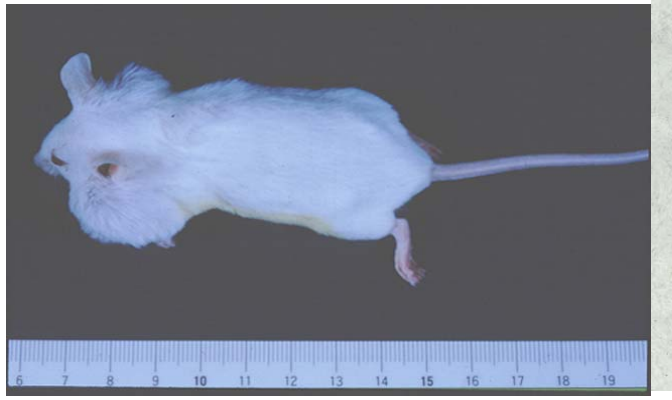
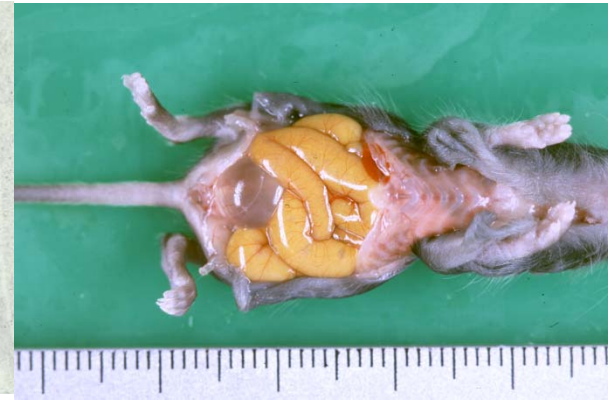
Transgenic Mice and Gene-targeting mice





In Biomedical Science

Techniques of transgenic and gene-targeting mice are indispensable for analyses of in vivo functions of particular genes and roles of their mutations in the development of human disease as well as genetic analyses of interaction of different genes.

Transgenic mouse

To investigate the effects of overexpression of genes in particular organs

Gene targeting mouse

1. Knock out mouse

   To investigate the physiological roles of genes during development

2. Knock-in mouse

   To investigate the effects of gene mutations found in diseases

3. Conditional knock out mouse

   To investigate the physiological roles of genes in particular organs at particular developmental stages

Design of Transgene

- Tissue-specific enhancer/promoter
- Effect of overexpression of cDNA in vivo
- Several copies~several hundred copies of transgene are tandemly integrated into chromosome

Advantage and disadvantage of transgenic mice

Advantage

1. Technique is not complicated.
2. Transgene can be overexpressed in particular organs

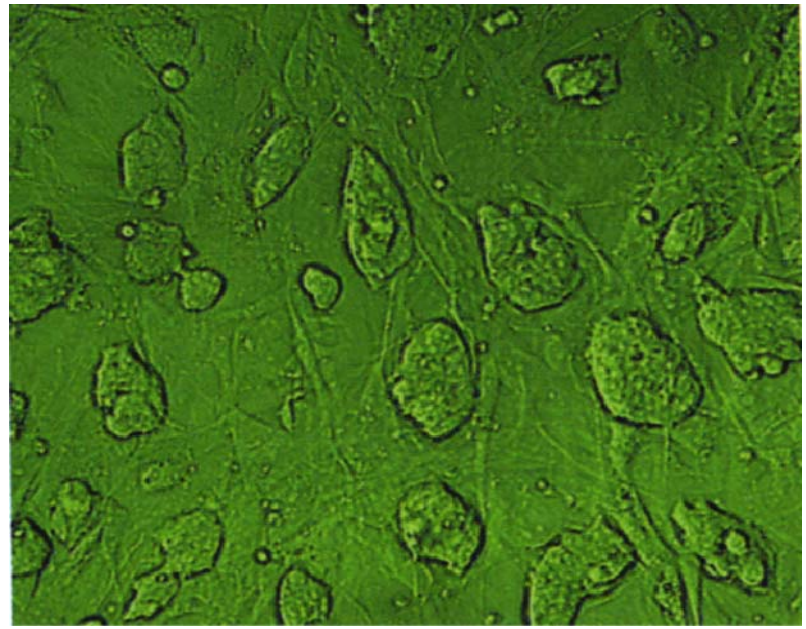
Disadvantage

1. Expression levels of transgene cannot be controlled
2. Integration site of the transgene is unpredictable

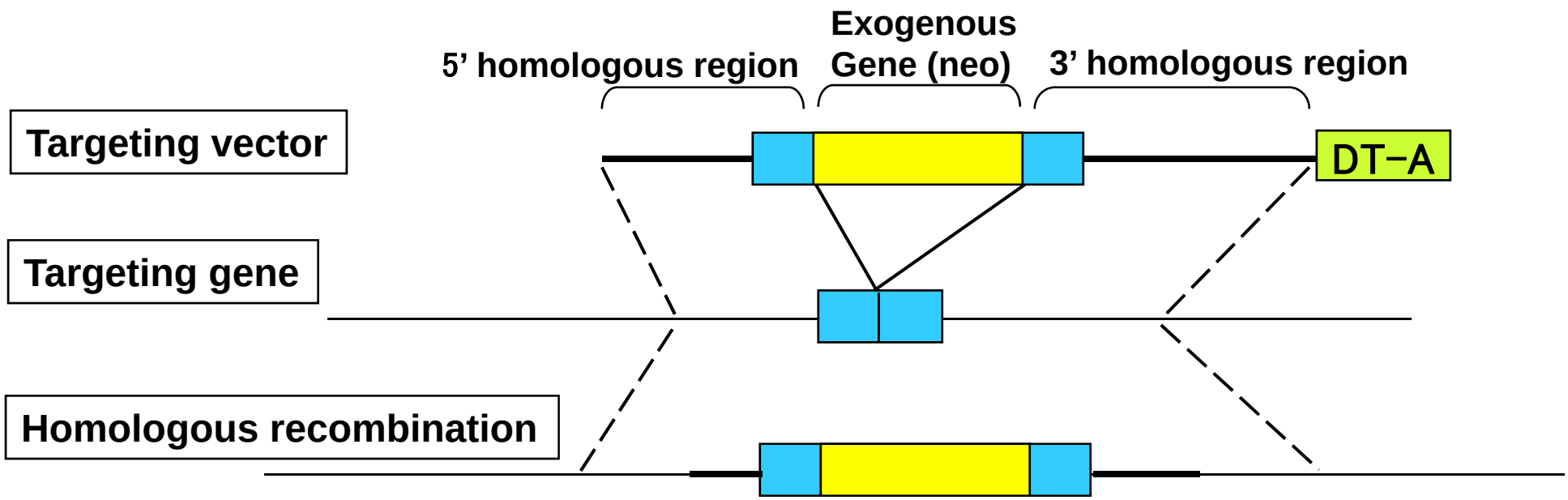
Mouse Embryonic Stem Cells (ES Cells)

ES cells are established from inner cells of mouse blastcysts

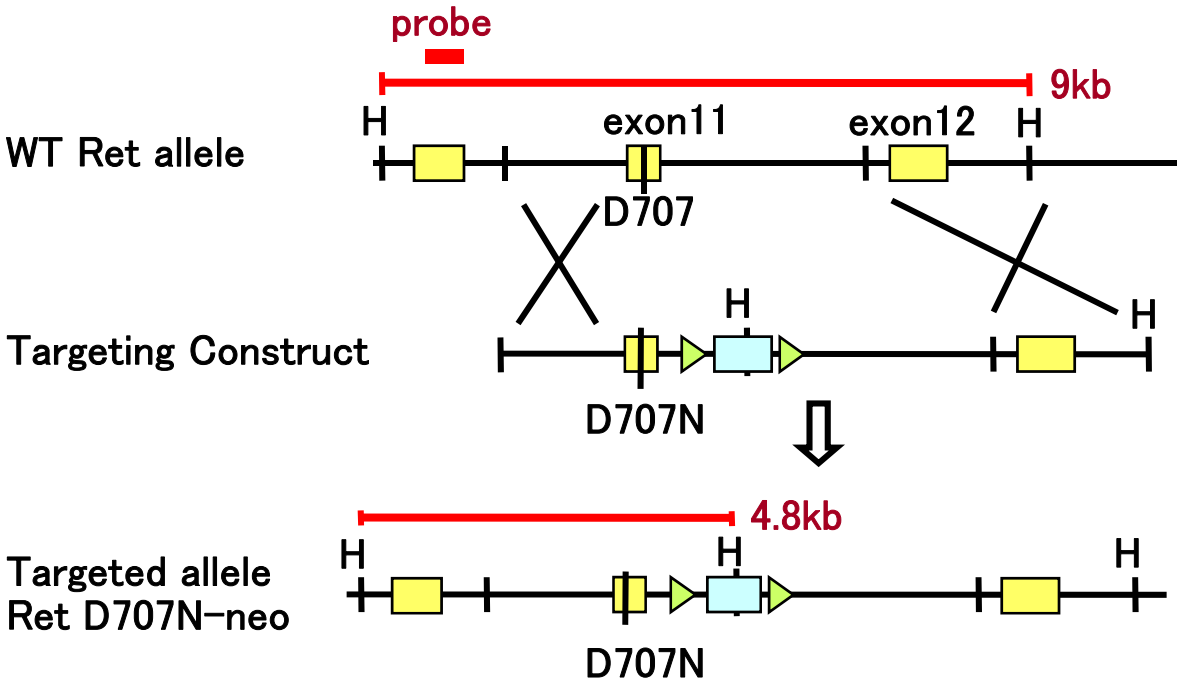
Culture conditions (including selection of serum) are critical to maintain the character of stemness

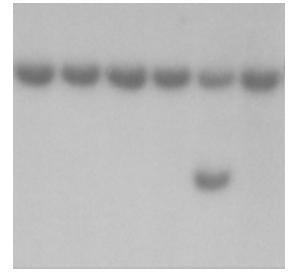


Generation of targeting vector



Selection of positive ES clones by Southern blotting

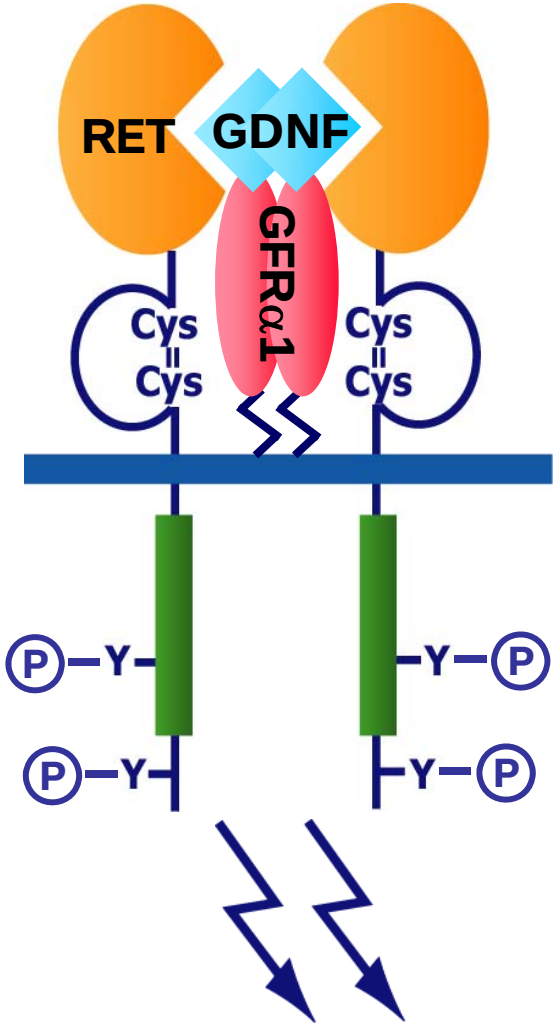




← 9.0k WT allele

← 4.8k targeted allele

GDNF/RET signaling



RET: receptor tyrosine kinase

Neuronal survival / differentiation
（eg. Enteric Nervous System)
Kidney development
(ureteric bud branching)
Spermatogenesis

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Activation of a Novel Human Transforming Gene, *ret*, by DNA Rearrangement

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Summary

A novel transforming gene was detected by transfection of NIH 3T3 cells with human lymphoma DNA. The tumor DNA induced a single focus in primary transfections, whereas DNAs of transformed NIH cells induced transformation with high efficiencies in secondary and tertiary assays. Molecular clones spanning about 37 kb of human sequence were isolated from tertiary transformant DNA. Blot hybridization indicated that the transforming gene consisted of two segments that were unlinked in both normal human and primary lymphoma DNAs. The two segments of human DNA were cotranscribed in transformed NIH cells but not in any human cells examined. The transforming gene thus appeared to be activated by recombination between two unlinked human DNA segments, possibly by cointegration during transfection.

