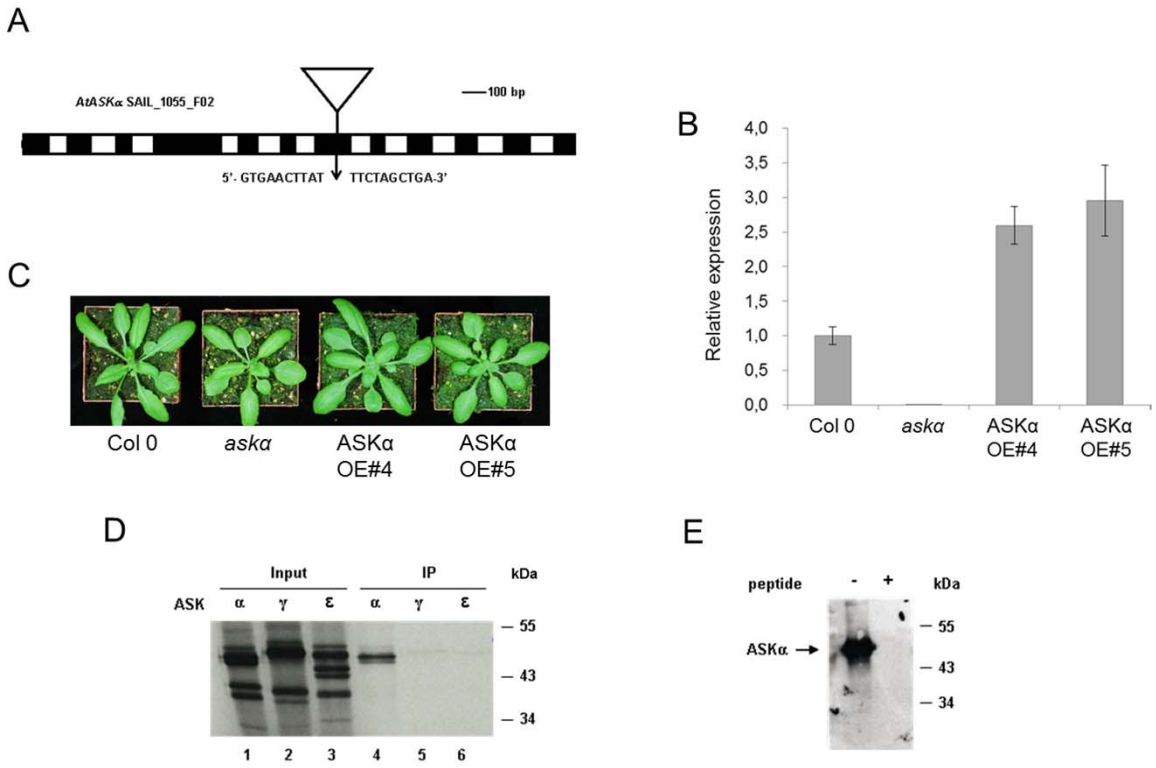


Supplemental Figure 1. The *askα* T-DNA insertion mutant and specificity of the anti-ASKα antibody.

(A) Schematic diagram of *ASKα* showing the site of T-DNA insertion in *askα*. Black boxes indicate coding and white boxes intron regions.

(B) qRT-PCR analysis of wild-type Col 0, *askα* and ASKα overexpressor lines OE#4 and Oe#5 showing the absence of a full-length *ASKα* transcript in *askα* plants and enhanced *ASKα* transcript levels in ASKα overexpressor lines. Data are means ± relative SD of three independent experiments.

