SUPPLEMENTARY MATERIALS

EFFECTS OF *GSTT1* GENOTYPE ON DETOXIFICATION OF 1,3-BUTADIENE DERIVED DIEPOXIDE AND FORMATION OF PROMUTAGENIC DNA-DNA CROSSLINKS IN HUMAN HAPMAP CELL LINES

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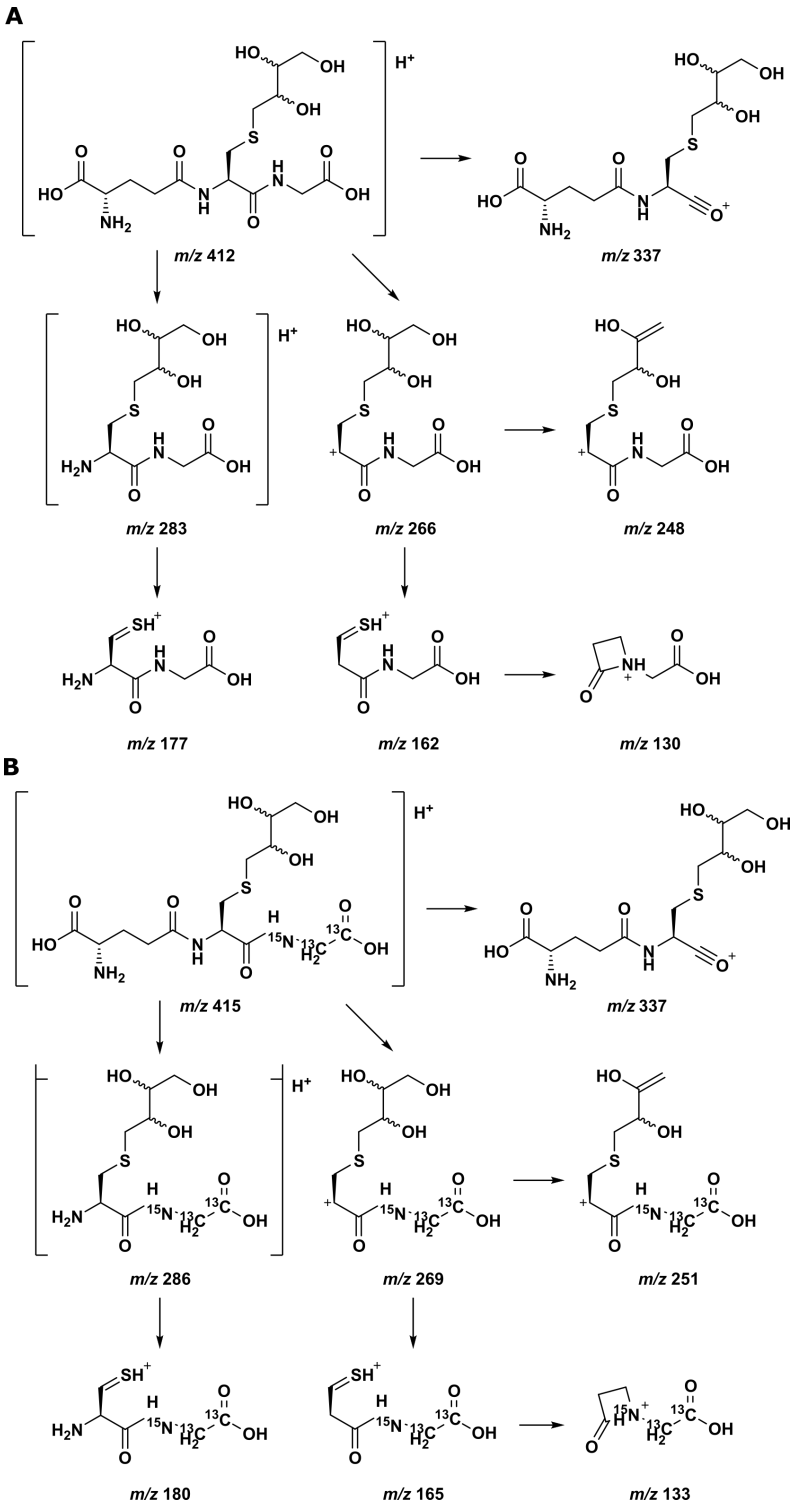

Figure S1. Proposed MS fragments for (A) THB-GSH and (B) [$^{15}N^{13}C_2$-glycine]THB-GSH.