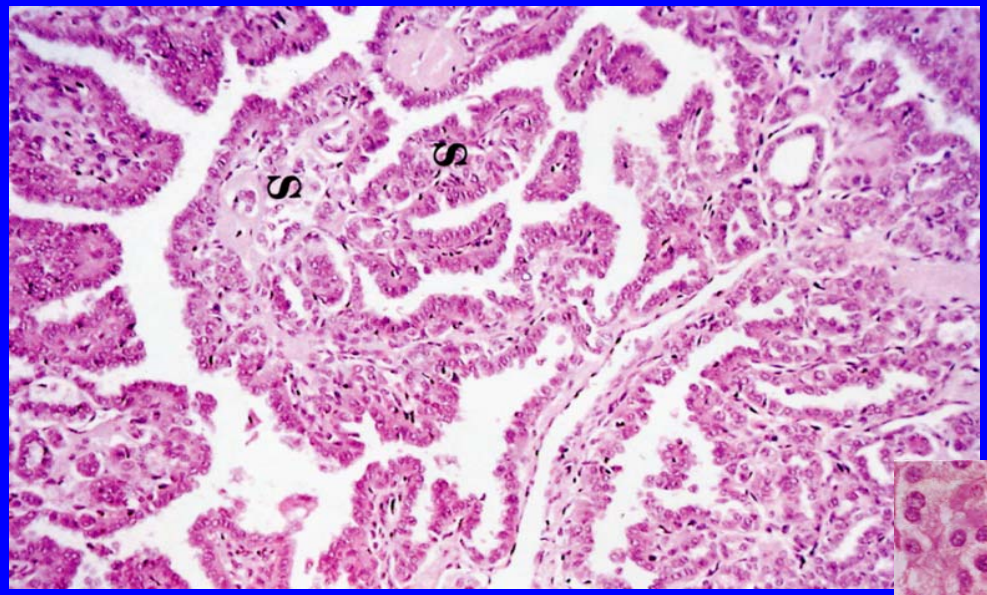
other transforming genes have not been established, blot hybridization analyses indicate that activation of *Blym-1*, *Tlym-1*, and *met* does not involve gene amplification or gross DNA rearrangements (Diamond et al., 1983; Lane et al., 1984; C. S. Cooper et al., 1984b).

Transfection of NIH 3T3 cells has also been used to investigate the potential transforming activity of genes of normal cells. Sonicated fragments (0.5-5 kb) of DNAs of normal chicken fibroblasts, nontransformed mouse fibroblasts (G. M. Cooper et al., 1980), and normal human lymphocytes (Schafer et al., 1984) induced transformation of NIH 3T3 cells with low efficiencies (approximately 0.001-0.003 transformants/μg DNA). In contrast, high efficiencies (0.1-1 transformants/μg DNA) of transformation were observed in secondary transfection assays of high molecular weight DNAs from NIH cells transformed by normal cell DNAs (G. M. Cooper et al., 1980; Schafer et al., 1984). Normal mouse DNAs were also reported to induce transformation of both NIH 3T3 and BALB/c 3T3 cells infected with murine leukemia viruses (Krump-Konvalinkova and van den Berg, 1980). Furthermore, cotransfection with normal NIH 3T3 DNA and retroviral LTR sequences was found to activate cellular transforming genes, one of which was the cellular homolog of the retroviral *raf* gene (Muller and Muller, 1984). Similarly, ligation of molecular clones of the normal cellular homologs of the retroviral oncogenes *mos* (Blair et al., 1981) and *ras*^{H} (DeFeo et al., 1981) to retroviral LTRs activated their transforming potential. These results indicate that potential

RET Mutations in Human Diseases

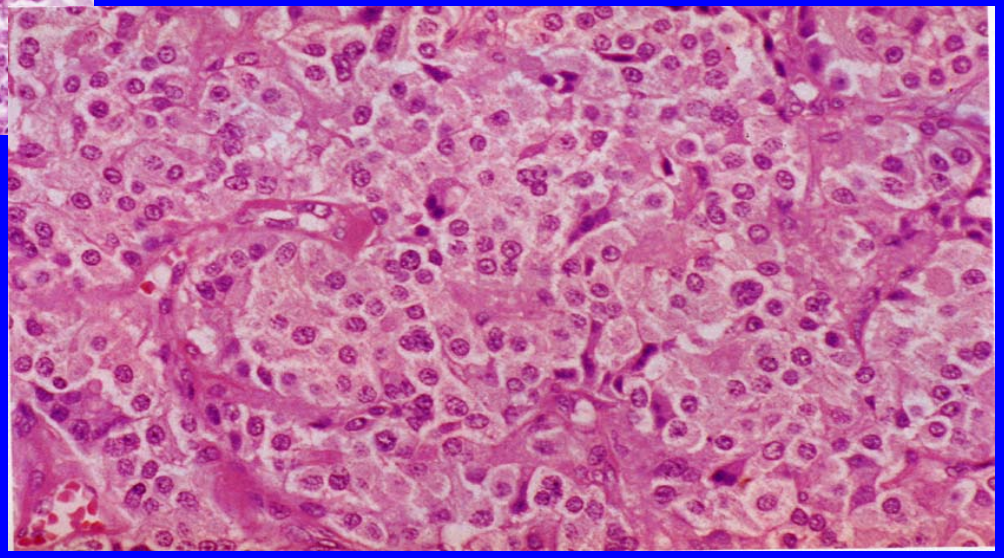
Disease	*RET* Mutations
Papillary Thyroid Carcinoma	Rearrangement Cell (1990)
MEN 2A & MEN 2B	Point Mutations (Nature 1993, 1994)
Hirschsprung's Disease	Point Mutations Frame Shift, Deletion (Nature 1994)

