

Butyrate may enhance toxicological defence in primary, adenoma and tumor human colon cells by favourably modulating expression of glutathione S-transferases genes, an approach in nutrigenomics

This accessory file to the manuscript submitted for publication contains additional data on GSTs and other genes which are related to drug metabolism and which were spotted on the c-DNA macro arrays

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Table 1a: **Phase I metabolising enzymes.** Baseline expression levels of in freshly excised colon tissues and in human colon cells cultivated in medium. Twenty five genes of the p450 family were spotted on the membrane; 16 genes yielded signals of over 10. For normalisation means of house keeping genes were set to equal 100%, means of negative reference spots were set to equal 0%.

Gene	Primary tissue		Primary cells		LT97 Adenoma cells ^A total culture period (h)		HT29 Tumour cells		HT29 Tumour cells ^A	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Phase I, p450 family										
CYP11A	6.3	4.9	3.4	0.7	3.6	1.6	3.8	0.8	0.0	1.4
CYP1A1	7.1	4.8	2.7	0.0	3.2	1.2	3.7	0.8	0.7	0.9
CYP1B1	-7.3	2.9	-4.7	2.2	-3.6	1.0	3.9	5.4	4.0	1.9
CYP24	-7.2	2.9	-5.4	1.5	-3.7	1.7	0.9	3.4	1.3	1.1
CYP2A6	13.4	7.2	2.4	1.1	3.5	2.1	3.5	1.0	1.7	1.8
CYP2B	60.3	21.8	*11.1	2.1	*14.3	9.4	#6.7	3.3	*#81.6	4.4
CYP2C19	19.1	7.1	6.8	0.8	7.6	3.7	6.6	3.3	3.0	2.4
CYP2C8	10.4	7.7	2.5	1.1	3.7	1.5	3.6	0.9	0.2	1.5
CYP2C9	16.2	4.2	8.6	0.5	9.2	4.8	6.4	3.5	3.0	3.2
CYP2D6	5.8	4.0	2.4	0.4	3.6	1.1	3.5	1.0	1.0	1.8
CYP2E	-6.0	2.3	-5.8	1.1	-3.6	0.9	3.4	5.7	0.6	2.0
CYP2F1	33.7	7.8	30.0	5.0	*11.4	10.3	#11.4	5.1	*#57.5	9.6
CYP3A4	36.3	17.5	16.9	6.3	3.8	1.5	3.2	1.3	13.2	4.9
CYP3A5	21.1	8.1	11.6	4.6	4.4	2.3	5.3	0.2	10.1	1.0
CYP3A7	33.2	9.7	10.0	3.5	*12.1	4.8	20.0	8.3	*43.9	2.5
CYP4A11	13.5	7.7	2.0	0.8	3.8	1.3	3.6	0.9	1.8	2.3
CYP4B1	5.8	4.0	3.1	1.1	3.8	1.5	3.6	0.9	1.3	1.1
CYP4F3	154.5	32.7	112.8	25.5	72.8	7.6	90.2	35.0	96.0	7.8
CYP7A1	7.8	4.4	22.1	6.7	-4.0	0.8	0.9	3.4	1.7	0.7
CYP7B1	-5.5	2.5	-3.7	0.5	-3.6	1.1	0.4	3.9	0.2	1.5
CYP8B1	6.8	4.7	2.7	1.4	3.4	1.4	3.1	1.3	0.8	1.2
LOC57404	8.5	4.8	6.1	1.9	3.8	1.7	3.8	0.8	14.3	4.5
P450RAI-2	2.8	3.3	12.3	6.0	3.8	1.3	2.9	1.6	0.6	1.7
POR	22.5	7.4	10.8	1.3	25.8	15.5	*4.6	0.4	33.8	2.4
TBXAS1	-4.2	2.2	-3.3	0.8	-2.5	1.5	4.4	4.9	10.1	3.7

Table 1b: **Phase II metabolising enzymes.** Baseline expression levels in freshly excised colon tissues and in human colon cells cultivated in medium. All genes of the acetyltransferases and of the glutathione S-transferases yielded signals of over 10 in at least one of the cell types. Fifteen of 21 genes of the group of sulfotransferases yielded signals of over 10.

Gene	Primary tissue		Primary cells		LT97 Adenoma cells ^A total culture period (h)		HT29 Tumour cells ^B		HT29 Tumour cells ^{A,B}	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Phase II, Acetyltransferases										
ACAT1	89.8	16.1	50.1	7.5	6.8	8.9	7.1	4.8	86.8	7.9
ACAT2	331.5	63.3	840.2	136.4	27.8	30.4	5.6	4.1	230.4	31.8
CHAT	134.8	44.2	79.9	16.7	*15.3	10.2	#4.2	0.5	*#33.5	2.3
CRAT	41.9	12.1	19.3	5.0	13.7	4.7	9.3	2.3	34.3	10.4
DLAT	27.8	7.4	16.5	7.6	6.2	1.9	4.9	0.6	90.7	9.7
HAT1	17.6	6.6	14.4	4.2	6.4	0.8	7.1	3.2	92.7	14.4
HBOA	3.2	4.7	6.3	2.4	-2.4	1.6	4.3	5.2	66.6	17.2
MORF	17.2	5.1	7.6	1.4	3.7	2.3	4.9	0.9	8.7	3.7
NAT1	82.4	16.6	50.2	9.9	9.8	4.8	3.0	1.6	5.5	1.2
LOC51126	48.2	12.9	102.8	33.2	9.3	2.9	9.2	2.0	109.7	11.0
Phase II, Glutathione S-transferases										
GSTA2	73.7	34.4	21.7	5.9	5.6	2.0	3.5	1.0	6.9	2.8
GSTA3	45.8	18.8	13.8	4.7	4.4	0.9	3.2	1.3	5.4	0.5
GSTA4	7.4	2.9	3.3	2.3	1.4	3.2	4.3	5.1	32.2	4.7
GSTM2	17.9	8.4	199.9	178.9	0.8	2.5	1.1	3.5	1.8	1.5
GSTM3	27.5	5.5	50.4	25.0	3.3	1.5	3.2	1.3	0.9	1.3
GSTM5	36.7	8.6	272.4	241.7	11.8	2.3	4.2	0.6	7.6	1.2
GSTP1	172.5	26.3	116.6	11.4	123.9	13.7	165.9	75.7	114.2	6.9
GSTT1	10.0	5.4	7.5	3.0	16.3	10.9	4.2	0.8	17.6	5.5
GSTT2	152.2	25.1	135.9	19.9	55.4	4.7	52.2	20.3	101.3	9.4
MGST1	35.1	9.7	12.3	5.6	25.1	9.7	38.3	14.8	91.0	16.2
MGST2	43.7	8.2	34.7	4.1	16.3	0.9	19.4	5.0	65.5	5.4
MGST3	99.3	43.3	159.7	64.1	17.1	8.6	4.0	2.9	75.8	18.2

