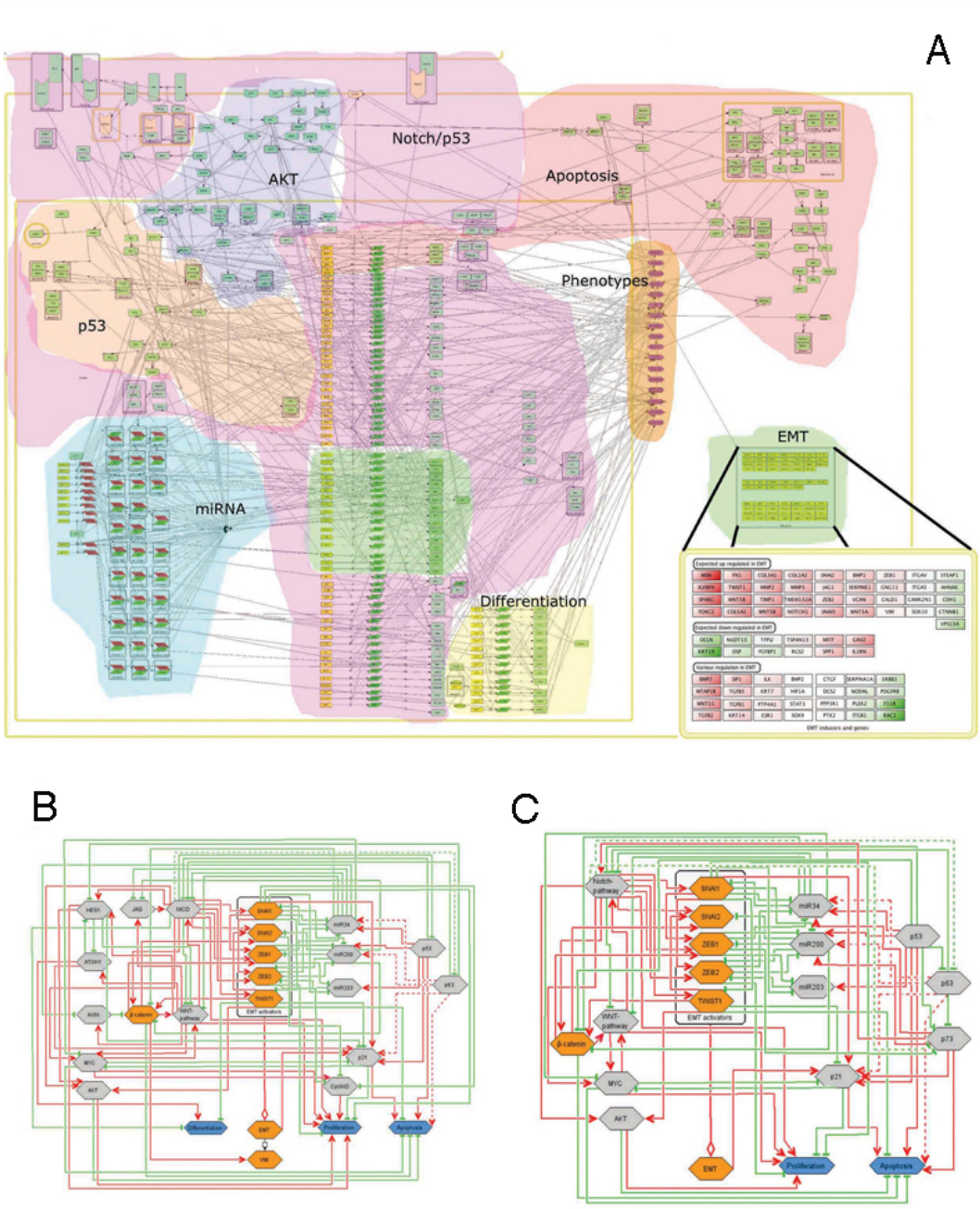
