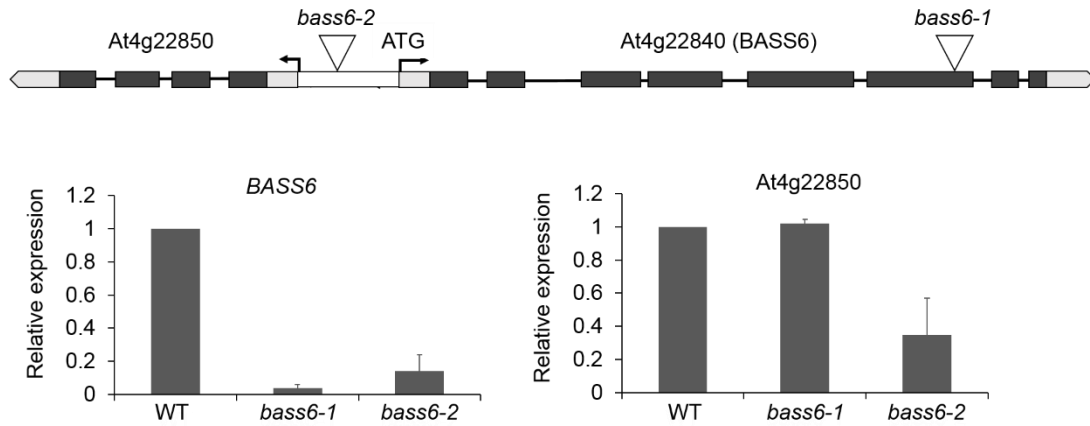


Supplemental Figure 1. BASS6 Is Necessary for Growth at Low CO₂.

(Supports Figure 2.)

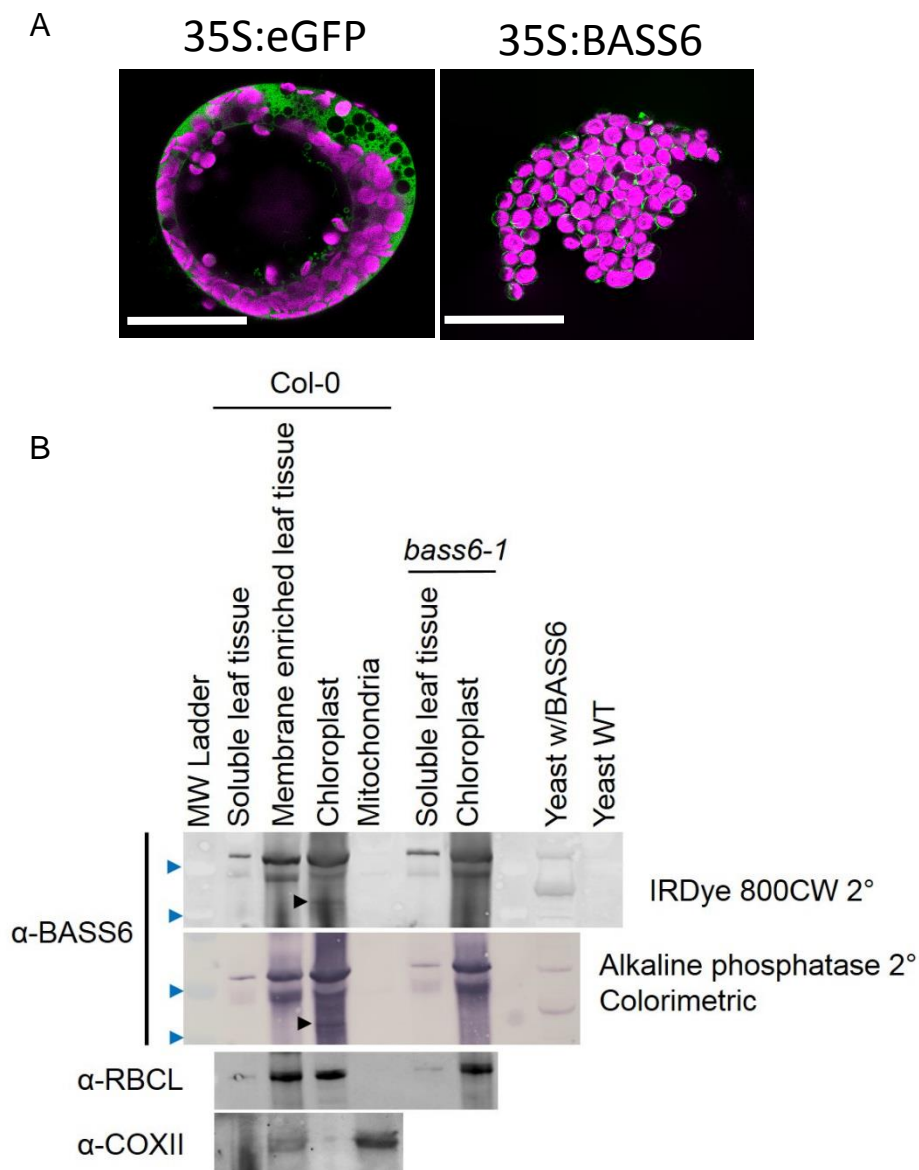
Growth curves at indicated CO₂ concentrations for WT (Col-0), *bass6-1*, and *plgg1-1* T-DNA Arabidopsis lines. Measurements were taken at indicated days after planting and are in mm² of rosette size. Measurements were stopped after leaf overlap at day 25. Error bars indicate standard deviation of at least 5 plants and 3 biological replicates each.



