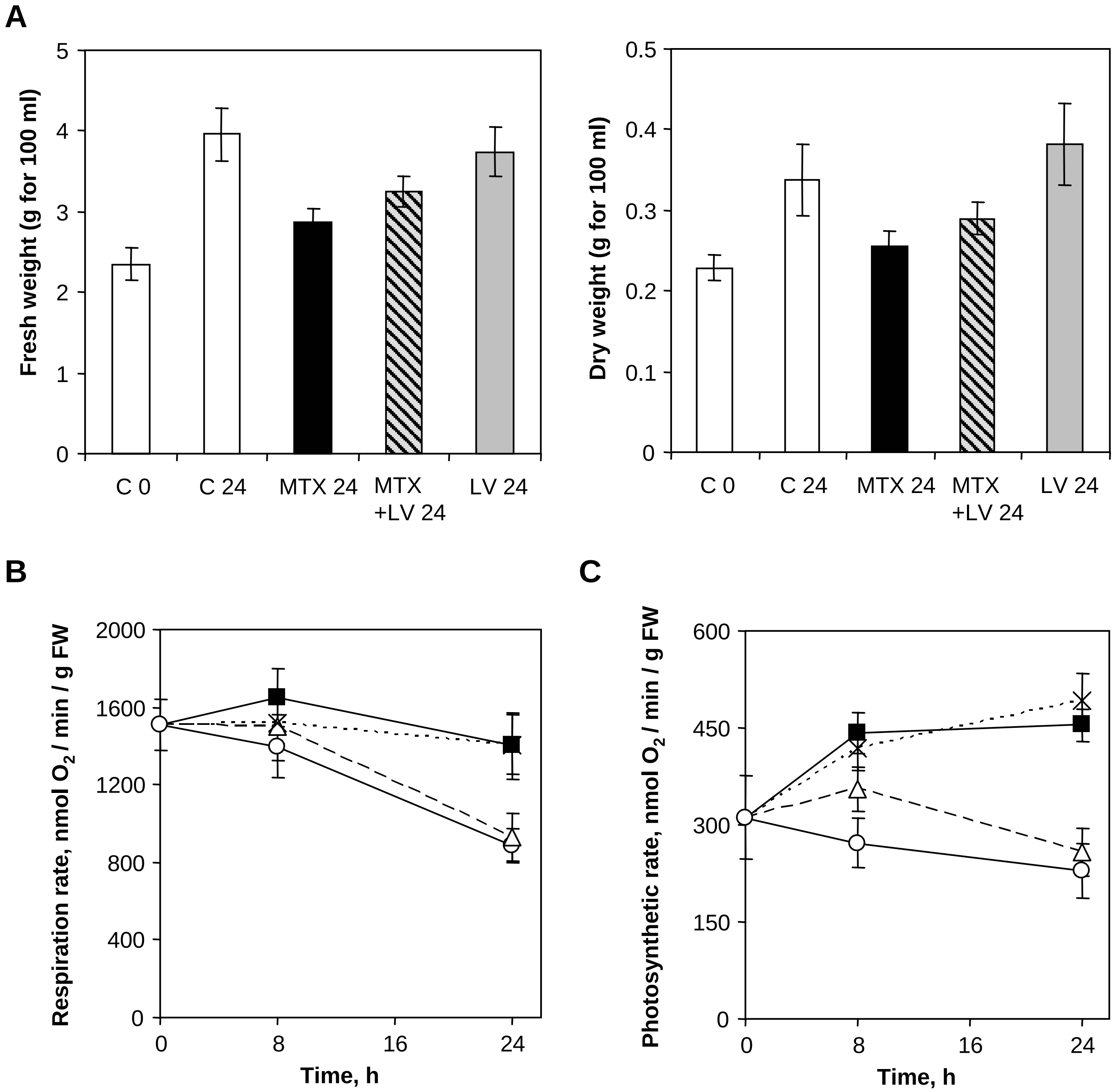
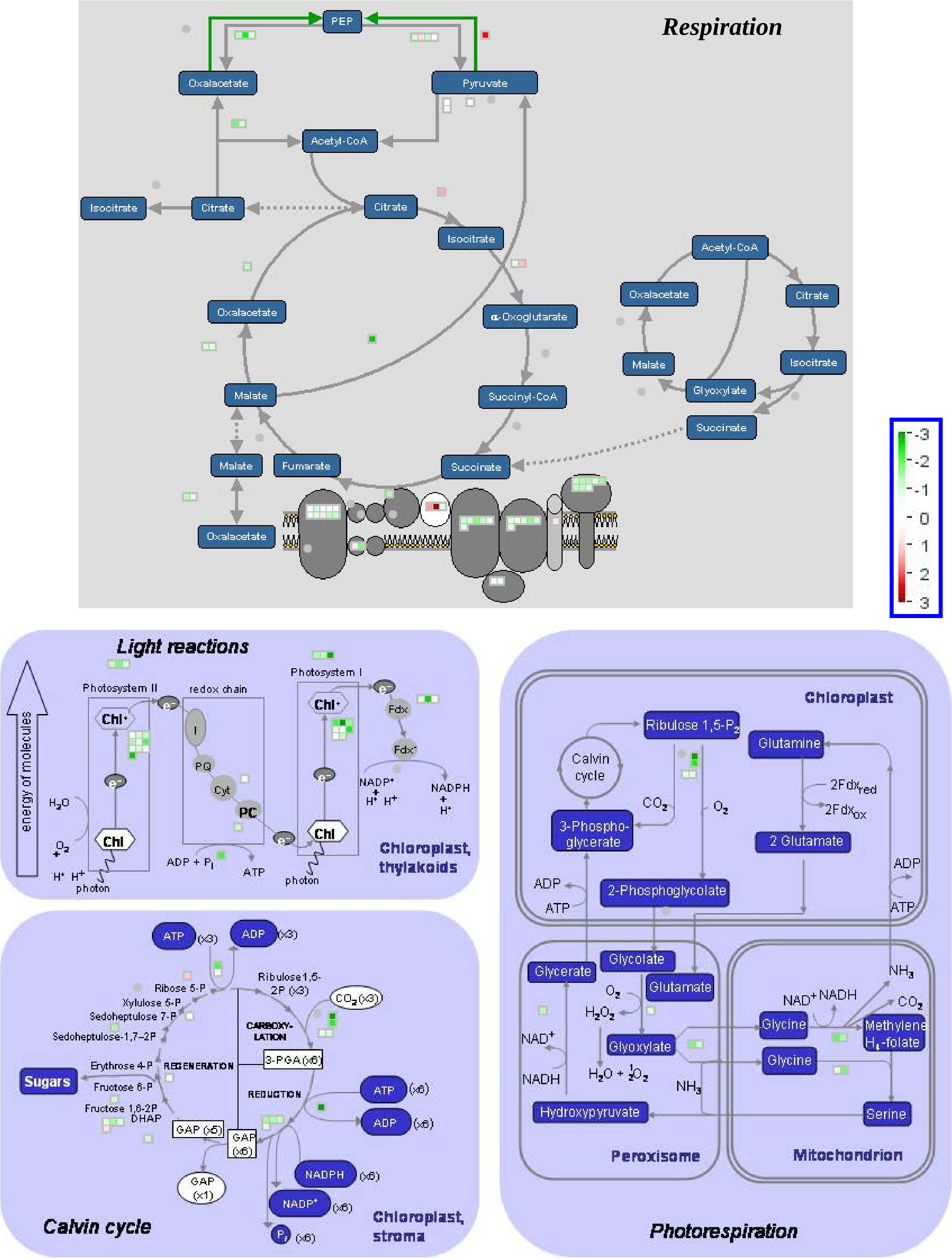


