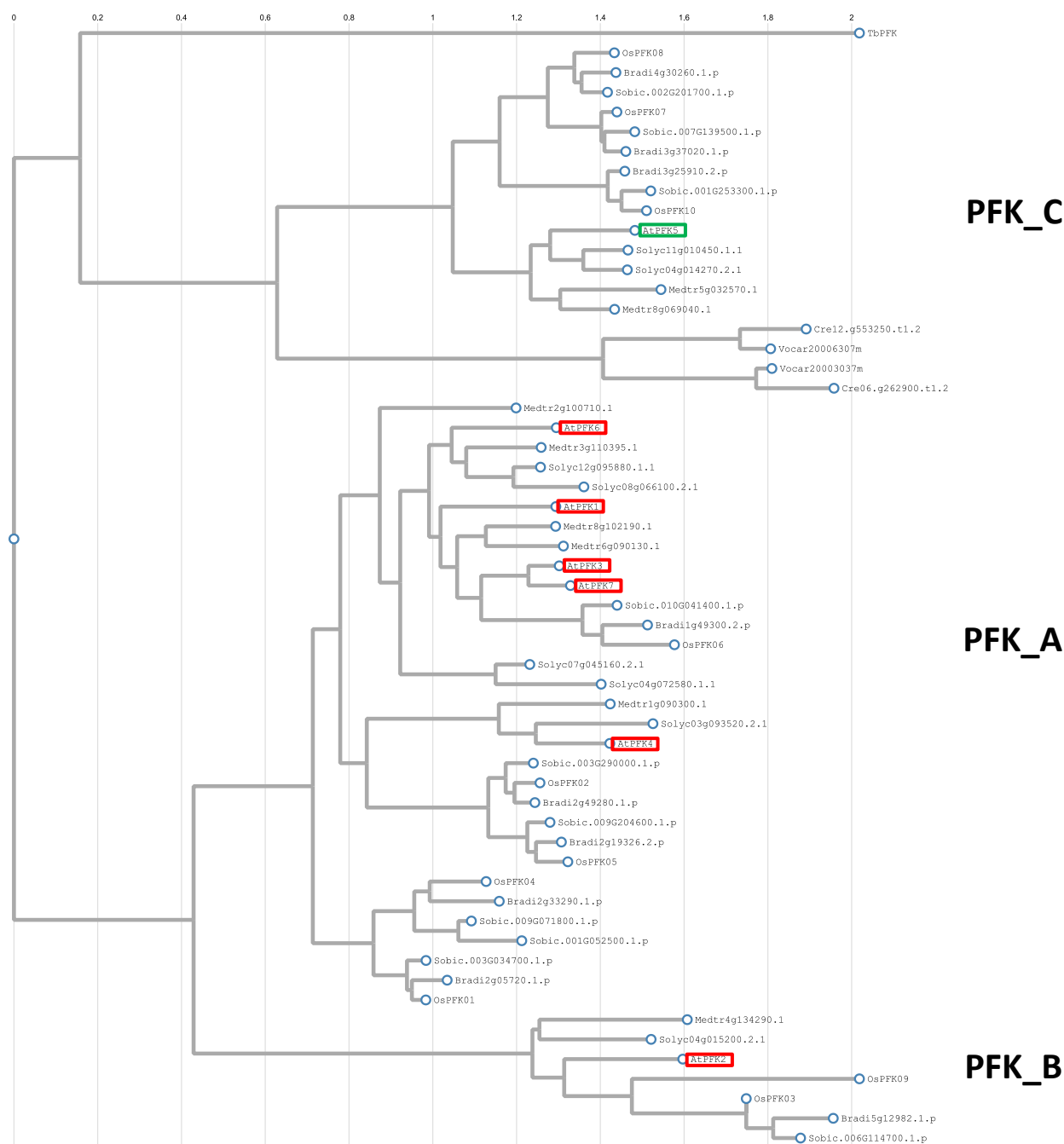


**The phosphofructokinase isoform AtPFK5 is a novel target of plastidic thioredoxin-f-dependent redox regulation**

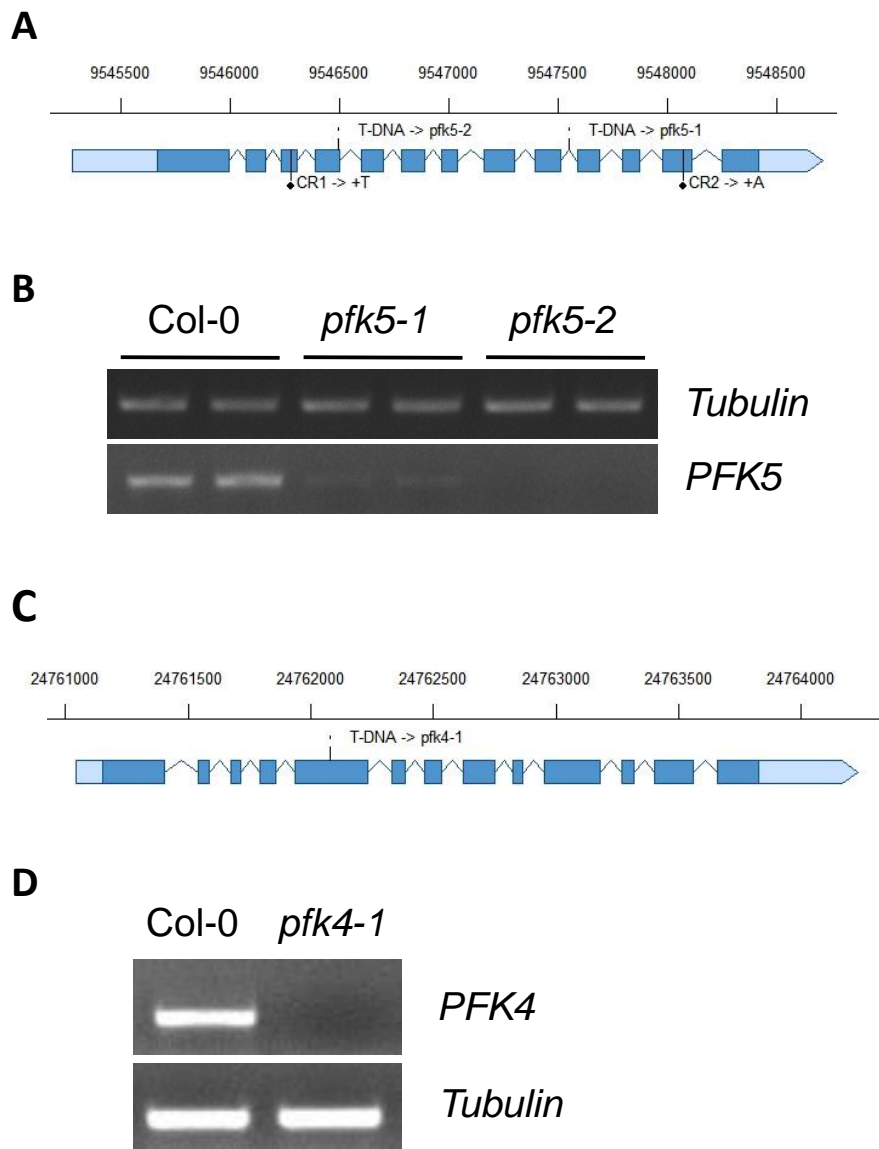
Natalia Hess, Simon Richter, Michael Liebthal, Karl-Josef Dietz, Angelika Mustroph

**SUPPLEMENTARY DATA**

**Supplementary Figures**



**Supplementary Figure S1:** Phylogeny of plant phosphofructokinase isoenzymes. PFK protein sequences were obtained from Phytozome and aligned using ClustalW using the platform <https://www.genome.jp/tools-bin/clustalw#clustalw.aln>. The phylogenetic tree was constructed with function "build" of ETE3 v3.1.1 (Huerta-Cepas et al., 2016) as implemented on the GenomeNet (<https://www.genome.jp/tools/ete/>). Species are abbreviated as follows: At, *Arabidopsis thaliana*; Bradi, *Brachypodium distachyon*; Cre, *Chlamydomonas reinhardtii*; Medtr, *Medicago truncatula*; Os, *Oryza sativa*; Sobic, *Sorghum bicolor*; Soly, *Solanum lycopersicum*; Tb, *Trypanosoma brucei*; Vocar, *Volvox carteri*. Protein IDs are found in Supplementary Table S1b. PFKs from Arabidopsis are highlighted in red, the target isoform of this study, AtPFK5, is highlighted in green.



