Supplemental Material to:

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Global hypomethylation and promoter methylation in small intestinal neuroendocrine tumors: An in vivo and in vitro study

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Supplementary Table S1. Methylation index (MetI) in SI-NET tumors and cell lines.

Tumor	**Gene specific promoter methylation**								**Global methylatio**	
sample	***WIF1***	***RASSF1A***	***CTNNB1***	***CXCL14***	***NKX2-3***	***P16***	***LAMA1***	***CDH1***	**ELISA**	**LINE1**
number	**MetI %**	**MetI %**	**MetI %**	**MetI %**	**MetI %**	**MetI %**	**MetI %**	**MetI %**		**MetI %**
SI-NET tumors										
1M	43	**20**	**19**	11	5	**33**	**17**	10	1.8	**63**
2M	16	**69**	4	9	4	NA	10	**22**	1.4	**71**
3P	**75**	3	**17**	12	**14**	2	13	8	NA	**54**
4P	36	**52**	**19**	**28**	**14**	2	15	8	NA	**61**
5M	**61**	**19**	8	11	**26**	2	9	7	**1.2**	**59**
6M	**72**	**37**	**17**	**39**	**16**	2	6	9	**1.0**	**55**
7P	**60**	**12**	10	**33**	**14**	2	8	12	**0.8**	**63**
8P	41	1	13	13	**10**	2	**19**	**18**	**0.7**	**68**
9M	**72**	**39**	**22**	10	**16**	**12**	9	3	1.3	**59**
10P	**92**	**22**	5	8	5	2	6	4	NA	**62**
11P	48	**13**	8	12	7	1	9	8	**0.9**	**58**
12P	**53**	4	12	8	**17**	2	7	6	**0.8**	**56**
13P	33	7	8	**16**	4	3	7	6	NA	**66**
14P	45	**38**	**25**	13	**28**	2	10	7	NA	**70**
15P	**68**	**18**	**24**	12	5	NA	10	6	NA	**68**
16P	19	**13**	9	7	**13**	2	7	5	NA	**68**
17P	**51**	6	8	13	**11**	2	**16**	**15**	NA	**69**
18P	**63**	**20**	10	11	**10**	2	**16**	8	NA	**59**
19P	32	6	10	7	5	4	12	8	NA	**66**
20P	26	1	**18**	8	2	2	14	**17**	**1.3**	76
20M	24	2	11	10	3	5	12	**19**	1.7	**74**
21M1	**55**	**25**	5	3	6	3	5	3	**0.7**	**57**
21M2	49	**21**	10	5	9	2	7	7	NA	**54**
22M	36	2	**24**	8	5	2	9	6	NA	76
23P	**72**	9	7	9	7	4	10	7	**0.6**	**66**
23M	**76**	5	7	6	7	3	8	6	**0.3**	**69**
24M1	**74**	3	6	**33**	**14**	4	11	4	NA	**61**
24M2	**50**	5	6	**36**	9	4	10	4	NA	**48**
25	**65**	**29**	7	**18**	4	9	9	8	NA	**68**
26P	43	**3**	6	15	**7**	2	11	4	**1.0**	**64**

26M	**55**	**24**	**24**	11	**17**	5	7	7	**0.9**	**68**
27P	42	3	**16**	14	6	4	13	14	**1.2**	**74**
27M	50	5	10	12	**19**	1	7	**10**	**0.7**	**67**
28P	**77**	**15**	10	5	3	4	4	3	**0.8**	**63**
28M	42	**7**	**19**	9	7	3	9	3	**0.9**	**69**
29P	**52**	2	8	9	5	1	7	7	NA	**63**
29M	34	1	**16**	10	5	2	15	**3**	NA	**70**
30P	**61**	3	4	**21**	**11**	3	4	5	**0.7**	**64**
30M	**54**	8	10	15	**17**	2	7	7	1.4	**67**
31P	32	**47**	**34**	12	3	**24**	5	5	1.7	**59**
31M	36	**13**	**20**	5	4	1	5	4	1.4	**62**
32P	34	9	11	**21**	3	7	**23**	8	1.8	**67**
32M	33	6	9	**19**	3	1	**24**	4	1.8	**64**
33M	41	**58**	10	15	**28**	NA	12	8	1.4	**74**
SI-NET cell lines										
HC45	38	2	7	6	**13**	7	5	**51**	NA	**55**
CNDT2	**99**	1	**44**	**97**	**95**	**96**	**86**	**88**	NA	**74**

