SUPPLEMENTAL DATA

Mice lacking three loci encoding fourteen glutathionetransferase genes: a novel tool for assigning function to the GSTP, GSTM, and GSTT families

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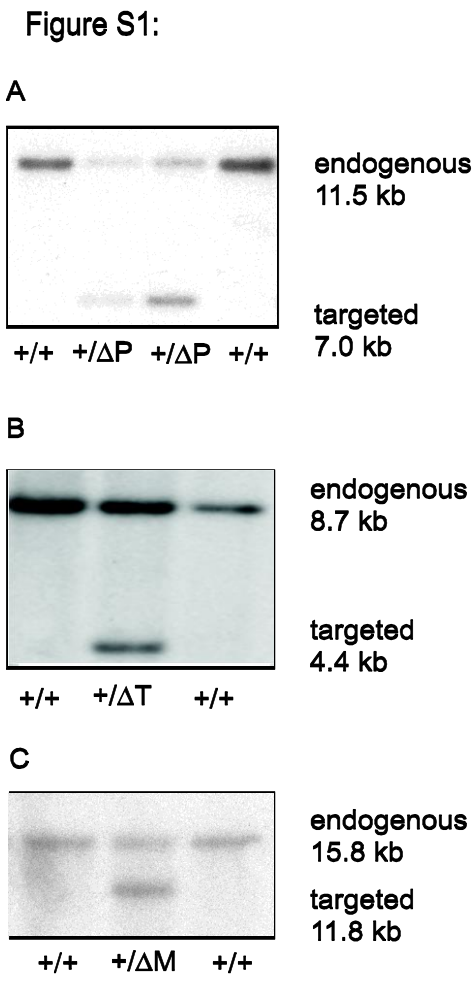
Figure S1. **Verification of integration of the *ΔGstp, ΔGstt* and *ΔGstm* targeting vectors by homologous recombination. (Related to Figure 1)** ES cell populations were first screened for integration of the targeting vectors by PCR analysis. Clones that met these criteria were subjected to further analysis by Southern blot, first using a probe located just outside of the region of DNA used to drive recombination event. **A**. DNA prepared from PCR positive clones obtained after electroporation of 129S6 ES cells with a vector designed to delete the *Gstp* locus was digested with *HindIII.* A probe corresponding to the region just centromeric to the *Gstp* locus recognizes an 11.5 kb fragment in the parental ES cells DNA. This band is also detected in all the PCR positive ES cell clones. However, a new *HindIII* fragment of 7 kb is observed on analysis of DNA prepared from the PCR positive clones. **B.** ES cells were electroporated with a targeting plasmid designed to delete the *Gstt* locus. ES cells identified as targeted based on PCR screening were expanded, DNA prepared and subjected to digestion with *EcoRI*. A probe corresponding to a region immediately telomeric to the *Gstt* locus detects an 8.7 kb band in DNA prepared from the starting ES cell population. The targeted integration of the vector results in a novel fragment of 4.4 kb. **C.** DNA was prepared from ES cells in which PCR analysis indicated that a vector designed to delete



the *Gstm* locus had integration by homologous recombination. *ApaI* digested DNA was analyzed using a probe located just centromeric to the *Gstm* locus. This probe detects a 15.8 kb DNA fragment originating from this locus in the parental ES cell and an 11.8 kb fragment from the targeted locus.