**Supplementary Figure S2:** Genotype characterization of T-DNA and CRISPR mutants for *AtPFK4* and *AtPFK5*. (A, C) Gene models for *AtPFK5* and *AtPFK4*, including site of mutations. (B, D) Transcript levels of *AtPFK5* and *AtPFK4* in wildtype Col-0 and T-DNA insertion mutant seedlings. Tubulin was used as the reference.

**Supplementary Figure S3 (continued over the next pages):** Alignment of plant phosphofructokinase isoenzymes, which is the basis for the phylogenetic tree presented in Supplementary Fig. S1. Cys residues are in red font, and conserved motifs are marked with asterisks above the alignment.

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**Clustal Consensus** 1

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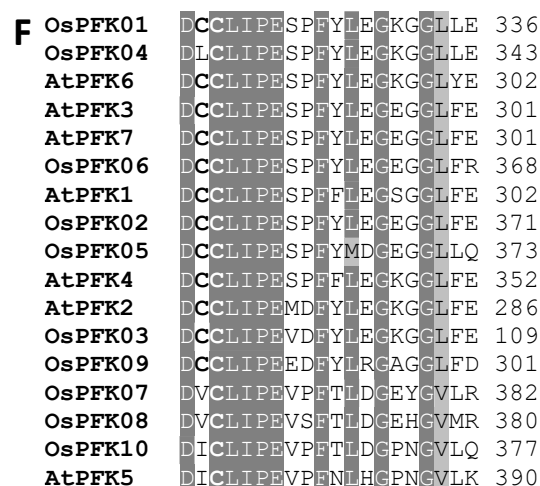
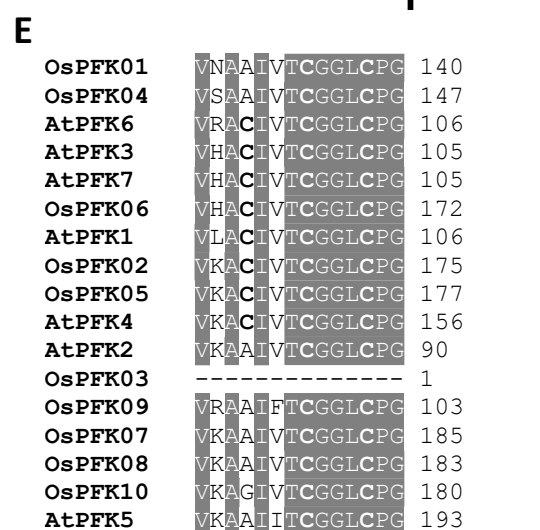
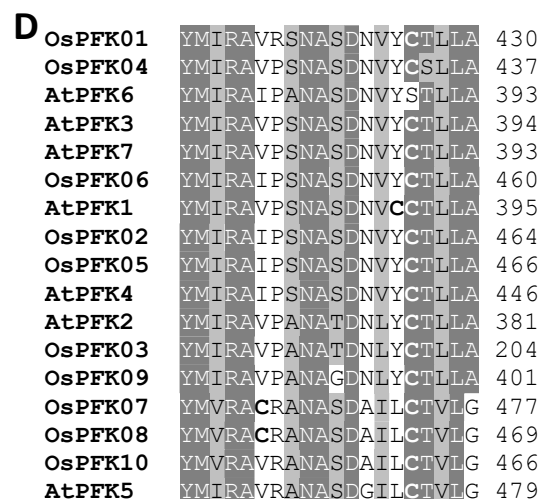
