

# Supplementary Materials: Mu Opioid Receptor 1 (MOR-1) Expression in Colorectal Cancer and Oncological Long-Term Outcomes: A Five-Year Retrospective Longitudinal Cohort Study

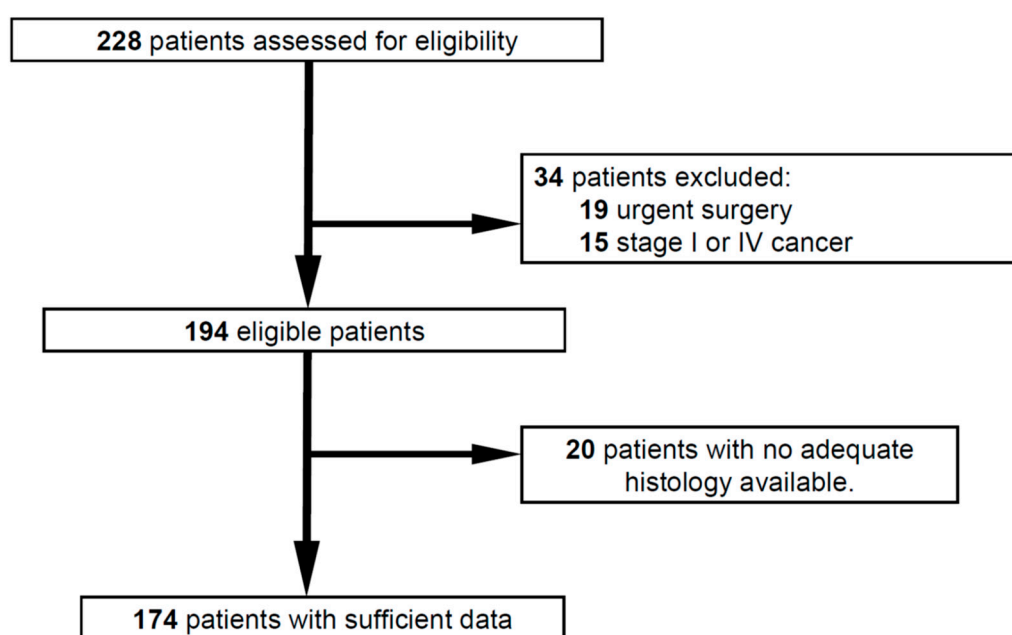


Figure S1. Patients' flowchart.

