Figure EV1. ROCK2 association with pancreas adenocarcinoma grade and expression in tumor cells in resection margin (related to Fig 1).
A Histoscores of ROCK2 staining (Fig 1A) in normal pancreas \( (n = 5) \), and pancreas adenocarcinoma grade 1 \( (n = 23) \), grade 2 \( (n = 27) \), and grade 3 \( (n = 13) \). Box (upper and lower quartiles divided by median value) and whisker (5\textsuperscript{th}–95\textsuperscript{th} percentile) plots show outliers as individual points.
B ROCK2-positive stained cells (indicated with arrows) found beyond the tumor in the resection margin of human PDAC.
Figure EV2. Tissue-selective expression of conditionally activated ROCK2 (related to Fig 1).

A A CAG-LSL-Rock2:ER expression cassette was targeted to the mutant mouse Hprt gene locus together with the promoter and first exon of human HPRT and the second exon of murine Hprt to reconstitute an active chimeric Hprt locus. Not to scale.

B Generation of LSL-ROCK2:ER; KPC (RKPC) mice.

C Conditional activation of the ROCK2:ER fusion protein.

Figure EV3. ROCK-induced collagen matrix invasion (related to Fig 2).

A H&E-stained sections of cell invasion into collagen matrix containing embedded fibroblasts after 8 days. Scale bar = 100 μm. Invasion index of KPfc cells treated with 1 μM 4HT. Means ± SEM (n = 6), one-way ANOVA with multiplicity adjusted exact P-value by post hoc Dunnett’s multiple comparison test.

B H&E-stained sections of cell invasion into collagen matrix after 8 days. Scale bar = 100 μm. Invasion index of KPfc cells treated with EtOH vehicle, 1 μM 4HT, or 1 μM 4HT + 10 μM H1152. Means ± SEM (n = 6; n = 5 for ROCK2:ER/4HT), one-way ANOVA with multiplicity adjusted exact P-value by post hoc Tukey multiple comparison test.

C Cell proliferation determined by Ki67 immunofluorescence. Scale bar = 20 μm.

D Quantification of cell number at the collagen matrix surface per 0.046 mm² field. Means ± SEM (n = 24), one-way ANOVA with multiplicity adjusted exact P-value by post hoc Tukey multiple comparison test.

E Ki67-positive cell percentages at the surface. Means ± SEM (n = 24), one-way ANOVA with multiplicity adjusted exact P-value by post hoc Tukey multiple comparison test.
Figure EV3.

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Figure EV4. Accumulation of MMP10 and MMP13 in bleblike protrusions (related to Fig 5).

A, B Confocal microscope images of KPfC cells expressing ROCK1:ER or ROCK2:ER as indicated co-stained for F-actin, MMP10 (A) or MMP13 (B), and DAPI following treatment with vehicle, 1 μM 4HT, or 1 μM 4HT + 1 μM H1152 for 24 h. Multiple z-planes were used to generate x–z and y–z images (bottom). Scale bar = 5 μm.