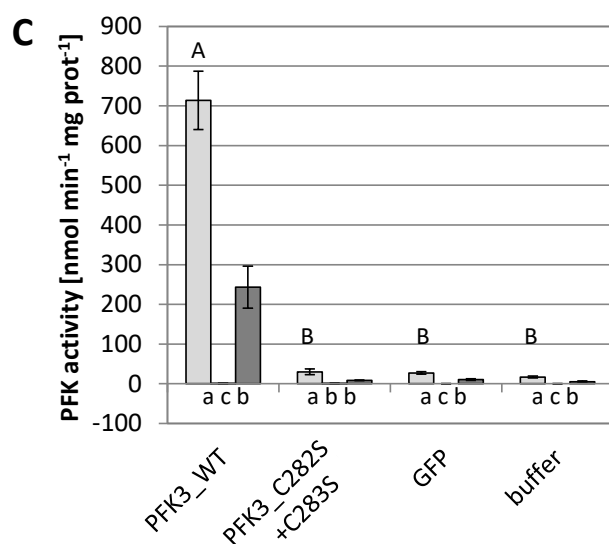
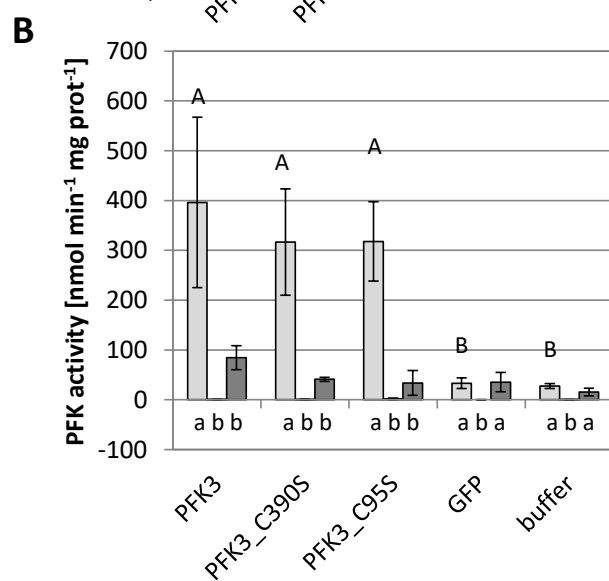
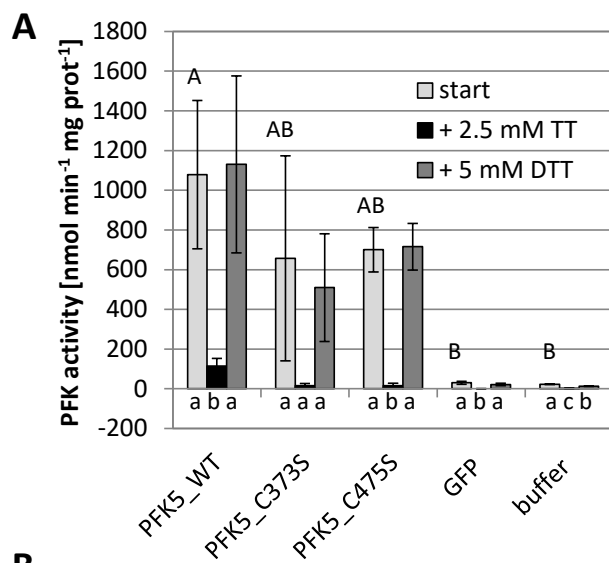
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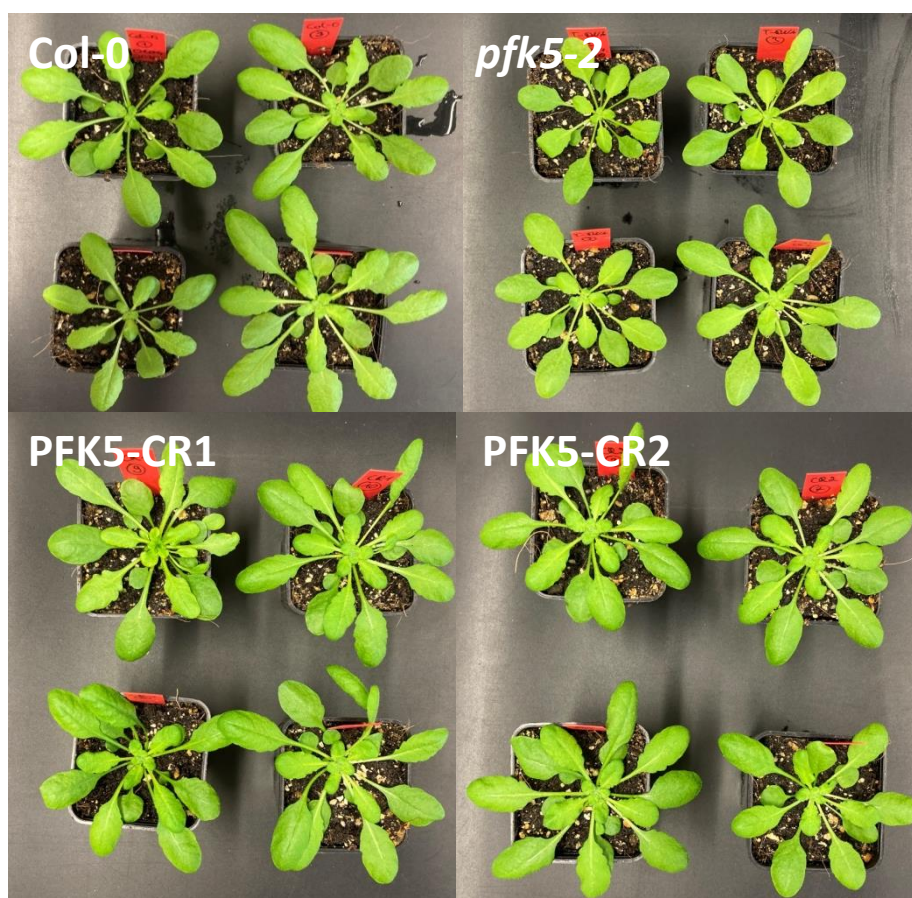
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Medtr2g100710.1 421 IEFHLINEGQKRVITDRMMARLLSSTHQPSTFVNPQHITBEAKVE----- 465
OsPFK02 489 IEFHRTVSTRNKVKITDRMMARLLSSTNQPSFLSQKDI DAAREADKLASKSPVNTKEHGENVKKPPANG 558
Bradi2g49280.1.p 488 IEFHRTVSTRNKVKITDRMMARLLSSTNQPSFLTQKDI DAAREADRLANRPVPAGTSELREHTEKHPAS 557
Sobic.003G290000.1.p 499 IEFHRTVSTRNKVKITDRMMARLLSSTNQPSFLSQKDI DAAREADKAANAKNQAPALANGEK----- 561
Sobic.009G204600.1.p 497 IEFYRVTSTRNKVKITDRMMARLLSSTNQPSFLSQKDI DAASEADRLANRPPLPTGASSRVARSFQSSS 566
Bradi2g19326.2.p 477 IEFYRVTSTRNKVKITDRMMARLLSSTNQPSFLSQKDI DAAREADRLANKPPLPSGANQSVANAFQGSAS 546
OsPFK05 491 IEFYRVTSTRNKVKITDRMMARLLSSTNQPSFLSQKDI DA AKENDRTANKPPLPTGLSHHVANSFDQAS 560
