**Table W1.** Microarray Results Leading to the Identification of Altered 5hmC Level of LZTS1.

Gene	Mean Intensities in Glucosylated Sample Group	Mean Intensities in Unglucosylated Sample Group	P Value
LZTS1	410.57	112.38	.0148
SOCS1	200.02	68.91	.0369
TP53	3564.11	905.54	.0404

The absolute signal intensities and P values of three candidate genes for qPCR validation are given in Table 1.
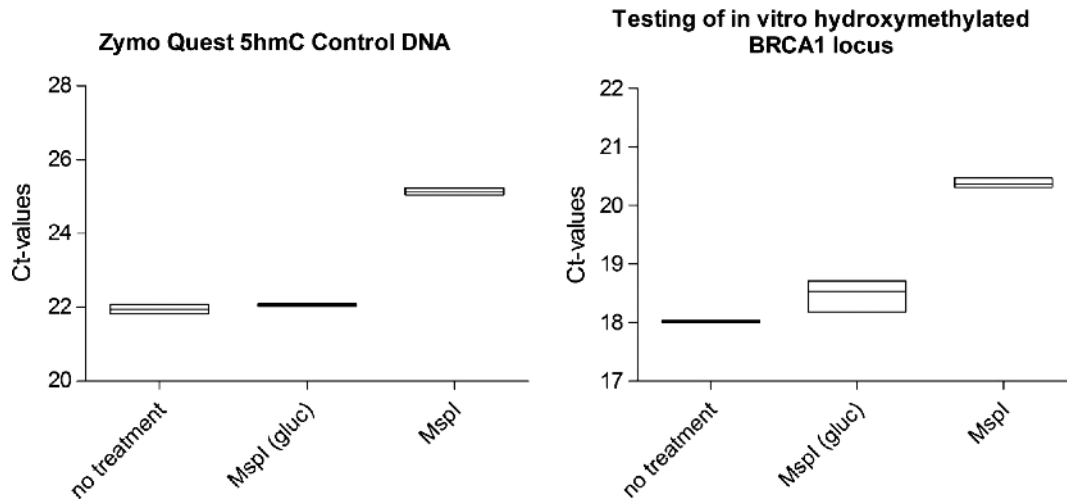

Figure W1. Quest 5-hmC Detection Kit testing using DNA supplied by Zymo Research and *in vitro* hydroxymethylated DNA of the BRCA1 PCR product. C_t values, unnormalized raw data; *Msp*I (gluc) indicates positive reaction, where 5-hmC glucosyltransferase enzyme was added to the reaction; *Msp*I indicates reactions with the same input DNA (*in vitro* hydroxymethylated) but without the enzyme.

Table W2. PCR Products plus Primer Sequences and PCR Efficiency.

| PCR Product	Target	Left Primer	Right Primer	Tm	Slope	d	R^2	PCR Efficiency
LZTS1_frag_A	DNA	cccagtgaatgtttgttgaat	tctgggcagtagagaaacaca	65	−3.20	26.55	0.997	105.20
LZTS1_frag_B	DNA	cttgctgccacagccttt	ccggagatgaggctactgac	65	−3.80	29.30	0.996	83.38
LZTS1_frag_C	DNA	ggcttcgcagtacaagctg	agtcctgggagaagccaaac	65	−4.26	35.41	0.981	71.58
LZTS1_frag_D	DNA	gcgtcagtagcctcatctcc	tcttgaggtgggaggacttg	65	−3.95	30.65	0.992	79.20
LZTS1_frag_E	DNA	atgggcaagagcgaagact	ctaaatccccgctggacagt	65	−3.34	26.77	0.997	99.28
LZTS1_frag_F	DNA	cagactcctcaaaaccagagc	acttctgcttcagggggact	65	−4.01	35.35	0.992	77.68
LZTS1_frag_G	DNA	ctggaagcacagatgaagagg	agggcagcaaatgagaagac	65	−3.46	27.38	0.999	94.43
LZTS1_frag_H	DNA	attcagtcctctgcccccttg	gccccttaatttgaaaagctg	65	−3.41	25.64	0.999	96.40
LZTS1_frag_I	DNA	caggagccatcctgctcact	gcttcagctcctgctcctt	65	−3.95	29.93	0.996	79.07
LZTS1_frag_J	DNA	caaggagcaggagctgaag	gctgctggtgctgtgtgt	65	−3.61	27.17	0.999	89.22
LZTS1_frag_K	DNA	ctgcagcttcagcaggaga	ctcgtaggacctgagcttgg	65	−4.24	35.11	0.961	72.16
control	DNA	gctctgcccatagatgcctttg	tccctggttttgacctggggga	65	−3.48	29.29	0.998	93.97
LZTS1	mRNA	gactgcttctctcattcctgc	acaatgtgttgcccaaccaaag	60	−4.14	34.76	0.996	74.21
TET1	mRNA	ccggcgcgagttggaaagtt	aaggtttttggtcgctggccg	60	−3.81	32.52	0.994	82.86
GAPDH	mRNA	atcactgccacccagaagac	atgaggtccaccaccctgtt	60	−3.63	30.04	0.993	88.41

