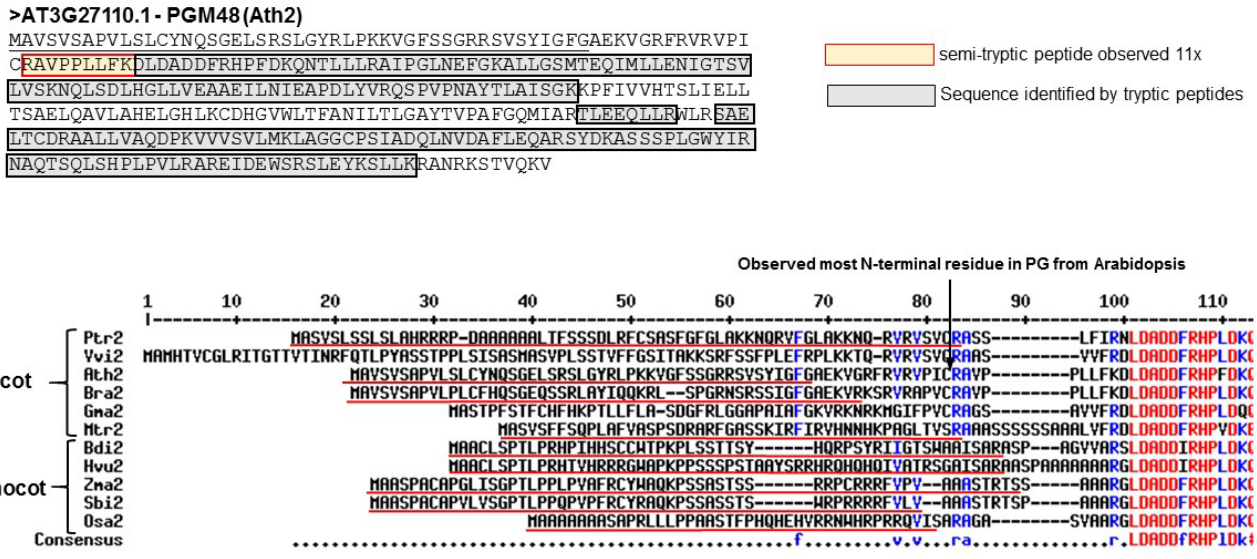
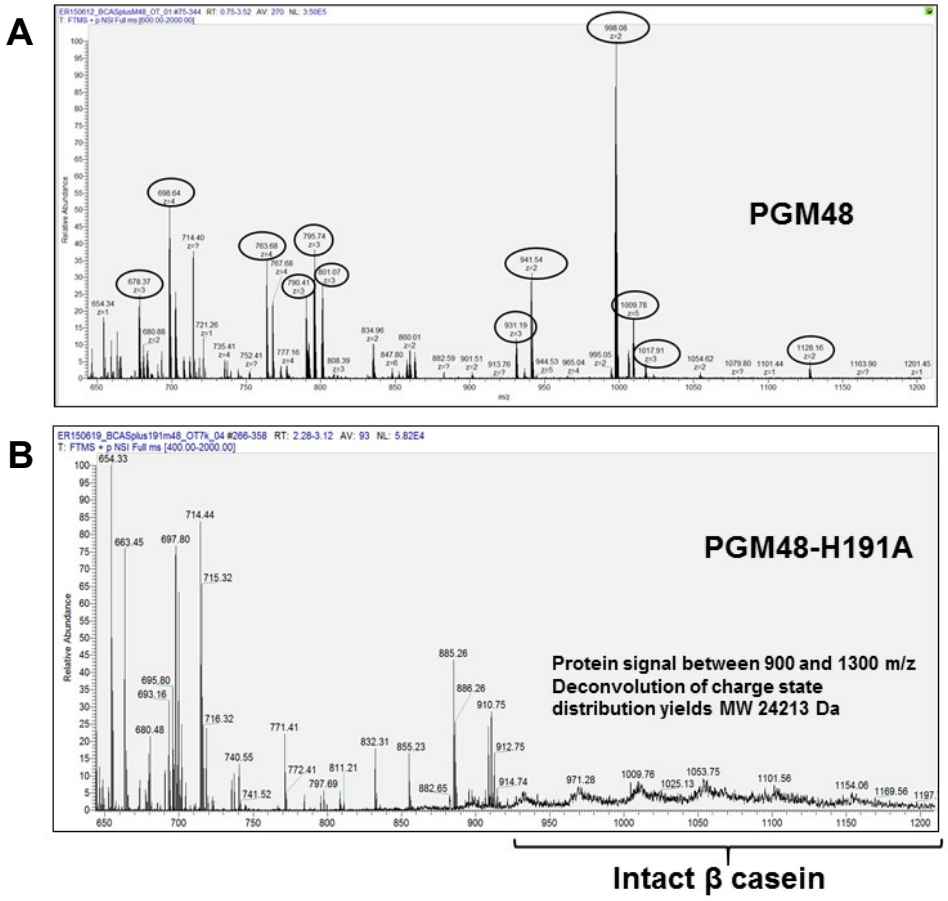
