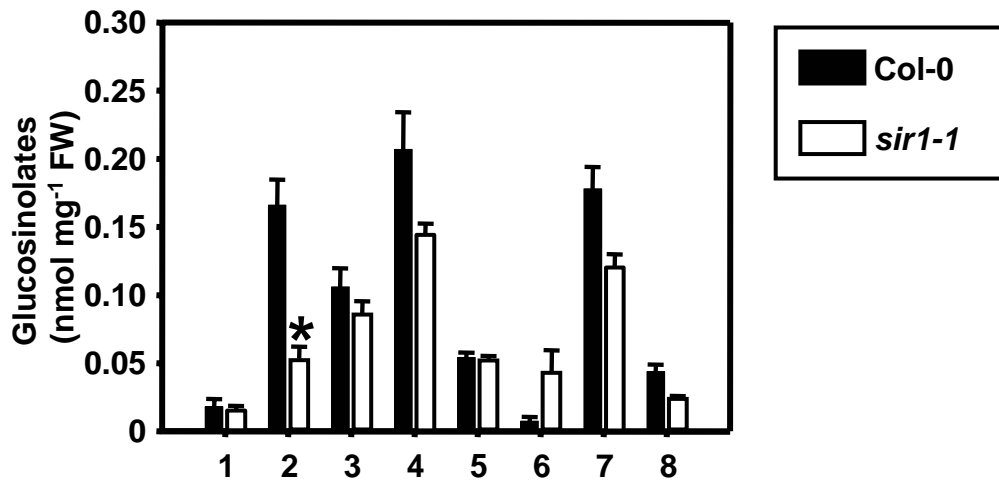
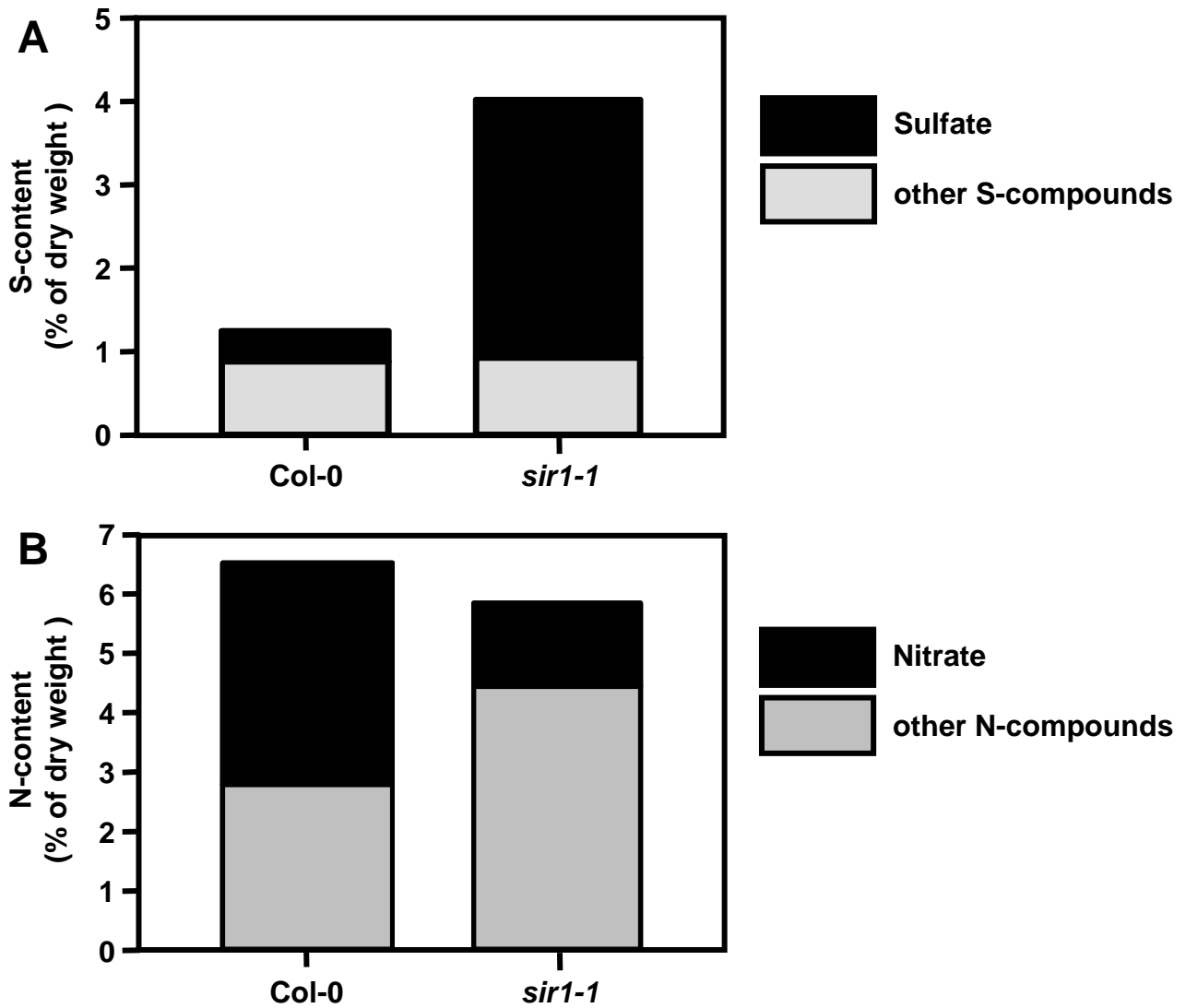


