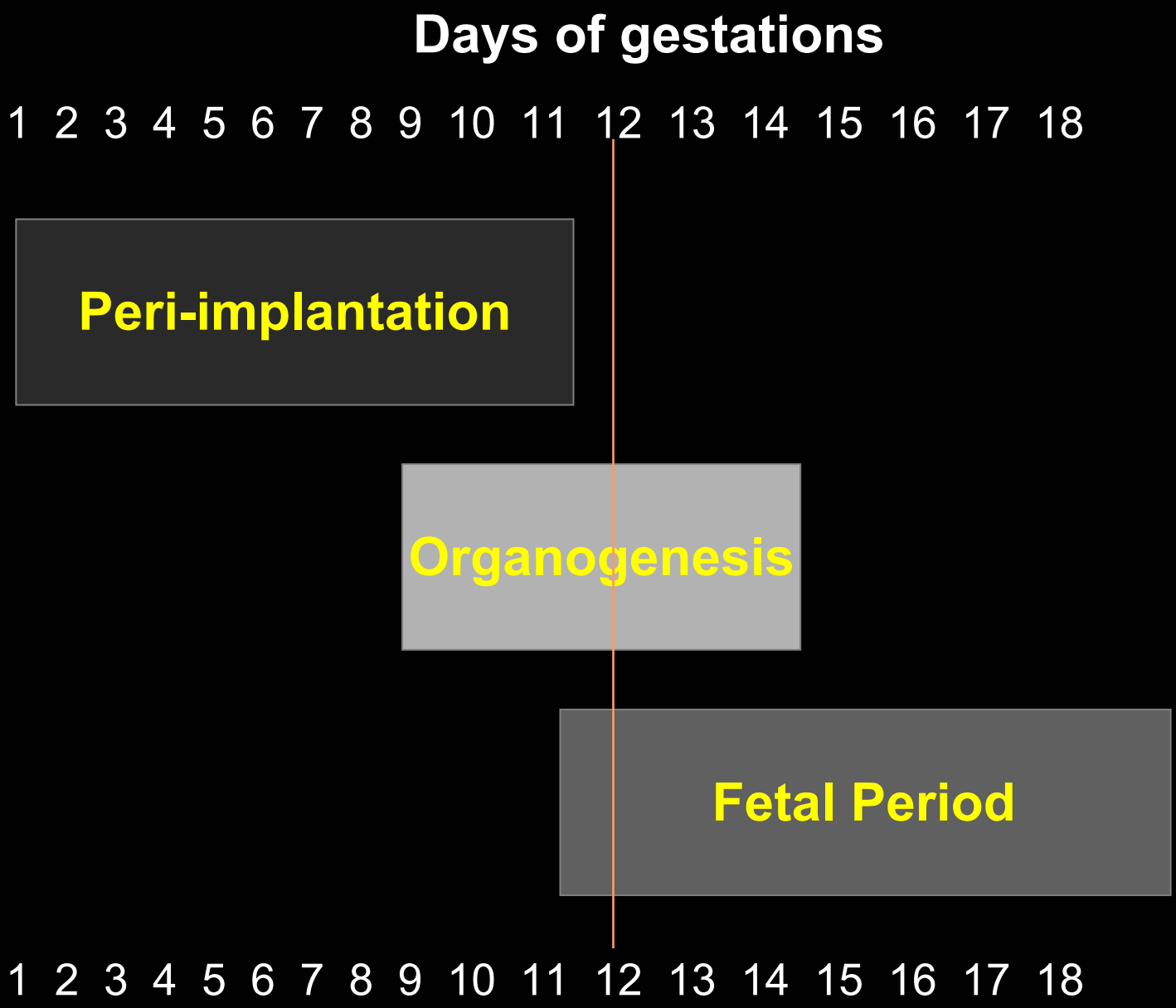
Lessons from the Genetically Altered Mouse

Clinical Examination
Pathologic Examination
Gene Expression

Viable
or
Embryonic Lethal

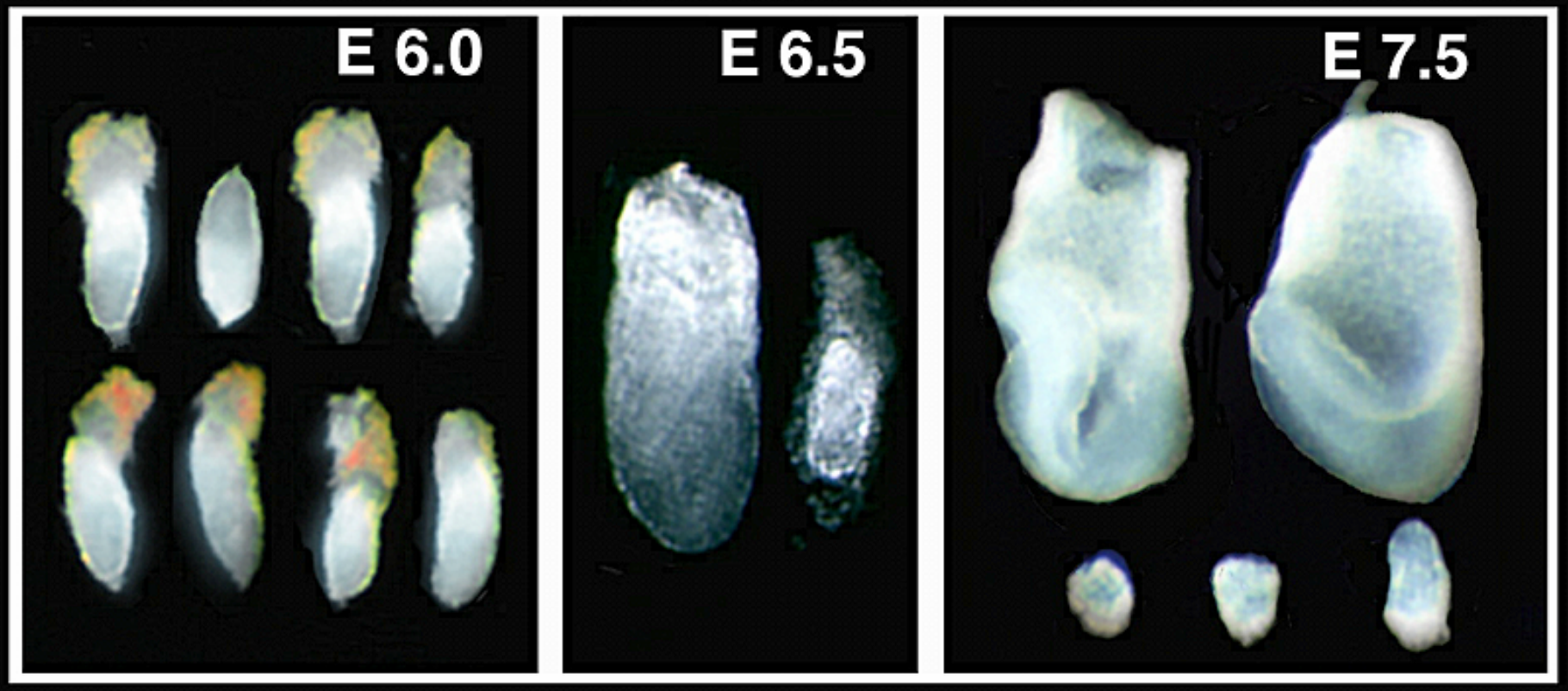

Days of gestations
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Peri-implantation
Organogenesis
Fetal Period
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

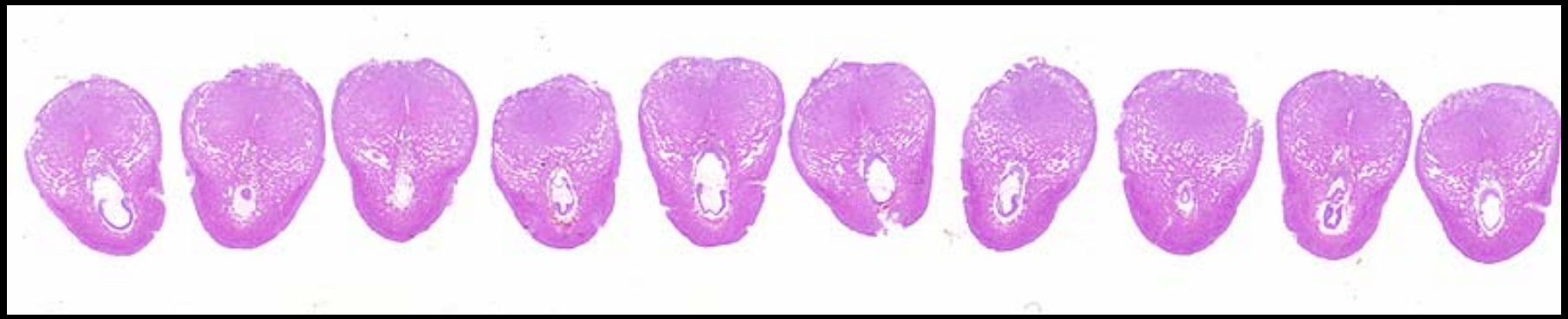
Peri-implantation

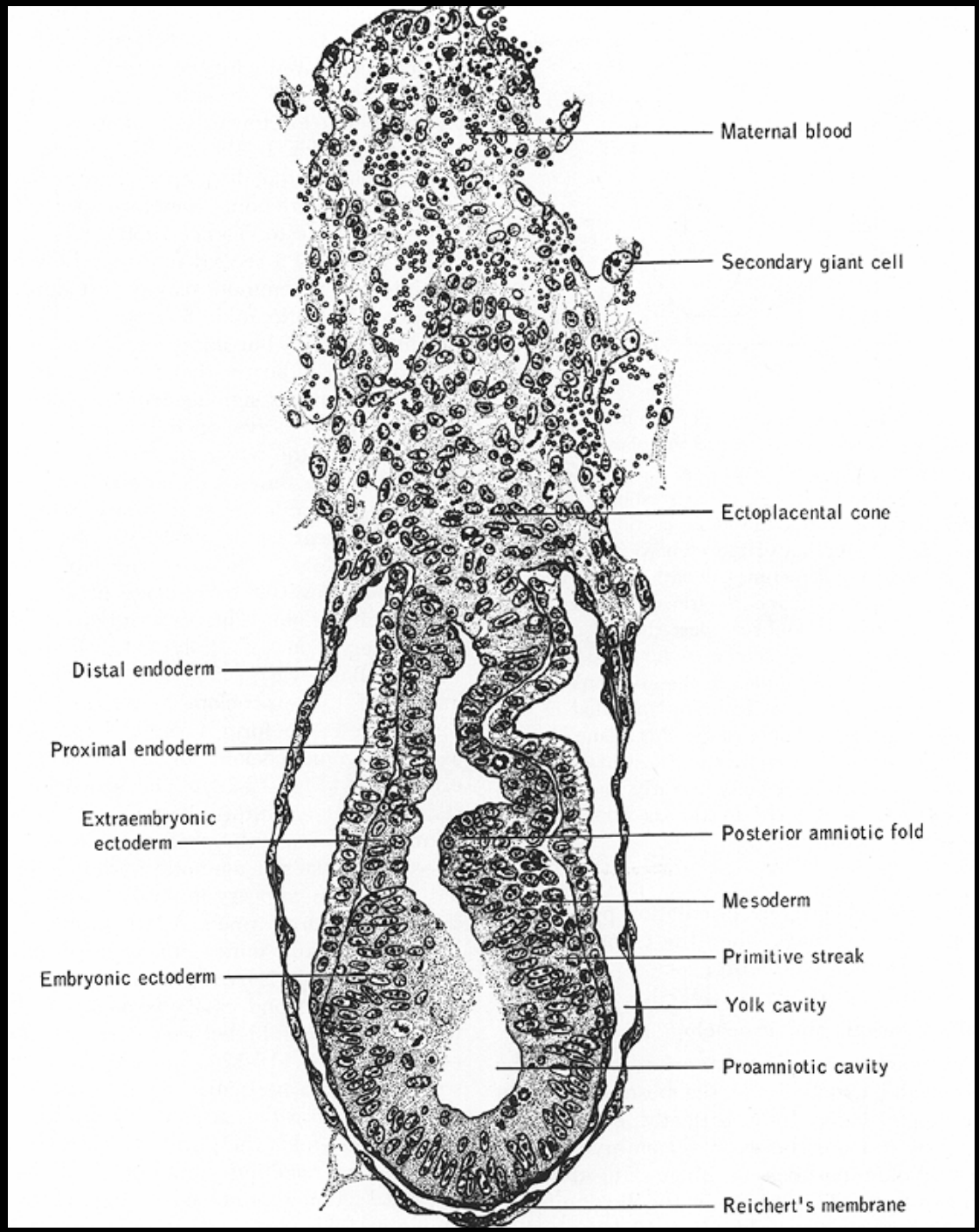
- Development of the blastocyst
- Functioning connection between the trophoblast and the maternal decidua
- Gastrulation

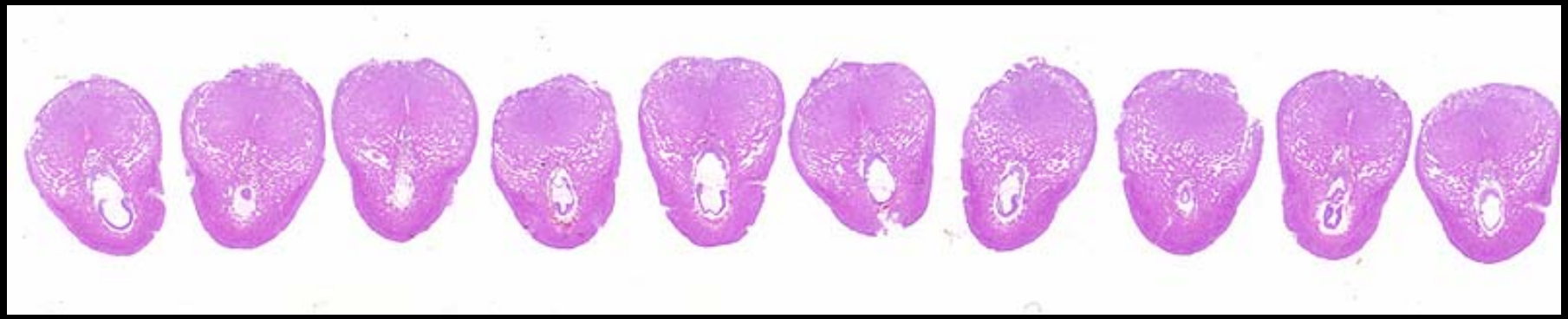
Genotype analysis of offspring from Scleraxis +/- intercrosses

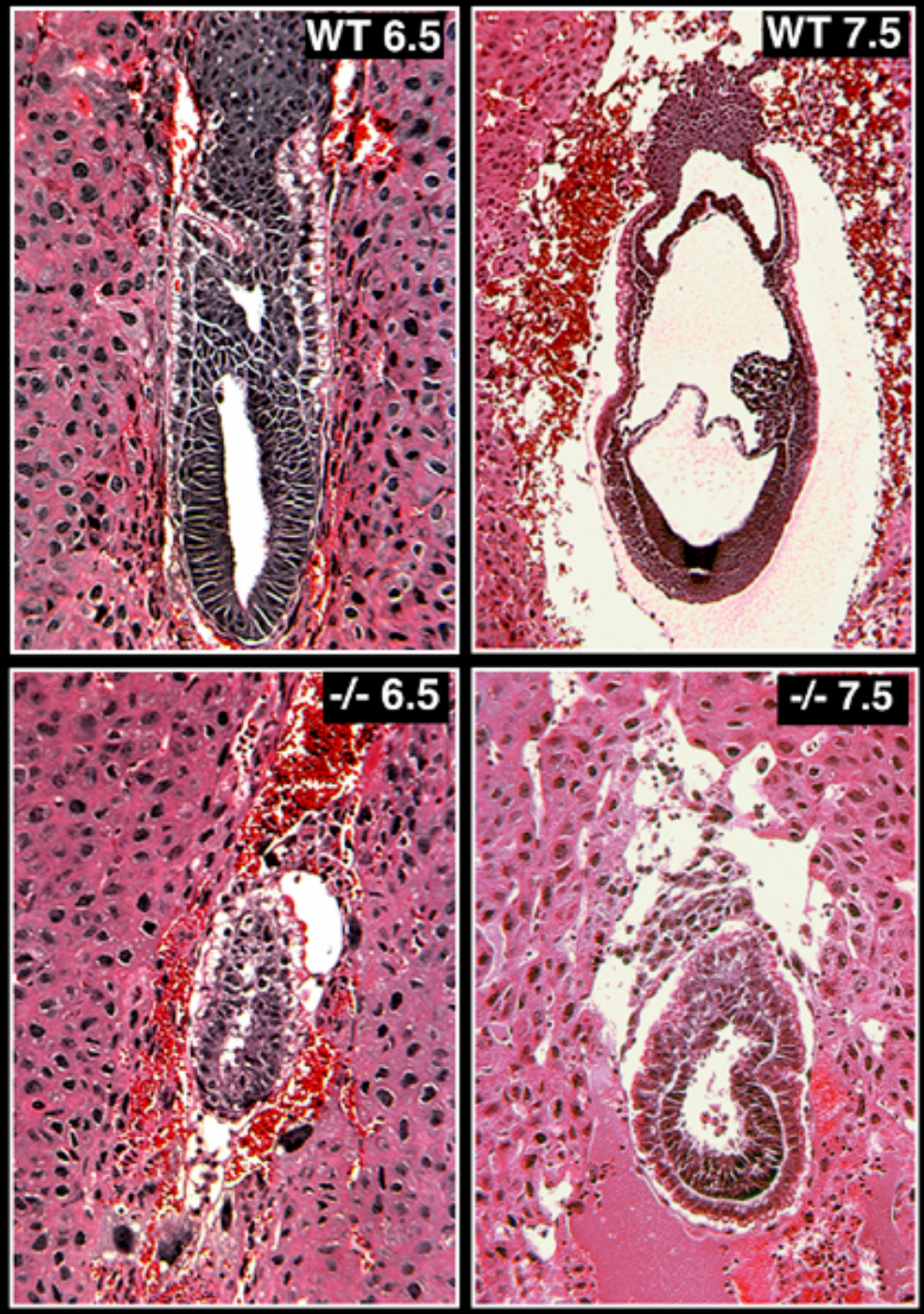
	Genotype		
Age	+/+	+/-	-/-
Neonates	98	212	0
E6.5	34(31)	46(43)	28(26)
E7.5	15(26)	28(49)	14(25)
E8.5	10(23)	27(61)	7(16)