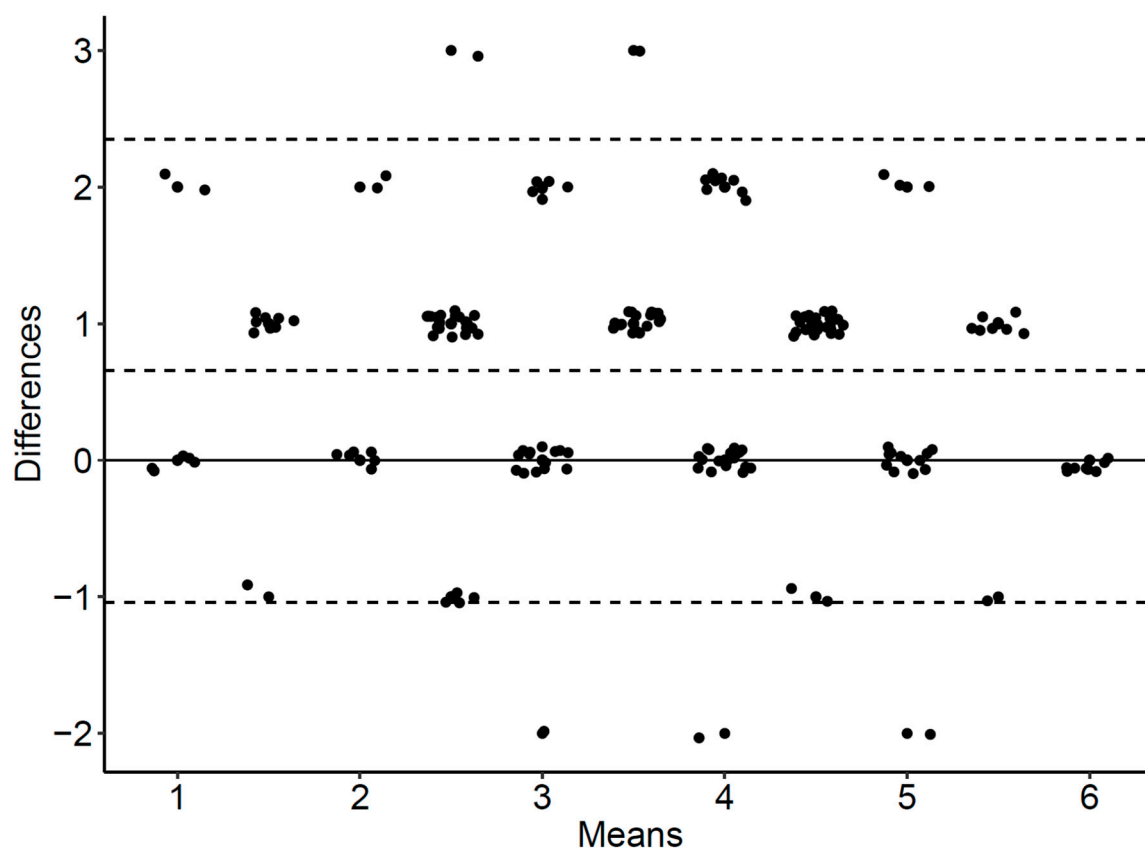
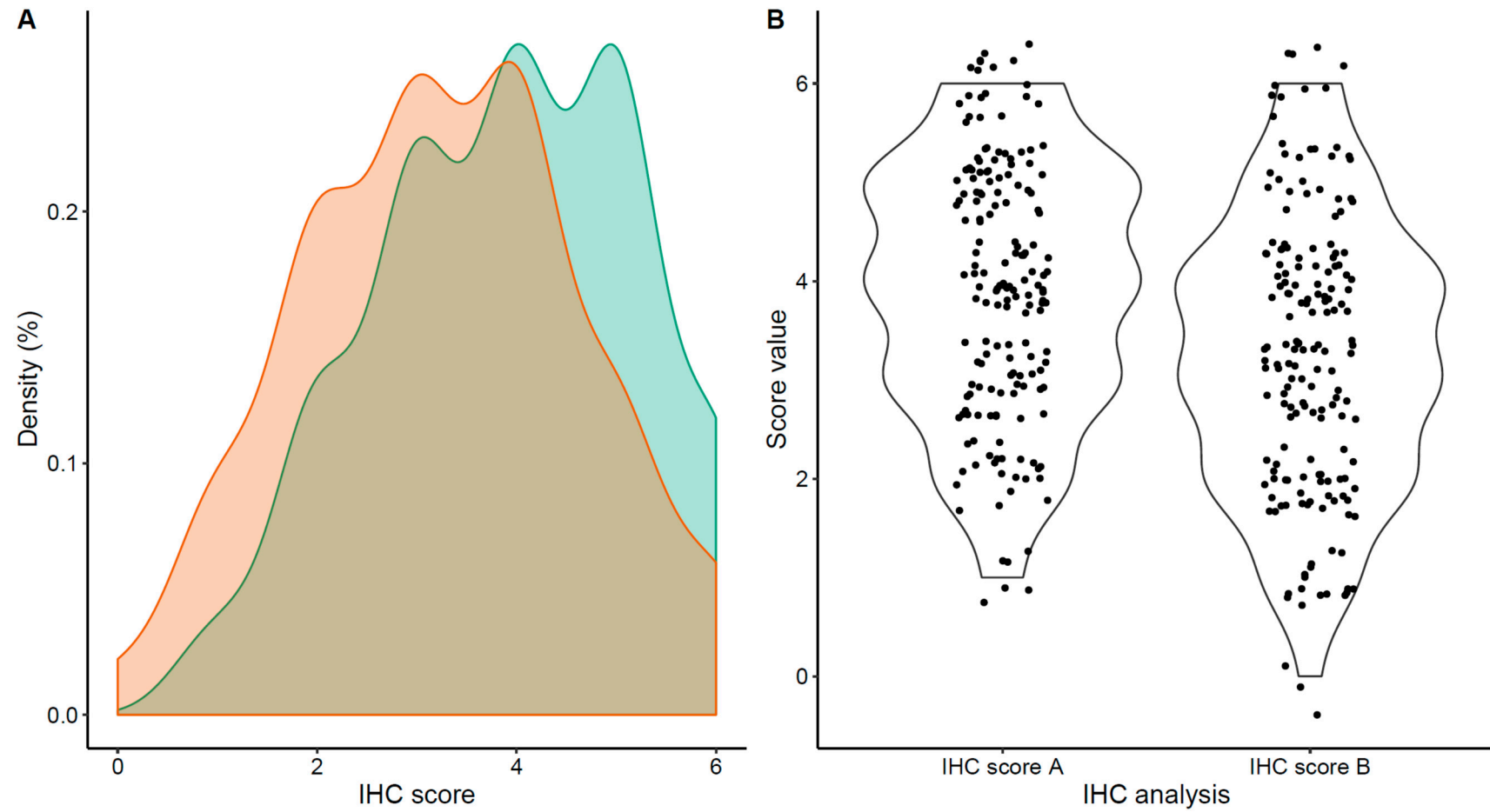


Figure S2. Bland–Altman plot of the agreement between two independent readings.



