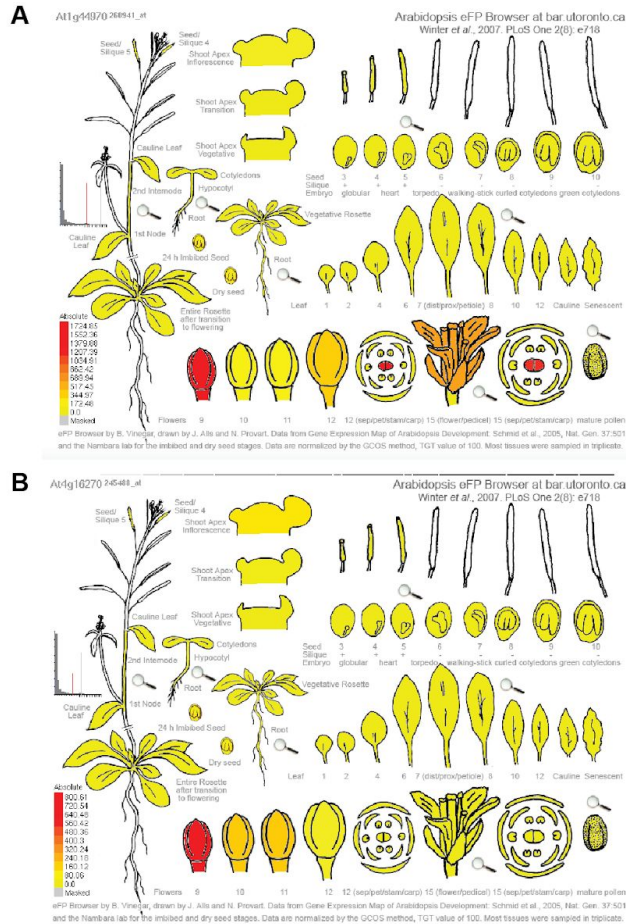


Supplemental Materials



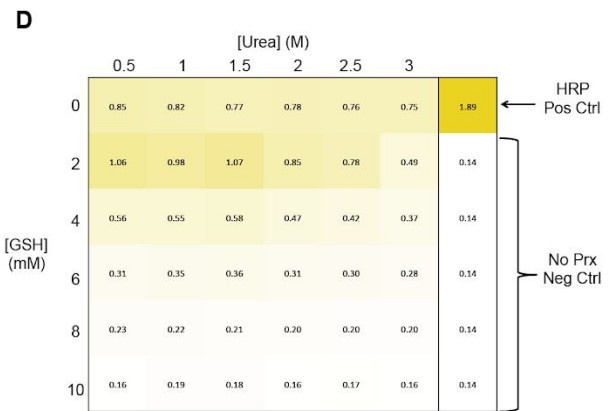
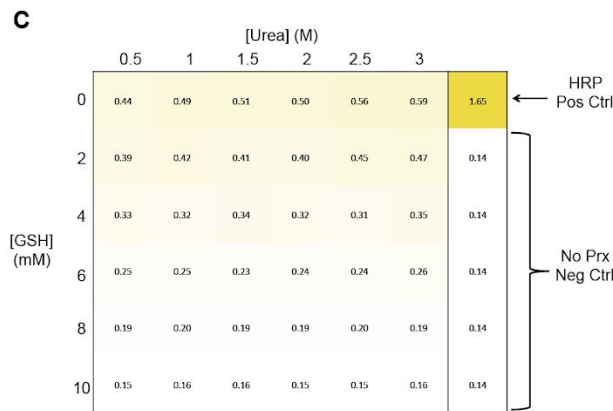
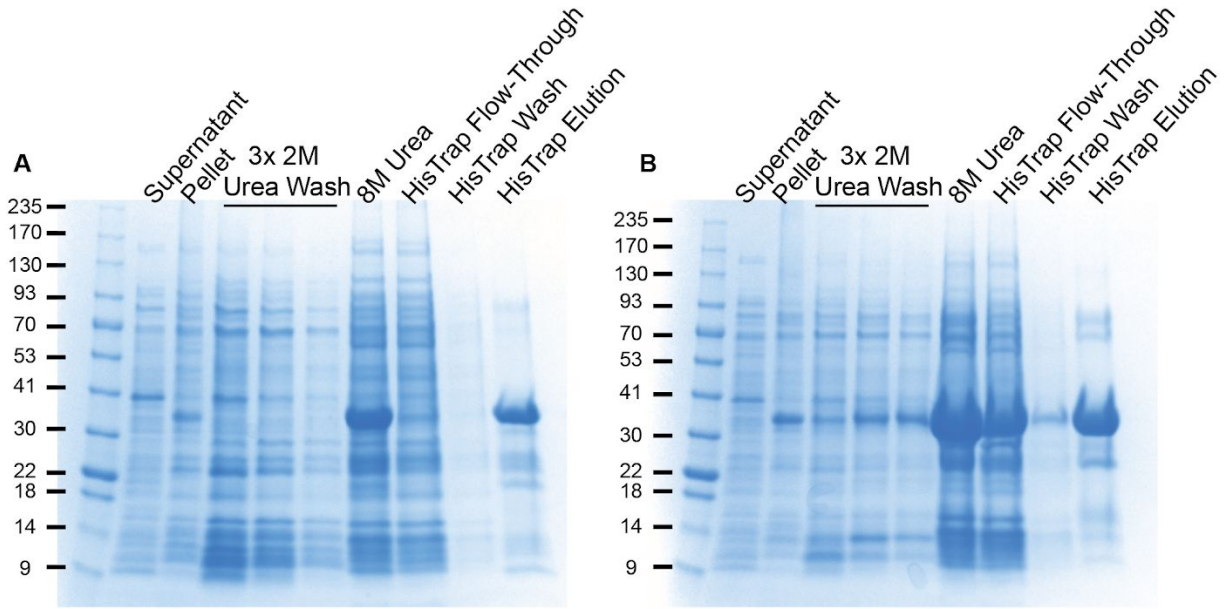
Supplemental Figure 1. BAR eFP predicts *PRX9* and *PRX40* are expressed in early flower buds.

Screen shots of Bioanalytic Resource (BAR) electronic fluorescent pictograph (eFP) (<http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>) (Waese and Provart 2017; Winter et al. 2007) expression patterns for *PRX9* (A) and *PRX40* (B). *PRX9* is expressed stage 9 flower buds and stage 12 - 15 carpels. *PRX40* is specifically expressed in stage 9 flower buds.



