

## Supplementary Tables and Figures

### Clinical performance of methylation as a biomarker for cervical carcinoma-in-situ and cancer diagnosis: a worldwide study

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**Supplementary Table 1** – S5 Classifier sensitivity rates at the predefined 0.80 cut-off in a CIN3 sample group, stratified per hrHPV status, age and country of origin.

	n / N*	S5 sensitivity at cut-off 0.80		P-value**
		%	95% CI	
<b>HPV***</b>				
HPV-positive	177 / 194	91.23	(86.26-94.19)	
HPV16	115 / 120	95.83	(91.44-97.94)	
HPV18	7 / 7	100.0	(88.57-100.0)	0.153 <sup>a</sup>
HPV31	25 / 30	83.33	(79.14-86.54)	<0.001 <sup>b</sup>
HPV33	7 / 7	100.0	(88.57-100.0)	
Other hr-HPV	23 / 30	76.66	(65.02-82.68)	
HPV-negative	9 / 10	90.00	(85.62-92.12)	
<b>Age</b>				
<25	13 / 14	92.85	(87.97-95.01)	
25-29	55 / 59	93.22	(88.79-95.92)	
30-39	75 / 81	92.59	(87.67-94.23)	0.365
40-49	27 / 30	90.00	(86.69-92.64)	
50-59	13 / 14	92.85	(87.97-95.01)	
>60	5 / 6	83.33	(77.89-89.92)	
<b>Country of Origin</b>				
Colombia	42 / 50	84.00	(80.24-87.46)	
Spain	46 / 50	92.00	(88.05-95.62)	0.127
United Kingdom	53 / 54	98.14	(96.36-99.13)	
USA (New Mexico)	45 / 50	90.00	(85.87-93.75)	
<b>Total</b>	<b>186 / 204</b>	<b>91.18</b>	<b>(86.49-94.92)</b>	

\* n = number of positive samples in a specified group; N = group total.

\*\* hrHPV genotype grouping performed by hierarchical genotype attribution, as detailed in materials and methods.

\*\*\* Determined by performing Fishers' exact test of independence.

<sup>a</sup> P-value among all subgroups in the HPV-positive group

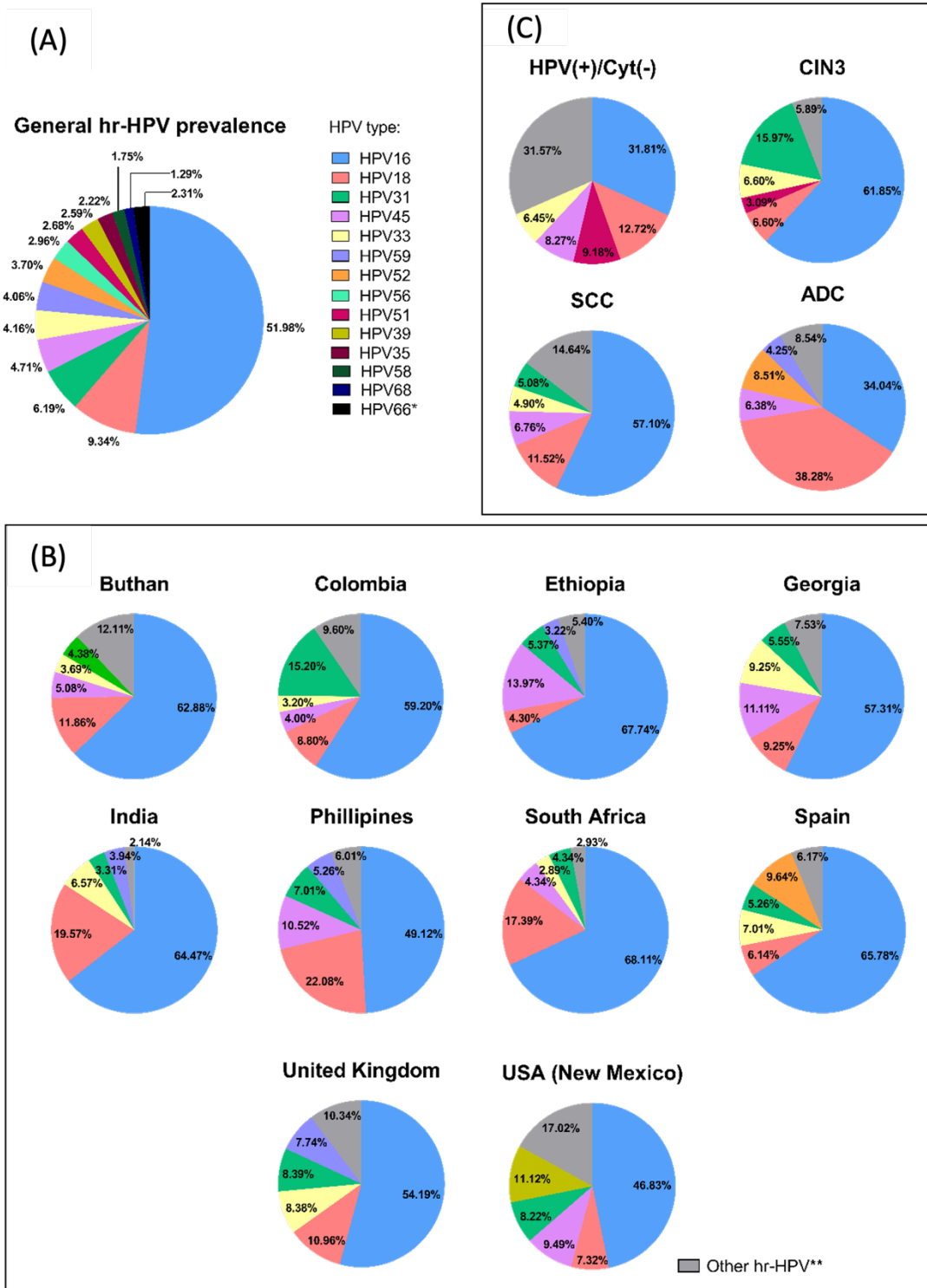
<sup>b</sup> P-value between HPV-positive and HPV-negative subgroups