**Supplemental Figure 1: Thiol levels of genetically complemented *sir1-1* plants** (A) Cysteine and (B) Glutathione steady state levels in leaves of 7 week old wild type (black), *sir1-1* (white) and three complemented *sir1-1* lines (gray) grown on soil under short day conditions. Mean  $\pm$  SE (n = 5) are shown. Asterisks indicate statistical significance (P < 0.05). FW, fresh weight



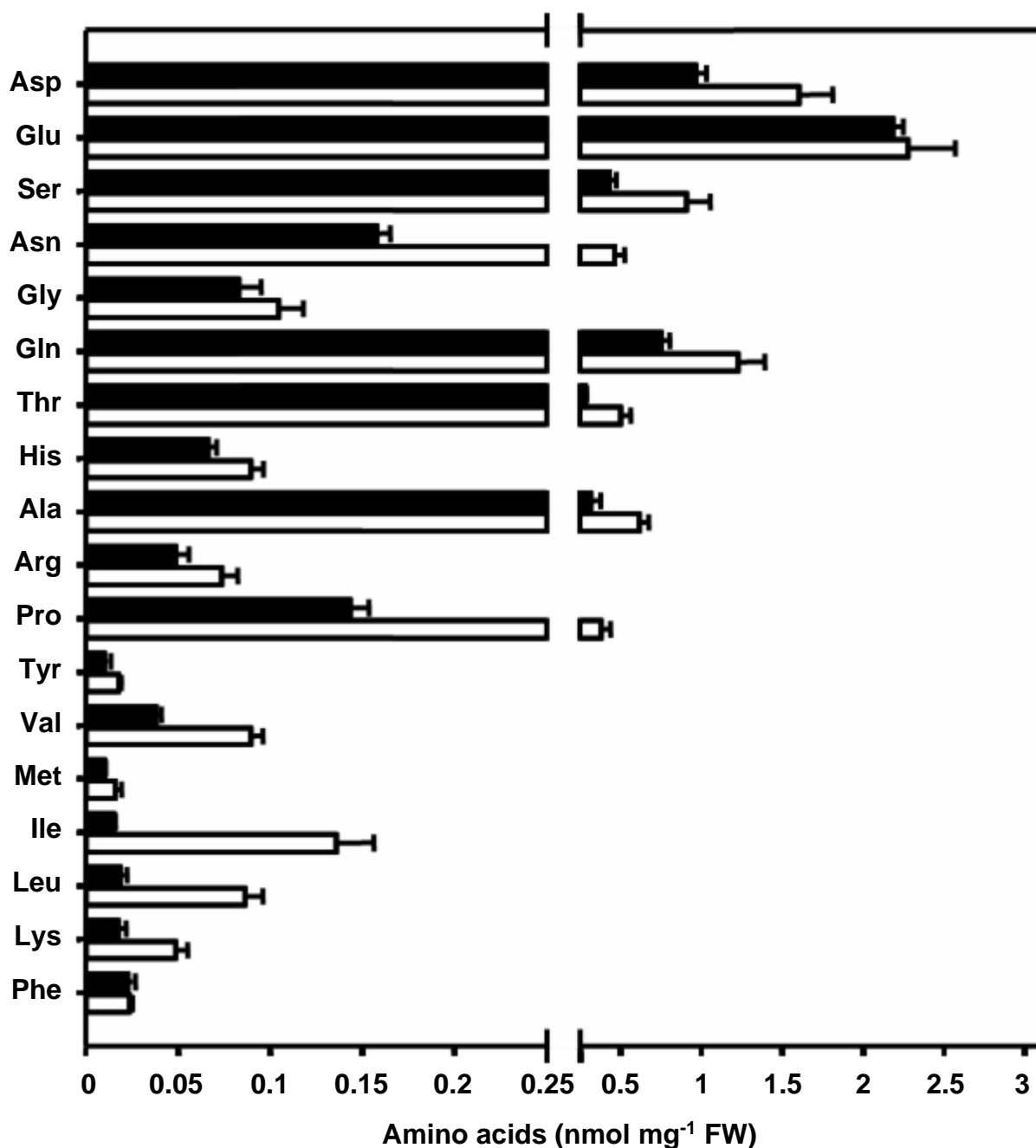
**Supplemental Figure 2: Spectrum of glucosinolates in *sir1-1* plants**

