KITP Workshop
in Physics and Mathematics of Cancer
May 21-July 13, 2012

Colon Cancer Progression:

Studies on Mouse Models

Kyoto Univ. Grad. Sch. Medicine (Pharmacology)

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Colon Cancer Studies Using Mouse Models

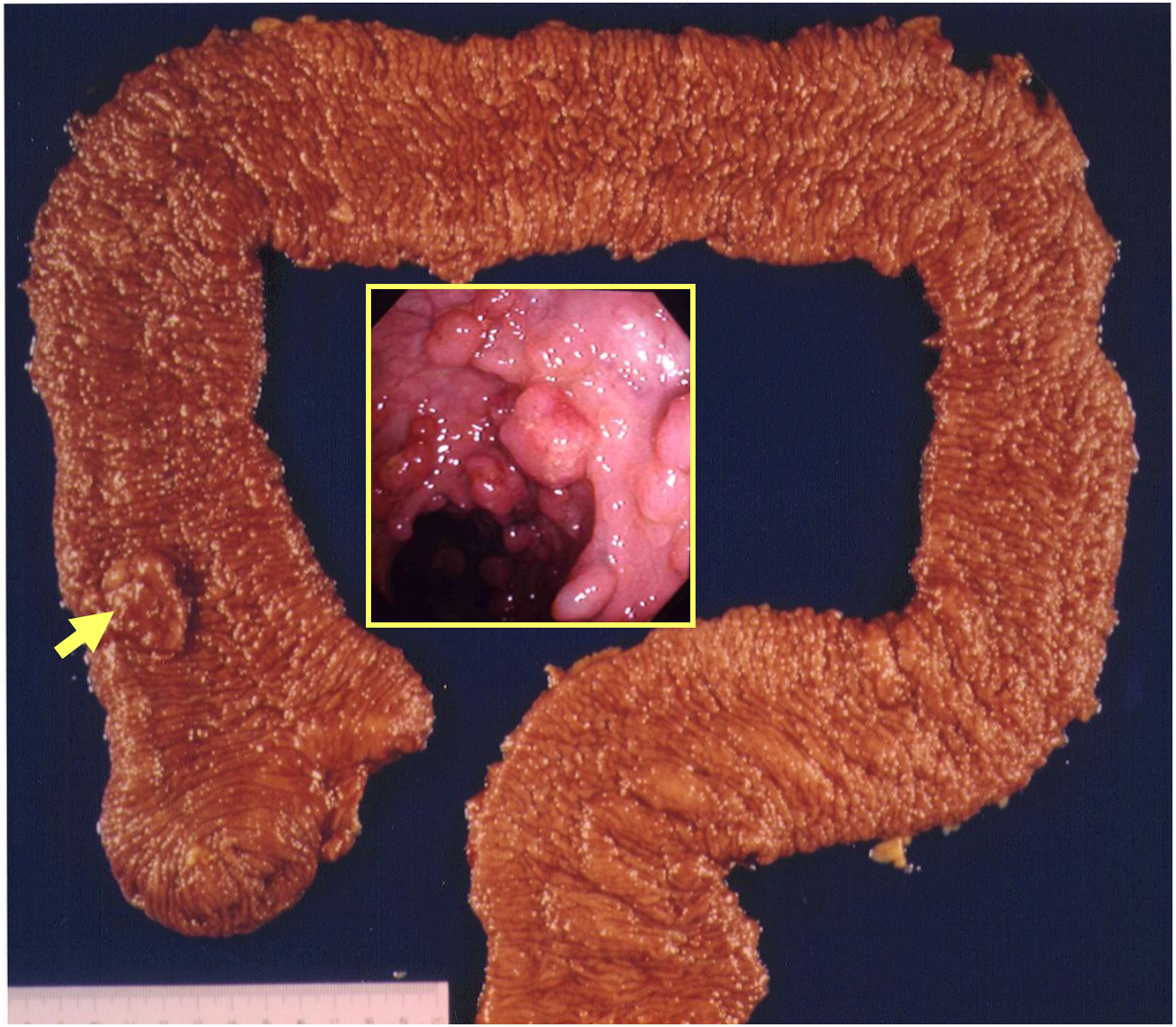
0. Introduction：Familial Adenomatous Polyposis（FAP），Wnt Signaling and Adenoma-Carcinoma Sequence in Human Colon Cancer Progression
1. Cyclooxygenase (COX-2) in *Apc*$^{\Delta 716}$ mice
2. Local Invasion Model (*Apc*/*Smad4*)

3a. Spleen—> Liver Metastatic Colonization (CCR1)

3b. Blood-born Metastasis Model（to Lv & Lg）(*Apc/Aes*)

4. Lymph Node Metastasis Model (CXCR3)

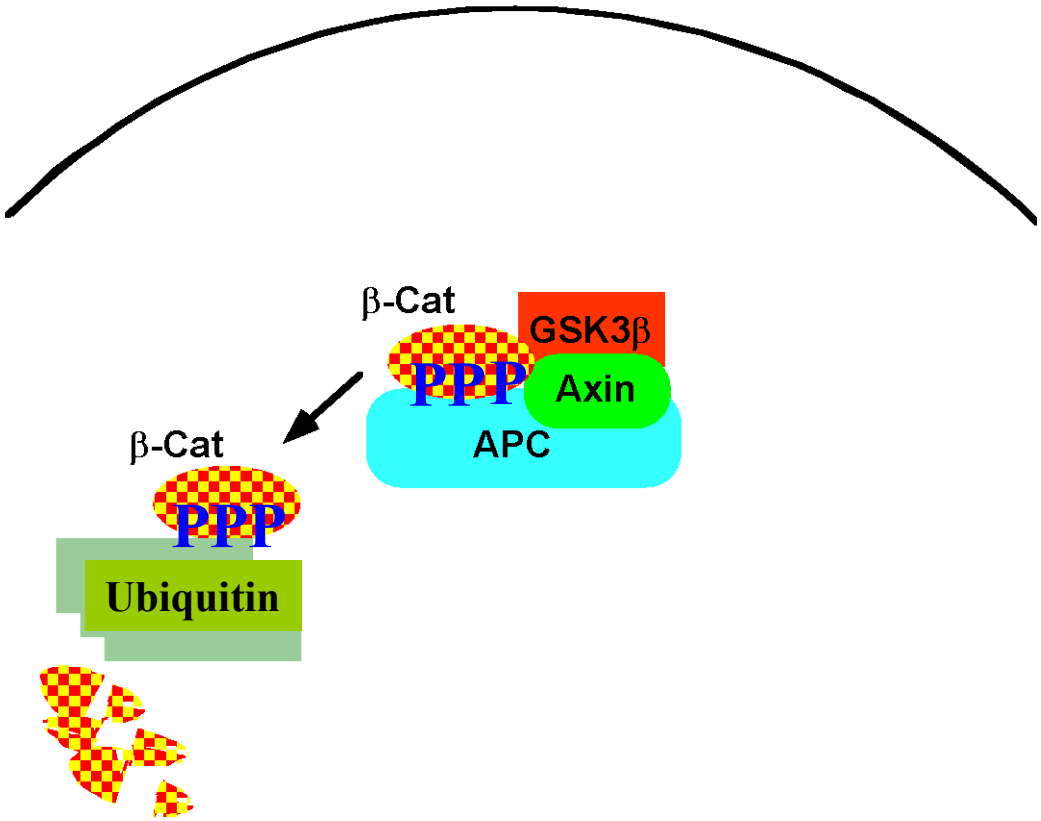
Familial adenomatous polyposis（FAP）: *APC* mutant



(Courtesy, Dr. K. Sugihara, Tokyo Medical and Dental Univ.: M.M. Taketo)

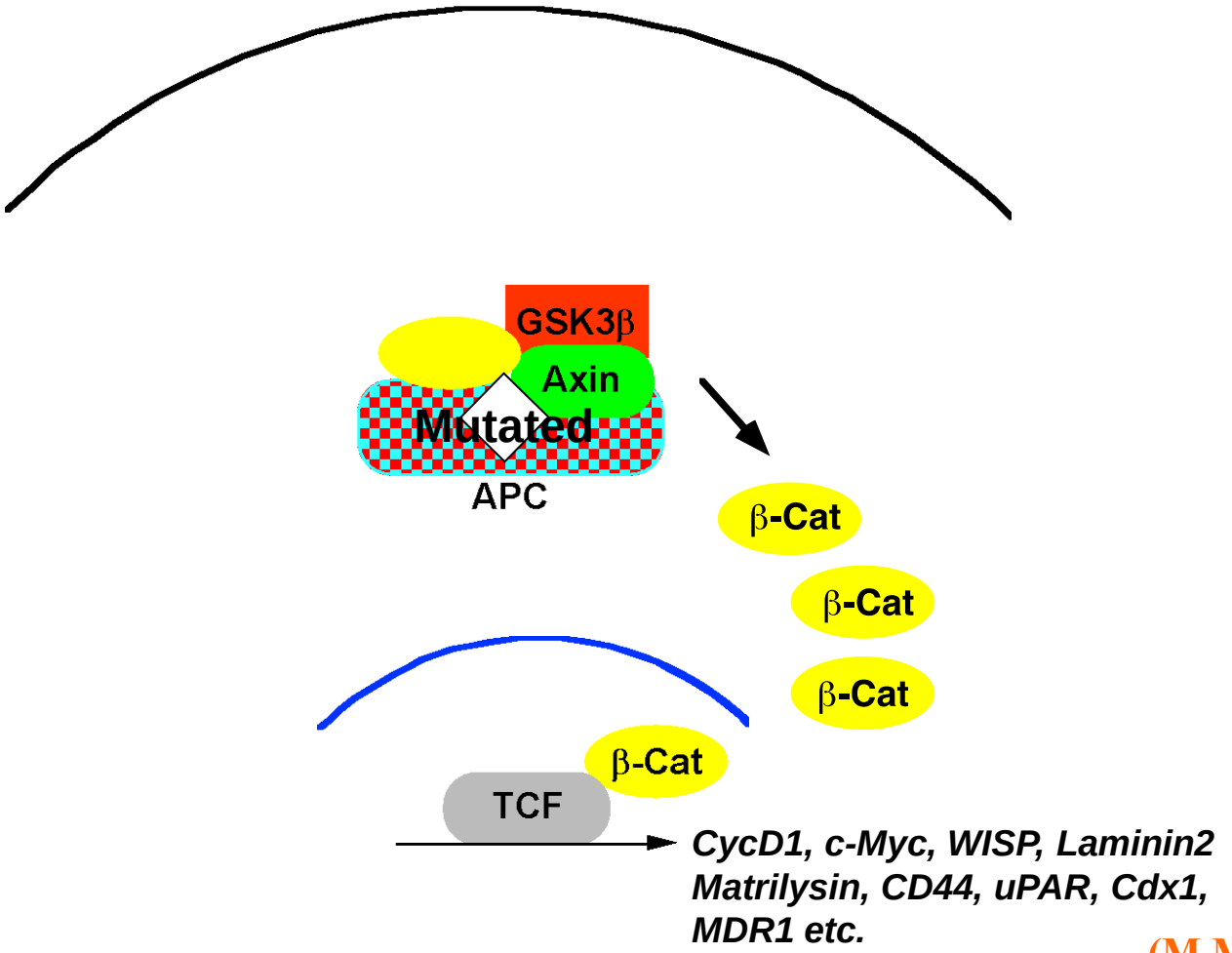
Minimalist's view of "un-activated"

Wnt Signaling Pathway



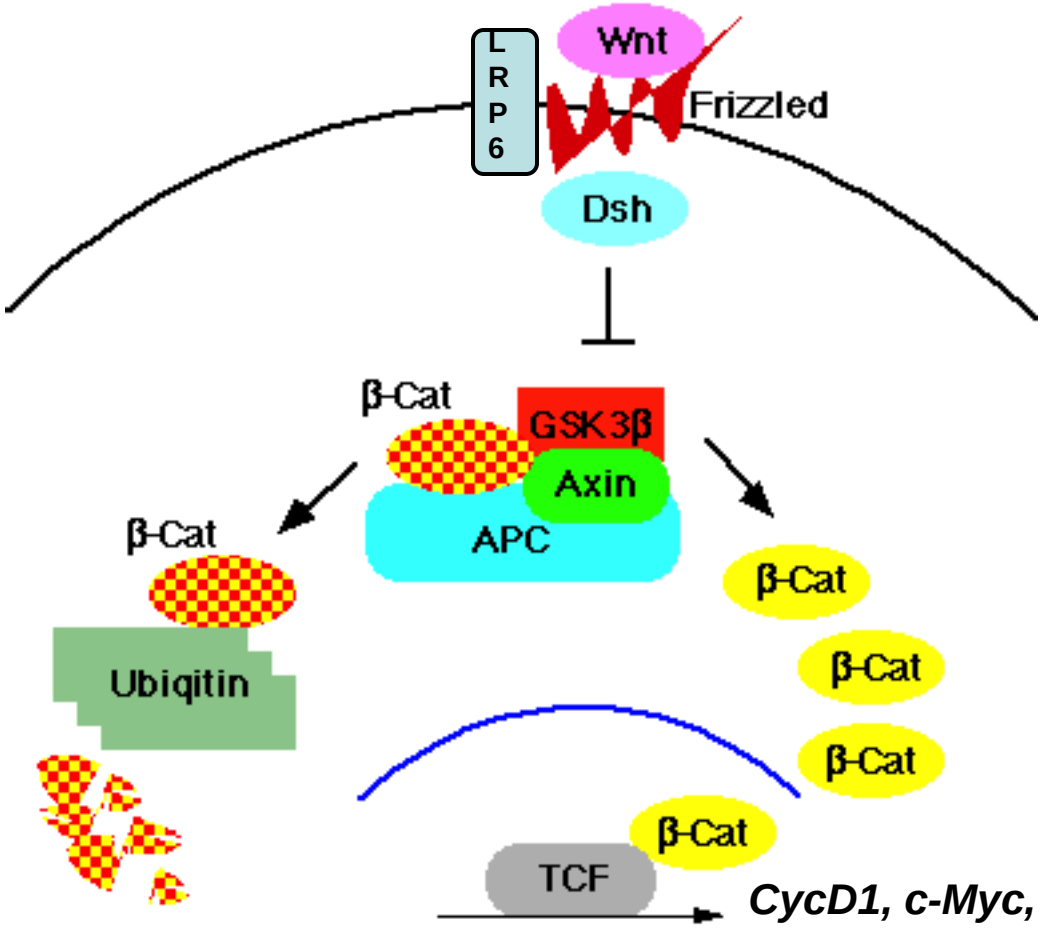
(M.M. Taketo)

Minimalist's view of Activation of Wnt Signaling Pathway



(M.M. Taketo)

Wnt Signaling Pathway

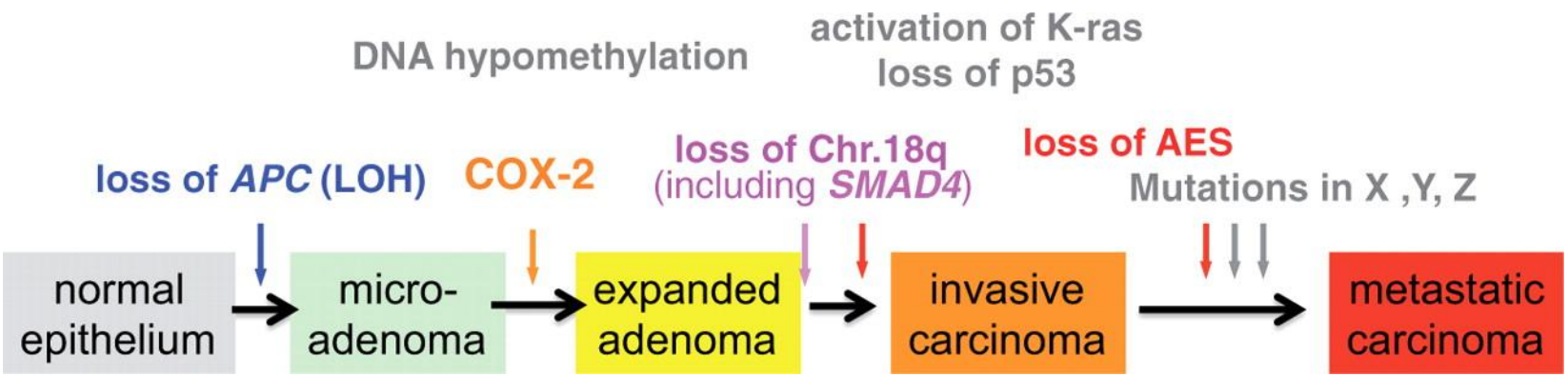


CycD1, c-Myc, WISP, Laminin2 Matrilysin, CD44, uPAR, Cdx1, MDR1 etc.

(M.M. Taketo)

0

Adenoma-Carcinoma Sequence in Colon Cancer Progression (Alternative)



Taketo M M J Biochem 2012;151:477-481

THE JOURNAL OF BIOCHEMISTRY

Why mouse models？

Laboratory mice are very different from field mice.