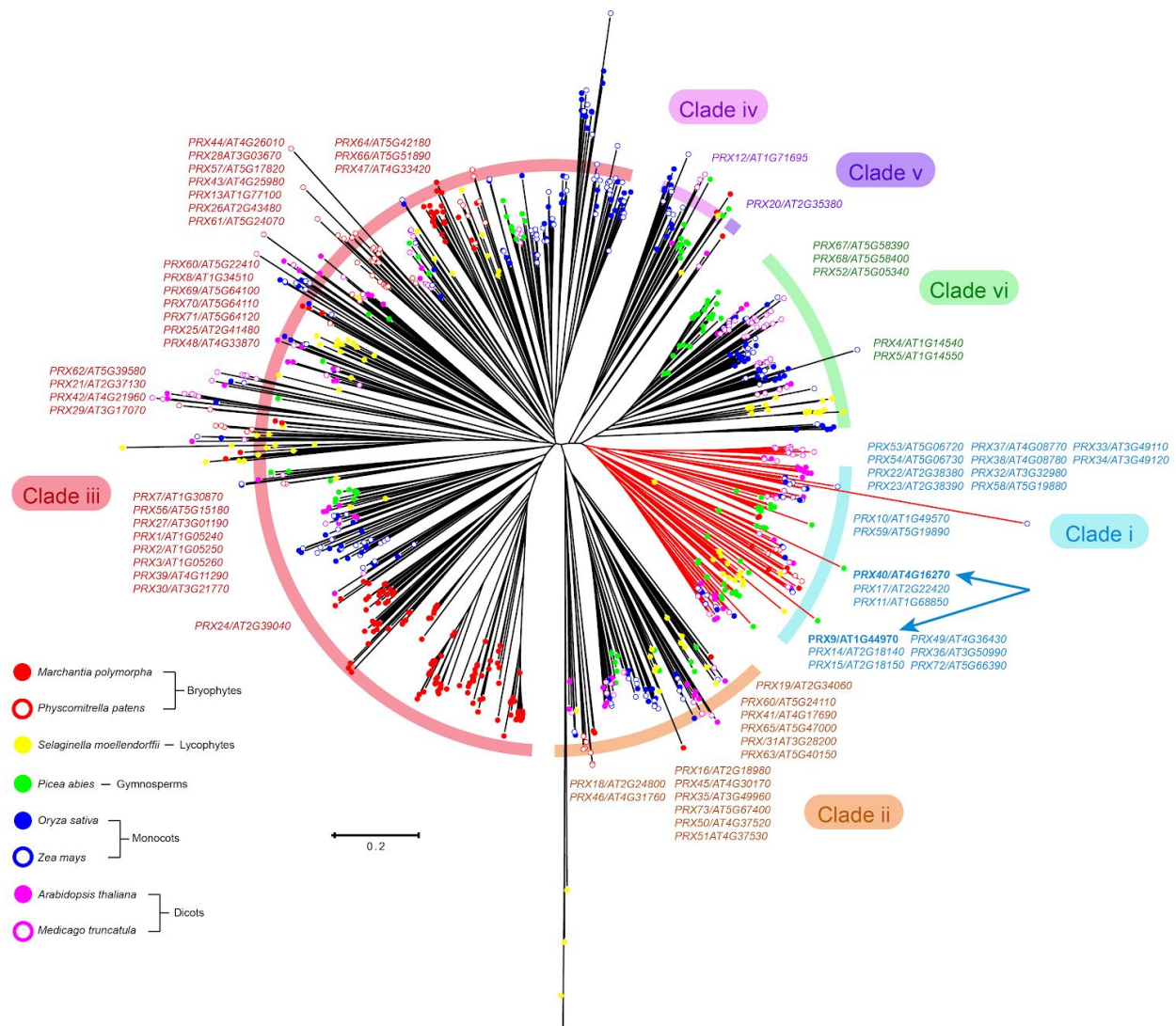
Supplemental Figure 2. Anther filaments from stage 14 *prx9-1 prx40-2* flower are not fully elongated.

Petals and sepals were removed from stage 14 wild-type (**Left Panel**) and *prx9-1 prx40-2* (**Right Panel**) flowers. Anther filaments from *prx9-1 prx40-2* flowers were shorter than those of wild-type flowers, indicated by the t-bars. Bars = 500 nm.



Supplemental Figure 3. Reconstituted enzyme activity can be obtained by solubilizing, purifying, and refolding protein from *E. coli* expressing PRX9 and PRX40.

Example SDS-PAGE gels visualizing steps in the purification of PRX9 (**A**) and PRX40 (**B**) by coomassie staining. Results from PRX9 (**C**) and PRX40 (**D**) refolding optimization. Refolding conditions were assayed in plate format and activity was determined by measuring the absorbance of the reactions at 420 nm with a plate reader. The absorbance in each condition is indicated by the color of the cells and by the number in the cells of the matrix. The top right cell is a positive control which used horseradish peroxidase. The remaining cells in the right-most columns were negative controls in which no peroxidase was added.



Supplemental Figure 4. Neighbor-joining phylogenetic analysis of class III peroxidases across land plants.

887 class III peroxidase protein sequences were collected, aligned, and used to produce a neighbor-joining tree with 500 bootstrap replications. The scale measures evolutionary distance in substitutions per amino acid. 6 distinct clades are seen in the phylogeny: clades i - vi. These clades are highlighted by various colors and the approximate locations of the 73 *Arabidopsis* class III peroxidases are placed around the phylogeny. PRX9 and PRX40 are placed in clade i, which have branches highlighted in red. PRX9 and PRX40 labels are bolded and indicated by blue arrows.