Steady state levels of the major glucosinolates in leaves of 7 week old wild type (black) and *sir1-1* (white) plants grown on soil in a growth chamber under short day conditions. Mean  $\pm$  SE (n = 5) are shown. Asterisks indicate statistical significance (P < 0.05). 1: glucoibarin, 2: glucoeurocin, 3: glucohirsutin, 4: glucobrassicin, 5: 4-methoxy.glucobrassicin, 6: neoglucobrassicin, 7: glucoiberin, 8: glucoalyssin.



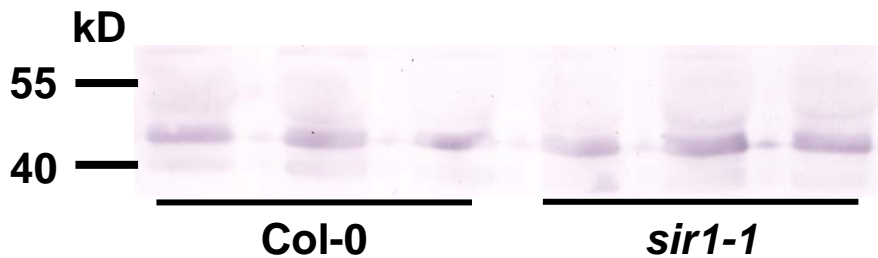
**Supplemental Figure 3: Contribution of anions to total sulfur and nitrogen content**

(A) The Content of sulfate in % of dry weight was calculated on the basis of means of sulfate steady-state levels in leaves of 7 week old plants grown on soil under short day conditions and a ratio of fresh to dry weight of 13.81 for wild type and 13.16 for *sir1-1* plants. (Fig. 4A). The contribution of other S-compounds was calculated by difference of sulfate content to the total content of sulfur in wild type and *sir1-1* (Fig. 4F, n = 5). (B) Same calculation for contribution of nitrate to total content of nitrogen as in A.



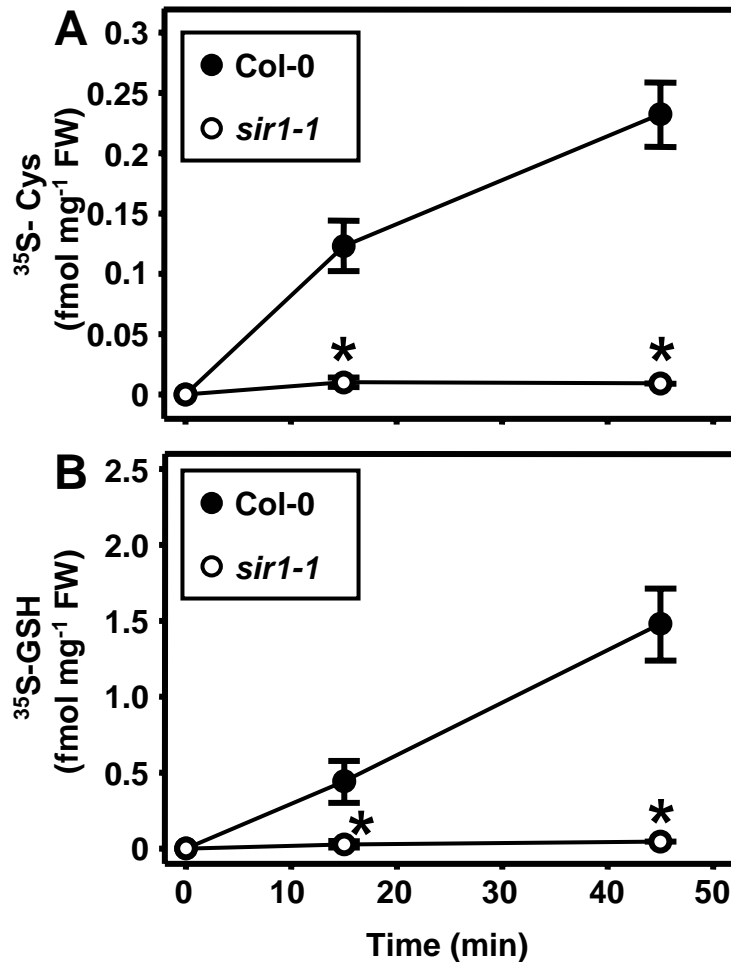
**Supplemental Figure 4: Spectrum of amino acids in *sir1-1* plants**

