Table S1. Component summary of buffering solutions used.

Name	Chemical Ingredients
Lysis Buffer	50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)/NaOH, pH 7.3, 0.5 M NaCl, 2 mM dithiothreitol (DTT), 1 μg/mL leupeptine, 0.1 mg/mL 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), 50 μg/mL DNaseI, and 20 mM MgCl
Binding Buffer A	50 mM HEPES/NaOH, pH 7.3, 0.5 M NaCl, 5 mM DTT
Binding Buffer B	50 mM HEPES/NaOH, pH 7.3, 0.5 M NaCl, 30 mM imidazole, 1 mM DTT
SE Buffer	50 mM HEPES/NaOH, pH 7.3, 0.5 M NaCl, 1 mM tris(2-carboxyethyl)phosphine (TCEP)
Assay Buffer A	50 mM HEPES/NaOH, pH 7.3, 500 mM NaCl
Assay Buffer B	20 mM HEPES/NaOH, pH 7.3, 20 mM NaCl
CS Assay Buffer	40 mM HEPES/NaOH, pH 7.8 40 μM Acetyl-CoA (Sigma-Aldrich), oxaloacetic acid (Sigma-Aldrich), 20 mM KOH, 50 mM KCl and 10 mM $(NH_4)_2SO_4$

Table S2. AtDJ-1B specific glyoxalase activities and corresponding observed reaction rates determined during glyoxalase assay.

AtDJ-1B Treatment	Specific Activity (nmol·min^{-1}·mg protein^{-1})	k_{obs} (min^{-1})
5 mM TCEP	596 ± 115	24.9 ± 4.8
2:1 H_2O_2	385 ± 120	16.1 ± 5.0
4:1 H_2O_2	220 ± 134	9.2 ± 5.6
6:1 H_2O_2	182 ± 56	7.6 ± 2.3
8:1 H_2O_2	99 ± 22	4.1 ± 0.9
10:1 H_2O_2	108 ± 108	4.5 ± 4.5
100:1 H_2O_2	14 ± 19	0.6 ± 0.8
5 mM diamide	99 ± 151	4.1 ± 6.3

Table S3. Arabidopsis thaliana DJ-1 specific activities [12].

AtDJ-1 Isoform	Specific Activity (nmol·min^{-1}·mg protein^{-1})	
	Methylglyoxal	Glyoxal
A	15 ± 4	250 ± 12
B	13 ± 3	310 ± 5
C	–	–
D	8600 ± 200	11 000 ± 600
E	6.2 ± 1.7	–
F	5.5 ± 1.1	–

AtDJ-1B open reading frame sequence without N-terminal chloroplastic targeting sequence and start codon, optimized for *E. coli.*

(Gene ID At1g53280)

```
GCGACGATGTCGAGCAGTACGAAAAAGGTACTTATTCCCGTTGCCCATGGAACAGAGCCGTTTGA
AGCAGTCGTTATGATCGATGTATTGCGCCGTGGAGGTGCCGACGTAACAGTAGCGTCCGTTGAAA
ATCAAGTGGGCGTTGATGCATGCCACGGTATCAAAATGGTAGCCGACACGCTTCTGAGCGATATC
```

ACCGATAGCGTGTTCGACTTGATCATGTTACCCGGCGGGCTGCCTGGAGGTGAGACGCTGAAAAA
TTGCAAGCCGCTTGAAAAAATGGTTAAGAAACAAGACACTGACGGGCGCTTGAACGCAGCAATCT
GCTGTGCTCCGGCCTTAGCATTTGGCACTTGGGGTTTACTGGAAGGGAAGAAAGCAACGTGCTAT
CCTGTGTTCATGGAGAAGTTAGCCGCCTGTGCTACAGCTGTAGAATCTCGTGTCGAGATCGACGG
AAAAATTGTTACGAGTCGCGGACCCGGGACCACGATGGAATTCTCGGTGACACTTGTAGAGCAGT
TATTGGGTAAAGAGAAGGCGGTGGAAGTTTCAGGGCCCCTGGTTATGCGCCCGAACCCAGGAGAC
GAGTACACAATCACGGAGCTTAATCAAGTATCCTGGTCTTTCGAGGGGACACCTCAAATCCTTGT
CCCCATTGCCGACGGGAGCGAGGAGATGGAAGCAGTCGCTATCATCGACGTTTTGAAACGCGCAA
AAGCTAACGTTGTCGTGGCCGCGTTGGGCAATTCTCTTGAGGTCGTTGCATCTCGTAAAGTGAAA
CTTGTCGCTGACGTATTATTAGATGAAGCGGAGAAAAACAGCTATGATTTAATCGTTTTGCCGGG
AGGTCTGGGCGGGGCTGAAGCATTCGCATCCTCAGAAAAATTGGTTAATATGCTTAAGAAACAAG
CAGAATCTAATAAGCCTTATGGTGCTATTTGTGCTTCCCCCGCTCTTGTGTTTGAGCCTCATGGA
CTTCTTAAGGGCAAAAAGGCTACTGCCTTCCCAGCTATGTGTTCGAAATTAACAGATCAGTCTCA
CATCGAACATCGTGTCTTGGTTGACGGGAACCTTATTACCTCCCGTGGACCGGGTACTTCATTGG
AATTTGCTTTGGCTATCGTCGAAAAATTTTACGGGCGTGAAAAGGGGTTGCAGTTATCGAAGGCA
ACTCTGGTG