Supplementary Table 1. List of primers used in this study.

Name	Sequence	Used For
JRJ0489F	ATGGCTATCTCAAAGCTTATTCCTACTC	PRX9 RT-PCR (Forward)
JRJ0490R	TTAGTTAATCACATGACAACCTTCCTCTG	PRX9 RT-PCR (Reverse)
JRJ0491F	ATGTTAAAACCTCAGAAGAAAGTGGAGTG	PRX40 RT-PCR (Forward)
JRJ0492R	TTAGTTAATCATTCTACAATTCTTCCTAATCTC	PRX40 RT-PCR (Reverse)
JRJ0497F	CGTACAACCGGTATTGTGCTG	ACTIN2 RT-PCR (Forward)
JRJ0498R	GAAACATTTTCTGTGAACGATTCC	ACTIN2 RT-PCR (Reverse)
JRJ0187F	CATGATTACGCCAAGCTTTCTAGAGAAAATTTTGTAGCAT TGAAACAAGC	proPRX9 Cloning for GUS reporter (Forward)
JRJ0188R	AACATAAGGGACTGACCTACCCGGGTTTTGTTACTTGGGA GTTTTAAGATGAG	proPRX9 Cloning for GUS reporter (Reverse)
JRJ0193F	CATGATTACGCCAAGCTTTCTAGAGAGAGGTCAAGCTCTA GTAAAGACTAAAGACT	proPRX40 Cloning for GUS reporter (Forward)
JRJ0194R	AACATAAGGGACTGACCTACCCGGGTCAATGGAGTTGGTA CTATATTAGAAAGCTA	proPRX40 Cloning for GUS reporter (Reverse)
JRJ0191F	GAAAACCTTGTACTTCCAGGCCCATGGCCATCCAGGTCTAG GATTTGGATG	PRX9 pHis8-4 Gibson Cloning (Forward)
JRJ0192R	CTCGAATTCGGATCCGCCATGGTTAGTTAATCACATGACA ACTCTTCCTG	PRX9 pHis8-4 Gibson Cloning (Reverse)
JRJ0197F	GAAAACCTTGTACTTCCAGGCCCATGGCGAGAATCCAACCA ATTTCAAGC	PRX40 pHis8-4 Gibson Cloning (Forward)
JRJ0198R	CTCGAATTCGGATCCGCCATGGTTAGTTAATCATTCTACAA TTCTTCCTAATCTC	PRX40 pHis8-4 Gibson Cloning (Reverse)
JRJ0019LP	TACTACCACCGGTATCCCTC	prx40-1 SALK_031680 Genotyping LP
JRJ0020RP	CACGGTGTTAGAGGATCCAAG	prx40-1 SALK_031680 Genotyping RP
JRJ0021LP	GAGCTGTAAACGAAGTGCACC	prx40-2_SALK_061827 Genotyping LP
JRJ0022RP	TTCCTTATGTTTTTCTTTGCAATG	prx40-2_SALK_061827 Genotyping RP
JRJ0219RP	GTTTTAAGAAACCGGTCTCGG	prx9-1 SALK_204557C Genotyping RP
JRJ0220LP	GAAGTTAGGCACATGGATTCCG	prx9-1 SALK_204557C Genotyping LP
JRJ0221RP	TCCAACGTAAAGGTTTGAACG	prx9-2 SAIL_875_A09 Genotyping RP
JRJ0222LP	TTTGCTTTTTCTTAAATCCGC	prx9-2 SAIL_875_A09 Genotyping LP
SALK_LBb1.3	ATTTTGCCGATTTCCGGAAC	SALK Line Genotyping BP
SAIL_LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	SAIL Line Genotyping BP

114r	CCAACGCTGATCAATTCCAC	CSHL GeneTrap Genotyping BP
JRJ0556LP	TGGTGGAGGAGGCGACTT	GT8324 Genotyping LP
12f	ACCATACCAGTTGCAGTGATGAC	GT8324 Genotyping RP

Supplemental Figure Legends

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