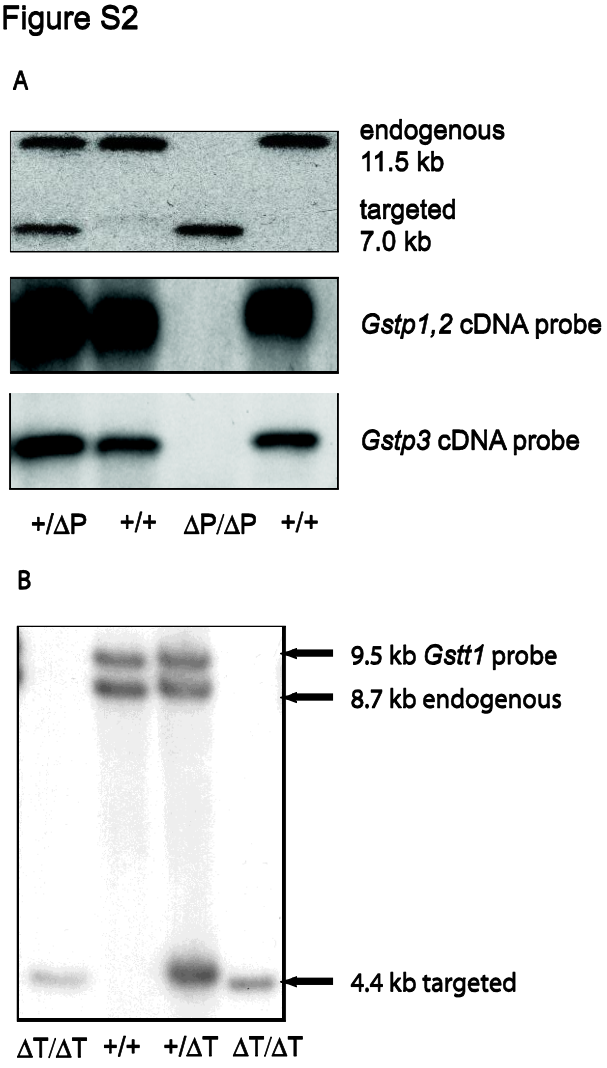
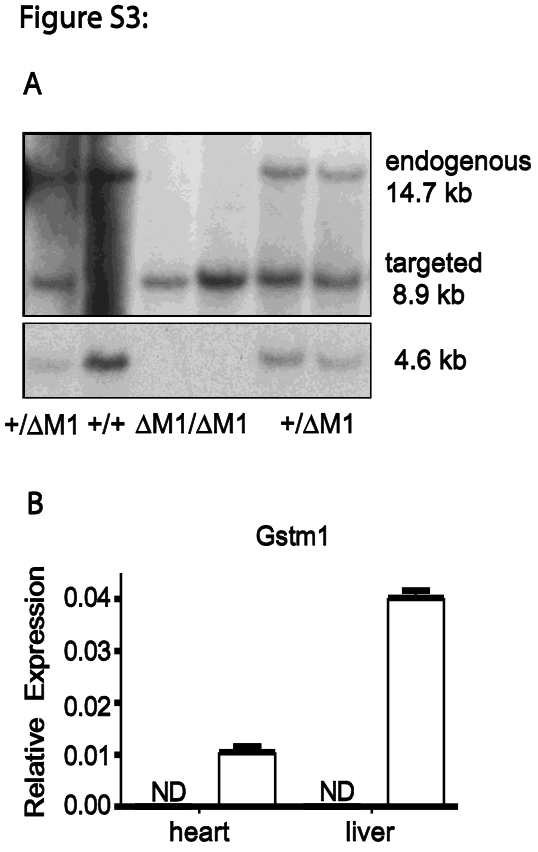


Figure S2. **Verification of the deletion of the *Gstp* and *Gstt* loci**. **(Related to Figure 3) A.** Analysis of *Gstp* locus of offspring generated from intercross of parents heterozygous for the *GstpΔ/Δ* locus. The top panel shows *HindIII* digest DNA analyzed with the probe located just outside the deleted region (identical to that used in the original screen of the ES cell colonies). This analysis identified lanes corresponding to DNA from pups homozygous and heterozygous for the modified locus. The nylon filter was then subjected to further analysis using a probes specific for the *Gstp1/2* genes (middle panel) and for the *Gstp3* gene (bottom panel). As expected the 4.9 kb *HindIII* fragment that contains the 3' portion of *Gstp2* and the entire *Gstp1* gene is absent in pups homozygous for the *GstpΔ/Δ* locus. The 5.9 kb fragment of DNA which contains the mouse *Gstp3* gene is also not present in DNA prepared from pups homozygous for the *GstpΔ/Δ* locus. **B.** A similar strategy was used to verify the deletion of the *Gstt* locus. DNA from offspring generated by the intercross of mice heterozygous for the modified locus was subjected to Southern analysis to identify pups homozygous for the targeted locus. The probe used for this analysis is identical to that described in Figure S1B. In samples from pups homozygous for the modified locus the 8.7 kb band corresponding to the endogenous *Gstt* locus is no longer detected. The filter was immediately subjected to a

second analysis with a probe corresponding to the *Gstt1* gene, which is centrally located in the locus. This probe binds to a 9.5 kb *EcoRI* fragment. As expected this band is observed lanes corresponding to wild type pups and pups heterozygous for the deleted locus, but not in pups that are homologous for *GsttΔ/Δ* allele.