AtDJ-1B protein sequence (after cleavage of GST fusion tag), catalytic cysteines are underlined

(UniProt Accession Q9MAH3)

GATMSSSTKK VLIPVAHGTE PFEAVVMIDV LRRGGADVTV ASVENQVGVD
ACHGIKMVAD TLLSDITDSV FDLIMLPGGL PGGETLKNCK PLEKMVKKQD
TDGRLNAAIC CAPALAFGTW GLLEGKKATC YPVFMEKLAA CATAVESRVE
IDGKIVTSRG PGTTMEFSVT LVEQLLGKEK AVEVSGPLVM RPNPGDEYTI
TELNQVSWSF EGTPQILVPI ADGSEEMEAV AIIDVLKRAK ANVVVAALGN
SLEVVASRKV KLVADVLLDE AEKNSYDLIV LPGGLGGAEA FASSEKLVNM
LKKQAESNKP YGAICASPAL VFEPHGLLKG KKATAFPAMC SKLTDQSHIE
HRVLVDGNLI TSRGPGTSLE FALAIVEKFY GREKGLQLSK ATLV

Table S4. Primers used for the study.

ID	Sequence	Use
	DJ-1 overexpression in E. coli	
DJ1B-attB1-TEV-Fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGAAAACCTGTATTTTCAGGGAGCGACGATGTCGAGCAGTACGAAAAAGGTACTTATT	PCR for gene amplification and adding TEV site
DJ1B-attB2-Rev	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACACCAGAGTTGCCTTCGATA	PCR for gene amplification and adding TEV site
SeqLA	CTCTCGCGTTAACGCTAGCATGGAT	Sequencing of the construct in pDONR221
SeqLB	GTAACATCAGAGATTTTGAGACAC	Sequencing of the construct in pDONR221
SeqFw2	GGTGGAAGTTTCAGGGCCCCTGGT	Sequencing of the construct in pDONR221

SeqRev2	ACCAGGGGCCCTGAAACTTCCACC	Sequencing of the construct in pDONR221
	Analysis of T-DNA insertion lines	
SALK_049637_DJ1A_F	CCTCCCTTTTCCCAATCATATC	Genotyping the *dj1a* (SALK_049637) T-DNA line
SALK_049637_DJ1A_R	TTTTTCGACCGGTTAACACTC	Genotyping the *dj1a* (SALK_049637) T-DNA line
SALK_093414_DJ1B_F	AGGCACAAATTGCTCCATATG	Genotyping the *dj1b-9* (SALK_093414) T-DNA line
SALK_093414_DJ1B_R	ACCATGGAATTCTCTGTCACG	Genotyping the *dj1b-9* (SALK_093414) T-DNA line
SALK_046449_DJ1B_F	GACGCATGAGCTCAGTAAAGC	Genotyping the *dj1b-4* (SALK_046449) T-DNA line
SALK_046449_DJ1B_R	AGCAAGACACTGATGGACGAC	Genotyping the *dj1b-4* (SALK_046449) T-DNA line
LB_SALK	ATTTTGCCGATTTCGGAAC	Genotyping the *dj1b-4* and *dj1b-9* SALK T-DNA lines
DJ1A_qPCR_F1	GGCGGGCAAAAGCAAATGTA	rt-qPCR analysis of DJ-1A expression
DJ1A_qPCR_R1	AAGACCGCCAGGTAACACAA	rt-qPCR analysis of DJ-1A expression
DJ1A_qPCR_F2	TGATTGTGTTACCTGGCGGT	rt-qPCR analysis of DJ-1A expression
DJ1A_qPCR_R2	AGGCTCGAAGACGTAAGCAG	rt-qPCR analysis of DJ-1A expression
DJ1B_qPCR_F1	GAAGCAGGCGGAATCAAACA	rt-qPCR analysis of DJ-1B expression
DJ1B_qPCR_R1	GTTGCCTTCTTACCCTTGAGT	rt-qPCR analysis of DJ-1B expression
DJ1B_qPCR_F2	GGTTTACTCAAGGGTAAGAAGGC	rt-qPCR analysis of DJ-1B expression
DJ1B_qPCR_R2	GAGATTGCCGTCCACCAAGA	rt-qPCR analysis of DJ-1B expression
DJ1B_qPCR_F3	CTCATGGTACGGAGCCGTTT	rt-qPCR analysis of DJ-1B expression
DJ1B_qPCR_R3	GGAAGTCCTCCAGGGAGCATA	rt-qPCR analysis of DJ-1B expression

