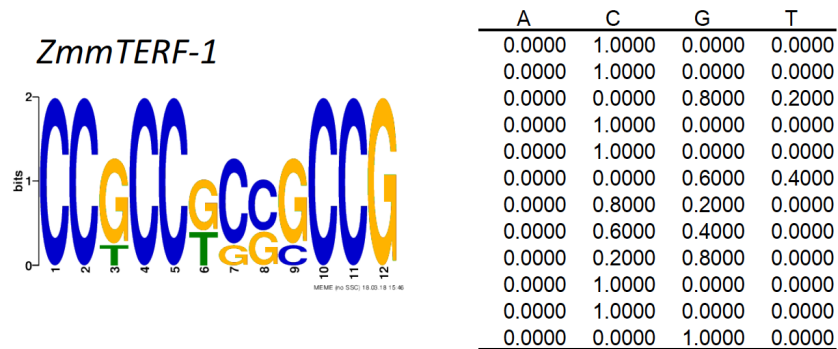
**Supplementary Figure S2.** Examples of gene expression profiles over the time-series transcriptomes under two conditions. Six gene expression profiles are shown over the time course from T00 to T72 (x-axis) under LD (blue line) and under TD (black line); the six TFs are *ZmABI3*, *ZmbZIP7*, *ZmWRKY39*, *ZmARF5*, *ZmSHR1*, and *ZmSCR1*. The up- and down-regulated time points of a gene are indicated by arrows (blue for LD and black for TD).



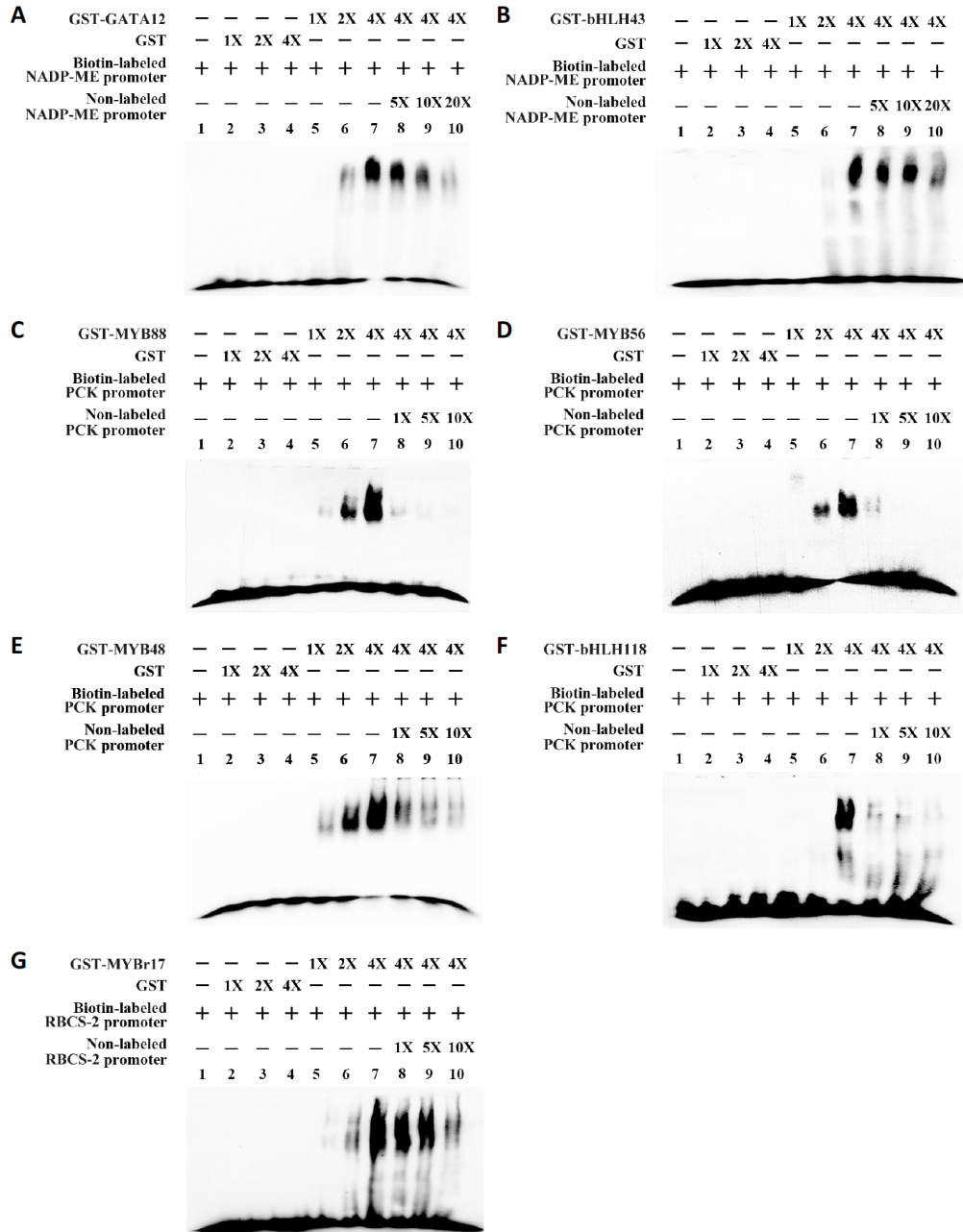
**Supplementary Figure S3.** The distributions of PCC values between TF genes over the time-series RPKM values under LD (blue bars) and under TD (red bars). The cutoff is set to 0.84 with  $p < 0.05$ .



