Figure S1. MLH1 promoter methylation analysis. (A) Primers used to amplify CpG sites at MLH1 promoter. Primer sequences were as follows: F, TTGTTTTTATTGGTTGGATATT (Tm=56.39) and R, AAATACCAATCAAATTTCTCAA (Tm=56.59). (B) Bisulfite targeted sequencing could determine the levels of MLH1 methylation in methylated control DNA samples *in vitro* in a quantitative manner. M100, M50, M15, M5 and UM represent 100, 50, 15 and 5% methylated and unmethylated control samples, respectively. F, forward; R, reverse; MLH1, DNA mismatch repair protein Mlh1; Tm, melting temperature.

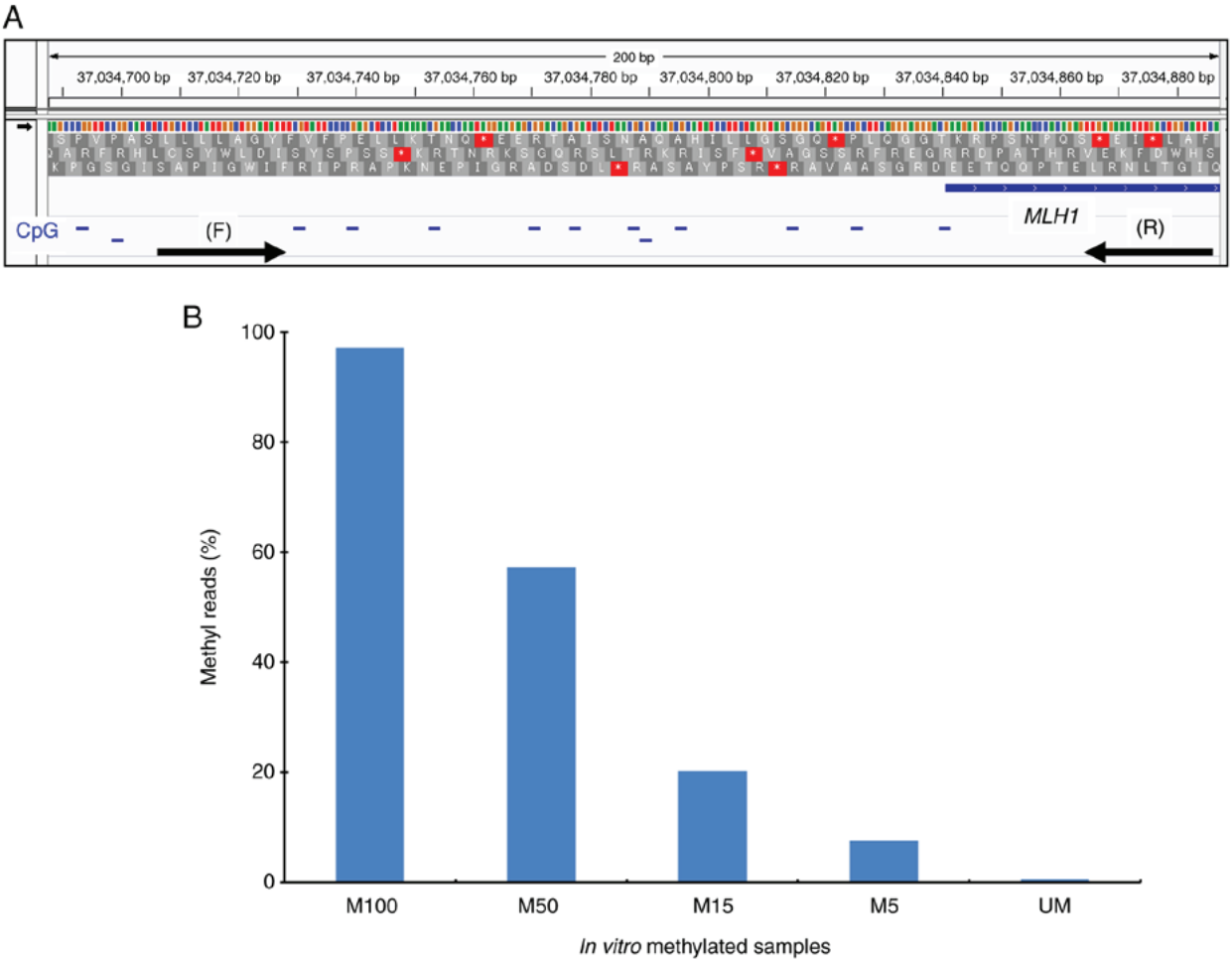

Figure S2. Representative images of immunohistochemistry in deficient mismatch repair colorectal cancers. (A, B) Histology of a rectal cancer sample from a patient with Lynch syndrome with an MSH2 germline mutation. (A) MSH2 and (B) MSH6 immunohistochemistry. The neoplastic epithelium shows loss of nuclear expression of MSH2 and MSH6, whereas the stroma and lymphocytes show expression of both. (C-E) A transverse colon cancer sample from a patient with positive MLH1 methylation and BRAF V600E mutation. Total loss of (C) MLH1 and (D) PMS2 expression were observed in the tumor cells. (E) BRAF V600E immunohistochemistry shows diffuse cytoplasmic staining of the tumor cells. Magnification, x200. MSH2, DNA mismatch repair protein Msh2; MSH6, DNA mismatch repair protein MSH6; MLH1, DNA mismatch repair protein Mlh1; PMS2, mismatch repair endonuclease PMS2.