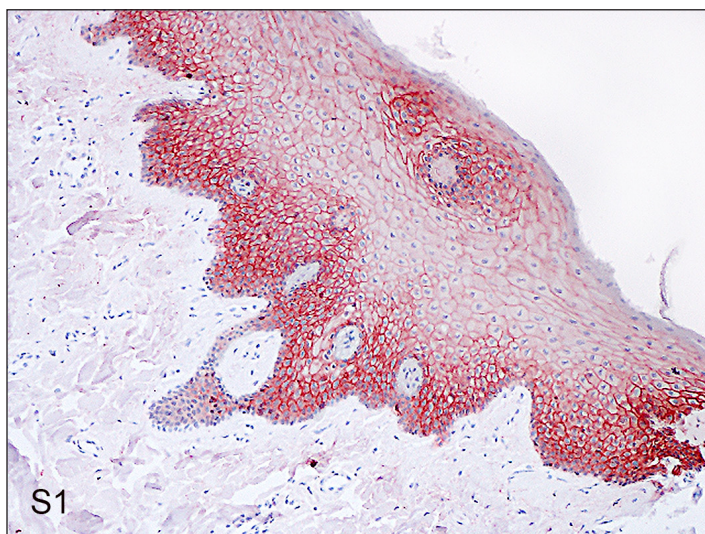
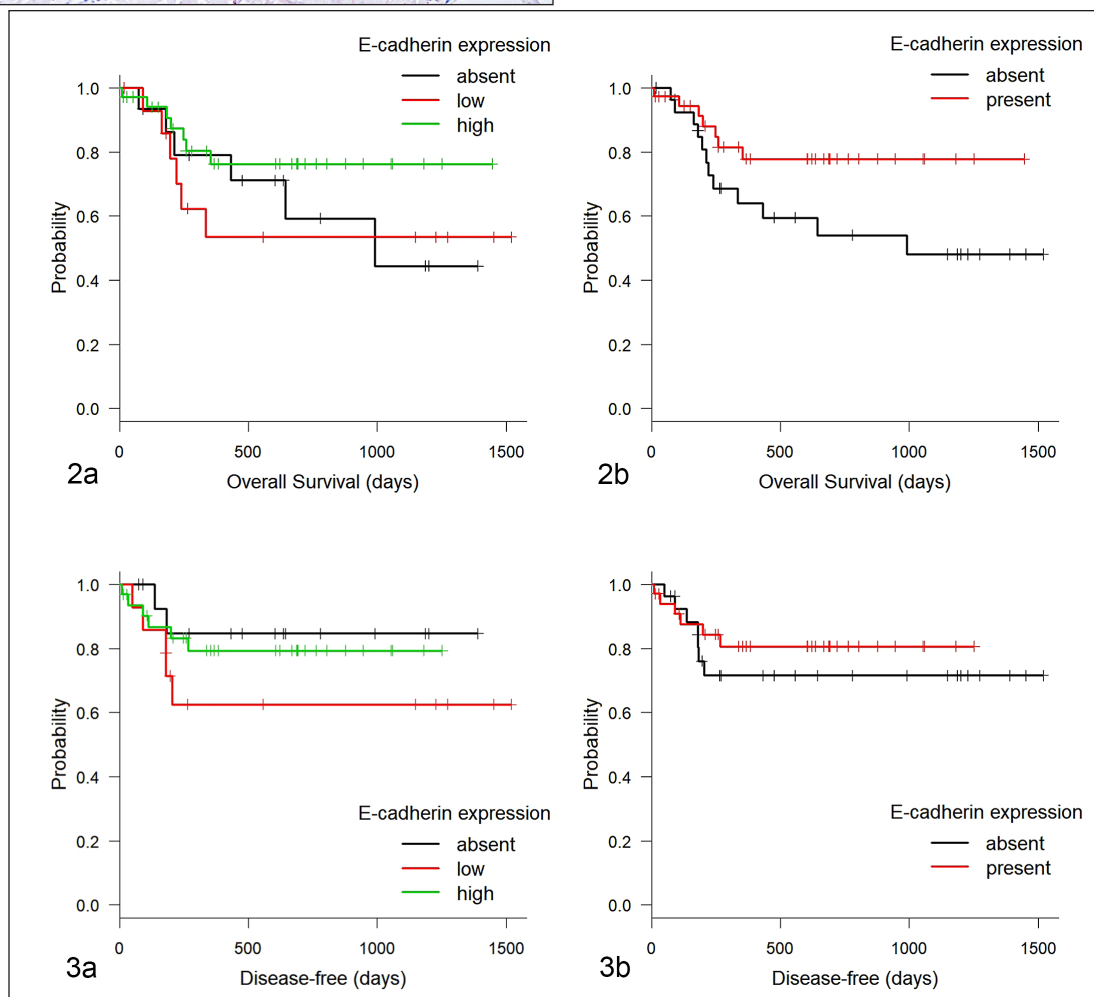