Supplemental Figure 2. Characterization of BASS6 T-DNA Insertions.

(Supports Figure 2.)

T-DNA insertion of *bass6-1* line is located in sixth exon of gene At4g22840 indicated by the white triangle. The T-DNA insertion for *bass6-2* is located within a shared promoter of gene At4g22850. Arrows indicate transcription start sites, dark gray boxes indicate exons, light gray 5' and 3' untranslated regions. Real Time PCR analysis of *BASS6* and the gene At4g22850 in the *bass6-1* and *bass6-2* T-DNA lines compared to WT. Error bars represent standard deviation. $n = 3$. Three biological replicates including three technical replicates each were used for all samples.

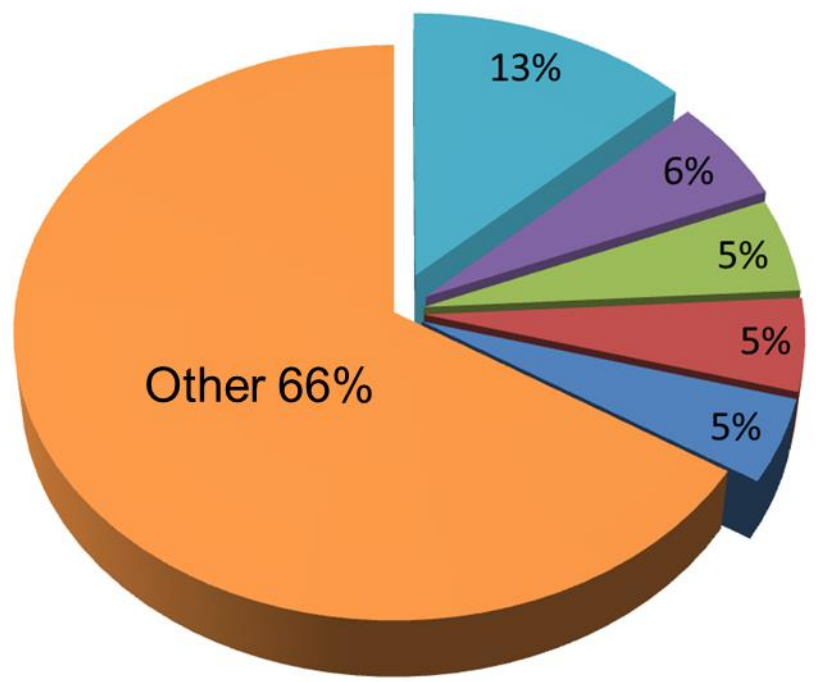


Supplemental Figure 3. BASS6 is Chloroplast-Localized.

(Supports Figure 4.)

(A) Confocal images of *Nicotiana benthamiana* protoplasts transiently expressing GFP protein (control) or Bass6-GFP expressed from the 35s promoter. GFP control is a single plane image and Bass6 GFP is a 3D rendering of 4 successive planes using Zeiss Zen software, Bar = 50µm.