**Supplementary Table 2** – S5 Classifier sensitivity rates at the predefined 3.70 cut-off in a CIN3 sample group, stratified per hrHPV status, age and country of origin.

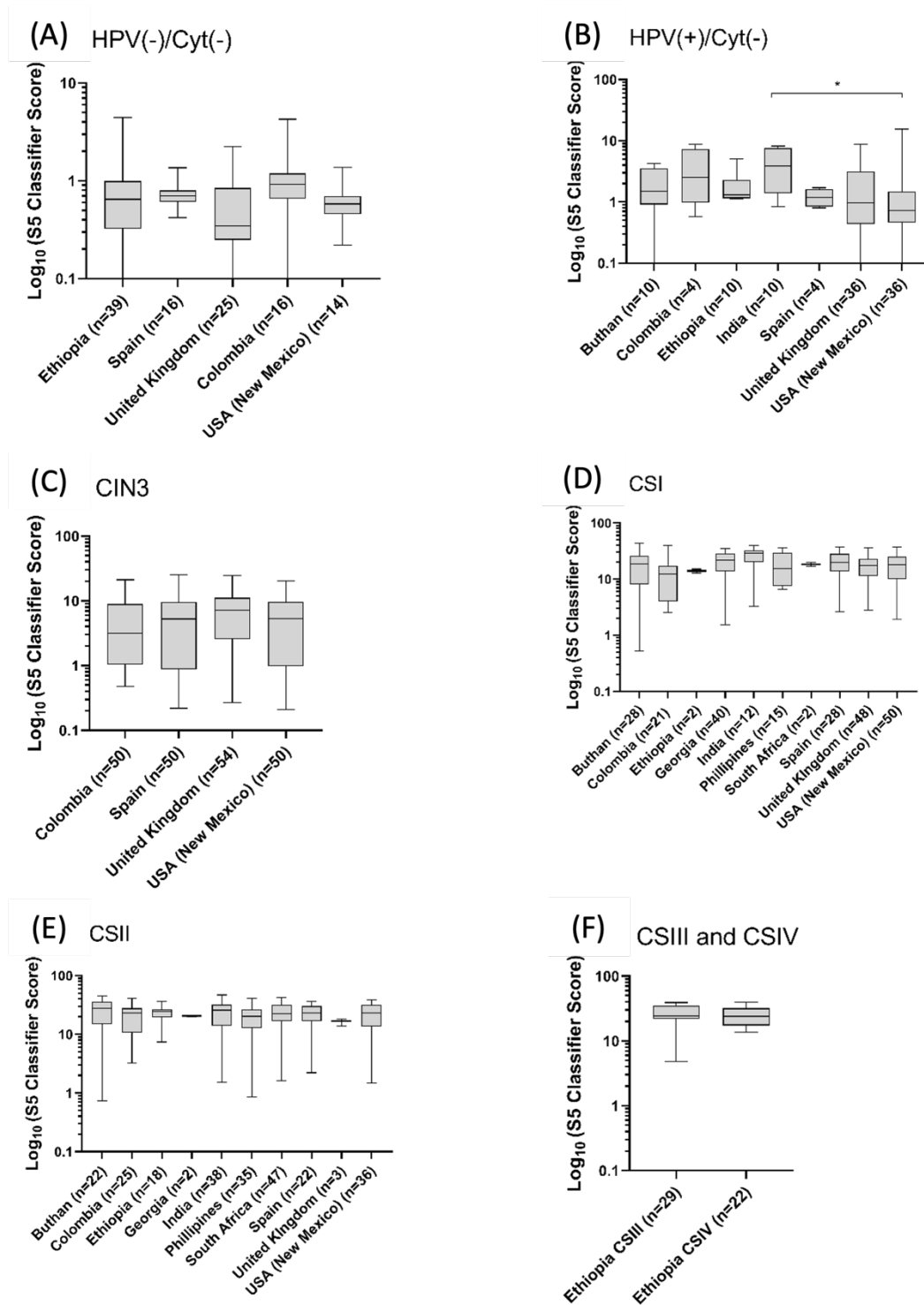
	S5 sensitivity at cut-off 3.70			P-value**
	n / N*	%	95% CI	
<b>HPV***</b>				
HPV-positive	116 / 194	59.79	(46.26-65.95)	
HPV16	90 / 120	75.00	(64.15-82.88)	
HPV18	5 / 7	71.42	(52.55-85.22)	0.083 <sup>a</sup>
HPV31	20 / 30	66.66	(40.56-79.23)	0.0094 <sup>b</sup>
HPV33	3 / 7	42.85	(20.65-58.26)	
Other hr-HPV	10 / 30	33.33	(15.82-56.34)	
HPV-negative	2 / 10	20.00	(5.54-39.12)	
<b>Age</b>				
<25	9 / 14	64.28	(35.22-75.10)	
25-29	38 / 59	64.40	(40.54-76.05)	
30-39	45 / 81	55.55	(41.14-68.67)	0.465
40-49	19 / 30	63.33	(38.76-78.13)	
50-59	10 / 14	71.42	(59.67-86.65)	
>60	4 / 6	66.66	(51.79-79.92)	
<b>Country of Origin</b>				
Colombia	28 / 50	56.00	(42.53-68.12)	
Spain	30 / 50	60.00	(44.15-72.35)	0.227
United Kingdom	41 / 54	75.92	(62.15-82.12)	
USA (New Mexico)	28 / 50	56.00	(42.53-68.12)	
<b>Total</b>	<b>128 / 204</b>	<b>62.74</b>	<b>(55.22-72.10)</b>	

**Supplementary Table 3 – Sensitivity and specificity of the S5 methylation classifier in identifying CIN3 and invasive cancer based on different classifier cut-offs.** 0.80 is the pre-defined cut-off developed in the UK and validated for developed countries, 2.62 is the Youden-J index cut-offs based on the methylation scores of invasive cervical cancers and 3.70 as cut-off explored in Mexico and proposed for underdeveloped countries.

