

International Symposium on Immunohistochemistry

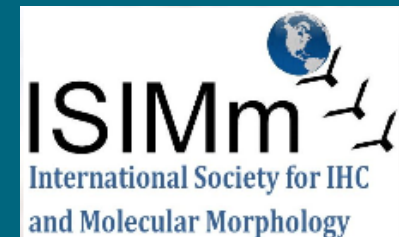
January 4th - 7th, 2018

Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India

In collaboration with NordiQC, Aalborg, Denmark and ISIMM, California, USA



Diagnostic IHC of endocrine, renal, prostate and germ cell tumor pathology



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Director of NordiQC
Aalborg University Hospital,
Aalborg, Denmark

Markers for endocrine tumours

Neuroendocrine

- Synaptophysin
- Chromogranin A
- *Hormones ...*

Unspecific "neuroendocrine"

- CD56
- neuron specific enolase
- PGP 9.5

Adrenal cortex, sex cord

- Calretinin
- Inhibin
- Melan A (A 103)
- Synaptophysin (adrenal)

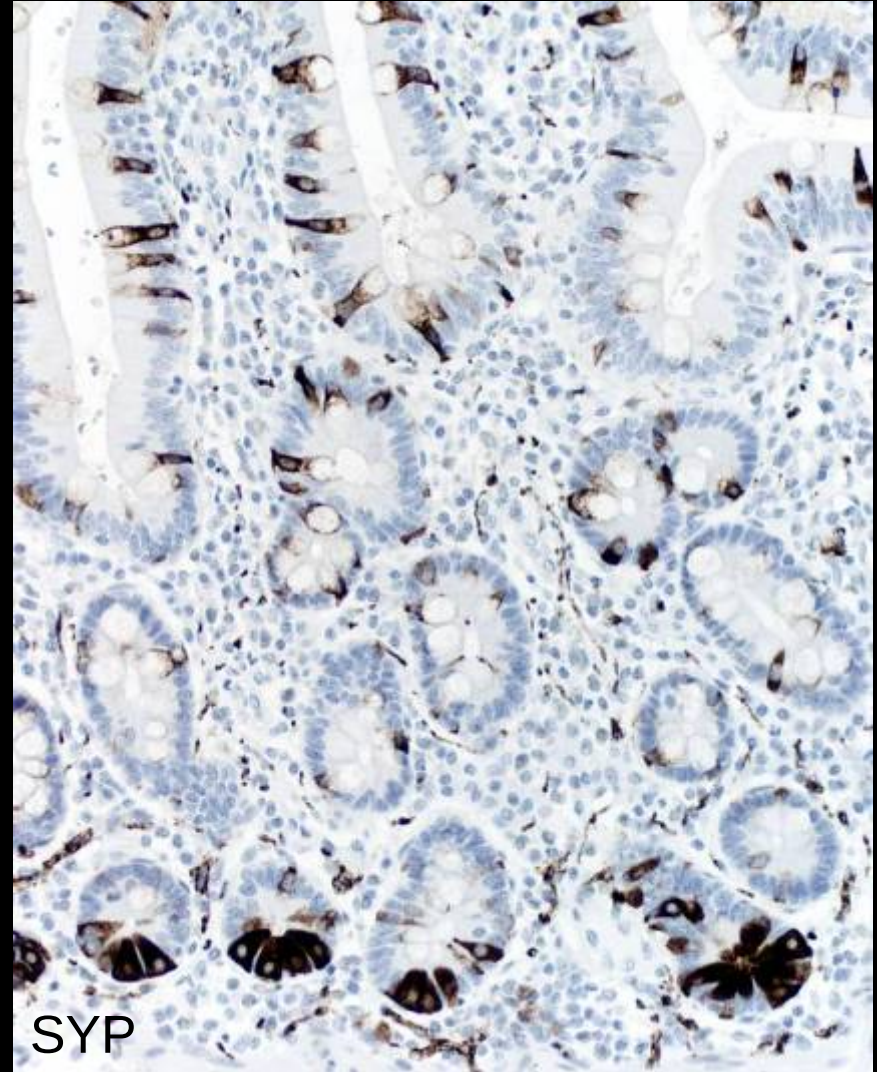
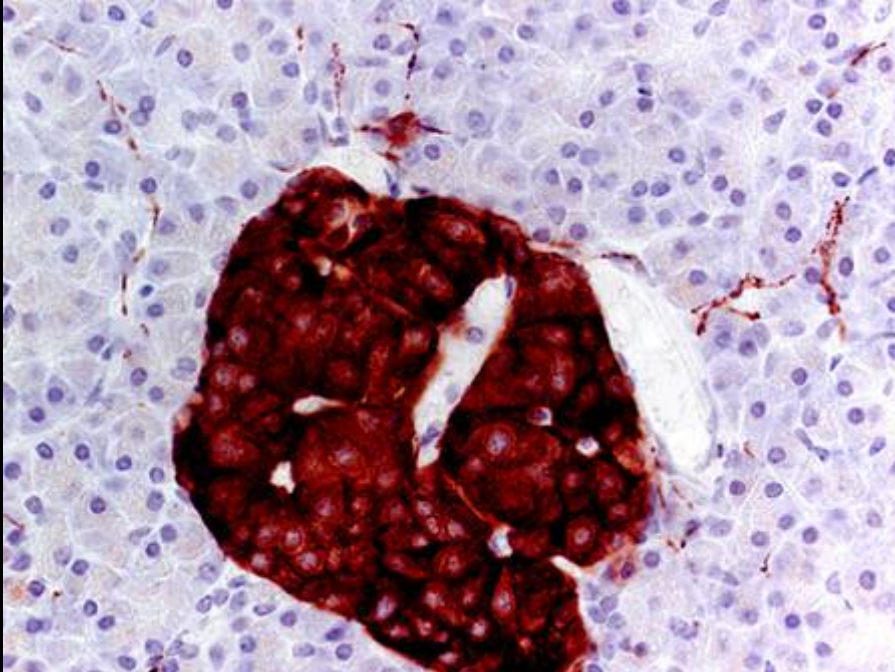
Thyroid

- TTF-1
- PAX8
- Thyroglobulin
- Thyroperoxidase

Synaptophysin

An integral-membrane glycoprotein of presynaptic vesicles

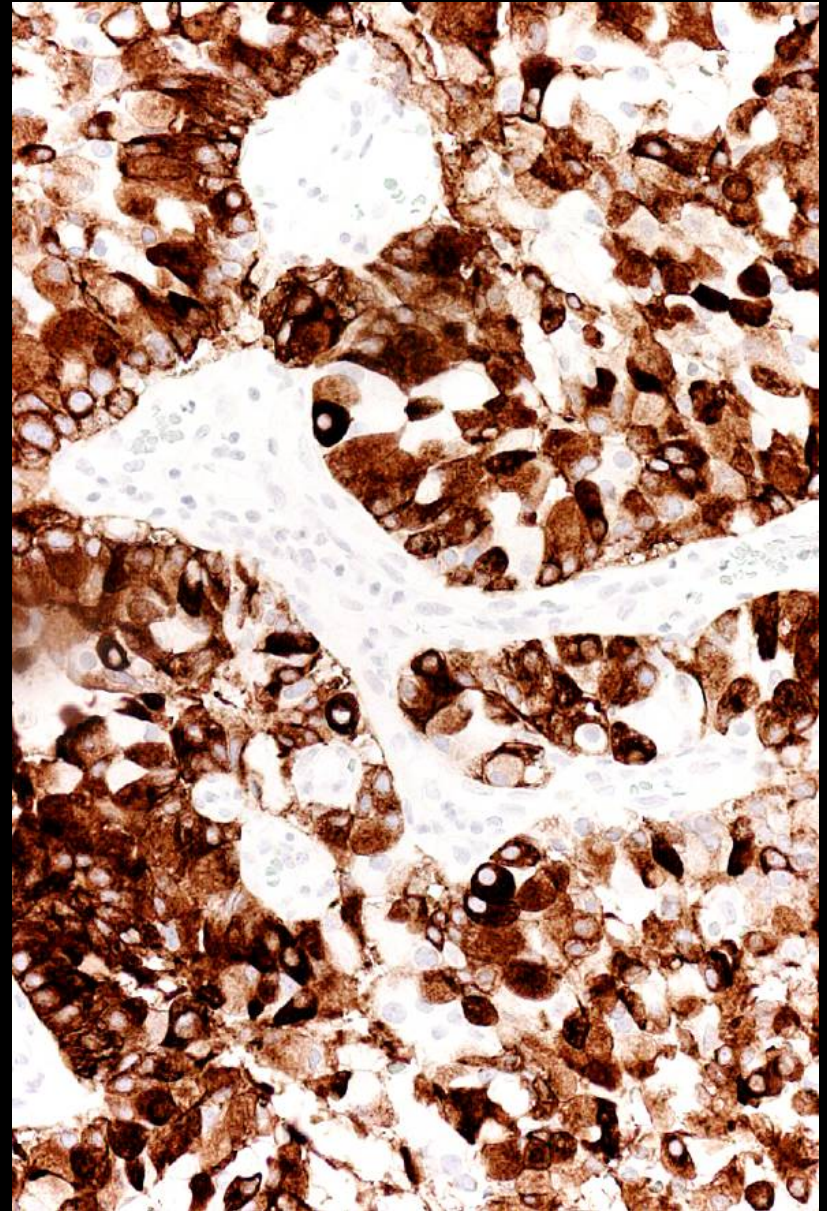
- Neurons
- Neuroendocrine cells
- (Adrenal cortical cells)
- (Goblet cells, Paneth cells)



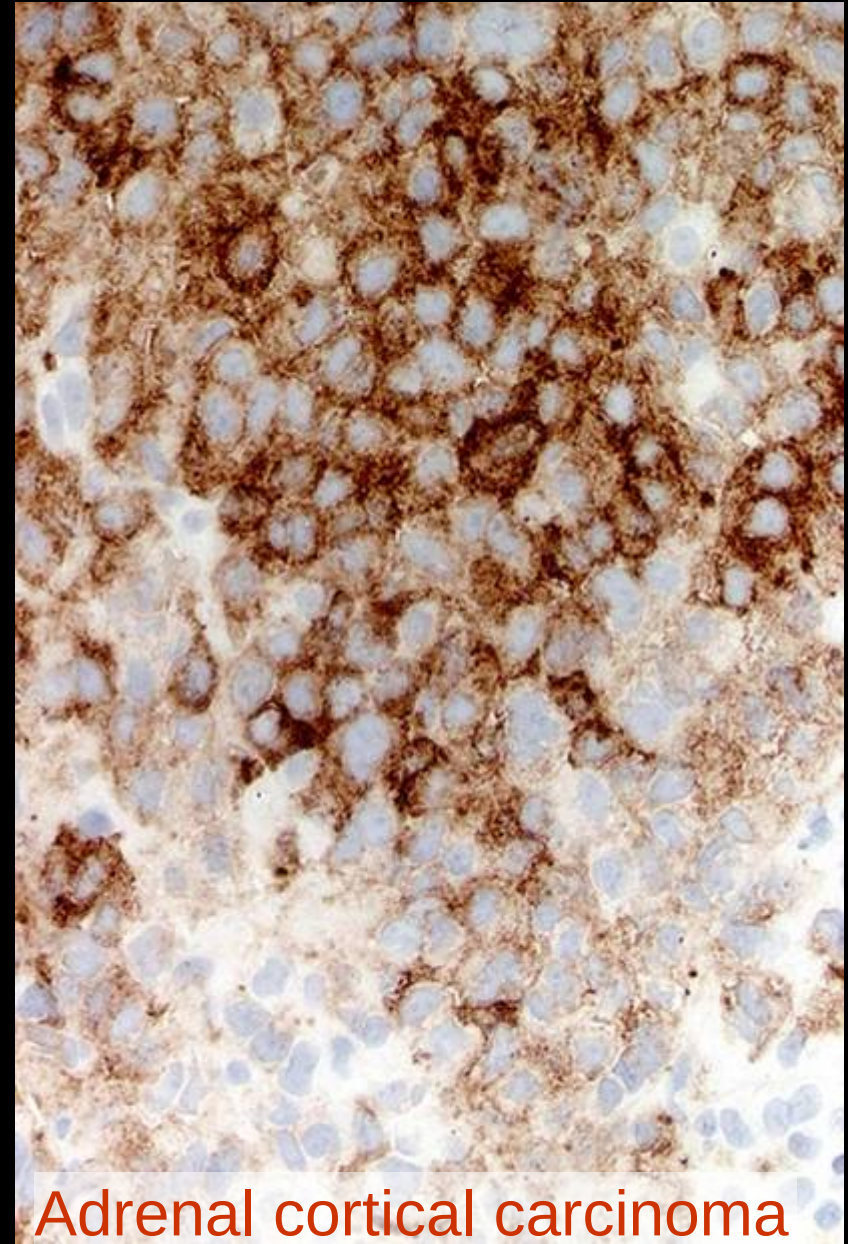
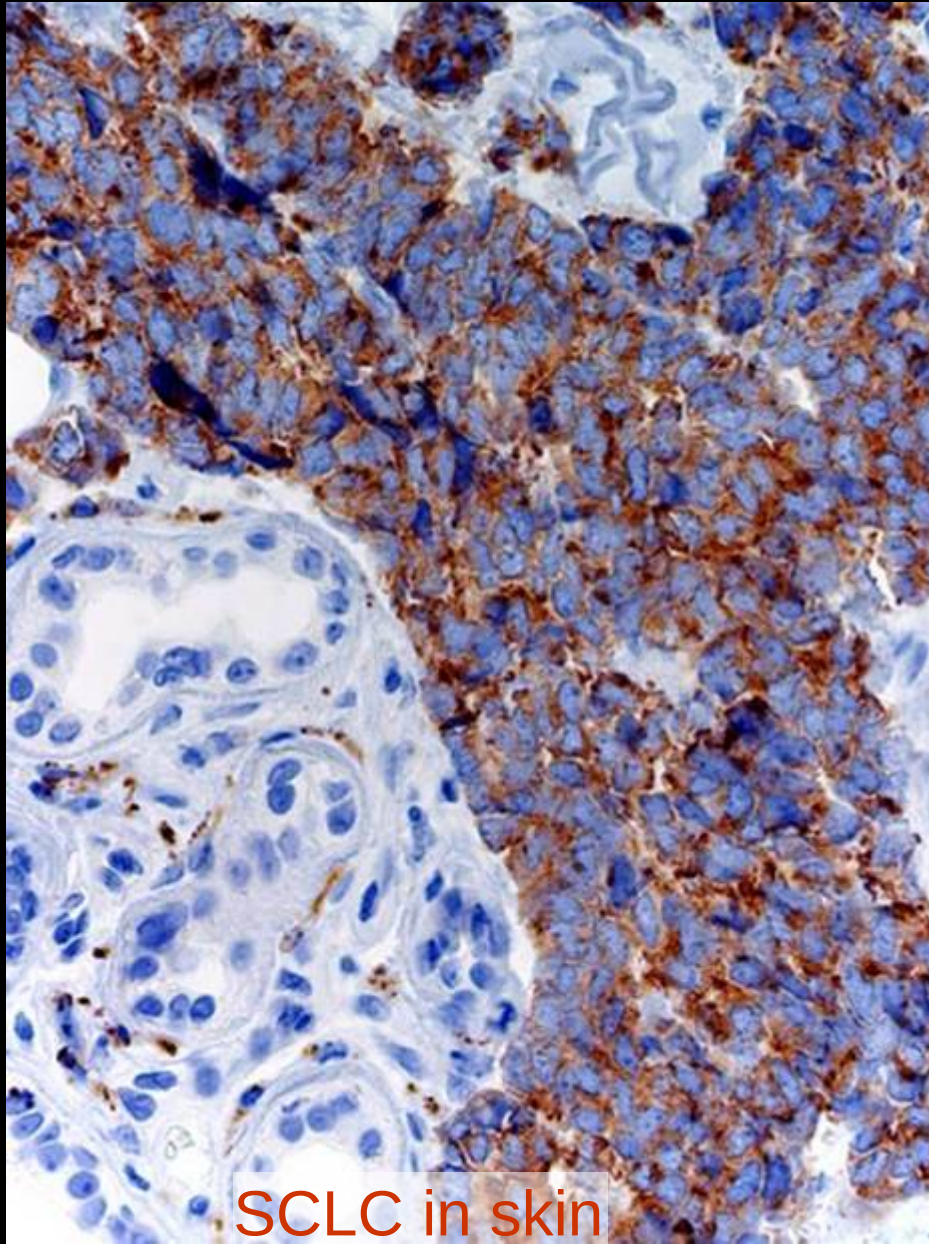
Synaptophysin in neoplasias

- Neural tumours
- NE tumours (gr. 1-2)
- NE carcinomas (gr. 3)
- Adrenal cortical tumours

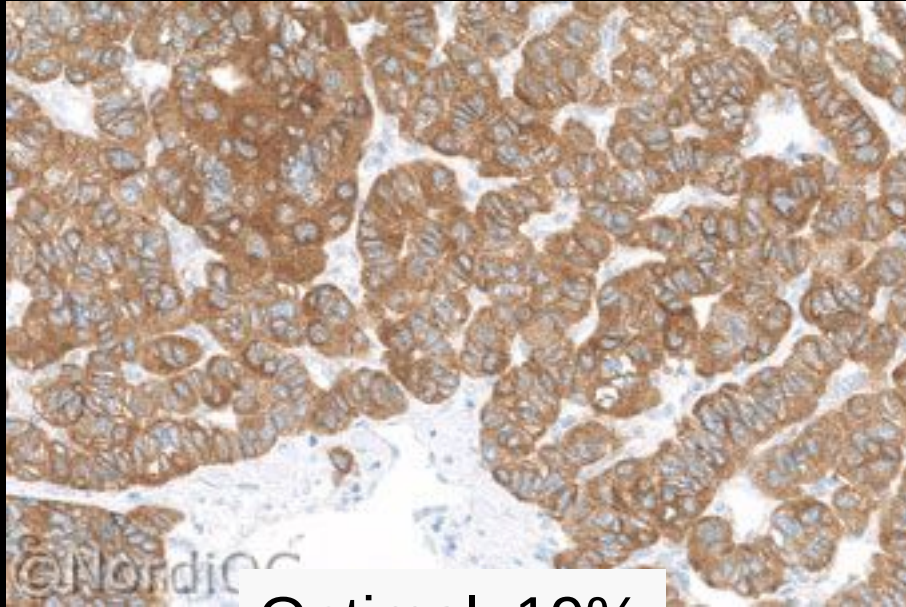
Lung NE large cell carcinoma



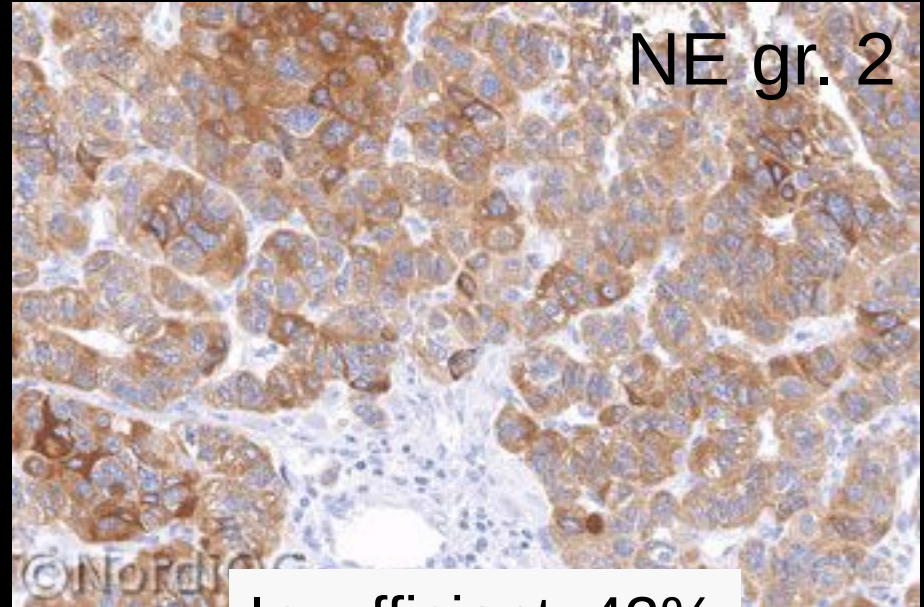
Synaptophysin in neoplasias



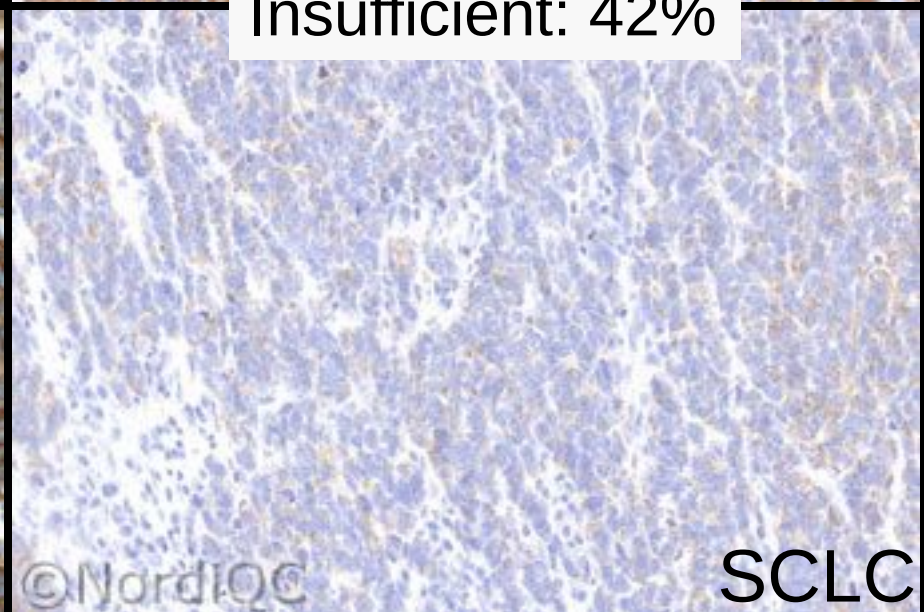
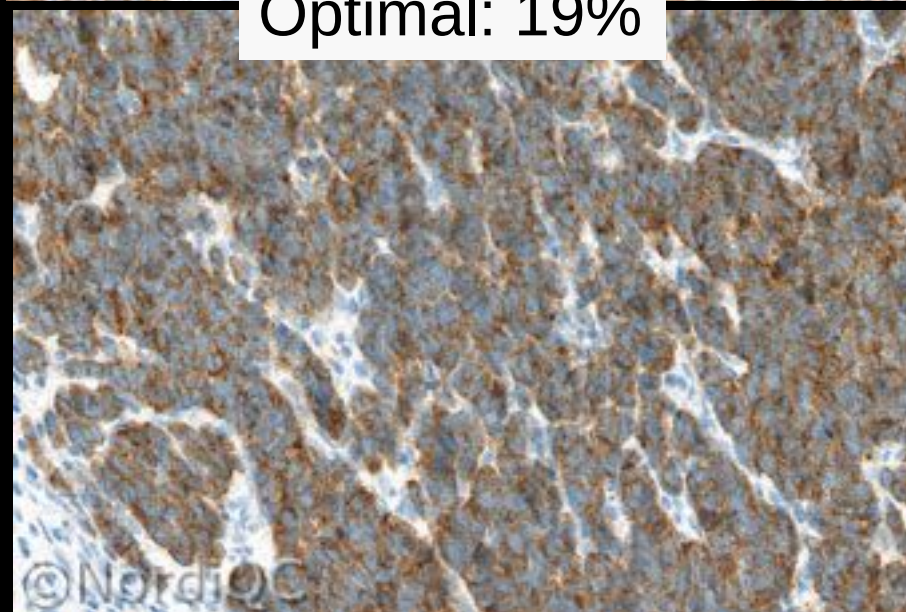
Synaptophysin – NordiQC run 37: 214 labs.



Optimal: 19%

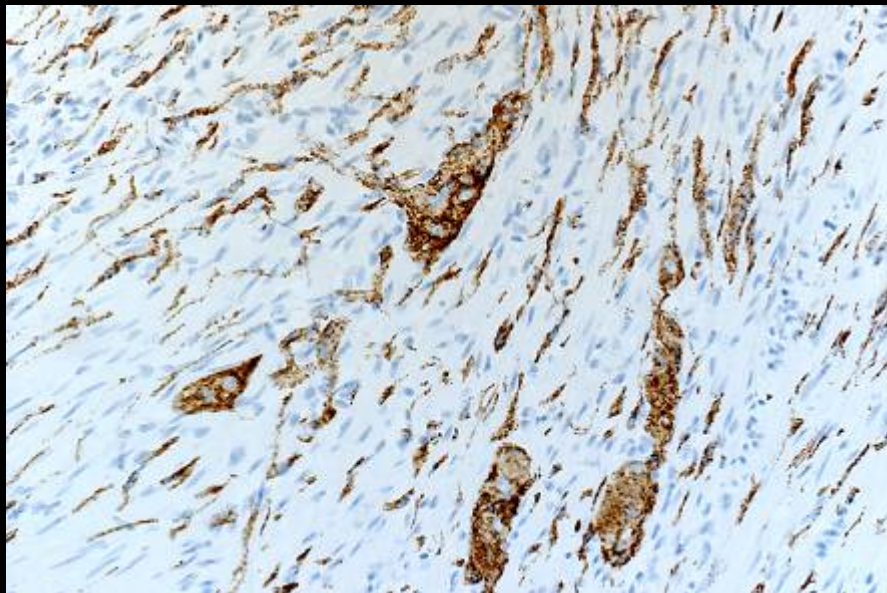


Insufficient: 42%

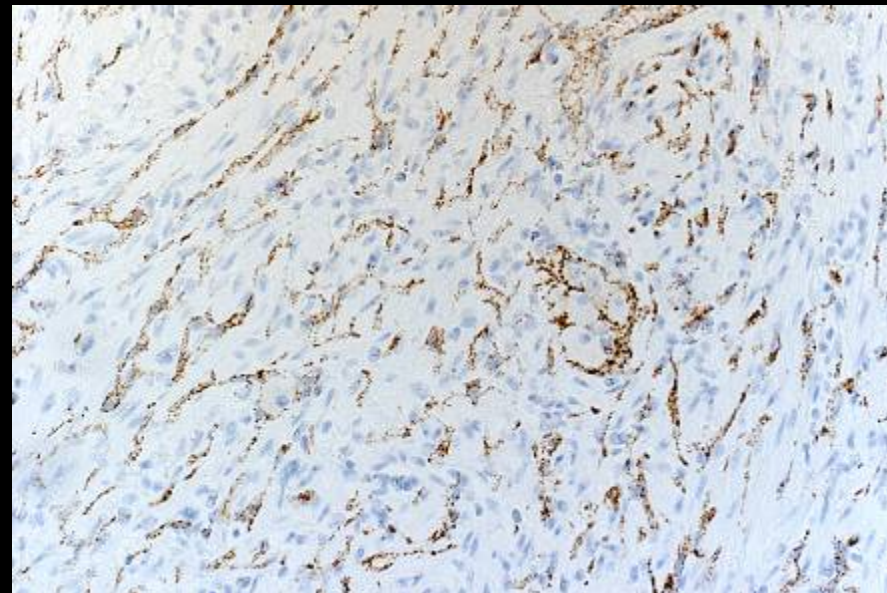


SCLC

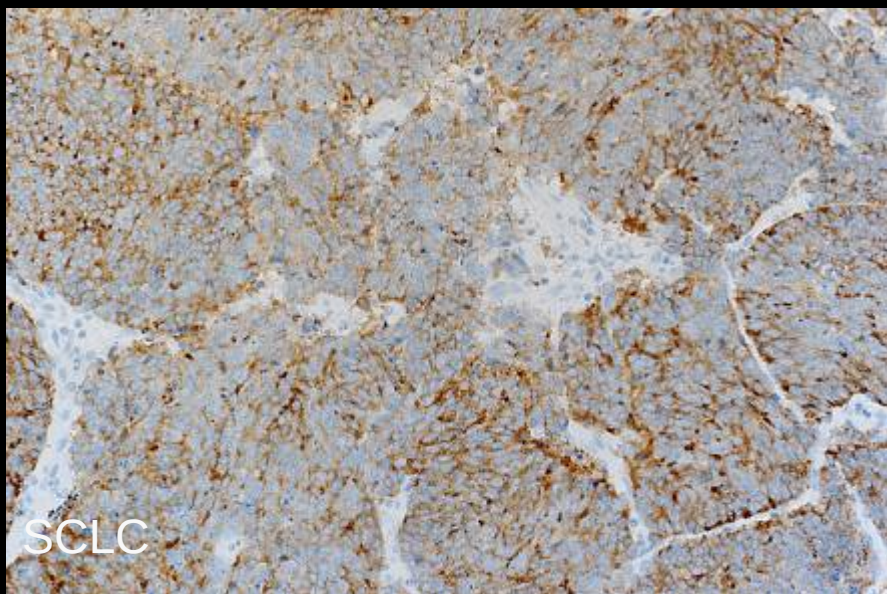
Less successful antibodies – Synaptophysin



