Supporting Information

Mitochondrial Glutathione Transferase Zeta 1 is Inactivated More Rapidly by Dichloroacetate than the Cytosolic Enzyme in Adult and Juvenile Rat Liver

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The supporting information presents details of the results described in the manuscript.

Table S1. Average GSTZ1 activity with DCA measured in liver cytosol and mitochondria of young rats.

	Cytosolic GSTZ1 Activity (nmol glyoxalate/min/mg)		**Mitochondrial GSTZ1 Activity** (nmol glyoxalate/min/mg)	
Time (h)	***Control***	***DCA-Treated***	***Control***	***DCA-Treated***
0.25		1.97 ± 0.19		0.133 ± 0.008
0.5	2.20 ± 0.43	1.56 ± 0.15	0.192 ± 0.026	0.117 ± 0.017
1	2.53 ± 0.31	0.98 ± 0.29	0.268 ± 0.031	0.068 ± 0.015
2	2.10 ± 0.66	0.66 ± 0.18	0.341 ± 0.040	0.037 ± 0.012
4	2.02 ± 0.37	0.22 ± 0.17	0.391 ± 0.049	0.024 ± 0.005
8	2.61 ± 0.54	0.09 ± 0.02	0.229 ± 0.034	0.036 ± 0.012
12	2.32 ± 0.61	0.27 ± 0.17	0.297 ± 0.041	0.026 ± 0.006
24	2.46 ± 0.32	0.36 ± 0.19	0.364 ± 0.040	0.038 ± 0.017

Values are presented as mean ± SD, n=6, for each DCA-treatment (8 h treated, n=5) and control time points for rat cytosol and mitochondria.

Table S2. Average GSTZ1 activity with DCA measured in liver cytosol and mitochondria of adult rats.

	Cytosolic GSTZ1 Activity (nmol glyoxalate/min/mg)		**Mitochondrial GSTZ1 Activity** (nmol glyoxalate/min/mg)	
Time (h)	***Control***	***DCA-Treated***	***Control***	***DCA-Treated***
0.25		1.54 ± 0.45		0.245 ± 0.051
0.5	1.51 ± 0.32	1.10 ± 0.25	0.468 ± 0.089	0.222 ± 0.056
1	2.39 ± 0.71	0.82 ± 0.22	0.495 ± 0.067	0.110 ± 0.020
2	3.03 ± 0.53	0.43 ± 0.19	0.548 ± 0.072	0.047 ± 0.010
4	2.72 ± 0.33	0.09 ± 0.02	0.572 ± 0.044	0.024 ± 0.007
8	2.22 ± 0.30	0.08 ± 0.01	0.444 ± 0.052	0.029 ± 0.004
12	1.89 ± 0.31	0.08 ± 0.01	0.516 ± 0.085	0.025 ± 0.004
24	2.16 ± 0.46	0.15 ± 0.10	0.432 ± 0.074	0.023 ± 0.007

Values are presented as mean ± SD, n=6 (8 h time point, n=5), for each DCA-treatment and control (1 and 4 h control, n=5) time points for rat cytosol and mitochondria.

Table S3. Average GSTZ1 expression levels in cytosol and mitochondria of young rats.

	Cytosolic GSTZ1 Expression (ng GSTZ1/μg protein)		**Mitochondrial GSTZ1 Expression** (ng GSTZ1/μg protein)	
Time (h)	***Control***	***DCA-Treated***	***Control***	***DCA-Treated***
0.25		0.662 ± 0.094		0.070 ± 0.015
0.5	0.690 ± 0.114	0.581 ± 0.107	0.074 ± 0.015	0.058 ± 0.018
1	0.544 ± 0.096	0.374 ± 0.112	0.126 ± 0.022	0.032 ± 0.011
2	0.581 ± 0.170	0.281 ± 0.060	0.143 ± 0.033	0.030 ± 0.002
4	0.606 ± 0.176	0.171 ± 0.105	0.140 ± 0.017	0.019 ± 0.006
8	0.716 ± 0.189	0.167 ± 0.072	0.096 ± 0.014	0.021 ± 0.006
12	0.569 ± 0.139	0.104 ± 0.053	0.125 ± 0.056	0.010 ± 0.003
24	0.690 ± 0.054	0.089 ± 0.049	0.135 ± 0.036	0.012 ± 0.011

Values are presented as mean ± SD, n=6, for each DCA-treatment (8 h treated, n=5) and control time points for rat cytosol and mitochondria.

