SUPPLEMENTAL METHODS

Animal Experiments and tumor samples. All animal experiments were conducted in accordance with institutional guidelines and approved protocols, as previously described [1, 2]. Briefly, tumor tissues resected from patients prior to any neoadjuvant radiation or chemotherapy were subsequently implanted into immunocompromised mice, to establish low passage tissue xenografts. These tumors were minced and serially implanted into greater number of nude mice to use for the *in vivo* analyses. Once tumors had reached a size of ~250mm^3 mice were randomly allocated to the respective treatment groups. In each group tumor size were measured weekly. A tumor size of 1cm^3 was considered the primary end point. Aqueous solution of gemcitabine (Lilly, Indianapolis, IN) was administered twice a week (125mg/kg i.p.) for 52 days. Chloroquine (Sigma-Aldrich, St. Louis, MO) was administered as PBS-based solution daily for three weeks (50mg/kg i.p.).

In vivo metastasis assay. Firefly luciferase expressing PDAC-354 cells were established by infecting cells with the CMV-Luciferase-RFP-TK Lentivector system from BioCat GmbH (Heidelberg, Germany). Cells were sorted for RFP expression with a FACS Influx instrument (BD, Heidelberg, Germany) and subsequently expanded *in vitro*. Then, 50µl of 5×10^5 sphere-derived 354-Luc cells were injected into the spleen of 6-8 week old NSG mice. Mice were randomized to control or chloroquine (50mg/kg) treatment until the end of the experiment. An IVIS Spectrum Imaging System (Caliper Life Sciences) was used for weekly *in vivo* luciferase imaging. Mice were anesthetized with isoflurane (2%) and injected intraperitoneally with 150 mg/kg of luciferin (Caliper Life Sciences) diluted 15 mg/mL in PBS. Sequential images were obtained after luciferin injection every 30 s (maximum light emission, □20 min after luciferin injection). Luciferase activity is in photons per second per square centimeter per steradian (p/s^{-1} cm^{-2} sr^{-1}). Living Image software (Caliper Life Sciences) was used for image analysis. For assessing final tumor burden explanted tissues were homogenized in a volume of 1X PBS equal to three times the weight of the tissue and subsequently lysed with passive lysis buffer (Promega,

Alcobendas, Spain). Following centrifugation at 9,000×g for 30min at 4°C, the supernatant was collected and firefly luciferase activity was measured using a Luciferase Assay System, according to the manufacturer's instructions (Promega). Total protein concentrations were determined using a BCA protein assay kit (Pierce).

Cells and cell cultures. *In vivo* expanded pieces of patient-derived cancer tissue were used to obtain primary cells for *in vitro* studies, as previously described [1, 2]. The identity of the primary cultures was tested by Short Tandem Repeat Profiling of the original xenografted tumors versus primary cells prior to the start of the experiments (University of Arizona, Genetics Core Facility, AZ). Cells were maintained in RPMI, 10%FBS, 100U/mlPen-Strep media (Invitrogen, Karlsruhe, Germany) for a maximum of 8 passages. To generate CSC-enriched spheres 10^3 cells/ml were seeded in ultra-low attachment plates (Corning B.V., Schiphol-Rijk, The Netherlands) in DMEM-F12 (Invitrogen) supplemented with B-27 (Gibco, Karlsruhe, Germany) and bFGF (PeproTech EC, London, UK), as described previously [2]. Human pancreatic cancer cell lines Panc1, BxPC3, and 8988T were cultured and maintained as previously described [1]. The selected concentration of aqueous solution of chloroquine (10μM; Sigma Aldrich) was based on the cytotoxicity analysis and administered daily (**Fig. S1**). After 7d control and treated spheres were collected and processed for RNA and protein isolation or dissociated to single cells for FACS analysis or to re-grow under non-adherent condition for the self-renewal studies.

Human primary PDAC-derived stromal cells were isolated and cultured as previously described [3]. The use of human material and isolation of primary cells were approved by the local ethics committee of the Technical University of Munich (Germany), and written informed consent was obtained from all patients. Immortalized human pancreatic stellate cells (RLT-PSCs) [4] and human foreskin fibroblasts (HFF-1) were maintained using 10%FBS, 100U/mlPen-Strep in RPMI or DMEM media. Sphere-conditioned media was collected after seven days of culture, centrifuged and filtered prior to incubation with PSCs or HFF-1 cells.

RNA preparation and RT-PCR. Total RNAs were extracted with TRIzol kit (Life Technologies, Alcobendas, Spain) according to the manufacturer's instructions. One microgram of total RNA was used for cDNA synthesis with SuperScript II reverse transcriptase (Life Technologies) and random hexamers. Quantitative real-time PCR was performed using SYBR Green PCR master mix (Qiagen, Barcelona, Spain), according to the manufacturer's instructions. Primers utilized in this study are depicted in **Table S1** or as previously published [2].

