Digestive tract carcinogenic toxicity in rodent models

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Preneoplastic lesions of the colon

Aberrant crypt foci (ACF)

β-Catenin accumulated crypts (BCAC)

Mucin depleted foci (MDF)

Tumors of the colon

Epithelial tumors

Polyps **(a) Hyperplastic** **(b) Adenoma**

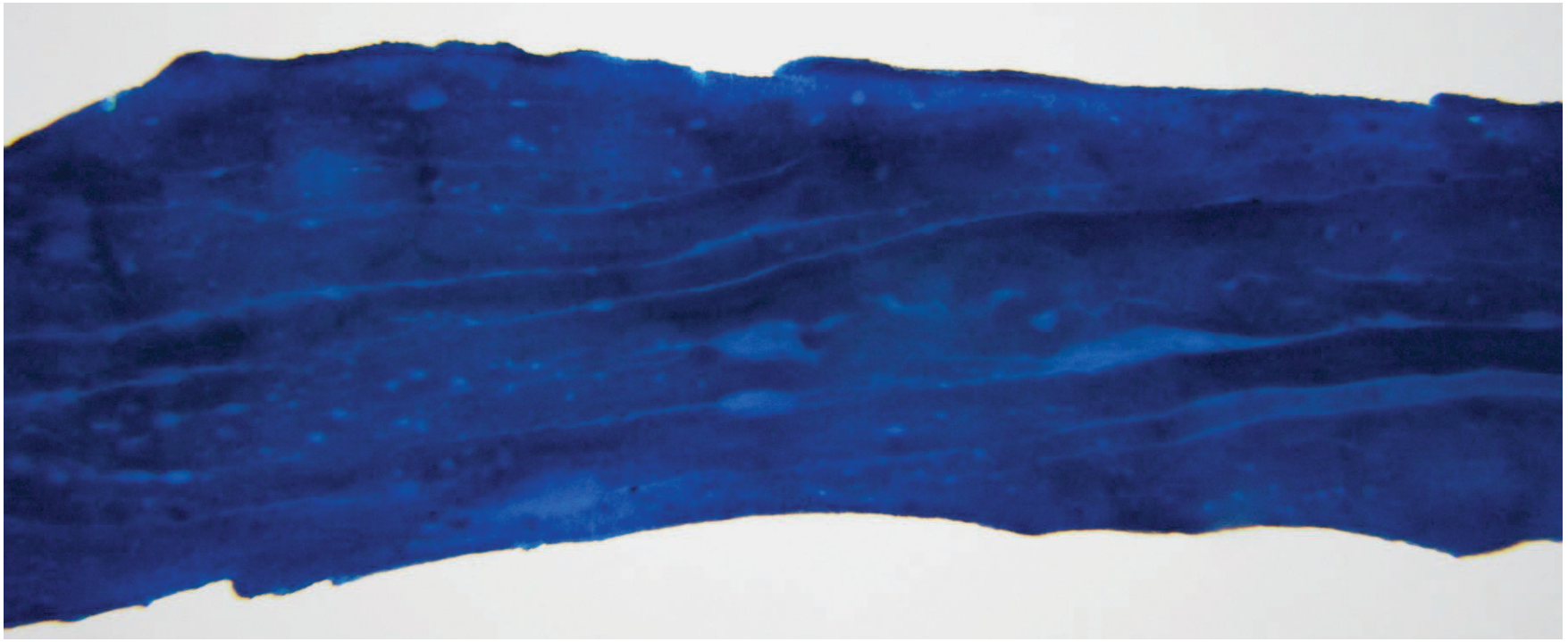
Carcinoma *in situ* (CIS)

Adenocarcinoma

Non-epithelial tumors

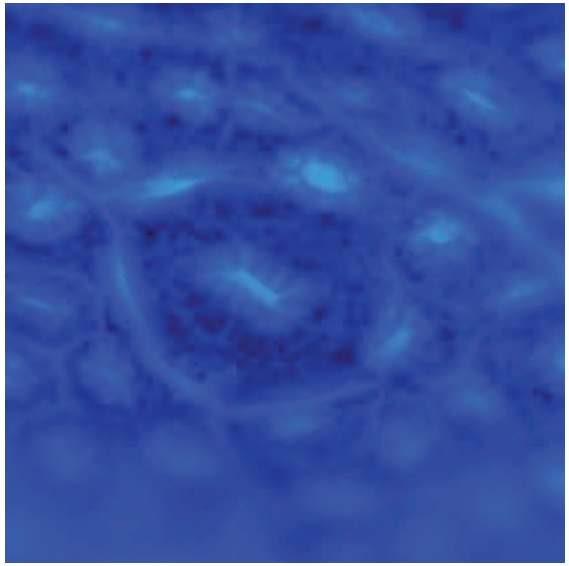
Sarcoma

Rat colonic mucosa

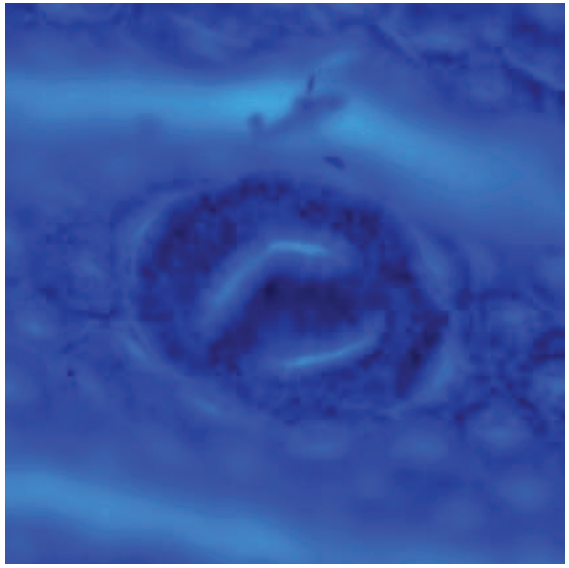


Methylene blue staining

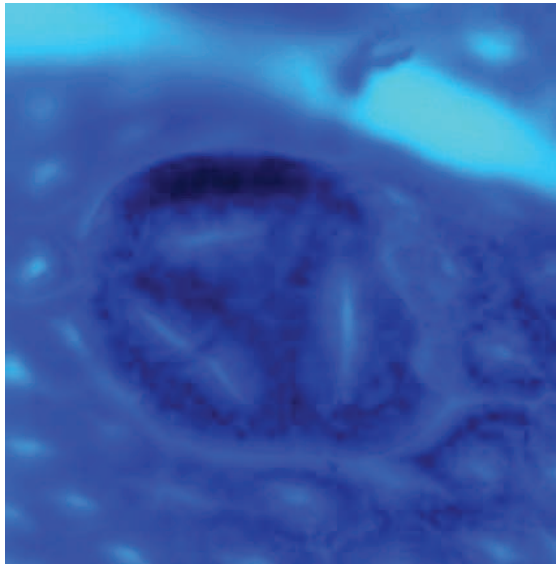
ACF containing more than two aberrant crypts



1 aberrant crypt



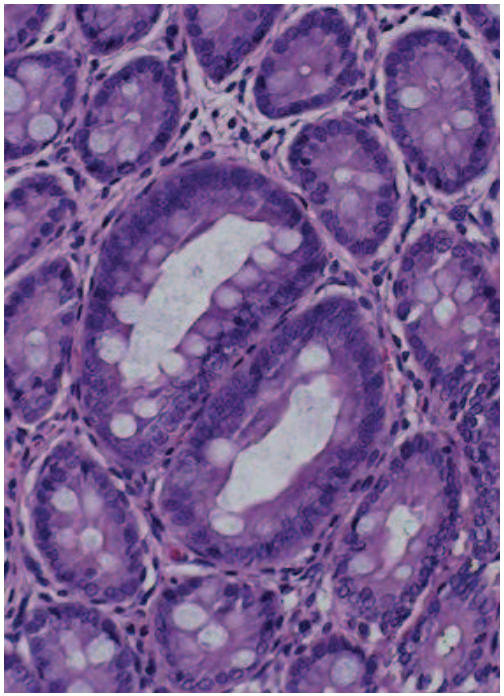
2 aberrant crypts



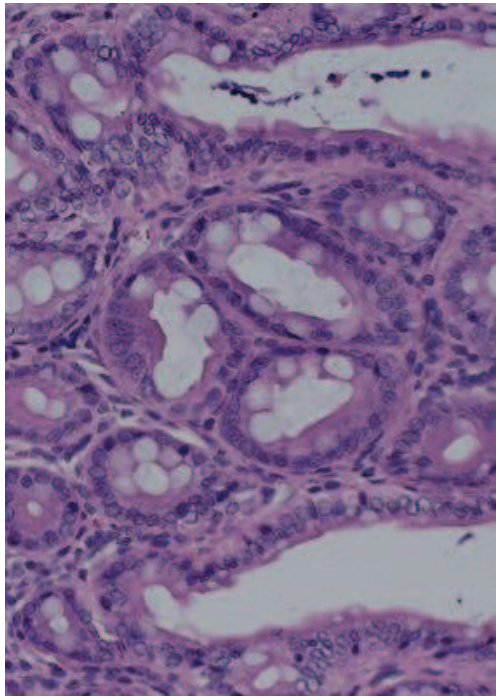
4 aberrant crypts

Methyelene blue staining, rat

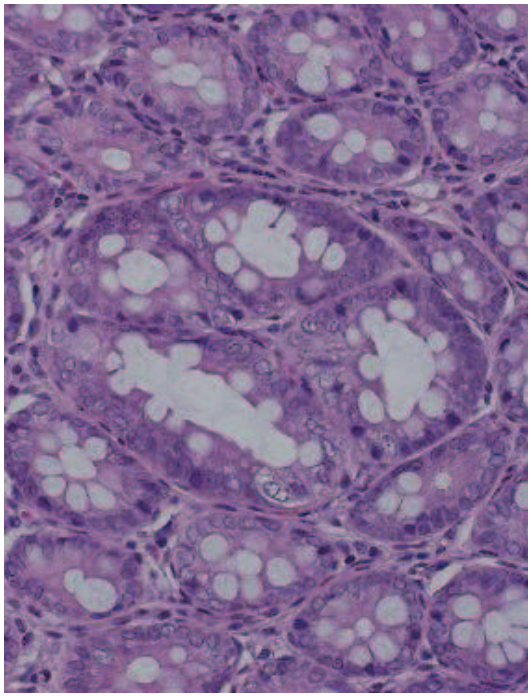
ACF containing more than two aberrant crypts



