Figure S1. Sequences of *SLC22A17* methylated DNA hotspot. (A) Bisulfite-converted sequence of *SLC22A17* and primers used for the PCR amplification and Sanger sequencing. Underlined sequence represents the *SLC22A17* hotspot analyzed in the present study. (B) *SLC22A17* sequence used to generate DNA unmethylated and methylated controls and to analyze the *SLC22A17* methylation status using the standard MSRE and the MSRE-droplet digital PCR protocols. (C) Sequence of Custom methCTRL. Primers and probes were highlighted in each sequence. MSRE, methylation-sensitive restriction enzyme.

Figure S2. Bisulfite sequencing of the *SLC22A17* hotspot in SK-MEL-23, A375 and A2058 cells. (A-C) Sanger sequencing chromatogram of the *SLC22A17* methylated DNA hotspot obtained from bisulfite-converted DNA of (A) SK-MEL-23, (B) A375 and (C) A2058 cells. The peak relative to internal cytosine of CCGG sequence within the *SL22A17* sequence is highlighted in red. The unconverted cytosine indicates complete methylation, while the conversion of cytosine to thymine (C>T) is indicative of hypomethylation of CpG dinucleotide. Bisulfite-converted sequence of *SLC22A17* is detailed in Fig. S1A.

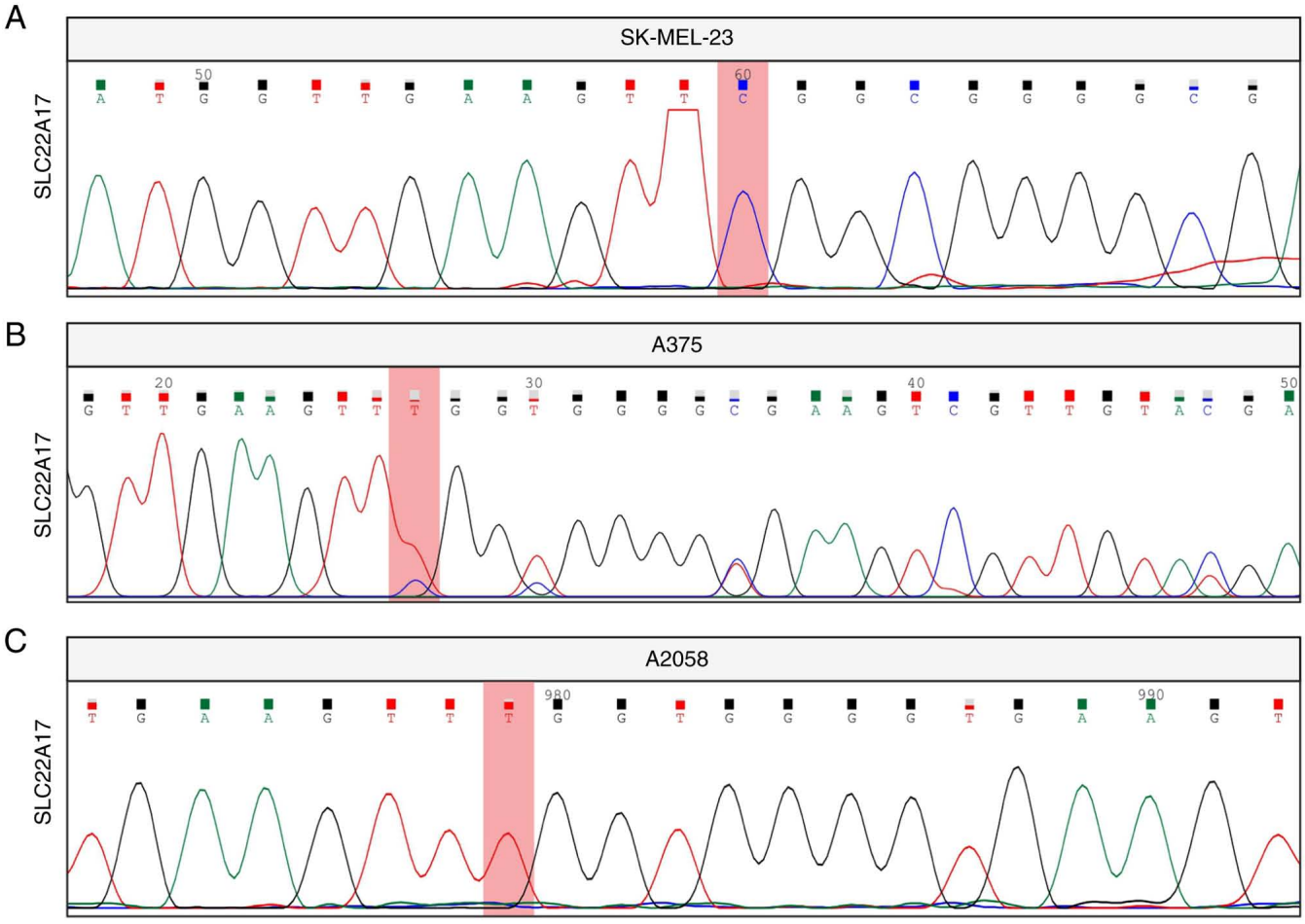

Figure S3. ddPCR amplification of the *SLC22A17* methDNA hotspot on standard MSRE-digested gDNA from SK-MEL-23. (A) FAM amplitude of *SLC22A17* hotspot detected in undigested, *HpaII* and *MspI* mix. (B) HEX amplitude of methCTRL. All experiments were performed in duplicate. For SK-MEL-23 MSRE mix (200 ng of gDNA in 10 μl final reaction volume), 1 μl of each digested sample was added to the ddPCR mix, while 5 μl of SK-MEL-23 diluted MSRE mix (20 ng of gDNA in 10 μl final reaction volume) were used for the following ddPCR amplification. ddPCR, droplet digital PCR; meth-, methylated; MSRE, methylation-sensitive restriction enzyme.

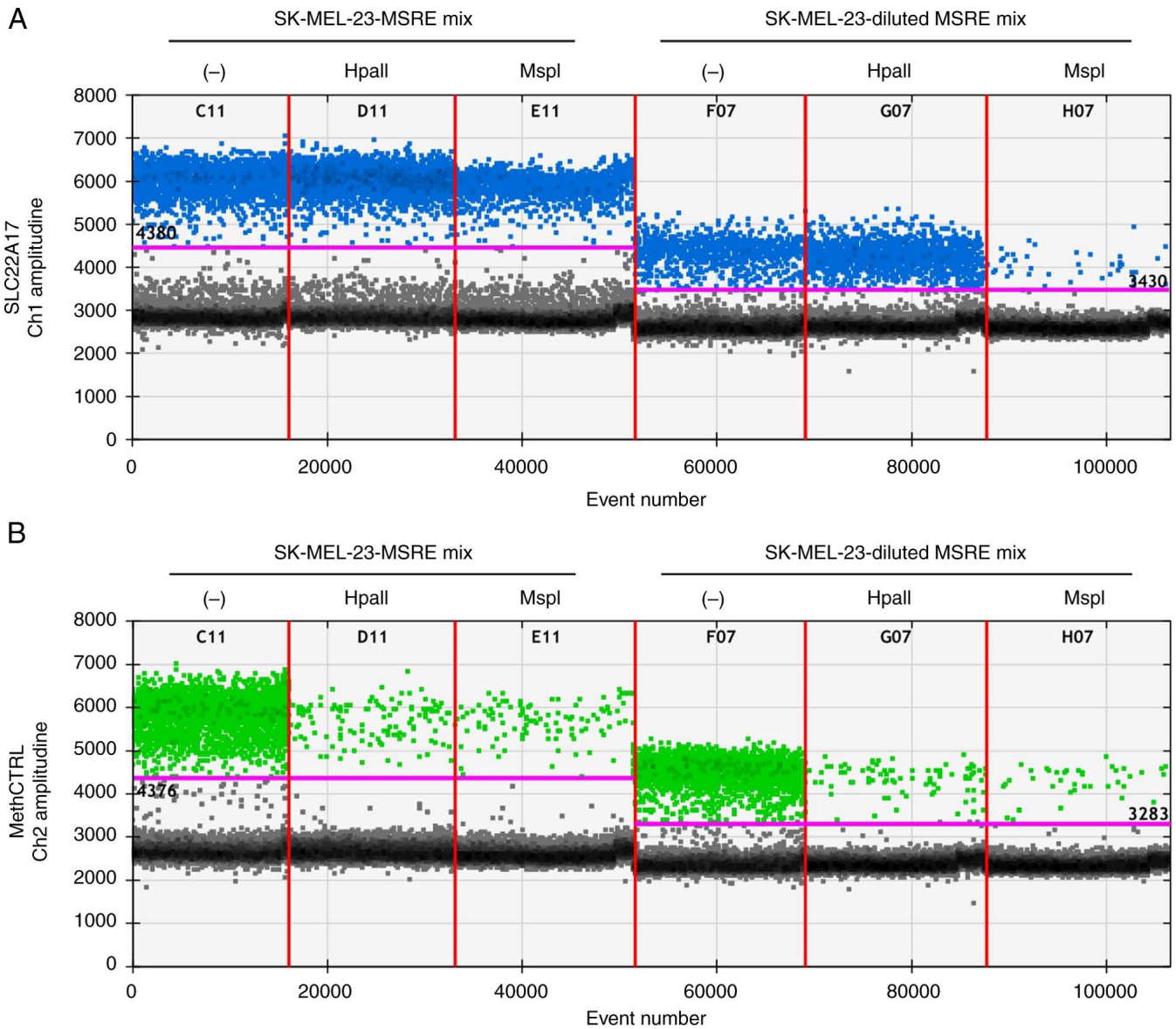

Figure S4. Methylation-sensitive restriction enzyme digestion buffer interference on droplet digital PCR amplification efficiency. (A) FAM amplitude of *SLC22A17* target. (B) HEX amplitude of methCTRL. (C) Amplitude plot of channel 1 (*SLC22A17*). (D) Amplitude plot of channel 2 (methCTRL). All experiments were performed in duplicate. meth-, methylated.