Steady state levels of amino acids in leaves of 7 week old wild type (black) and *sir1-1* (white) plants grown on soil in a growth chamber under short day conditions. Mean  $\pm$  SE (n = 5) are shown. Asp: aspartic acid, Glu: glutamic acid, Ser: serine, Asn: asparagine, Gly: glycine, Gln: glutamine, Thr: threonine, His: histidine, Ala: alanine, Arg: arginine, Pro: proline, Tyr: tyrosine, Val: valine, Met: methionine, Ile: isoleucine, Leu: leucine, Lys: lysine, Phe: phenylalanine, FW, fresh weight



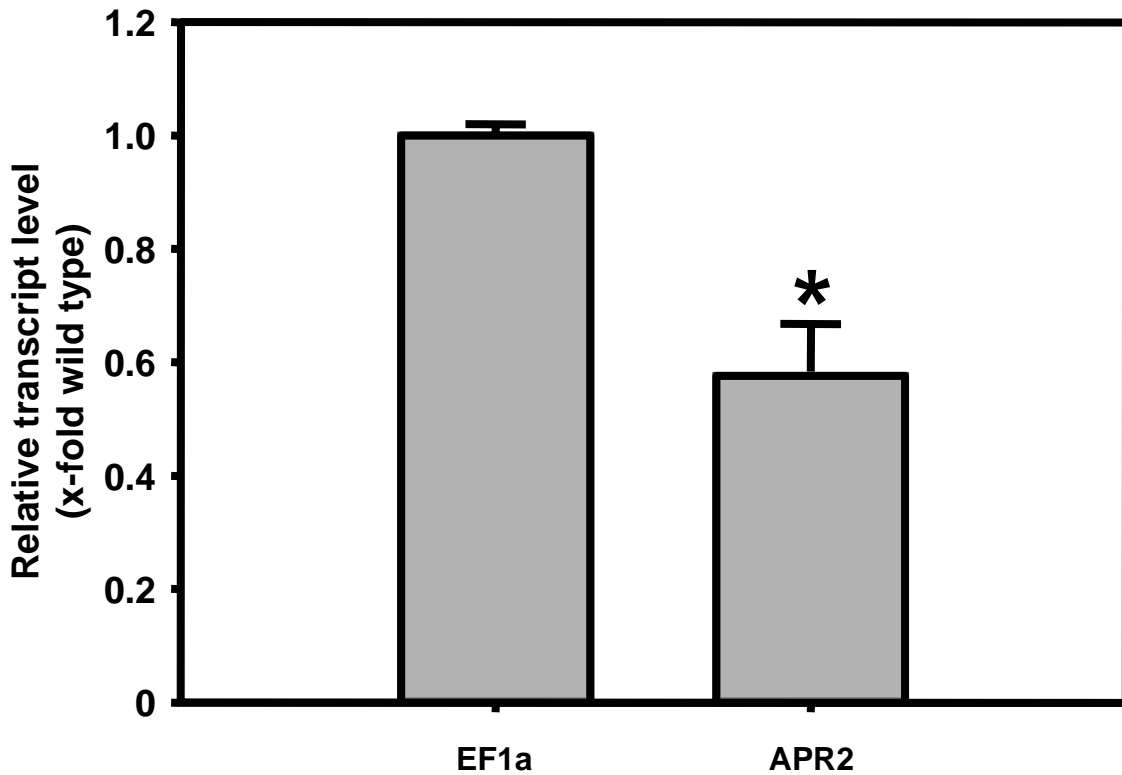
**Supplemental Figure 5: Immunological detection of sulfite oxidase (SO) in *sir1-1* plants**