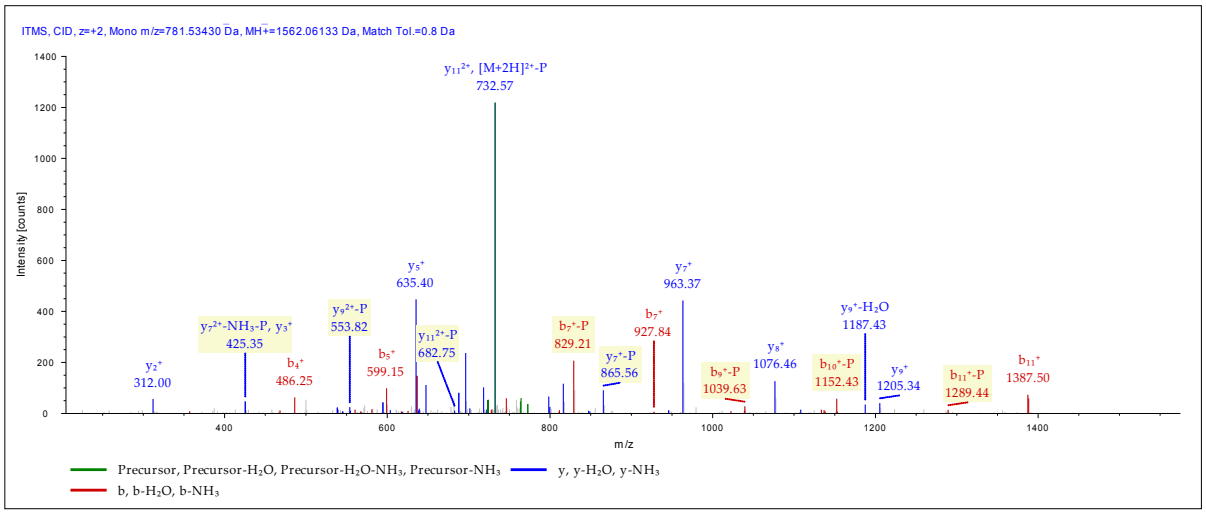
(C) Four-week-old *A. thaliana* wild-type Col 0, *askα* and ASKα-overexpressor lines OE#4 and OE#5 grown under control conditions in soil.

(D) ^{35}S-methionine-labelled *in vitro*-translated proteins ASKα, ASKγ and ASKε (lanes 1 to 3, respectively) and immunoprecipitations (IP) of the *in vitro*-translated proteins ASKα, ASKγ and ASKε with anti-ASKα antibody (lanes 4 to 6, respectively).

(E) Immunoprecipitation blot analysis of *Arabidopsis* leaf protein extract. ASKα was immunoprecipitated with ASKα-specific antibodies from 100 µg of total protein extract. Subsequent analysis was performed with an anti-ASKα antibody with (+) or without (-) prior blocking of the antibody with the N-terminal ASKα peptide.

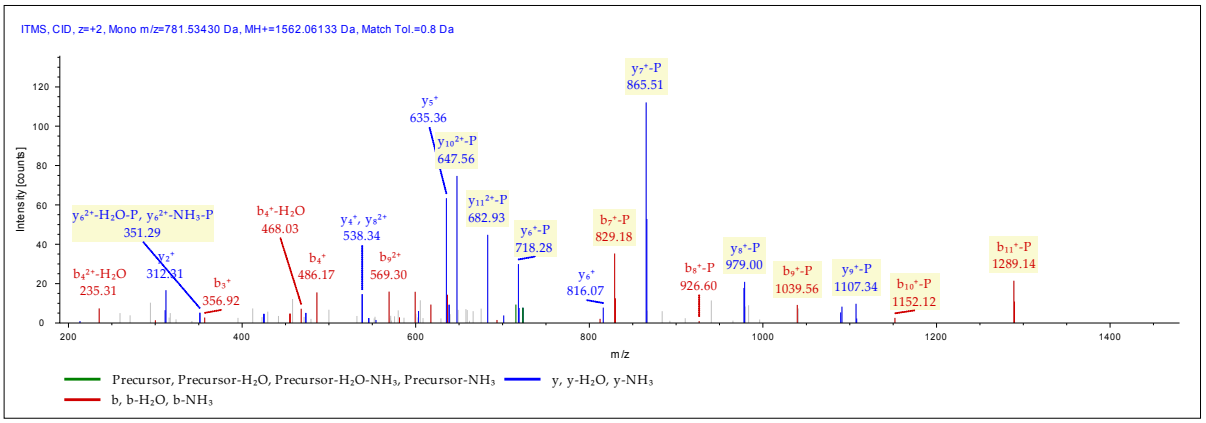
A

CID MS/MS spectrum of VAWEIF#TPLLHR (m/z 781.53 Da); Xcorr:3.41; phospho RS score:114



B

CID MS/MS/MS spectrum of VAWEIF#TPLLHR (m/z 781.53 Da); Xcorr:2.18



C

MS/MS of m/z 781.53 Da

#1	b⁺	b²⁺	Seq.	y⁺	y²⁺	#2
1	100.07570	50.54149	V			12
2	171.11282	86.06005	A	1462.72432	731.86580	11
3	357.19214	179.09971	W	1391.68720	696.34724	10
4	486.23474	243.62101	E	1205.60788	603.30758	9
5	599.31881	300.16304	I	1076.56528	538.78628	8
6	746.38723	373.69725	F	963.48121	482.24424	7
7	927.40124	464.20426	T-Phospho	816.41279	408.71003	6
8	1024.45401	512.73064	P	635.39878	318.20303	5
9	1137.53808	569.27268	L	538.34601	269.67664	4
10	1250.62215	625.81471	L	425.26194	213.13461	3
11	1387.68106	694.34417	H	312.17787	156.59257	2
12			R	175.11896	88.06312	1