Table S5. Log2 fold change values of DJ-1 mRNA expression levels, as visualised on Figure 9.

Gene	AtDJ-1A	AtDJ-1B	AtDJ-1C	AtDJ-1D	AtDJ-1E	AtDJ-1F
	At3g14990	**At1g53280**	**At4g34020**	**At3g02720**	**At2g38860**	**At3g54600**
***Pseudomonas syringae* infection (Stael et al., personal communication)**	1.376	−0.901	−2.673	−1.516	2.601	−4.146
3h high light, *cat2-2* [27]	2.859	−0.357	−1.201	−0.456	1.86	−2.137
Methyl viologen, 24 h (He et al., submitted)	0.873	0.117	−0.04	0.046	0.263	0.363
***cat2-2* vs. Col-0 [30]**	1.688	0.105	−0.033	0.155	1.214	0.277
***cat2-2* 24h RGCL [30]**	2.945	−0.204	−0.112	−0.56	1.877	−3.988
Col-0 25h RGCL [30]	3.115	0.615	0.473	−0.209	0.888	−2.354
50 µM Antimycin A [28]	0.958	−0.155	−0.275	−0.353	0.282	−1.357

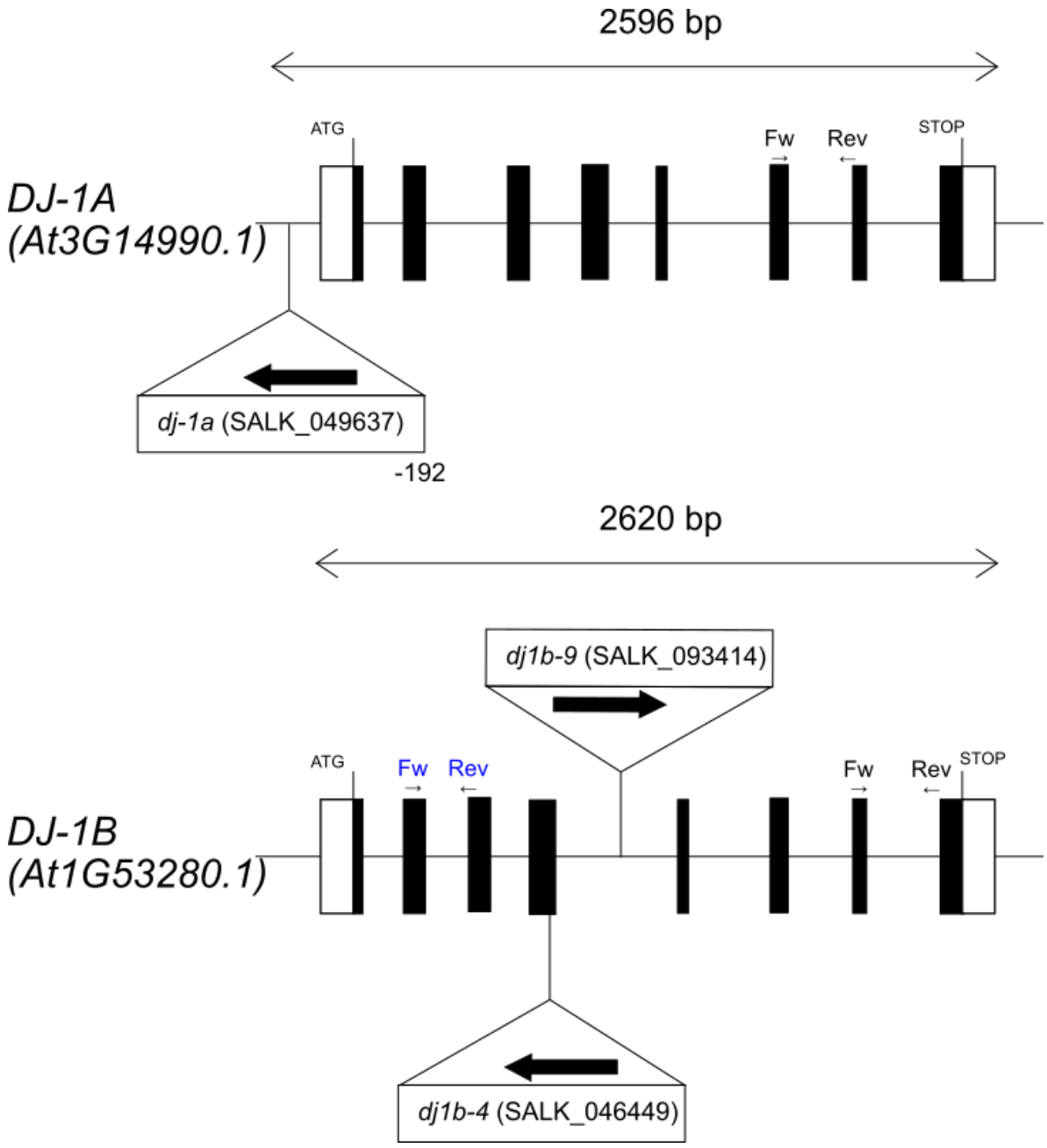