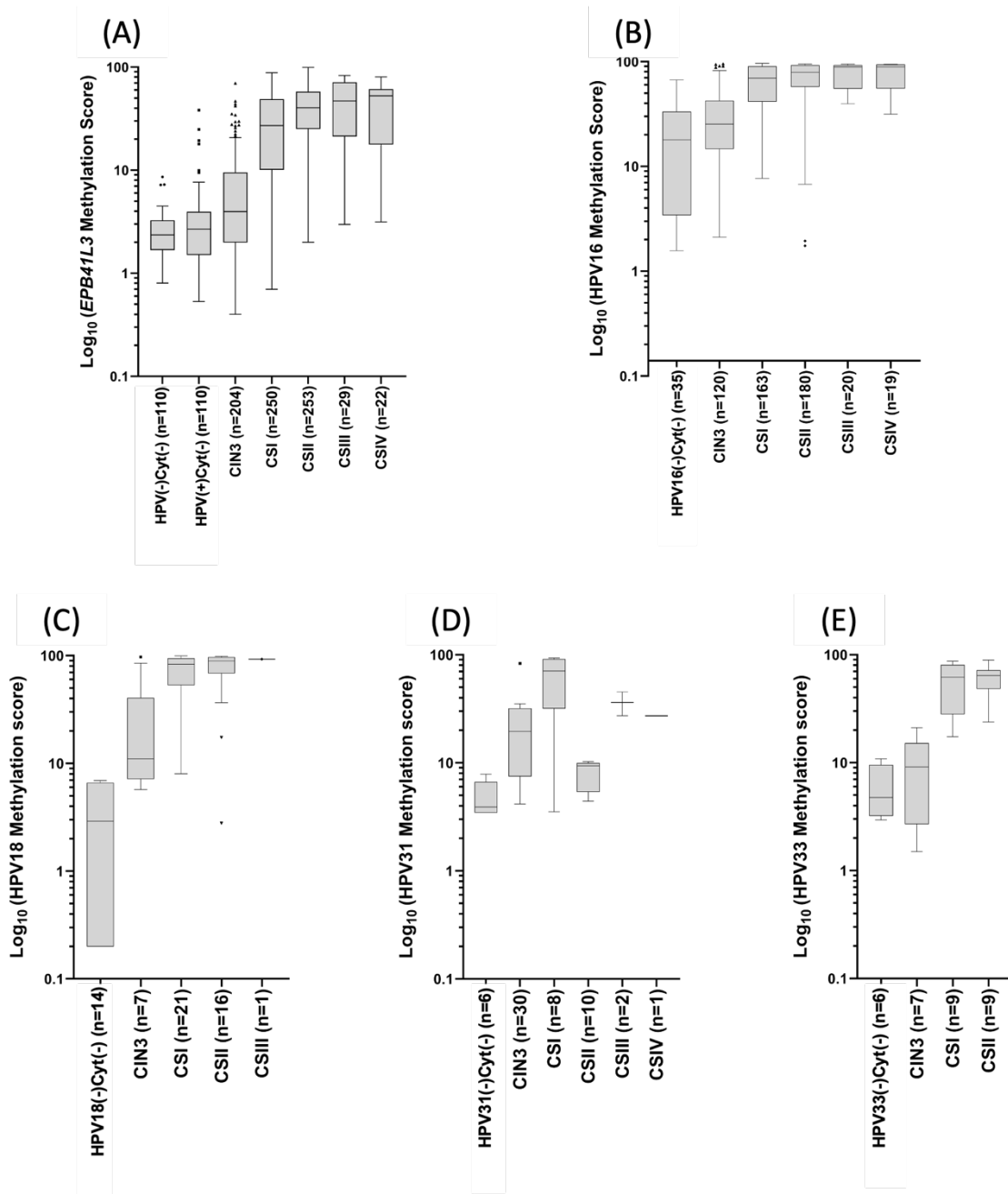
		<b>AUC</b>	<b>95% CI</b>	<b>Cut-off</b>	<b>Sensitivity (%)</b>	<b>95% CI</b>	<b>McNemar p-value*</b>	<b>Specificity (%)</b>	<b>95% CI</b>	<b>McNemar p-value*</b>	<b>Women missed N (% total)</b>	
<b>HPV(-)/Cyt(-) vs</b>	CIN3 (n=204)	<b>0.90</b>	0.87 0.93	-	0.80	91.18	86.48 - 94.16		65.12	54.59 – 74.35		16 (8.22)
					2.62	68.13	61.46 – 74.15	<0.0001	100	95.19 - 100	<0.0001	65 (31.87)
					3.70	62.74	55.93 – 69.09	<0.0001	100	95.72 - 100	<0.0001	76 (37.26)
	Cancer (n=544)	<b>0.99</b>	0.98 0.99	-	0.80	99.81	98.56 – 99.99		65.12	54.59 – 74.35		2 (0.36)
					2.62	95.21	93.12 – 96.68	0.036	100	95.19 – 100	0.0025	27 (4.88)
					3.70	93.26	90.89– 95.05	0.002	100	95.72 - 100	<0.0001	38 (6.74)
<b>HPV(+)/Cyt(-) vs</b>	CIN3 (n=204)	<b>0.80</b>	0.76 0.84	-	0.80	91.18	86.48 - 94.16		48.81	41.36 - 56.31		16 (8.22)
					2.62	68.13	61.46 – 74.15	<0.0001	78.57	71.76 – 84.10	<0.0001	65 (31.87)
					3.70	62.74	55.93 – 69.09	<0.0001	83.33	76.97 – 88.21	<0.0001	76 (37.26)
	Cancer (n=544)	<b>0.97</b>	0.96 0.98	-	0.80	99.81	98.56 – 99.99		50.60	43.11 – 58.06		2 (0.36)
					2.62	95.21	93.12 – 96.68	0.036	78.57	71.76 – 84.10	0.0051	27 (4.79)
					3.70	93.26	90.89– 95.05	0.002	83.33	76.97 – 88.21	<0.0001	38 (6.74)