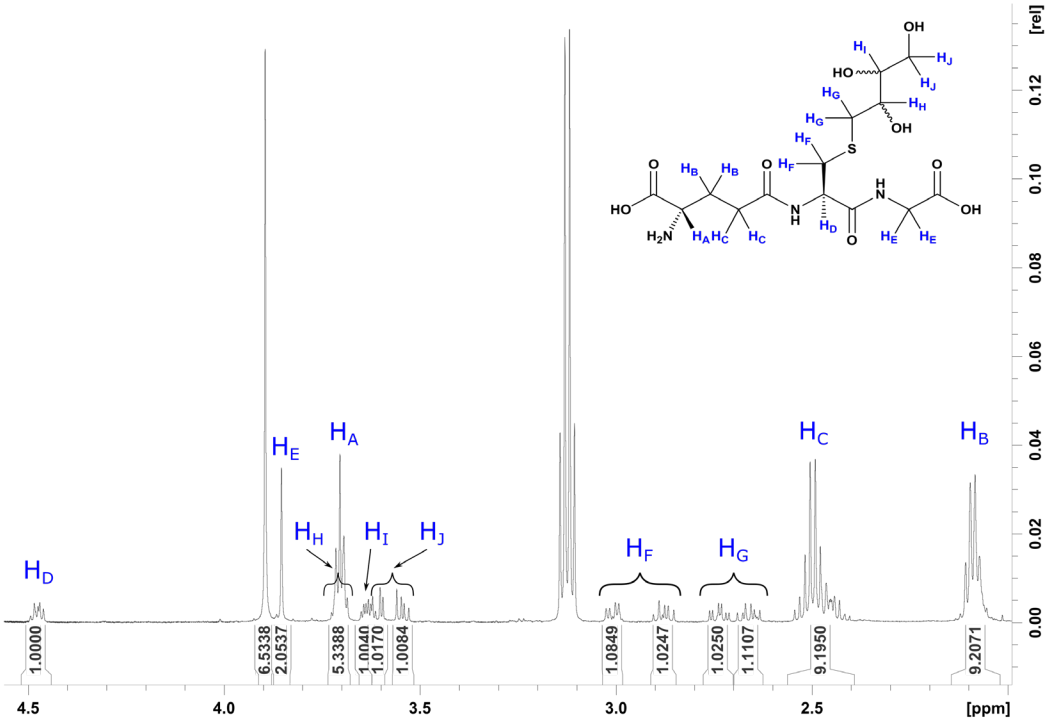

Figure S2. ^{1}H-NMR spectrum of *S*-(1,2,3-trihydroxybutyl)-GSH (THB-GSH) prepared from L-glutathione and racemic DEB as a mixture of diastereomers. The NMR spectra was acquired in D_2O at ambient temperature using a Bruker (600.64 MHz) Avance Neo spectrometer equipped with a 5 mm TCI cryoprobe at the Minnesota NMR Center and Bruker zg30 pulse program with a sweep width of 11,904.76 Hz, 65536 data points, 128 scans, recycle delay of 1.0 sec, and 32 receiver gain.

