Supplemental methods

Transduction of human PDA-derived cells with lentiviral vectors.

$5x10^4$ Capan-2 or Mia PaCa-2 PDA-derived cells were plated in 48-well clusters and transduced overnight with lentiviral vectors encoding for miR-21 antisenses or Lucia, respectively at the multiplicity of infection of 5 in 250µl of transduction medium (complete culture medium + 4µg/ml Protamine Choay, Sanofi Aventis France). Multiplicity of infection (MOI) is the number of lentiviral particles added per cells during transduction. In addition, Capan-2 cells transduced with LV(a/miR-21) were selected using puromycin. Capan-2 a/miR-21 and Mia PaCa-2 Lucia cells were cloned by serial dilution. Efficient knockdown of miR-21 was verified by qRTPCR and Lucia production was measured in 5µl of culture medium using coelenterazine (0.5µM) as a substrate.

Orthotopic implantation of Mia PaCa-2 cells.

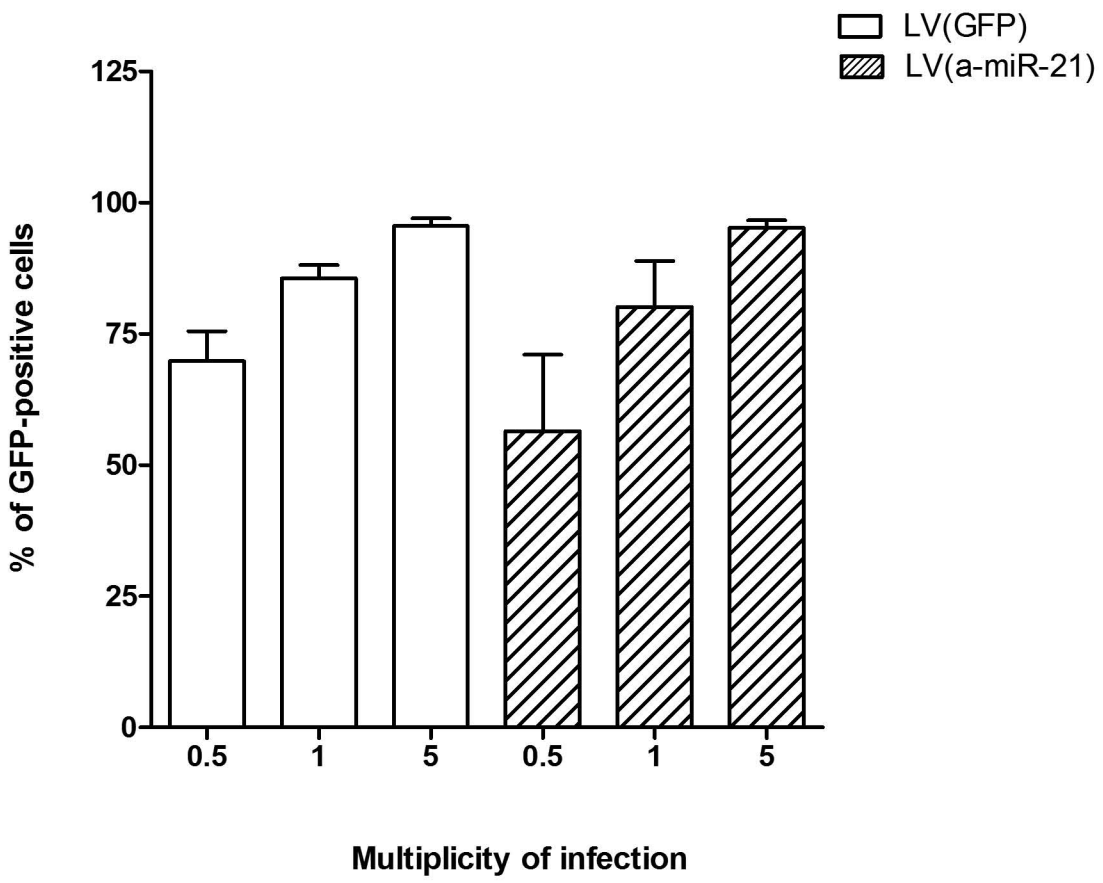
The protocol was approved by the Anexplo UMS006 institutional animal use committee. Mia PaCa-2 Lucia cells were harvested from subconfluent cultures, washed twice in serum-free medium and resuspended in PBS. Sixt to eight weeks old SCID CB 17 mice were anesthetized with isofluoran, a small left abdominal flank incision was made, and 20×10^6 cells in 50µL PBS were injected into the subcapsular region of the pancreas using a 27-gauge needle and a calibrated push button–controlled dispensing device (Hamilton Syringe). To prevent leakage, a cotton swab was held cautiously for 1 min over the site of injection. The abdominal wound was closed in one layer. Sixty days later, tumors were removed, digested by collagenase and cultured *in vitro* to give Mia PaCa-2 Lucia F1 cells. Next, $1x10e^6$ Mia PaCa-2 Lucia F1 cells were collected in 50µl sterile PBS and implanted into the pancreas of SCID CB 17 mice as previously described. Blood samples were collected every 2 to 3 days during the course of the experiment.