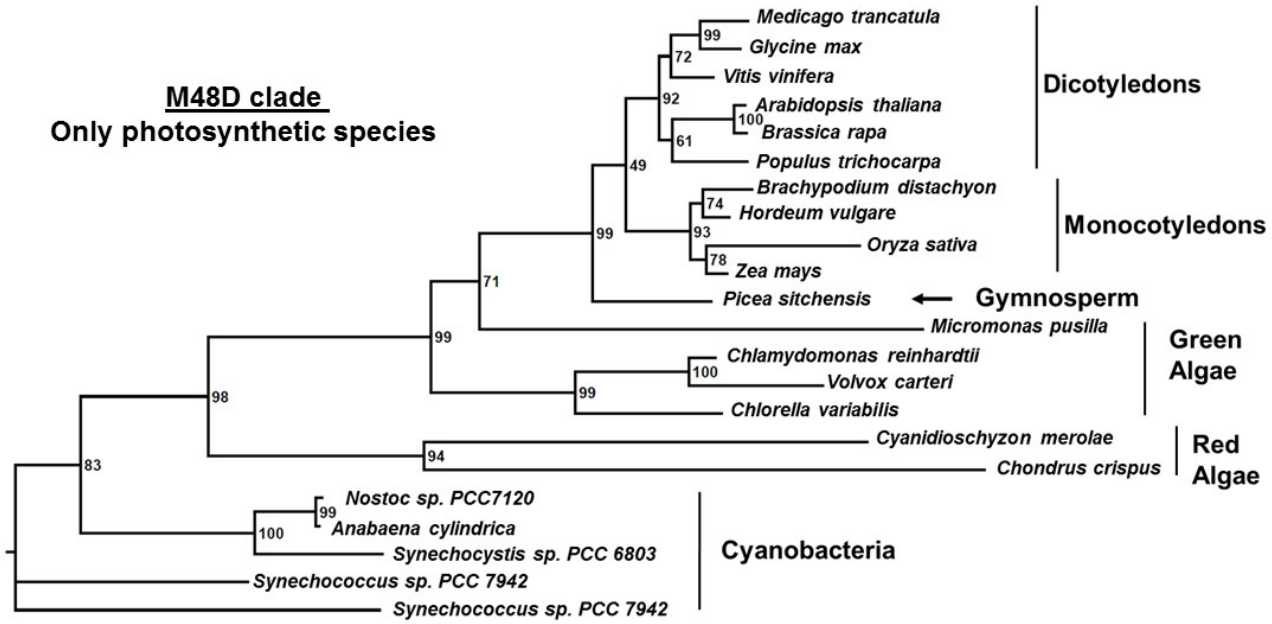