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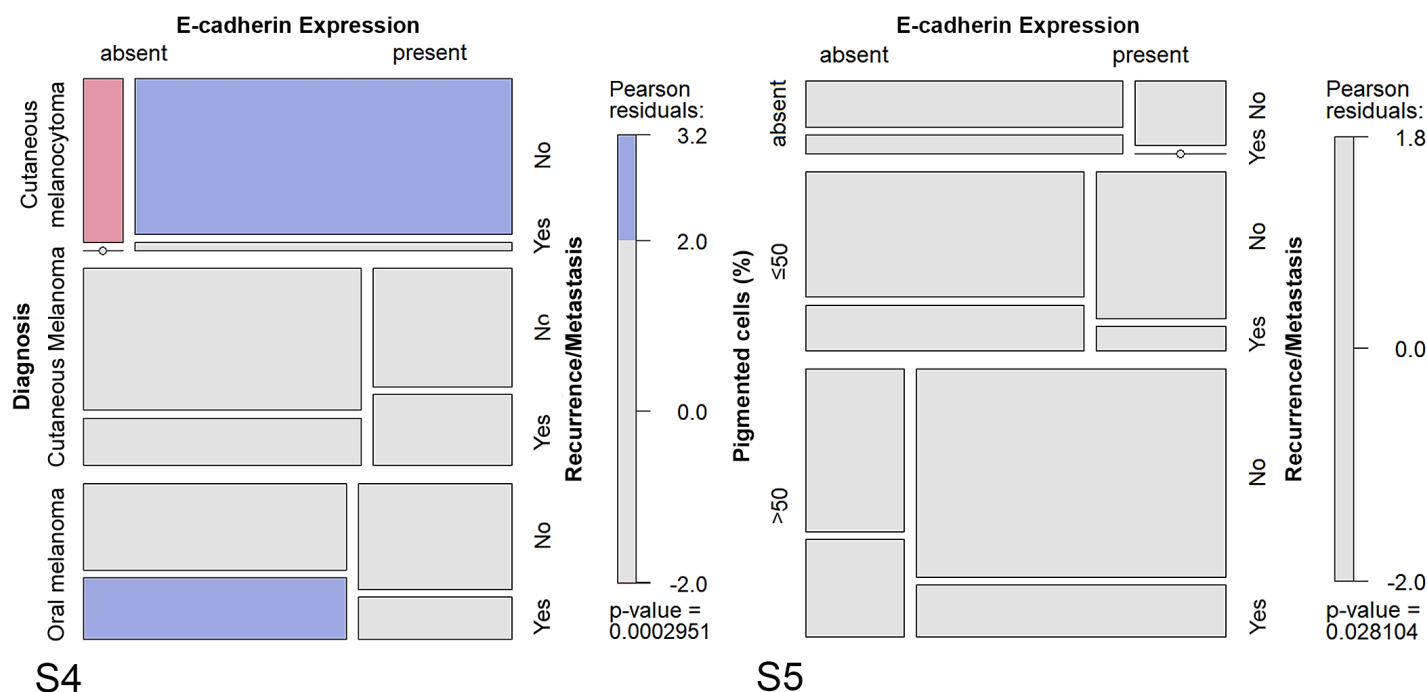


Supplemental Figure S1. Normal skin, dog. Immunohistochemistry for E-cadherin. Epidermal keratinocytes showed the typical E-cadherin pattern (clearly membranous and more intense in the basal layers); the dermis shows no immunolabeling (negative internal control).