Supplemental Figure S1. Physiological parameters of Arabidopsis cells treated with MTX and leucovorin. Cells were grown for up to 24h in standard conditions or in the presence of MTX (100 μM) and leucovorin (LV, 500 μM), alone or in combination. **A** – Cell growth was estimated through measurements of fresh and dry weights of cultures after 24h of exposure to the different chemicals. C0, control cells at time zero. **B, C** – Respiratory and photosynthetic rates were determined in freshly prepared culture medium containing 50 mM bicarbonate in the darkness or with a white light source of 2000 μmol of photons.m^{-2}.s^{-1}, respectively. Control cells (■) and cells treated with MTX (○), MTX and LV (△), and LV (×) were analyzed after 0, 8, and 24h of treatment. Data are means of two biological replicates and SD.



Supplemental Figure S2. MapMan-generated overview of MTX-responsive genes involved in respiration and photosynthesis. Genes that are differentially expressed between MTX-treated and control cells after 24h of treatment were analyzed using the MapMan tool with maps downloaded from the MapMan website (Thimm et al, 2004). Differentially expressed genes are represented by colored boxes according to the $\log_2$(ratio) values. Genes induced by MTX are shown as red, the one repressed by MTX as green.

Supplemental Table S1. Folate measurements in Arabidopsis cells used for the transcriptomic studies (A) and the rescue experiments (B).

In two independent series of experiments, Arabidopsis cells were grown in standard conditions or exposed to MTX (100 µM) and/or leucovorin (LV, 500 µM) over a 24h period. Folates were analyzed by LC-MS/MS as described by Zhang et al. (2005). Using this procedure, 5,10-methylene-THF is converted to THF and 10-formyl-THF is oxidized to 10-formyl-folic acid or converted to 5,10-methenyl-THF (De Brouwer et al, 2007). Also, dihydrofolate is partly converted to folic acid or degraded. Values are in nmol folates or MTX per gram of fresh weight. Data are means of three biological replicates and SD. nd, not detected.

A - Cells used for transcriptomic studies

Treatment	**Time**	**THF + 5,10-methylene-THF**	**5-methyl-THF**	**5-formyl-THF**	**10-formyl-folic acid**	**5,10-methenyl-THF**	**Folic acid**	**Total**	**MTX**
Control	2 h	6.61 ± 0.38	2.38 ± 0.33	0.52 ± 0.16	nd	2.83 ± 1.22	0.03 ± 0.01	12.36 ± 2.10	nd
	6 h	7.67 ± 0.27	2.43 ± 0.46	0.58 ± 0.24	nd	2.52 ± 1.27	0.03 ± 0.01	13.23 ± 2.25	nd
	24 h	6.13 ± 1.24	2.20 ± 0.36	0.47 ± 0.27	nd	2.49 ± 1.24	0.02 ± 0.01	11.32 ± 3.11	nd
+ MTX	2 h	5.26 ± 1.28	0.92 ± 0.36	0.32 ± 0.10	0.07 ± 0.01	2.43 ± 0.90	0.67 ± 0.14	9.67 ± 2.80	5.72 ± 0.90
	6 h	3.36 ± 1.78	0.14 ± 0.03	0.16 ± 0.07	0.09 ± 0.02	1.40 ± 0.53	0.49 ± 0.0.5	5.77 ± 2.69	9.47 ± 1.04
	24 h	1.79 ± 0.43	0.01 ± 0.00	0.06 ± 0.03	0.03 ± 0.01	0.43 ± 0.19	0.27 ± 0.04	2.60 ± 0.69	8.51 ± 0.82

B - Cells used for rescue experiments

Treatment	**Time**	**THF + 5,10-methylene-THF**	**5-methyl-THF**	**5-formyl-THF**	**10-formyl-folic acid**	**5,10-methenyl-THF**	**Folic acid**	**Total**	**MTX**
Control	6 h	3.67 ± 0.93	6.38 ± 0.60	1.84 ± 0.38	0.03 ± 0.02	5.40 ± 0.70	0.01 ± 0.00	17.32 ± 2.63	nd
	12 h	2.91 ± 0.76	6.33 ± 0.76	2.03 ± 0.44	0.17 ± 0.19	6.05 ± 1.31	0.01 ± 0.00	17.50 ± 3.47	nd
	24 h	2.09 ± 0.30	7.22 ± 1.20	2.19 ± 0.73	0.34 ± 0.07	6.09 ± 0.32	0.03 ± 0.01	17.96 ± 2.63	nd
+ MTX	6 h	1.76 ± 0.39	2.00 ± 0.43	0.61 ± 0.15	0.11 ± 0.07	1.59 ± 0.53	0.66 ± 0.06	6.73 ± 1.62	8.23 ± 1.60
	12 h	0.57 ± 0.24	0.53 ± 0.08	0.32 ± 0.08	0.08 ± 0.04	0.59 ± 0.21	0.45 ± 0.07	2.54 ± 0.73	10.26 ± 3.15
	24 h	0.33 ± 0.15	0.13 ± 0.04	0.23 ± 0.03	0.03 ± 0.01	0.25 ± 0.06	0.34 ± 0.06	1.30 ± 0.35	11.05 ± 1.86
+ MTX + LV	6 h	2.26 ± 0.68	3.74 ± 0.63	6.58 ± 1.70	0.36 ± 0.12	2.07 ± 0.66	0.84 ± 0.19	15.86 ± 3.98	9.00 ± 0.70
	12 h	1.58 ± 0.36	2.37 ± 0.77	12.58 ± 0.93	0.20 ± 0.22	3.38 ± 0.38	0.69 ± 0.05	20.79 ± 2.70	16.17 ± 0.28
	24 h	1.16 ± 0.12	2.86 ± 0.42	16.83 ± 0.58	0.09 ± 0.06	1.10 ± 0.47	0.58 ± 0.04	22.63 ± 1.68	20.60 ± 1.44
+ LV	6 h	5.25 ± 0.99	7.45 ± 0.34	11.12 ± 1.17	0.05 ± 0.02	7.58 ± 0.78	0.02 ± 0.01	31.47 ± 3.30	nd
	12 h	4.25 ± 1.04	11.59 ± 3.38	15.35 ± 0.69	0.52 ± 0.73	10.14 ± 2.50	0.05 ± 0.02	41.90 ± 8.35	nd
	24 h	4.65 ± 2.31	12.07 ± 2.27	26.46 ± 3.05	0.45 ± 0.28	12.31 ± 4.96	0.10 ± 0.03	56.04 ± 12.90	nd

Supplemental Table S2. Validation of the microarray data by quantitative RT-PCR.

