Ang-Tie angiogenic pathway is distinctively expressed in benign and malignant adrenocortical tumors
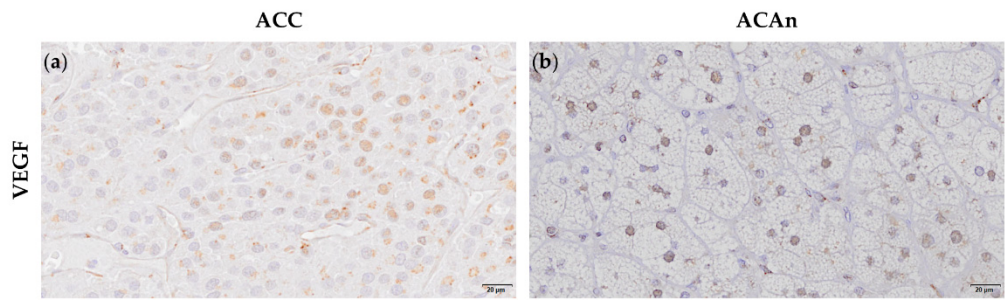

Figure S1. Immunochemistry staining of adrenocortical carcinomas (ACC) (**a**) and non-functioning adrenocortical adenoma (ACAn) (**b**) for VEGF. VEGF nuclear staining in ACC and ACAn (**a**, **b**).
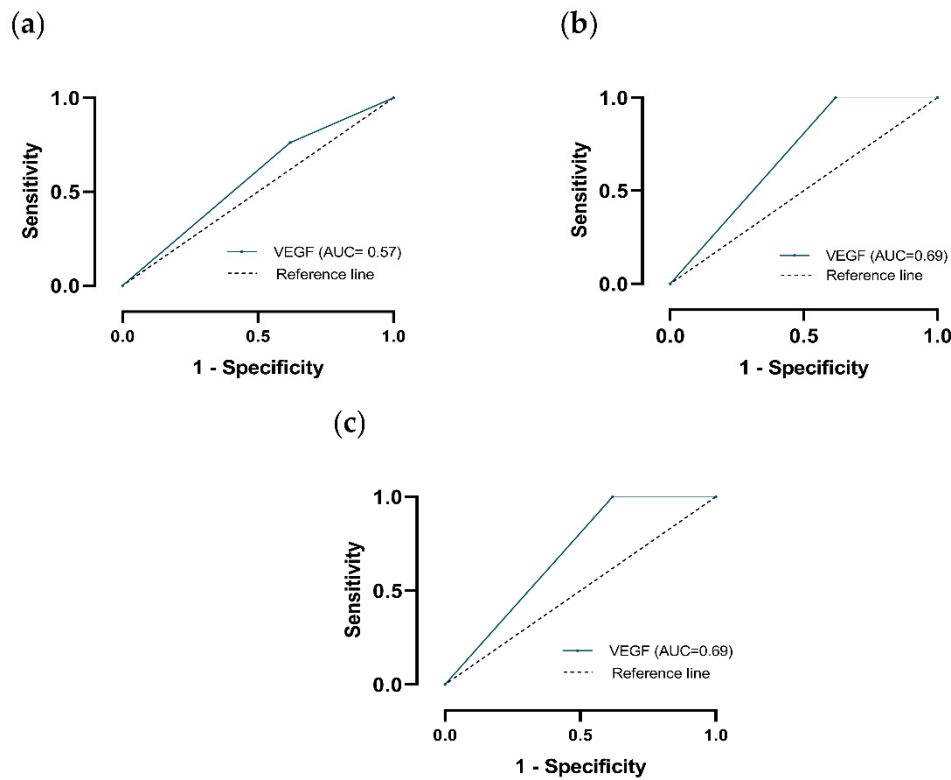
Figure S2. ROC curves to assess the accuracy of VEGF to distinguish between adrenocortical carcinomas (ACC) and total adrenocortical adenomas (ACAt) (**a**); ACC and adrenocortical adenomas with Cushing syndrome (ACAc) (**b**); ACC and non-functioning adrenocortical adenoma (ACAn) (**c**) with the respective area under the curve (AUC).

Table S1. Correlation between the angiogenic markers and patient/tumor characteristics in ACC.