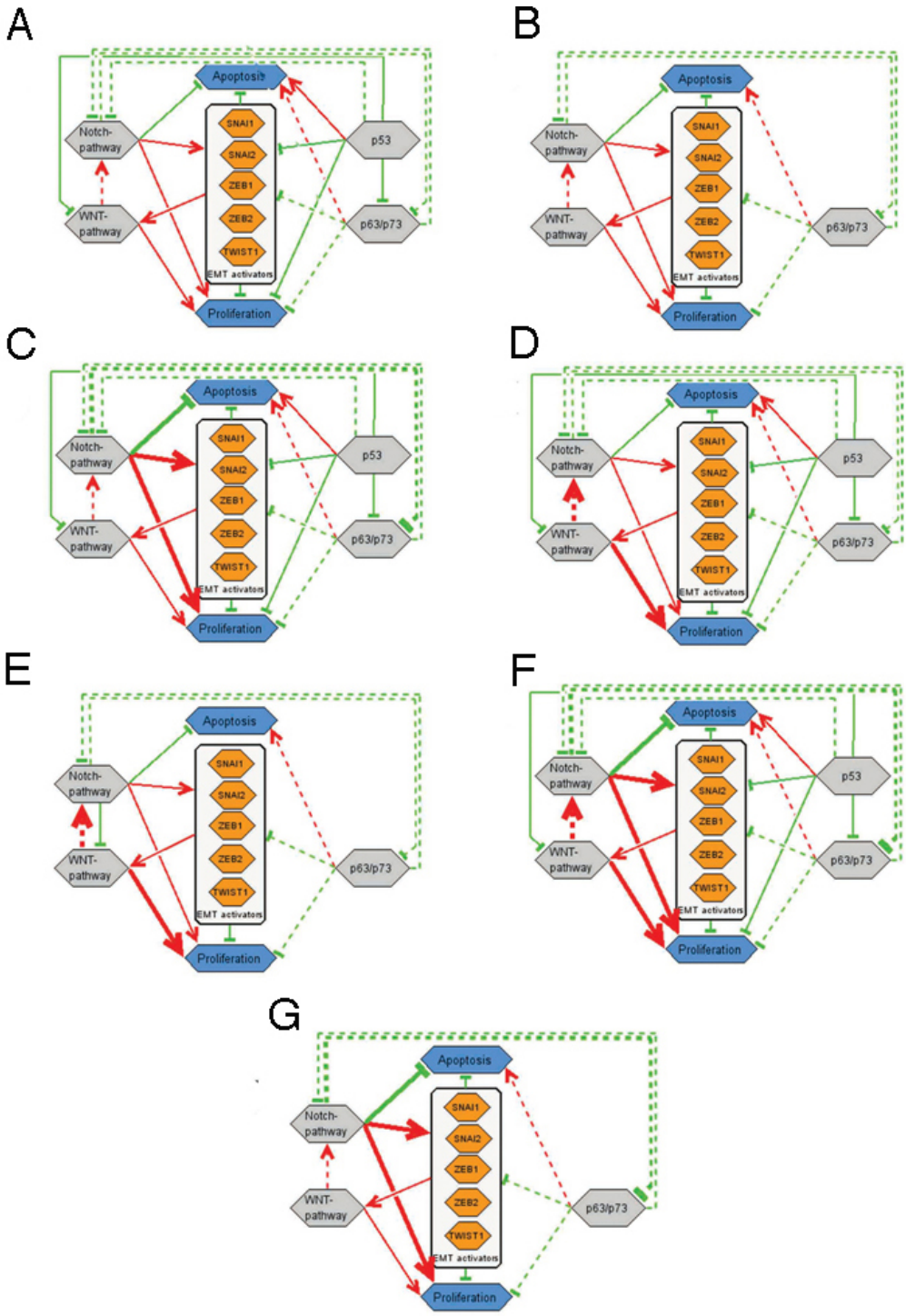