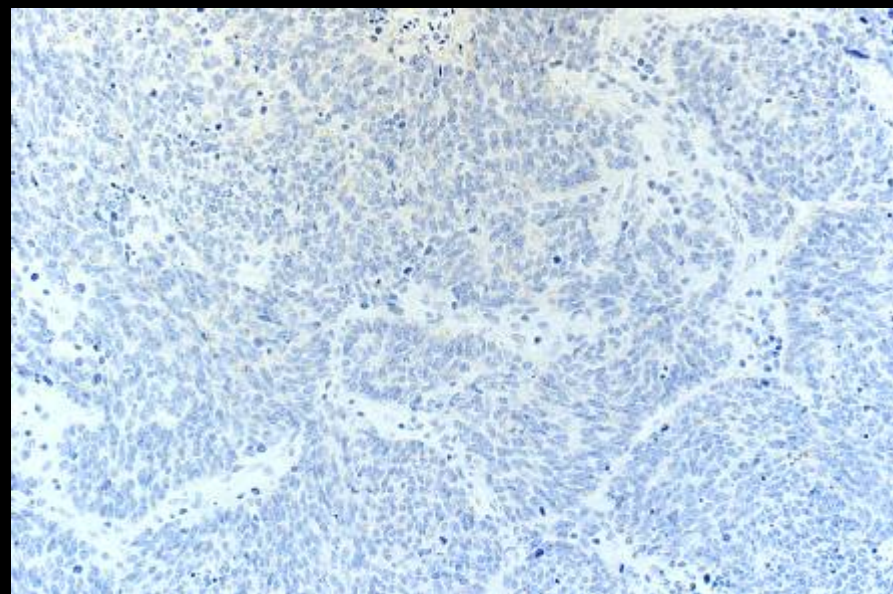
27G12



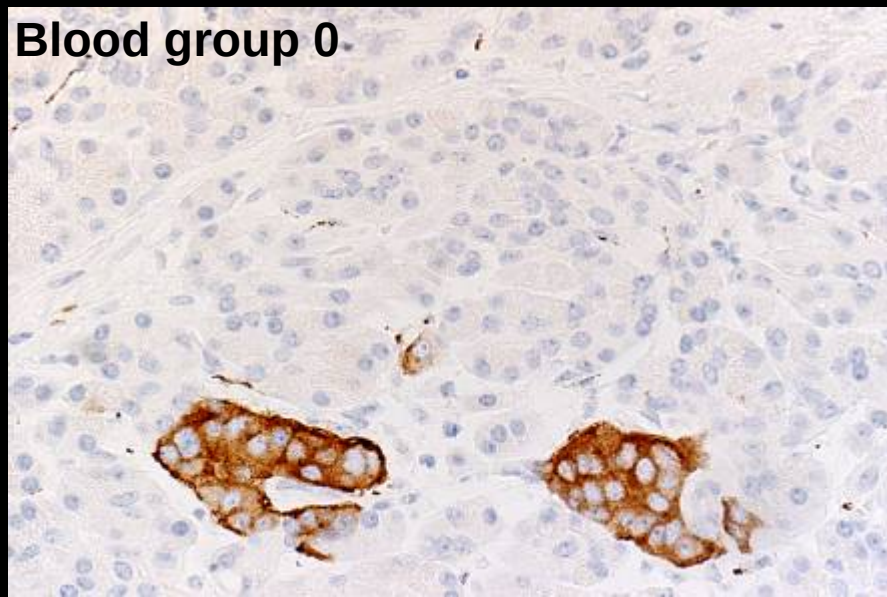
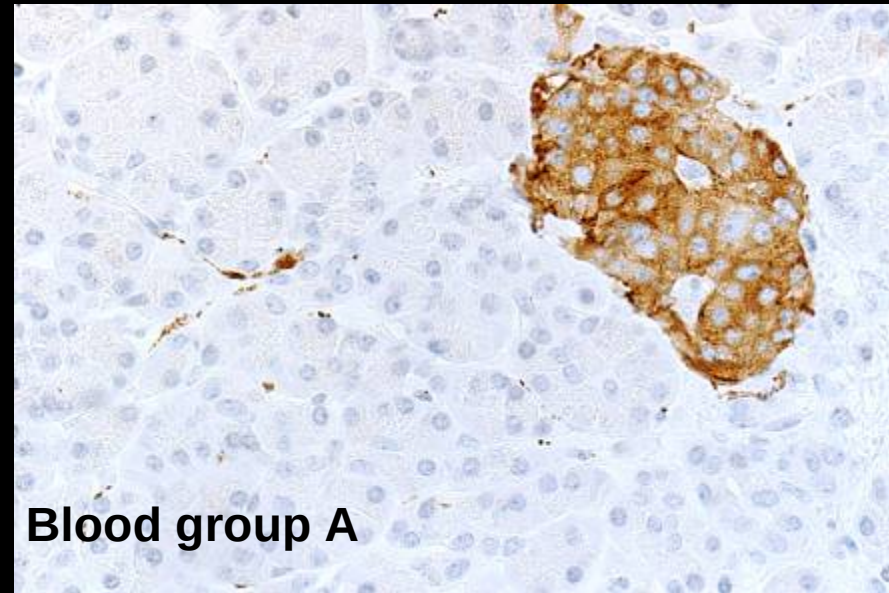
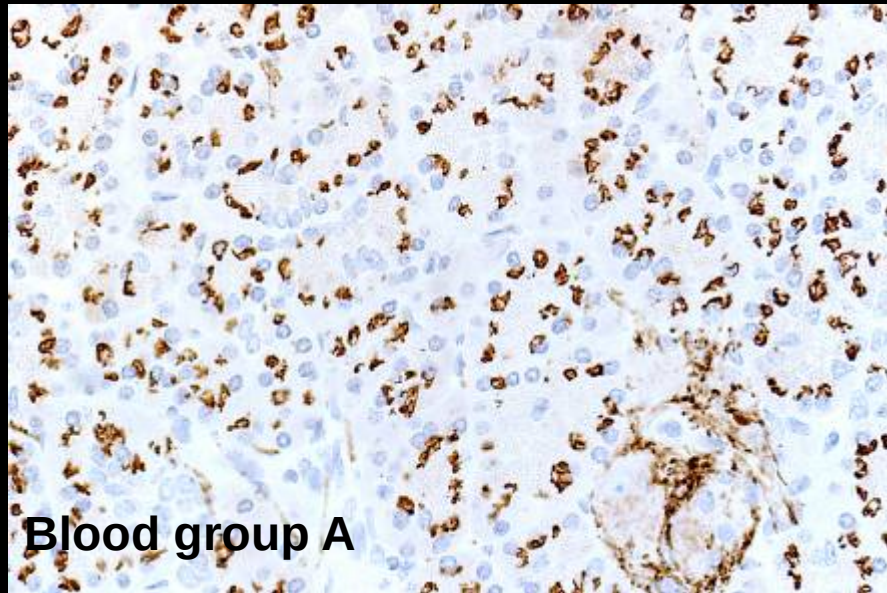
Sy38



SCLC

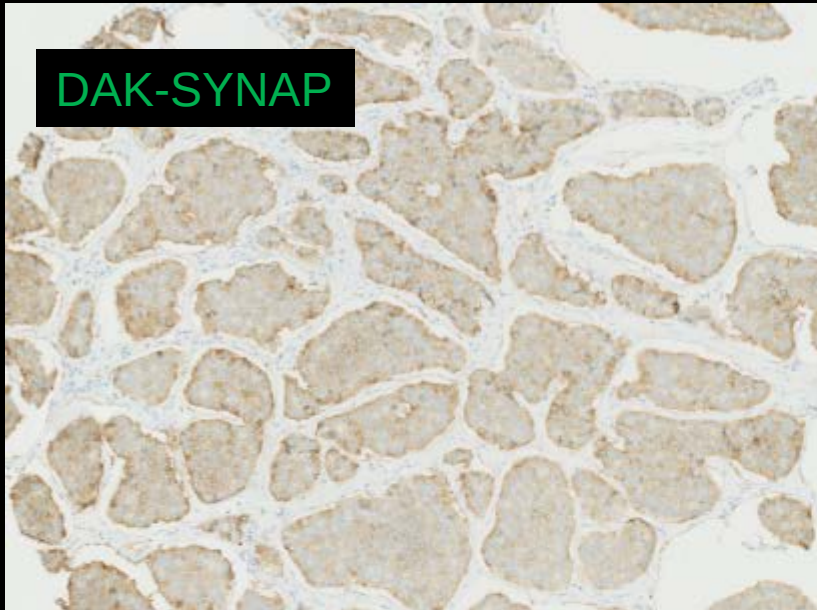


Less successful antibodies – Synaptophysin

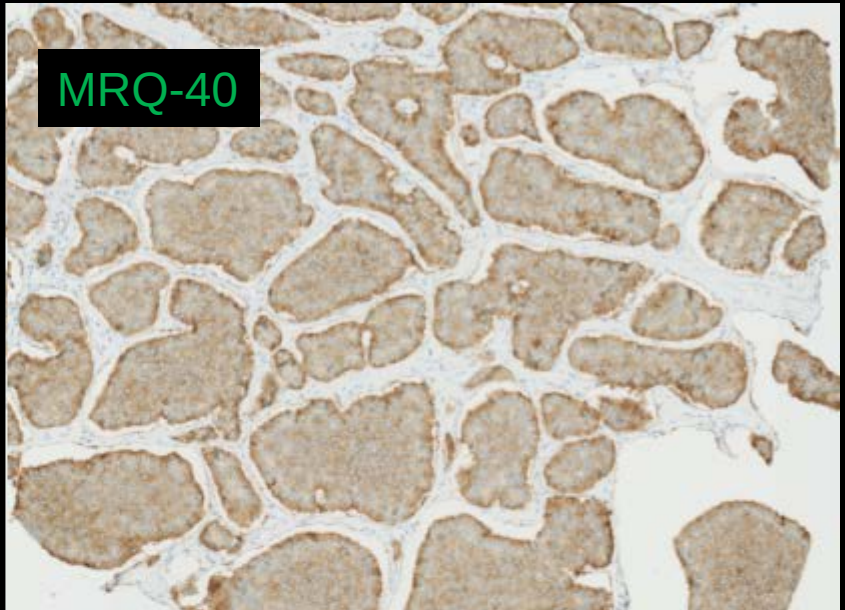


Synaptophysin – endocrine tumor

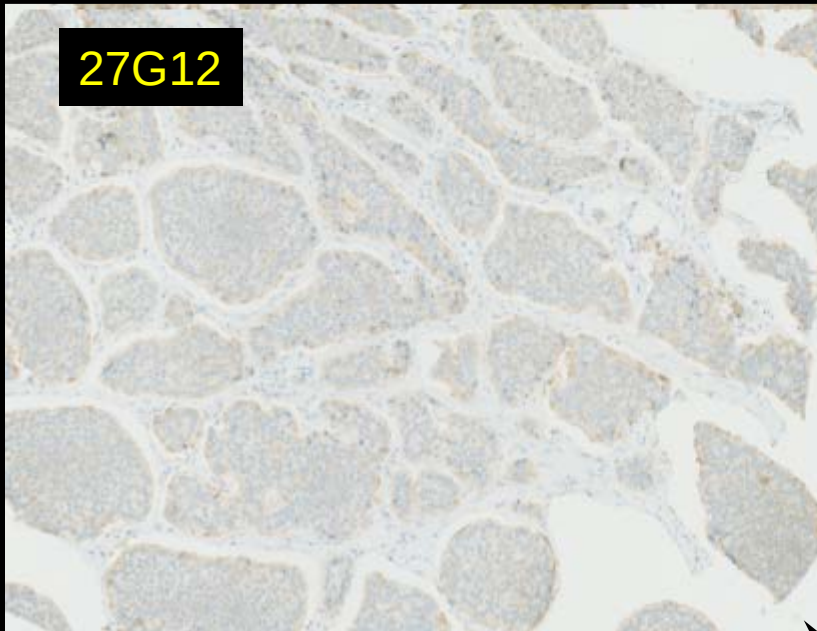
DAK-SYNAP



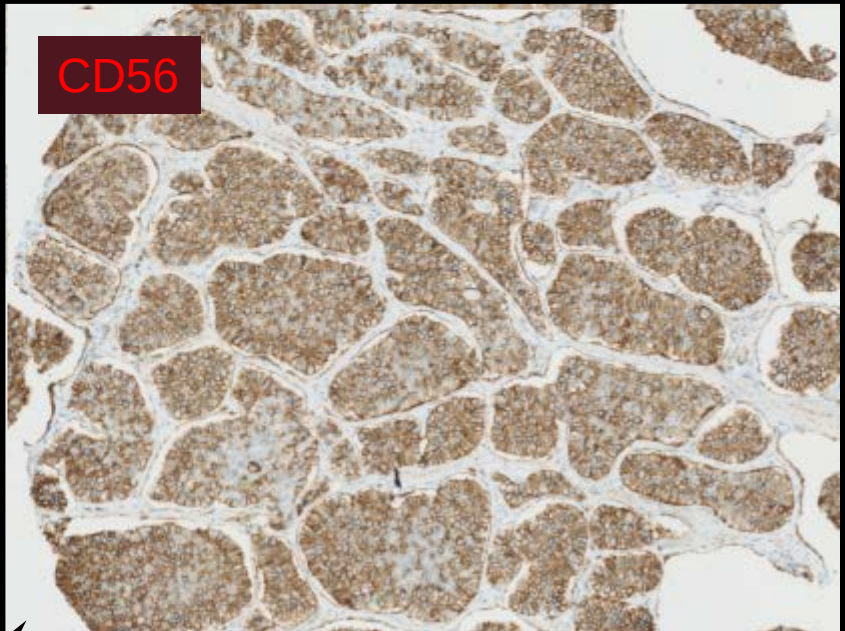
MRQ-40



27G12



CD56



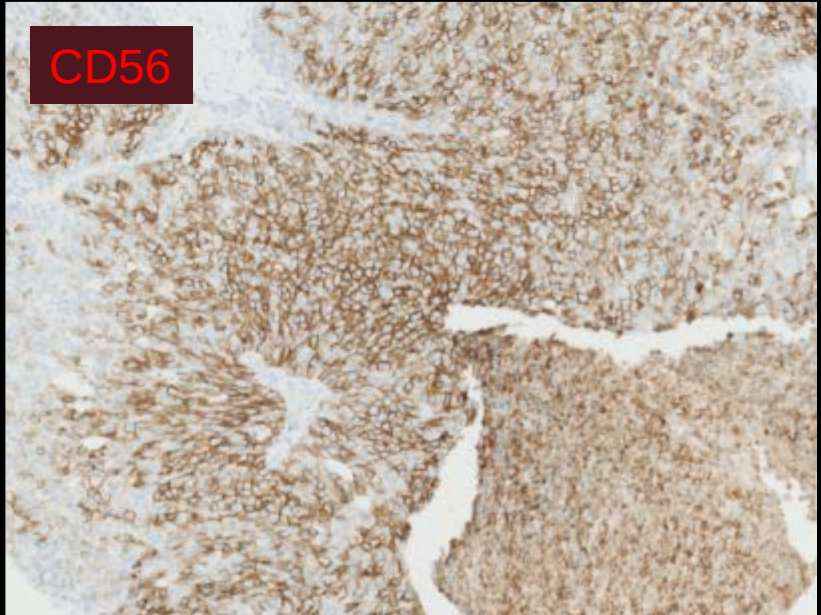
Synaptophysin – Breast carcinoma

DAK-SYNAP

MRQ-40

27G12

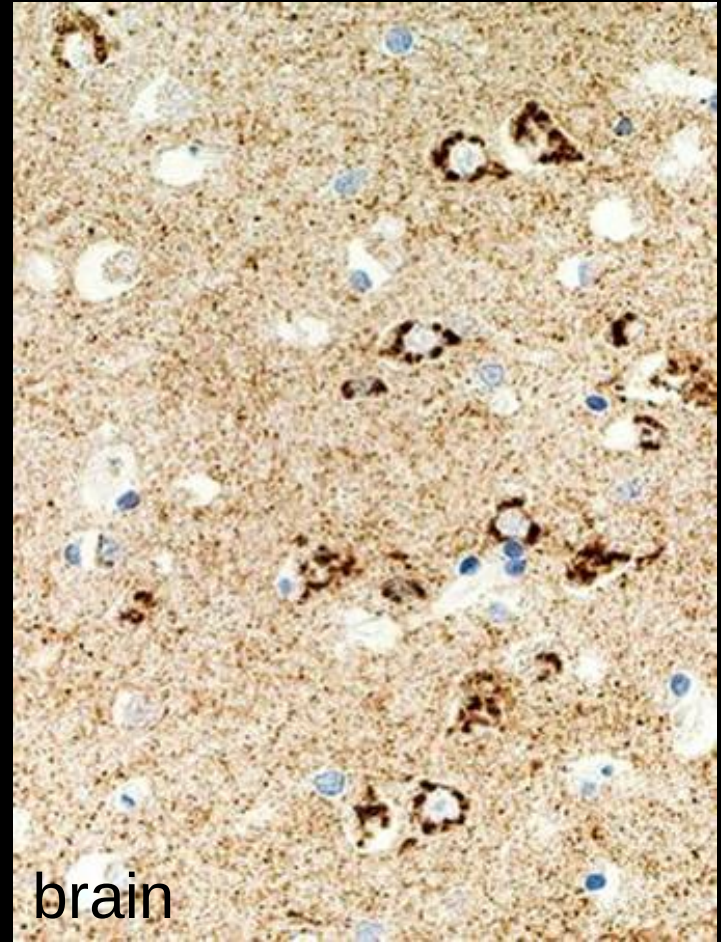
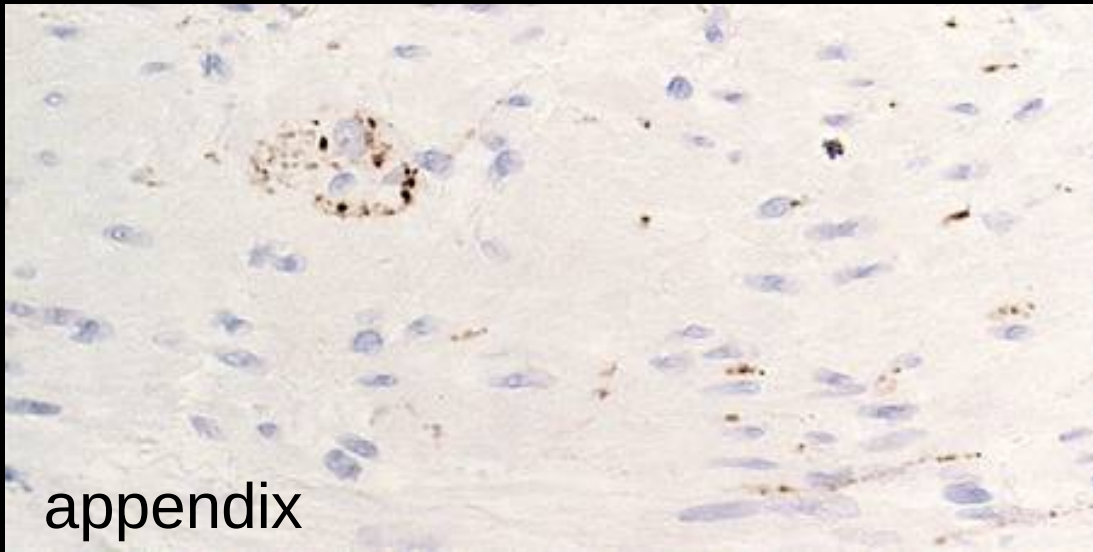
CD56



Chromogranin

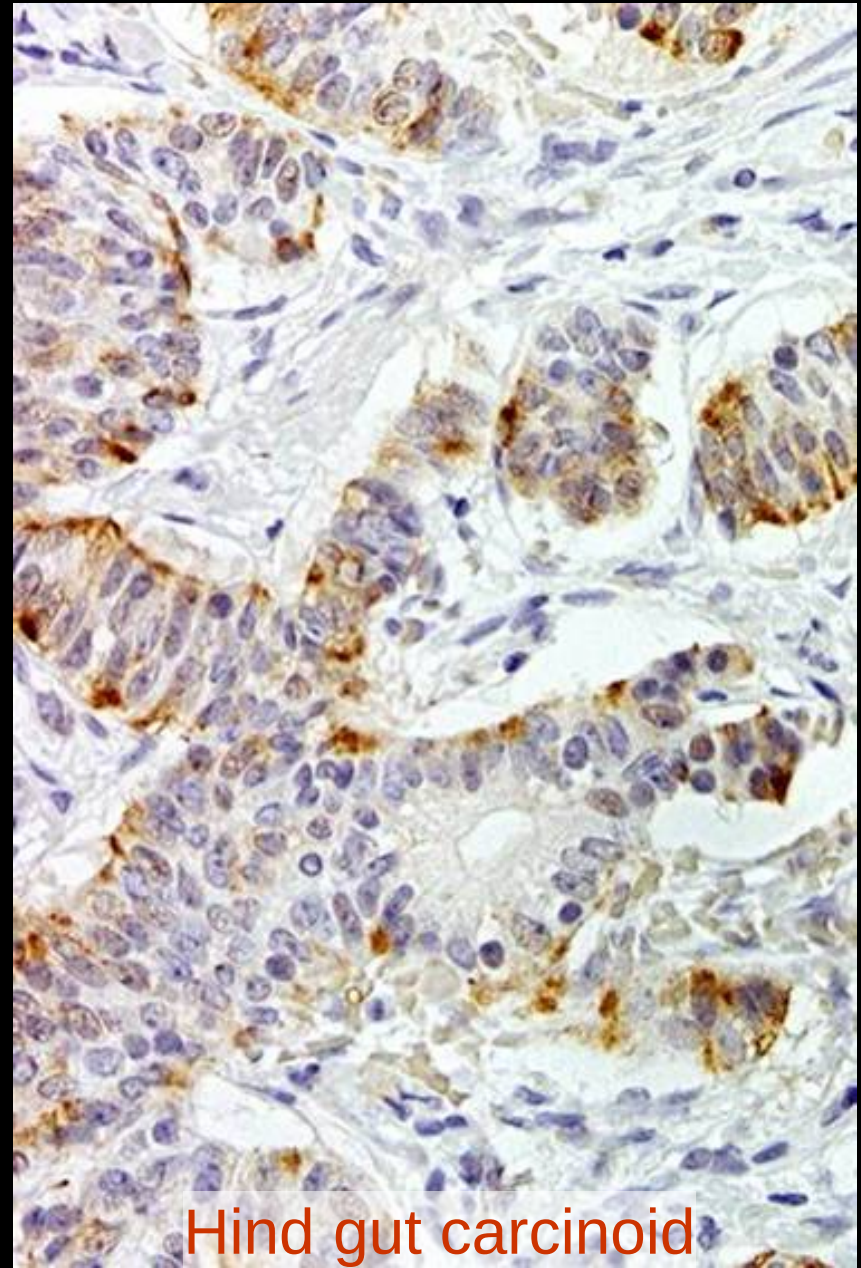
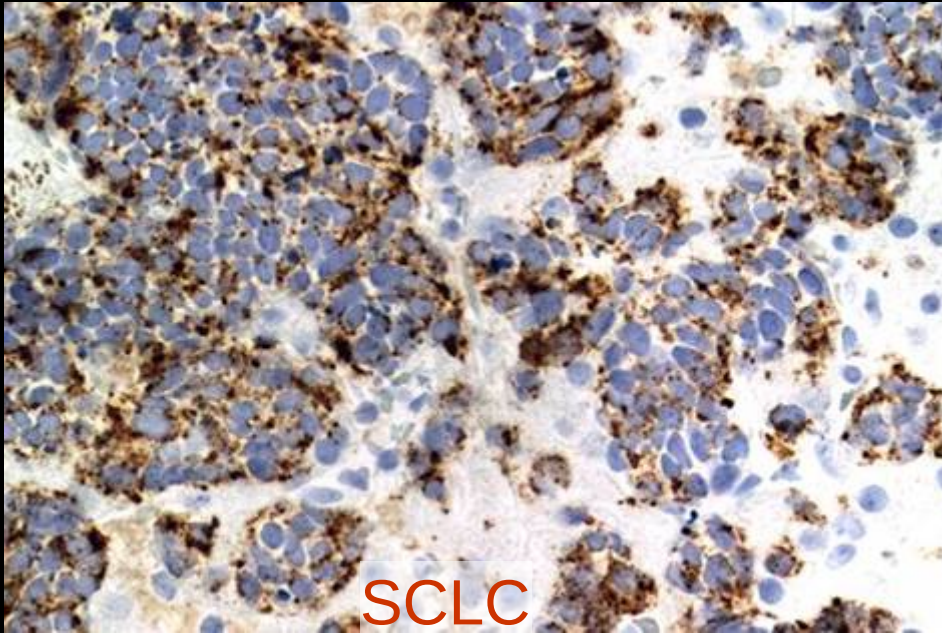
Acid calcium-binding glycoprotein closely associated with the matrix of dense-core neurosecretory granules, probably involved in packaging and processing of neuropeptides and peptide hormones