Microarray data were obtained from comparisons between MTX-treated and control cells after 2, 6, and 24h of treatment (see Fig. 2). A statistical cut-off *P* <0.05 after Bonferroni correction was used to determined which genes were differentially expressed in response to MTX. The average ratio (MTX vs. control transcript levels) and *P* -value for 2 independent biological repeats of the experiment is shown. Real-time qPCR was done as described in the Materials and methods section. Values ± SD correspond to relative transcript levels in treated vs. control cells, as determined for 3 biological repeats.

			Microarray data						**Quantitative RT-PCR**		
			MTX / control 2h		MTX / control 6h		MTX / control 24h		MTX / control 2h	MTX / control 6h	MTX / control 24h
Accession number	**Gene description**	**Gene name**	Ratio	P-value	Ratio	P-value	Ratio	P-value	Ratio ± SD	Ratio ± SD	Ratio ± SD
AT1G19770	purine permease	PUP 14	0.93	1.00E+00	14.37	0.00E+00	1.00	1.00E+00	1.33 ± 0.21	27.65 ± 4.31	2.02 ± 0.37
AT1G36370	serine hydroxymethyltransferase (plastidial)	cpSHMT	0.94	1.00E+00	8.21	0.00E+00	1.49	1.00E+00	1.21 ± 0.20	8.85 ± 1.23	4.73 ± 0.90
AT1G50480	10-formyltetrahydrofolate synthetase	FTHFS	1.21	1.00E+00	8.42	0.00E+00	1.74	1.13E-02	1.31 ± 0.20	4.3 ± 0.52	1.69 ± 0.19
AT1G74590	glutathione transferase	GSTU10	1.04	1.00E+00	3.06	0.00E+00	8.26	0.00E+00	1.16 ± 0.75	3.00 ± 0.70	16.70 ± 1.71
AT3G02470	S-adenosylmethionine decarboxylase	SAMdc	1.26	1.00E+00	4.34	0.00E+00	7.24	0.00E+00	1.39 ± 0.17	2.18 ± 0.25	11.89 ± 2.86
AT3G22370	alternative oxidase 1a	AOX1a	1.07	1.00E+00	18.62	0.00E+00	11.82	0.00E+00	1.04 ± 0.21	28.82 ± 5.62	16.59 ± 3.57
AT3G23810	S-adenosyl-L-homocysteine hydrolase	SAHH2	1.08	1.00E+00	0.77	1.00E+00	0.31	0.00E+00	1.38 ± 0.44	1.02 ± 0.19	0.59 ± 0.16
AT3G55630	folylpolyglutamate synthetase (cytosolic)	FPGSD	1.00	1.00E+00	2.49	0.00E+00	0.57	1.09E-02	1.23 ± 0.22	4.93 ± 0.31	0.90 ± 0.27
AT4G04610	5'-adenylylsulfate reductase	APR1	0.98	1.00E+00	19.83	0.00E+00	3.17	0.00E+00	2.24 ± 0.48	28.28 ± 9.55	5.21 ± 2.48
AT4G37930	serine hydroxymethyltransferase (mitochondrial)	mtSHMT	0.96	1.00E+00	0.75	1.00E+00	0.28	0.00E+00	1.00 ± 0.15	1.08 ± 0.28	0.49 ± 0.08
AT5G10180	sulfate transporter	SULTR2.1	0.92	1.00E+00	1.48	1.00E+00	0.39	0.00E+00	0.94 ± 0.09	2.16 ± 0.40	0.35 ± 0.02
AT5G17920	vitamin-B12-independent methionine synthase	MS1	1.03	1.00E+00	1.11	1.00E+00	0.48	8.71E-07	0.79 ± 0.17	1.13 ± 0.32	0.51 ± 0.06

Supplemental Table S3. Selected MTX-responsive genes that may be involved in the regulation of cell cycle and response to stress.

Microarray data are expressed in log2(ratio) obtained from comparisons between MTX-treated and control cells after 2, 6, and 24h of treatment (see Fig. 2). A statistical cut-off $P<0.05$ after Bonferroni correction was used to determined which genes were differentially expressed in response to MTX. Positive (red boxes) and negative (green boxes) ratio indicate that the genes are induced or repressed by MTX, respectively. The average log2(ratio) and P-value for 2 independent biological repeats of the experiment is shown.

