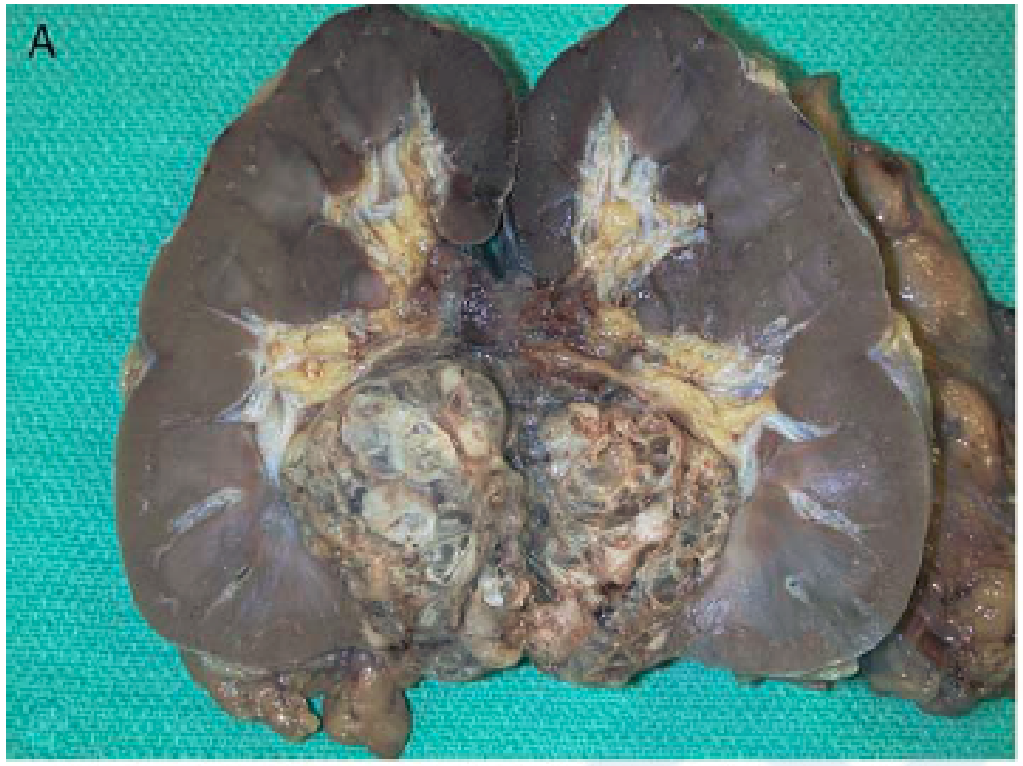
Am J Surg Pathol Volume 43, Number 1, January 2019

Somatic Mutations of TSC2 or MTOR Characterize a Morphologically Distinct Subset of Sporadic Renal Cell Carcinoma With Eosinophilic and Vacuolated Cytoplasm

汇报人：许秀丽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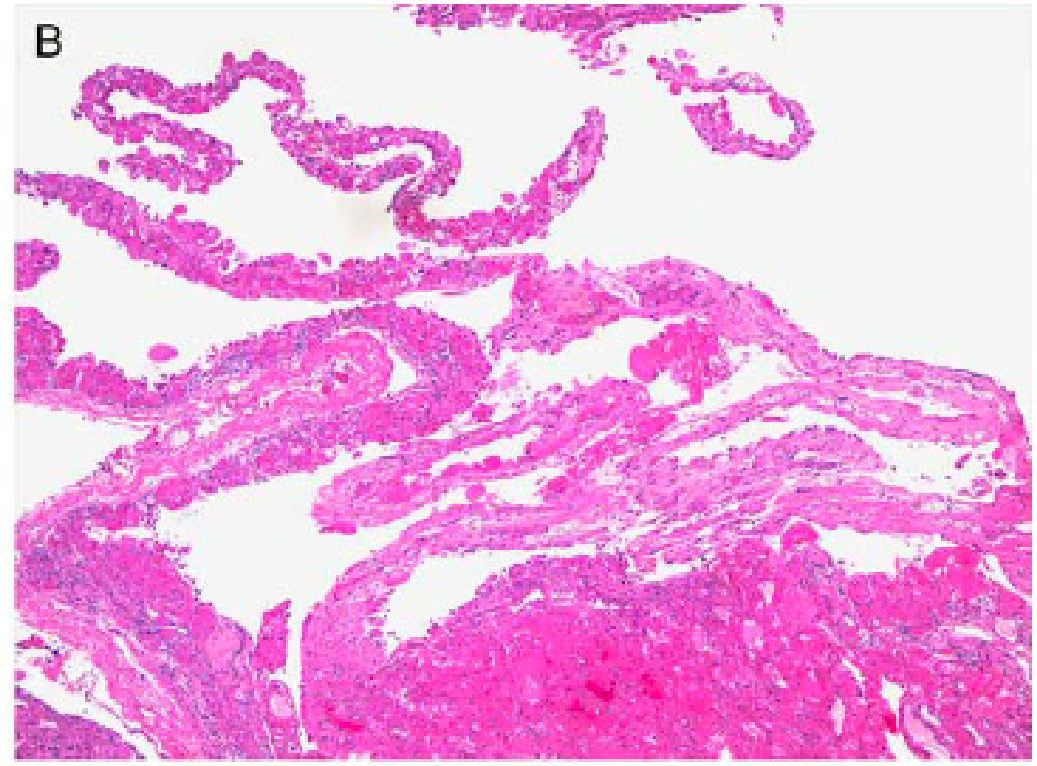
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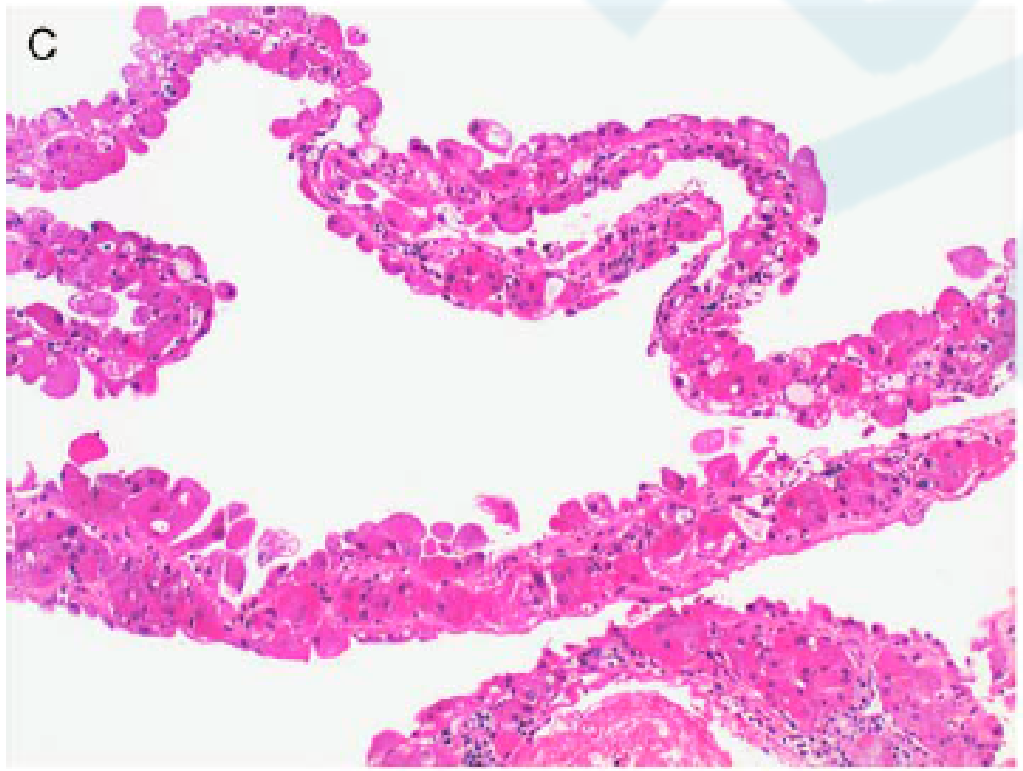
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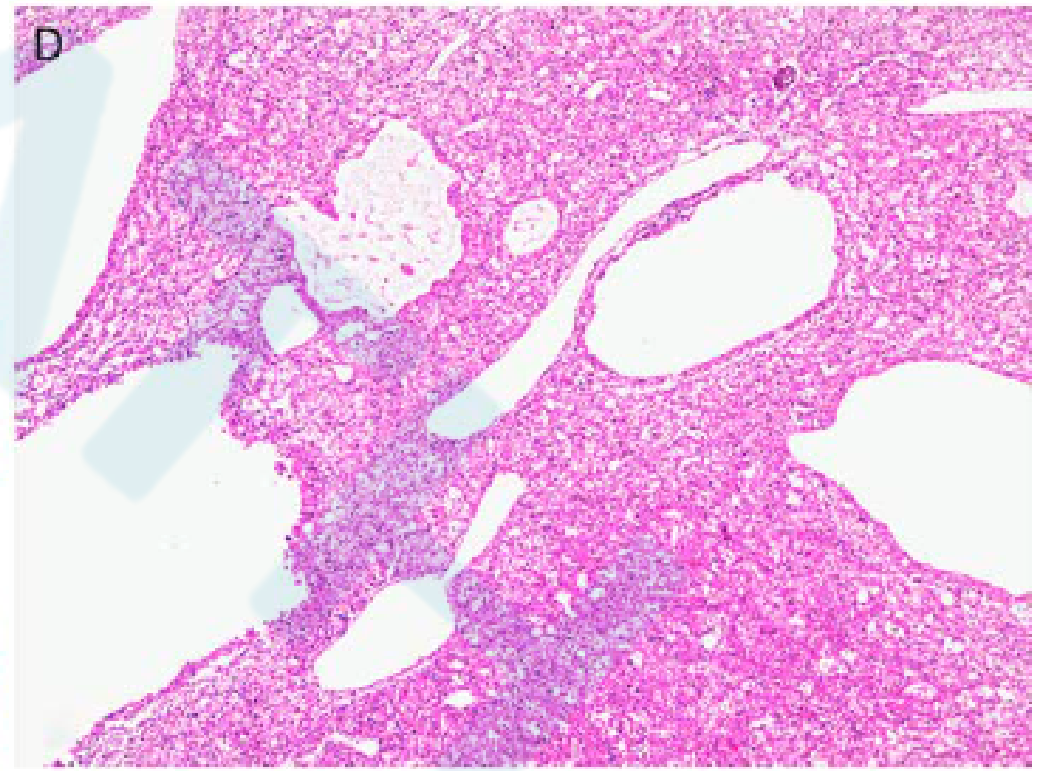
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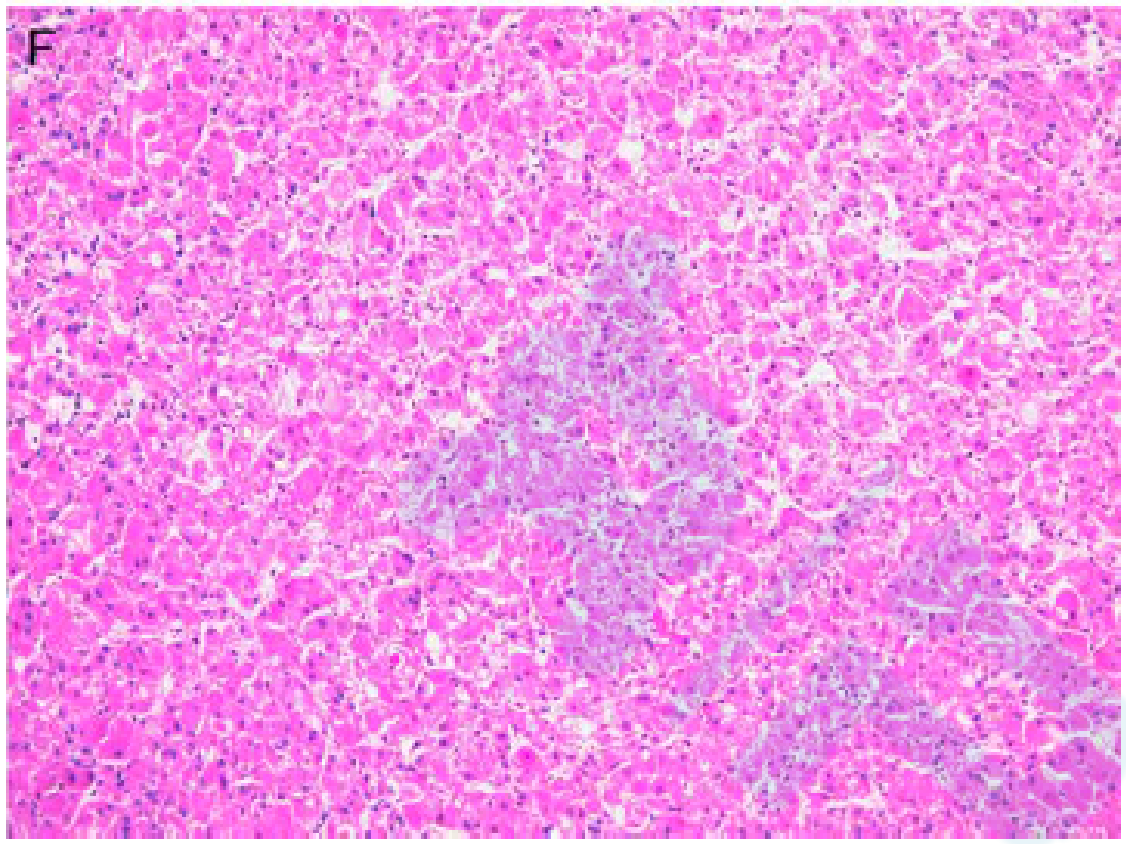