Supplementary Figure 1: Models of Notch-p53-Wnt signalling network and cell phenotypes.

A) Modular master model of signalling network of EMT regulation and cell phenotypes. Manually constructed network based on information extracted from 140 scientific papers. It contains about 400 chemical species. Regulatory circuits are formed by approximately 400 biochemical reactions with positive and negative regulatory loops. Modules: Apoptosis, AKT, Notch/p53, p53 specific, EMT, miRNAs, Differentiation and Phenotypes. The Notch-p53-Wnt signalling network is browsable in NaviCell tool for signaling maps navigation at **(https://navicell.curie.fr/pages/signalling_network_emt_regulation_description.html)**

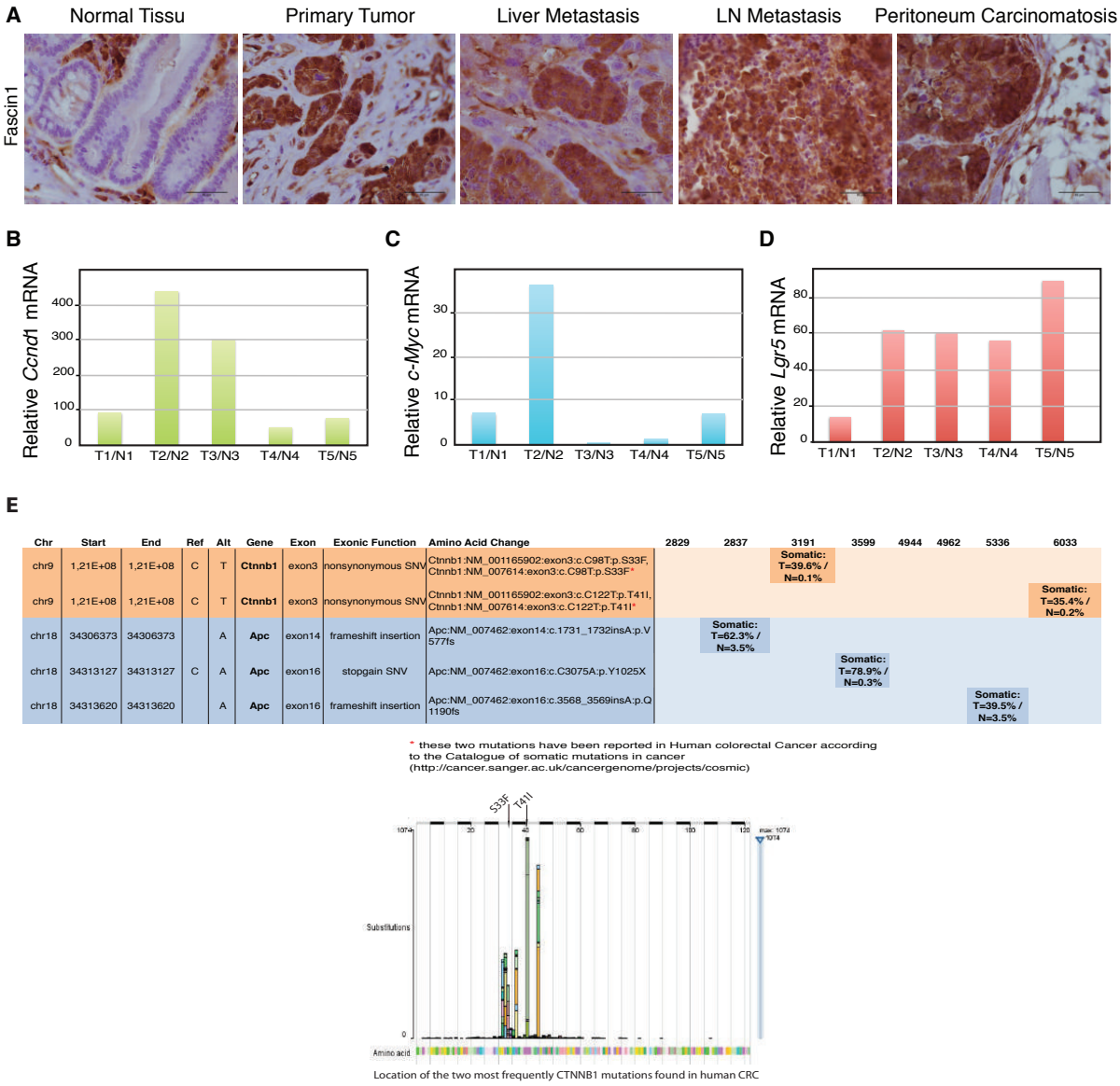
B) Model reduction level 1.