Table 1b: *continued*

Gene	Primary tissue		Primary cells		LT97 Adenoma cells ^A total culture period (h)		HT29 Tumour cells ^B		HT29 Tumour cells ^{A,B}	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Phase II, Sulfotransferases										
CHST1	12.5	9.2	4.0	1.5	6.5	2.1	3.7	0.8	0.4	1.5
CHST2	14.6	9.7	3.3	1.9	5.4	1.8	3.9	0.7	4.1	2.9
CHST3	10.6	8.6	2.6	1.5	3.6	1.6	3.8	0.8	0.3	1.4
CHST4	10.3	6.9	3.3	0.4	6.0	1.4	6.9	3.2	4.3	4.4
CHST5	37.8	18.2	3.5	0.3	*26.2	8.5	42.4	16.4	*74.7	6.6
CHST6	188.7	24.9	273.7	38.2	*58.3	31.3	#34.4	13.5	*#136.2	21.4
CHST7	72.1	45.8	18.2	14.8	*37.8	13.6	#48.2	18.8	*#162.1	11.4
CHST8	10.6	7.7	3.3	1.9	3.8	1.7	3.7	0.8	2.1	1.3
CST	11.3	7.5	3.5	0.3	6.1	1.6	4.6	0.5	6.2	3.5
HNK-1ST	56.4	7.9	58.6	12.0	*17.2	21.8	#1.2	3.2	*#73.4	12.4
STE	6.5	2.1	5.9	1.0	3.5	1.5	2.6	1.8	0.8	0.1
SULT1A1	31.8	11.9	13.1	8.2	1.8	1.7	5.8	4.2	29.9	15.0
SULT1A2	27.5	9.5	15.8	3.6	0.2	1.4	6.3	3.6	34.5	23.1
SULT1B1	52.4	20.4	9.0	2.6	3.2	2.1	2.7	1.6	1.3	2.0
SULT1C1	14.5	4.5	7.9	1.6	8.7	4.0	6.9	3.2	6.7	2.7
SULT1C2	7.3	4.0	5.9	1.0	2.8	1.4	2.7	1.6	0.3	0.9
SULT2A1	4.1	2.5	5.0	2.2	3.3	1.2	2.8	1.6	-0.3	1.3
SULT2B1	7.8	2.2	4.5	1.0	6.5	1.7	4.5	0.5	8.1	4.1
SULT4A1	7.0	2.1	1.3	0.1	4.6	1.6	2.6	1.8	9.7	1.4
TPST1	40.2	4.8	95.9	38.1	7.5	3.0	6.6	3.3	31.4	3.8
TPST2	3.3	1.8	2.0	0.8	3.3	2.0	5.8	3.8	-0.5	0.8

Table 1b: *continued*

Gene	Primary tissue		Primary cells		LT97 Adenoma cells ^{A,B} total culture period (h)		HT29 Tumour cells		HT29 Tumour cells ^{A,B}	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Phase II, Miscellaneous										
EPHX1	23.8	7.5	13.2	8.6	8.8	2.7	13.6	4.4	67.4	12.1
EPHX2	8.6	5.0	11.0	6.8	5.4	1.5	3.5	0.9	1.4	1.7
LABH1	11.0	4.0	6.5	1.6	3.1	1.7	3.4	1.1	1.7	0.8
LTA4H	12.9	5.4	7.7	3.2	5.4	1.5	4.8	1.1	32.7	2.9
UGT1A1	151.5	54.0	124.1	32.4	*27.0	9.2	#25.7	3.1	*.#116.9	12.6
UGT2A1	28.0	10.0	5.2	1.6	3.6	1.3	5.4	4.0	1.2	0.5
UGT2B	59.2	9.7	34.2	9.8	*26.5	4.1	#23.0	3.0	*.#123.9	1.2
UGT2B10	105.5	15.8	57.5	13.0	15.3	8.1	2.2	1.6	22.8	3.8
UGT2B4	38.8	13.7	1.2	0.9	-1.8	1.0	2.7	5.4	4.5	1.5
COMT	32.4	8.6	18.6	5.0	13.2	4.7	5.6	0.6	23.8	6.8
HNMT	169.9	20.9	169.3	90.1	*9.6	3.7	"5.3	0.4	*."109.6	13.0
NNMT	87.8	18.3	310.1	136.4	3.4	2.0	3.0	1.4	11.6	3.1
TPMT	33.6	25.7	-2.5	1.7	30.6	9.8	77.0	28.4	104.0	17.1

Table 1c: **Phase III metabolising enzymes.** Baseline expression levels of in freshly excised colon tissues and in human colon cells cultivated in medium. All 8 genes of the metallothioneins yielded signals of over 10. Four of 7 genes of the p-glycoprotein group yielded signals of over 10.