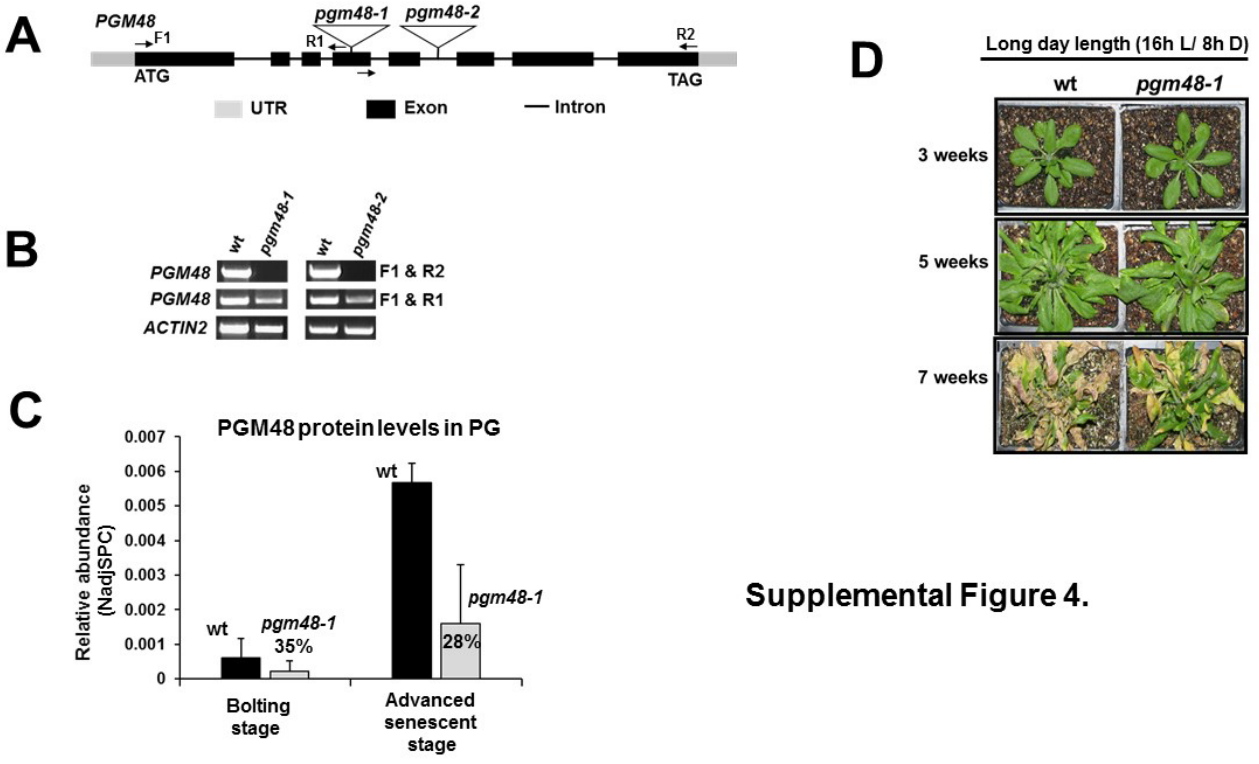
Supplemental Figure 1. N-terminus of Arabidopsis PGM48 and cTP predictions for Arabidopsis and homologs in other dicots and monocots. ***Vitis vinifera (Vvi); Populus trichocarpa (Ptr); Medicago truncatula (Mtr); Brassica rapa)Bra); Arabidopsis thaliana (Ath); Glycine max (Gma); Zea mays (Zma); Brachypodium distachyon (Bdi); Hordeum vulagere (Hvu); Oryza sativa (Osa); Sorghum bicolor (Sbi).***