Table S4. Average GSTZ1 expression levels in cytosol and mitochondria of adult rats.

	Cytosolic GSTZ1 Expression (ng GSTZ1/μg protein)		**Mitochondrial GSTZ1 Expression** (ng GSTZ1/μg protein)	
Time (h)	***Control***	***DCA-Treated***	***Control***	***DCA-Treated***
0.25		0.516 ± 0.114		0.092 ± 0.019
0.5	0.497 ± 0.110	0.506 ± 0.139	0.093 ± 0.024	0.127 ± 0.028
1	0.598 ± 0.088	0.379 ± 0.052	0.216 ± 0.037	0.076 ± 0.019
2	0.667 ± 0.067	0.282 ± 0.114	0.212 ± 0.034	0.062 ± 0.029
4	0.601 ± 0.074	0.098 ± 0.040	0.185 ± 0.038	0.026 ± 0.007
8	0.593 ± 0.119	0.133 ± 0.048	0.134 ± 0.045	0.024 ± 0.012
12	0.541 ± 0.083	0.060 ± 0.033	0.150 ± 0.032	0.016 ± 0.004
24	0.554 ± 0.075	0.087 ± 0.054	0.120 ± 0.021	0.018 ± 0.012

Values are presented as mean ± SD, n=6 (8 h time point, n=5), for each DCA-treatment and control (1 and 4 h control, n=5) time points for rat cytosol and mitochondria.

