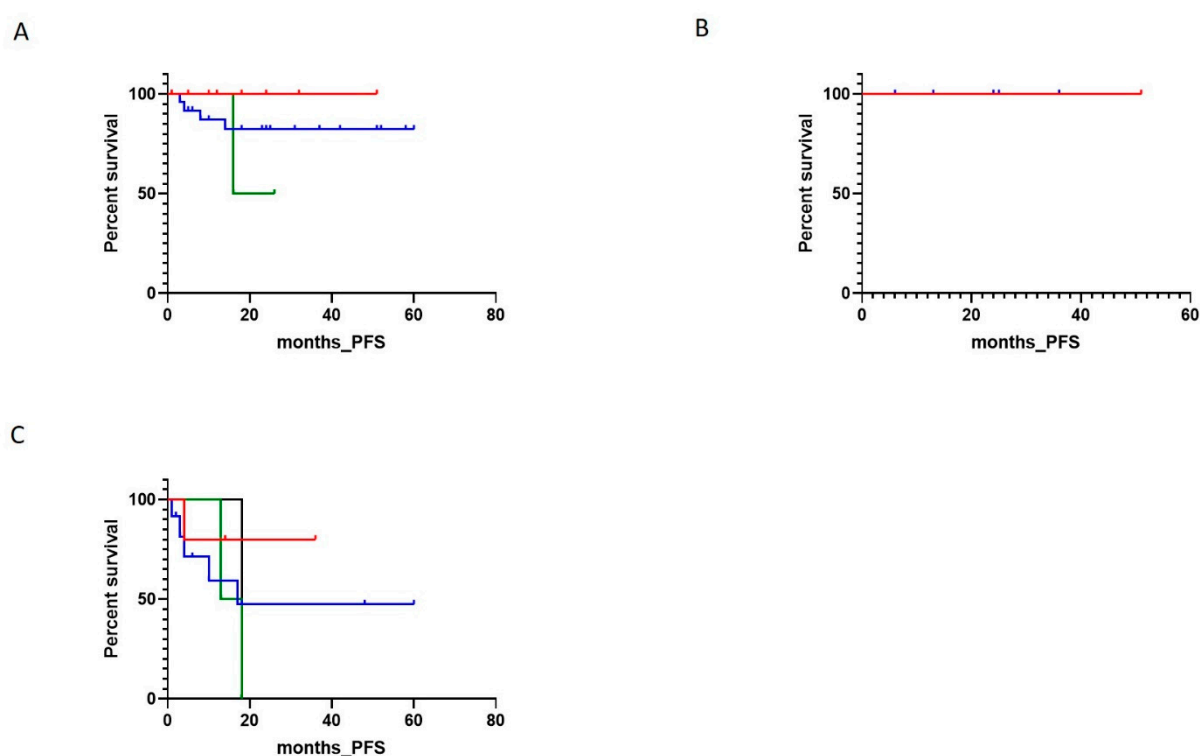
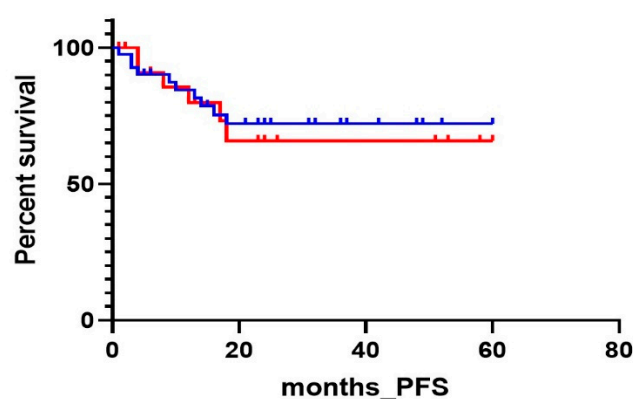


# Supplementary Material: Neonatal Nav1.5 Protein Expression in Human Colorectal Cancer: Immunohistochemical Characterization and Clinical Evaluation