Supplemental Figure S2-S3. Kaplan-Meier curves for E-cadherin expression. The vertical thin dashes represent censored patients. **Figure S2a.** Overall survival time according to the 3-tier classification system ($P=0.336$). **Figure S2b.** Overall survival time according to the 2-tier classification system ($P=0.072$). **Figure S3a.** Disease-free survival time according to the 3-tier classification system ($P=0.335$). **Figure S3b.** Disease-free survival time according to the 2-tier classification system ($P=0.462$).

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Supplemental Figure S4-S5. Mosaic plots and log-linear model analysis results (based on Pearson chi-square statistic). Box dimensions are proportional to the number of subjects in each category. Boxes in blue indicate observed frequencies higher than expected in the independence model, while those in red indicated a frequency lower than expected (light blue or light red: $P < 0.05$); gray boxes indicate no differences between observed and expected frequencies. **Figure S4.** E-cadherin expression (2-tier classification system), diagnosis and presence of recurrence/metastasis. **Figure S5.** E-cadherin expression (2-tier classification system), percentage of pigmented cells and presence of recurrence/metastasis.

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Supplemental Table S1. Comparison of the associations of E-cadherin expression with histological features, clinical outcome and recurrence/metastasis among the classification systems used.

EXPRESSION OF E-CADHERIN									
FEATURES	Percentage of positive neoplastic cells		3-tier system				2-tier system		
	<i>P value</i> ^A		Absent	Low	High	<i>P value</i> ^A	Absent	Present	<i>P value</i> ^A
Histological diagnosis	Median (IQR) (%)		n (%)				n (%)		
Cutaneous melanocytoma	80.0 (50.0-100.0) ^a	0.009	4(16.0) ^a	3 (12.0) ^a	18(72.0) ^a	0.026	5(20.0) ^a	20(80.0) ^a	0.0005
Cutaneous melanoma	20.0 (0.0-70.0) ^b		10 (34.5) ^a	11 (37.9) ^a	8 (27.6) ^b		21 (72.4) ^b	8 (27.6) ^b	
Oral melanoma	50.0 (10.0-80.0) ^{a,b}		5 (21.7) ^a	7(30.4) ^a	11(47.8) ^{a,b}		12(52.2) ^{a,b}	11(47.8) ^{a,b}	
Tumor thickness	rho (ρ)		Median (IQR) (cm)				Median (IQR) (cm)		
	-0.293	0.053	0.50 (0.30-0.85)	0.80 (0.55-1.00)	0.40 (0.20-0.60)	0.123	0.75 ^a (0.50-1.00)	0.40 ^b (0.20-0.60)	0.017
Dichotomous modified Clark level	Median (IQR) (%)		n (%)				n (%)		
I-III level	80.0 (52.5-92.5)	0.057	3(15.0) ^a	2 (10.0) ^a	15 (75.0) ^a	0.029	3(15.0) ^a	17(85.0) ^a	0.0009
I-IV level	20.0 (5.0-80.0)		7 (28.0) ^a	9 (36.0) ^a	9 (36.0) ^b		16 (64.0) ^b	9 (36.0) ^b	
Necrosis	Median (IQR) (%)		n (%)				n (%)		
Present	15.0 (0.0-80.0) ^a	0.045	11 (42.3) ^a	5 (19.2) ^a	10 (38.5) ^a	0.036	15(57.7)	11(42.3)	0.296
Absent	70.0 (20.0-80.0) ^b		8 (15.7) ^a	16 (31.4) ^a	27 (52.9) ^a		23(45.1)	28(54.9)	
Ulceration	Median (IQR) (%)		n (%)				n (%)		
Present	50.0 (7.5-80.0)	0.135	9 (25.7)	9 (25.7)	17(48.6)	0.683	18 (51.4)	17(48.6)	0.631
Absent	70.0 (20.0-90.0)		6 (17.1)	10(28.6)	19(54.3)		15 (42.9)	20(57.1)	

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Supplemental Table S1. Continued.