Mice were bred as pets for a long time in Asia.（Esp. albinos; below）

Some of them were transported to Britain in 19C where they were bred for genetic studies by brother-sister mating for many more generations.

Each strain is syngeneic (identical genetic/genomic constitution; clonal).

They breed fast (3-week gestation), occupy relatively small cage space.

With small brains, and little emotional life（different from dogs or cats.）

“Mice transcribing a book” by Gyosai Kawanabe（1831-1889）（Smithsonian Institution）

Mice practicing reading and writing：the albino mice（with white coat & red eyes）wear kimonos, whereas wild (field) mice at the corner do not.

（From Dr. Shiroishi, T., Natl. Inst. Genet.）

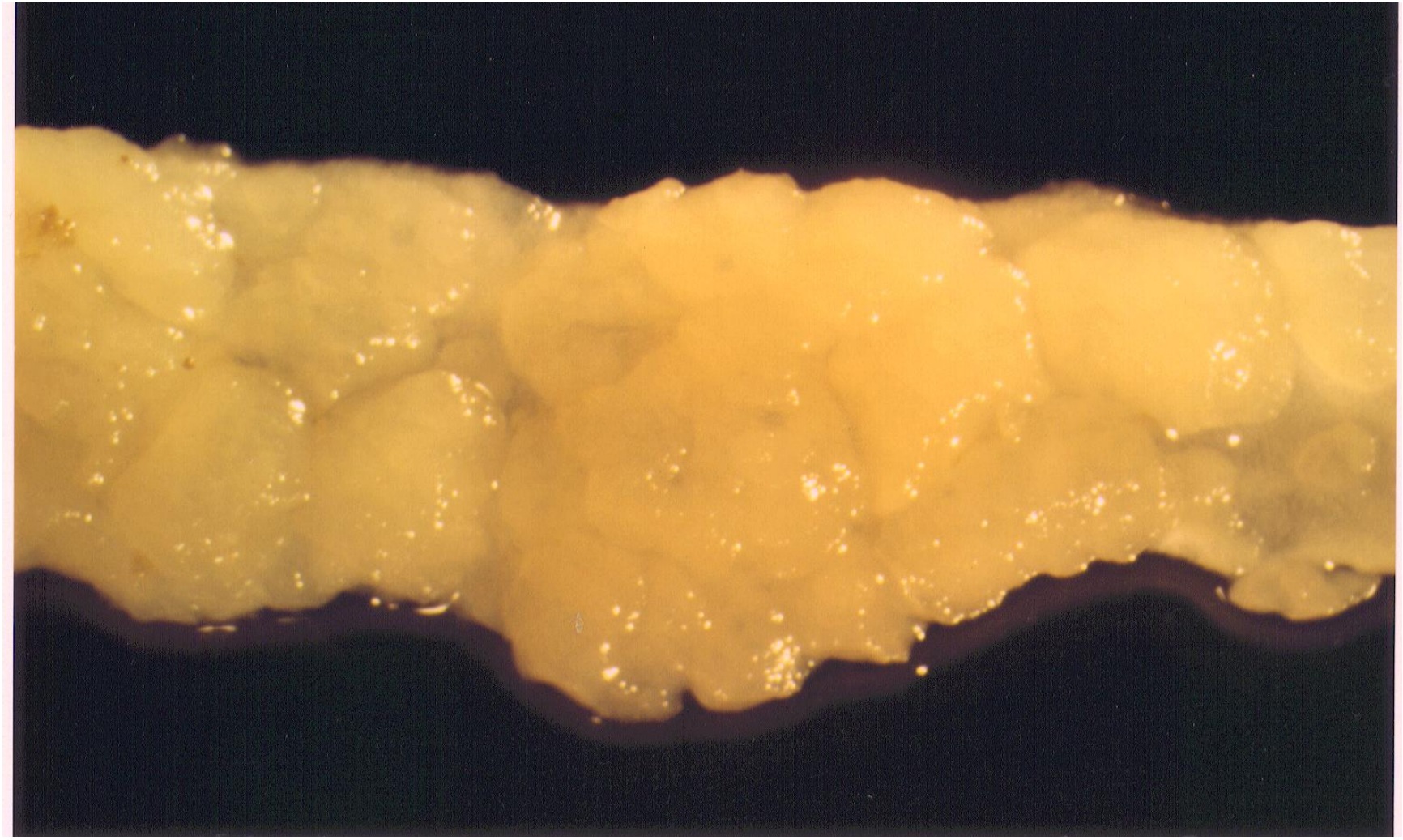
0

Germ-line chimera of *Apc*$^{+/\Delta 716}$ mutant ES cells

(M.M. Taketo)

0

Small intestinal polyps in *Apc*$^{+/\Delta 716}$ mouse



(M.M. Taketo)

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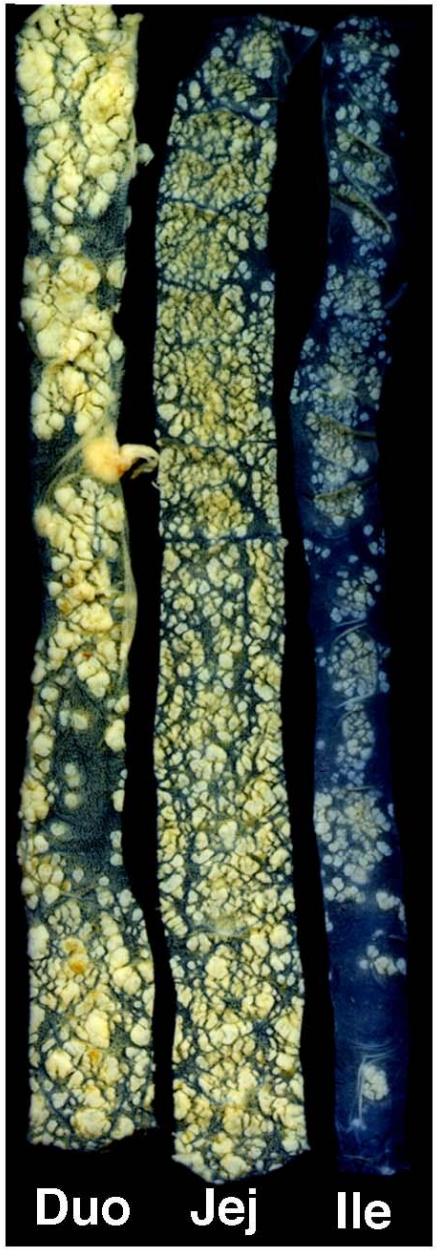
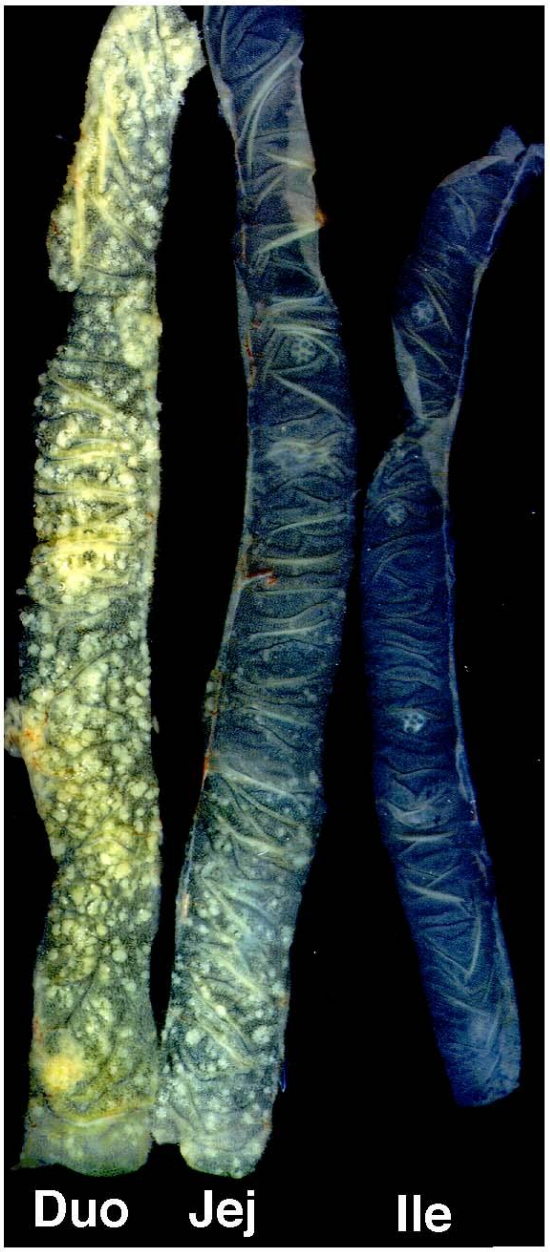
Intestinal Polyposis in Stable β-Catenin (*Catnb*$^{\Delta ex3}$) Mutants

K19-*cre*
~3,000 polyps



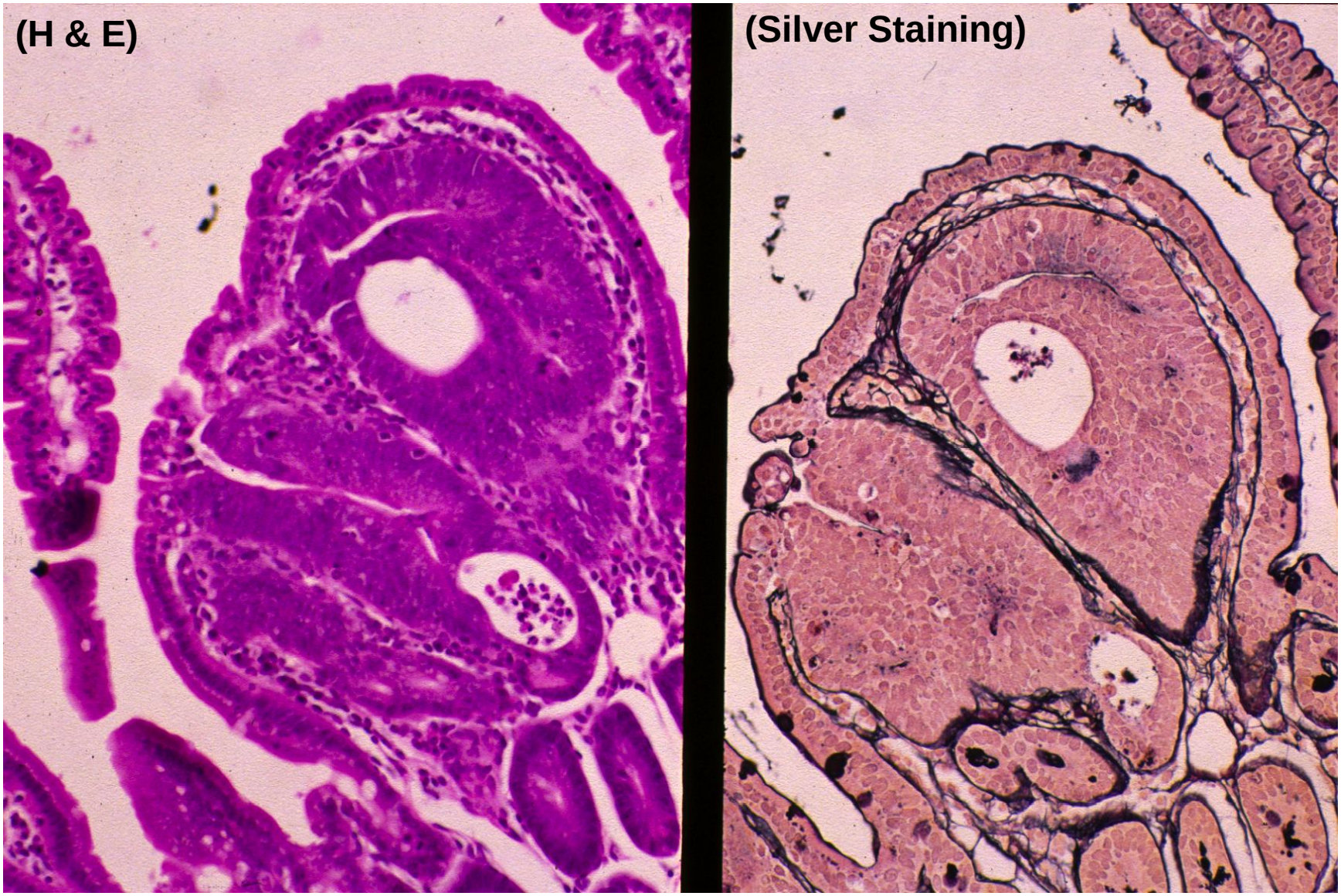


FABP-*cre*
~700 polyps

(Harada et al., *EMBO J. 18* : 5931-5942, 1999)

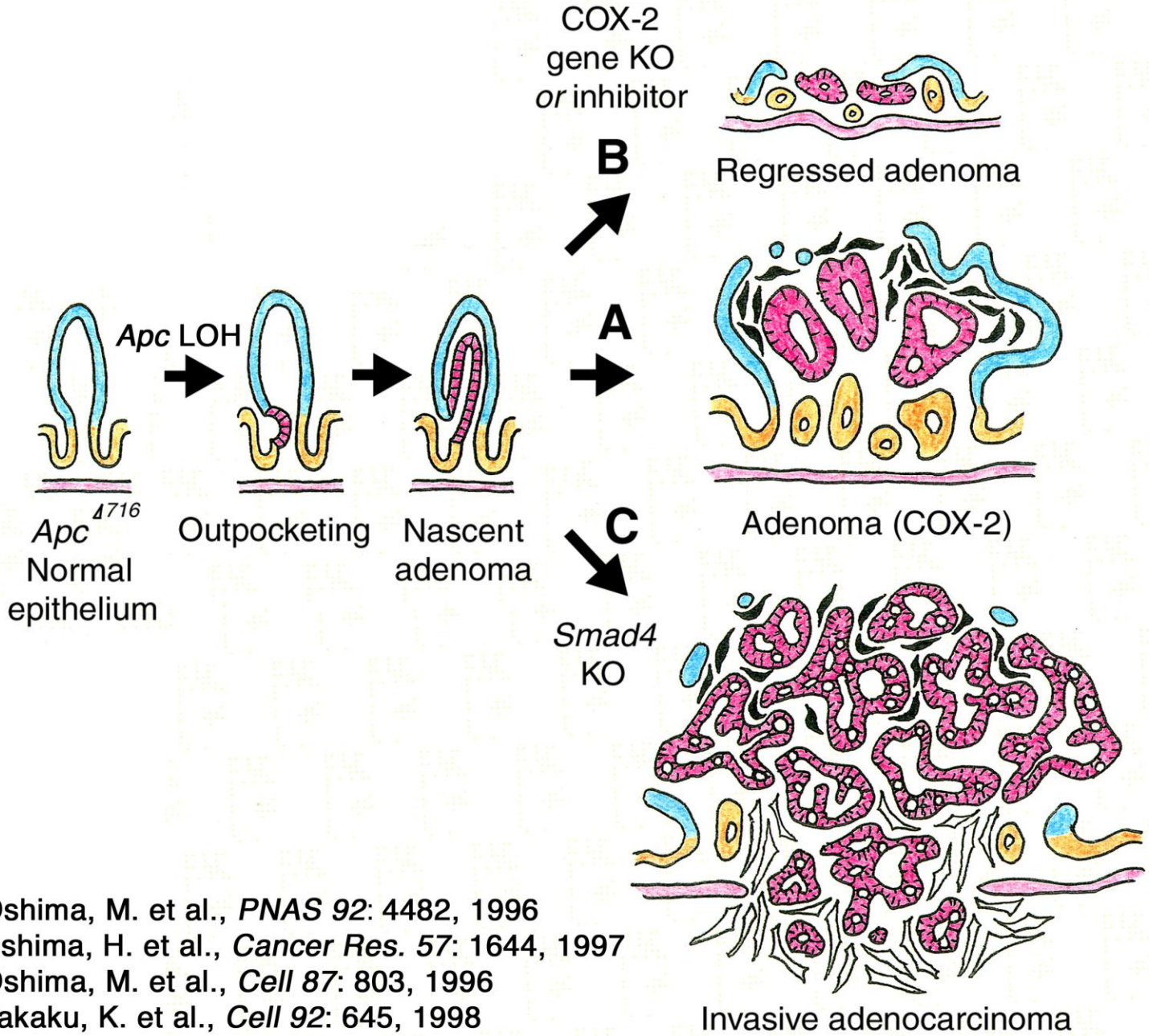
0

Polyp adenoma in *Apc*$^{+/\Delta 716}$ mouse intestine



(Oshima, H. et al. *Cancer Res. 57* : 1644-1649, 1997)

