Supporting Information

Zorde Khvalevsky et al. 10.1073/pnas.1314307110

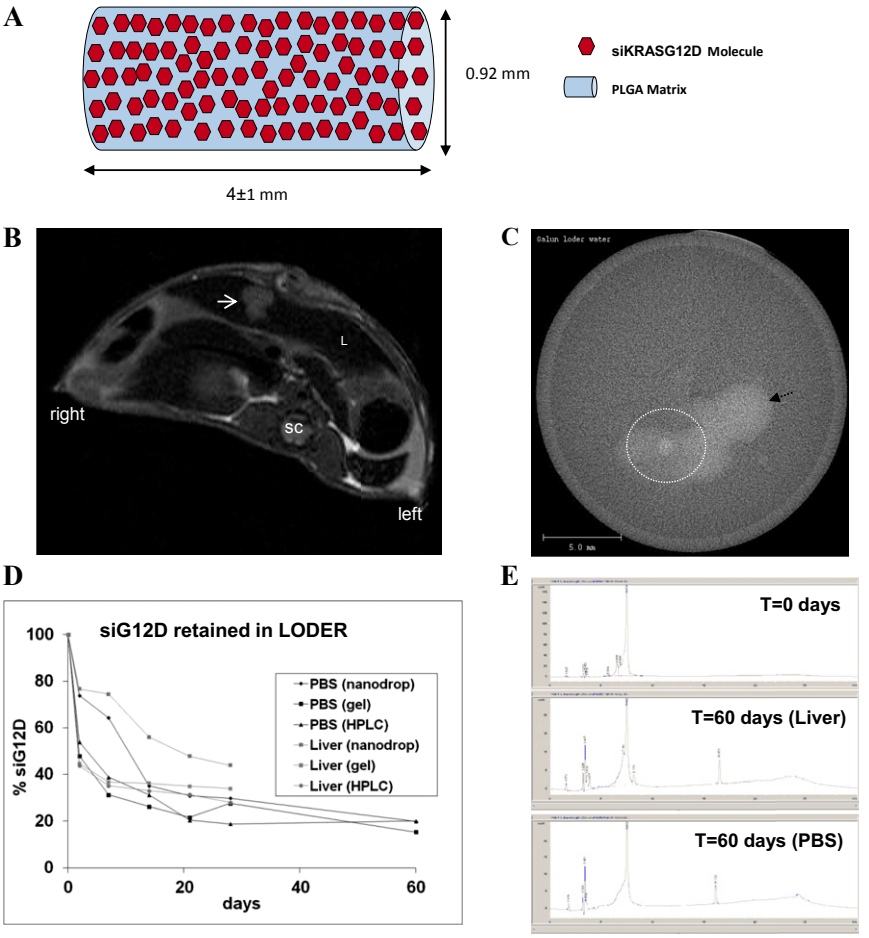

Fig. S1. LODER characteristics. (*A*) Diagram of the siG12D LODER matrix. PLGA, poly(lactic-co-glycolic) acid. (*B*) Magnetic resonance imaging (MRI) detects the LODER in the liver of mice; arrow points to the three inserted LODERs (one lower LODER and two above). L, liver; sc, spinal cord. (*C*) LODER visualization using microcomputer tomography (μCT). LODERs were implanted into xenograft tumors originating from the human pancreatic cancer cell line Capan1. At 57 d following implantation, mice were killed and tumor tissue was extracted and visualized by μCT. LODER (in the circle) located within the tumor (black arrow). (*D*) siRNA retained in LODER: siG12D LODERs were incubated in phosphate buffed saline (PBS) (pH = 7.4) or mouse liver tissue ex vivo at 37 °C. siG12D was extracted from LODERs at different time points and the amount of embedded siG12D was measured using three different methods: high-performance liquid chromate (HPLC), gel electrophoresis, and NanoDrop (absorption). (*E*) LODERs protect siG12D from degradation: protection of LODER-embedded siG12D from degradation was assessed by HPLC. Presented are HPLC graphs showing siG12D: T = 0 d, pure siG12D; T = 60 d (liver), siG12D extracted from LODER after 60 d incubation in liver at 37 °C (ex vivo); T = 60 d (PBS), siG12D extracted from LODERs after 60 d incubation in PBS at 37 °C.

