

Maml2 3'UTR-Luc s: 5'- ATAATGCCGGCAAGAAAGGGAAGACAATTTACAAACTC
as: 5'- ATAATACGCGTTTTTTTGTTTAATCACTAGACACAGCAT

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'- CCAGCTTGTCAAACCTTAAATTACAGGGATTCTAAGTCAGCAAC

site 2:

s: 5'- GCTATGCAAAAAGCTAGTCAACAGTTGTCCCCTTGCAG
as: 5'- CTGCAAGGGGACAACCTGTTGACTAGCTTTTTGCATAGC

site 3:

s: 5'-GTTCAAGTATTCAACTAGCTTATTAGCCCTAAATGCAGTAGATTAAAAAAAAA
as: 5'- TTTTTTTTAATCTACTGCATTTAGGGCTAATAAGCTAGTTGAATACTTGAAC

site 4:

s: 5'- CATCAAAGATGCATTTGTATGTTTCATATAATAGGACAATACAAAGTATCTTCAC
as: 5'- GTGAAGATACTTTGTATTGTCTTATTATATGAACATACAAATGCATCTTTGATG

site 5:

s: 5'- GTAATATTTATTAAATTTTTTTGTATGAAAACATGGATGGCCTCTTCTGAGCTTAC
as:5'-GTAAGCTCAGAAGAGGCCATCCATGTTTTCATACAAAAAAATTTAATAAATATTAC

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'- GAATGGCAACAAAACCTTGCAT
as:5'- AGCCTTGTTCGGCAAATAGC

Maml2 s: 5'- GTGGTGGGATAAACGGAGAG
as: 5'- TCTTTTCAAGGAACCCTGGAG

Maml3 s: 5'- CGGAGCAGAGGAACCACA
as: 5'- CATTCTGCTGGTCTCCATTAAGT

hs E-cadherin s: 5'- GTCCTGGGCAGACTGAATTT
as: 5'- GACCAAGAAATGGATCTGTGG

hs Vimentin s: 5'- CGAGGAGAGCAGGATTTCTC
as: 5'- GGTATCAACCAGAGGGAGTGA

hs β -Actin s: 5'- GCCCTGAGGCACTCTTCCA
as: 5'- TTGCGGATGTCCACGTCA

For transient **knock down** the following siRNAs were used

hs ZEB1 5'- AGAUGAUGAAUGCGAGUCGdTT

Jag1 (a) 5' - GAAUGUGAGGCCAAACCUdTT

Jag1 (b) 5' – CAUCGAUUAUUGUGAGCCUdTT

Maml2 5'- CUCUAACCAGGCUUUGGCAdTT

Maml3 5'- GUUGGAAGGAGCUCGAUCAdTT

si ctrl 5'- GCUACCUGUCCAUGGCCAdTT

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(*)mAmUmCmUmUmUmAmCmCmAmGmAmCmAmGmUmG(*)mU(*)
mU(*)mA(*)-3'Cholesterole