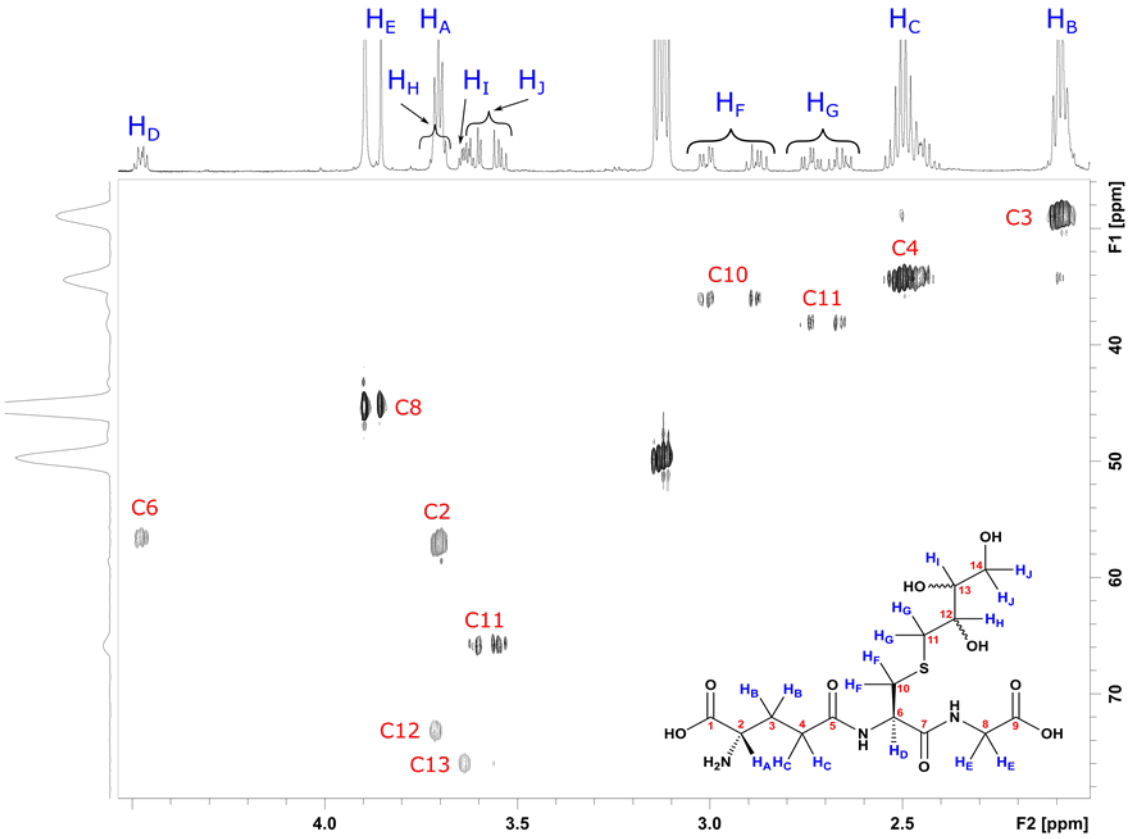

Figure S3. ^{1}H-^{13}C HSQC spectrum of *S*-(1,2,3-trihydroxybutyl)-GSH (THB-GSH) prepared from L-glutathione and racemic DEB as a mixture of diastereomers. The NMR spectra was acquired in D_2O at ambient temperature using a Bruker (600.64 MHz) Avance Neo spectrometer equipped with a 5 mm TCI cryoprobe at the Minnesota NMR Center and Bruker hsqcedetgpsisp2.3 pulse program with 4096 x 128 data matrix, 32 dummy scans, 16 scans, recycle delay of 1.0 sec, and 101 receiver gain.