Soluble proteins were extracted from leaves of 7 week old wild type (Col-0) and *sir1-1* plants grown on soil in a growth chamber under short day conditions (n=3). Total soluble proteins (10  $\mu$ g) were separated by SDS-PAGE (12% acrylamide) and transferred to a nitrocellulose membrane as described in the Methods. The abundance of Sulfite oxidase was tested with a polyclonal antiserum according to Lang et al., (2007).



**Supplemental Figure 6: Incorporation rates of sulfate in wild type and *sir1-1* plants grown on soil**

Leaf pieces of 7 week old wild type (black circles) and *sir1-1* (white circles) from plants grown on soil at short day conditions were incubated with  $^{35}\text{SO}_4^{2-}$ -spiked  $\frac{1}{2}$  Hoagland solution for 15 min (pulse) and subsequently kept on the same medium without radiolabel for 30 min (chase). Time point 0 was defined by transfer of leaf pieces to  $^{35}\text{SO}_4^{2-}$ -spiked  $\frac{1}{2}$  Hoagland solution. Samples were taken at indicated time points. (A) Cysteine and (B) GSH were extracted and separated by HPLC. The incorporated  $^{35}\text{S}$ -label was quantified by scintillation counting. The mean  $\pm$  SE from 4 independent extractions of wild type and *sir1-1* are shown. Asterisks indicate statistical significance ( $P < 0.05$ ). FW, fresh weight



**Supplemental Figure 7: Transcript levels of *APR2* in *sir1-1* determined by qRT-PCR**

Total mRNA was extracted from leaves of *sir1-1* and wild type plants grown on soil under short day conditions for 7 weeks ( $n = 3$ ) and analysed for transcript levels of *APR2* by qRT-PCR as described in the Methods. Ef1a served as reference. Means  $\pm$  SE are shown. Asterisks indicate statistically significant differences.

