Biomedical research has the goal of making us better

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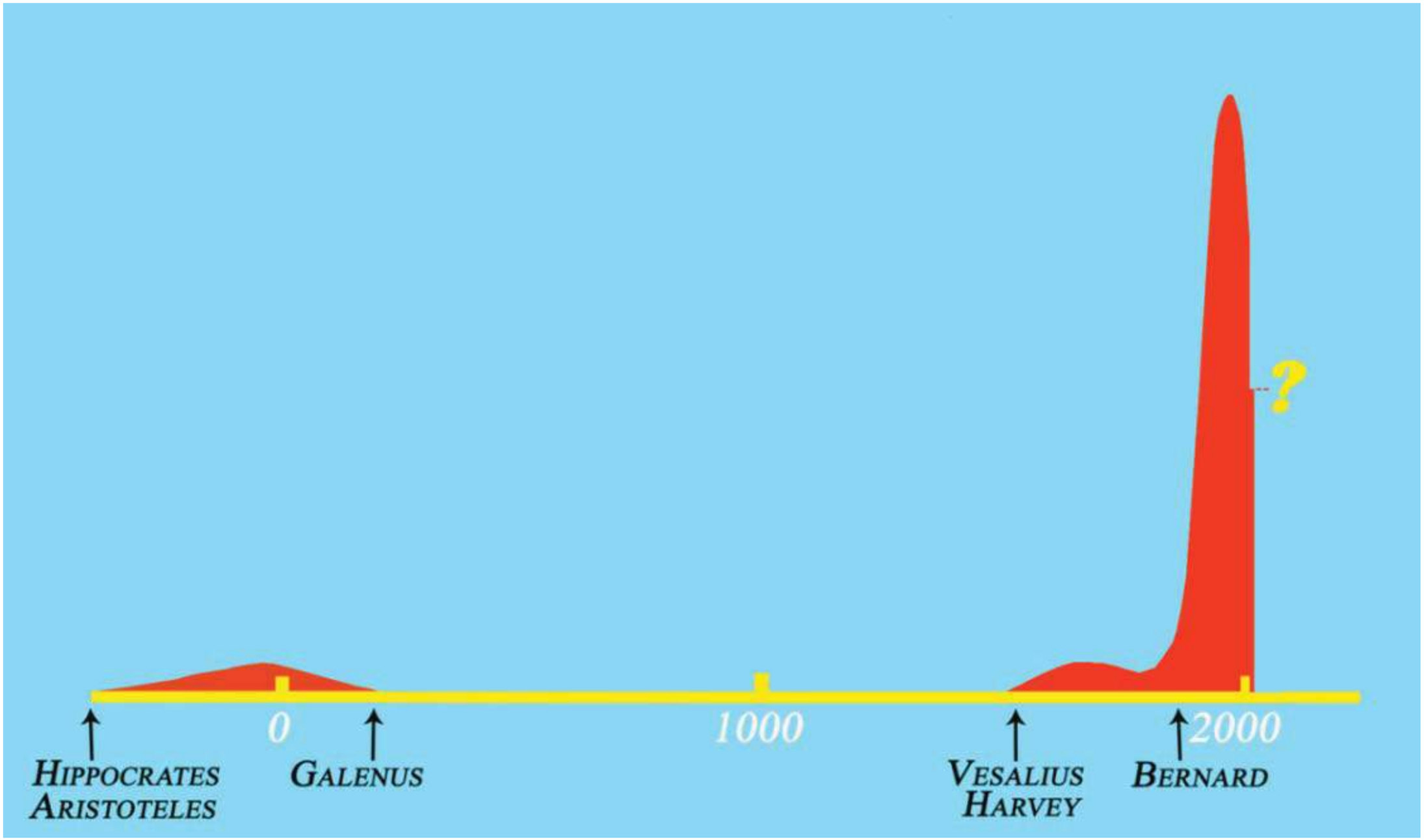
To do this we need to better understand humankind biology

Outline

- Discuss the benefits of and how to choose a model system.
- Go through a case study of using a zebrafish model to understand how melanomas form

Use of animal model systems has exploded in the last 100 years



Reasons for using model systems:

Identify genes that are causative mutations for birth defects

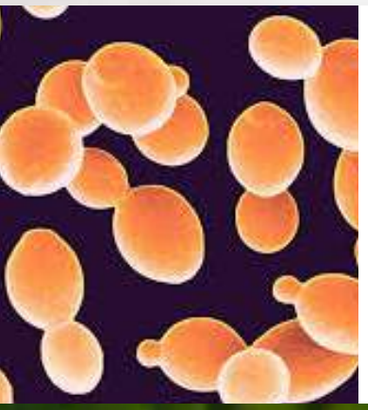
Identify genes that are causative mutations for genetic diseases (CF, Diabetes, cancer, etc)

Understand the biology of critical molecular pathways

Build genetic models of a disease to screen for novel compounds that might alleviate problem

Screen molecules for potential therapeutic value

What is the right animal model system?




1 cm
v.d.



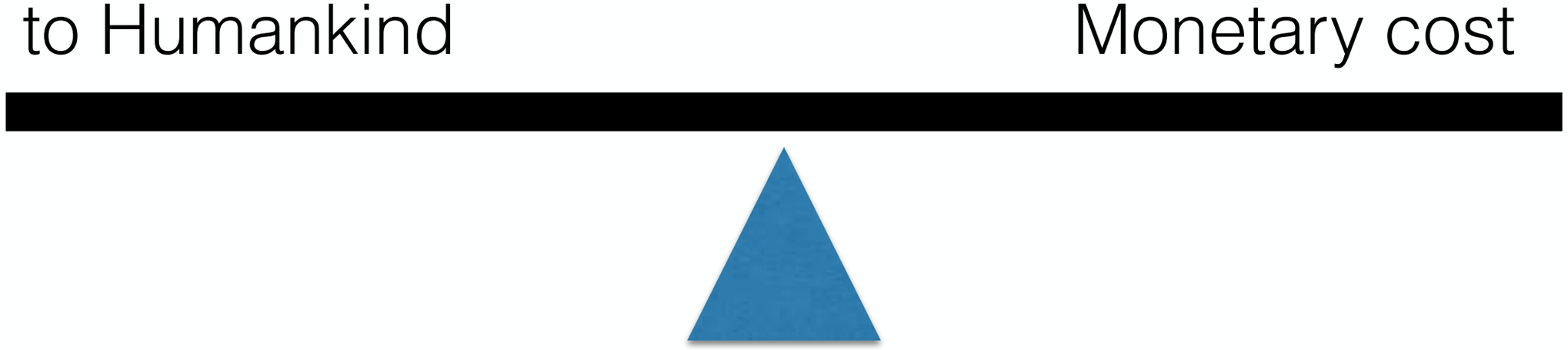

These are all great model systems. Each one is useful depending on the biology being investigated