Thyroid carcinoma

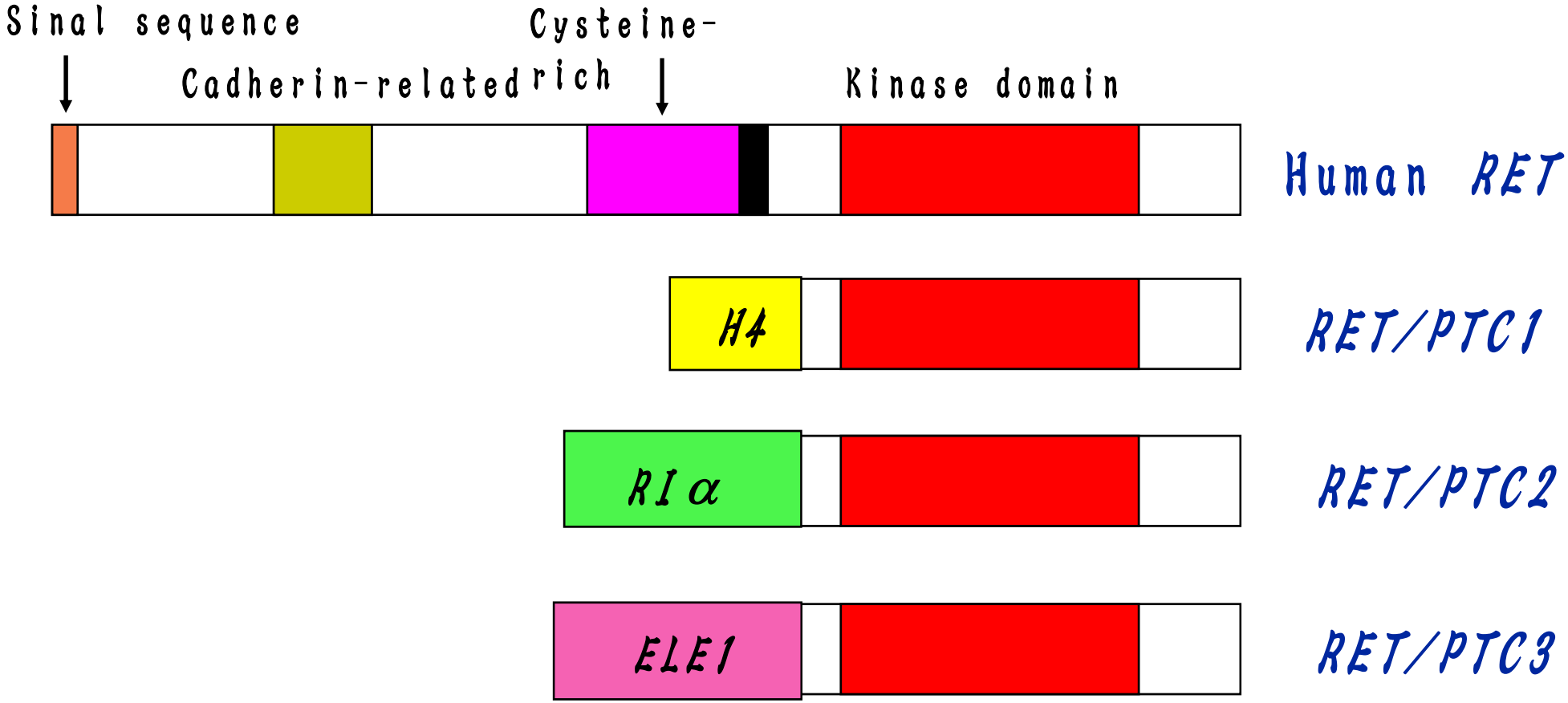


Papillary carcinoma

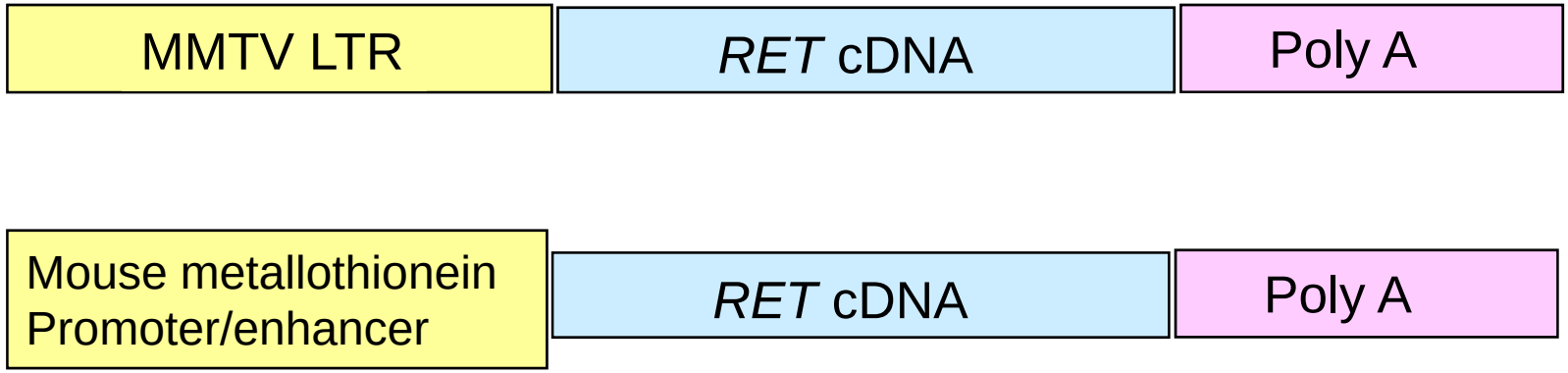
Medullary carcinoma

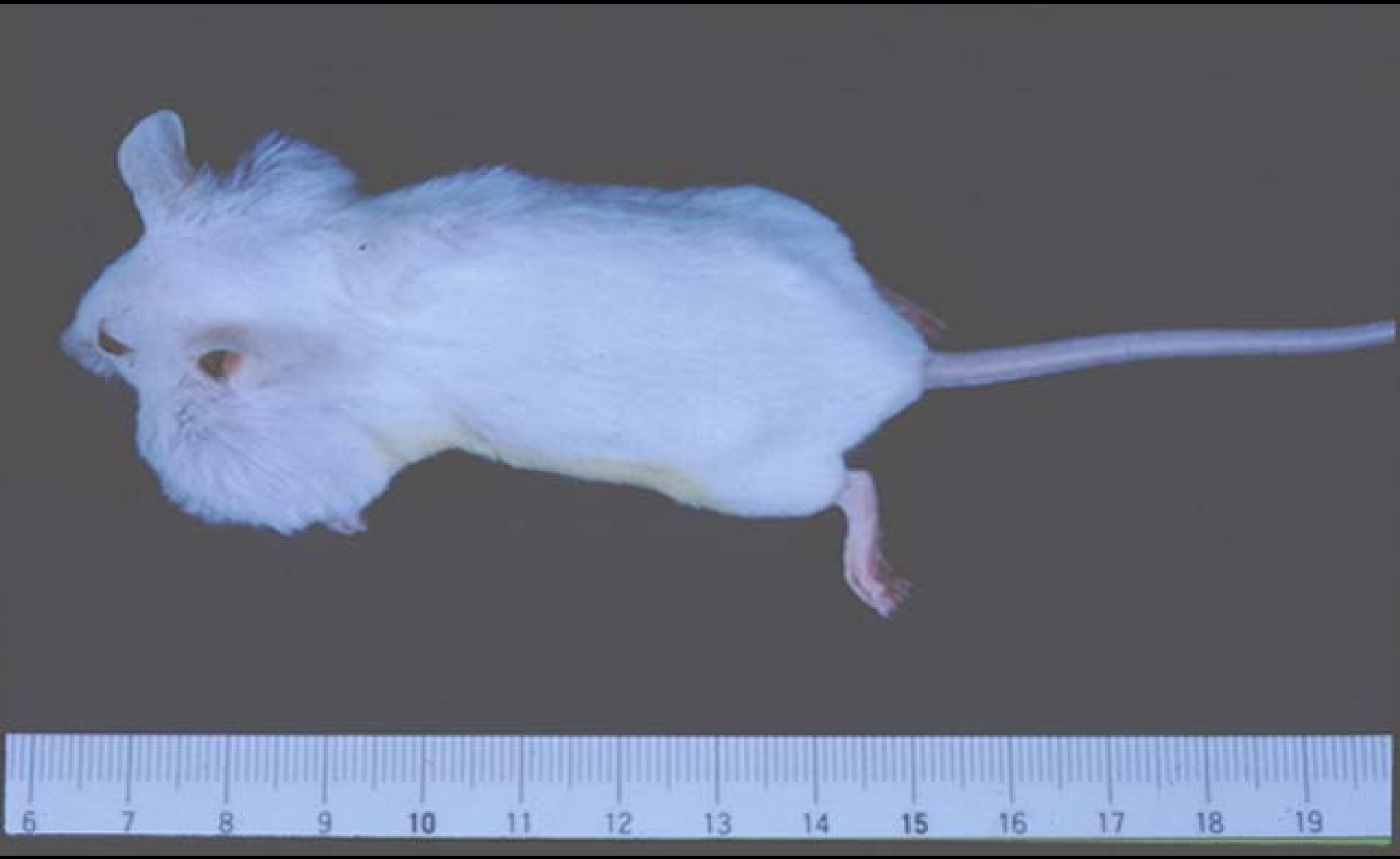


RET Rearrangement in Papillary Thyroid Carcinoma



Design of *RET* Transgene

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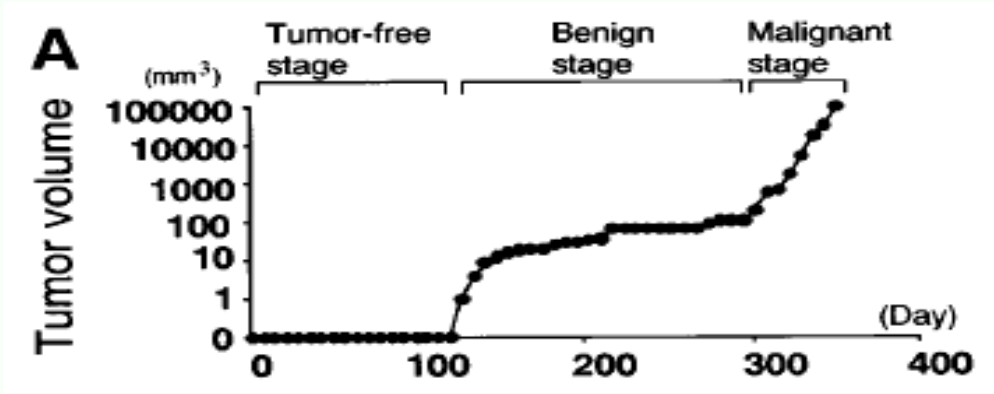
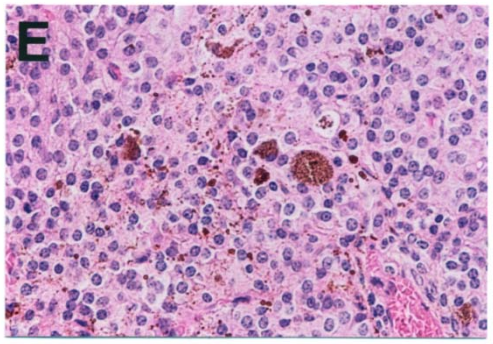
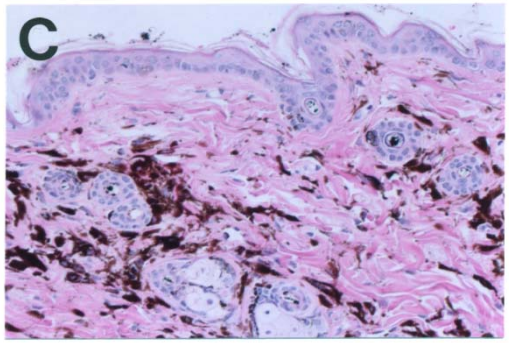
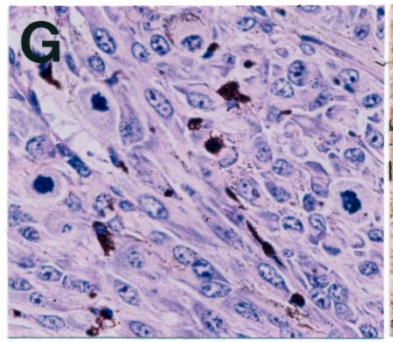
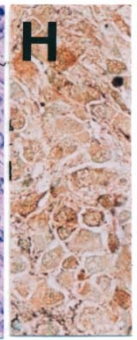
Pigment cell tumor developed in RET-transgenic mice







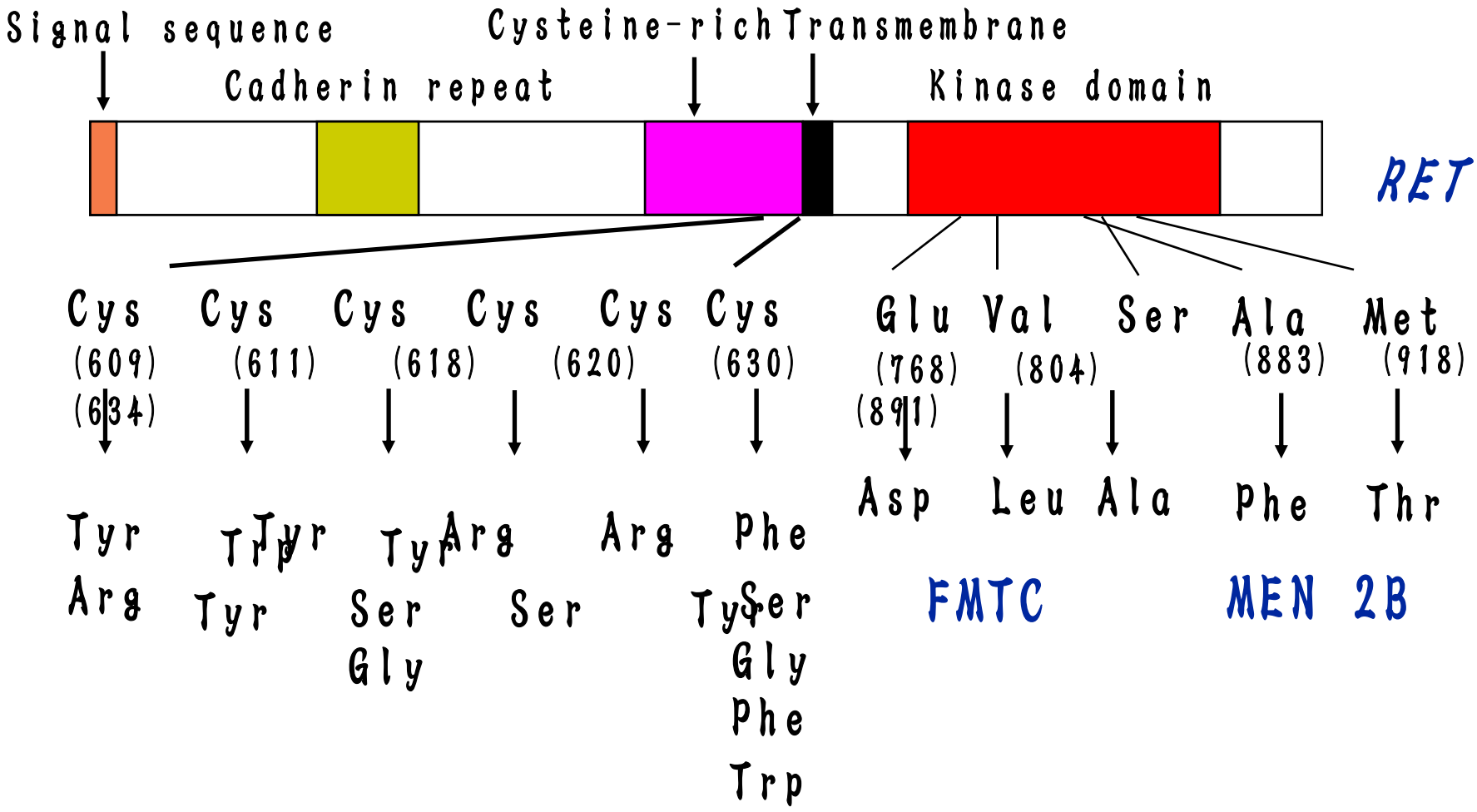


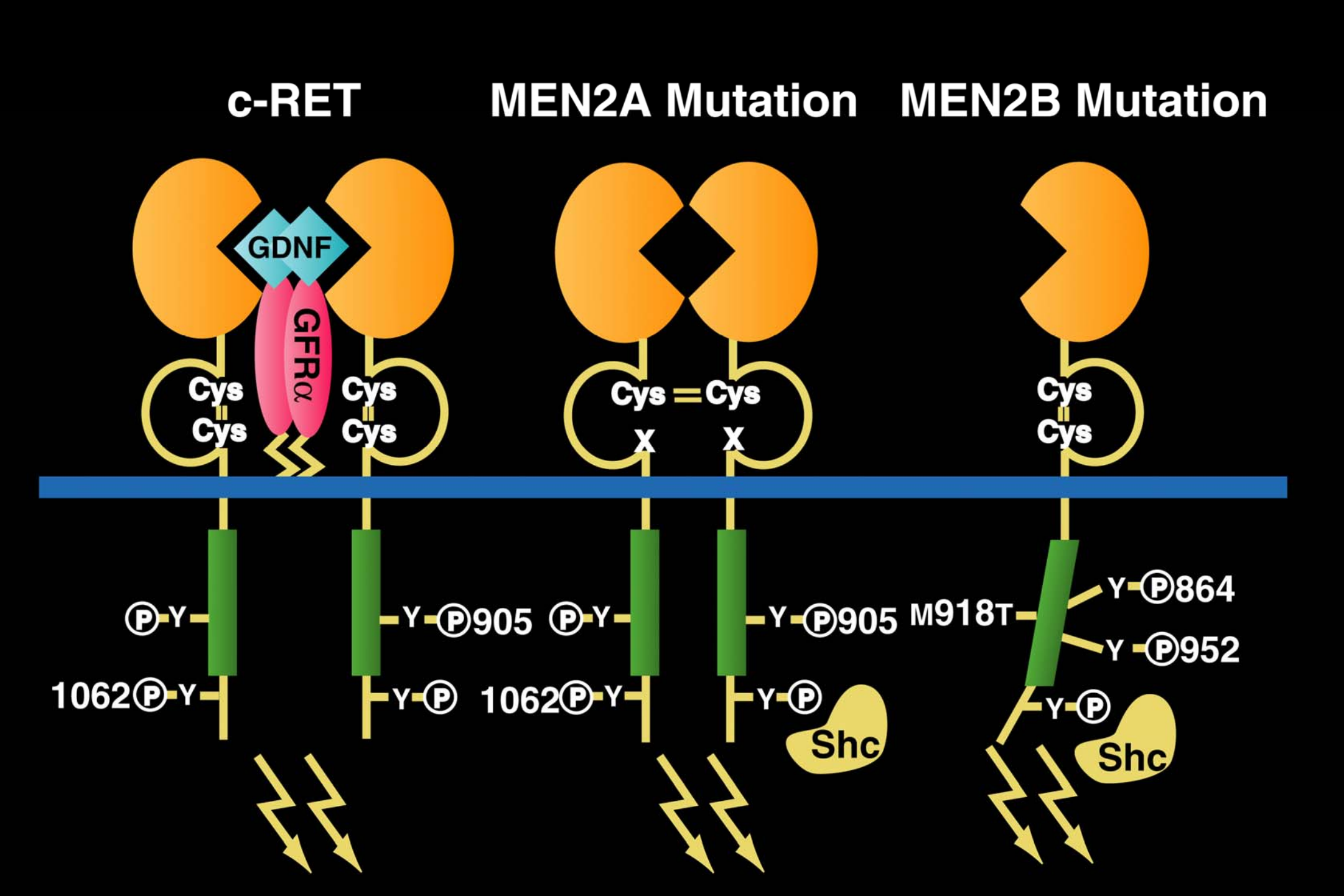


Clinical phenotype in Multiple Endocrine Neoplasia 2

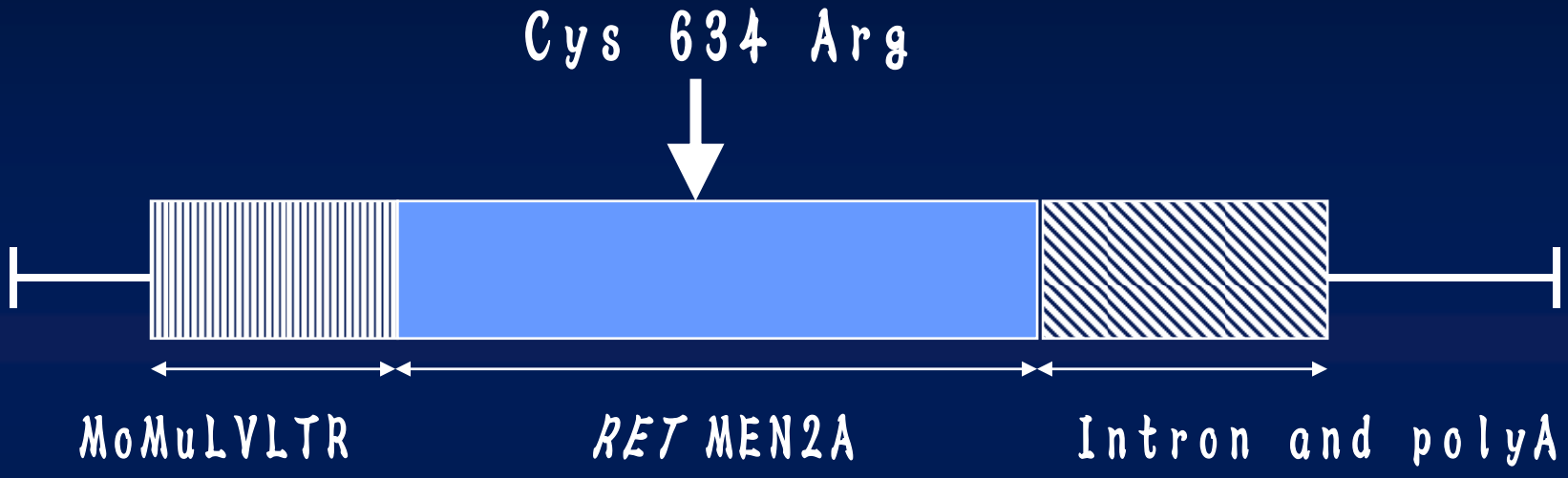
MEN 2A	MEN 2B	FMTC
Medullary Thyroid Carcinoma	Medullary Thyroid Carcinoma	Medullary Thyroid Carcinoma
Pheochromocytoma	Pheochromocytoma	
Parathyroid Hyperplasia	Mucosal Neuroma	
	Hyperganglionosis of the GI Tract	
	Marfanoid Habitus	

