

Expanded View Figures

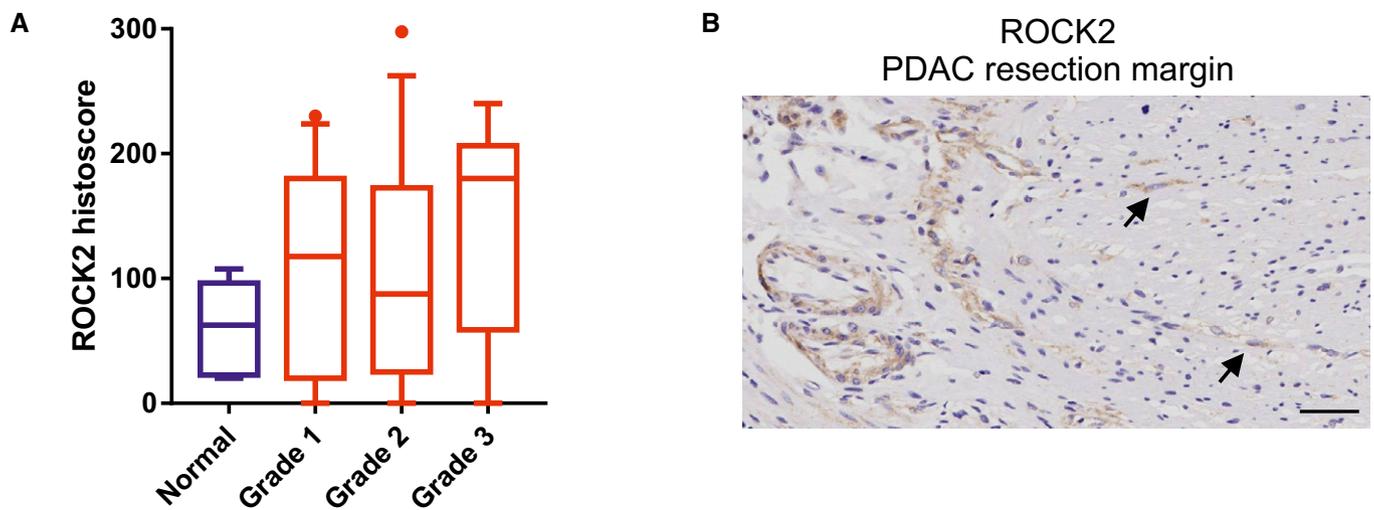


Figure EV1. ROCK2 association with pancreas adenocarcinoma grade and expression in tumor cells in resection margin (related to Fig 1).

- 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 (5th–95th 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.