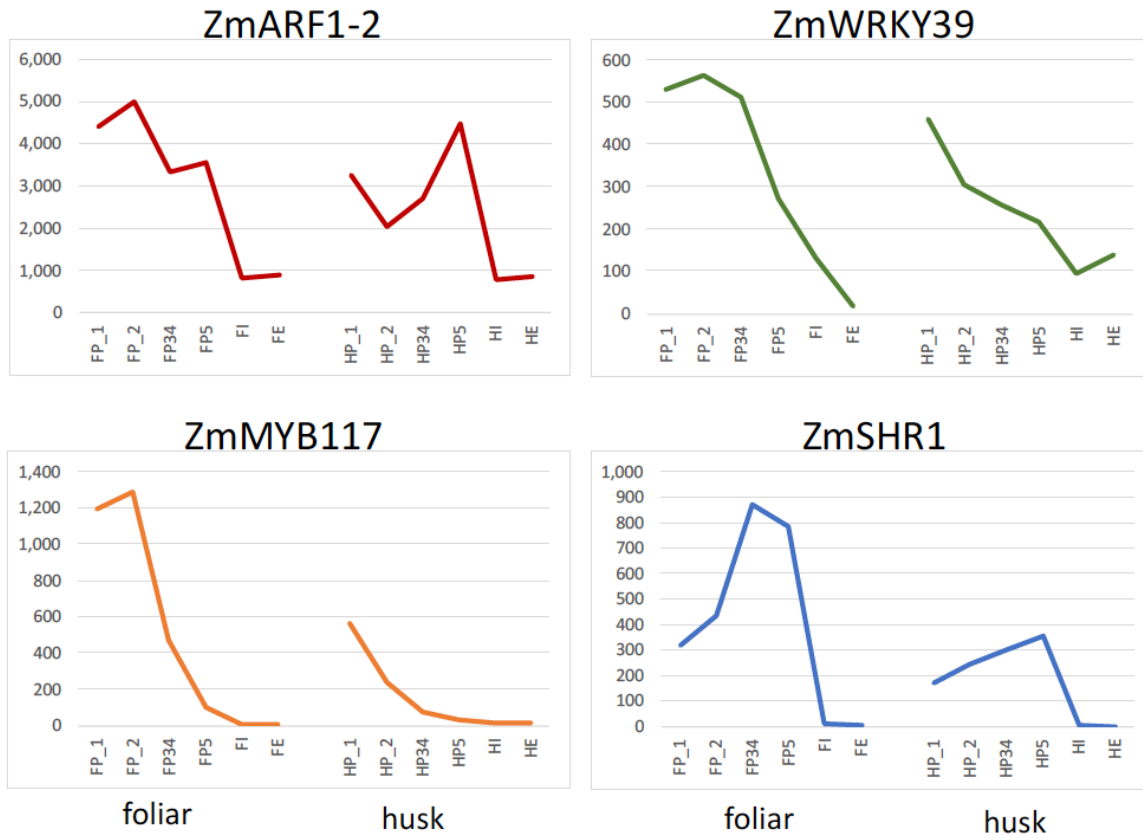
**Supplementary Figure S4.** TF-TF gene coexpression networks with LD+TD+ coexpression relationships (1,275 nodes and 16,281 edges). Each blue node and grey edge in the GCNs represent a TF gene and a coexpression relationship, respectively. The GCNs are visualized using the Cytoscape tool (<http://cytoscape.org>).



