Supplemental Data

uPAR Messenger RNA Determination

RNA was extracted from semiconfluent cell cultures, from BxPC-3 xenografts, and from PANC-1 liver metastases using the RNeasy Mini RNA Extraction Kit (Qiagen). One microgram of total RNA was reverse-transcribed using Moloney Murine Leukemia Virus reverse transcriptase (Ambion, Austin, TX) and 1/10 of this reaction was linearly amplified for 23 cycles (cell lines) or 28 cycles (tumor samples) after denaturation (30 seconds at 95°C), annealing (30 seconds at 60°C), and extension (30 seconds at 72°C) in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, London, UK). The following primers were used: forward 5′ CAGGACCTCTGCAGGACCAC 3′, reverse 5′ CCTTGCAGCTGTAACACTGG 3′. Total RNA was normalized using QuantumRNA 18S Internal Standards at a 1:4 ratio (Ambion).

Biodistribution Studies

BALB/c nude male mice (Charles River France, Lyon, France) received 2×10^{10} vp of AdCMVGFPLuc or AduPARLuc or saline solution through tail vein injection in a final volume of 200 µl. At days 3, 5, 11, 20, 31 and 50 after adenoviral transduction, luciferase activity was visualized and quantified using an *in vivo* bioluminescent imaging system (IVIS; Xenogen). At day 50, immediately after imaging, mice were killed and organs were removed and frozen. When stated, frozen tissues were mechanically homogenized, 100 mg was used for protein extraction using the Cell Culture Lysis Reagent (Promega) for 15 minutes at 25°C, and 10 µl was assayed for luciferase activity.

Metastasis Model by Intrasplenic Injection of Tumor Cells

Metastatic pancreatic adenocarcinoma animal models were generated following the protocol previously described [1]. Briefly, BALB/c nude mice were anesthetized with isoflurane and placed in the right lateral decubitus. Thereafter, a left subcostal 1-cm incision was made, and 4×10^6 PANC-1 or PANC-1-Luc cells in 50-µl saline solution were injected into the spleen with a 29-G needle. To maintain hemostasis and prevent leakage of tumor cells outside the splenic capsule, a cotton-tipped applicator was applied to the puncture site. After surgery, mice were monitored daily for health and survival.

References

[1] Tseng JF, Farnebo FA, Kisker O, Becker CM, Kuo CJ, Folkman J, and Mulligan RC (2002). Adenovirus-mediated delivery of a soluble form of the VEGF receptor Flk1 delays the growth of murine and human pancreatic adenocarcinoma in mice. *Surgery* **132**, 857–865.

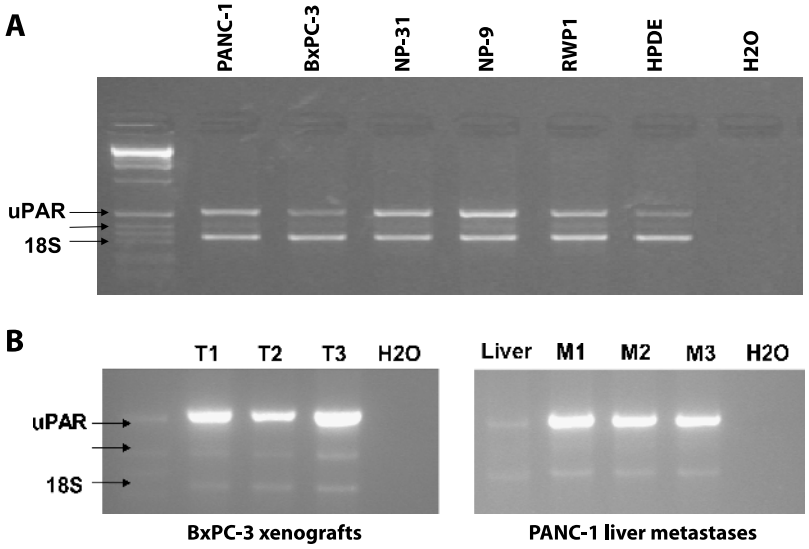

Figure W1. uPAR expression in pancreatic cancer cells, normal cells and tumors. We have analyzed, by semiquantitative RT-PCR analysis, the uPAR expression in five pancreatic cancer cell lines and in normal pancreatic ductal epithelium cell HPDE (A) and in two tumor models (B). Two different transcripts were detected corresponding to the entire form (534-bp fragment) or to one variant lacking one of the L6/uPAR/α-neurotoxin domains of the receptor (400-bp fragment).