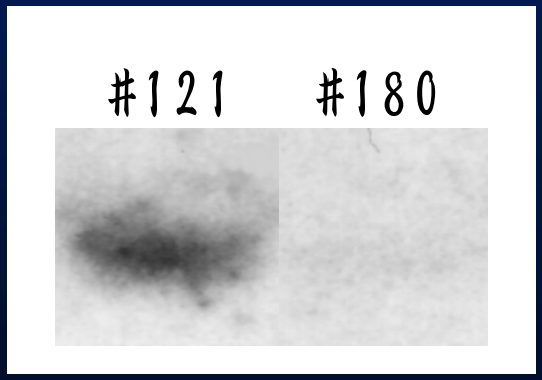
RET Mutations in MEN 2A, MEN 2B and FMTC



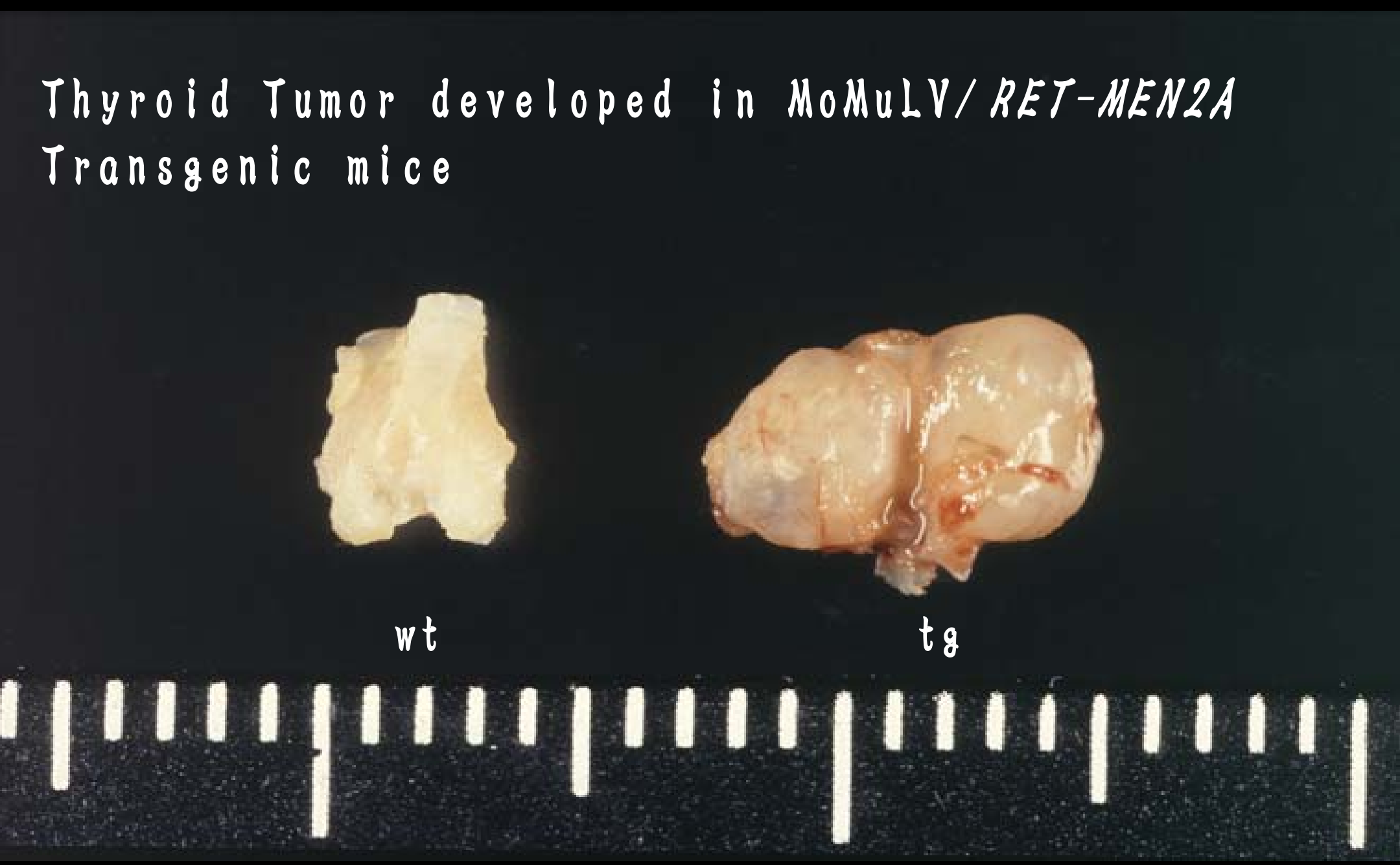

c-RET
MEN2A Mutation
MEN2B Mutation
GDNF
GFRα
Cys
Cys
Cys
Cys
Cys
Cys
Cys
Cys
X
X
P Y
1062 P Y
Y P 905
Y P
P Y
1062 P Y
Y P 905
Y P
Shc
M918T
Y P 864
Y P 952
Y P
Shc

Construct of *RET-MEN2A* gene for Transgenic Mice

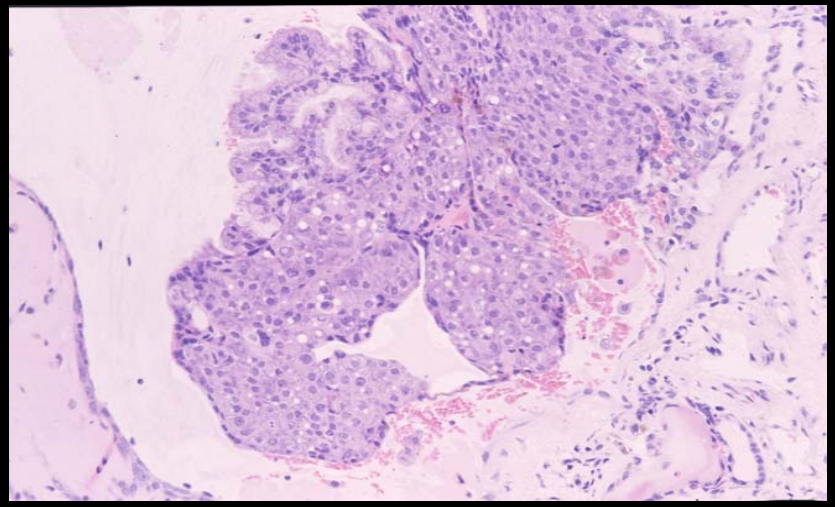




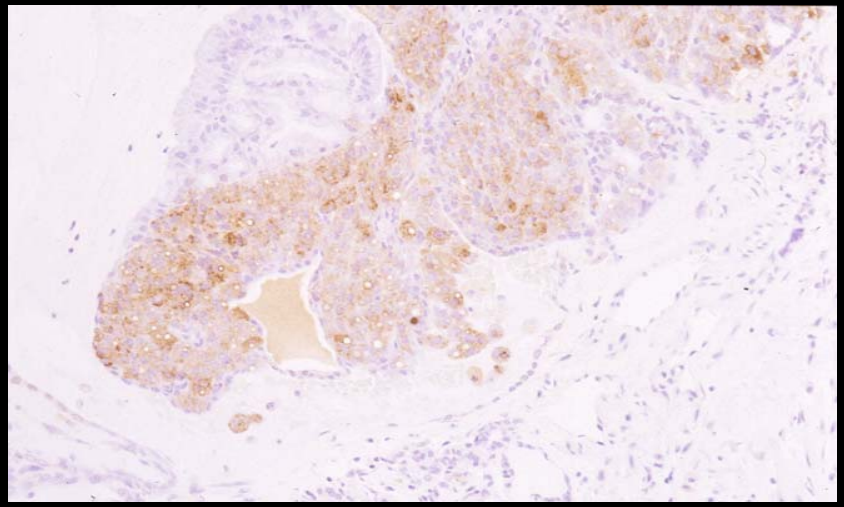
Thyroid Tumor developed in MoMuLV/*RET-MEN2A* Transgenic mice



Medullary Thyroid Carcinoma developed in RET-MEN2A Transgenic Mice



HE Staining

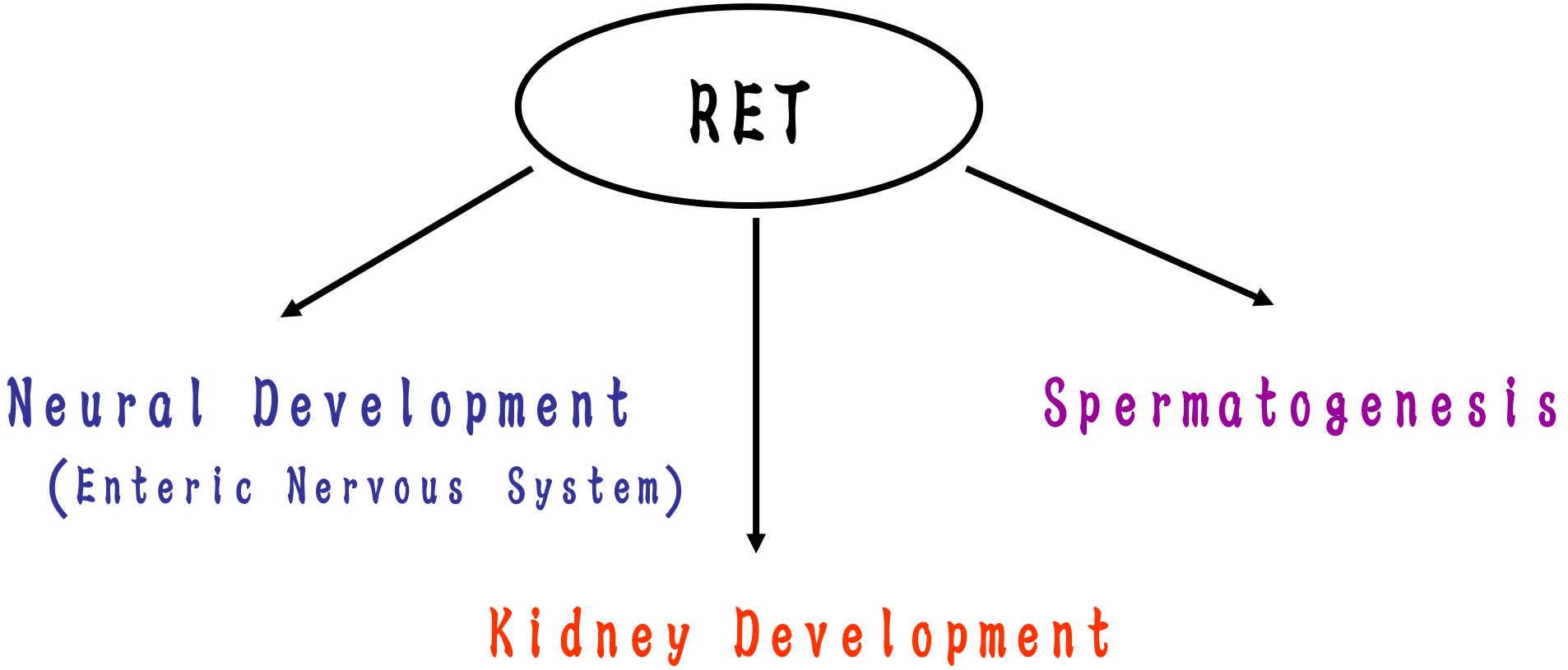


Anti-calcitonin antibody

Serum Calcitonin Level in Transgenic Mice

phenotype	No. animal	Serum calcitonin level (pg/ml)
Bilateral MTC	9	320±192
Unilateral MTC	4	138±58
CCH	6	83±48
Normal control	17	42±13

Physiological functions of RET



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Hirschsprung's Disease	Point Mutations Frame Shift, Deletion (Nature 1994)