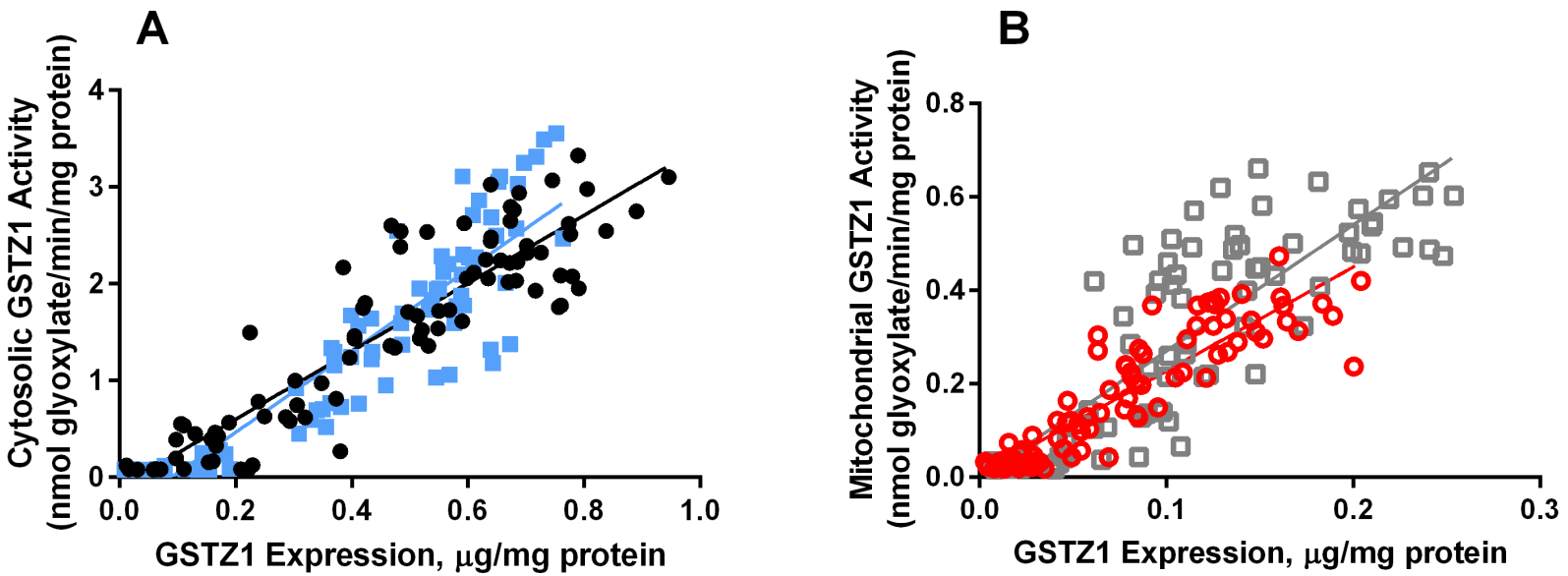

Figure S1: Relationship between GSTZ1 protein expression and activity. A) Correlation of expression and activity in young (black circle) and adult (blue square) rat cytosol. B) Young (open red circle) and adult (open grey square) rat mitochondrial GSTZ1 expresssion and activity correlation. All DCA-treated and control rats were included in this analysis, n=89 for young rat cytosol and mitochondria; n=88 for adult rat cytosol and mitochondria

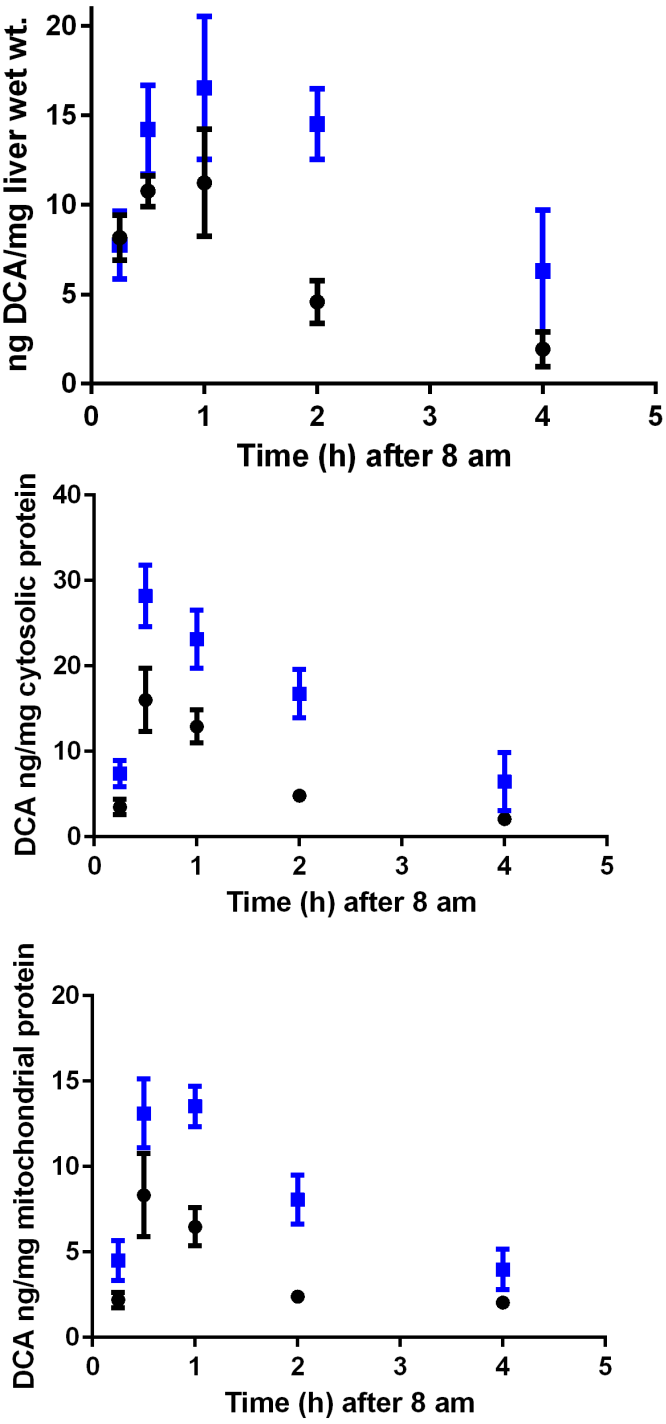

Figure S2: DCA concentrations in liver, cytosol and mitochondria of young (black circles) and adult (blue squares) rats at different times after the dose of DCA. Data shown are mean ± S.D., n=6. Where no error bars are visible, replicates were very close. Concentrations were undetectable after 4h.