2 aberrant crypts



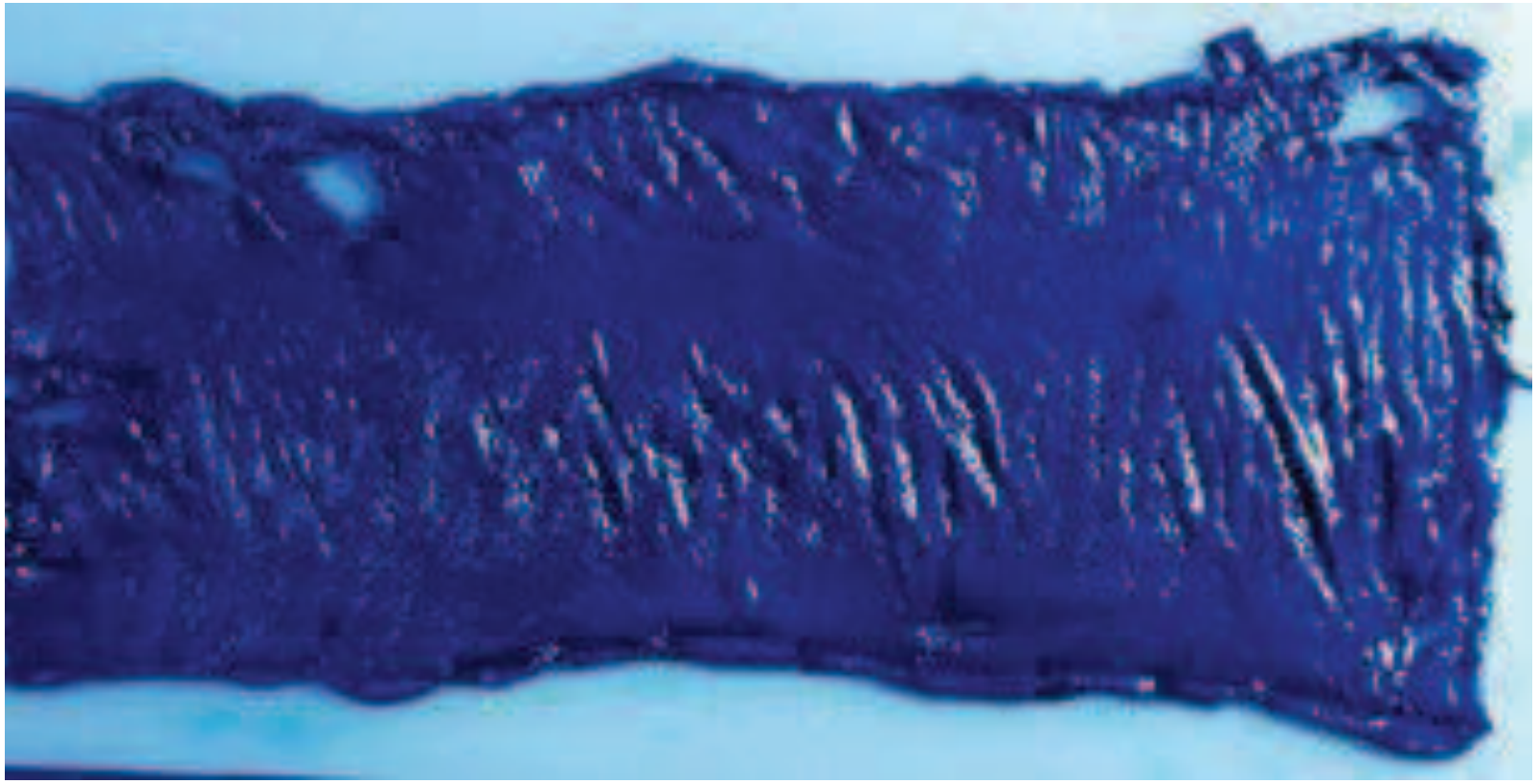
3 aberrant crypts



4 aberrant crypts

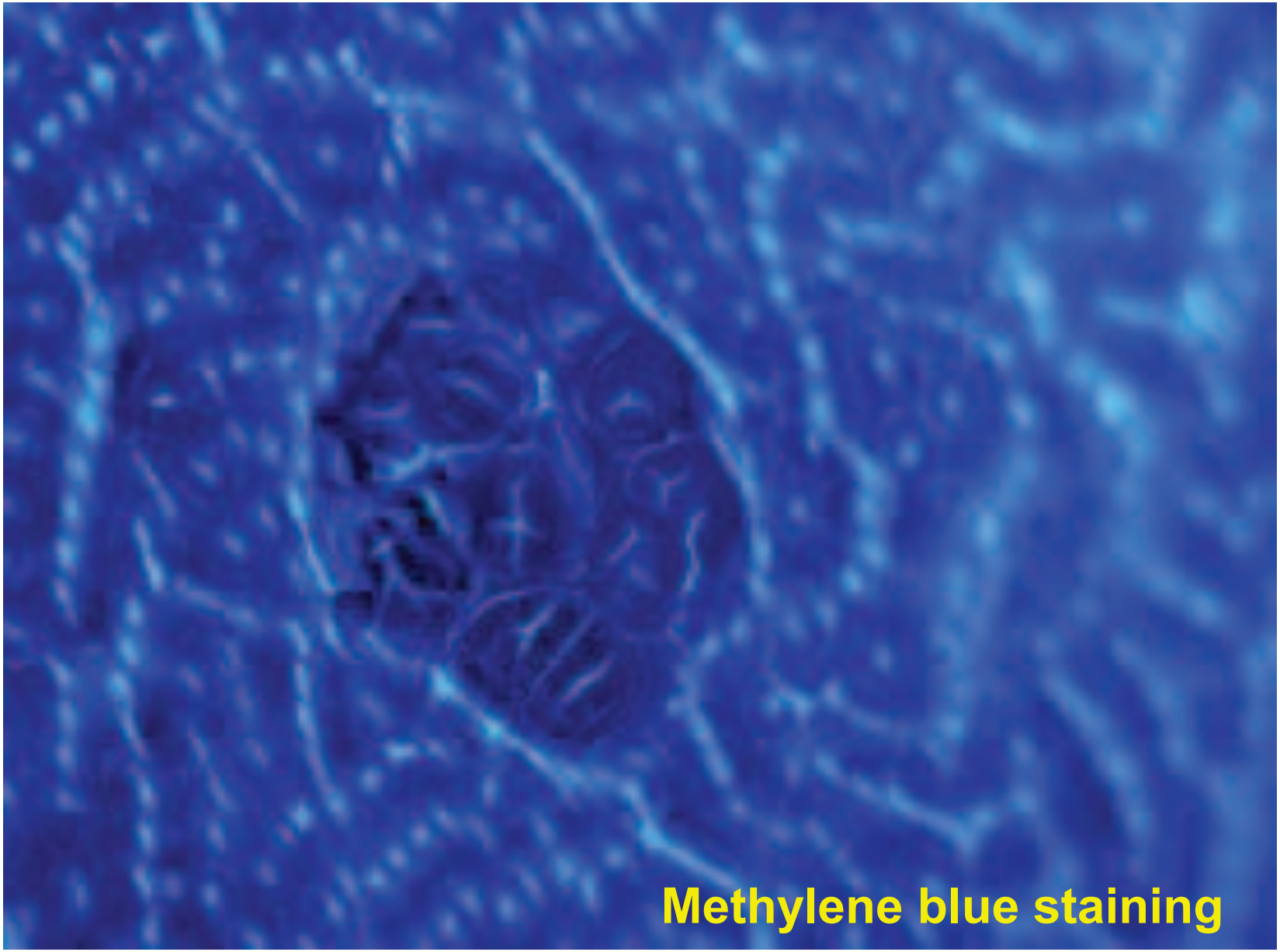
HE staining, rat

Human colon mucosa

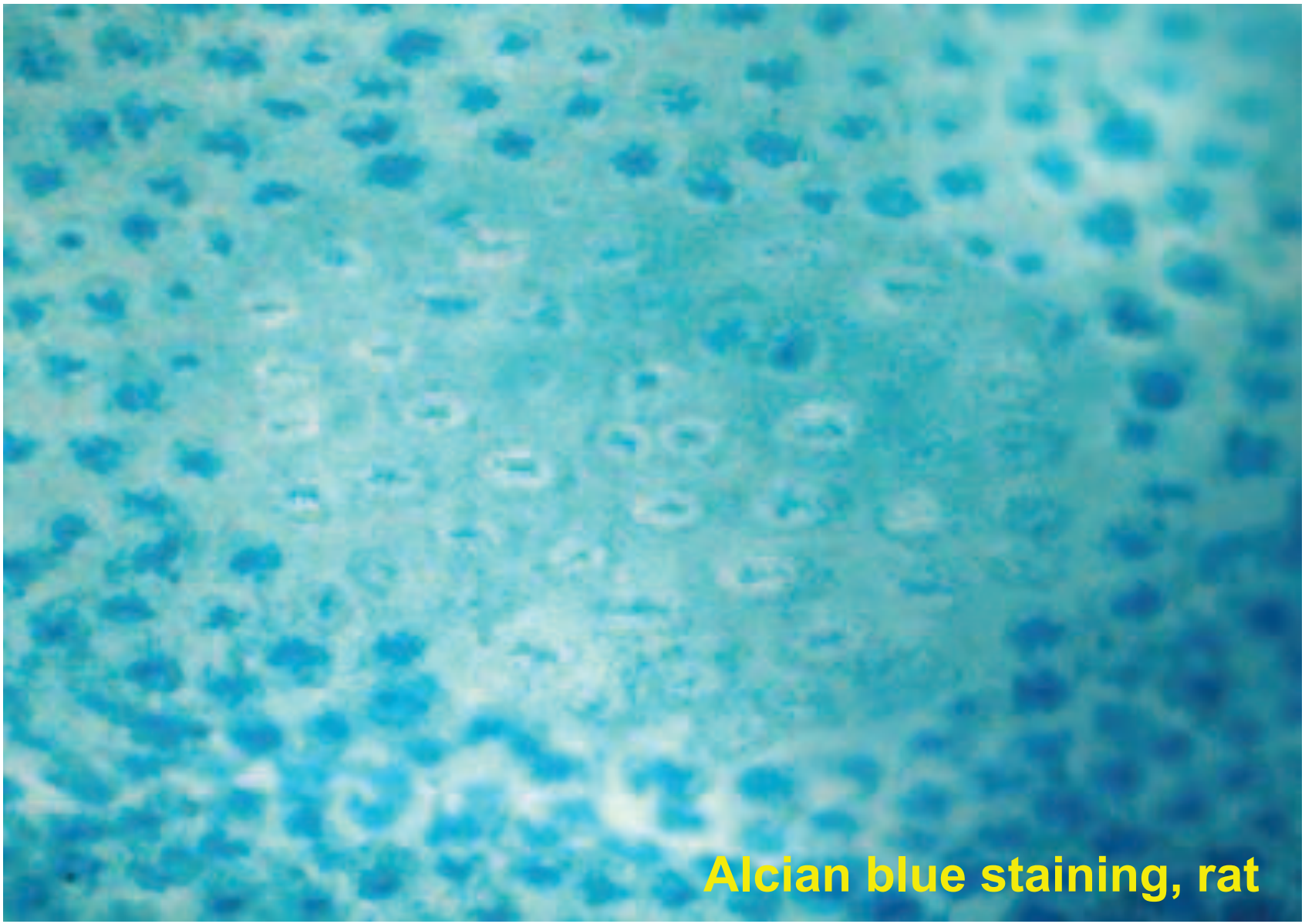


Methylene blue staining

Aberrant crypt foci (ACF) in human colon



Mucin depleted foci (MDF)



Mutation status of β-catenin gene in BCAC/ACF/MDF

	Frequency	Mutation	
ACF	7/30 (23%)	^{32}A→G (Asp → Gly)	
		^{34}G→T (Gly → Val)	
		^{36}C→T (His → Tyr)	
BCAC	10/15 (67%)	^{28}A→T (Gln → His)	^{34}G→A (Gly → Glu)
		^{29}C→G (Ser → Cys)	^{34}G→T (Gly → Stop)
		^{30}T→C (Tyr → His)	^{41}A→T (Thr → Ile)
		^{32}G→A (Asp → Asn)	
MDF	7/28 (25%)	^{32}G→A (Asp → Asn)	
		^{33}C→T (Ser → Phe)	
		^{37}C→T (Ser → Phe)	
		^{41}C→T (Thr → Ile)	

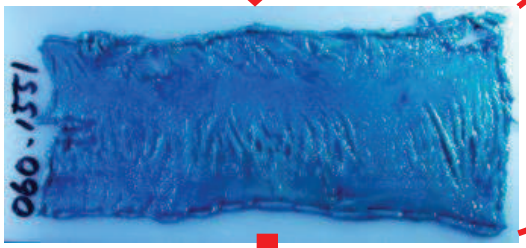
Ochiai et al. Am J Pathol 2003
Suzui et al. J Toxicol Sci 2014 (review)

Yamada et al. Cancer Res 2000
Suzui et al. J Toxicol Sci 2014 (review)

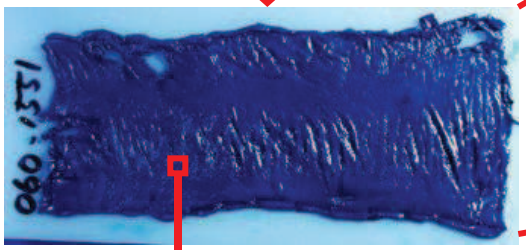
Femia et al. Int J Cancer 2005
Suzui et al. J Toxicol Sci 2014 (review)