- Neurons
- Neuroendocrine cells

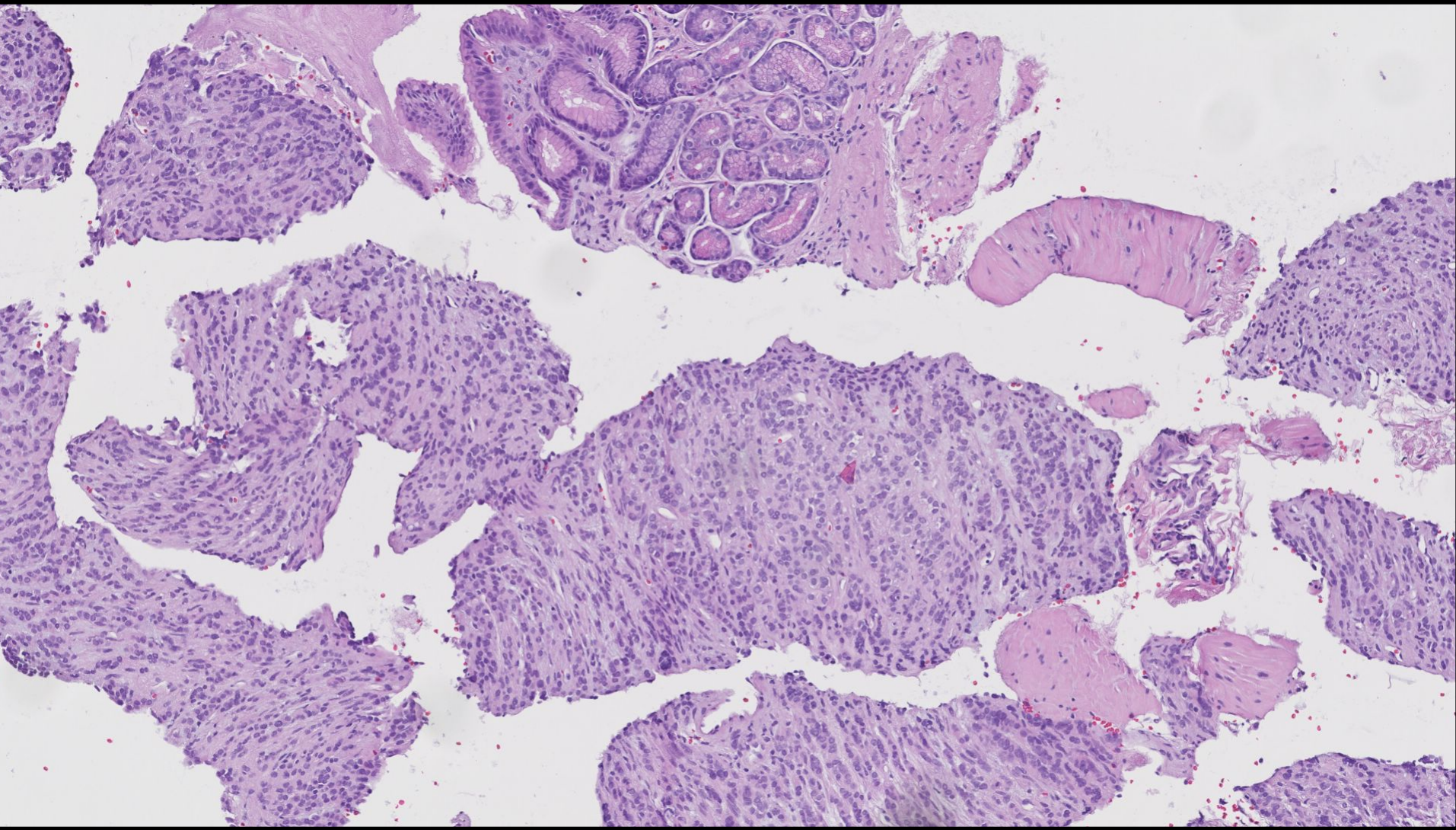


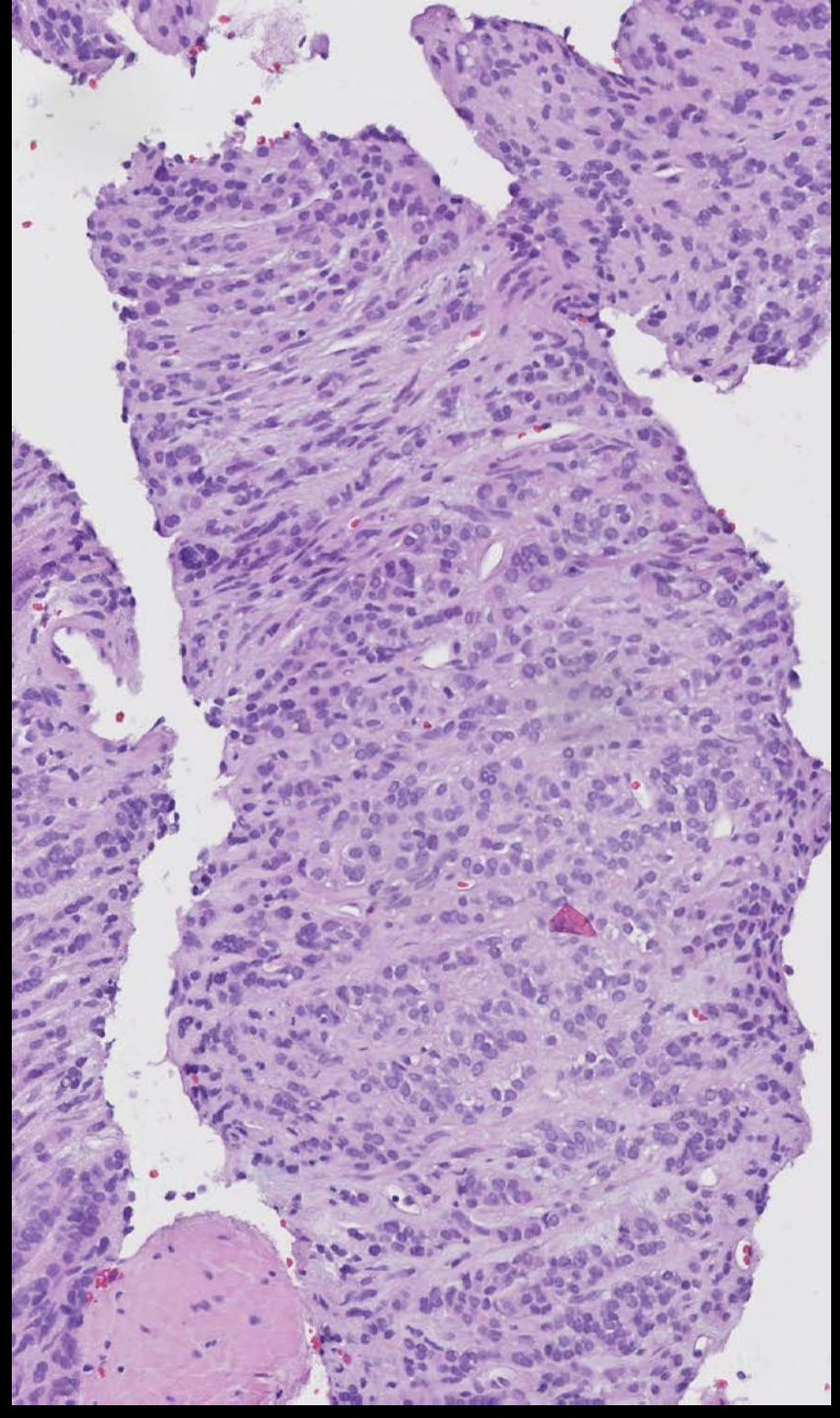
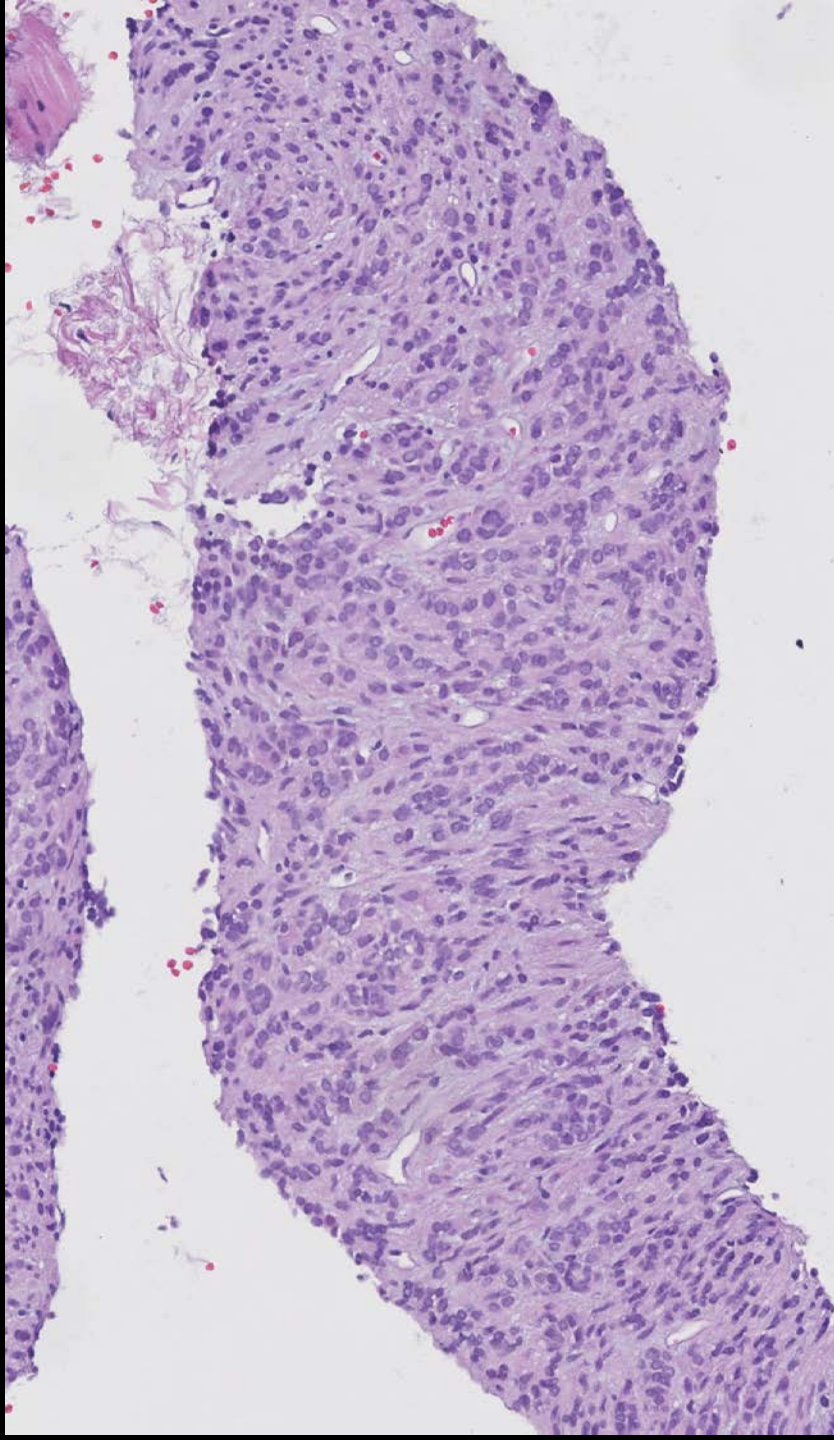
Chromogranin A

- Neuronal tumours
 - ganglioneuroblastoma
 - paraganglioma
- Neuroendocrine neopl.
 - Neuroendocrine tumours
 - Neuroendocrine carcinoma
 - Small cell carcinoma

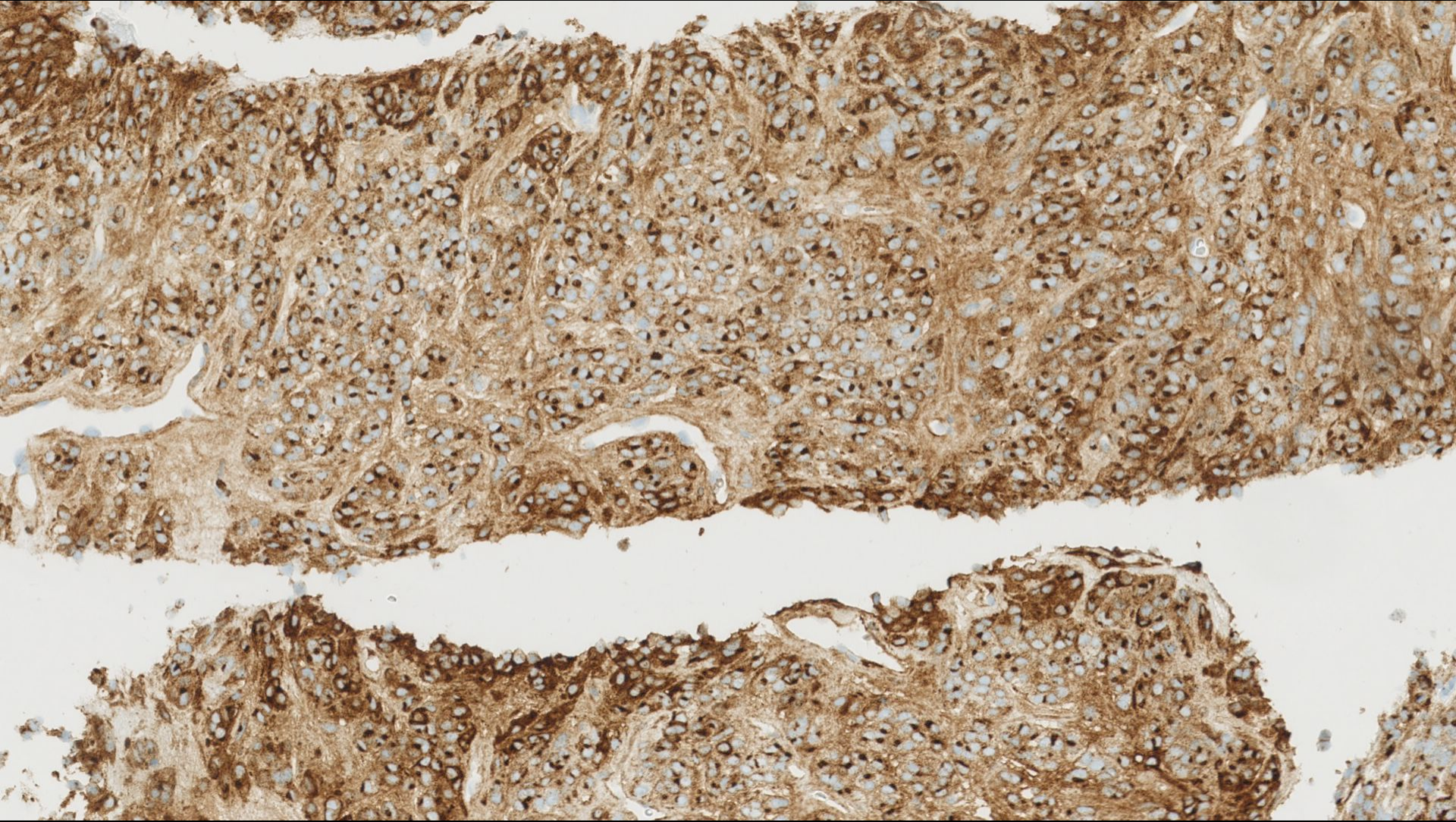


Male 32, suspicion of endocrine pancreastumour

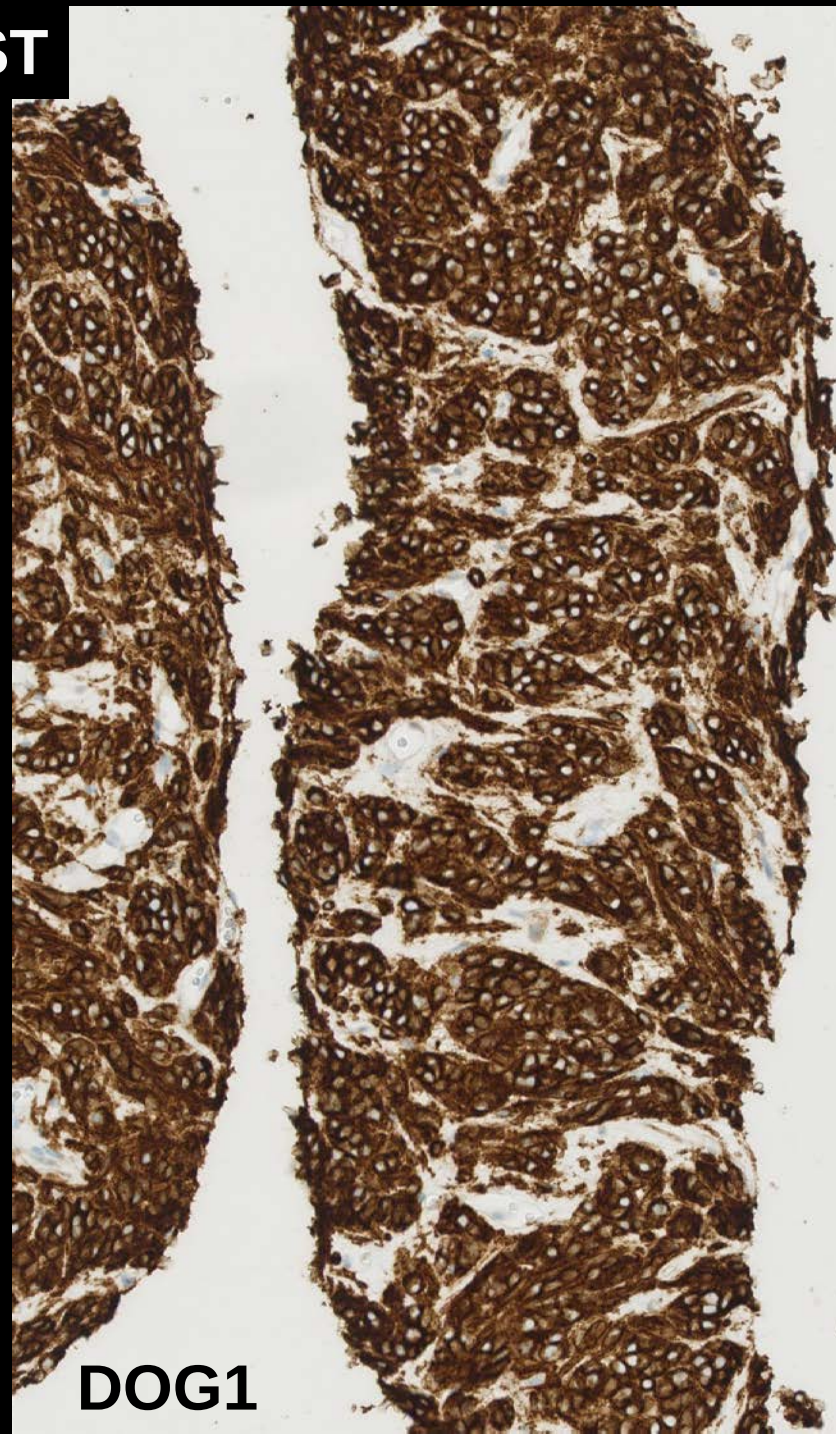
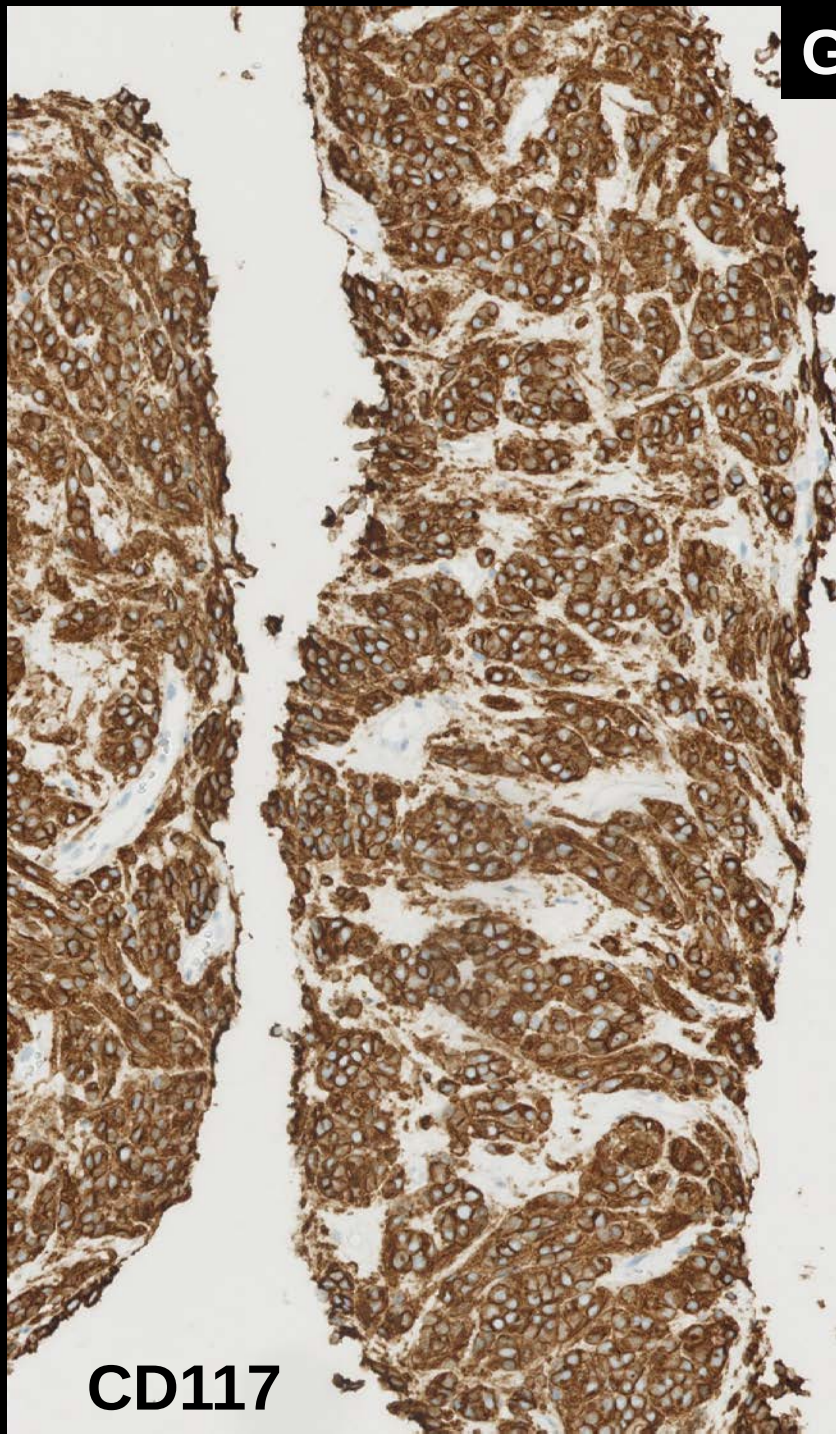




Chromogranin A



GIST



Chromogranin – NordiQC run 46

Table 1. Antibodies and assessment marks for CGA, run 46

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff. ¹	Suff. OPS ²
mAb clone 5H7	4	Leica/Novocastra	0	0	3	1	-	-
mAb clone DAK-A3	36	Dako/Agilent	0	2	17	17	6%	-
mAb clone LK2H10	22	Thermo/Neomarkers	24	31	0	4	93%	98%
	18	Cell Marque						
	6	Immulologic						
	3	Biogenex						
	2	Millipore						
	2	Zytomed						
	1	Abcam						
	1	A.Menarini						
	1	Diagnostic Biosystems						
	1	Europroxima						
	1	Monosan						
	1	Unknown						
mAb clone PHE5	1	Unknown	0	0	1	0	-	-
mAb clones LK2H10+PHE5	6	Thermo/Neomarkers	3	8	0	0	100%	100%
rmAb clone EP38	1	Epitomics	0	1	0	0	-	-

Poor RTU formats: Chromogranin A

Medullary carcinoma

LK2H10 REF

pAb **RTU**
Company 1

mAb LK2H10 **RTU**
Company 2

mAb LK2H10 **RTU**
Company 3

Poor RTU formats: CGA

Small cell carcinoma

LK2H10 REF

pAb **RTU**
Company 1

mAb LK2H10 **RTU**
Company 2

mAb LK2H10 **RTU**
Company 3

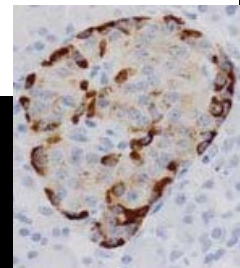
Misleading datasheets

Table 1. Recommended Staining Protocols for Anti-Chromogranin A (LK2H10)

Procedure Type	Platform/Method	
	ES or NexES IHC	BenchMark or BenchMark XT
Deparaffinization	Off Line	Selected
Cell Conditioning (Antigen Unmasking)	None Required	None Required
Enzyme (Protease)	None Required	None Required

Positive Tissue Control

A tissue with weak positive staining is more suitable for optimal quality control and for detecting minor levels of reagent degradation. An example of a positive control with Anti-Chromogranin A (LK2H10) Primary Antibody is pancreas. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly.



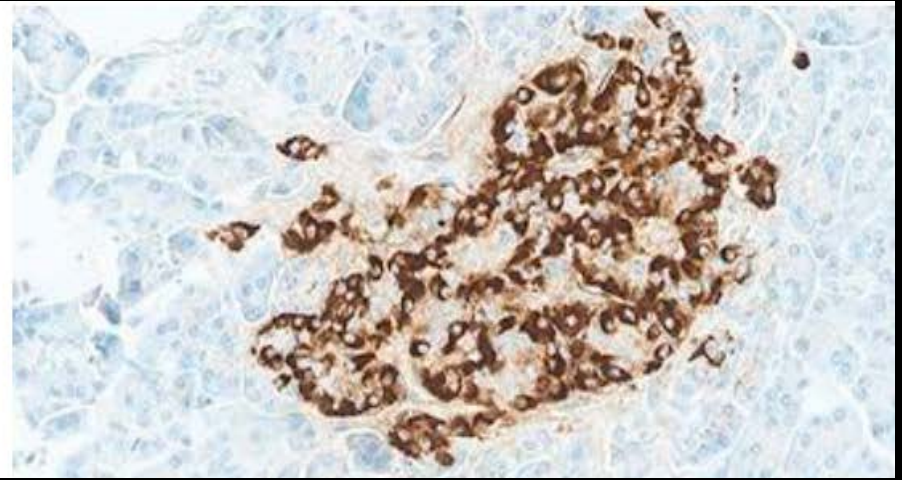
Giving false negative results in low expressing cells and tumours

CGA – run 46, 2016, 242 labs

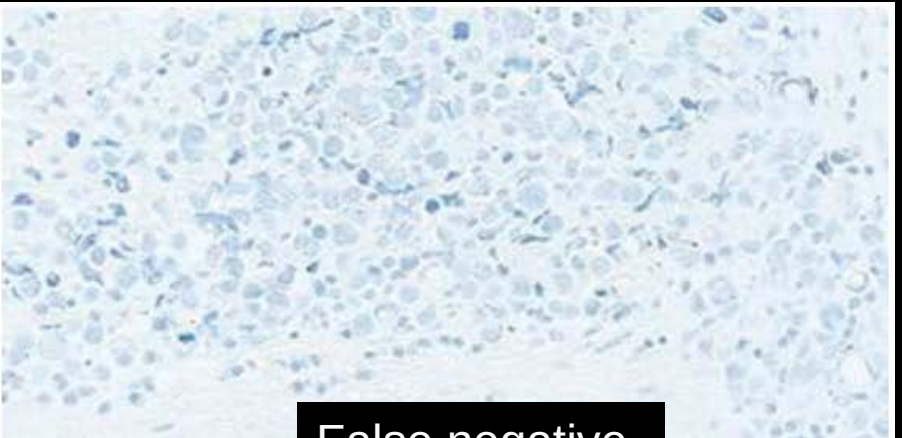
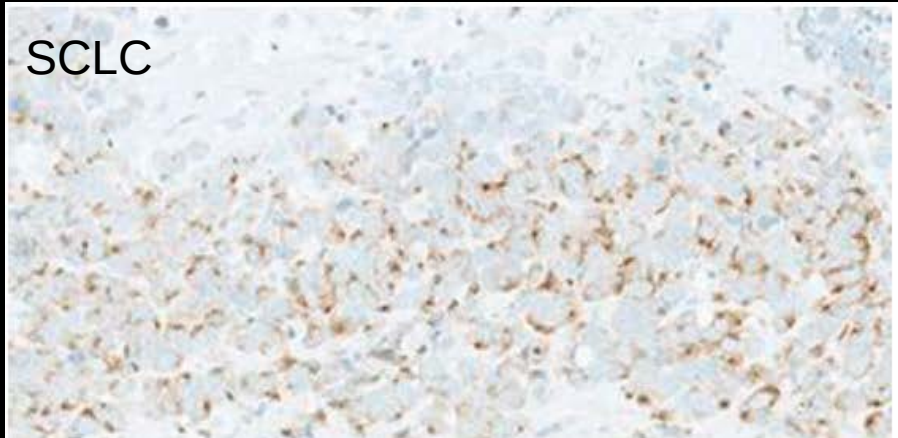
Table 1. Antibodies and assessment marks for CGA, run 46

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mAb clone 5H7	4	Leica/Novocastra	0	0	3	1	-	-
mAb clone DAK-A3	36	Dako/Agilent	0	2	17	17	6%	-
mAb clone LK2H10	22	Thermo/Neomarkers	24	31	0	4	93%	98%
	18	Cell Marque						
	6	Immulologic						
	3	Biogenex						
	2	Millipore						
	2	Zytomed						
	1	Abcam						
	1	A.Menarini						
	1	Diagnostic Biosystems						
	1	Europroxima						
	1	Monosan						
	1	Unknown						
mAb clone PHE5	1	Unknown	0	0	1	0	-	-
mAb clones LK2H10+PHE5	6	Thermo/Neomarkers	3	8	0	0	100%	100%
	5	Biocare						
rmAb clone EP38	1	Epitomics	0	1	0	0	-	-
rmAb clone SP12	1	Master Diagnostica	0	0	0	2	-	-
	1	Thermo/NeoMarkers						
pAb A0430*	38	Dako/Agilent	8	17	8	5	63%	-
pAb NB120-17064	1	Novus Biologicals	0	1	0	0	-	-
pAb RB-9003	1	Thermo/NeoMarkers	0	1	0	0		

Langerhans islet



SCLC



Appendix - nerves



Steroid producing cell markers

- Inhibin
- Calretinin
- Melan A (A103)
- (Synaptophysin – adrenal cortex)

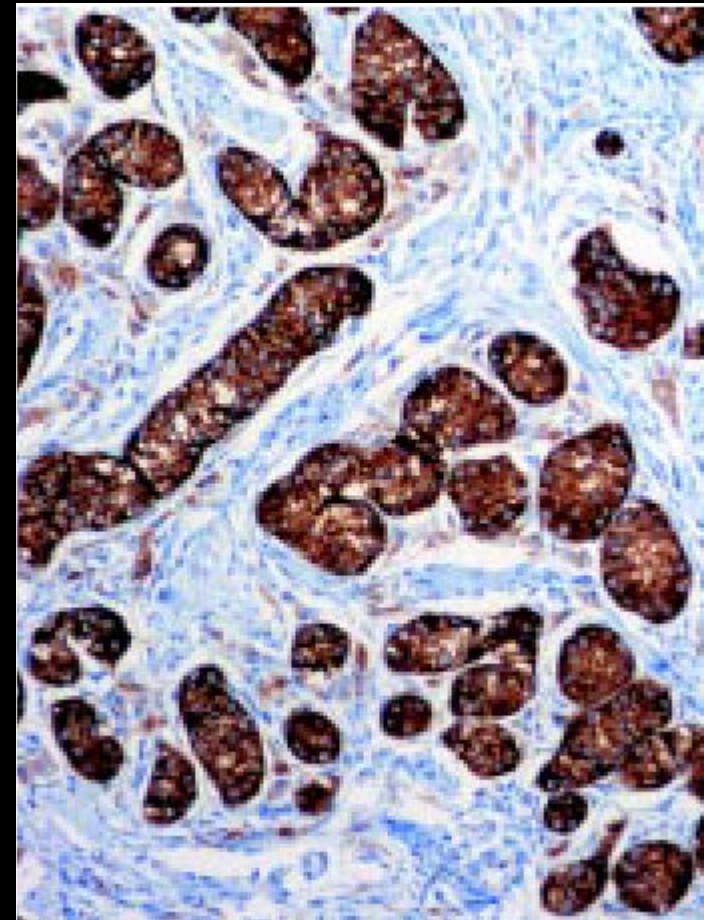
Inhibin

Transforming growth factor β -family

Inhibiting the production and secretion of gonadotropins

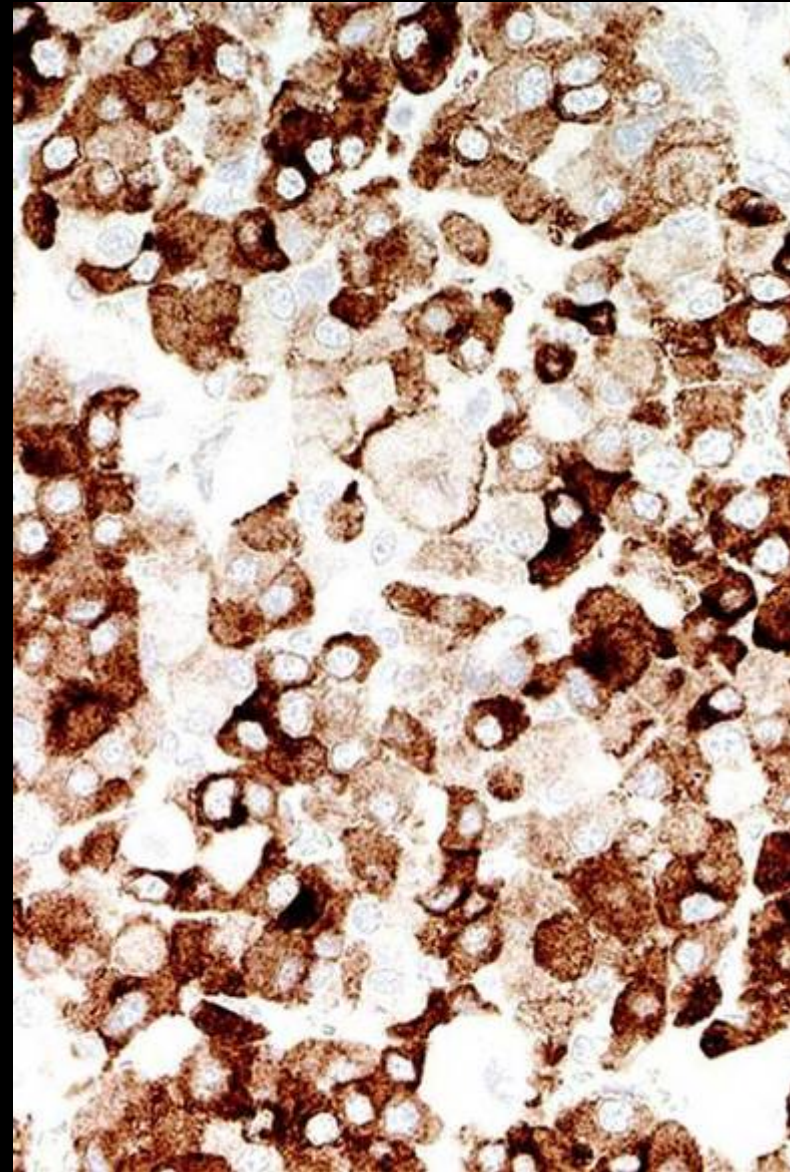
- Granulosa & theca interna cells
- Syncytiotrophoblast
- Sertoli & Leydig cells
- Germinal cells
- Adrenal cortical cells