C) Model reduction level 3



Supplementary Figure 2: Molecular mechanisms of wild type and mutants predicted by signalling network analysis

Molecular mechanism of wild type and single and double mutants predicted by signalling network analysis shown on a level 3 model reduction-signalling network of Notch-p53-Wnt with cell phenotypes: Proliferation, EMT and Apoptosis. A) WT, B) *p53*$^{-/-}$ mutant, C) *NICD* mutant, D) *Apc*$^{-/-}$ mutant, E) *Apc*$^{-/-}$,*p53*$^{-/-}$ mutant, F) *NICD/Apc*$^{-/-}$ mutant, G) *NICD/p53*$^{-/-}$ mutant.

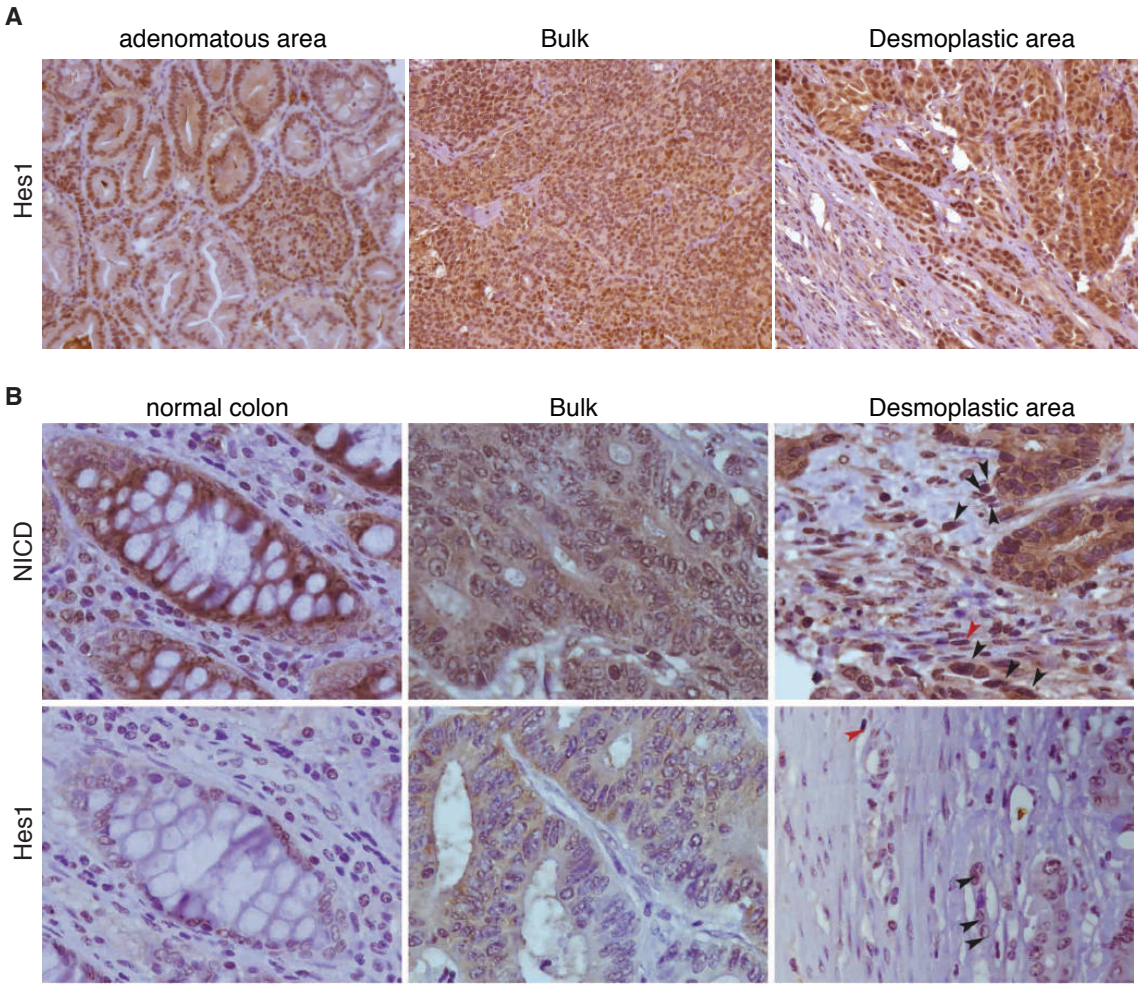


Supplementary Figure 3: Wnt pathway is altered in *NICD/p53*$^{-/-}$ primary tumours and metastases.