Procedure of detecting ACF or MDF

Alcian blue stain

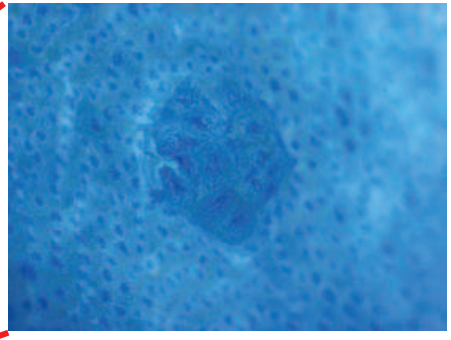


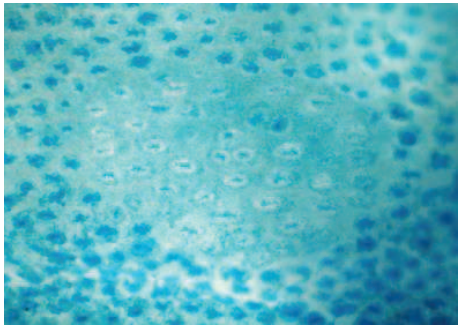
Methylene blue stain

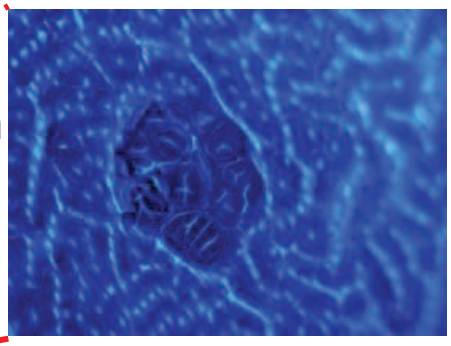


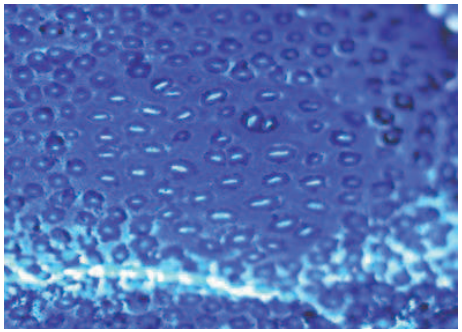
ACF

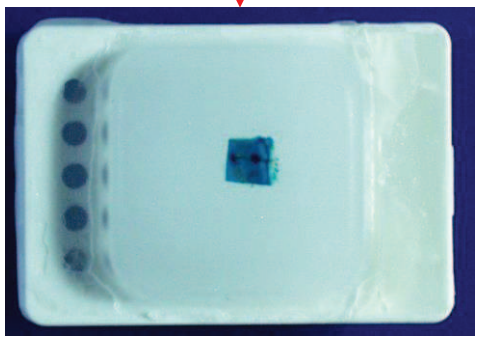
MDF

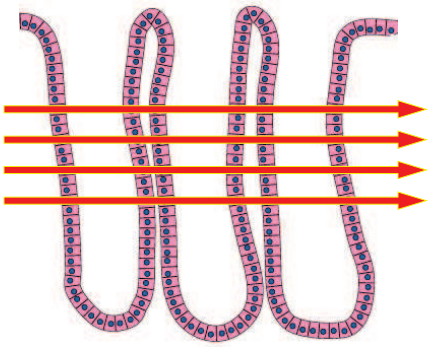


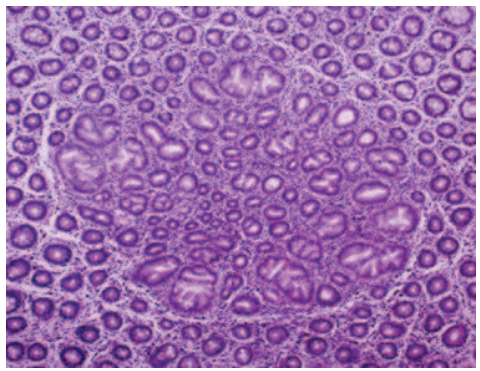






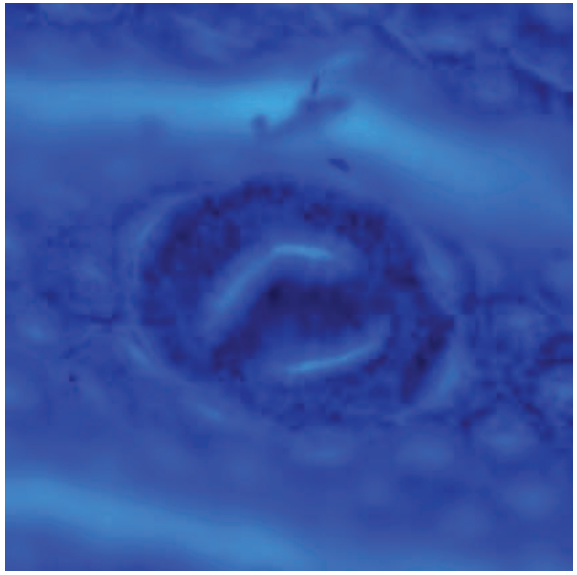




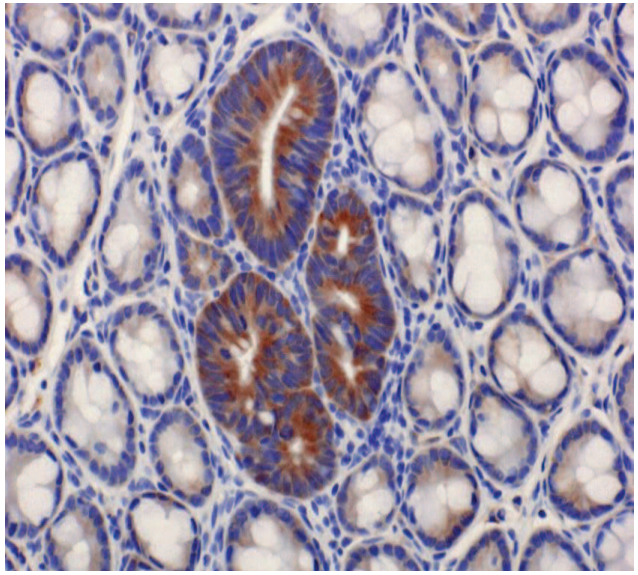


HE stain

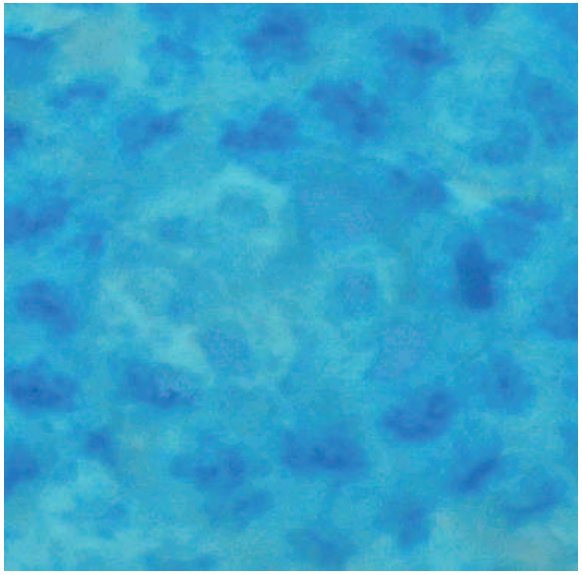
ACF



BCAC



MDF



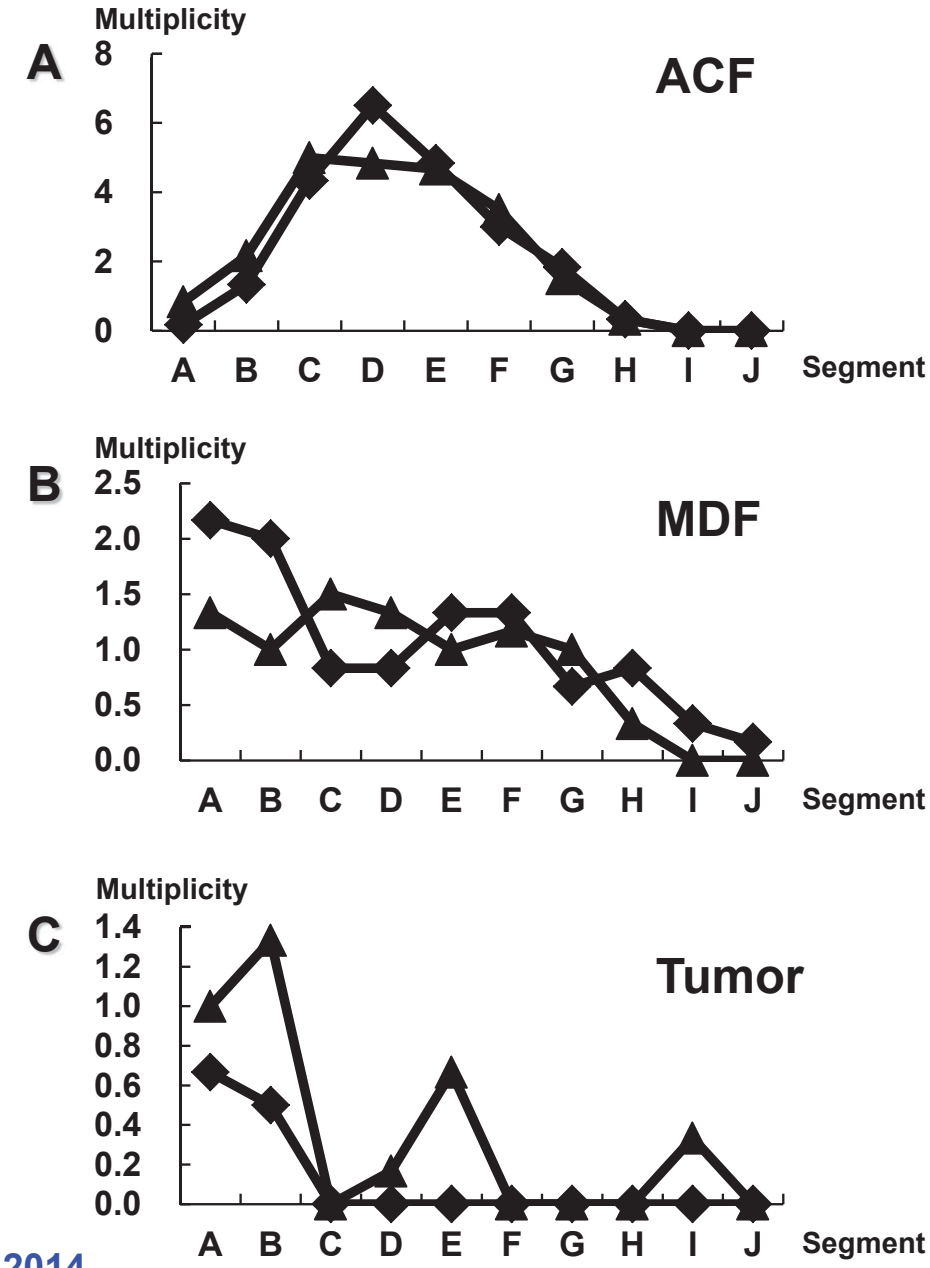
ACF: aberrant crypt foci
BCAC: β-catenin accumulated crypts
MDF: mucin depleted foci

Summary of preneoplastic lesions in the animal model

	Staining method	Macroscopic/ microscopic findings
ACF	Methylene blue (0.2%)	Increased cryptical size Thicker epithelial lining Increased pericryptical zone Hypercellularity of cells with or without dysplasia
BCAC	IHC	Accumulation of cytoplasmic/nuclear β-catenin protein
MDF	High-iron diamine Alcian blue (1%, pH2.5)	Foci of crypts with scarce or absent mucin, when colon tissue was stained with HID-AB