Accession number	Gene description	MTX / control 2h		MTX / control 6h		MTX / control 24h	
		$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value
Cell cycle							
AT1G76540	CDKB2;1; kinase_ cell division control protein, putative	-0.03	1.00E+0	-0.32	1.00E+0	-1.60	0.00E+0
AT2G27960	CKS1; cyclin-dependent protein kinase_ cyclin-dependent kinase / CDK (CKS1)	0.04	1.00E+0	-1.61	0.00E+0	-1.56	0.00E+0
AT2G27970	CKS2; cyclin-dependent protein kinase_ cyclin-dependent kinase, putative / CDK, putative	0.10	1.00E+0	-0.96	1.74E-7	-2.26	0.00E+0
AT1G44110	CYCA1;1; cyclin-dependent protein kinase regulator	0.11	1.00E+0	-0.12	1.00E+0	-1.98	0.00E+0
AT2G26760	CYCB1;4 (CYCLIN 3); cyclin-dependent protein kinase regulator	0.03	1.00E+0	-0.12	1.00E+0	-2.14	0.00E+0
AT2G17620	CYCB2;1; cyclin-dependent protein kinase regulator	-0.13	1.00E+0	-0.36	1.00E+0	-1.11	6.37E-8
AT5G03455	CDC25_ rhodanese-like domain-containing protein	0.06	1.00E+0	-1.44	0.00E+0	-0.84	3.41E-3
AT5G42190	ASK2 (ARABIDOPSIS SKP1-LIKE 2); ubiquitin-protein ligase_ E3 ubiquitin ligase SCF complex subunit SKP1/ASK1	-0.10	1.00E+0	0.39	1.00E+0	-1.50	0.00E+0
AT1G75950	SKP1 ; ubiquitin-protein ligase_ E3 ubiquitin ligase SCF complex subunit SKP1/ASK1	0.10	1.00E+0	-1.15	5.62E-12	-0.05	1.00E+0
AT1G07370	PCNA1 (PROLIFERATING CELLULAR NUCLEAR ANTIGEN)	0.10	1.00E+0	-0.54	1.00E+0	-1.65	0.00E+0
AT2G30410	KIS (KIESEL); unfolded protein binding_ tubulin folding cofactor A (KIESEL)	0.01	1.00E+0	-1.08	3.49E-10	-1.72	0.00E+0
AT5G23860	TUB8_ tubulin beta-8 chain (TUB8) (TUBB8)	0.06	1.00E+0	-0.84	5.31E-5	-1.42	0.00E+0
AT5G12250	TUB6 (BETA-6 TUBULIN)_ tubulin beta-6 chain (TUB6)	0.00	1.00E+0	-0.70	1.49E-2	-1.56	0.00E+0
AT4G14960	TUA6_ tubulin alpha-6 chain (TUA6)	-0.01	1.00E+0	0.10	1.00E+0	-2.76	0.00E+0
AT4G20890	TUB9; GTP binding / GTPase/ structural molecule_ tubulin beta-9 chain (TUB9)	0.01	1.00E+0	-0.42	1.00E+0	-1.10	1.22E-7
AT2G29550	TUB7_ tubulin beta-7 chain (TUB7)	0.02	1.00E+0	-0.55	1.00E+0	-3.32	0.00E+0
AT1G04820	TUA4_ tubulin alpha-2/alpha-4 chain (TUA4)	0.07	1.00E+0	-0.18	1.00E+0	-3.99	0.00E+0
AT1G50010	TUA2 unknown protein	0.04	1.00E+0	-0.20	1.00E+0	-3.58	0.00E+0
Alternative oxidases							
AT1G32350	alternative oxidase 1d (AOX1D)	0.10	1.00E+0	0.89	4.20E-6	1.49	0.00E+0
AT3G22370	alternative oxidase 1a (AOX1A)	0.10	1.00E+0	4.22	0.00E+0	3.56	0.00E+0
Ethylene and polyamine metabolism							
AT4G26200	1-aminocyclopropane-1-carboxylate synthase, putative / ACC synthase 7 (ACS7)	0.13	1.00E+0	3.77	0.00E+0	2.61	0.00E+0
AT1G01480	1-aminocyclopropane-1-carboxylate synthase 2 / ACC synthase 2 (ACS2)	0.09	1.00E+0	3.59	0.00E+0	2.73	0.00E+0
AT1G62380	1-aminocyclopropane-1-carboxylate (ACC) oxidase (ACO2)	0.11	1.00E+0	-1.10	1.24E-10	-1.36	0.00E+0
AT2G25450	1-aminocyclopropane-1-carboxylate (ACC) oxidase	-0.10	1.00E+0	0.46	1.00E+0	2.22	0.00E+0
AT5G43450	1-aminocyclopropane-1-carboxylate (ACC) oxidase	-0.01	1.00E+0	2.93	0.00E+0	1.42	0.00E+0
AT1G05010	1-aminocyclopropane-1-carboxylate (ACC) oxidase (EFE)	0.03	1.00E+0	1.57	0.00E+0	0.73	9.26E-2
AT3G45410	Ethylene receptor	0.08	1.00E+0	1.29	0.00E+0	0.95	4.99E-5
AT5G15950	S-adenosylmethionine decarboxylase	-0.06	1.00E+0	0.56	1.00E+0	1.69	0.00E+0
AT3G02470	S-adenosylmethionine decarboxylase	0.34	1.00E+0	2.12	0.00E+0	2.86	0.00E+0
AT2G16500	arginine decarboxylase (ADC1)	0.35	1.00E+0	1.02	7.13E-9	1.51	0.00E+0
AT4G34710	arginine decarboxylase (ADC2)	0.20	1.00E+0	-0.32	1.00E+0	1.14	1.89E-8
Cytochrome P450 family							
AT5G42580	a member of the cytochrome P450 family	0.10	1.00E+0	-1.08	2.81E-10	-0.01	1.00E+0
AT5G45340	CYP707A3; heme binding / iron ion binding / monooxygenase/ oxygen binding	-0.06	1.00E+0	1.37	0.00E+0	-0.46	1.00E+0
AT2G34500	CYP710A1; heme binding / iron ion binding / monooxygenase/ oxygen binding	-0.11	1.00E+0	-0.52	1.00E+0	2.22	0.00E+0
AT2G34490	CYP710A2; heme binding / iron ion binding / monooxygenase/ oxygen binding	0.10	1.00E+0	-1.51	0.00E+0	-1.16	6.82E-9
AT4G39950	CYP79B2; heme binding / iron ion binding / monooxygenase/ oxygen binding	-0.11	1.00E+0	0.67	4.66E-2	2.44	0.00E+0