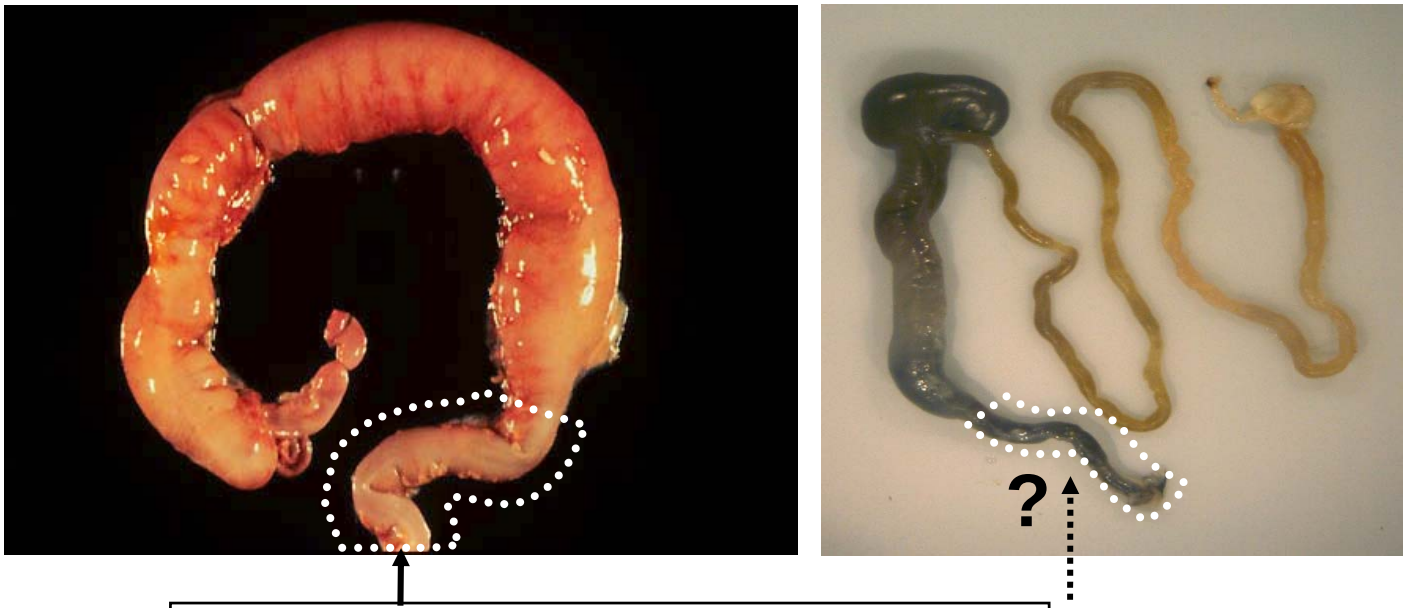
Hirschsprung's disease

A common pediatric disorder
with an incidence of 1/5000 births

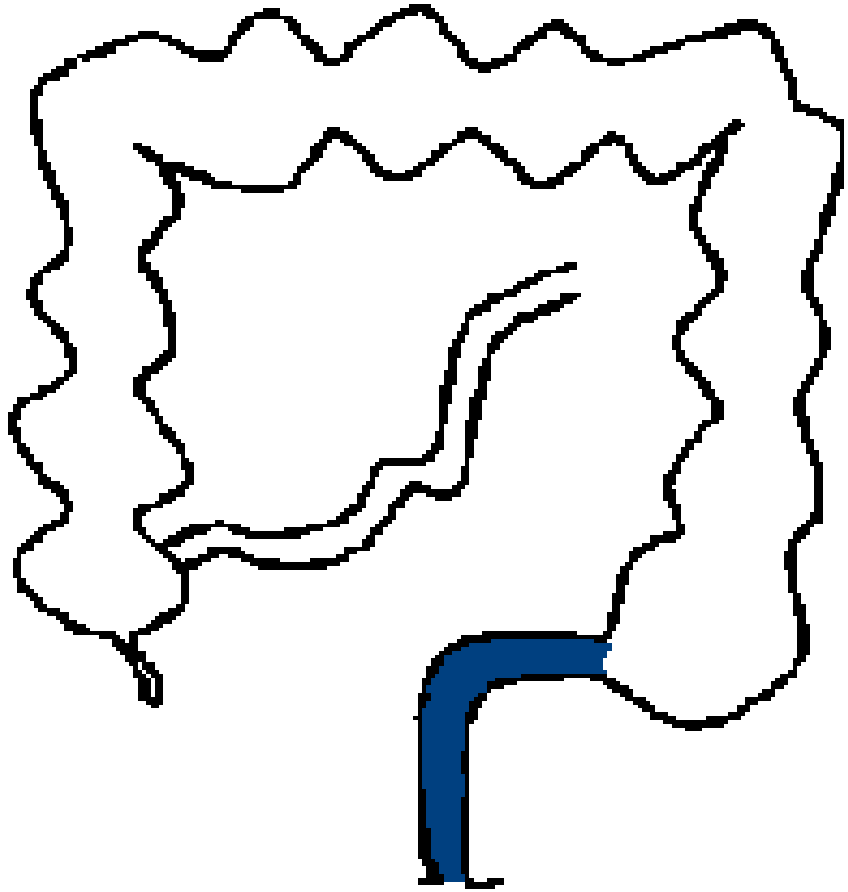
The congenital absence of enteric neurons
in the distal region of the colon

A marked intestinal dilation (megacolon)

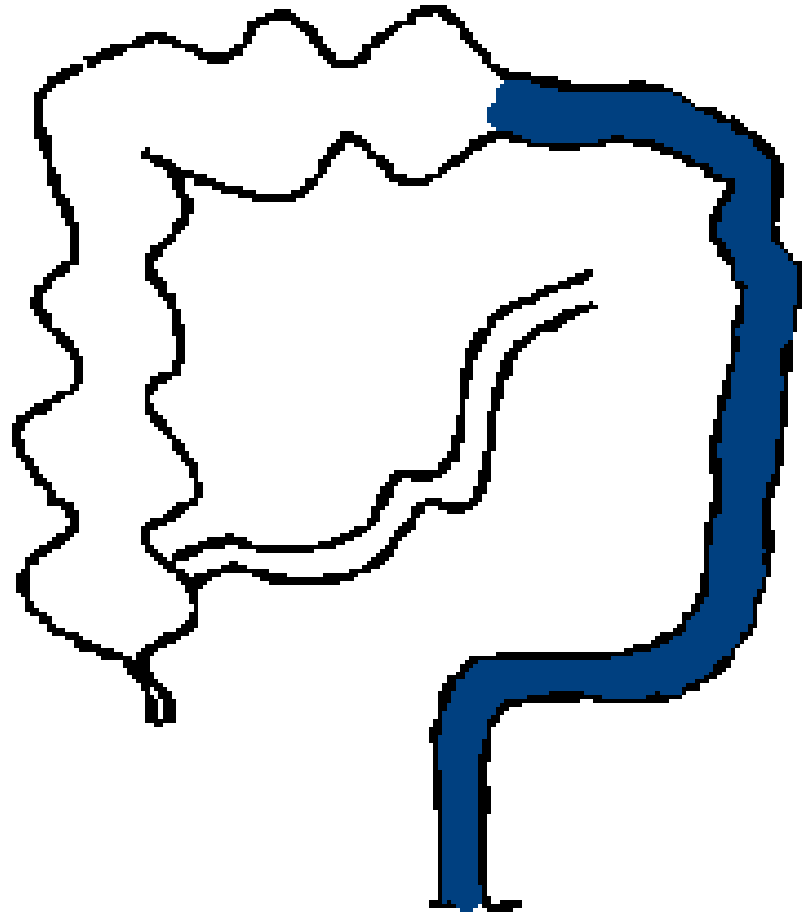
Hirschsprung's disease **D707N mice**



absence of enteric neurons

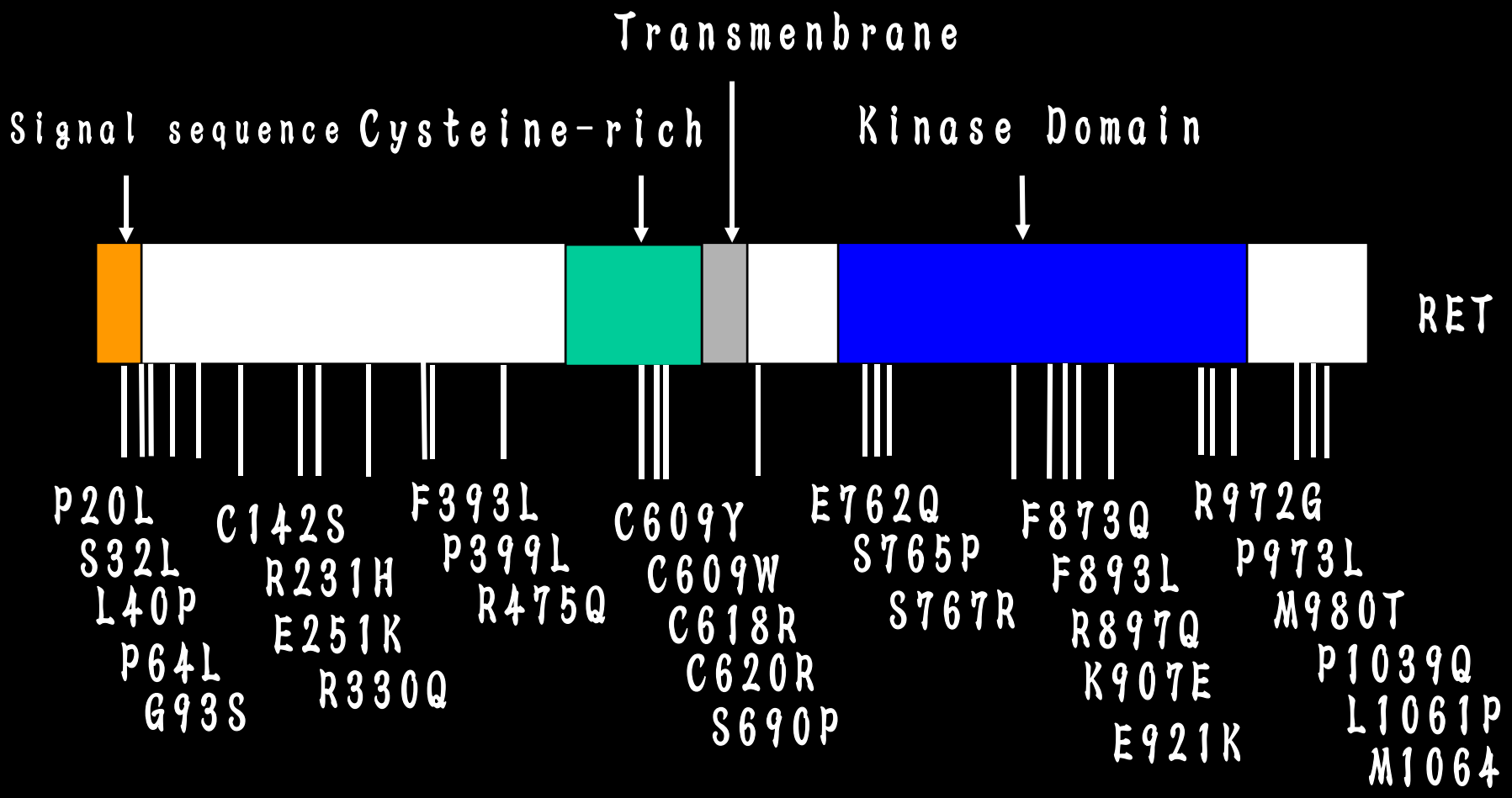


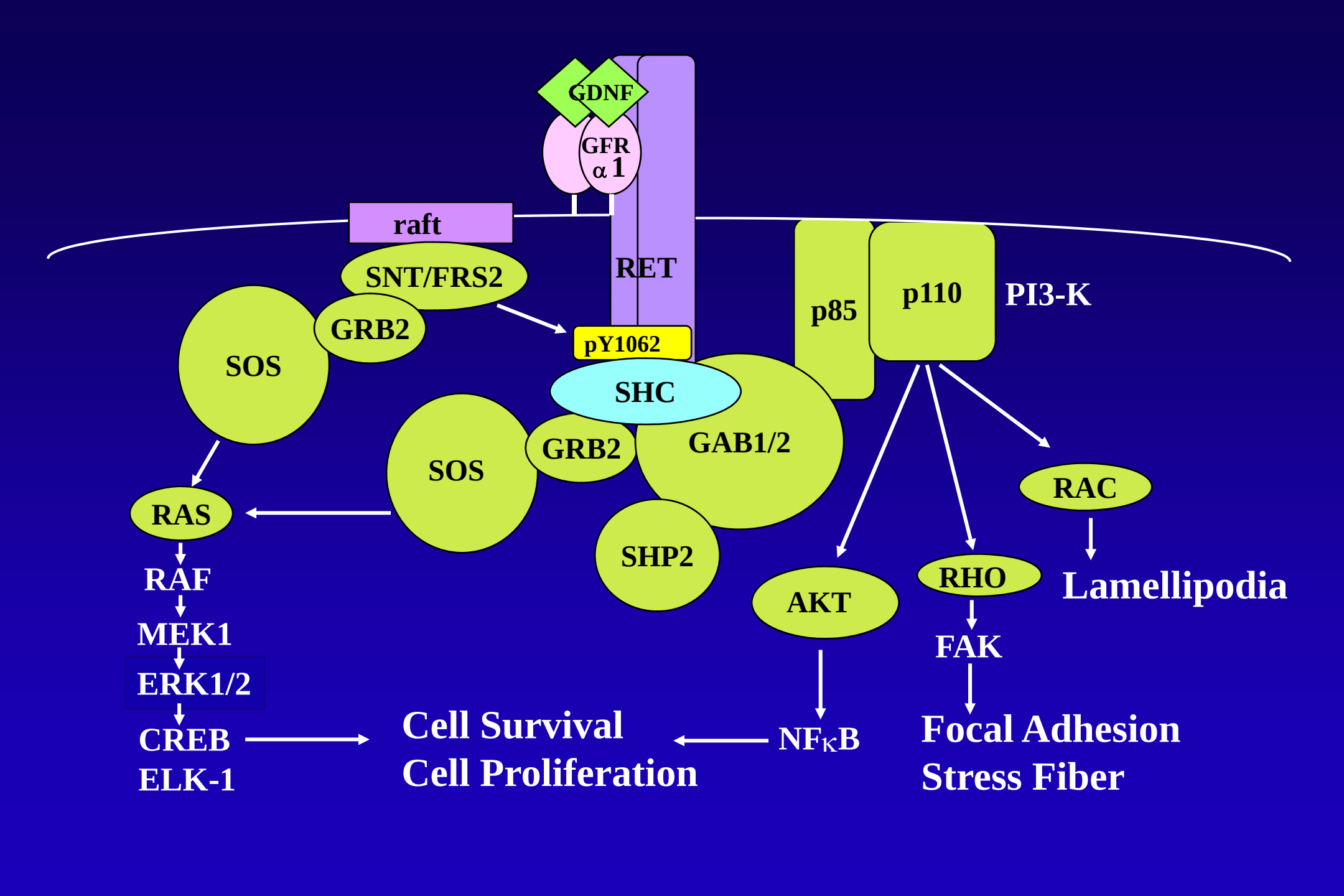
Short Segment Type (80%)



