Figure S1. Vanillin downregulates NNMT activity and phospho-STAT3 in SW480 cell lines. (A) NNMT mRNA was determined by RT-qPCR in SW480/NC and SW480/NNMT cells after treatment with vanillin for 48 h and displayed as a histogram. The untreated group of SW480/NC cells was used as the control group which was normalized to 1. (B) NNMT and p-STAT3 proteins were determined by western blotting after treatment with vanillin at different concentrations for 48 h in SW480/NC and SW480/NNMT cells. (C) NNMT protein levels were displayed as a histogram after treatment with vanillin for 48 h in SW480/NC and SW480/NNMT cells. The untreated group of SW480/NNMT was used as the control group which was normalized to 1. (D) Phospho-STAT3 protein levels were displayed as a histogram after treatment with vanillin for 48 h in SW480/NC and SW480/NNMT cells. The untreated group of SW480/NC was used as the control group which was normalized to 1. (E) Relative 1-MNA level after treatment with vanillin at different concentrations for 48 h in SW480/NNMT cell lines. The untreated group of SW480/NNMT cells was used as the control group which was normalized to 1. Data are represented as means ± SD, n=3; *P<0.05, ***P<0.001. Van, vanillin; NNMT, nicotinamide N-methyltransferase; p-STAT3, phosphorylated signal transducer and activator of transcription 3; 1-MNA, 1-methylnicotinamide.

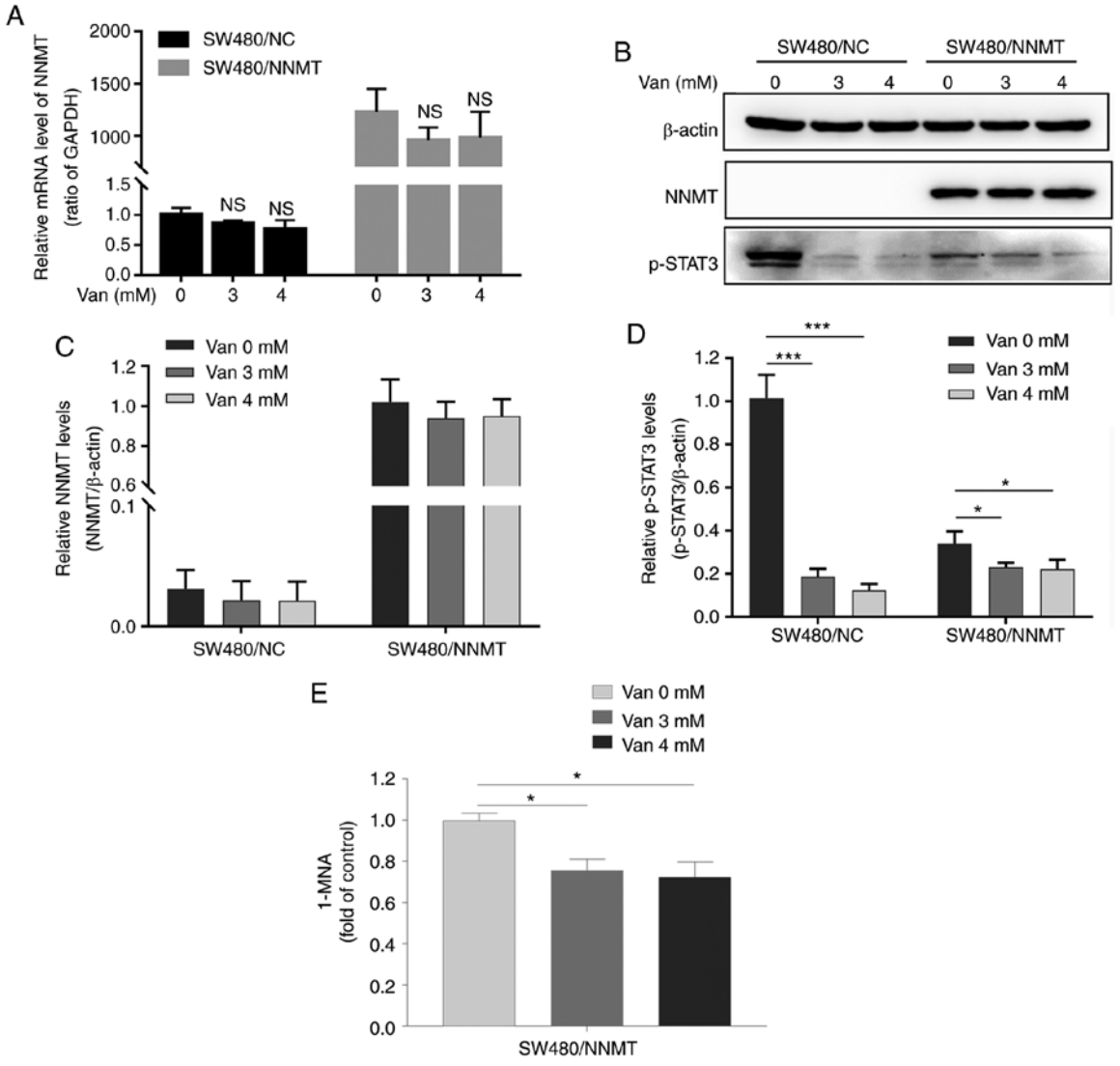

Figure S2. The apoptosis induced by vanillin was partly reversed by 1-MNA. CRC cell lines, HT-29/NC, HT-29/shNNMT, SW480/NC and SW480/NNMT, were treated with 1-MNA and/or vanillin for 48 h. (A and C) Cell apoptosis was analyzed by flow cytometry in HT-29/NC and HT-29/shNNMT (A) and SW480/NC and SW480/NNMT (C) cell lines. (B and D) Cell apoptosis was displayed as a histogram in HT-29/NC and HT-29/shNNMT (B) and SW480/NC and SW480/NNMT (D) cell lines. Data are represented as means ± SD, n=3; *P<0.05, **P<0.01. Van, vanillin; CRC, colorectal cancer; NNMT, nicotinamide N-methyltransferase; 1-MNA, 1-methylnicotinamide.