Gene	Primary tissue		Primary cells		LT97 Adenoma cells ^{A,B} total culture period (h)		HT29 Tumour cells ^{A,C}		HT29 Tumour cells ^{B,C}	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Phase III, Metallothioneins										
MT1A	125.5	33.1	121.6	37.2	23.9	11.8	5.3	0.8	60.7	6.8
MT1E	54.1	8.6	36.1	2.4	30.6	6.7	32.6	12.3	43.8	1.8
MT1G	257.1	23.2	632.0	193.9	45.1	18.4	4.8	0.4	46.3	9.0
MT1H	196.7	32.6	297.4	41.6	23.7	13.5	3.8	1.1	25.8	3.3
MT1L	267.5	34.1	373.3	60.0	33.5	8.7	9.7	1.8	100.5	14.1
MT2A	87.9	23.6	105.9	43.8	9.5	7.9	4.6	5.2	72.4	4.1
MT3	21.2	4.3	13.9	3.2	4.7	9.1	1.2	3.2	35.2	5.2
MTIX	386.0	42.6	697.6	156.2	43.1	19.1	6.2	0.9	105.6	5.8
Phase III, p-Glycoproteins										
ABCB1	6.8	5.7	8.8	9.1	0.1	1.9	3.7	5.4	0.9	1.3
ABCB4	-5.2	2.4	-4.4	1.1	-3.0	1.6	3.4	5.7	-0.1	1.4
ABCC1	-4.3	3.1	-5.4	1.5	0.2	1.1	7.4	3.1	14.0	3.3
ABCC2	127.1	70.4	60.1	20.4	-3.8	1.2	1.6	3.0	9.3	5.3
ABCC3	45.7	13.0	38.0	14.8	17.9	5.9	23.9	9.2	102.2	18.2
ABCC5	-6.1	3.3	-2.3	0.9	-3.7	1.0	4.2	5.1	5.1	2.3
ABCG2	131.0	39.6	79.4	29.1	-1.0	3.9	4.8	4.9	119.8	16.9

Table 1d: **Housekeeping genes and negative reference spots.** Baseline expression levels of the in freshly excised colon tissues and in human colon cells cultivated in medium.

Gene	Primary tissue		Primary cells		LT97 Adenoma cells total culture period (h)		HT29 Tumour cells		HT29 Tumour cells	
	0		12		72		72		48	
	means	SEM	means	SEM	means	SEM	means	SEM	means	SEM
Housekeeping Genes										
GAPD	311.2	30.3	380.5	20.6	267.0	81.0	223.1	17.5	124.8	14.1
GAPD	275.3	17.7	343.0	51.8	246.5	76.5	211.5	16.0	141.3	16.8
PPIA	89.5	7.6	65.0	13.3	14.8	12.1	158.4	112.3	159.8	11.5
PPIA	88.4	11.0	64.1	10.1	16.6	12.5	35.4	21.4	129.7	4.8
PPIA	97.7	6.8	64.4	1.5	17.2	11.3	37.6	19.7	115.6	8.9
PPIA	107.0	4.9	63.1	1.3	19.0	12.1	36.1	21.6	121.9	14.1
RPL13A	-0.3	5.1	-2.9	2.6	26.3	15.1	56.6	40.2	41.1	18.7
RPL13A	0.9	4.4	-2.2	1.9	24.9	13.3	52.0	38.0	40.5	17.5
ACTB	15.3	7.1	15.6	9.1	85.8	41.7	91.9	7.5	58.3	3.9
ACTB	14.9	7.6	9.4	3.0	82.5	39.2	97.4	11.3	66.8	3.0
Negative reference spots										
PUC18	-7.5	2.9	-5.8	1.1	-4.9	1.4	-7.4	6.0	0.4	0.7
PUC18	1.1	0.3	0.6	0.8	0.4	0.5	2.2	1.6	-0.5	0.3
PUC18	1.2	0.3	0.9	0.4	0.2	0.3	2.1	1.7	-0.6	0.3
Blank	2.1	1.0	1.3	0.1	1.1	0.1	2.0	1.7	1.0	1.5
Blank	0.9	0.5	1.3	0.1	1.6	1.0	3.7	0.9	-1.7	0.3
Blank	2.2	1.0	1.7	0.3	1.5	0.7	-2.7	3.1	1.4	1.4

Legend to Table 1a-d: Data are the relative signals obtained after normalisation from 6 (primary tissues), 3 (primary, LT97, HT29 cells) independent samples. Differences were assessed between primary tissues and primary cells or between primary cells, LT97 cells and HT29 cells. Identical symbols in each row depict differences between individual genes (two-way ANOVA with Bonferroni's post tests). Identical capital letters depict differences between samples on a group basis (paired and unpaired t-test analysis, as appropriate). *The data of this table was used to identify the differentially expressed genes presented in Table 1 of the manuscript.*

Table 2a: **Primary human colon cells**. Modulation of expression of genes for drug metabolizing enzymes by 10mM butyrate. Butyrate was added to the epithelial stripes which were incubated for a total of 12 hours.