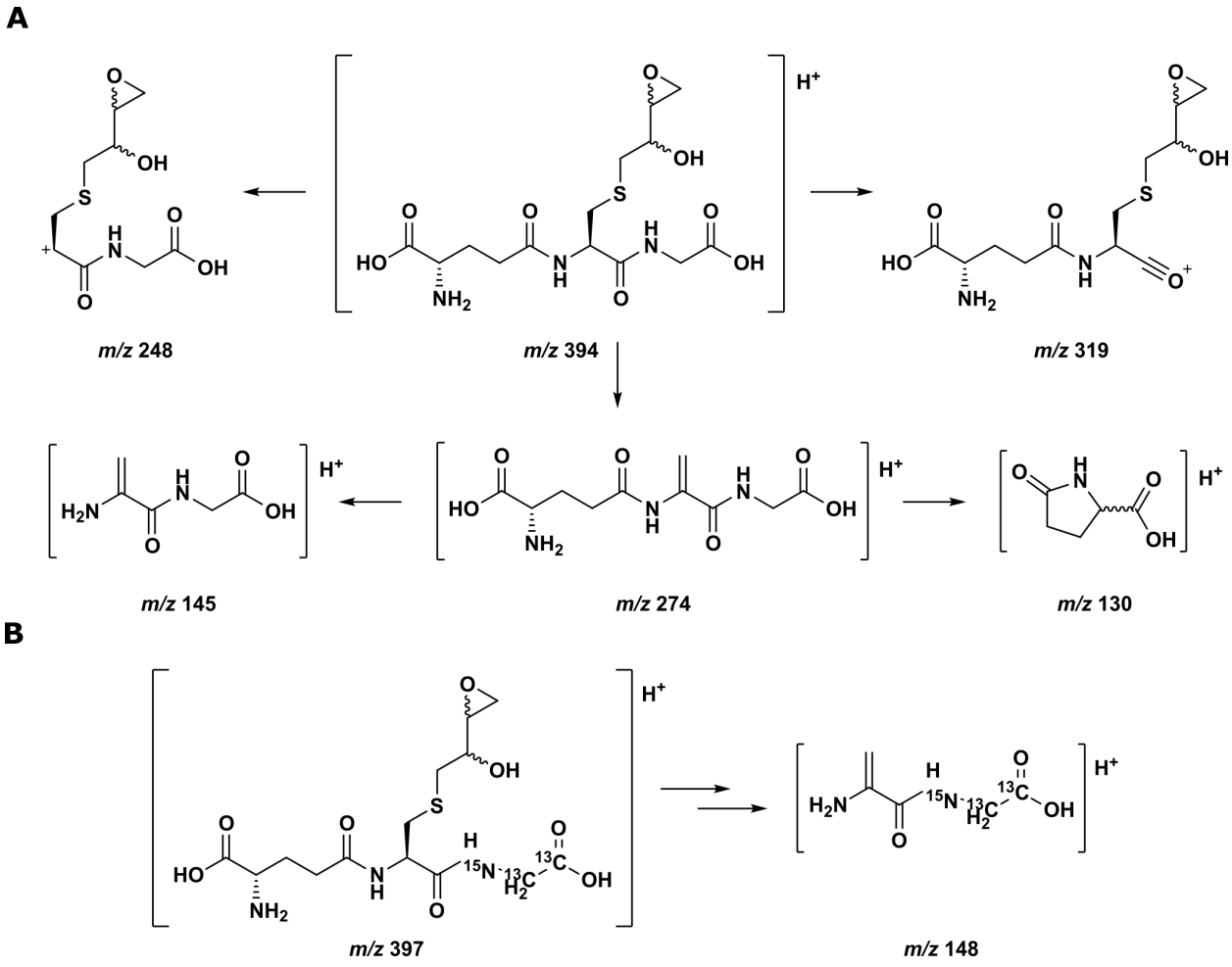

Figure S4. Proposed MS fragments for (A) HEB-GSH and (B) [$^{15}N^{13}C_2$-glycine]HEB-GSH.

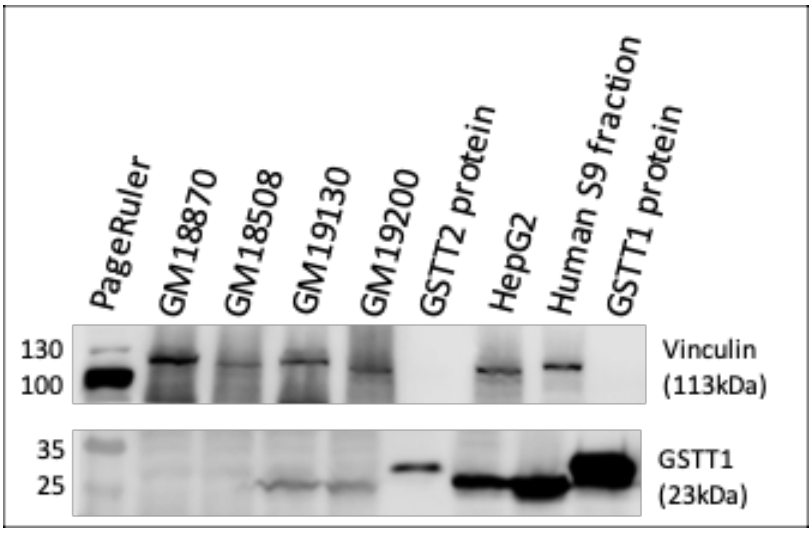

Figure S5. A representative immunoblot of GSTT1 protein (Sigma GSTT1 Antibody: ABS1653) and loading control, Vinculin (Sigma Vinculin antibody: SAB1404522) using HapMap cell lysates and controls.

