



Supplementary Materials: Dark-induced senescence protein Tic55 involved in MYB108 linked ANAC signaling pathway in *Arabidopsis thaliana*

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Table S1. Primers used in this study.

Target genes	Primer name	Oligo sequence (5' to 3') ¹	Purpose
<i>atTic55</i>	atTic55-RT-F	GAGCATGCATAGTCCCAATTCCGCCTT	Semi qRT-PCR forward primer
	atTic55-RT-R	GAGAATCTGATGGATCCTGCTCATGTT	Semi qRT-PCR reverse primer
<i>atActin 2</i>	ACT2-RT-F	ACATTGCAAAGAGTTTCAAGGT	Semi qRT-PCR forward primer
	ACT2-RT-R	CTAAGCTCTCAAGATCAAAGG	Semi qRT-PCR reverse primer
peaTic55	psTic55S-F-NcoI	CATGCCATGGAGTTGGGTCTCAAAGTATA TGAT	yeast two-hybrid analysis, Tic55S PCR forward primer
	psTic55S-R-XhoI	CCGCTCGAG TCACAATCTCCTATGTACCCT	yeast two-hybrid analysis, Tic55S PCR reverse primer
peaTic40	psTic40S-F1-NcoI	CATGCCATGGAGCAGATGAATACACAAA ATAAT	yeast two-hybrid

			analysis, Tic40S/ Tic40TPR PCR forward primer yeast two-hybrid
	psTic40S-F2-NcoI	CATGCCATGGAGGGGCTTTCTCCACAAGA AGTC	analysis, Tic40Hip/Ho p PCR forward primer yeast two-hybrid
	psTic40S-R1-PstI	AACTGCAGTCAAGGGGGGCCTGAAACCC C	analysis, Tic40S/ Tic40Hip/Ho p PCR reverse primer yeast two-hybrid
	psTic40S-R2-PstI	AACTGCAGAATTTGATCAAATTGTTGTTT	analysis, Tic40TPR PCR reverse primer yeast two-hybrid
peaTic 110	psTic110S-F1-NcoI	CATGCCATGGAGAATGCTGCTGCACCGCA AGTT	analysis, Tic110S/ Tic110N PCR forward primer yeast two-hybrid
	psTic110S-F2-NcoI	CATGCCATGGAGCTCATCTCATTTAAGAA CCAT	analysis, Tic110C PCR forward primer yeast two-hybrid
	psTic110S-R1-PstI	AACTGCAGCTAGAATACAAACTTCTCTTC	analysis, Tic110S/

			Tic110C PCR reverse primer yeast two-hybrid analysis, Tic110N PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer Real-time qRT-PCR
	psTic110S-R2-PstI	AACTGCAGTAAATCATTAAATGCCAATAC	
APG7	APG7-qRT-F	CTGGTTTAACCGAGCTTAAGAA	
	APG7-qRT-R	CATCGTCTTCCCAGTCGAGGTT	
ASP3	ASP3-qRT-F	AGCTCAAGTCTCCTTCATGACT	
	ASP3-qRT-R	ATCCTCCCGTCAGATGTCATGT	
DIN11	DIN11-qRT-F	TGCCAAAAGAGTCGTCTATGGA	
	DIN11-qRT-R	TTTGCAAAGGTTGTCAGGACTT	
DIN2	DIN2-qRT-F	CGTTGCTCCTTTGATTTTCCTC	
	DIN2-qRT-R	TGGGTTCTCTATGCCTTGCAAA	
SAG12	SAG12-qRT-F	TGCAGTAACTGCGATTGGATAC	

Gene	Primer	Sequence	Direction
SAG12	SAG12-qRT-R	TTGATGATCCAATACTTTGATC	reverse
	SAG12-qRT-F	TTGATGATCCAATACTTTGATC	forward
SAG13	SAG13-qRT-F	ATTTAGATGTGTCCACATGTTC	forward
	SAG13-qRT-R	CCACGCAAGCATAAATATCTAA	reverse
YLS9	YLS9-qRT-F	TTTAAGTACCTCAAACGGAAC	forward
	YLS9-qRT-R	AAGTCGCACTTGATCGGGAAAA	reverse
ANAC003	AtNAC003-qRT-F	TGATACCGCCGAGAAGAAGAA	forward
	AtNAC003-qRT-R	TGACCCAATTTATGCTCACCA	reverse
ANAC010	AtNAC010-qRT-F	CGGGTATGAGCATGGTGGTAA	forward
	AtNAC010-qRT-R	TCGCCTTCACGAACTACCAA	reverse
ANAC042	AtNAC042-qRT-F	TAGATTCCGACCACACGAGC	forward
	AtNAC042-qRT-R	ATTCTGTGGCTGTGGAAGCG	reverse