Justifying choice of model system

Overall Benefit to Humankind

Ethical cost + Monetary cost



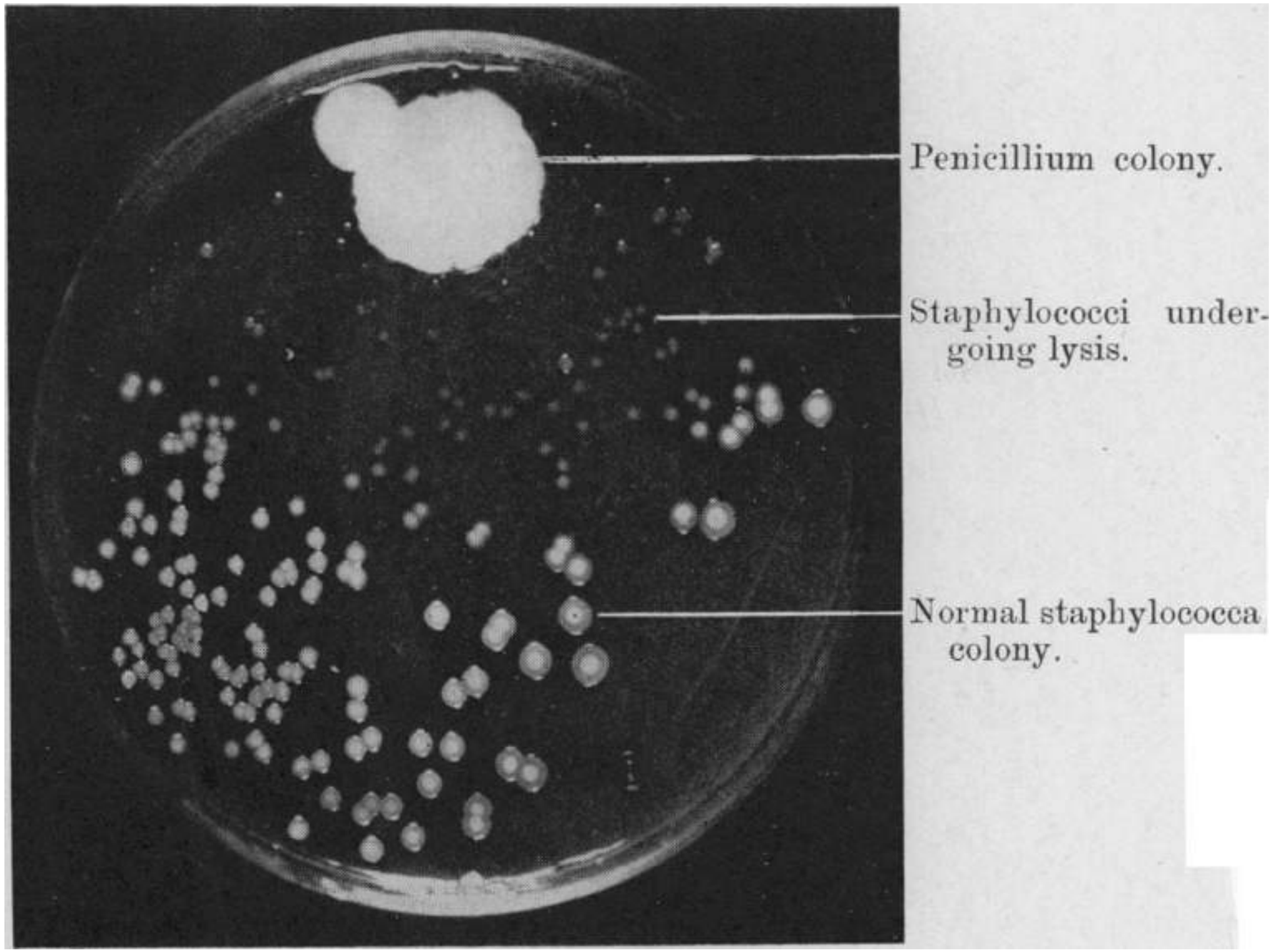
Choosing a model system

1. **is the animal's biology appropriate for the question**

2. is there a better ethical choice for a model system

3. is the question being asked valuable enough to overcome the ethical and financial obstacles.

Penicillin is good - but we could have gotten it wrong



Alexander Fleming

TOXICITY OF PENICILLIN.

The toxicity to animals of powerfully antibacterial mould broth filtrates appears to be very low. Twenty c.c. injected intravenously into a rabbit were not more toxic than the same quantity of broth. Half a c.c. injected intraperitoneally into a mouse weighing about 20 gm. induced no toxic symptoms. Constant irrigation of large infected surfaces in man was not accompanied by any toxic symptoms, while irrigation of the human conjunctiva every hour for a day had no irritant effect.

Is penicillin toxic?

Had Penicillin been injected into guinea pigs, we probably would have been delayed in finding our first antibiotic.

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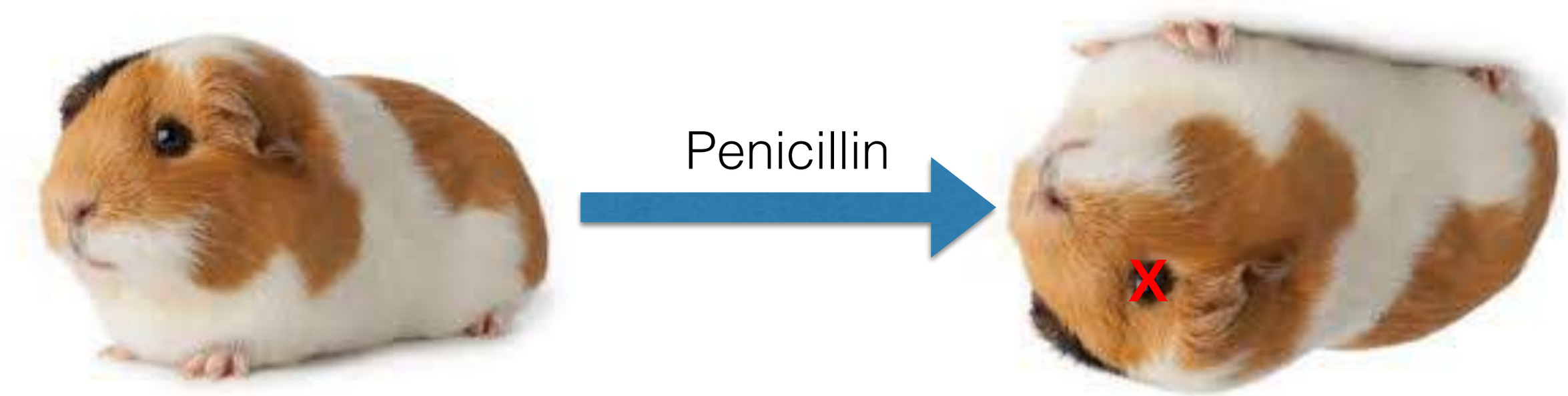