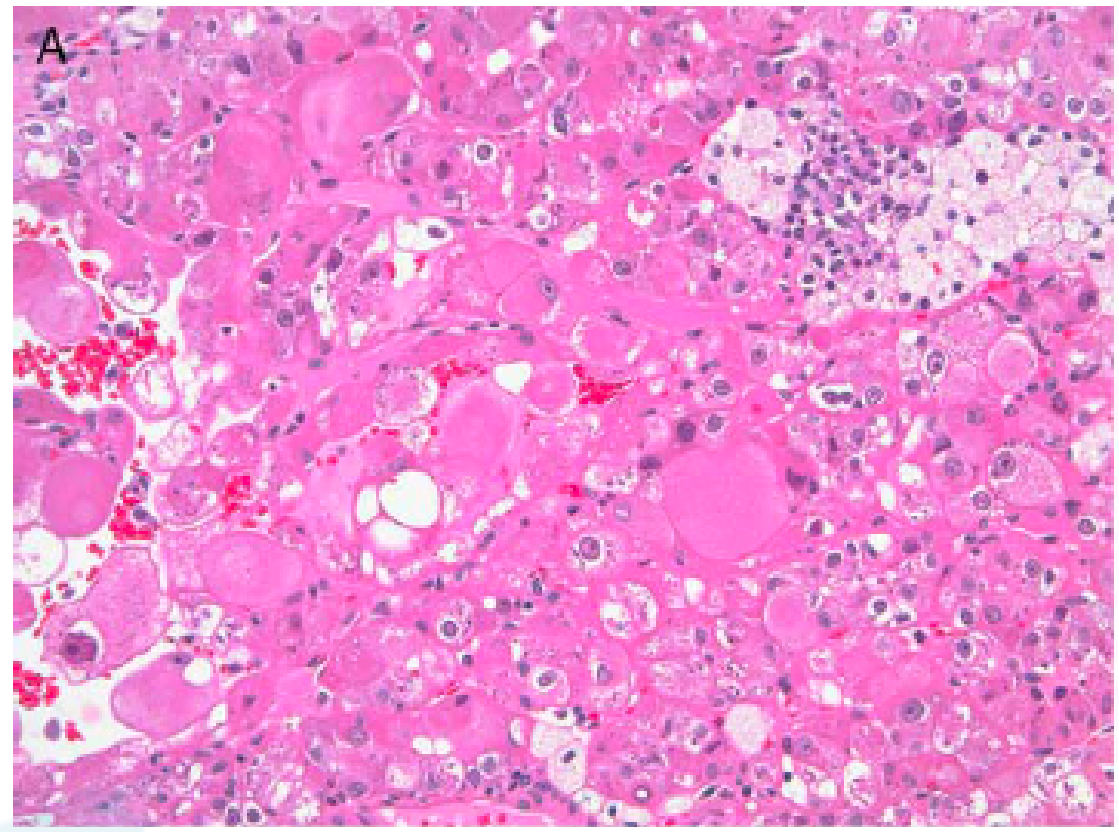

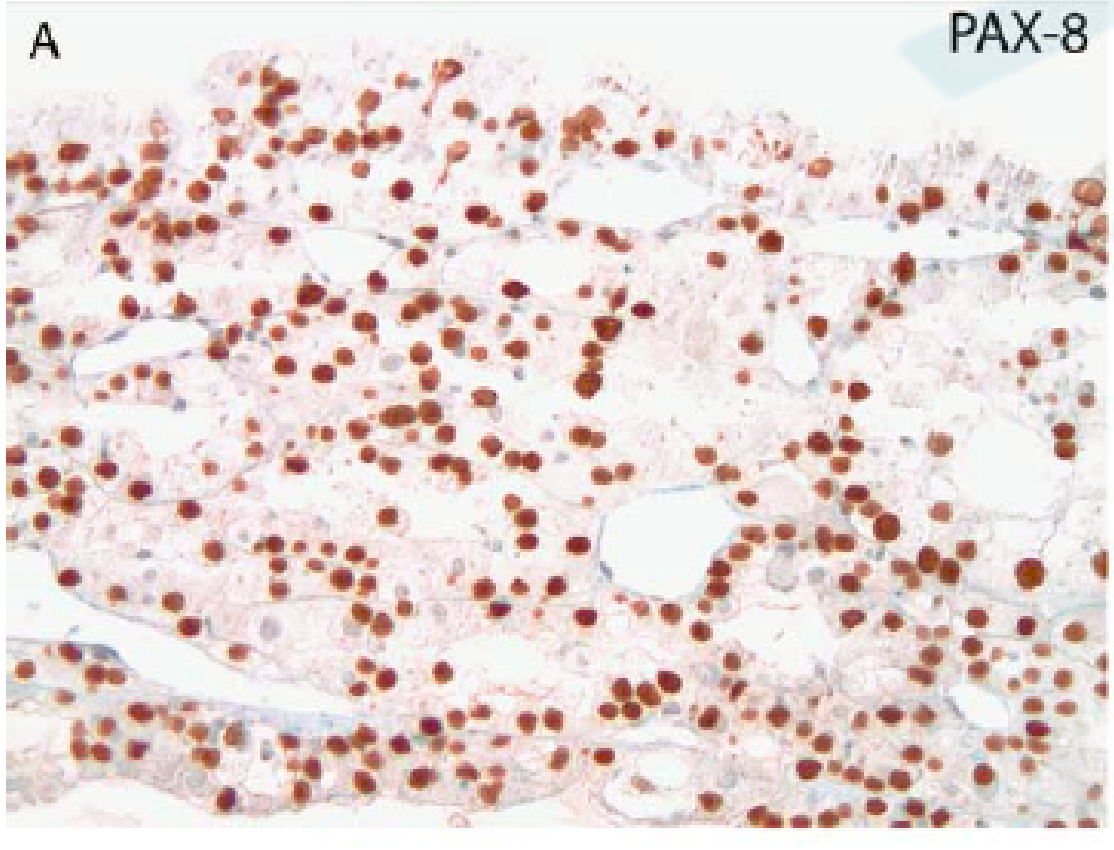

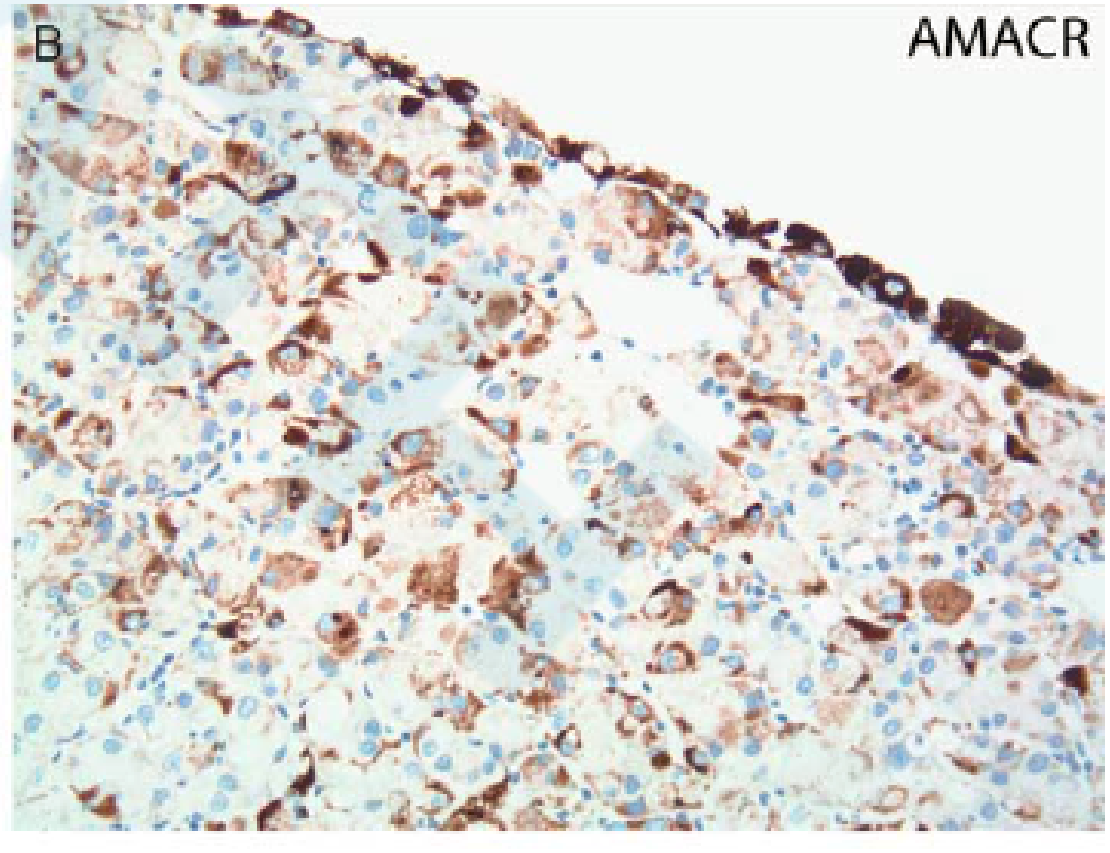

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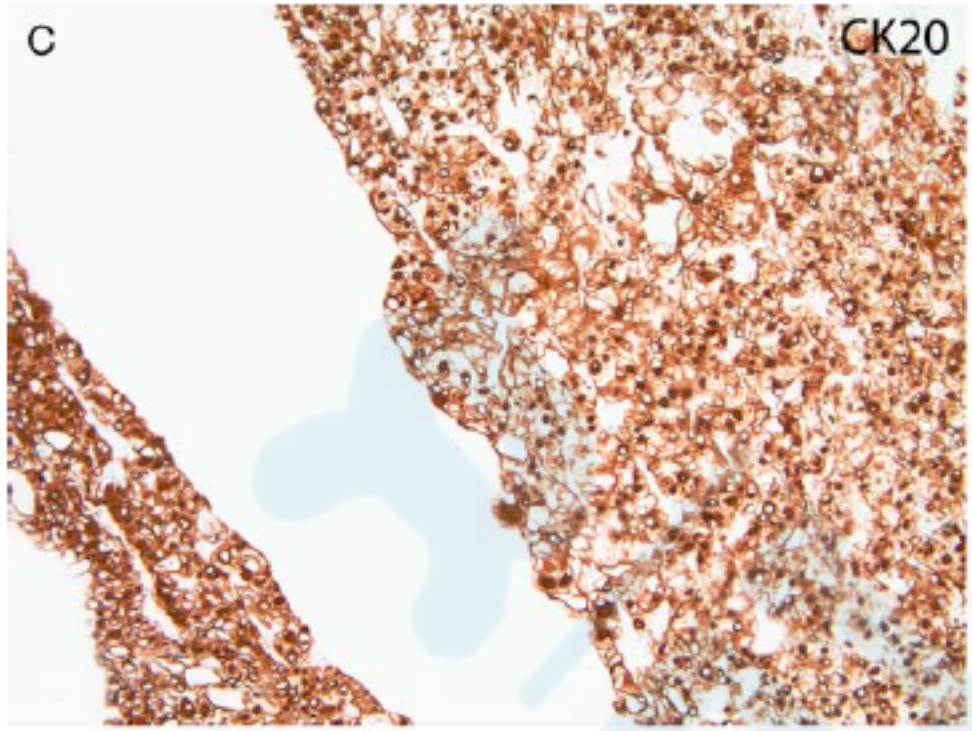