Testis: Sertoli cells only



Inhibin in neoplasms

- Sex cord-stromal tumours
 - Granulosa cell tumour +
 - Sertoli-Leydig cell tumour +
 - Fibrothecoma +/-
- Adrenal cortical tumours +/-
- Choriocarcinoma +/-
- Other carcinomas -(+)



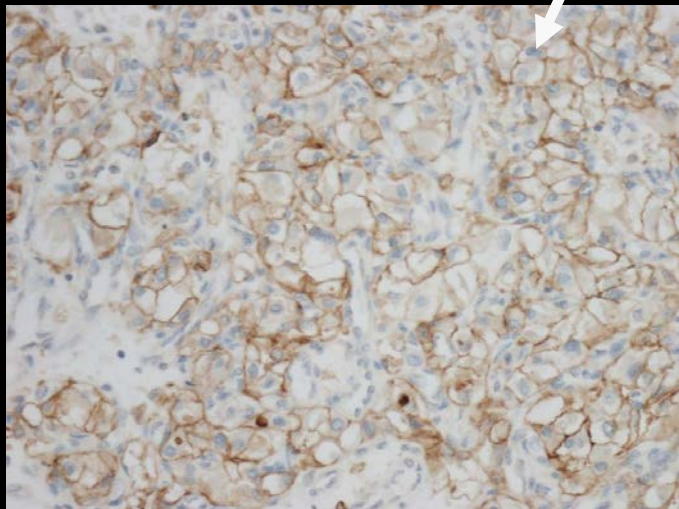
Adrenal cortical carcinoma

Renal cell markers

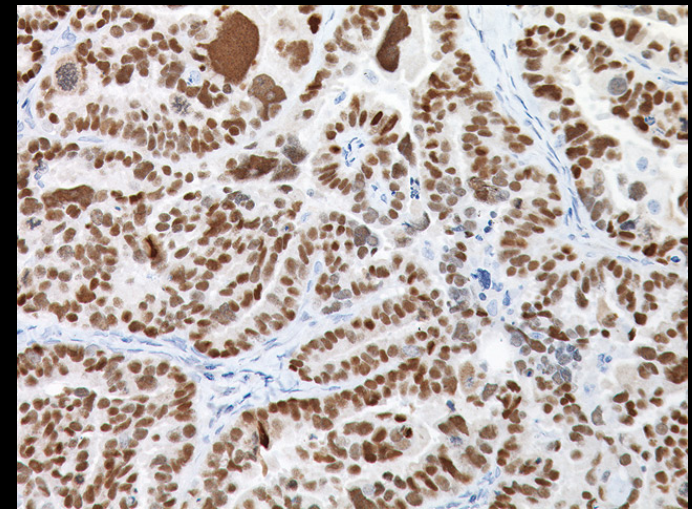
- PAX8
- Cytokeratins
- Vimentin
- CD117
- AMACR
- Napsin A
- Carboanhydrase IX
(CA IX)

ADULT RENAL CELL TUMOURS

	Clear cell RCC	Papillary RCC	Chromophobe RCC	Oncocytoma
CK 8/18	+	+	+	+ (globs)
CK 7	—	(+) patchy	+	(+) focal
Pax8	+	+	+	+
Vimentin	+	+	—	—
CD117	—	—	+	+
AMACR	(+)	+	—	+ ?
Napsin A	(+) focal	+ focal	—	- ?
CA IX	+ diffuse	(+) focal	—	—



CA IX



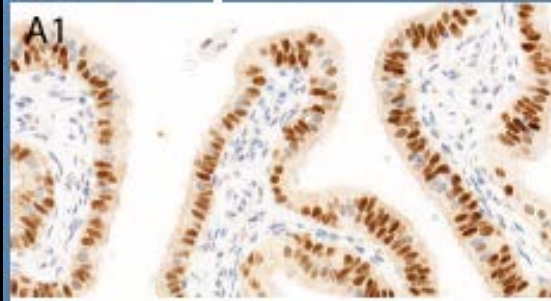
PAX8

PAX8 - NordiQC Run 51 (to be published in AIMM April 2018)

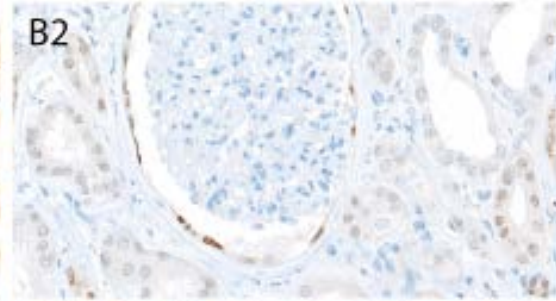
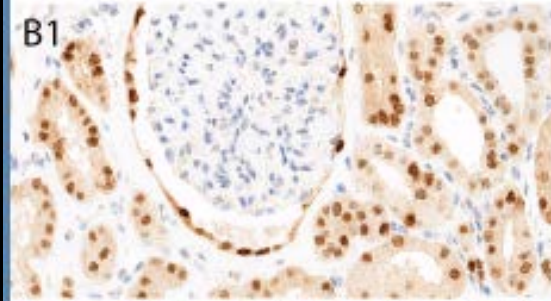
Optimal

Insufficient

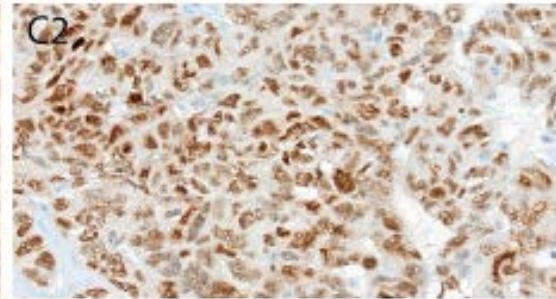
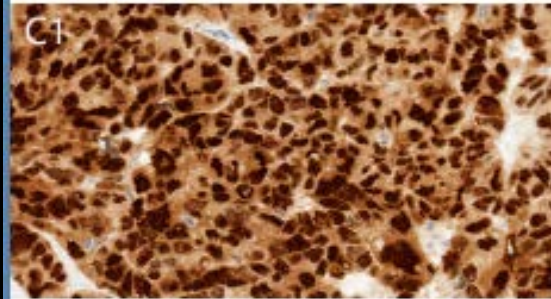
Tube



Kidney



**Ovarian
serous C**



CC RCC

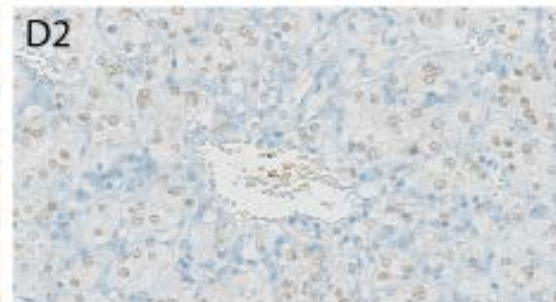
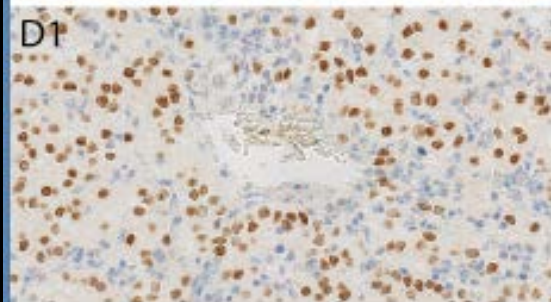


Table 3. Proportion of optimal results for PAX8 for the most commonly used antibodies as concentrate on the 4 main IHC systems*

Concentrated antibodies	Dako Autostainer Link / Classic		Dako Omnis		Ventana BenchMark GX / XT / Ultra		Leica Bond III / Max	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb MRQ-50	6/15** (40%)	-	0/3	0/1	1/22 (5%)	0/2	8/12 (67%)	0/2
mAb BC12	0/1	-	-	0/1	0/2	-	0/4	-
pAb 10336-1-AP	3/5 (60%)	-	0/4	0/1	5/15 (33%)	0/1	0/3	-

Table 4. Proportion of sufficient and optimal results for PAX8 for the most commonly used RTU IHC systems

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT mAb MRQ-50 760-4618	0% (0/1)	0% (0/1)	40% (4/10)	0% (0/10)
VMS Ultra/XT mAb MRQ-50 363M-18	25% (1/4)	0% (0/4)	36% (20/56)	2% (1/56)

rmAb clone EP298	4 2 1	Cell Marque Epitomics BIO SB	3	3	0	1	86%	100%
rmAb clone SP348	1	Spring Biosciences	1	0	0	0	-	-
rmAb clone ZR-1	3 1 1	Zeta Corporation Abcam Nordic Biosite	2	2	1	0	80%	100%

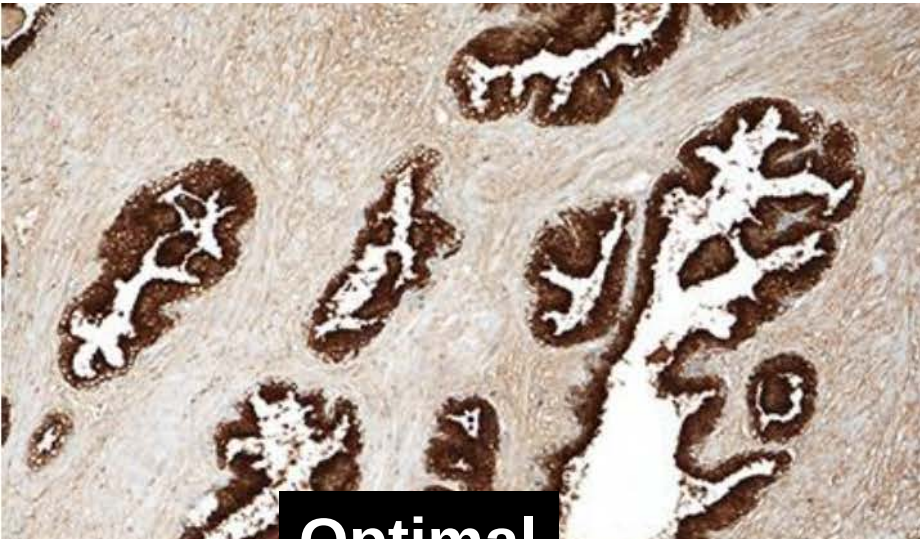
Prostate cell markers

- Prostate specific antigen (PSA)
 - Prostein (P501S)
 - NKX3.1
 - AMACR
 - P63/p40/CK5
 - ERG

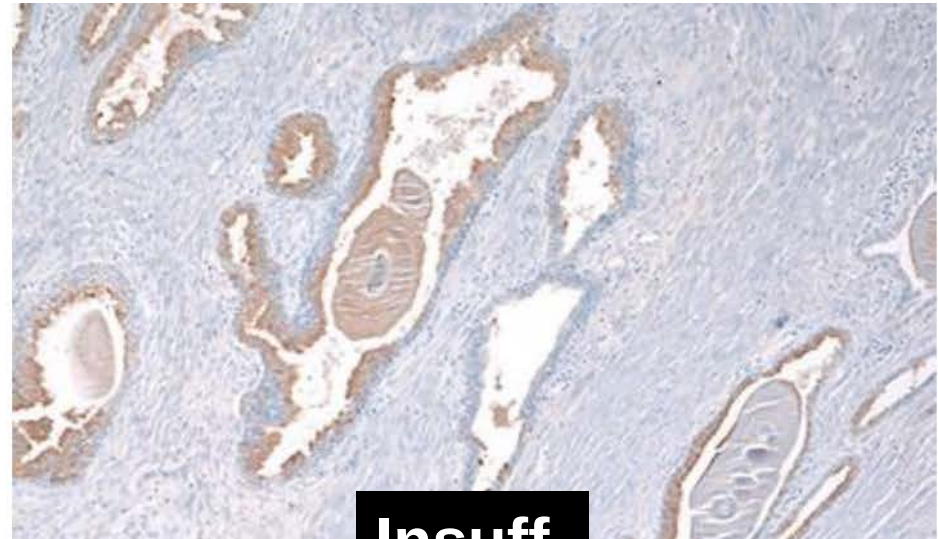
**Double
stain**

Assessment Run 49 2017

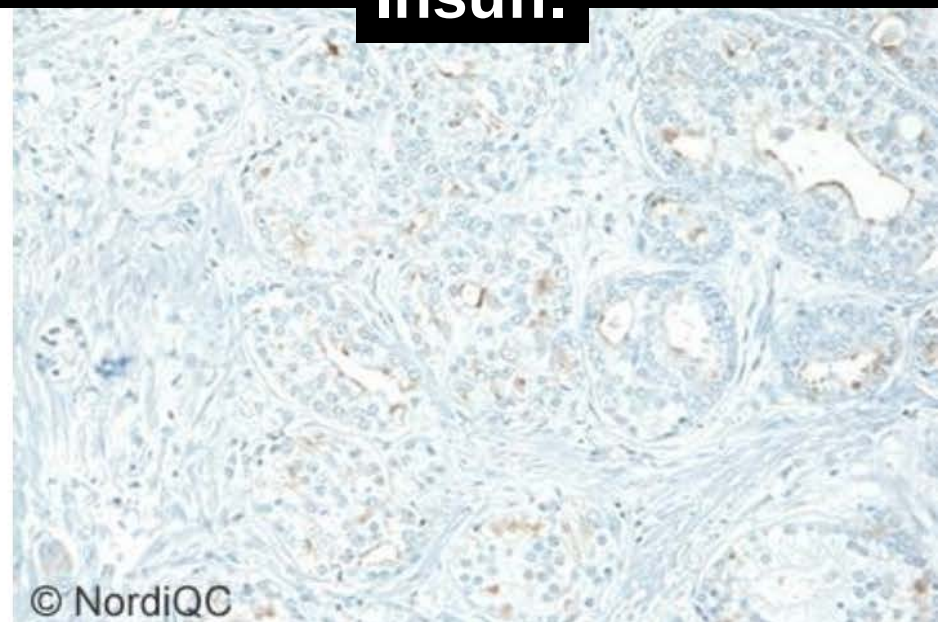
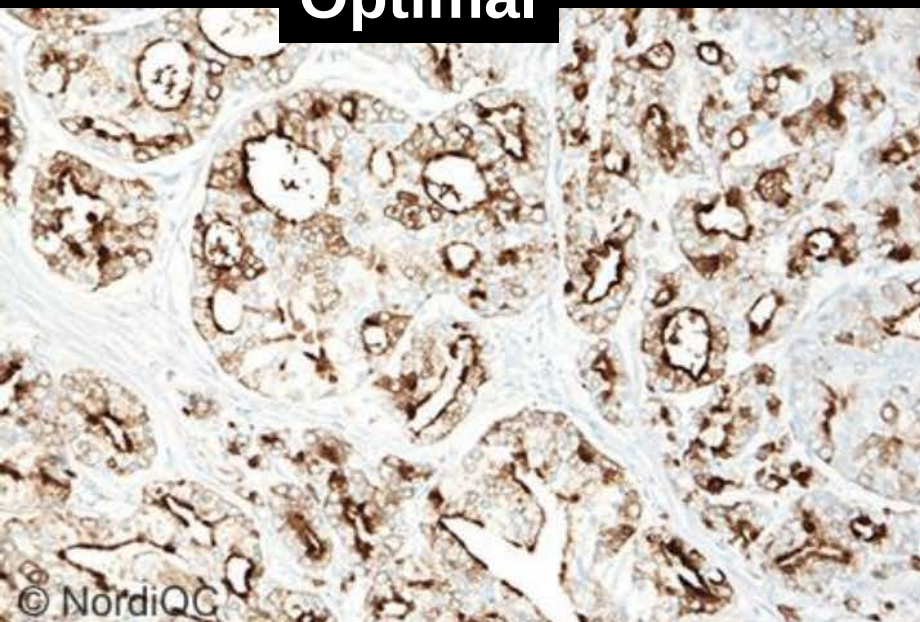
Prostate Specific Antigen (PSA)



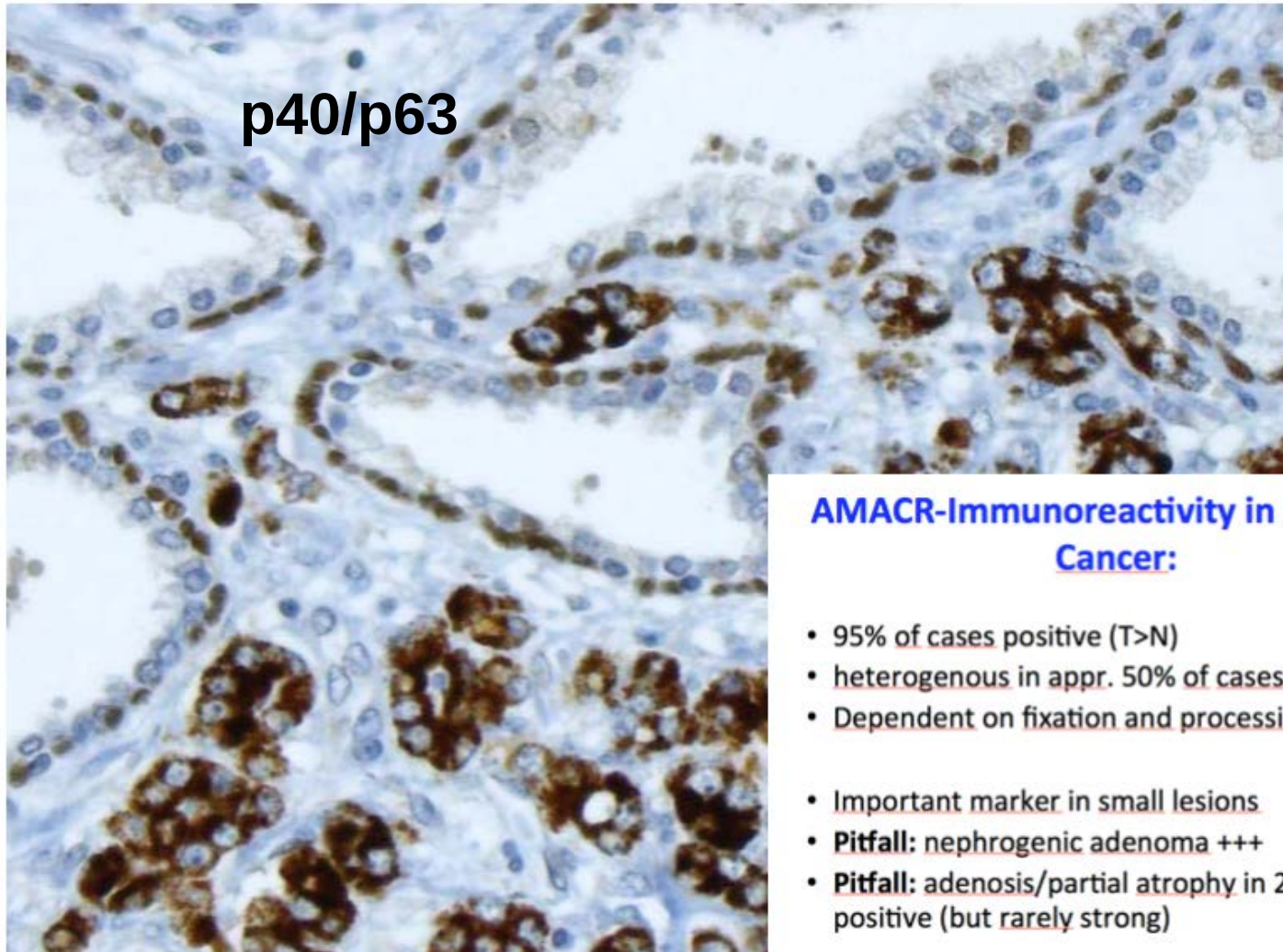
Optimal



Insuff.



Positive Markers of Malignancy: AMACR



AMACR-Immunoreactivity in Prostate Cancer:

- 95% of cases positive (T>N)
- heterogenous in appr. 50% of cases
- Dependent on fixation and processing
- Important marker in small lesions
- **Pitfall:** nephrogenic adenoma +++
- **Pitfall:** adenosis/partial atrophy in 20%-30% positive (but rarely strong)

Confirmation of Prostatic Origin: NKX3.1

NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumors

Bora Gurel, MD, Tehmina Z. Ali, MD,† Elizabeth A. Montgomery, MD,* Shahnaz Begum, PhD,*
Jessica Hicks, BA,* Michael Goggins, MD,*‡ Charles G. Eberhart, MD, PhD,*‡
Douglas P. Clark, MD,*‡ Charles J. Bieberich, PhD,§ Jonathan I. Epstein, MD,*‡||
and Angelo M. De Marzo, MD, PhD*‡||*

Am J Surg Pathol • Volume 34, Number 8, August 2010

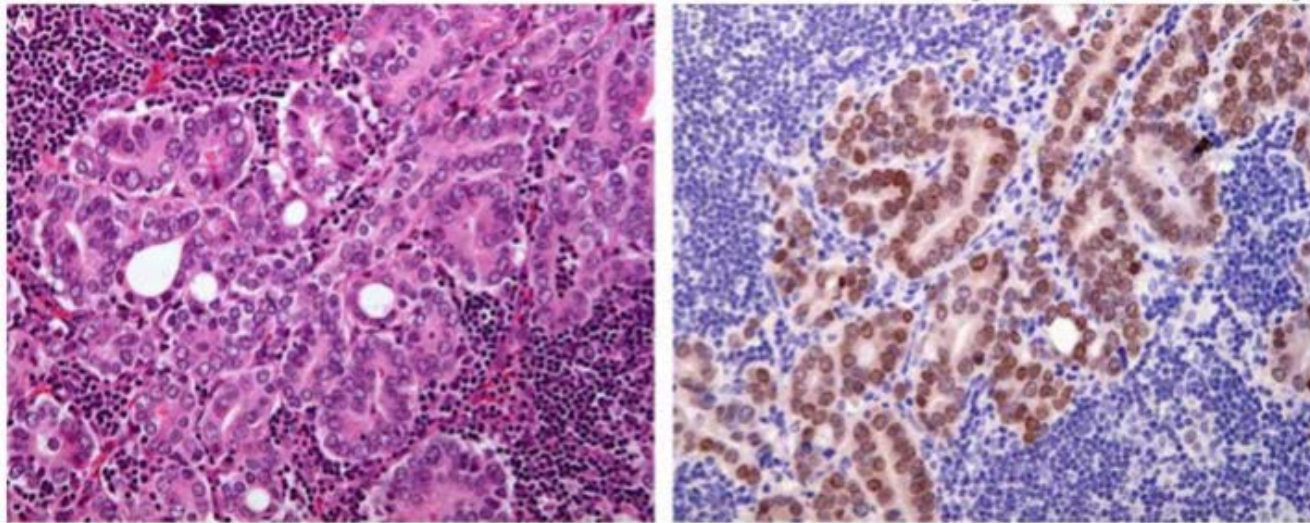
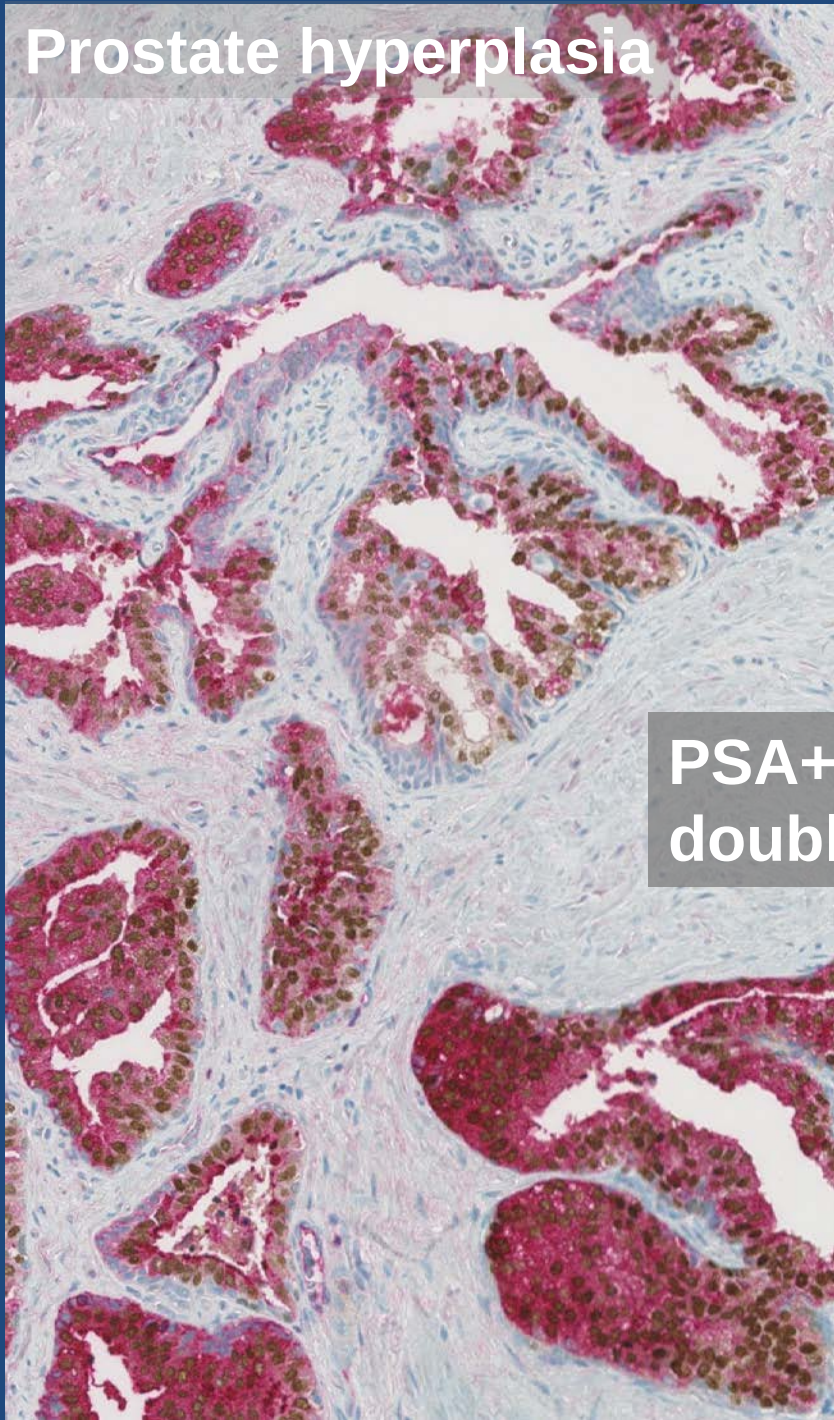


