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Supplemental Information

Novel Non-integrating DNA Nano-S/MAR Vectors Restore Gene Function in Isogenic Patient-Derived Pancreatic Tumor Models

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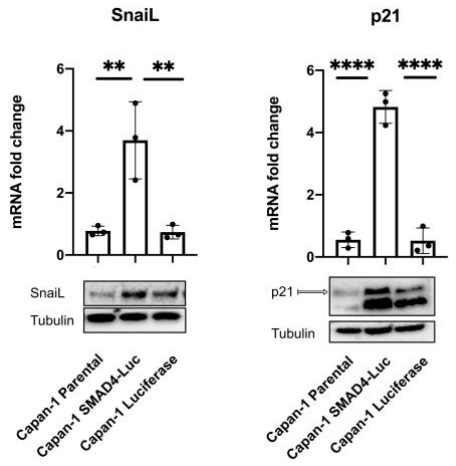
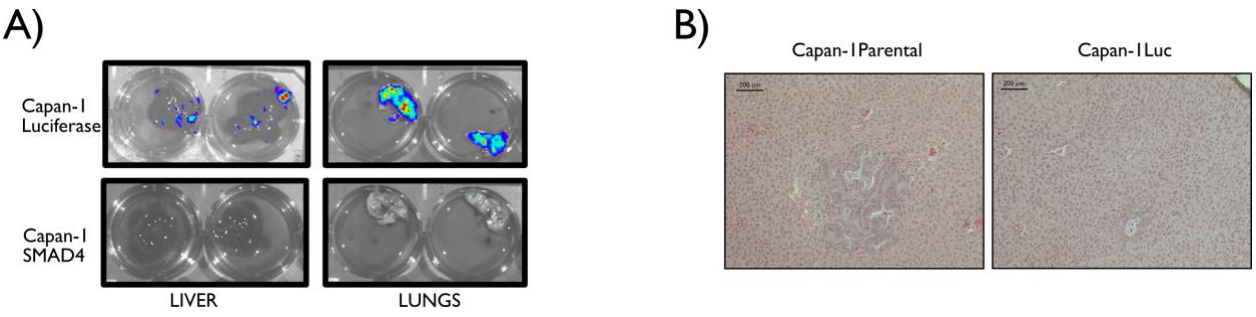
Supplementary Figure 1



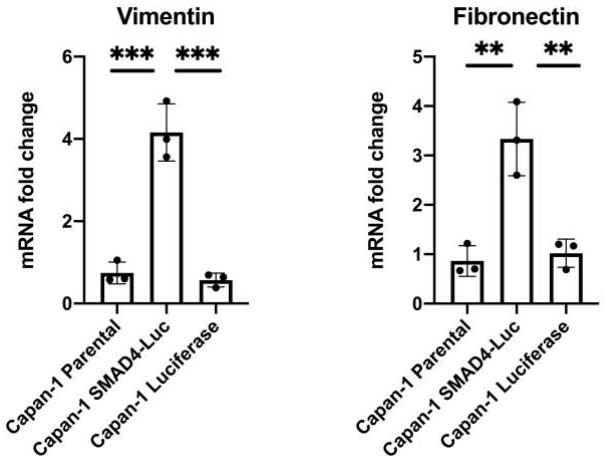
Supplementary Figure 1. Quantitative Real Time PCR analysis of SnaiL and p21 in Capan-1. The expression of SnaiL and p21 was addressed in genetically modified Capan-1 with SMAD4 and Luciferase, in comparison to parental unmodified cells through qRT-PCR and Western Blot.