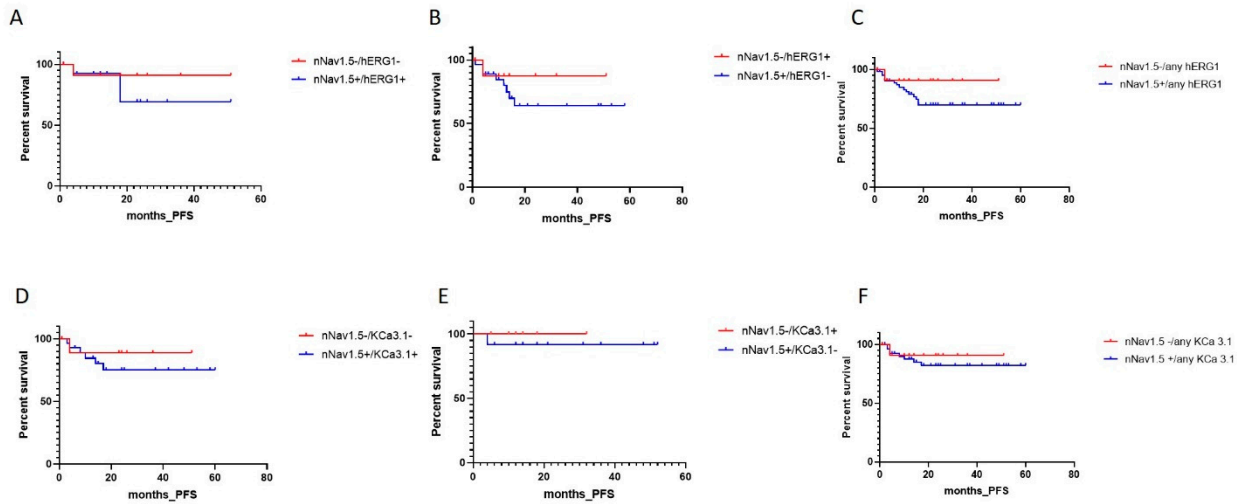
Elena Lastraioli, Scott P. Fraser, R. Mine Guzel, Jessica Iorio, Lapo Bencini, Emanuela Scarpi, Luca Messerini, Vincenzo Villanacci, Giulia Cerino, Niccolò Ghezzi, Giuseppe Perrone, Mustafa B. A. Djamgoz and Annarosa Arcangeli



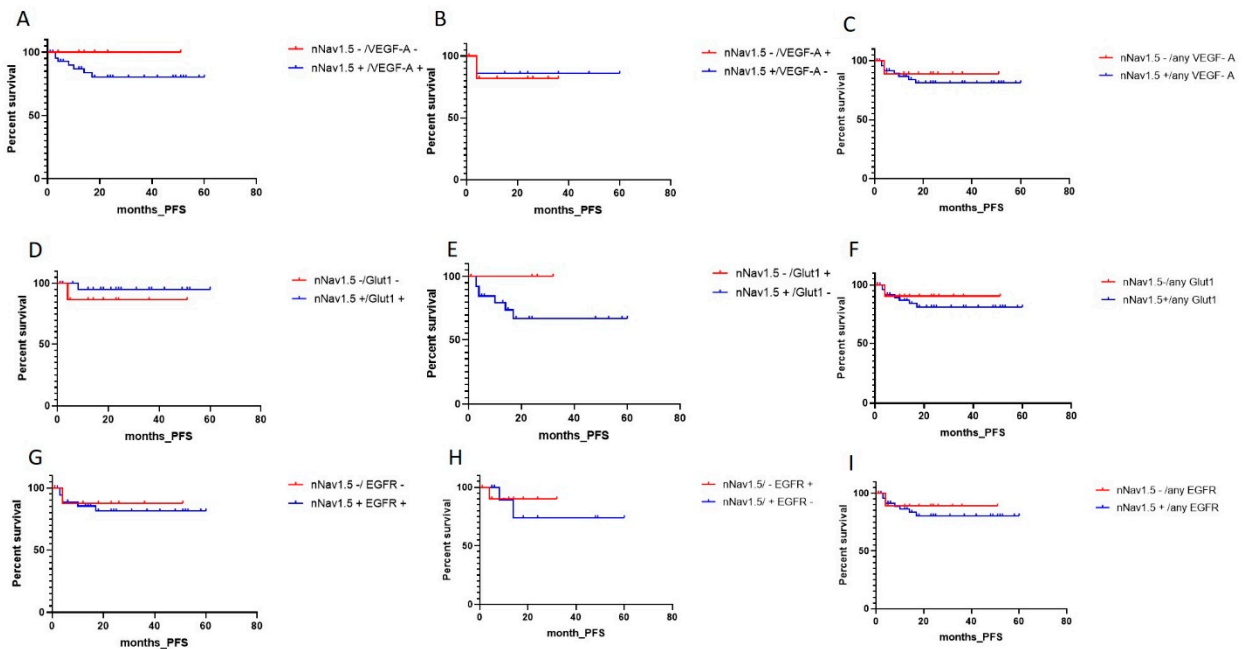
**Figure S1.** Kaplan-Meier plots of PFS according to nNav1.5 scoring at different localisations in colon. A, right colon; B, transverse colon; and C, rectum. The data for the scores are denoted with different colours: Score 0 (red); Score 1 (blue); Score 2 (green) and Score 3 (black).