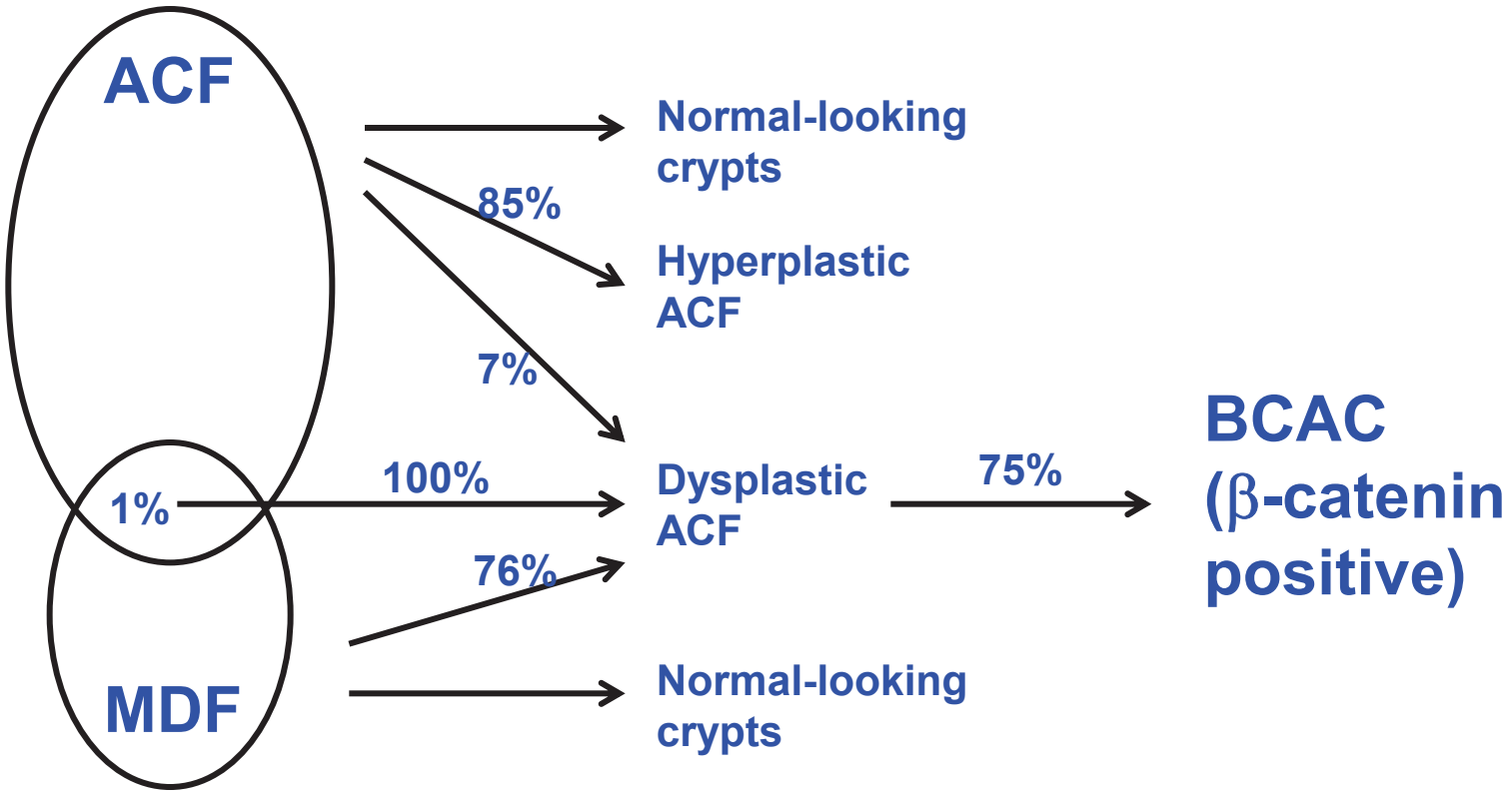
Suzui et al. J Toxicol Pathol 2014

Distribution of ACF/MDF/tumor



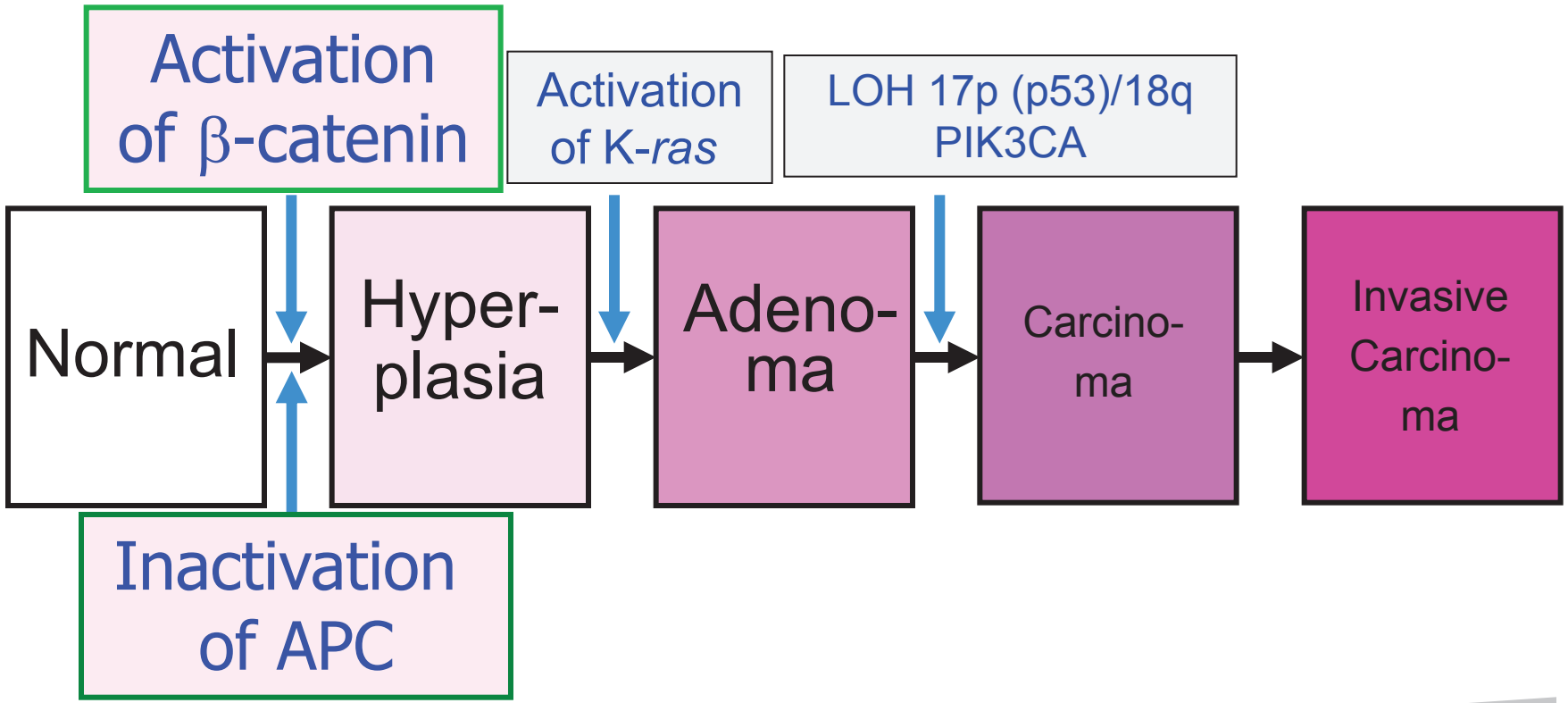
Suzui et al. J Toxicol Pathol 2014

Possible relationship of preneoplastic lesions

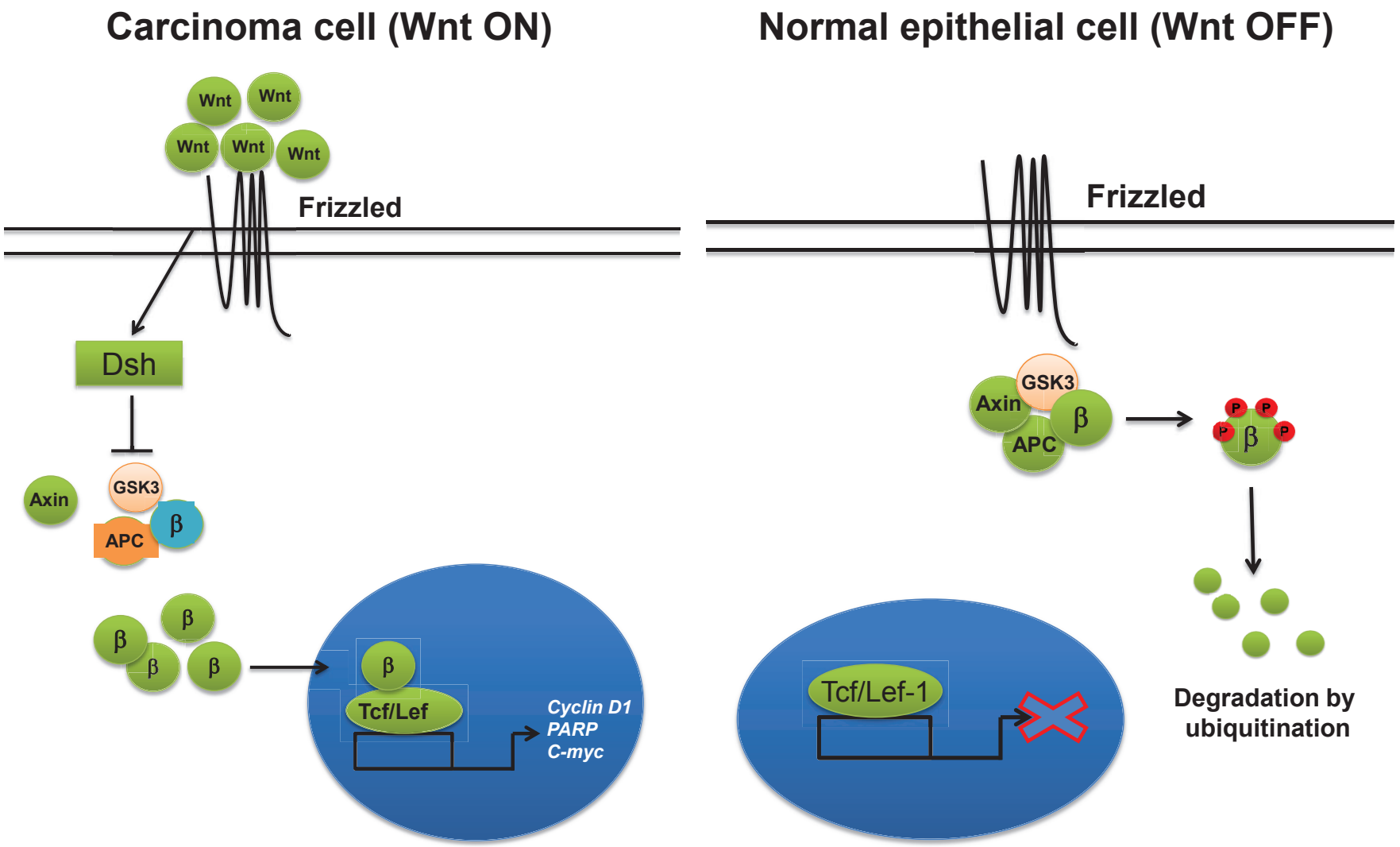


Yoshimi, Suzui et al. Cancer Sci 2004

Multistage model of colon carcinogenesis

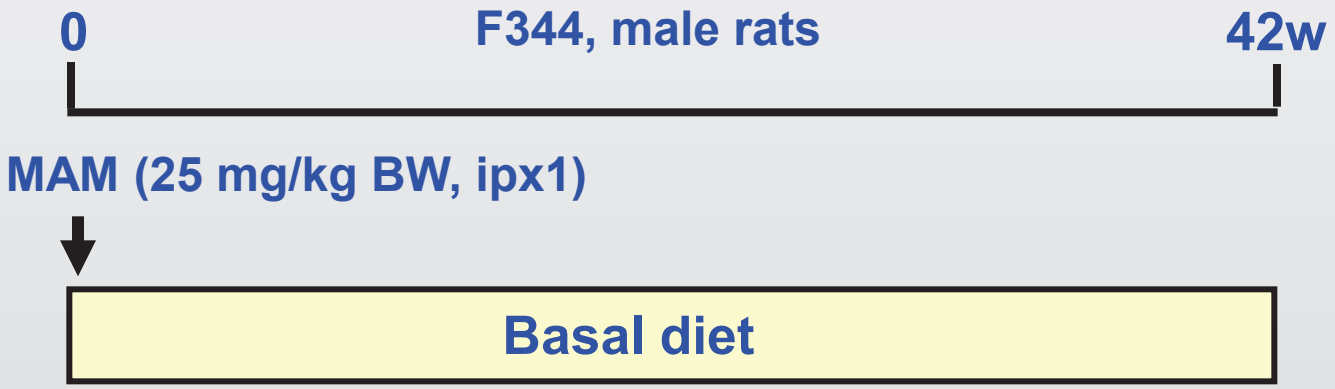


β-Catenin-TCF signaling pathway



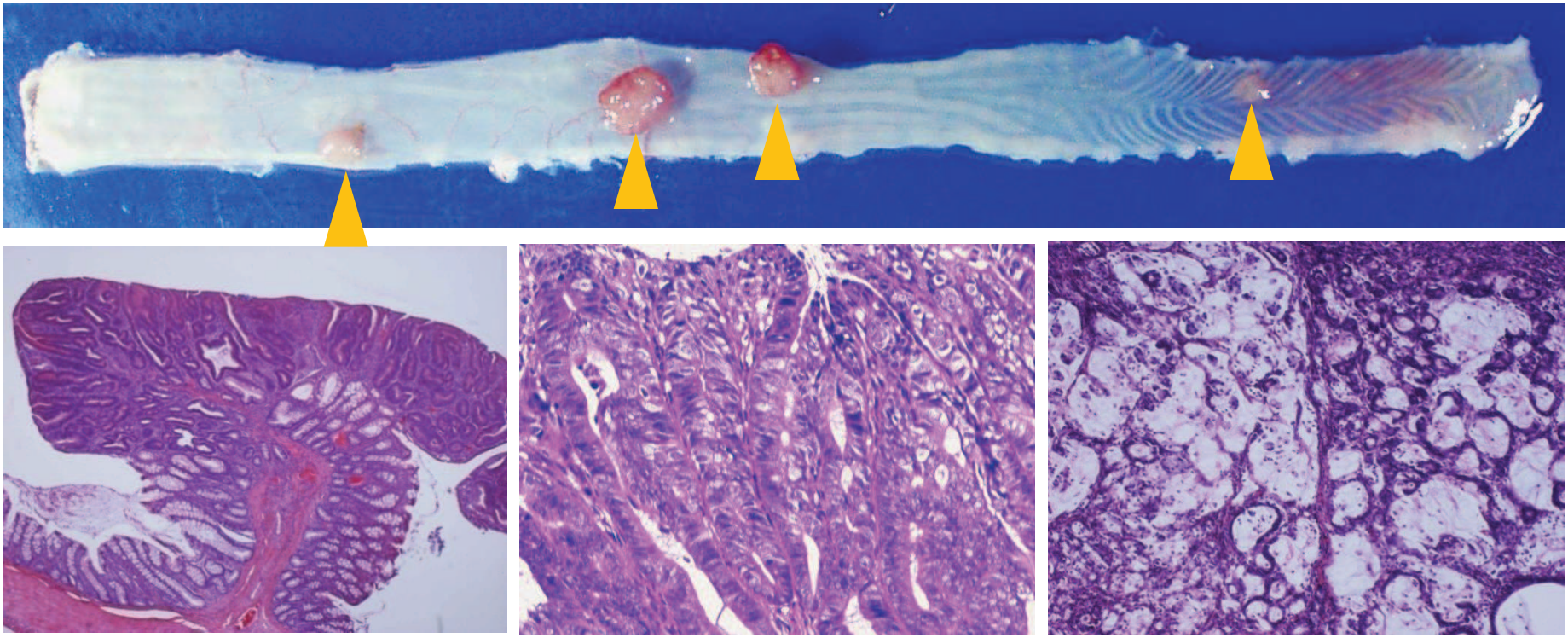
Carcinogenesis model-1

Experimental protocol



MAM: methylazoxymethanol acetate

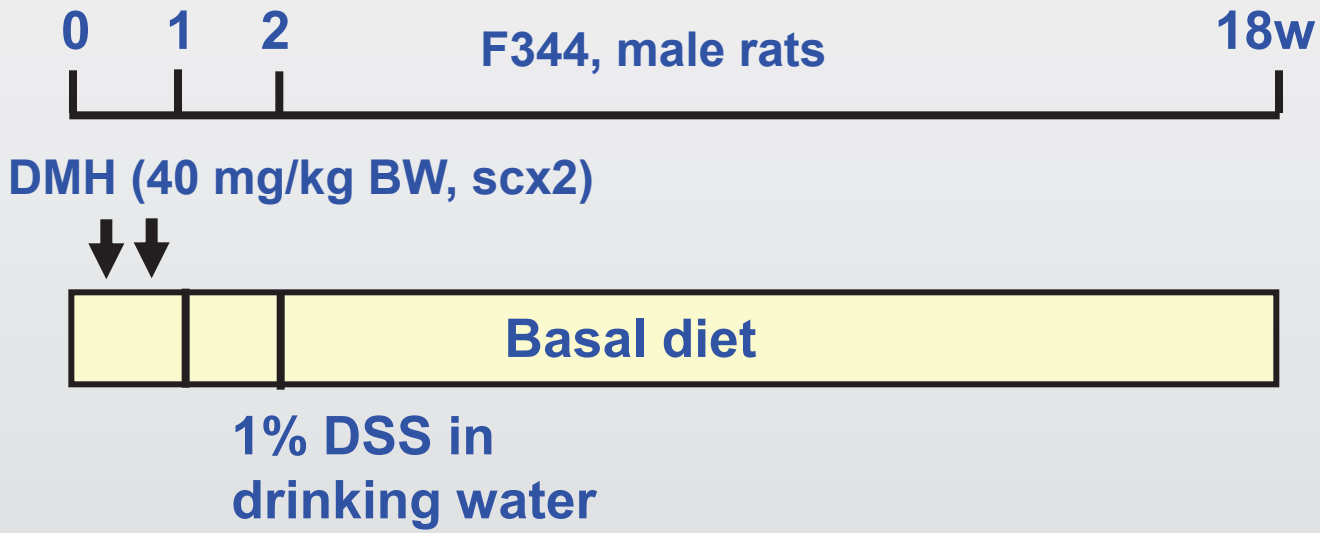
MAM-induced rat colon carcinoma



Suzui et al. Mol Carcinog, Cancer Lett, 1995, 1997, 1999, 2001, 2002

Carcinogenesis model-2

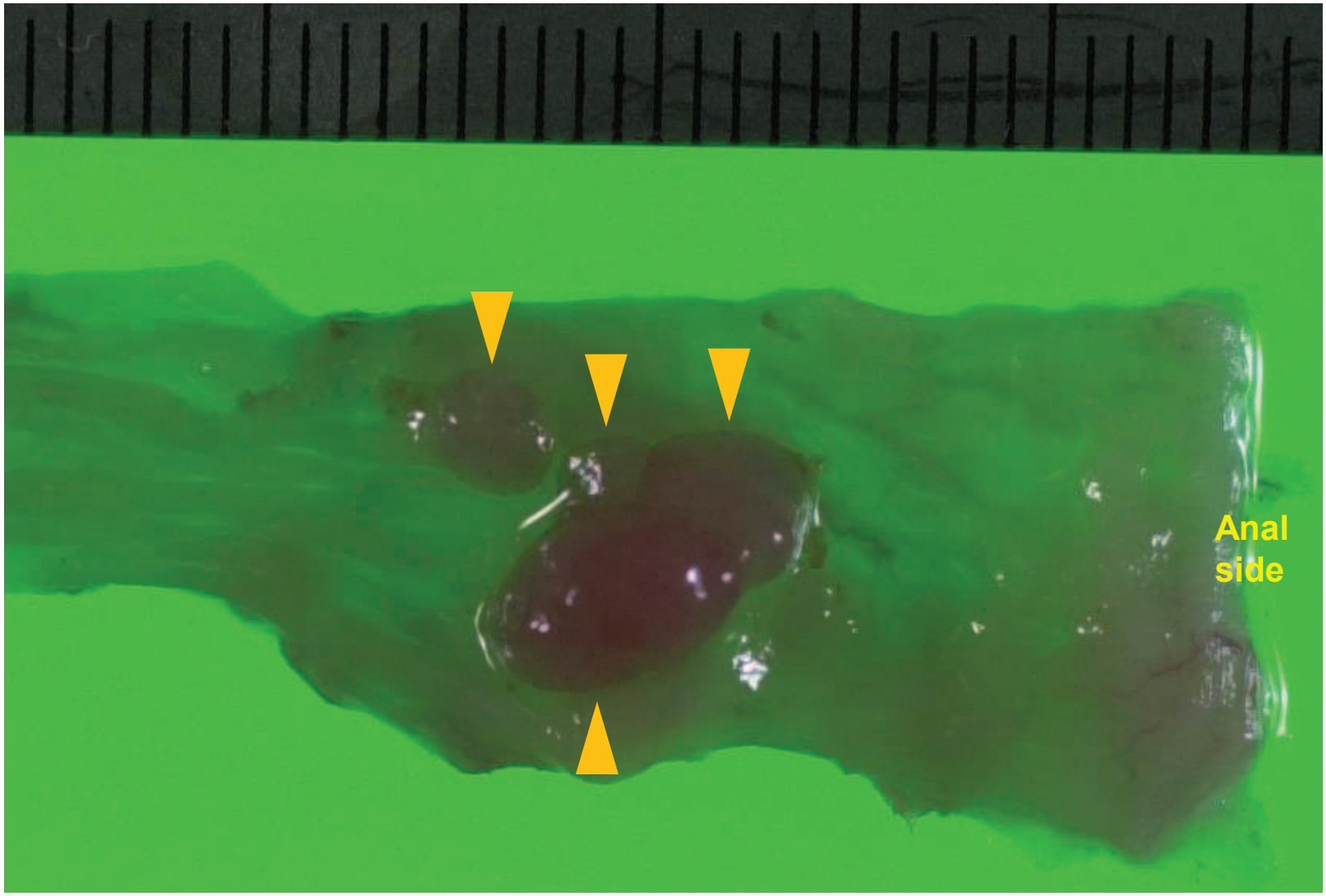
Experimental protocol