Supplementary Figure 2



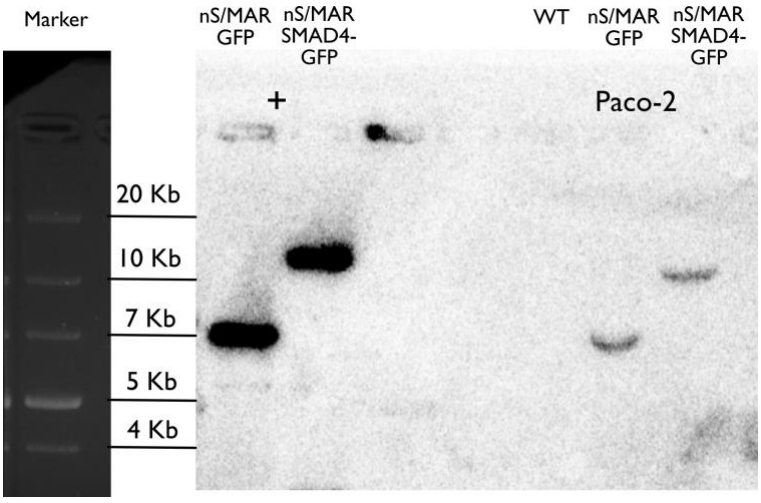
Supplementary Figure 2. Bioluminescent detection of metastasis. (A) The formation of metastasis in the liver and in the lungs of mice injected with Capan-1 labelled with the reporter gene Luciferase were detected through Bio Luminescent Imaging (BLI) imaging and (B) their morphology was assessed through H&E staining.

Supplementary Figure 3



Supplementary Figure 3. Analysis of EMT markers. The expression of Vimentin and Fibronectin in modified and unmodified Capan-1 cells was investigated through qRT-PCR.

Supplementary Figure 4

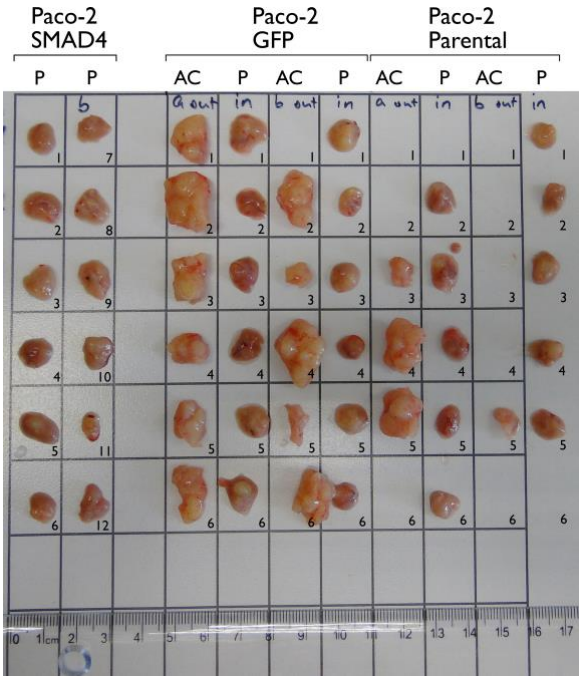


Supplementary Figure 4. Southern Blot analysis of PACO2 cells modified with Nano-S/MAR vectors.

The molecular integrity and the episomal maintenance of the two Nano-S/MAR constructs were evaluated by Southern Blot analysis. Control Nano-Vectors (first two lanes) and the total DNA

from the modified PACO2 cells was digested with the restriction enzyme BamHI and separated on a 1 % agarose gel before being transferred on a nylon membrane. The GFP reporter gene was used to generate the radioactive probe. The plasmid isolated via total DNA extraction from established cells showed an identical size to their respective reference controls. The DNA molecular weight marker is shown as a reference.

Supplementary Figure 5



Supplementary Figure 5. PACO2 cells macroscopic evaluation of tumour growth.

PACO2 Parental and GFP cells form tumours in the pancreas (P) and in the abdominal cavity (AC) of the injected mice. Paco-2 SMAD4 cells did not form clear tumour masses in the injection site and abdominal cavity.