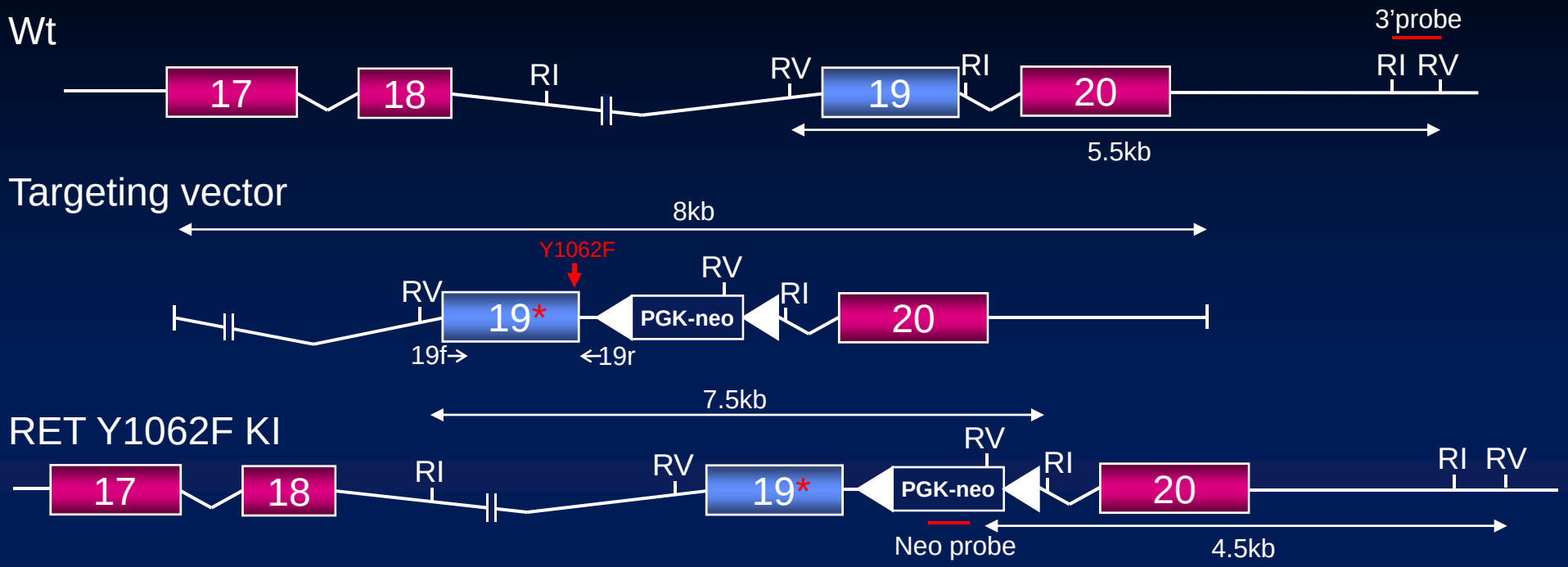
Long Segment Type (12%)

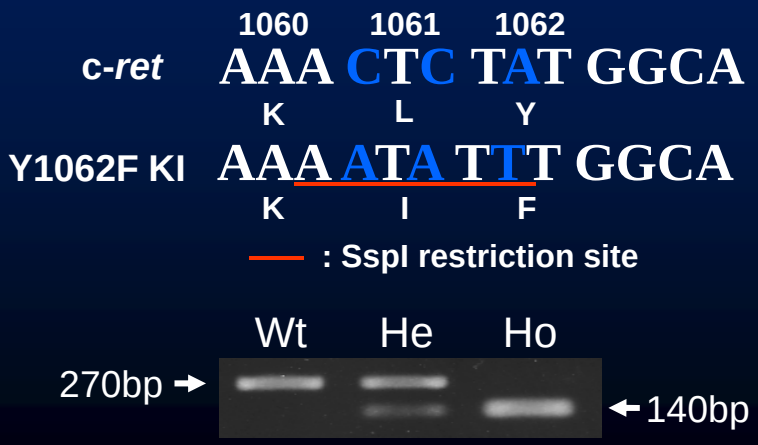
RET Mutations in Hirschsprung's Disease

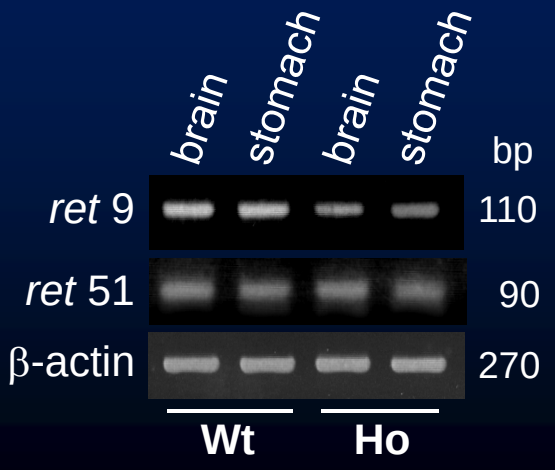



GDNF
GFR α1
raft
RET
SNT/FRS2
GRB2
SOS
pY1062
SHC
p85
p110
PI3-K
SOS
GRB2
GAB1/2
RAS
SHP2
RAC
RAF
MEK1
ERK1/2
AKT
RHO
Lamellipodia
FAK
CREB
ELK-1
Cell Survival
Cell Proliferation
NFκB
Focal Adhesion
Stress Fiber

