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 antagonirnrs 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’- AATTACGCAGTCATCGTATAGCAGACCGCG as: 5’- AATTATTCGAATTCAGTATTCACACTTGCT
human Jag1 expression vector s: 5’- ATTAGCTAGCATGCGTCCCCACGGACG as: 5’- CAGCGCGGCGCGCTATACGATGTACTCCATTCCGTTTAAGC
Maml2 3’UTR-Luc  s: 5’- ATAATGCCGGCAAGAAAGGGAAGACAATTTACAAACTC  
as: 5’- ATAATACGCGTTTTTTGTTTAATCACTAGACACACAGCAT

Maml3 3’UTR-Luc  s: 5’- ATAATGCCGGCCTCTGCAATTGACGCACATC  
as: 5’- ATAATACGCGTCAGTGAGGACCCCTCCCTACCT

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’- CTGCAAGGGGACAACACTGGACTAGCTTTTTCATAGC

site 3:  
s: 5’- GTTTCAAGTATTCAACTAGCTTTAGCCCTAAATGCAGTAGATTTTTAAAAAA  
as: 5’- TTTTTTTAATCTACTGCATTAGGGCTAATAAGCTAGTTGGAATACTTGAAC

site 4:  
s: 5’- CATCAAAGATGCATTTGTATGTGCATATAATAGGACAATACAAATGATCTTTCG  
as: 5’- GTGAAGATACTTTGTATTGTCCTATTATATGGAACATAAACATCCTCTCTGAGCTTAC

site 5:  
s: 5’- GTAATATTATTTAATTTTTTTTGATGAAACATGGATGGCCTCTCTGAGCTTAC  
as: 5’- GTAAGCTCACAGAAGGAGCCATCCATGTTTTCATACAAAAAATTTAAATAATATTAC

For quantitative real time RT-PCR, the following primers were used:
hs ZEB1  s: 5’- AAGAATTCACAGTGGAGAGAAGCCA  
as: 5’- CGTTTCTTGCAAGTGGCCATT

Hey1  s: 5’- AGGGAGCCACAGCATGAA  
as: 5’- ATGGAACCTAGAGCCGAA

Jagged1  s: 5’- GAATGGCAACAAAACTTGCAT  
as: 5’- AGCCTTGTCGGCAAATAGC

Maml2  s: 5’- GTGCTGGGGATAAACCGGAGAG  
as: 5’- TCTTTTCAAGGAACCCTGGAG
Maml3 s: 5’- CGGAGCAGAGGAACCACA  
as: 5’- CATTCTGCTGGTCTCCATTAAGT
hs E-cadherin s: 5’- GTCCTGGGAGACTGAATTT  
as: 5’- GACCAAGAAATGGATCTGTGG
hs Vimentin s: 5’- CGAGGAGAGCAGGATTCTC  
as: 5’- GGTATCAACCAGAGGGAGTGA
hs β-Actin s: 5’- GCCCTGAGGCACTCTTCCA  
as: 5’- TTGCGGATGTCACGCTCA

For transient knock down the following siRNAs were used:

hs ZEB1 5’- AGAUGAUGAAGCAUGACGUCdTT
Jag1 (a) 5’- GAAUGUGAGGCCAAACCUdTT
Jag1 (b) 5’- CAUCGAUUAUUGUGAGCCUCdTT
Maml2 5’- CUCUAACCAGGUUUGGCAAdTT
Maml3 5’- GUUGGAAGGAGCGAUUCAAdTT
si ctrl 5’- GCUACCUGUUCCAUGGCCAdTT

The following antagonirs were constructed:

anta-mir-200c (1):
5’mU(*)mC(*)mCmAmUmCmAmUmAmCmCmCmGmAmGmUmGmUmAmAmAmCmAmCmU(*)mA(*)mU(*)mU(*)mA-3’Cholesterol

anta-mir-200c (2):
5’-mU(*)mC(*)mCmAmUmCmAmUmAmCmCmCmGmAmGmUmAmAmAmCmAmCmGmUmGmUmAmAmAmCmAmCmU(*)mA-3’Cholesterol

anta-mir-141:
5’-mC(*)mC(*)mAmUmCmUmAmCmCmCmCmCmGmAmGmAmGmUmG(*)mU(*)mU(*)mA-3’Cholesterol

anta-mir-ctrl: (see Yi et al., Nature 2008;452:225-9)
5’-mU(*)mC(*)mUmCmGmUmGmUmCmAmAmAmAmAmAmAmCmA(*)mU(*)mC(*)mA-3’Cholesterol
**Antibodies used in this study:**

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

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<th>Antibody Description</th>
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<th>Dilution</th>
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<td>mouse anti β-Actin</td>
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<tr>
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<td>for IB</td>
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<td>Deciphergen Biotech., #SRP00383</td>
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<td>goat-anti-rabbit Alexa 488</td>
<td>MolProbes, #A11-008</td>
<td>1:500</td>
<td>for IF</td>
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Brabletz et al., suppl. Fig. 1
A) MiaPaCa2

B) MCF7

C) C

D) D

Brabletz et al., suppl. Fig. 2