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Animal Models of Tuberculosis: Guinea Pigs

Simon Clark, Yper Hall, and Ann Williams

Microbiology Services, Public Health England, Porton Down, Salisbury SP4 0JG, United Kingdom

Correspondence: ann.rawkins@phe.gov.uk

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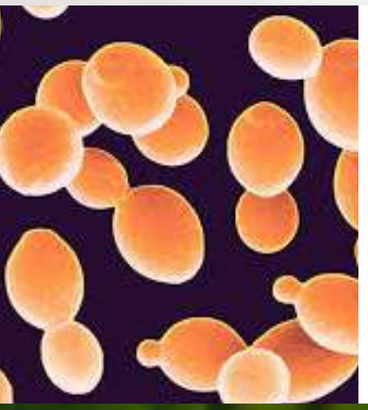
Not all of these require the use of vertebrate animals. In some cases, the use of vertebrates isn't justified




1 cm
v.d.

"You've heard about some of these pet projects, they really don't make a whole lot of sense and sometimes [tax] dollars go to projects that have little or nothing to do with the public good,…….. things like fruit fly research in Paris, France. I kid you not."

-Politician

"You've heard about some of these pet projects, they really don't make a whole lot of sense and sometimes [tax] dollars go to projects that have little or nothing to do with the public good,…….. things like fruit fly research in Paris, France. I kid you not."

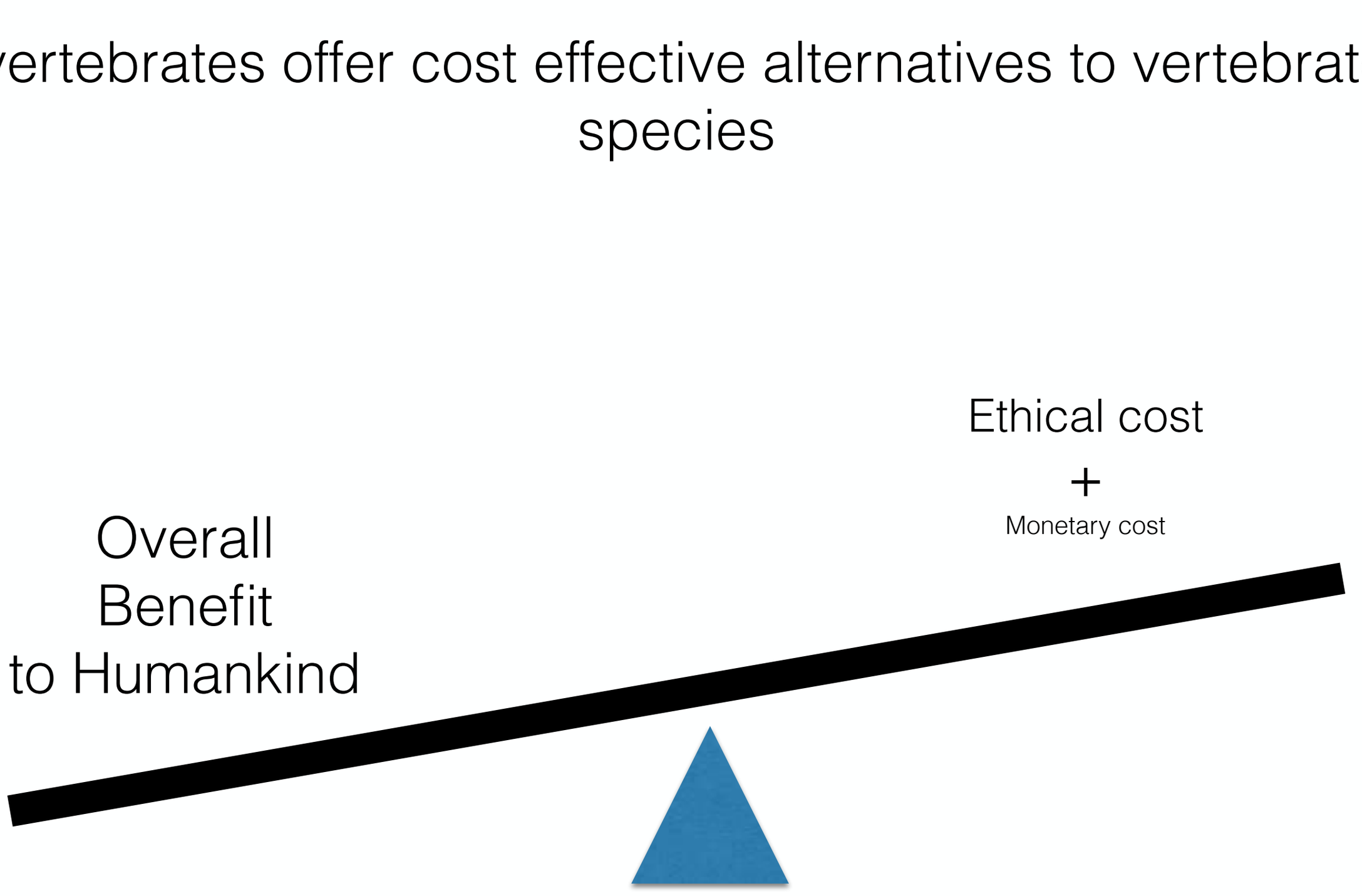
-Politician

This is a dangerous statement from an ignorant person!

Invertebrates offer cost effective alternatives to vertebrates species

Overall
Benefit
to Humankind

Ethical cost
+
Monetary cost



Drosophila melanogaster (fruit fly)