AtPFK4 472 IPTSQVTEVTNTVKLITDRMMARLLSSTNQPSFLTGEALQNVIDMETQEKIDNMKISSI----- 530
Solyc03g093520.2.1 482 IEFNRVTEVTNTVNMTRGRMMARLLSSTNQPSFVN--HQTLRERVDKNTIDAINNMKINST----- 539
Medtr1g090300.1 488 IPIACVTERPNTVQLTGRMMARLLSSTNQPSFVS---SDQQRVEKEMVEDTNKMNITSI----- 543
Sobic.006G114700.1.p 448 IPLEDVAVAKNPVDVNDHKKAWVRSVFNQPDFLKSQA----- 484
Bradi5g12982.1.p 431 IPLEDVAVAKNPVDVNDHKKAWVRSVFNQPDFLKPQDY----- 468
OsPFK03 230 IPLEDVAVAKNPVDVNDHKKAWVRSVFNQPDFMFKPKY----- 266
AtPFK2 407 IPLEEVAQTKNQVNTDRHKKAWVRSVFNQPDFETNVKG----- 444
Medtr4g134290.1 426 IPLEDVAVAKNPVNTKDHNNQSWVRSVFNQPDFLKS----- 460
Solyc04g015200.2.1 422 IVDVAVAKARKVDTRHKKAWVRSVFNQPDFFIRS----- 456
OsPFK09 427 IEMDEVAEAKNPVDTRHKKAWVRSVFNQPDFIRAGPTS----- 465
ThPFK 425 VETKVAATSVRRVLDLRGQLRQVREITVLDLGSVDVRLARKLEIRELEAINRNRDRLHEELAKL----- 487
Clustal Consensus 79 .*. . : . * : 83