Gene	Primary cells					fold change
	Total culture period (12h)					
	medium means	SEM	10mM butyrate means	SEM		
Phase I, p450 Family 4 of 25						
CYP2F1	30.0	5.0	64.1	20.4	2.1	
CYP3A4	16.9	6.3	42.1	28.6	2.5	
CYP4F3	112.8	25.5	227.7	107.2	2.0	
CYP7A1	16.6	6.7	68.9	50.4	4.1	
Phase II, Acetyltransferases 2 of 10						
ACAT1	50.1	7.5	172.1	131.9	3.4	
CHAT	79.9	16.7	138.3	53.5	1.7	
Phase II, Glutathione S-transferases 2 of 12^a						
GSTA2 ^a	21.7	5.9	44.4	17.2	2.0	
GSTT2	135.9	19.9	275.5	136.5	2.0	
Phase II, Sulfotransferases 1 of 21						
HNK1-ST	58.6	12.0	89.8	46.9	1.5	
Phase II, Miscellaneous 0 of 13						
Phase III, Metallothioneins 3 of 8						
MT1E	36.1	2.4	67.9	32.7	1.9	
MT1L	373.3	60.0	639.3	295.1	1.7	
MT3	13.9	3.2	27.4	9.1	2.0	
Phase III, p-Glycoproteins 3 of 7						
ABCC2	60.1	20.4	126.9	55.2	2.1	
ABCC3	38.0	14.8	100.8	82.6	2.6	
ABCG2	79.4	29.1	195.3	148.9	2.5	

Table 2b: **LT97 human colon adenoma cells**. Modulation of expression of drug metabolizing enzymes in cells treated with 1mM and 2mM butyrate. Butyrate treatment was for 24 hours and was added 48 hours after plating (total culture period 72 hours).

LT97 Adenoma cells								
Gene	Total culture period (72h)							
	medium		1mM butyrate		fold change	2mM butyrate		fold change
Phase I, p450 Family 4 of 25								
CYP2B	14.4	9.4	27.8	21.9	1.9	23.1	19.0	1.6
CYP2F1	11.5	10.3	13.8	12.0	1.2	17.2	7.9	1.5
CYP3A7	12.2	4.8	20.4	9.4	1.7	27.0	12.9	2.2
CYP4F3	*#72.7	7.6	*§124.4	13.9	1.7	#§173.8	53.0	2.4
Gene	medium ^{A,B}		1mM butyrate ^A		fold change	2mM butyrate ^B		
	means	SEM	means	SEM		means	SEM	
Phase II, Acetyltransferases 7 of 10								
ACAT1	6.8	8.9	27.5	29.4	4.0	24.2	26.7	3.5
ACAT2	27.8	30.4	33.3	35.4	1.2	45.5	48.3	1.6
CRAT	13.7	4.7	32.9	21.0	2.4	35.5	21.5	2.6
DLAT	6.2	1.9	24.2	16.7	3.9	30.4	22.7	4.9
HAT1	6.4	0.8	15.5	12.3	2.4	17.0	11.2	2.7
NAT1	9.8	4.8	34.9	22.2	3.6	43.7	29.8	4.5
LOC51126	9.3	2.9	31.8	23.9	3.4	40.7	28.2	4.4
Gene	medium ^{A,B}		1mM butyrate ^A		fold change	2mM butyrate ^B		
	means	SEM	means	SEM		means	SEM	
Phase II, Glutathione S-transferases 8 of 12^a								
GSTA2 ^a	5.6	2.0	9.2	5.4	1.6	7.0	2.3	1.2
GSTA4 ^a	1.4	3.2	8.5	11.3	6.1	3.7	9.1	2.7
GSTM2 ^a	0.8	2.5	2.6	7.5	3.2	2.9	8.1	3.5
GSTM3	3.3	1.5	19.2	14.6	5.8	23.8	20.5	7.3
GSTM5	11.8	2.3	18.1	5.8	1.5	19.7	6.1	1.7
GSTP1 ^a	123.9	13.7	144.4	37.4	1.2	147.8	43.6	1.2
GSTT1 ^a	16.3	10.9	16.3	10.1	1.0	17.8	9.9	1.1
GSTT2	*55.3	4.7	84.4	8.8	1.5	*112.1	26.0	2.0
MGST3	17.1	8.6	33.2	21.4	2.0	45.8	35.5	2.7

Table 2b: *continued*

LT97 Adenoma cells								
Gene	Total culture period (72h)							
	medium		1mM butyrate		fold change	2mM butyrate		fold change
Phase II, Sulfotransferases 4 of 21								
CHST5	*26.2	8.5	36.2	13.9	1.4	*52.8	25.4	2.0
CHST7	*37.9	13.6	46.5	6.2	1.2	*59.0	11.5	1.6
SULT1C1	8.7	4.0	14.0	4.3	1.6	13.3	4.9	1.5
TPST1	7.5	3.0	20.4	12.3	2.7	28.8	15.5	3.9
Gene	medium ^{A,B}		1mM butyrate ^A		fold change	2mM butyrate ^B		fold change
	means	SEM	means	SEM		means	SEM	
Phase II, Miscellaneous 7 of 13								
EPHX1	8.8	2.7	25.7	8.6	2.9	25.4	7.2	2.9
LTA4H	5.4	1.5	17.4	9.3	3.2	19.7	7.0	3.7
UGT1A1	*27.0	9.2	*66.7	17.0	2.5	49.7	13.0	1.8
UGT2B	26.5	4.1	41.2	16.1	1.6	50.4	11.2	1.9
COMT	13.2	4.7	30.0	19.5	2.3	32.2	22.7	2.5
HNMT	9.6	3.7	19.9	14.7	2.1	19.0	14.2	2.0
TPMT	*30.6	9.8	49.1	22.1	1.6	*67.4	35.5	2.2
Gene	medium ^{A,B}		1mM butyrate ^{A,C}		fold change	2mM butyrate ^{B,C}		fold change
	means	SEM	means	SEM		means	SEM	
Phase III, Metallothioneins 8 of 8								
MT1A	23.9	11.8	89.6	50.5	3.8	68.9	13.3	2.9
MT1E	30.6	6.7	49.6	8.7	1.6	55.9	13.1	1.8
MT1G	45.1	18.4	122.5	59.5	2.7	104.3	11.8	2.3
MT1H	23.7	13.5	61.5	23.0	2.6	53.1	12.0	2.2
MT1L	33.5	8.7	167.3	89.0	5.0	146.2	32.3	4.4
MT2A	9.5	7.9	64.5	30.5	6.8	46.5	10.3	4.9
MT3	4.7	9.1	12.0	15.9	2.6	19.6	23.6	4.2
MTIX	43.1	19.1	175.1	101.0	4.1	162.8	39.1	3.8
Gene	medium		1mM butyrate		fold change	2mM butyrate		fold change
	means	SEM	means	SEM		means	SEM	
Phase III, p-Glycoproteins 1 of 7								
ABCC3	17.9	5.9	12.6	16.4	0.7	6.4	9.4	0.4