Vector production.

Control lentiviral plasmids encoding for copGFP (pCDH-CMV-MCS-EF1-copGFP) and lentiviral plasmids targeting miR-21 (pmiRZip miR-21) were purchased from System

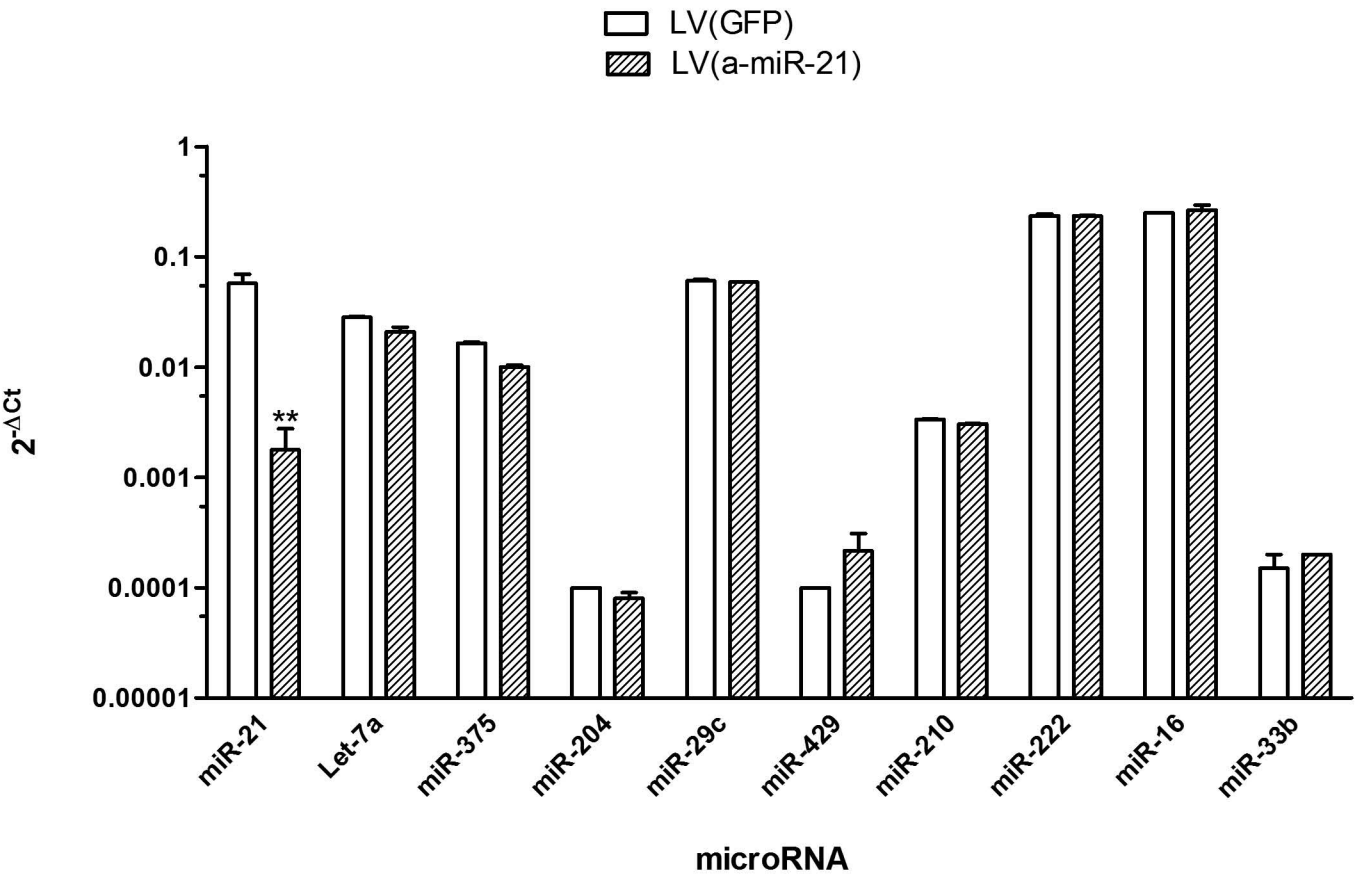
Biosciences. pmiRZip miR-21 encodes for two tandem antisenses targeting miR-21. Lucia encoding lentiviral plasmid was from Invivogen. Lentiviral particles were produced in a BSL-3 facility (vectorology platform, INSERM U1037, Toulouse) using Lenti-SmartINT kit (Invivogen) following the manufacturer's recommendations. For *in vitro* use, lentiviral particles were concentrated using Vivaspin filter devices (Vivaspin). For *in vivo* use, lentiviral vectors were concentrated by ultracentrifugation and stored in Phosphate Buffer Saline (PBS) at -80°C. The viral titers were determined on HT1080 cells and expressed in transduction unit/ml (TU/ml) as described elsewhere [10]. Vector concentrations were quantified by p24 ELISA (Ingen). All batches were checked replicative virus-free.

