

Supplementary Material

Kras/ADAM17-dependent Jag1-ICD reverse signalling sustains colorectal cancer progression and chemoresistance

Maria Pelullo^{1*}, Francesca Nardoza^{2*}, Sabrina Zema^{2*}, Roberta Quaranta^{2‡}, Carmine Nicoletti³, Zein Mersini Besharat⁴, Maria Pia Felli⁴, Bruna Cerbelli⁵, Giulia d'Amati⁵, Rocco Palermo², Carlo Capalbo², Claudio Talora², Lucia Di Marcotullio⁶, Giuseppe Giannini⁶, Saula Checquolo⁷, Isabella Screpanti² and Diana Bellavia²

Supplementary Figure S1. Nature of Kras, Braf, APC and CTNNB1 mutations in CRC cell lines.

Supplementary Figure S2. Jagged1 affects EMT-related genes expression.

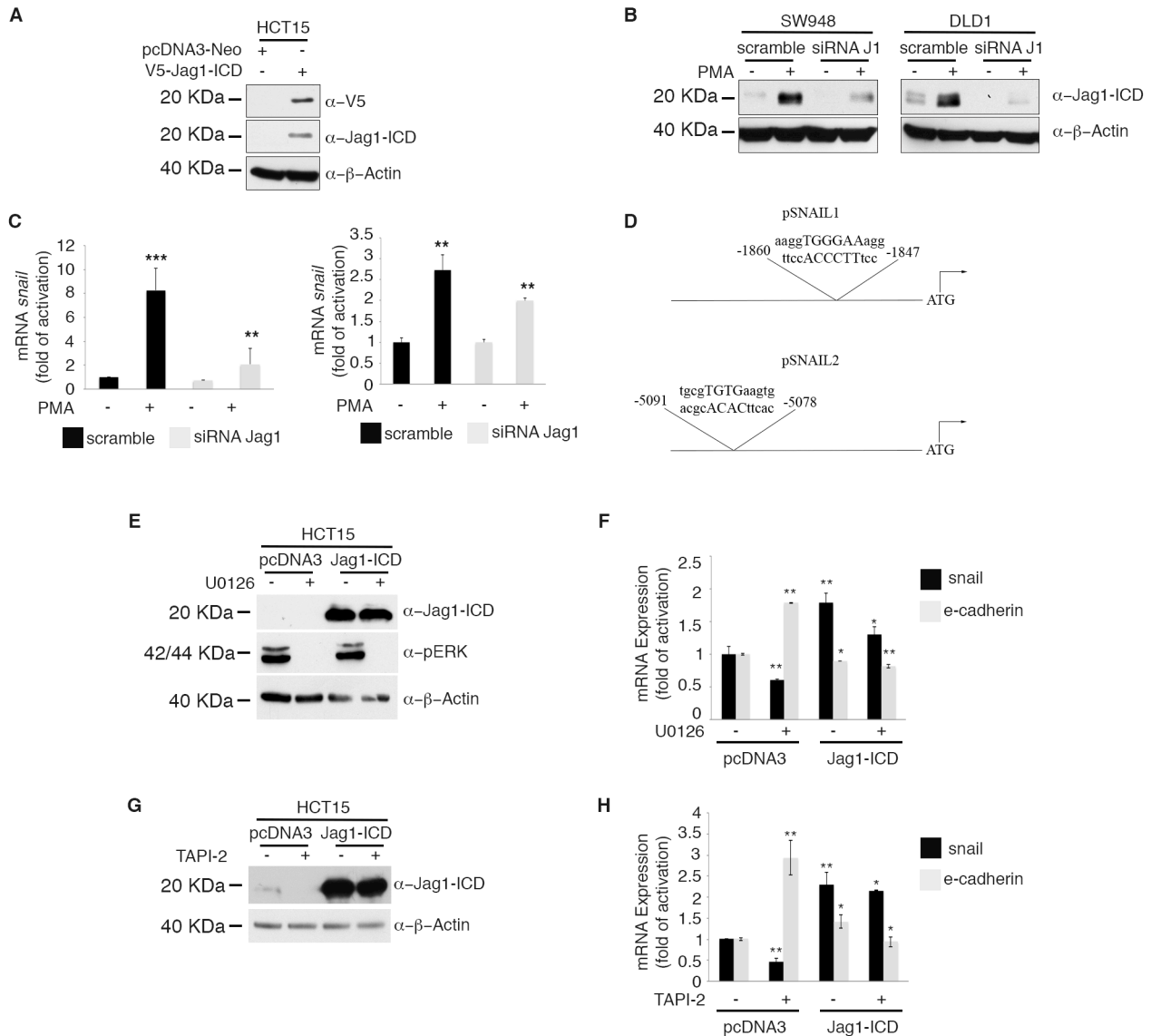
Supplementary Figure S3. Jag1-ICD confers 5-FU resistance in DLD1 cells, a CRC cell line with high levels of endogenous Jagged1.