Thomas Hunt Morgan - Nobel Prize for Genetic Studies in *Drosophila*

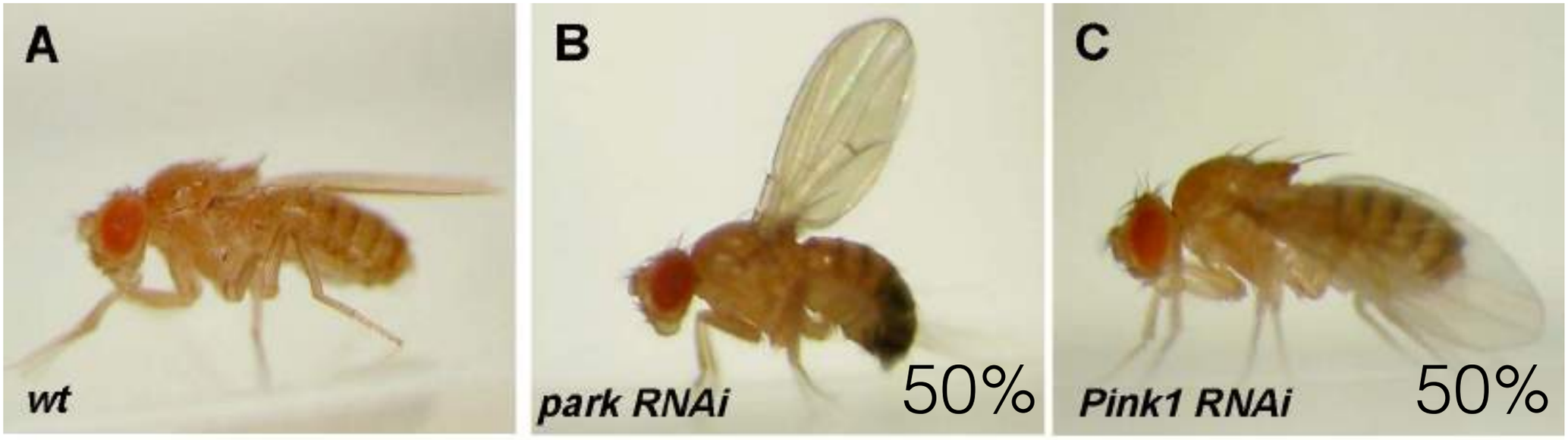
Drosophila melanogaster (fruit fly)

Thomas Hunt Morgan - Nobel Prize for Genetic Studies in *Drosophila*

Many molecular pathways associated with a particular disease are deeply conserved even when the phenotype at the end of those pathways does not resemble the disease.

Here is where invertebrate systems are incredibly useful!

Using forward genetics to screen for novel interacters with *park and pink*



II III II

park and pink1 are both genes that when mutated in us result in increased probability in developing parkinson's disease

Fernandes and Rao *Molecular Brain* 2011, **4**:17
http://www.molecularbrain.com/content/4/1/17

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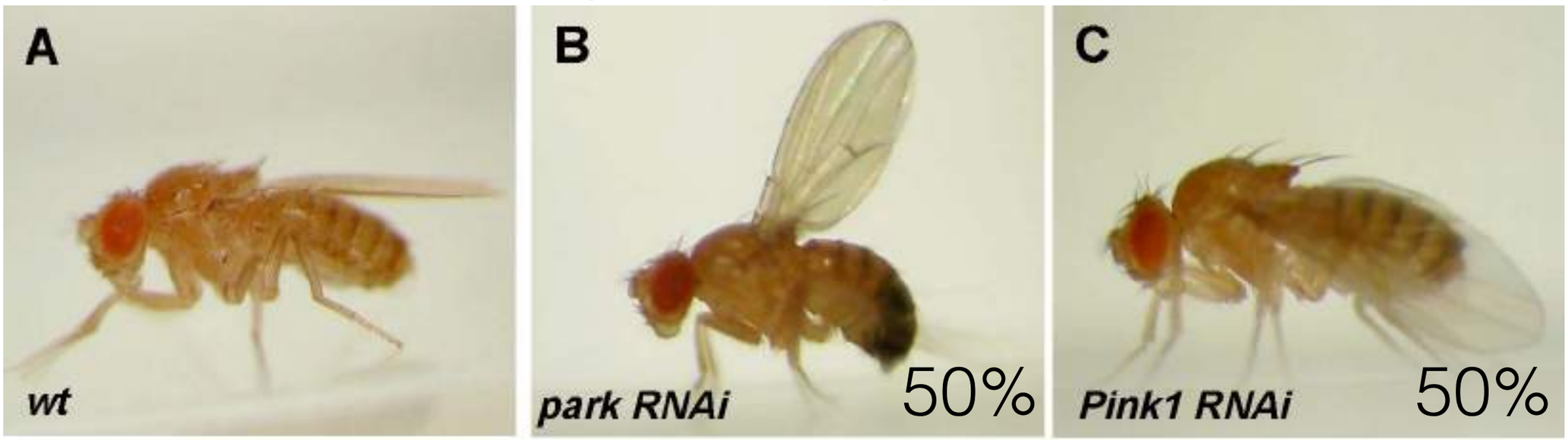

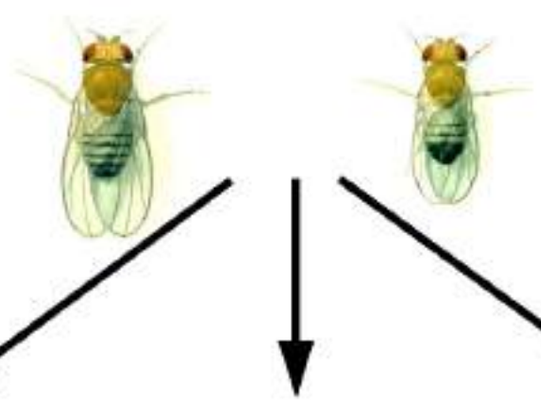

↓ Abnormal wing posture phenotype

[UAS-park-RNAi] [tub-GAL4]
Su*

suppressor

no change

[UAS-park-RNAi] [tub-GAL4]
*

no effect

↑ Abnormal wing posture phenotype

[UAS-park-RNAi] [tub-GAL4]
En*

enhancer

Fernandes and Rao *Molecular Brain* 2011, **4**:17
http://www.molecularbrain.com/content/4/1/17

Table 7 Analysis of the interaction between a *Pink1* null mutation and cytological regions that modified both *park-RNAi* and *pink1-RNAi* wing phenotype