FIGURE S1



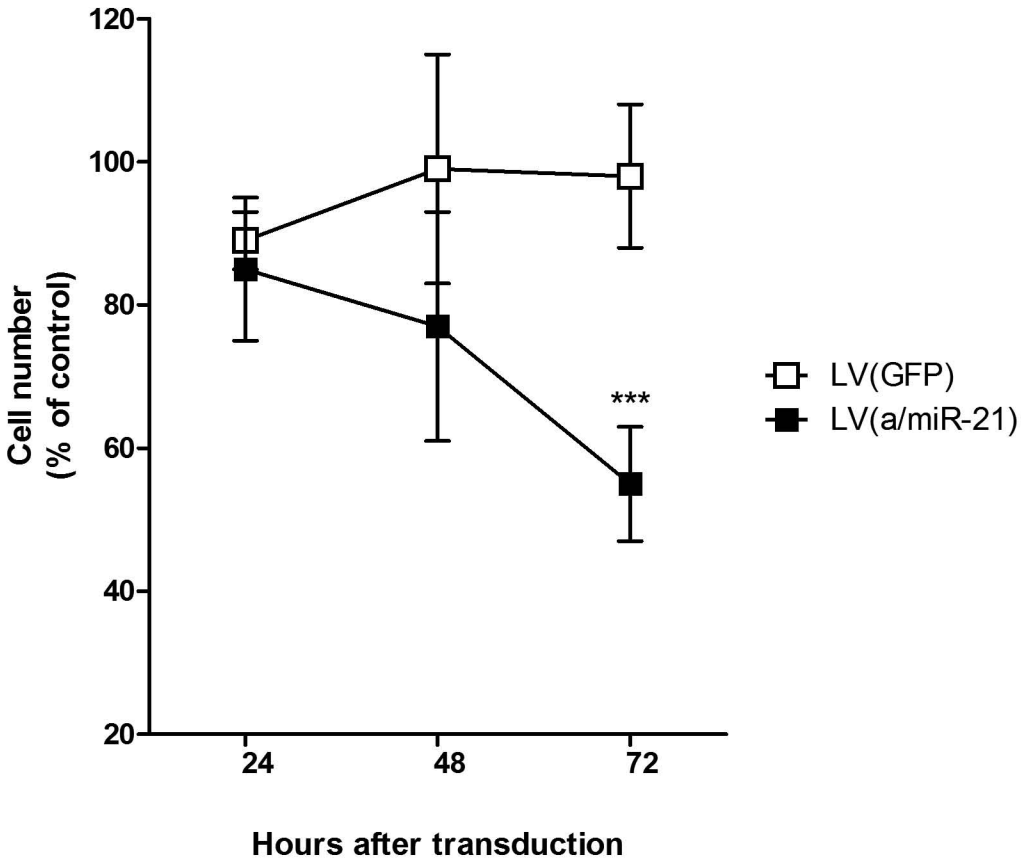
Lentiviral vectors transduce human PDA-derived cell lines with high efficacy. Mia PaCa-2cells were transduced with LV(GFP) or LV(a/miR-21) at the indicated MOI. GFP-positive cells were quantified by FACS 72h later. Results are mean +/- s.e.m. of 3 independant experiments done in triplicate with different batches of vector.