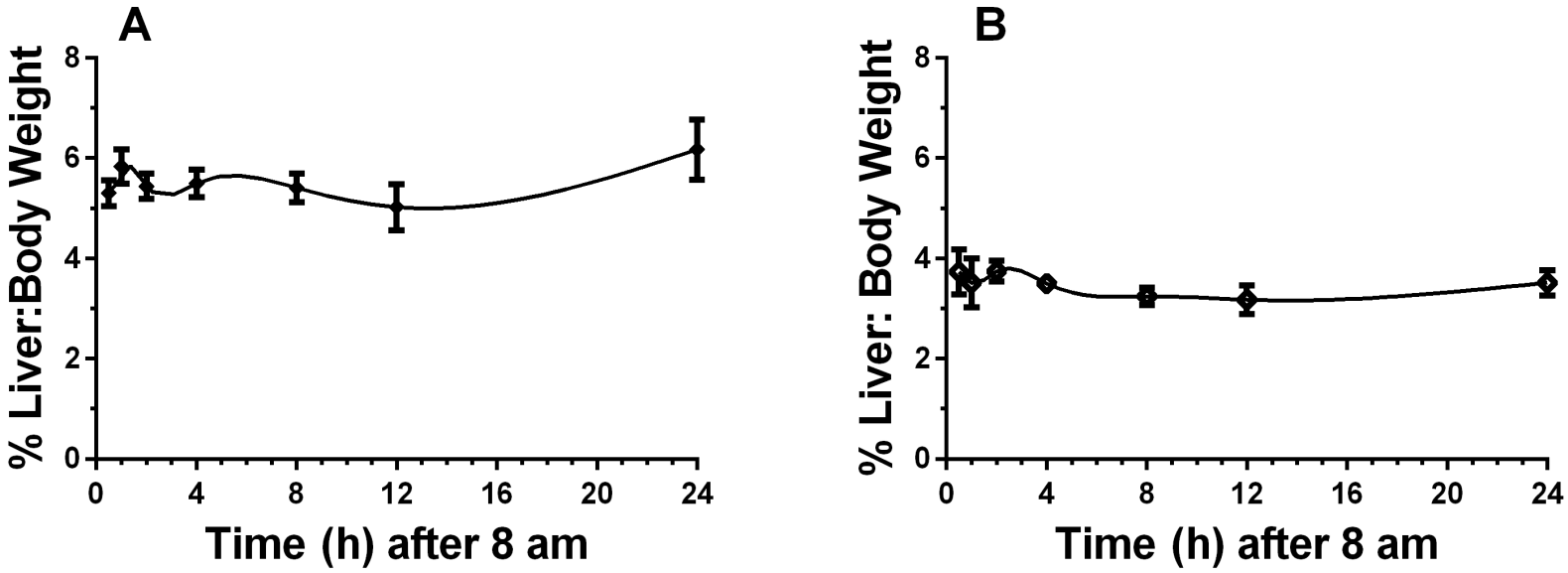

Figure S3: Values for liver to body weight ratio in control (sodium acetate-treated) rats over the 24 hour period after the dose. Panel A shows data for the 4-week old rats and panel B for the 52-week old rats. Values shown are mean ± S.D., n=6