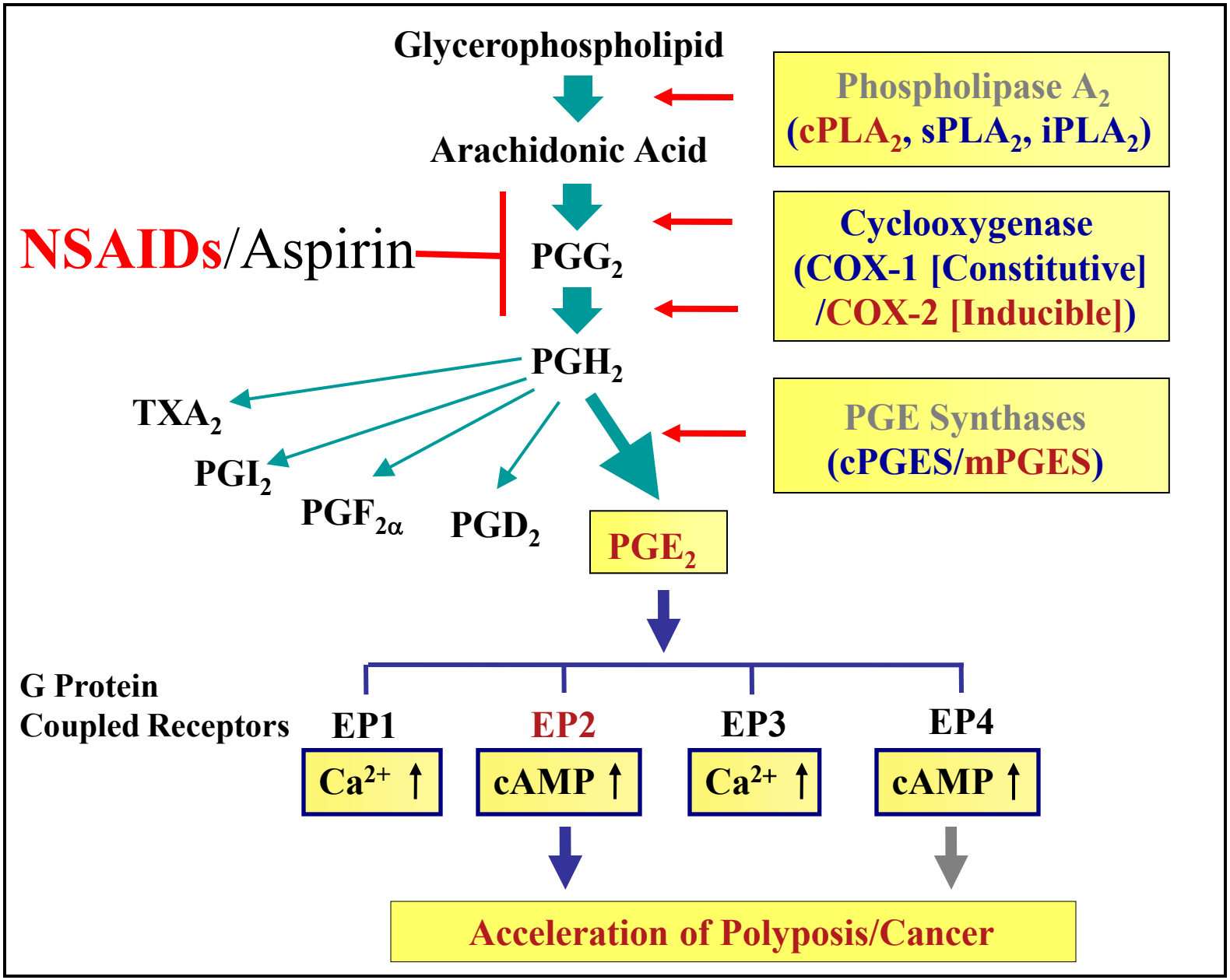
Three Mouse Models for Colon Polyposis & Cancer



A. Oshima, M. et al., *PNAS 92*: 4482, 1996
Oshima, H. et al., *Cancer Res. 57*: 1644, 1997
B. Oshima, M. et al., *Cell 87*: 803, 1996
C. Takaku, K. et al., *Cell 92*: 645, 1998

1

Arachidonic Acid Metabolism in Intestinal Polyposis



Cyclooxygenase Isozymes and Inhibitors

COX-1	COX-2
Constitutive Expression	Inducible Expression
House-Keeping	Inflammation
Homeostasis	Tumorigenesis

Inhibitors

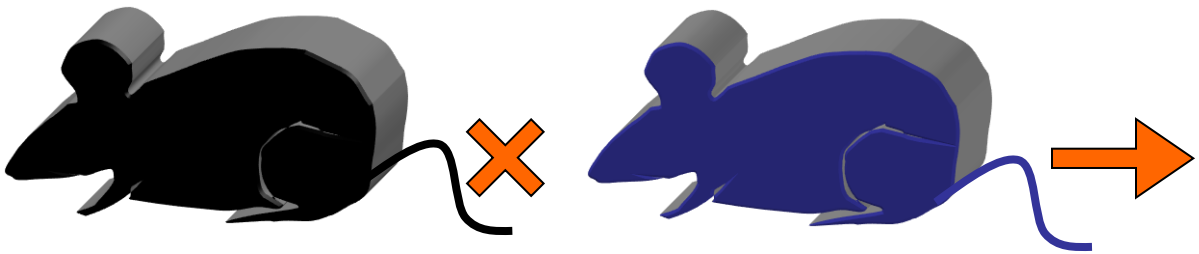
Aspirin	Coxibs

NSAIDs

(M.M. Taketo)

1

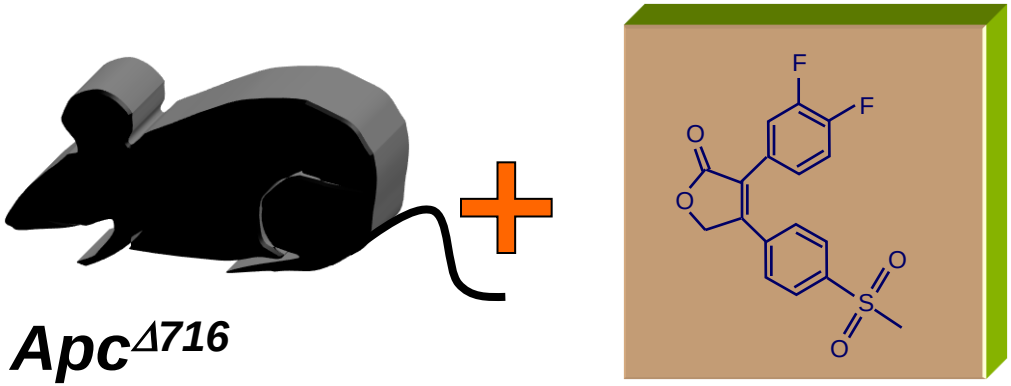
Suppression of intestinal polyposis by inhibition of COX-2



***Apc*$^{\Delta 716}$ Polyposis**

COX-2 -/- Mutant

Suppression of intestinal polyposis



***Apc*$^{\Delta 716}$ Polyposis**

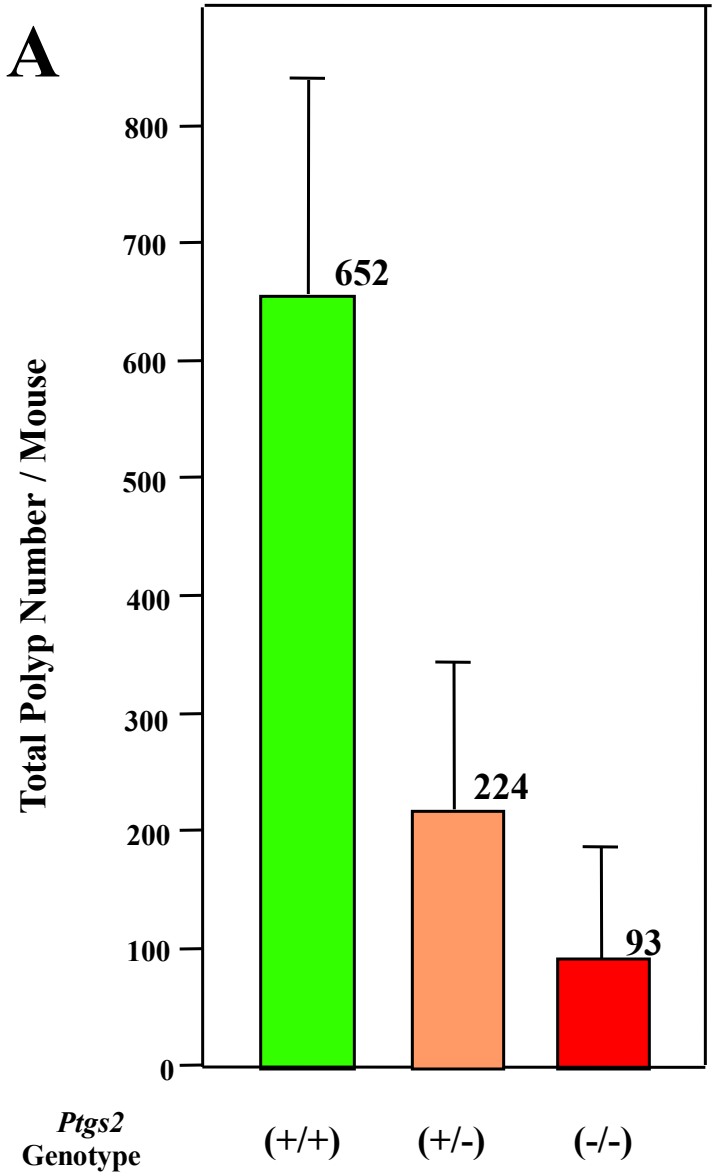
COX-2 Inhibitor

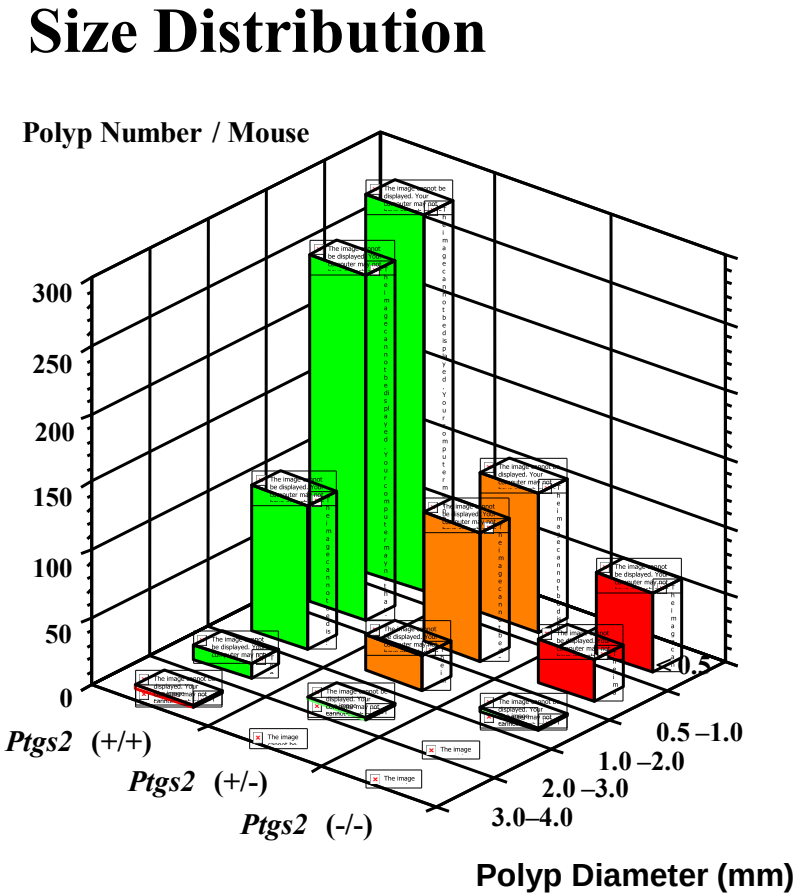
Suppression of intestinal polyposis

(M.M. Taketo)

1

Suppression of Intestinal Polyposis by Mutation in the COX-2 Gene (*Ptgs2*)



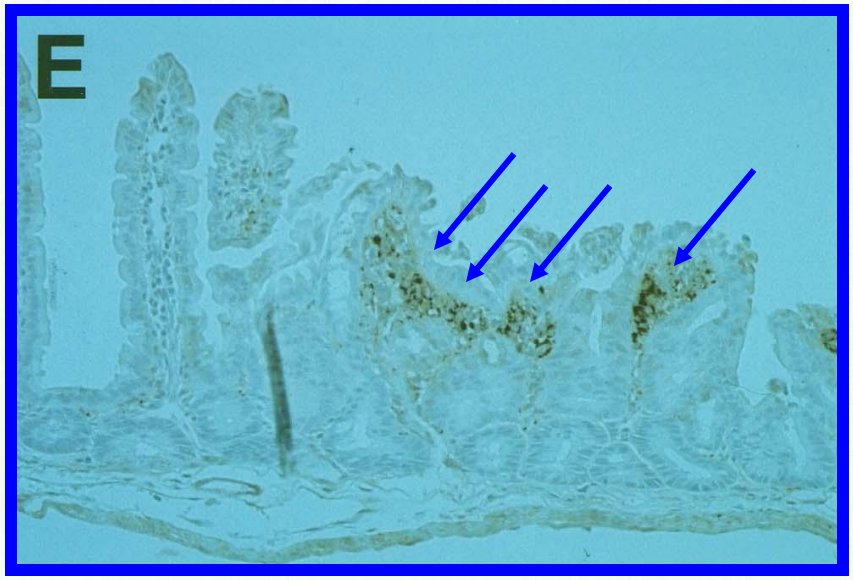


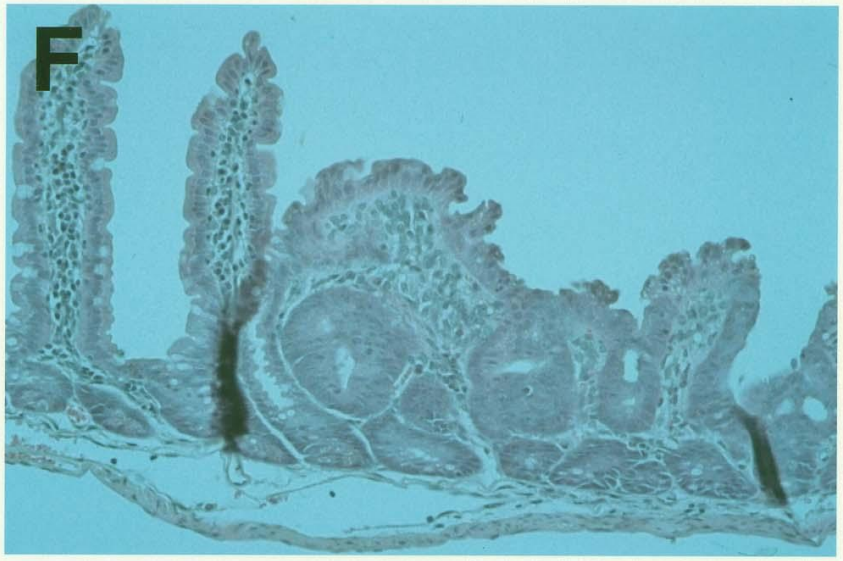
(Oshima, M. et al., *Cell 87*: 803-809, 1996)

1

COX-2 is expressed in tumor stroma in adenomas

β-Gal Expression from COX-2 Gene Promoter



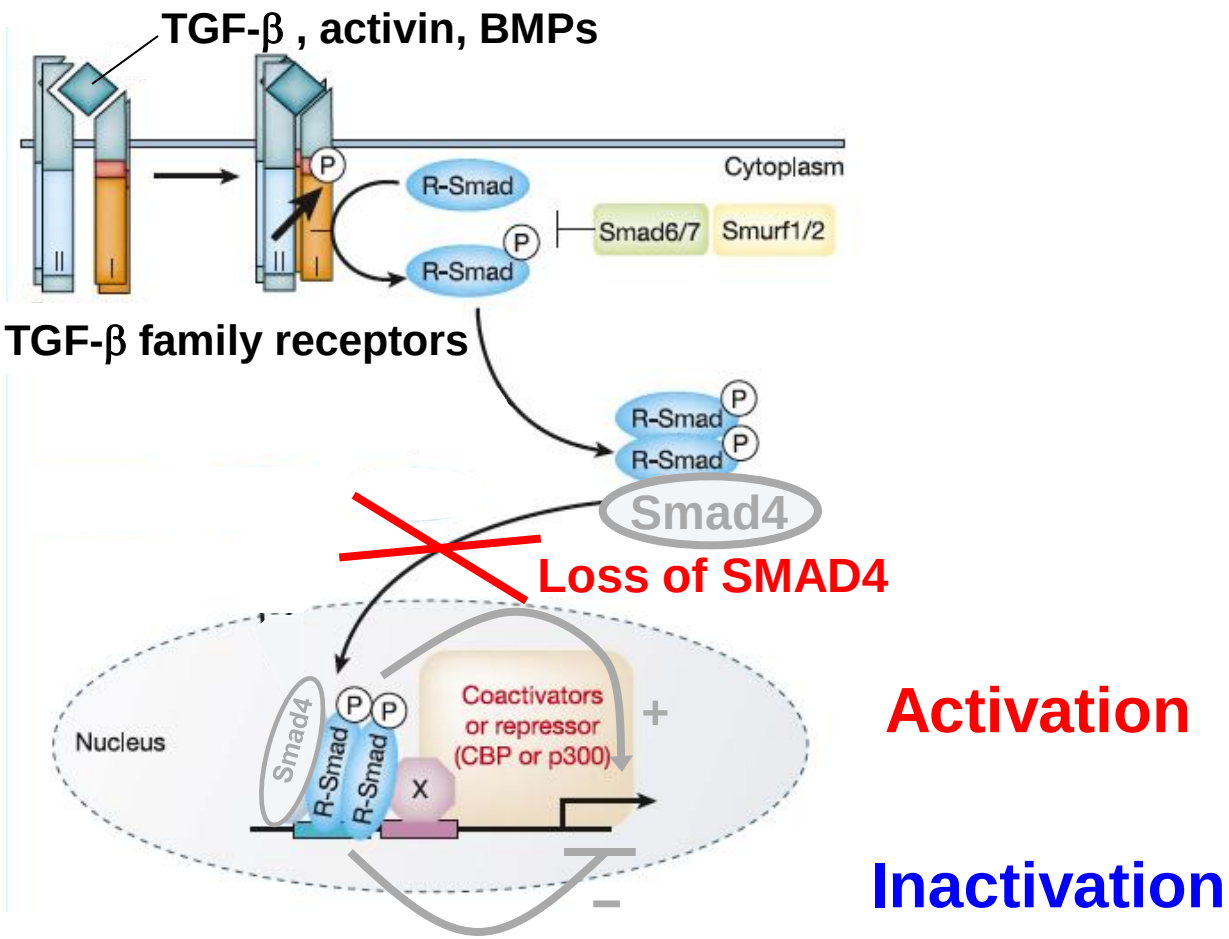


(Immunostaining)