AT2G24180	cytochrome P450 monooxygenase	0.09	1.00E+0	1.19	0.00E+0	1.46	0.00E+0
AT1G64900	cytochrome P450 (CYP89A2).	-0.18	1.00E+0	0.58	8.12E-1	1.33	0.00E+0
AT4G30210	NADPH-cytochrome P450 reductase	-0.12	1.00E+0	1.74	0.00E+0	-0.57	1.00E+0
AT2G45510	member of CYP704A	-0.01	1.00E+0	-0.83	6.90E-5	-1.05	1.06E-6
AT3G20960	member of CYP705A	-0.08	1.00E+0	0.27	1.00E+0	1.10	1.00E-7
AT4G27710	member of CYP709B	-0.09	1.00E+0	1.07	5.17E-10	0.26	1.00E+0
AT4G37320	member of CYP81D	-0.10	1.00E+0	1.14	5.62E-12	0.22	1.00E+0
AT5G57220	member of CYP81F	-0.05	1.00E+0	2.88	0.00E+0	0.59	1.00E+0
AT4G31500	oxime-metabolizing enzyme	-0.06	1.00E+0	1.32	0.00E+0	-1.19	1.36E-9
Glutathione S-transferases							
AT1G69930	glutathione transferase belonging to the tau class of GSTs	-0.09	1.00E+0	2.72	0.00E+0	3.70	0.00E+0
AT1G74590	glutathione transferase belonging to the tau class of GSTs	0.05	1.00E+0	1.62	0.00E+0	3.05	0.00E+0
AT4G02520	glutathione transferase belonging to the phi class of GSTs.	0.01	1.00E+0	1.42	0.00E+0	3.34	0.00E+0
AT1G78370	glutathione transferase belonging to the tau class of GSTs	0.02	1.00E+0	1.34	0.00E+0	0.66	8.69E-1
AT2G47730	glutathione transferase belonging to the phi class of GSTs	0.01	1.00E+0	1.02	7.35E-9	0.50	1.00E+0
AT2G29420	glutathione transferase belonging to the tau class of GSTs	0.05	1.00E+0	0.65	8.71E-2	1.51	0.00E+0
AT3G09270	glutathione transferase belonging to the tau class of GSTs	-0.07	1.00E+0	0.59	5.95E-1	1.82	0.00E+0
AT1G78380	glutathione transferase belonging to the tau class of GSTs	-0.04	1.00E+0	0.02	1.00E+0	1.53	0.00E+0
AT1G17190	glutathione transferase belonging to the tau class of GSTs	-0.01	1.00E+0	-1.11	4.50E-11	-1.26	5.04E-11
Glucosyl- and sulfo-transferases							
AT2G28080	glycosyltransferase family protein	0.15	1.00E+0	0.65	8.96E-2	1.60	0.00E+0
AT1G05670	UDP-glucoronosyl/UDP-glucosyl transferase family protein	0.06	1.00E+0	3.08	0.00E+0	3.50	0.00E+0
AT5G12890	UDP-glucoronosyl/UDP-glucosyl transferase family protein	0.13	1.00E+0	1.89	0.00E+0	1.17	4.03E-9
AT1G07240	UDP-glucoronosyl/UDP-glucosyl transferase family protein	-0.03	1.00E+0	1.74	0.00E+0	1.25	7.85E-11
AT1G07260	UDP-glucoronosyl/UDP-glucosyl transferase family protein	-0.10	1.00E+0	0.59	6.73E-1	1.08	2.68E-7
AT3G11340	UDP-glucoronosyl/UDP-glucosyl transferase family protein	-0.29	1.00E+0	0.52	1.00E+0	3.32	0.00E+0
AT3G21800	UDP-glucoronosyl/UDP-glucosyl transferase family protein	0.00	1.00E+0	0.16	1.00E+0	1.33	0.00E+0
AT2G30140	UDP-glucoronosyl/UDP-glucosyl transferase family protein	0.04	1.00E+0	-0.10	1.00E+0	1.34	0.00E+0
AT3G21780	UDP-glucosyl transferase	-0.02	1.00E+0	0.33	1.00E+0	1.55	0.00E+0
AT2G15490	UGT73B4; UDP-glycosyltransferase	-0.28	1.00E+0	3.31	0.00E+0	3.03	0.00E+0
AT1G74100	sulfotransferase family protein	-0.10	1.00E+0	1.40	0.00E+0	0.62	1.00E+0
AT2G03760	High similarity to flavonol sulfotransferases (FSTs)	0.08	1.00E+0	-0.39	1.00E+0	5.31	0.00E+0
ABC transporters and MATE efflux proteins							
AT1G30400	MRP1, glutathione S-conjugate transporting ATPase (tonoplast)	0.03	1.00E+0	-0.28	1.00E+0	-0.02	1.00E+0
AT2G47800	MRP4, ATPase transporter, multidrug transport (plasma membrane)	0.05	1.00E+0	-0.07	1.00E+0	0.15	1.00E+0
AT3G62150	MDR17 multidrug restistance transporter	0.02	1.00E+0	1.14	1.12E-11	0.07	1.00E+0
AT2G47000	MDR4 multidrug restistance transporter	0.01	1.00E+0	1.96	0.00E+0	0.55	1.00E+0
AT1G02520	MDR8 multidrug restistance transporter	-0.08	1.00E+0	2.88	0.00E+0	1.09	1.51E-7
AT2G34660	MRP2 multidrug resistance protein	0.09	1.00E+0	-0.21	1.00E+0	1.11	7.47E-8
AT3G13080	MRP3 multidrug resistance protein	-	-	1.36	0.00E+0	0.86	1.60E-3
AT1G04120	MRP5 multidrug resistance protein	0.06	1.00E+0	-0.45	1.00E+0	-1.02	3.01E-6
AT2G37280	PDR5 pleiotropic drug resistance	0.09	1.00E+0	-0.17	1.00E+0	-1.66	0.00E+0
AT1G59870	PDR8 pleiotropic drug resistance	0.18	1.00E+0	2.19	0.00E+0	0.62	1.00E+0
AT5G39040	TAP2 transporter antigen processing	-0.10	1.00E+0	0.85	2.85E-5	2.21	0.00E+0
AT3G25620	WBC21 white-brown complex homolog	0.08	1.00E+0	0.28	1.00E+0	1.01	4.96E-6
AT3G52310	WBC28 white-brown complex homolog	0.05	1.00E+0	2.29	0.00E+0	-2.07	0.00E+0
AT2G38330	antiporter/ drug transporter/ transporter DNA binding / transcription factor	-0.10	1.00E+0	0.91	1.94E-6	1.18	2.70E-9
AT1G61890	antiporter/ drug transporter/ transporter_ MATE efflux family protein	0.07	1.00E+0	2.68	0.00E+0	0.08	1.00E+0
AT2G04100	antiporter/ drug transporter/ transporter_ MATE efflux family protein	0.04	1.00E+0	1.83	0.00E+0	-0.03	1.00E+0
AT1G71140	antiporter/ drug transporter/ transporter_ MATE efflux family protein	0.04	1.00E+0	0.05	1.00E+0	2.93	0.00E+0
AT4G39030	EDS5 enhanced disease susceptiblility protein 5	-0.04	1.00E+0	1.69	0.00E+0	3.58	0.00E+0
AT1G58340	ZF14; antiporter/ drug transporter/ transporter_ MATE efflux protein-related	-0.05	1.00E+0	0.22	1.00E+0	1.33	0.00E+0

Supplemental Table S4. Expression of genes associated with C1-metabolism in Arabidopsis cells treated with MTX

Microarray data are expressed in log2(ratio) obtained from comparisons between MTX-treated and control cells after 2, 6, and 24h of treatment (see Fig. 2). A statistical cut-off *P* <0.05 after Bonferroni correction was used to determined which genes were differentially expressed in response to MTX. Positive (red boxes) and negative (green boxes) ratio indicate that the genes are induced or repressed by MTX, respectively. The average log2(ratio) and P-value for 2 independent biological repeats of the experiment is shown.