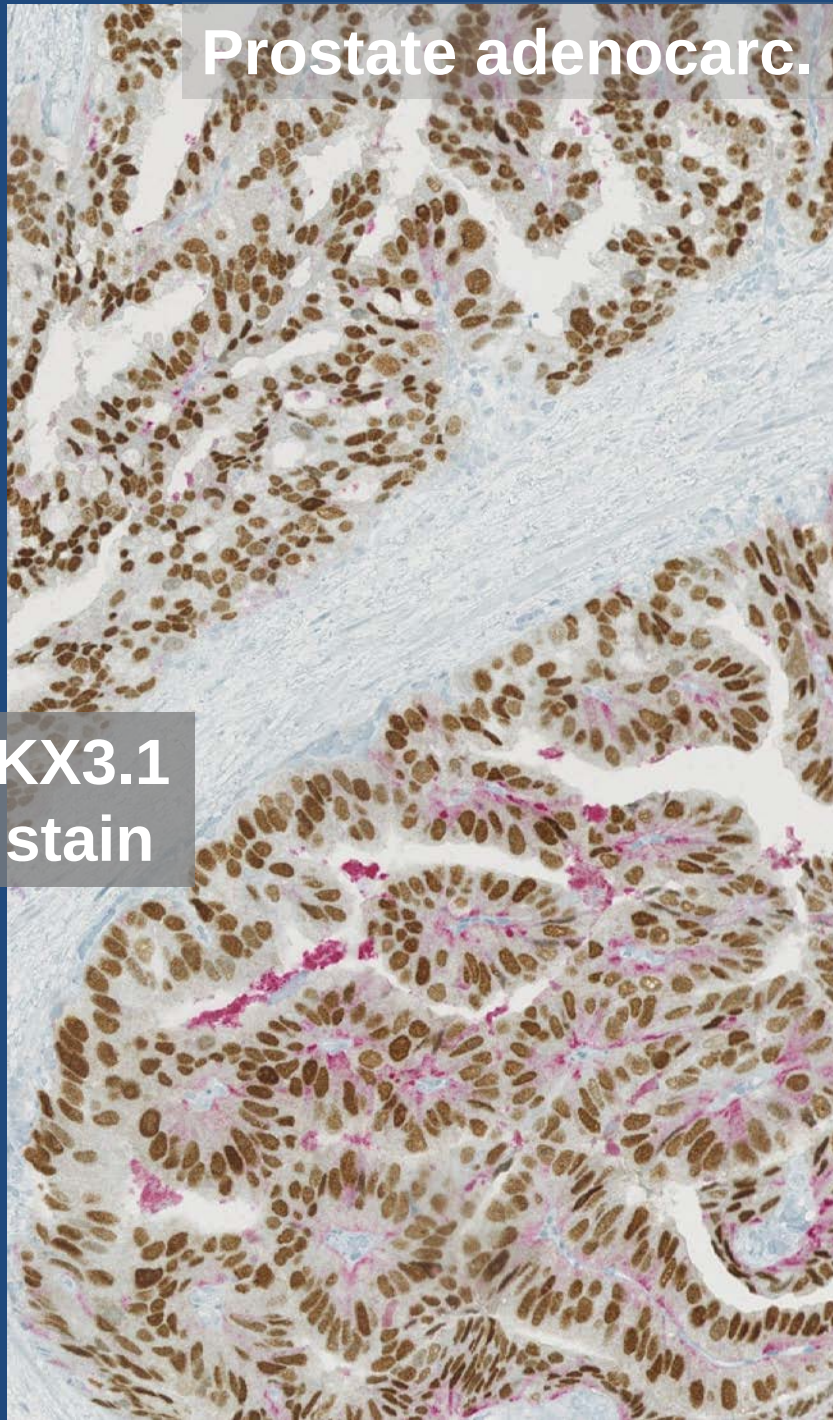
TABLE 3. The Average Percentage of Positively Stained Cells and the Calculated Staining Scores of NKX3.1, PSA and PSAP for Normal Prostate, Primary and Metastatic Prostate Carcinoma

	NKX3.1		PSA ^a		PSAP	
	% Positive (Range)	Staining Score(SD)	% Positive (Range)	Staining Score(SD)	% Positive (Range)	Staining Score(SD)
Normal Prostate	92.0 (38.3-100)	218.3 (85.22)	965.0 (0-100)	241.7 (76.88)	97.6 (0-100)	287.8 (50.96)
Primary Ca	84.7 (25-100)	179.1 (77.84)	87.3 (10-100)	180.7 (91.36)	98.6 (85-100)	249.2 (64.35)
Lymph Node Met	74.2 (0-100)	155.4 (84.78)	80.1 (0-100)	1743.0 (99.14)	94.4 (0-100)	235.6 (78.24)
Distant Site Met	54.0 (0-88.3)	111.4 (85.36)	30.8 (0-100)	50.8 (92.83)	74.2 (0-100)	162.0 (116.49)
Average	80.5	175.9 (87.83)	83.1	186.0 (101.29)	95.0	248.5 (77.32)

Prostate hyperplasia

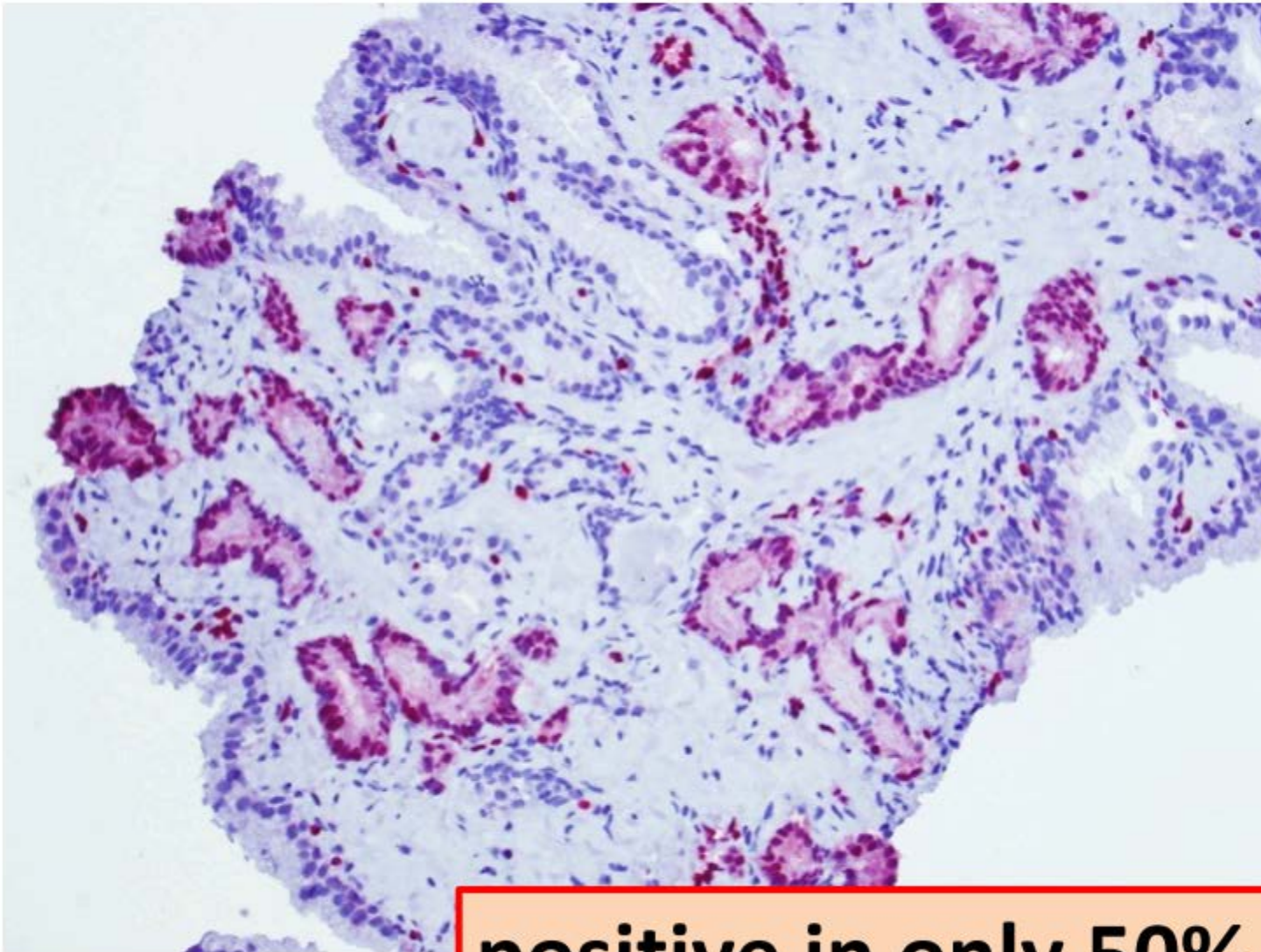


Prostate adenocarc.



PSA+NKX3.1
double stain

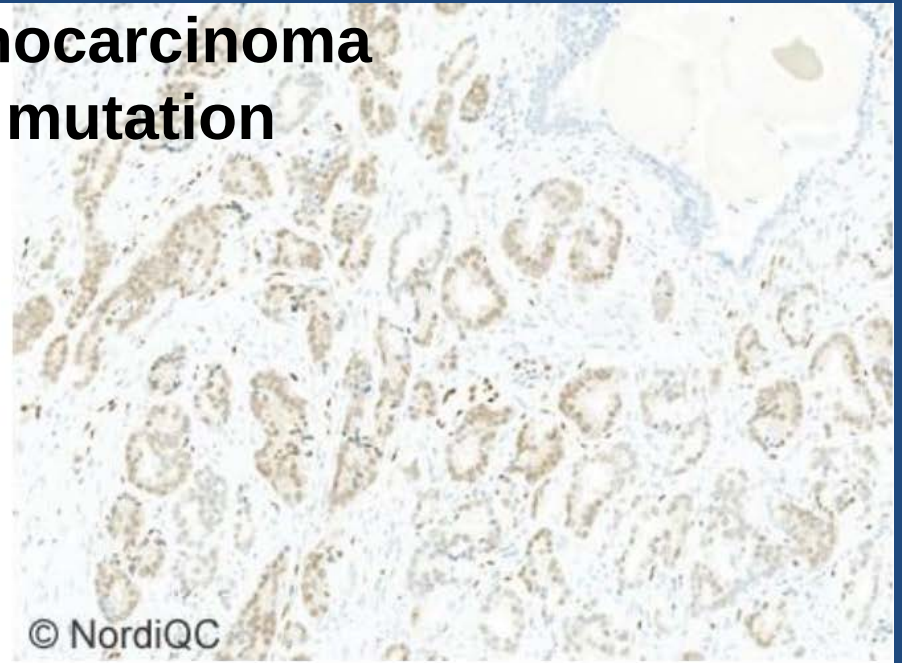
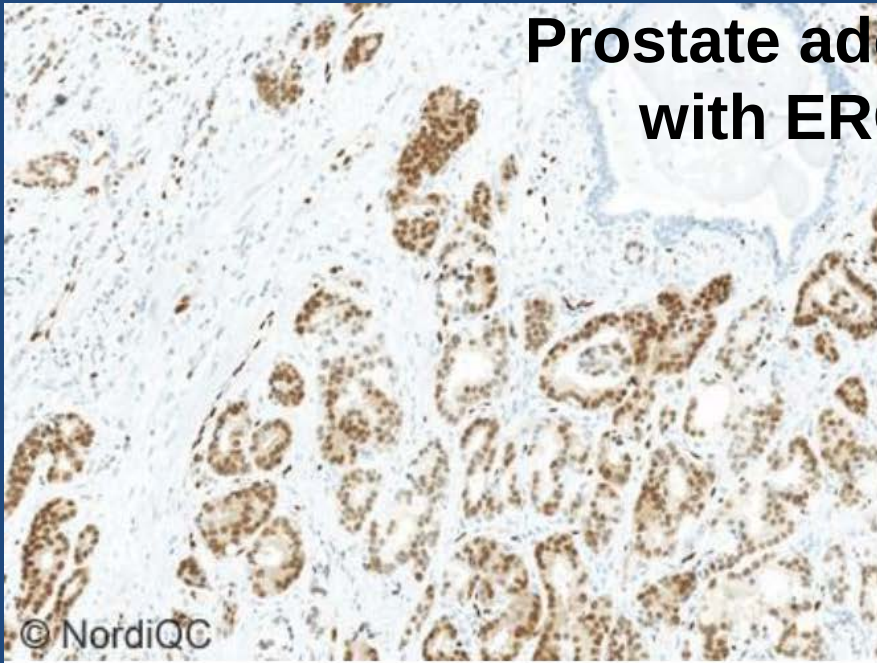
Alternatives to AMACR: 4. ERG



positive in only 50% of PCa

ERG – NordiQC run 50

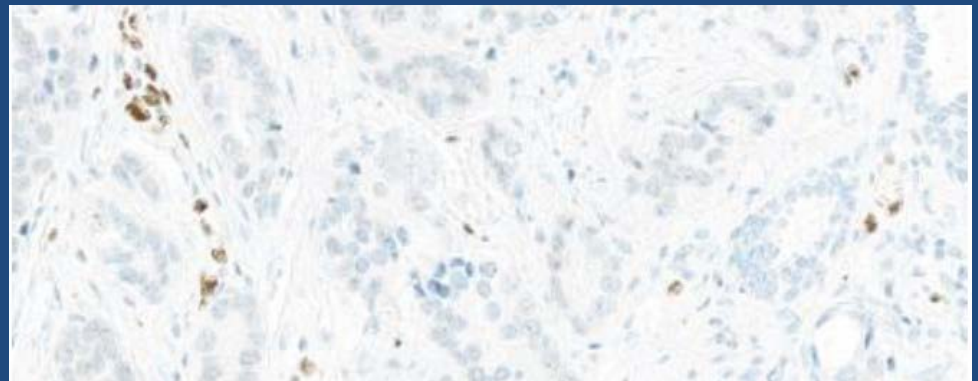
Prostate adenocarcinoma with ERG mutation

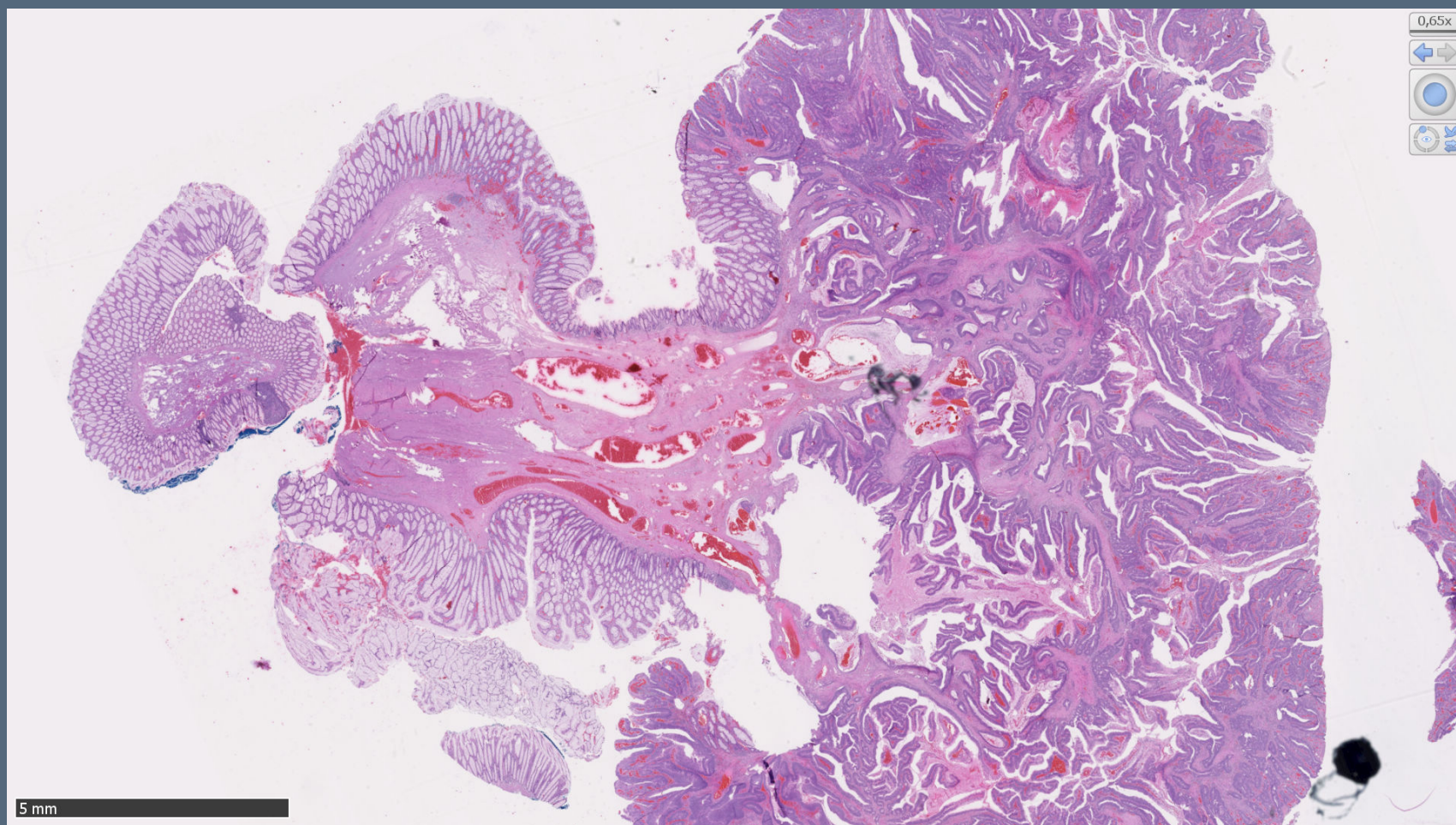


Optimal: 38%
Clones EP111 and EPR3864
with sensitive protocols

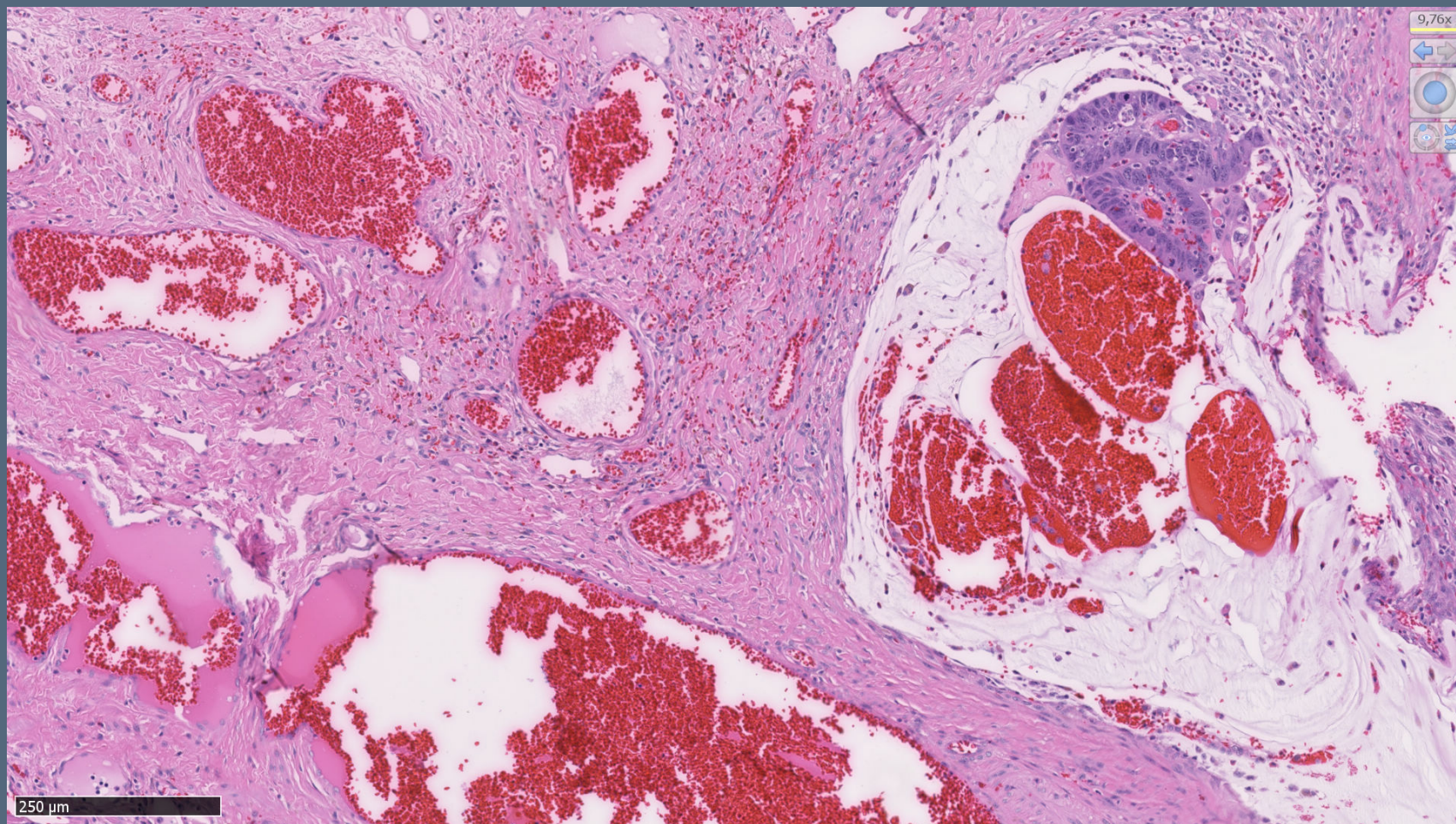
Insufficient: 33%
e.g., EPR3864 RTU (VMS)

Clone FY9





16029806

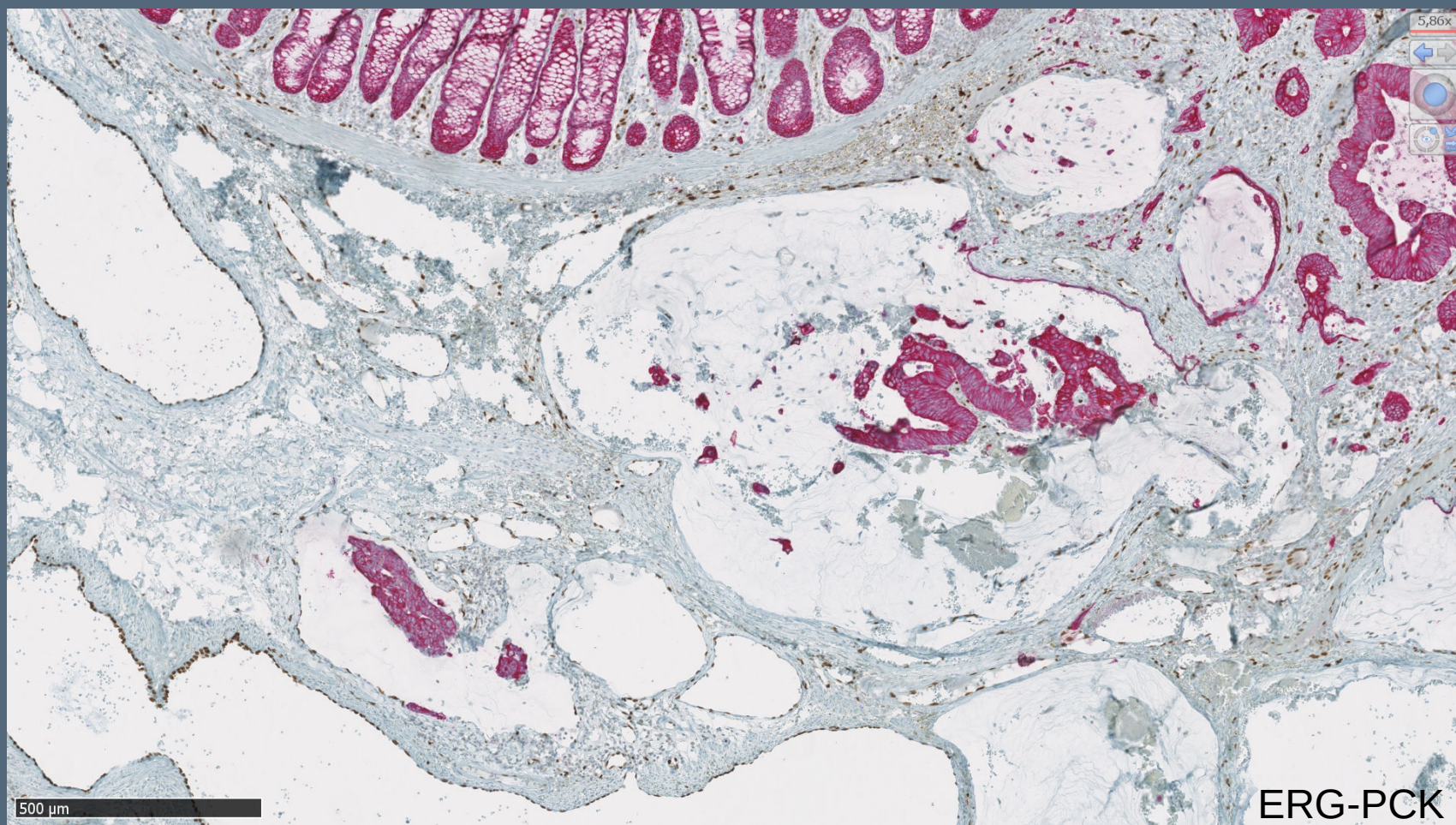




Risk of lymph node metastasis (>10%) in colorectal "malignant adenoma"

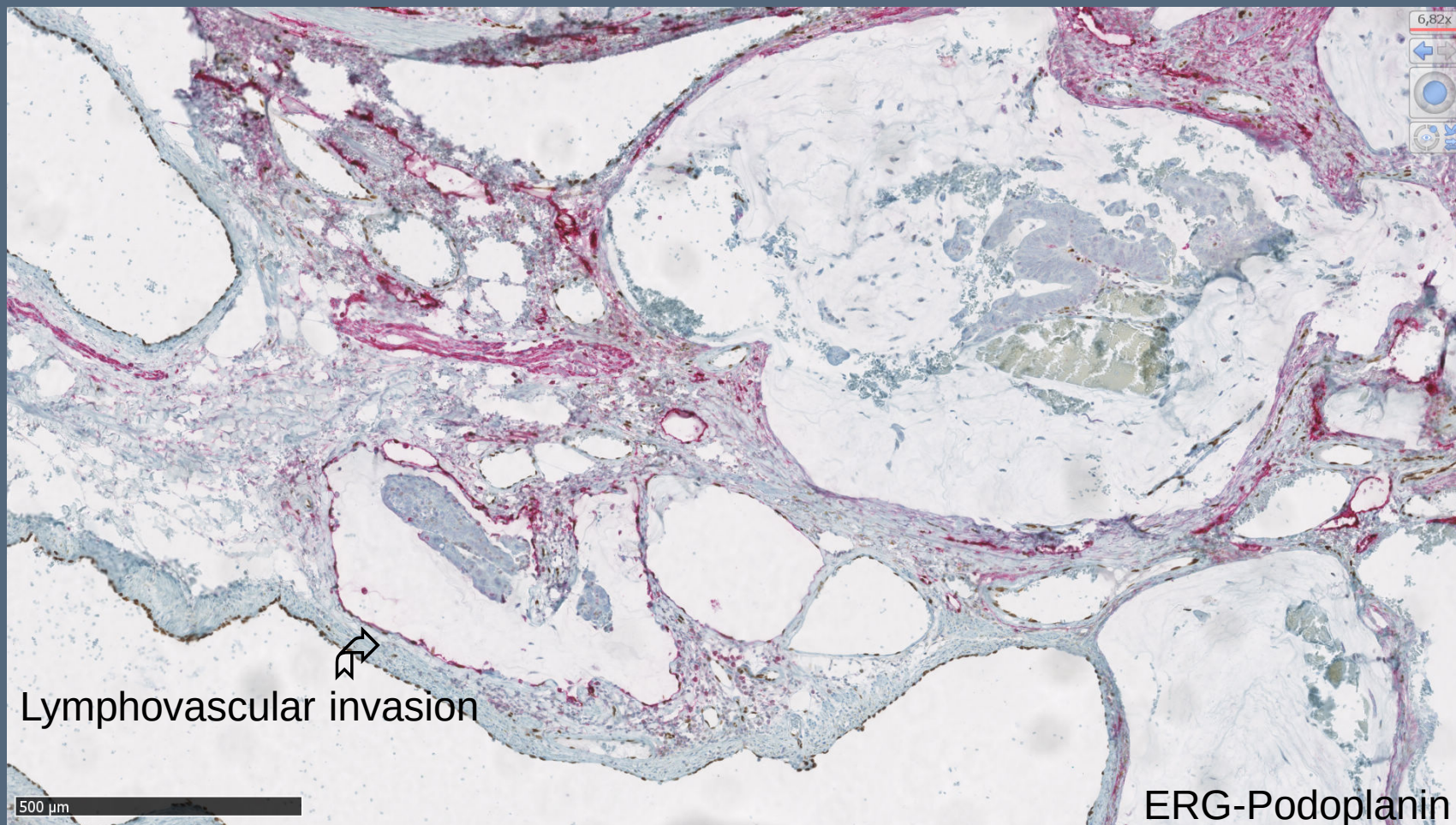
- Tumor budding 42%
- Low/mucinous diff. 35%
- Res. marg. <1mm 33%
- Kikuchi sm3 23%
- Haggitt level 4 17%

- Lymphovasc. invasion 35%



500 μm

ERG-PCK



Lymphovascular invasion

“Germinal cell markers”:

SALL4

OCT3/4

PLAP

CD30

AFP

Podoplanin

GLP3

CD117

GERM CELL



NO DIFFERENTIATION

Seminoma/Dysgerminoma/Germinom

EMBR. DIFFERENTIATION

Embryonal carcinoma

EXTRAEMBRYONIC DIFFERENT.

Endodermal sinus/Yolk sac tumour

**EMBRYONIC
TISSUE**

Teratoma

TROPHOBLAST

Choriocarcinoma

Germ cell tumours

	AFP	CD 30	CD 117	CK LMW	GLP 3	OCT 3/4	PL AP	SALL 4
Seminoma/ dysgerminoma	-	-	+	+/- dot	-	+	+	+
Embryonal carcinoma	-/+	+	-/+	+	-/+	+	+/-	+
Yolk sac tumour	+/-	-/+	-/+	+	+/- focal	-	-/+	+
Chorio- carcinoma	-	-	-	+	+	-	+/-	+cyto -syn

Cao et al. AJSP 2009, 33:1065.