EXPRESSION OF E-CADHERIN									
FEATURES	Percentage of positive neoplastic cells		3-tier system				2-tier system		
	<i>P value</i> ^A		Absent	Low	High	<i>P value</i> ^A	Absent	Present	<i>P value</i> ^A
Percentage of pigmented cells	Median (IQR) (%)		n (%)				n (%)		
Absent	0.0 (0.0-10.0) ^a		6(66.7) ^a	1(11.1) ^a	2 (22.2) ^a	0.004	7 (77.8) ^a	2 (22.2) ^a	0.007
≤50%	40.0 (10.0-80.0) ^{a,b}		5(17.9) ^a	13 (46.4) ^a	10 (35.7) ^a		18 (64.3) ^{a,b}	10 (35.7) ^{a,b}	
>50%	80.0 (20.0-90.0) ^b		8(20.0) ^a	7 (17.5) ^a	25(62.5) ^a		13 (32.5) ^b	27(67.5) ^b	
Predominant cell type	Median (IQR) (%)		n (%)				n (%)		
Polygonal/epithelioid	60.0 (5.0-80.0)		8(36.4)	3 (13.6)	11(50.0)	0.193	9(40.9)	13(59.1)	0.213
Spindle/fibromatous	30.0 (5.0-80.0)		7 (25.9)	10(37.0)	10 (37.0)		17(63.0)	10 (37.0)	
Mixed	75.0 (35.0-80.0)		3 (12.5)	6 (25.0)	15(62.5)		9 (37.5)	15(62.5)	
Dendritic	40.0 (0.0-100.0)		1 (33.3)	1 (33.3)	1 (33.3)		2 (66.7)	1 (33.3)	
Balloon type	10.0 (10.0-10.0)		0 (0.00)	1 (100)	0 (0.00)		1 (100.0)	0 (0.0)	
Junctional activity	Median (IQR) (%)		n (%)				n (%)		
Present	50.0 (10.0-80.0)		13(23.2)	16(28.6)	27(48.2)	0.831	27 (48.2)	29(51.8)	0.867
Absent	70.0 (12.5-85.0)		3 (27.3)	2 (18.2)	6(54.5)		5 (45.5)	6(54.5)	
Intraepithelial neoplastic cells	Median (IQR) (%)		n (%)				n (%)		
Present	50.0 (10.0-80.0)		10 (24.4)	11 (26.8)	20 (48.8)	0.958	17 (44.7)	21(55.3)	0.773
Absent	70.0 (20.0-90.0)		6 (20.0)	8 (26.6)	16 (53.3)		14 (48.3)	15(51.7)	

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Supplemental Table S1. Continued.

EXPRESSION OF E-CADHERIN								
FEATURES	Percentage of positive neoplastic cells		3-tier system				2-tier system	
		<i>P value</i> ^A	Absent	Low	High	<i>P value</i> ^A	Absent	Present
	Median (IQR) (%)		n (%)				n (%)	
Stratified nuclear atypia								
≤20%	45.0 (10.0-82.5)	0.695	8(22.2)	11(30.6)	17(47.2)	0.802	17 (47.2)	19(52.8)
>20%	50.0 (5.0-80.0)		11 (26.8)	10 (24.4)	20(48.8)		21 (51.2)	20(48.8)
Cellular pleomorphism								
	Median (IQR) (%)		n (%)				n (%)	
Mild	70.0 (10.0-100)	0.102	6(24.0) ^a	4 (16.0) ^a	15(60.0) ^a	0.038	8(32.0)	17(68.0)
Moderate	50.0 (20.0-80.0)		5 (13.9) ^a	14(38.9) ^a	17 (47.2) ^a		19(52.8)	17 (47.2)
Marked	8.0 (0.0-75.0)		8 (50.0) ^a	3 (18.8) ^a	5(31.3) ^a		11 (68.8)	5(31.2)
Mitotic count								
	rho (ρ)		Median (IQR) (n)				Median (IQR) (n)	
	-0.218	0.059	15.0 (1.0-45.0)	13.0 (4.0-53.0)	2.5 (0.0-41.5)	0.190	18.0 ^a (2.5-45.75)	2.0 ^b (0.0-38.75)
Vascular invasion								
	Median (IQR) (%)		n (%)				n (%)	
Present	45.0 (31.25-62.5)	0.888	1 (25.0)	2 (50.0)	1 (25.0)	0.566	3 (75.0)	1 (25.0)
Absent	55.0 (8.75-80.0)		18 (25.0)	18 (25.0)	36 (50.0)		34 (47.2)	38 (52.8)
Clinical outcome								
	Median (IQR) (%)		n (%)				n (%)	
Tumor-related death	30.0 (0.0-80.0)	0.063	7 (35.0)	6 (30.0)	7(35.0)	0.256	13 (65.0)	7(35.0)
Others	70.0 (10.0-90.0)		10 (20.0)	12(24.0)	28(56.0)		20(40.0)	30(60.0)
Recurrence/Metastasis								
	Median (IQR) (%)		n (%)				n (%)	
Present	30.0 (7.30-80.0)	0.614	4 (26.7)	5 (33.3)	6 (40.0)	0.507	9 (60.0)	6 (40.0)
Absent	70.0 (5.0-80.0)		13 (26.5)	10 (20.4)	26 (53.1)		21 (42.9)	28 (57.1)