Table 2c: **HT29 human colon tumour cells.** Modulation of expression of drug metabolizing enzymes in cells treated for 24 hours with 4mM butyrate. Butyrate treatment was for 24 hours and was added 48 (total culture period 72 hours) and 24 (total culture period 48 hours) hours after plating.

HT29 Tumour cells											
Gene	Total culture period (h)										
	72				fold change	48				fold change	
	medium	means	SEM	4mM butyrate		means	SEM	medium ^A	4mM butyrate ^A		
means	SEM	means	SEM	means	SEM	means	SEM	means	SEM	change	
Phase I, p450 Family 5 of 25											
CYP2B	*6.9	3.3		#1.7	0.6	0.2	*81.6	4.4	"99.6	15.6	1.2
CYP2F1	*11.4	5.1		#11.4	6.9	1.0	*57.6	9.6	"69.6	6.3	1.2
CYP3A5	5.3	0.2		3.8	0.9	0.7	10.1	1.0	20.7	6.3	2.1
CYP3A7	20.0	8.3		#21.1	9.8	1.1	44.0	2.5	"60.5	8.5	1.4
POR	4.6	0.4		31.6	21.6	6.9	33.8	2.4	52.7	4.6	1.6
Phase II, Acetyltransferases 8 of 10											
ACAT1	*7.0	4.8		#6.3	3.3	0.9	*86.8	7.9	"97.4	14.4	1.1
ACAT2	*5.6	4.1		#3.2	3.2	0.6	*230.4	31.8	"252.6	32.4	1.1
CHAT	4.2	0.5		#2.9	1.3	0.7	33.5	2.3	"45.2	3.8	1.4
CRAT	9.3	2.3		#11.6	0.6	1.3	34.3	10.4	"76.8	16.4	2.2
DLAT	*4.9	0.6		#5.2	1.9	1.1	*90.7	9.7	"102.8	11.1	1.1
HAT1	*7.1	3.2		#3.8	0.9	0.5	*92.7	14.4	"79.9	6.8	0.9
HBOA	*4.3	5.2		#-0.2	2.6	-0.1	*66.6	17.2	"61.8	11.8	0.9
LOC51126	*9.2	2.0		#5.5	3.3	0.6	*109.7	11.0	"130.3	8.8	1.2
Phase II, Glutathione S-transferases 10 of 12^a											
GSTA2 ^a	3.5	1.0		0.8	1.0	0.2	6.9	2.8	13.2	5.8	1.9
GSTA4 ^a	4.3	5.1		1.7	2.8	0.4	32.2	4.7	53.1	8.3	1.7
GSTM2 ^a	1.1	3.5		1.4	3.1	1.3	1.8	1.5	25.2	4.7	14.1
GSTM3	3.2	1.3		1.7	0.3	0.5	0.9	1.3	4.7	2.1	5.4
GSTM5	4.2	0.6		4.6	1.6	1.1	7.6	1.2	23.1	2.2	3.0
GSTP1 ^a	*165.9	75.7		*#358.2	100.6	2.2	114.2	6.9	"122.8	6.4	1.1
GSTT1 ^a	4.2	0.8		-0.1	2.6	0.0	17.6	5.5	22.1	9.2	1.3
GSTT2	52.2	20.3		71.6	28.8	1.4	101.3	9.4	96.3	6.0	1.0
MGST1	38.3	14.8		19.6	9.0	0.5	91.0	16.2	47.5	3.9	0.5
MGST3	4.0	2.9		"5.21	5.0	1.3	75.8	18.2	"140.3	26.2	1.9