(B) Immunoblot analysis of *Arabidopsis* subcellular fractions. 15 µg of protein loaded per lane for α-BASS6 antiserum, 5 µg per lane for α-RBCL, and 25 µg per lane for α-COXII immunoblots. Black arrows indicate BASS6 detection between the 55 and 37 (blue arrows) kilodalton molecular markers (predicted MW 44KDa). Antibody dilution was 1:1000 overnight incubation at 4°C for α-BASS6 and α-COXII and 1:5000 for 4 hours at room temperature for α-RBCL. Detection on PVDF-F membranes using an Odyssey CLX (LiCor Biosciences) or through colorimetric detection. Soluble leaf tissue is total soluble protein. Membrane enriched fraction is insoluble and organellar proteins isolated from whole leaf tissue. Chloroplast and mitochondrial fractions are from extraction of whole organelle isolation including soluble and membrane fractions.



- Gluconeogenesis & Glyoxylate cycle
- Fermentation
- TCA cycle
- Nitrogen Metabolism
- Transport

Supplemental Figure 4. Co-Expression Analysis of *BASS6* in Arabidopsis.

(Supports Figure 1.)

Percentage of genes with GO terms associated with co-expression using At4g22840 (*BASS6*) in the query from CSB.DB (<http://csbdb.mpimp-golm.mpg.de>). The Spearman and Pearson co-expression coefficients for all genes tested were <0.7 .

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Nt-Bass6M      -----MSLNskfLLQqqQYHQGfKILHQKSLPCFDQHPFVsfPKKwKCFHSGGN
Nt-Bass6C      -----MQSTL-----ASGN
At-Bass6       MSVITTPiETLHLKSTLRLLPRAVYRSQRIQVfPPNIFs---NTSLSSPLRIDPISQVGG
                                     .:                                     *.

Nt-Bass6M      SRLlISRCISEKfSDSLGpSDSESlENLkQkENTllTILKANSfLPHVVLGStILALIY
Nt-Bass6C      SRLlISRCISKkFpDSLgPsdSESlENLkQkENTfLTILKANSfLPHVVLGStILALIY
At-Bass6       SRNLWRYASDNfSEMGLDPGADPFkVIEKp--SIVDRMkKANsILPHVVlASTILALIY
** * * * *.:*.:  .:.:.:  :.:  :.:  :* ***:*****.*****

Nt-Bass6M      PPSFTWfTNRYySPALGfLmFAVGVNSSEKDFLEAFkKPAaIFAGYIqGFavKPLlGyLF
Nt-Bass6C      PPSFTWfTNRYyAPALGfLmFAVGVNSSEKDFLEAFkKPAaIFAGYIqGFavKPLlGyLF
At-Bass6       PPSFTWfTSRYfVPALGfLmFAVGINSNEKDFLEAFkRPKAILLgYVgQyLVkPVLGfIF
*****.*.: *****:*.*****:*. *****:*. *****: *****:*.

Nt-Bass6M      GTLAMtVfGLPtSLAAGIMlTScVSGAQLsNYATfLTDpQMAPLSIVMtsLStATAVfVt
Nt-Bass6C      GTLAMtVfDLPtSLAAGIMlTScVSGAQLsNYATfLTDpQMAPLSIVMtsLStATAVfVt
At-Bass6       GLAAVSLfQLPtPIgAGIMlVScVSGAQLsNYATfLTDpALAPLSIVMtsLStATAVlVt
* *.:*: * *.:* *.:* *.:* *.:* *.:* *.:* *.:* *.:* *.:* *.:*

Nt-Bass6M      PILSllLIgKkLPVDVKGMVsnIlQIVVAPVAGllLNRFfPRICNAIRPlLPPlSVfVt
Nt-Bass6C      PILSllLIgKkLPVDVKGMVsnIlQIVVAPVAGllMNRfFPQICNAIRPlLPPlSVfVt
At-Bass6       PMLSllLIgKkLPVDVKGMISsilQVVIAPiAAGllLNkLFpkVSNaiRPfLPILSVlDt
*:*****:*****:*.*****:*.*****:*.*****:*.*****:*. *****: *

Nt-Bass6M      ALCVGAPLAINIEslVSPSGMSVllLMIAfHLSAfILGyLLSGIAfHKAPDVKPlQRTLS
Nt-Bass6C      ALCVGAPLAINIEslVSPSGMSVllLVIAfHLSAfILGyLLSGIAfHKAPDVkALQRTLS
At-Bass6       ACCVGAPLAlNINsvMSPfGAtIlLLVtMfHLSAfLAGYfLTGsvFRNAPDAkAMQRTLS
* *****:*****:*.*****:*. *****: * *.:* *.:* *.:* *.:* *.:*

Nt-Bass6M      YETGMQSSllALALANKffQDPLVgVPPAISVViMSlMGfSLVmlWAKkKETViG
Nt-Bass6C      YETGMQSSllALALANKffQDPLVgVPPAISVViMSlMGfSLVmlWAKkKETViG
At-Bass6       YETGMQSSllALALATkffQDPLVgIPPAISTVVMslMGfTLVLIWSKEKSNTf-
*****.*****:*****.*:*****:*.*****:*.*****:*.*****:

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Supplemental Figure 5. BASS6 Amino Acid Sequence Alignments.