**Figure S3.** MOR-1 expression scoring: (a) Probability density plot of MOR-1 score, Green: IHC score A, Orange: IHC score B; (b) Scatterplot and Violin plot of score distribution by analysis.

**Table S1.** Association between Mu–opioid receptor expression score and oncological features.

Characteristics	N	Correlation	Lower– Upper 95% CI	P value
<b>Ca 19–9 (U·mL<sup>-1</sup>)</b>	124	–0.07*	–0.26 – 0.12	0.448
<b>CEA (U·mL<sup>-1</sup>)</b>	163	0.05*	–0.09 – 0.22	0.497
<b>Number of positive lymph nodes</b>	174	0.15*	0.003 – 0.29	0.044
<b>Duke stage</b>		0.19**	0 – 0.31	0.119
A	2			
B	78			
C	71			
D	2			
<b>Cancer stage</b>		0.15**	0 – 0.28	0.053
II	96			
III	78			
<b>Resection margin</b>		0.008**	0 – 0.13	0.926
R+	25			
R0	110			
<b>Tumoral tissue differentiation</b>		0.06**	0 – 0.18	0.717
Poor/Undifferentiated	20			
Moderately differentiated	132			
Well differentiated	17			

*Ca 19-9*, Gastrointestinal Cancer Antigen; *CEA*, Carcinoembryonic antigen; *R+*, positive margin; *R0*, negative margin; *CI*, Confidence interval. \* Spearman correlation coefficient. \*\* square root of ANOVA  $\eta^2$

**Table S2.** Multivariable logistic regression model for complications at 28 postoperative days.

	Odds Ratio	Lower – Upper 95%CI	P value
<b>MOR expression tumor tissues</b>	0.711	0.340 – 1.310	0.305
<b>MOR expression non–tumour tissue</b>	0.555	0.188 – 1.400	0.237
<b>First postoperative 96 hours opioid dose (morphine equivalents)</b>	1.012	0.989 – 1.039	0.342
<b>First postoperative 96 hours transfusion (yes)</b>	0.819	0.123 – 5.055	0.828
<b>Gender (Female)</b>	0.391	0.052 – 2.100	0.299
<b>ASA (Reference category = 1)</b>			
2	–0.147	–2.438 – 1.510	0.996
≥3	0.224	–2.066 – 2.395	0.995
<b>Surgery duration (minutes)</b>	1.004	0.991 – 1.018	0.539
<b>Arterial Hypertension (yes)</b>	4.383	0.717 – 42.894	0.141
<b>Number of affected lymph nodes</b>	3.610	1.546 – 10.789	0.008
<b>Serum total proteins (g·dL<sup>-1</sup>)</b>	1.024	0.832 – 1.920	0.879
<b>Smoker (yes)</b>	0.779	0.026 – 9.585	0.86
<b>Age (years)</b>	1.007	0.921 – 1.107	0.878

*MOR*, Mu–opioid receptor; *ASA*, American Society of Anesthesiology; *CI*, Confidence Interval.

**Table S3.** Study's REMARK profile.

<b>A. Patients, treatment and variables</b>	
Study and marker	Remarks
Marker	MOR-1: Immunohistochemical cell staining assessed on an ordinal scale from 0 to 6 (see Table 3) in tumour tissue
Further variables	v1= Gender v2= Anaesthetic agent (intravenous vs. halogenated) v3= Complication in the first 28 postoperative days (yes/no) v4= Amount of opioid drugs administered in the first 96 postoperative hours (in morphine equivalents) v5= Blood transfusion in the first 96 postoperative hours v6= Epidural analgesia (yes/no) v7= neo-adjuvant radiotherapy v8 = Neo-adjuvant chemotherapy v9 = Adjuvant chemotherapy v10= Adjuvant radiotherapy v11= ASA risk score v12 = Duration of surgery v13= Preoperative Haemoglobin value v14= Stage III cancer (%) v15 = Reintervention v16= MOR-1 expression in non-tumor tissue v17= Carcinoembryonic antigen value at diagnosis (log scale) v18= Number of positive lymph nodes (log scale) v19= Age v20 = Arterial Hypertension v21= Diabetes v23= Smoker

## v24= Preoperative Plasma Total Protein

Patients	N	Remarks
Assessed for eligibility		<i>Disease:</i> Colorectal cancer stage II and III.
	228	<i>Patients:</i> Surgery 2010 to 2013, Hospital Universitario la Fe, Valencia, Spain. <i>Sample:</i> Archived specimens available.
Excluded	54	
Included	174	
With outcome events	174	