Table 2c: *continued*

HT29 Tumour cells										
Gene	Total culture period (h)									
	72		48		fold change	medium ^A		4mM butyrate ^A		fold change
	means	SEM	means	SEM		means	SEM	means	SEM	
Phase II, Sulfotransferases 5 of 21										
SULT1A1	5.8	4.2	16.5	5.6	2.9	29.9	15.0	37.8	12.2	1.3
SULT1A2	6.3	3.6	#9.5	1.9	1.5	34.5	23.1	#44.7	18.3	1.3
SULT1C1	6.9	3.2	8.1	2.8	1.2	6.7	2.7	10.4	2.7	1.5
SULT2B1	4.5	0.5	16.7	9.2	3.7	8.1	4.1	19.0	9.0	2.4
TPST1	6.6	3.3	#3.5	1.0	0.5	\$31.4	3.8	#,\$78.4	2.9	2.5
Gene	medium		4mM butyrate		fold change	medium		4mM butyrate		fold change
	means	SEM	means	SEM		means	SEM	means	SEM	
Phase II, Miscellaneous 3 of 13										
UGT2B	#23.0	3.0	\$35.9	14.0	1.6	#123.9	1.2	\$102.5	8.3	0.8
COMT	5.6	0.6	\$7.7	0.7	1.4	23.8	6.8	\$58.5	10.2	2.5
TPMT	*77.0	28.4	*130.3	55.8	1.7	104.0	17.1	136.8	7.8	1.3
Gene	medium ^A		4mM butyrate ^A		fold change	medium ^B		4mM butyrate ^B		fold change
	means	SEM	means	SEM		means	SEM	means	SEM	
Phase III, Metallothioneins 8 of 8										
MT1A	#5.30	0.8	\$35.1	17.1	6.6	#60.7	6.8	\$111.3	5.4	1.8
MT1E	32.6	12.3	53.4	24.4	1.6	43.8	1.8	68.7	2.2	1.6
MT1G	4.8	0.4	37.3	16.0	7.8	46.3	9.0	71.5	8.9	1.6
MT1H	3.8	1.1	18.5	9.9	4.9	25.8	3.3	41.4	3.3	1.6
MT1L	*#9.7	1.8	*\$74.5	24.6	7.7	#100.5	14.1	\$156.1	22.2	1.6
MT2A	#4.6	5.2	\$34.3	15.2	7.5	#72.4	4.1	\$88.8	6.8	1.2
MT3	1.2	3.2	\$1.0	3.0	0.9	35.2	5.2	\$55.5	4.5	1.6
MTIX	*#6.2	0.9	*\$87.4	26.2	14.2	#105.6	5.8	\$206.9	24.2	2.0
Gene	medium ^A		4mM butyrate ^A		fold change	medium ^B		4mM butyrate ^B		fold change
	means	SEM	means	SEM		means	SEM	means	SEM	
Phase III, p-Glycoproteins 2 of 7										
ABCC2	1.6	3.0	1.3	3.3	0.8	9.3	5.3	17.8	7.0	1.9
ABCC5	4.2	5.1	1.4	2.7	0.3	5.1	2.3	18.0	8.5	3.5

Legend to Table 2a-c: Only those genes are shown for which the baseline expression reached a signal of ≥ 10 , and for which there was either a significant difference compared to the medium control or for which there was a butyrate mediated change ≥ 2 fold (up regulation) and ≤ 0.5 (down regulation). These genes are highlighted in bold lettering. ^aExceptions to these criteria are selected genes belonging to the family of glutathione S-transferases with signals ≤ 10 , but for which other data on mRNA and protein level are available. Data are the relative signals obtained after normalisation from 3 independently reproduced experiments. Differences were assessed between the treatment groups. Identical symbols in each row depict differences between individual genes (two-way ANOVA with Bonferroni's posttests). Identical capital letters depict differences between samples on a group basis (paired and unpaired t-test analysis, as appropriate). The value "fold change" was calculated by dividing the mean of the values of butyrate treated samples by the mean values of the corresponding medium controls. *The data of this table was used to identify the butyrate-modulated genes presented in Table 1 of the manuscript.*

Table 3: Comparison of the gene expression in LT97 cells after treatment with 1 and 2mM butyrate (Affymetrix® versus Superarray®). **Bold lettering** depicts at least 2 fold expression in comparison to medium control *This table contains more extensive information than Table 3 of the manuscript, in particular it additionally presents Affymetrix® values for genes present but not modulated on Superarray®*

Gene	Superarray		Gene	Fold change		Affymetrix
	1mM	2mM		1mM	2mM	
Phase I, p-450 family						
CYP4F3	1.7	2.4	CYP4F3	3.7	6.5	206514_s_at
			CYP2C19	0.1	0.3	216058_s_at
			CYP2D6	0.1	0.6	217468_at
			CYP2J2	0.4	0.4	205073_at
			CYP3A7	1.0	0.2	205939_at
			P450RAI-2	21.0	28.5	219825_at
			POR	6.2	6.1	208928_at
Phase II, Acetyltransferases						
ACAT1	4.0	3.5	ACAT1	1.7	1.9	205412_at
			ARD1	1.4	1.5	203025_at
			HAT1	0.6	1.0	203138_at
			MYST2 (HBOA)	0.7	0.4	200049_at
Phase II, Glutathione S-transferases						
GSTM3	5.8	7.3	GSTM3	3.9	4.3	202554_s_at
MGST3	1.9	2.7	MGST3	1.1	1.1	201403_s_at
			MGST2	0.3	0.2	204168_at
			GSTA4	2.2	1.7	202967_at
			GSTM2	0.6	0.7	204418_x_at
Phase II, Sulfotransferases						
TPST2	NC	3.9	TPST2	2.8	2.9	204079_at
CHST5	NC	2.0	CHST5	0.8	0.5	219182_at
CHST7	NC	1.6	CHST4	0.7	0.6	220446_s_at
			CHST6	2.2	2.6	221059_s_at
			SULT1A1	0.9	0.7	203615_x_at
			SULT1B1	0.7	0.5	207601_at
			SULT1C2	1.4	1.5	205812_s_at
Phase II, Miscellaneous						
TPMT	19.5	26.7	TPMT	1.7	1.4	203671_at, 203672_x_at
			EPHX1	2.6	1.5	202017_at
			LTA4H	1.9	2.3	208771_s_at
			COMT	0.3	0.4	208818_s_at
			HNMT	0.4	0.2	204112_s_at, 211732_x_at
Phase II, Metallothioneins						
MT1G	2.7	2.3	MT1G	1.9	4.5	204745_x_at, 210472_at
MT1H	2.6	2.2	MT1H	4.9	6.1	206461_x_at
MT1X	4.1	3.8	MT1X	6.3	8.8	204326_x_at, 208581_x_at
MT2A	6.8	4.9	MT2A	4.8	5.7	212185_x_at, 212859_x_at
Phase III, p-Glycoproteins						
ABCB1	169.7	205.4	ABCB1	2.4	1.5	209994_s_at, 209993_s_at
			ABCG2	2.2	2.0	209735_at

NC - no change in control (medium) and under treatment

Bold - signal detected in both control and treatment with fold change ≥ 2 or ≤ 0.5

Fold change - is mean value of probe sets named in Affymetrix array

Figure 1a: Individual expression levels in freshly excised colon tissue of 6 different individuals (control spots/genes. and phase I and phase III). This figure corresponds to Figure 1 of the manuscript.