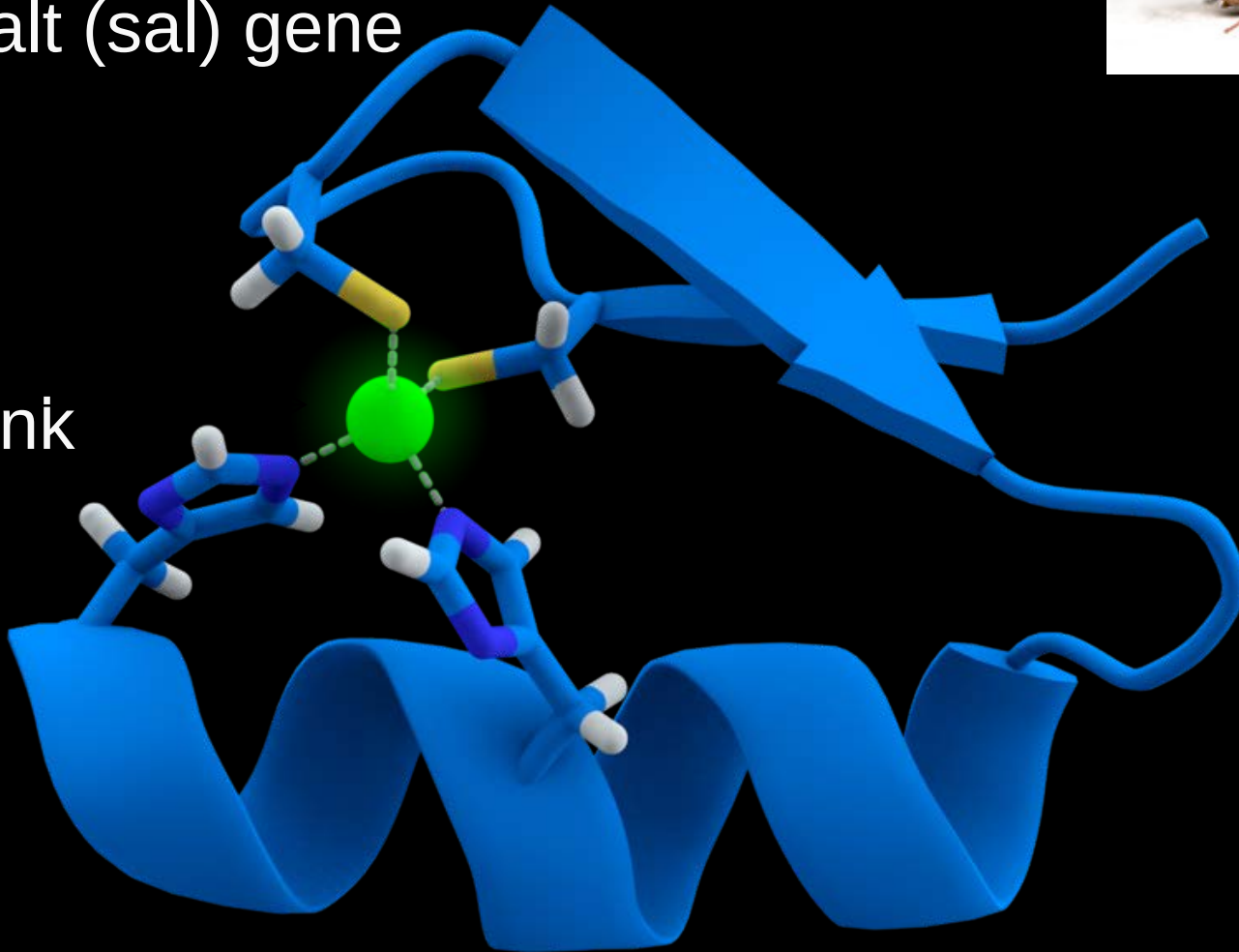
Trinh et al. JOGR 2012, 38:841

SALL4

- A zinc finger transcription factor that shares homology to the *Drosophila* spalt (sal) gene



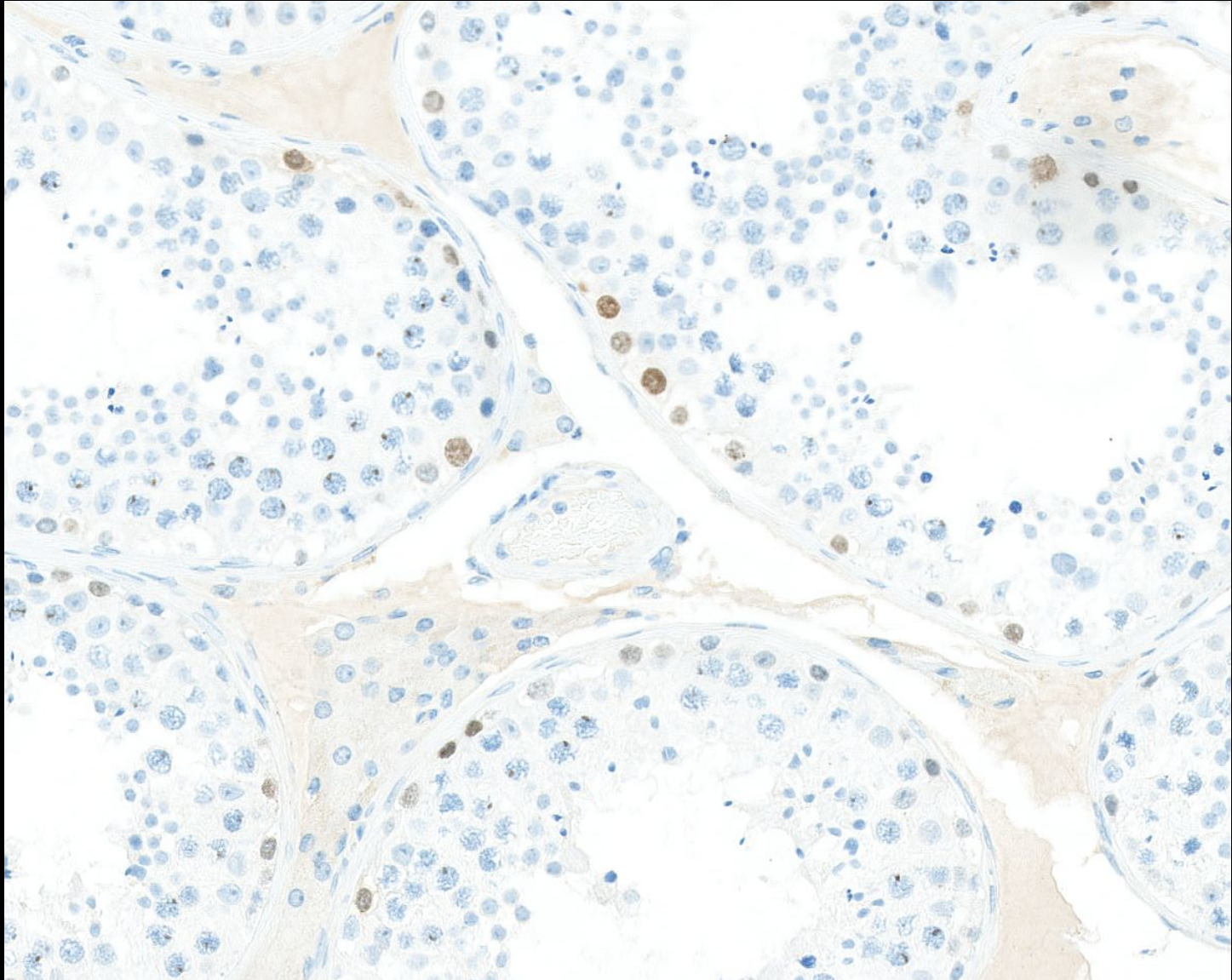
Zink



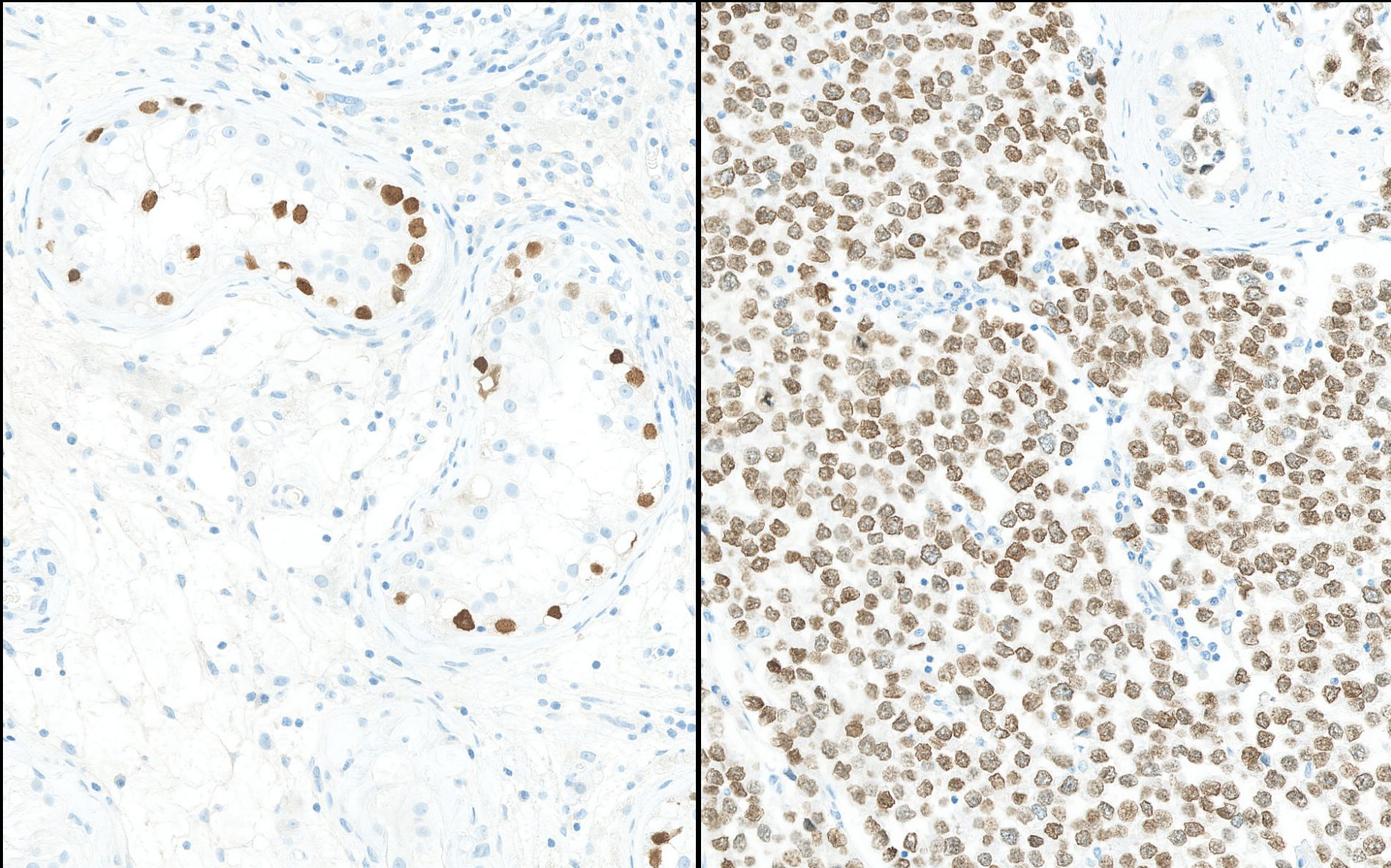
SALL4

- Transcription factor essential to early embryogenesis in all species - plays an important role in the specification of head and tail regions
- In human located on chromosome 20q13.13–13.2
- Forms a regulatory circuit with OCT4, NANOG, and SOX2 to **maintain embryonic stem cell pluripotency and self-renewal**
- By IHC detected in all types of non-teratoma germinal cell tumours
- Rarely, weak and focal reaction is seen in non-germinal cell carcinomas

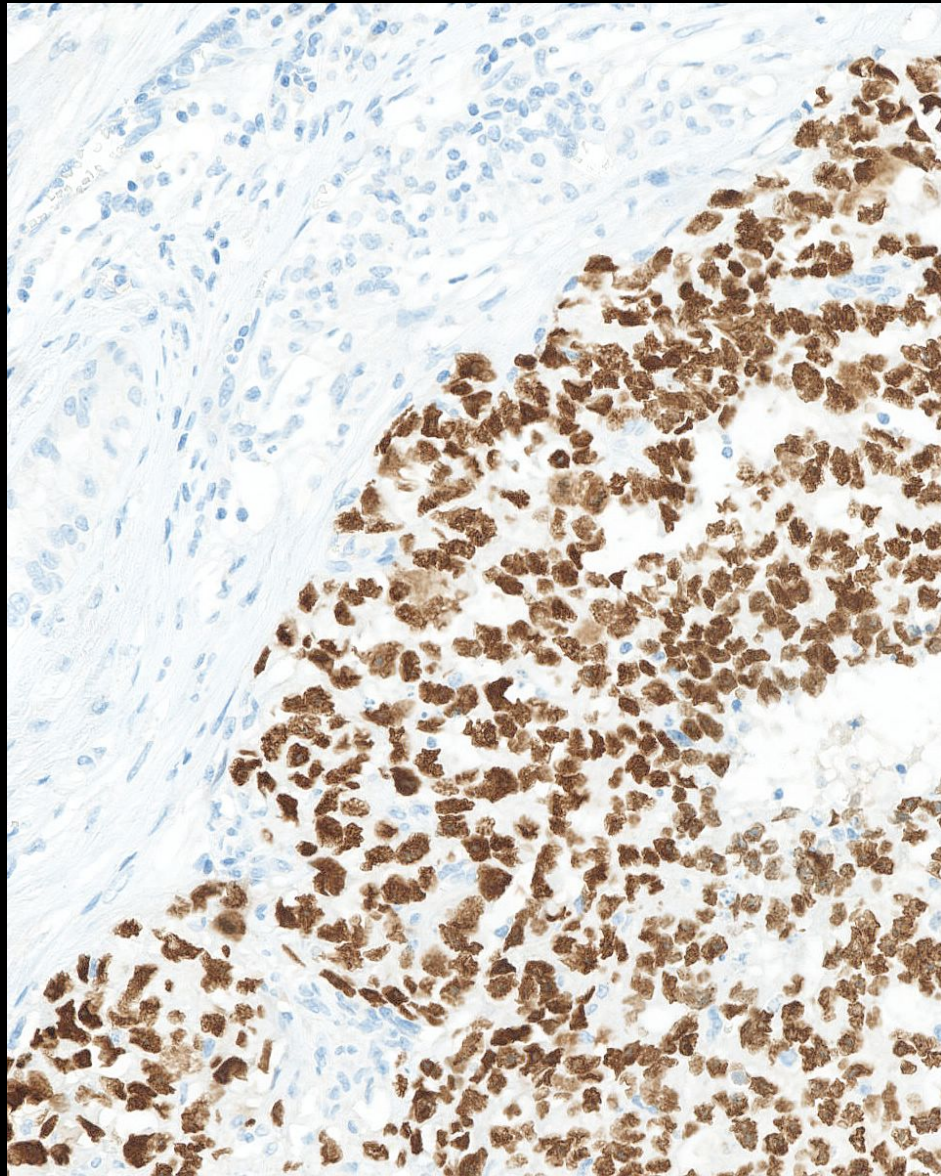
SALL4 – Testis



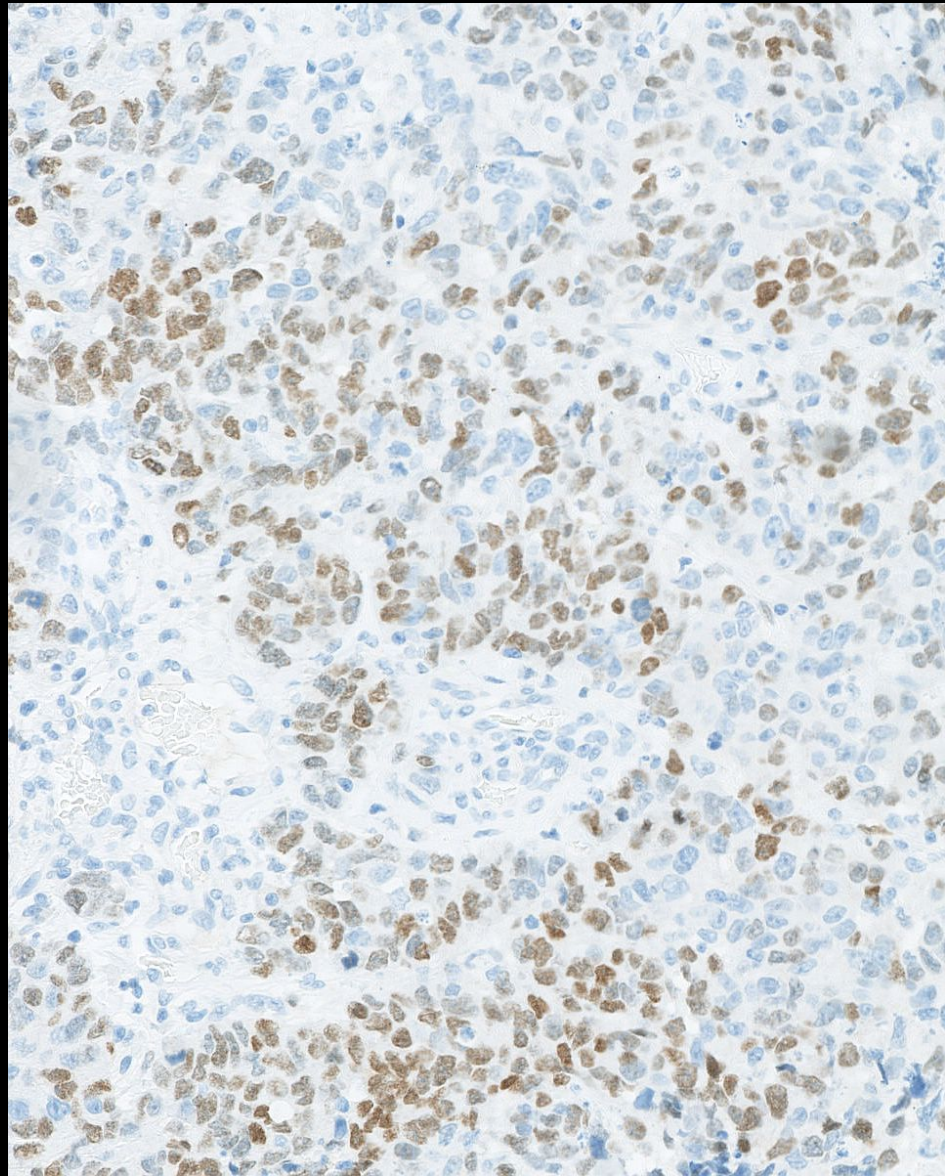
SALL4 – GCNIS and seminoma



SALL4 – Embryonal carc.



Lung adenocarcinoma !



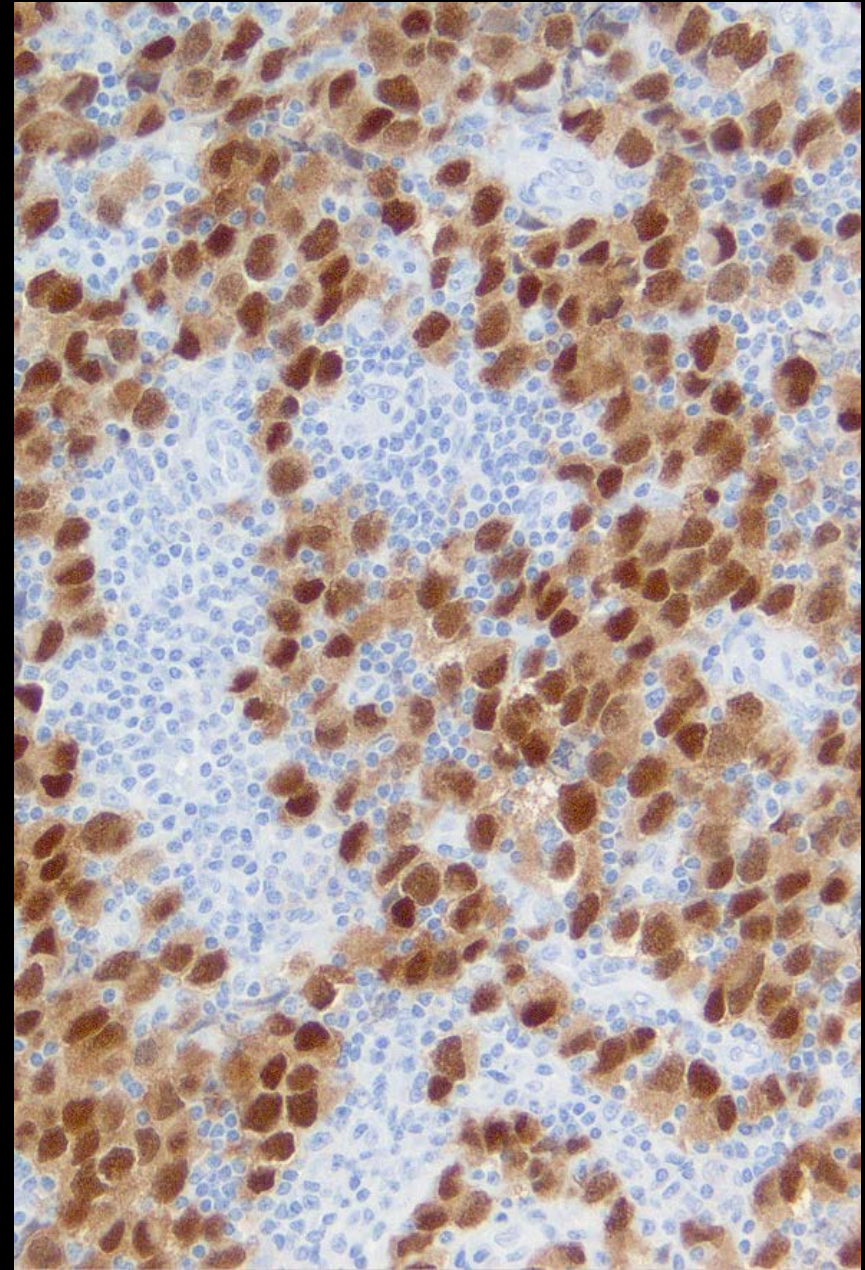
OCT3/4

Transcription factor expressed in in early embryonic cells, germ cells and stem cells maintaining cell replication

- Seminoma +
- Germinal cell neoplasia in situ (GCNIS) +
- Dysgerminoma +/-
- Embryonal carcinoma +

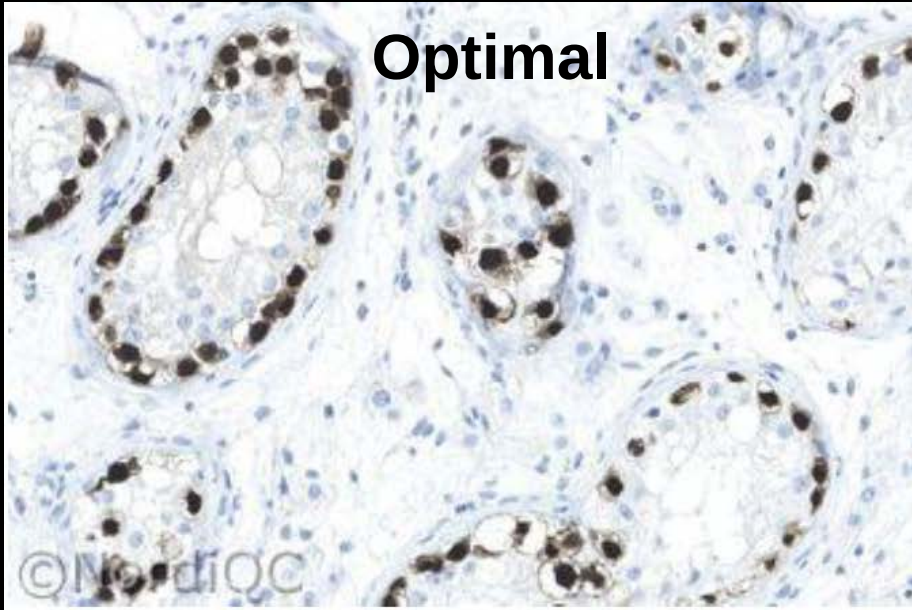
>90% of nuclei, + sometimes a dot-like cytoplasmic reaction