**B. Statistical analyses of outcomes**

Analysis	Patients	Events	Variable considered	Results/Remarks
A1: MOR-1 expression in tumour and non-tumour tissue	174	-	MOR-1 , v18	Text (Page 5,Line 76)
A2: DFS Univariable	174	40	MOR-1	Text (Page 4,Line 88)
A3: DFS Multivariable. Variable selection process	109	25	MOR-1,v1 to v24	Elastic net with hyperparameters estimated by cross validation $\alpha = 0.8 \lambda = 0.1$
A4a: Adjusted Effect of MOR -1on DFS	135	30	MOR-1,v5,v11,v13,v17 to v19	Table 2
A4b: Adjusted Effect of MOR-1 on DFS with missing values imputation	174	40	MOR-1,v5,v11,v13,v17 to v19	Table 2
A5: OS Univariable	174	40	MOR-1	Text (Page 4, Line 90)
A6: OS Multivariable. Variable selection process	109	26	MOR-1,v1 to v24	Elastic net with hyperparameters estimated by cross validation $\alpha = 0.8 \lambda = 0.08$
A7: Adjusted Effect of MOR -1 on OS	135	29	MOR-1,v5,v11,v13,v17 to v19	Table 2
A7b: Adjusted Effect of MOR-1 on OS with missing values imputation	174	40	MOR-1,v5,v11,v13,v17 to v19	Table 2

A8: Complications Univariable	174	32	MOR-1	Text (Page 6, Line 113)
A9: Complications Multivariable	87	11	MOR-1, v1 to v24 omitting v15 which is a complication	Elastic net with hyperparameters estimated by cross validation $\alpha = 0.8 \lambda = 0.08$
A10: Adjusted Effect of MOR-1 on complications	74	16	MOR-1, v1,v4,v5,v11,v12,v15, v18,v20,v23,v24	Table S2

**Table S4.** Multivariable Cox regression model for disease free survival and overall survival at 5 years follow-up. Sensitivity analysis with chemotherapy and tumor stage as covariables.

		<b>Complete cases model</b>		
<b>Disease free survival model</b>		<b>N = 132 Events = 30</b>		
		Hazard Ratio	Lower – Upper 95%CI	P –value
<b>MOR expression</b>		0.786	0.597 – 1.036	0.087
<b>First postoperative 96 hours transfusion (yes)</b>		1.069	0.418 – 2.73	0.889
<b>ASA (Reference category = 1)</b>				
2		0.587	0.122 – 2.816	0.506
3		0.855	0.176 – 4.147	0.845
4		1.126	0.132 – 9.603	0.913
<b>Preoperative Hemoglobin (g·dL<sup>-1</sup>)</b>		1.050	0.850 – 1.297	0.649
<b>Number of affected lymph nodes</b>		1.871	1.177 – 2.974	0.008
<b>CEA at diagnosis (U·mL<sup>-1</sup>)</b>		1.200	0.830 – 1.735	0.333
<b>Age (years)</b>		1.021	0.978 – 1.066	0.349
<b>Stage III (yes)</b>		0.793	0.287 – 2.194	0.655
<b>Adjuvant chemotherapy (yes)</b>		1.304	0.528 – 3.22	0.565
<b>Overall survival model</b>		<b>N = 132 Events = 29</b>		
<b>MOR expression</b>		0.982	0.754 – 1.278	0.891
<b>First postoperative 96 hours transfusion (yes)</b>		1.555	0.649 – 3.726	0.322
<b>ASA score (Reference category = 1)</b>				
2		0.993	0.119 – 8.272	0.995
3		1.969	0.241 – 16.092	0.527
4		2.526	0.215 – 29.625	0.461
<b>Preoperative Hemoglobin (g·dL<sup>-1</sup>)</b>		0.916	0.734 – 1.143	0.435
<b>Number of affected lymph nodes</b>		1.340	0.800 – 2.245	0.265
<b>CEA at diagnosis (U·mL<sup>-1</sup>)</b>		1.639	1.156 – 2.324	0.006
<b>Age (years)</b>		1.036	0.992 – 1.082	0.114
<b>Stage III (yes)</b>		1.236	0.426 – 3.588	0.696
<b>Adjuvant chemotherapy (yes)</b>		1.380	0.567 – 3.363	0.478



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MOR-1 expression is introduced in both models as a 0 to 6 ordinal variable. The effect estimate is thus to be interpreted as the difference in hazard in the monitored time period when MOR-1 expression increases one level.

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