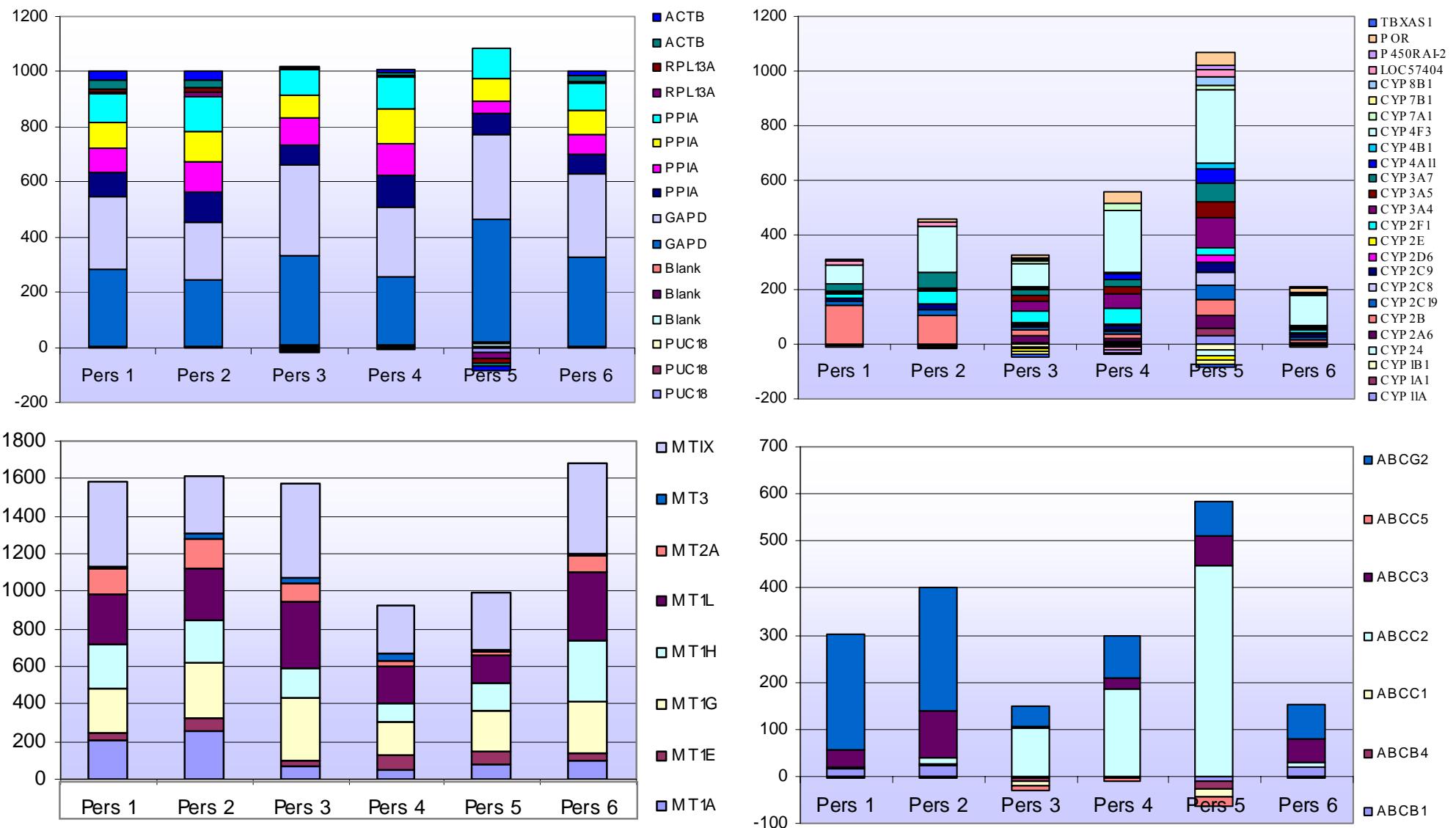


Figure 1b: Individual expression levels in freshly excised colon tissue of 6 different individuals (phase II)