E 6.0
E 6.5
E 7.5

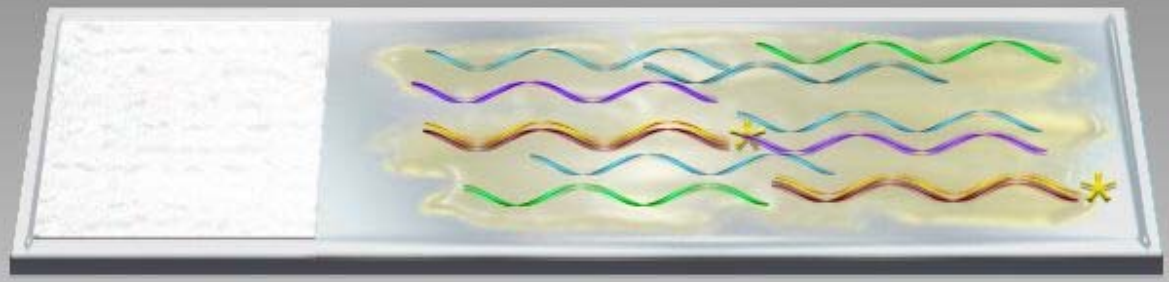



Maternal blood
Secondary giant cell
Ectoplacental cone
Distal endoderm
Proximal endoderm
Extraembryonic ectoderm
Posterior amniotic fold
Mesoderm
Primitive streak
Embryonic ectoderm
Yolk cavity
Proamniotic cavity
Reichert's membrane



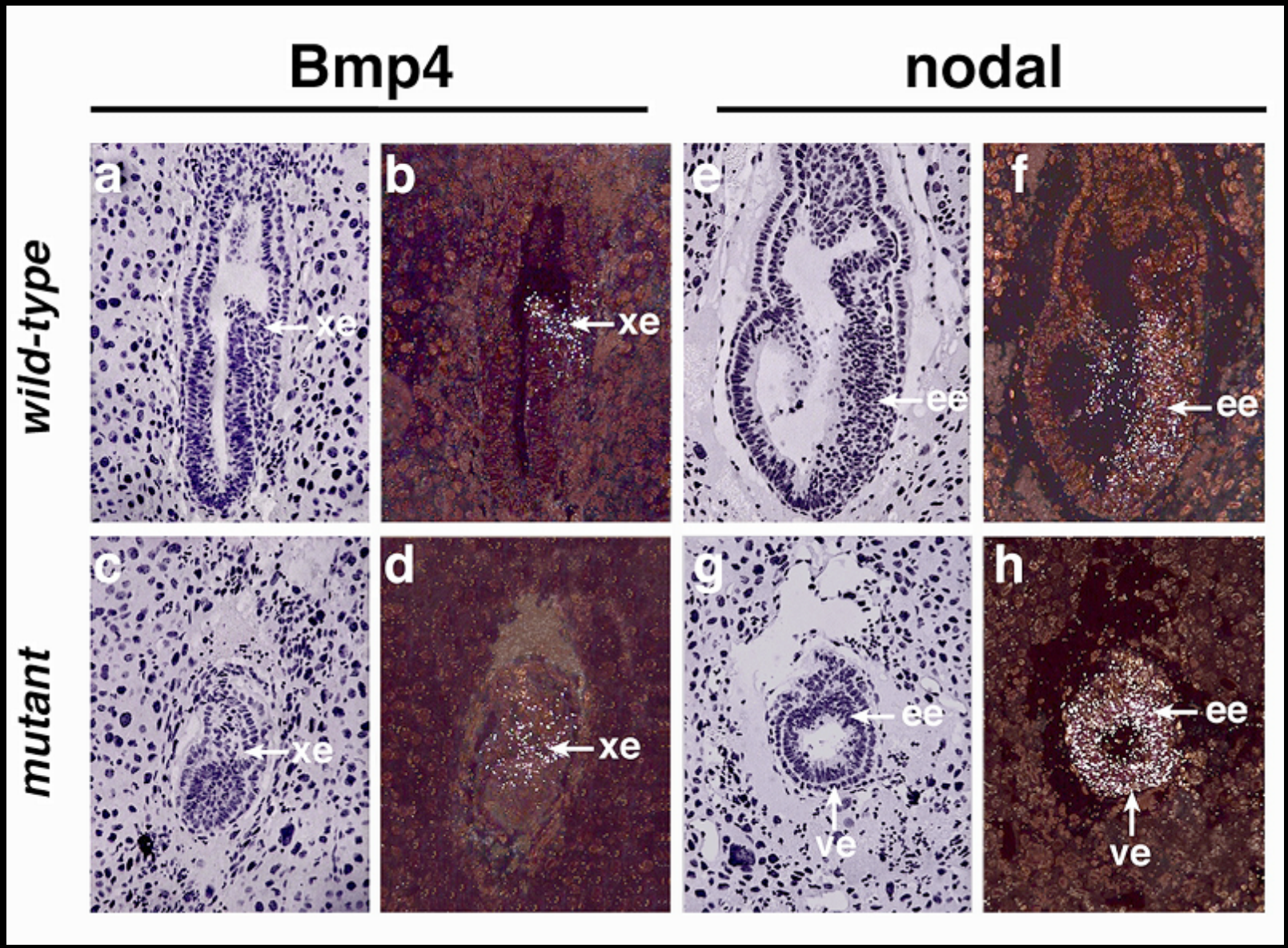

WT 6.5
WT 7.5
-/- 6.5
-/- 7.5

3. Exposure to Emulsion

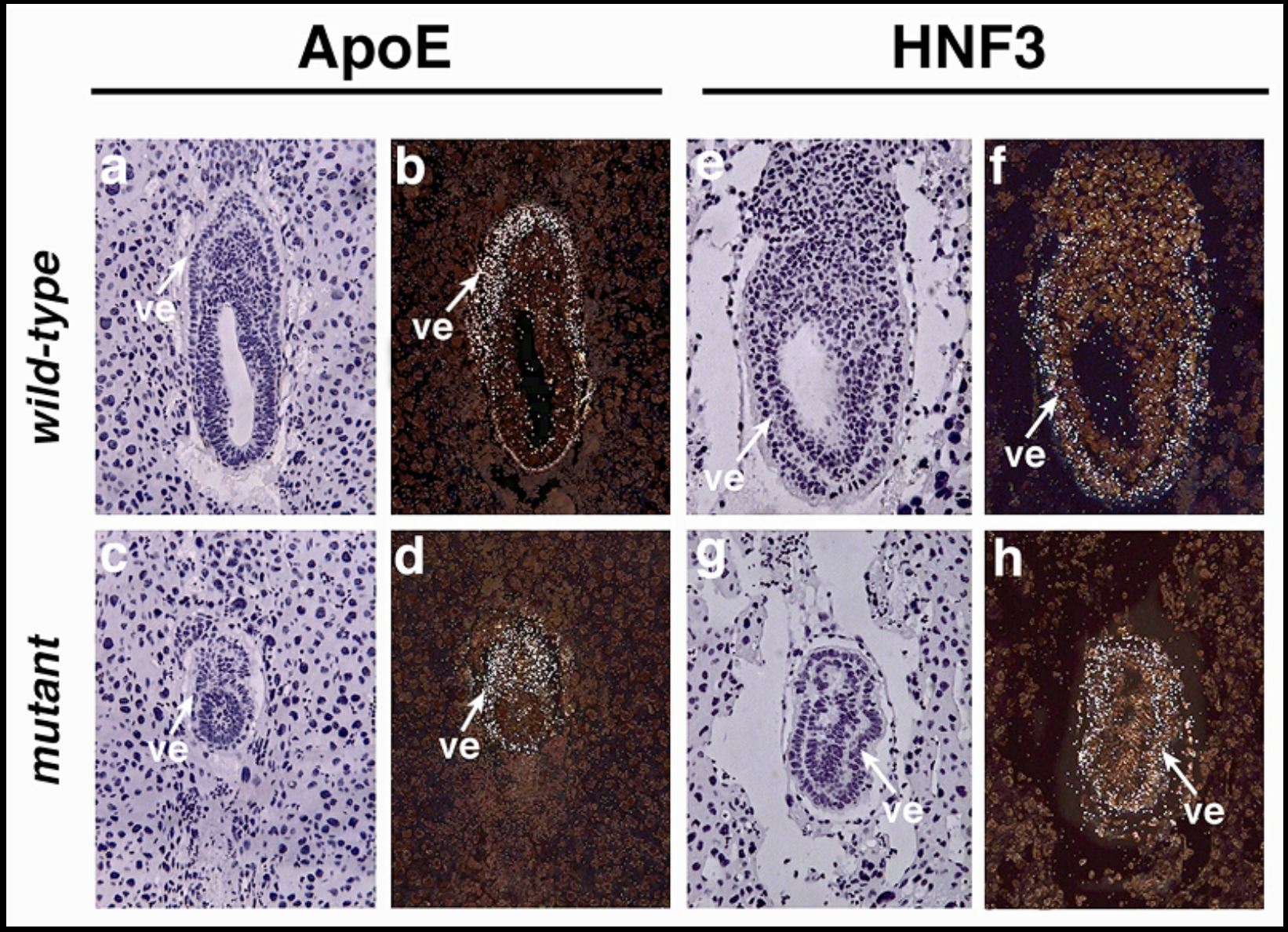


Target mRNA

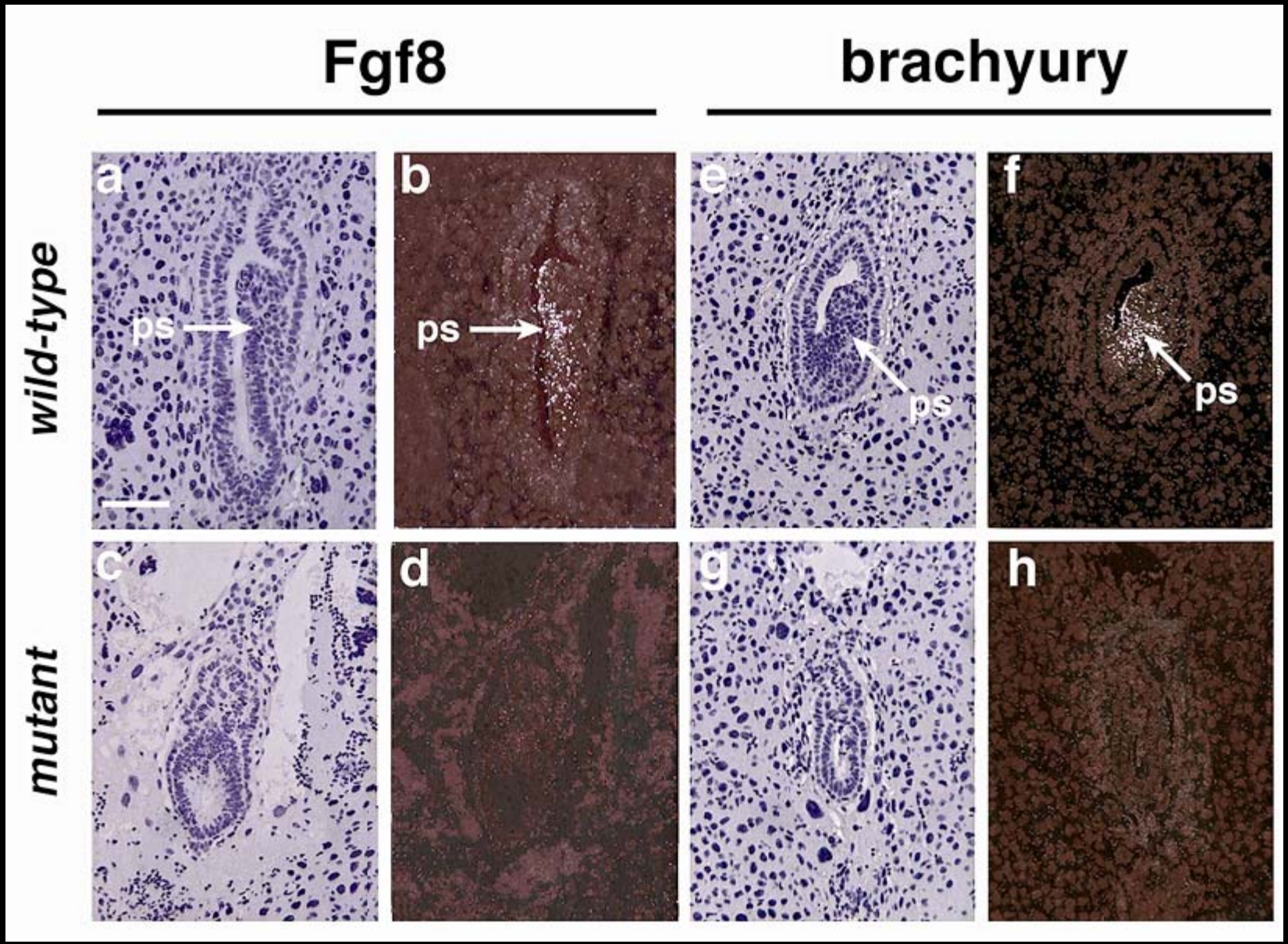
Ectodermal Markers



Endodermal Markers



Mesodermal Markers



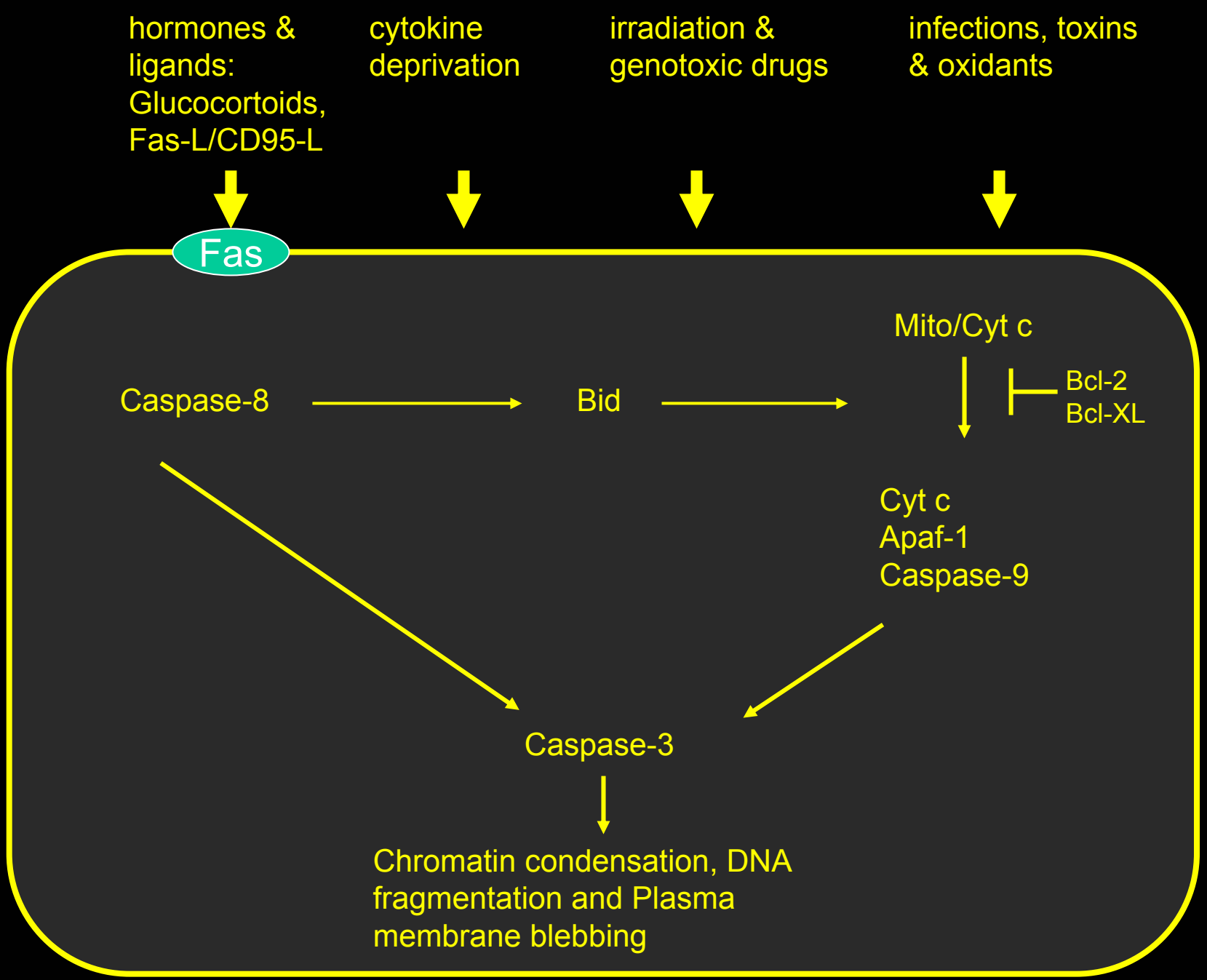
Scleraxis is Necessary for Gastrulation

Early Organogenesis

Is Cytochrome c Involved in Apoptosis?