DMH: 1,2-dimethylhydrazine

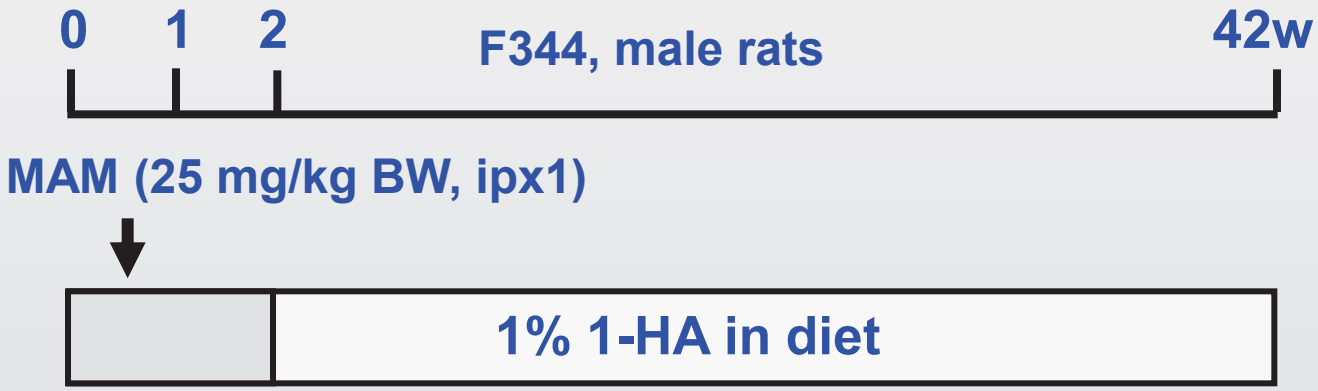
DSS: dextran sodium sulfate

Colon tumors



Carcinogenesis model-3

Experimental protocol

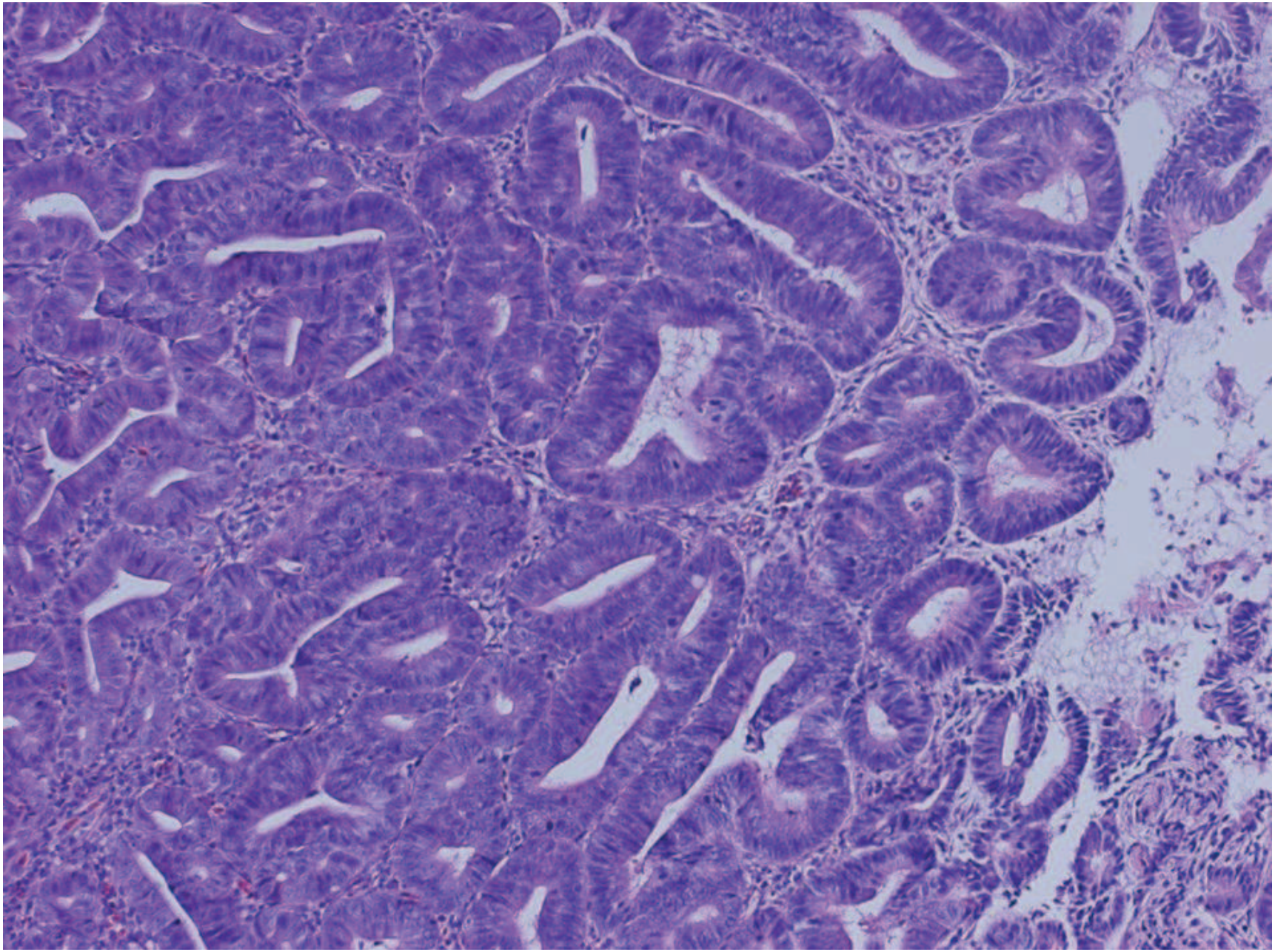


MAM: methylazoxymethanol acetate

1-HA: 1-hydroxyanthraquinone

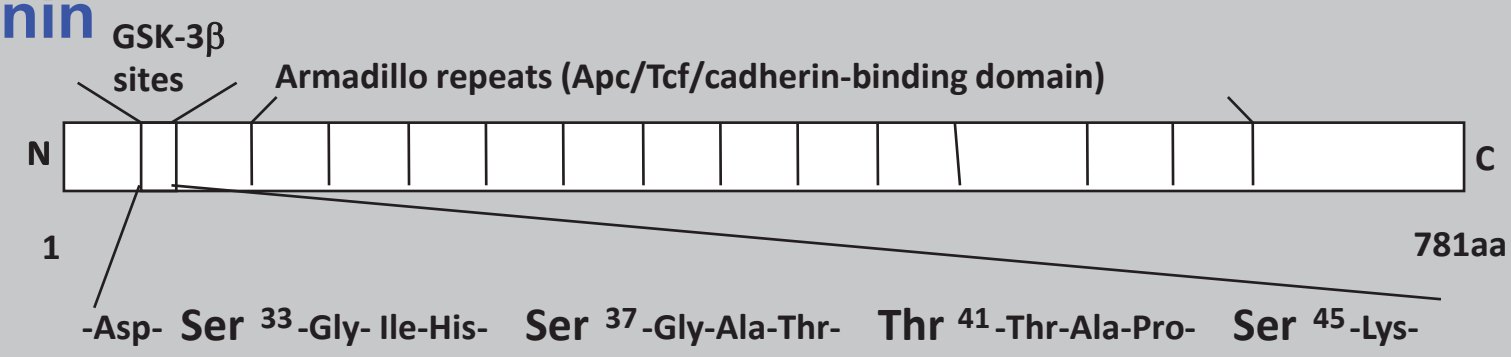
Suzui et al. Mol Carcinog 1999, 2001

Adenocarcinoma

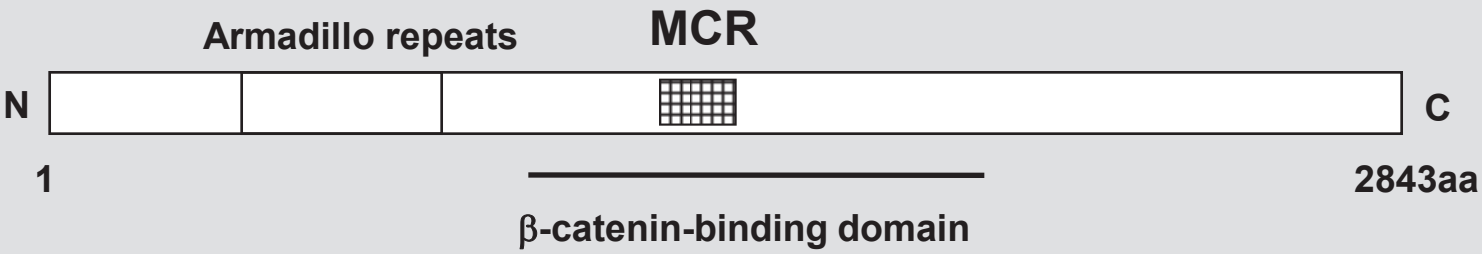


β-CateninAPC mutations

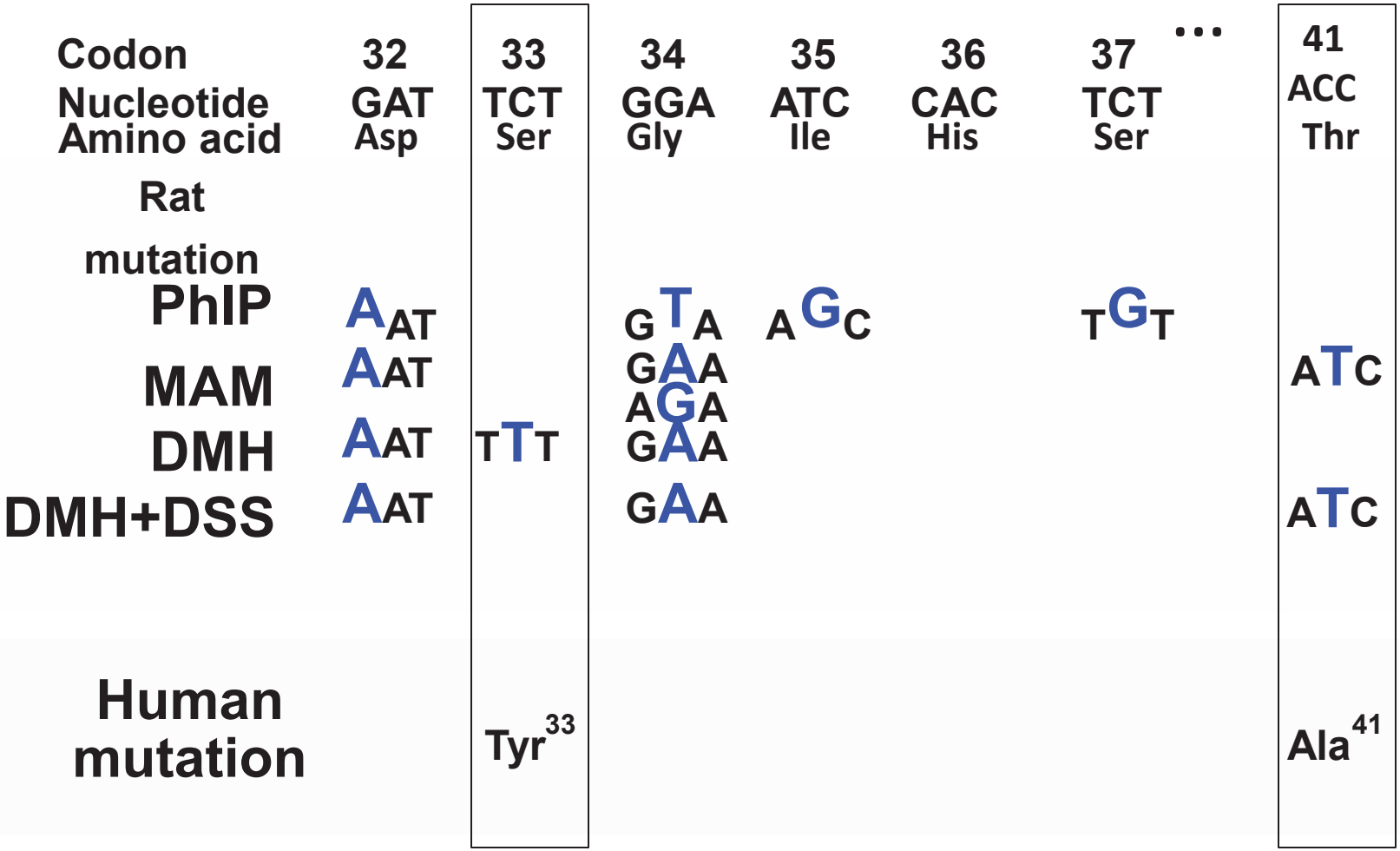
β-Catenin



APC



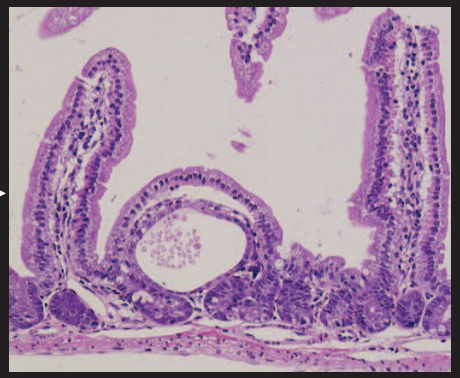
β-Catenin mutation spectrum in the rat colon tumor



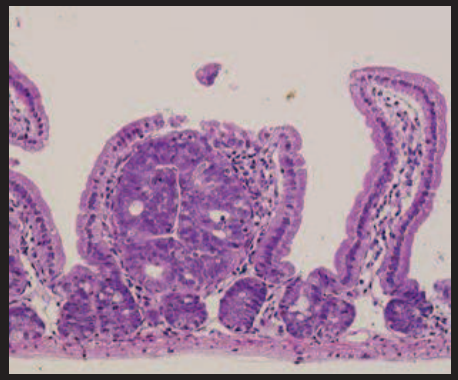
Suzui et al. Mol Carcinog 1999, 2001, Dashwood, Suzui et al. Cancer Res 1998
Suzui, Yoshimi. New insights into molecular carcinogenesis 2005