(H&E histopathology)

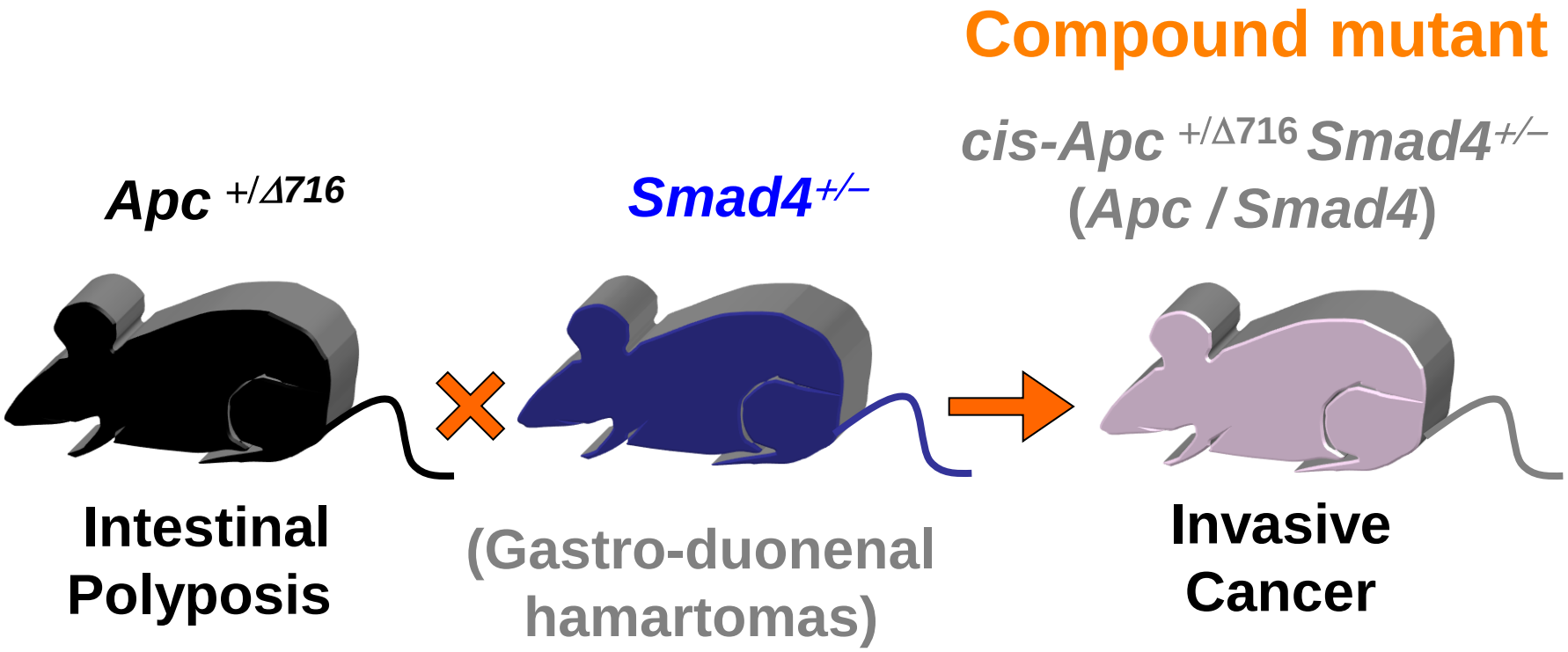
2 SMAD4 plays a key role in the TGF-β family signaling

Transcriptional Activation and Inactivation



NATURE | VOL 425 | 9 OCTOBER 2003 |

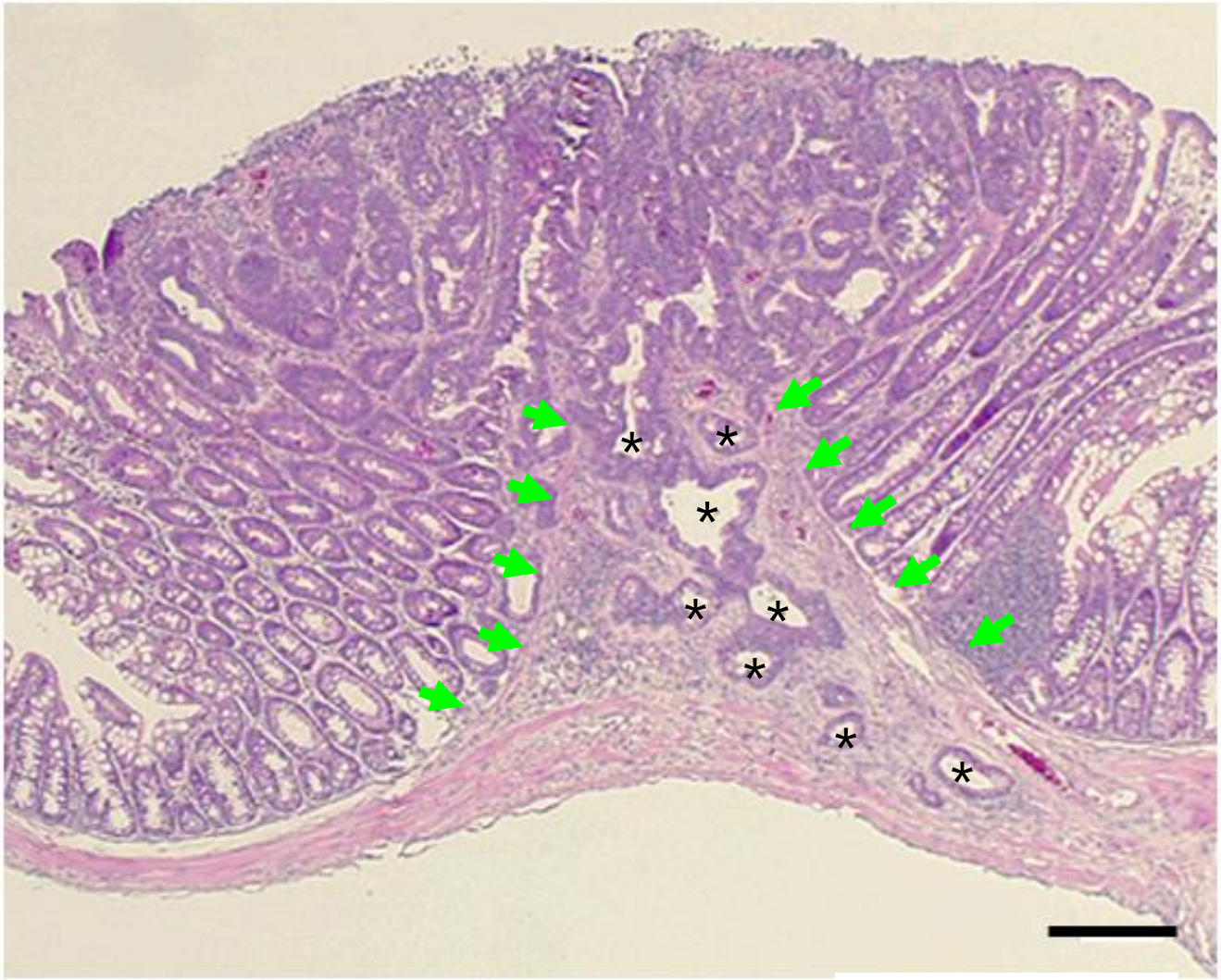
A mouse model for colon cancer invasion



(M.M. Taketo)

2

Marked invasion* of intestinal adenocarcinoma in *Apc / Smad4* mouse

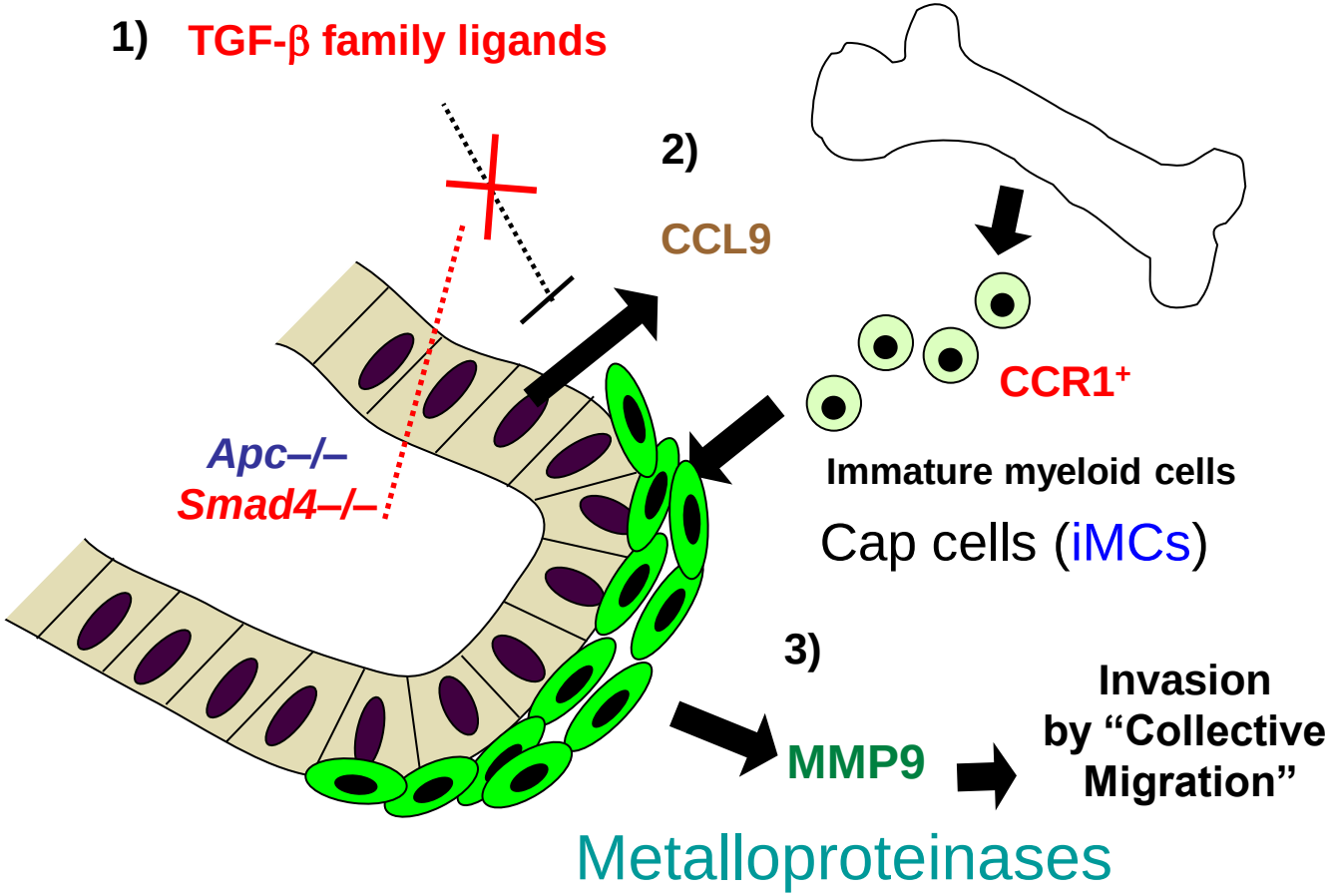


Trabeculation

(M.M. Taketo)

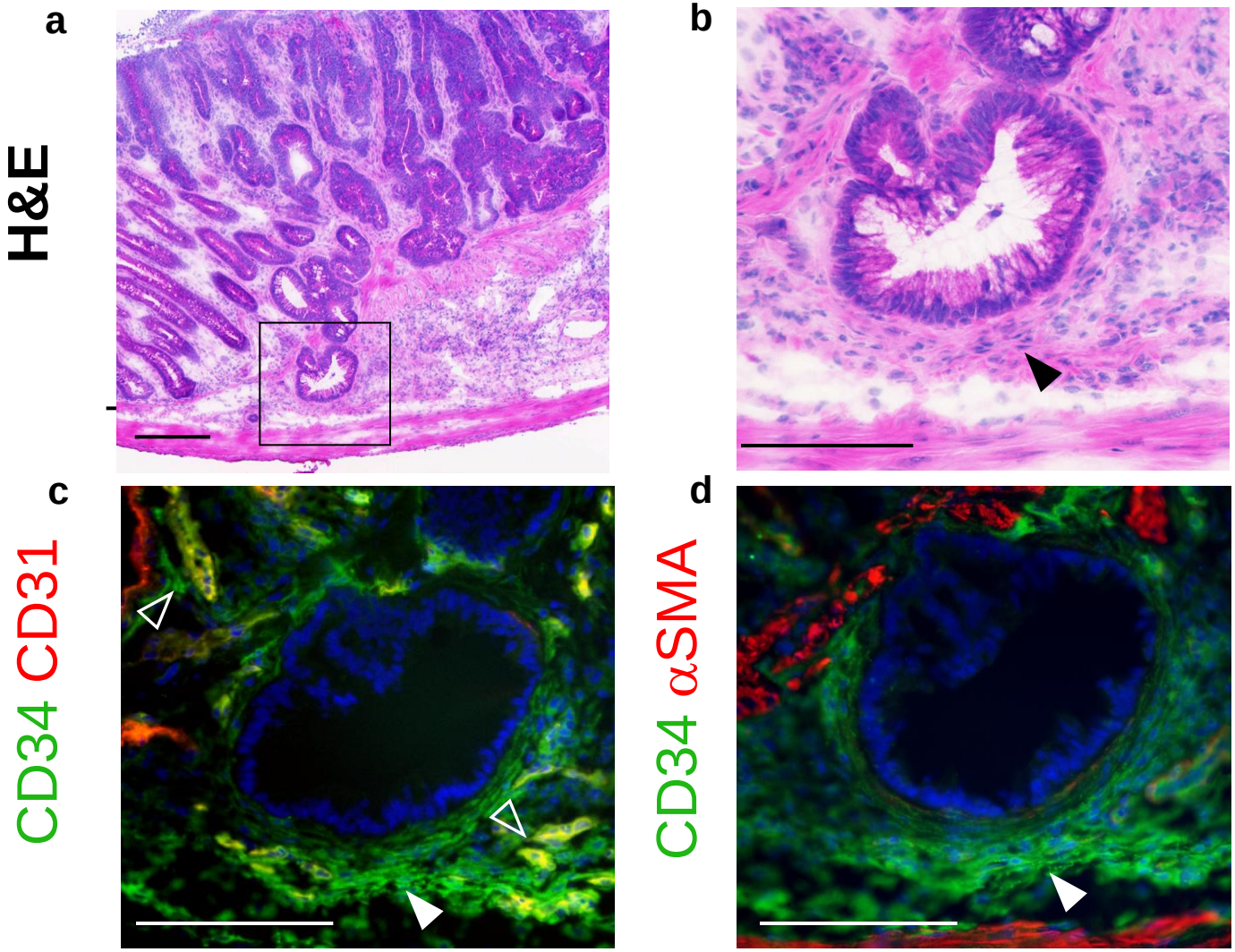
Conclusion first!