D

MS/MS/MS of m/z 781.53 Da

#1	b⁺	b²⁺	Seq.	y⁺	y²⁺	#2
1	100.07570	50.54149	V			12
2	171.11282	86.06005	A	1462.72432	731.86580	11
3	357.19214	179.09971	W	1391.68720	696.34724	10
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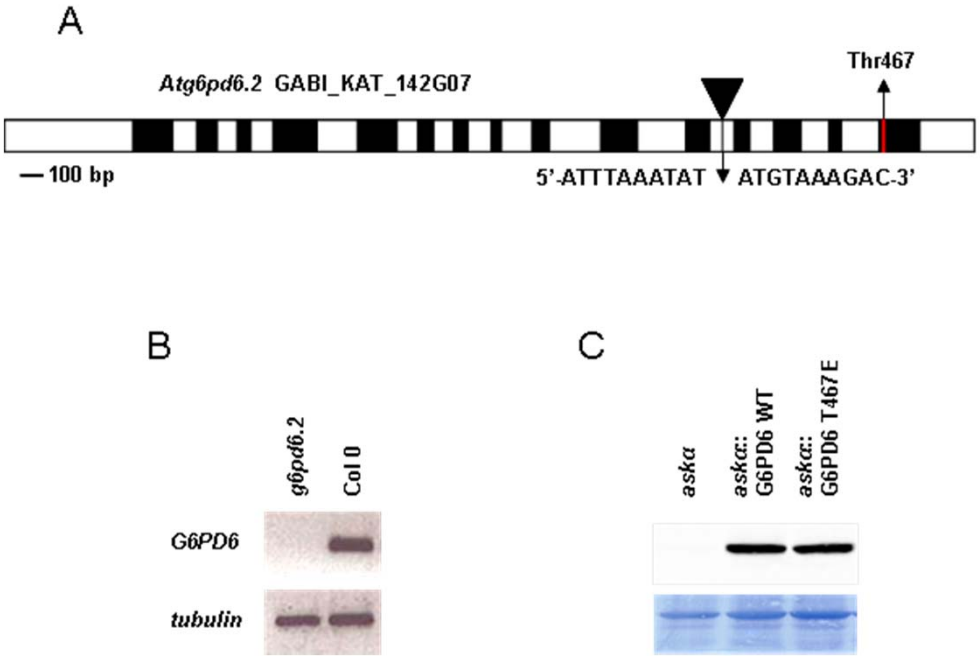
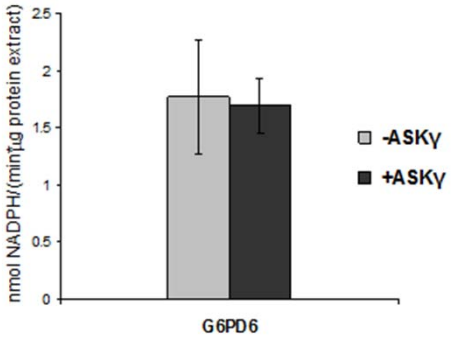
11	1387.68106	694.34417	H	312.17787	156.59257	2
12			R	175.11896	88.06312	1

Supplemental Figure 2. Mass spectrometry (MS) analysis of G6PD6 phosphorylation by ASKα. (**A**) CID (collision induced decay) MS/MS and (**B**) CID MS/MS/MS spectrum of VAWEIF#TPLLHR. (**C, D**) List of fragment ions of MS/MS (C) and MS/MS/MS (D) of m/z 781.53 Da. Detected fragment ions are labeled red or blue for b or y ions, respectively.