FIGURE S2



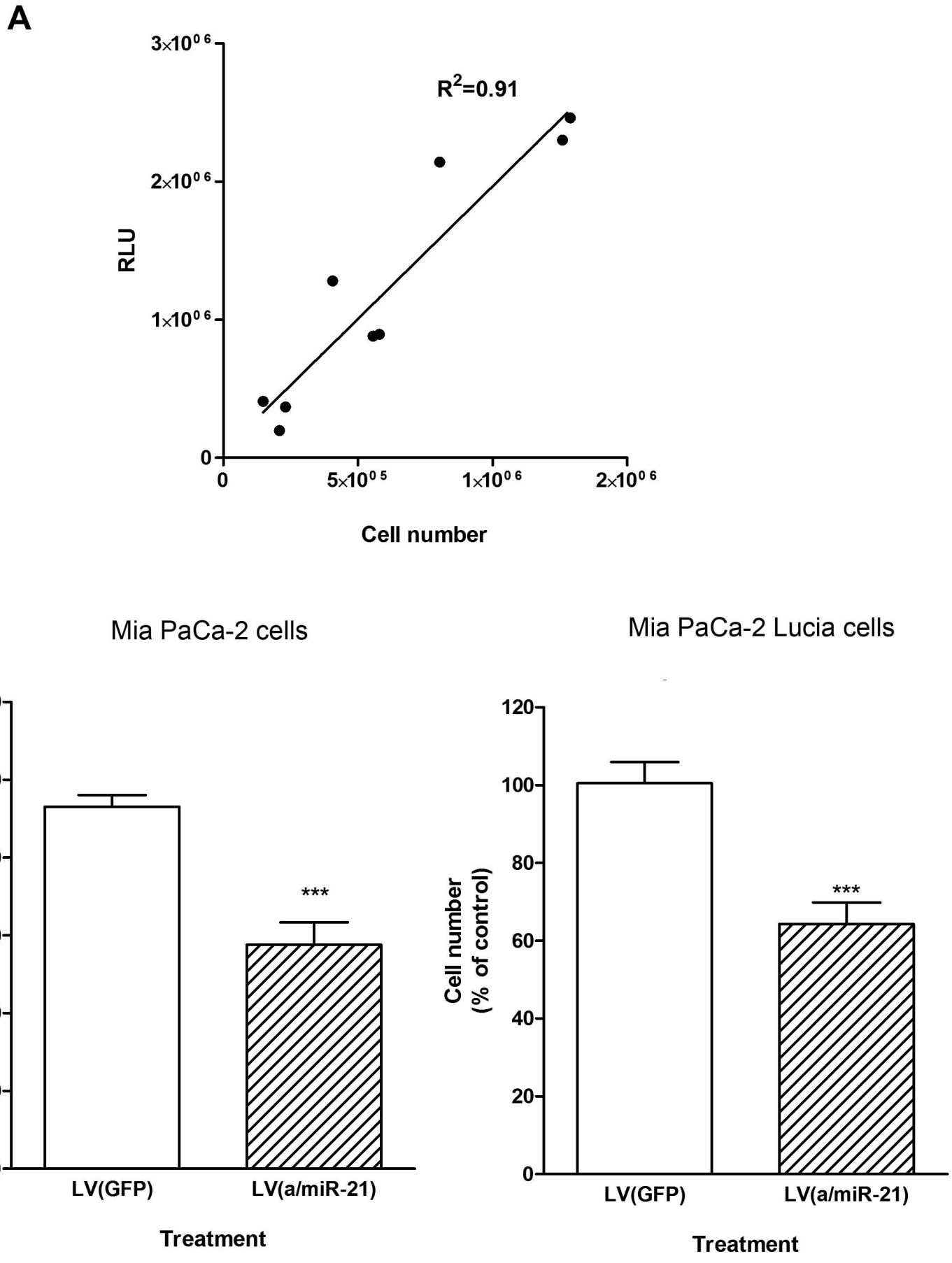
Targeting miR-21 doesn't impact on other cellular miRNA expression. Mia PaCa-2 cells were transduced with LV(GFP) or LV(a/miR-21) at MOI=5. miRNA expression was measured by q(RT)PCR in the transduced cells. Results are mean +/- s.e.m. of 3 independant experiments done in duplicate (**: $p<0.01$)

FIGURE S3



Kinetics of inhibition of cell proliferation following targeting of miR-21 in PDA-derived cells. Mia PaCa-2 cells were transduced with LV(GFP) or LV(a/miR-21) at MOI=5. Cell counting was performed at day 1, 2 and 3 following transduction. Results are mean+/- s.e.m. of 3 independant experiments done in triplicate (***: $p<0.005$).