Supplemental Figure 2. Ms spectra of digests by active PGM48 (A) and mutant inactive PGM48 (PGM48-H191A) (B).

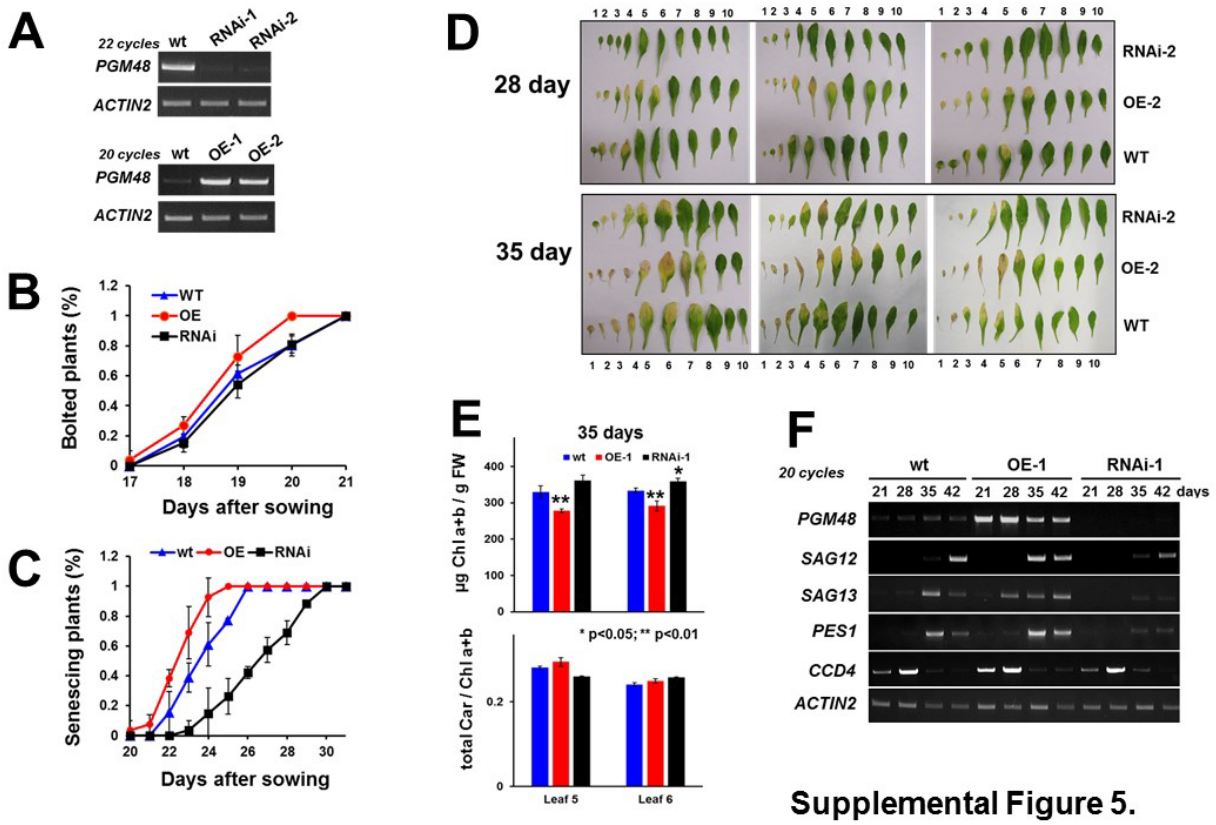


Supplemental Figure 3. Phylogenetic tree of clade M48D with PGM48 homologs. This cladogram is a close up of the M48D clade shown in Figure 2. Cladogram of PGM48 across cyanobacteria, green algae, gymnosperm, monocots and dicots. The analysis includes 22 proteins from different species including 10 angiosperms, one gymnosperm, four green- and two red algae and five cyanobacteria. RAxML bootstrap support values are shown at the nodes of the tree.



Supplemental Figure 4.

Supplemental Figure 4. Characterization of T-DNA insertion mutants in PGM48 and PGM48 is a senescence induced protein in Arabidopsis.
(A) PGM48 gene structure and position of T-DNA insertions in lines pgm48-1 (SALK_082409) and pgm48-2 (GABI_324A06).
(B) mRNA levels of PGM48 and ACTIN2 in pgm48-1 and pgm48-2 as determined by RT-PCR (28 cycles). RNA was collected from 5 weeks olds plants grown at 16h light/8 h dark condition at 130 µmol photons m-2 s-1 light intensity The positions of the forward primer F1 and reverse primers R1 and R2 are indicated in (A).
(C) PGM48 relative protein accumulation levels determined by label-free spectral counting quantitative proteomics of isolated PG at bolting stage and advanced senescence stage in wt and pgm48-1. Plants were grown under short day conditions (10 h light/14h dark). Results from two replicates of independent PG preparations were analyzed; relative abundance was normalized to the total amount of PG core protein (see Methods). Supplemental Dataset 2 provides values for all PG proteins.
(D) Phenotypes of wt and pgm48-1 plants after 3, 5 and 7 weeks growth under long day conditions (16h light/8h dark, 130 µmol photons m-2 s-1 light intensity). At 7 weeks of growth, pgm48-1 plants showed delayed senescence compared to wt plants. Similar results were obtained for allele pgm48-2.



Supplemental Figure 5.

Supplemental Figure 5. Additional phenotypic analysis of RNAi and OE lines(A) mRNA levels of PGM48 in wt, and independent overexpression (OE-1,2) and RNAi (RNAi-1,2) lines determined by RT-PCR.

(A) RNA was collected from 18 days old plants grown at 18h light/6h dark condition 130 µmol photons m^{-2} s^{-1} light intensity. The cycle number was higher (22x) to evaluate the RNAi lines to determine if there was any PGM48 expression, and lower (20x) to better estimate the overexpression of PGM48-StrepII.