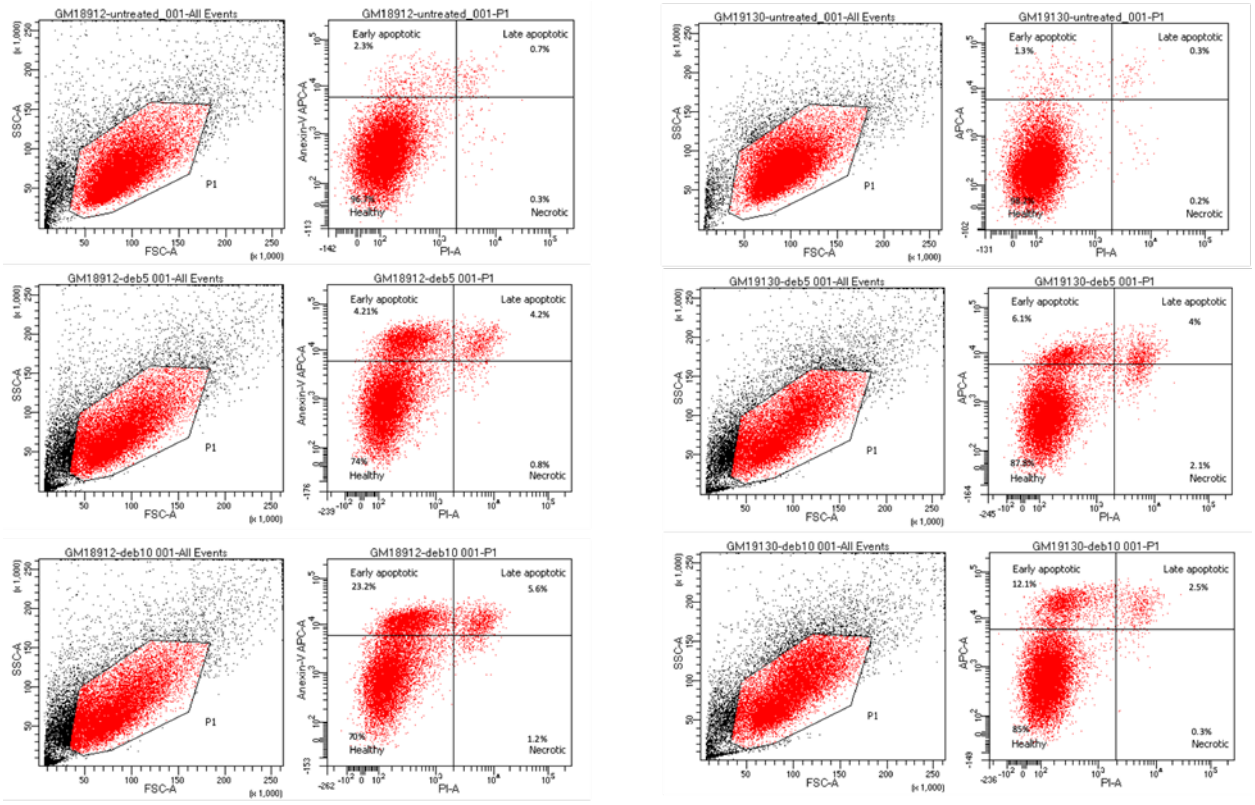

Figure S6. Representative flow cytometry plots using annexin V, allophycocyanin (APC) and propidium iodide (PI) staining for apoptosis measurement. During early apoptotic stage, cells stain with annexin V but still exclude PI. In later stages of apoptosis, cells stain with both APC and PI. Together, early and late apoptotic cells sum up to give total apoptosis induced in the population.

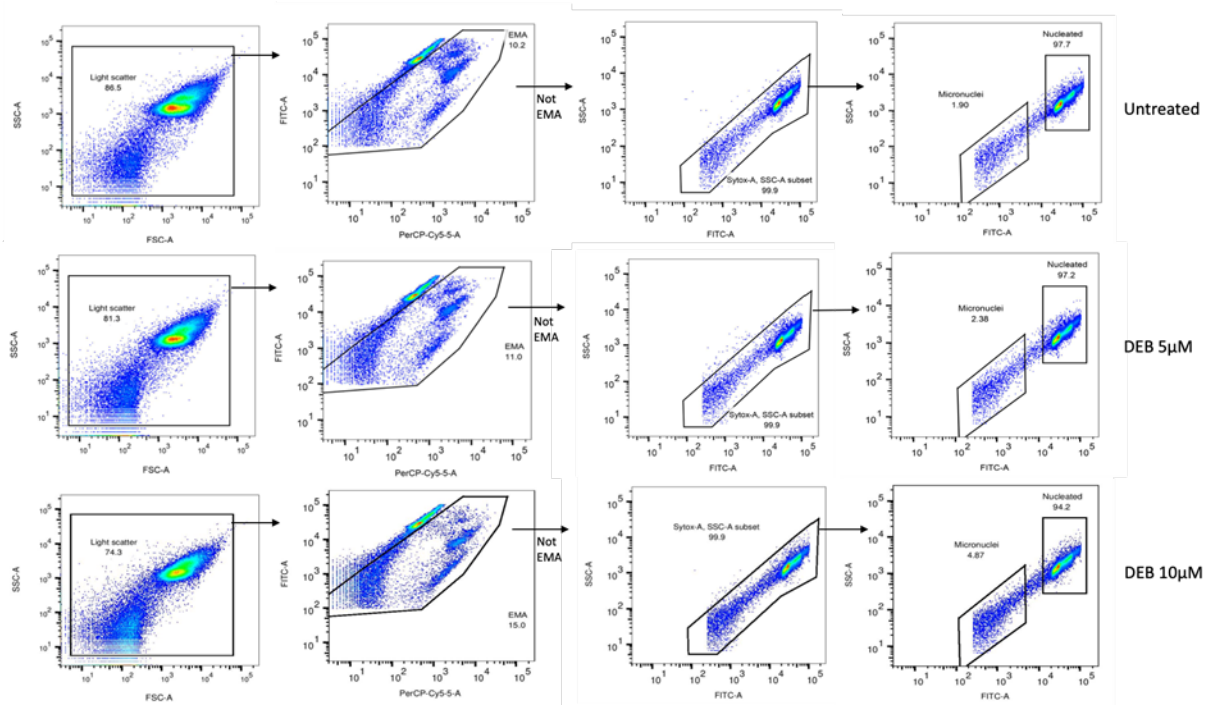

Figure S7. Representative flow cytometry plots for micronuclei formation measurement (cell line: GM12812). A liberal "Light Scatter" region is drawn as MN could have got excluded otherwise based on their small size. These events are further gated to exclude EMA positive events, denoting chromatin from dead/dying cells. Only the "not EMA" events are further gated to determine MN frequency.