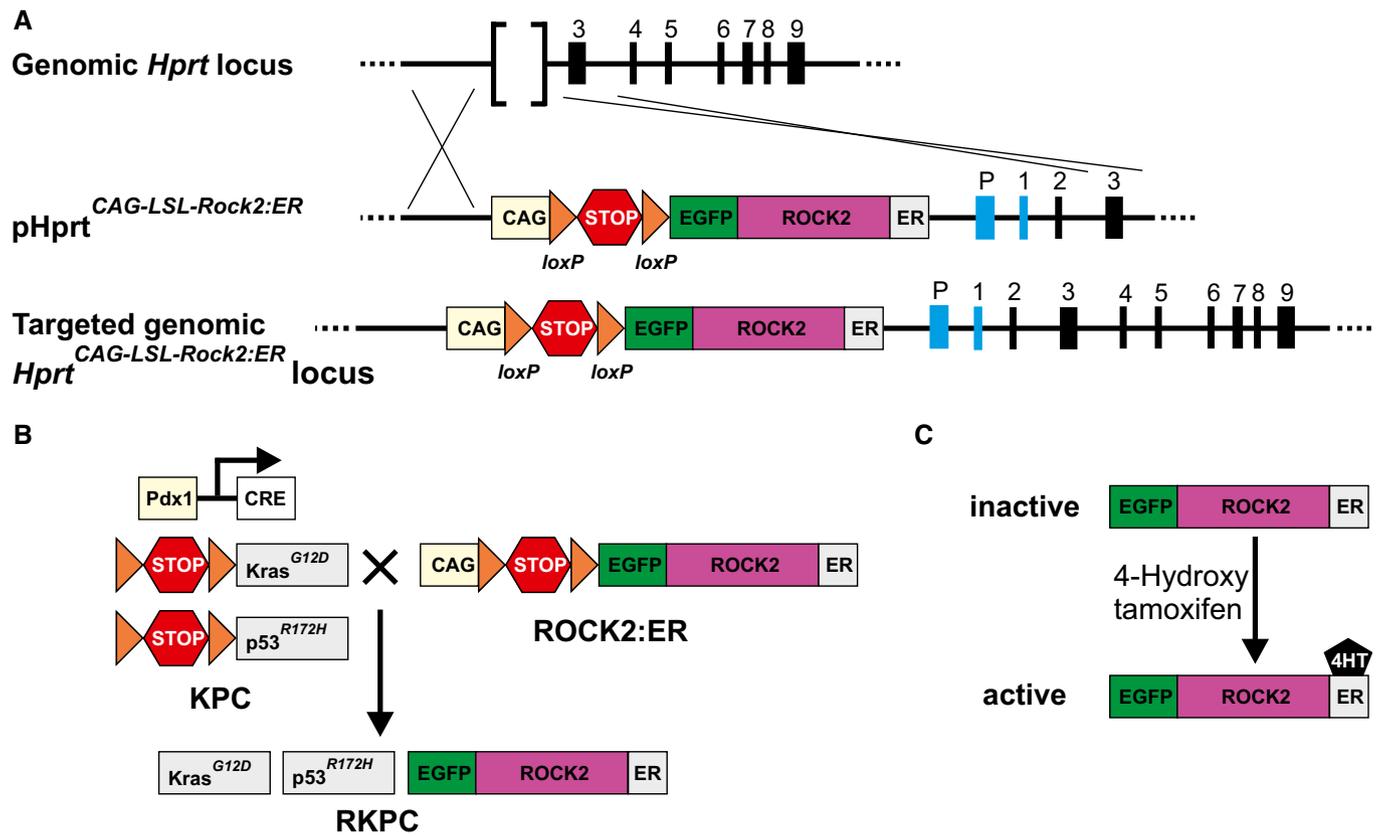


Figure EV2. Tissue-selective expression of conditionally activated ROCK2 (related to Fig 1).

- 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 μ m. Invasion index of KPfC cells treated with 1 μ M 4HT. Means \pm 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 μ m. Invasion index of KPfC cells treated with EtOH vehicle, 1 μ M 4HT, or 1 μ M 4HT + 10 μ M H1152. Means \pm 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 μ m.
 D Quantification of cell number at the collagen matrix surface per 0.046 mm² field. Means \pm 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 \pm SEM ($n = 24$), one-way ANOVA with multiplicity adjusted exact *P*-value by *post hoc* Tukey multiple comparison test.