Apoptosis

- Programed cell death.
- Characterized by condensation and fragmentation of the nucleus.
- DNA is digested by specific enzymes called caspases that cut DNA at specific sites.
- Digested DNA forms a ladder when run on a gel

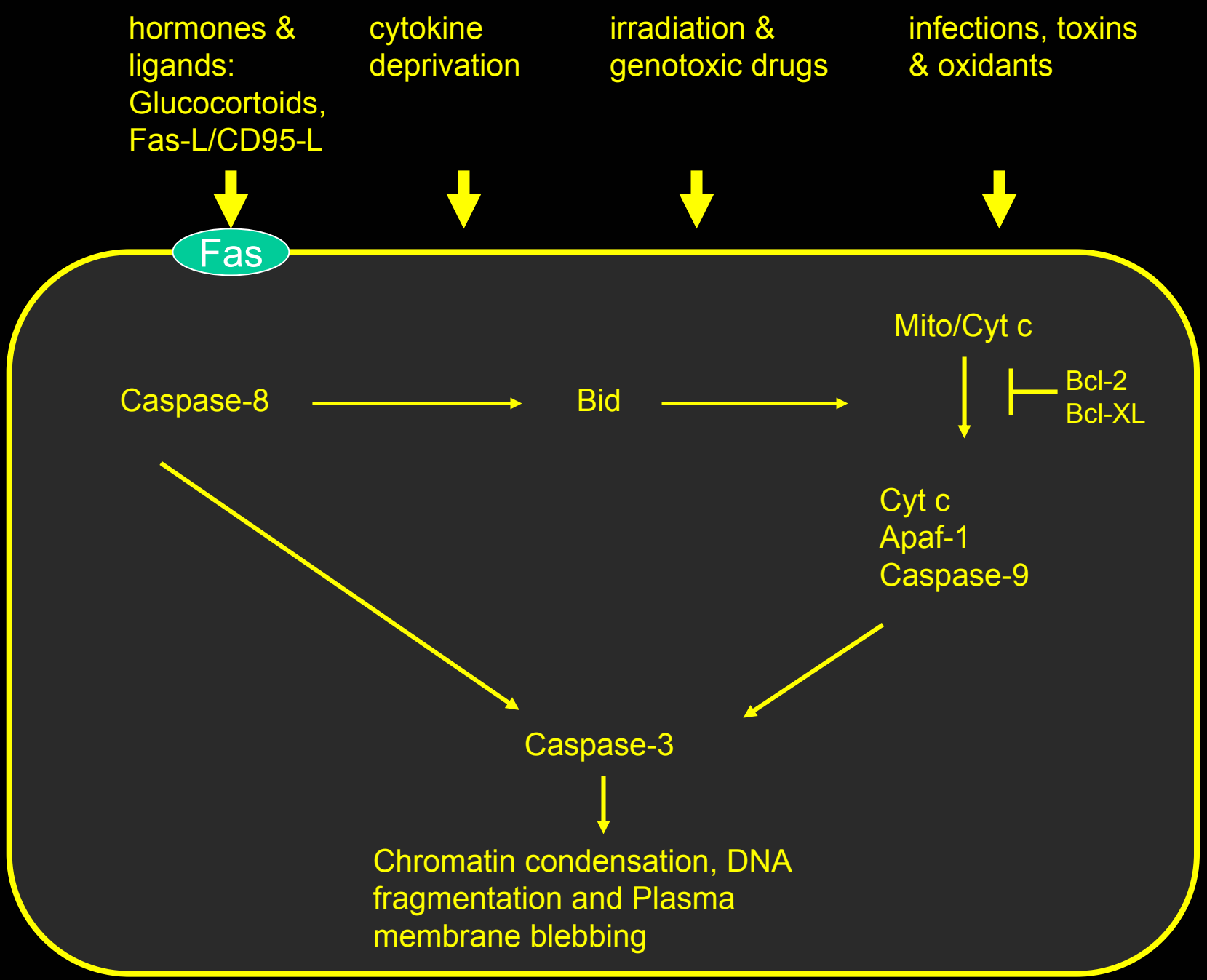

hormones & ligands: Glucocortoids, Fas-L/CD95-L
cytokine deprivation
irradiation & genotoxic drugs
infections, toxins & oxidants
Fas
Mito/Cyt c
Caspase-8
Bid
Bcl-2
Bcl-XL
Cyt c
Apaf-1
Caspase-9
Caspase-3
Chromatin condensation, DNA fragmentation and Plasma membrane blebbing

Analysis of Embryos from intercrosses Cyt c+/- mice

Stage	Total Embryos	Petite / Total Embryos	Normal		Petite	
			Cyt c +/+	+/-	-/-	N/A
E8.5	104	24%	25	54	17	8
E9.5	106	14%	31	60	8	7
E10.5	102	7%	32	63	7*	
E11.5	97	0%	32	65		

N/A : not assayed
* : not viable


Cyt c -/-
Cyt c +/+
ysc
h
h
a
ysc
E9.5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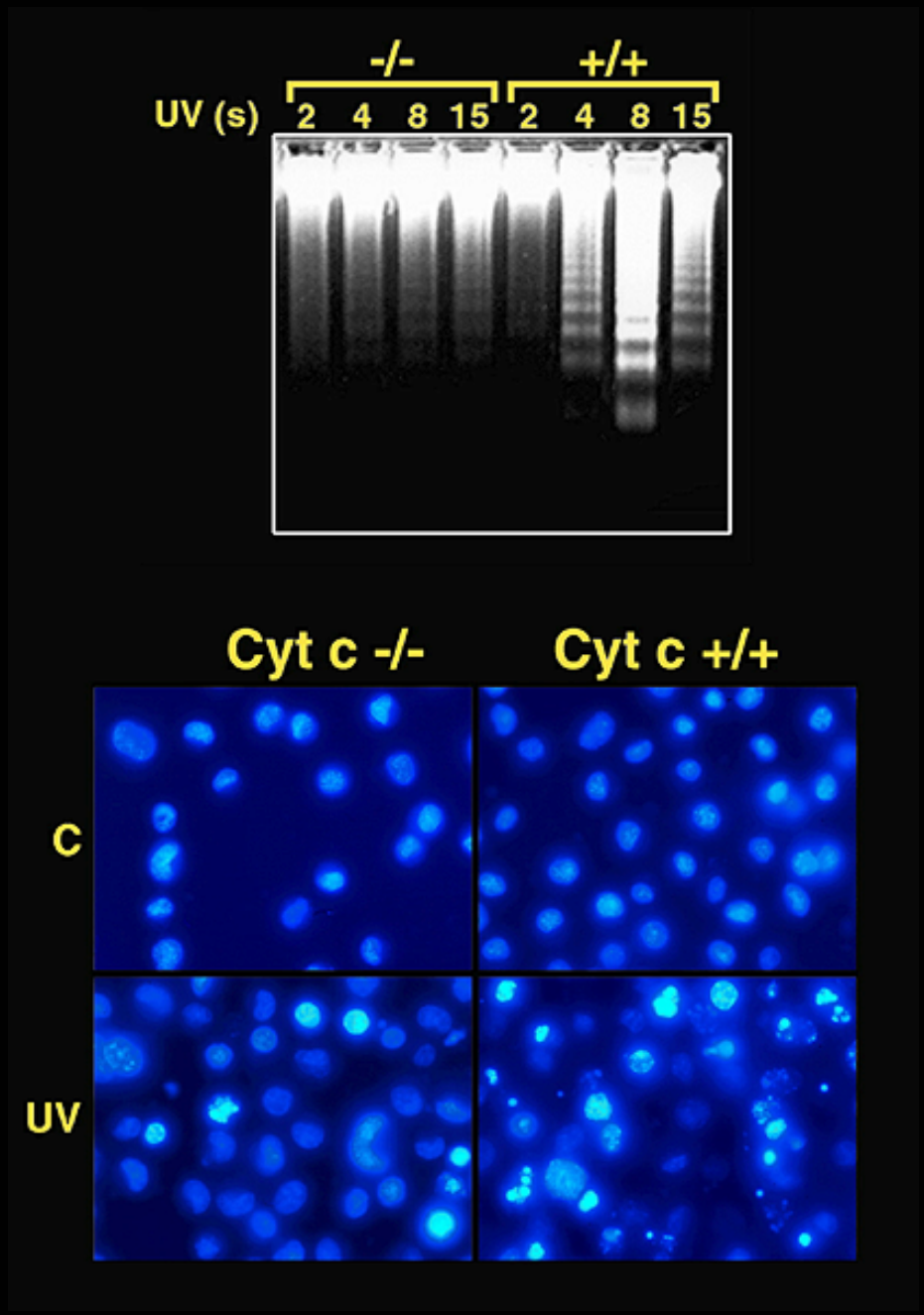
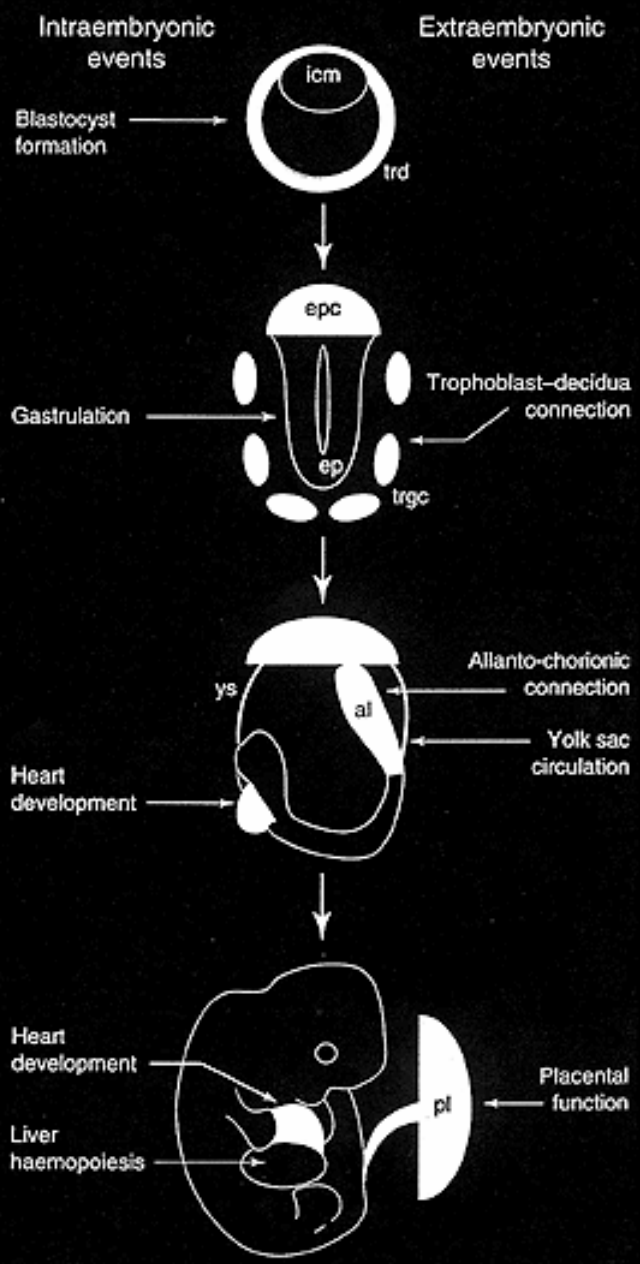
Chromatin condensation, DNA fragmentation and Plasma membrane blebbing


-/-
+/+
UV (s)
2 4 8 15 2 4 8 15
Cyt c -/-
Cyt c +/+
C
UV

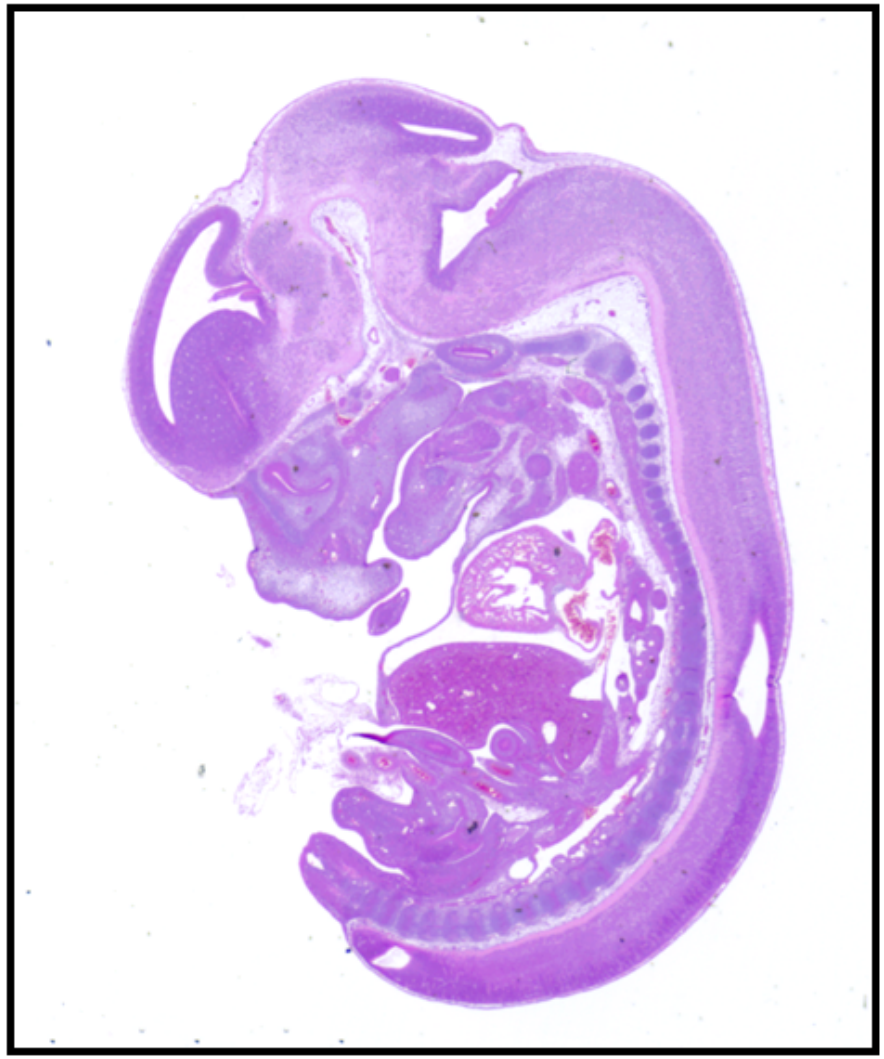
Cytochrome c is necessary for apoptosis in vivo

You Got to Have Heart


Intraembryonic events
Extraembryonic events
icm
Blastocyst formation
trd
epc
Gastrulation
Trophoblast–decidua connection
ep
trgc
Allanto-chorionic connection
ys
al
Yolk sac circulation
Heart development
Heart development
Placental function
pl
Liver haemopoiesis