"Cap cells" pilot cancer cell invasion



2

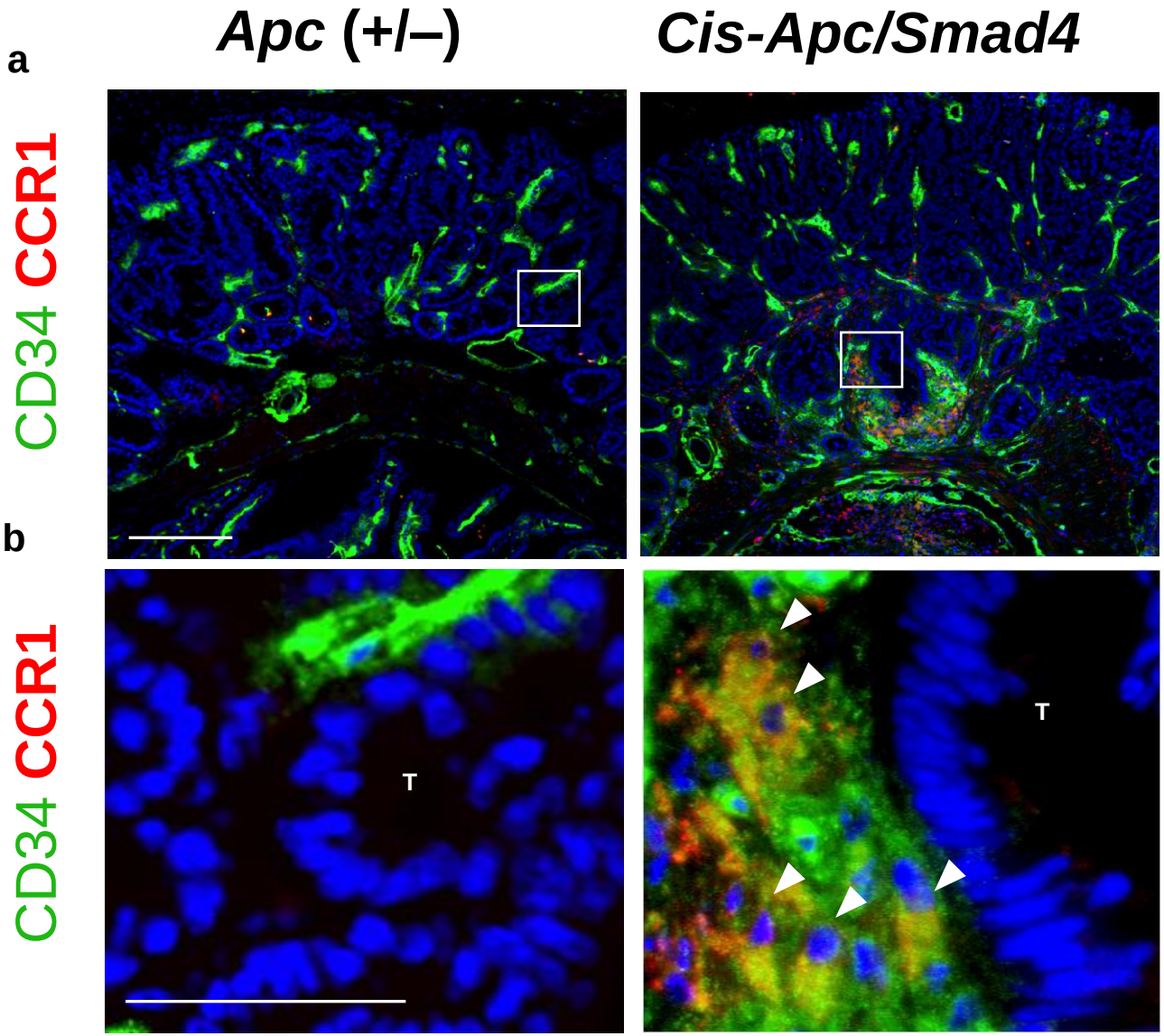
Cap cells found in colonic polyp of *Apc / Smad4* mouse



(Kitamura et al., *Nat.Genet.* 39: 467-475, 2007)

2

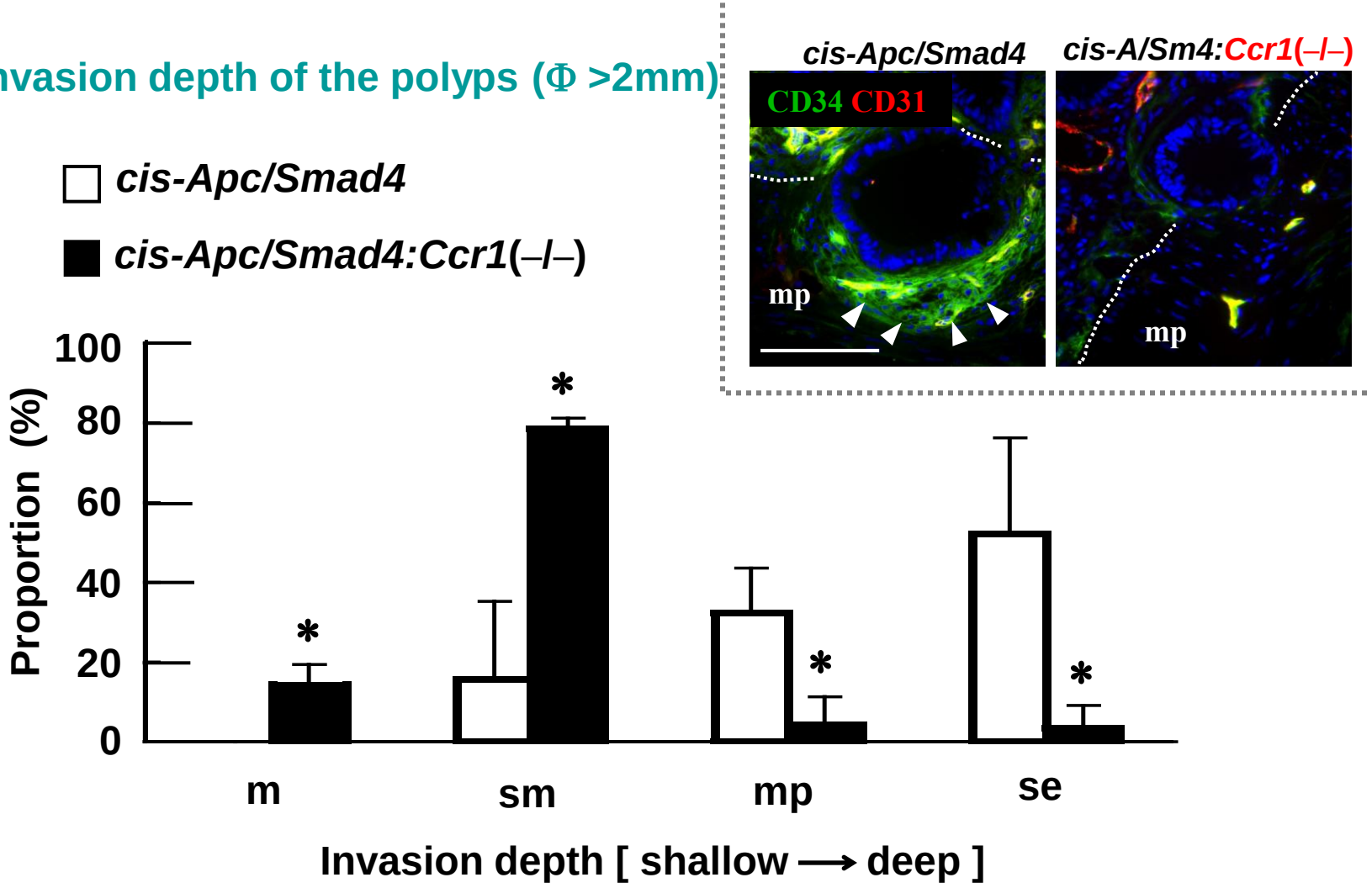
The Cap Cells express cognate CCL9 receptor CCR1



(Kitamura et al., *Nat.Genet.* 39: 467-475, 2007)

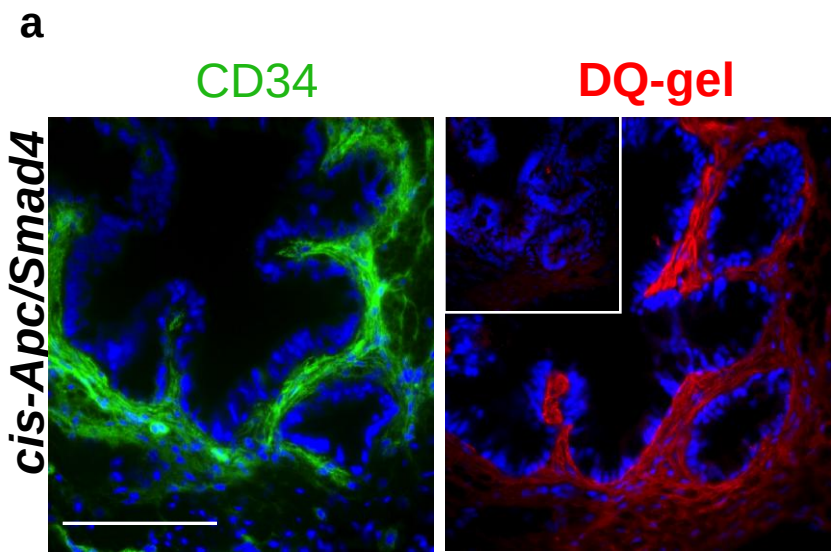
2

Cap Cells promote tumor invasion in the *cis-Apc/Smad4* polyps



2

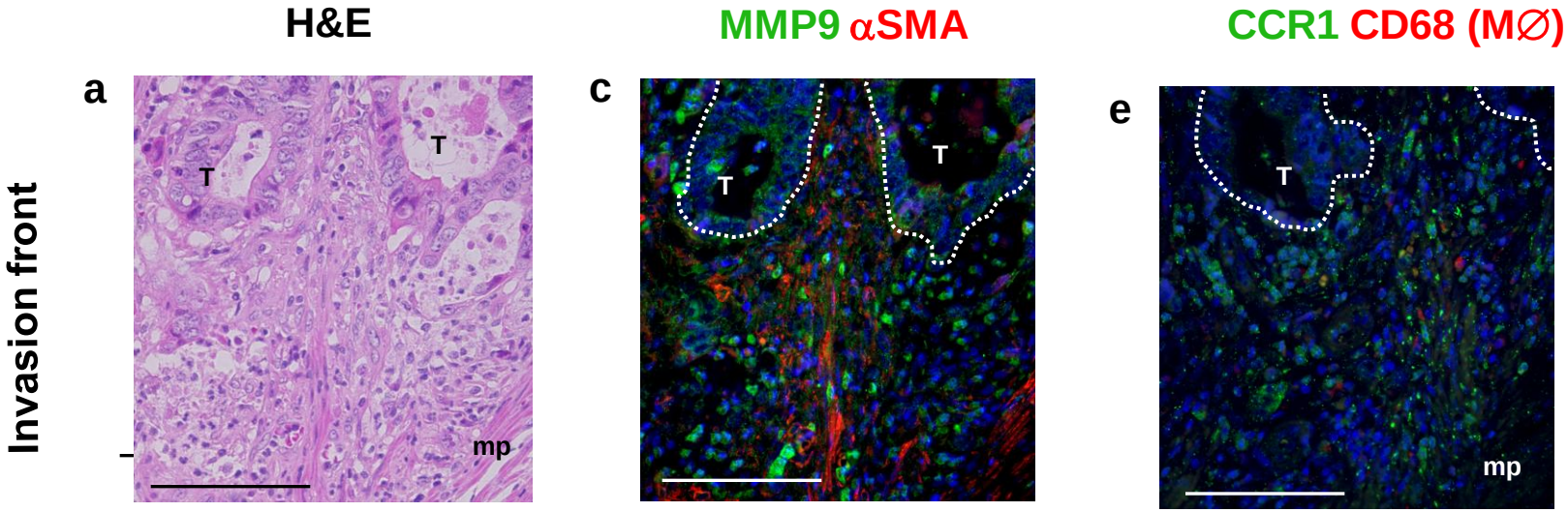
The Cap Cells in *cis-Apc/Smad4* mice produce gelatinases (MMPs) for tumor invasion



(Kitamura et al., *Nat.Genet.* 39: 467-475, 2007)

2

CCR1+ cells express MMP9 at the invasion front in human CRC*



*Right-side colon cancer with *TGFBRII* mutation $(A)_{10} \rightarrow (A)_9$ that cannot be corrected by DNA mismatch repair in the patients lacking the system (e.g., HNPCC).

(Kitamura et al., *Nat.Genet.* 39: 467-475, 2007)

Significance in Basic Research

- In contrast to previous impression that immune cells help protect the host by attacking cancer cells, these results show that they can aggravate cancer by helping cancer cells to invade through chemokine–chemokine receptor interaction.

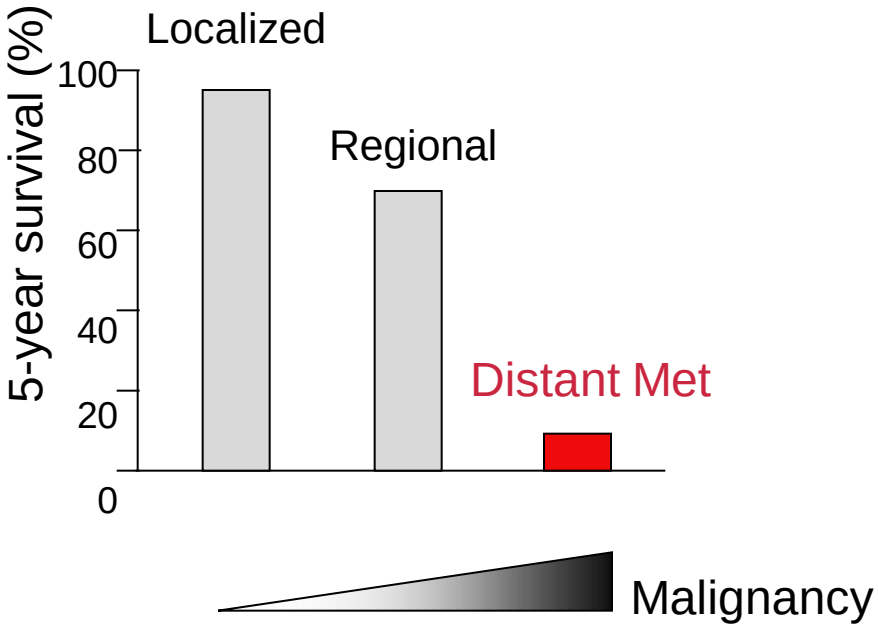
- Because local invasion is the earliest step in cancer metastasis, it is possible that the same chemokine–chemomkine receptor axis may be involved in the metastasis of colon cancer → **3.**

3a

Colon Cancer Metastasis and Prognosis （5-yr Survival）

w/o Metastasis

w/ Metastasis

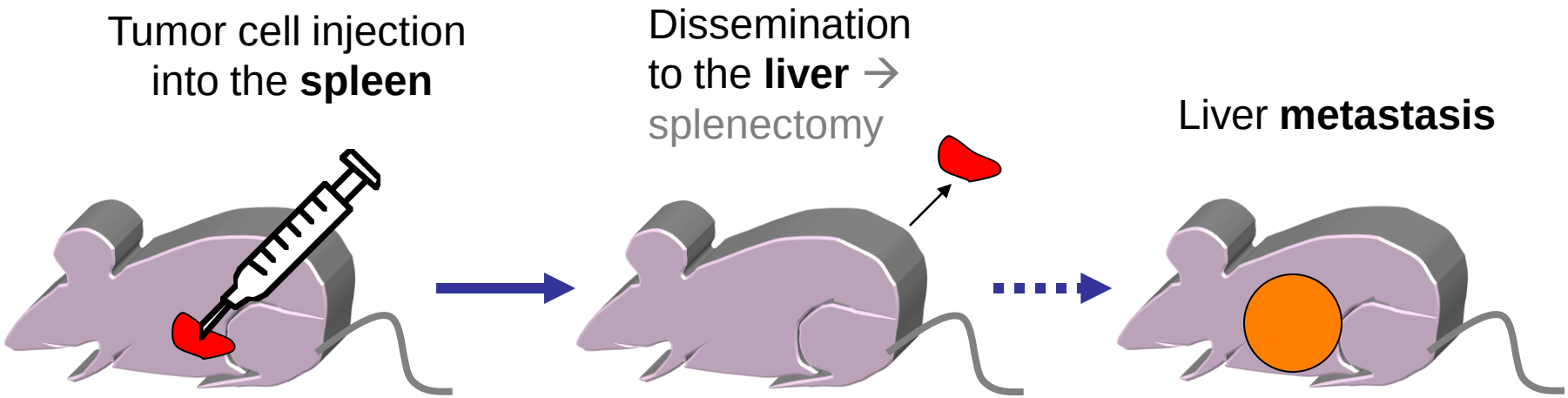


(Modified from *Cancer Statistics in Japan*, 2008)

3a

Q: Do CCR1^{+} iMCs stimulate metastasis ?