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Solyc04g014270.2.1	533	-----	533
Solyc11g010450.1.1	532	-----	532
AtPFK5	537	-----	537
Medtr5g032570.1	533	-----	533
Medtr8g069040.1	529	-----	529
OsPFK07	535	-----	535
Bradi3g37020.1.p	530	-----	530
Sobic.007G139500.1.p	527	-----	527
Sobic.002G201700.1.p	529	-----	529
Bradi4g30260.1.p	531	-----	531
OsPFK08	527	-----	527
OsPFK10	524	-----	524
Sobic.001G253300.1.p	524	-----	524
Bradi3g25910.2.p	526	-----	526
Cre06.g262900.t1.2	529	-----	529
Vocar20003037m	523	-----	523
Cre12.g553250.t1.2	512	-----	512
Vocar20006307m	513	-----	513
Medtr6g090130.1	488	CLAPLAVPVLRTGGISGV-----	505
Medtr8g102190.1	475	-----	475
AtPFK3	489	K-----	489
AtPFK7	485	K-----	485
Sobic.010G041400.1.p	539	-----	539
Bradi1g49300.2.p	548	NGVK-----	551
OsPFK06	556	AVK-----	558
AtPFK1	473	-----	473
Solyc08g066100.2.1	492	LLPCLSCGQ-----	500
Solyc12g095880.1.1	485	-----	485
Medtr3g110395.1	492	PTQLMEGDKSKDNQKSRNLADSDSCIKK	519
AtPFK6	462	-----	462
Solyc04g072580.1.1	480	INEANKNKEPPELSDGETN-----	499
Solyc07g045160.2.1	489	TF-----	490
OsPFK01	525	NGTAAAPV-----	531
Sobic.003G034700.1.p	527	NGTV-----	530
Bradi2g05720.1.p	536	NGFAAPL-----	542
OsPFK04	529	ANAGDRAALCNGAA-----	542
Bradi2g33290.1.p	523	MNGHGHF-----	530
Sobic.001G052500.1.p	528	DVQRANGNHL-----	538
Sobic.009G071800.1.p	526	NGNHLCSGAA-----	536
Medtr2g100710.1	465	-----	465
OsPFK02	559	EK-----	560
Bradi2g49280.1.p	558	ESANGEK-----	564
Sobic.003G290000.1.p	561	-----	561
Sobic.009G204600.1.p	567	SSNGEI-----	573
Bradi2g19326.2.p	547	SSSNGEI-----	553
OsPFK05	561	SSSNSQI-----	567
AtPFK4	530	-----	530
Solyc03g093520.2.1	539	-----	539
Medtr1g090300.1	543	-----	543
Sobic.006G114700.1.p	484	-----	484
Bradi5g12982.1.p	468	-----	468
OsPFK03	266	-----	266
AtPFK2	444	-----	444
Medtr4g134290.1	460	-----	460
Solyc04g015200.2.1	456	-----	456
OsPFK09	465	-----	465
TbPFK	487	-----	487
Clustal Consensus	83	-----	83