		Effects of modification		
Deficiencies	**Breakpoints**	***Pink1-RNAi***	***park-RNAi***	***Pink1^{B9}***
Enhancers				
Df(2L)net-PMF	21A1;21B7-8	++	++	n/d
Df(2L)BSC17	30C3-5;30F1	++	++	n/d
Df(2L)BSC50	30F5;31B1	+++	++++++	En
Df(2R)nap9	42A1-2;42E6-F1	++	+++++	En
Df(2R)cn9	42E;44C	++	++	En
Df(2R)BSC39	48C5-D1;48D5-E1	++	++++	En
Df(3R)BSC47	83B7-C1;83C6-D1	++	++	En
Df(3R)Tpl10	83C1-2;84B1-2	++	++	No
Suppressors				
Df(2L)BSC106	21B7;21C2	—	——	Su
Df(2L)dp-79b	22A2-3;22D5-E1	———	——	No
Df(2L)ed1	24A2;24D4	—	——	n/d
Df(2L)BSC109	25C4;25C8	——	——	Su
Df(2L)E110	25F3-26A1;26D3-11	——	–	n/d
Df(2L)BSC142	28C3;28D3	——	——	Su
Df(2L)BSC143	31B1;31D9	—	–	No
Df(2R)Exel7131	50E4;50F6	———	——	Su
Df(2R)BSC550	53C1;53C6	——	–	No
Df(2R)robl-c	54B17-C4;54C1-4	—	–	n/d
Df(2R)P34	55E2-4;56C1-11	——	——	Su
Df(3L)XDI98	65A2;65E1	—	–	n/d
Df(3L)BSC33	65E10-F1;65F2-6	—	–	n/d
Df(3L)66C-G28	66B8-9;66C9-10	—	—	No
Df(3L)Scf-R6	66E1-6;66F1-6	———	–	Su
Df(3L)BSC10	69D4-5;69F5-7	—	——	Su
Df(3L)ME107	77F3;78C8-9	—	—	No
Df(3R)p-XT103	85A2;85C1-2	———	——	Su
Df(3R)sbd104	89B5;89C2-7	—	–	n/d
Df(3R)P115	89B7-8;89E7	——	—	Su
Df(3R)crb-F89-4	95D7-D11;95F15	—	—	No
Df(3R)Exel6202	96C9;96E2	—	—	No
Df(3R)Exel6203	96E2;96E6	——	——	Su

> 40 genetic regions that interact with both *pink1* and *park*

Potentially new places to look for mutations associated with Parkinson's disease

Potentially new therapeutic targets

"[tax] dollars go to projects that have little or nothing to do with the public good — things like fruit fly research in Paris, France. I kid you not."

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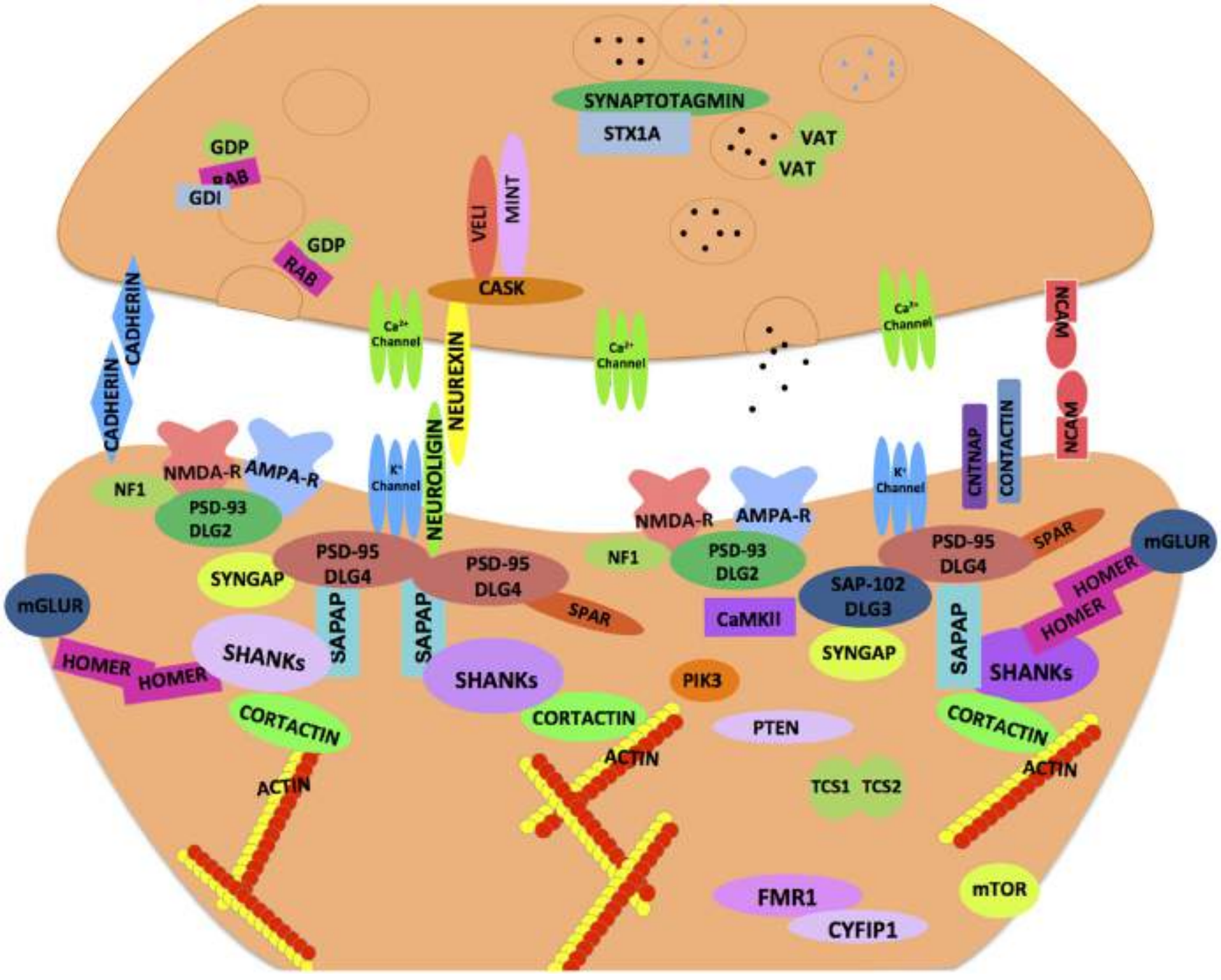
Sometimes just general curiosity in simple systems can build foundational knowledge necessary for rapid understanding of the molecular basis of a disease.