NA = Not Assessed

Supplementary Table S2. Correlations between methylation index (MetI) for individual gene promoters and global methylation

		Gene specific promoter methylation								Global methylation	
Assay	value	*WIF1*	*RASSF1A*	*CTNNB1*	*CXCL14*	*NKX2-3*	*P16*	*LAMA1*	*CDH1*	LINE1	ELISA
WIF1	r	1	n.s.	n.s.	n.s.	**0.451**	n.s.	-0.279	**-0.402**	**-0.47**	-0.491
	P					**<0.001**		0.027	**0.001**	**<0.001**	0.003
RASSF1A	r	n.s.	1	n.s.	n.s.	n.s.	0.42	n.s.	n.s.	-0.292	n.s.
	P						0.002			0.026	
CTNNB1	r	n.s.	n.s.	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	P										
CXCL14	r	n.s.	n.s.	n.s.	1	n.s.	n.s.	**0.42**	0.328	n.s.	n.s.
	P							**0.001**	0.009		
NKX2-3	r	**0.451**	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	n.s.	n.s.
	P	**<0.001**									
P16	r	n.s	0.42	n.s.	n.s.	n.s.	1	n.s.	n.s.	n.s.	n.s.
	P		0.002								
LAMA1	r	-0.279	n.s.	n.s.	**0.42**	n.s.	n.s.	1	**0.582**	**0.51**	0.498
	P	0.027			**0.001**				**<0.001**	**<0.001**	0.002
CDH1	r	**-0.402**	n.s.	n.s.	0.328	n.s.	n.s.	**0.582**	1	**0.574**	n.s.
	P	**0.001**			0.009			**<0.001**		**<0.001**	
LINE1	r	**-0.47**	-0.292	n.s.	n.s.	n.s.	n.s.	**0.51**	**0.574**	1	n.s.
	P	**<0.001**	0.026					**<0.001**	**<0.001**		
ELISA	r	-0.491	n.s.	n.s.	n.s.	n.s.	n.s.	0.498	n.s.	n.s.	1
	P	0.003						0.002			

n.s. = not significant (*P*-value >0.05)

Statistically significant correlations (*P*-value ≤0.001, bold) and suggestive correlations (*P*-values ≤0.05) are indicated

Supplementary Table S3. Clinical data for the 33 patients with SI-NET in the study.

Case	Sample(s)	Age at	Gender	Sporadic	Metastatic disease			Follow-up	
no.	studied	diagnosis	F / M	Familial	liver	lymph	other	outcome	months
1	Dist met	63	F	Sporadic	x	x		Dead	-
2	Dist met	59	F	Sporadic	x	x		Dead	222
3	Prim	74	M	Sporadic	x	x		Dead	-
4	Prim	57	F	Sporadic	x	x		Dead	-
5	Dist met	69	M	Sporadic	x	x		Dead	155
6	Dist met	73	F	Sporadic	x	x		Dead	12
7	Prim	44	F	Sporadic	x	x		Dead	98
8	Prim	69	M	Sporadic	x	x		Dead	15
9	Dist met	65	F	Sporadic	x	x		Alive	218
10	Prim	71	M	Sporadic	x	x	x	Dead	155
11	Prim	75	F	Sporadic		x		Dead	124
12	Prim	39	M	Sporadic	x	x		Alive	196
13	Prim	50	M	Sporadic	x	x		Dead	44
14	Prim	46	F	Sporadic	x	x		Dead	86
15	Prim	77	M	Sporadic	x	x		Dead	7
16	Prim	71	M	Sporadic	x			Alive	185
17	Prim	63	M	Sporadic		x		Alive	175
18	Prim	64	F	Sporadic	x	x		Dead	124
19	Prim	80	M	Sporadic	x	x		Dead	69
20	Prim + Reg met	52	M	Sporadic	x	x		Alive	93
21	Dist met x 2	76	F	Sporadic		x	x	Dead	64
22	Reg met	71	M	Sporadic	x	x		Dead	19
23	Prim + Reg met	65	F	Sporadic	x	x		Alive	97
24	Reg met x 2	78	F	Sporadic	x	x		Dead	53
25	Prim	61	M	Sporadic	x	x	x	Alive	88
26	Prim + Reg met	66	F	Sporadic	x	x		Dead	35
27	Prim + Reg met	74	F	Sporadic	x	x	x	Dead	73
28	Prim + Reg met	78	F	Sporadic	x	x		Dead	84
29	Prim + Reg met	51	M	Sporadic		x		Alive	57
30	Prim + Reg met	69	M	Sporadic		x		Alive	68
31	Prim + Reg met	75	F	Sporadic	x	x		Alive	61
32	Prim + Reg met	66	M	Sporadic	x	x	x	Alive	57
33	Reg met	74	F	Familial		x		Alive	28

