Figure S1. Western blot analysis of K-Ras V12, B-Raf V600E, and PI3KCA H1047R cell lysates and effects of U0126 or PIK-90 inhibitors. (A) 2D cultures expressing control (pQCXIP GFP), K-Ras V12 (pQCXIP GFP K-Ras V12), B-Raf V600E (pQCFLAP B-Raf V600E), or PI3KCA H1047R (pQCFLAP PI3KCA H1047R) were lysed and analyzed by Western blotting with the indicated antibodies. (B) 2D cultures expressing control (pQCXIP GFP) or K-Ras V12 (pQCXIP GFP K-Ras V12) and treated with 5, 10, or 20 µM of the MEK inhibitor U0126 or 0.5, 1.0, or 2.0 µM of the PI3K inhibitor PIK-90 were lysed and analyzed by Western blotting with the indicated antibodies.
Figure S2.  

c-myc up-regulation and RNAi depletion in K-Ras V12 and B-Raf V600E cells. (A) Quantification of c-myc protein levels normalized to actin from two independent Western blot experiments of control (pQCXIP GFP), K-Ras V12 (pQCXIP GFP K-Ras V12), and B-Raf V600E (pQCFLAP B-Raf V600E) day 1 3D cultures. The means ± SEM are shown. (B) Day 1 3D cultures expressing control (pQCXIP GFP), B-Raf V600E (pQCFLAP B-Raf V600E), and B-Raf V600E + 20 µM U0126 were lysed and analyzed by Western blotting with the indicated antibodies. (C) RNA extracted from day 1 3D control (pQCXIP GFP), K-Ras V12 (pQCXIP GFP K-Ras V12), and B-Raf V600E (pQCFLAP B-Raf V600E) was converted to cDNA and analyzed by quantitative PCR to determine c-myc levels relative to glyceraldehyde 3-phosphate dehydrogenase. The means ± SEM of three independent experiments are shown. (D) 2 d after infection with either control shRNA, shmyc 1, or shmyc 2 lentivirus, cells were selected with puromycin for 4 d to make stable cell pools. These cells were superinfected with control (pQCXIP GFP), K-Ras V12 (pQCXIP GFP K-Ras V12), or B-Raf V600E (pQCFLAP B-Raf V600E) retrovirus. 4 d later, cells were lysed and analyzed by Western blotting with the indicated antibodies.
Figure S3. Promoting proliferation in control cells or inhibiting proliferation in K-Ras V12 cells does not affect 3D morphology. (A) Day 1 3D cultures expressing control [pQCXIP GFP], K-Ras V12 [pQCXIP GFP K-Ras V12], B-Raf V-600E [pQCFLAP B-Raf V600E], or c-myc [pBabe blast c-myc] were lysed and analyzed by Western blotting with the indicated antibodies. The positive control (+ control) for the p16, p21, and p27 Western blot is a lysate from retinal pigment epithelial cells treated for 3 h with 50 µM MG132 and 5 Gy ionizing radiation (gift from D. Ciznadija, Memorial Sloan-Kettering Cancer Center, New York, NY). (B) Single cells expressing control [pQCXIP GFP], K-Ras V12 [pQCXIP GFP K-Ras V12], or cyclin D1 [pBABE puro cyclin D1] were embedded in matrix. After 2 d, cells were fixed and stained for aPKC (red) and DNA (blue). Representative images are shown. Bar, 50 µm. (C) At least 50 two-cell structures were imaged for each condition in three independent experiments and analyzed for proper apical localization of aPKC. (D) 3D cultures expressing control [pQCXIP GFP] or K-Ras V12 [pQCXIP GFP K-Ras V12] were continuously treated with 0 or 1.25 µM of the cdk4 inhibitor PD0332991. At day 10, these structures were fixed and stained for aPKC (red) and DNA (blue). The midplanes of ≥50 structures were imaged for each condition in three independent experiments. Both the proportion of structures with normal morphology and the number of nuclei in the midplane of each structure were quantified. (E) 3D day 10 cultures expressing control [pQCXIP GFP], K-Ras V12 [pQCXIP GFP K-Ras V12], or cyclin D1 [pBABE puro cyclin D1] were lysed and analyzed by Western blot with the indicated antibodies. (F) 3D day 3 cultures expressing control [pQCXIP GFP] or K-Ras V12 [pQCXIP GFP K-Ras V12] treated with 0 or 1.25 µM PD0332991 were lysed and analyzed by Western blotting with the indicated antibodies. The means ± SEM are shown. **, P < 0.005.
Video 1. **Morphology of control Caco-2 3D structure.** A Caco-2 3D culture expressing control (pQCXIP GFP) was fixed at day 10 and stained for actin (red) and DNA (blue). 135 images comprising a z stack spanning 74.25 µm were acquired with a spinning-disk confocal microscope (UltraVIEW ERS) and used to make a stack video with MetaMorph.

Video 2. **Morphology of K-Ras V12 Caco-2 3D structure.** A Caco-2 3D culture expressing K-Ras V12 (pQCXIP GFP K-Ras V12) was fixed at day 10 and stained for actin (red) and DNA (blue). 200 images comprising a z stack spanning 106 µm were acquired with a spinning-disk confocal microscope (UltraVIEW ERS) and used to make a stack video with MetaMorph.