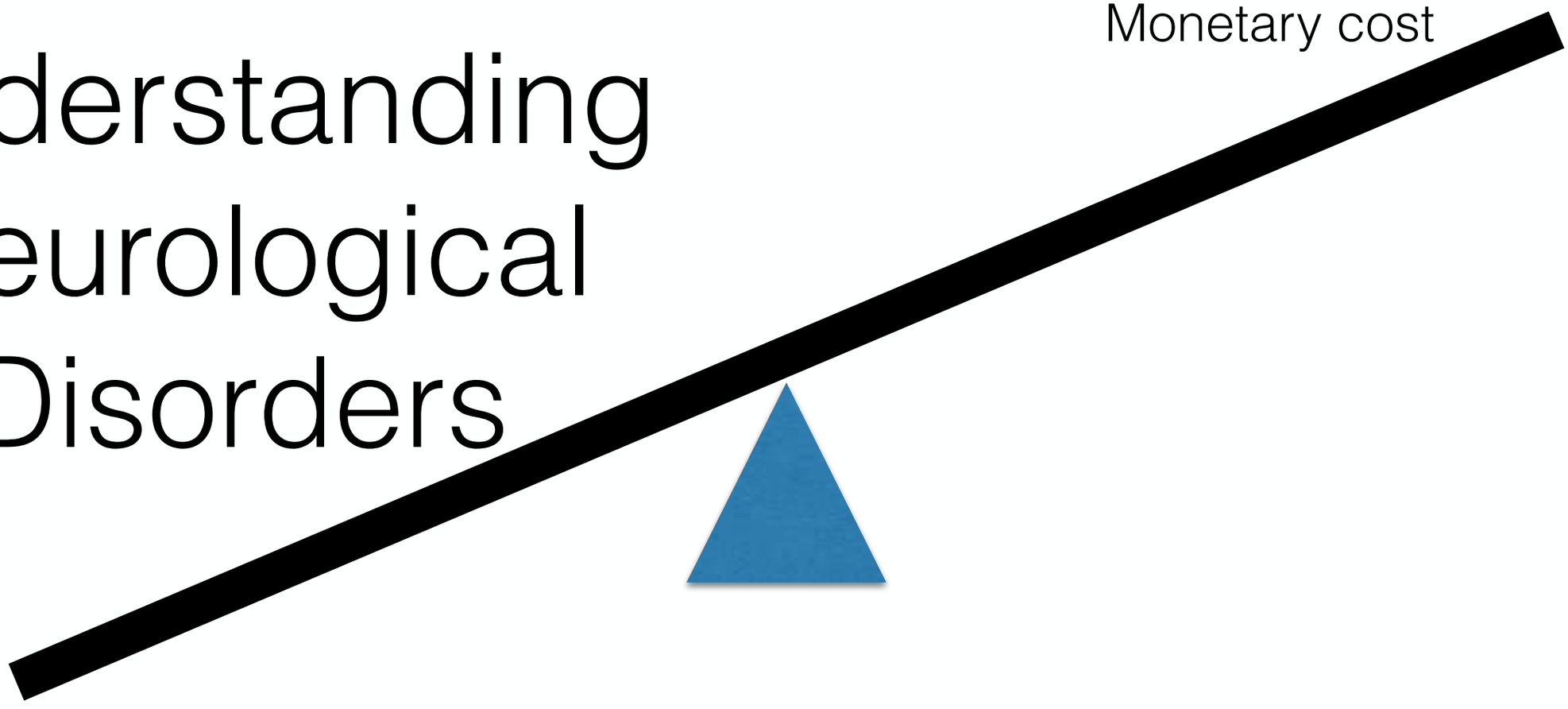
Synaptic proteins mostly discovered through work in invertebrates



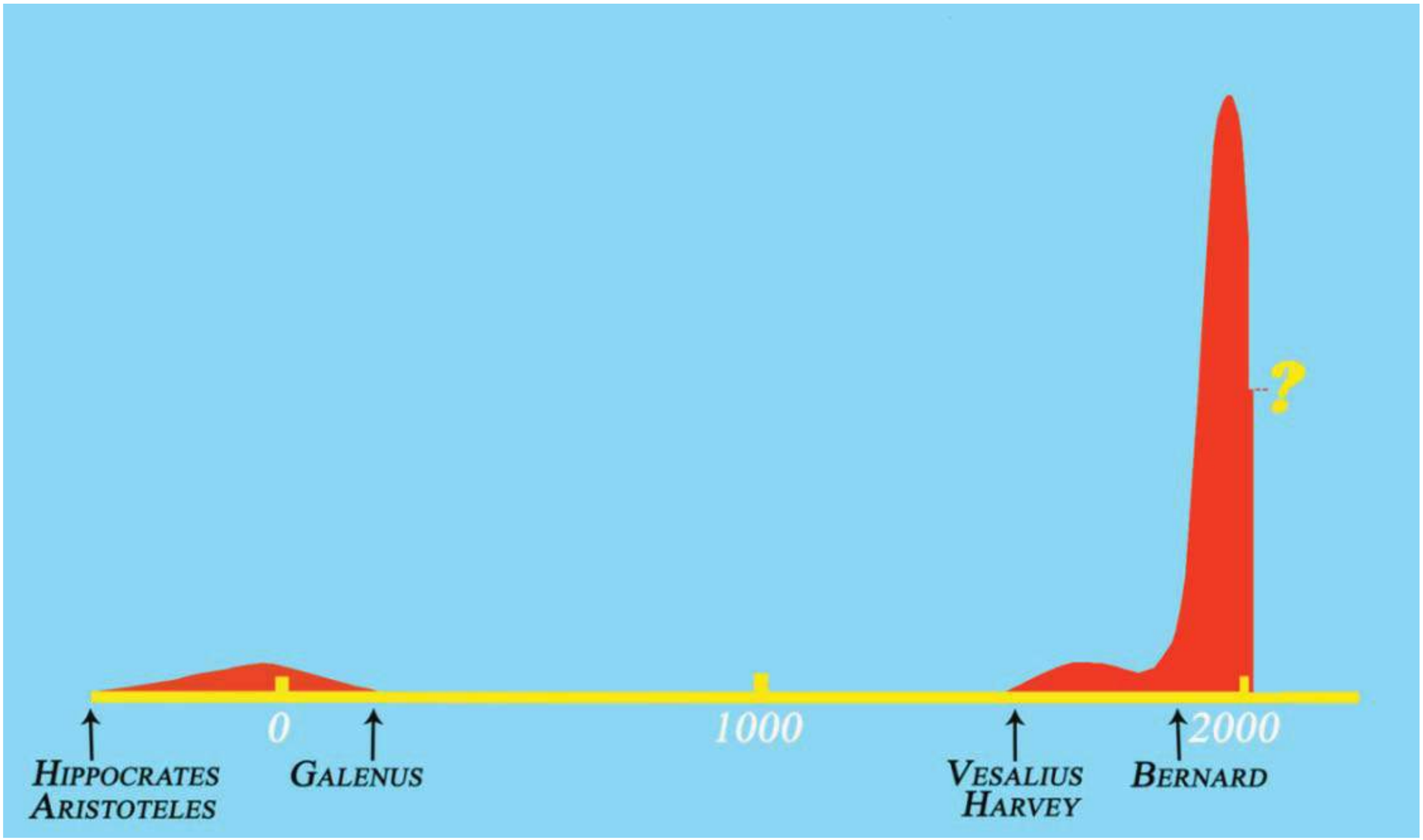
Many of these genes are linked to neurological disorders

Use of invertebrate systems can improve speed, cost, and reduce some ethical concerns