			qRT-PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer Real-time qRT-PCR forward primer Real-time qRT-PCR reverse primer yeast one-hybrid analysis, genomic DNA PCR forward primer yeast one-hybrid analysis, genomic DNA PCR reverse primer yeast one-hybrid analysis, genomic DNA PCR forward primer yeast one-hybrid analysis,
<i>ANAC</i> <i>075</i>	AtNAC075-qRT-F	ATGGGGAACCAACAAGAAGCA	
	AtNAC075-qRT-R	TGTCAGAAGAGTCAGGGGCT	
<i>Tubulin</i> <i>2</i>	TUB2/qRT-F	TGGCATCAACTTTCATTGGA	
	TUB2/qRT-R	ATGTTGCTCTCCGCTTCTGT	
<i>ANAC</i> <i>003</i> promoter	AtNAC003p-F-Sac I	TCGAGCTCGCCAAAATAATAAAATCGCGT	
	AtNAC003p-R-Sp eI	GGACTAGTCTCTGCAGCGATCAAAAAAG A	
<i>ANAC</i> <i>010</i> promoter	AtNAC010p-F-Sac I	TCGAGCTCGGTCTACTTTAAGACACAGAT	
	AtNAC010p-R-Sp eI	GGACTAGTCCTTCTTATTTTACTGATCTC	

			genomic DNA PCR reverse primer yeast one-hybrid analysis,
ANAC 042 promoter	AtNAC042p-F-Sac I	TCGAGCTCGGAATGCAGTTGAAGGAATTG	genomic DNA PCR forward primer yeast one-hybrid analysis,
	AtNAC042p-R-Sp eI	GGACTAGTCTTCACTACATCGATCTCTTT	genomic DNA PCR reverse primer yeast one-hybrid analysis,
ANAC 075 promoter	AtNAC075p-F-Sac I	TCGAGCTCGTTAATAGCGATGAAAATTTC	genomic DNA PCR forward primer yeast one-hybrid analysis,
	AtNAC075p-R-Sp eI	GGACTAGTCTCAATCTCGAATATCTTTGA	genomic DNA PCR reverse primer yeast one-hybrid analysis,
MYB 108	AtMYB108-F-NcoI	CATGCCATGGATGAAAAAGGAAGAAGCT TGAAG	analysis, RT-PCR forward primer yeast one-hybrid analysis,
	AtMYB108-R-ClaI	CCATCGATCAGAAGCTACCATTATTGTTG AACTG	RT-PCR reverse

primer

¹ Underlined sequences represent respective restriction enzyme sites.**Table S2.** Number of genes that upregulated or downregulated (2-fold differentially expressed transcripts) according to the GO classification scheme in the dark-induced leaf senescence identified by microarray analysis.

Category	GO Term	No. of upregulated transcripts (Positive values)	No. of downregulated transcripts (Negative values)
Biological_process	GO:0007568 aging	3	-7
	GO:0009072 aromatic amino acid family metabolic process	0	-5
	GO:0019438 aromatic compound biosynthetic process	0	-12
	GO:0009851 auxin biosynthetic process	0	-3
	GO:0010120 camalexin biosynthetic process	0	-3
	GO:0052317 camalexin metabolic process	0	-3
	GO:0046394 carboxylic acid biosynthetic process	5	-11
	GO:0009830 cell wall modification during abscission	0	-2
	GO:0022411 cellular component disassembly	0	-3
	GO:0070301 cellular response to hydrogen peroxide	2	-5
	GO:0034599 cellular response to oxidative stress	2	-6
	GO:0034614 cellular response to reactive oxygen species	2	-6
	GO:0033554 cellular response to stress	5	-14
	GO:0006825 copper ion transport	1	-3
	GO:0006952 defense response	13	-30
	GO:0042742 defense response to bacterium	4	-7
	GO:0009814 defense response, incompatible interaction	1	-7
	GO:0042744 hydrogen peroxide catabolic process	2	-5
	GO:0042743 hydrogen peroxide metabolic process	2	-5
	GO:0006955 immune response	1	-12
	GO:0046219 indolalkylamine biosynthetic process	0	-3
	GO:0006586 indolalkylamine metabolic process	0	-4