Correlation	p

Angiogenic marker	Patient/ Tumor characteristics	Coefficient correlation (r)	
CD34	Age	0.080	ns
	Tumor size	-0.138	ns
	Weiss score	0.006	ns
	ENSAT score	0.285	ns
VEGF-R2	Age	-0.188	ns
	Tumor size	0.136	ns
	Weiss score	0.279	ns
	ENSAT score	0.441	ns
Ang1	Age	0.376	ns
	Tumor size	0.055	ns
	Weiss score	0.128	ns
	ENSAT score	0.043	ns
Ang2	Age	-0.004	ns
	Tumor size	0.317	ns
	Weiss score	0.152	ns
	ENSAT score	0.336	ns
Tie1	Age	0.096	ns
	Tumor size	0.092	ns
	Weiss score	0.060	ns
	ENSAT score	0.006	ns
Tie2	Age	-0.126	ns
	Tumor size	0.202	ns
	Weiss score	-0.175	ns
	ENSAT score	-0.158	ns

Ang1: Angiopoietin 1; Ang2: Angiopoietin 2; ENSAT: European Network for the Study of Adrenal Tumors; ns: non-significant; Tie1: Tyrosine kinase with immunoglobulin-like and EGF-like domain 1; Tie2: Tyrosine kinase with immunoglobulin-like and EGF-like domain 2; VEGF-R2: Vascular endothelial growth factor receptor 2.

Ang-Tie angiogenic pathway is distinctively expressed in benign and malignant adrenocortical tumors

Table S2. Correlation between the angiogenic markers and patient/tumor characteristics in ACAc.

Correlation		Coefficient correlation (r)	p
Angiogenic marker	Patient/ Tumor characteristics		
CD34	Age	-0.538	ns
	Tumor size	0.655	ns
	Weiss score	0.131	ns
VEGF-R2	Age	0.357	ns
	Tumor size	-0.509	ns
	Weiss score	0.393	ns
Ang1	Age	0.429	ns
	Tumor size	0.436	ns
	Weiss score	0.655	ns
Ang2	Age	0.500	ns
	Tumor size	0.127	ns
	Weiss score	0.393	ns
Tie1	Age	-0.250	ns
	Tumor size	0.164	ns

	Weiss score	-0.655	ns
Tie2	Age	0.143	ns
	Tumor size	0.291	ns
	Weiss score	0.393	ns

Ang1: Angiopoietin 1; Ang2: Angiopoietin 2; ENSAT: European Network for the Study of Adrenal Tumors; ns: non-significant; Tie1: Tyrosine kinase with immunoglobulin-like and EGF-like domain 1; Tie2: Tyrosine kinase with immunoglobulin-like and EGF-like domain 2; VEGF-R2: Vascular endothelial growth factor receptor 2.

Ang-Tie angiogenic pathway is distinctively expressed in benign and malignant adrenocortical tumors

Table S3. Correlation between the angiogenic markers and patient/tumor characteristics in ACAn.

Correlation		Coefficient correlation (r)	p
Angiogenic marker	**Patient/ Tumor characteristics**		
CD34	Age	0.073	ns
	Tumor size	-0471	ns
	Weiss score	-	
VEGF-R2	Age	0.093	ns
	Tumor size	0.311	ns
	Weiss score	-	
Ang1	Age	0.091	ns
	Tumor size	0.471	ns
	Weiss score	-	-
Ang2	Age	0.030	ns
	Tumor size	0.546	ns
	Weiss score	-	
Tie1	Age	0.358	ns
	Tumor size	0.101	ns
	Weiss score	-	

Ang1: Angiopoietin 1; Ang2: Angiopoietin 2; ENSAT: European Network for the Study of Adrenal Tumors; ns: non-significant; Tie1: Tyrosine kinase with immunoglobulin-like and EGF-like domain 1; VEGF-R2: Vascular endothelial growth factor receptor 2. Correlation between expression of the angiogenic markers and the Weiss score as not performed, since the score for all ACAn was 0.

Ang-Tie angiogenic pathway is distinctively expressed in benign and malignant adrenocortical tumors

Table S4. Percentage of ACC samples with different VEGF expression localization pattern according to various parameters including ENSAT score, metastasis, capsular, venous and sinusoidal invasion.

		Cytoplasm + Nucleus expression	Cytoplasm expression
ENSAT score	1-2	50.00%	50.00%
	3-4	50.00%	50.00%
	p	>0.99	
Metastasis	Yes	33.33%	16.67%
	No	66.67%	83.33%
	p	>0.99	
Capsular Invasion	Yes	85.71%	71.43%
	No	14.29%	28.57%
	p	>0.99	

Venous Invasion	Yes	28.57%		55.56%
	No	71.43%		44.44%
	p		0.36	
Sinusoidal Invasion	Yes	57.14%		87.50%
	No	42.86%		12.50%
	p		0.28	

ENSAT: European Network for the Study of Adrenal Tumors.