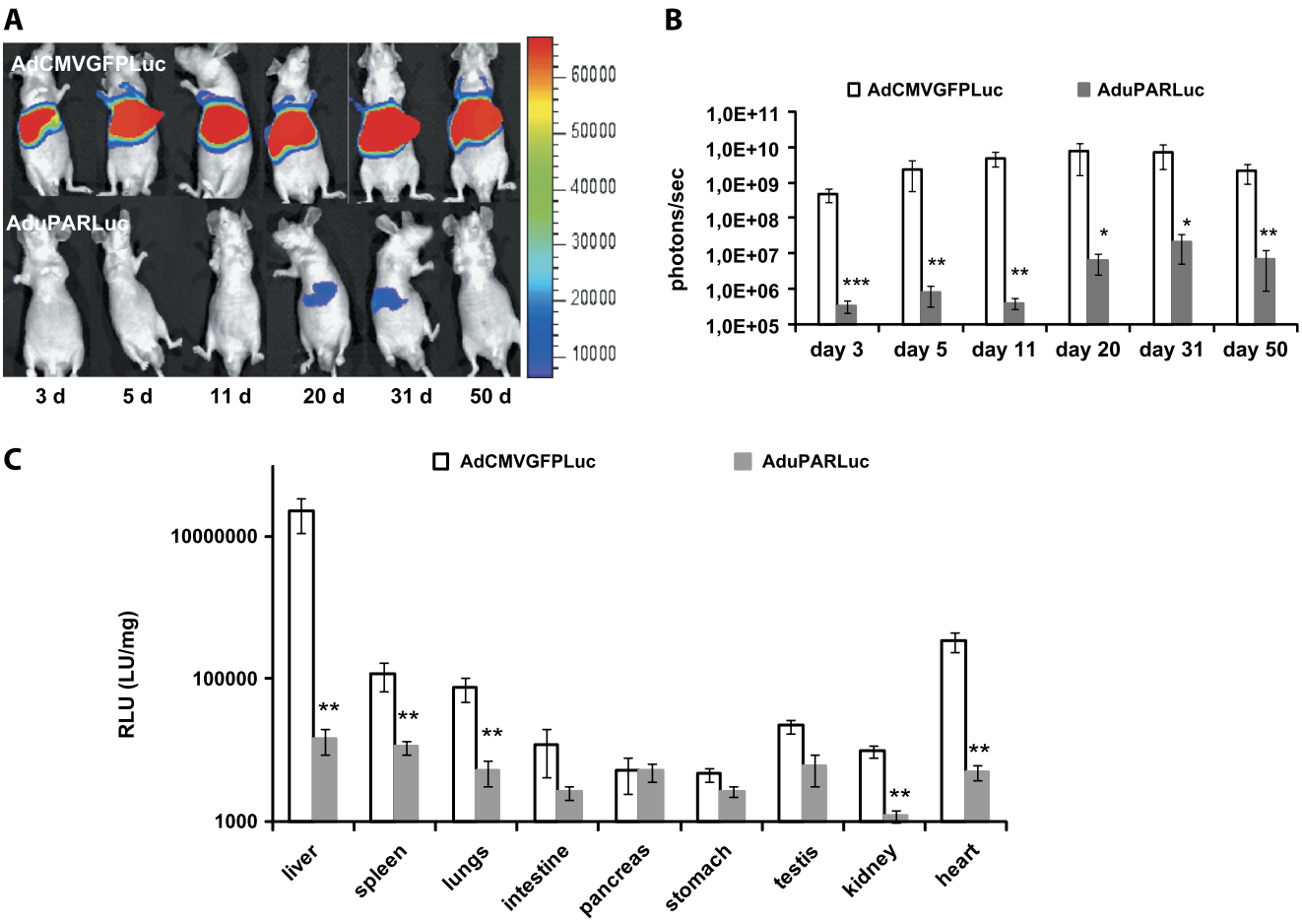

Figure W2. AduPARLuc biodistribution studies. BALB/c nude mice were injected IV with either saline solution ($n = 3$) or 2×10^{10} vp of AdCMVGFPLuc ($n = 4$) or 2×10^{10} vp of AduPARLuc ($n = 5$). Bioluminescent activity was measured in anesthetized animals at days 3, 5, 11, 20, 31, and 50 after viral injection. (A) Representative images of bioluminescent emission. (B) Quantification of bioluminescence emission was performed by measuring the total amount of emitted light captured by the camera. Results are expressed as photons per second. Values are represented as mean ± SEM. ***$P < .001$, **$P < .01$, *$P < .05$. (C) At day 50, animals were killed and organs were collected. Luciferase activity from tissue extracts was determined and normalized to total protein. Luciferase activity from the saline group was considered