**Figure S2.** Kaplan-Meier plot of PFS according to the pattern of nNav1.5 expression for all TNM stages. Red lines, delineated expression; blue lines, diffuse expression.



**Figure S3.** Kaplan-Meier plots of PFS according to the combined expression of nNav1.5 and hERG1 (A-C); and nNav1.5 and KCa3.1 (D-F).



**Figure S4.** Kaplan-Meier plots of PFS according to expression of nNav1.5 in combination with VEGF-A (A-C); Glut1 (D-F); and EGFR (G-I).

**Table S1.** Quantitative data and statistical analysis from the immunohistochemistry of nNav1.5 expression in adenomas with low- and high-grade dysplasia (Mann Whitney Test). Data are shown as mean  $\pm$  SEM. Symbols defined in main text.

Adenoma	Parameter	P value
Low- grade dysplasia (LGD-A)	Extent, E	61.50 $\pm$ 6.78
	Staining Intensity, SI	2.05 $\pm$ 0.22
	Median Total Staining, TS	152.50 $\pm$ 20.44
	Median Delineating Factor, DF	0
	Median Extent, E	69.00 $\pm$ 6.96
High- grade dysplasia (HGD-A)	Median Staining Intensity, SI	2.20 $\pm$ 0.21
	Median Total Staining, TS	176.50 $\pm$ 22.77
	Median Delineating Factor, DF	0

**Table S2.** Association between nNav1.5 expression and other molecular biomarker expression in CRCa biopsy tissues (Chi-Square or Fisher Exact Test). Statistically significant associations (p<0.05) are highlighted in bold.

		nNav1.5 negative	nNav1.5 positive	P value
<b>hERG1</b>	<i>negative</i>	25 (38.46)	40 (61.54)	<b>0.046</b>
	<i>positive</i>	18 (22.78)	61 (77.22)	
<b>KCa3.1</b>	<i>negative</i>	26 (47.27)	29 (52.73)	<b>&lt;0.001</b>
	<i>positive</i>	11 (15.07)	62 (84.93)	
<b>CA IX</b>	<i>negative</i>	12 (21.05)	45 (78.95)	0.199
	<i>positive</i>	10 (34.48)	19 (65.52)	
<b>VEGF-A</b>	<i>negative</i>	8 (47.06)	9 (52.94)	<b>0.032</b>
	<i>positive</i>	14 (20.29)	55 (79.71)	
<b>Glut1</b>	<i>negative</i>	18 (33.33)	36 (66.67)	<b>0.041</b>
	<i>positive</i>	4 (12.50)	28 (87.50)	
<b>Ki67</b>	<i>negative</i>	20 (27.40)	53 (72.60)	1.000
	<i>positive</i>	0 (0.00)	2 (100.00)	
<b>P53</b>	<i>negative</i>	15 (28.85)	37 (71.15)	0.611
	<i>positive</i>	7 (21.88)	25 (78.13)	
<b>Bcl2</b>	<i>negative</i>	19 (27.14)	51 (72.86)	1.000
	<i>positive</i>	51 (72.86)	5 (83.33)	
<b>EGFR</b>	<i>negative</i>	10 (43.48)	12 (20.00)	<b>0.050</b>
	<i>positive</i>	13 (56.52)	48 (80.00)	

