cancers

MDPI

Supplementary Materials

Sulforaphane Inhibits the Expression of Long Noncoding RNA H19 and its Target APOBEC3G and Thereby Pancreatic Cancer Progression

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Table S1. Primer sequences.

	Forward	Reverse
H19	GCACCTTGGACATCTGGAGT	TTCTTTCCAGCCCTAGCTCA
MALAT	CTCCCCACAAGCAACTTCTC	TTCAACCCACCAAAGACCTC
HOTAIR	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
HOTTIP	CCTAAAGCCACGCTTCTTTG	TGCAGGCTGGAGATCCTACT
PVT1	GCCCCTTCTATGGGAATCACTA	GGGGCAGAGATGAAATCGTAAT
A3G	GGGACCCAGATTACCAGGAG	GCAGATTATTCCAAGGCTCAA
β-actin	AATCGTGCGTGACATTAAGGAG	ACTGTGTTGGCGTACAGGTCTT

Table S2. Identification of 17 candidate genes.

Gene Symbol	Gene Name	Tumor type	Function
OSGIN1	Oxidative stress induced growth inhibitor	Breast cancer, Hepatocellular carcinoma	Tumor suppressor
TNFSF9	TNF superfamily member 9	Hepatocellular carcinoma	Tumor suppressor
ADI1	Acireductone dioxygenase 1	Hepatoma, Prostate cancer	Tumor suppressor
TRIM16	Tripartite motif containing 16	Breast cancer, Ovarian cancer, Prostate Cancer	Tumor suppressor
TMEM127	Transmembrane protein 127	Pheochromocytomas, Renal cancer	Tumor suppressor
APOBEC3G	**Apolipoprotein B MRNA Editing Enzyme Catalytic Subunit 3G**	**Pancreatic cancer, Mesenchymal gliomas**	**Tumor promotor, associated with immunity**
ASAH1	N-Acylsphingosine Amidohydrolase 1	Breast cancer	Tumor promotor
TARS	Threonyl-tRNA synthetase	Breast cancer, Pancreatic cancer, Ovarian cancer	Tumor promotor
BIRC3	Baculoviral IAP repeat containing 3	Pancreatic cancer, Breast cancer, Colorectal cancer	Tumor promotor
APOBEC3B	Apolipoprotein B MRNA Editing Enzyme Catalytic Subunit 3B	Breast cancer, Hepatocellular carcinoma	Tumor promotor
RAPB3IP	RAB3A interacting protein	Esophageal squamous cell carcinoma	Tumor promotor
MYO1B	Myosin IB	Cervical cancer, Prostate cancer	Tumor promotor
TCF7L2	Transcription factor 7 like 2	Cervical cancer, Gastric cancer, Breast cancer	Tumor promotor
DTX3L	Deltex E3 ubiquitin ligase 3L	Melanoma, Glioma	Tumor promotor
WARS	Tryptophanyl-tRNA synthetase	Oral cancer, Gastric cancer	Tumor promotor
CLU	Clusterin	Prostate cancer, Colorectal cancer	Tumor promotor
CDKN2AIP	CDKN2A interacting protein	Ovarian cancer, Lung cancer	Tumor promotor

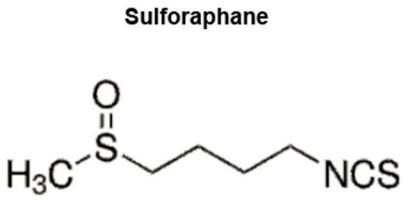

Figure S1. Chemical structure of sulforaphane.

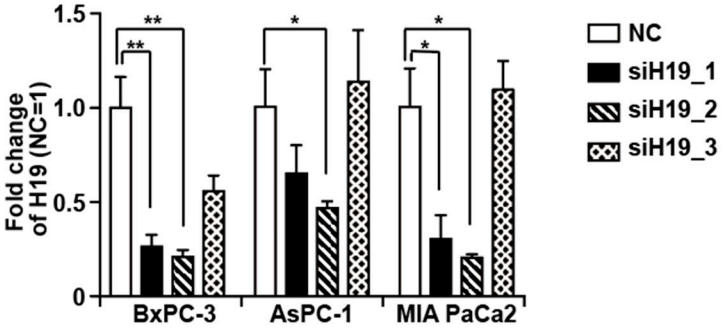

Figure S2. Identification of siH19_2 as the most efficient lncRNA H19 inhibitor. (**A**) The three different siRNA constructs targeting H19, namely, siH19_1, siH19_2 and siH19_3, along with a nonsense siRNA control (NC), were lipo-transfected into BxPc-3, AsPC-1 and MIA-PaCa2 cells at 20 μg each. Twenty-four hours later, the expression of H19 was detected by RT-qPCR. The fold change of H19 expression in the knockdown group was normalized to that in the nonsense siRNA control group, which was set to 1. * $p < 0.05$, ** $p < 0.01$.