**Supplementary Figure 1 – HPV prevalence in the study.** (A) general hr-HPV prevalence (B) stratified prevalence per country of origin and (C) histology diagnosis of women in the study. Each analysed group and subgroup investigate HPV positive (all 13 types of hr-HPV and HPV66\* now regarded as low-risk) only women. (B) and (C) show the percentages of the 5 most prevalent hr-HPV type in women per subgroup, any other hrHPV types different than the top 5 were marked as Other hr-HPV (\*\*). Only the most abundant cervical cancer subgroups were considered: squamous cell carcinoma (SCC, n=510) and adenocarcinoma (ADC, n=29). The adenosquamous cell carcinomas (n = 1) and neuroendocrine small cell carcinoma (n = 4) were deliberately not included. Abbreviations: HPV(+)/Cyt(-), HPV positive with cytology negative results; CIN, cervical intraepithelial neoplasia (of grade 3); SCC, squamous cell carcinoma; ADC, adenocarcinoma.



**Supplementary Figure 2** – S5-classifier score distribution in log scale per country, measured at different endpoints: **(A)** (HPV(-)/Cyt(-), **(B)** HPV(+)/Cyt(-), **(C)** CIN3, **(D)** CSI, **(E)** CSII, **(F)** CSIII and CSIV. Data is shown as box plots. Significant differences between countries are marked on top of the graph with “\*” for  $p < 0.01$  and “\*\*\*” for  $p < 0.001$ . Abbreviations: HPV(-/+)/Cyt(-), HPV positive/negative and cytology negative; CIN, cervical intraepithelial neoplasia (of grade 3); CSI-IV, cervical cancer stages I-IV.



**Supplementary Figure 3 - Distribution of *EPB41L3*, HPV16, HPV18, HPV31L1 and HPV33L2 methylation scores based on the histopathological diagnostic of the patient.** Absolute methylation was calculated as an average of the selected CpGs on: *EPB41L3* (A), HPV16 L1 and L2 late genes (B) HPV18 L2 (C), HPV31 L1 (D) and HPV33 L2 (E) and plotted as log10 of methylation score. Significant differences were found between the following group comparisons in HPV(-)/Cyt(-) vs CIN3 ( $p = 0.049$ ), HPV(-)/Cyt(-) vs CSI-IV (all,  $p < 0.0001$ ), HPV(+)/Cyt(-) vs CSI-IV (all,  $p < 0.0001$ ), CIN3 vs CSI-IV (all,  $p < 0.0001$ ) and CSI vs CSII ( $p < 0.0001$ ); in HPV16(+)/Cyt(-) vs CSI-IV (all,  $p < 0.0001$ ) and CIN3 vs CSI-IV (all,  $p < 0.0001$ ). HPV18(+)/Cyt(-) vs CSI ( $p = 0.013$ ), HPV18(+)/Cyt(-) vs CIN3 ( $p = 0.023$ ), HPV18(+)/Cyt(-) vs CSII ( $p = 0.003$ ), CIN3 vs CSII ( $p = 0.010$ ); in HPV31(+)/Cyt(-) vs CSI ( $p < 0.001$ ); in HPV33(+)/Cyt(-) vs CSI ( $p = 0.025$ ), HPV33(+)/Cyt(-) vs CSII ( $p = 0.007$ ), CIN3 vs CSI ( $p = 0.007$ ), CIN3 vs CSII ( $p < 0.001$ ). Other comparisons were not significant.