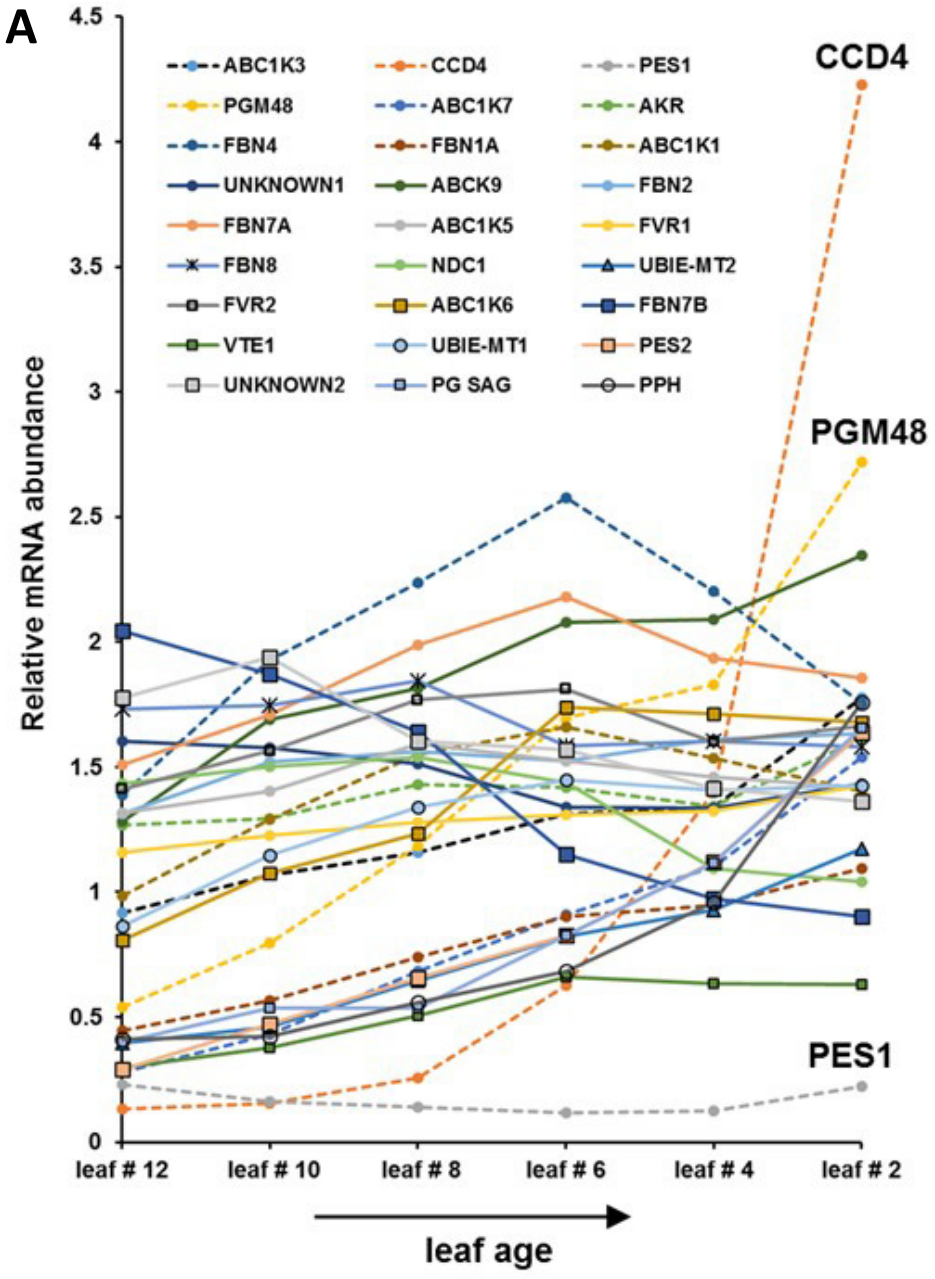
(B) PGM48 expression levels do not significantly affect the rate of bolting. Plants were grown on soil under 18h light / 6h dark, 130 µmol photons m-2 s-1 light intensity and carefully inspected for the switch from vegetative to reproductive stage (defined as bolting). The percentage of bolted plants for each of three genotypes (wt, OE and RNAi) was recorded and plotted. Three independent series of plants for each genotype were grown, each with 8 or 9 plants per genotype. Standard deviations across series are plotted (n=3).

(C) PGM48 expression levels do affect the rate of natural senescence. Observations were carried out for the same series of plants as panel (E) and each leaf rosette was carefully inspected visible senescence during the flowering stage. Plants were counted as senescing as soon as leaf senescence was visible (typically the oldest true leaf). The percentage of senescing plants for each of three genotypes (wt, OE and RNAi) was recorded and plotted. Three independent series of plants for each genotype were grown, each with 8 or 9 plants per genotype. Standard deviations across series are plotted (n=3).

(D) Examples of progression of natural leaf senescence of wt, OE-2 and RNAi-2 lines under 18h light / 6h dark, 130 µmol photons m-2 s-1 light intensity. Individual leaves were separated from plants and shown from 1 to 10 leaf (numbered from old to young) at day 28 and 35.