Prim = Primary; Reg met = Regional metastasis (including mesenterial, regional lymph node, omentum)

Dist met = Distant metastasis (including liver and ovarial metastasis)

Supplementary Table S4. Genotypes obtained at STR profiling of the HC45 and CNDT2 cell lines.

STR marker	Genotypes	
locus	HC45	CNDT2
D8S1179	13 / 16	9 / 13 / 14
D21S11	29 / 32.2	27 / 31
D7S820	8 / 8	8 / 10
CSF1PO	9 / 11	8 / 11
D3S1358	14 / 17	16 / 19
TH01	6 / 7	6 / 10
D13S317	10 / 11	8 / 11
D16S539	12 / 12	12 / 13
D2S1338	23 / 25	16 / 16
D19S433	12 / 15	14 / 14
vWA	15 / 16	17 / 18 / 25
TPOX	8 / 12	10 / 11
D18S51	16 / 16	11 / 13
AMEL	X / X	X / X
D5S818	12 / 13	11 / 14
FGA	21 / 24	20 / 22

STR = Short tandem repeat

Supplementary Table S5. Details of assays used for Pyrosequencing and qRT-PCR.

Assay Gene	Gene name	Purchased from	Assay ID or catalogue no.	No. of CpGs
Methylation by Pyrosequencing				
APC	Adenomatous polyposis coli	Biomeres	00108429-1	10
CDH1	Cadherin 1	Qiagen	Hs_CDH1_02_PM	5
CDH3	Cadherin 3	Qiagen	Hs_CDH3_04_PM	3
CTNNB1	Catenin beta 1	Qiagen	Hs_CTNNB1_01_PM	4
CXCL14	Chemokine C-X-C motif ligand 14	Qiagen	Hs_CXCL14_02_PM	4
HIC1	Hypermethylated in cancer 1	Qiagen	Hs_HIC1_05_PM	4
LAMA1	Laminin alpha 1	Qiagen	Hs_LAMA1_02_PM	7
NKX2-3	NK2 homeobox 3	Qiagen	Hs_NKX2-3_01_PM	4
P14	Cyclin-dependent kinase inhibitor 2A	Biomeres	00103418-9	13
P14	Cyclin-dependent kinase inhibitor 2A	Biomeres	00078588-12	7
P14	Cyclin-dependent kinase inhibitor 2A	Qiagen	Hs_CDKNA_02_PM*	6
RASSF1A	Ras association domain family member 1	Biomeres	00103418-3	5
RASSF1A	Ras association domain family member 1	Qiagen	Hs_RASSF1A_03_PM**	5
SMAD2	SMAD family member 2	Qiagen	Hs_SMAD2_02_PM	4
SMAD4	SMAD family member 4	Qiagen	Hs_SMAD4_01_PM	7
WIF1	WNT inhibitor factor 1	Qiagen	Hs_WIF1_01_PM	2
LINE1	Long INterspersed Elements 1	Biomeres	970042	3
Expression by qRT-PCR				
CDH1	Cadherin 1	ABI	Hs01023894_m1	
CTNNB1	Catenin beta 1	ABI	Hs00355049_m1	
CXCL14	Chemokine C-X-C motif ligand 14	ABI	Hs01557413_m1	
LAMA1	Laminin alpha 1	ABI	Hs00300550_m1	
NKX2-3	NK2 homeobox 3	ABI	Hs00414553_g1	
P16	Cyclin-dependent kinase inhibitor 2A	ABI	Hs00923894_m1	
RASSF1A	Ras association domain family member 1	ABI	Hs00200394_m1	
WIF1	WNT inhibitor factor 1	ABI	Hs00183662_m1	
WIF1	WNT inhibitor factor 1	ABI	Hs01548031_m1	
ACTB	Actin, beta	ABI	Hs99999903_m1	

P14 and *P16* are from the *CDKN2A* locus; ABI = Applied Biosystems

*used for cell lines ** used for cell lines and pooled blood samples