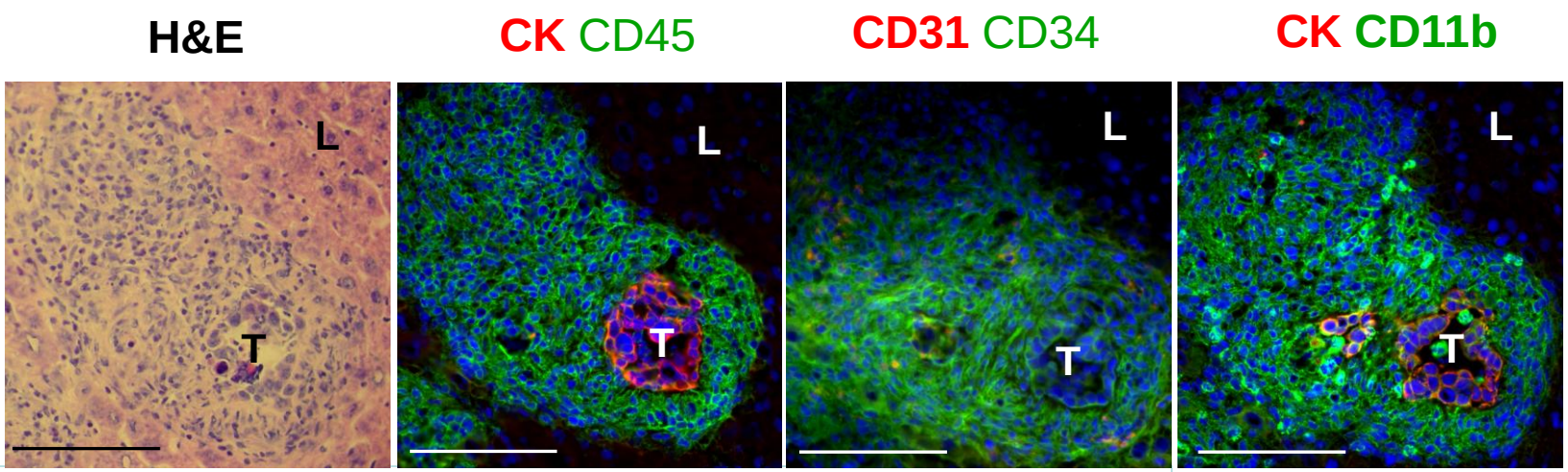
Method: Colon cancer cell line injected into the spleen of the syngeneic mice —> Intrahepatic dissemination —> Metastatic expansion



3a

Colon cancer cells that disseminated to the liver are associated with Cap Cells (iMCs)

Metastatic foci of CMT93 mouse colon cancer cells in the liver



CK : tumor cells

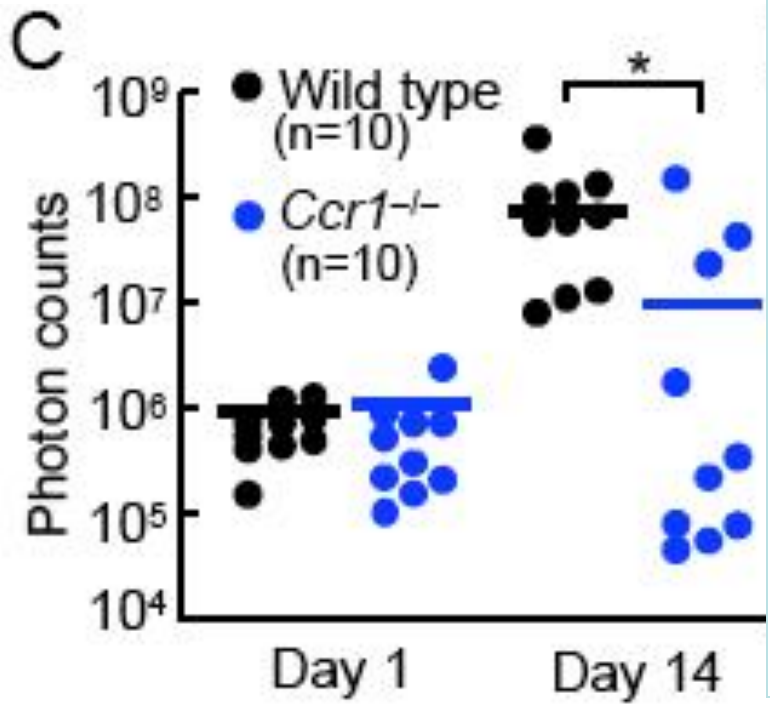
CD45, CD34, CD11b } iMC markers

F4/80 : macrophages
B2.20 : B-cells
CD3ε : T-cells
αSMA : myofibroblasts

L: liver T: tumor gland

3a

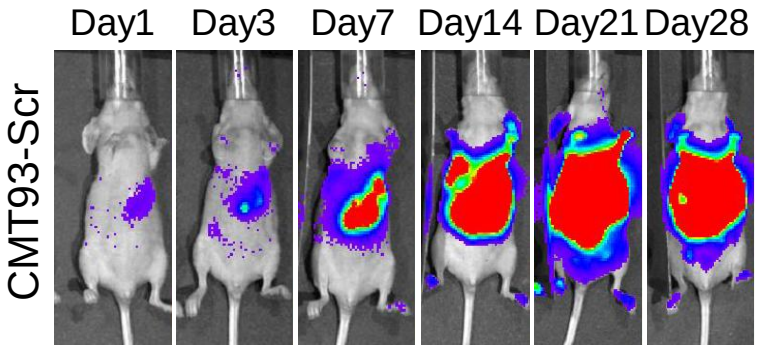
In CCR1 knockout hosts, expansion of the metastatic lesions is suppressed.



3a

Expansion of metastatic lesions is suppressed if cancer cells are inhibited for CCL9 production

In vivo bioluminescent images of mice injected with luciferase-expressing CMT93 cells

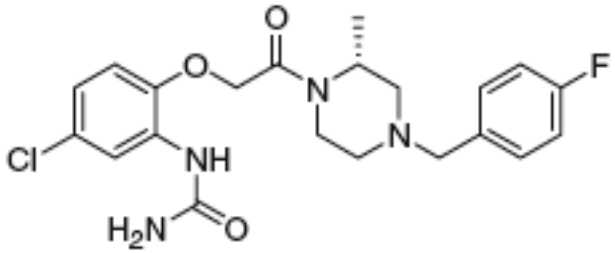


Fluoroscent intensity
(Low ⟶ High)

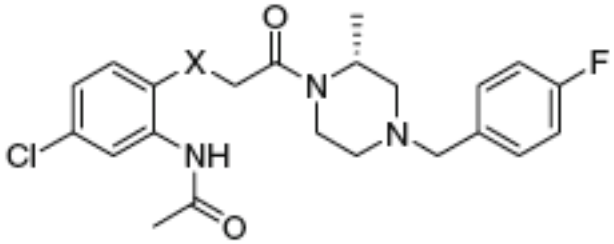
3a

Are iMCs involved in human colon cancer metastasis in the liver?

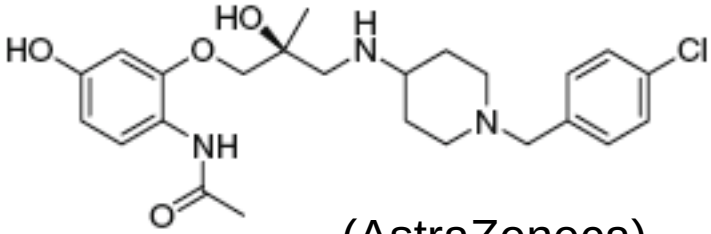
Can **CCR1 inhibitors** block metastasis?



16: BX-471 (Berlex)



17a: X = CH_2
17b: X = NH (Novartis)



25 (AstraZeneca)

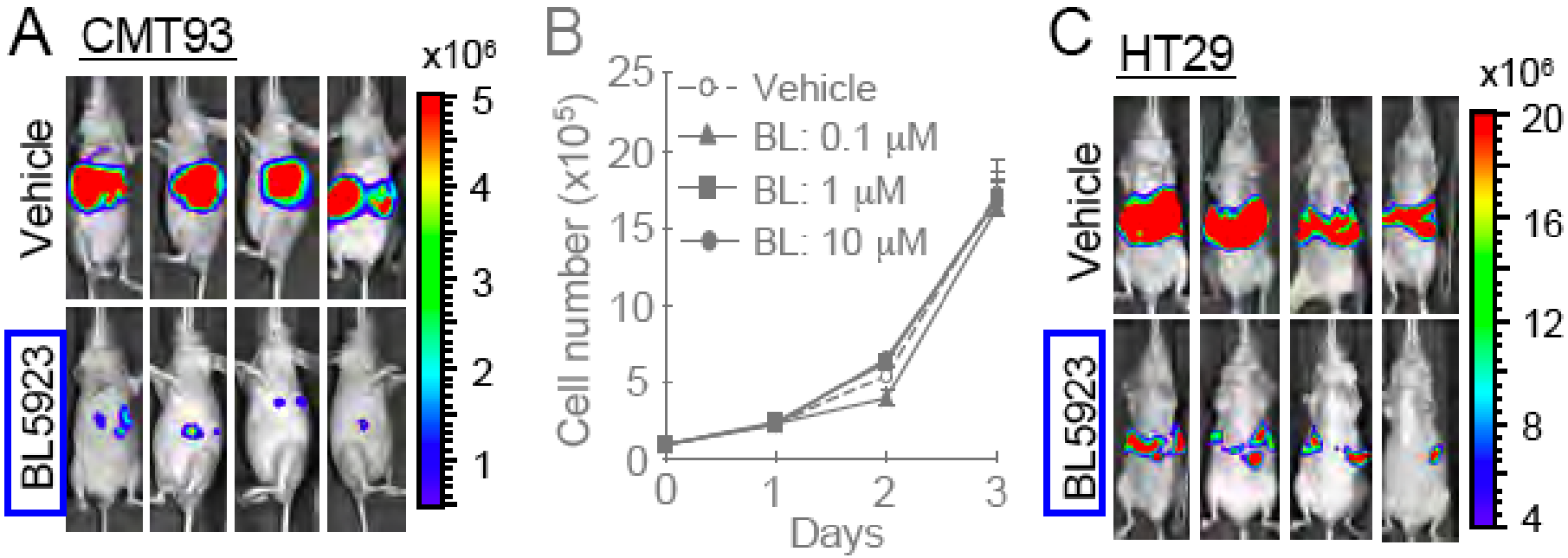
(Cheng, J.-F. and Jack, R. *Mol. Divers.* 12:17, 2008)

3a

CCR1 inhibitor BL5923 suppresses metastatic expansion of colon cancer cells in the liver

mouse

human



(BL5923 has no effects on in vitro proliferation)

3a

Significance of this study in clinical research

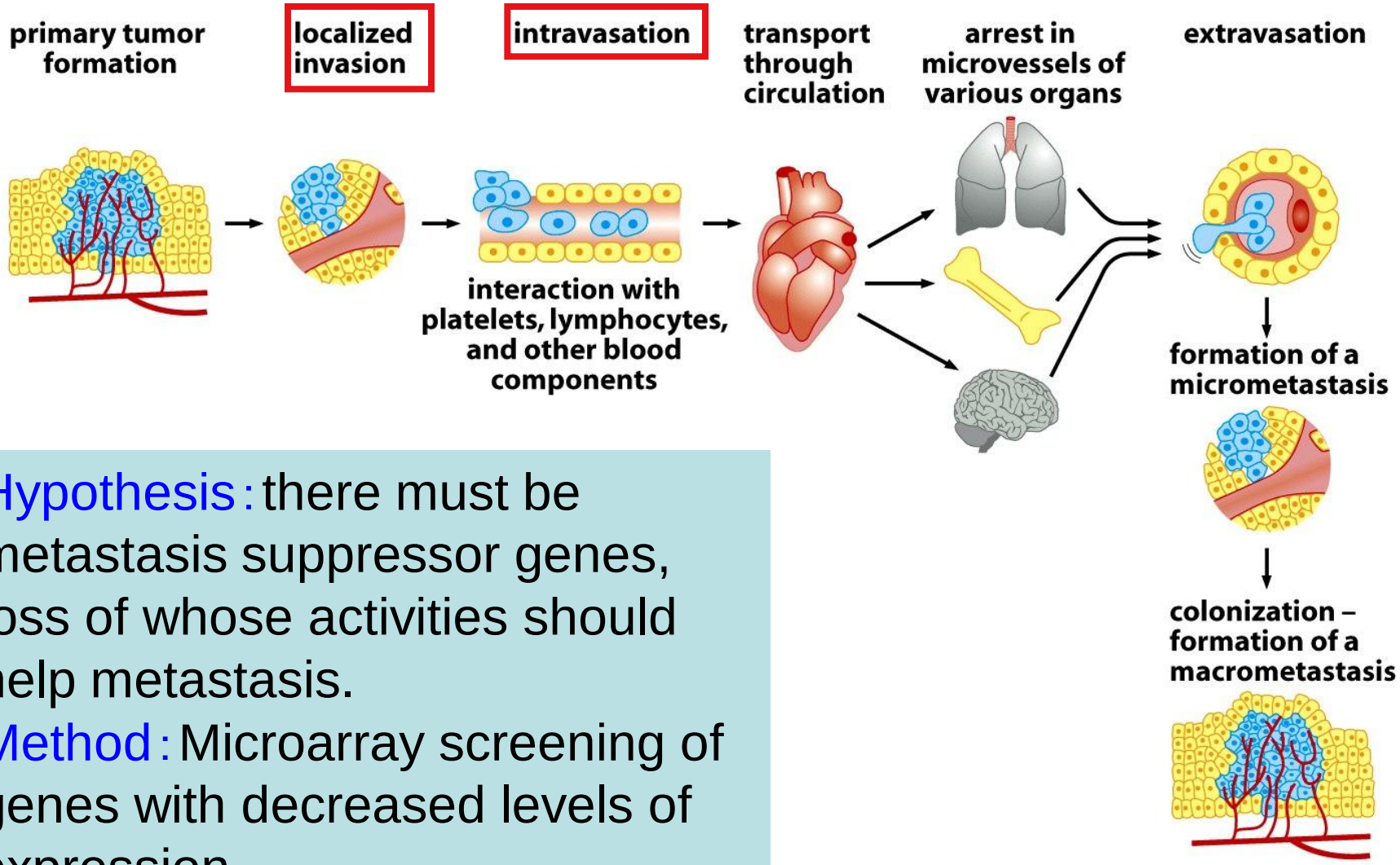
It is known that various proteases, especially metalloproteinases are involved in cancer invasion. In fact, many pharmaceutical companies developed inhibitors of MMPs, and more than ten compounds were tested in clinical trials recently. Unfortunately, however, none succeeded in the trials due to severe side effects.

Our present results suggest the possibility that we can block cancer invasion/metastasis by inhibiting the recruitment of MMP-producing iMCs with CCR1 antagonists, rather than by direct inhibition of MMPs systemically:

"Cellular Targeting Therapy"

3b

Elementary processes in cancer metastasis



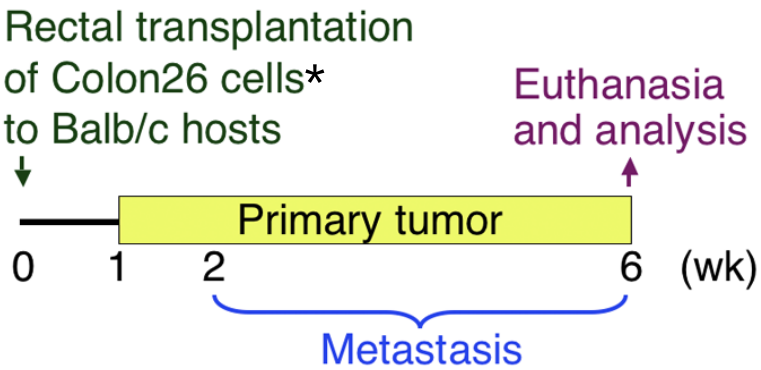
Hypothesis : there must be metastasis suppressor genes, loss of whose activities should help metastasis.