Supplemental Data. Khan et al. Plant Cell. (2010). 10.1105/tpc.110.074088

MIPs code	NCBI GeneID:	Identity, description	Category	median fitted data Col-0	median fitted data sir	Change	Change in % of wild type	P-value
At3g19710	GeneID:821508	branched-chain amino transferase	Glucosinolat-synthesis	5594	9639	1,72	172,0	0,004
At5g25980	GeneID:832667	myrosinase	Glucosinolat-synthesis	35276	56689	1,61	161,0	0,031
At5g26000	GeneID:832669	myrosinase	Glucosinolat-synthesis	5726	9084	1,59	159,0	0,004
At4g13770	GeneID:827011	cytochrom P450	Glucosinolat-synthesis	7864	12020	1,53	153,0	0,022
At1g16400	GeneID:838210	cytochrom P450, CYP79F2	Glucosinolat-synthesis	2083	2957	1,42	142,0	0,022
At4g03060	GeneID:828102	AOP2	Glucosinolat-synthesis	1948	2476	1,27	127,0	0,006
At1g24100	GeneID:839022	glucosyl transferase	Glucosinolat-synthesis	3025	3591	1,19	119,0	0,016
At1g54040	GeneID:841842	epithiospecifier protein,	Glucosinolat-synthesis	2408	2751	1,14	114,0	0,03
At2g22330	GeneID:816765	cytochrome P450 CYP79B3	Glucosinolat-synthesis	1627	1829	1,12	112,0	0,04
At3g56060	GeneID:824772	mandelonitrile lyase-like protein	Glucosinolat-synthesis	3126	2400	-1,3	54,3	0,034
At5G63980	GeneID:836519	PAPS Phosphatase	Sulfur-metabolism	3942	5354	1,36	136,0	0,038
AT3G22740	GeneID:821845	AtHMT-3	Sulfur-metabolism	1580	1937	1,23	123,0	0,03
At4G01850	GeneID:826987	SAM2	Sulfur-metabolism	5572	6779	1,22	122,0	0,033
At1g62180	GeneID:842514	APR2	Sulfur-metabolism	6278	5390	-1,16	86,2	0,034
At5g65720	GeneID:836701	cysteine desulhydrilase	Sulfur-metabolism	3258	2731	-1,19	84,0	0,043
At5g44070	GeneID:834430	Phytochelatinsynthase	Sulfur-metabolism	4958	4081	-1,21	82,6	0,032
AT3G56300	GeneID:824797	Cysteinyl-tRNA synthetase	Sulfur-metabolism	37265	29949	-1,24	80,6	0,048
At5g10180	GeneID:830882	Sultr2;1	Sulfur-metabolism	3738	2728	-1,37	73,0	0,018
AT1G13420	GeneID:837902	Sulfotransferase family protein	Sulfur-metabolism	12743	8940	-1,43	69,9	0,03
At5g43780	GeneID:834400	APS4	Sulfur-metabolism	3554	1932	-1,84	54,3	0,003
AT5G04590	GeneID:830336	SIR	Sulfur-metabolism	16134	5008	-3,22	31,1	0
AT2G25450	GeneID:817083	similar to ACC oxidase	Sulfur-induced	6902	11797	1,71	171,0	0,036
AT4G12470	GeneID:826859	lipid transfer protein family protein	Sulfur-induced	3877	3121	-1,24	80,6	0,014
ATCG01270	GeneID:6686170	chloroplast encoded hypothetical prot	Sulfur-induced	15366	9567	-1,6	62,5	0,042
At5g24770	GeneID:832546	VEGETATIVE STORAGE PROTEIN 2	Sulfur-induced	2675	30425	11,37	1137,0	0,001
At5g24780	GeneID:832547	VEGETATIVE STORAGE PROTEIN 1	Sulfur-induced	2104	18883	8,98	898,0	0,001
At2g47880	GeneID:819400	Glutaredoxin	REDOX	2618	4220	1,61	161,0	0,032
At2g03980	GeneID:814924	hydrolase family protein	REDOX	2887	3421	1,18	118,0	0,034
At2g32880	GeneID:817849	MATH domain-containing protein	REDOX	2032	2324	1,14	114,0	0,03
At4g23150	GeneID:828414	pad2 regulateded	REDOX	1753	1587	-1,1	90,9	0,047
At2g43570	GeneID:818959	chitinase	REDOX	3112	2681	-1,16	86,2	0,025
At1g03680	GeneID:839436	Thioredoxin	REDOX	21720	18502	-1,17	85,5	0,038
At3g54660	GeneID:824631	Glutathione Reductase I	REDOX	7168	6124	-1,17	85,5	0,039
At5g63030	GeneID:836423	Glutaredoxin	REDOX	3430	2900	-1,18	84,7	0,03
At2g05380	GeneID:815086	glycine-rich protein	REDOX	19826	16057	-1,23	81,3	0,012
At2g29580	GeneID:817507	zinc finger family protein	REDOX	4009	3118	-1,29	77,5	0,018
At4g31870	GeneID:829316	AtGpx7	REDOX	9410	7139	-1,32	75,8	0,017
At5g06290	GeneID:830517	2-Cys Prx B	REDOX	42866	31255	-1,37	73,0	0,004
AT2G41680	GeneID:818766	dihydropolyl dehydrogenase	REDOX	12743	8940	-1,43	69,9	0,03
At3g11630	GeneID:820335	2-Cys Prx A	REDOX	33458	23286	-1,44	69,4	0,027
At4g03520	GeneID:825653	Thioredoxin	REDOX	19556	13243	-1,48	67,6	0,022
AT3G25250	GeneID:822119	OXI1	REDOX	2742	1716	-1,6	62,5	0,022
At2g14610	GeneID:815949	PR1	REDOX	2742	1716	-1,6	62,5	0,022
At3g57260	GeneID:824893	PR2	REDOX	18484	10710	-1,73	57,8	0,004
At2g44290	GeneID:819037	Lipid transfer protein	REDOX	6112	3441	-1,78	56,2	0,004
At2g29450	GeneID:817494	ATGST U5	GSH-Transfer	4869	7458	1,53	153,0	0,035
At2g02930	GeneID:814822	putative glutathione S-transferase	GSH-Transfer	3718	2873	-1,29	77,5	0,024
At2g25080	GeneID:817046	putative glutathione peroxidase AtGpx	GSH-Transfer	13950	9001	-1,55	64,5	0,021
At4g02520	GeneID:827931	ATGST F2	GSH-Transfer	33328	15340	-2,17	46,1	0,009
AT1G19670	GeneID:838554	Chlorophyllase	pathogens-related	6112	13274	2,17	217,0	0,002
At1g66100	GeneID:842924	THI1.1	pathogens-related	3790	5138	1,36	136,0	0,038
AT2G06050	GeneID:815160	OPR3	pathogens-related	2692	3296	1,22	122,0	0,011
At1g72260	GeneID:843558	THI2.1	pathogens-related	1486	1767	1,19	119,0	0,031
AT5G13160	GeneID:831155	AVRPPHB SUSCEPTIBLE 1	pathogens-related	2697	2342	-1,15	87,0	0,038
AT1G59870	GeneID:842281	ATP binding cassette transporter	pathogens-related	21777	15576	-1,4	71,4	0,024
AT3G20770	GeneID:821625	ETHYLENE-INSENSITIVE3	pathogens-related	9919	6722	-1,48	67,6	0,007
AT4G16860	GeneID:827395	RPP4	pathogens-related	7455	4951	-1,51	66,2	0,009
AT3G44480	GeneID:823573	RPP10	pathogens-related	6297	4170	-1,51	66,2	0,025
AT4G16950	GeneID:827403	RPP5	pathogens-related	16675	10827	-1,54	64,9	0,012
AT1G75040	GeneID:843842	PR5	pathogens-related	6586	4134	-1,59	62,9	0,006
At5g18170	GeneID:831935	glutamate dehydrogenase	amino acid synthesis	3356	2820	-1,19	84,0	0,022
At5g05730	GeneID:830457	ASA1	amino acid synthesis,	4182	3420	-1,22	82,0	0,033
At4g39540	GeneID:830108	shikimate kinase - like protein	amino acid synthesis	3686	2922	-1,26	79,4	0,033
AT3G44300	GeneID:823555	nitrilase	Auxin-Biosynthese	6014	9007	1,5	150,0	0,006
AT5G15230	GeneID:831375	gibberellin-regulated (GASA4)	Hormon induced/related	2778	3778	1,36	136,0	0,01
AT3G44310	GeneID:823556	NI1	Auxin-Biosynthese	7552	9348	1,24	124,0	0,022
AT4G24620	GeneID:828564	Phosphoglucose isomerase	Asc-biosynthesis	5388	4293	-1,26	79,4	0,022
AT4G14560	GeneID:827103	auxin induced gene (IAA1)	Hormon induced/related	3725	2836	-1,31	76,3	0,033