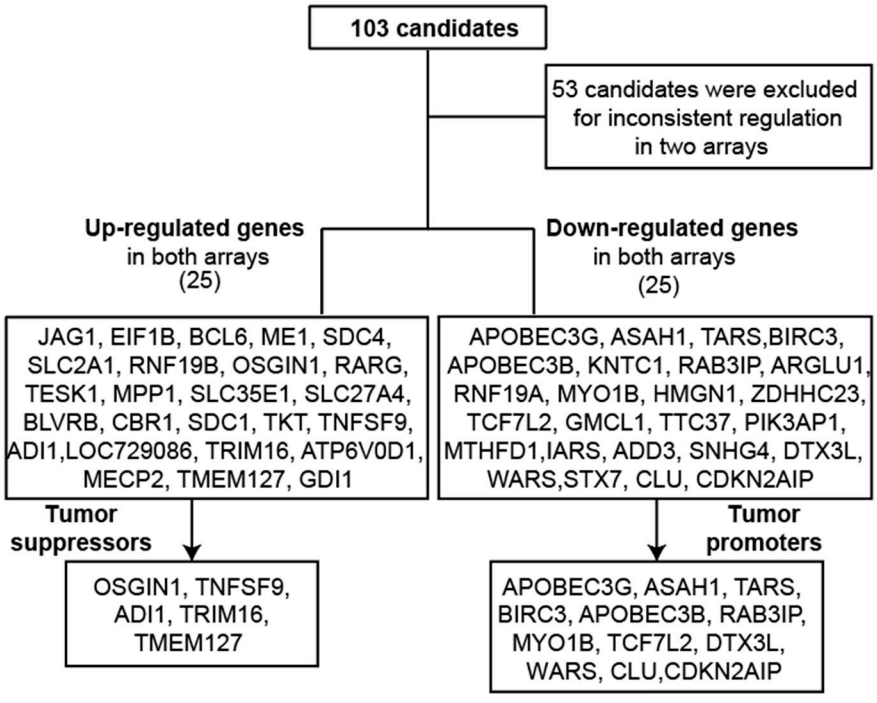

Figure S3. *In* silico identification of 17 H19-related candidate genes. A total of 103 candidates were selected according to the threshold of adjusted p value < 0.01 in two gene arrays as described in Fig. 3. Among them, 53 candidates were excluded because of inconsistent expression trends within the two gene arrays. Among the resulting 50 candidates, 25 genes were upregulated and 25 genes were downregulated in both gene arrays. By the use of the PubMed and Web of Science databases and the key words "cancer promoter" or "cancer suppressor", 17 candidates were selected, including 5 tumor suppressors and 12 tumor promoters.