as the background and subtracted from the viral groups. Results are expressed as light units per milligram of tissue (LU/mg). Values are represented as mean ± SEM. **$P < .01$.

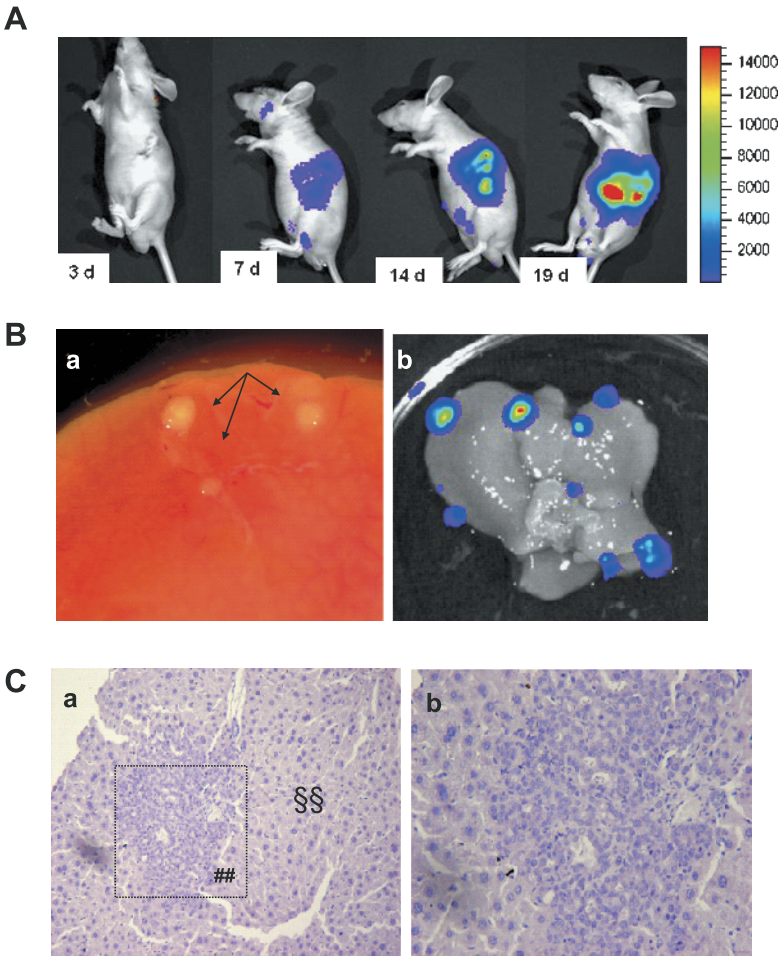

Figure W3. Liver metastases model. Liver metastases were generated by intrasplenic injection of PANC-1-Luc cells into BALB/c nude mice. Luciferase activity was monitored in live animals. At day 19 after tumor implantation, after bioluminescent acquisition, animals were killed, and liver was collected and analyzed for luciferase expression and metastases formation. (A) Representative images of liver. (a) Light image. Original magnification, ×0.8. (b) Bioluminescent image. (B) Histologic analysis of liver tissue by hematoxylin and eosin staining. (a) [§§]Liver area, [##]tumoral area. Original magnification, ×10. (b) Original magnification, ×20.