Supplementary Figure 6

A) SMAD4 open frame cDNA

ATGGACAATATGTCTATTACGAATACACCAACAAGTAATGATGCCTGTCTGAGCATTGTGCATAGTTTGA
TGTGCCATAGACAAGGTGGAGAGAGTGAAACATTTGCAAAAAGAGCAATTGAAAGTTTGGTAAAGAAG
CTGAAGGAGAAAAAAGATGAATTGGATTCTTTAATAACAGCTATAACTACAAATGGAGCTCATCCTAGT
AAATGTGTTACCATACAGAGAACATTGGATGGGAGGCTTCAGGTGGCTGGTCGGAAAGGATTTCCTCAT
GTGATCTATGCCCGTCTCTGGAGGTGGCCTGATCTTCACAAAAATGAACTAAAACATGTTAAATATTGTC
AGTATGCGTTTGACTTAAAATGTGATAGTGTCTGTGTGAATCCATATCACTACGAACGAGTTGTATCACC
TGGAATTGATCTCTCAGGATTAACACTGCAGAGTAATGCTCCATCAAGTATGATGGTGAAGGATGAATA
TGTGCATGACTTTGAGGGACAGCCATCGTTGTCCACTGAAGGACATTCAATTCAAACCATCCAGCATCCA
CCAAGTAATCGTGCATCGACAGAGACATACAGCACCCCAGCTCTGTTAGCCCCATCTGAGTCTAATGCTA
CCAGCACTGCCAACTTTCCCAACATTCCTGTGGCTTCCACAAGTCAGCCTGCCAGTATACTGGGGGGCAG
CCATAGTGAAGGACTGTTGCAGATAGCATCAGGGCCTCAGCCAGGACAGCAGCAGAATGGATTTACTG
GTCAGCCAGCTACTTACCATCATAACAGCACTACCACCTGGACTGGAAGTAGGACTGCACCATACACACC
TAATTTGCCTCACCACCAAAACGGCCATCTTCAGCACCACCCGCCTATGCCGCCCCATCCCGGACATTACT
GGCCTGTTCACAATGAGCTTGCATTCCAGCCTCCCATTTCCAATCATCCTGCTCCTGAGTATTGGTGTTCC
ATTGCTTACTTTGAAATGGATGTTCAGGTAGGAGAGACATTTAAGGTTCCTTCAAGCTGCCCTATTGTTA
CTGTTGATGGATACGTGGACCCTTCTGGAGGAGATCGCTTTTGTTTGGGTCAACTCTCCAATGTCCACAG
GACAGAAGCCATTGAGAGAGCAAGGTTGCACATAGGCAAAGGTGTGCAGTTGGAATGTAAAGGTGAA
GGTGATGTTTGGGTCAGGTGCCTTAGTGACCACGCGGTCTTTGTACAGAGTTACTACTTAGACAGAGAA
GCTGGGCGTGCACCTGGAGATGCTGTTCATAAGATCTACCCAAGTGCATATATAAAGGTCTTTGATTTGC
GTCAGTGTCATCGACAGATGCAGCAGCAGGCGGCTACTGCACAAGCTGCAGCAGCTGCCCAGGCAGCA
GCCGTGGCAGGAAACATCCCTGGCCCAGGATCAGTAGGTGGAATAGCTCCAGCTATCAGTCTGTCAGCT
GCTGCTGGAATTGGTGTTGATGACCTTCGTCGCTTATGCATACTCAGGATGAGTTTTGTGAAAGGCTGG
GGACCGGATTACCCAAGACAGAGCATCAAAGAAACACCTTGCTGGATTGAAATTCACTTACACCGGGCC
CTCCAGCTCCTAGACGAAGTACTTCATACCATGCCGATTGCAGACCCACAACCTTTAGAC

B) Illumina probe for SMAD4 transcript

GCAGCGTCACTCTACCTAATGTCTCACTGTTCTGCAAAGGTGGCAATGCT

Supplementary Figure 6. Sequences for microarray analysis

A) SMAD4 open reading frame was codon-optimized and stop codon was removed to allow the tandem expression of the tumour suppressor with a reporter gene. This sequence is not recognized by the Illumina probe (B) responsible for the detection of SMAD4 endogenous transcript.

TABLE

Table 1. Differentially expressed genes in Capan-1 Luciferase *vs* Capan-1 unmodified parental cells with a fold change greater than 2 (*n* = 351)

Table 2. Differentially expressed genes in Capan-1 SMAD4 Luciferase *vs* Capan-1 Luciferase cells (*n* = 189 genes)

Table 3. Differentially expressed genes in PACO2 GFP Vs PACO2 Parental cells with a fold change greater than 2 (n = 2)

Table 4. Differentially expressed genes in PACO2 GFP-SMAD4 *vs* PACO2 GFP cells with a fold change greater than 2 (n = 169)

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