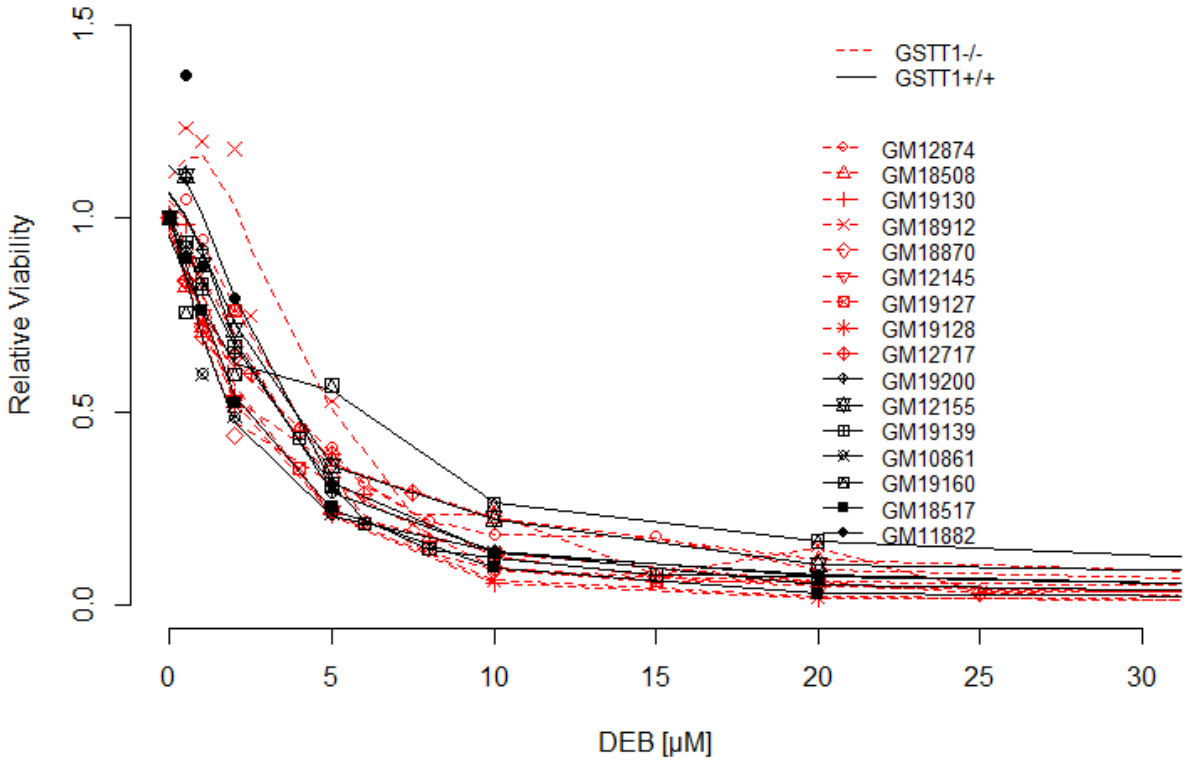

Figure S8. Cytotoxicity curves for sixteen lymphocyte cell lines. Lymphocyte cell lines were seeded (2,000 cells per well) in triplicate in 96-well plates (final volume:100 µL). After 24 h, the cells were treated with 0 - 50 µM DEB for 72 h. Cell viability was determined with CellTiter Glo (Promega, Madison, WI) according to the manufacturer's instructions. Cell survival was normalized to the luminescence of the cells treated with 0 µM. Standard deviation for replicate analysis were <20%; error bars are omitted for clarity.

Table S1. GSTT1 and GSTM1 copy number in the cell lines.[1]		
Cell line	GSTT1	GSTM1
GM10861	0	0
GM10851	0	0
GM18872	0	2
GM18870	0	1
GM19139	0	1
GM18508	0	0
GM12874	0	0
GM18912	0	1
GM18517	0	2
GM19128	0	1
GM19211	1	0
GM12812	1	0
GM19200	2	2
GM12145	1	0
GM18516	1	1
GM12155	2	0
GM10860	1	0
GM19130	2	2
[1]As reported in *Nat. Genet. 38*, 86-92, 2006.		

