

## Supplemental data

### International histopathology consensus for unilateral primary aldosteronism

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Adrenal sample	Histopathologic findings	Genotype	Outcomes after adrenalectomy		Classical or non-classical histopathologic findings of unilateral primary aldosteronism				Final score	
			Clinical success	Biochemical success	Group 1 pathologists (n=5)		Group 2 pathologists (n=6)			
					Classical	Non-classical	Classical	Non-classical		
#1	Relatively large, well-circumscribed APA composed of clear cells.	KCNJ5-G151R	Partial	Complete	5	0	6	0	C	
#2	Well-circumscribed APA composed predominantly of compact cells. Adjacent cortex shows multiple APMs and weak CYP11B2 immunoreactivity in the ZG.	NMD	No FU	No FU	5	0	6	0	C	
#3	Relatively large, well-circumscribed APA composed of clear cells. Adjacent cortex shows multiple APMs in the hyperplastic ZG.	NMD	Partial	Complete	5	0	6	0	C	
#4	APDH with several APNs composed of clear cells.	ND	Absent	Partial	1	4	0	6	NC	
#5	Multiple APNs composed of clear and/or compact cells. Weak CYP11B2 immunoreactivity in the ZG.	ND	Partial	Partial	1	4	1	5	NC	
#6	Multiple APNs composed of clear and/or compact cells. Weak CYP11B2 immunoreactivity in the ZG.	ND	Partial	Absent	1	4	1	5	NC	
#7	Multiple APNs composed predominantly of clear cells and APMs	ND	Absent	Absent	2	3	1	5	NC	
#8	Large, well-circumscribed nonfunctioning adenoma composed of clear and compact cells with lipomatous degeneration and intratumoural lymphocyte infiltration. Adjacent cortex shows APDH with multiple APMs.	PRKACA-L206R	Partial	Complete	2	3	0	6	NC	
#9	APDH with multiple APMs and a small well-circumscribed APN composed of clear cells.	KCNJ5-G151R	Partial	Absent	2	3	2	4	NC	
#10	Well-circumscribed APN composed of lobulated clear cells. Adjacent cortex shows smaller APNs and APDH.	KCNJ5-G151R	Absent	Complete	2	3	5	1	C	
#11	Well-circumscribed APA composed of clear cells. Adjacent cortex shows multiple APMs in a partly hyperplastic ZG	KCNJ5-L168R	No FU	No FU	2	3	6	0	C	
#12	Well-circumscribed APA composed of clear cells undergoing pseudoglandular formation. Adjacent cortex shows multiple APMs	NMD	Complete	Complete	2	3	6	0	C	
#13	Well-circumscribed, relatively large APA composed of clear cells with degenerative changes (pseudoglandular formation and stromal fibrosis). Adjacent cortex shows an APN and APMs with paradoxical ZG hyperplasia.	KCNJ5-G151R and T158P*	Partial	Partial	3	2	6	0	C	
#14	Well-circumscribed APA composed of small compact cells. Adjacent cortex shows APMs.	ND	Absent	Complete	3	2	5	1	C	

#15	Well-circumscribed APA composed predominantly of clear cells and a larger nonfunctioning adenoma composed predominantly of compact cells with lipomatous degeneration. Adjacent cortex shows APMs and paradoxical ZG hyperplasia.	CACNA1D-F747V	Partial	Partial	3	2	6	0	C
#16	Relatively large well-circumscribed APA composed of clear cells. Adjacent cortex shows APDH.	KCNJ5-L168R	Partial	Partial	3	2	6	0	C
#17	Relatively large APN composed of clear cells with focal lipomatous degeneration. Adjacent cortex shows APDH.	KCNJ5-L168R	Absent	Complete	3	2	4	2	C
#18	Nonfunctioning adenoma (larger lesion) composed of clear and compact cells with lipomatous degeneration. Well-circumscribed APA (smaller lesion) composed of clear cells with pseudoglandular formation representing degeneration. Adjacent cortex shows multiple nodule formation with distorted basic structure of adrenal cortex.	CACNA1D-G403R	Partial	Complete	2	3	3	3	D
#19	Well-circumscribed nonfunctioning adenoma composed of clear cells. Adjacent cortex shows APDH of the ZG with multiple APMs.	NMD	Absent	Complete	1	4	-	-	NC
#20	Relatively well-circumscribed APA composed of clear and compact cells. Adjacent cortex shows paradoxical ZG hyperplasia.	NMD	Partial	Complete	4	1	-	-	C
#21	III-defined APN showing gradient of CYP11B2 immunoreactivity and multiple APNs in the ZG.	NMD	Partial	Complete	1	4	-	-	NC
#22	Relatively large well-circumscribed APA composed of clear cells and hyalinized stroma with cells undergoing lobulated degeneration. Adjacent cortex shows multiple cortical nodules of the ZG including APMs	ATP1A1-L104R	Complete	Complete	5	0	-	-	C
#23	Relatively well-circumscribed APA composed predominantly of clear cells. Adjacent cortex shows paradoxical ZG hyperplasia.	KCNJ5-L168R	Partial	Complete	5	0	-	-	C
#24	Relatively large, well-circumscribed, partly encapsulated APA composed of clear cells.	KCNJ5-L168R	Complete	Partial	5	0	-	-	C
#25	Well-circumscribed APA, partially positive for CYP11B2 immunoreactivity, composed predominantly of clear cells and heterogeneous elements of eosinophilic cells and degenerative changes. Adjacent cortex shows paradoxical ZG hyperplasia.	NMD	Partial	Complete	5	0	-	-	C
#26	Relatively well-circumscribed partly encapsulated APA composed of both clear and compact cells. Adjacent cortex shows one APN and paradoxical ZG hyperplasia	NMD	Partial	Complete	5	0	-	-	C