**Supplementary Figure S5.** The predicted TFBSs of four upstream regulators of *ZmSHR1*. The four regulators are *ZmmTERF-1*, *ZmARF1-2*, *ZmWRKY39*, and *ZmMYB117* and in each case the motif logo and position weight matrix are shown in the left and right hand panel, respectively.



**Supplementary Figure S6.** EMSA validation of predicted upstream regulators of the  $C_4$  enzyme genes. The regulators tested were ZmGATA12, ZmbHLH43, ZmMYB88, ZmMYB56, ZmMYB48, ZmbHLH118, and ZmMYBr17. GST- ZmGATA12 (A), GST- ZmbHLH43 (B), GST- ZmMYB88 (C), GST- ZmMYB56 (D) and GST- ZmMYB48 (E), GST- ZmbHLH118 (F), GST- ZmMYBr17 (G), were isolated from *E. coli* and were separately incubated with probes corresponding to the predicted TF binding site in the *ZmNADP-ME* (A and B), *ZmPCK* (C-F), and *ZmRbcS2* (G) promoters, respectively. In each case EMSA was conducted with the biotin-labeled probe alone (lane 1), with increasing amounts of purified GST (lanes 2-4) or with GST-TF (lanes 5-7) and the biotin-labeled probe. The DNA-binding specificity of a TF was tested by adding excess of the unlabeled probe (lanes 8-10).



**Supplementary Figure S7.** Expression profiles of four TF genes in maize foliar (C<sub>4</sub>) and husk (C<sub>3</sub>-type) embryonic leaves. The analysis used the data from Wang et al. (1).



## Supplementary tables

**Supplementary Table S1.** Read count statistics of the RNA sequencing data for the 12 time points under total darkness (TD). The data were obtained using Illumina HiSeq2000 with the paired-end 2\*101nt format.

Sample (TD)	Quality filtering		Mappable reads	
	Total reads	Rate (%)	Total reads	Rate (%)
T06	622,278,182	92.8	502,136,866	80.7
T12	631,190,482	92.9	529,141,296	83.8
T18	654,440,380	92.6	550,918,931	84.2
T24	507,710,021	94.3	422,194,745	83.2
T30	564,950,902	91.6	477,864,256	84.6
T36	520,170,828	94.0	441,941,333	85.0
T42	589,910,974	92.7	499,591,439	84.7
T48	441,648,581	95.1	370,185,791	83.8
T54	478,353,359	95.0	403,373,361	84.3
T60	619,109,440	93.0	524,564,651	84.7
T66	594,676,837	93.7	500,542,333	84.2
T72	732,667,006	91.9	621,034,074	84.8

**Supplementary Table S2.** Numbers of genes and TF-gene pairs in eight expression correlation types under LD and TD growth conditions.

	Coexpression type							
	LD+TD+	LD+TD0	LD0TD+	LD0TD-	LD-TD0	LD-TD-	LD+TD-	LD-TD+
<b># genes</b>	17,457	22,227	22,645	23,096	22,460	15,874	294	1,384
<b>(# TF genes)</b>	(1,538)	(1,698)	(1,702)	(1,703)	(1,654)	(1,398)	(102)	(329)
<b># TF-gene pairs</b>	431,974	547,762	671,020	546,822	704,620	539,624	239	3,710
<b>(#TF-TF pairs)</b>	(16,281)	(18,984)	(25,907)	(14,394)	(29,727)	(15,951)	(9)	(160)

Note that the coexpression type of LD0TD0 means no coexpression under both conditions, so we exclude it in this study.