E13.5

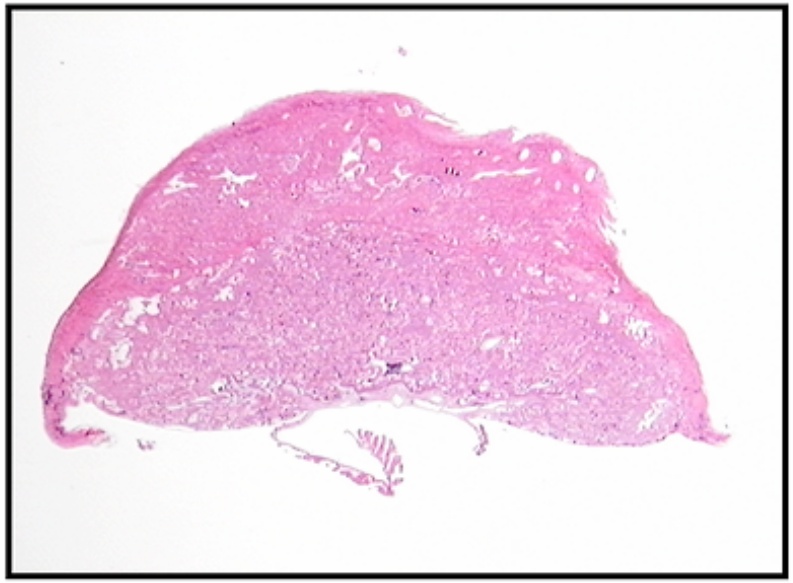
+/+ –/–

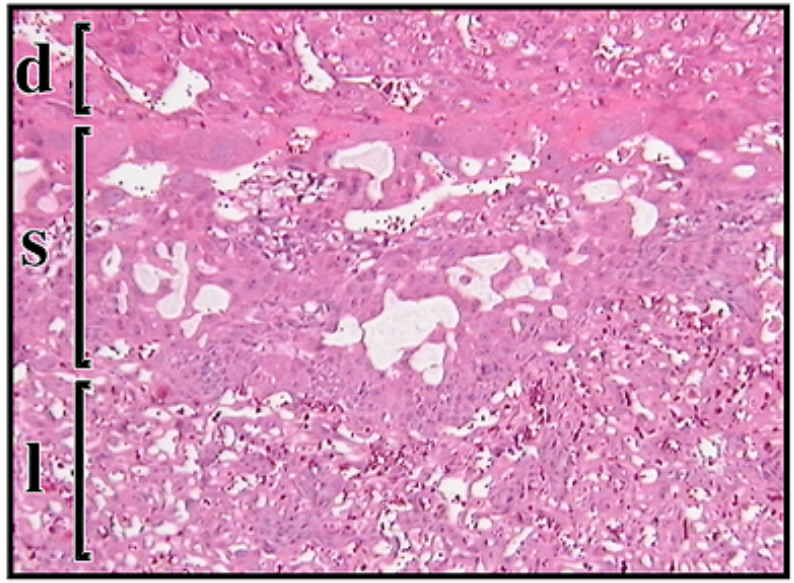


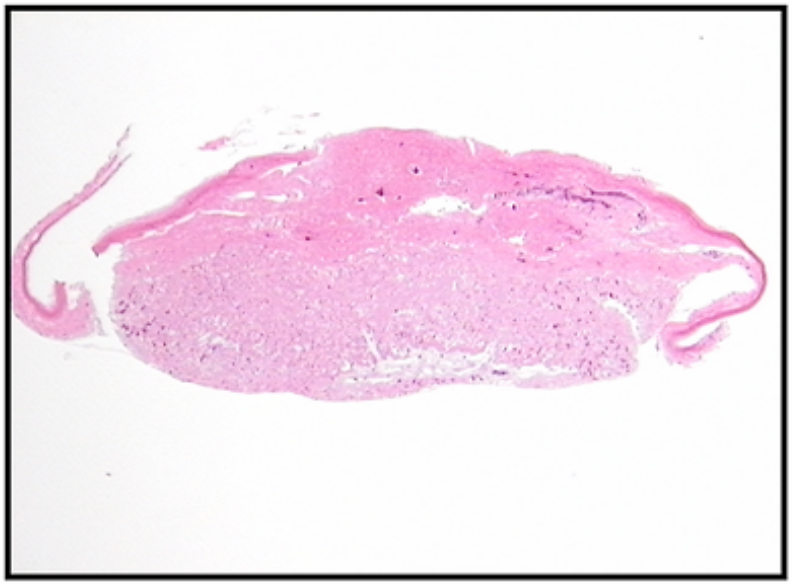
+/+

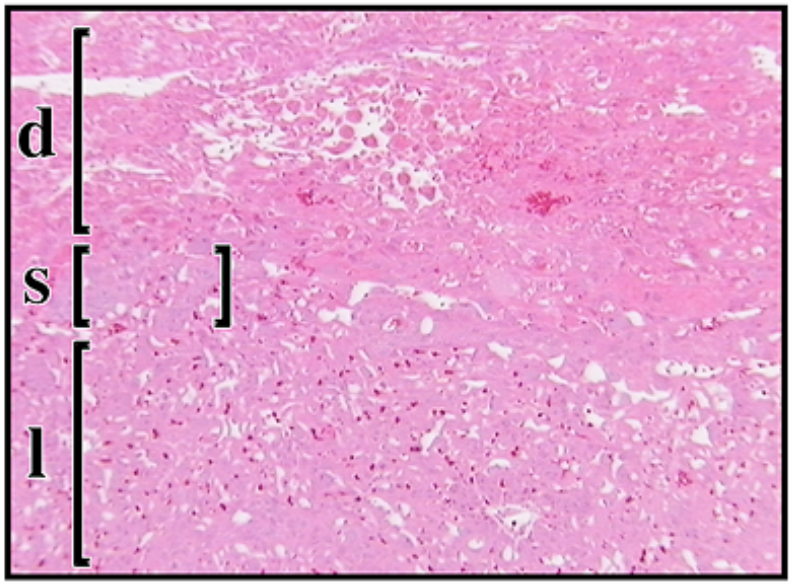
13.5

–/–



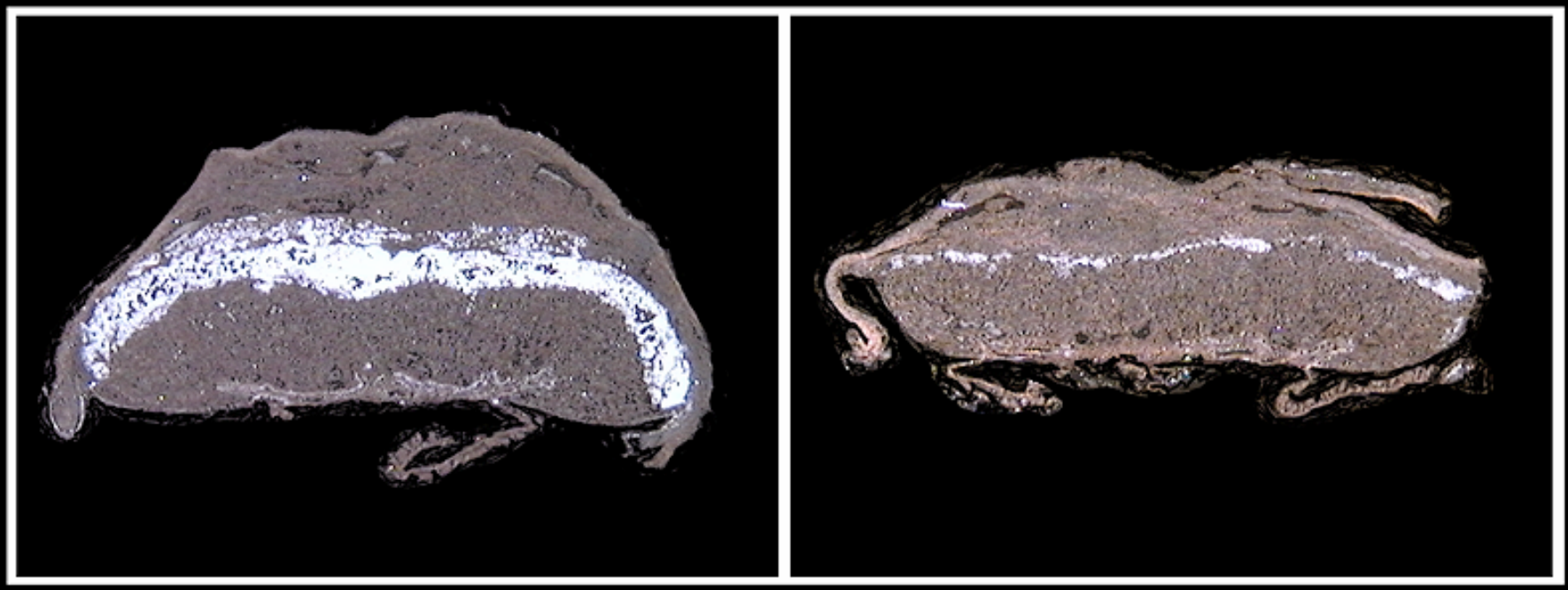






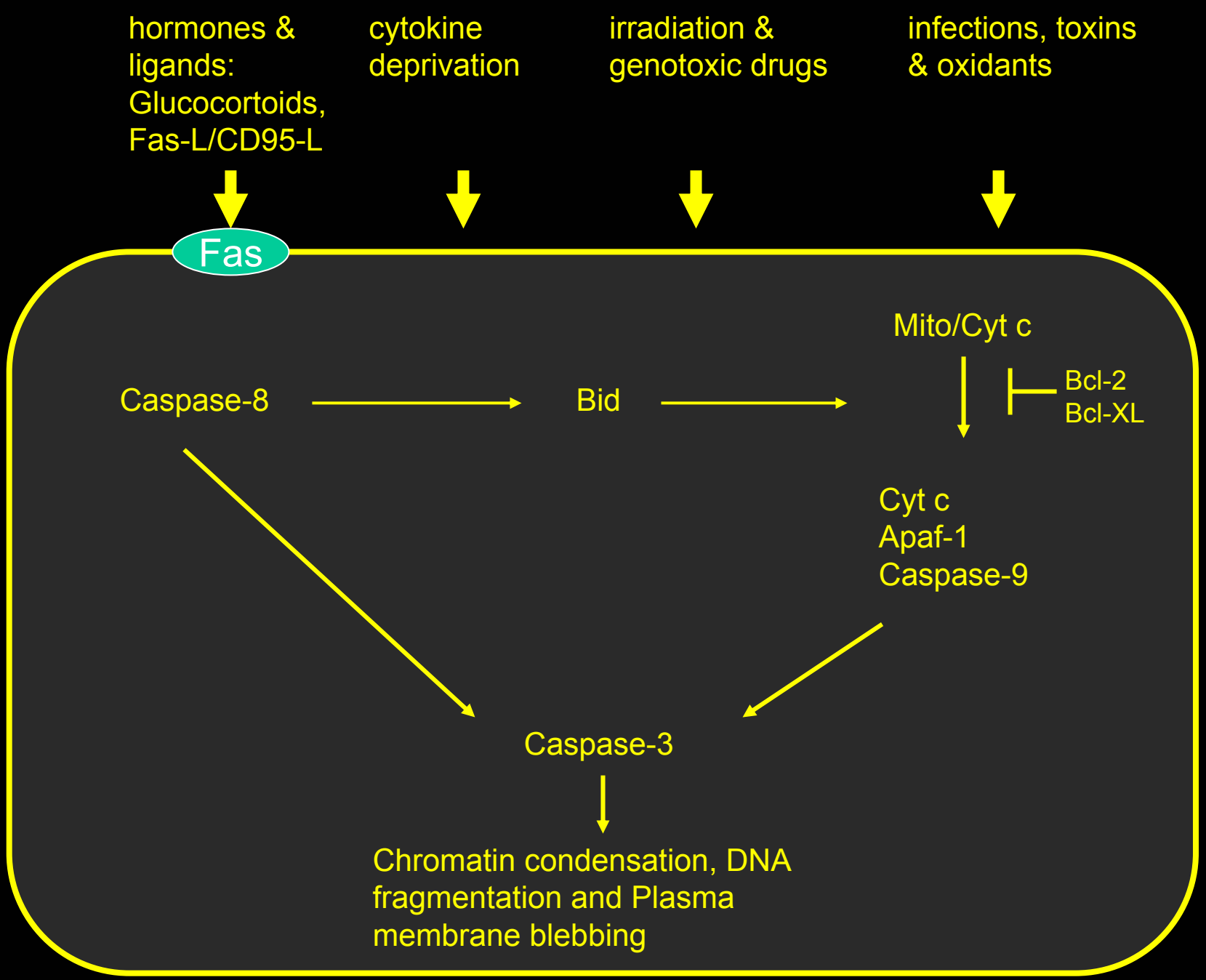
flt-1

+/+ –/–



Hsf-1 is Necessary for Normal Placental Development

