Supporting Information

Petrova and Resh

Hedgehog Acyltransferase is a target for inhibiting pancreatic cancer cell growth

Supplementary Table 1

Supplementary Figures

Supplementary Figure 1. Hhat knockdown inhibits Shh signaling in Panc-1 and Panc 05.04 **cells.** (**a, b**) Hhat was depleted in Panc-1 cells using lentivirally delivered shRNAs, and the cells were selected in puromycin. Hhat (**a**) and Gli-1 (**b**) mRNA levels were determined by qPCR. Bars represent mean ± SD (n=2). The experiment was performed three times. (**c,d**) Panc 05.04 cells were treated as described in A. Hhat (**c**) and Gli-1 (**d**) mRNA levels were determined by qPCR. Bars represent mean ± SD (n=2).

Supplementary Figure 2. Quantification of Shh knockdown in Panc-1 experiments from Figure 1i. mRNA levels of Shh were measured in the cells used in the experiment from Figure 1i. The corresponding Hhat levels, following Hhat knockdown are shown in Supplementary Figure 1A. Bars represent mean ± SD (n=2).

Supplementary Figure 3. Hhat knockdown decreases proliferation of Panc-1 and Panc 05.04 cells. (**a**) Panc-1 cells stably expressing scrambled or Hhat shRNA were plated at $4x10^4$ cells/ well in a 6-well plate. Cells were grown for 6 days and counted. Bars are mean ± SD (n=2). The

experiment was performed three times. (**b**) Panc 05.04 cells were stably transfected as described in A. $1x10^5$ cells were plated/ well in a 6-well plate, grown for 4 days, then counted. Bars are mean ± SD (n=2). The experiment was performed three times.

Supplementary Figure 4. RU-SKI 43 treatment does not affect proliferation of Hs766t pancreatic cancer cells. Hs766t cells were seeded at $5x10^4$ cells/well in 6-well plates. 24h later, DMSO, 10μM RU-SKI 43 or 10μM LDE-225 was added to the media. Cells were grown in the presence of drugs until they were counted on Day 4 or Day 7. Each point is the mean ± SD (n=2).

Supplementary Figure 5. Gene knockdown in Panc-1 cells used for the pancreatic cancer xenograft experiments in mice. Panc-1 cells were transfected with shRNAs (scrambled, Shh or Hhat) and selected in puromycin. mRNA levels of Shh and Hhat were measured by qPCR. Each bar represents mean ± SD (n=2).

Supplementary Table 1. List of targets tested with the PathScan® Intracellular Signaling Array Kit. + indicates presence of the modification, - indicates absence.

TARGET	MODIFICATION	DMSO	RU-SKI 43
ERK1/2	Thr202/Tyr204 Phosphorylation	-	-
Stat1	Tyr701 Phosphorylation	-	-
Stat3	Tyr705 Phosphorylation	-	-
Akt	Thr308 Phosphorylation	+	-
Akt	Ser473 Phosphorylation	+	-
AMPKα	Thr172 Phosphorylation	+	+
S6 Ribosomal Protein	Ser235/236 Phosphorylation	+	-
mTOR	Ser2448 Phosphorylation	+	-
HSP27	Ser78 Phosphorylation	-	-
Bad	Ser112 Phosphorylation	+	-
P70 S6 Kinase	Thr389 Phosphorylation	-	-
PRAS40	Thr246 Phosphorylation	+	-
p53	Ser15 Phosphorylation	+	-
p38	Thr180/Tyr182 Phosphorylation	+	+
SAPK/JNK	Thr183/Tyr185 Phosphorylation	-	-
PARP	Asp214 Cleavage	-	-
Caspase-3	Asp175 Cleavage	-	-
GSK-3β	Ser9 Phosphorylation	+	-