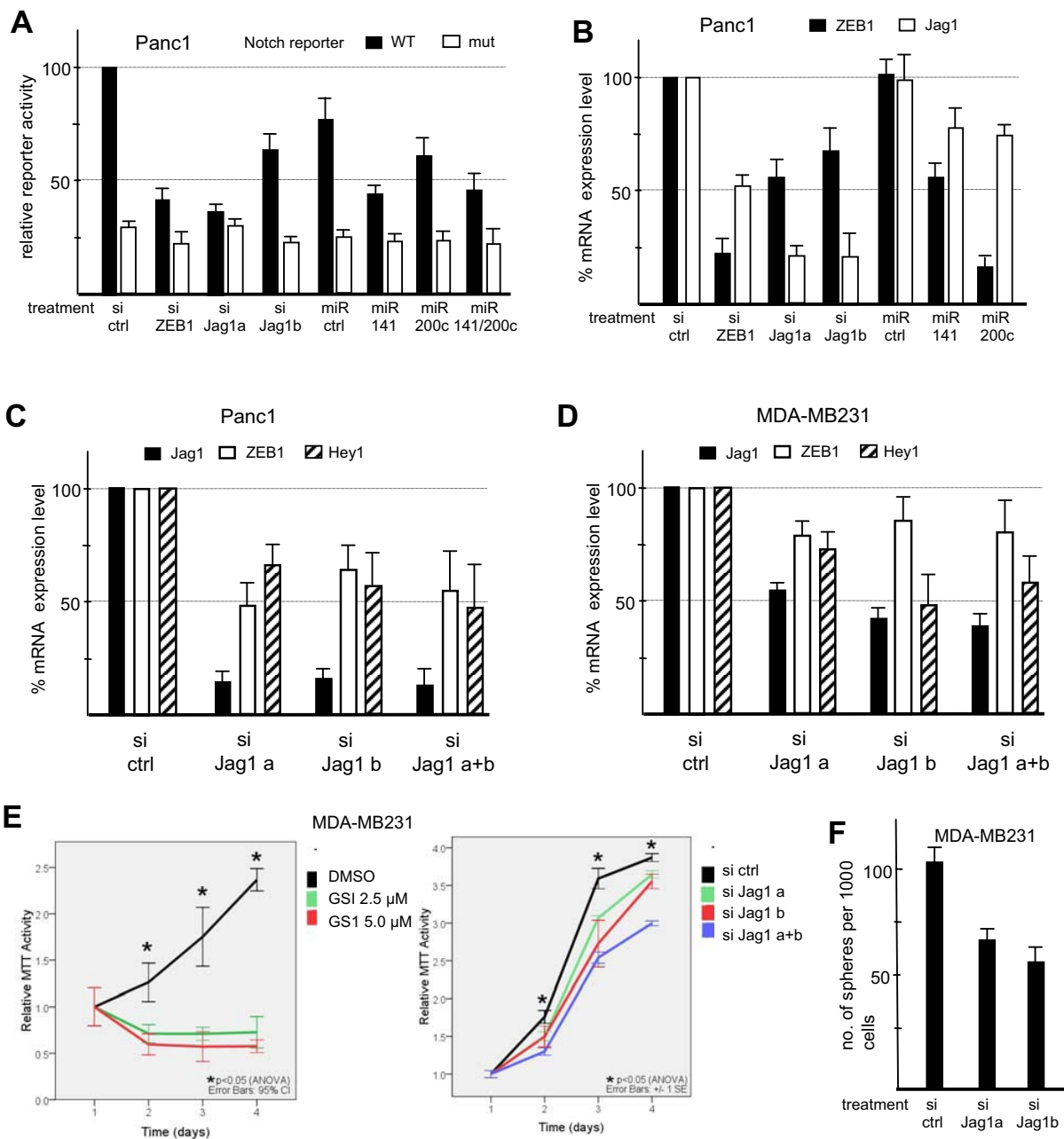
anta-mir-ctrl: (see Yi et al., Nature 2008;452:225-9)

5'-mU(*)mC(*)mUmCmGmUmGmUmUmCmAmUmAmAmAmCmAmCmU(*)mU(*)
mC(*) mA(*)-3'Cholesterole

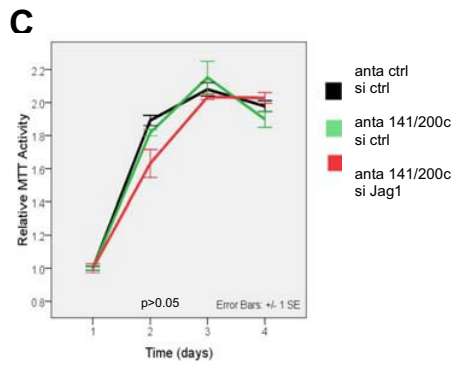
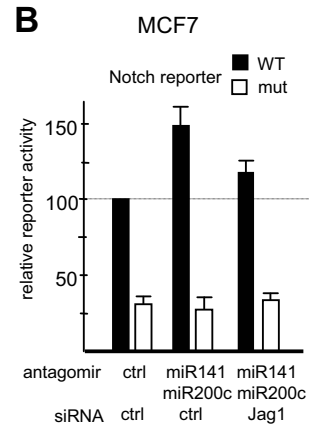
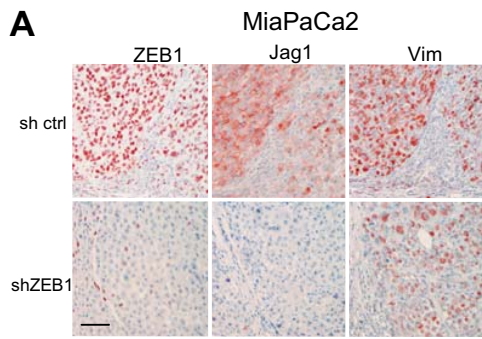
Antibodies used in this study:

For immunohistochemistry (IHC), immunofluorescence (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. #SRP00383	1:500	for IHC, IF
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



Brabletz et al., suppl. Fig. 1



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	n	ZEB1 +	correlated Jag1
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