#27	Relatively well-circumscribed APA composed of clear cells with weak CYP11B2 immunostaining. Adjacent cortex shows paradoxical ZG hyperplasia.	NMD	Partial	Complete	5	0	-	-	C
#28	Well-circumscribed APA composed of clear cells with lobulation. Adjacent cortex shows small APMs and paradoxical ZG hyperplasia.	NMD	Absent	Complete	5	0	-	-	C
#29	APDH of the ZG with multiple APMs.	NMD	Partial	Partial	1	4	-	-	NC
#30	Multiple APMs with paradoxical ZG hyperplasia.	ND	Partial	Complete	1	4	-	-	NC
#31	Multiple APNs with paradoxical ZG hyperplasia.	NMD	Partial	Absent	1	4	-	-	NC
#32	Relatively large, well-circumscribed and encapsulated APA composed of clear cells. Adjacent cortex shows APMs and paradoxical ZG hyperplasia.	KCNJ5-G151R	Complete	Complete	4	1	-	-	C
#33	Multiple APNs composed of clear cells and several APMs in markedly hyperplastic ZG	ATP1A1 c.299_313del	Partial	Absent	1	4	-	-	NC
#34	Relatively large, CYP11B2 negative nodule composed of clear and compact cells. Adjacent cortex shows multiple APNs, APMs and paradoxical ZG hyperplasia.	ND	Absent	Absent	4	1	-	-	C
#35	Large well-circumscribed APA composed predominantly of clear cells. Adjacent cortex shows several APMs and paradoxical ZG hyperplasia.	KCNJ5-G151R	Partial	Complete	4	1	-	-	C
#36	Relatively large, lobulated, and well-circumscribed APA with marked intratumoural heterogeneity composed predominantly of clear cells. Adjacent cortex shows weak CYP11B2 immunoreactivity in the ZG.	KCNJ5-G151R	Partial	Complete	4	1	-	-	C
#37	An APA composed of clear cells. Adjacent cortex shows paradoxical ZG hyperplasia.	NMD	Partial	Complete	4	1	-	-	C