Figure S3. **Generation of mice carrying a null *Gstm1* allele**.



(Related to Figure 4) A. A null mutation was introduced into 129S6 ES cells using a standard targeting vector. Targeted ES cells were identified by PCR and used to generate mice heterozygous for the modified locus. DNA from pups born to *Gstm1*+/- parents was digested with *EcoRV*, and subjected to Southern analysis with a probe corresponding to a region just outside of the *Gstm1* gene (top panel). This probe recognizes a 14.7 kb fragment corresponding to the wild type *Gstm1* locus, and an 8.9 kb fragment generated by the targeted locus. A probe corresponding to the *Gstm1* gene itself failed to bind to DNA from *Gstm1*-/- pups (bottom panel), while it recognized the expected band of 4.6 kb in DNA in wild type and *Gstm1*+/- mice.

B. Verification of loss of expression of *Gstm1* in mice homozygous for the targeted allele. Total RNA was isolated from the heart and liver of wild type and *Gstm1*-/- mice and analyzed for expression of *Gstm1* by qPCR. Transcripts were easily detected in RNA prepared from both tissues obtained from wild type mice, while expression of this gene could not be detected in samples prepared from the *Gstm1*-/- animals. Reverse transcription of total RNA to cDNA for quantitative qPCR was performed using a high-capacity cDNA archive kit (Applied Biosystems). Primers and probes specific for *Gstm1* were purchased from Applied Biosystems. All reactions were performed with TaqMan PCR Universal Master Mix (Applied Biosystems) using the Applied Biosystems 7900 HT Fast Real-Time PCR System. Reactions were carried out in doublets. Expression levels were normalized to 18S RNA. Data was analyzed using the comparative C_T method (ΔCT) as described by

Applied Biosystems. Data shows mean obtained from three samples ± SEM. ND (not detected) indicates that the level of the transcript was below the level of sensitivity of the assay.

