Supplemental Table 1. Affymetrix gene array analysis of HT29 cells after transfection by non-targeting control (NTC) siRNA or NFAT5 siRNA. HT29 cells were transfected with control siRNA or siRNA targeting NFAT5. Forty-eight h after transfection, Total RNA was extracted and the quality of RNA samples was checked using the Agilent 2100 Bioanalyzer. Total RNA (250 ng) was labeled using the Ambion WT Expression kit (cat # 4411974). Single-stranded cDNA (ss cDNA) (5.5 ug) was fragmented and terminal labeled using Affymetrix WT Terminal Labeling kit (cat# 900671). Fragmented and labeled ss cDNA (2.0 ug) were hybridized to the arrays for 16 h. The arrays were then washed and stained using the Affymetrix GeneChip Fluidics Station 450 and scanned using the Affymetrix 7G scanner. Data was collected using Affymetrix Expression Console software. Genes upregulated or downregulated by a mean of at least 2-fold are shown.

Supplemental Figure 1. Knockdown of NFAT5 using shRNA targeting NFAT5 decreases REDD1 expression in HT29 cells. HT29 cells were infected with the NTC shRNA or human NFAT5 shRNA lentivirus particles. Stably expressing cells were selected with puromycin at a concentration of 2 μg/ml. Total protein was extracted and analyzed by Western blotting using anti-REDD1, NFAT5 and anti–β-actin antibodies. MISSION NTC shRNA (Non-Target shRNA Control Transduction Particles, # SHC002V) and NFAT5 shRNA (TRCN0000020020) Lentiviral Particles were purchased from Sigma (St. Louis, MO). The NTC shRNA contains four base-pair mismatches within the short-hairpin sequence to any known human or mouse genes.

Supplemental Figure 2. Treatment with NaBT results in the inhibition of mTOR signaling in HT29 cells. HT29 cells were treated with NaBT for 24 h. Total protein was extracted and Western blotting was performed using the indicated antibodies.

Supplemental Figure 3. Repression of mTOR signaling is associated with spontaneous differentiation of Caco-2 cells. Caco-2 cells, that spontaneously differentiate to an enterocyte-like phenotype with post-confluence, were cultured and harvested at different time points: preconfluency (pre) or 3, 6 and 12 d post-confluency (post). Total protein was extracted and mTOR activity was determined by Western blotting using anti-p-mTOR and anti-p-S6 antibodies.