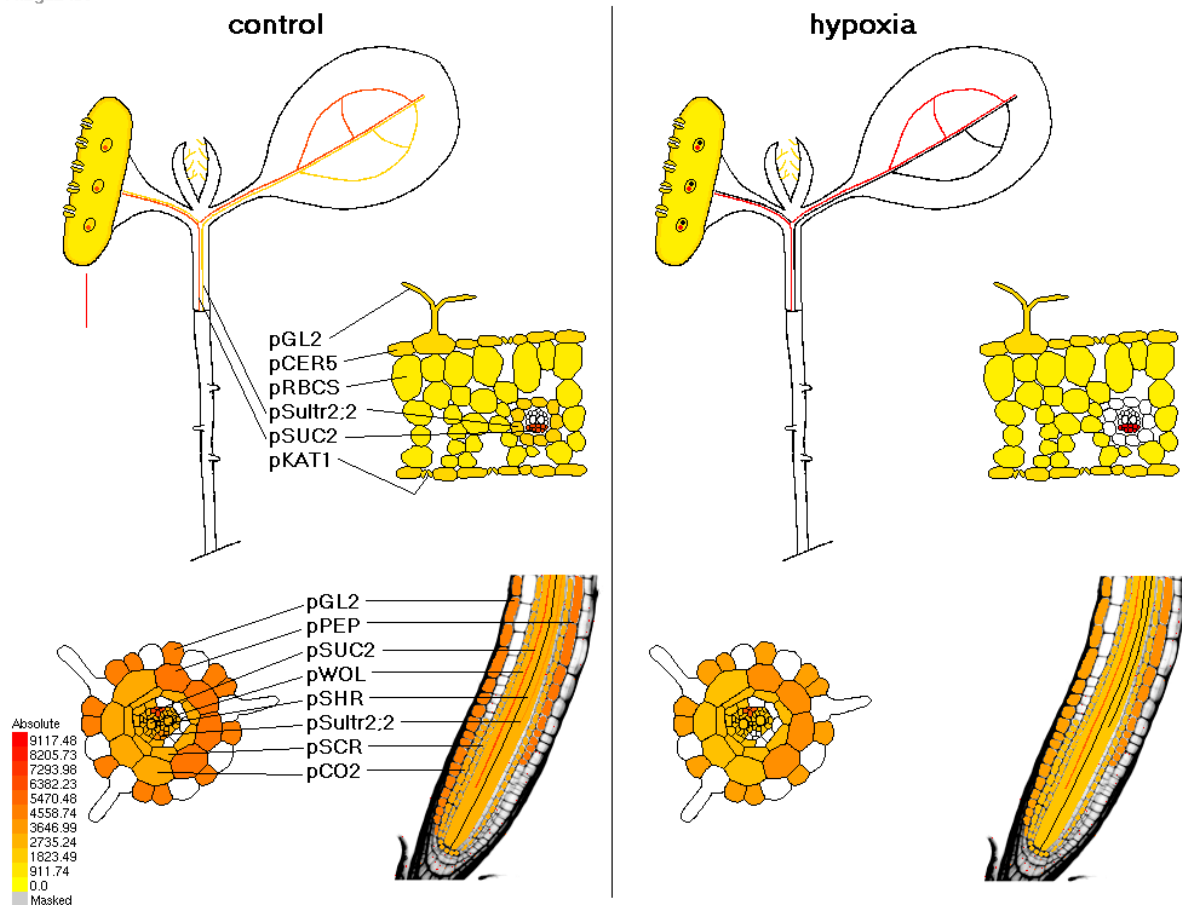
**Supplementary Figure S4 (next page):** Significance of the remaining conserved Cys for redox regulation. Cys to serine single and double mutations were introduced into *A. thaliana* AtPFK5 (A) and AtPFK3 (B;C). The variants were transiently overexpressed in tobacco leaves. PFKs from the tobacco leaf samples were extracted with buffer containing 5 mM  $\beta$ -mercaptoethanol ( $\beta$ -ME). After initial activity determination, samples were oxidized with 2.5 mM sodium tetrathionate (TT) for 20 min, and afterwards re-reduced with 5 mM dithiothreitol (DTT). Upper case letters indicate significant differences for each isoform to buffer and GFP control, lower case letters indicate significant differences among treatments for each isoform at  $p < 0.05$  (ANOVA, Post-hoc Tukey test,  $n=3-6$ ). The results are means  $\pm$  SD. (D-F) Details of the protein alignment of conserved Cys in PFKs from *A. thaliana* and *O. sativa*. The mutated Cys are marked with an arrow.