Perinatal Lethality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Apaf-1
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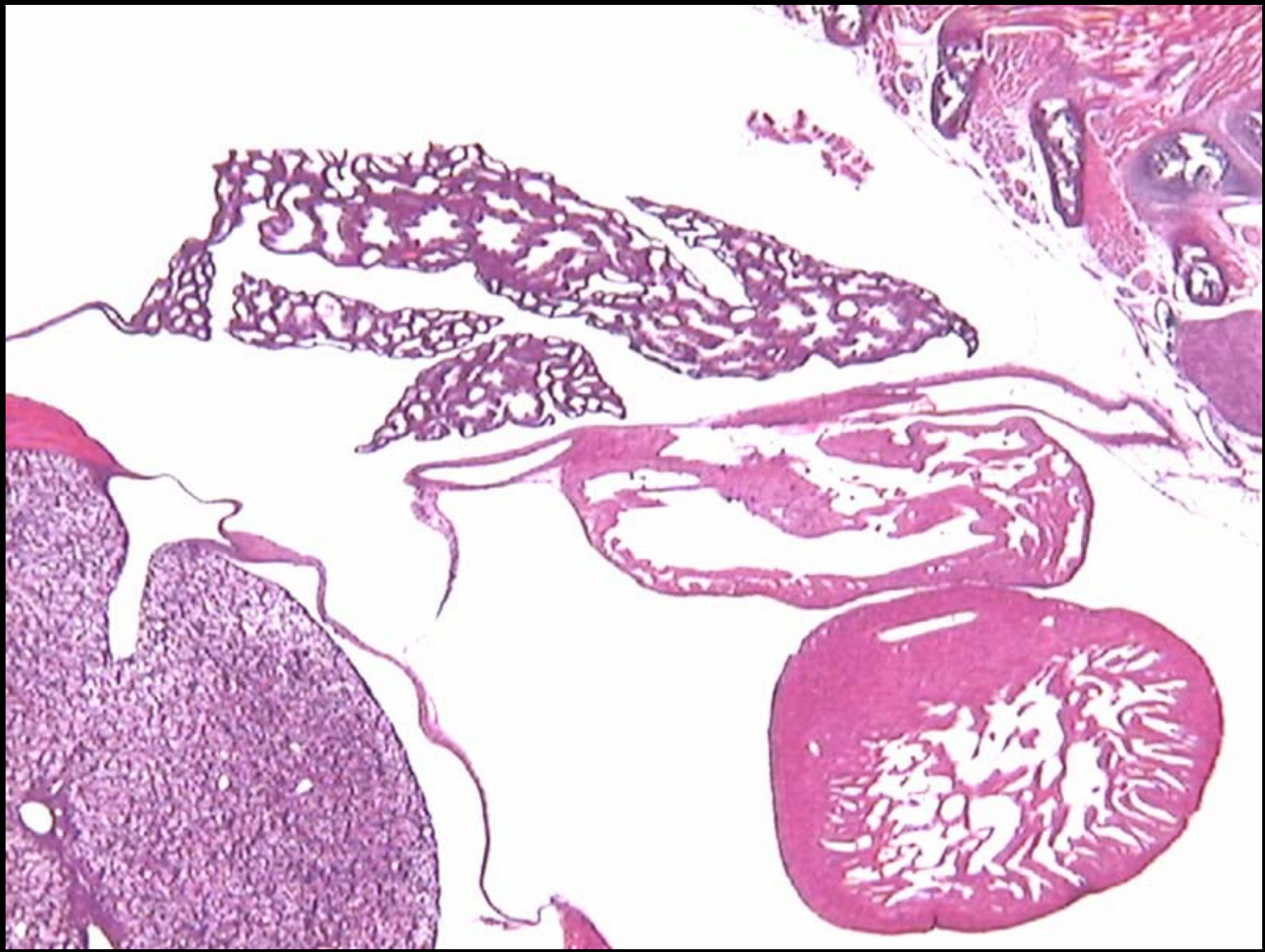
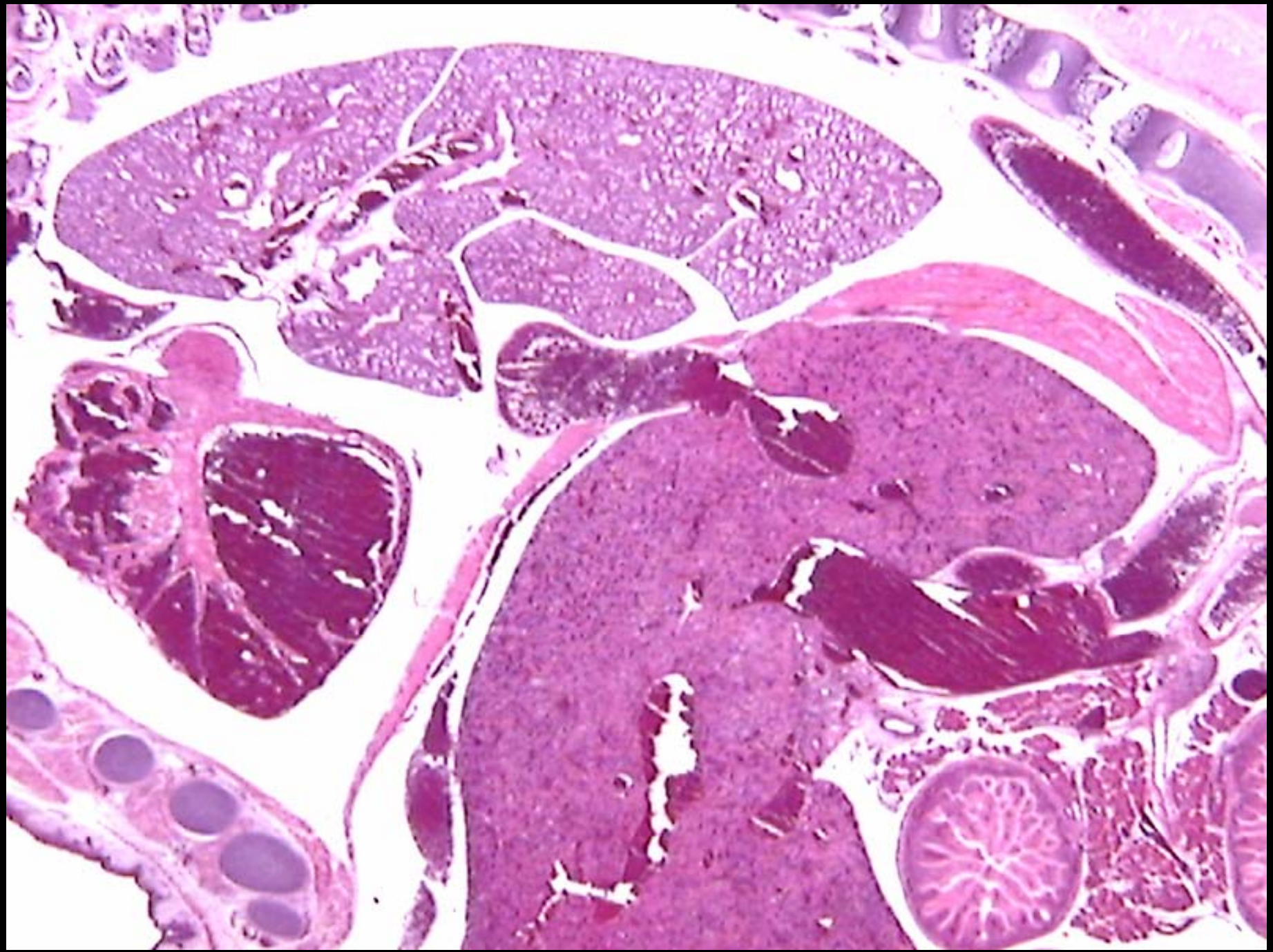
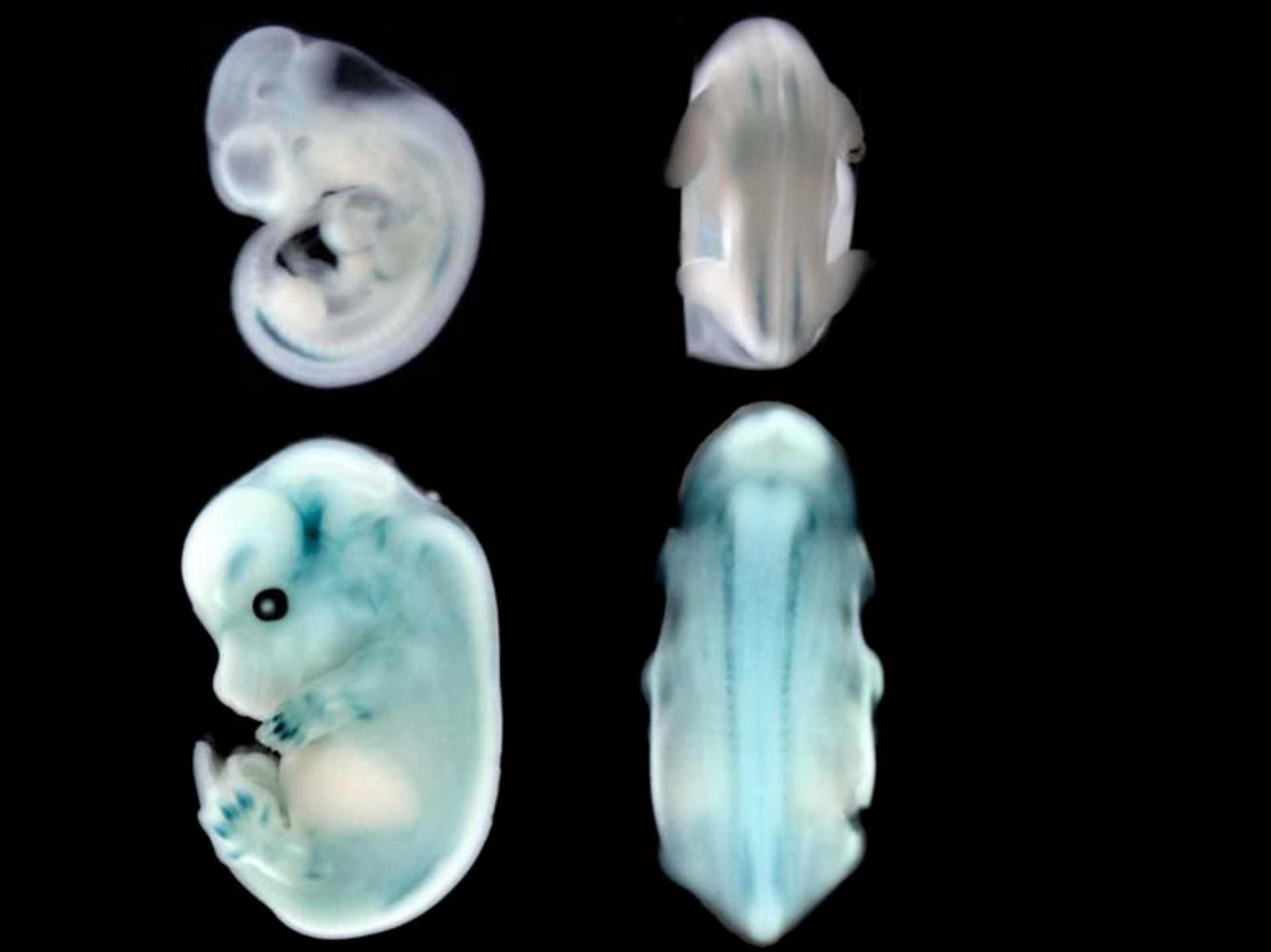
Caspase-3
Chromatin condensation, DNA fragmentation and Plasma membrane blebbing