Kinjo, Suzui et al. Anticancer Res & J Exp Clin Cancer Res 2006

Carcinogenesis process of Min mouse

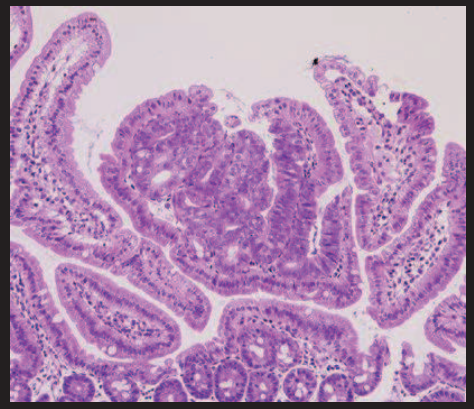
Normal mucosa



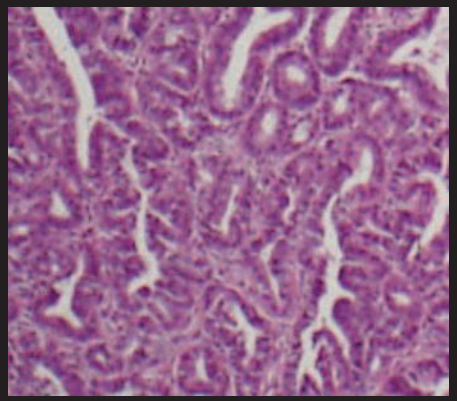
Unicryptic adenoma



Unicryptic adenoma
(advanced)



Microadenoma



Adenoma

Carcinogenesis model-4

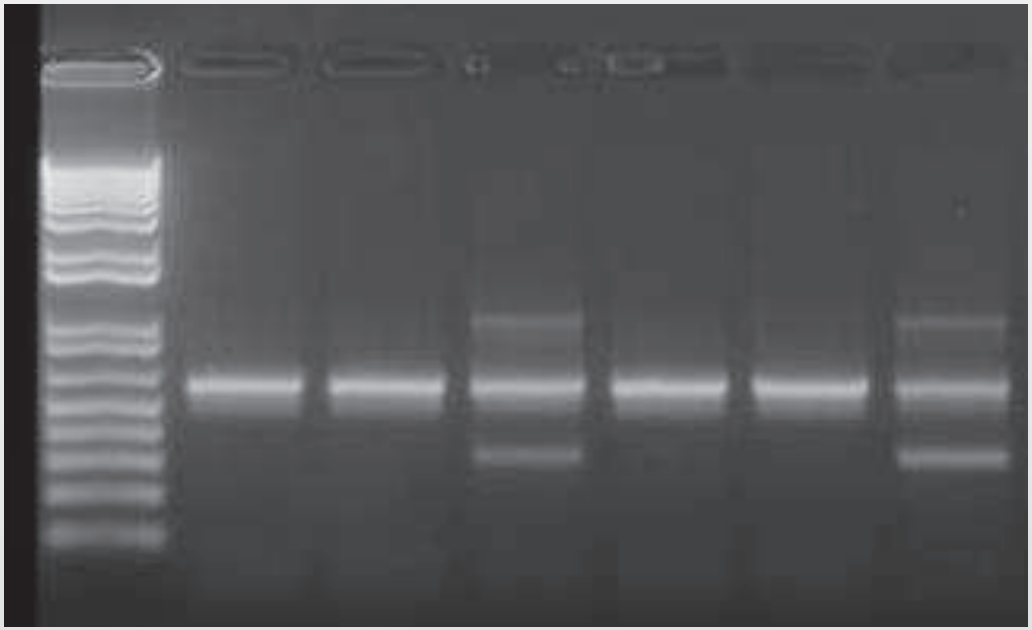
Experimental protocol

AOM: azoxymethane

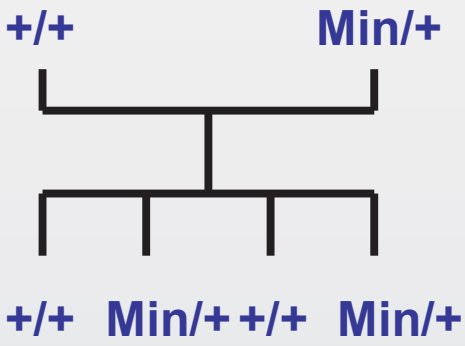
Suzui et al. Cancer Lett 2002

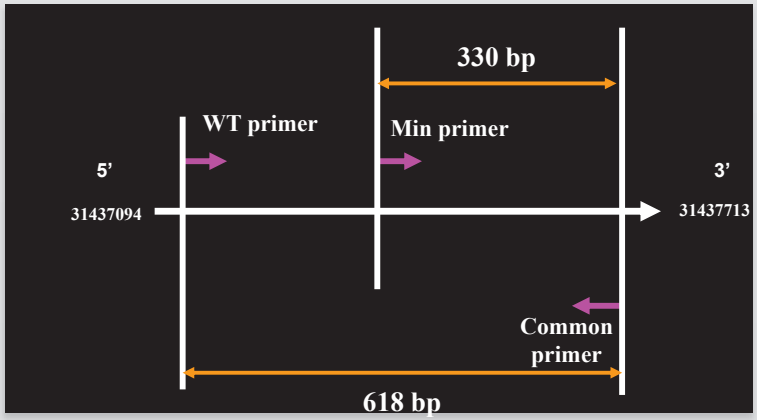
Genotyping of Min mice

+/+ +/+ Min/+ +/+ WT Min



618-bp
330-bp





Incidence of colon tumors in AOM-treated Min mice

Treatment	Incidence			
	Total	Ad	CIS	Adc
AOM	14/15	5/15	5/15	14/15*
None	10/15	4/15	2/15	6/15

	Multiplicity			
	Total	Ad	CIS	Adc
AOM	2.2±1.4*	0.3±0.4	0.3±0.4	1.6±1.0*
None	1.0±0.9	0.4±0.8	0.1±0.3	0.4±0.7