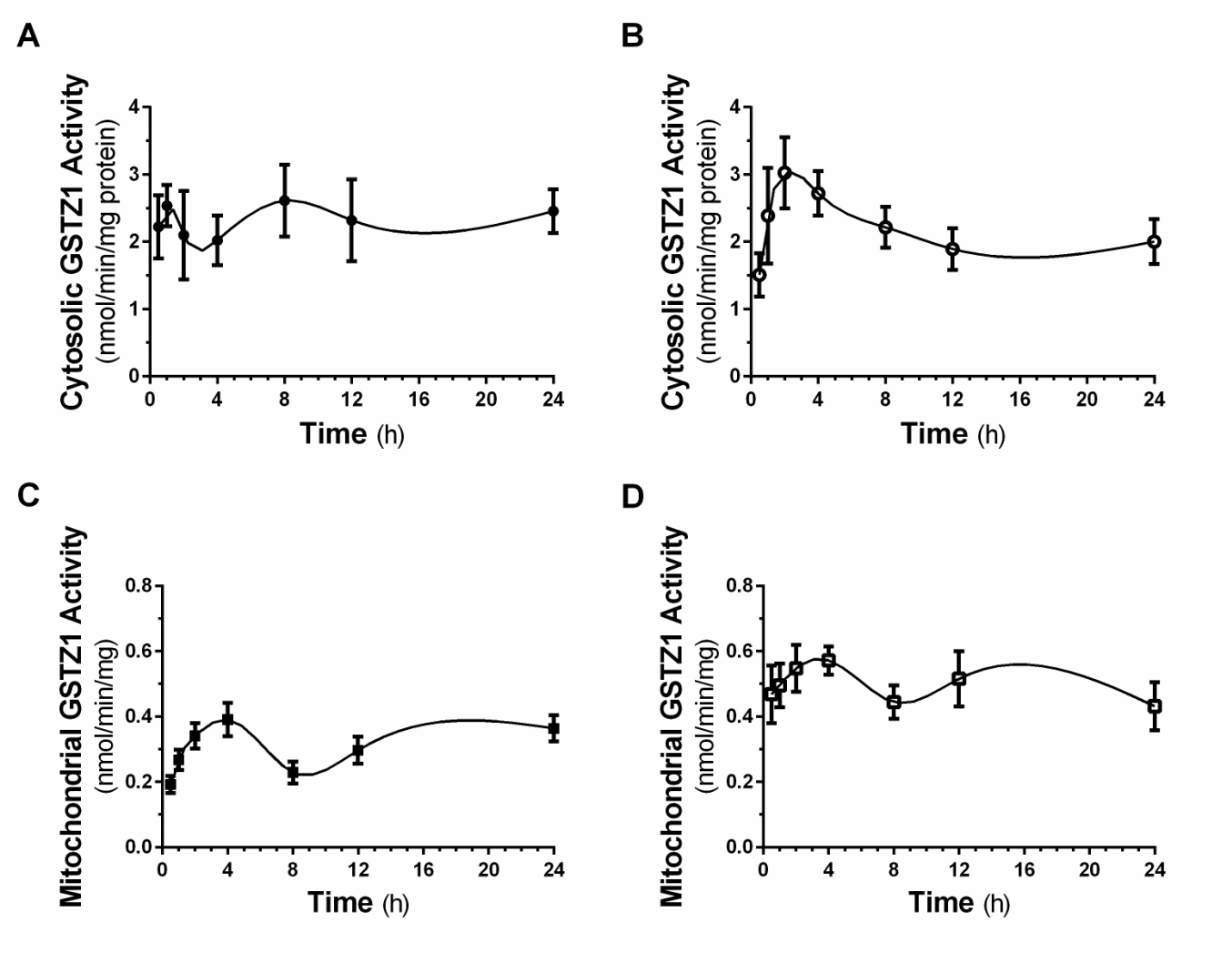

Figure S4: GSTZ1 activity with DCA as substrate in control rats at the times shown after the dose of sodium acetate. Data for young control rats is shown in panels A (cytosol) and C (mitochondria). Data for adult control rats are shown in panels B (cytosol) and D (mitochondria). Values shown are mean ± S.D., n=6.