A) Immunohistochemical staining of Fascin1 showing its activation in cancer cells compared with normal epithelial tissues. Scale bars= 40 μm

B) C) and D) Quantification by real time qPCR of the Wnt target genes *Ccnd1* (B), *c-Myc* (C) and *Lgr5* (D) in 5 independent *NICD/p53*$^{-/-}$ primary tumours relative to adjacent normal tissue, showing up-regulation of Wnt-target genes in the tumours. Data were normalized using *b2-microglobulin* as reference gene.

E) Analysis by exome sequencing for the presence of mutations in *Ctnnb1* and *Apc* gene in 8 independent *NICD/p53*$^{-/-}$ primary tumours. The two mutations labelled with * have been reported as two hot spots of CTNNB1 mutations in human colorectal cancer according to the catalogue of somatic mutations in cancer (COSMIC, http://www.sanger.ac.uk/cosmic).



Supplementary Figure 4: Notch signals are activated in EMT-like cells invading stroma of mouse and human CCR.

A) Hes1 staining on NICD/p53-/- tumours confirms activation of Notch in the tumour cells.

B) Immunohistochemical stainings of NICD and Hes1 in human colorectal adenocarcinomas and adjacent normal tissues. *Upper panels*: NICD is lightly expressed in the nuclei of normal epithelial cells and in the bulk of the tumour. It is overexpressed in invading tumour cells undergoing EMT-like processes. *Lower panels*: nuclear expression of Hes1 in normal epithelial cells is lost in the bulk of the adenocarcinoma but turns back on in cells exiting the tumour in the desmoplastic area. Black arrows pinpoint isolating cells with an epithelial morphology; red arrows show isolated cells with fibroblast morphology.

Supplementary Table 1: Pathological characteristics of tumours in *NICD/p53*$^{-/-}$ mice

A) Histopathological analysis of 103 H&E primary tumours arising from 30 *NICD/p53*$^{-/-}$ mice analysing the type, the grade and the invasion status of the tumours. FD=focal dysplasia; MA= microadenoma; AD= adenoma; ISC= in situ carcinoma; MADK= microadenocarcinoma; ADK= adenocarcinoma. WD= well differentiated; MD= moderately differentiated; PD= poorly differentiated. pT0= no invasion; pT1= muscularis mucosa invasion; pT2= muscularis invasion; pT3= serosa invasion. Grading was determined for the 91 malignant tumours (CIS, MADK and ADK).

B) Characterisation of the most aggressive primary tumour, defined by its invasiveness. 17 over 30 analyzed animals have developed metastasis. 96.7% of animals develop adenocarcinoma invading the serosa. *: the overall vascular invasion is 9.7% (10/103 tumours), however 2 (2.1%) of the tumours are well-differentiated with low invasive potential and a further 2 presented poor differentiation with moderate invasion. Vascular invasion was not determined on 5 cases due to the exclusive presence of tumour cells on the examined slides.

C) Metastasis quantification and determination of metastasis differentiation status in the 30 animals.

a- Primary tumours (n = 103)					
Type		**Grade**		**Invasion**	
FD	5	WD	30	pT0	20
MA	5	MD	25	pT1	12
AD	2	PD	36	pT2	11
CIS	8			pT3	54
MADK	3				
ADK	80				

b- Most aggressive tumour per mice (n = 30)											
Location		**Size in mm²**		**Grade**		**Invasion**		**Desmoplasia**		**Vascular invasion**	
Duod	2	<100	9	WD	2	pT0	O	Low	11	observed	3/30
Jej	19	100-200	3	MD	8	pT1	1	Moderate	6		
Ileum	8	>200	17	PD	20	pT2	0	Severe	11		
Colon	1					pT3	29				

c- Metastases per mice (n = 30)					
Lymph node		**Distant organs (Liver)**		**Carcinosis**	
observed	6/30	observed	3/30	observed	15/30
WD	0	WD	0	WD	2
MD	2	MD	1	MD	3
PD	4	PD	2	PD	10