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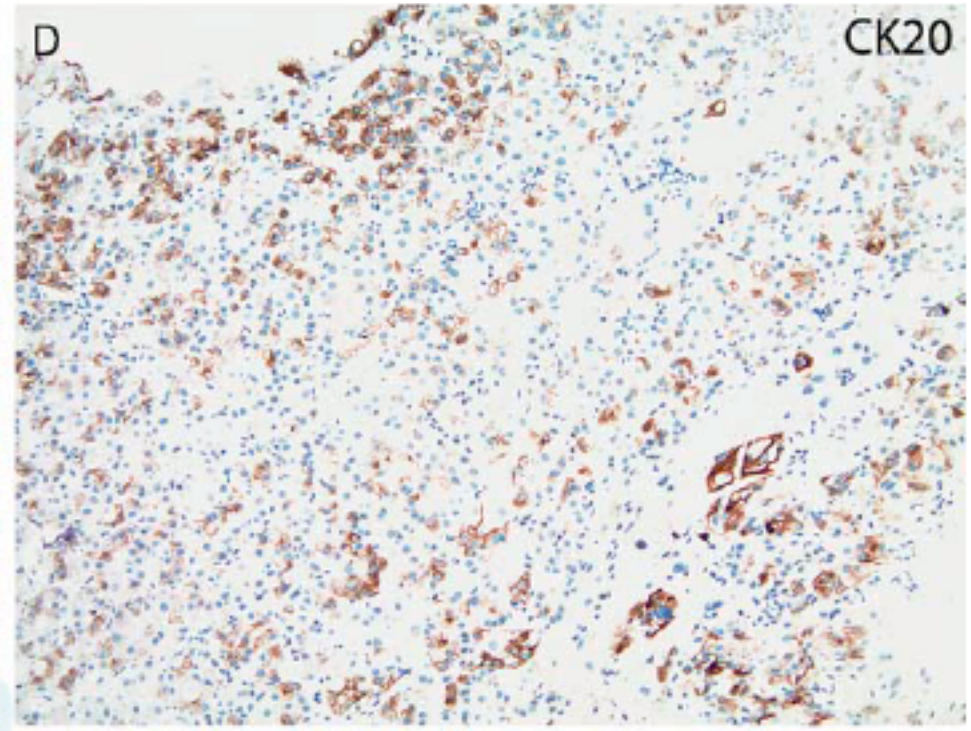
CK20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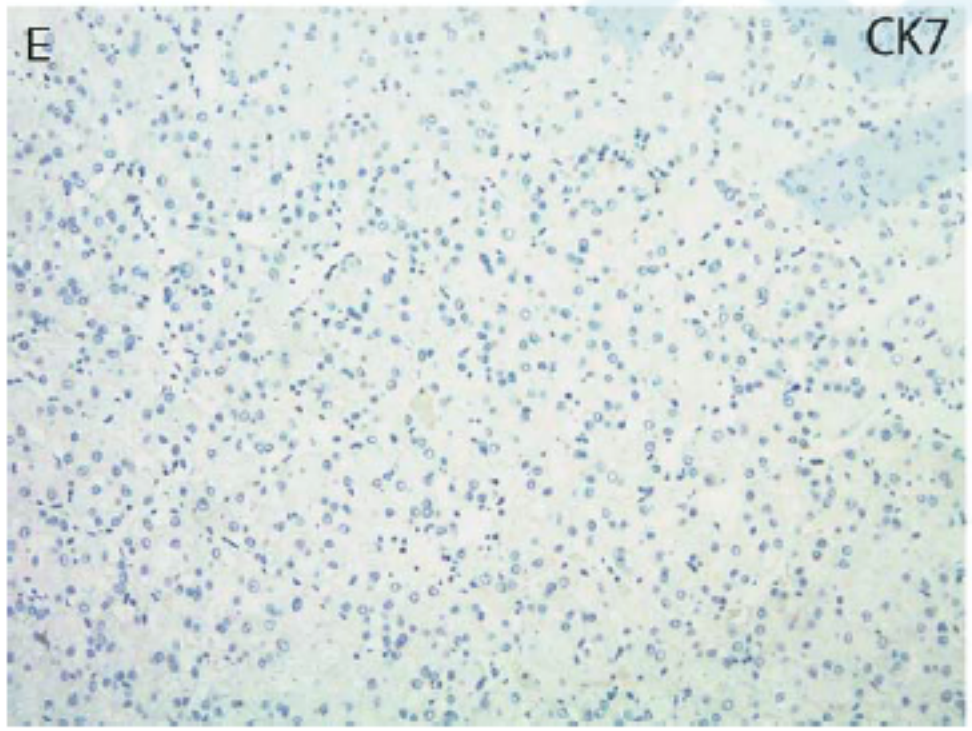
CK20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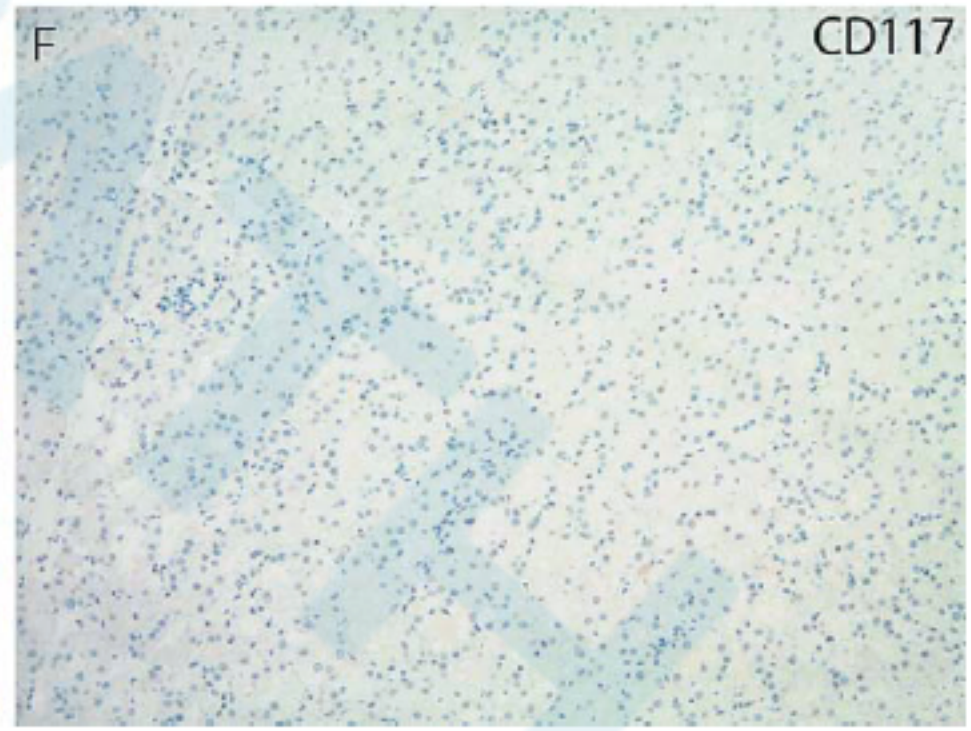
CK7