**Supplementary Table S3.** First to fourth order upstream TF candidates of *ZmSHR1*.

Gene ID AGPv4	TF family	TO-GCN level	Order of candidate	Gene name	TFBS ID
Zm00001d043969	GATA	11	1st	<i>GATA20</i>	C2C2gata_tnt.GATA19_colamp_a_m1
Zm00001d020037	C2H2	11	1st	<i>DOT5</i>	C2H2_tnt.WIP5_colamp_a_m1
Zm00001d012731	ARF	11	1st	<i>ARF3-2</i>	ETT_TGTCGGAA
Zm00001d037117	GRF	11	1st	<i>GRF5</i>	GRF_tnt.AtGRF6_col_a_m1
Zm00001d022295	HSF	11	1st	<i>HSFB4</i>	HSF_tnt.HSFB4_col_a_m1
Zm00001d049443	HD-ZIP	11	1st	<i>ML1</i>	M0852_1.02
Zm00001d034204	AP2	11	1st	<i>ANT1</i>	M2364_1.02
Zm00001d052256	TCP	11	1st	<i>TCP14</i>	MA1063.1
Zm00001d025669	WRKY	11	1st	<i>WRKY2</i>	MA1078.1
Zm00001d048493	NAC	11	1st	<i>NAC086</i>	NAC_tnt.ANAC028_col_a_m1
Zm00001d031463	NAC	11	1st	<i>CUC3</i>	NAC_tnt.CUC3_col_a_m1
Zm00001d022613	bZIP	11	1st	<i>FD</i>	pTH7315_AREB2_AT3G19290
Zm00001d002075	AP2	11	1st	<i>AP2</i>	TOE1_CCTCGTAC
Zm00001d002829	YABBY	11	1st	<i>YAB3</i>	<b>unknown</b>
Zm00001d004889	WOX	11	1st	<i>WOX3</i>	<b>unknown</b>
Zm00001d017784	ZF-HD	11	1st	<i>ZFHD5</i>	<b>unknown</b>
Zm00001d025944	YABBY	11	1st	<i>YAB1</i>	<b>unknown</b>
Zm00001d027991	HD-ZIP	11	1st	<i>HB6</i>	<b>unknown</b>
Zm00001d028371	C2H2	11	1st	<i>ZFP4</i>	<b>unknown</b>
Zm00001d036356	MYB-related	11	1st	<i>SANT1</i>	<b>unknown</b>
Zm00001d040186	bHLH	11	1st	<i>bHLH110</i>	<b>unknown</b>
Zm00001d042588	ERF	11	1st	<i>CRF3</i>	<b>unknown</b>
Zm00001d048309	bHLH	11	1st	<i>bHLH</i>	<b>unknown</b>
Zm00001d051471	B3	11	1st	<i>NGA2-3</i>	<b>unknown</b>
Zm00001d002794	WRKY	11	1st	<i>WRKY12</i>	WRKY12_CGTTGACC
Zm00001d002562	B3	10	1st	<i>NGA2-1</i>	ABI3VP1_tnt.NGA4_col_a_m1
Zm00001d017618	B3	10	1st	<i>NGA2-2</i>	ABI3VP1_tnt.NGA4_col_a_m1
Zm00001d013699	HD-ZIP	10	1st	<i>HB14</i>	HB_tnt.ATHB15_col_a_m1
Zm00001d041489	HD-ZIP	10	1st	<i>PHB</i>	M2366_1.02
Zm00001d032194	MYB/MYB-related	10	1st	<i>MYB117</i>	MYB_tnt.MYB105_col_a_m1
Zm00001d014814	bHLH	10	1st	<i>bHLH93</i>	<b>unknown</b>
Zm00001d020774	ZF-HD	10	1st	<i>ZHD1-2</i>	ZFHD_tnt.ATHB25_col_a_m1
Zm00001d015243	ARF	10	2nd	<i>ARF20</i>	ARF_ecoli.MP_col_m1
Zm00001d034163	Dof	10	2nd	<i>OBP2</i>	Dof5.7_ACGTTAAC
Zm00001d039006	ARF/B3	10	2nd	<i>ARF3-1</i>	ETT_TGTCGGAA

