# Supplemental online data

#### **Supplemental Figure Legends:**

## Suppl. Fig. 1:

(A) Notch luciferase reporter assay showing reduced activity after transient knockdown of ZEB1, Jag1, and overexpression of miR-141 and miR-200c. (B) Reduced Jag1 mRNA expression after knockdown of ZEB1 and Jag1. Overexpression of miR-141 and miR-200c only had a weak effect on Jag1 mRNA level. Note that Jag1 knockdown also affects ZEB1 expression and that miR-200c strongly reduces ZEB1 expression. (C, D) Knockdown of Jag1 affects expression of the Notch target gene Hey1 and to a lesser extend of ZEB1 in pancreatic (C) and breast (D) cancer cells. (E) GSI treatment and to a lesser extend the knockdown of Jag1 reduces the proliferative capacity of the breast cancer cell line MDA-MB231. X-axis indicates days after cell seeding. Asterisks indicate significance of control vs. treated cells. (F) Knockdown of Jag1 affects the sphere forming capacity of MDA-MB231 in the second sphere generation.

#### Suppl. Fig. 2:

(A) Representative immunohistochemistry showing reduced expression of ZEB1, Jag1 and vimentin in tumors derived from ZEB1 knockdown clones of the pancreatic cell line MiaPaCa2. Size bar 20µm. (B) Inhibition of endogenous miR-141 and miR-200c in differentiated MCF7 breast cancer cells increases Notch reporter activity, which is only partially reversed by siRNA-mediated knockdown of Jag1. (C) Proliferation of differentiated MCF7 breast cancer cells is not significantly affected by antagomirs and Jag1 knockdown. (D) High expression ratio of ZEB1 in the indicated undifferentiated primary human tumor types is significantly associated with Jag1 expression, as scored by immunohistochemistry.

# **Supplemental Materials and Methods:**

## Oligonucleotides used in this study:

For **cloning** the following primers were used for PCR : Jag1 3'UTR-Luc s: 5'- AATTACGCGTCATCGTATAGCAGACCGCG as: 5'- AATTATTCGAATTCAAGCAGTATTCACACTTGCT

human Jag1 expression vector s: 5'- ATTAGCTAGCATGCGTTCCCCACGGACG as: 5'- CAGCGCGGCCGCCTATACGATGTACTCCATTCGGTTTAAGC

# Maml2 3'UTR-Luc s: 5'- ATAATGCCGGCAAGAAAGGGAAGACAATTTACAAACTC as: 5'- ATAATACGCGTTTTTTGTTTAATCACTAGACACAGCAT

Maml3 3'UTR-Luc s: 5'- ATAATGCCGGCCTCTGCAATTGACGCACATC as: 5'- ATAATACGCGTCAGTGGAGCCTCCCTACCT

For **mutagenesis** of the Jag1 3'UTR the following primers were used for PCR: site 1:

s: 5'- GTTGCTGACTTAGAATCCCTGTAATTTAAGTTTTGACAAGCTGG

as: 5'- CCAGCTTGTCAAAACTTAAATTACAGGGATTCTAAGTCAGCAAC site 2:

s: 5'- GCTATGCAAAAAGCTAGTCAACAGTTGTCCCCTTGCAG

as: 5'- CTGCAAGGGGACAACTGTTGACTAGCTTTTTGCATAGC site 3:

s: 5'- CATCAAAGATGCATTTGTATGTTCATATAATAGGACAATACAAAGTATCTTCAC as: 5'- GTGAAGATACTTTGTATTGTCCTATTATATGAACATACAAATGCATCTTTGATG site 5:

For quantitative **real time RT-PCR**, the following primers were used:

hs ZEB1	s: 5'- AAGAATTCACAGTGGAGAGAAGCCA
	as: 5'- CGTTTCTTGCAGTTTGGGCATT
Hey1	s: 5'- AGGGAGCCAGCATGAA
	as: 5'- ATGGAACCTAGAGCCGAA
Jagged1	s: 5'- GAATGGCAACAAAACTTGCAT
	as:.5'- AGCCTTGTCGGCAAATAGC
Maml2	s: 5'- GTGGTGGGATAAACGGAGAG
	as: 5'- TCTTTTCAAGGAACCCTGGAG

Maml3s: 5'- CGGAGCAGAGGAACCACA<br/>as: 5'- CATTCTGCTGGTCTCCATTAAGThs E-cadherin s:5'- GTCCTGGGCAGACTGAATTT<br/>as:s:5'- GACCAAGAAATGGATCTGTGGhs Vimentins:s:5'- CGAGGAGAGCAGGATTTCTC<br/>as:as:5'- GGTATCAACCAGAGGGAGTGAhs  $\beta$ -Actins:s:5'- GCCCTGAGGCACTCTTCCA<br/>as:s:5'- TTGCGGATGTCCACGTCA

For transient knock down the following siRNAs were used

hs ZEB1 5'- AGAUGAUGAAUGCGAGUCGdTT

Jag1 (a) 5' - GAAUGUGAGGCCAAACCUUdTT

Jag1 (b) 5' – CAUCGAUUAUUGUGAGCCUdTT

Maml2 5'- CUCUAACCAGGCUUUGGCAdTT

Maml3 5'- GUUGGAAGGAGCUCGAUCAdTT

si ctrl 5'- GCUACCUGUUCCAUGGCCAdTT

The following antagomirs were constructed:

anta-mir-200c (1):

5'mU(\*)mC(\*)mCmAmUmCmAmUmUmAmCmCmCmGmGmCmAmGmU(\*)mA(\*)mU(\*) mU(\*)mA-3'Cholesterole

anta-mir-200c (2):

5'-mU(\*)mC(\*)mCmAmUmCmAmUmUmAmCmCmCmGmGmCmAmGmUmA(\*)mU(\*) mU(\*)mA(\*)-3'Cholesterole

anta-mir-141: 5'-mC(\*)mC(\*)mAmUmCmUmUmAmCmCmAmGmAmCmAmGmUmG(\*)mU(\*) mU(\*)mA(\*)-3'Cholesterole

anta-mir-ctrl: (see Yi et al., Nature 2008;452:225-9) 5'-mU(\*)mC(\*)mUmCmGmUmGmUmCmAmUmAmAmAmCmAmCmU(\*)mU(\*) mC(\*) mA(\*)-3'Cholesterole

# Antibodies used in this study:

For immunohistochemistry (IHC), immunoflourescence (IF) and immunoblots (IB):

mouse anti β-Actin	Sigma, #A5541	1:5000	for IB
rabbit anti Jagged1	Cell Sig. Tech #2620	1:500, 1:75	for IB, IHC
rabbit anti Maml2	Bethyl Lab., #A300-682	1:1000	for IB
rabbit anti Maml3	Bethyl Lab., #A300-683	1:5000	for IB
rabbit anti Notch1	Abcam ab8925	1:500	for IHC
rabbit anti Notch2	Deciphergen Biotech.	1:500	for IHC, IF
	#SRP00383		
mouse anti Vimentin	Dako, #M0725	1:20	for IHC
rabbit anti ZEB1	Sigma, #HPA027524	1:5000, 1:600	for IB, IHC
goat-anti-rabbit Alexa 488	MolProbes, #A11-008	1:500	for IF







Time (days)

treatment si si si ctrl Jag1a Jag1b

Brabletz et al., suppl. Fig. 1

Time (days)







	D	n	ZEB1 +	correlated Jag1
0	basal type breast cancers	8	6 (75%)	6/6 (100%) p<0.05
	undifferentiated (G3-4) pancreatic adenocarcinomas	12	8 (67%)	7/8 (87%) p<0.05

Brabletz et al., suppl. Fig. 2