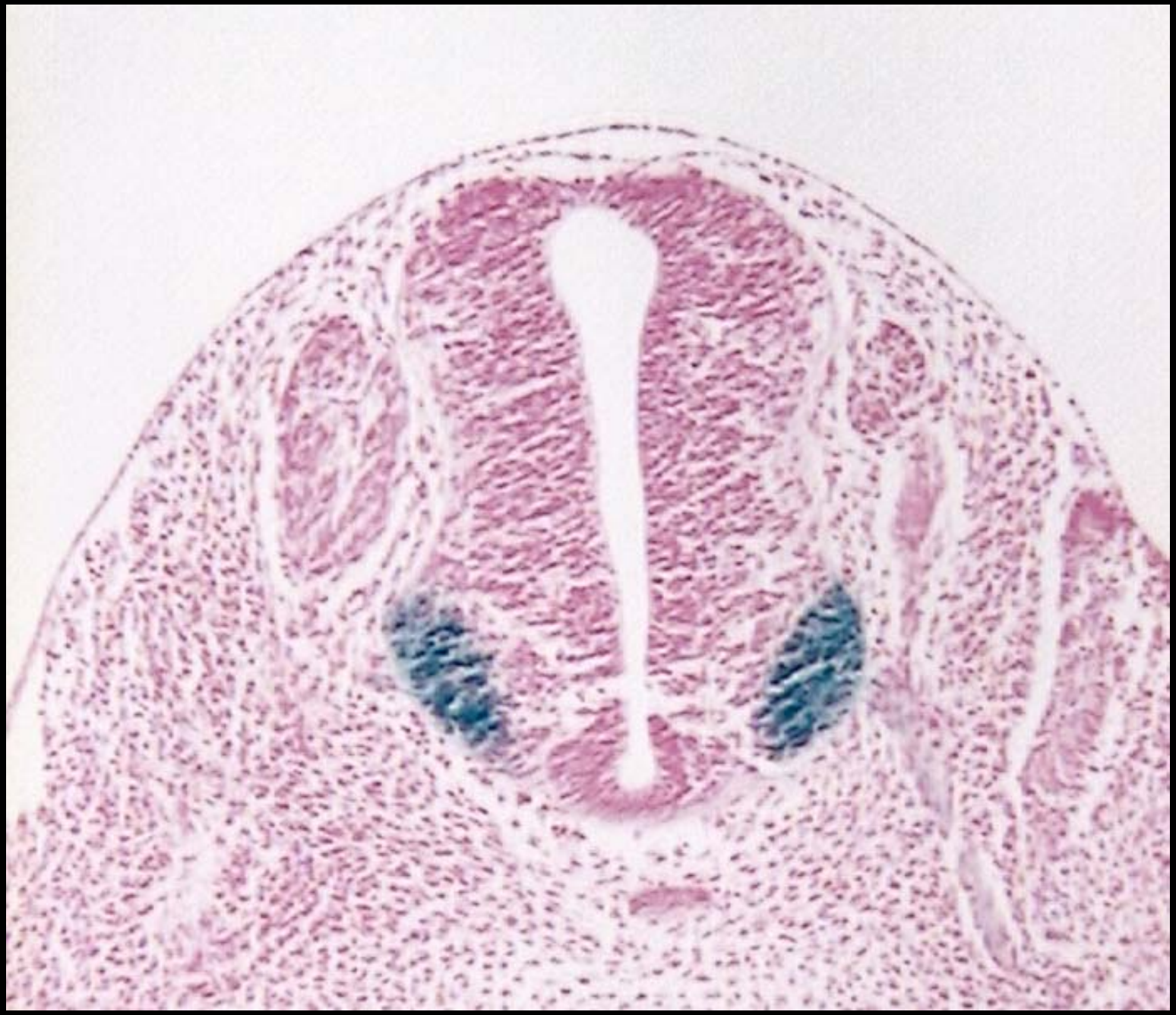
No Brain

Not Enough Lung

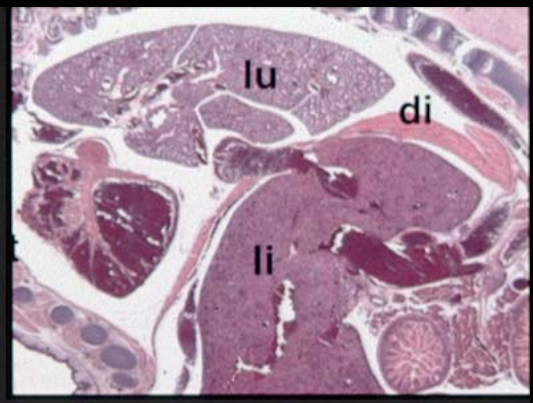






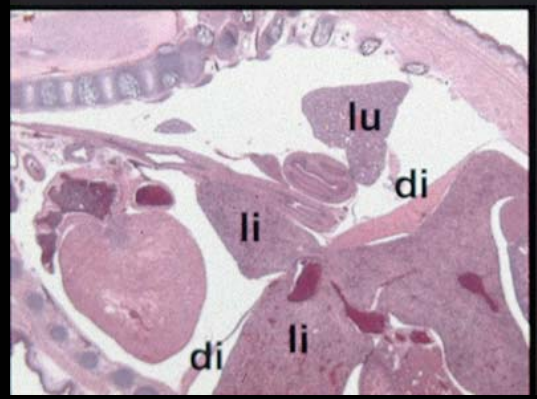


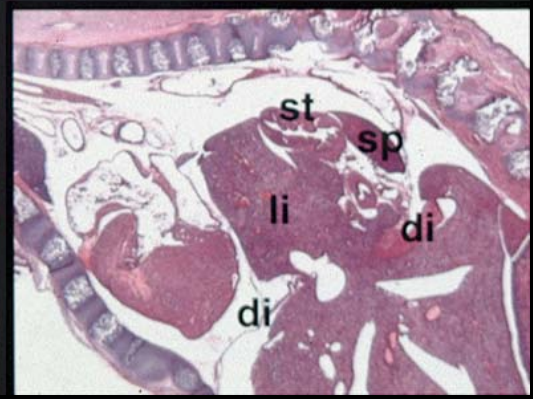
wild type



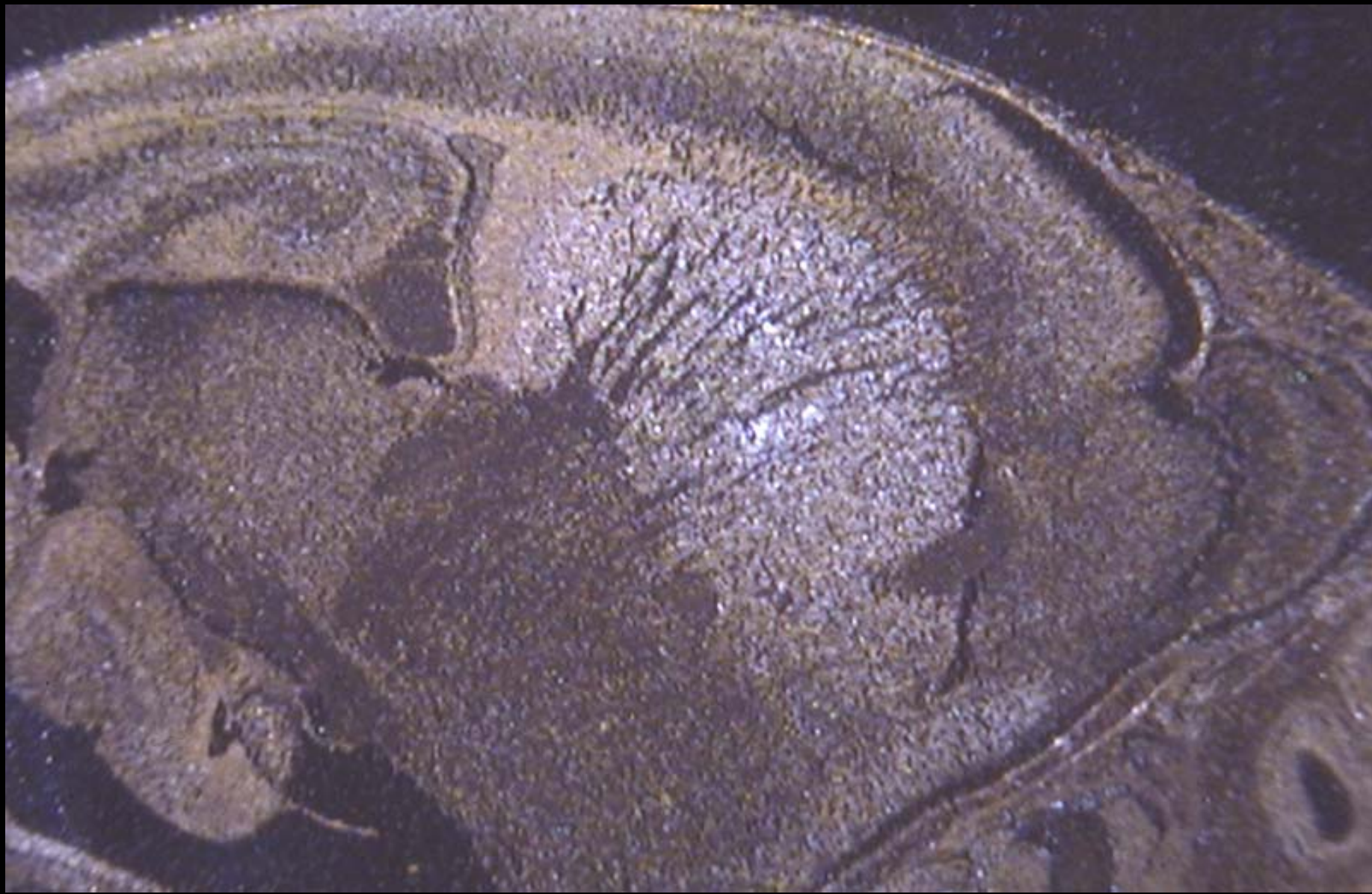
mutant
no hernia

mutant
small hernia





mutant
large hernia



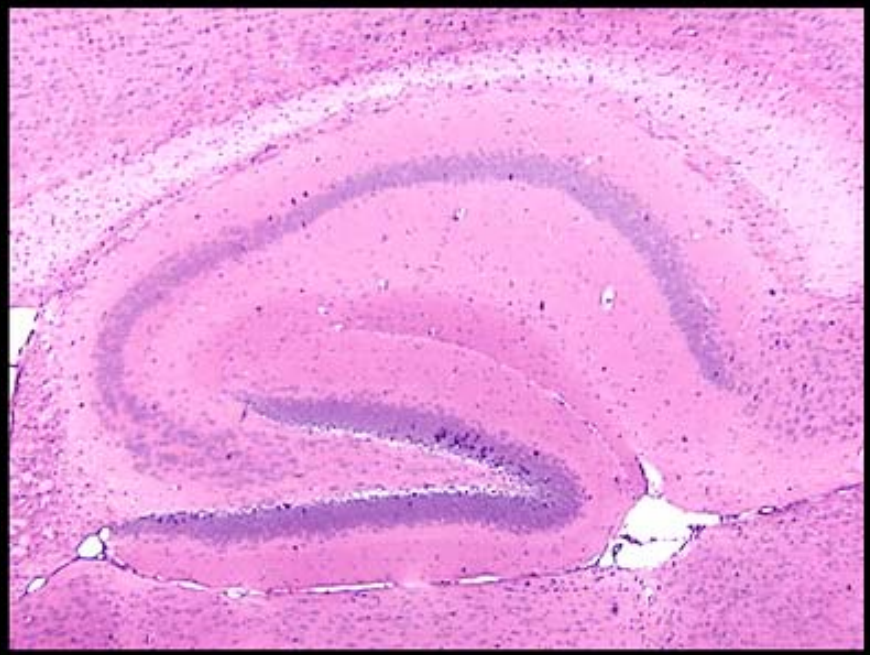
Pathologists Don’t Know Everything

Maybe It’s Been Done Before

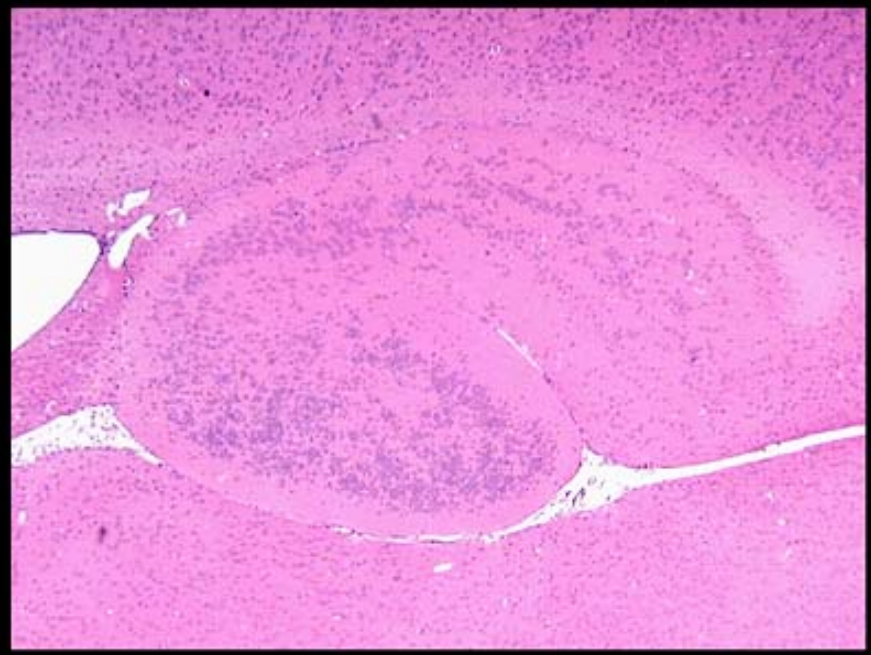
REELER

Hippocampus

wt

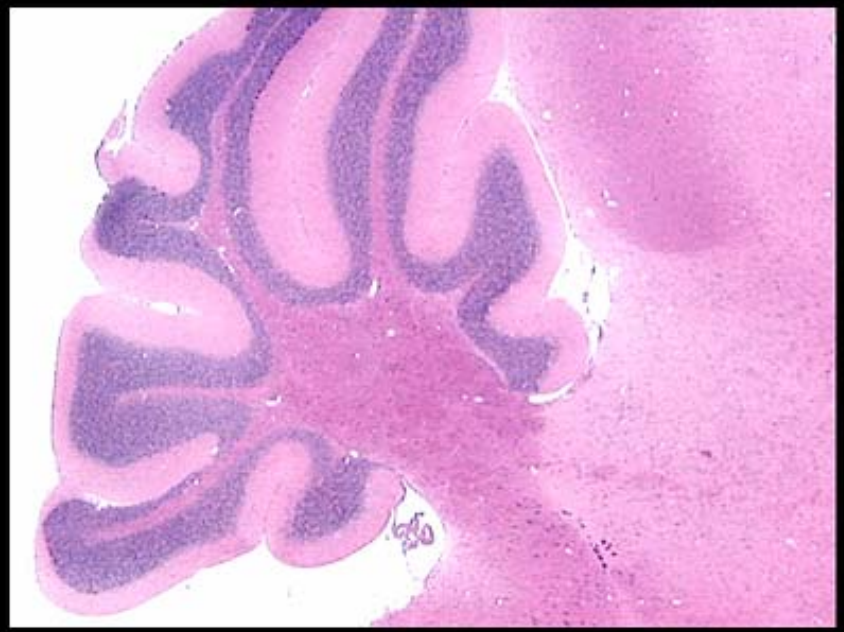


reeler

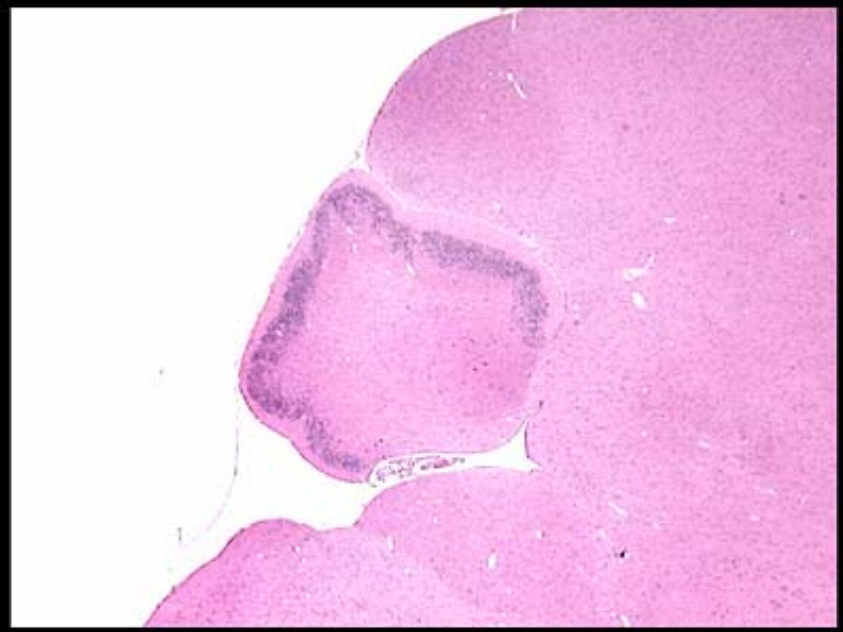


Cerebellum

wt

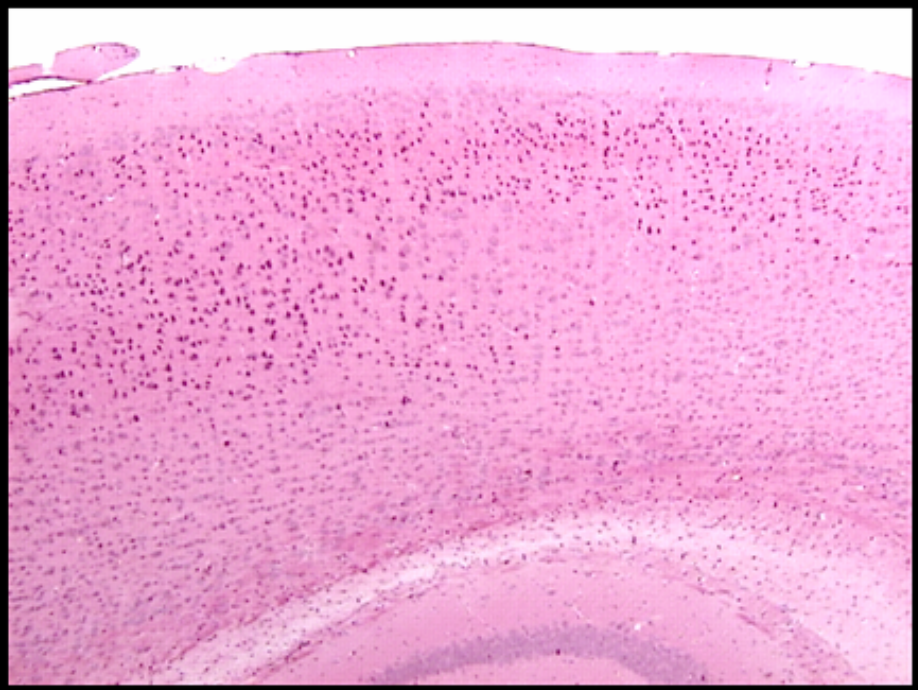


reeler

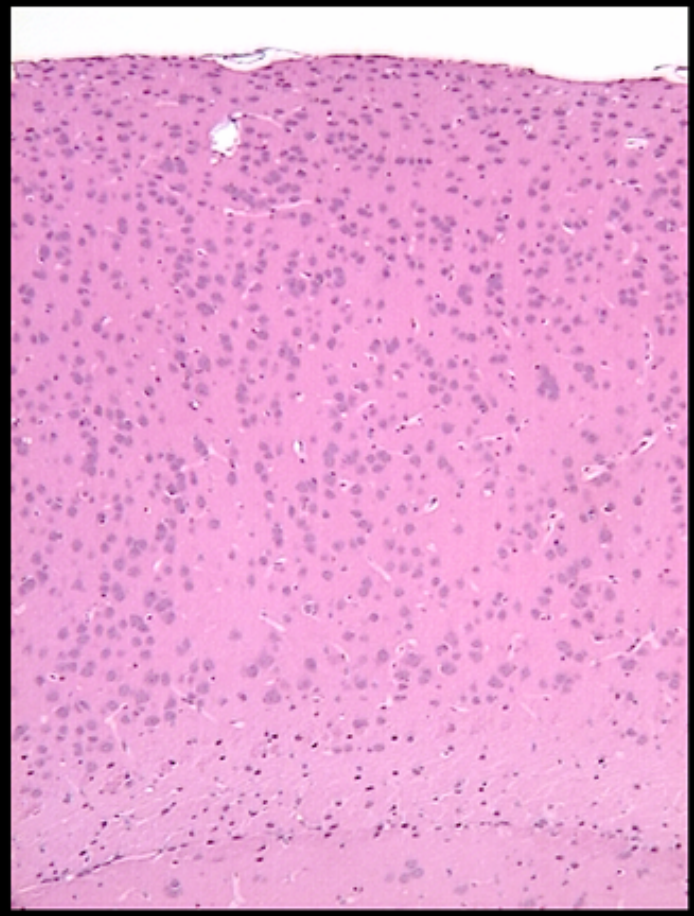


Cortex

wt



reeler



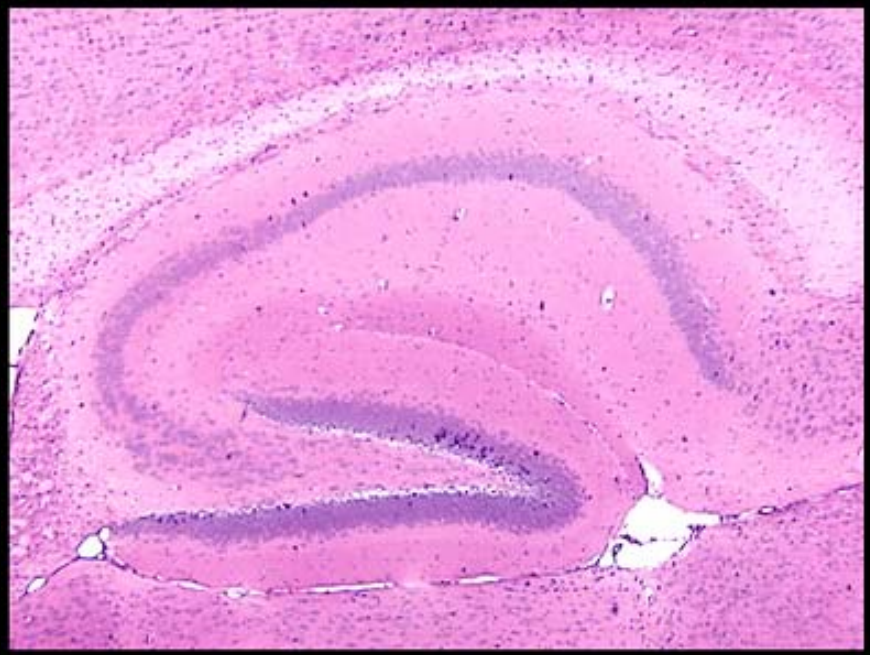
The Reeler Mouse

- **Mutations in the Extracellular Matrix Associated Signaling Molecule *Reelin***
- **Specific Defect in Neuronal Migration Along the Radial Glial Network Causes abnormal cortical layering and abnormal cerebellar foliation**