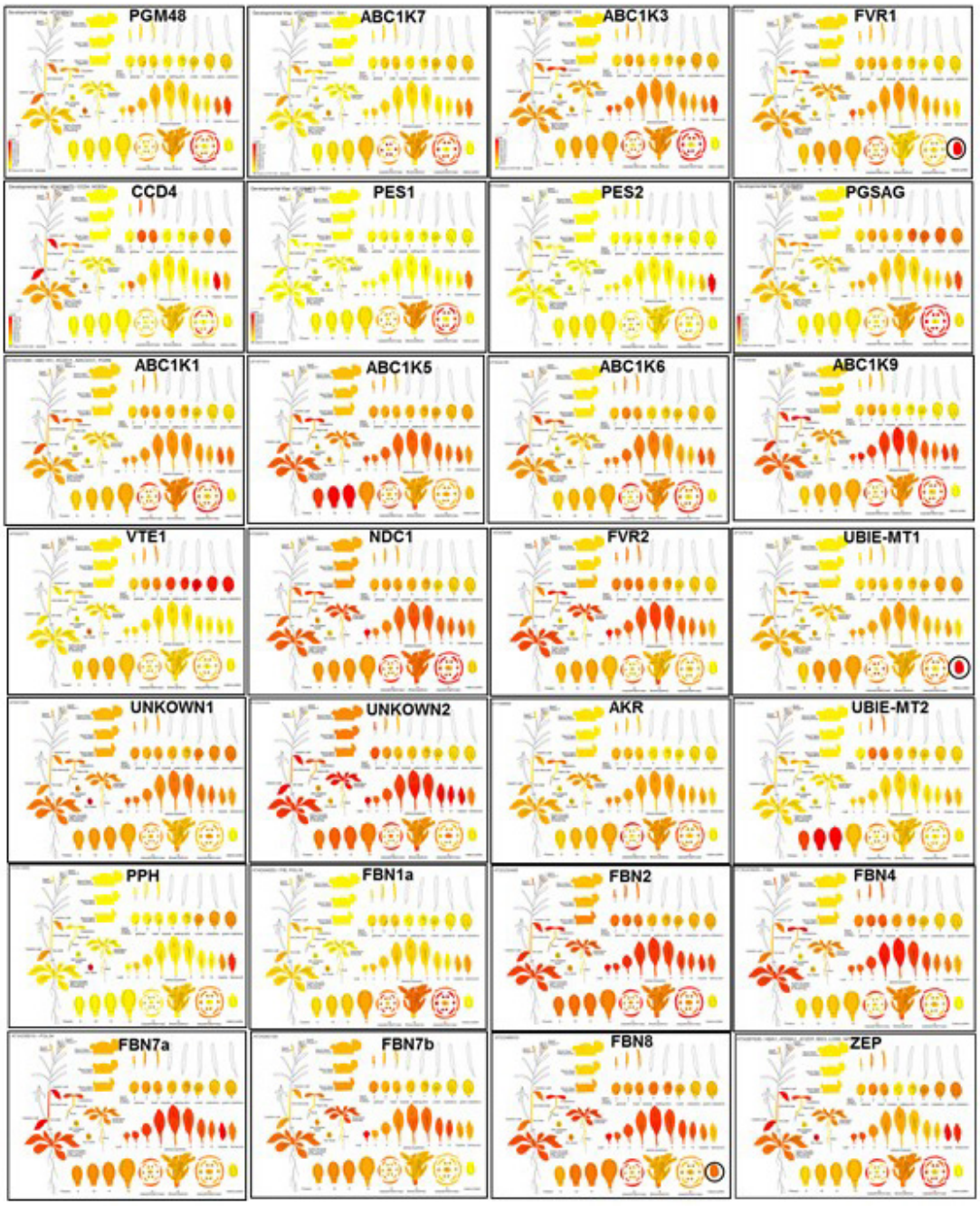
(E) Chlorophyll a + b content on fresh weight bases and total carotenoid/total chlorophyll ratios for leaf 5 and 6 in the 3 genotypes at 35 days. Significance levels are indicated - * p<0.05; ** p<0.01.

(F) mRNA levels of PGM48, SAG12, SAG13, PES1, CCD4 in rosettes of wt, OE-1 and RNAi-1 lines after 21, 28, 35 and 42 days growth under 18h light/6h dark, 130 µmol photons m-2 s-1 light intensity. ACTIN2 mRNA was used as normalizer across the samples.