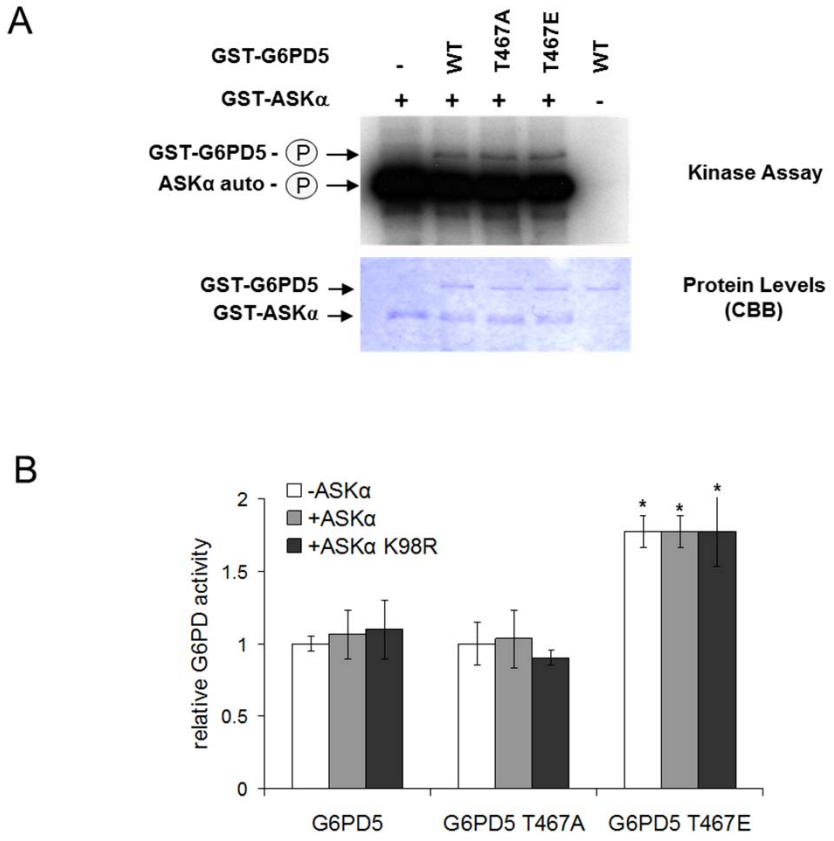
Supplemental Figure 3. G6PD6 threonine 467 is conserved.
Partial protein alignment of eukaryotic G6PDs was performed with ClustalW using Blosum as protein weight matrix, 10 as GAP Open and 0,1 as Gap Extension. Thr467 of G6PDs is boxed. Asterisks denote identical residues, colons indicate conserved amino acid substitutions and periods designate semi-conservative substitutions. AtG6PD6 and AtG6PD5, *Arabidopsis thaliana*, Q9FJI5 and Q9LK23, respectively; Potato, *Solanum tuberosum*, P37830; Poplar, *Populus trichocarpa*, Q2L9V8; Grapevine, *Vitis vinifera*, D7UBH2; Rice, *Oryza sativa* subsp. japonica, Q8RY51; Wheat, *Triticum aestivum*, Q9LR19; *Medicago truncatula*, G7KS28; Pea, *Pisum sativum*, A6XIG0; *Chlamydomonas reinhardtii*, A8J6N3; *Physcomitrella patens* subsp. patens, A9TA54; Yeast, *Saccharomyces cerevisiae*, P11412; Drosophila, *Drosophila mojavensis*, B4L7Z3; Mouse, *Mus musculus*, P97324; Human, *Homo sapiens*, P11413.



Supplemental Figure 4. ASKα and G6PD6 function in the same signaling pathway.
(A, B) *g6pd6.2* T-DNA insertion mutant. Schematic diagram of G6PD6 showing the site of T-DNA insertion in the *g6pd6.2* mutant (A). Black boxes indicate coding and white boxes indicate non-coding regions. The position of Thr467 is depicted in red. RT-PCR analysis of wild-type (WT) and *g6pd6.2* (B). No full length *G6PD6* transcript was present in *g6pd6.2* plants in three independent biological replicas.
(C) Wild-type G6PD6-HA and G6PD6T467E-HA protein levels in *askα* plants. Upper: Wild-type G6PD6-HA and G6PD6T467E-HA protein levels were detected by western blot analysis using an anti-HA antibody. Lower: Coomassie stained gel.



Supplemental Figure 5. ASKγ does not enhance G6PD6 activity *in vivo*.
In vivo G6PD activity of *Arabidopsis* protoplasts transformed with G6PD6-HA or co-transformed with G6PD6-HA and ASKγ-myc. Data are means ± SD with n = 3. The assays were performed three times independently and showed similar results.



Supplemental Figure 6. Characterization of Thr467 of G6PD5.
(A) *In vitro* kinase assay with GST-ASKα and GST-G6PD5, GST-G6PD5 T467A or GST-G6PD5 T467E. The experiment was repeated three times showing comparable results.
(B) G6PD5 T467E displays enhanced enzymatic activity. Enzymatic activity of G6PD5, G6PD5 T467A and G6PD5 T467E. G6PD6 activity was quantified after a phosphorylation reaction with ASKα, ASKα K98R or without kinase. Data are means ± relative SD. The assay was performed four times using fresh proteins from independent purifications. Asterisk indicates a significant difference (*P<0.05) tested by Student´s t-test for pairwise comparison to the un-phosphorylated control.