**Supplemental Table 1: Significantly regulated transcripts in leaves of 7 week old *sir1-1* plants**

The table represents significantly up- (white) and down-regulated (red) transcript levels in leaves of *sir1-1* in comparison to the wild type (Col-0). MIPS code (unique identifier of genes in *Arabidopsis thaliana*), NCBI GeneID (unique identifier of cDNAs), Identity, description (common name or function of gene product), Category (classification in metabolic network), median fitted data (raw data sets), Change (difference in transcript level in x-fold of wild type), Change in % of wild type (difference in transcript level in percent of wild type), P-value (indicator of statistical significance determined by M-CHiPS).



### **Supplemental Methods: Processing of microarray data**

Signal intensities were normalized by loglinear regression as described [1]. All hybridizations showed correlation coefficients higher than 0.8 between the two channels. The normalized data were used to compute P-values via the limma package [1]. In limma, a contrast matrix defines the comparisons of interest between samples in the experiment. We used a complete pairwise-comparisons contrast matrix to test differential expression across all samples.

Regression coefficients were estimated using a least squares linear model fitting procedure and tested for differential expression with moderated Student's t-statistic via the empirical Bayesian statistics described in the limma package. P-values computed for the *F-statistic* [2] were adjusted for multiple testing to control the FDR at 5% [3]. The adjusted p-values can serve to accept or reject the nullhypothesis based on a significance level. We selected genes showing intensity levels of more than 1000 in at least one of the conditions and also exhibit p-values smaller than 0.05.

[1] Fellenberg, K., Hauser, N.C., Brors, B., Neutzner, A., Hoheisel, J.D., Vingron, M. (2001). Correspondence analysis applied to microarray data. Proc. Natl. Acad. Sci. USA. **98**(19): 10781-10786

[2] Smyth, G.K. (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments, Stat Appl Genet Mol Biol, 3, Article3

[3] Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B, 1995, **57**: 289–300