Supplemental Figure 6. mRNA expression data from publicly available microarray data for leaves from wt (col-0) for all genes encoding for the PG core proteome, except for FBN1B, HBP3 and ESTERASE1 for which no data are available; data are downloaded from http://bar.utoronto.ca/.
(A) mRNA abundance levels of individual leaves from rosettes of 17 day old (continuous light), with leaf 2 being the oldest and leaf 12 the youngest. CCD4 and PGM48 clearly stand-out in their steep increase in aging leaves.
(B) Screen captures for the developmental expression atlas for all available PG genes and for ZEP.

B



No data: HBP3 ESTERASE1; FBN

Supplemental Figure 6. mRNA expression data from publicly available microarray data for leaves from wt (col-0) for all genes encoding for the PG core proteome, except for FBN1B, HBP3 and ESTERASE1 for which no data are available; data are downloaded from http://bar.utoronto.ca/.
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(B) Screen captures for the developmental expression atlas for all available PG genes and for ZEP.

Supplemental Table 1. Mass spectrometry analysis of β-casein digests with recombinant wt PGM48.

mono-isotopic m/z (experimental)	charge (z)	mono-isotopic mass (Da)	peptide sequence	oxidized peptide form detected	N-terminal cleavage	C-terminal cleavage	MS/MS identification
790.07	3	2367.2	MHQPHQPLPPTVMFPPQSVLS *	yes	LLQSW|MHQPH	QSVLS|LSQSK	yes
1006.17	5	5025.8	QSKVLPVPQKAVPYPQRDMPIQAFLLYQEPVLGPVRGPFPI	yes	QSVLS|LSQSK	none	no
930.85	3	2789.5	LSQSKVLPVPQKAVPYPQRDMPIQA *	yes	QSVLS|LSQSK	MPIQA|FLLYQ	yes
698.39	4	2789.5	LSQSKVLPVPQKAVPYPQRDMPIQA*	yes	QSVLS|LSQSK	MPIQA|FLLYQ	yes
763.43	4	3049.7	LSQSKVLPVPQKAVPYPQRDMPIQAFL*	yes	QSVLS|LSQSK	IQAFL|LYQEP	no
1127.66	2	2253.3	FLLYQEPVLGPVRGPFPIIV	no	MPIQA|FLLYQ	none	no
997.58	2	1993.1	LYQEPVLGPVRGPFPIIV	no	IQAFL|LYQEP	none	yes
941.04	2	1880.1	YQEPVLGPVRGPFPIIV	no	QAFLL|YQEPV	none	yes
834.96	2	1667.9	LYQEPVLGPVRGPFP	no	IQAFL|LYQEP	GPFP|IIV-	yes

* also observed with methionine residues oxidized

Supplemental Table 2. Distribution of M48 members and assignment M48 subfamilies across species in different phylogenetic groups within the tree of life.