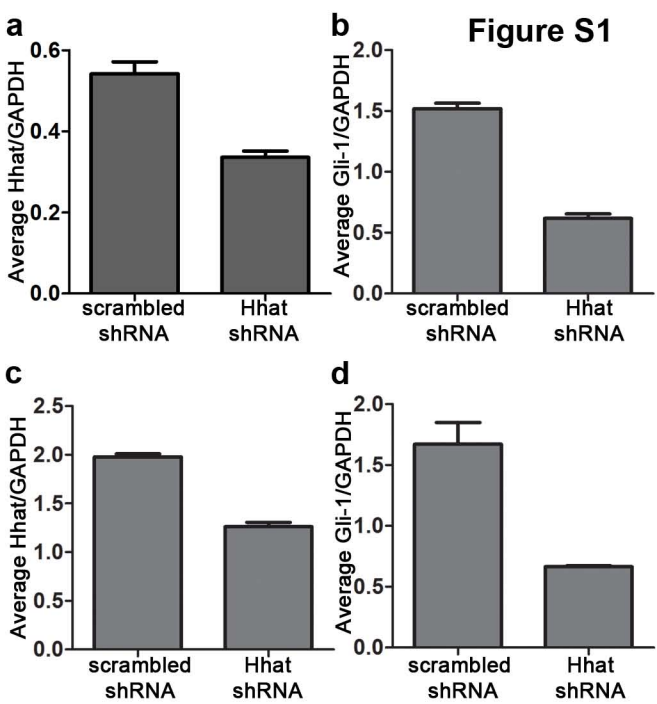
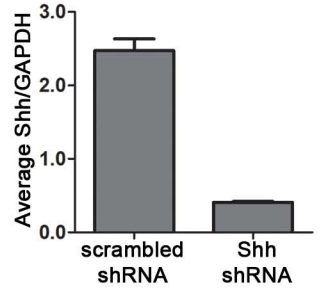

a
Average Hhat/GAPDH
0.6
0.4
0.2
0.0
scrambled shRNA
Hhat shRNA
b
Figure S1
Average Gli-1/GAPDH
2.0
1.5
1.0
0.5
0.0
scrambled shRNA
Hhat shRNA
c
Average Hhat/GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
scrambled shRNA
Hhat shRNA
d
Average Gli-1/GAPDH
2.0
1.5
1.0
0.5
0.0
scrambled shRNA
Hhat shRNA

Figure S2



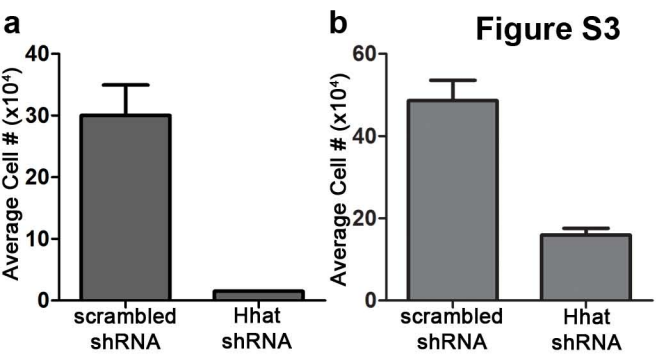

a
b
Figure S3
Average Cell # (x10⁴)
40
30
20
10
0
scrambled shRNA
Hhat shRNA
Average Cell # (x10⁴)
60
40
20
0
scrambled shRNA
Hhat shRNA

Figure S4

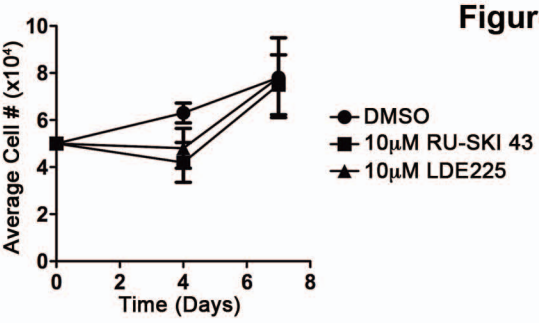
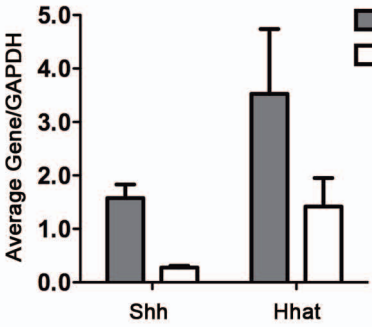

Average Cell # (x10⁴)
10
8
6
4
2
0
0
2
4
6
8
Time (Days)
DMSO
10μM RU-SKI 43
10μM LDE225

Figure S5


scrambled shRNA
target shRNA
Average Gene/GAPDH
5.0
4.0
3.0
2.0
1.0
0.0
Shh
Hhat