F
CD117

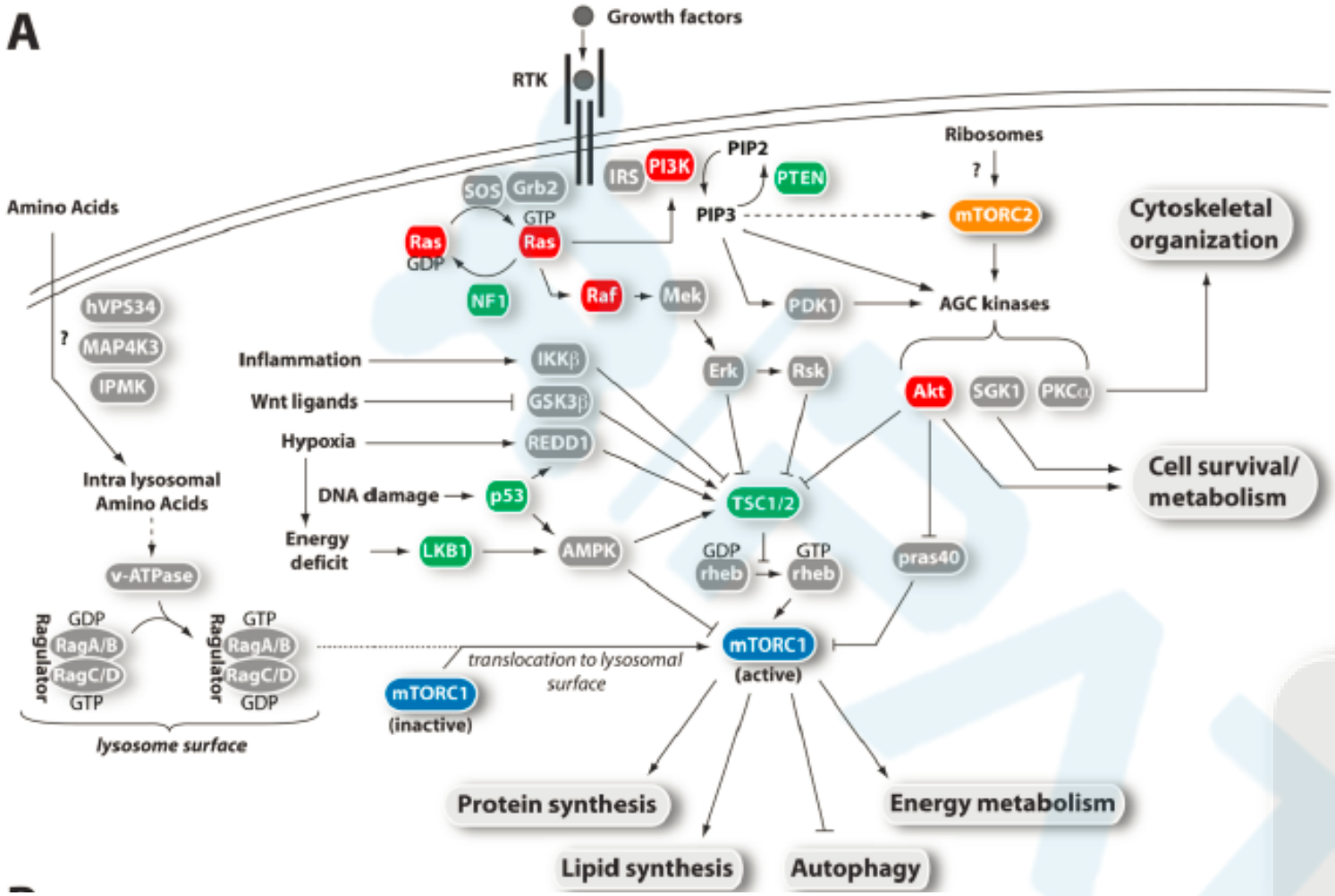
Eosinophilic, solid, and cystic renal cell carcinoma" (ESC RCC)

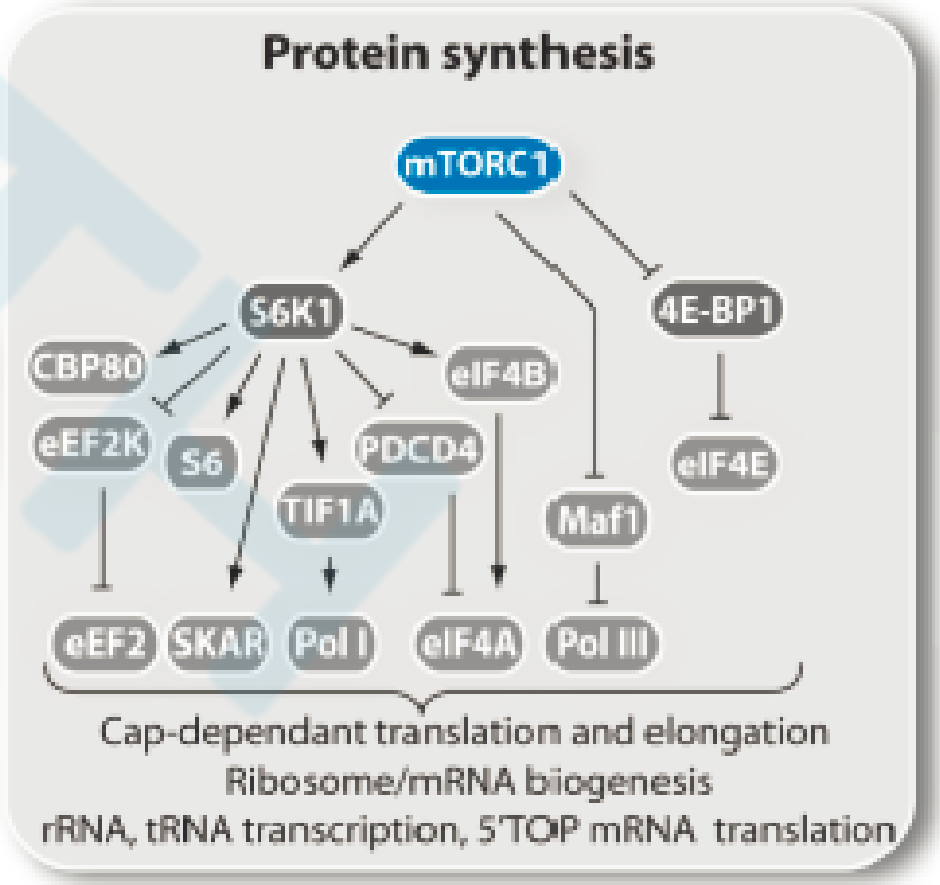
TABLE 2. Summary of the Key Features of ESC RCC

Clinical	Females, usually low stage, good prognosis
Gross	Solid and cystic or solid (minority), tan, single tumors
Light microscopy	Architecture: Solid and cystic. Hobnail arrangement of cells lining septa. Diffuse or tightly compact acinar or nested growth in solid foci. Capsule absent. Cytology: Eosinophilic, voluminous cytoplasm with granular stippling, round to oval nuclei, and prominent nucleoli. Scattered foamy histiocytes, lymphocytes, and multinucleated cells.
IHC	Positive: PAX8, $CK20^{+}/CK7^{-}$ phenotype most common, Vimentin, AMACR (+/−), CD10 (+/−) Negative: CA9, CD117, HMB45
Electron microscopy	Abundant rough endoplasmic reticulum
Molecular karyotype	LOH: 16p and Xq (3/3 cases); 11p (2/3 cases) CN gains: 1p, 7p-q, 10q, 13q, 16p-q (2/3 cases) CN losses: 19p, 19q, Xp, Xq (2/3 cases)
aCGH	Gain of Chr 16 (only 1 case analyzed)

CN indicates copy number.

Cell. 2012 April 13; 149(2): 274–293.





MATERIALS AND METHODS

➢Case Selection and Histologic Review

The study included **7** retrospectively identified cases with similar histomorphology.

➢Immunohistochemistry

Pax8、CK7、CK20 、CD117 、TFE3 、HMB45、Melan A、cathepsin-K、 FH、 SDHB、 phospho-S6、 phospho-4E-BP1

➢Molecular Analysis

The **5 cases** with molecula analysis, 3 had paired tumor and normal DNA samples, and 2 had only tumor DNA available

RESULTS

➢Clinical Features

TABLE 1. Clinicopathologic Characteristics of 7 Cases in the Cohort

Pt	Age (y) /Sex	Presentation	Imaging	Personal /Family History*	Size (cm)	pT	Growth Pattern	Nuclei	Cytoplasm Vacuolization	Thick-walled Vessels	Calc	Foamy Histiocytes /Lymphocytes	F/U (mo)
1	55/F	Incidental	R/solitary	None/none	2.5	pT1a	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	Yes	No	NED (128)
2	40/F	Incidental	R/solitary	None/none	5	pT1b	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	Yes	No	NED (21)
3	40/M	Right flank pain and urinary frequency	L/solitary	None/none	2.5	pT1a	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	No	No	NED (14)
4	68/F	UTI	L/solitary	Osteoporosis /none	4.4	pT1b	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	Yes, exten-sive	No	NED (13)
5	59/M	UTI	R/solitary, one simple cyst (2.3 cm)	Hypertension /none	3.6	pT1a	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	Yes	No	NED (10)
6	52/M	Trauma	R/solitary	Trauma/NA	1.5	pT1a	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	No	No	NA
7	62/F	Incidental	R/solitary	NA	4.2	pT1b	Nested, with dispersed single cells	Prominent nucleoli, multinucleation	Extreme	Yes	No	No	NA

*History includes any past medical history raising concerns for TSC, other significant past medical history, and family history of renal cancer or any syndromic condition.
Calc indicates calcifications; F, female; F/U, follow-up; L, left; M, male; NA, not available; NED, no evidence of disease; R, right; UTI, urinary tract infection.