(Supports Figure 4.)

Clustal W alignments between two Bass6 homolog translations. N-terminal alignment showing one of two Bass6 proteins has an N-terminal extension that predicts transport to the mitochondria (Bass6M). (*) and blue text show 100% conservation, (:) and green text show similar amino acids. Nt = *Nicotiana tabacum*, At = *Arabidopsis thaliana*

Supplemental Table 1. Oligonucleotides Used in this Study.

Primer name	Primer type	Sequence
p35s seq F	Sequencing	CTCTCTGCCGACAGTGGT
t35s seq R	Sequencing	CTTATATGCTCAACACATGAGCG
Salk_053469 LP	T-DNA verification	ATAACCGCGAGATAGAGAGGC
Salk_053469RP	T-DNA verification	CCCATGGCTACTCTTTTAGCC
LBb1.3	T-DNA verification	ATTTTGCCGATTTTCGGAAC
Plgg1-005F	Yeast cloning	GCCGGATCCATGGCTTCGTGCTCTAAGATCCGTTTCG
Plgg1-006R	Yeast cloning	GCCCTCGAGTCAGCCGACGACCGCTAGC
Bass6-001F	Yeast cloning	GCTCTAGAATGAGCGTGATCACAACCTCC
Bass6-002R	Yeast cloning	GACTCGAGTTAAAATGTGTTACTCTTTTC
Plgg1-RT 1F	QRT-PCR	CTACTCTTTTAGCCACTCCTATCTTC
Plgg1-RT 2R	QRT-PCR	AGATTCAACTTCTGGGCACC
Bass6-RT 1F	QRT-PCR	TCGCAGTCAACGGATTCAAG
Bass6-RT-2R	QRT-PCR	TCTACGCCACAAATTCCTCG
VDE-RT-1F	QRT-PCR	TGAGTTCAACGAGTGTGCTG
VDR-RT-2R	QRT-PCR	ACTTGTAATGTACCACTTCCCG
At4g22850-1F	QRT-PCR	GGTCAAGAATCAGAGCAGGAG
At4g22850-2R	QRT-PCR	ACCGATCCAAATGTAACCCAC

Supplemental Table 2. Plasmids Used in this Study.

Plasmid	Inserted genes	Promoter	Vector	Source
EC50505	none		EC50505	ENSA (project ensa.ac.uk)
EC27349	<i>p19, GFP</i>	<i>2x35s</i>	EC50505	This study
EC27357	<i>p19, BASS6-GFP</i>	<i>2x35s</i>	EC50505	This study
p415 ADH1	none		p415 ADH1	ATCC-87374
P415 BASS6	<i>BASS6</i>	<i>ADH1</i>	p415 ADH1	This study
P415 PLGG1	<i>PLGG1</i>	<i>ADH1</i>	p415 ADH1	This study
pMDC-P19	<i>P19</i>	<i>2x35s</i>	pMDC32	Rolland et al 2016
pMDC-PLGG1	<i>PLGG1-mGFP6</i>	<i>2x35s</i>	pMDC32	Rolland et al 2016
pMDC-BASS6	<i>BASS6-mGFP6</i>	<i>2x35s</i>	pMDC32	This Study
EC15325	<i>BAR</i>	<i>NOS</i>	EC50505	ENSA (project ensa.ac.uk)
EC27403	<i>BAR, PLGG1</i>	<i>NOS, PLGG1</i>	EC15325	This study
EC27404	<i>BAR, BASS6</i>	<i>NOS, BASS6</i>	EC15325	This study
EC27406	<i>BAR, BASS6</i>	<i>NOS, PLGG1</i>	EC15325	This study

Supplemental Table 3. Yeast Strains Used in this Study.

Yeast Strains	Genotype	Reference
BY4741	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0</i>	GE Open biosystems
<i>ady2Δ</i>	<i>MATα his3Δ leu2Δ0 LYS2 met15Δ0 ura3Δ0 ady2Δ</i>	GE Open Biosystems