Supplementary Table 2: EMT array results with colour code and expected expression in EMT

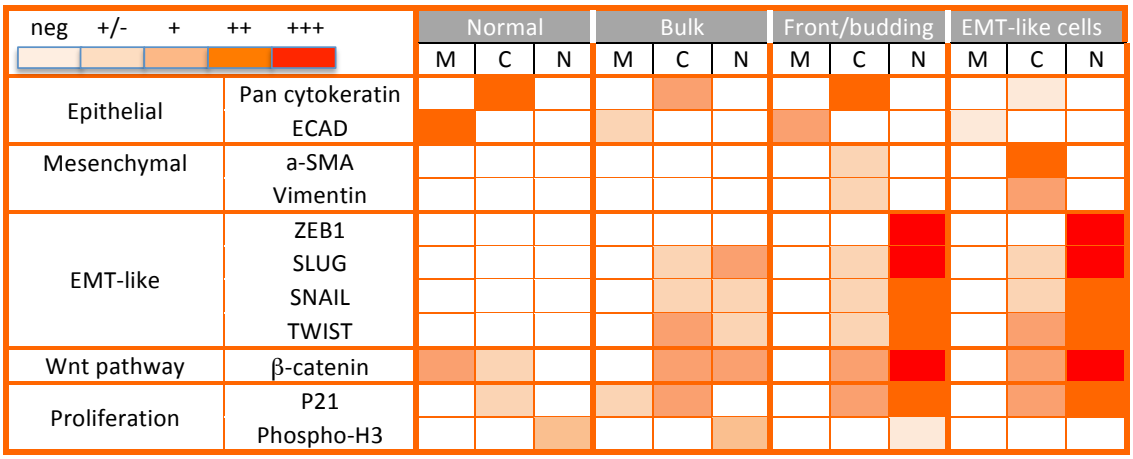
For each gene we report the result of Student's t-test, used to compare gene expression in tumour versus normal samples. The t-test values have been colour-coded with red indicating high relative expression in tumours versus samples and green indicating low relative expression. The last column specifies the expected expression of the gene during EMT.

HUGO	t-score	p-value	Expected EMT expression
Msn	4.723	0.007	Exp-EMT-up
Tcf7l1	3.686	0.032	NA
Igfbp4	3.669	0.034	Exp-EMT-up
Sparc	3.412	0.018	Exp-EMT-up
Tcf4	2.949	0.059	Exp-EMT-up
Bmp7	2.787	0.068	NA
Foxc2	2.615	0.079	Exp-EMT-up
Wnt5b	2.496	0.087	Exp-EMT-up
Mtap1b	2.454	0.09	NA
Twist1	2.441	0.091	Exp-EMT-up
Wnt11	2.366	0.099	NA
Cav2	2.341	0.098	Exp-EMT-down
Col5a2	2.309	0.102	Exp-EMT-up
Fn1	2.309	0.102	Exp-EMT-up
Mmp2	2.097	0.125	Exp-EMT-up
Col3a1	1.977	0.124	Exp-EMT-up
Timp1	1.971	0.143	Exp-EMT-up
Sip1	1.933	0.109	NA
Snai3	1.933	0.117	Exp-EMT-up
Tgfb2	1.889	0.149	NA
Mmp3	1.686	0.187	Exp-EMT-up
Wnt5a	1.678	0.19	Exp-EMT-up
Il1rn	1.622	0.203	Exp-EMT-down
Col1a2	1.603	0.207	Exp-EMT-up
Tmem132a	1.58	0.21	Exp-EMT-up
Notch1	1.578	0.212	NA
Mitf	1.574	0.208	Exp-EMT-down
Spp1	1.549	0.219	Exp-EMT-down
Zeb2	1.498	0.197	NA
Vcan	1.39	0.217	Exp-EMT-up
Snai2	1.387	0.259	Exp-EMT-up
Serpine1	1.375	0.219	Exp-EMT-up
Fzd7	1.332	0.269	NA
Tgfb1	1.325	0.277	NA
Tmeff1	1.305	0.283	NA
Bmp1	1.3	0.276	Exp-EMT-up
Mmp9	1.267	0.294	Exp-EMT-up
Cdh2	1.223	0.309	NA
Tgfb3	1.2	0.316	NA
Jag1	1.192	0.317	NA
Krt14	1.065	0.365	NA
Gsc	0.896	0.435	NA