Supplementary Table S1. List of primers utilized in this study

Supplementary Table S2. List of antibodies utilized in this study

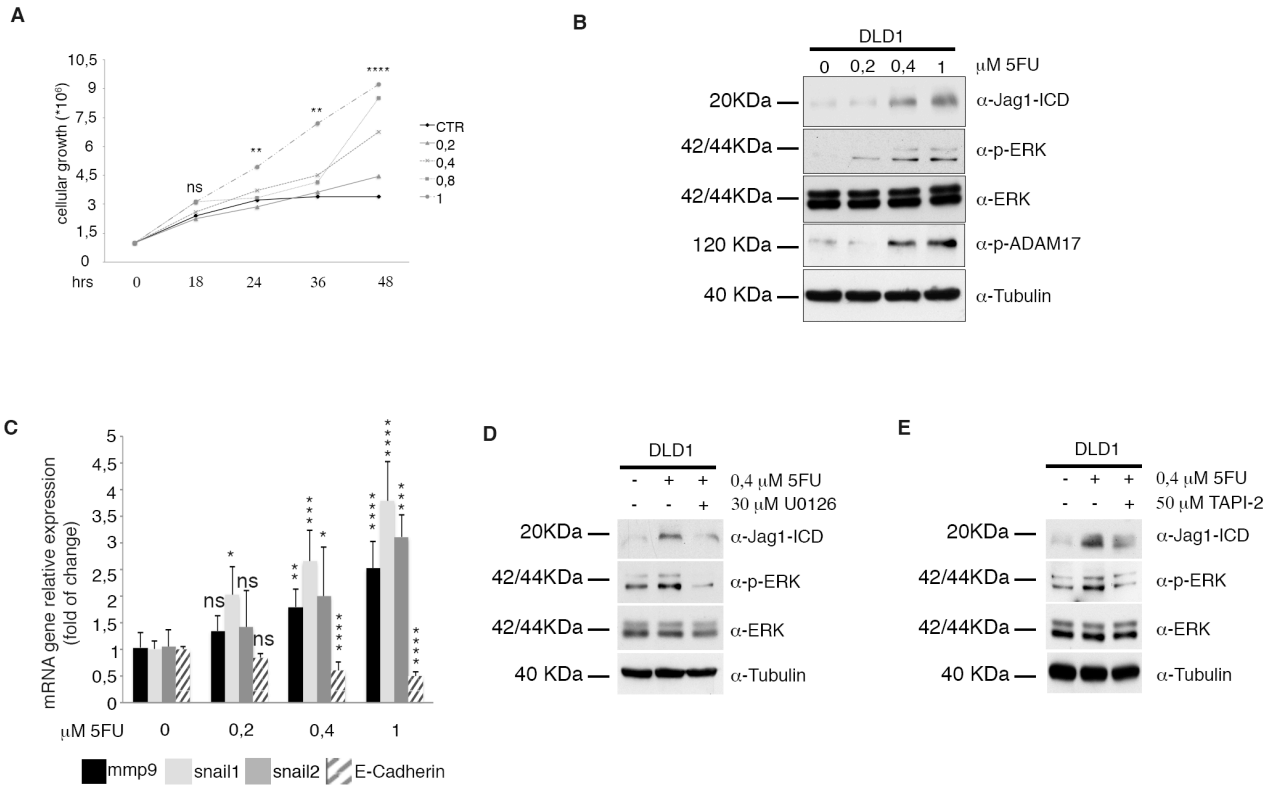
CRC cell line	Kras	Braf	APC	CTNNB1
HT-29	wt	c. 1799T>A	c.2557G>T; c.4666_4667insA	wt
HCT15	c.38G>A	wt	c.4248delC; c.6496C>T	wt
DLD1	c.38G>A	wt	c.4248delC	wt
HCT116	c.38G>A	wt	wt	c.133_135delTCT
LS174T	c.35G>A	wt	wt	c.134C>T
LoVo	c.38G>A	wt	c.3340C>T; c.4290delC	wt
RKO	wt	c. 1799T>A	wt	wt
SW1116	c.35G>C	wt	c.790C>T; c.4287_4296delA ACCATGCCA	wt
SW948	c.182A>T	wt	c.3340C>T; c.4285C>T	wt

Supplementary Figure S1. Nature of Kras, Braf, APC and CTNNB1 mutations in CRC cell lines.