Figure S1. *AtDJ-1A* and *AtDJ-1B* gene models. For *AtDJ-1A* and *AtDJ-1B* two pairs of primers complementary to C-terminal fragment of the transcript were used (plasmids with suffix: qPCR_F1/R1/F2/R2), their positions marked in black (Fw, Rev). For *AtDJ-1B* an additional primer pair complementary to the N-terminal fragment was used: qPCR_F3/R3, marked in blue.

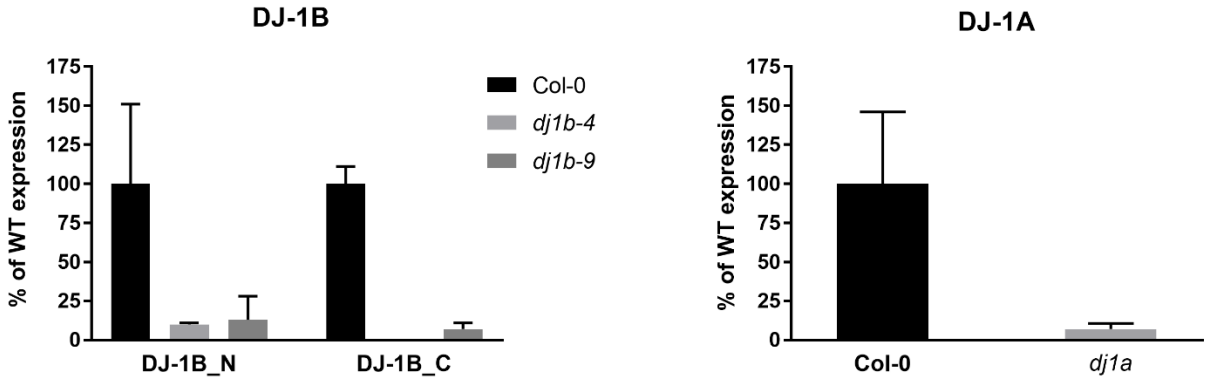

Figure. S2. *AtDJ-1B* and *DJ-1A* transcript levels (left and right, respectively) in WT and KO T-DNA lines. DJ1B_N represents the transcript detected with primers complementary to N-terminal fragment of DJ-1B mRNA (blue arrows, Figure S1), DJ1B_C represents the transcript detected with primers complementary to C-terminal fragment of *AtDJ-1B* mRNA (black arrows, Figure S1). RNA was extracted from pooled 11-day-old plants grown *in vitro* and used to quantify gene expression levels by RT-qPCR. Values are means ± SD.