Seminoma

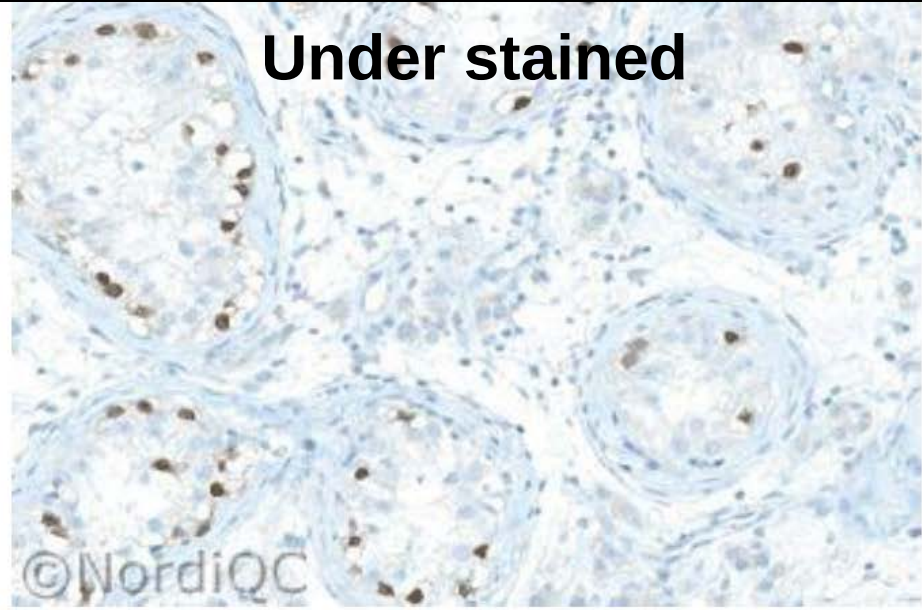


OCT3/4 – NordiQC run 35

Optimal

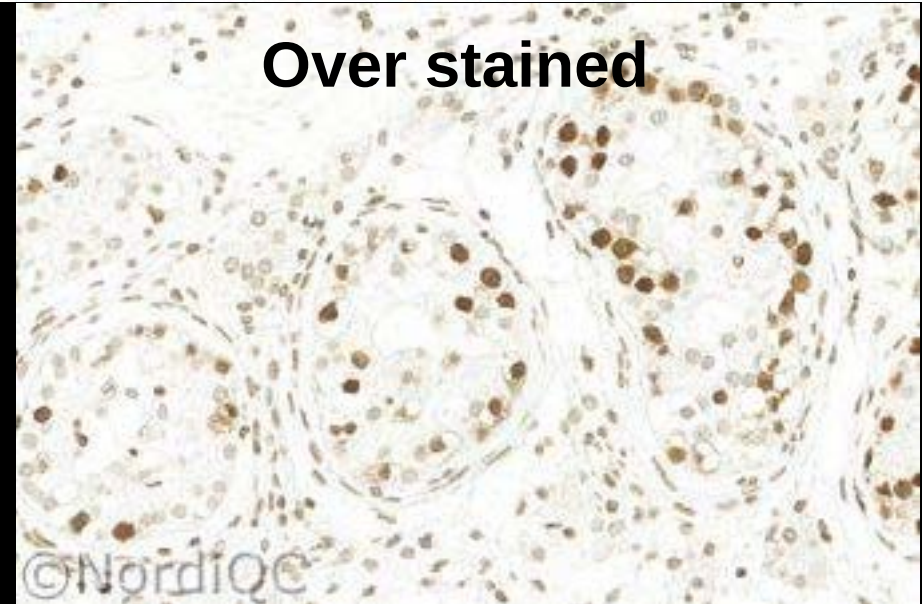


Under stained

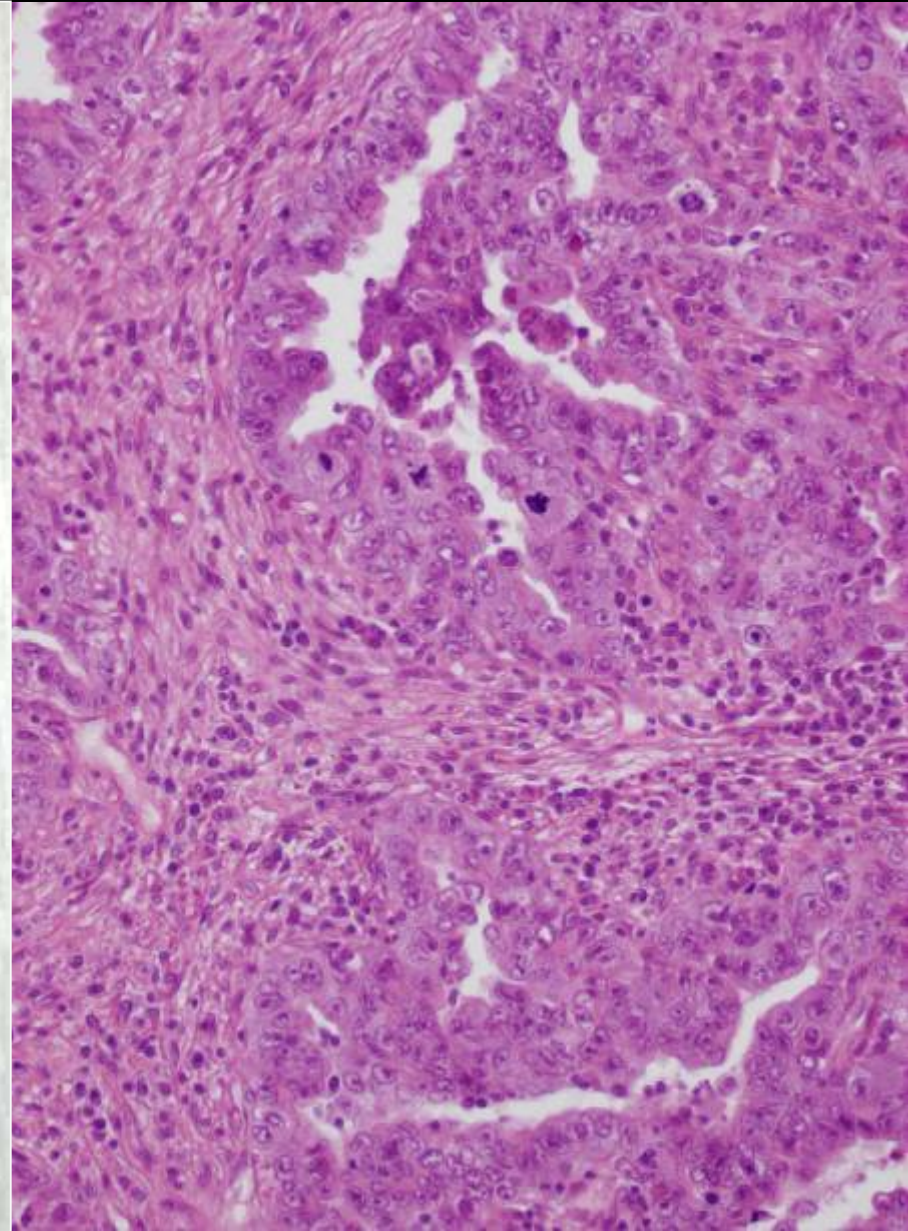


Intratubular germ
cell neoplasia

Over stained



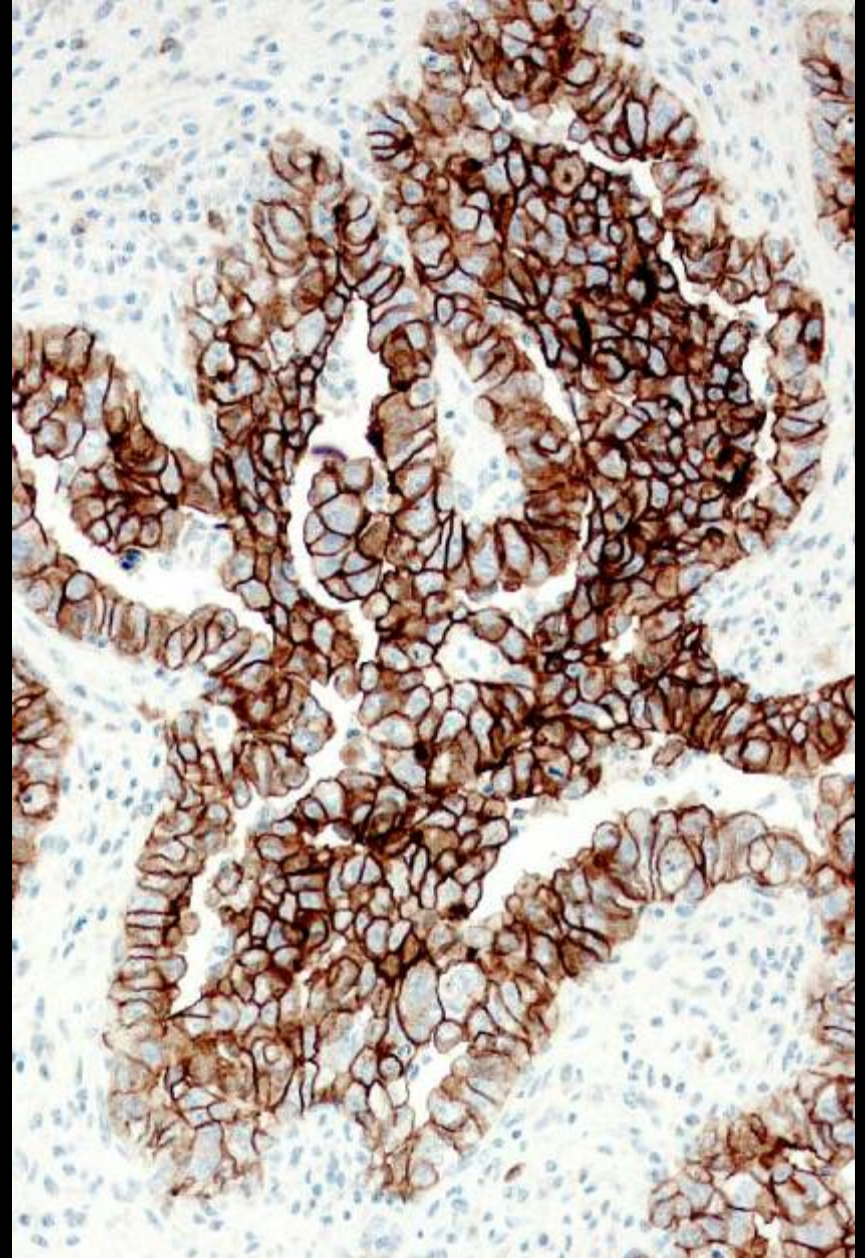
Embryonal carcinoma



CD30

Cytokine receptor, mediating cell proliferation

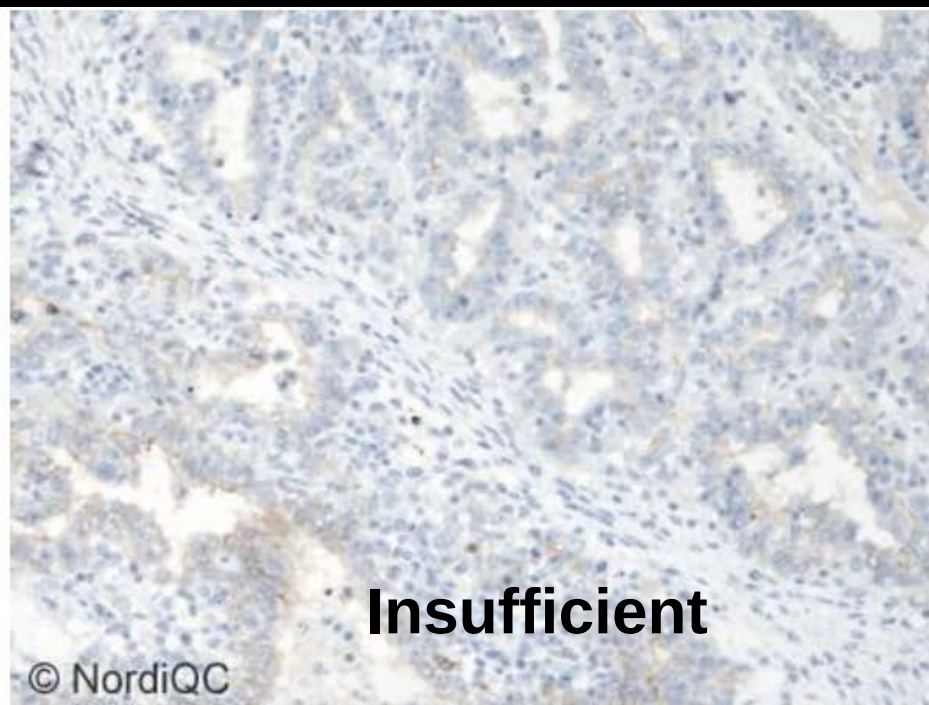
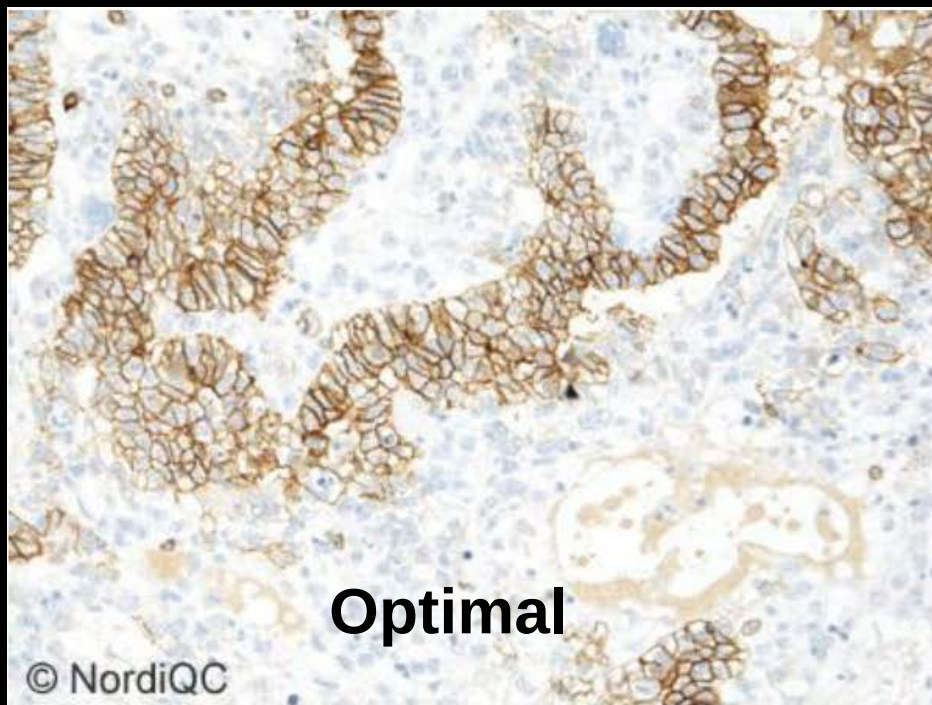
- Embryonal carcinoma +
- Yolk sac tumour -/+
- Seminoma -
- Salivary carcinoma +/-
- Other carcinomas -/+
- ALCL +
- Other lymphomas +/-
-



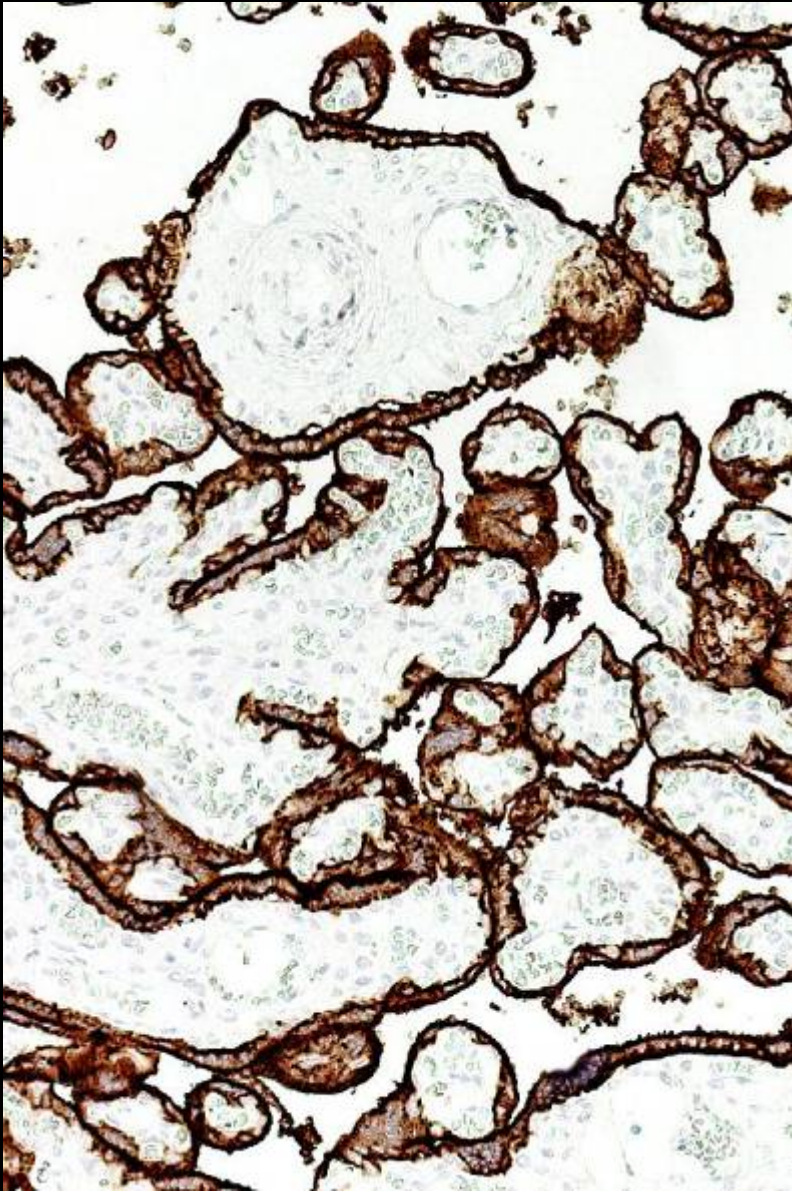
CD30 – NordiQC run 51

Table 4. **Proportion of sufficient and optimal results for CD30 for the most commonly used RTU IHC systems**

RTU systems	Recommended protocol settings*		Laboratory modified protocol settings**	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb Ber-H2 IS/IR602	100% (3/3)	100% (3/3)	96% (23/24)	50% (12/24)
VMS Ultra/XT mAb Ber-H2 790-4858	66% (6/9)	22% (2/9)	96% (23/24)	49% (11/24)
Leica BOND MAX/III mAb JCM182 PA0790	83% (5/6)	50% (3/6)	100% (4/4)	100% (4/4)



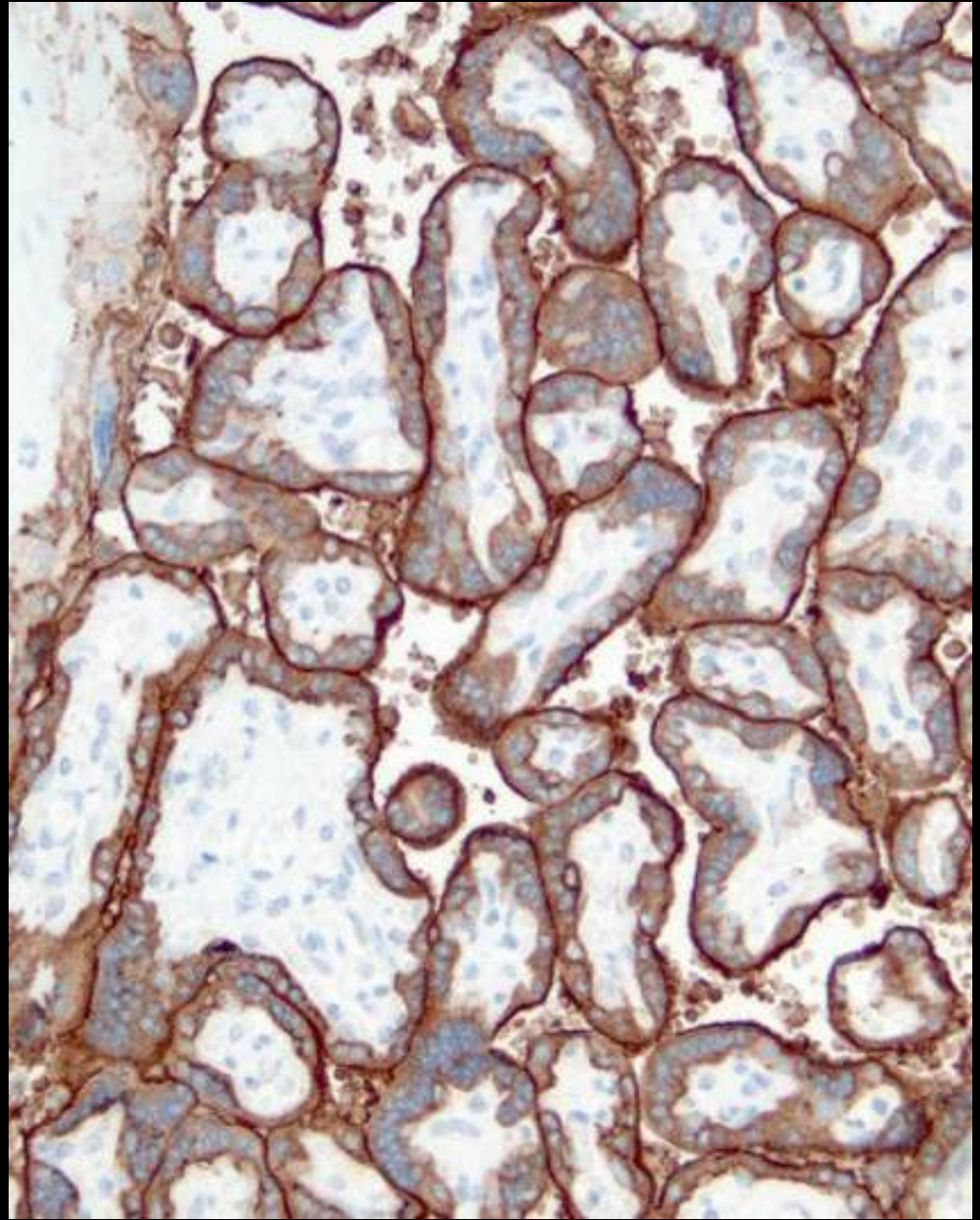
Placental alkaline phosphatase (PLAP)



- PLAP belong to heterogeneous group of glycoproteins confined to the cell membranes.
- PLAP plays a role in guiding migratory cells and transport specific molecules over the plasma membrane.
- PLAP is expressed in placenta from the 8th week of gestation.

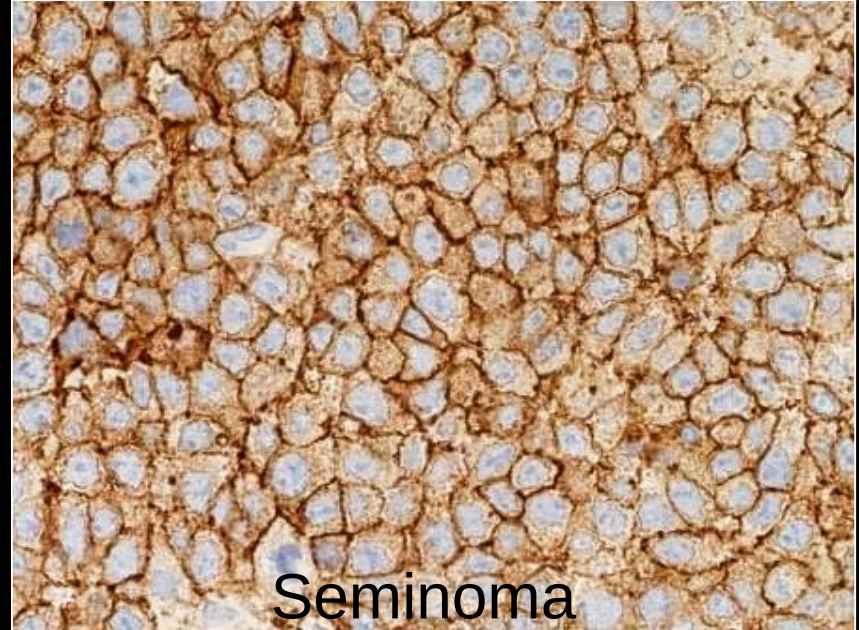
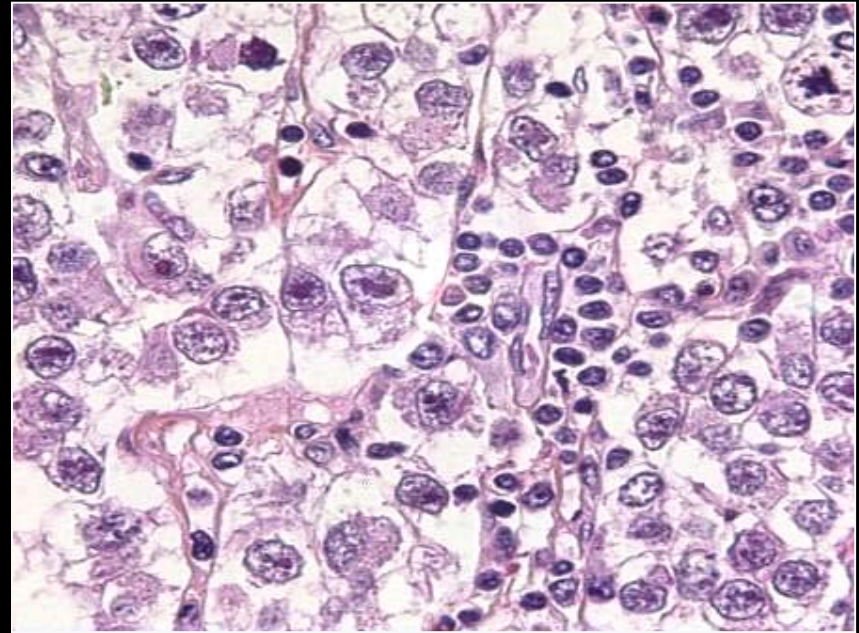
Placental alkaline phosphatase (PLAP)

- Placental syncytiotrophoblast
- Ovary
- Testis
- Fallopian tube
- Uterine cervix
- Respiratory tract
- Thymus



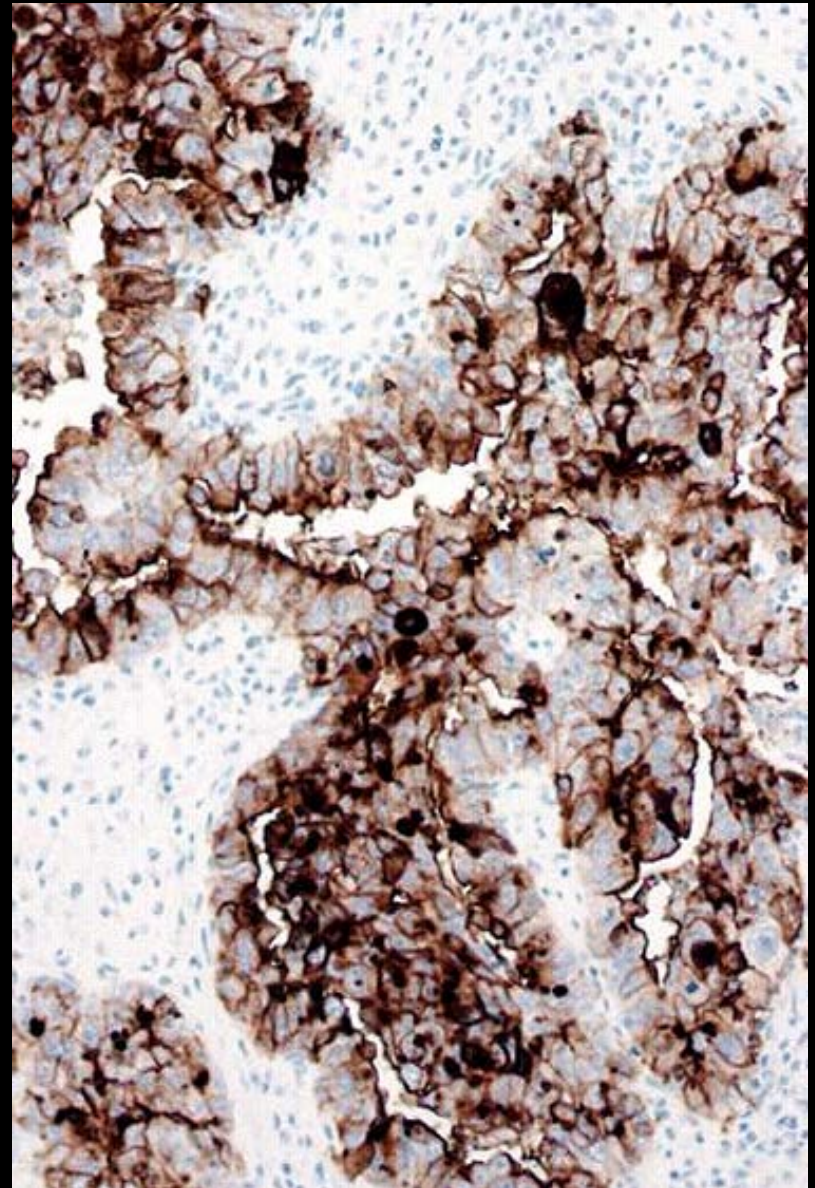
Placental alkaline phosphatase (PLAP)

- Seminoma/dysgerminoma +
 - Except spermatocytic sem.
- Choriocarcinoma +/-
- Embryonal carcinoma +/-
 - Often focal
- Yolk sac tumour -/+
- Serous ovarian carcinoma +/-
- Adenocarcinomas
 - Various types –(+)



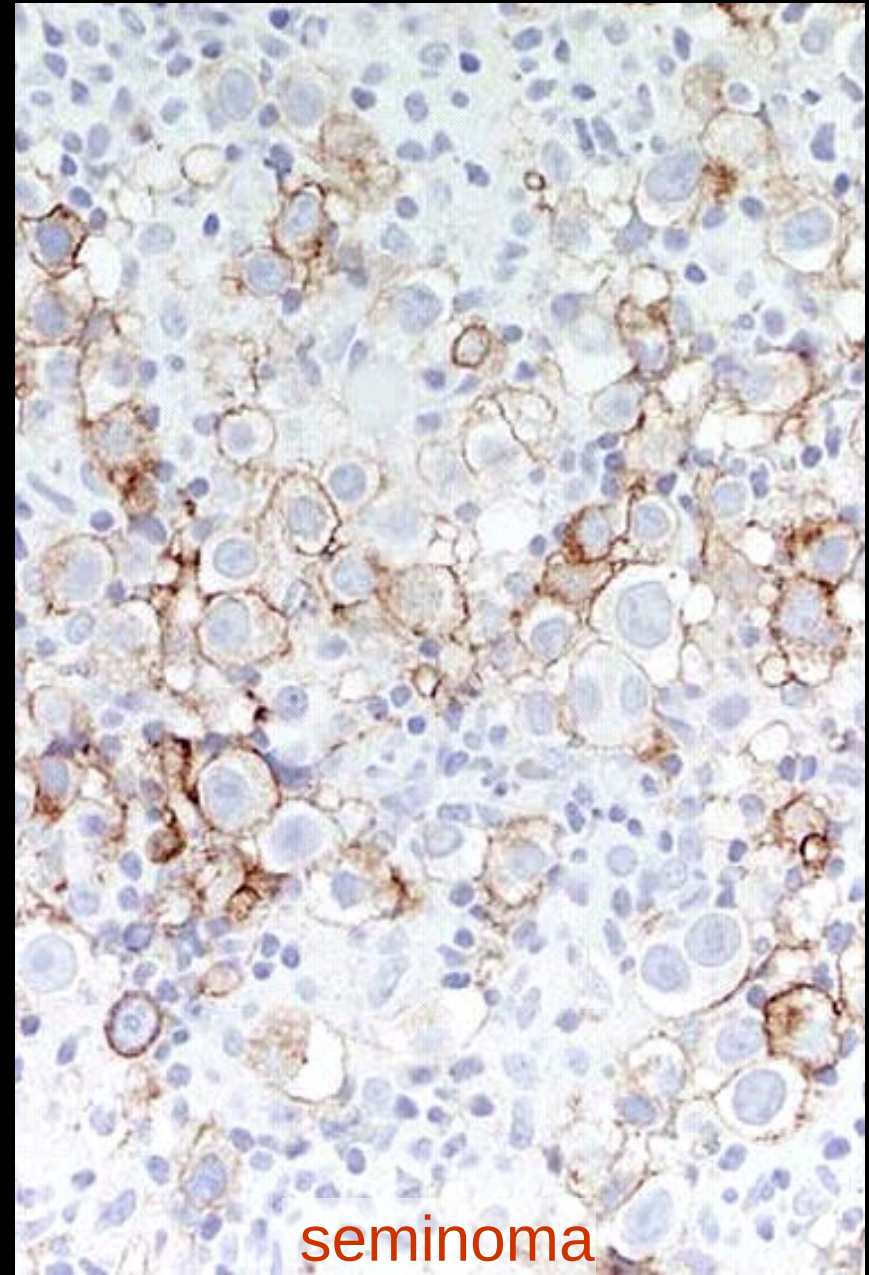
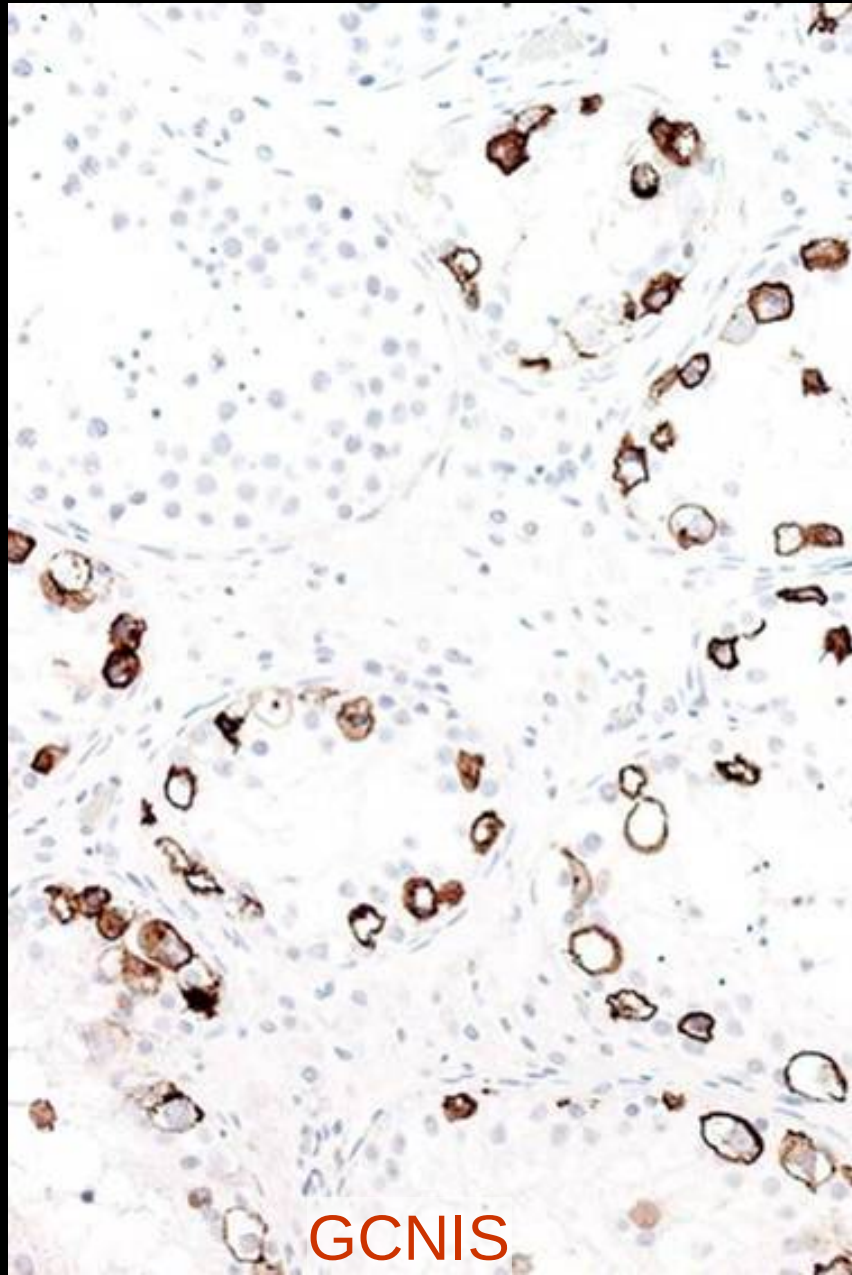
Placental alkaline phosphatase (PLAP)