Understanding Neurological Disorders



Plot of animal usage in research over time



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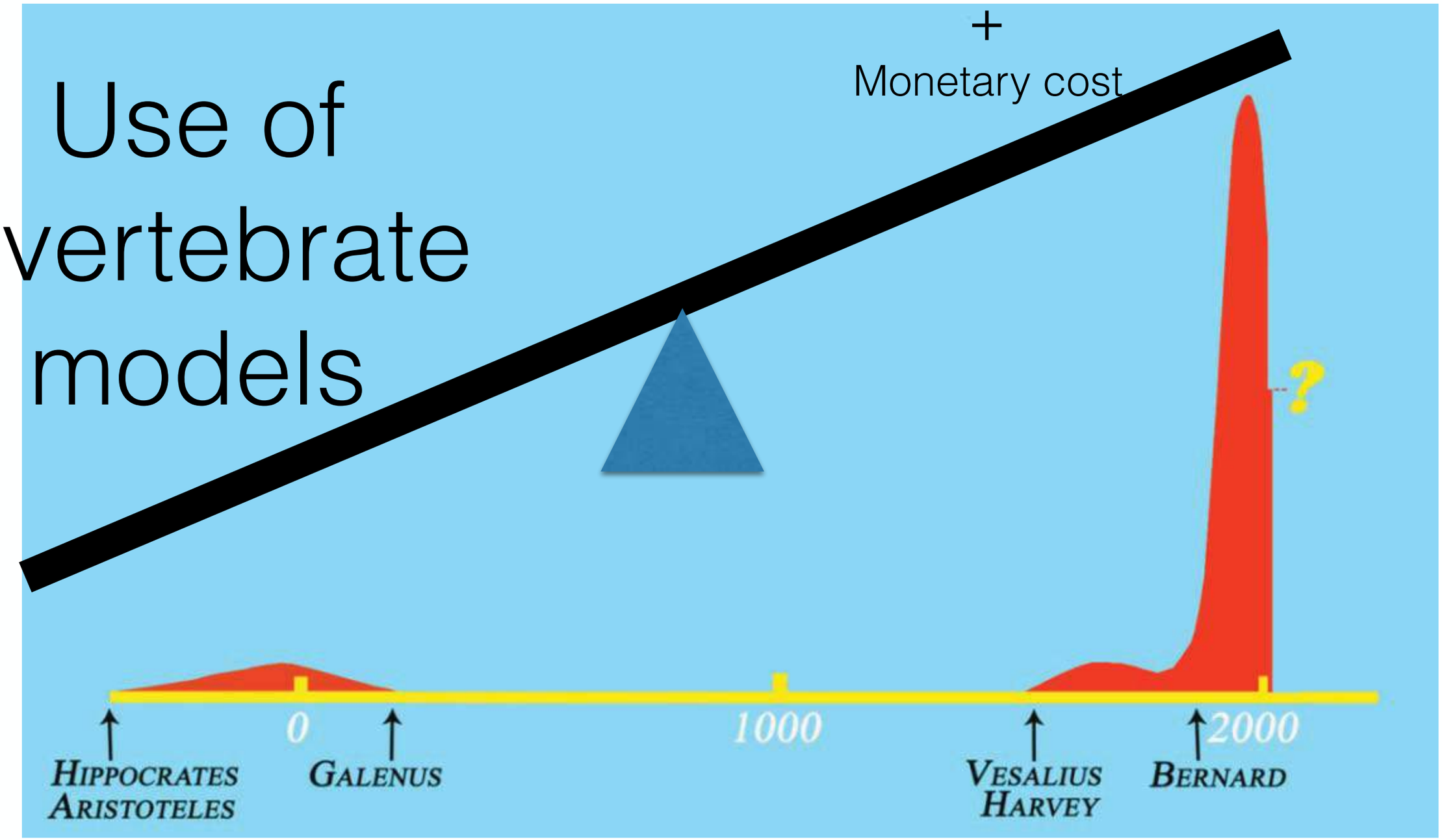

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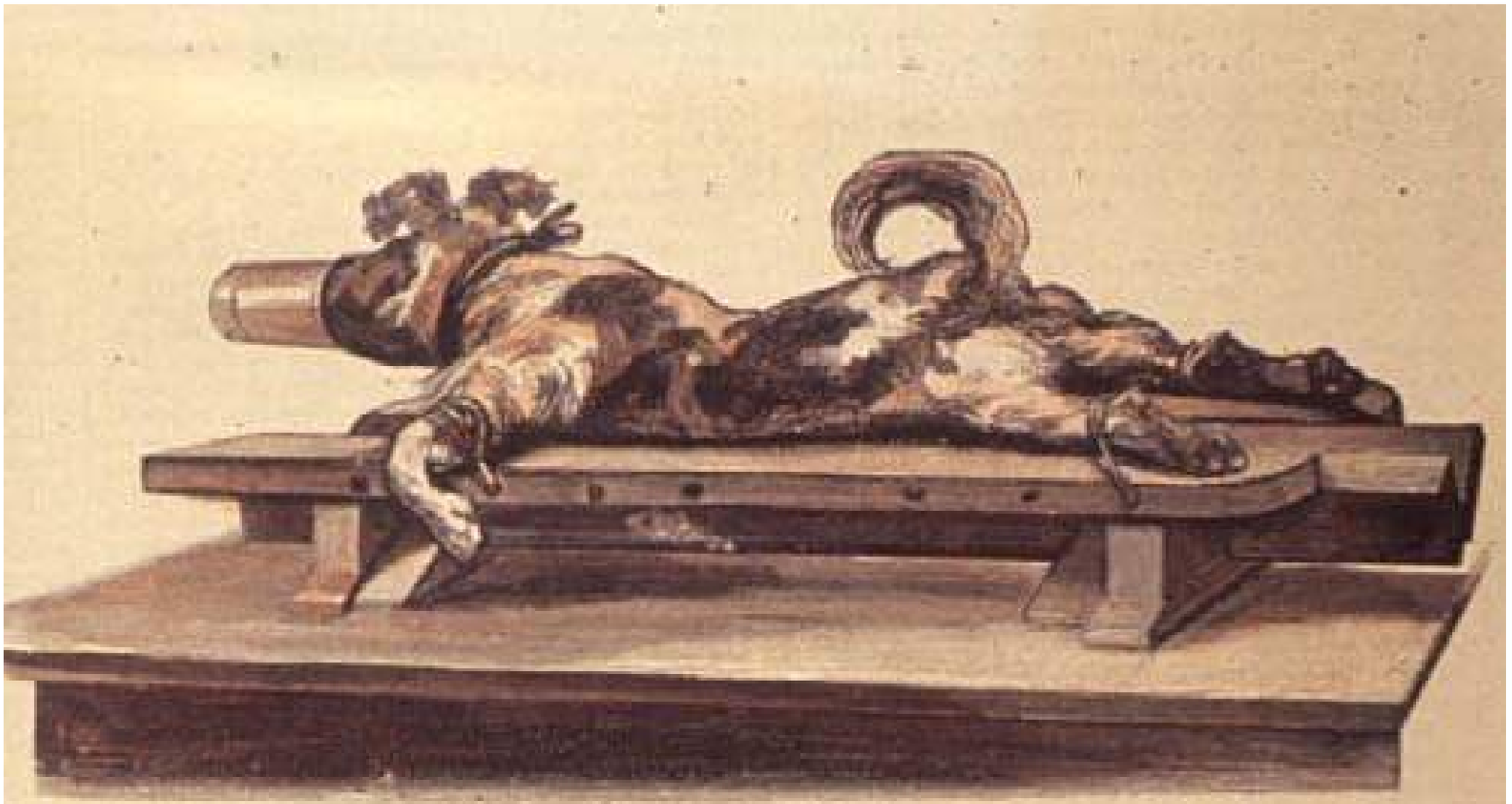