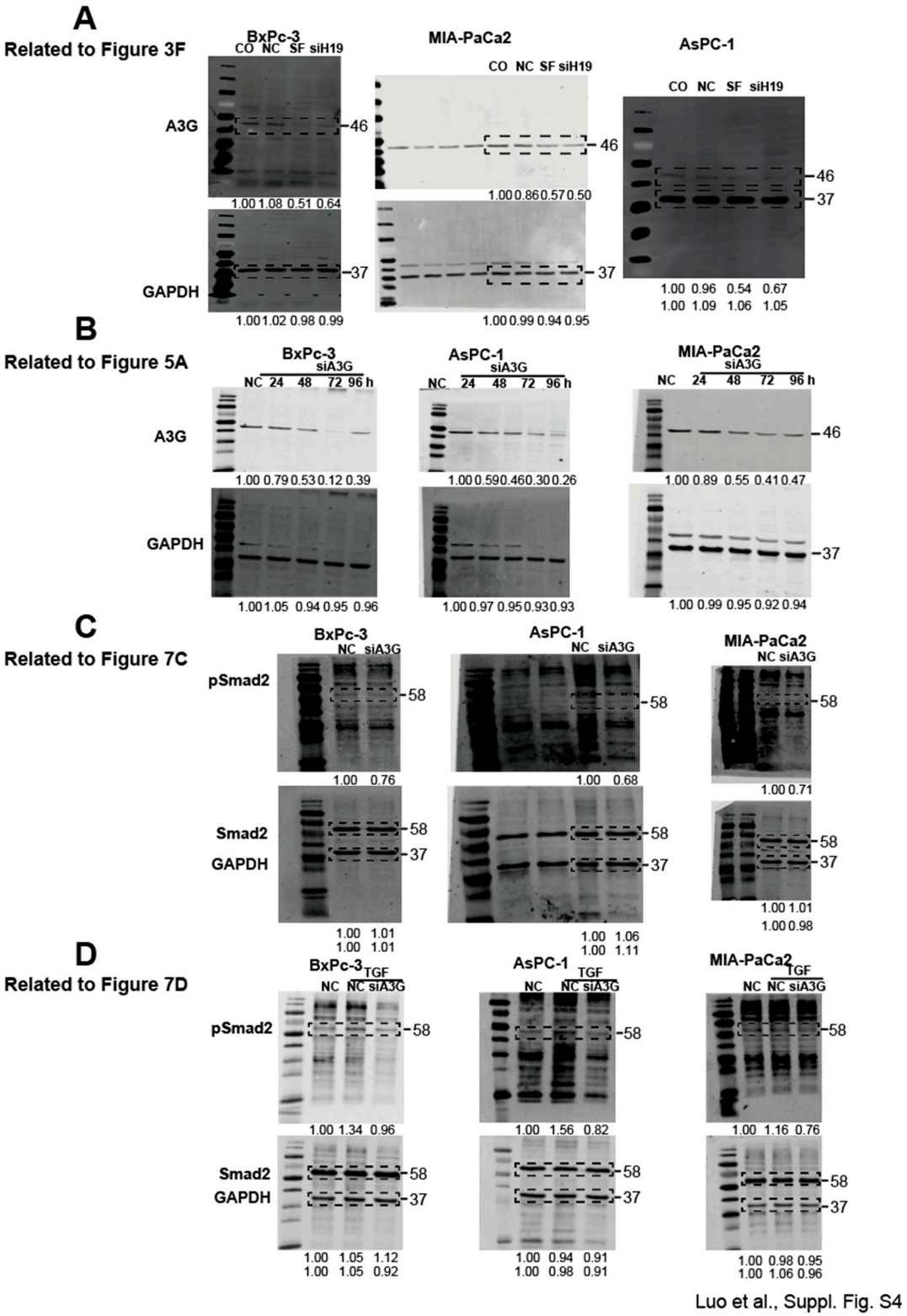

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Figure S4. Original, crude Western blot images with molecular weight markers. (**A**)Related to Fig. 3F. (**B**) Related to Fig. 5A. (**C**) Related to Fig. 7C. (**D**) Related to Fig. 7D.

Figure S5. Treatment scheme. After cleaning, the eggs were incubated to start the development of the chick embryo, which is scheduled at day 1 at the first day of incubation. At day 3 of development, a hole was cut into the egg shell, to open it for xenotransplantation. At the developmental day 9, 10^6 MIA-PaCa2 cells in 50 µl Matrigel™, were transplanted onto the scratched CAM. At day 14, 100 µl of a 100 µM sulforaphane solution, or 100 µl saline, was injected directly into the CAM vessels that supplied the xenograft tumors. At 18 day of chick development, all embryos were humanely euthanized.

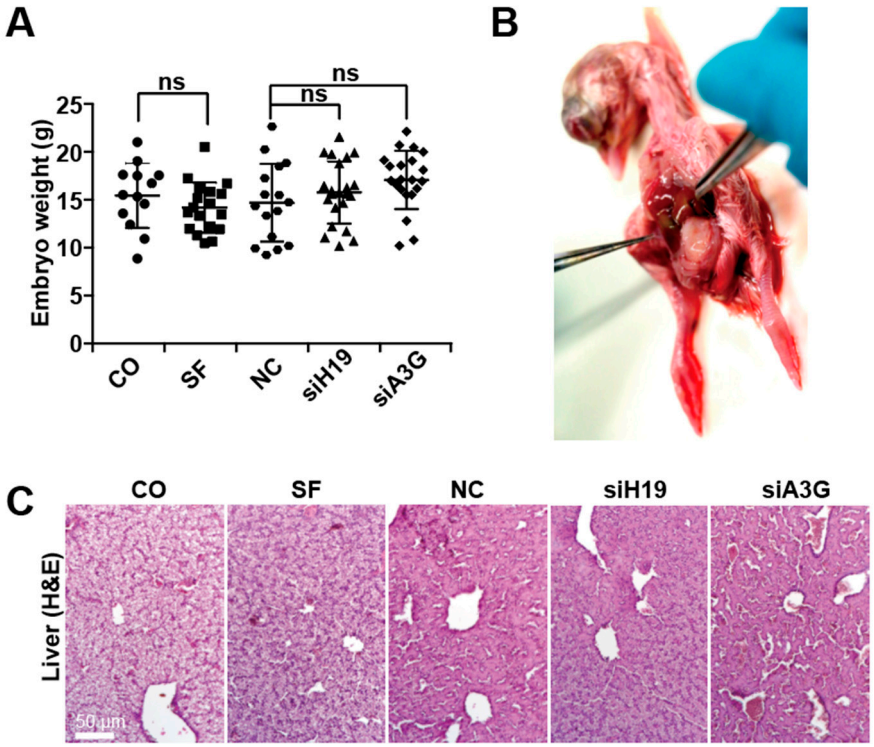

Figure S6. siH19, siA3G or sulforaphane treatment did not induce side effects in vivo. (**A**) After resection of xenograft tumors as described in Fig. 6, the weight of each chick embryo was determined and is presented as a black dot, and the mean weights per group are given. (**B**) Representative image of a chick embryo at day 18 of development and liver resection. (**C**) Representative H&E staining of frozen liver tissue sections from each group.