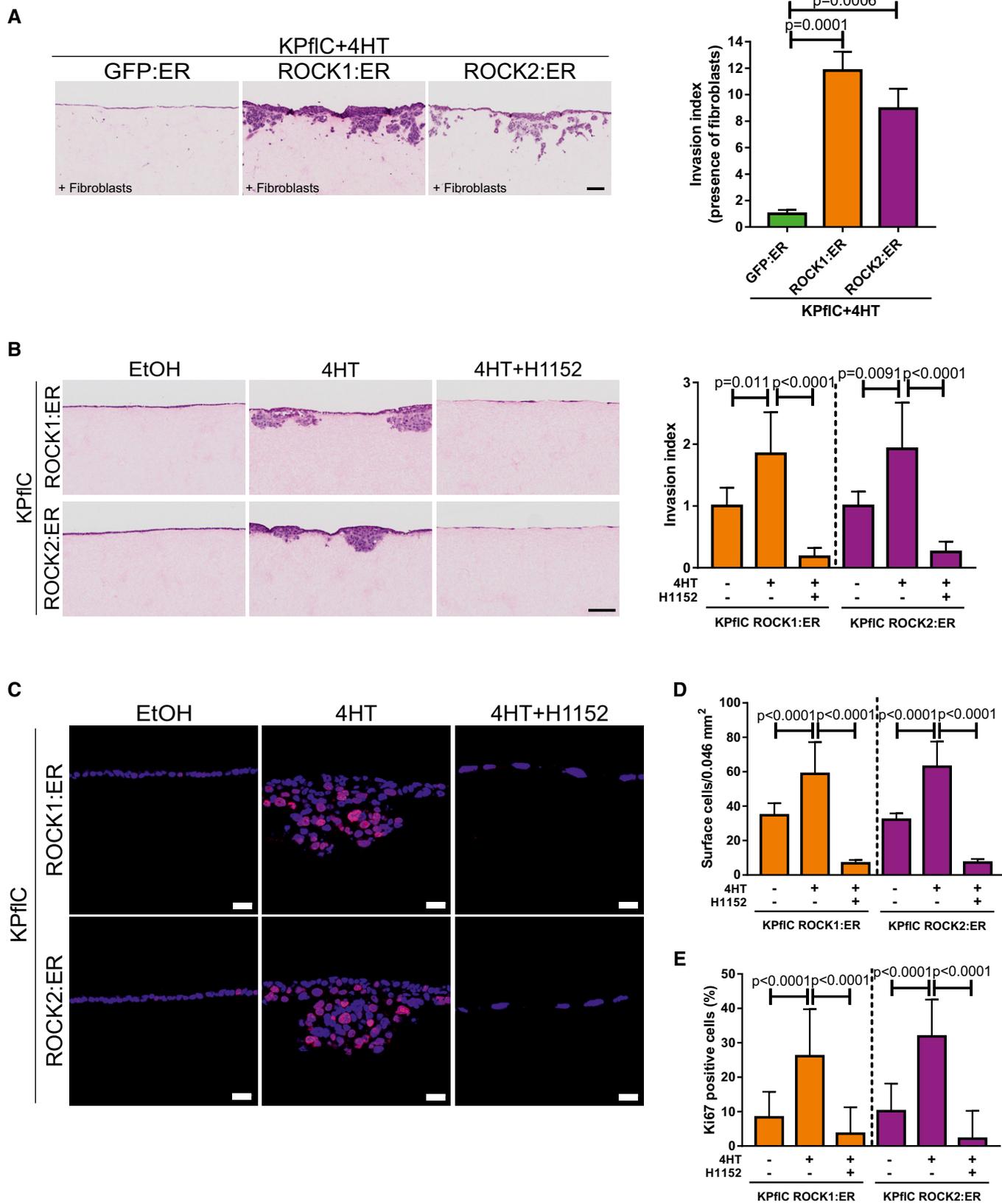


Figure EV3.

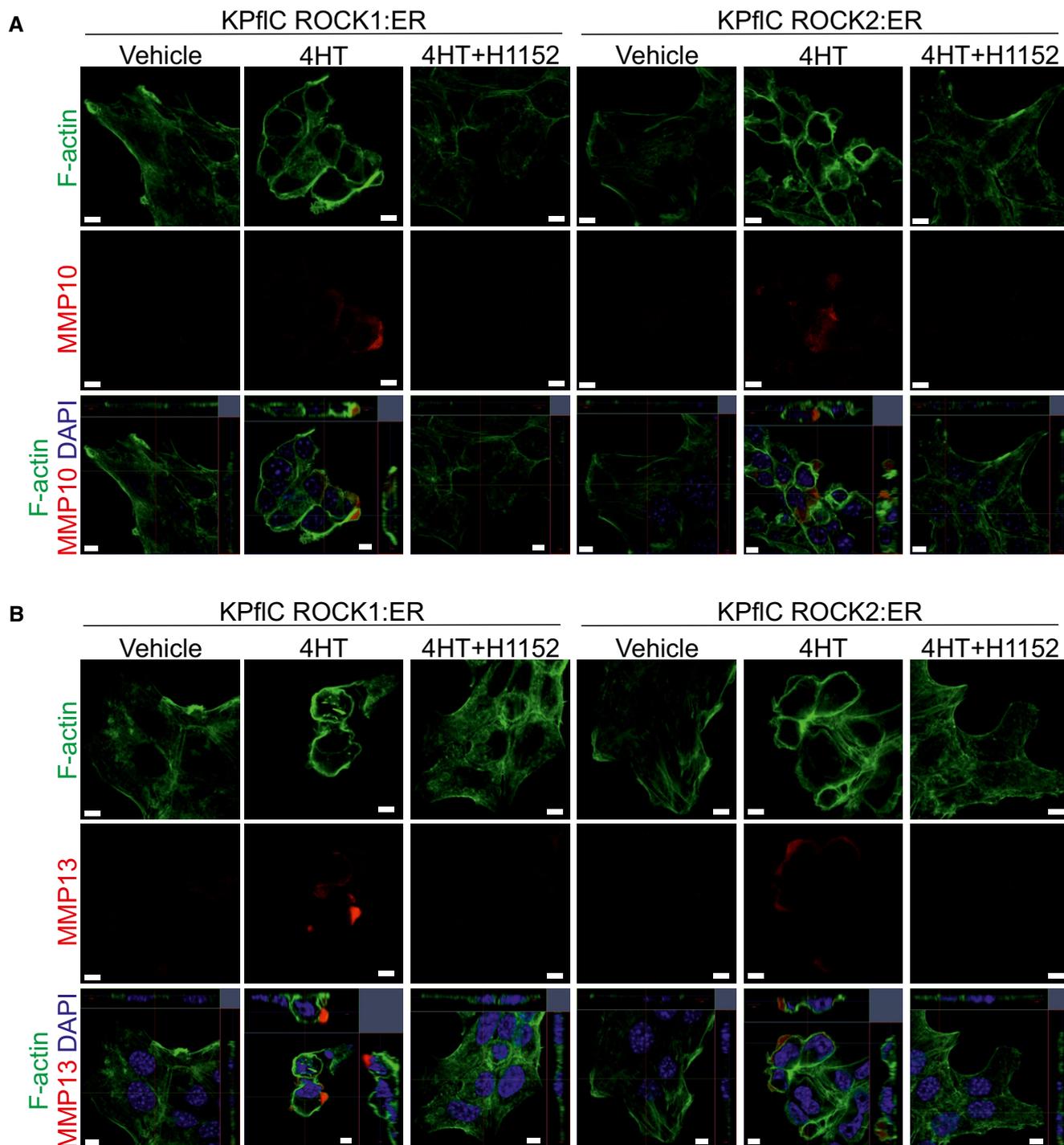


Figure EV4. Accumulation of MMP10 and MMP13 in bleblike protrusions (related to Fig 5).

A, B Confocal microscope images of KPfIC 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 μ M 4HT, or 1 μ M 4HT + 1 μ M H1152 for 24 h. Multiple z-planes were used to generate x-z and y-z images (bottom). Scale bar = 5 μ m.