Zm00001d002005	SBP	10	2nd	<i>SPL8</i>	M2371_1.02
Zm00001d001879	ARF	10	2nd	<i>ARF8</i>	MA0944.1
Zm00001d012067	bHLH	10	2nd	<i>bHLH62</i>	pTH7309_AT1G68920_AT1G68920
Zm00001d017350	TCP	10	2nd	<i>TCP21</i>	TCP_tnt.At5g08330_colamp_a_m1
Zm00001d021236	HD-ZIP	9	2nd	<i>ANL2</i>	HB_tnt.ANL2_colamp_a_m1
Zm00001d051527	G2-like	9	2nd	<i>KAN2-2</i>	KAN1_CGAATAT.T
Zm00001d030727	Dof	9	2nd	<i>DOF5.1-1</i>	M2712_1.02
Zm00001d026590	ARF	9	2nd	<i>ARF6</i>	MA0944.1
Zm00001d041866	TCP	9	2nd	<i>TCP8-2</i>	MA1063.1
Zm00001d011953	ARF	9	2nd	<i>ARF2-2</i>	ARF_tnt.ARF2_col_v31_m1
Zm00001d039439	BES1	9	2nd	<i>BEH4</i>	BZR_tnt.At4g18890_colamp_a_m1
Zm00001d017702	G2-like	9	2nd	<i>KAN2-1</i>	KAN1_CGAATAT.T
Zm00001d039907	RAV	9	2nd	<i>RAV1</i>	M0013_1.02
Zm00001d006110	bHLH	9	2nd	<i>bHLH137</i>	M0166_1.02
Zm00001d041549	Dof	9	2nd	<i>DOF5.1-2</i>	M2712_1.02
Zm00001d033965	WRKY	9	2nd	<i>WRKY39-2</i>	WRKY_tnt.WRKY7_colamp_a_m1
Zm00001d023565	TCP	8	3rd	<i>TCP8-1</i>	MA1063.1
Zm00001d049824	SBP	8	3rd	<i>SPL13B-2</i>	SBP_tnt.SPL13_col_a_m1
Zm00001d021285	SRS	8	3rd	<i>SRS3</i>	<b>unknown</b>
Zm00001d013307	WRKY	8	3rd	<i>WRKY39</i>	WRKY_tnt.WRKY7_colamp_a_m1
Zm00001d003601	ARF	8	4th	<i>ARF1-2</i>	ARF_tnt.ARF2_col_v31_m1
Zm00001d038698	ARF/B3	8	4th	<i>ETT-1</i>	ETT_TGTCGGAA
Zm00001d015585	mTERF	8	4th	<i>mTERF-2</i>	<b>unknown</b>
Zm00001d024468	AP2/ERF	8	4th	<i>AIL1</i>	<b>unknown</b>
Zm00001d038158	mTERF	8	4th	<i>mTERF-3</i>	<b>unknown</b>
Zm00001d050781	ARF	7	4th	<i>ARF1-1</i>	MA0942.1
Zm00001d012015	SBP	7	4th	<i>SPL13B-1</i>	SBP_tnt.SPL13_col_a_m1
Zm00001d031533	mTERF	7	4th	<i>mTERF-1</i>	<b>unknown</b>
Zm00001d005931	ZF-HD	7	4th	<i>ZHD1-1</i>	ZFHD_tnt.ATHB25_col_a_m1

**Supplementary Table S4.** Sequences of gene cloning primers for protoplast transient assay (PTA).

Gene ID	Level in the TO-GCN: gene name	Forward primer	Reverse primer
Zm00001d003601	L8: ZmARF1-2	GAATTCATGGACGCGCC CAACCCT	GAGCTCTCATTGAGACA GTGAGTTCGCAGC
Zm00001d013307	L8: ZmWRKY39	GAATTCATGGAGGCGA TGGAGGAGG	AAGCTTCAGGCTGGCTG GGCTAGAA
Zm00001d032194	L10: ZmMYB117	GAATTCATGCCGTGCTC GTCGCC	AAGCTTTCATGTGGCTC CGACCCC
Control	GFP	ATGGTGAGCAAGGGCG AGGAG	TACTTGACAGCTCGTC CATGC