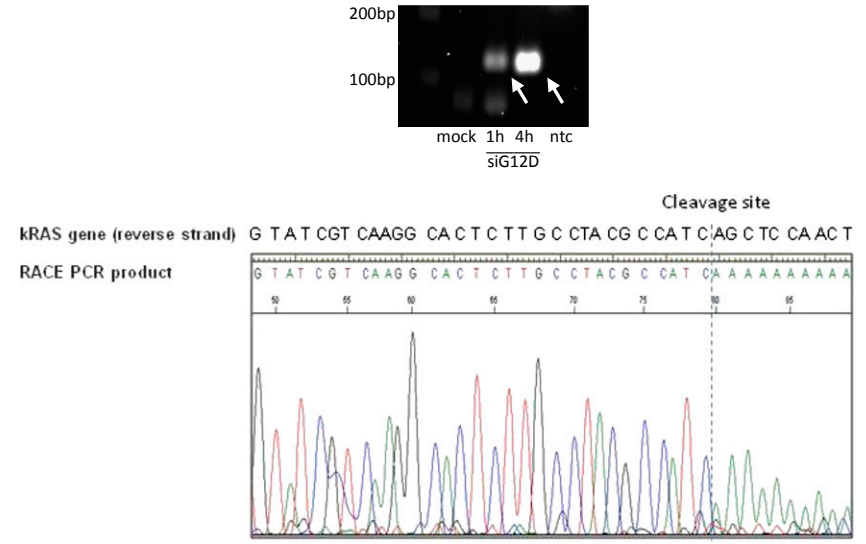

Fig. S2. RACE: A site-specific, siG12D-directed cleavage of KRAS message. Panc1 cells were transfected with siG12D and harvested after 1 and 4 h. cDNA was made using KRAS-specific primer. A 5′ poly-A tail was added with terminal transferase and dATP. PCR amplification of the RACE-specific target was done using 3′ KRAS-specific primer and an oligo-dT primer containing 4 bp at the 3′ complementary to the 5′ nick site. The results show specific amplification of the treated samples and a time-dependent activity with a peak at 4 h posttransfection (*Upper*). Sequencing results confirm cleavage at a specific site (*Lower*).