Accession number	**Gene name**	**Gene description**	**MTX / control 2h**		**MTX / control 6h**		**MTX / control 24h**	
			$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value
THF synthesis, degradation and recycling								
AT2G28880	ADCS	ADC synthase	-0.11	1.00E+0	0.03	1.00E+0	0.13	1.00E+0
AT5G57850	ADCL	ADC lyase	0.05	1.00E+0	-0.10	1.00E+0	0.07	1.00E+0
AT3G07270	GTPCHI	GTP cyclohydrolase I	0.10	1.00E+0	0.45	1.00E+0	0.37	1.00E+0
AT3G11750	DHNA1	Dihydroneopterin aldolase	ns	ns	ns	ns	ns	ns
AT5G62980	DHNA2	Dihydroneopterin aldolase	0.03	1.00E+0	-0.06	1.00E+0	0.28	1.00E+0
AT3G21730	DHNA3	Dihydroneopterin aldolase	ns	ns	ns	ns	ns	ns
AT4G30000	HPPK-DHPS 1	Dihydropterin pyrophophokinase-dihydropteroate synthas	0.07	1.00E+0	0.03	1.00E+0	-0.04	1.00E+0
AT1G69190	HPPK-DHPS 2	Dihydropterin pyrophophokinase-dihydropteroate synthas	ns	ns	ns	ns	ns	ns
AT5G41480	DHFS	Dihydrofolate synthetase	-0.01	1.00E+0	0.05	1.00E+0	-0.09	1.00E+0
AT2G16370	DHFR-TS 1	Dihydrofolate reductase-thymidylate synthase (thy1	0.25	1.00E+0	0.11	1.00E+0	0.27	1.00E+0
AT2G21550	DHFR-TS 3	Dihydrofolate reductase-thymidylate synthase	0.02	1.00E+0	0.30	1.00E+0	0.08	1.00E+0
AT4G34570	DHFR-TS 2	Dihydrofolate reductase-thymidylate synthase (thy2	0.04	1.00E+0	-0.07	1.00E+0	-0.05	1.00E+0
AT5G05980	FPGS B	Folylpolyglutamate synthethase	0.07	1.00E+0	-0.25	1.00E+0	-0.40	1.00E+0
AT3G10160	FPGS C	Folylpolyglutamate synthethase	-0.01	1.00E+0	-0.83	6.79E-5	-0.30	1.00E+0
AT3G55630	FPGS D	Folylpolyglutamate synthethase	0.00	1.00E+0	1.32	0.00E+0	-0.80	1.09E-2
AT1G78680	GGH1	Gamma-glutamyl hydrolase	0.05	1.00E+0	0.17	1.00E+0	0.34	1.00E+0
AT1G78660	GGH2	Gamma-glutamyl hydrolase	0.05	1.00E+0	0.38	1.00E+0	0.03	1.00E+0
AT1G78670	GGH3	Gamma-glutamyl hydrolase	0.15	1.00E+0	0.28	1.00E+0	0.23	1.00E+0
AT1G10310		Pterin reductase	-0.16	1.00E+0	-0.26	1.00E+0	-0.27	1.00E+0
Synthesis and interconversion of C1-THF								
AT1G50480	FTHFS	10-formyl THF synthetase	0.28	1.00E+0	3.07	0.00E+0	0.80	1.13E-2
AT5G13050	5FCL	5-formyl THF cycloligase	ns	ns	ns	ns	ns	ns
AT2G38660	DECY1	Methylene THF dehydrogenase / methenyl THF cyclohydrolas	0.01	1.00E+0	-0.38	1.00E+0	-0.42	1.00E+0
AT4G00620	DECY2	Methylene THF dehydrogenase / methenyl THF cyclohydrolas	0.07	1.00E+0	-0.69	1.85E-2	-0.68	4.89E-1
AT3G12290	DECY3	Methylene THF dehydrogenase / methenyl THF cyclohydrolas	0.03	1.00E+0	0.17	1.00E+0	-0.04	1.00E+0
AT4G00600	DECY4	Methylene THF dehydrogenase / methenyl THF cyclohydrolas	ns	ns	ns	ns	ns	ns
AT3G59970	MTHFR 1	Methylene THF reductase	-0.01	1.00E+0	-0.26	1.00E+0	-1.12	4.04E-8
AT2G44160	MTHFR 2	Methylene THF reductase	0.08	1.00E+0	0.01	1.00E+0	-1.62	0.00E+0
AT4G17360	FTHFD1	10-formyl THF deformylase	ns	ns	ns	ns	ns	ns
AT5G47435	FTHFD2	10-formyl THF deformylase	-0.03	1.00E+0	0.07	1.00E+0	-0.04	1.00E+0
AT1G66520	PDE194	formyltetrahydrofolate deformylase	ns	ns	ns	ns	ns	ns
AT4G17360		formyltetrahydrofolate deformylase	ns	ns	ns	ns	ns	ns
AT5G47435		formyltetrahydrofolate deformylase	-0.03	1.00E+0	0.07	1.00E+0	-0.04	1.00E+0
AT4G37930	SHMTmt1	Serine hydroxymethyltransferase	-0.06	1.00E+0	-0.41	1.00E+0	-1.86	0.00E+0
AT5G26780	SHMTmt2	Serine hydroxymethyltransferase	0.05	1.00E+0	-0.51	1.00E+0	-0.42	1.00E+0
AT4G32520	SHMTcp1	Serine hydroxymethyltransferase	0.07	1.00E+0	-0.36	1.00E+0	0.27	1.00E+0
AT4G13930	SHMTcy1	Serine hydroxymethyltransferase	0.13	1.00E+0	-0.48	1.00E+0	-0.14	1.00E+0
AT4G13890	SHMTcy2	Serine hydroxymethyltransferase	ns	ns	ns	ns	ns	ns
AT1G36370	SHMTcp2	Serine hydroxymethyltransferase	-0.09	1.00E+0	3.04	0.00E+0	0.57	1.00E+0
AT1G22020	SHMTcy3	Serine hydroxymethyltransferase	0.09	1.00E+0	0.03	1.00E+0	0.11	1.00E+0
AT2G26080	P	Glycine decarboxylase - P protein	-0.02	1.00E+0	0.64	1.14E-1	0.00	1.00E+0
AT4G33010	P	Glycine decarboxylase - P protein	0.06	1.00E+0	-0.14	1.00E+0	-1.14	1.55E-8
AT1G11860	T	Glycine decarboxylase - T protein	0.15	1.00E+0	-0.27	1.00E+0	-1.43	0.00E+0