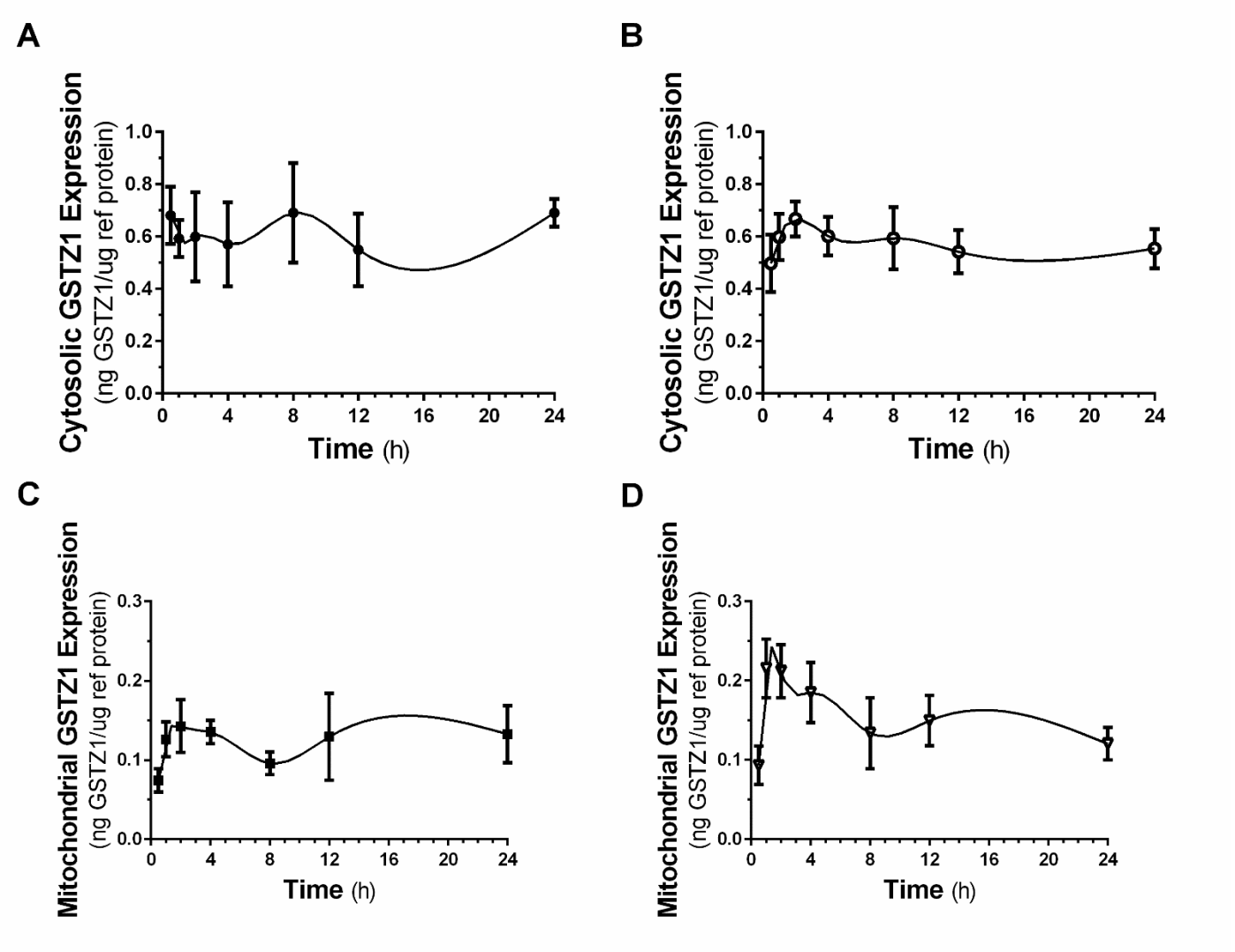

Figure S5: GSTZ1 expression in control rats at the times shown after the dose of sodium acetate. Data for young control rats is shown in panels A (cytosol) and C (mitochondria). Data for adult control rats are shown in panels B (cytosol) and D (mitochondria). Values shown are mean ± S.D., n=6.