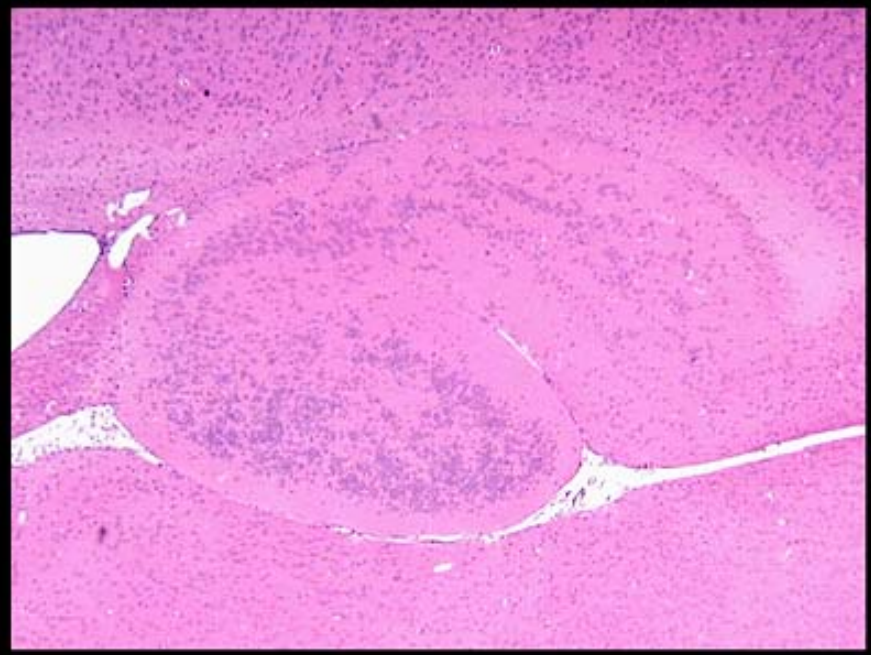
SCRAMBLER

Hippocampus

wt

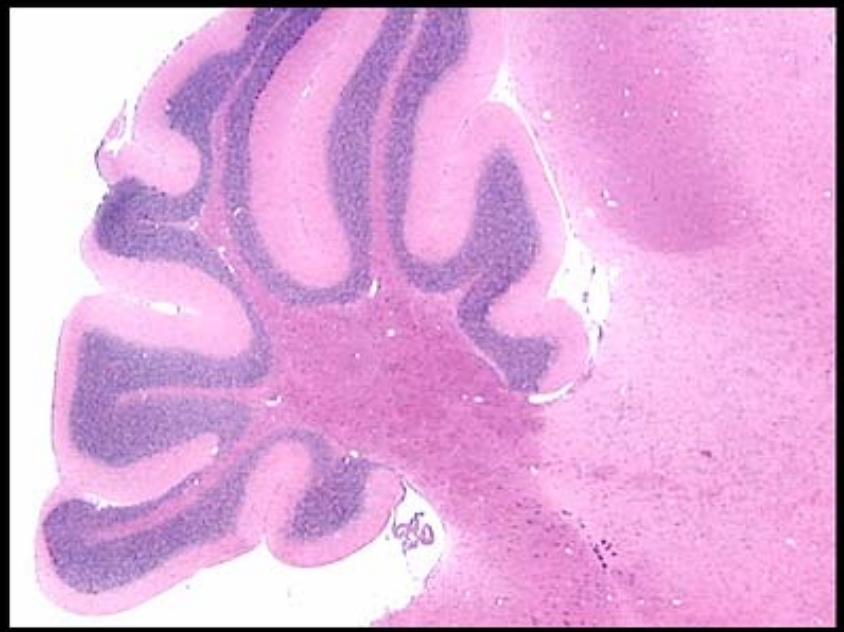


reeler

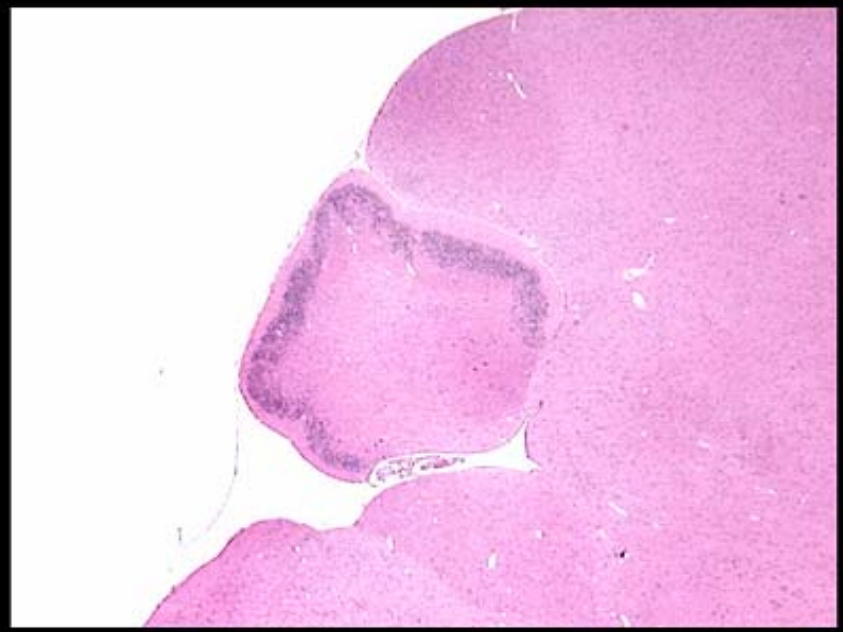


Cerebellum

wt

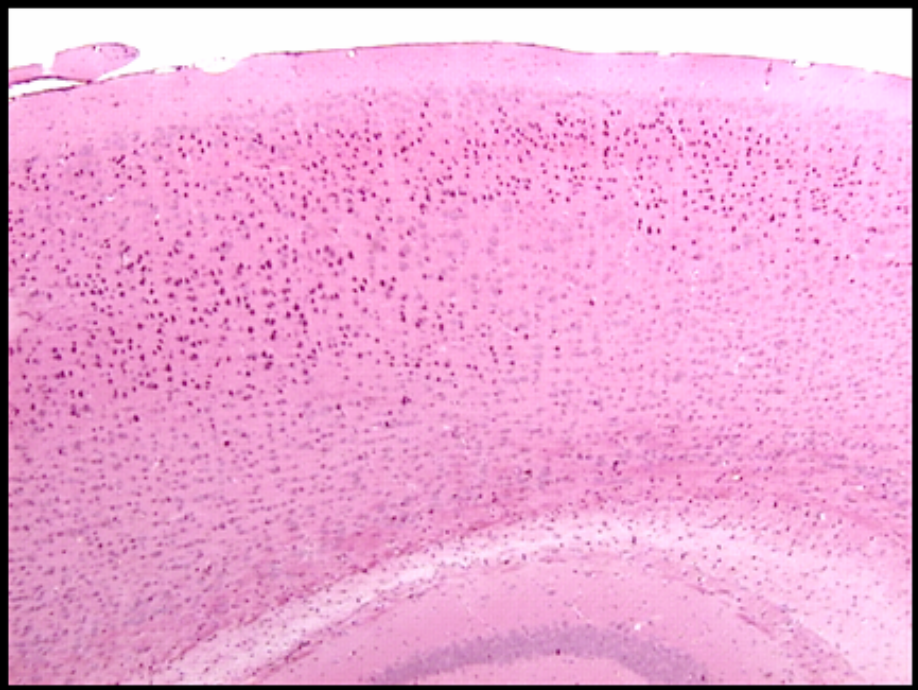


reeler

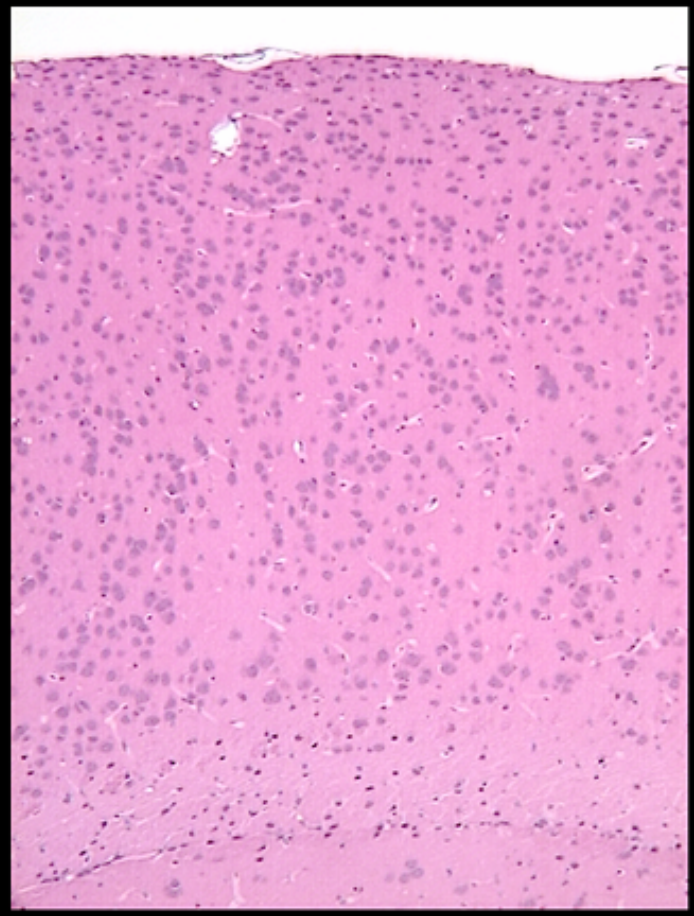


Cortex

wt



reeler



The Scrambler Mouse

- Same Neurological and Neuroanatomical Phenotype as Reeler, but Caused by a Mutation in the mDab1 Gene
- mDab1 encodes an intracytoplasmic adaptor protein

VLDL Receptor Knockout

- Generated by Conventional Knockout
- No Abnormalities of Lipid Metabolism
- Growth Rate Slightly Reduced Compared to Wild Type Control Animals
- No Other Significant Abnormalities Detected
- No Apparent Neurological Phenotype

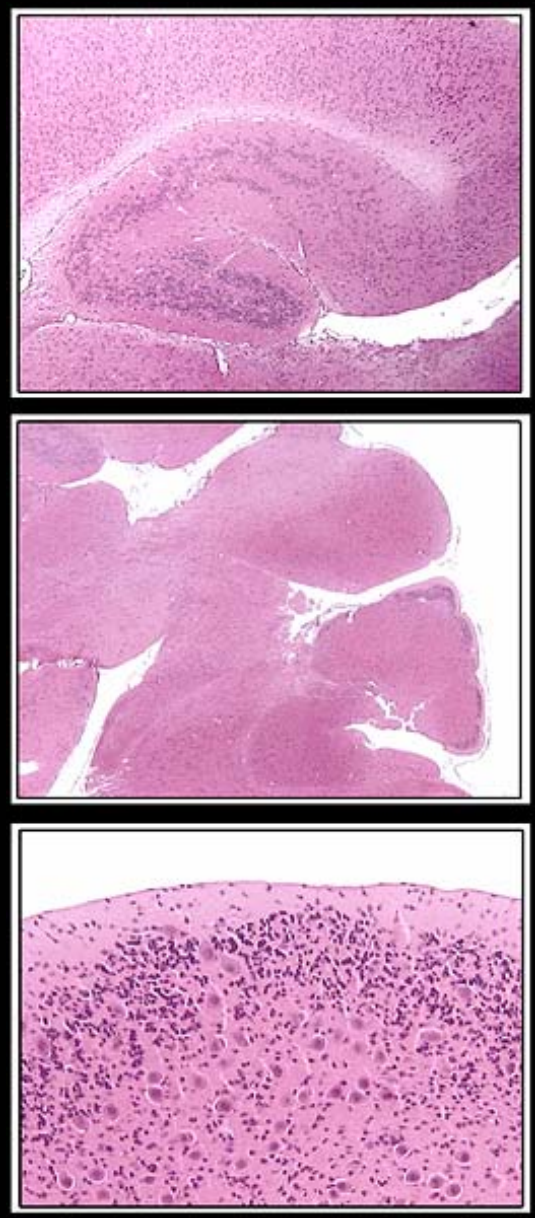
ApoE Receptor 2 Knockout

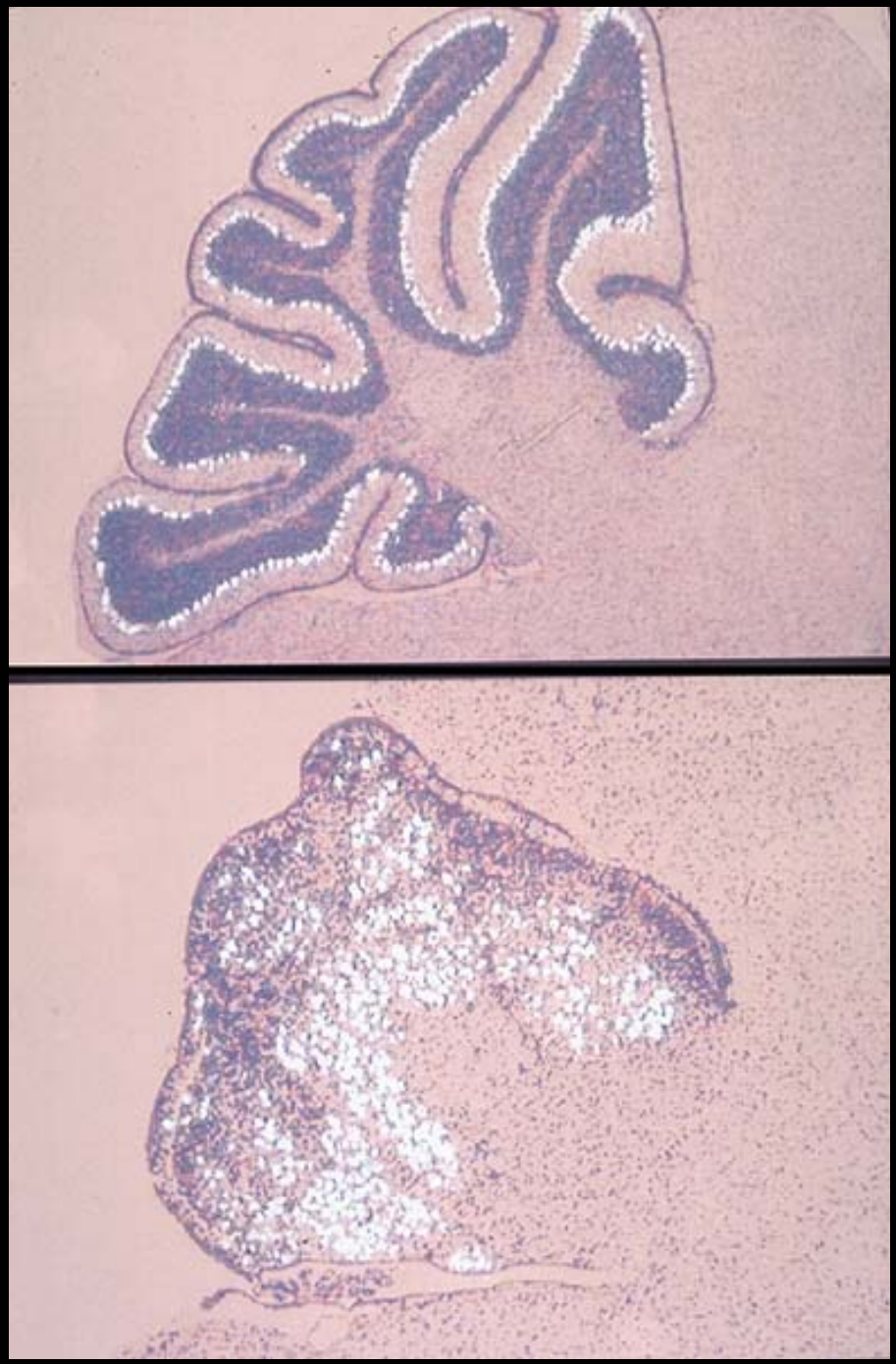
- Generated by Conventional Knockout
- Superficially Normal
- No Apparent Neurological Phenotype

Phenotype of VLDLR/ApoER2 Double Knockout

- Failure to Thrive
- Ataxia
- Wide Gait
- Animals Frequently Flip onto Their Back

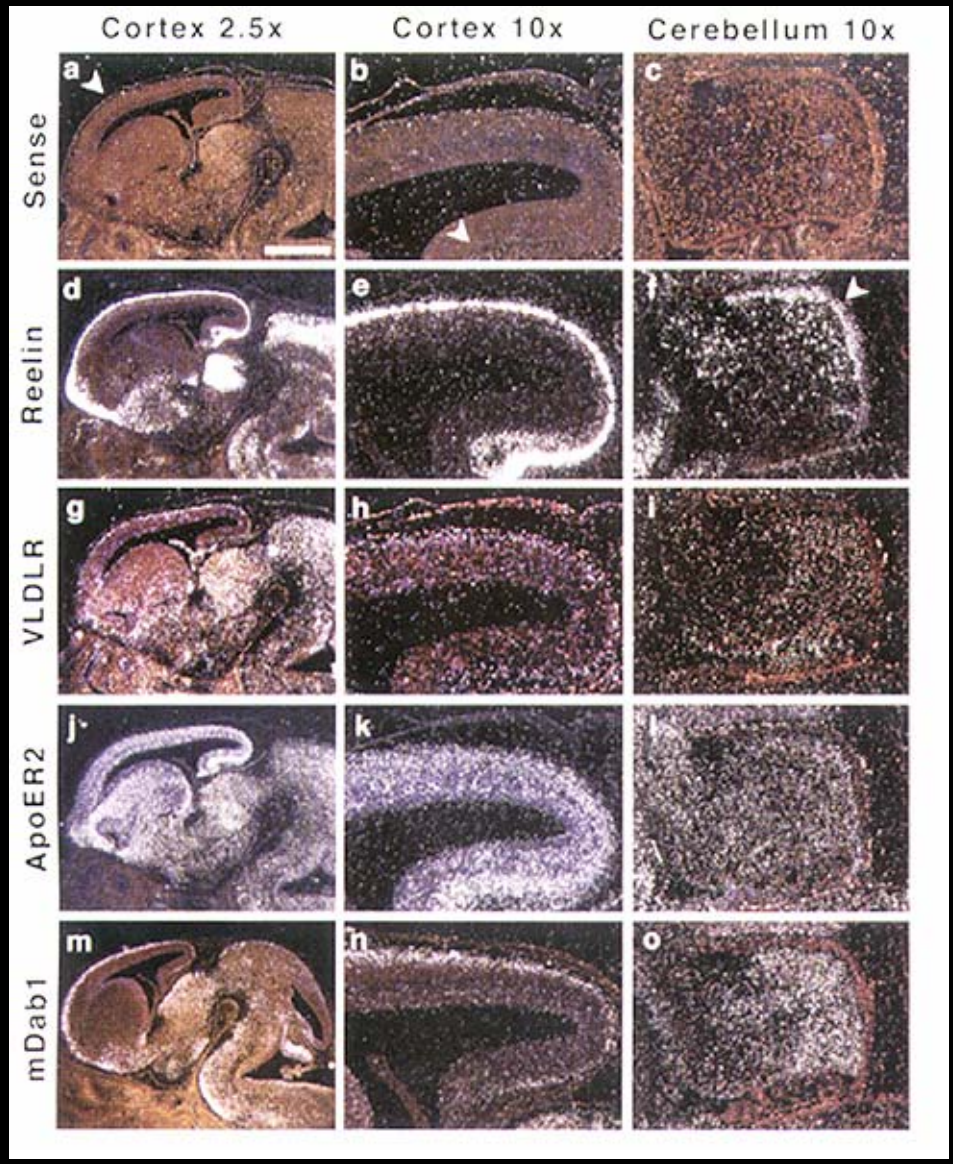
VLDLR / ApoER –/–





The Neurological Phenotype of VLDLR/ApoER2 Knockout Mice is Indistinguishable From That of REELER and SCRAMBLER Mice