AT1G60990	T	Glycine decarboxylase - T proteir	0.00	1.00E+0	0.23	1.00E+0	-0.45	1.00E+0
AT1G48030	L (LPD1)	Glycine decarboxylase - L proteir	ns	ns	ns	ns	ns	ns
AT3G17240	L (LPD2)	Glycine decarboxylase - L proteir	0.13	1.00E+0	0.13	1.00E+0	-0.06	1.00E+0
AT3G16950	L	Glycine decarboxylase - L proteir	0.15	1.00E+0	-0.19	1.00E+0	-0.37	1.00E+0
AT4G16155	L	Glycine decarboxylase - L proteir	ns	ns	ns	ns	ns	ns
AT2G35120	H	Glycine decarboxylase - H proteir	-0.11	1.00E+0	-0.89	5.17E-6	-1.49	0.00E+0
AT2G35370	H	Glycine decarboxylase - H proteir	ns	ns	ns	ns	ns	ns
AT1G32470	H	Glycine decarboxylase - H proteir	0.09	1.00E+0	-1.12	3.37E-11	-1.96	0.00E+0
Folate carriers								
AT5G66380	AtFOLT1	Folate transporter (plastids)	0.04	1.00E+0	-0.08	1.00E+0	-0.13	1.00E+0
AT2G32040	FBT1	Folate transporter (plastids)	0.00	1.00E+0	0.29	1.00E+0	0.32	1.00E+0
AT1G30400	AtMRP1	glutathione S-conjugate transporting ATPase (tonoplast	0.03	1.00E+0	-0.28	1.00E+0	-0.02	1.00E+0
AT2G47800	AtMRP4	ATPase transporter, multidrug transport (plasma membrane)	0.05	1.00E+0	-0.07	1.00E+0	0.15	1.00E+0
Methionine synthesis and recycling								
AT3G01120	CGS1	Cystathionine gamma-synthase	0.08	1.00E+0	0.30	1.00E+0	0.55	1.00E+0
AT3G57050	CBL	Cystathionine beta-lyase	-0.05	1.00E+0	-0.31	1.00E+0	-0.25	1.00E+0
AT5G17920	MS1	Methionine synthase	0.04	1.00E+0	0.14	1.00E+0	-1.05	8.71E-7
AT3G03780	MS2	Methionine synthase	0.04	1.00E+0	-0.36	1.00E+0	-1.19	1.55E-9
AT5G20980	MS3	Methionine synthase	-0.09	1.00E+0	-0.31	1.00E+0	-0.15	1.00E+0
AT1G64660	MGL	Methionine gamma-lyase	0.04	1.00E+0	-0.18	1.00E+0	1.71	0.00E+0
AT1G66520	FMT	Methionyl-tRNA formyltransferase	ns	ns	ns	ns	ns	ns
AT4G01850	SAMS2	S-adenosylmethionine synthetase	-0.08	1.00E+0	-0.92	1.29E-6	-0.05	1.00E+0
AT3G17390	SAMS3	S-adenosylmethionine synthetase	ns	ns	ns	ns	ns	ns
AT1G02500	SAMS1	S-adenosylmethionine synthetase	-0.10	1.00E+0	-0.17	1.00E+0	-0.65	9.37E-1
AT2G36880	SAMS4	S-adenosylmethionine synthetase	-0.14	1.00E+0	0.77	1.06E-3	-0.71	2.23E-1
AT4G13940	SAHH1	S-adenosylhomocysteine hydrolase	0.07	1.00E+0	-0.10	1.00E+0	-0.40	1.00E+0
AT3G23810	SAHH2	S-adenosylhomocysteine hydrolase	0.11	1.00E+0	-0.37	1.00E+0	-1.71	0.00E+0
AT5G49810	MMT	Methionine S-methyl transferase	0.04	1.00E+0	-0.08	1.00E+0	-1.05	9.33E-7
AT3G25900	HMT1	Homocysteine S-methyltransferase	-0.06	1.00E+0	0.67	4.21E-2	0.40	1.00E+0
AT3G63250	HMT2	Homocysteine S-methyltransferase	0.00	1.00E+0	-0.12	1.00E+0	-0.04	1.00E+0
AT3G22740	HMT3	Homocysteine S-methyltransferase	-0.04	1.00E+0	-0.08	1.00E+0	0.55	1.00E+0
AT3G09820	ADK1	Adenosine kinase	0.00	1.00E+0	-0.01	1.00E+0	0.04	1.00E+0
AT5G03300	ADK2	Adenosine kinase	-0.04	1.00E+0	-0.43	1.00E+0	-2.60	0.00E+0
AT4G39460	SAMT1	S-adenosylmethionine transporter (plastids	0.01	1.00E+0	0.28	1.00E+0	0.02	1.00E+0
AT1G34065	SAMT2	S-adenosylmethionine transporter (mitochondrial, putative	0.03	1.00E+0	-0.41	1.00E+0	-0.27	1.00E+0
S-adenosylmethionine-dependent methyltransferases								
AT1G04870		arginine N-methyltransferase family protein	-0.08	1.00E+0	-0.07	1.00E+0	1.74	0.00E+0
AT1G76090	SMT3	S-adenosyl-methionine-sterol-C-methyltransferase	-0.18	1.00E+0	0.77	8.52E-4	1.63	0.00E+0
AT1G20330	SMT2	sterol-C24-methyltransferase	0.06	1.00E+0	0.89	5.73E-6	1.20	9.14E-10
AT3G26410		methyltransferase/ nucleic acid binding	0.01	1.00E+0	0.49	1.00E+0	1.18	2.28E-9
AT1G64970	G-TMT	gamma-tocopherol methyltransferase (g-TMT)	0.08	1.00E+0	-1.44	0.00E+0	-0.89	4.38E-4
AT5G49020		protein arginine N-methyltransferase family proteir	0.00	1.00E+0	-0.55	1.00E+0	-1.32	0.00E+0
AT1G48600		phosphoethanolamine N-methyltransferase 2	-0.03	1.00E+0	-0.34	1.00E+0	-2.19	0.00E+0
AT5G13710	SMT1	SMT1 controls the level of cholesterol in plants	-0.04	1.00E+0	-0.24	1.00E+0	-3.08	0.00E+0
AT3G18000	NMT1	N-methyltransferase-like protein	-0.01	1.00E+0	-0.14	1.00E+0	-3.17	0.00E+0
Other genes coding folate-dependent enzymes								
AT1G31220	GART	Glycinamide ribonucleotide formyltransferase	0.03	1.00E+0	0.16	1.00E+0	-0.01	1.00E+0
AT2G35040	AICART	Aminoimidazole-carboxamide ribonulceotide formyltransferase	0.04	1.00E+0	-0.01	1.00E+0	0.16	1.00E+0
AT2G46110	KPHMT1	Ketopantoate hydroxymethyltransferase	0.15	1.00E+0	-0.08	1.00E+0	-0.08	1.00E+0
AT3G61530	KPHMT2	Ketopantoate hydroxymethyltransferase	0.01	1.00E+0	0.04	1.00E+0	0.96	4.08E-5

Supplemental Table S5. Expression of genes involved in the sulfate assimilatory pathway in Arabidopsis cells treated with MTX.

Microarray data are expressed in log2(ratio) obtained from comparisons between MTX-treated and control cells after 2, 6, and 24h of treatment (see Fig. 2). A statistical cut-off P<0.05 after Bonferroni correction was used to determined which genes were differentially expressed in response to MTX. Positive (red boxes) and negative (green boxes) ratio indicate that the genes are induced or repressed by MTX, respectively. The average log2(ratio) and P-value for 2 independent biological repeats of the experiment is shown.