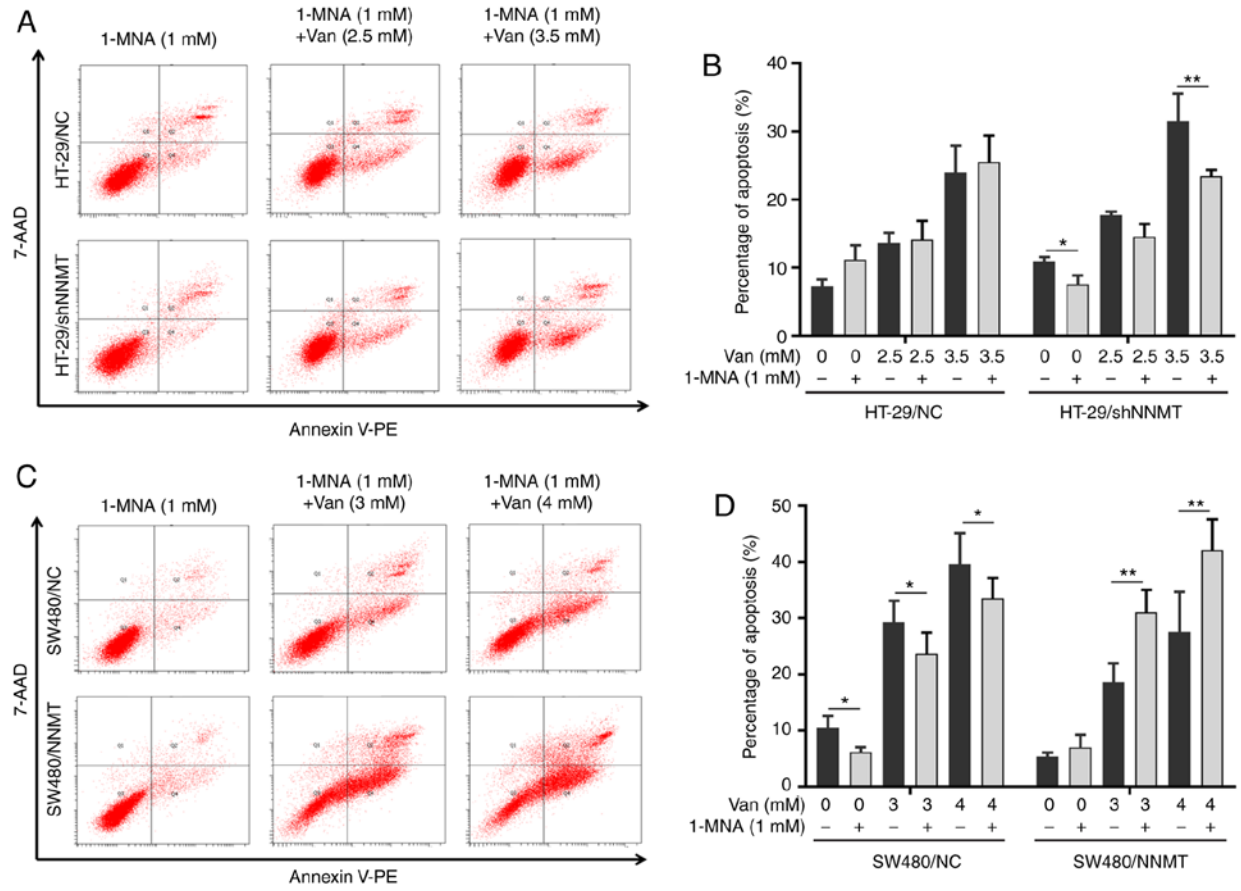

Figure S3. Vanillin increases cell apoptosis by inducing mitochondrial damage and ROS production in HT-29 cell lines. HT-29 cells were transfected with siNNMT or siNC for 48 h, and then treated with vanillin for 48 h, or were pretreated with 10 mM NAC for 2 h followed by treatment with vanillin for 48 h. (A and B) NNMT protein levels were determined by western blot and displayed as a histogram after treatment with vanillin for 48 h. The untreated group of HT-29/siNC cells was used as the control group which was normalized to 1. (C and D) ROS were detected by flow cytometry using the fluorescent probe DCFH-DA and displayed as a histogram. (E and F) Measurement of mitochondrial transmembrane potential by JC-1 fluorescence and displayed as a histogram. (G) Cell apoptosis was displayed as a histogram in HT-29/NC and HT-29/shNNMT cell lines. Data are represented as means ± SD, n=3; *P<0.05, **P<0.01, ***P<0.001. Van, vanillin; NNMT, nicotinamide N-methyltransferase; ROS, reactive oxygen species.