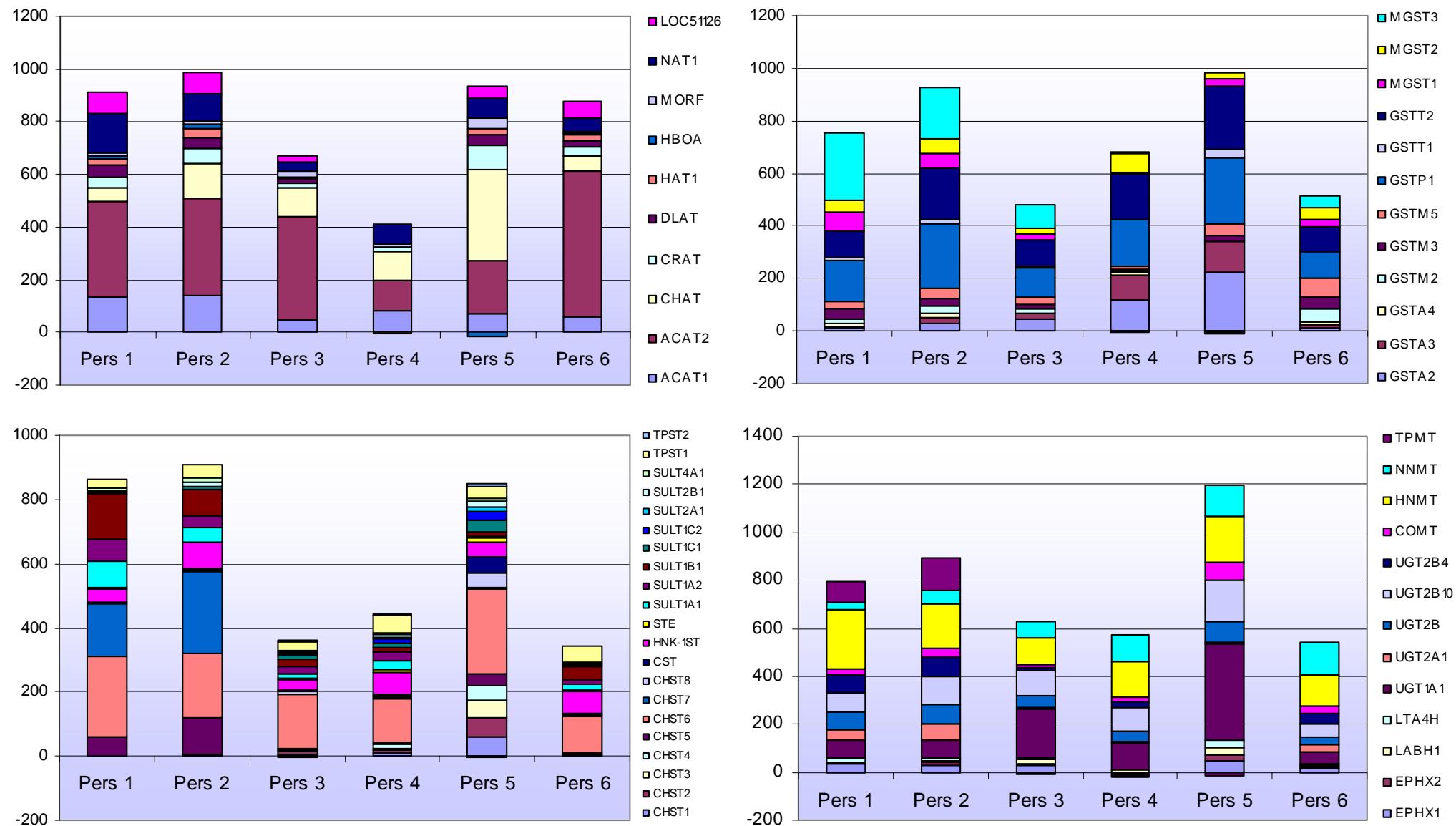


Figure 2: These scatter plots display the modulation of gene expression in LT97 cells treated with butyrate (1mM and 2mM)

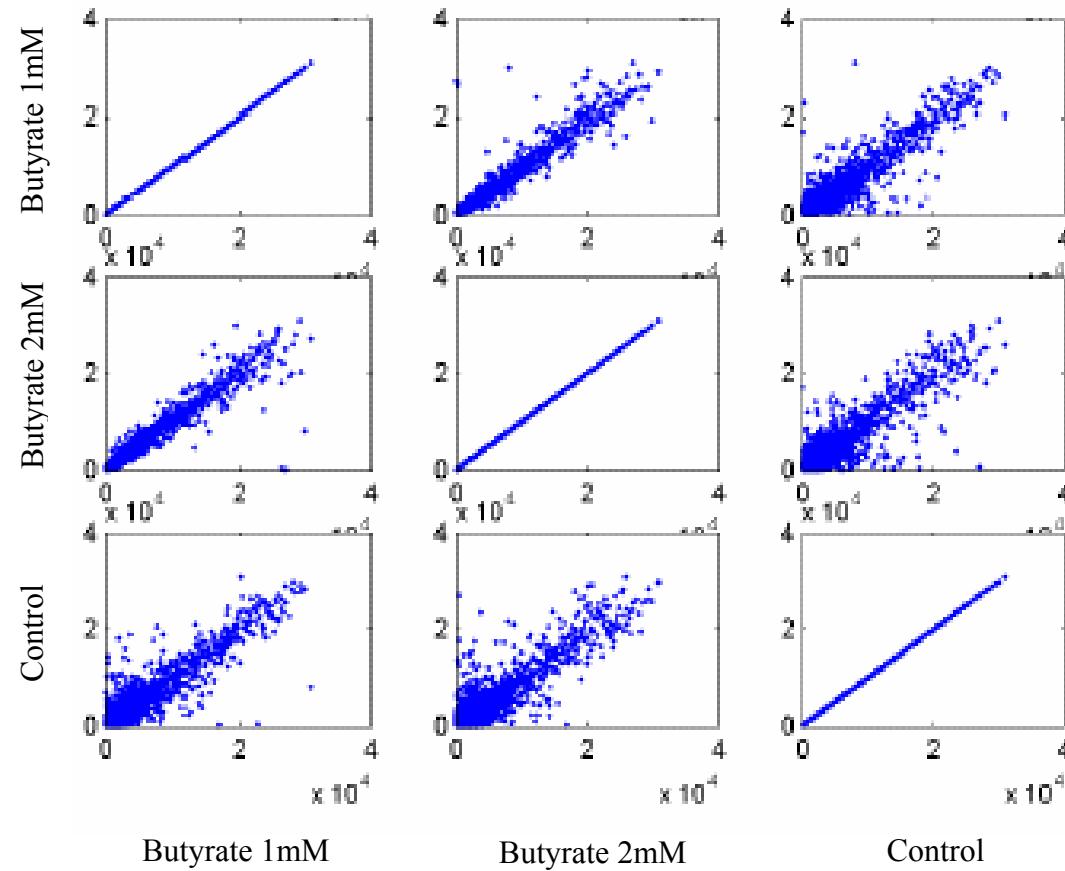


Figure 3: Comparison of regulation of gene expression by butyrate. Graphs depict the numbers of genes from designated functional groups which expression is regulated.