- **1.The mean age at presentation was 54 years (range: 40 to 68 y) with a male to female ratio of 3:4;**
- **2.Tumor size ranged from 1.5 to 5 cm (mean: 3.4 cm);**
- **3. All 5 patients with clinical follow-up data had no evidence of disease at the last visit. The median follow-up time was 14 months.**

➢Pathologic Features

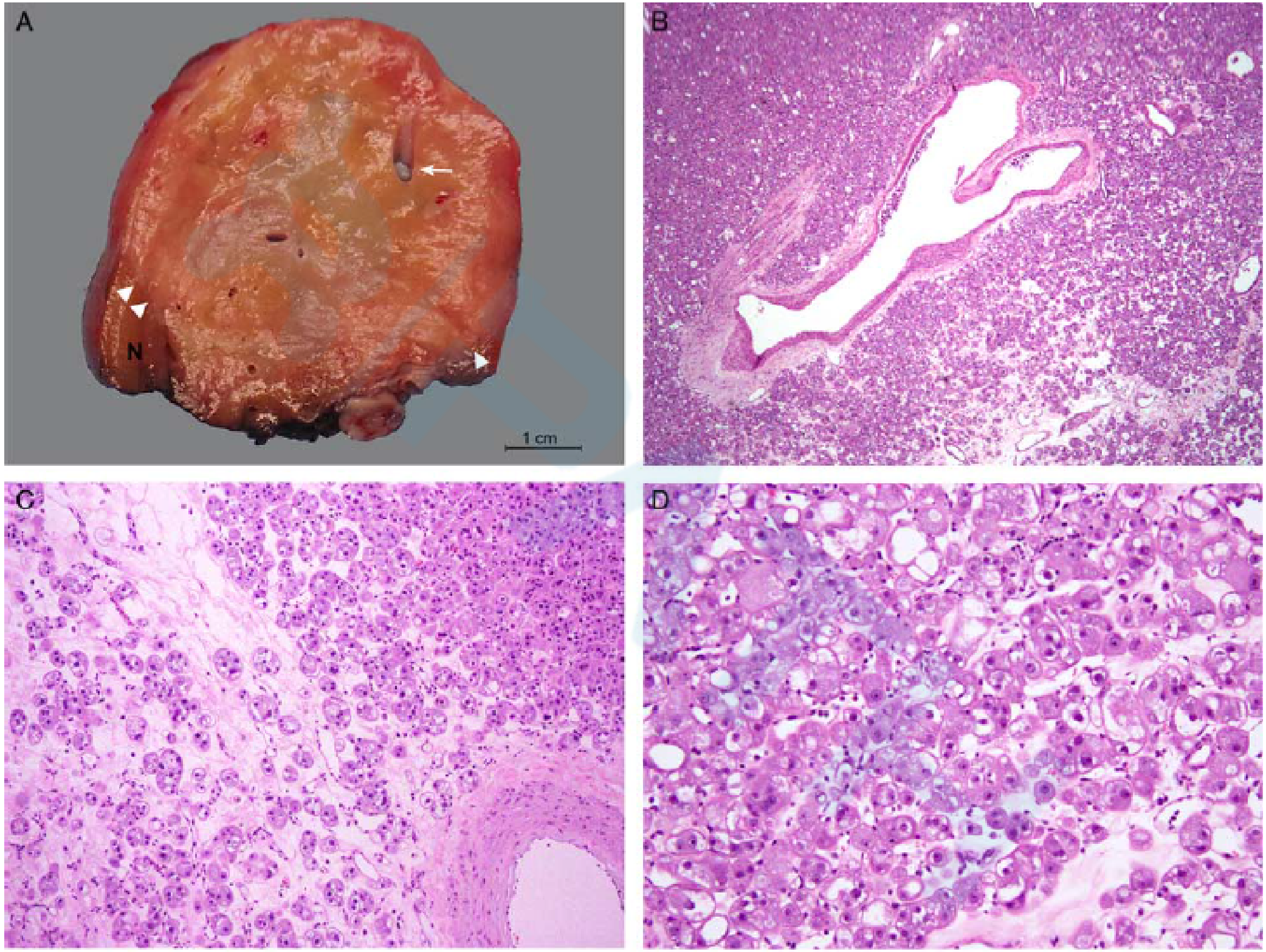
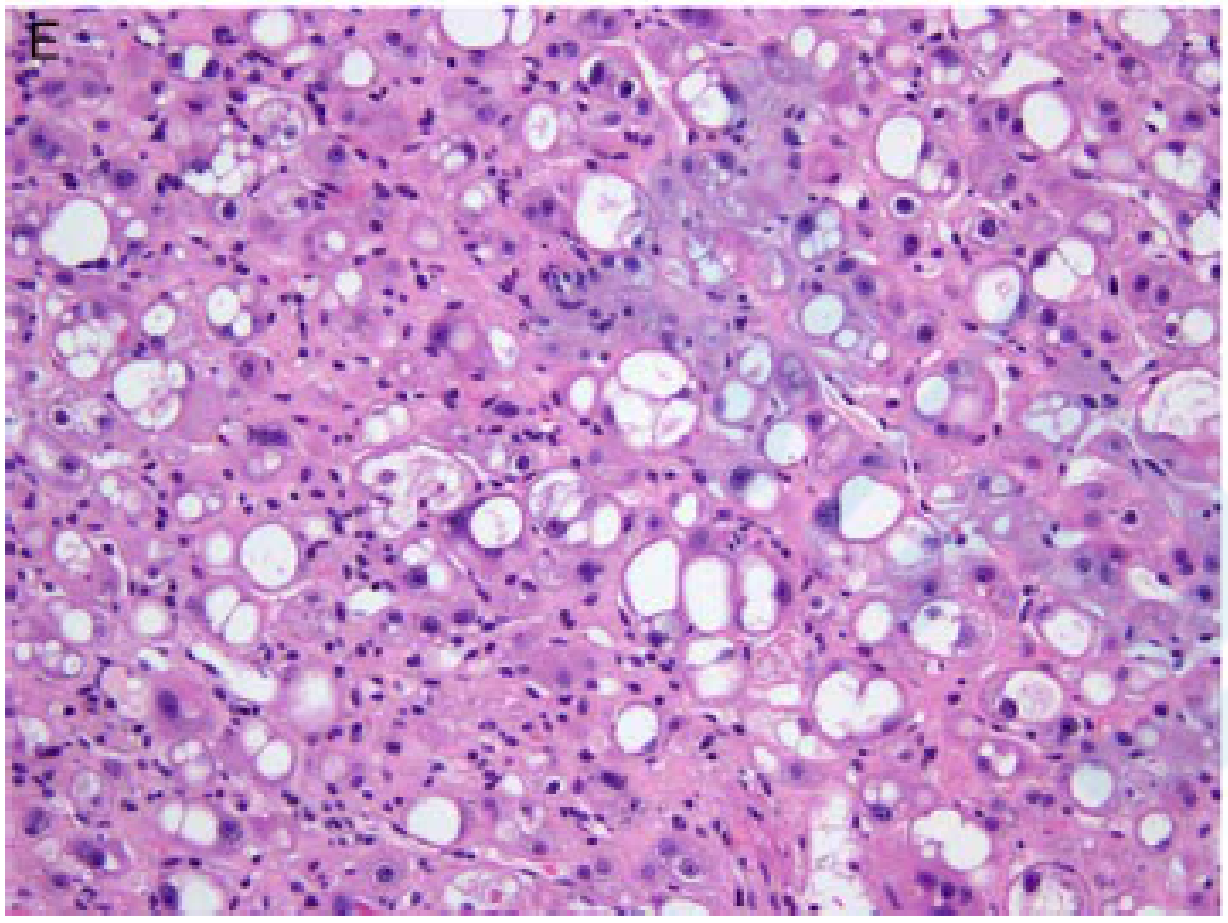
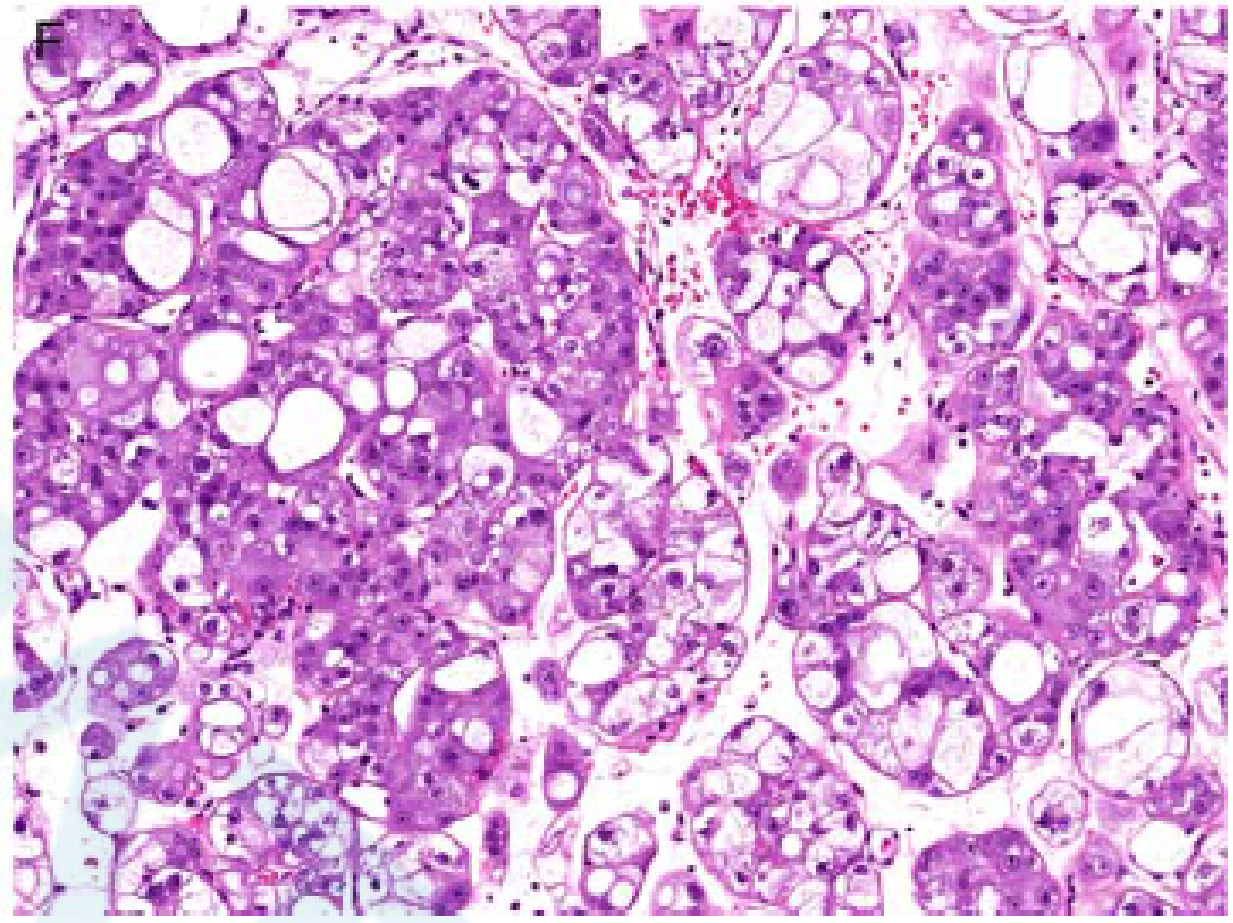


FIGURE 1. Macroscopic and microscopic features of a distinct group of RCC with eosinophilic and vacuolated cytoplasm. D, Case 1.