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^ASpearman correlation coefficient, Kruskal-Wallis test, chi-square test or Fisher's exact test. Values in bold are statistically significant ($\alpha=0.05$). When $P>0.05$, no multiple comparisons were performed (no letters displayed). Groups not sharing any superscript lowercase letter represent subsets whose medians or **rows** proportions are significantly different after multiple comparisons and Bonferroni correction ($P\leq 0.05$).

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Supplemental Table S2. Survival analysis of E-cadherin expression depending on overall survival time. The table shows the results of Cox proportional regression analysis on E-cadherin expression evaluated according to the three classification systems we used and on each of the features considered for characterizing E-cadherin expression.

E-CADHERIN EXPRESSION	HR	95%CI	<i>P value</i>
Percentage of positive neoplastic cells	0.991	0.900-1.003	0.152
3-tier system			0.351 ^a
Low vs absent	1.156	0.372-3.597	0.802
High vs absent	0.544	0.182-1.622	0.275
2-tier system (present vs absent)	0.434	0.170-1.107	0.081
Intensity of the staining			0.392 ^a
Mild vs absent	0.880	0.254-3.048	0.841
Moderate vs absent	0.744	0.235-2.353	0.615
Marked vs absent	0.247	0.048-1.276	0.095
Pattern of expression			0.688 ^a
Cytoplasmic vs both	1.668	0.523-5.323	0.387
Membranous vs both	0.000	0.00-inf	0.998
Intensity of membranous staining (low vs intense)	1.819	0.636-5.202	0.264
Intensity of cytoplasmic staining (low vs intense)	0.525	0.175-1.571	0.249
Distribution of positive cells			0.281 ^a
Focal vs diffuse	0.490	0.059-4.070	0.509
Multifocal vs diffuse	2.028	0.652-6.309	0.222
Main localization of positive cells			0.519 ^a
Central vs diffuse	2.877	0.345-23.980	0.329
Peripheral vs diffuse	1.486	0.372-5.948	0.575
Random vs diffuse	2.571	0.641-10.320	0.183

^a overall P value from Wald statistics.

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Supplemental Table S3. Survival analysis of E-cadherin expression depending on disease-free time. The table shows the results of Cox proportional regression analysis on E-cadherin expression evaluated according to the three classification systems we used and on each of the features considered for characterizing E-cadherin expression.

E-CADHERIN EXPRESSION	HR	95%CI	<i>P value</i>
Percentage of positive neoplastic cells	0.999	0.985-1.014	0.975
3-tier system			0.357 ^a
Low vs absent	2.898	0.561-14.970	0.204
High vs absent	1.471	0.297-7.289	0.636
2-tier system (present vs absent)	0.667	0.223-1.985	0.465
Intensity of the staining			0.391 ^a
Mild vs absent	4.358	0.509-37.340	0.179
Moderate vs absent	1.573	0.163-15.140	0.695
Marked vs absent	2.710	0.303-24.250	0.372
Pattern of expression			0.432 ^a
Cytoplasmic vs both	2.251	0.658-7.697	0.196
Membranous vs both	1.423	0.175-11.590	0.742
Intensity of membranous staining (low vs intense)	2.639	0.837-8.323	0.098
Intensity of cytoplasmic staining (low vs intense)	0.545	0.159-1.864	0.333
Distribution of positive cells			0.193 ^a
Focal vs diffuse	0.688	0.077-6.161	0.738
Multifocal vs diffuse	2.738	0.770-9.736	0.119
Main localization of positive cells			0.467 ^a
Central vs diffuse	3.491	0.406-30.010	0.255
Peripheral vs diffuse	1.589	0.379-6.662	0.527
Random vs diffuse	2.719	0.646-11.430	0.172

^a overall P value from Wald statistics.

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Supplemental Table S4. First independence model. The table shows the results regarding the log linear model between E-cadherin expression (2-tier classification system), histological diagnosis and clinical outcome.

