Supporting Information

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SI Materials and Methods

Cell Lines and Drug Treatment. Colo320DM and primary fibroblasts (HDF) were grown in DMEM + 10% FBS. HCT116 and HCT116 DKOs were grown in McCoy's 5A modified medium + 10% FBS. UW228 was grown in EMEM + 10% FBS. For 5-aza-2′-deoxycytidine experiments, UW228 cells were treated with either 5 μM (from 2 mM stock in DMSO) 5-aza-2′-deoxycytidine (Sigma) or with vehicle alone (DMSO) for a total of 72 h, with fresh media containing either DMSO or 5-aza-2′-deoxycytidine being changed once every 24 h. RNA was harvested using TRIzol (Invitrogen) after 72 h of drug treatment.

Bisulfite Sequencing. Genomic DNA (1 μg) was treated with bisulfite per manufacturer's protocol (Qiagen EpiTect Bisulfite kit) and eluted in a total of 40 μL elution buffer. Using 2 μL of bisulfite-converted DNA as template in a 25-μL PCR, cycling conditions were as follows: 96°C for 6 min; 5 cycles of 96°C for 45 sec, 50°C for 90 sec, and 72°C for 2 min; 35 cycles of 96°C for 45 sec, 50°C for 90 sec, and 72°C for 90 sec; and a final extension of 72°C for 7 min. Bisulfite PCR products were gel purified and cloned into pCR4-TOPO (Invitrogen), and independent clones were subjected to sequencing (ABI). Sequence analysis was visualized using MethTools (1). See Table S6 for primer sequences.

Comparison of DAMD to MeDIP and MBD. For the DAMD assay, 2 μg of DNA from HCT116 or HCT116 DKO was used as input per the protocol described in the main text with the exception that after S1 inactivation using phenol and recovery of DNA by ethanol precipitation, DNA was resuspended in 100 μL of TE. DNA at this step was used as input (3.3 μL) for qPCR. The MBD method was performed using the MethylMiner Methylated DNA Enrichment kit (Invitrogen), and 2 μg of sonicated genomic DNA was used as input per manufacturer's protocol. DNA was eluted using 2 M NaCl, and after ethanol precipitation, DNA was resuspended in 100 μL TE and 3.3 μL was used as input for a given qPCR reaction. MeDIP was performed exactly as described (2). As input, 4 μg of sonicated genomic DNA was used. Antibody to 5mC was obtained from Eurogentec, and Dynabeads M-280 Sheep anti-mouse IgG were from Invitrogen. Each method was run in triplicate. For qPCR, 10 μL of 2× FastStart Universal SYBR Green Master Mix (Rox) from Roche, 0.6 μL of 10 μM forward and reverse primers (see Table S6), 6.1 μL water, and 3.3 μL template were used in an ABI 7900HT machine (40 cycles of 95°C for 15 sec followed by 60°C for 1 min). Fivefold serial dilutions (100–0.16 ng) were prepared from input DNA and standard curves were generated for each primer pair. Fold enrichment was calculated using the formula [(HCT116 Gene X ng/HCT116 H3b ng)/(DKO Gene X ng/DKO H3b ng)] except for H3b, which used (HCT116 H3b/DKO H3b). Each sample was performed in triplicate and the mean and standard deviation were calculated.

RT-PCR. RNA was isolated using TRIzol per manufacturer's protocol (Invitrogen) and quantitated using a NanoDrop spectrophotometer. Normal cerebellum RNA was obtained from Biochain. First-strand cDNA synthesis was performed using random hexamers on 1 μg of total RNA as input, using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen) in a final total volume of 20 μL. For PCR, 2 μL of cDNA was used as template in a 25-μL PCR [2.5 μL 10× PCR buffer, 2.5 μL 2 mM dNTPs, 1 μL 10 μM forward and reverse primers (Table S6), 5 μL GC-rich solution, 0.2 μL FastStart Taq (Roche), and 11.8 μL water], using the following cycle parameters: 96°C for 6 min; 32 cycles of 96°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min; and a final extension of 72°C for 7 min.

1. Grunau C, Schattevoy R, Mache N, & Rosenthal A (2000) MethTools—a toolbox to visualize and analyze DNA methylation data. *Nucleic Acids Res* 28 (5):1053–1058.

2. Mohn F, Weber M, Schübeler D, & Roloff T-C (2009) Methylated DNA immunoprecipitation (MeDIP). *Methods Mol Biol* 507:55–64.

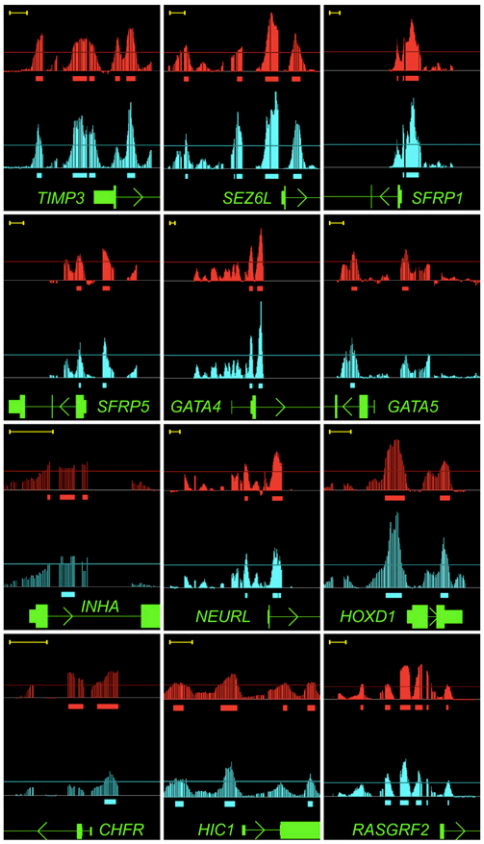

Fig. S1. DAMD identifies CpG methylation at previously described loci in HCT116 cells. Promoter tiling array analysis is shown of DAMD-positive regions in HCT116 compared to DKO cells. Graphically displayed are signal [$\log_2$(signal ratio), red] and *P* values (−10 $\log_{10}$, blue), and the solid bars below each graph depict $\log_2$(signal ratio) > 1.2 where HCT116 > DKO and (−10 $\log_{10}$) *P* values >30 (= $P < 0.001$). Gene names and mRNA structures (exon, large rectangle; intron, thin line; UTR, small rectangle; arrow, direction of transcription) are shown in green at the bottom of each panel. (Scale bars, ~1 kb.)

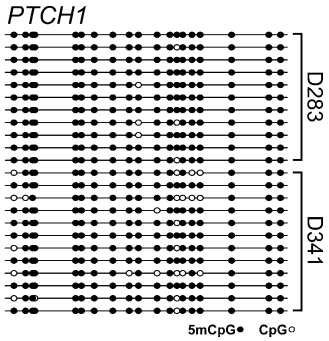

Fig. S2. *PTCH1* hypermethylation in human medulloblastoma cell lines. Bisulfite sequence analysis of the *PTCH1-1C* DAMD-positive promoter region is shown for the human medulloblastoma cell lines D283 and D341. Solid circles represent CpG methylation, and open circles depict unmodified CpG dinucleotides.

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)
Table S3 (DOC)
Table S4 (DOC)
Table S5 (DOC)
Table S6 (DOC)