E, Case 4.

F, Case 6.

D–F, Tumor cells show round nuclei with conspicuous to prominent nucleoli and eosinophilic, granular, and vacuolated cytoplasm. Vacuolization varies from numerous small intracytoplasmic vesicles to large spaces almost occupying the entire cytoplasm.

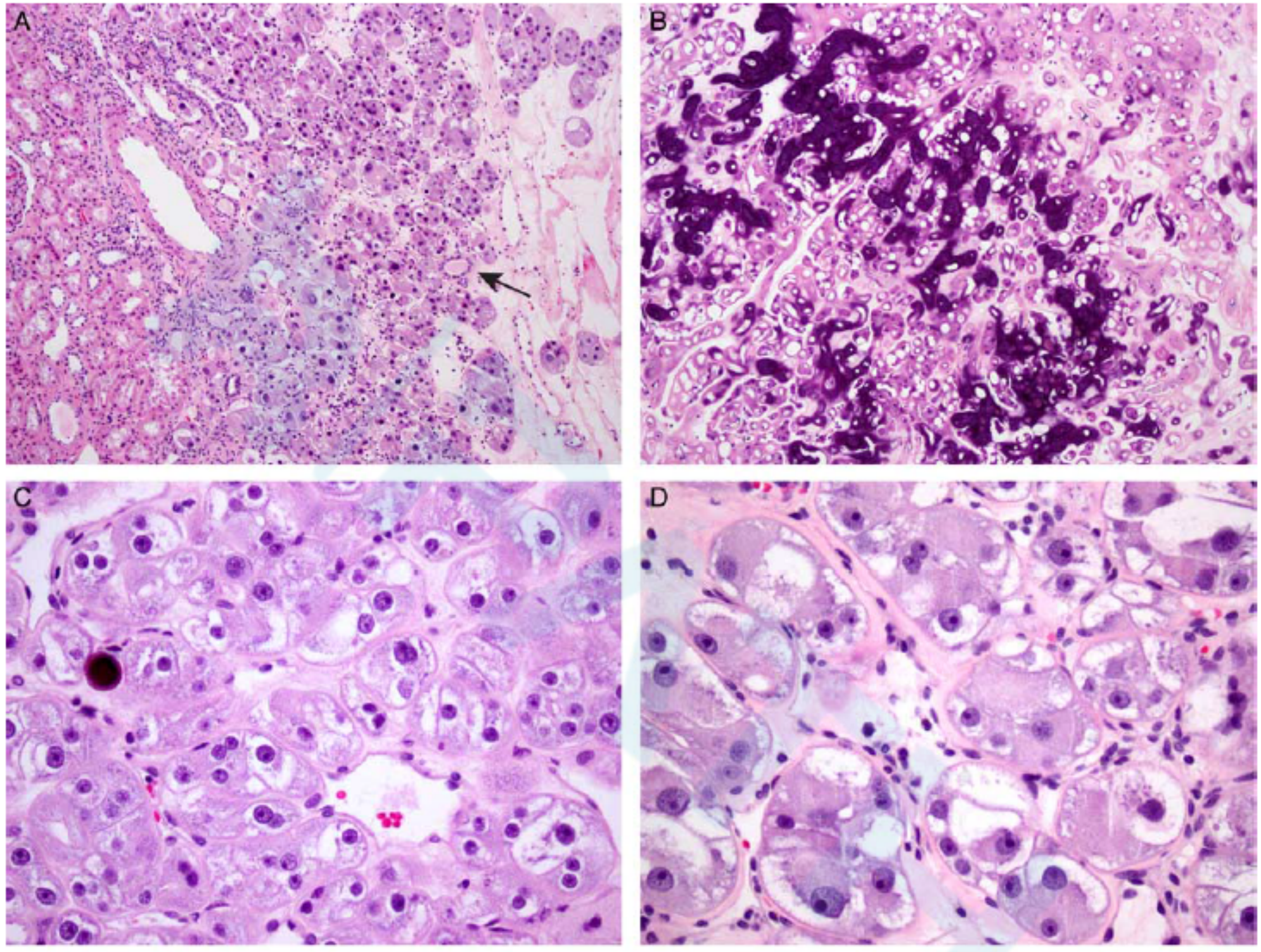
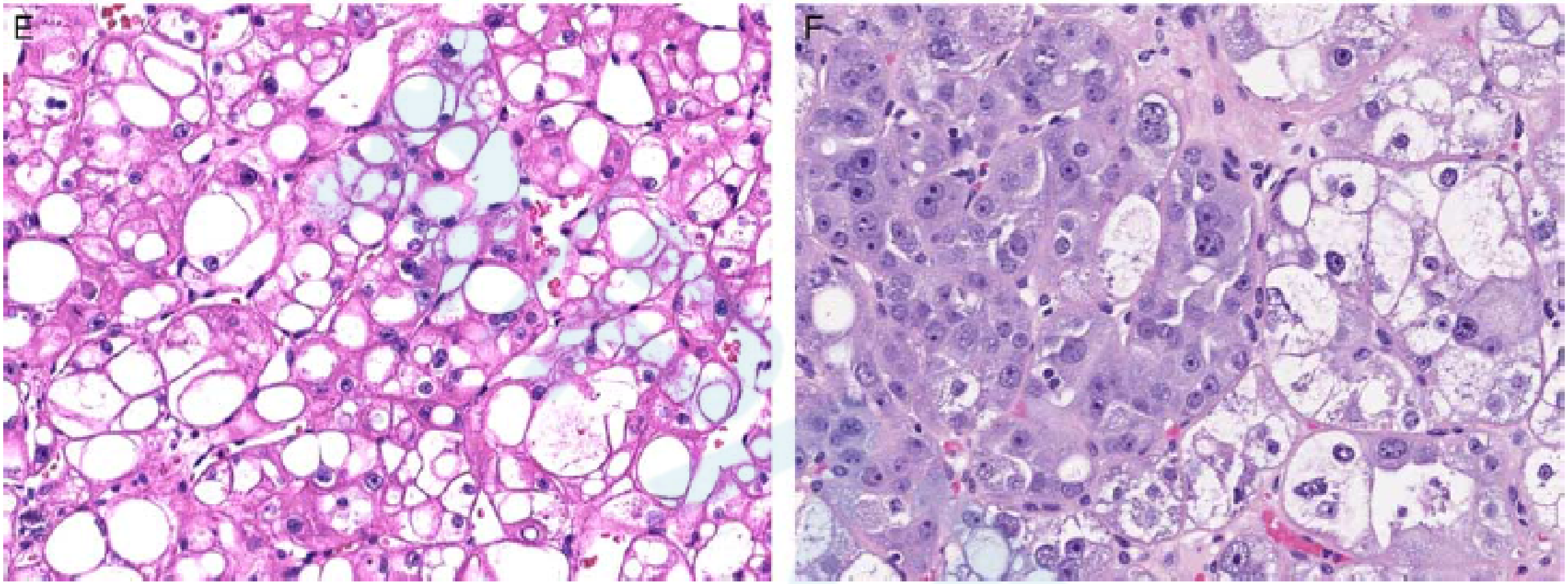


FIGURE 2. Additional histologic features include: lack of tumor capsule and entrapped renal tubules (arrow) (A); extensive calcifications (B); microscopic calcifications and perinuclear cytoplasmic clearing, mimicking chromophobe RCC (C); marked cytoplasmic eosinophilia with filamentous material and focal cytoplasmic stippling (D)



(E) cribriform or sieve-like appearance in areas with tightly packed nests and extensive vacuolization; (F)abrupt transition between tumor cells with prominent vacuolization and those with dense, granular cytoplasm.