Appendix

Digestive tract carcinogenic toxicity in rodent models

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Normal structure of gastrointestinal (GI) tract

1. Tongue

2. Esophagus

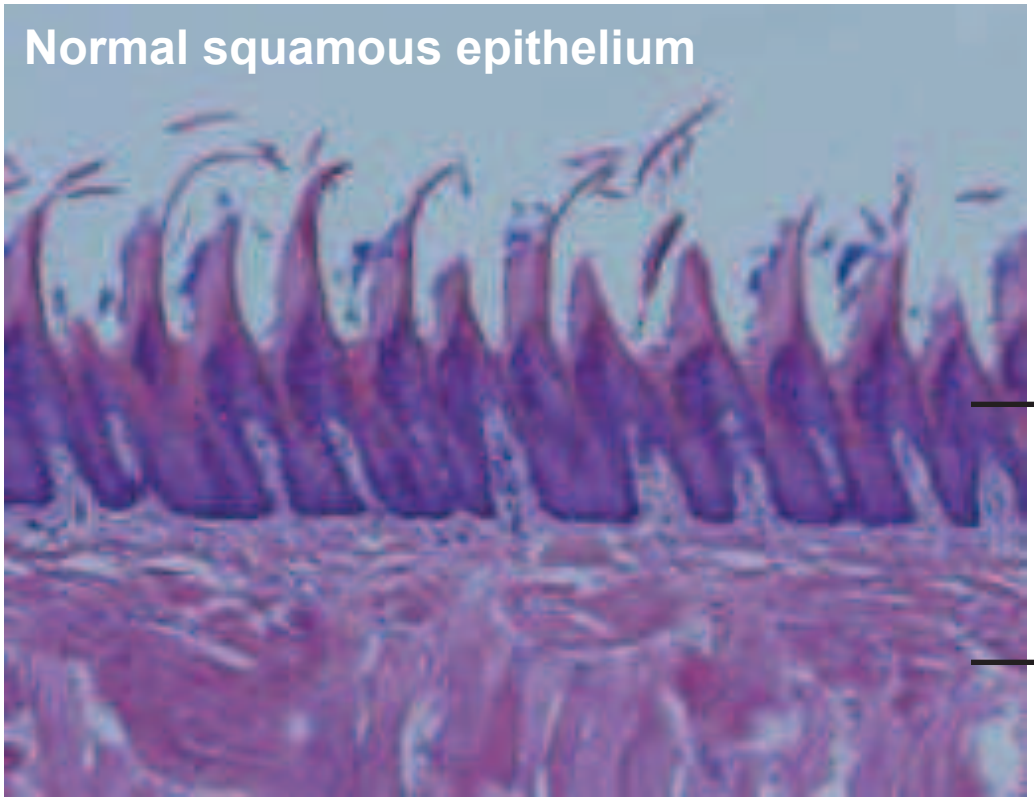
3. Stomach

4. Small/large intestine

GI tract (rat)


Esophagus
Duodenum
Forestomach
Glandular stomach
Small intestine
Mesentery
Cecum
Colon

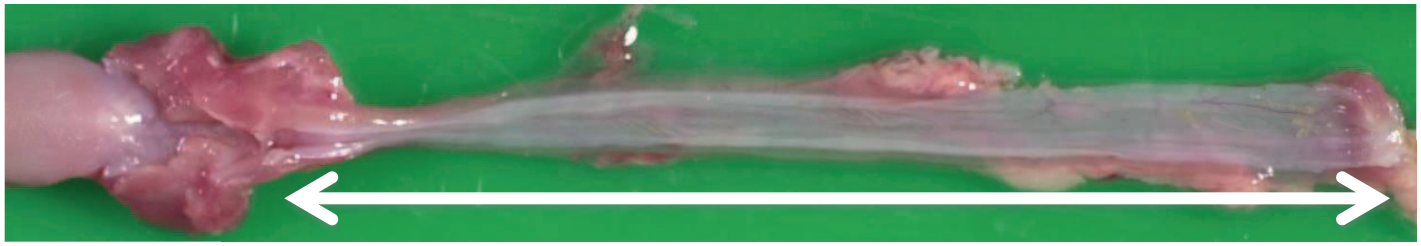
Tongue (rat)

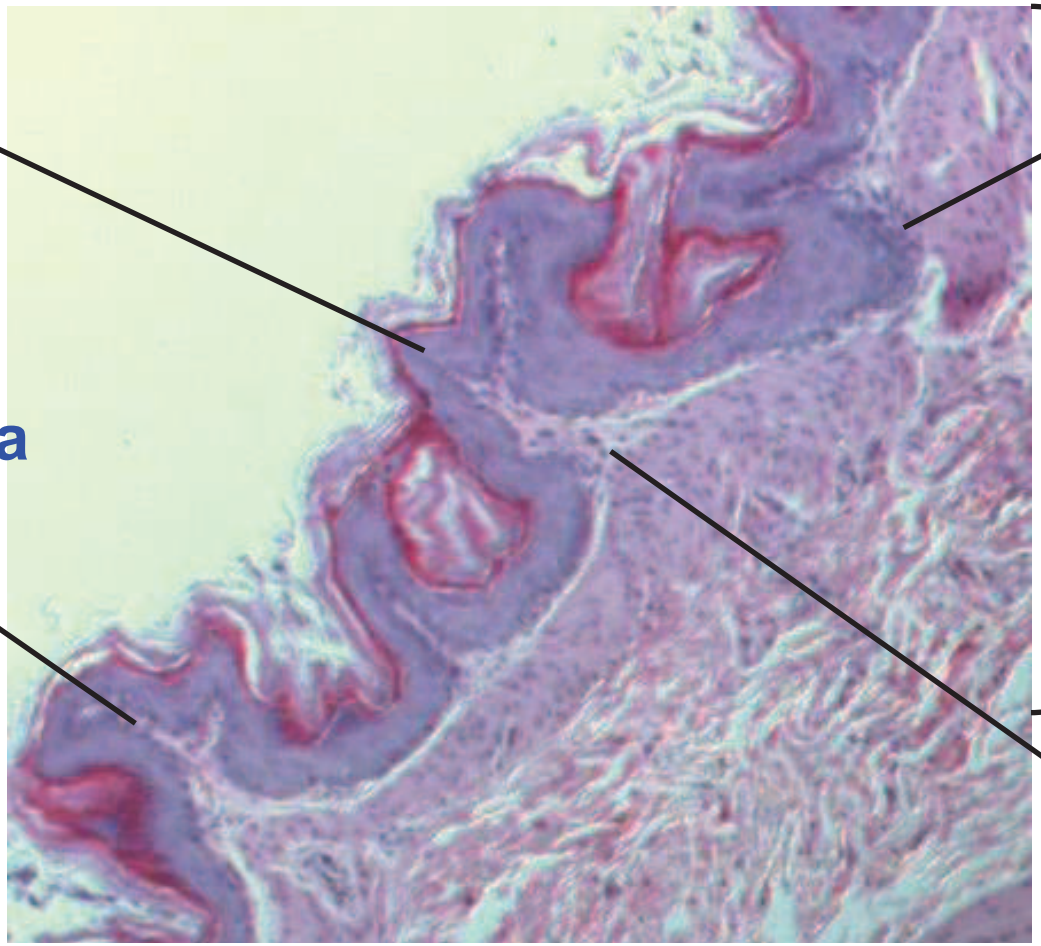


Squamous epithelium

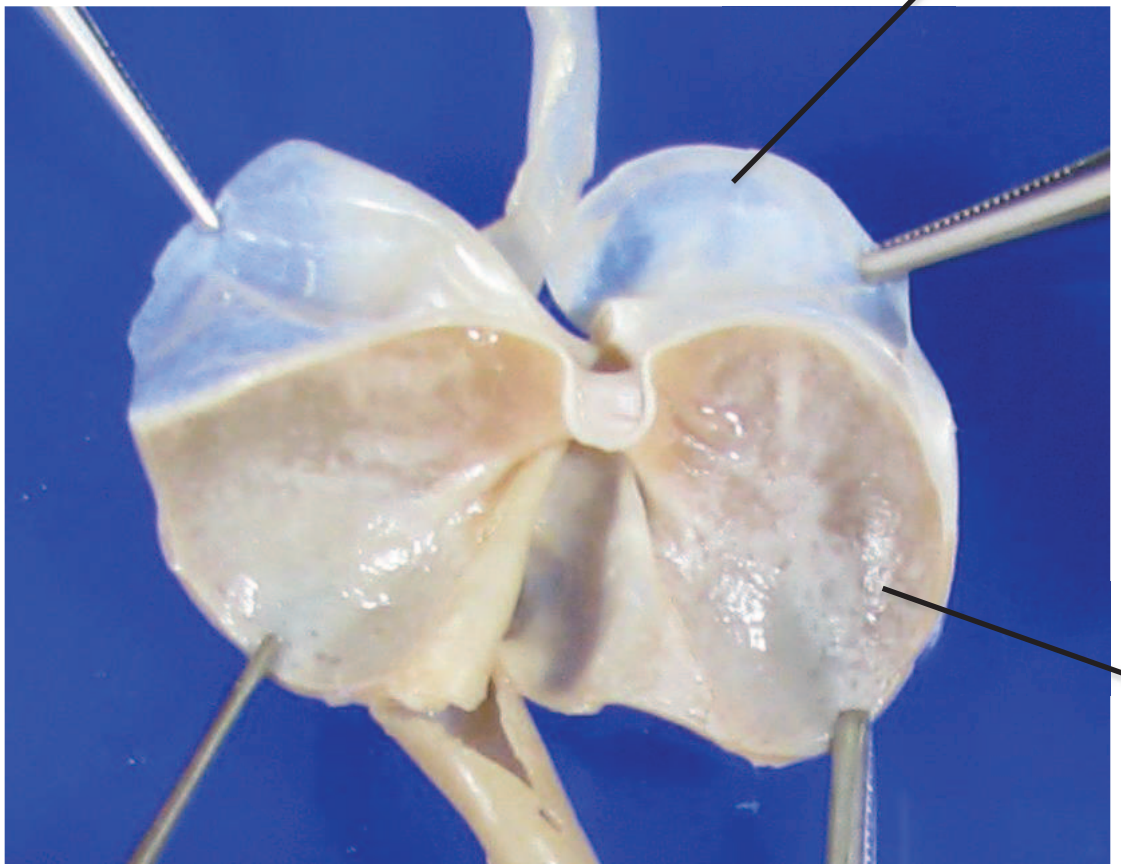
Muscular layer

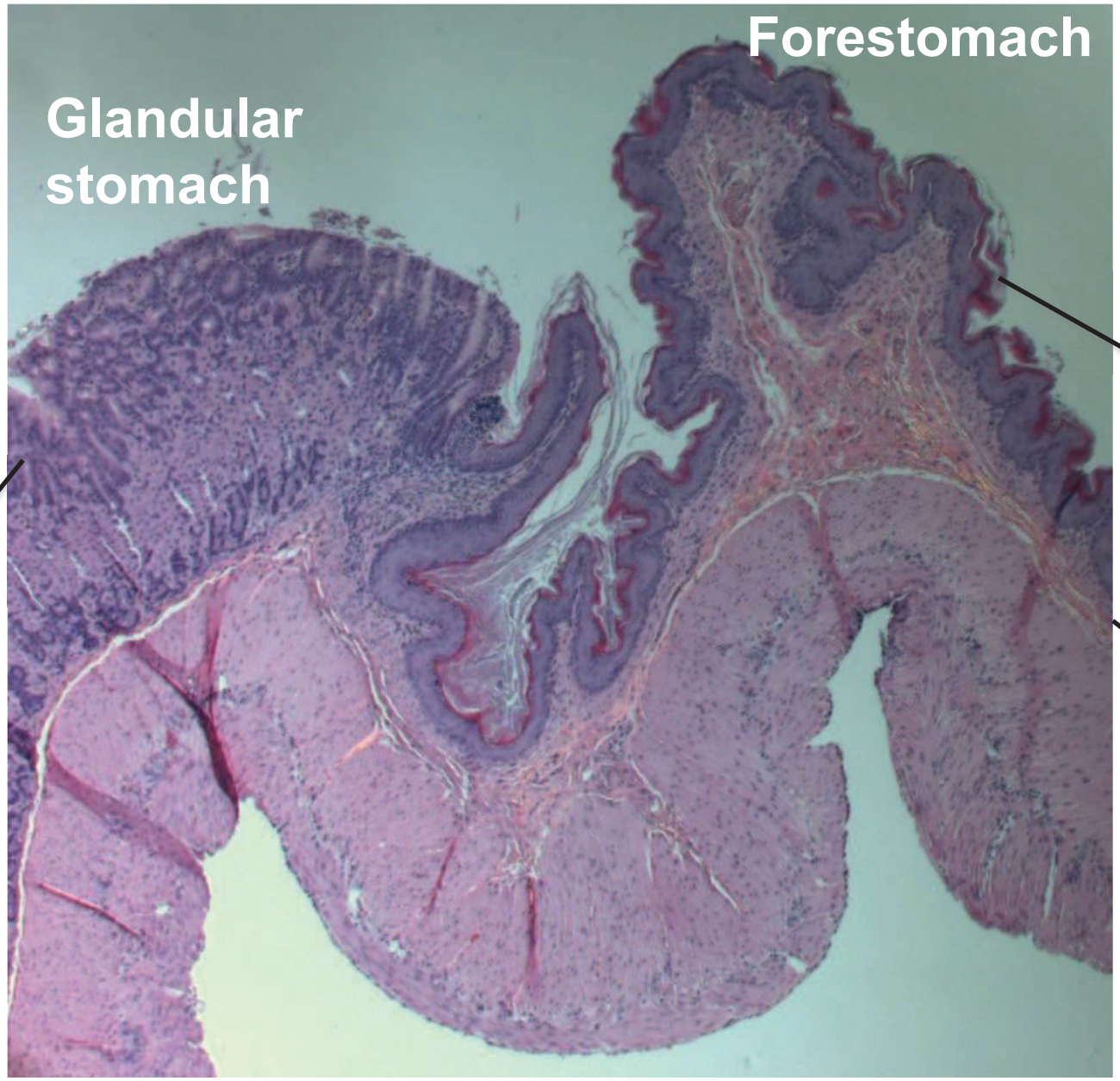
Esophagus (rat)