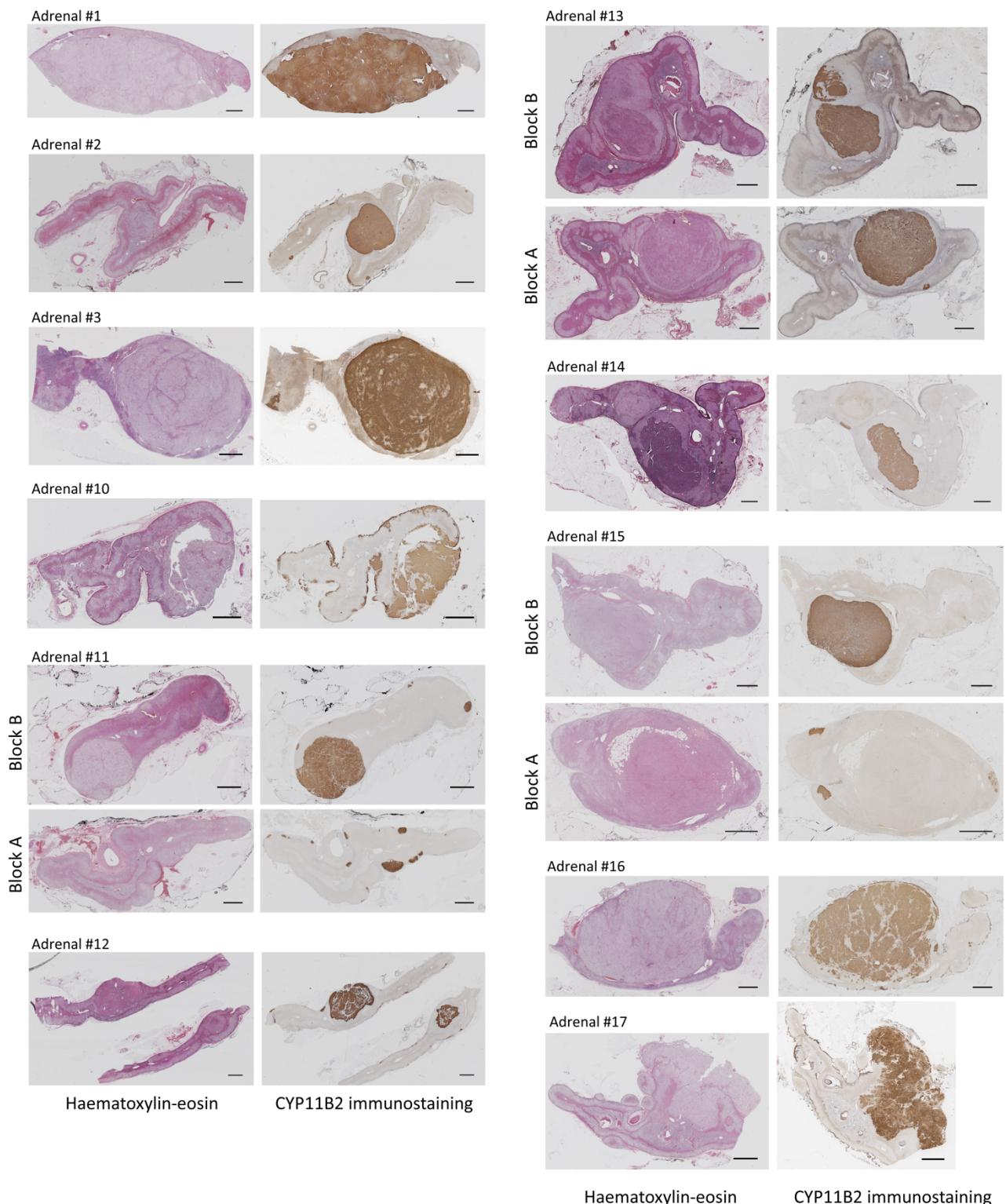
**Table S1. Histopathologic findings of surgically removed adrenal specimens from patients diagnosed with unilateral primary aldosteronism**

Table showing level of agreement or disagreement between Group 1 and Group 2 pathologists for the histopathologic assessment of 18 adrenals. There were 5 pathologists in group 1 and 6 pathologists in group 2. Numbers indicate number of pathologists reaching the indicated evaluation. Post-surgical outcomes assessed in accordance with the PASO criteria (1). CYP11B2, aldosterone synthase (encoded by the *CYP11B2* gene); FU, follow-up; NMD, no mutation detected in KCNJ5, CACNA1D, ATP1A1, ATP2B3 and CTNNB1; UD, undetermined; APA, aldosterone-producing adenoma; APDH, aldosterone-producing diffuse hyperplasia; APN, aldosterone-producing nodule; APM, aldosterone-producing micronodule; ZG, zona glomerulosa. Genotypes were determined as described previously (2). Final score refers to the categorization of the histopathologic assessment: C, classical; NC, non-classical and D, disagreement and not categorized. Final scores were assigned when four or more of the five pathologists in group 1 agreed, or 4 or more of the six pathologists in group 2. \*Adrenal #13 comprised a KCNJ5-G151R mutation in the APA and a KCNJ5-T158P mutation in the APN (**Figure S1**)