GO:0042430	indole and derivative metabolic process	0	-6
GO:0042435	indole derivative biosynthetic process	0	-6
GO:0042434	indole derivative metabolic process	0	-6
GO:0042431	indole metabolic process	0	-3
GO:0009700	indole phytoalexin biosynthetic process	0	-3
GO:0046217	indole phytoalexin metabolic process	0	-3
GO:0045087	innate immune response	1	-11
GO:0010386	lateral root primordium development	0	-3
GO:0010150	leaf senescence	1	-4
GO:0009809	lignin biosynthetic process	0	-4
GO:0016042	lipid catabolic process	5	-8
GO:0010876	lipid localization	7	-3
GO:0006869	lipid transport	7	-3
GO:0030001	metal ion transport	2	-9
GO:0009556	microsporogenesis	1	-3
GO:0010260	organ senescence	1	-4
GO:0016053	organic acid biosynthetic process	5	-11
GO:0055114	oxidation reduction	9	-41
GO:0006800	oxygen and reactive oxygen species metabolic process	2	-6
GO:0052315	phytoalexin biosynthetic process	0	-3
GO:0052314	phytoalexin metabolic process	0	-3
GO:0048236	plant-type spore development	1	-3
GO:0010817	regulation of hormone levels	2	-6
GO:0009733	response to auxin stimulus	13	-11
GO:0009617	response to bacterium	6	-12
GO:0009719	response to endogenous stimulus	23	-38
GO:0009723	response to ethylene stimulus	3	-9
GO:0009725	response to hormone stimulus	20	-30
GO:0042542	response to hydrogen peroxide	2	-5
GO:0009753	response to jasmonic acid stimulus	4	-12
GO:0010033	response to organic substance	24	-46
GO:0006979	response to oxidative stress	3	-11
GO:0010193	response to ozone	0	-4
GO:0000302	response to reactive oxygen species	2	-7
GO:0009751	response to salicylic acid stimulus	2	-7
GO:0009636	response to toxin	1	-3
GO:0009611	response to wounding	2	-13

	GO:0048364	root development	3	-8
	GO:0022622	root system development	3	-8
	GO:0009863	salicylic acid mediated signaling pathway	1	-3
	GO:0019748	secondary metabolic process	1	-16
	GO:0010149	senescence	2	-4
	GO:0000041	transition metal ion transport	2	-4
	GO:0000162	tryptophan biosynthetic process	0	-3
	GO:0006568	tryptophan metabolic process	0	-4
Cellular_component	GO:0048046	apoplast	11	-8
	GO:0005618	cell wall	14	-18
	GO:0012505	endomembrane system	37	-99
	GO:0030312	external encapsulating structure	14	-19
	GO:0005576	extracellular region	23	-27
	GO:0044421	extracellular region part	0	-4
	GO:0009505	plant-type cell wall	5	-8
Molecular_function	GO:0003993	acid phosphatase activity	2	-2
	GO:0004022	alcohol dehydrogenase (NAD) activity	0	-3
	GO:0005275	amine transmembrane transporter activity	1	-4
	GO:0016209	antioxidant activity	2	-7
	GO:0004190	aspartic-type endopeptidase activity	2	-4
	GO:0070001	aspartic-type peptidase activity	2	-4
	GO:0005509	calcium ion binding	5	-11
	GO:0004091	carboxylesterase activity	5	-14
	GO:0043169	cation binding	22	-96
	GO:0045551	cinnamyl-alcohol dehydrogenase activity	0	-3
	GO:0048037	cofactor binding	1	-18
	GO:0015082	di-, tri-valent inorganic cation transmembrane transporter activity	1	-4
	GO:0019104	DNA N-glycosylase activity	2	-1
	GO:0009055	electron carrier activity	4	-34
	GO:0004175	endopeptidase activity	3	-10
	GO:0004857	enzyme inhibitor activity	1	-9
	GO:0050660	FAD binding	1	-10
	GO:0046527	glucosyltransferase activity	0	-8
	GO:0020037	heme binding	3	-22
	GO:0016799	hydrolase activity, hydrolyzing	2	-1

N-glycosyl compounds			
GO:0043167	ion binding	22	-96
GO:0005506	iron ion binding	5	-32
GO:0008289	lipid binding	7	-6
GO:0016165	lipxygenase activity	0	-3
GO:0046872	metal ion binding	18	-86
GO:0045735	nutrient reservoir activity	4	-3
GO:0016717	oxidoreductase activity, acting on paired donors, with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water	2	-1
GO:0016684	oxidoreductase activity, acting on peroxide as acceptor	2	-6
GO:0016701	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	0	-5
GO:0019825	oxygen binding	1	-15
GO:0046910	pectinesterase inhibitor activity	0	-6
GO:0004601	peroxidase activity	2	-6
GO:0080043	quercetin 3-O-glucosyltransferase activity	0	-4
GO:0080044	quercetin 7-O-glucosyltransferase activity	0	-3
GO:0046906	tetrapyrrole binding	6	-22
GO:0046914	transition metal ion binding	12	-71
GO:0035251	UDP-glucosyltransferase activity	0	-8