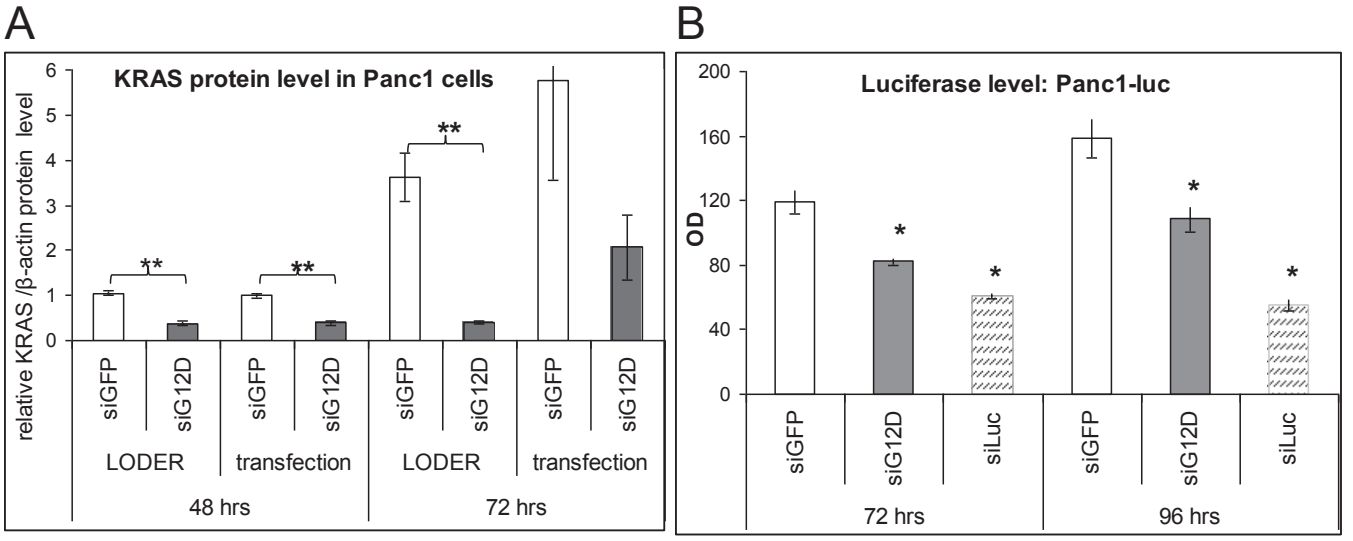

Fig. S3. The silencing effect of the siG12D LODER in human pancreatic cancer cells. (*A*) Panc1-Luc cells were incubated with LODERs containing either siGFP or siG12D in the presence of Lipofectamine 2000 or transiently transfected with these siRNAs using Lipofectamine 2000. After 48 h, cells were lysed and total level of KRAS protein was assessed by Western blot analysis. Representative quantification results (TINA software) of the protein levels (normalized to the level of β-actin) of four independent experiments (± SEM) are shown. (*B*) Panc1-Luc cells were incubated with LODERs containing siGFP or siLuc or siG12D in the presence of Lipofectamine 2000. After 72 and 96 h, the cells were harvested and the luciferase level was assessed. The representative result of four different samples is shown. The results are shown ± SEM; *P* value was calculated relative to siGFP of the corresponding time point. **P* < 0.05; ***P* < 0.01 according to the Student *t* test.

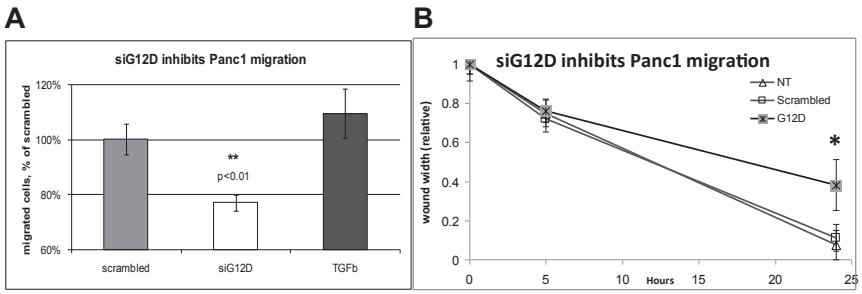

Fig. S4. siG12D reduces epithelial–mesenchymal transition in Panc1 cells. (*A*) siG12D reduces the migration ability of Panc1 cells. Panc1 cells were transfected with siG12D, nontargeting control (scrambled), or treated with TGFβ (positive control). The migration ability was assessed using a modified Boyden chamber assay. The presented percentage of migrated cells relative to untreated cells is presented. (*B*) Scratch assay: Panc1 cells were transfected with siG12D or nontargeting control (scrambled). The next day, cells were counted and reseeded to ∼90% confluency and the scratch assay was performed. The graph represents average wound width.