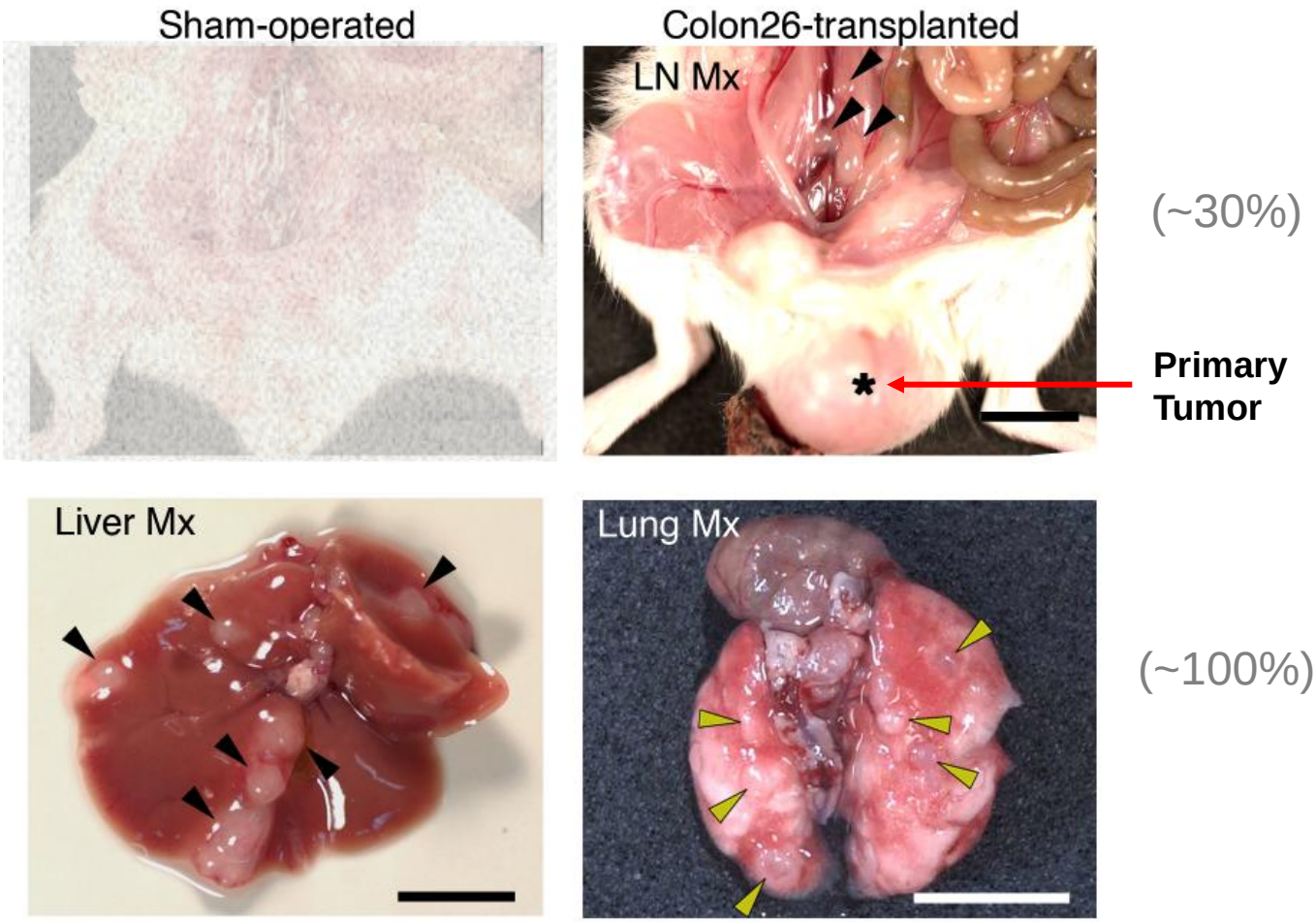
Method : Microarray screening of genes with decreased levels of expression.

3b

Model for colon cancer metastasis: rectal transplantation

*Ras-activated





3b

— Part I —

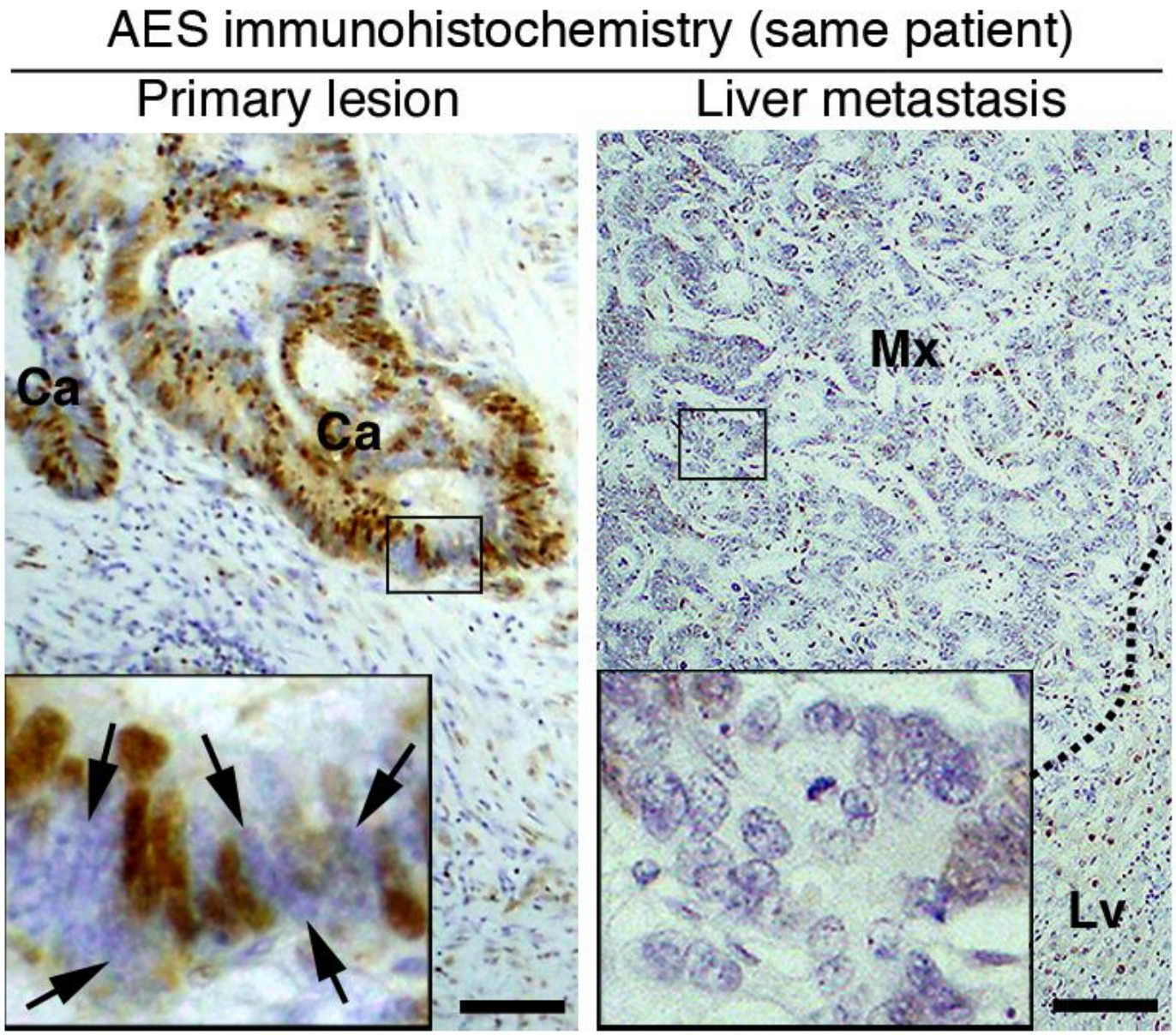
Identification of *Aes* as a metastasis suppressor

Microarray profile comparison between **primary** and **metastatic** tumors.

Focus on the "transcription regulator activity" (Gene Ontology), because some genes in this group such as *CRSP3* and *Twist* were reported to regulate metastatic potential of cancer cells.

3b

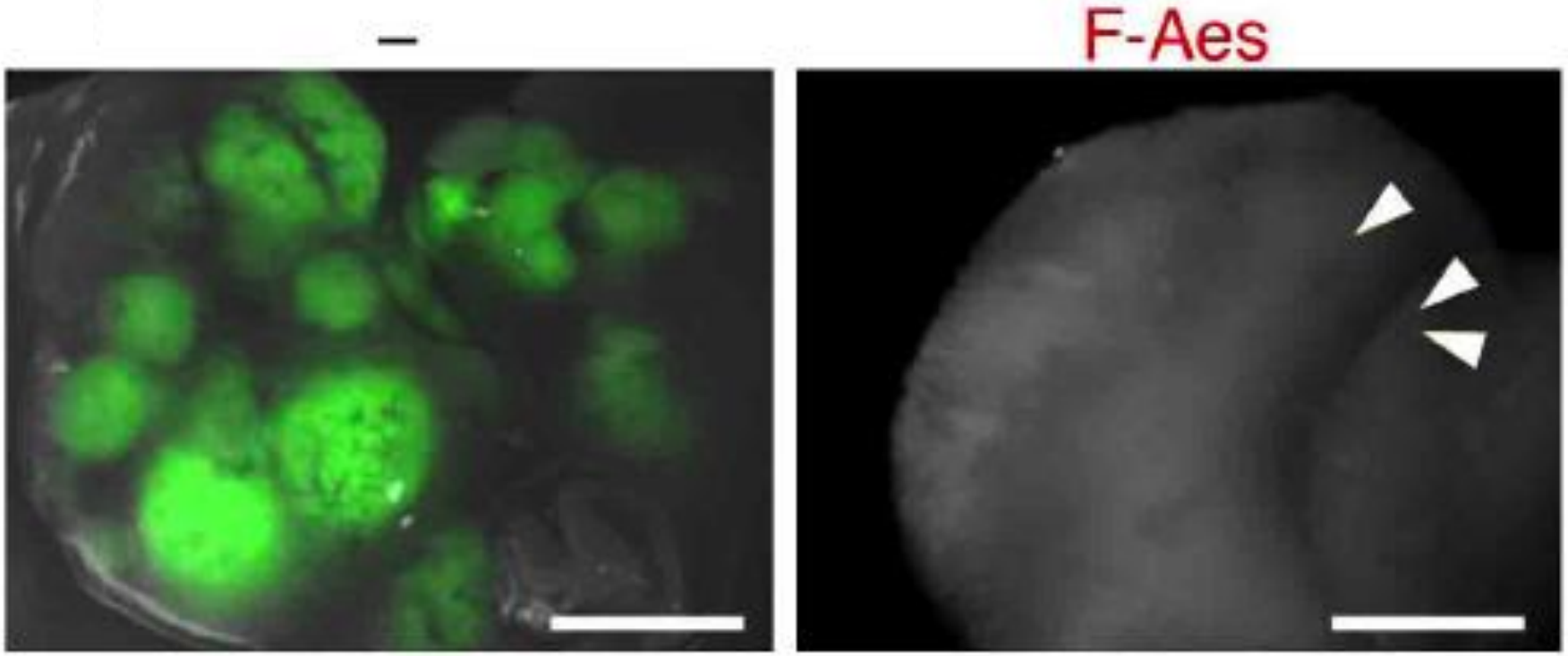
Reduced expression of Aes in colon ca met.



3b

Forced expression of *Aes* suppresses liver met

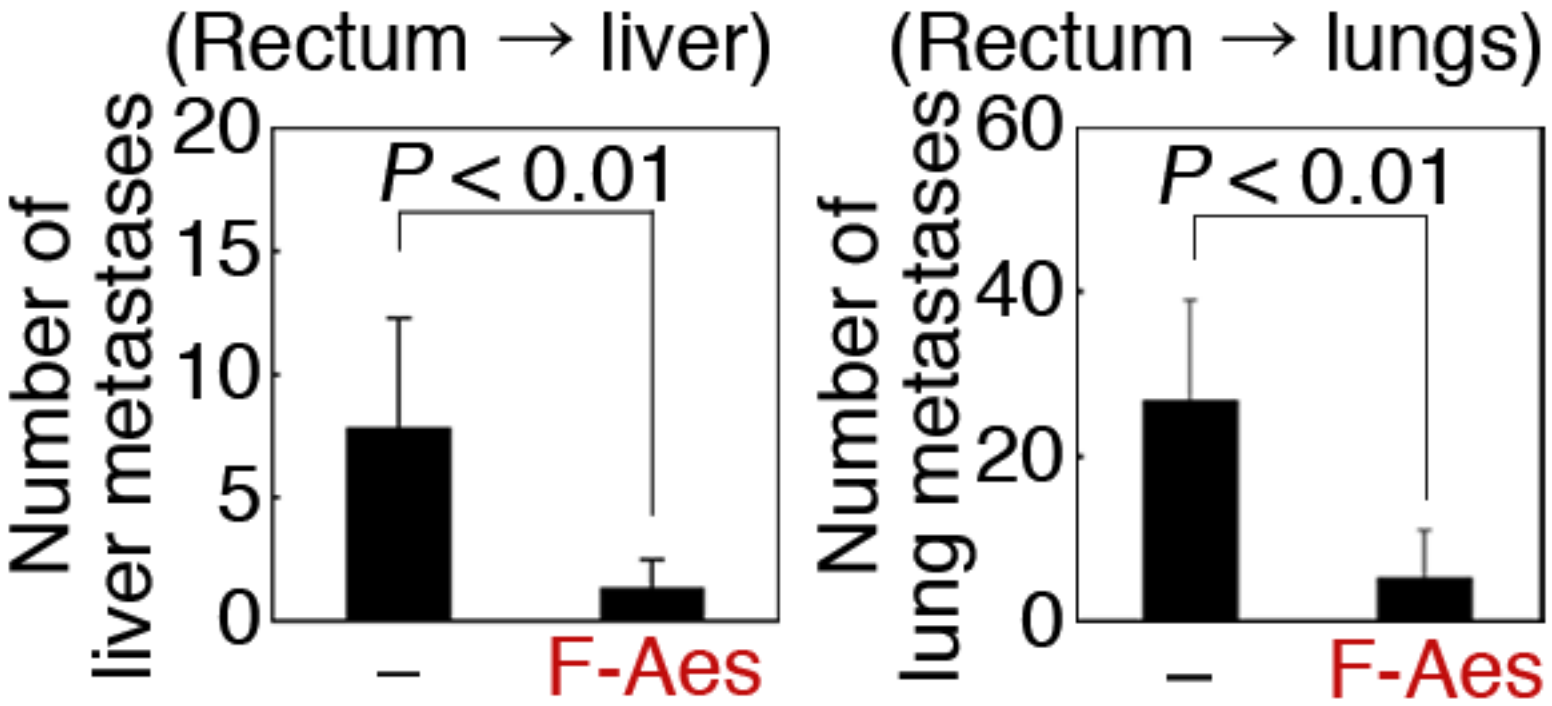
(Syngeneic mouse colon cancer cell transplantation model)



3b

Forced expression of *Aes* suppresses liver met （quantified data）

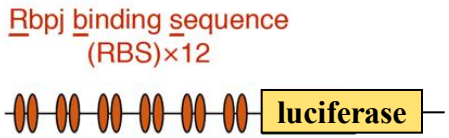
(Syngeneic mouse colon ca cell transplantation model)



