Figure S1. MS-PCR analysis detects methylated alleles of SLC5A8 in cervical cancer cell lines. (A) Schematic diagram of the location of the SLC5A8 CGI (black frame) in the 5' region of the gene. The TSS is indicated by the right-angled arrow at nucleotide position +1. The thin vertical red lines under the plot represent the location of each CpG site. The grey shaded area in the CGI indicates the region selected for the MS-PCR analysis, and the boxes underneath indicate the position of the primer pairs selected for the methylated (MF1-MR1) and unmethylated (UF1-UR1) sequence. (B) MS-PCR analysis in cervical cancer cell lines (HeLa, CaLo, SiHa, CaSki and C-33A) and NCT for the U-SLC5A8 or M-SLC5A8 region of interest. HEK and HCT were used as unmethylated and methylated controls, respectively. MS-PCR, methylation-specific polymerase chain reaction; SLC5A8, Na^+-coupled monocarboxylate transporter 1; CGI, CpG island; TSS, transcriptional start site; NCT, non-tumour cervical tissue; U-SLC5A8, unmethylated SLC5A8; M-SLC5A8, methylated SLC5A8; HEK, 293 cells; HCT, HCT116 cells; UTR, untranslated region; Neg, no template control.

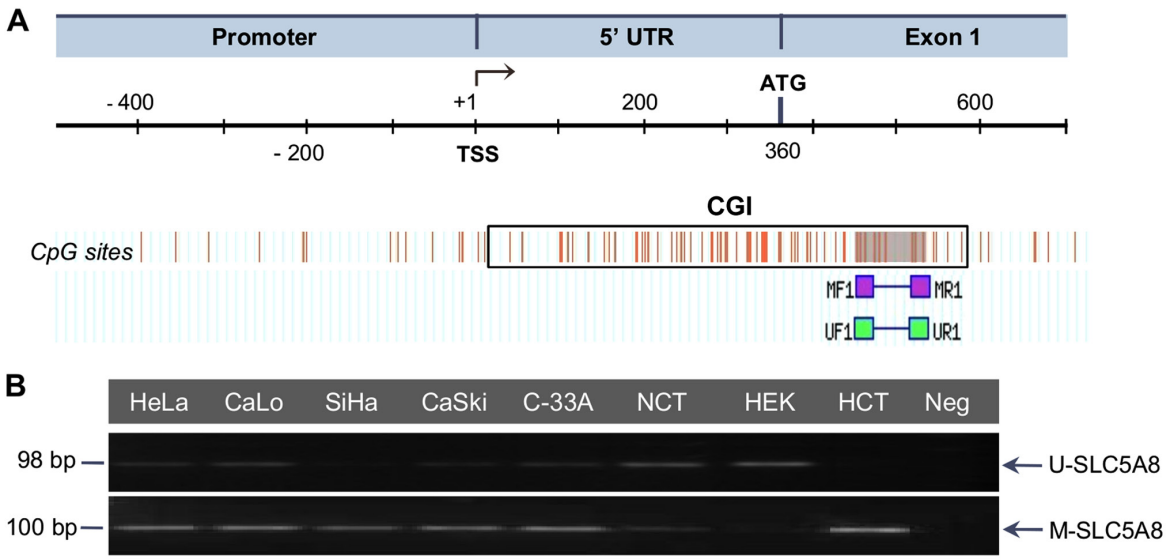

Figure S2. Aggregated profiles demonstrating the patterns of SLC5A8 first exon hypermethylation in cervical cancer cell lines. The diagram represents a summary of the bisulphite-sequencing analysis in cervical cancer cells. HEK and HCT were used as unmethylated and methylated controls, respectively. Each row represents the aggregated methylation profile of 5 clones per sample. The boxes represent each of the CpG sites analysed within the SLC5A8 CpG island and the colour ratio corresponds to the proportion of methylated and unmethylated sites at that position. The colour code and score for each CpG site were assigned as follows: Uniformly methylated CpG site (yellow box; score, 2), uniformly unmethylated CpG site (blue box; score, 0), semi-methylated CpG site, predominantly methylated (mainly yellow box; score, 1.5), and semimethylated CpG site, predominantly unmethylated (mainly blue box; score, 0.5). SLC5A8, Na^+-coupled monocarboxylate transporter 1; HEK, 293 cells; HCT, HCT116 cells; NCT, non-tumour cervical tissue; UTR, untranslated region.

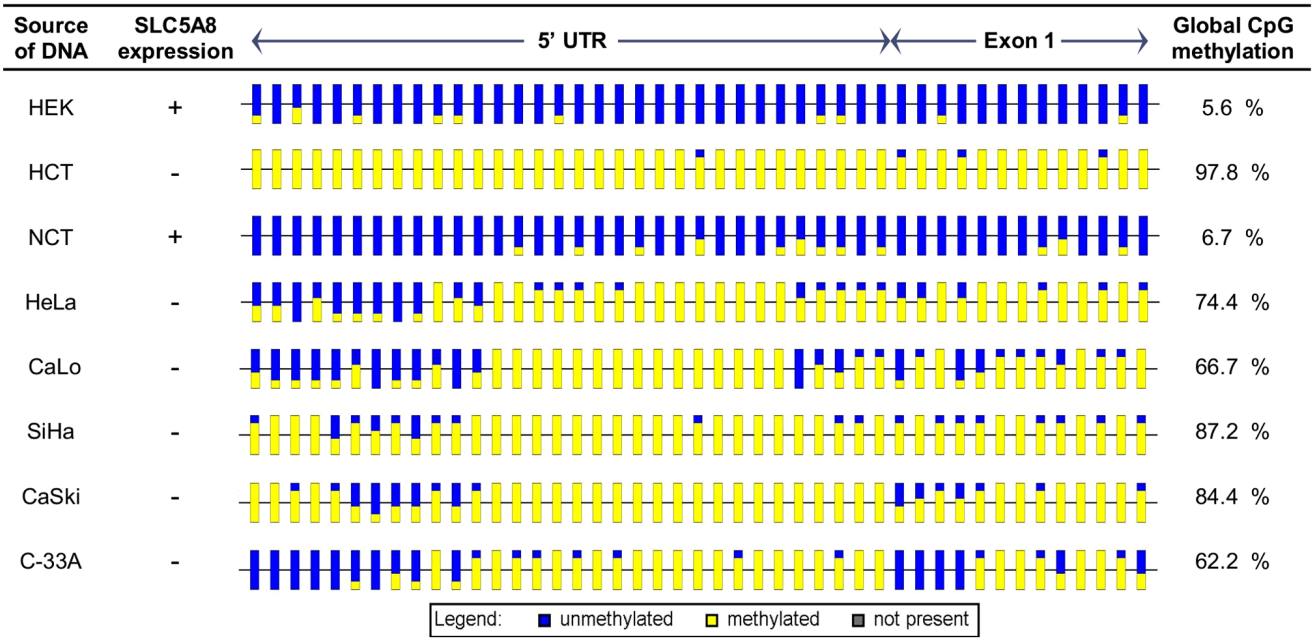

Figure S3. Methylation profiles of the SLC5A8 CpG island by bisulphite-sequencing in cervical cancer. Tumour samples were categorized in groups, according to their level of SLC5A8 expression: (A) High and medium, (B) low and (C) null expression. (D) Five NCTs were used as controls. Each individually cloned and sequenced allele is indicated by a horizontal line. Each circle on the graph represents the methylated state of each CpG: White, unmethylated site; black, methylated site. Overall, 3-4 clones per sample were analysed. SLC5A8, Na^+-coupled monocarboxylate transporter 1; NCT, non-tumour cervical tissue.