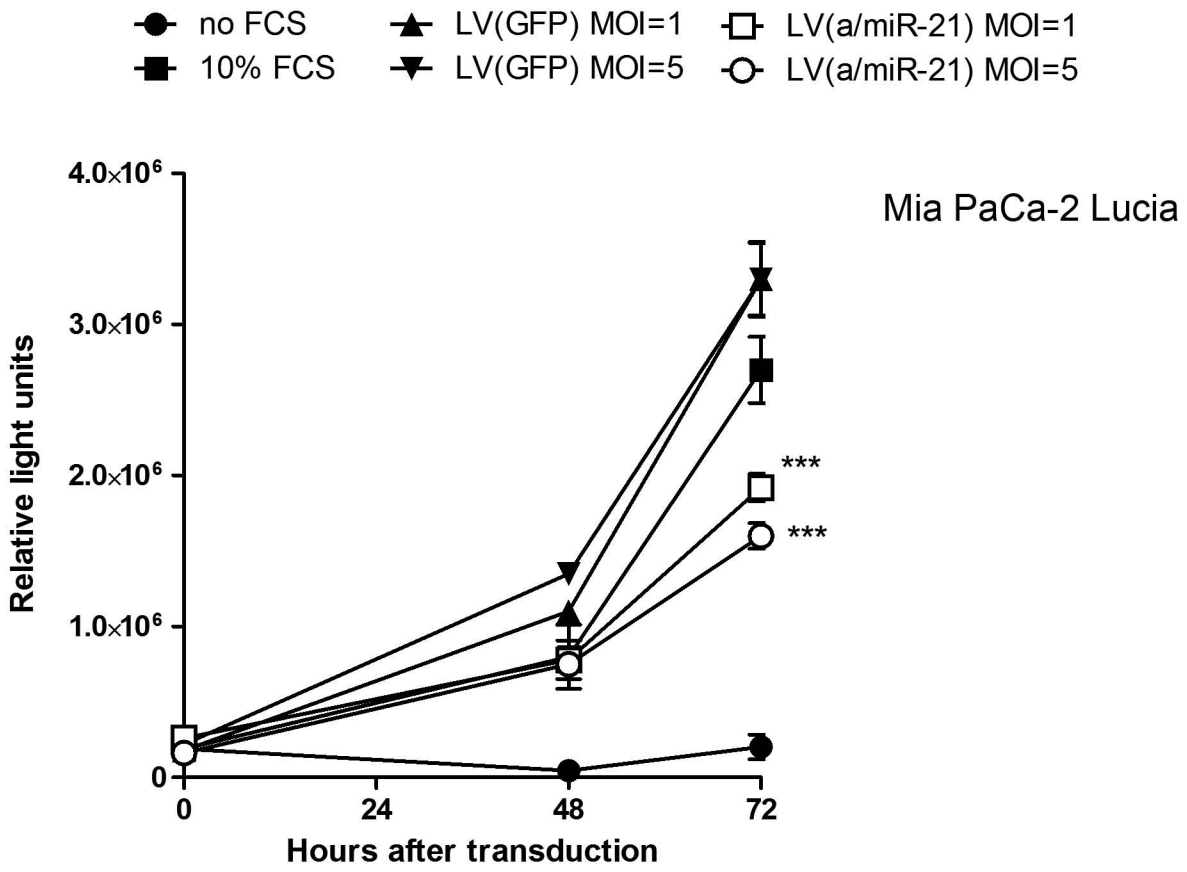
FIGURE S4



Lucia expression correlates with tumor cell proliferation and response to treatment.
A. Mia PaCa-2 Lucia cells were seeded in triplicate in 6-well clusters. Seventy-two hours later, Lucia was assayedin cell supernatants and cells were counted. The experiment was repeated 3 times. R2 was calculated using GraphPad prism software. **B.** Mia PaCa-2 expressing or not Lucia were transduced with LV(a/miR-21). Control cells received LV(GFP). Proliferation was measured by cell counting 72h later.Results are mean+/- s.e.m. of 3 independant experiments done in triplicate. ***: $p<0.005$.