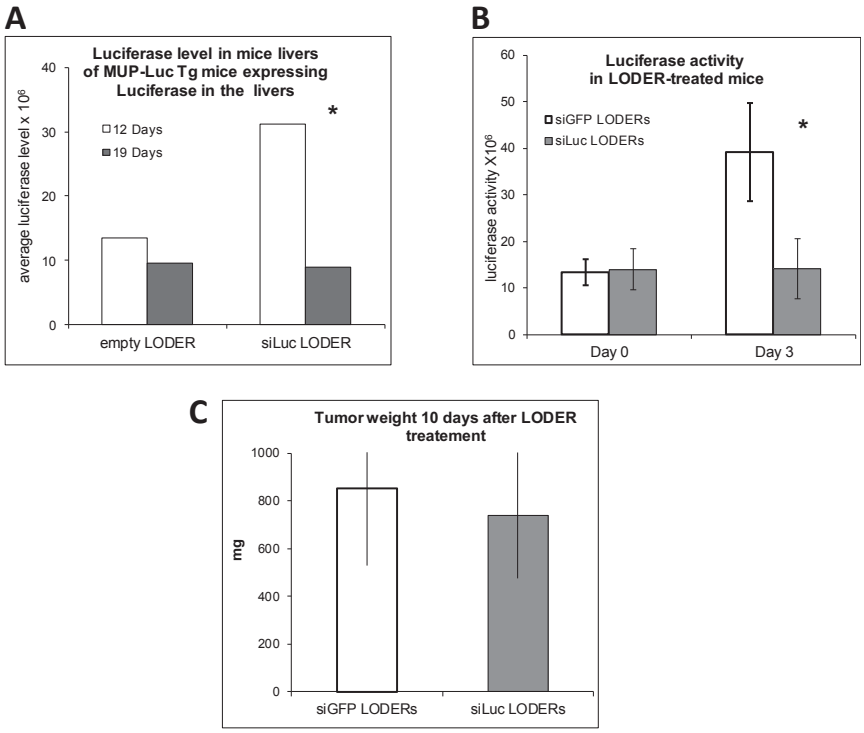

Fig. S5. The in vivo silencing effects of siLuc LODERs. (*A*) Luciferase activity in the livers of mice that stably express luciferase (Luc) in the liver after implantation of an empty or siLuc LODERs. (*B*) Luciferase activity in mice bearing s.c. syngeneic CT26-Luc tumors after intratumoral implantation of two siLuc or siGFP LODERs. (*C*)Tumor weight in the group treated with the siLuc LODERs in comparison with the siGFP LODERs group. *$P < 0.05$ according to the Student *t* test.