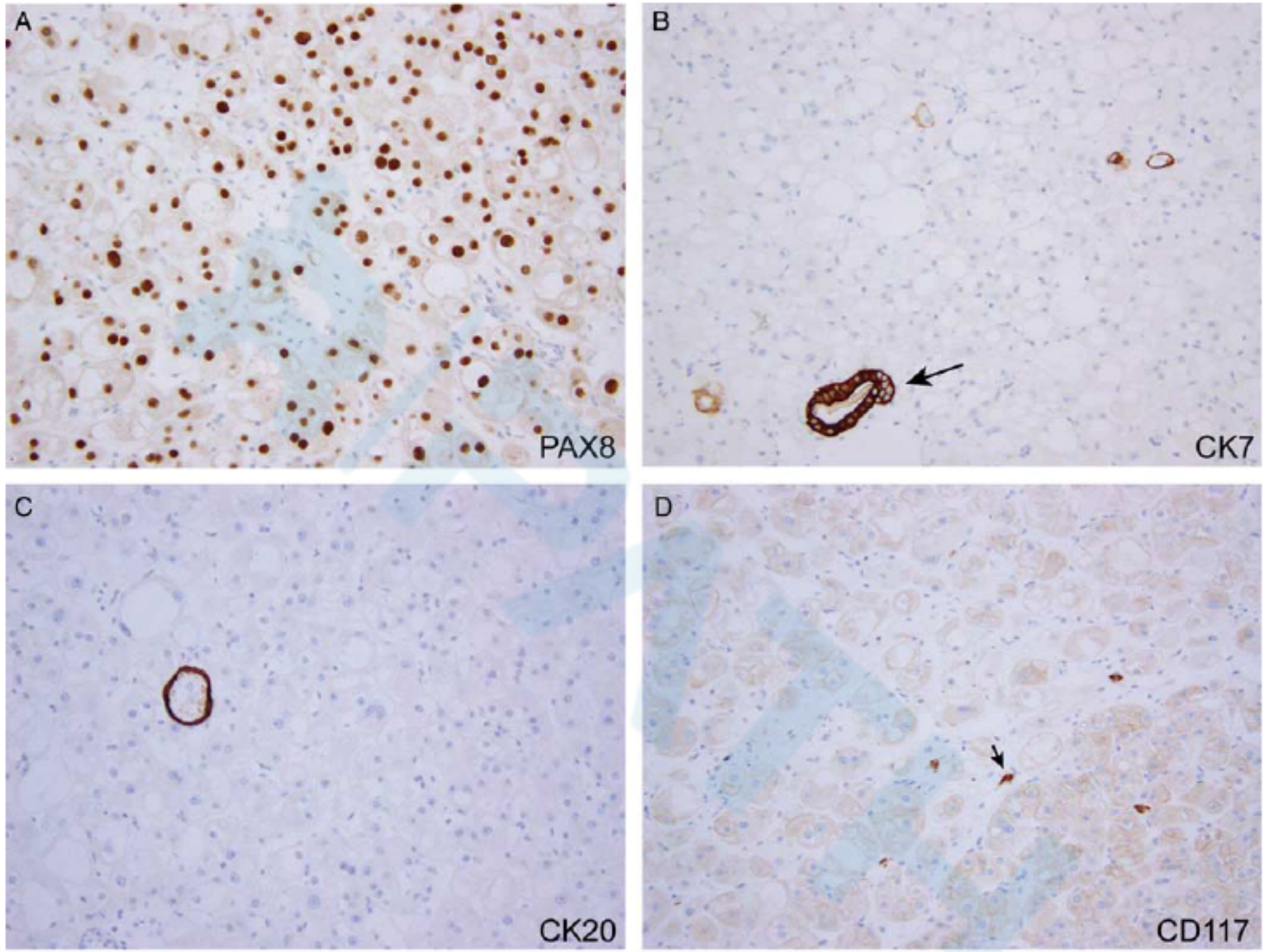
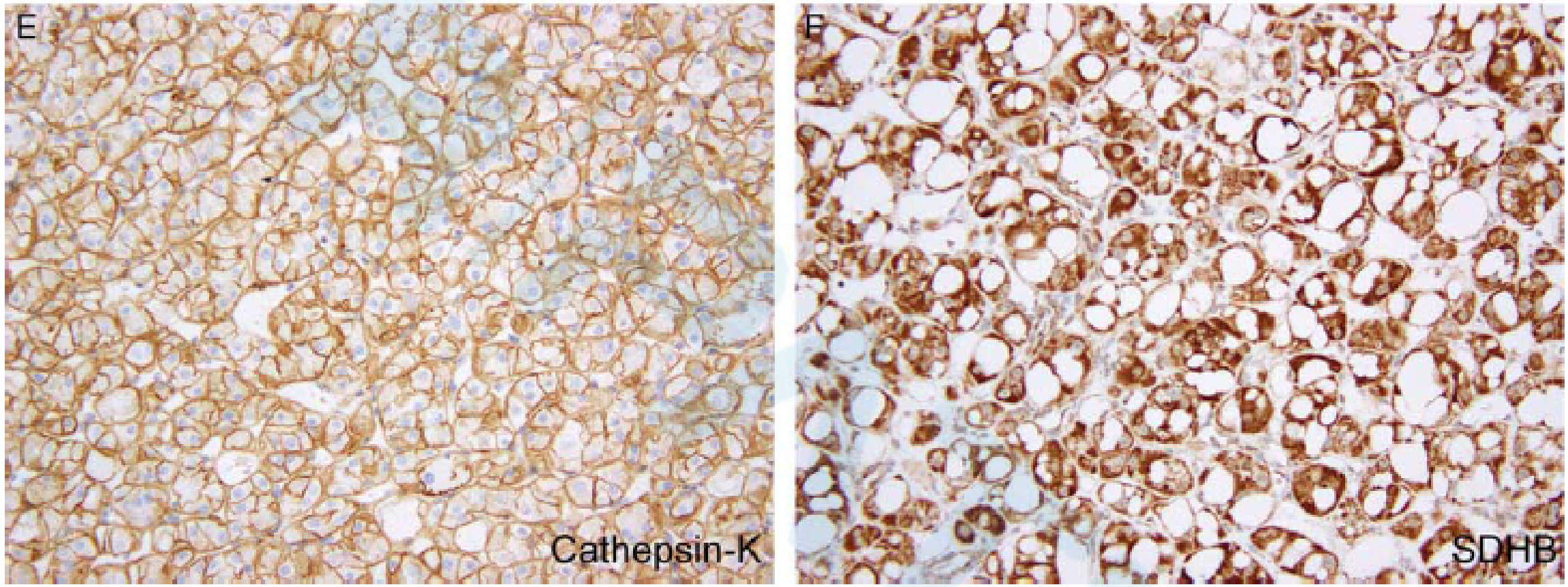


FIGURE 3. Immunohistochemical features. A, Positive nuclear staining for PAX8. B and C, Tumor cells negative for CK7 (B) and CK20 (C) except for scattered rare cells/vacuoles; arrow in (B) marks an entrapped renal tubule. D, Weak membranous staining for CD117; arrow marks a mast cell.



E, Diffuse cathepsin-K immunoreactivity. F, Retained SDHB expression.

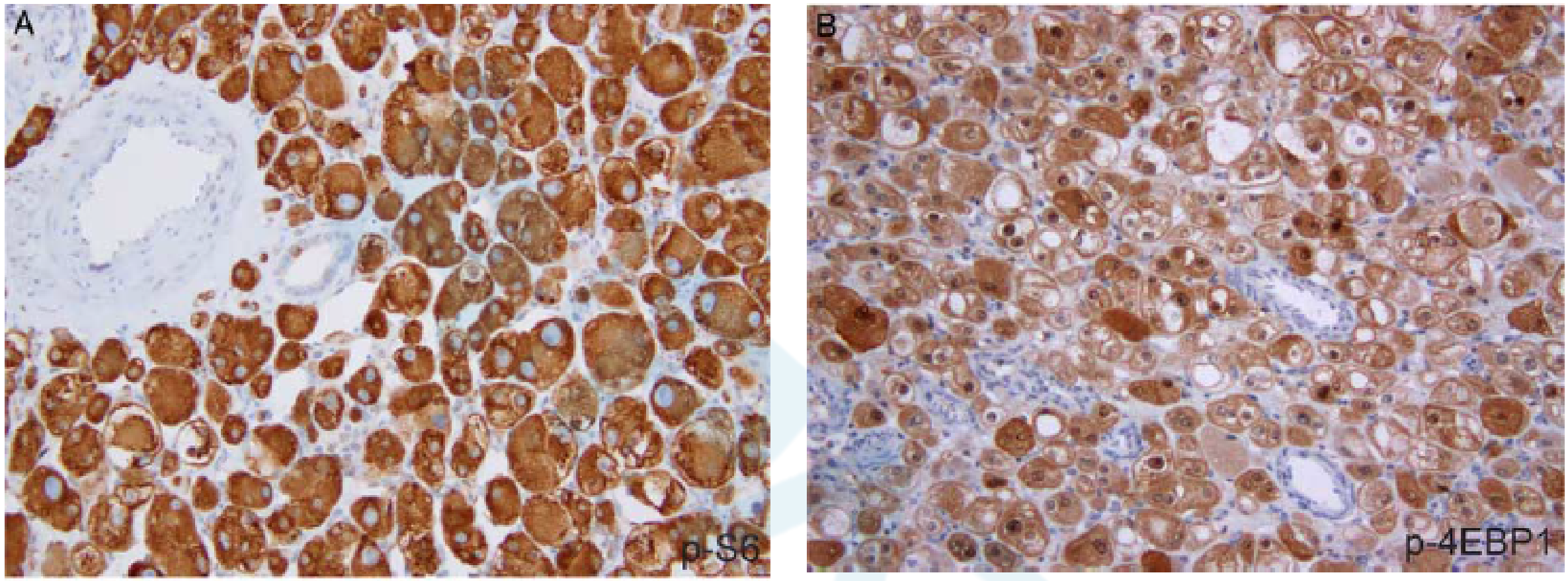


FIGURE 4. Representative images of p-S6 (A) and p-4EBP1 (B) immunostains.

TABLE 2. Immunohistochemical Results and Molecular Alterations Detected

	Immunohistochemistry													
Pt	PAX8	CK7	CK20	CD117	Cathepsin K	SDHB	FH	TFE3	HMB45	Melan A	p-S6*	p-4EBP1	Somatic Mutations†	Copy Number Alterations
1	+	−	−	−	+	R	R	−	−	−	300	300	**TSC2 p.R1138***	ND
2	+	−	−	Weak (+)	+	R	R	−	−	−	300	300	**TSC2 p.X373_splice** **TSC2 p.Q510Sfs***	Focal losses 1p36.3, 1p35 Focal gains 5q35, 6p22, 9q31-34 Minor clone(s) with gains of 4q, 5, 8q, 12§
3	+	−	−	Weak (+)	−	R	R	−	−	−	300	300	ND	ND
4	+	−	−	−	+	R	R	−	−	−	300	300	**MTOR p.L2427R**	Loss of 1
5	+	−	NA	Weak (+)	NA	R	R	−	−	−	NA	NA	**MTOR p.L2427R** NOTCH2 p.D1306N (VUS)‡	Loss of 1 Loss of 6p-q24 LOH and gain of 21 Gain of 6q25-26
6	+	NA	−	−	+	R	R	−	NA	NA	300	230	**TSC2 p.X534_splice** **TSC2 p.K506Sfs*** PTPRD p.T988S (VUS)‡	Loss of 21q Focal gain 10q25-26
7	+	−	−	Weak (+)	Focal (+)	R	R	−	−	−	250	230	ND	ND

*Immunohistochemical stain result is shown in H-scores [H = intensity (0−3)×percentage of positive cells (1−100)].
†Mutations with known or likely oncogenic significance are marked in bold fonts.
‡On the basis of variant allele frequency, the possibility of this VUS represents a germline single nucleotide polymorphism cannot be excluded.
§The estimated minor clone fraction is about 9%.
LOH indicates loss of heterozygosity; NA, not available; ND, not determined; R, retained; VUS, variant of unknown significance.