Table S2. Apoptosis measurements in DEB-treated *GSTT1* null HapMap cell lines.[1]

Cell line	GSTT1 Genotype	μM DEB	Early apoptosis (AnnexinV+/PI-)	Late apoptosis (AnnexinV+/PI+)	Total apoptosis (Early+Late)
GM10861	-/-	0	0.4% ± 0.07%	0.4% ± 0.04%	0.8% ± 0.03%
		5	1.2% ± 0.09%	0.7% ± 0.04%	1.8% ± 0.1%
		10	1.2% ± 0.2%	0.8% ± 0.07%	2.0% ± 0.1%
GM10851	-/-	0	0.5% ± 0.08%	0.2% ± 0.02%	0.7% ± 0.1%
		5	1.3% ± 0.07%	0.3% ± 0.05%	1.6% ± 0.08%
		10	2.0% ± 0.2%	0.5% ± 0.1%	2.6% ± 0.1%
GM18872	-/-	0	1.3% ± 0.1%	0.4% ± 0.06%	1.8% ± 0.2%
		5	2.1% ± 0.09%	0.8% ± 0.01%	2.9% ± 0.09%
		10	4.1% ± 0.2%	1.7% ± 0.2%	5.8% ± 0.4%
GM18870	-/-	0	1.6% ± 0.08%	1.0% ± 0.06%	2.5% ± 0.08%
		5	4.8% ± 0.2%	2.6% ± 0.2%	7.4% ± 0.3%
		10	6.1% ± 0.1%	5.5% ± 0.3%	11.6% ± 0.4%
GM19139	-/-	0	1.0% ± 0.4%	1.5% ± 0.7%	2.5% ± 0.6%
		5	4.5% ± 1.8%	3.1% ± 2.2%	7.6% ± 3.9%
		10	5.9% ± 1.9%	5.9% ± 1.7%	11.7% ± 1.1%
GM18508	-/-	0	0.5% ± 0.2%	0.9% ± 0.8%	1.4% ± 0.8%
		5	5.7% ± 0.1%	3.0% ± 0.09%	8.7% ± 0.07%
		10	6.8% ± 2.3%	6.5% ± 3.7%	13.3% ± 4.1%
GM12874	-/-	0	1.3% ± 0.2%	0.8% ± 0.4%	2.1% ± 0.5%
		5	8.1% ± 0.9%	6.4% ± 1.6%	14.5% ± 2.6%
		10	8.6% ± 0.8%	7.1% ± 4.8%	15.7% ± 5.1%
GM18912	-/-	0	1.7% ± 0.3%	0.6% ± 0.03%	2.4% ± 0.3%
		5	14.6% ± 0.6%	7.5% ± 3.4%	22.1% ± 3.9%
		10	15.7% ± 1.3%	11.8% ± 4.9%	27.5% ± 6.1%
GM18517	-/-	0	1.0% ± 0.2%	0.3% ± 0.1%	1.3% ± 0.1%
		5	14.3% ± 2.6%	12.6% ± 2.9%	26.9% ± 5.4%
		10	13.2% ± 3.0%	17.4% ± 0.4%	30.6% ± 3.2%
GM19128	-/-	0	2.0% ± 0.9%	1.0% ± 0.3%	3.0% ± 0.6%
		5	17.5% ± 3.4%	10.4% ± 2.6%	27.9% ± 6.0%
		10	19.7% ± 2.1%	19.0% ± 2.1%	38.8% ± 4.2%

[1]Lymphoblastoid cell lines were treated with 0, 5 or 10 μM DEB for 24 h in triplicate. See The percent of apoptotic cells was determined based on annexin V marker expression on the cell surface as detected by allophycocyanin (APC) labeled annexin-V. During early apoptotic stage, cells stain with APC but still exclude propidium iodide (PI), whereas cells in later stages of apoptosis stain with both APC and PI. Together early and late apoptotic cells sum up to give total apoptosis induced in the population.

Table S3. Apoptosis measurement in DEB-treated *GSTT1* Positive HapMap cell lines.[1]

Cell line	GSTT1 Genotype	μM DEB	Early apoptosis (AnnexinV+/PI-)	Late apoptosis (AnnexinV+/PI+)	Total apoptosis (Early+Late)
GM19211	-/+	0	0.8% ± 0.06%	0.3% ± 0.01%	1.1% ± 0.06%
		5	1.6% ± 0.1%	0.4% ± 0.05%	2.1% ± 0.2%
		10	2.0% ± 0.2%	0.5% ± 0.1%	2.6% ± 0.2%
GM12812	-/+	0	1.0% ± 0.07%	0.3% ± 0.02%	1.4% ± 0.05%
		5	1.5% ± 0.09%	0.7% ± 0.03%	2.2% ± 0.1%
		10	2.5% ± 0.9%	1.1% ± 0.4%	3.7% ± 1.3%
GM12145	-/+	0	1.4% ± 1.4%	1.3% ± 0.9%	2.8% ± 0.8%
		5	5.1% ± 0.6%	3.3% ± 0.7%	8.4% ± 1.2%
		10	4.1% ± 2.2%	4.2% ± 1.7%	8.3% ± 3.7%
GM18516	-/+	0	1.2% ± 0.1%	0.4% ± 0.03%	1.6% ± 0.09%
		5	4.2% ± 0.1%	1.2% ± 0.2%	5.4% ± 0.3%
		10	6.5% ± 0.1%	1.7% ± 0.01%	8.2% ± 0.1%
GM10860	-/+	0	2.3% ± 0.2%	1.5% ± 0.1%	3.8% ± 0.2%
		5	4.7% ± 0.2%	2.6% ± 0.4%	7.4% ± 0.4%
		10	6.0% ± 0.3%	4.1% ± 0.3%	10.1% ± 0.5%
GM19200	+/+	0	0.7% ± 0.4%	0.7% ± 0.3%	1.4% ± 0.2%
		5	2.6% ± 0.1%	0.8% ± 0.04%	3.4% ± 0.1%
		10	2.6% ± 0.6%	2.4% ± 1.4%	5.1% ± 0.9%
GM12155	+/+	0	0.8% ± 0.2%	0.6% ± 0.3%	1.4% ± 0.3%
		5	4.2% ± 1.3%	3.3% ± 1.2%	7.5% ± 2.5%
		10	5.5% ± 2.4%	3.2% ± 2.0%	8.6% ± 4.2%
GM19130	+/+	0	1.1% ± 0.4%	0.7% ± 0.7%	1.8% ± 0.4%
		5	5.3% ± 0.5%	3.3% ± 0.8%	8.6% ± 1.0%
		10	7.7% ± 0.9%	4.1% ± 1.2%	11.8% ± 1.1%