(continue)			
HUGO	t-score	p-value	Expected EMT expression
Smad2	0.883	0.431	NA
Ilk	0.847	0.457	NA
Gng11	0.769	0.479	Exp-EMT-up
Zeb1	0.732	0.506	NA
Ptp4a1	0.653	0.557	NA
Esr1	0.605	0.579	NA
Krt7	0.583	0.585	NA
Tspan13	0.577	0.592	NA
Cald1	0.45	0.681	Exp-EMT-up
Gsk3b	0.326	0.755	NA
Vim	0.295	0.785	Exp-EMT-up
Rgs2	0.174	0.87	Exp-EMT-down
Egfr	0.139	0.894	NA
Stat3	0.123	0.907	NA
Itgav	-0.115	0.913	Exp-EMT-up
Camk2n1	-0.15	0.886	Exp-EMT-up
Ptk2	-0.187	0.858	NA
Akt1	-0.19	0.856	NA
Sox10	-0.268	0.803	Exp-EMT-up
Itga5	-0.374	0.73	Exp-EMT-up
Tfpi2	-0.447	0.674	Exp-EMT-down
Snai1	-0.469	0.659	Exp-EMT-up
Nodal	-0.996	0.393	NA
Steap1	-1.037	0.341	Exp-EMT-up
Plek2	-1.182	0.317	NA
Fgfbp1	-1.248	0.282	Exp-EMT-down
Itgb1	-1.282	0.248	NA
Ctnnb1	-1.397	0.244	NA
Dsp	-1.552	0.175	Exp-EMT-down
Pppde2	-1.556	0.183	NA
Pdgfrb	-1.572	0.167	NA
Nudt13	-1.633	0.155	Exp-EMT-down
Ahnak	-1.69	0.142	Exp-EMT-up
Cdh1	-1.723	0.137	Exp-EMT-down
Erbb3	-1.734	0.137	NA
Dsc2	-2.221	0.096	NA
Vps13a	-2.509	0.047	Exp-EMT-up
Ocln	-2.894	0.028	Exp-EMT-down
Mst1r	-3.812	0.011	NA
F11r	-5.472	0.002	NA
Rac1	-5.857	0.001	NA
Krt19	-6.86	0.003	Exp-EMT-down

Supplementary Table 3: IHC quantification and localisation in *NICD/p53*$^{-/-}$ tumours

This overview of the different IHC experiments highlights the overexpression of nuclear EMT-like related proteins and mesenchymal markers in the front of the tumours and in EMT-like cells. This is associated with a decrease in epithelial marker expression (M: membrane; C: cytoplasmic; N: nuclear staining).



Supplementary Table 4: Quantification of the triple immunofluorescence images presented in Figure 5 and Figure 6

This tables shows the total count of cells for each staining in each categories, leading to the figure 5C and 6F. Statistical analysis was performed on this contingency table using a chi-square test. P-value denotes the probability of rejecting the hypothesis of independence.
Images on which the quantification was performed are accessible on https://cid.curie.fr , login readerNCom password Welcome!1

among GFP+ only — Figure 5C left

	CK+	CK-	total
SMA+	6	126	*132*
SMA-	537	18	*555*
total	*543*	*144*	687

	E+	E-	total
ZEB1+	4	241	*245*
ZEB1-	425	21	*446*
total	*429*	*262*	691

	E+	E-	total
VIM+	30	101	*131*
VIM-	356	5	*361*
total	*386*	*106*	492

among GFP+ only — Figure 5C right

	ZEB+	ZEB-	total
SMA+	60	8	*68*
SMA-	72	128	*200*
total	*132*	*136*	268

among GFP+ only — Figure 6 F

	E+	E-	total
Ph3+	71	6	*77*
Ph3-	514	268	*782*
total	*585*	*274*	859