Figure S1. Morphological changes of C2C12 myoblast with other GIC cells CM. Representative microscopic images of C2C12 cells cultured with CM from other GIC cell lines for 5 days. CM from these cell lines showed no apparent ability to inhibit myoblast differentiation compared with the cell lines shown in Fig. 1. Scale bar, 200 μm. GIC, gastrointestinal cancer; CM, conditioned medium.

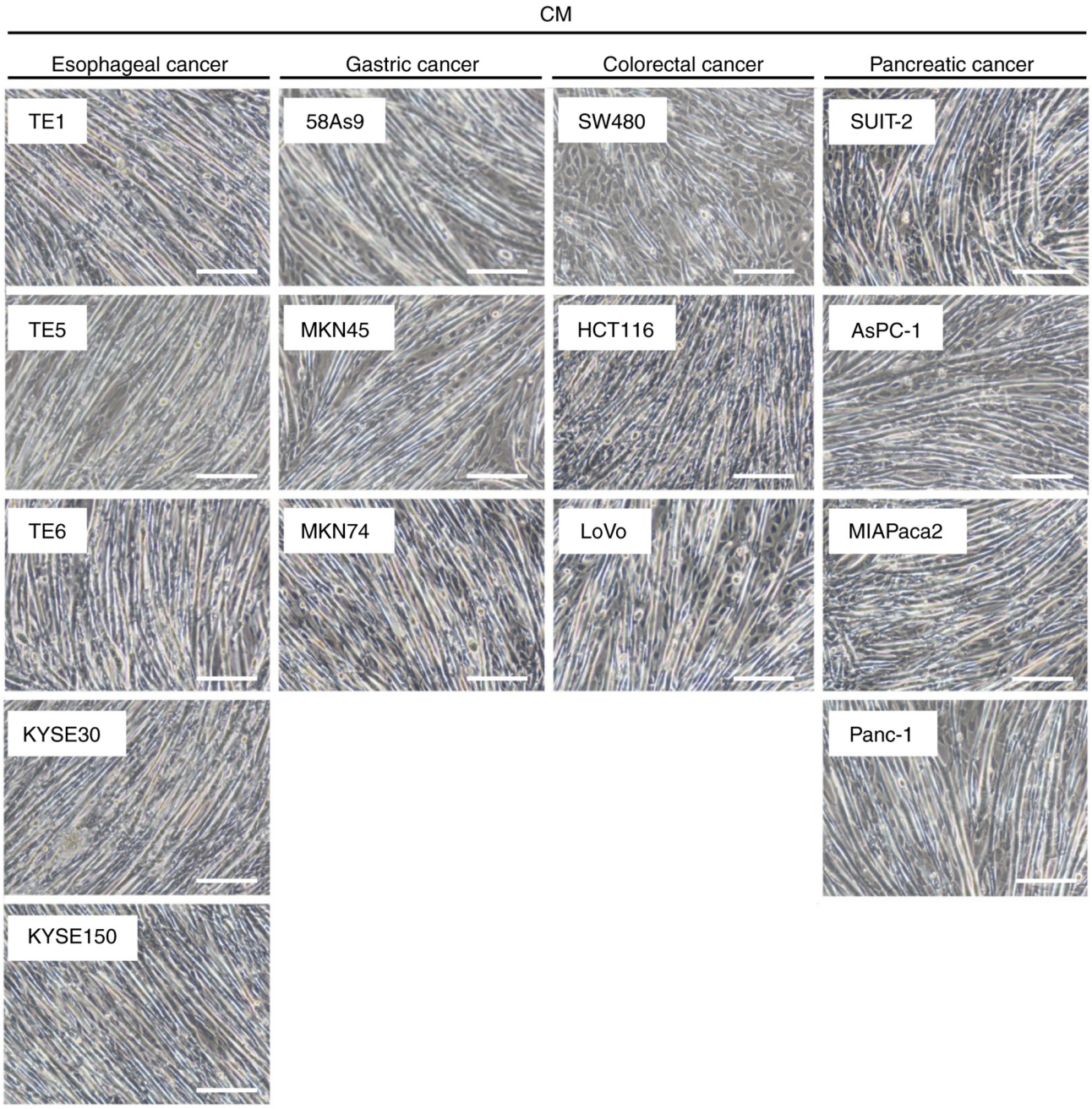

Figure S2. mRNA expression of BMPs in three gastrointestinal cancer cells. Representative reverse transcription-quantitative PCR of BMP-2, -6 and -7 mRNA expression in 58As9, HT29 and DLD1 cells (n=3). These experiments were independently repeated three times. BMP, bone morphogenetic protein.

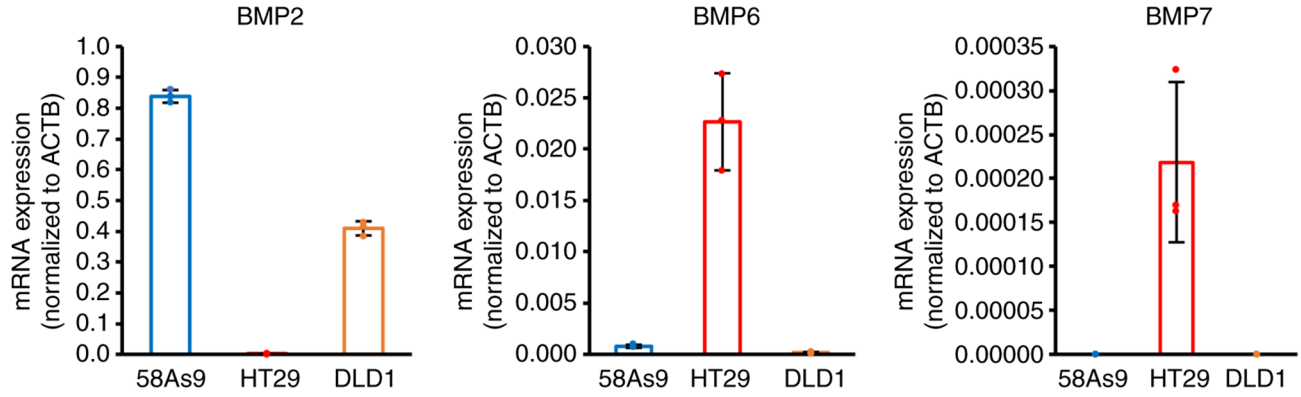

Figure S3. Protein degradation by ubiquitin-proteasome system and autophagy-lysosome system were activated in the differentiated myotube by CM treatment from 44As3 and C26, but not HT29 or DLD1. (A) Diagram demonstrating the experimental design. After C2C12 cells were cultured in DM for 3 days, the completely differentiated myotubes were treated with control DM or cancer cell CM from day 3 to day 5. (B) Representative immunofluorescence staining images on day 5. (C) The mean myotube diameter was plotted. The diameter of 50 myotubes were measured per group, in which the mean diameter was estimated by measuring 3 sites in each myotube. Data are presented as mean ± SD, and statistical significance was analyzed compared with DM using one-way ANOVA with the Dunnett's post hoc test. ns not significant, ***P<0.001. (D) Representative western blotting of MuRF-1 and LC3B protein expressions. (E) Reverse transcription-quantitative PCR analysis of IL-6 mRNA expression in indicated human cancer cells (n=3). Data are presented as mean ± SD, and statistical significance was determined using one-way ANOVA with Tukey's post hoc test. DM, differentiation medium; CM, conditioned medium; GM, growth medium; GIC, gastrointestinal cancer.