Generation of RET Y1062F Knock in mice

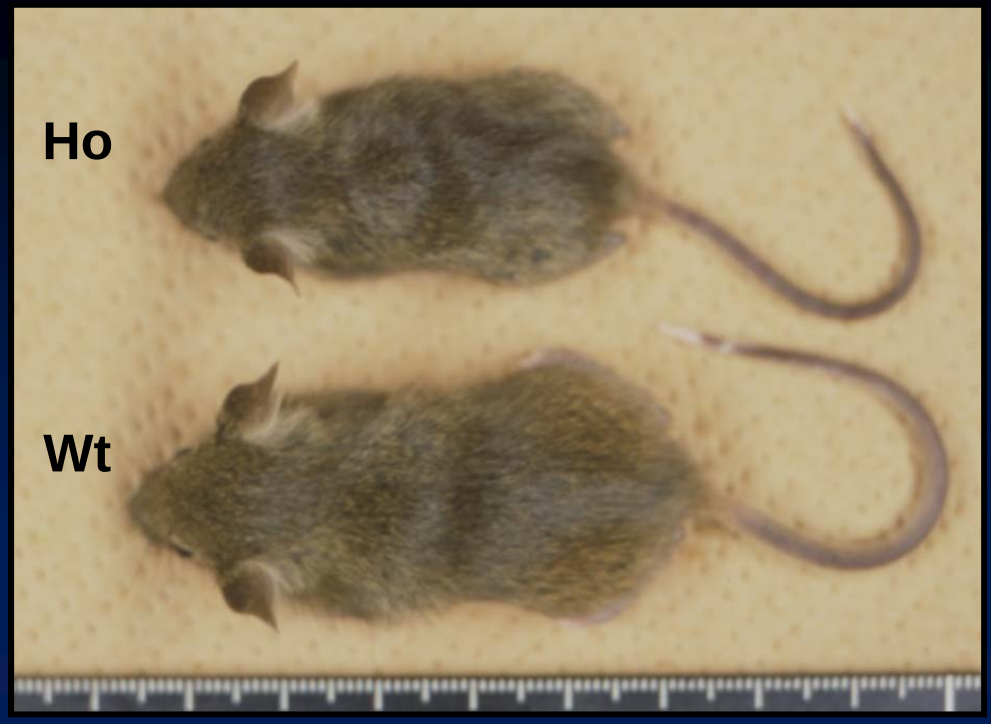




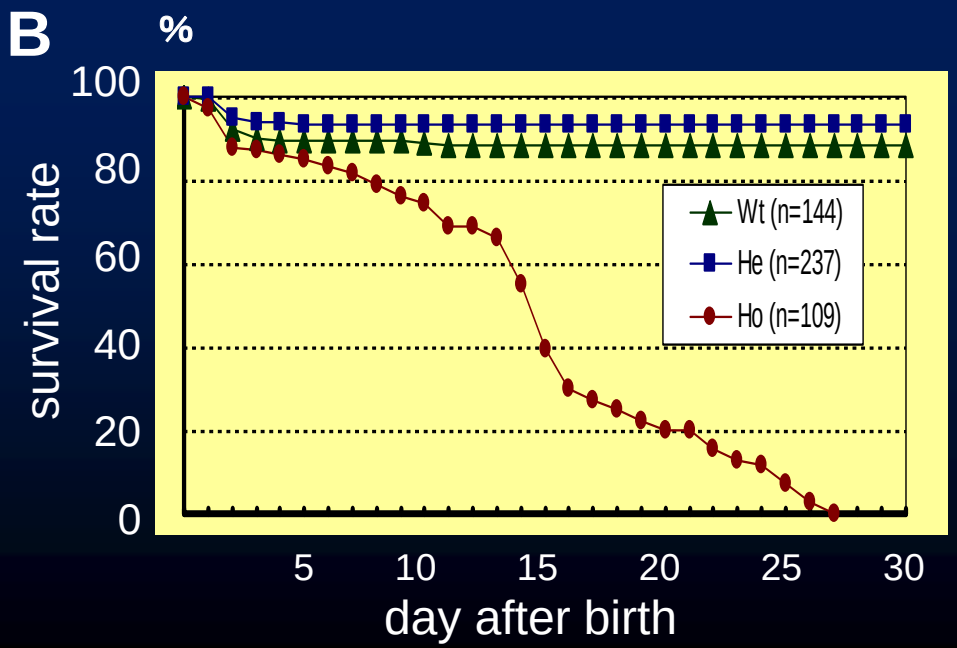


Gross Appearance and Survival Rate of RET Y1062F Knock-in Mice

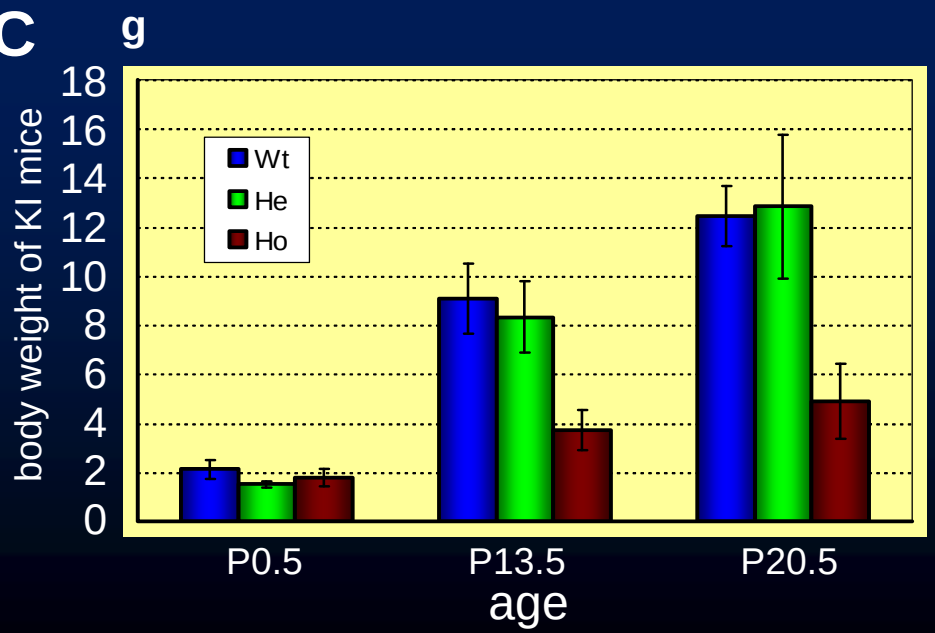
A

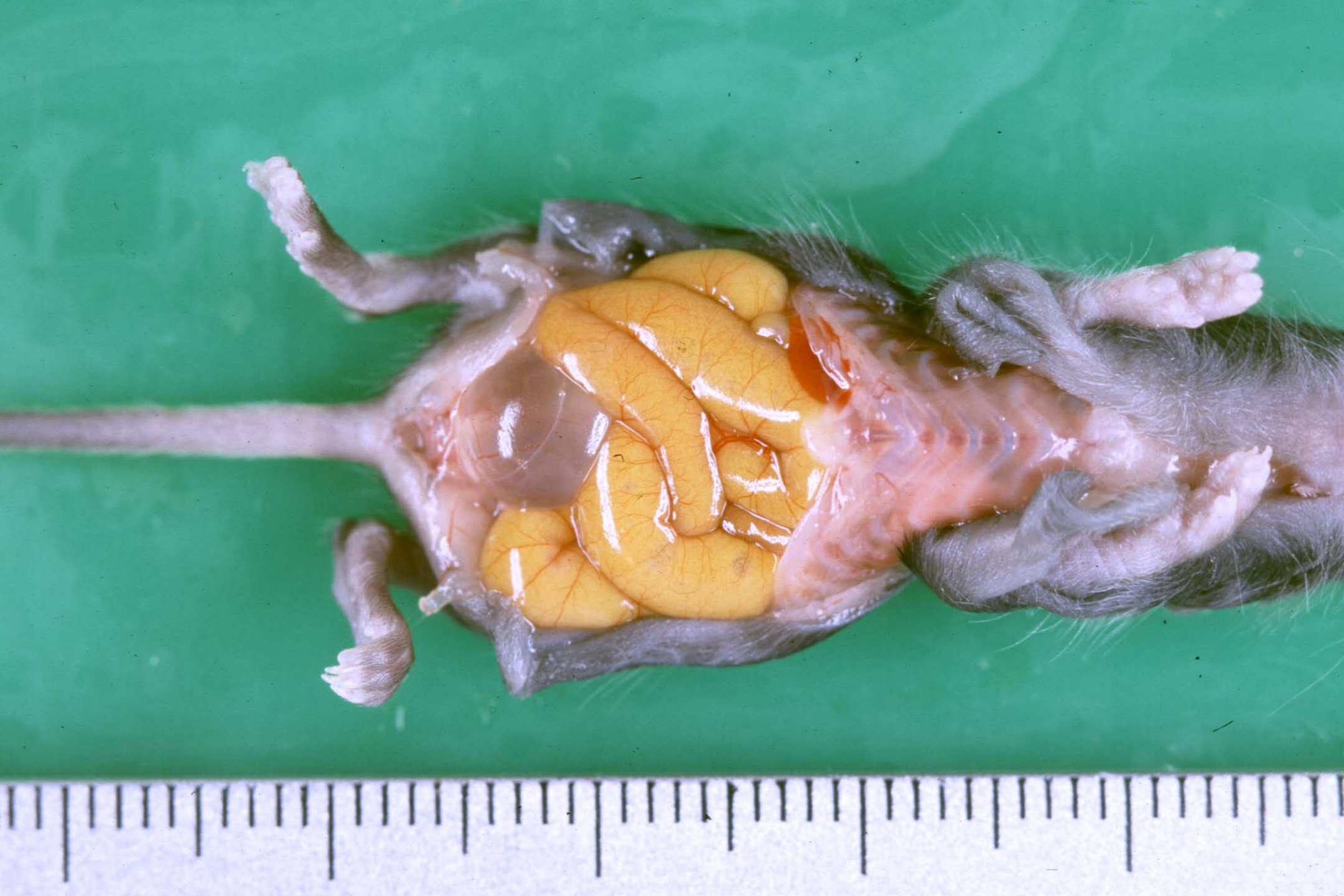


B

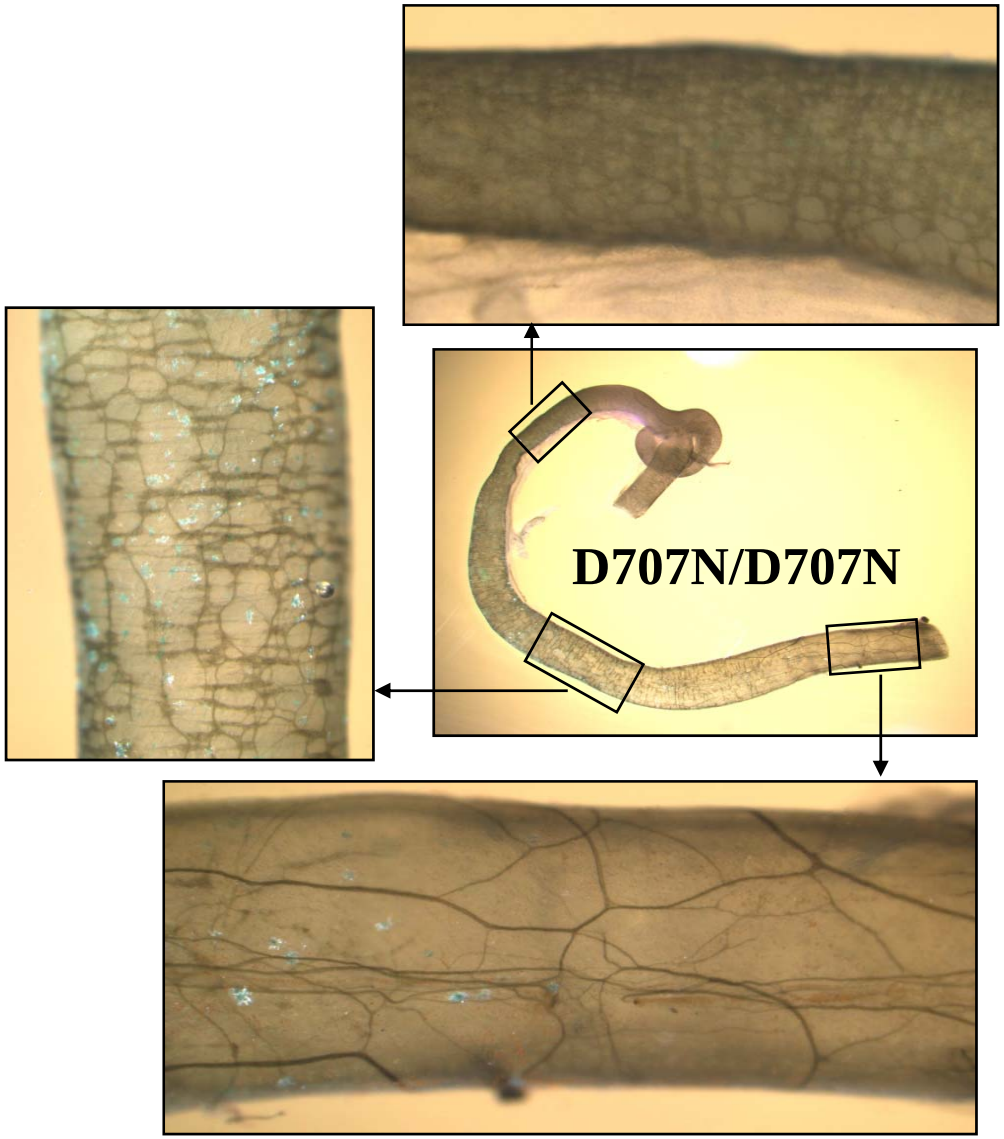


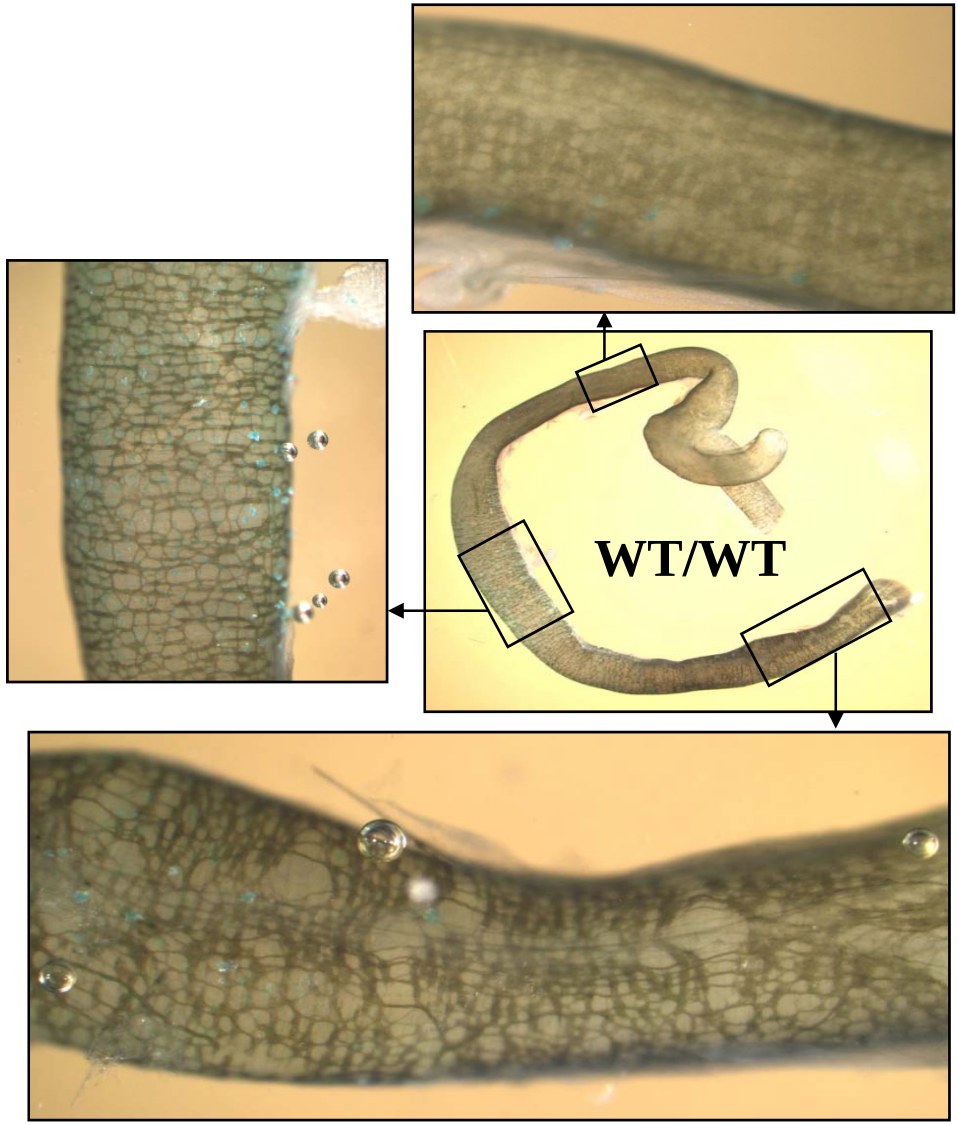
C



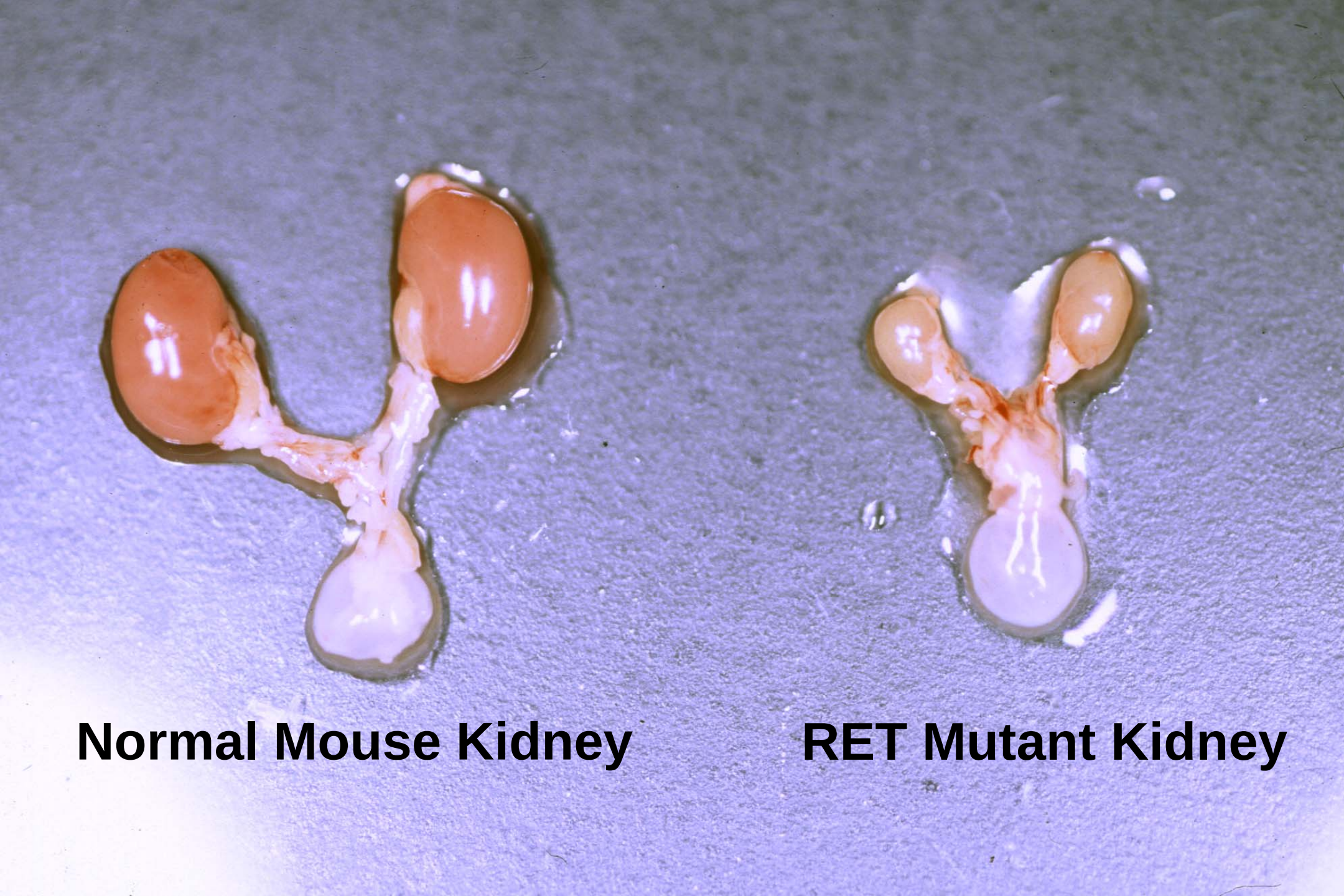


D707N mice lack enteric neurons in the distal portion of the colon (2)



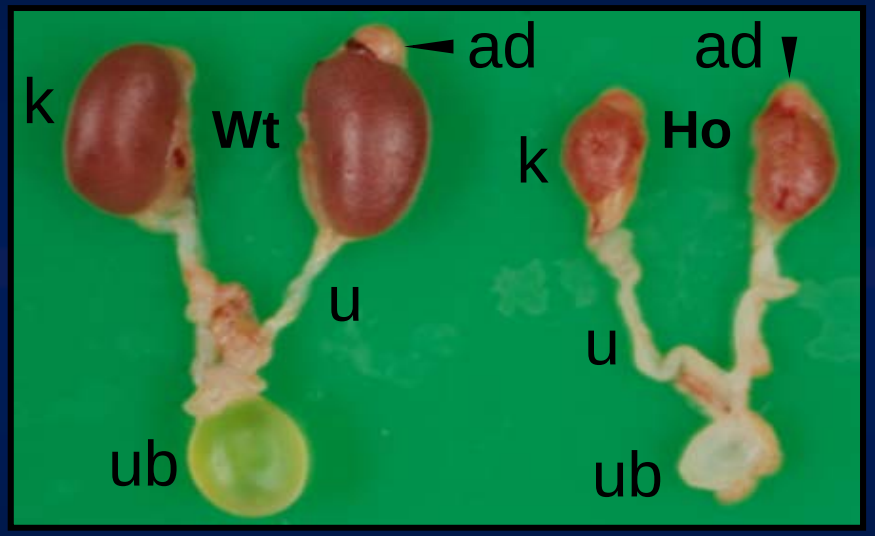


Acetylcholinesterase staining （P3)