Gene	Primer Name	Primer Sequence	Purpose
At-*ASKα*	qRT_ASKα_11/12ex	(F) 5'-AAGAGACCCAAACGCACGTCTACC-3'	qRT-PCR
		(R) 5'-ACACCTTTCAGCTCGTGAGGCTT-3'	qRT-PCR
	ASKα cDNA	(F) 5'-GGATCCTTATGGCGTCAGTGGGTATAGCTCC-3'	Cloning
		(R) 5'-GGATCCGCGGCCGCAAACCGAGCCAAGGACACTGC-3'	Cloning
	ASKα K98R	(F) 5'-GTTGCGATAAGGAAAGTTTTACAAGATAGG-3'	Mutagenesis
		(R) 5'-CCTATCTTGTAAAACTTTCCTTATCGCAAC-3'	Mutagenesis
	ASKα1 5'	(F) 5'-TGGCTGAGCGTGTTGTTGGTCA-3'	Genotyping
	ASKα 9ex 3'	(R) 5'-GAATTTGAATTCCGTGTAGTTTGG-3'	Genotyping
At-*G6PD6*	G6PD6 cDNA	(F) 5'-GCATGGGATCTGGTCAATGGCACGTTGAG-3'	Cloning pGEX4T1
		(R) 5'-TAGTGTAGGAGGGATCCAGATATAGCC-3'	Cloning pGEX4T1
	G6PD6 cDNA1	(F) 5'-GGAGGTGGATCCATGGGATCTGGTCAATGG-3'	Cloning pGWR8
		(R) 5'-ACCTCCGGATCCTAGTGTAGGAGGAATCCAG-3'	Cloning pGWR8
	G6PD6 T467A	(F) 5'-GCGTGGGAGATCTTCGCGCCGCTACTCCACAGG-3'	Mutagenesis
		(R) 5'-CCTGTGGAGTAGCGGCGCGAAGATCTCCCACGC-3'	Mutagenesis
	G6PD6 T467E	(F) 5'-GCGTGGGAGATCTTCGAGCCGCTACTCCACAGG-3'	Mutagenesis
		(R) 5'-CCTGTGGAGTAGCGGCTCGAAGATCTCCCACGC-3'	Mutagenesis
	G6PD6 9ex	(F) 5'-TTGCCATGGAGAAACCAATATCTC-3'	Genotyping
	G6PD6 13ex	(R) 5'-ACCTTGATACCGTTGCCCATACG-3'	Genotyping
At-*G6PD5*	G6PD5 cDNA	(F) 5'-GCATGGGTTCTGGTCAATGGCATATGGAG-3'	Cloning pGEX4T1
		(R) 5'-CAATGTAGGAGGGATCCAAATGTAGCC-3'	Cloning pGEX4T1
	G6PD5 cDNA1	(F) 5'-GGAGGTGGATCCATGGGTTCTGGTCAAT-3'	Cloning pGWR8
		(R) 5'-ACCTCCGGATCCCAATGTAGGAGGAATCC-3'	Cloning pGWR8
At-*β-Tubulin*	RT-TUB	(F) 5'-ACCACTCCTAGCTTTGGTGATCTG-3'	RT-PCR
		(R) 5'-AGGTTCACTGCGAGCTTCCTCA-3'	RT-PCR
At-*PP2A*	qRT-PP2A	(F) 5'-TAACGTGGCCAAAATGATGC-3'	qRT-PCR
		(R) 5'-GTTCTCCACAACCGCTTGGT-3'	qRT-PCR
At-*GAPDH*	qRT-GAPDH	(F) 5'-TTGGTGACAACAGGTCAAGCA-3'	qRT-PCR
		(R) 5'-AAACTTGTCGCTCAATGCAATC-3'	qRT-PCR
Gabi T-DNA LB	GK_LB	(R) 5'-CCCATTTGGACGTGAATGTAGACAC-3'	Genotyping
Sail T-DNA LB	SAIL_LB1	(R) 5'-TAGCATCTGAATTTCATAACCAATCTCGA-3'	Genotyping

Supplemental Table 1. List of primers.
Mutated nucleotides are depicted in red. The BamHI site is shown in pink, the NotI site in green, and in blue nucleotides added to maintain the right frame.