Molecular Analysis

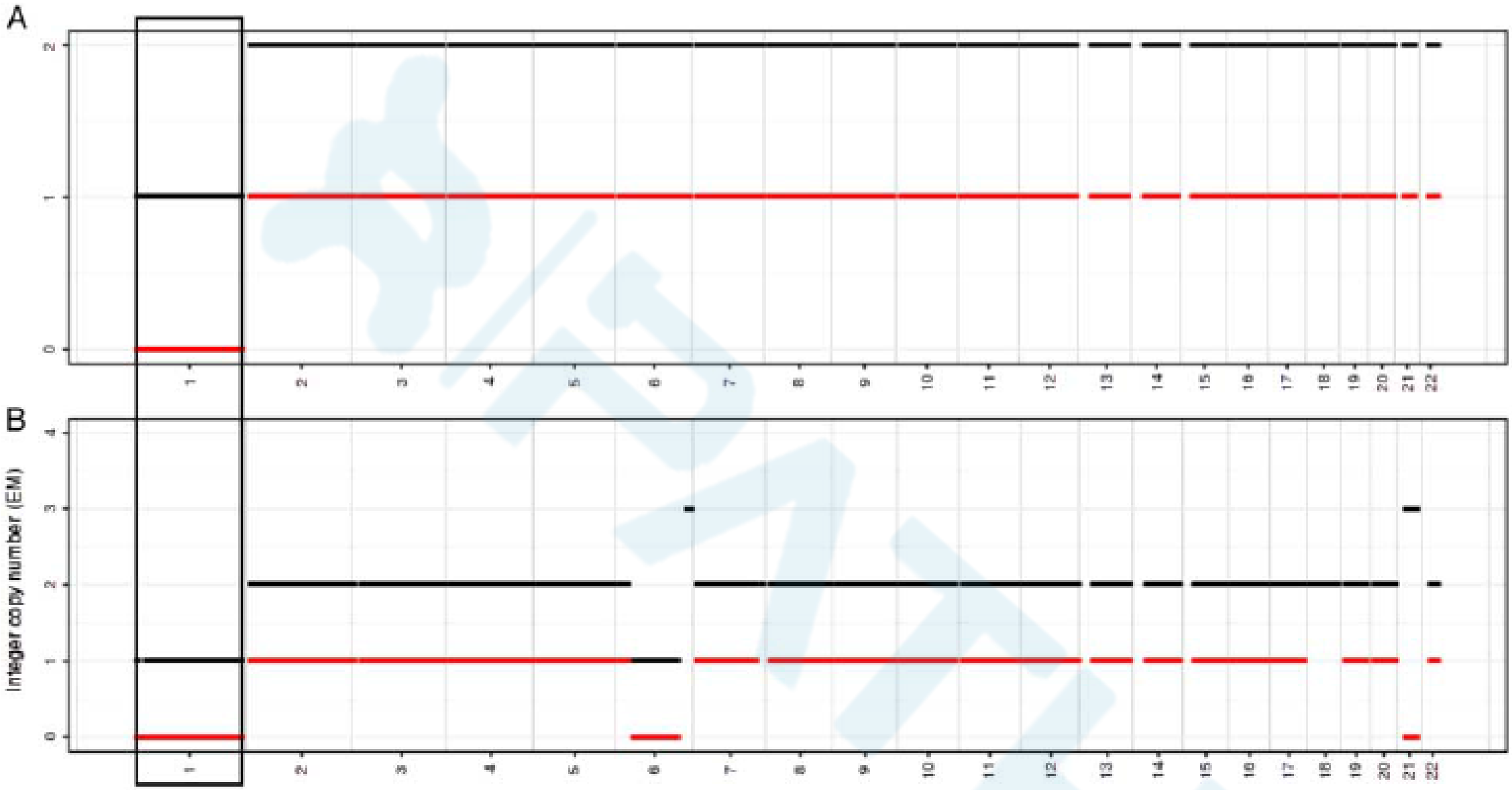
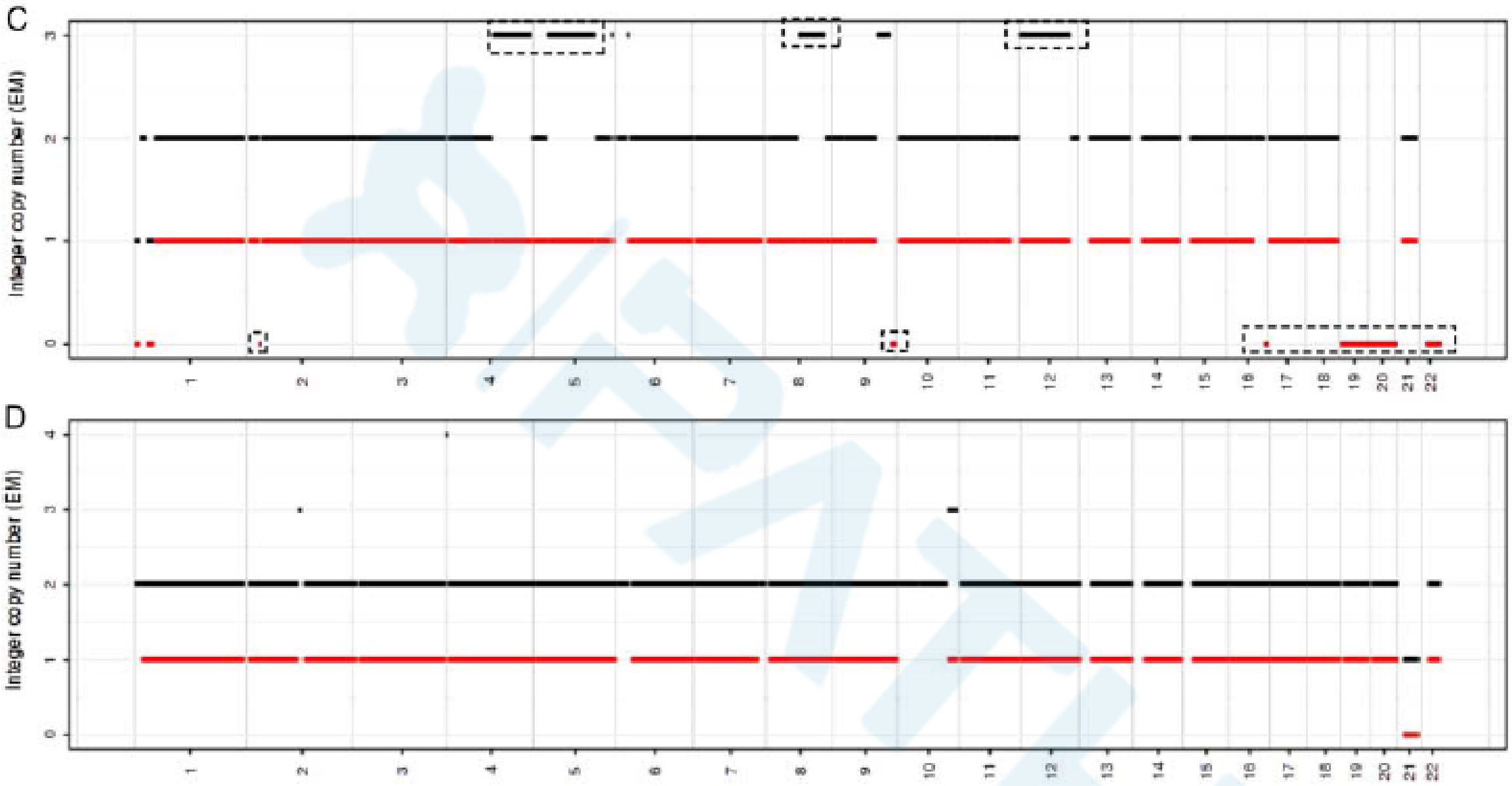


FIGURE 5. FACETS analysis in 4 cases. The integer copy number (copy number call corrected for tumor purity and ploidy) is plotted on the y-axis. Diploid corresponds to n =2. Chromosomes 1 to 22 are plotted on the x-axis. Black line—total copy number, red line—minor allele. A and B, Cases 4 (A) and 5 (B) (MTOR p.L2427R) show recurrent chromosome 1 loss (solid box).



C and D, Cases 2 (C) and 6 (D) (biallelic TSC2 mutations) have no shared gains/losses. Dashed boxes in (C) mark copy number changes detected in minor clone(s) with an estimated clone fraction $\leqslant$ 9%.

DISCUSSION

- Differential Diagnosis: ESC RCC

First, ESC RCC typically shows solid and cystic architecture, with a confluent growth of sheets or less commonly nests of tumor cells with almost invariably admixed small aggregates of histiocytes and lymphocytes.

Second, ESC RCC exhibits a predominant CK20(+)/CK7(−) immunophenotype, whereas our cases were all CK20(−)/CK7(−).

Third, ESC RCC occurs predominantly in women.

TABLE 3. Key Features and Immunostains Helpful in Distinguishing ESC RCC From Other Renal Tumors

Diagnosis	Key Distinguishing Features	IHC
ESC RCC	Female individuals, solid and cystic growth, voluminous eosinophilic cytoplasm, granular cytoplasmic stippling, usually low stage	$CK20^{+}/CK7^{-}$, $CD117^{-}$, $PAX8^{+}$, $PanCK^{+}$, $HMB45^{-}$, $CA9^{-}$ (no membranous reactivity)
Chromophobe RCC, eosinophilic	Solid and uniform architecture, irregular nuclear membranes, perinuclear halos	$CD117^{+}$, $CK7^{+}$, $CK20^{-}$
Oncocytoma	Uniform cytology, lacks macrocysts	$CD117^{+}$, $CK7^{-/+}$, $CK20^{-}$
Epithelioid angiomyolipoma	Epithelioid cells that may be pleomorphic, lacks macrocysts	$PAX8^{-}$, $HMB45^{+}$, $PanCK^{-}$, $CK7^{-}$, $CK20^{-}$
Papillary RCC, oncocytic	Papillary formations (at least focal), uniform cytology	$CK7^{+}$, $CK20^{-}$
Clear cell RCC, eosinophilic morphology	Focal clear cell areas, delicate vasculature, may contain macrocysts	$CA9^{+}$, $CK20^{-}$
MiT translocation RCC	Large cells with clear (or eosinophilic) morphology, focal papillary and nested growth, lack cysts (usually)	$TFE3^{+}$, $TFEB^{+}$, $HMB45^{+}$, $PanCK^{-}$
SDH-deficient RCC	Lacks macrocysts, uniform low-grade oncocytic cells with flocculent to densely eosinophilic cytoplasmic vacuoles	$CD117^{-}$, $SDHB^{-}$, $SDHA^{+}$, $CK20^{-}$

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In Summary

- Tumors were well-circumscribed, unencapsulated, and comprised of nests of eosinophilic cells in a hypocellular and often edematous stroma.
- Tumor cells had round nuclei with prominent nucleoli and granular cytoplasm with striking vacuolization. Thickwalled vessels and calcifications were also frequently present, **whereas increased mitotic activity, necrosis, foamy histiocytes or lymphocytic infiltrates were not identified.**
- All cases were positive for PAX8, had retained expression of SDHB and FH, and exhibited a **CK7–/CK20–** phenotype.
- Molecularly, these tumors are characterized by **somatic mutations of TSC2 or MTOR genes and hyperactive mTORC1 signaling.**

谢谢！