**Table S3.** Associations between nNav1.5 staining pattern and clinicopathological parameters. Case numbers are shown with the percentage in brackets (Chi-Square or Fisher Exact Test). Statistically significant associations (p<0.05) are highlighted in bold.

		nNav1.5 Negative	nNav1.5 Apical/Basal	nNav1.5 All-over	P value
<b>Gender</b>	<i>Female</i>	27 (31.03)	24 (27.59)	36 (41.38)	0.836
	<i>Male</i>	25 (31.25)	25 (31.25)	30 (37.50)	
<b>Localisation</b>	<i>Right colon</i>	33 (40.74)	17 (20.99)	31 (38.27)	<b>0.027</b>
	<i>Transverse</i>	0 (0.00)	<b>6 (60.00)</b>	4 (40.00)	
	<i>Left colon</i>	12 (24.49)	19 (38.78)	18 (36.73)	
	<i>Rectum</i>	7 (25.93)	7 (25.93)	13 (48.15)	
<b>Grading</b>	<i>G1</i>	5 (55.56)	2 (22.22)	2 (22.22)	0.325
	<i>G2</i>	43 (31.39)	43 (31.39)	0 (0.00)	
	<i>G3</i>	1 (25.00)	0 (0.00)	3 (75.00)	
<b>TNM stage</b>	<i>I</i>	11 (37.93)	11 (37.93)	7 (24.14)	0.510
	<i>II</i>	13 (24.53)	16 (30.19)	24 (45.28)	
	<i>III</i>	20 (35.09)	15 (26.32)	22 (38.60)	
	<i>IV</i>	8 (28.57)	7 (25.00)	13 (46.43)	
<b>Metastases</b>	<i>No</i>	43 (31.39)	41 (29.93)	53 (38.69)	0.905
	<i>Yes</i>	9 (30.00)	8 (26.67)	13 (43.33)	

**Table S4.** Bivariate analyses of the co-expression of nNav1.5 and other biomarkers (log-rank tests). Only nNav1.5 + Glut1 co-expression reached statistical significance (p<0.05), highlighted in bold.

Co-expression	Number of cases ('events')	P value
<b>nNav1.5- / hERG1-</b>	1	0.463
<b>nNav1.5- / hERG1+</b>	2	
<b>nNav1.5+ / hERG1-</b>	10	
<b>nNav1.5+ / hERG1+</b>	10	
<b>nNav1.5- / KCa3.1-</b>	1	0.765
<b>nNav1.5- / KCa3.1+</b>	1	
<b>nNav1.5+ / KCa3.1-</b>	2	
<b>nNav1.5+ / KCa3.1+</b>	8	
<b>nNav1.5- / CA IX-</b>	2	0.654
<b>nNav1.5- / CA IX +</b>	1	
<b>nNav1.5+ / CA IX -</b>	9	
<b>nNav1.5+ / CA IX +</b>	1	
<b>nNav1.5- / VEGF A-</b>	1	0.940
<b>nNav1.5- / VEGF A+</b>	2	
<b>nNav1.5+ / VEGF A-</b>	1	
<b>nNav1.5+ / VEGF A+</b>	9	
<b>nNav1.5- / Glut1-</b>	3	<b>0.043</b>
<b>nNav1.5- / Glut1+</b>	0	
<b>nNav1.5+ / Glut1 -</b>	9	
<b>nNav1.5+ / Glut1 +</b>	1	