**Supplementary Table S5.** Statistics of level order changes with different initial seeds

TF gene ID of initial seed	TF family	Gene name	Proportion of TFs with level change	Mean of level change <sup>a</sup>	STDEV of level change
Zm00001d013074	bZIP	ZmbZIP25	16%	-0.06	0.39
Zm00001d018941	HSF	ZmHSFA6B	10%	-0.05	0.32
Zm00001d040775	GATA	ZmGATA16	11%	-0.11	0.32
Zm00001d046974	MYB_related	ZmRVE6	13%	-0.12	0.34
Zm00001d050242	NF-YB	ZmNF-YB12	8%	-0.07	0.28
Zm00001d013202	G2-like	ZmHHO5	17%	-0.12	0.39
Zm00001d030907	B3	ZmABI46	9%	-0.09	0.29
Zm00001d043992	bZIP	ZmGBF3	14%	-0.06	0.38
Zm00001d001837	MYB	ZmMYB133	13%	-0.08	0.34
Zm00001d031723	bZIP	ZmbZIP127	15%	-0.12	0.37
Zm00001d007339	C2H2		10%	0.04	0.32

<sup>a</sup> For each node, the positive and negative number represent its level changes to lower or higher level, respectively, in the new TO-GCN against the original one. Zero represents no change between two TO-GCN. For example, a node belongs to level 5 in the original TO-GCN and changes to level 4 in the new one then the level change number is -1.

**Supplementary Table S6.** Sequences of qRT-PCR primers.

Gene ID	Level in the TO-GCN: gene name	Forward primer	Reverse primer
Zm00001d021973	L11: ZmSHR1	CCAACGCATCCTAC AACTAC	TGTTGCCGCATTCTTCTT
Zm00001d032194	L10: ZmMYB117	CCTTTGAAGGGACG ACAAGA	AAGCTTTCATGTGGCTC CGACCCC
Zm00001d013307	L8: ZmWRKY39	GAGATGGTTAAGAG GAGCAATC	ATGTCTGAGAGCCACTA ACT
Control	GFP	AAGCAGCACGACTT CTTC	CACCTTGATGCCGTTCTT
Control	ZmACT	CTGAGGTTCTATTC CAGCCATCC	CCACCACTGAGGACAAC ATTACC

**Supplementary Table S7.** EMSA primer sequences for regulator-target pairs in the upstream regulatory pathway of *ZmSHR1*.

TF Level: Gene name	Target gene Level in the TO-GCN: Gene name	Probe	
		Forward primer	Reverse primer
L8: ZmARF1-2	L8: ZmWRKY39	ACGCTCGCTCGTCGT CACCACCA	ATGGTGGTGACGACG AGCGAGCG
L8: ZmWRKY39	L10: ZmMYB117	ATGGCCAGTCAAGC GTTGCAGAG	ACTCTGCAACGCTTG ACTGGCCA
L10: ZmMYB117	L11: ZmSHR1	AATCCGAACCATTC CTTAG	AACTAAGGAATGGTT CGGA