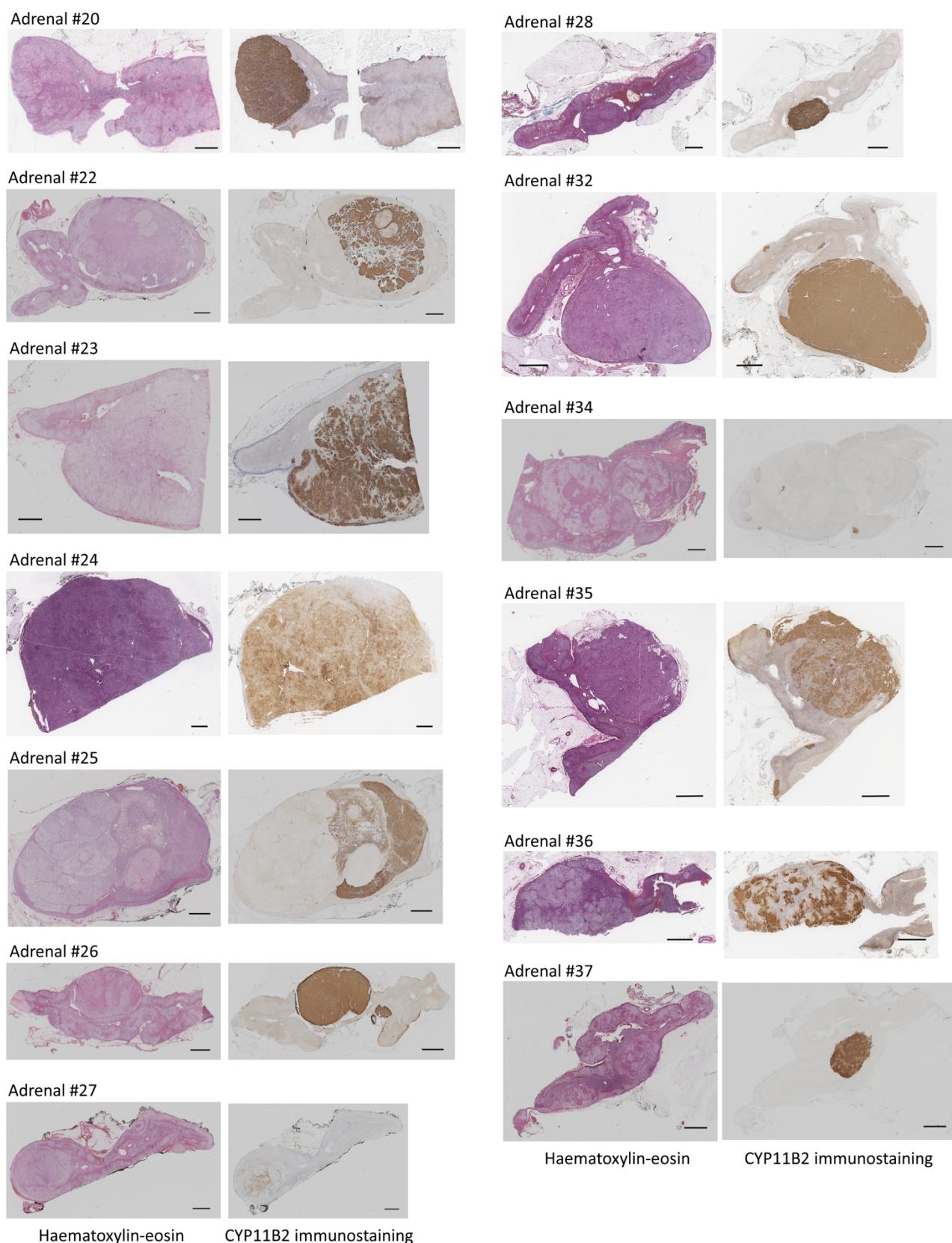
Pathologist	Classical/nonclassical histopathologic features of unilateral PA		Histopathologic diagnosis
	Classical	Non-classical	
<b>Group 1 pathologists</b>			
1	CYP11B2 positive adenoma with paradoxical hyperplasia	None	Classical
2	CYP11B2 positive adenoma	None	Classical
3	None	Multiple nodules positive for CYP11B2	Non-classical
4	None	Nodular hyperplasia and adenoma of the zona glomerulosa	Non-classical
5	None	Multiple CYP11B2 positive nodules	Non-classical
<b>Group 2 pathologists</b>			
1	APN with diffuse CYP11B2 positivity	Single APM in background	Classical
2	Two demarcated APN composed of eosinophilic cells.	Two APN	Non-classical
3	Absence of diffuse hyperplasia	Multifocal CYP11B2 expressing adenomas	Non-classical
4	APA	NFA and APMs	Classical
5	None	NFA and CYP11B2-positive micronodules (APM)	Non-classical
6	Presence of dominant nodule or adenoma which could be producing aldosterone	Diffuse and nodular adrenocortical hyperplasia	Classical