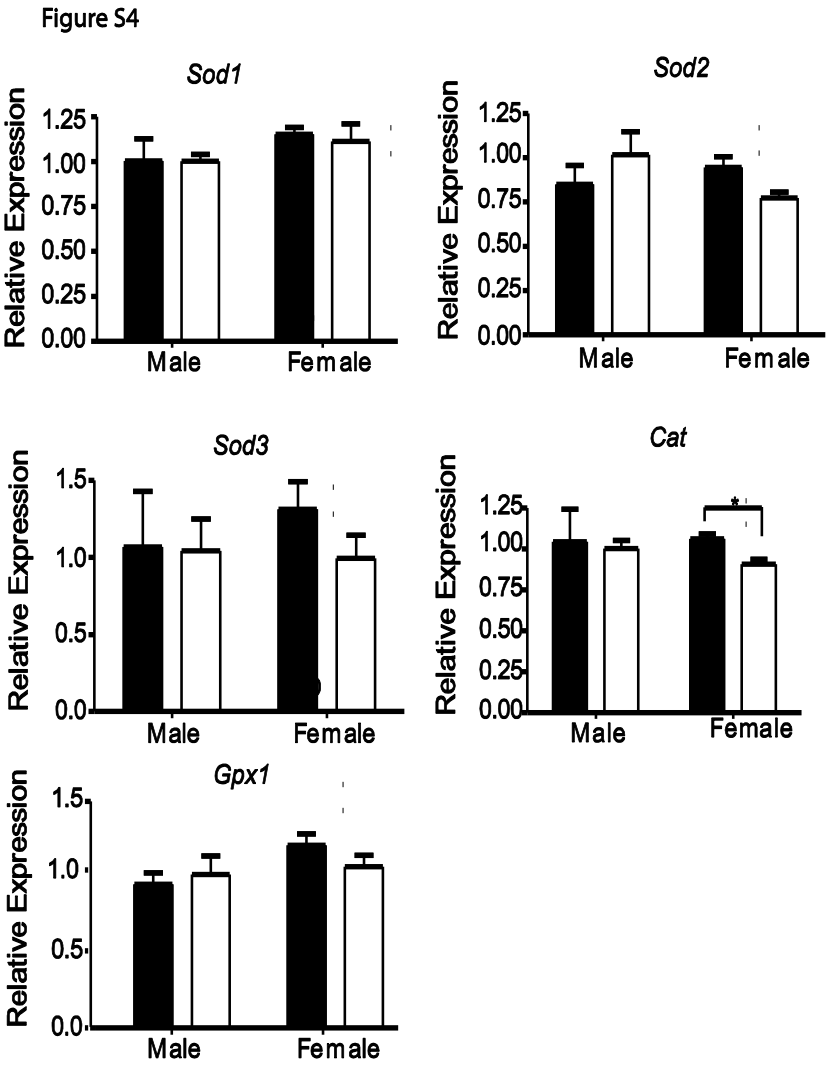


Figure S4 Relative abundance of transcripts of genes for antioxidant enzymes. (Related to Figure 5) Total RNA was prepared from liver of male and female *ΔPMT* (solid bars) and wild type controls (open bars) and the level of transcripts for the gene, indicated in each panel, determined by qPCR. Primers and probes for specific for these transcripts were obtained from Applied biosystems. Expression levels were normalized to 18S RNA. No difference was detected using this method in the expression of these enzymes with the exception of an increase of about 20% catalase transcripts. This increase was, however, not observed on comparison of samples from the *ΔPMT* males. Data was analyzed using the comparative C_T method (ΔΔCT). Values represent the mean of samples from three animals ± SEM. Asterisk indicates $p<0.05$ (unpaired t test). *Sod1-3:* superoxide dismutase, *Cat*: catalase, *Gpx1:* glutathione peroxidase.

Supplementary methods:

Primer sets for mice genotyping:

Gstm,

common: TTTGTTTTCTTGGGAGGAATCAAC

endogenous: GGGTGTCTTGGGTTCTATAAGAGG

delta locus: AAATGCCTGCTCTTTACTGAAGG

Gstt

common: ATGCTTTGCGCAGCGGACTCTCG

endogenous: TTCGCATCAGACTTCCTGGTATTC

delta locus: AAATGCCTGCTCTTTACTGAAGG

Gstp

endo upper: GGGCCTGGGCTCGCTCACGA

endogenous lower: TAAGGATTTGGGGACAGCATTCAG

delta locus upper: AAATGCCTGCTCTTTACTGAAGG

delta locus lower: ATGGCGTTTGCTTTCTTGGGTTGT

Gstm1

common: TCAACCTGGGATTCTTACAGAAGA

endogenous: TTTTAATGAGCCTGCTTAGCTGAG

mutant: AAATGCCTGCTCTTTACTGAAGG

Supplemental Table 1 (related to Figure 4):

Homology to universal probe for *Gstm* region. The universal probe is a 397 bp PCR product corresponding to mouse genomic DNA extending from the middle of exon 3 to just downstream of exon 4 of *Gstm6.* It shares sufficient homology to allow detection by Southern blot of 8 regions in the *Gstm* gene cluster as well as four other regions in the mouse genome (shaded).

Size (bp)	Match (%)	chromosome	gene
29467	90.1	1	*Gstm2*
15840	96.5	1	*Gstm3*
14717	100.0	1	*Gstm7-6*
12598	92.0	1	*Gstm1*
11038	92.4	1	*Gstm5-7*
8535	90.4	1	*Gstm6-3*
6711	69.3	1	*Gstm4*
6326	87.7	1	*Gstm1-4*
18068	92.2	5	
13393	93.0	18	
11870	94.9	1	
5318	97.6	10	

Supplemental Table 2 (related to Figure 5):

Body weight of +/+ and *ΔPMT* mice at 8 weeks of age, n for each group is indicated in parentheses.