**Supplementary Table S8.** Predicted TFBSs with cross-species conservation.

Target C4 enzyme	Regulator gene name	Arabidopsis (DBD identity %) <sup>a</sup>	Motif ID	Conservation <sup>b</sup>
BS: ZmNADP-ME	ZmGATA12	AT5G49300 (70%)	C2C2gata_tnt.GATA16_col_a_m1	1
BS: ZmNADP-ME	ZmbHLH43	AT1G09530 (79%)	M2863_1.02	3
BS: ZmNADP-ME	ZmERF	AT1G36060 (86%)	AP2EREBP_tnt.At1g36060_col_a_m1	4
BS: ZmNADP-ME	ZmNAC	AT5G18270 (73%)	NAC_tnt.ANAC087_col_v3_a_m1	2
BS: ZmPCK	ZmMYB48	AT5G59780 (86%)	MYB59_GTTAGGTA	1
BS: ZmPCK	ZmMYB88	AT5G59780 (83%)	MYB59_GTTAGGTA	1
BS: ZmPCK	ZmMYB56	AT5G59780 (86%)	MYB59_GTTAGGTA	1
BS: ZmPCK	ZmbHLH118	AT5G54680 (71%)	M0167_1.02	2
BS: ZmRbcS2	ZmMYBr17	AT5G47390 (82%)	MYBrelated_tnt.At5g47390_colamp_a_m1	3
M: ZmCA	ZmABI33	AT1G13260 (73%)	RAV_tnt.RAV1_colamp_a_m1	1
M: ZmCA	ZmRAV	AT1G13260 (77%)	RAV_tnt.RAV1_colamp_a_m1	1
M: ZmPEPC	ZmRAV	AT1G13260 (77%)	RAV_tnt.RAV1_colamp_a_m1	1

<sup>a</sup> DNA-binding domain (DBD) sequence identity between a maize TF and its best homologous Arabidopsis TF

<sup>b</sup> Four reference species were used, including *Brachypodium distachyon*, *Oryza sativa*, *Sorghum bicolor*, and *Setaria italica*. The number refers to the number of species in which the TFBS was observed.

**Supplementary Table S9.** Sequences of the cloning primers for predicted regulator genes of C<sub>4</sub> enzyme genes.

Regulator Gene ID	Clone ID	Regulator gene name	Forward cloning primer	Reverse cloning primer
Zm00001d037605	PUT6147	ZmGATA12	GAATTCATGATGATGG ATTCCGCGGA	AAGCTTCTAGCCATG TAGCAGCGTGGATCT
Zm00001d033267	PUT4131	ZmbHLH43	GAATTCATGAACCAGTT CGTGCCGGA	AAGCTTTCATCTAAG AGCGCCCATCTGCAG
Zm00001d048623	PUT5992	ZmMYB88	GAATTCATGGTGACTGT GAGAGAGGAGATGC	AAGCTTCCCACCCTTT CACATCATGATTTTC
Zm00001d030678	PUT5678	ZmMYB56	GAATTCCTTCACCAT GGTGACAGTGAG	AAGCTTTCAGAAGCA GGGCCAACTCCTTC
Zm00001d038357	PUT3433	ZmbHLH118	GAATTCCTTCACCATGAG CTGCGCGGG	CTCGAGACCCTTCTAG GCGACCGGCGG
Zm00001d044409	PUT3161	ZmMYBR17	GAATTCATGGCTAGGA AGTGCTCACACTGTG	AAGCTTTCATGTTCGG ATGGATGGGGCA

**Supplementary Table S10.** EMSA primer sequences for TF genes and their target C<sub>4</sub> enzyme genes.

TF gene name	Target gene name	Target gene ID	Forward EMSA primer	Reverse EMSA primer
ZmGATA12	ZmNADP-ME	Zm00001d000316	ACATCATCACCAG CAACATATAAAG	TATCTTTATATGTT GCTGGTGATGA
ZmbHLH43	ZmNADP-ME	Zm00001d000316	AACGTCACACGTG AAAAGC	AAGCTTTTCACGTG TGACG
ZmMYB88	ZmPCK	Zm00001d028471	AAATGACCGAACA TCAG	AACTGATGTTCGGT CAT
ZmMYB56	ZmPCK	Zm00001d028471	AAATGACCGAACA TCAG	AACTGATGTTCGGT CAT
ZmbHLH118	ZmPCK	Zm00001d028471	AATAACACGTGAG TAGGG	AACCCTACTCACGT GTTA
ZmMYBR17	ZmRbcS2	Zm00001d004894	AATCAGGATAGGC CAGGC	AAGCCTGGCCTATC CTGA

1. Wang P, Kelly S, Fouracre JP, & Langdale JA (2013) Genome-wide transcript analysis of early maize leaf development reveals gene cohorts associated with the differentiation of C<sub>4</sub> Kranz anatomy. *Plant J* 75(4):656-670.