**Supplemental Figure 1. Quantitative image based analysis of pERK.**  (A) SW48-KRasG12D cells were grown in 8-well glass bottom dishes. Cells were starved (0% FBS) for 18 h in the presence of fatty acid and subsequently stimulated with EGF (25 ng/ml) for 5 min. Cells were then immediately fixed in ice cold 100% methanol. (A) merged DIC and Hoechst images; (B) CellMaskTM Green Plasma Membrane Stain was used to define (C) binary whole cell masks, which was used to create (D) masked images of pERK. Scale bar = 20 µM.

**Supplemental Figure 2. Western blot of pERK in the DKOB8 oncogenic KRasG21D overexpression model.** Representative western blots from lysates of DKOB8 cells grown in 6-well plates and treated with fatty acid (50 μM) for 72 h. Cells were incubated with ponasterone A (10 μM) for the final 48 h, and then were serum starved (0% FBS) over the final 18 h in the presence of fatty acid or, where indicated, MEK inhibitor (U0126, 10 μM). (A) Incubation with ponasterone A results in a dramatic increase in Ras protein along with pERK. Note that oncogenic KRasG12D is myc-tagged and migrated at a higher molecular weight than the endogenous wild type Ras, which is faintly visible below. (B) Total protein on the membrane was visualized using the Stain Free system (BioRad).

**Supplemental Figure 3. Holidic diet containing corn oil does not disrupt Ras spatiotemporal dynamics in *Drosophila* midguts**. Nanoclustering-FRET analysis (illustrated in schemes) in *Drosophila* midgut stem cells co-expressing GFP- and RFP-tagged (A) truncated KRas (tK) or (B) truncated HRas (tH). (B and D) 1-2 d old *Drosophila* were placed on holidic diet containing no lipid (red) or corn oil (green) for 5 d prior to dissection. Midguts were mounted for microscopy. In all graphs (B and D), the apparent FRET efficiency was calculated from FLIM data (mean ± SEM, n= (B) 2 or (D) 1 independent experiment(s)). Values in the bars indicate the number of analyzed FOVs. No statistical significance (P>0.05) was observed between treatments (n.s.) as determined by an unpaired t-test.