- Seminoma/dysgerminoma +
 - Except spermatocytic sem.
- Choriocarcinoma +/-
- Embryonal carcinoma +/-
 - Often focal
- Yolk sac tumour -/+
- Serous ovarian carcinoma +/-
- Adenocarcinomas
 - Various types -(+)



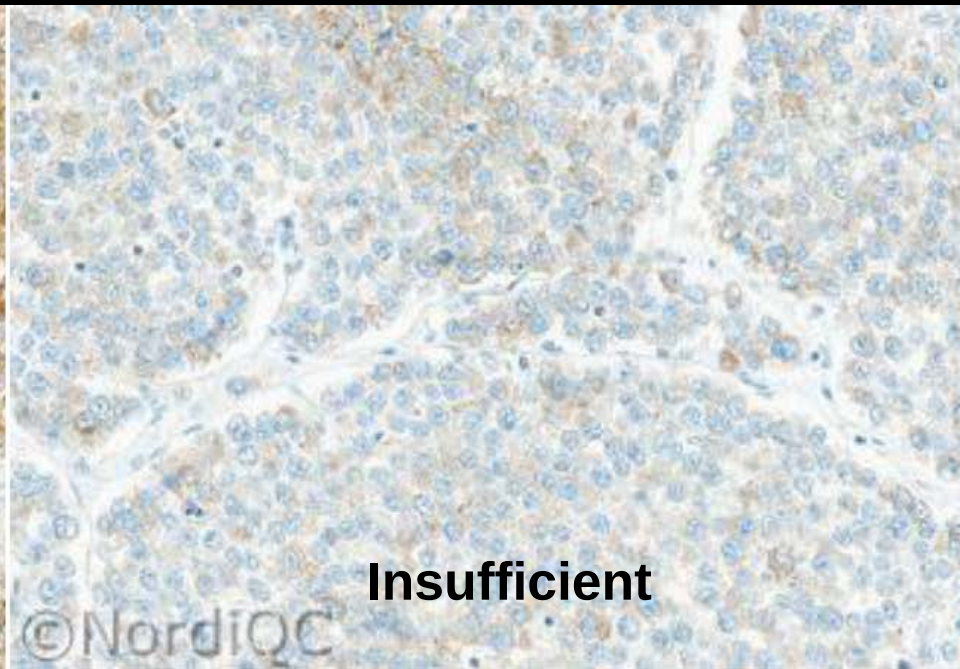
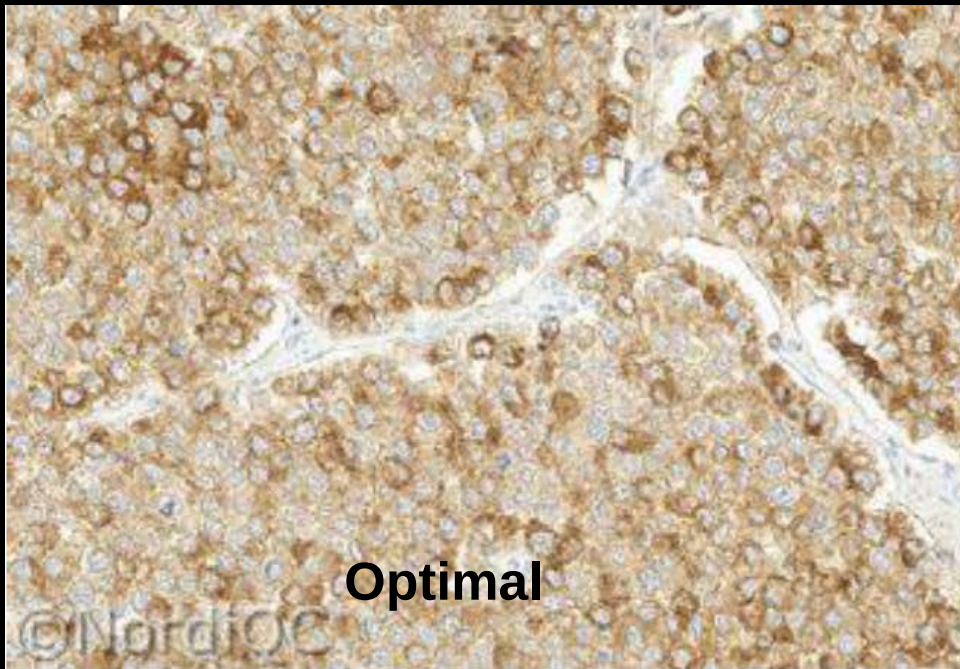
Embryonal carcinoma

Placental alkaline phosphatase (PLAP)

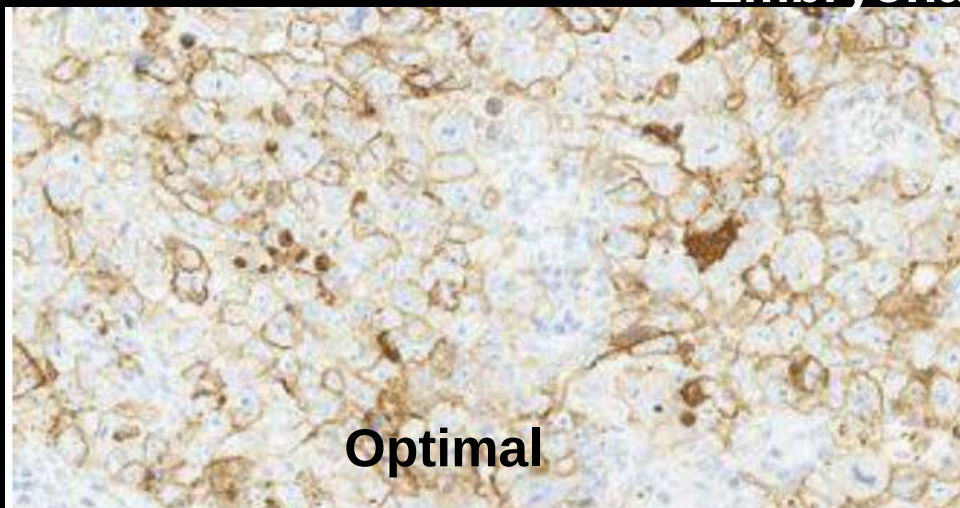


PLAP – NordiQC run 35

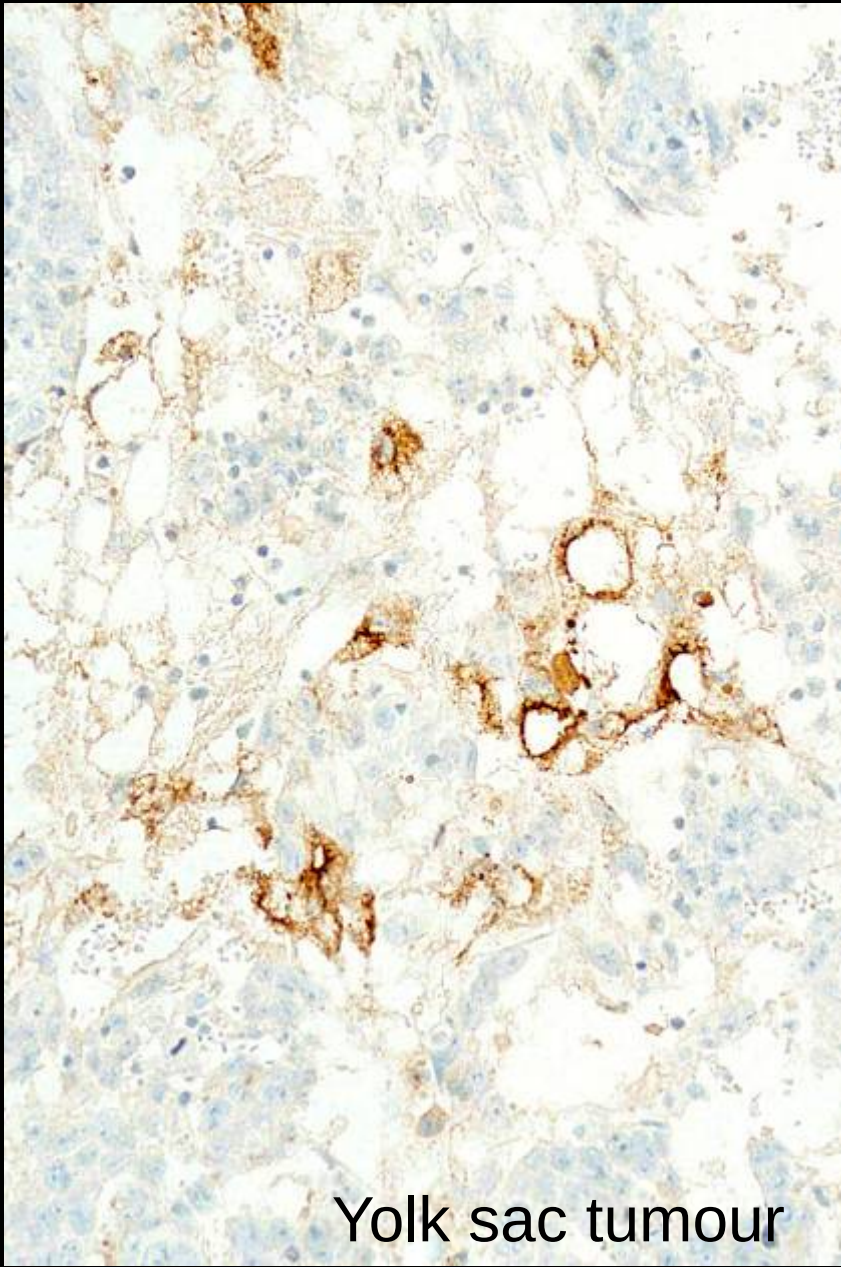
Seminoma



Embryonal carcinoma



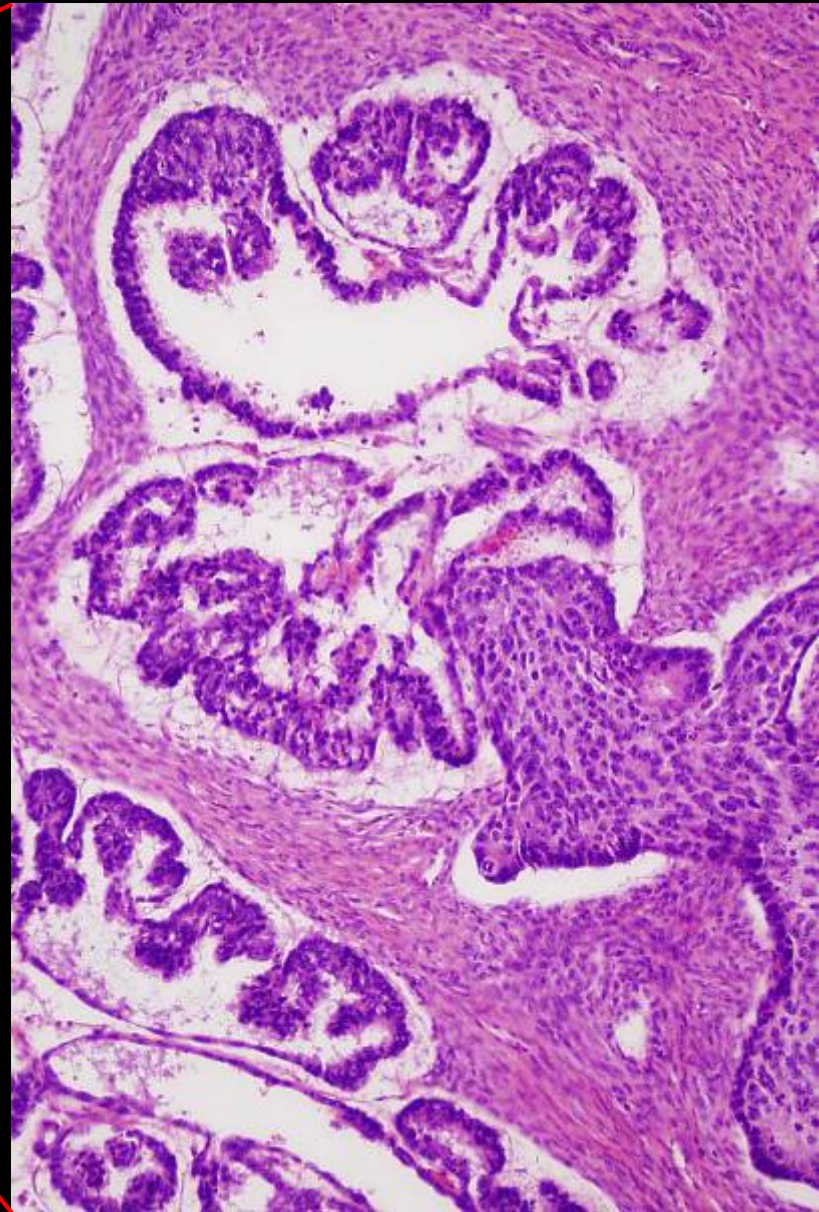
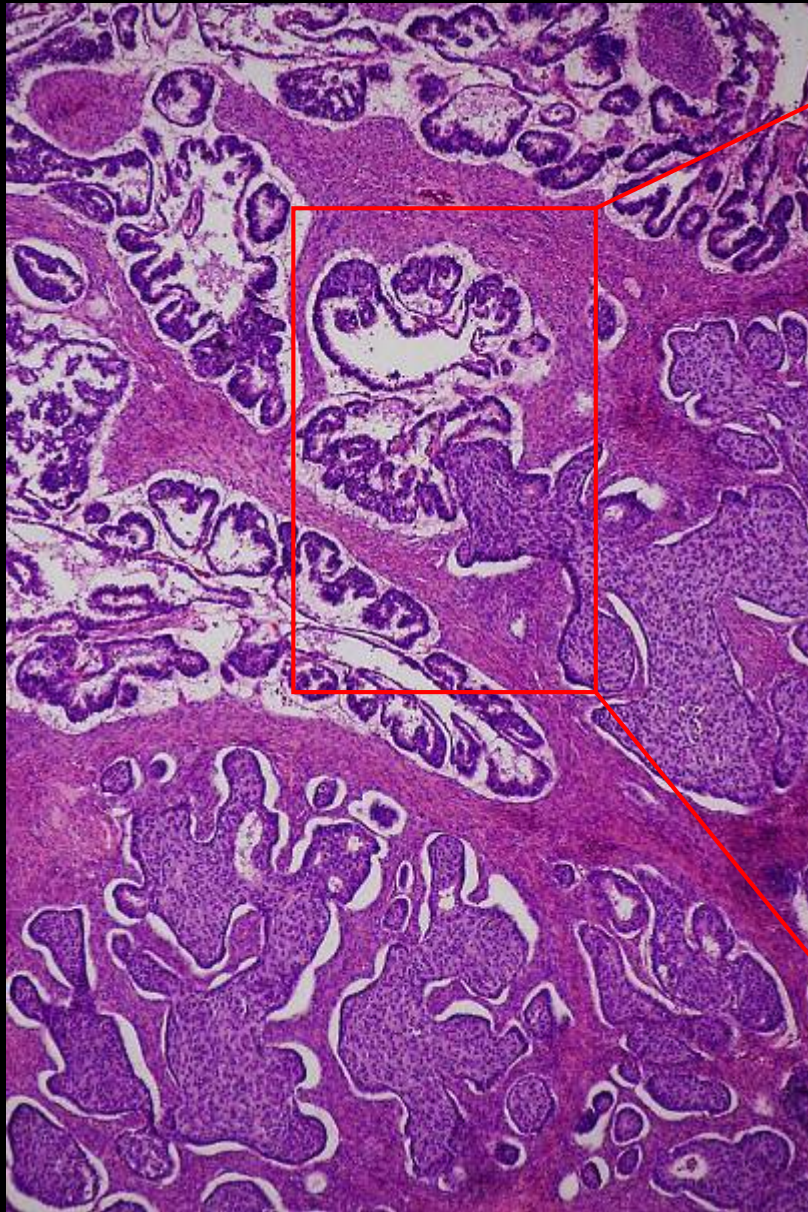
Alpha-fetoprotein (AFP)



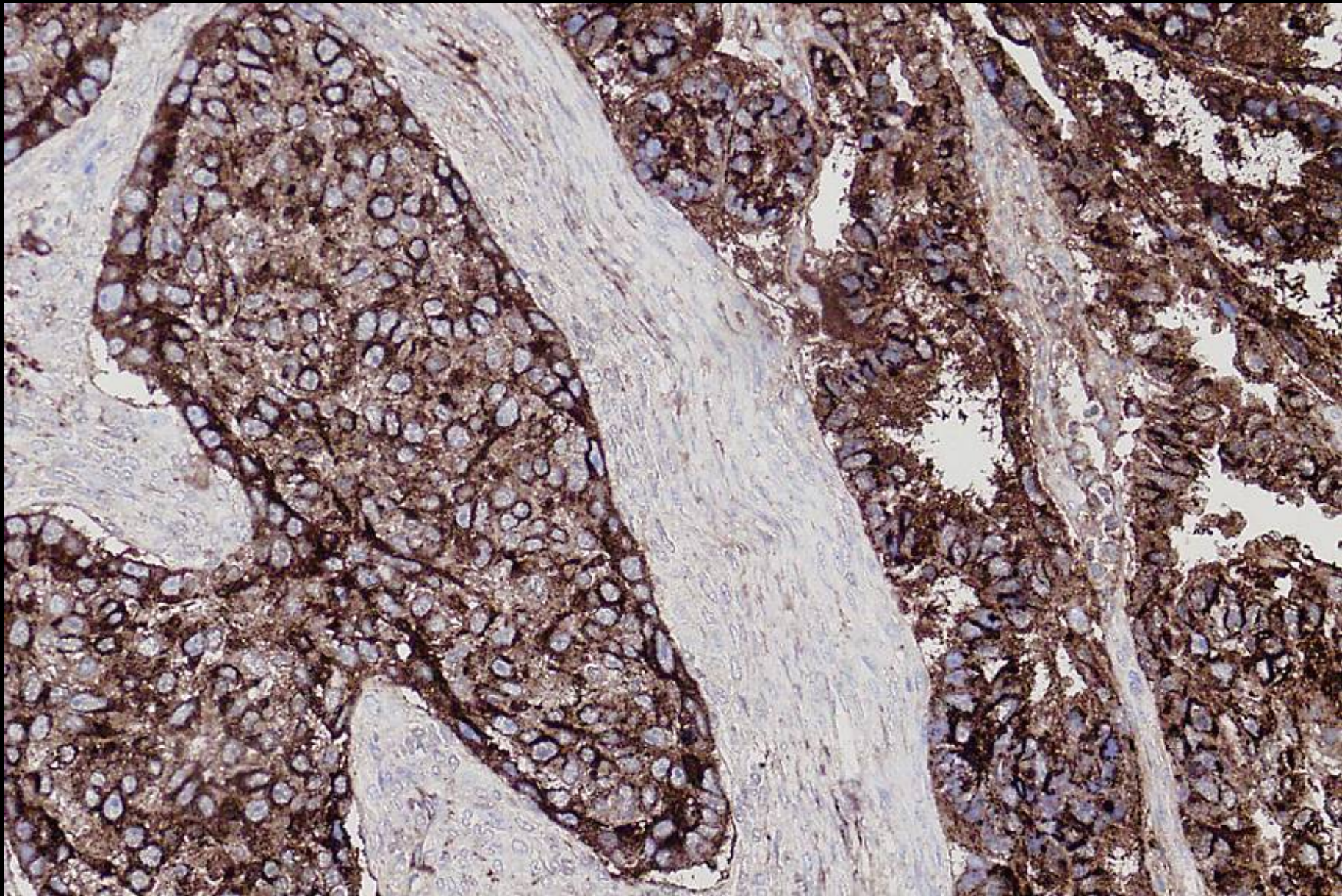
- Yolk sac tumour +/—
- Embryonal carc. —/+
- Choriocarcinoma —
- Seminoma —

- Hepatoblastoma +
- Hepatocellular carc. —/+
- Other carcinomas —(+)

Yolk sac tumour

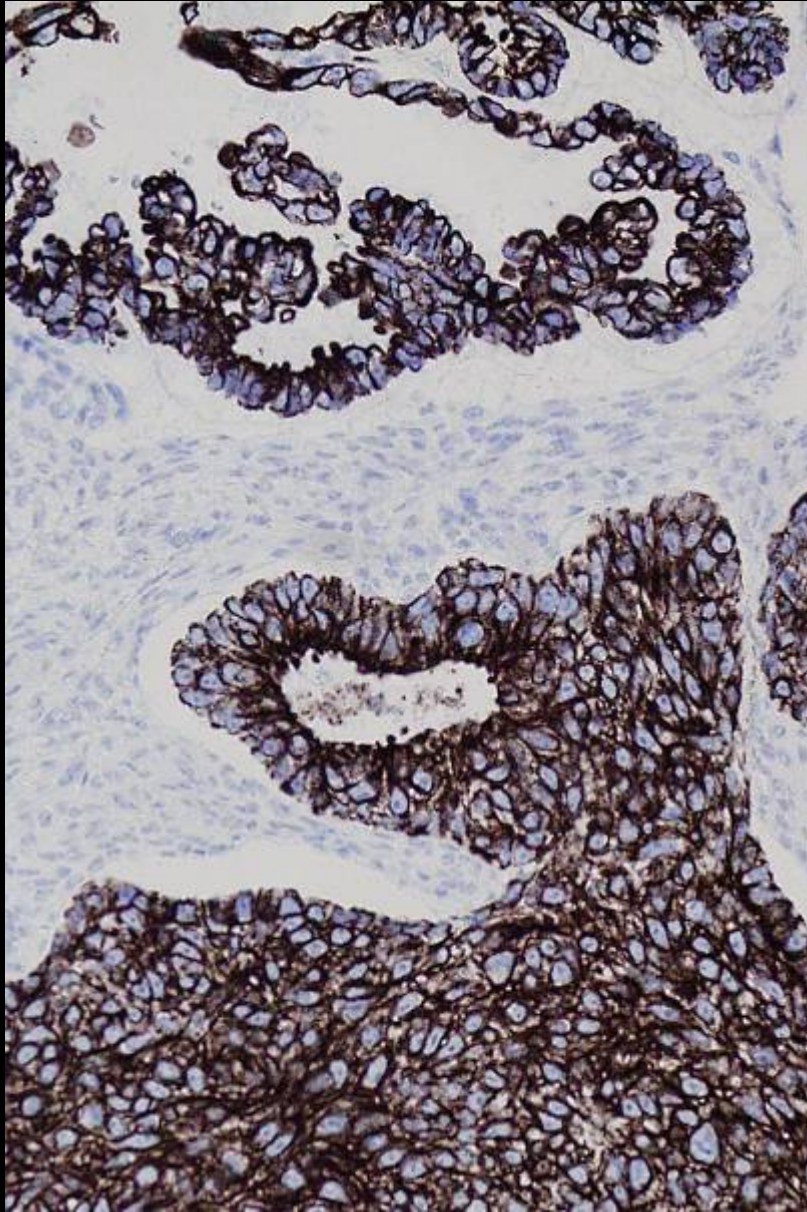


Yolk sac tumour

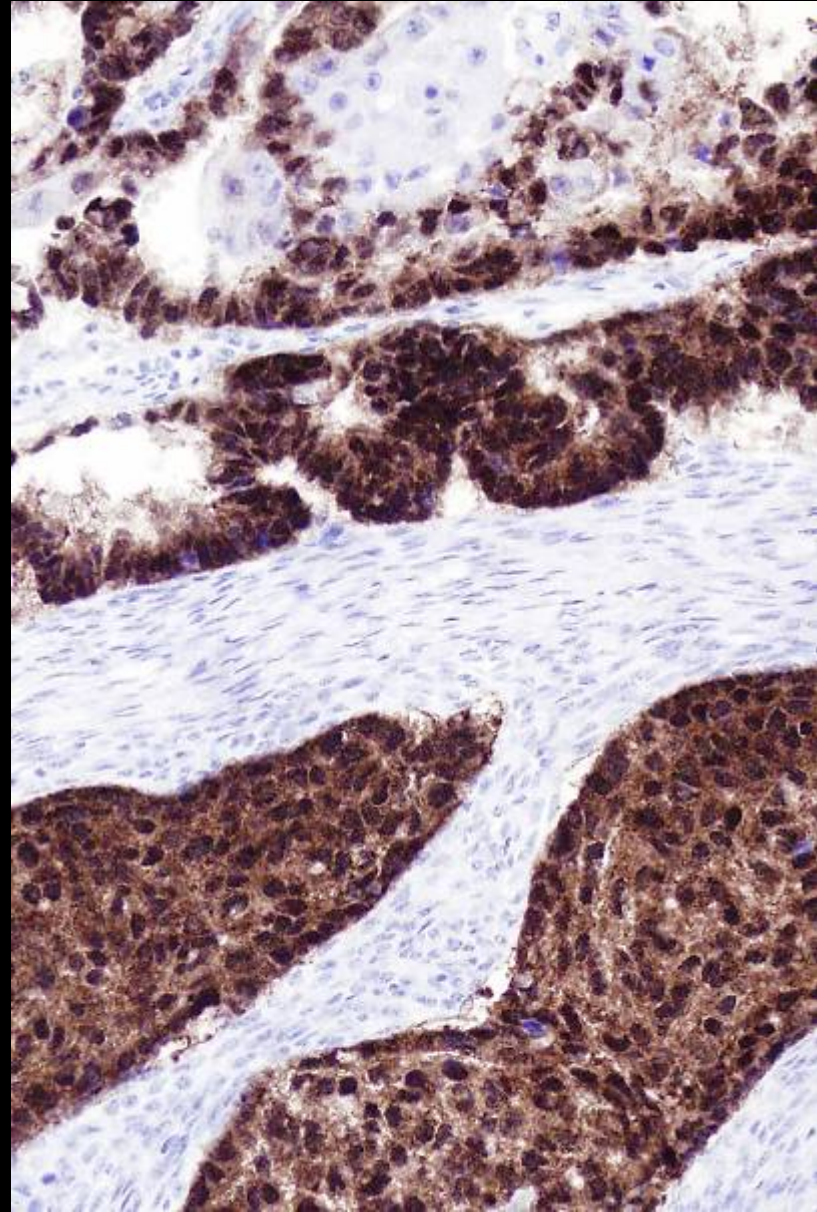


AFP

Yolk sac tumour



CK19



CDX2

International Symposium on Immunohistochemistry

January 4th - 7th, 2018

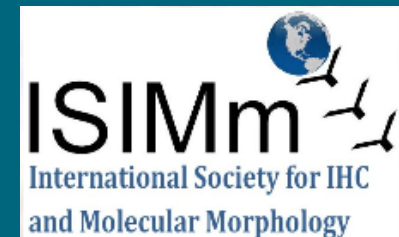
Hosted by Dept. of Histopathology, Tata Medical Center, Kolkata, India

In collaboration with NordiQC, Alborg, Denmark and ISIMM, California, USA



Diagnostic IHC of endocrine, renal, prostate and germ cell tumor pathology

**Thank you for
your attention**



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