**Table S2. Individual assessments of adrenal #18 with between-pathologist disagreement**

APA, aldosterone-producing adenoma; APM, aldosterone-producing micronodule; APN, aldosterone-producing nodule; CYP11B2, aldosterone synthase; NFA, nonfunctioning adenoma

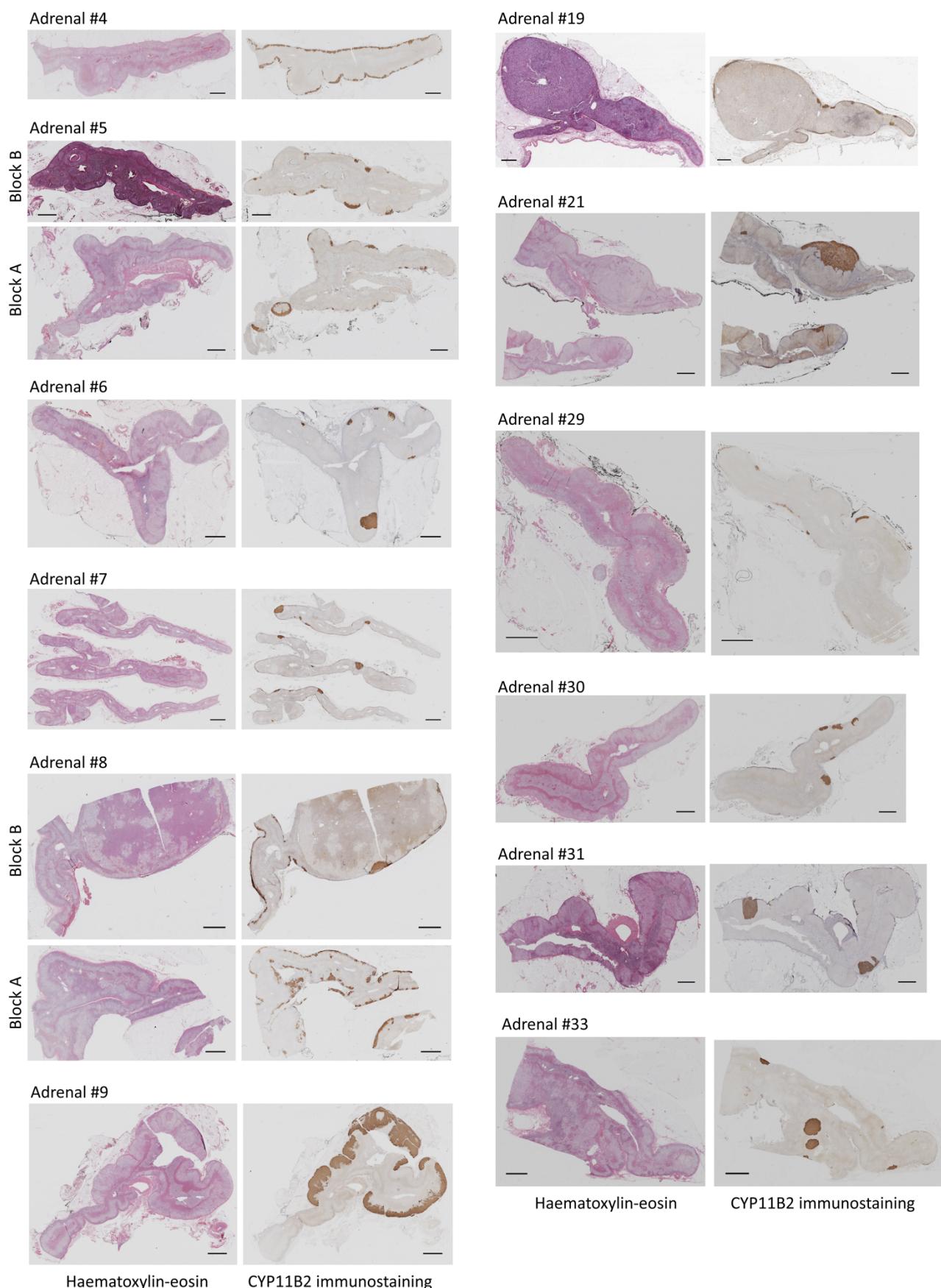


**Figure S1, part A. Haematoxylin-eosin staining and CYP11B2 immunostaining of adrenals with histopathologic findings of classical unilateral primary aldosteronism**  
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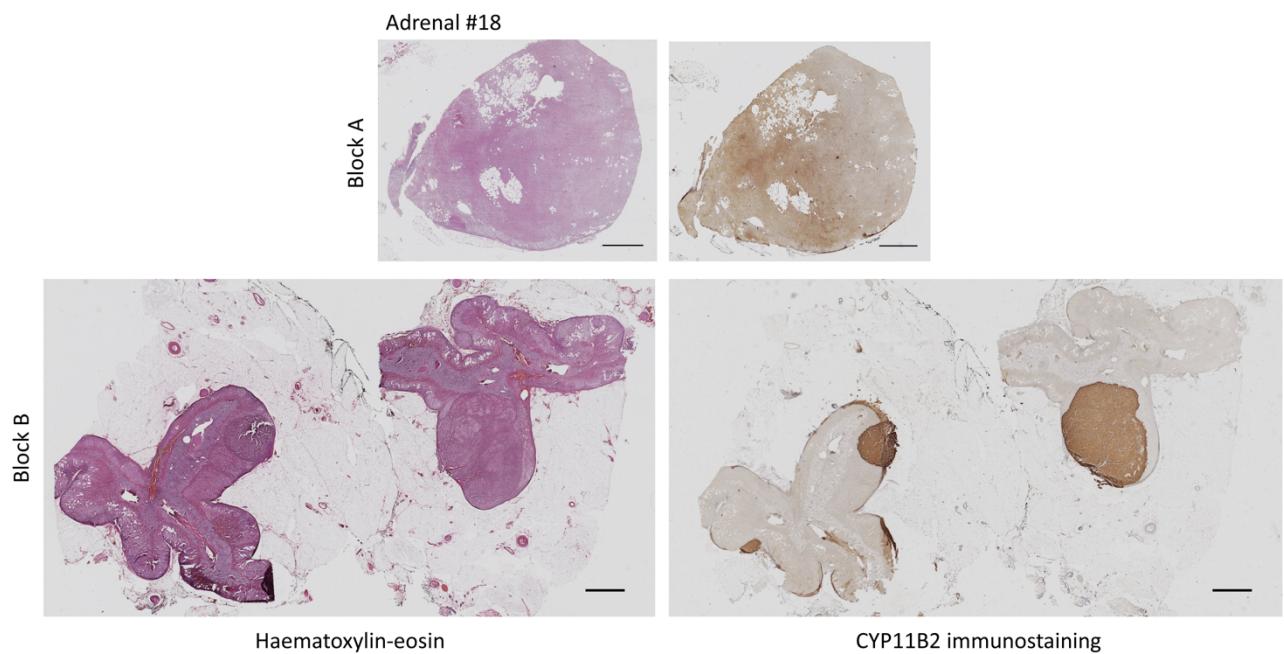


**Figure S1, part B. Haematoxylin-eosin staining and CYP11B2 immunostaining of adrenals with histopathologic findings of classical unilateral primary aldosteronism**

Scale bar, 2 mm



**Figure S2. Haematoxylin-eosin staining and CYP11B2 immunostaining of adrenals with histopathologic findings of non-classical unilateral primary aldosteronism**  
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**Figure S3. Haematoxylin-eosin staining and CYP11B2 immunostaining of the adrenal with disagreement between group 1 and group 2 pathologists for histopathologic findings**

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## References

- (1) Williams TA, Lenders JWM, Mulatero P, Burrello J, Rottenkolber M, Adolf C, Satoh F, Amar L, Quinkler M, Deinum J, Beuschlein F, Kitamoto KK, Pham U, Morimoto R, Umakoshi H, Prejbisz A, Kocjan T, Naruse M, Stowasser M, Nishikawa T, Young WF Jr, Gomez-Sanchez CE, Funder JW, Reincke M; Primary Aldosteronism Surgery Outcome (PASO) investigators. Outcomes after adrenalectomy for unilateral primary aldosteronism: an international consensus on outcome measures and analysis of remission rates in an international cohort. *Lancet Diabetes Endocrinol.* 2017; **5**: 689-699.
- (2) Yang Y, Burrello J, Burrello A, Eisenhofer G, Peitzsch M, Tetti M, Knösel T, Beuschlein F, Lenders JWM, Mulatero P, Reincke M, Williams TA. Classification of microadenomas in patients with primary aldosteronism by steroid profiling. *J Steroid Biochem Mol Biol.* 2019; **189**: 274-282.