Supplementary Figure S2. Jagged1 affects EMT-related genes expression. **A**, WCE derived from HCT15 cells stably transfected with V5-Jag1-ICD or negative control (pcDNA3) analysed by Western blots assay for Jagged1-ICD and V5-tag. **B**, Representative immunoblots of Jag1-ICD in WCE from SW948 (left panel) and DLD1 (right panel) cells after 48 hours of silencing for Jagged1 or scramble control. The cells were treated with PMA or vehicle alone for 4 hours. Protein levels normalized relative to β -Actin. **C**, qRT-PCR analysis of *snail* gene expression in Jagged1-silenced cells compared with scramble, upon PMA treatment. The data are presented as fold change respect

to DMSO and graphed after intrasample normalization respect to the GAPDH. **, P<0,01; ***, P<0,001. **D**, Schematic representation of putative RBP-Jk binding site on *Snail1* and *Snail2* promoters ranging between -1860/-1847 bps and -5091/-5078 bps respectively upon ATG start site. All data are representative of at least three independent experiments, each in triplicate. **E and G**, Representative immunoblots of Jag1-ICD and pERK in HCT15 cells stably transfected with V5-Jag1-ICD or negative control (pcDNA3) treated with U0126 (E) or TAPI-2 (G) or vehicle alone. Protein levels normalized relative to β -Actin. **F and H**, qRT-PCR analysis of *snail* and *e-cadherin* gene expression in cells derived respectively from E and G. The data are presented as fold change respect to DMSO and graphed after intrasample normalization respect to the GAPDH. *, P<0,05, **, P<0,01.



Supplementary Figure S3. Jag1-ICD confers 5-FU resistance in DLD1 cells, a CRC cell line with high levels of endogenous Jagged1. **A**, Proliferation rate of DLD1 cells treated with increasing doses of 5-FU, for the indicated time (hrs). **B**, Representative immunoblots of Jag1-ICD, pERK, total ERK and pADAM17 in WCE derived from DLD1 cells, treated or not with an increasing amount of 5-FU. **C**, DLD1 cell line treated or not with 5-FU shows the modulation of *mmp9*, *snail1*, *snail2* and *E-Cadherin* genes by qRT-PCR. Data are reported as fold changes \pm SD after intrasample normalization to the level of *GAPDH*. **D** and **E**, Representative Western blot of Jag1-ICD, pERK and total ERK in WCE derived from DLD1 cells treated for 18 hours with 5-FU alone or in combination with U0126 (D) or TAPI-2 (E). The protein levels normalized respective to α -Tubulin. All data are representative of at least three independent experiments, each in triplicate. *, $P < 0,05$; **, $P < 0,01$; ***, $P < 0,001$; ****, $P < 0,0001$.

Gene	Taqman Ref
Jagged1	Hs01070032_m1
PCNA	Hs00427214_g1
Cyclin D2	Hs00153380_m1
MMP9	Hs00234579_m1
Snail1	Hs00195591_m1
Snail2	Hs00161904_m1
E-Cadherin	Hs01023894_m1
GAPDH	Hs02758991_g1

Gene	Sequence
Jag1-ICD-V5 (fow)	5'-ATG AGG AAG CGG CGG AAG-3'
Jag1-ICD-V5 (rev)	5' TAC GAT GTA TTC CAT CCG GTT-3'
Snail1 (fow)	5'- GAGGCTGAGCAGTTAGTGAA-3'
Snail1 (rev)	5'-CAGAGTAAAAGCCAAAGTCC-3'
Snail2 (fow)	5'-GCAAAATAAGCTACTTTGGAGGCA-3'
Snail2 (rev)	5'-AGTGCCCAACAGTGTGTGG-3'

Supplementary Table S1: List of primers utilized in this study

Primary antibody	Source	Reference	Dilution
Jagged1	Sigma-Aldrich	HPA021555	1:1000
Jagged1	Cell Signaling	#2155	1:1000
Jagged1	Abcam	Ab192767	1:100
Kras	Abnova	H00003845-M02	1:1000
ADAM17	Abcam	Ab2251	1:1000
Total ERK	Cell Signaling	#4695	1:1000
p-ERK	Santa Cruz Biotchenology	#7383	1:1000
Phospho Serine	Abcam	AB1607	1:500
Snail	Cell Signaling	#3895	1:1000
Vimentin	Santa Cruz Biotchenology	SC-373717	1:500
N-Cadherin	Santa Cruz Biotchenology	SC-271386	1:100
Notch1 Val1774	Cell Signaling	#4147	1:1000
Notch2 Val1694	Sigma-Aldrich	SAB4502022	1:1000
Notch3	Cell Signaling	#2889	1:1000
β -actin	Sigma-Aldrich	A5441	1:20000
α -tubulin	Santa Cruz Biotchenology	SC-8035	1:500
Lamin B1	Santa Cruz Biotchenology	SC-6217	1:500
RBP-J κ	Santa Cruz Biotchenology	SC-8213	1:1000

Secondary antibody	Source	Reference	Dilution
Donkey anti rabbit-HRP	Bethyl	A120-108P	1:30000
Donkey anti goat-HRP	Santa Cruz Biotchenology	SC-2020	1:3000
Goat anti mouse-HRP	Bethyl	A90-116P	1:30000

Supplementary Table S2: List of antibodies utilized in this study