N°	Log linear Model	Clinical outcome	Diagnosis	E-cadherin expression (n)		Pearson chi-square statistic		
				Absent	Present	χ^2	df	P value
1	~ Histological diagnosis + E-cadherin expression + Clinical Outcome	Favorable	Cutaneous melanocytoma	2 (7.1)	<u>19 (7.9)</u>	56.062	7	9.178 e⁻¹⁰
			Cutaneous melanoma	<u>16 (9.1)</u>	6 (10.2)			
			Oral melanoma	2 (7.4)	5 (8.3)			
		Unfavorable	Cutaneous melanocytoma	0 (2.8)	0 (3.2)			
			Cutaneous melanoma	3 (3.6)	2 (4.1)			
			Oral melanoma	<u>10 (3.0)</u>	5 (3.3)			

In the table, the absolute frequencies are shown. The expected frequencies are in brackets.

Values in blue have an observed frequency higher than expected if the model fitted ($P < 0.05$); values underlined in blue have an observed frequency higher than expected ($P < 0.0001$).

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Supplemental Table S5. Second independence model. The table shows the results regarding the log linear model between E-cadherin expression (2-tier classification system), histological diagnosis and presence of recurrence/metastasis.

N°	Log linear Model	Recurrence/ Metastasis	Diagnosis	E-cadherin expression (n)		Pearson chi-square statistic		
				Absent	Present	χ^2	df	P value
2	~ Histological diagnosis + E-cadherin expression + Recurrence/Metastasis	No	Cutaneous melanocytoma	2 (7.5)	18 (8.5)	27.293	7	2.950 e ⁻⁴
			Cutaneous melanoma	12 (8.6)	5 (9.8)			
			Oral melanoma	7 (6.8)	5 (7.7)			
		Yes	Cutaneous melanocytoma	0 (2.3)	1 (2.6)			
			Cutaneous melanoma	4 (2.6)	3 (3.0)			
			Oral melanoma	5 (2.1)	2 (2.4)			

In the table, the absolute frequencies are shown. The expected frequencies are in brackets.

Values in blue have an observed frequency higher than expected if the model fitted ($P<0.05$); values in red have an observed frequency lower than expected ($P<0.05$).

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Supplemental Table S6. Third independence model. The table shows the results regarding the log linear model between E-cadherin expression (2-tier classification system), percentage of pigmented cells and clinical outcome.

N°	Log linear Model	Clinical outcome	Pigmentation (%)	E-cadherin expression (n)		Pearson chi-square statistic		
				Absent	Present	χ^2	df	P value
3	~ Percentage of Pigmented Cells + E-cadherin expression + Clinical Outcome	Favorable	Absent	1 (3.0)	1 (3.4)	35.732	7	8.142 e⁻⁶
			≤ 50%	11 (8.4)	6 (9.4)			
			> 50%	8 (12.1)	<u>23 (13.6)</u>			
		Unfavorable	Absent	<u>6 (1.2)</u>	1 (1.4)			
			≤ 50%	5 (3.4)	3 (3.8)			
			> 50%	2 (4.8)	3 (5.4)			

In the table, the absolute frequencies are shown. The expected frequencies are in brackets.

Values in blue have an observed frequency higher than expected if the model fitted ($P < 0.05$); values underlined in blue have an observed frequency higher than expected ($P < 0.0001$).

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Supplemental Table S7. Fourth independence model. The table shows the results regarding the log linear model between E-cadherin expression (2-tier classification system), percentage of pigmented cells and presence of recurrence/metastasis.

N°	Log linear Model	Recurrence/ Metastasis	Pigmentation (%)	E-cadherin expression (n)		Pearson chi-square statistic		
				Absent	Present	χ^2	df	P value
4	~ Percentage of Pigmented Cells + E-cadherin expression + Recurrence/Metastasis	No	Absent	5 (3.2)	2 (3.7)	15.690	7	2.810 e⁻²
			≤ 50%	11 (7.9)	6 (8.9)			
			> 50%	5 (11.8)	20 (13.4)			
		Yes	Absent	2 (1.0)	0 (1.1)			
			≤ 50%	4 (2.4)	1 (2.7)			
			> 50%	3 (3.6)	5 (4.1)			

In the table, the absolute frequencies are shown. The expected frequencies are in brackets.