FIGURE S4

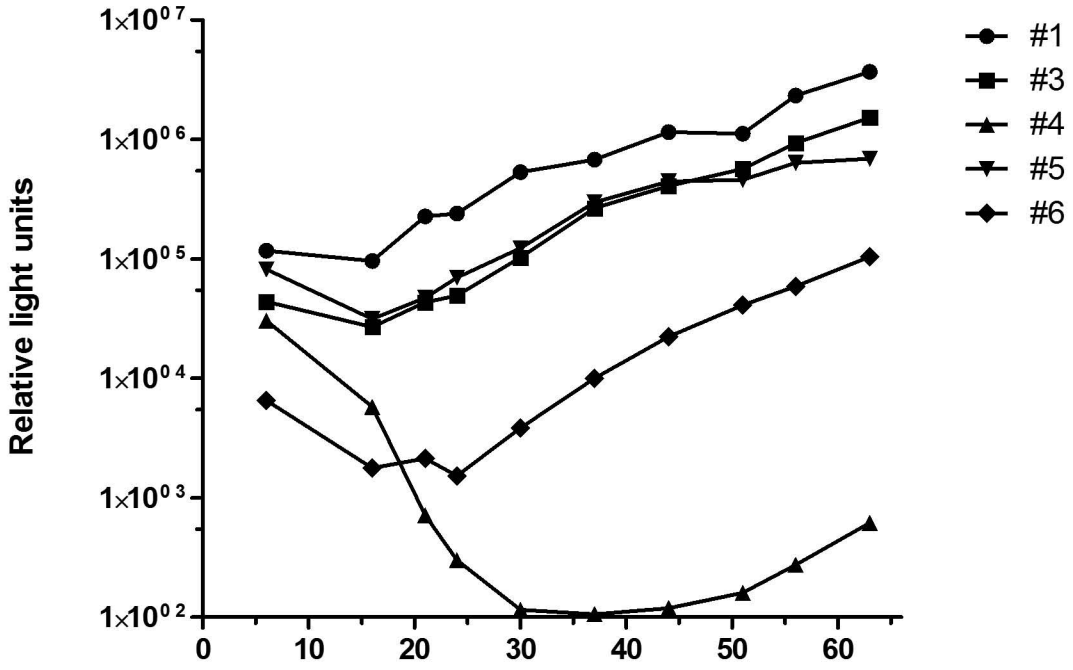
C



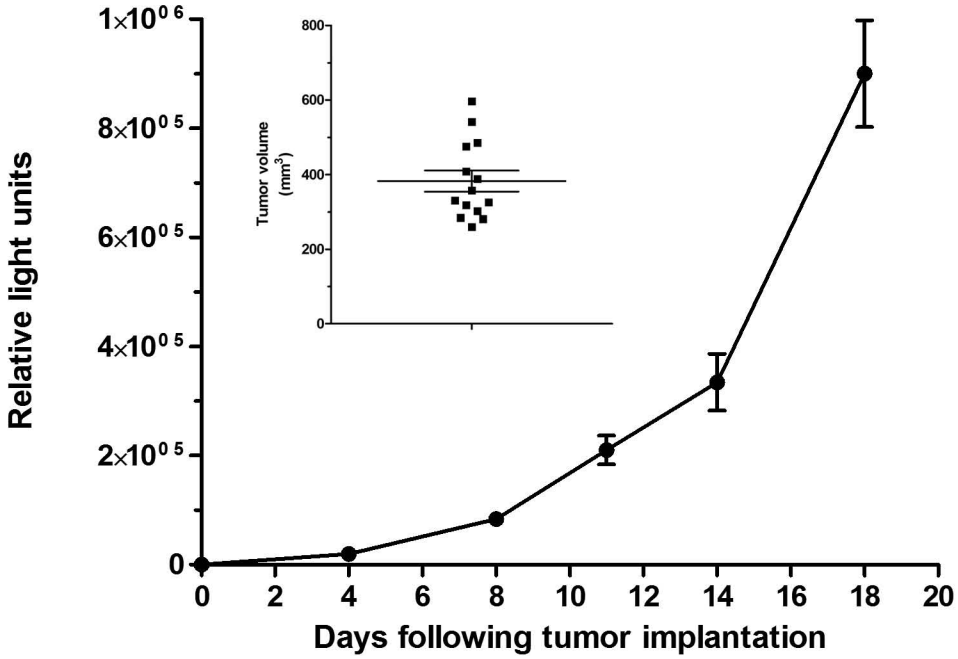
C. Mia PaCa-2 Lucia cells were transduced by LV(a/miR-21) Control cells received LV(GFP). Lucia was assayed in the cell supernatant 48 and 72h following transduction. Results are mean+/- s.e.m. of 3 independant experiments done in triplicate. ***: $p<0.005$.

FIGURE S5

A



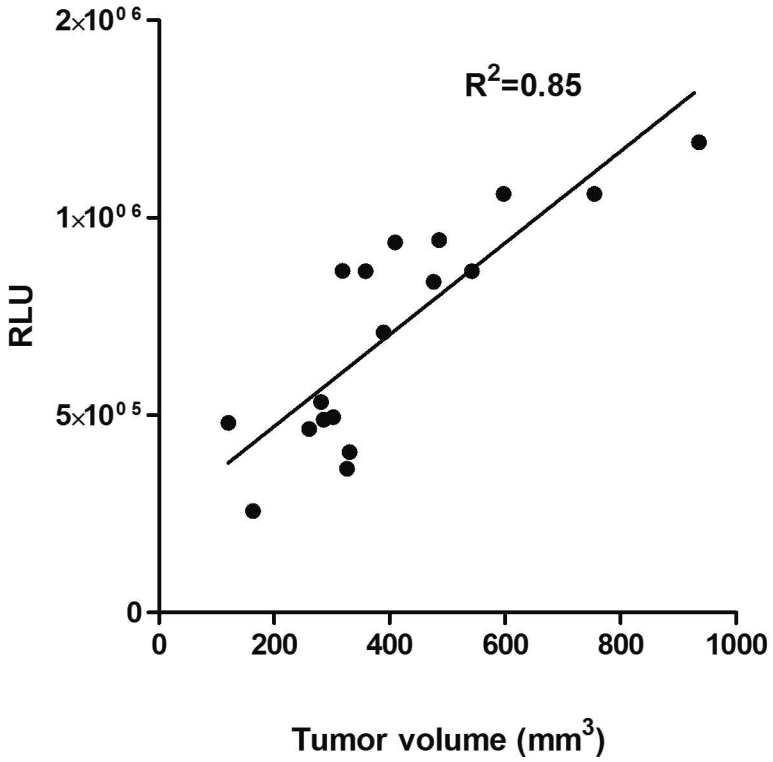
B



A. Mia PaCa-2 Lucia cells were injected in the pancreas of SCID mice. Lucia was assayed in the mice serum up to 63 days following tumor induction. **B.** Mia PaCa-2 Lucia F1 cells were injected in the pancreas of SCID mice.Lucia was assayed in the mice serum up to 18 days following tumor induction. Results are mean +/- sem of Lucia expression in the serum of 14 mice bearing tumors. Insert: Tumor volume was measured 18 days following PDA-derived cell injection.