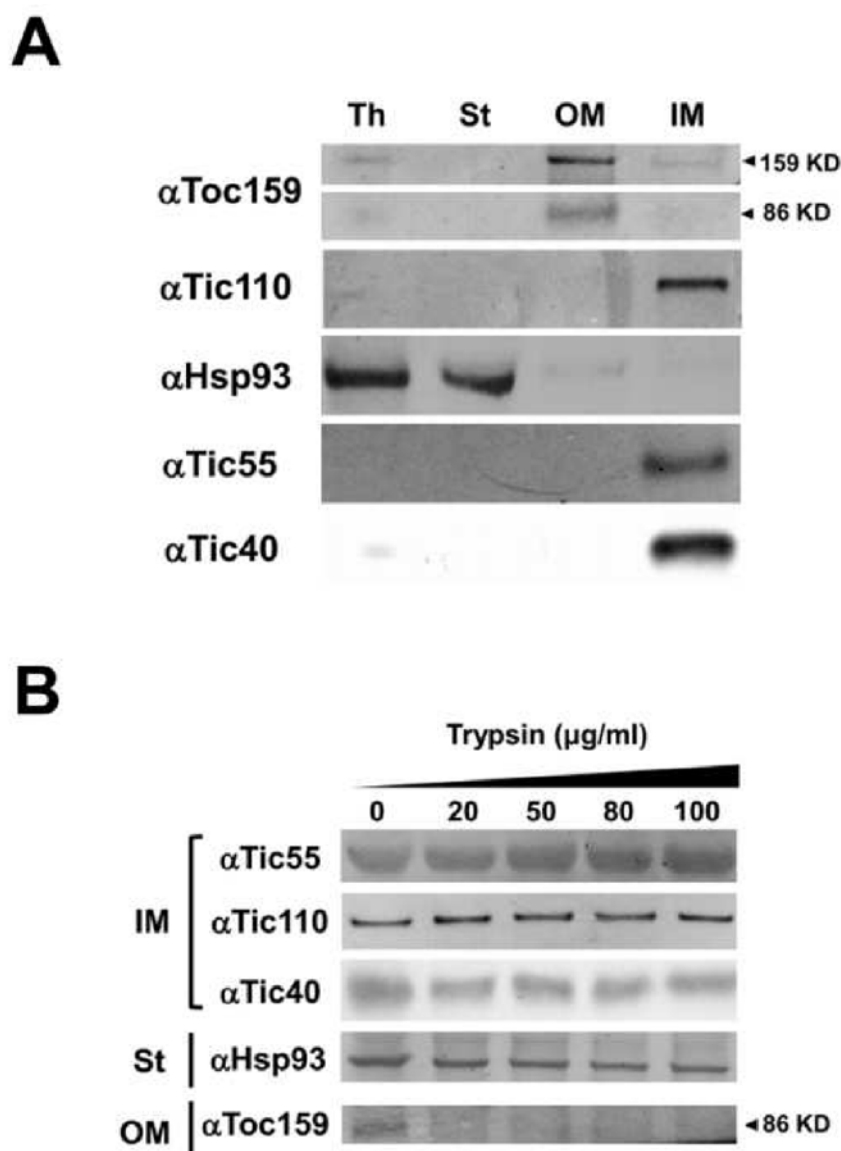


Figure S1. Tic55 situates at the inner membrane of chloroplast. (A) Fractionation analysis of chloroplast was carried out as described in the Materials and Methods section. Thylakoid proteins (Th), Stoma proteins (St), Outer membrane proteins (OM), and Inner membrane proteins (IM) were successfully separated; protein gel blots were performed using specific antibodies against the translocon proteins Toc159, Tic110, Hsp93, Tic55, and Tic40, respectively. (B) Protease Trypsin treatment to determine the protein orientation of Tic55 at the inner membrane of chloroplast. Isolated chloroplasts were treated with different concentrations of trypsin (0 μg/mL–100 μg/mL), followed by protein gel blot analysis with specific antibodies. IM: inner membrane. St: stroma. OM: outer membrane.