Phylogenetic group	Species	HTPX (M48B)	STE24 (M48A)	OMA1 (M48C)	PGM48 (M48D)
Angiosperms - dicots	*Vitis vinifera (Vvi)*	0	1	1	1
	Populus trichocarpa (Ptr)	0	1	1	1
	Medicago truncatula (Mtr)	0	1	2	1
	Brassica rapa (Bra)	0	1	1	1
	Arabidopsis thaliana (Ath)	0	1	1	1
	Glycine max (Gma)	0	1	1	1
Agiosperms - monocots	*Zea mays (Zma)*	0	1	1	1*
	Brachypodium distachyon (Bdi)	0	1	1	1
	Hordeum vulgare (Hvu)	0	1	0	1
	Oryza sativa (Osa)	0	1	1	1
	Sorghum bicolor (Sbi)	0	1	1	1
Gymnosperm	*Picea sitchensis (Psi)*	0	1	0	1
Lycopod	*Selaginella moellendorffii (Smo)*	0	1	1	0
Moss	*Physcomitrella patens (Ppa)*	0	1	1	0
Liverworts	*Marchantia polymorpha*	0	1**	1**	1**
Red algae	*Cyanidioschyzon merolae (Cme)*	0	0	1	1
	Chondrus crispus (Ccr)	0	1	0	1
Green algae	*Volvox carteri (Vca)*	0	1	1	1
	Chlamydomonas reinhardtii (Cre)	0	1	1	1
	Chlorella variabilis (Cva)	0	1	1	1
	Micromonas pusilla (Mpu)	0	0	1	1
Cyanobacteria	*Synechocystis sp. PCC6803 (Syne)*	0	0	0	1
	Anabaena cylindrica (Acy)	1	0	0	1
	Nostoc sp. PCC7120 (Nos)	1	0	0	1
	Synechococcus sp. PCC 7942 (Syn)	1	0	0	2
Metazoa/Fungi	*Homo sapiens (Hsa)*	0	1	1	0
	Penicillium marneffei (Pma)	0	1	0	0
	Saccharomyces cerevisiae (Sce)	0	1	1	0
	Saccharomyces mikatae (Smi)	0	1	0	0
Alpha-proteobacteria	*Agrobacterium tumefaciens (Atu)*	1	0	1	0
Beta-proteobacteria	*Nitrosomonas europaea (Neu)*	1	0	1	0
Gama-proteobacteria	*Escherichia coli (Eco)*	1	0	0	0
	Vibrio parahaemolyticus (Vpa)	1	0	1**	0
Epsilon-proteobacteria	*Sulfuricurvum kujiens (Sku)*	1	0	1	0
Delta-proteobacteria	*Geobacter sulfurreducens (Gsu)*	1**	0	1	0
Actinobacteria	*Streptomyces griseus (Sgr)*	1	1	0	0
Firmicutes	*Bacillus subtilis (Bsu)*	1	1	1	0
Spirochaetes	*Leptospira interrogans (Lin)*	1	1	0	0
	Leptospira biflexa (Lbi)	1	0	1	0
Crenarchea	*Sulfolobus islandicus (Sis)*	2	0	0	0
	Metallosphaera sedula (Mse)	2	0	0	0
Euryarchaea	*Haladaptatus paucihalophilus (Hpa)*	1	0	0	0
	Natrinema pellirubrum (Npe)	1	0	0	0

* 2 additional genes, but very long; not shown in tree

** not shown in the tree

Supplemental Table 3. Primers used in this study.

Primer name	Sequence (5'-3')
PGM48-Anti-BamHI-FW	GGATCCCTCGATGCTGATGATTTCCGACAT
PGM48-Anti-XhoI-RV	TCTCGAGACTCCTGGACCACTCATCAATCTC
PGM48-M-BamH1-FW	GGATCCTTCGGTGCGGAGAAAGTTGGA
PGM48-M-SalI-RV	GTCGACAACCTTCTGTACTGTGCTTTTTC
PGM48H191A-FW	TTAGCCGCTGAACTAGGCCATC
PGM48H191A-RV	GATGGCCTAGTTCAGCGGCTAA
PGM48E192D-FW	TTAGCCCATGATCTAGGCCATC
PGM48E192D-RV	GATGGCCTAGATCATGGGCTAA
PGM48-OE-FW	ATGGCGGTTTCAGTCTCAGCTC
PGM48-OE-strepII-STOP-RV	CTACTTCTCGAATTGAGGATGAGACCAAACCTTCTGTACTGTGCTTTTTC
ihpRNAi-GG-PGM48-FW	accaggtctcaggagATGGCGGTTTCAGTCTCAGCTC
ihpRNAi-GG-PGM48-RV	accaggtctcatcgtGAGCTTTGCCGAATTCGTTCAAC
PGM48-TDNA-RP (genotyping)	TGTCTTTTGATGCAGAACACG
PGM48-TDNA-LP (genotyping)	TACAACAACCTCTTGGAAGCG
PGM48-RTPCR-F1	ATGGCGGTTTCAGTCTCAGCTC
PGM48-RTPCR-R1	CAAACCGTGAAGATCAGAGAG
PGM48-RTPCR-F2	GCAATAAGCGGTAAGAAGCCGT
PGM48-RTPCR-R2	AACCTTCTGTACTGTGCTTTT
CCD4-EcoR1-FW	GAATTCTCTCCAATCACAAACCCAAGCGAC
CCD4-XhoI-RV	CTCGAGAAGCTTATTAAGGTCACTTTCCTT
PES1-EcoR1-FW	GAATTCGCTCAAATCTCTGGTGAAAACAAGAAG
PES1-Xho1-RV	CTCGAGTGGCTCGAAAGATGGAACATGAGTTG
ABC1K3-EcoR1-FW	GAATTCAGATTGGCGCGTGCGGCCCTGGTA
ABC1K3-Sal1-RV	GTCGACTGGGGATGGTGCAGAAGATGGTGT
PG-SAG-EcoR1-FW	GAATTCTCATCATCTCCGGCGATC
PG-SAG-Sal1-RV	GTCGACACTCTCATCTTGTTGTTGAAG