		MTX / control 2h		MTX / control 6h		MTX / control 24h	
Accession number	**Gene description**	$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value	$\log_2$(ratio)	P-value
Sulfate transporters							
AT1G22150	sulfate transporter (SULTR1.3)	0.05	1.00E+0	0.41	1.00E+0	-0.11	1.00E+0
AT1G23090	sulfate transporter (AST91)	0.05	1.00E+0	0.08	1.00E+0	0.07	1.00E+0
AT1G77990	sulfate transporter (AST56)	0.02	1.00E+0	0.20	1.00E+0	-0.13	1.00E+0
AT1G78000	sulfate transporter (SULTR1.2)	-0.14	1.00E+0	0.43	1.00E+0	0.20	1.00E+0
AT1G80310	sulfate transporter	0.05	1.00E+0	0.90	4.04E-6	-0.19	1.00E+0
AT2G25680	sulfate transporter	0.13	1.00E+0	-0.06	1.00E+0	0.02	1.00E+0
AT3G12520	sulfate transporter (SULTR4.2)	-0.08	1.00E+0	0.08	1.00E+0	-0.02	1.00E+0
AT3G15990	sulfate transporter (SULTR3.4)	0.17	1.00E+0	0.84	4.79E-5	-1.81	0.00E+0
AT3G51895	sulfate transporter (SULTR3.1)	0.01	1.00E+0	0.01	1.00E+0	0.04	1.00E+0
AT4G02700	sulfate transporter (SULTR3.2)	0.11	1.00E+0	0.01	1.00E+0	0.03	1.00E+0
AT4G08620	sulfate transporter (SULTR1.1)	-0.01	1.00E+0	0.07	1.00E+0	-0.09	1.00E+0
AT5G10180	sulfate transporter (SULTR2.1; AST68)	-0.12	1.00E+0	0.56	1.00E+0	-1.36	0.00E+0
AT5G13550	sulfate transporter (SULTR4.1)	0.06	1.00E+0	0.83	9.03E-5	0.55	1.00E+0
AT5G19600	sulfate transporter (SULTR3.5)	0.01	1.00E+0	0.07	1.00E+0	-0.10	1.00E+0
Sulfate activation and reduction							
AT1G19920	ATP sulfurylase (APS2)	0.11	1.00E+0	-0.47	1.00E+0	-1.05	1.05E-6
AT5G43780	ATP sulfurylase (APS4)	-0.08	1.00E+0	-0.39	1.00E+0	-0.46	1.00E+0
AT3G22890	ATP sulfurylase (APS1)	0.10	1.00E+0	2.31	0.00E+0	2.61	0.00E+0
AT4G14680	ATP sulfurylase (APS3)	0.03	1.00E+0	-0.06	1.00E+0	-0.01	1.00E+0
AT1G62180	adenosine 5'-phosphosulfate reductase (APR2)	0.10	1.00E+0	-0.01	1.00E+0	0.88	7.50E-4
AT4G04610	adenosine 5'-phosphosulfate reductase (APR1)	-0.04	1.00E+0	4.31	0.00E+0	1.66	0.00E+0
AT4G21990	adenosine 5'-phosphosulfate reductase (APR3)	0.02	1.00E+0	0.26	1.00E+0	-0.05	1.00E+0
AT5G04590	sulfite reductase	0.03	1.00E+0	0.31	1.00E+0	0.67	6.03E-1
AT2G14750	adenylylsulfate kinase (AKN1)	0.05	1.00E+0	0.96	1.48E-7	-0.57	1.00E+0
Cysteine and glutathione biosynthesis							
AT2G43750	O-acetylserine (thiol) lyase (oasB)						
AT3G03630	O-acetylserine (thiol) lyase (CS26)	0.01	1.00E+0	-0.01	1.00E+0	-0.01	1.00E+0
AT3G04940	O-acetylserine (thiol) lyase (CYSD1)	0.03	1.00E+0	-0.49	1.00E+0	-0.90	3.69E-4
AT3G59760	O-acetylserine (thiol) lyase (oasC)	0.05	1.00E+0	0.14	1.00E+0	0.07	1.00E+0
AT3G61440	O-acetylserine (thiol) lyase (CYSC1)	0.25	1.00E+0	0.81	2.19E-4	-0.19	1.00E+0
AT4G14880	O-acetylserine (thiol) lyase (oasA1)	0.18	1.00E+0	0.94	4.15E-7	0.15	1.00E+0
AT5G28020	O-acetylserine (thiol) lyase (CYSD2)	0.04	1.00E+0	-0.06	1.00E+0	0.03	1.00E+0
AT1G55920	serine O-acetyltransferase (SAT2.1)	-0.11	1.00E+0	2.35	0.00E+0	0.96	3.79E-5
AT2G17640	serine O-acetyltransferase (SAT3.1)	0.05	1.00E+0	0.32	1.00E+0	0.20	1.00E+0
AT3G13110	serine O-acetyltransferase (SAT2.2)	0.02	1.00E+0	0.12	1.00E+0	-0.03	1.00E+0
AT4G35640	serine O-acetyltransferase (SAT3.2)	0.03	1.00E+0	0.04	1.00E+0	0.02	1.00E+0
AT5G56760	serine O-acetyltransferase (SAT1.1)	-0.02	1.00E+0	-0.35	1.00E+0	-0.19	1.00E+0
AT5G27380	glutathione synthetase (GSH2)	-0.02	1.00E+0	0.18	1.00E+0	-0.15	1.00E+0
AT4G23100	glutamate-cysteine ligase (GSH1)	-0.12	1.00E+0	0.70	1.56E-2	0.12	1.00E+0

Supplemental Table S6. Sequences of primers used for qPCR.

Accession number	Gene description	Gene name	Forward primer	Reverse primer
AT1G19770	purine permease	PUP 14	GGATTCTCGAAAGGGAAAGG	GCGCTTAAGACGGCAGTAAC
AT1G36370	serine hydroxymethyltransferase (plastidial)	cpSHMT	CGAGAAAGTGTGTGAGATGTGCC	CCATGCTCTCTCTGCAACGC
AT1G50480	10-formyltetrahydrofolate synthetase	FTHFS	GAAGGGAAGCGTAGTTTCAGTGAC	TGACCAATAGTAATCTTCCTCAAGAACC
AT3G02470	S-adenosylmethionine decarboxylase	SAMdc	CTCCCCTCGGTTACAGCATTG	GGAGAGAACGATTGGATCCG
AT3G22370	alternative oxidase 1a	AOX1a	ATGTTCCTGCTCCGGCTATTG	CCTTTAGTTCACGACCTTGGTAGTG
AT3G23810	S-adenosyl-L-homocysteine hydrolase	SAHH2	GACTGGAGCTCTCTTGTTCCCTG	CACTATGACACGAGCACCCG
AT3G55630	folylpolyglutamate synthetase (cytosolic)	FPGSD	GAAATTGCTGCAGAGAAAGCC	GAAATGTAGAGCACAACGCAAC
AT4G04610	5'-adenylylsulfate reductase	APR1	CCAGAAGGAGTTTGCTAAGCAGG	CTAGCAATCTGGAACGAGACGAAG
AT4G37930	serine hydroxymethyltransferase (mitochondrial)	mtSHMT	CTGTCTTCCCTGGTCTTCAAGG	GCAATGTGAACAGCTTCCAACAC
AT5G10180	sulfate transporter	SULTR2.1	TGATCAACGTCGATACATCGGG	GCCACCGATTCTGTCGACG
AT1G74590	glutathione transferase	GSTU10	CCCGGTGCACAAGAAGATTCC	GGACATGACTTGACCCATGACCTC
AT5G09810	actin 2/7	ACT2/7	ACATCGTTCTCAGTGGTGGTTC	ACCTGACTCATCGTACTCACTC
AT5G17920	vitamin-B12-independent methionine synthase	MS1	CTGTGGTCTCAAGACCCGT	CCTTGTTCAAATCAAGCTTTTC