[1]Lymphoblastoid cell lines were treated with 0, 5 or 10 μM DEB for 24 h in triplicate. The percent of apoptotic cells was determined based on annexin V marker expression on the cell surface as detected by allophycocyanin (APC) labeled annexin-V. During early apoptotic stage, cells stain with APC but still exclude propidium iodide (PI), whereas cells in later stages of apoptosis stain with both APC and PI. Together early and late apoptotic cells sum up to give total apoptosis induced in the population.

Table S4. Average extent of apoptosis in *GSTT1* negative and positive cell lines based on *GSTT1* genotype.[1]				
GSTT1 genotype	µM DEB	Early apoptosis (Annexin V+/PI-)	Late apoptosis (Annexin V+/PI+)	Total apoptosis (Early+Late)
-/-	0	1.1% ± 0.5%	0.7% ± 0.4%	1.8% ± 0.8%
	5	7.4% ± 6.0%	4.7% ± 4.3%	12% ± 10%
	10	8.3% ± 6.1%	7.3% ± 6.5%	16% ± 12%
-/+	0	1.4% ± 0.6%	0.8% ± 0.6%	2.2% ± 1.2%
	5	3.4% ± 1.7%	1.6% ± 1.3%	5.1% ± 2.9%
	10	4.2% ± 2.0%	2.3% ± 1.7%	6.5% ± 3.2%
+/+	0	0.9% ± 0.1%	0.9% ± 0.6%	1.8% ± 0.6%
	5	3.3% ± 1.5%	2.0% ± 1.2%	5.2% ± 2.6%
	10	4.1% ± 2.4%	3.1% ± 1.3%	7.3% ± 3.3%
[1]Values represent mean ± SD. PI = propidium iodide.				

Table S5. Univariate responses of apoptosis, MN, DEB-GSH formation or between *GSTT* positive and *GSTT* negative (*GSTT+/-*) cell lines, *GSTM1* genotypes and between DEB treatment levels.[1]

ANOVA		SS	F	df	P(>F)
Apoptosis	**Treatment**	0.099	10.583	2	**< 0.001**
	GSTT1	0.036	7.767	1	**0.008**
	GSTM1	0.014	2.9491	1	0.093
MN	**Treatment**	0.002	12.194	2	**< 0.001**
	GSTT1	0.000	2.321	1	0.135
	GSTM1	0.000	0.601	1	0.442
DEB-GSH	***GSTT1***	0.006	20.121	1	**<0.001**
	GSTM1	0.002	5.245	1	0.037[2]
***bis*-N7G-BD**	***GSTT1***	0.054	0.169	1	0.687
	GSTM1	0.057	0.679	1	0.679

[1]The significance of the change in apoptosis and MN for *GSTT1* and *GSTM1* genotypes were measured using Analysis of Variance using the model: *apoptosis~ GSTT1 *treatment*GSTM1 or MN~ GSTT1 *treatment*GSTM1*, DEB-GSH (5uM DEB treatment) ~GSTT1*GSTM1, or *bis*-N7G-BD (5 μM DEB treatment)~GSTT1*GSTM1. (SS) sum of squares, (F) F test, (df) degrees of freedom, (P(>F)) p level. For the genotype analyses, heterozygotes and homozygote were pooled and compared to GST negatives.

[2]The average level of DEB-GSH in GSTM1 negative cells is 298 ± 165 fmol/mg protein and average level of DEB-GSH GSTM1 positive cells is 171 ± 161 fmol/mg protein.