Western Blot Analysis. Total protein extracts were obtained by lysis with Laemmli buffer (65.8mM Tris-HCl, pH6.8, 2.1%SDS, 26.3%(w/v)glycerol, 0.01%bromophenol blue; 2xsolution) and 25-50μg of protein was separated by SDS/PAGE and transferred to nitrocellulose membranes that were probed with antibodies depicted in **Table S2.** After incubation with secondary peroxidase-conjugated goat anti-mouse or anti-rabbit Ig antibody (Sigma) the membranes were visualized by enhanced chemiluminescence (Amersham, Barcelona, Spain). Pierce Cell Surface Protein Isolation Kit (Thermo Fisher Scientific, Waltham, MA) was used according to manufacturer's instructions to obtain membrane fraction of the protein lysate. Loading amount was determined by silver staining using SilverQuest™ Silver Staining Kit (Life technologies). Stained gels were scanned and ImageJ was used to quantify the band signals for each sample.

Flow Cytometry. Cells derived from human primary cancer cells, or seven day old sphere cultures or from freshly isolated tumors were incubated with primary antibodies (or appropriate isotype-matched control antibodies) listed in **Table S2** for 30 min (on ice) and than processed by flow cytometry, using a FACS Canto II (Becton Dickenson, Heidelberg, Germany), and data were analyzed with FloJo 9.5.2 (Treestar, Ashland, OR). DAPI staining was used to exclude the dead cells. For the analysis of cell death cells were incubated with Annexin V fluorescein isothiocyanate (FITC) staining kit (Becton Dickenson) according to the manufacturers instructions. To analyze cell cycle cells were collected and fixed with 100% cold ethanol overnight at 4^{o}C and stained with DAPI. Analysis was performed the following day.

Migration and invasion assay. Migration and invasion assays were performed as previously described [5]. Briefly, 50,000 cells pretreated with chloroquine or AMD were transferred to the each Matrigel™-coated inserts (Becton Dickenson). Serum-free medium with or without recombinant human CXCL12, SHH, Nodal, or TGFb1 were added to the lower chamber. After 22h incubation at 37°C invaded cells were fixed in 4% PFA, stained with DAPI and counted.

Histology. Tumor samples were paraformaldehyde-fixed, paraffin-embedded, sectioned and processed for immunohistochemistry. Sections were incubated with primary antibody against pERK (9101, Cell Signaling), Cytokeratine 19 (CNIO Monoclonal Antibody Core Unit) and GLI1 (sc-20687, Santa Cruz Biotech, Santa Cruz, CA). Detection was made following incubation with appropriate anti-rabbit (pERK and Gli1) and anti-rat HRP-conjugated antibody (Cytokeratin 19) with DAB substrate (Vector Labs, Burlingame, CA). Nuclear counterstaining was performed using hematoxylin.

Immunofluorescence. Primary pancreatic cancer cells were seeded on glass coverslips and cultured overnight with 10μM/ml chloroquine. Following day cells were washed with cold PBS and fixed with 4% PFA (20min at 4°C). Fixative was removed by washing with cold PBS-TritonX 0.1% and samples were then blocked using 5% BSA in PBS-TritonX 0.1%. Incubation with primary antibodies against SMO (ab38686; Abcam) and acetylated tubulin (Sigma-Aldrich) was performed overnight at 4°C. Cells were then washed with cold PBS and incubated with Alexa-Fluor-conjugated secondary antibodies against mouse or rabbit (Invitrogen) at room temperature for 45min in the dark. Samples were mounted in Vectashield containing DAPI and analyzed using an SP5 confocal microscope (Leica, Heidelberg, Germany).

Plasmids and transfections. Plasmids used were as follows: LC3II-GFP reporter plasmid (a kind gift of Dr. M. Soengas), Gli-Luc reporter plasmid and GLI1 expression plasmid. Plasmid containing *GLI1* encoding cDNA was obtained from I.M.A.G.E. Consortium CloneID 3531657 [6]. The protein-encoding domain was amplified by PCR introducing restriction sites and cloned into

the expression vector pCDNA3.1, confirmed by sequencing. Primary pancreatic cancer cells were transfected with lentiviral viruses as previously described [2]. Activity of the reporter constructs was performed using the Opera system (GFP) or Dual-Luciferase® Reporter Assay System (Promega, Alcobendas, Spain) according to manufacturer's instructions.

Statistical Analysis. Results for continuous variables are expressed as as mean ± standard deviation (S.D.) unless stated otherwise. Overall comparison of continuous variables was performed with the Kruskal-Wallis test followed by post hoc pairwise comparison using the Mann-Whitney U test. Survival was compared using a Log Rank test. P values < 0.05 were considered statistically significant. All analyses were performed with SPSS 19 (SPSS Inc., Chicago, IL).

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