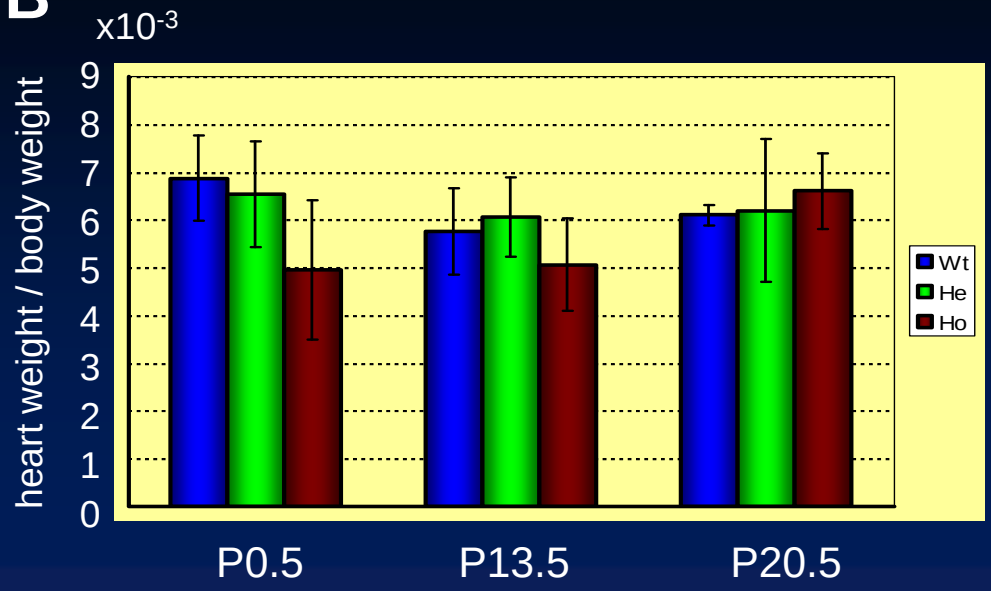

Normal Mouse Kidney
RET Mutant Kidney

Findings of RET Y1062F Knock in Mice Kidney

A



B



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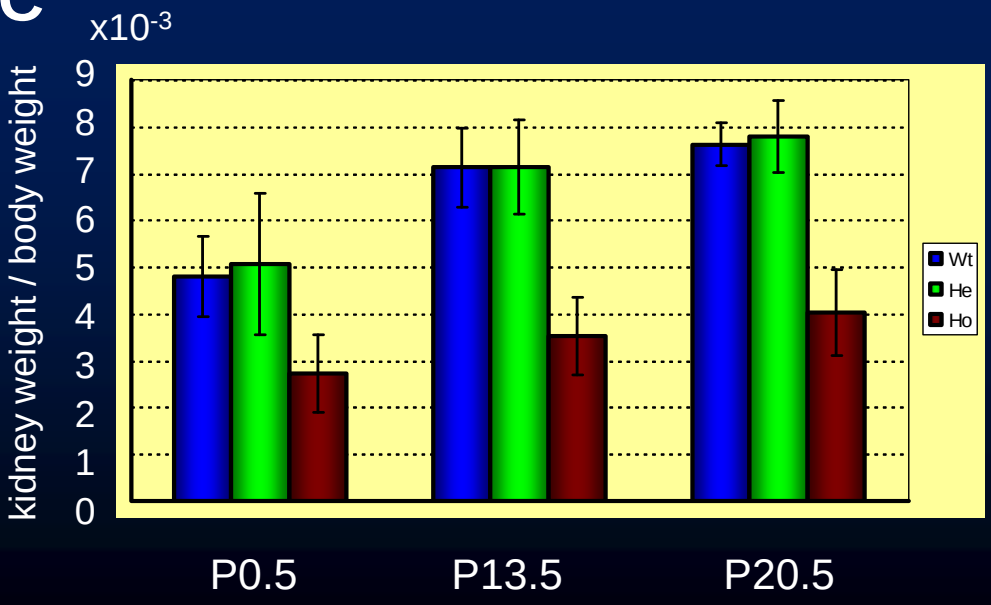
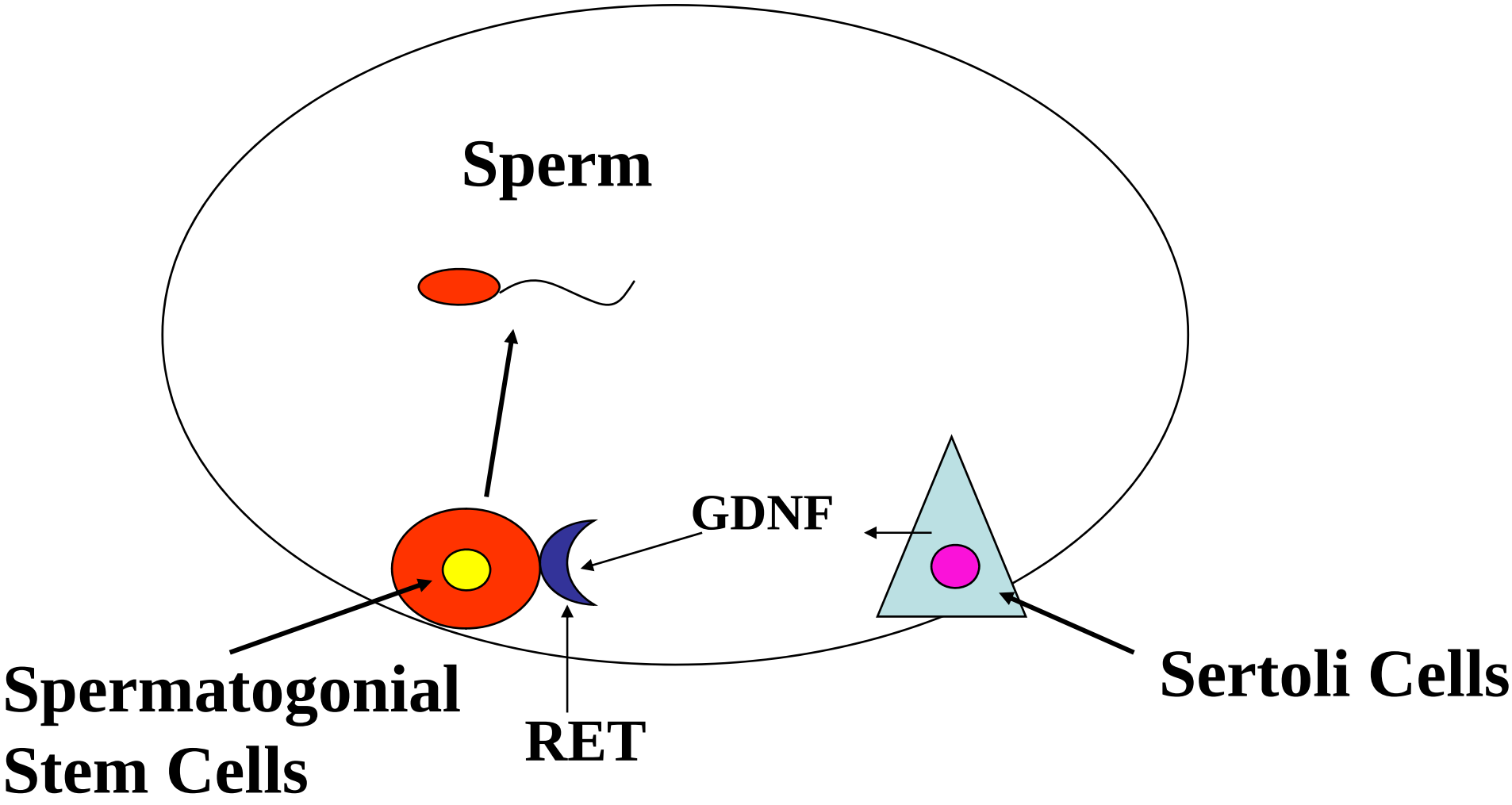
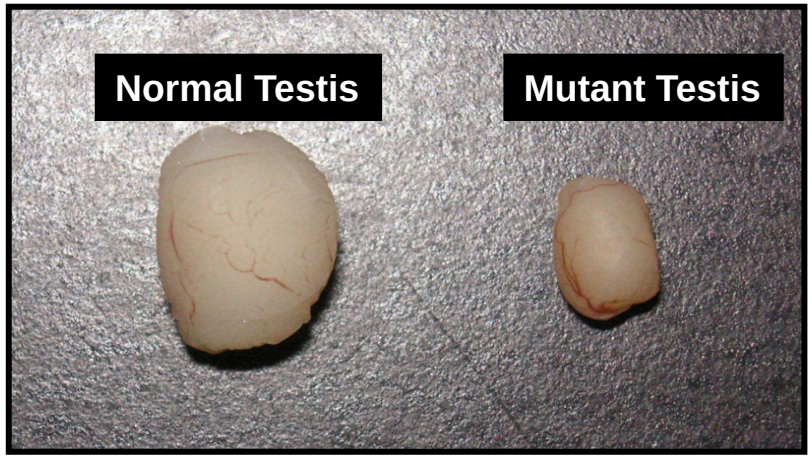
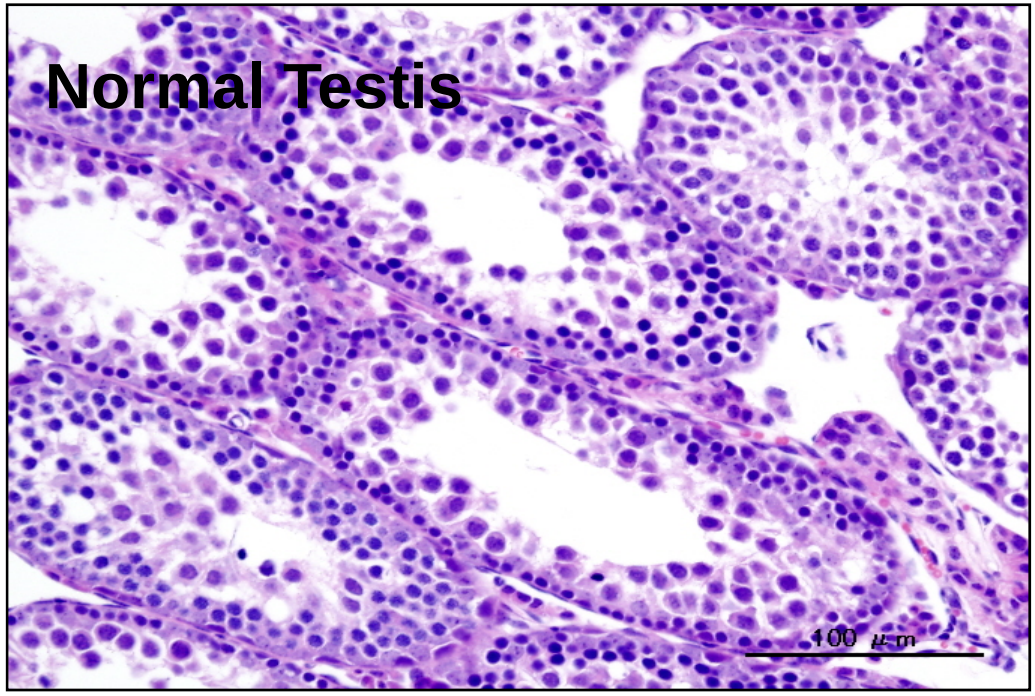


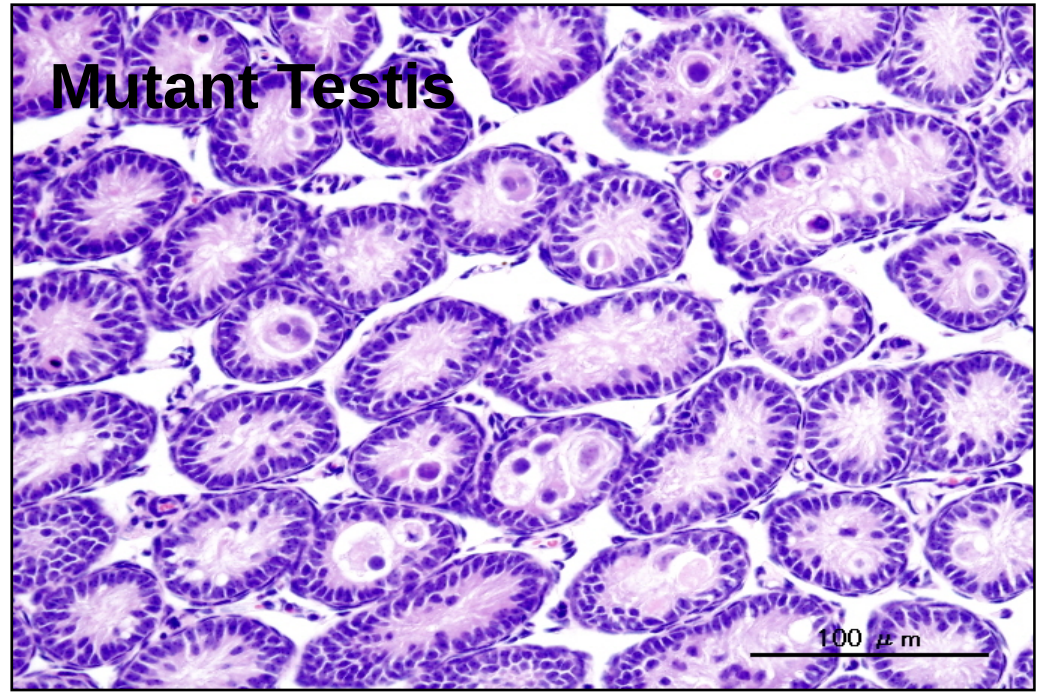
GDNF/RET signaling is essential for germ cell development





Hypoplasia of Testis in Y1062F mutant mouse





No sperm observed