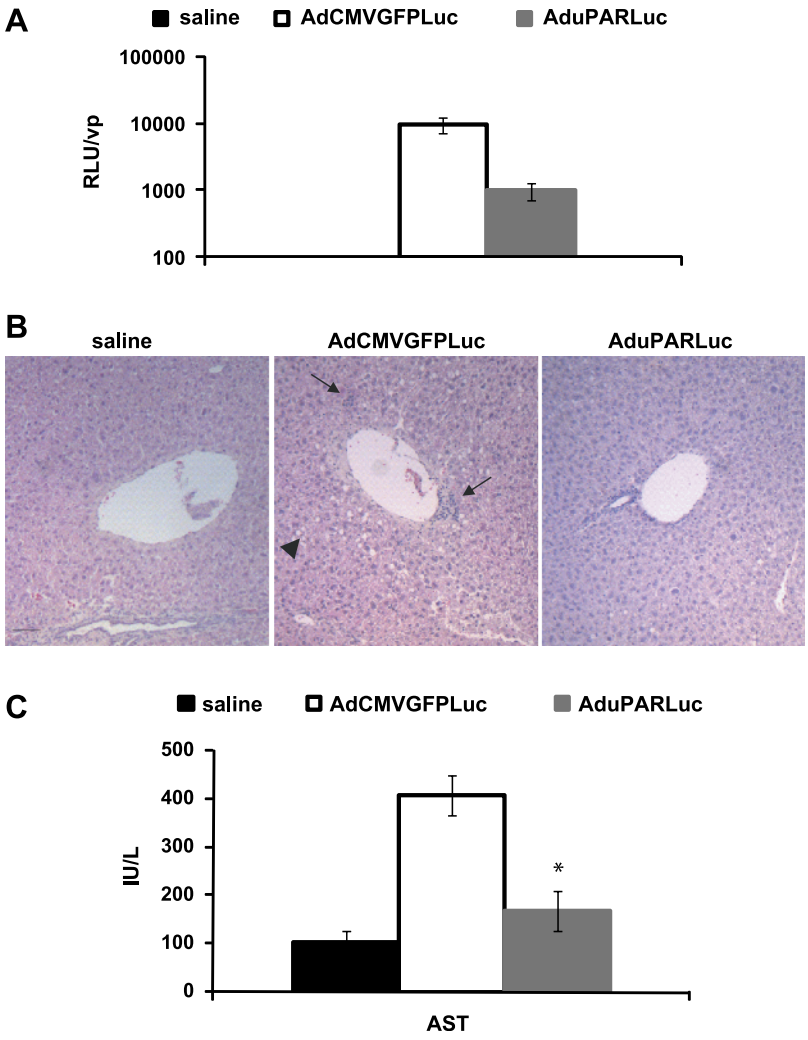

Figure W4. AduPARLuc toxicity studies. BALB/c mice were injected through the tail vein with either saline solution ($n = 4$) or 2×10^{10} vp of AdCMVGFPLuc ($n = 4$) or 2×10^{10} vp of AduPARLuc ($n = 4$). Five days later, blood samples were collected. Next, animals were killed, and the liver tissue was excised. Liver portions were either frozen for luciferase or viral DNA determination, or fixed and embedded in paraffin. (A) Luciferase activity from the liver extracts. Results were normalized to the number of viral particles per milligram of tissue determined by real-time PCR. Results are expressed as light units per milligram (RLU) relative to the number of viral particles (RLU/vp). Values are shown as mean ± SEM. (B) Hematoxylin and eosin staining of liver tissue. Lymphocyte infiltrates (arrows) and necrotic hepatocytes (arrowhead) were only detected in the AdCMVGFPLuc-injected mice. Scale bar, 0.5 mm. (C) AST determination. AST values expressed as international units (IU) per liter. *$P = .02$.