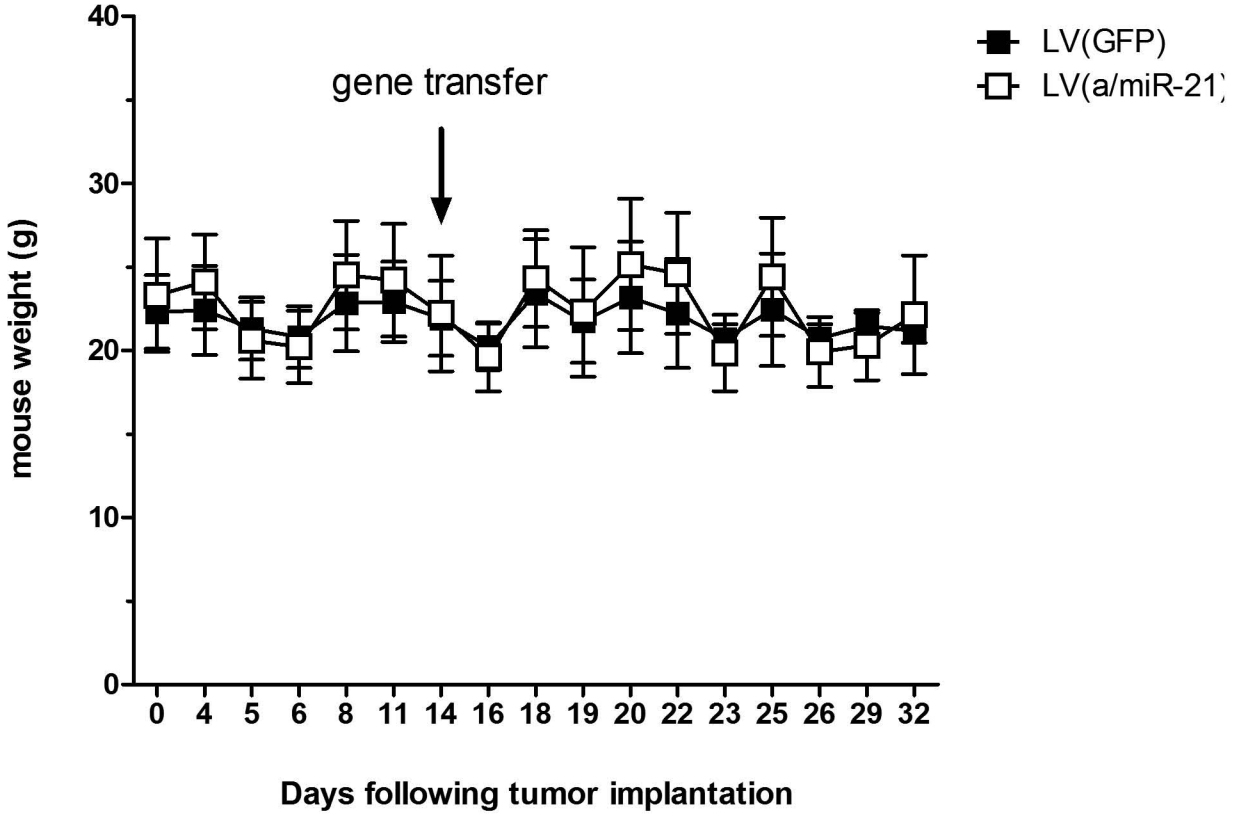
FIGURE S5

C



C. Correlation of Lucia levels in serum with the tumor volume, 18 days following injection of Mia PaCa-2 cells in the pancreas of nude mice (n=18). R2 was calculated using Graphpad Prism software.