"Target" indicates if primer is specific to mRNA of DNA; "Tm" reflects the annealing temperature that was applied to perform qPCR; "slope" represents slope of standard curve; d gives the intercept point of the standard curve and the y-axis; R^2 indicates correlation coefficient.
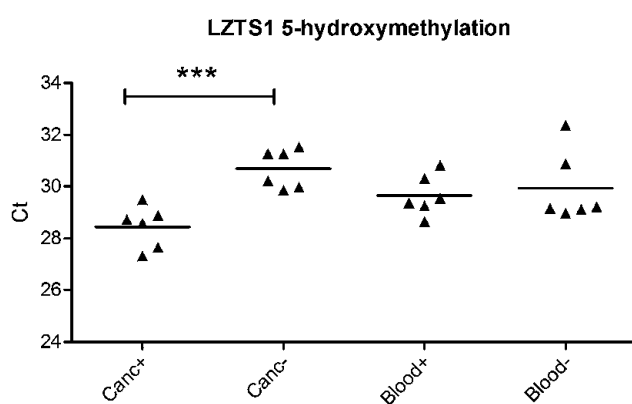
Figure W2. qPCR validation of LZTS1 on the discovery sample set (previous section) shows changed 5hmC levels in cancer compared to no change in 5hmC content in blood of healthy donors. Canc+ indicates positive reaction inclusive of glucosyltransferase enzyme; canc− indicates negative reaction without the enzyme.
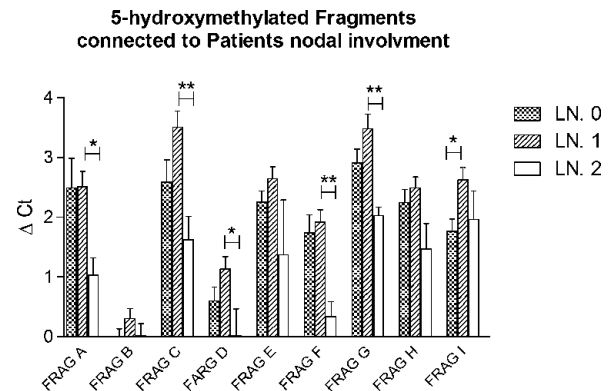
Figure W3. LZTS1 5-hydroxymethylation in cancerous breast tissue. 5-Hydroxymethylated fragments correlate to patient's lymph node affection. ΔC_t method was applied to minimize the experimental bias and varying DNA concentrations. ΔC_t values were obtained by qPCR. The negative reaction of each sample was then subtracted from the positive (glucosylated) sample and plotted as the mean difference of each sample group. Fragments A to I reflect the different qPCR-tested regions of the LZTS1 locus. LN0, patients with no lymph node involvement detected ($n = 26$); LN1, patients with lymph node affection of clinical grade 1 ($n = 39$); LN2, patients with local lymph node affection of grade 2 ($n = 8$); error bars indicate the SEM; $*P < .05$ and $**P < .01$.
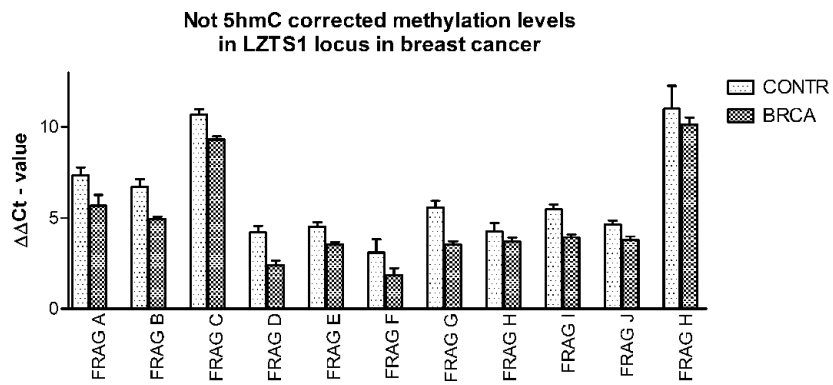
Figure W4. Methylation levels of LZTS1 locus; identical qPCR assays (fragments A-K) such as for 5hmC assessment were used. ΔC_t methodology was applied to minimize the experimental bias and varying DNA concentrations. $\Delta \Delta C_t$ values were obtained by subtraction of the negative untreated sample from the *Hpa*II digested sample values. The effect of 5hmC was not considered. The plot reflects a combination of 5hmC and 5mC of the LZTS1 locus.