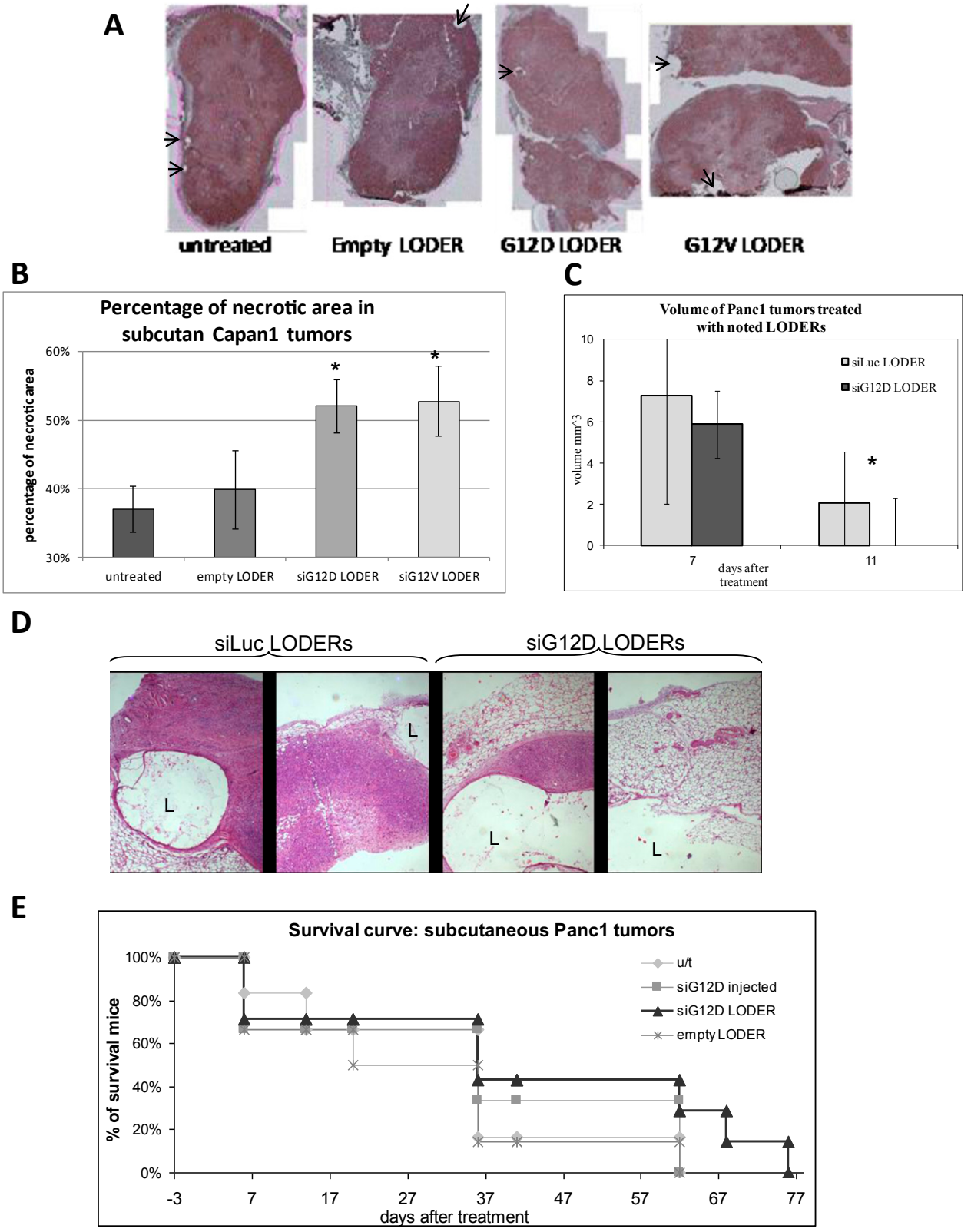

Fig. S6. Silencing of KRAS inhibited s.c. tumor growth of human pancreatic cancer cells. (*A* and *B*) CDC47 immunostaining of s.c. Capan-1 tumors from the different treatment groups: untreated or implanted with empty LODERs, siG12D LODER, or siG12V LODER. (*A*) Representative slide pictures; arrows point to the LODERs' location. (*B*) Calculation of percentage of necrotic area. The calculation was done using ImageJ program. (*C*) Tumor volume of Panc1 s.c. tumors after implantation of siLucs or siG12D LODERs. (*D*) Representative slide pictures of Panc1 s.c. tumors 20 d after implantation of siLuc- or siG12D LODERs. L, LODER location. (*E*) Survival curve of mice bearing Panc1 s.c. tumors from the different treatment groups: untreated, intraperitoneally injected with siG12D, or implanted with empty LODERs or siG12D LODERs $*P < 0.05$ according to the Student *t* test.

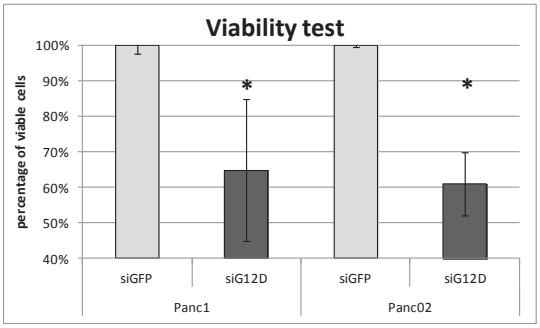

Fig. S7. The silencing effect of the siG12D in pancreatic cancer cells. Panc1 and PancO2 cells were transfected with siG12D or nontargeting control, siGFP, using Lipofectamine 2000. After 72 h, cell viability was assessed by methylene blue test. Shown is average percentage of viable cells (± SEM). *$P < 0.05$.