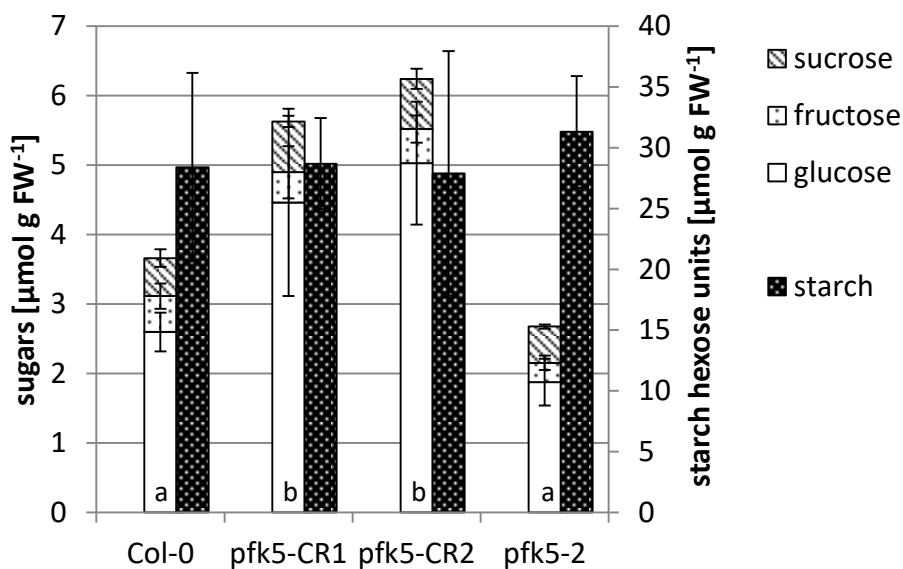
**Supplementary Figure S5:** Representative pictures of five-week-old *pfk5-CR1*, *pfk5-CR2*, *pfk5-2* and wildtype Col-0 plants grown on soil (representative pictures from the experiment in Figure 9C). One pot has the width of 5.3 cm.

At2g22480 264044\_at



**Supplementary Figure S6:** Expression atlas of *AtPFK5* from the eFP browser (<http://bar.utoronto.ca/>, Winter et al. 2007, Mustroph et al. 2009).





**Supplementary Figure S7:** Sugar content in leaves of five-week-old *pfk5-CR1*, *pfk5-CR2*, *pfk5-2* and wildtype Col-0 plants grown on soil, harvested at the end of the day. Data are means  $\pm$  SD of  $n=4$ . Letters indicate significant differences at  $p < 0.05$  for glucose content (ANOVA, Post-hoc Tukey test). Fructose, sucrose and starch contents were not significantly different between genotypes.

**Supplementary Table S1 (separate Excel file):** Primer sequences (a) and Protein IDs (b) used in this study

#### Supplementary References:

- Huerta-Cepas J, Serra F, Bork P (2016) ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. *Mol Biol Evol* 33: 1635-1638.
- Mustroph A, Zanetti ME, Jang CJ, Holtan HE, Repetti PP, Galbraith DW, et al. (2009) Profiling translomes of discrete cell populations resolves altered cellular priorities during hypoxia in Arabidopsis. *Proc Natl Acad Sci USA* 106: 18843-18848.
- Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. *PLoS One* 2: e718.