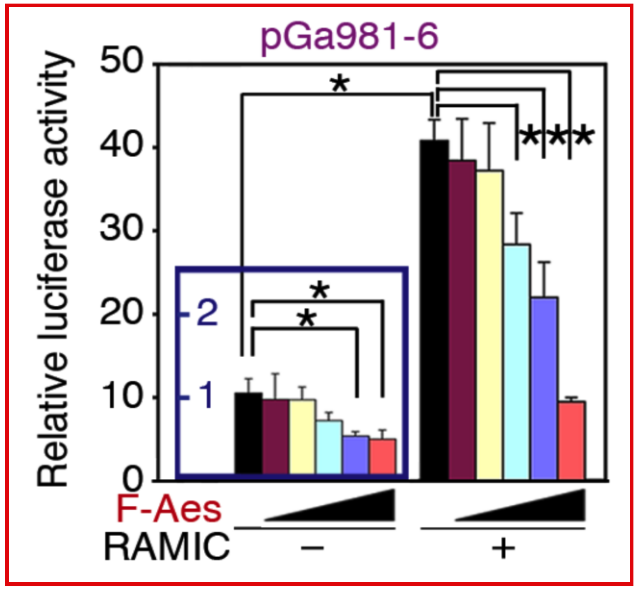
3b

Aes inhibits Notch signaling

Over-expression



pGa981-6 reporter

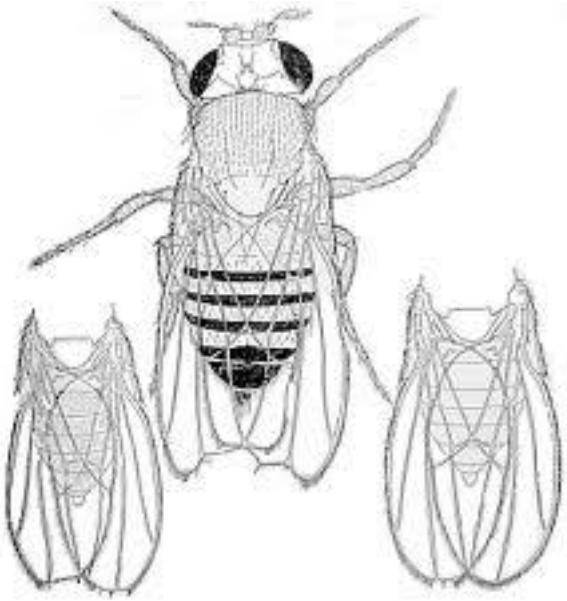


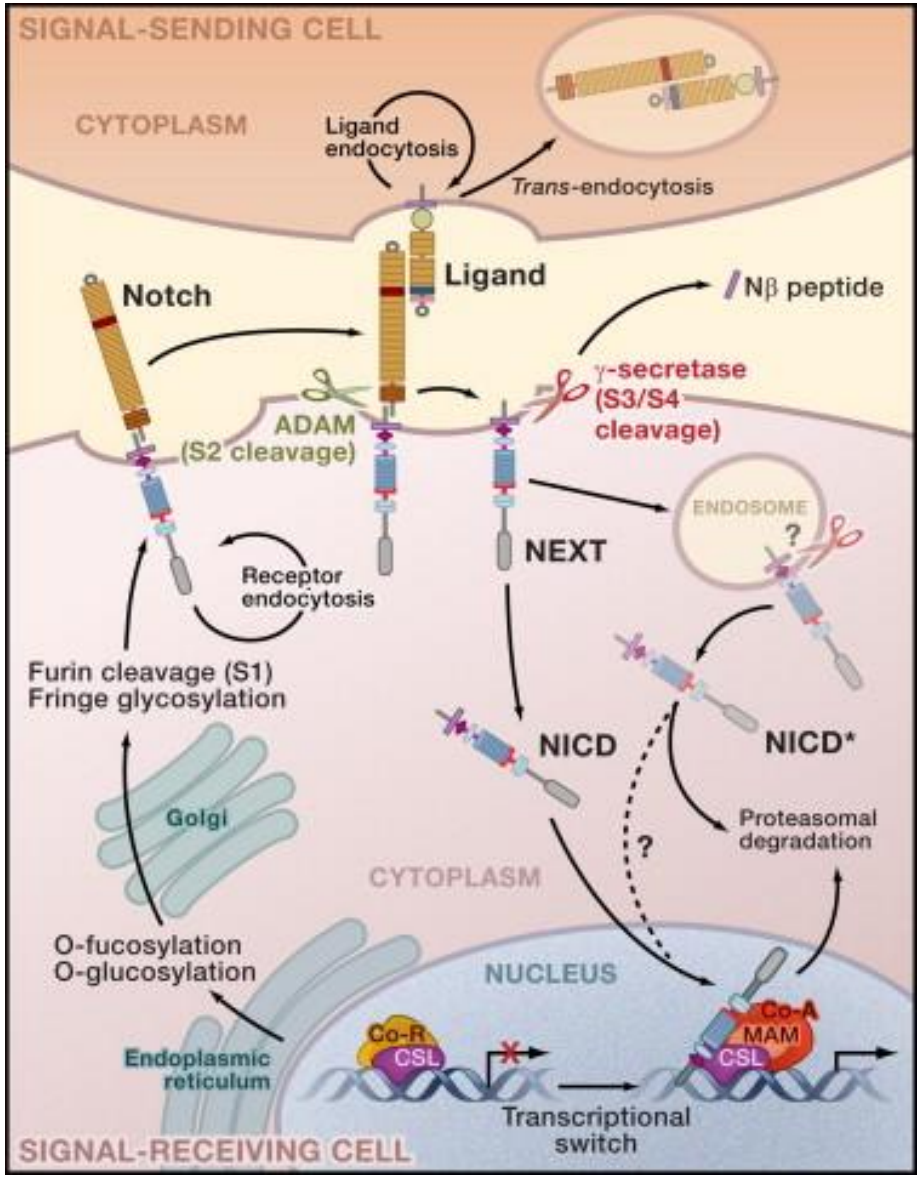
RAMIC: Rbpj-associated molecule domain and intracellular domain of the Notch receptor; equivalent to Notch intracellular domain **NICD**.

(Sonoshita, M. et al., Cancer Cell 19: 125-137, 2011)

3b

Canonical Notch Signaling Pathway





(Kopan & Ilagan, Cell, 2009)

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— Part I —

Aes suppresses colon cancer metastasis

Aes inhibits Notch signaling

— Part II —

Q. Does Notch signaling stimulate metastasis?

Are proteins other than Aes expressed in cancer?

e.g., ligands Jagged1 and/or Dll4

Q. Does inhibition of Notch signaling other than at Aes suppress metastasis?

e.g., RBPJ-KD, GSI (inhibit NICD cleavage)

Q. How does the inhibition of Notch signaling suppress metastasis?

Q. What is the phenotype of *Aes* knockout mice?

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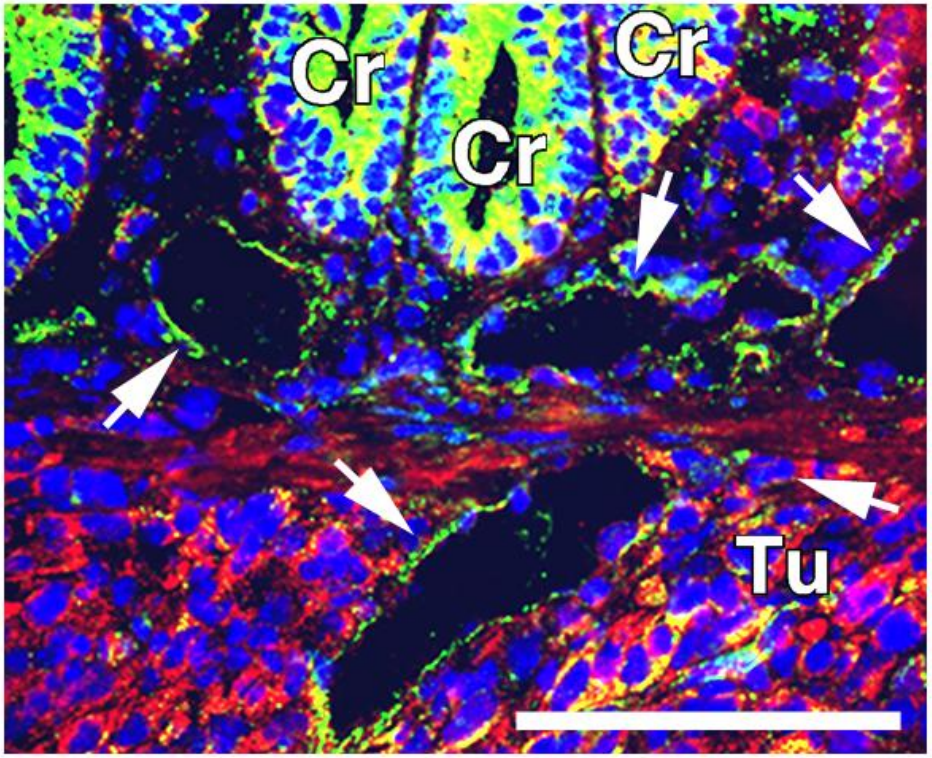
Notch ligand Jagged1 is expressed on vessels of primary colon cancer

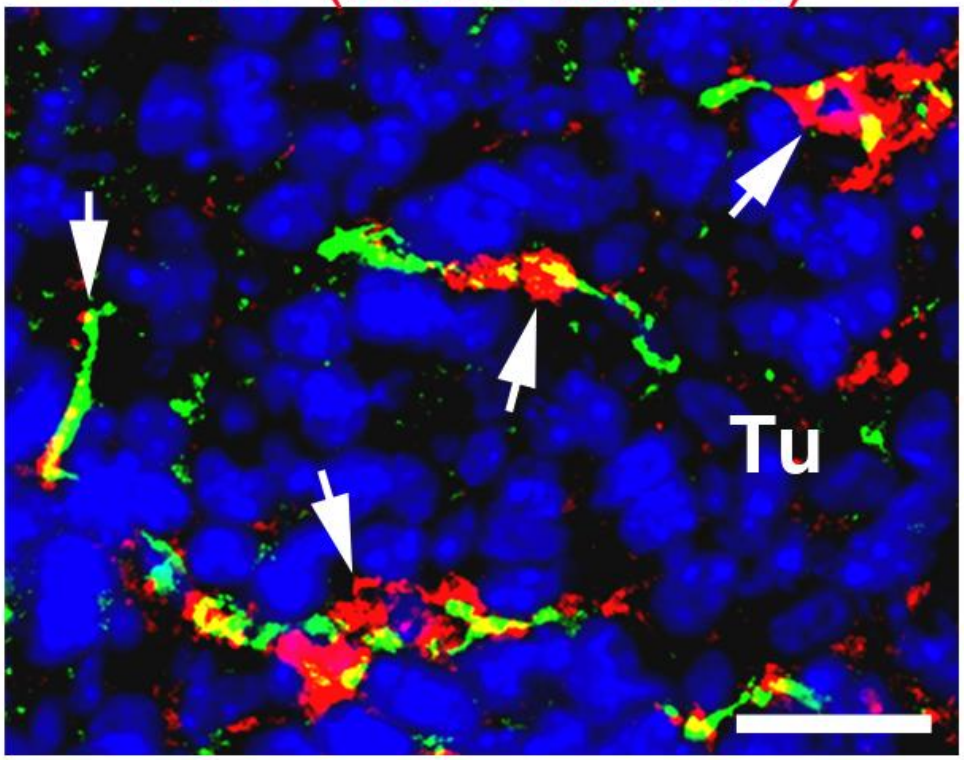
Primary; Jagged1 /DAPI

Notch1



Bar; 100 μm

vWF (blood vessels)



Bar; 10 μm

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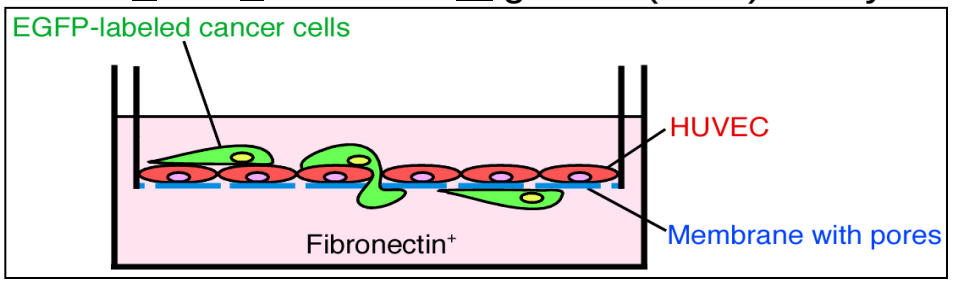
Q. What is the mechanism of metastasis suppression by Notch signaling inhibition?

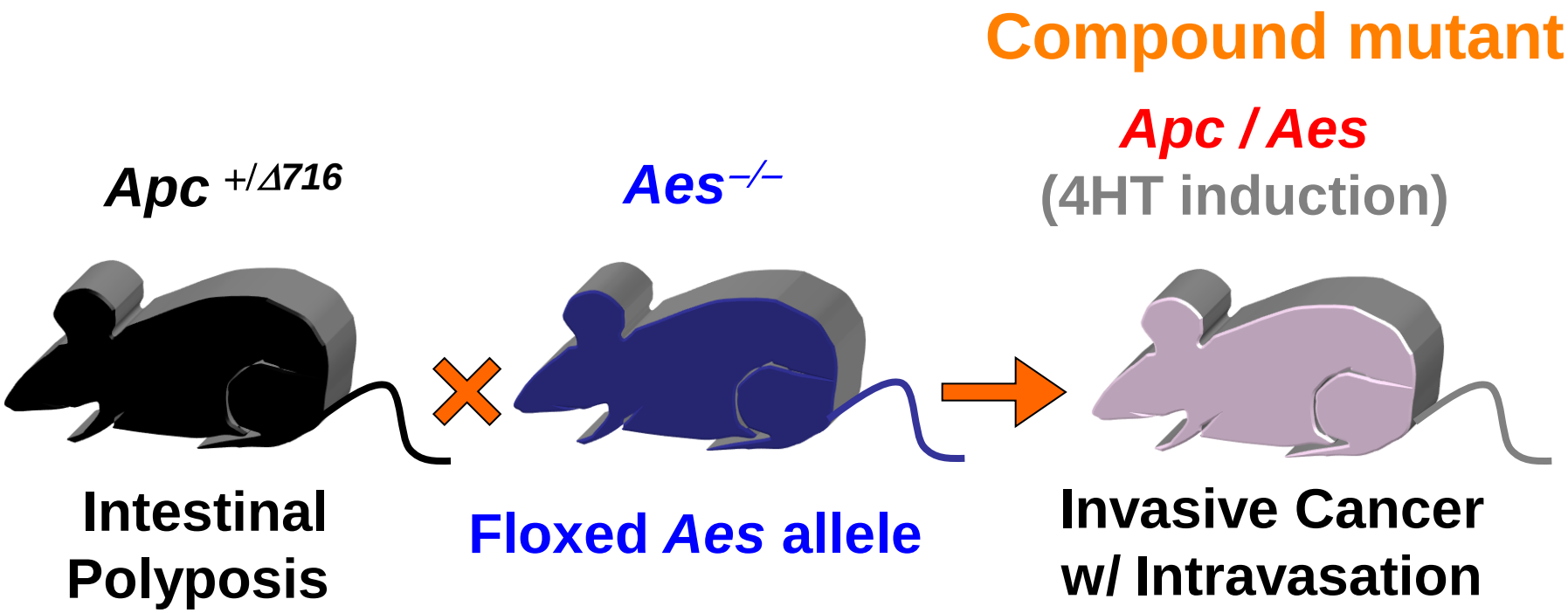
A. Trans-endothelial migration (TEM) relevant to intra-vasation & extra-vasation)

Transendothelial migration (TEM) assay



3b

Whole animal phenotype of *Apc/Aes* mutation in the intestines



(M.M. Taketo)

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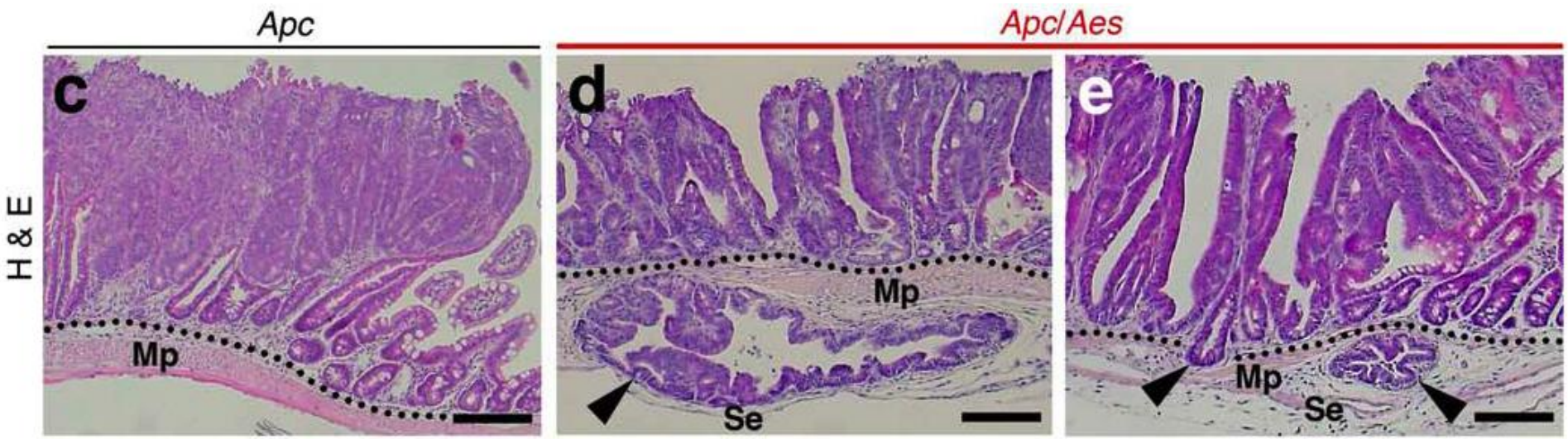
Local invasion and intravasation of *Apc*/*Aes* mouse tumors



Adenoma

Strong local invasion

Dotted lines: *Muscularis propria*

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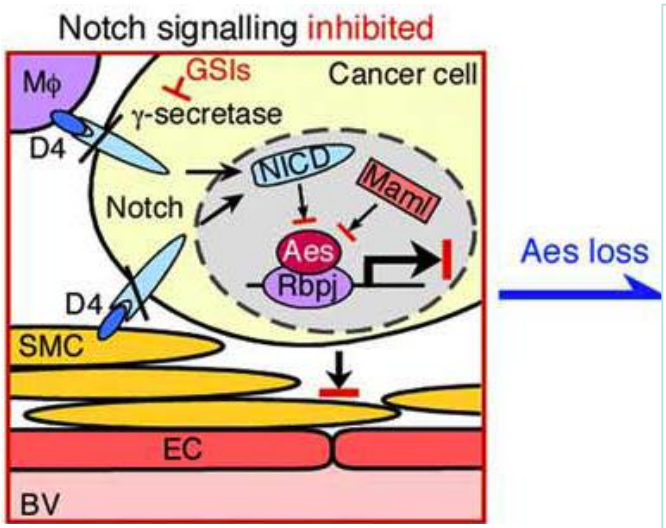
Notch signaling inhibition and metastasis suppression by Aes



Summary

Although colon cancer arises from the mucosal epithelium, the stromal cells play key roles in the expansion of microadenomas（e.g., COX-2 induction).

In locally invasive colon cancer, $CCR1^+$ iMCs are recruited to the invasion front by chemokines (CCL9/15) secreted by the tumor epithelium and produce proteases MMP9/2. Similar iMCs play key roles in metastatic expansion of colon cancer cells disseminated to the liver.

In another model where colon cancer cells metastasize to the liver, lungs and lymph nodes, Notch receptors are activated by the ligands expressed on the stromal cells such as blood vessels, smooth muscle etc. However, if the cancer cells express Aes, transcriptional activation of Notch signaling is inihibited. If Aes is lost in cancer cells, Notch signaling is activated and the cancer cells actively move into (or out of) the blood vessels.

These results collectively indicate that heterotypic interactions of cancer cells with the stroma (i.e., the microenvironment) play key roles in cancer progression.

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