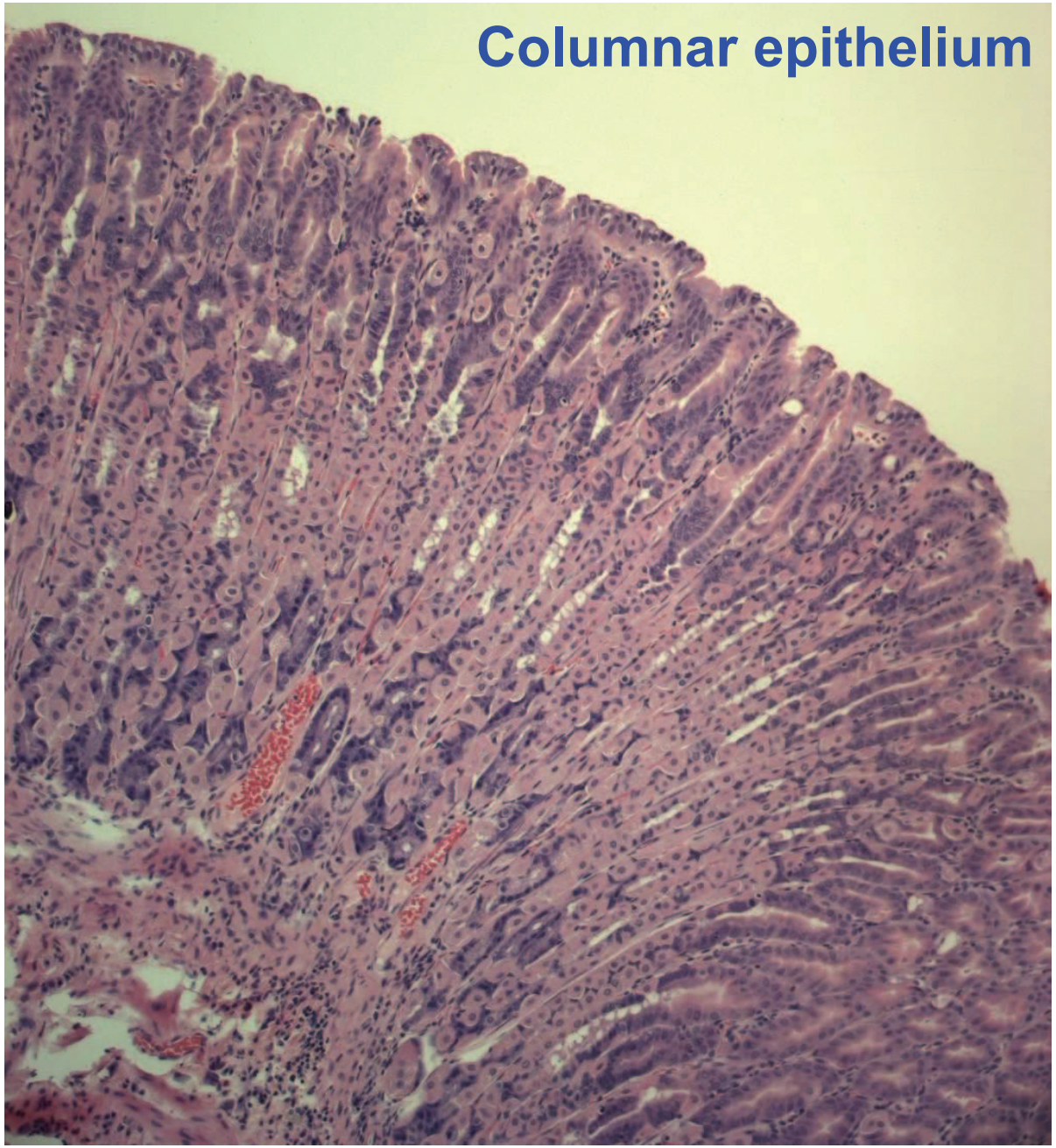




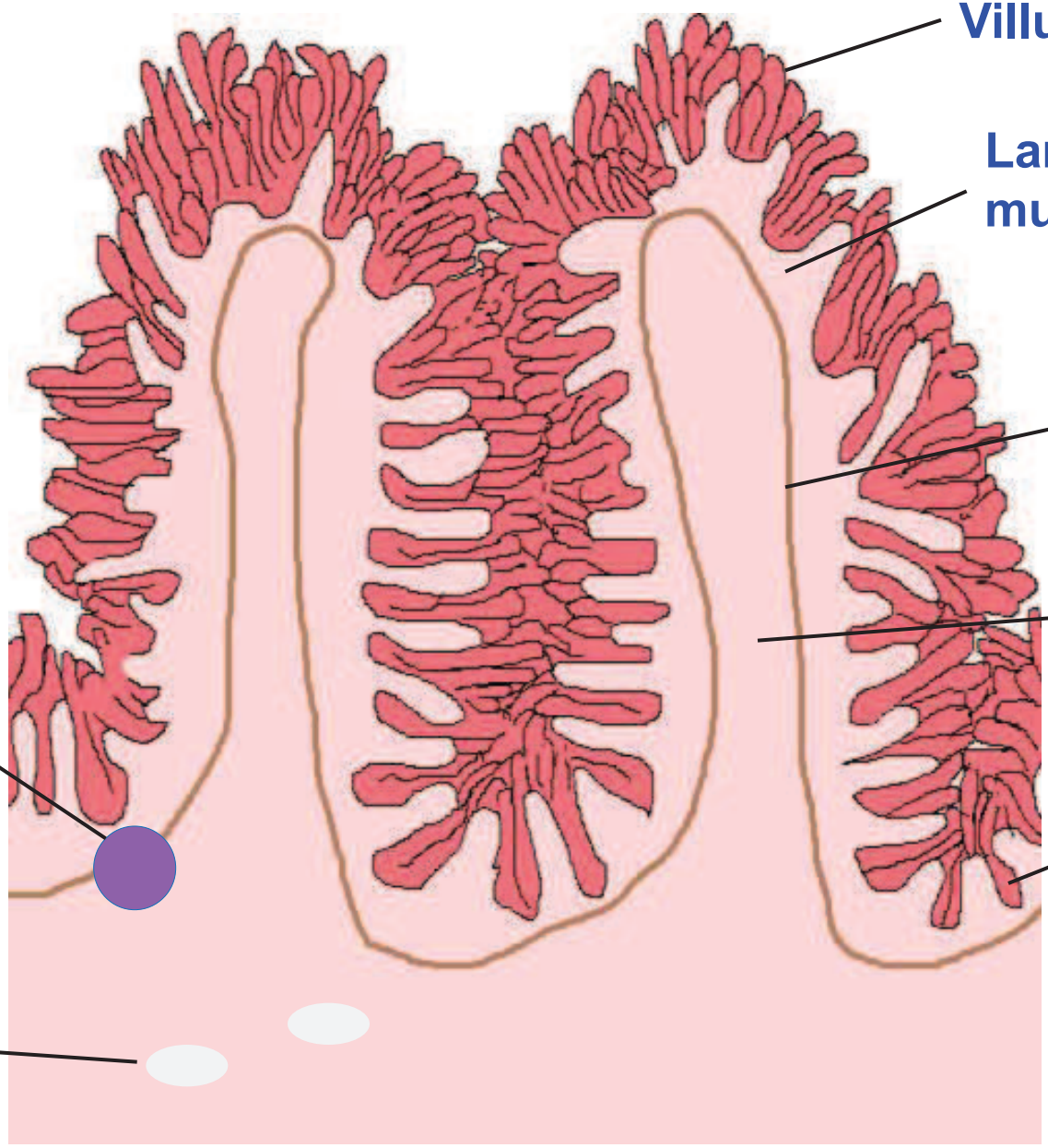
Stomach (rat)



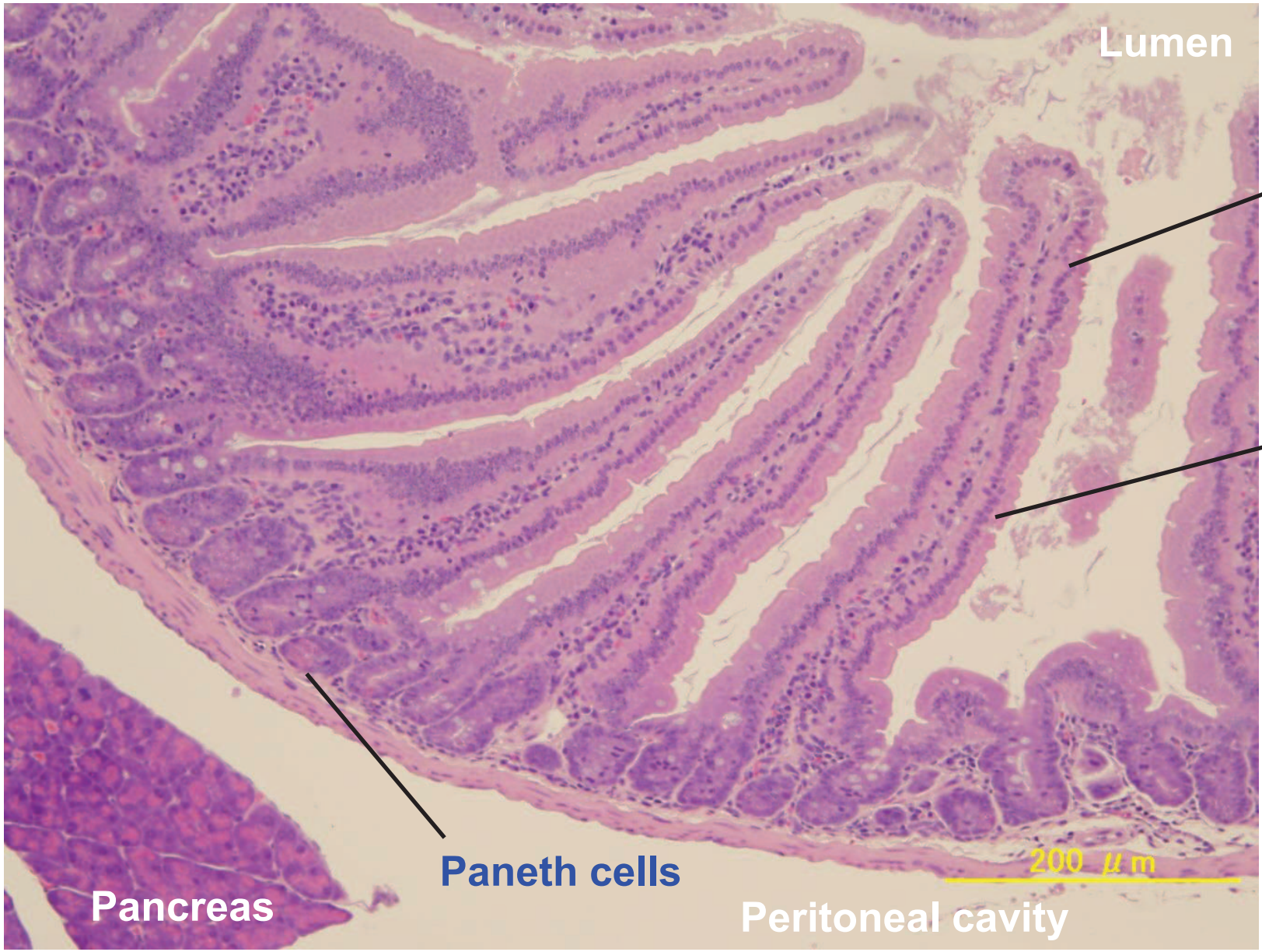

Forestomach
Glandular stomach
Stratified squamous epithelium
Columnar epithelium
Muscularis mucosa
Muscularis propria


Columnar epithelium
Parietal cells
Chief cells
Submucosa

Small intestine



Small intestine (mouse)



Colon


Villus
Crypt
Lamina propria mucosa
Goblet cells
Lymphatic follicle
Muscularis mucosa
Myenteric plexus
Submucosa

Colon (rat)