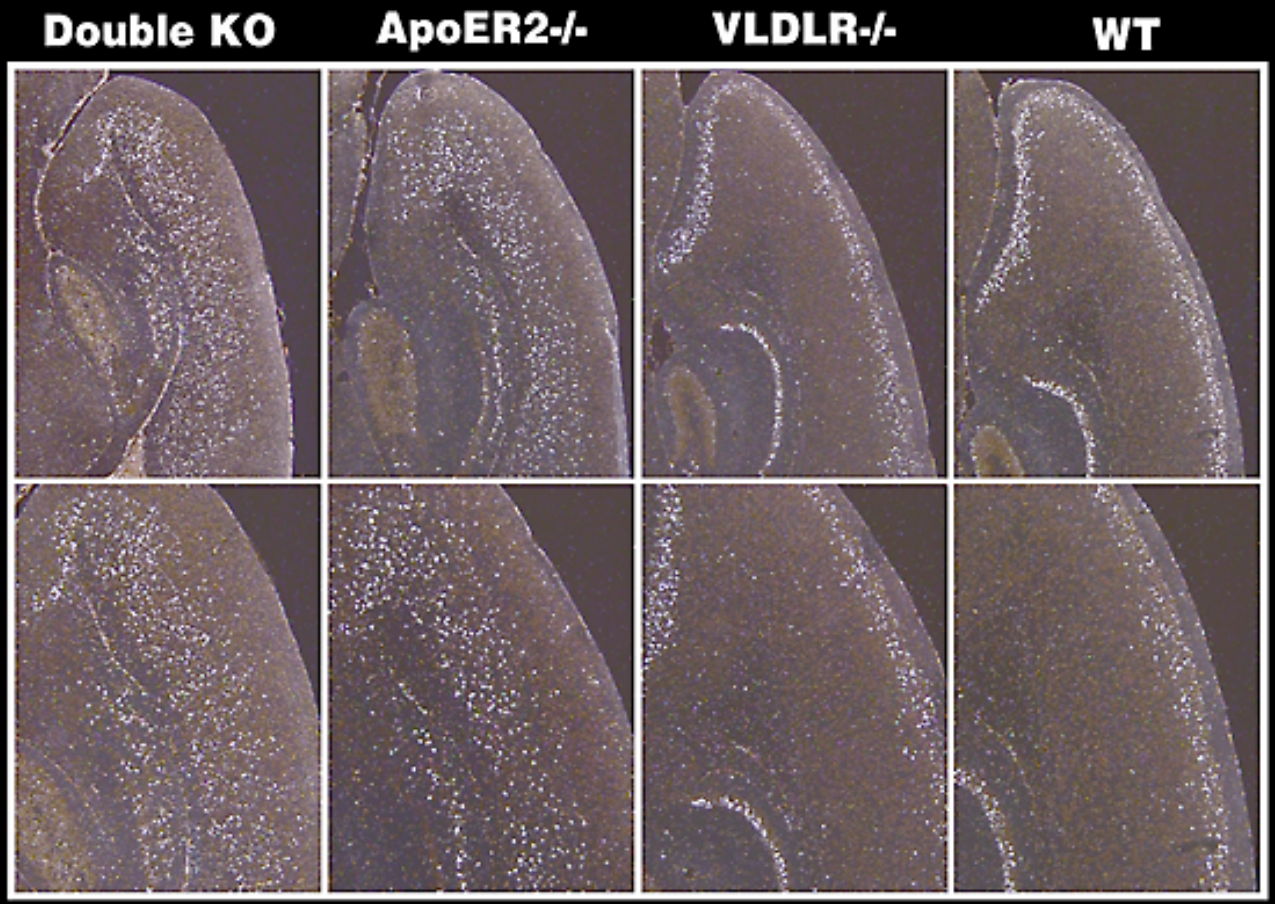
in situ 13.5 Mouse Brain

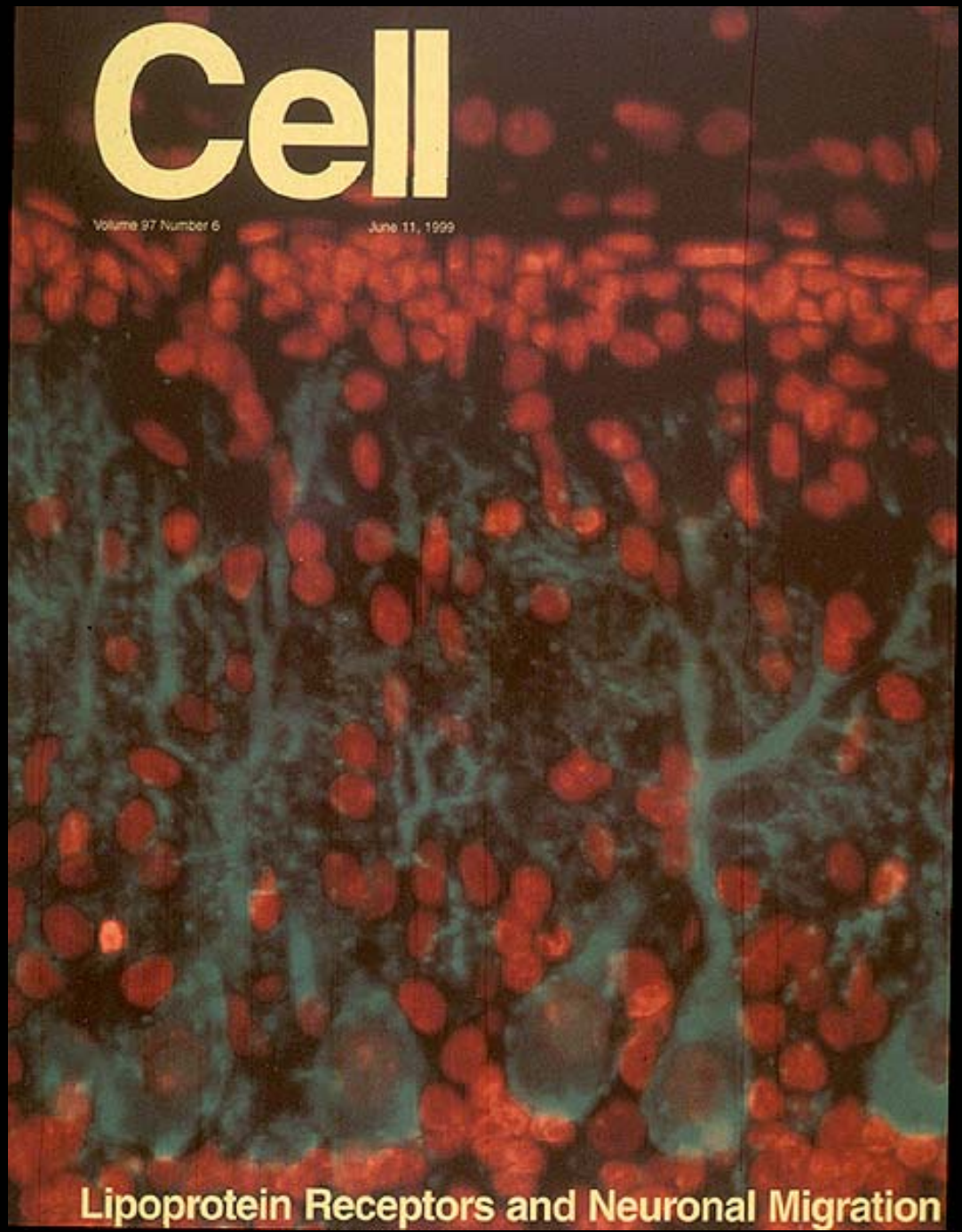


The Cerebral Cortex in Reeler and Scrambler Mice is Inside Out

- In the Normal Animal,Younger Neurons Migrate Past Older Neurons
- Are the VLDLR/ApoER2 brains inside out?

- Label Dividing Neurons with Radioactive Hydrogen at E15
- Euthanize animals at 20 days of age
- Expose sections of brain to photographic emulsion

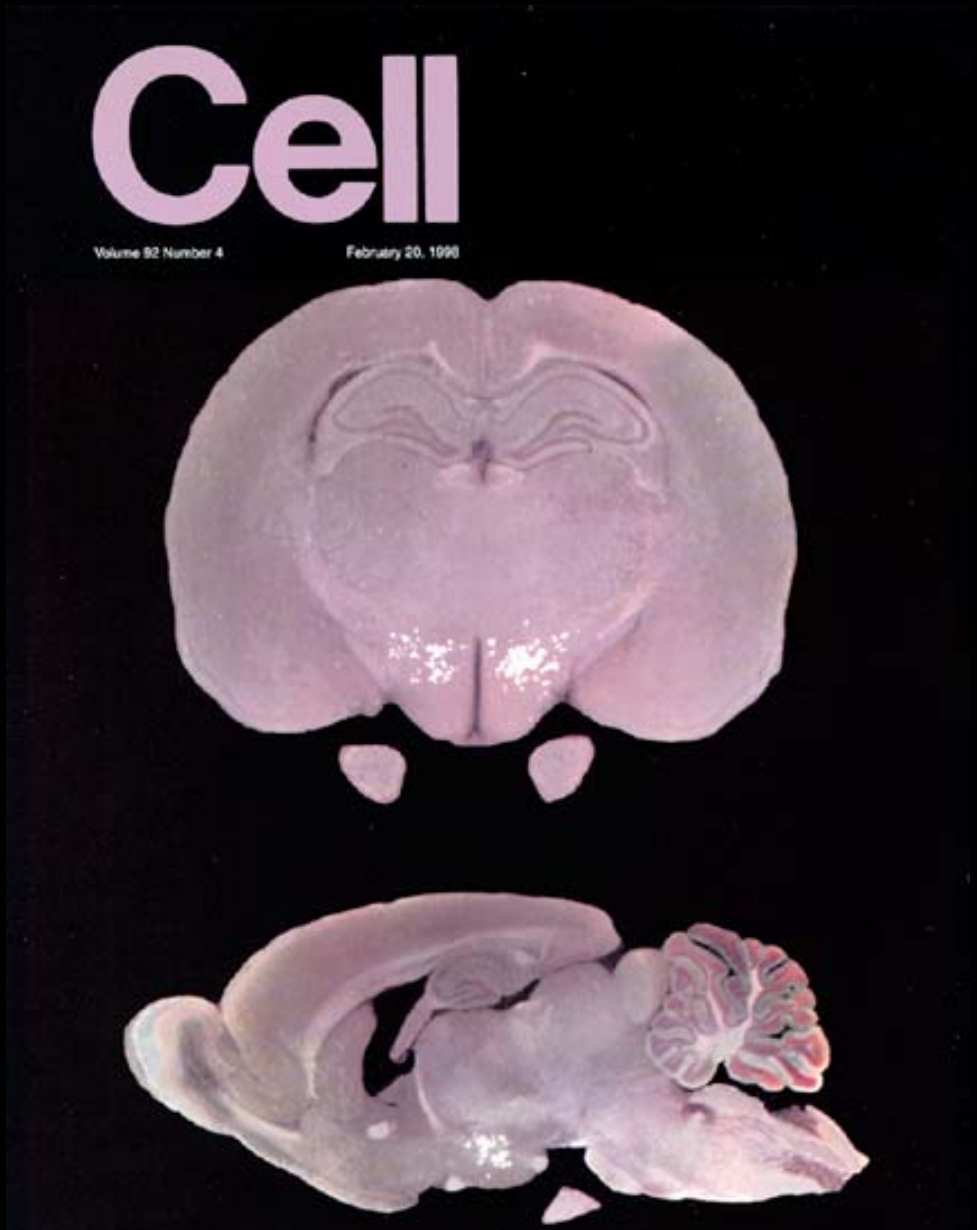

Double KO
ApoER2-/-
VLDLR-/-
WT
E 15


Cell
Volume 97 Number 6
June 11, 1999
Lipoprotein Receptors and Neuronal Migration

OREXIN

Cell

Volume 92 Number 4 February 20, 1998



Neuropeptides that Control Feeding Behavior

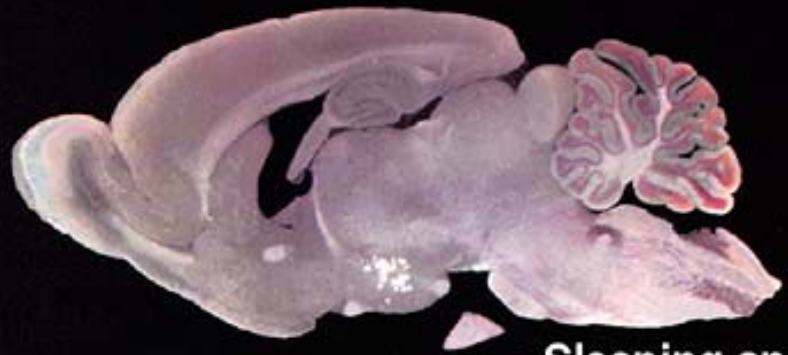
Viable
No Phenotype Related to Weight

Look at Night

- **Narcoleptic Mice**
  - **are missing the orexin ligand**
- **Narcoleptic dogs**
  - **are missing orexin 2 receptor**

Cell

Volume 92 Number 4 February 20, 1998



Sleeping and

Neuropeptides that Control Feeding Behavior

A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains

Christelle Peyron[1], Juliette Faraco[1], William Rogers[1], Beth Ripley[1], Sebastiaan Overeem[1,2], Yves Charnay[3], Sona Nevsimalova[4], Michael Aldrich[5], David Reynolds[6], Roger Albin[5], Robin Li[1], Marcel Hungs[1], Mario Pedrazzoli[1], Muralidhara Padigaru[6], Melanie Kucherlapati[6], Jun Fan[7], Richard Maki[7], Gert Jan Lammers[2], Constantin Bouras[3], Raju Kucherlapati[6], Seiji Nishino[1], & Emmanuel Mignot[1]

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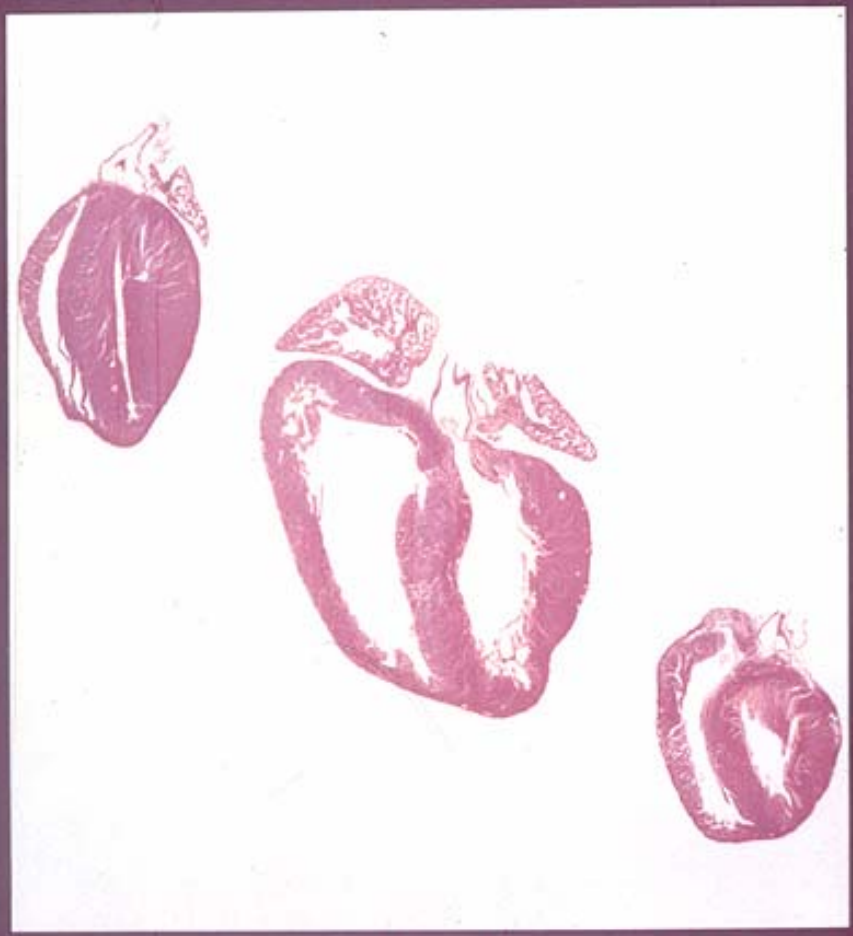
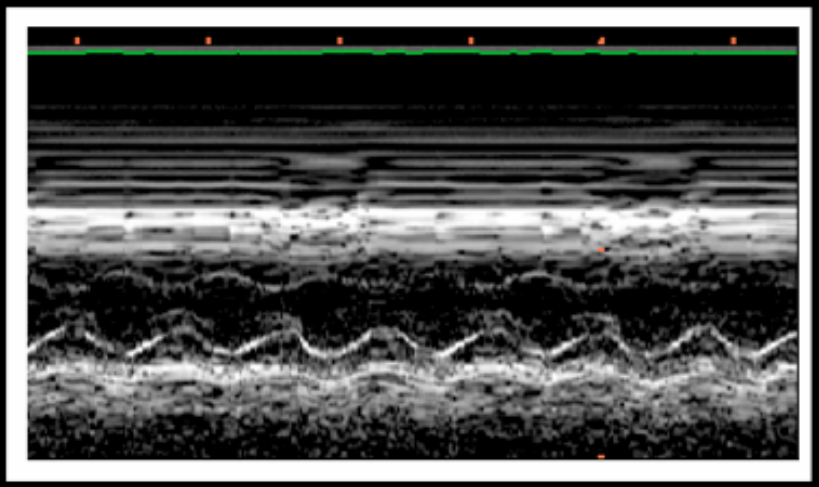


Mouse Model for Heart Failure

In Vivo Assesment of Cardiac Contractability

Control



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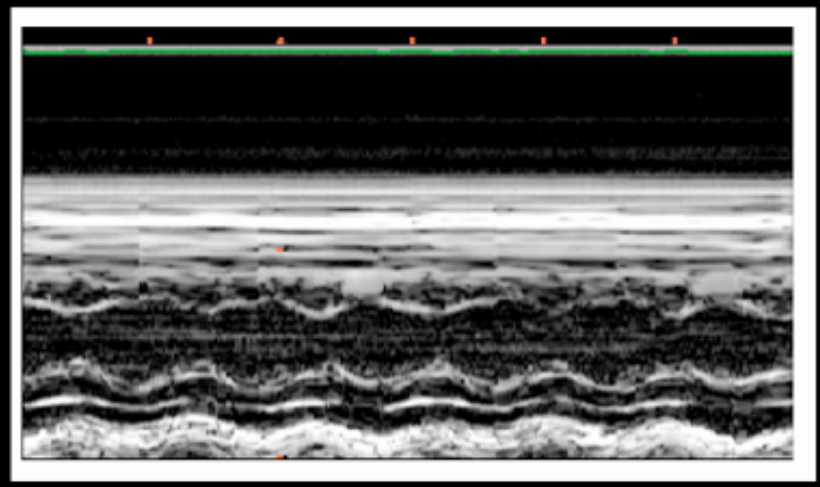

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