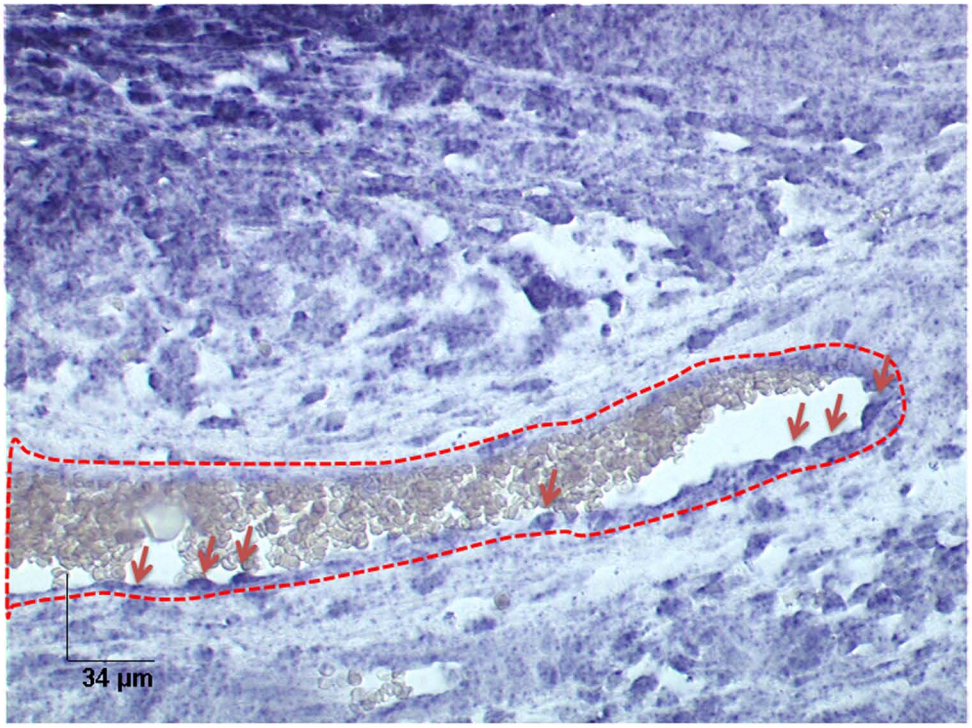
FIGURE S6



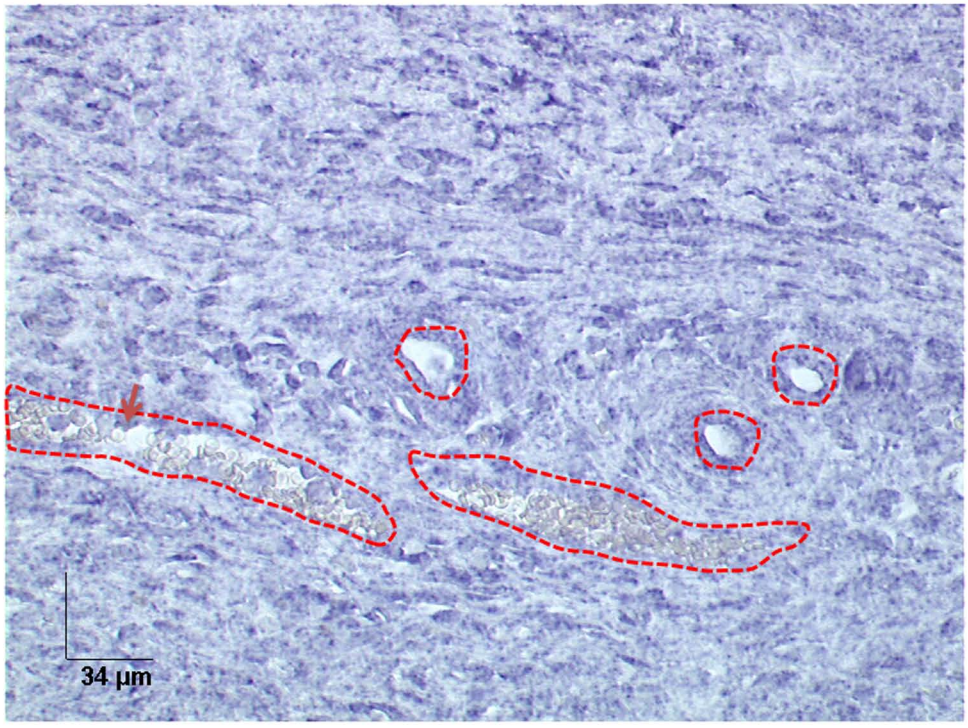
Tumor growth and intrapancreatic injection of lentiviral vectors are well tolerated in SCID mice. Exponentially growing Mia PaCa-2 Lucia F1 were inoculated in the pancreas of SCID mice. Fourteen days later, tumors were transduced with either 150ng of p24 of LV(GFP) or LV(a/miR-21). Mouse weight was recorded during the course of the experiment. Ten mice were used per group.

FIGURE S7

A



B



miR-21 is down-regulated in peripheral blood vessels following LV(a/miR-21) gene transfer. Pancreatic tissue was harvested 12 days following LV(GFP) (A) or LV(a/miR-21) (B) gene transfer for analysis of miR-21 expression by *in situ* hybridization. Results are representative of 5 different high power fields from 3 different tumors for each group. Blood vessels are highlited in red. Endothelial cells positive for miR-21 are indicated by a red arrow

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