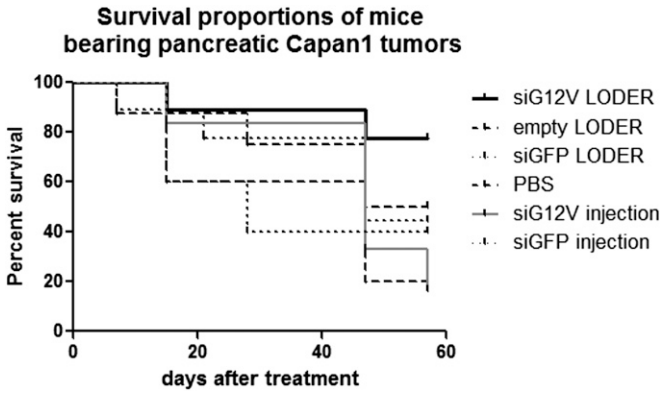

Fig. S8. siG12V LODERs inhibit growth of pancreatic tumors of human origin. Survival curve in mice bearing Capan1 pancreatic tumors from the different treatment groups: implanted with siG12V, empty, or siGFP LODERs intraperitoneally (i.p.) injected with PBS, siG12V, or siGFP.

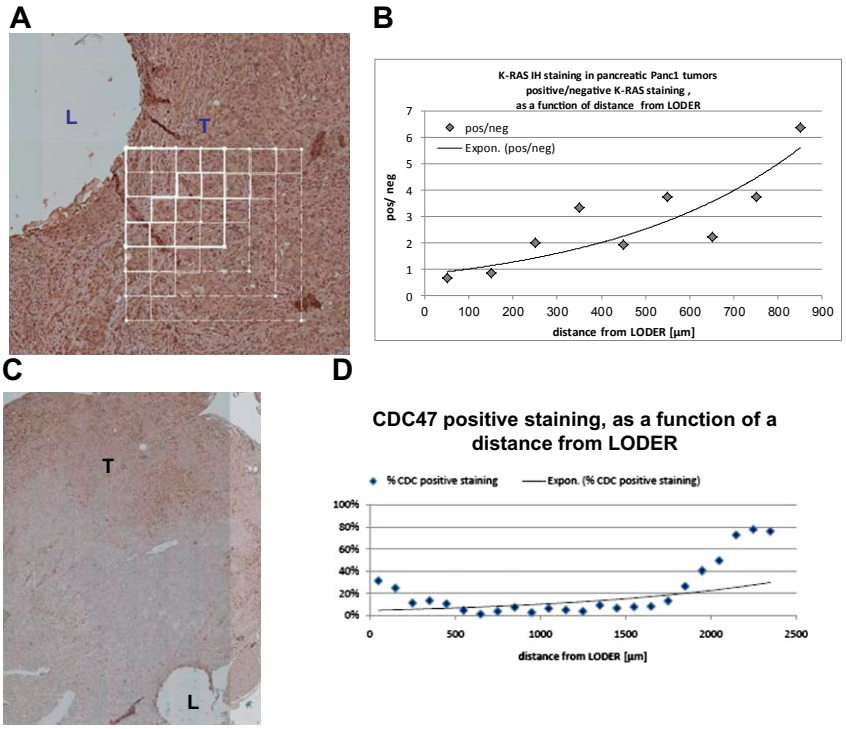

Fig. S9. siG12D LODER inhibits growth of pancreatic tumors of human origin. Immunostaining (IH) was performed on pancreatic tumors of human origin treated with the siG12D LODER, 24 d posttratment. (*A*) Representative pictures of KRAS IH. Shown squares of 0.1×0.1 mm^2 at growing distances from LODER are used for calculations. (*B*) Graph represents ratio of KRAS positively stained vs. unstained cells as a function of distance from LODER. (*C*) CDC47 IH. L, LODER place; T, tumor tissue. (*D*) Graphs represent percentage of CDC47 stained vs. unstained cells as a function of distance from LODER.