Sex (n)	**+/+**	**ΔPTM**
Male (11)	24.64 ± 0.31	24.25 ± 0.59
Female (9)	21.22 ± 0.59	20.92 ± 0.61

Supplemental Table 3 (related to Figure 5):

Analysis of the development of immune cell populations in mice lacking GSTP, GSTT and GSTM (ΔPMT). Cells from whole blood (white blood cells), spleen, thymus and lymph nodes were collected, and analyzed for number of T cells (CD3+, CD4+, CD8+) , B cells (B220+), myeloid (CD11+, CD11+ GR1+) cells as described in method section. All leukocytes were observed in both with wild type and mutant mice, although a small but significant decrease in the number of B cells was observed in the *ΔPMT* mice. No difference was observed in the composition of B cells, T cells, macrophages and neutrophils in the spleen, and B cells in the lymph nodes of wild type and *ΔPMT* mice. The ratio of CD4+ and CD8+ T cells in the thymus also did not differed. Mice analyzed were between 6-8 weeks of age and were sex matched. Data shows means from 6 mice ± SEM. P<0.5*, determined by unpaired t test.

Peripheral blood				
	CD3+	**B220+**	**CD11b+**	**CD11b+GR-1+**
+/+	37.3 ± 4.2	48.6 ± 11.5*	38.1 ± 11.7	5.6 ± 3.9
ΔPMT	39.4 ± 5.6	33.6 ± 11.2*	24.6 ± 9.5	7.8 ± 1.9
Spleen				
+/+	46.9 ± 1.4	64.1 ± 5.6	10.6 ± 1.7	2.1 ± 0.2
ΔPMT	43.3 ± 1.1	58.1 ± 6.9	9.9 ± 0.7	2.5 ± 0.7
Thymus				
	CD4+	**CD8+**		
+/+	9.0 ± 0.5	3.3 ± 1.3		
ΔPMT	8.3 ± 1.5	3.4 ± 0.3		
Lymph nodes				
	B220+			
+/+	63.9 ± 8.1			
ΔPMT	54.7 ± 3.2			

Supplemental Table 4 (related to Figure 5):

Blood samples were collected in EDTA (5 mM) from deeply anesthetized wild type and *ΔPMT* animals by cardiac puncture and analyzed using Heska's Animal blood counter. Clinical chemistry was carried out on plasma prepared from the blood using an Automatic Chemical Analyzer (Johnson and Johnson's V350). Data shows means from 3 mice ± SEM. P<0.5*, determined by unpaired t test.

	+/+	**ΔPMT**
General Health Test & Liver Test		
Alb g/dL	2.5 ± 0.20	2.63 ± 0.09
BUN mg/dL	25.67 ± 1.33	23.33 ± 1.20
Crea mg/dL	0.2 ± 0	0.2 ± 0
Glu mg/dL	199.7 ± 13.54	170.0 ± 7.0
AST U/L	50.33 ± 1.45	66.33 ± 14.75
ALT U/L	33.0 ± 1.15	40.33 ± 10.41
ALKP U/L	76.0 ± 2.31*	59.67 ± 4.81*
Hematology		
WBC - 10^3/ul	1.13 ± 0.33	0.83 ± 0.13
LYMF - 10^3/ul	0.67 ± 0.22	0.50 ± 0.10
GRAN - 10^3/ul	0.20 ± 0.06	0.17 ± 0.03
MONO - 10^3/ul	0.27 ± 0.07	0.17 ± 0.03
LYMF - %	59.77 ± 6.90	61.47 ± 2.41
GRAN - %	25.3 ± 3.48	25.0 ± 1.19
MONO - %	14.93 ± 3.59	13.50 ± 1.86
HCT - %	51.07 ± 2.24	43.53 ± 4.87
MCV - fl	55.87 ± 1.12	53.70 ± 0.36
RBC - 10^6/ul	9.14 ± 0.40	8.09 ± 0.87
HGB - g/dl	14.87 ± 0.59	12.93 ± 1.32
MCH - pg	16.27 ± 0.48	16.07 ± 0.23
MCHC - g/dl	29.10 ± 0.21	29.93 ± 0.43
RDW%	21.87 ± 2.59	19.83 ± 0.76
MPV - fl	5.97 ± 0.09	5.97 ± 0.09
PLT - 10^3/ul	566.0 ± 52.0	611.7 ± 74.58