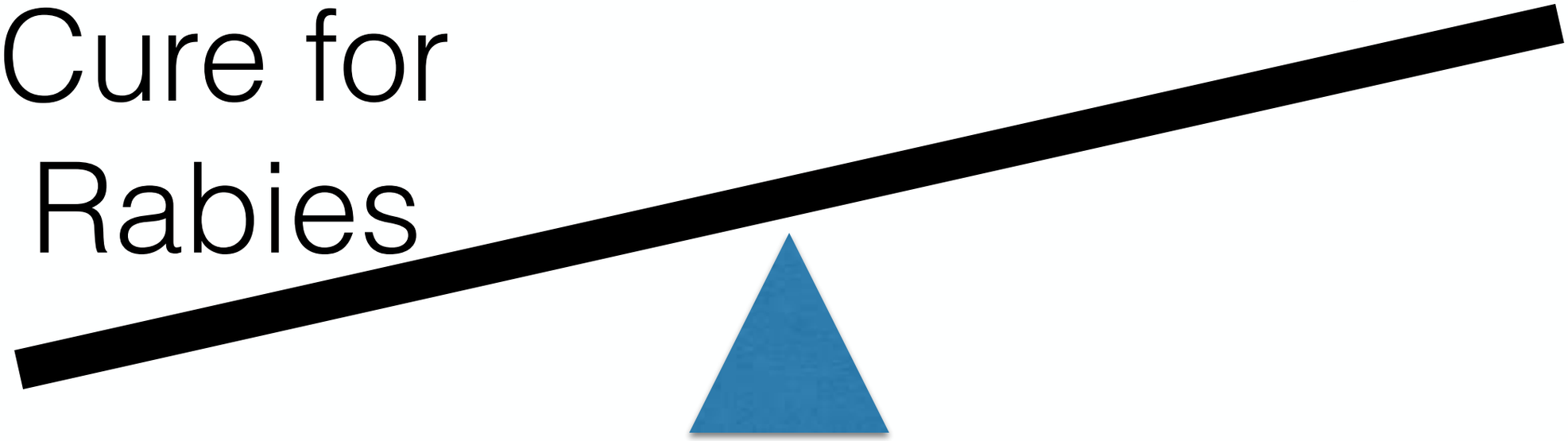
A DOG AWAITING INOCULATION.

Humans And Animals Would Still Be Dying From Rabies If Pasteur Hadn't Experimented With Dogs.

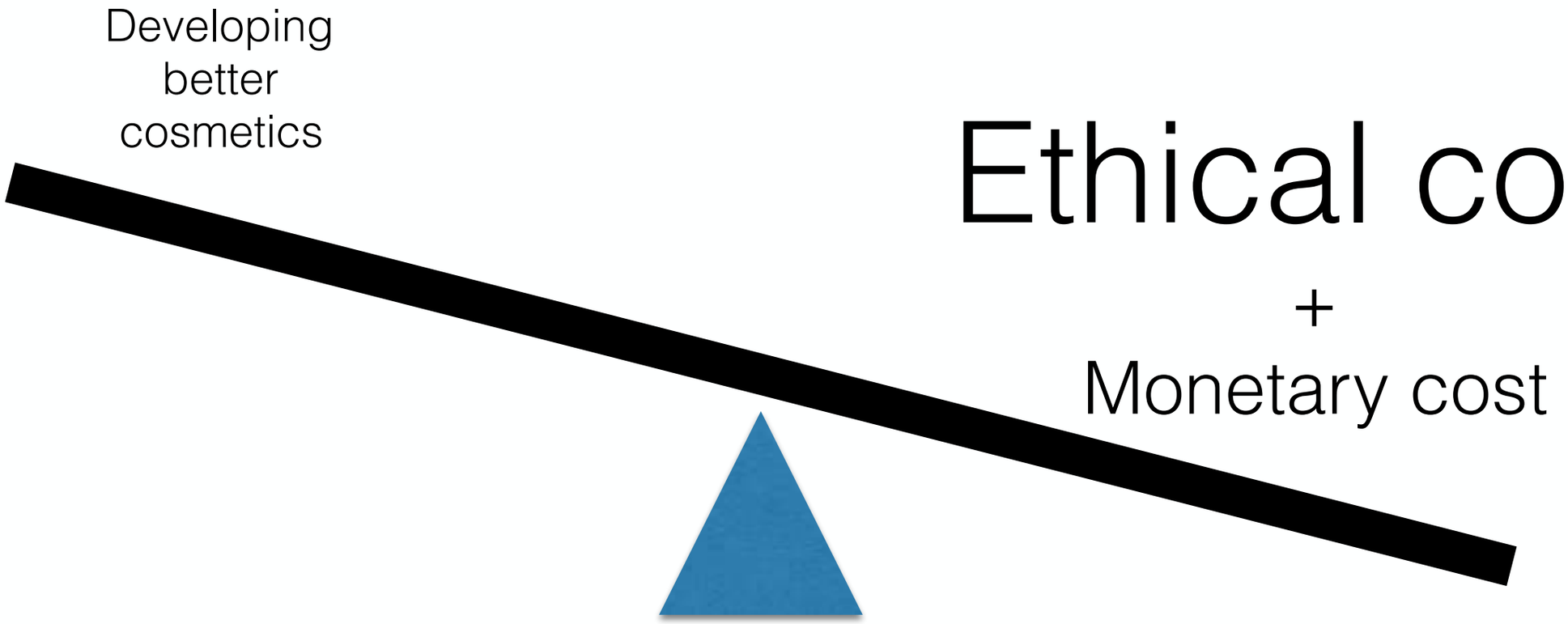
Developing rabies vaccination was BIG deal

Ethical cost
+
Monetary cost

Cure for
Rabies



Testing cosmetics on animals doesn't really justify use of animal models for most people



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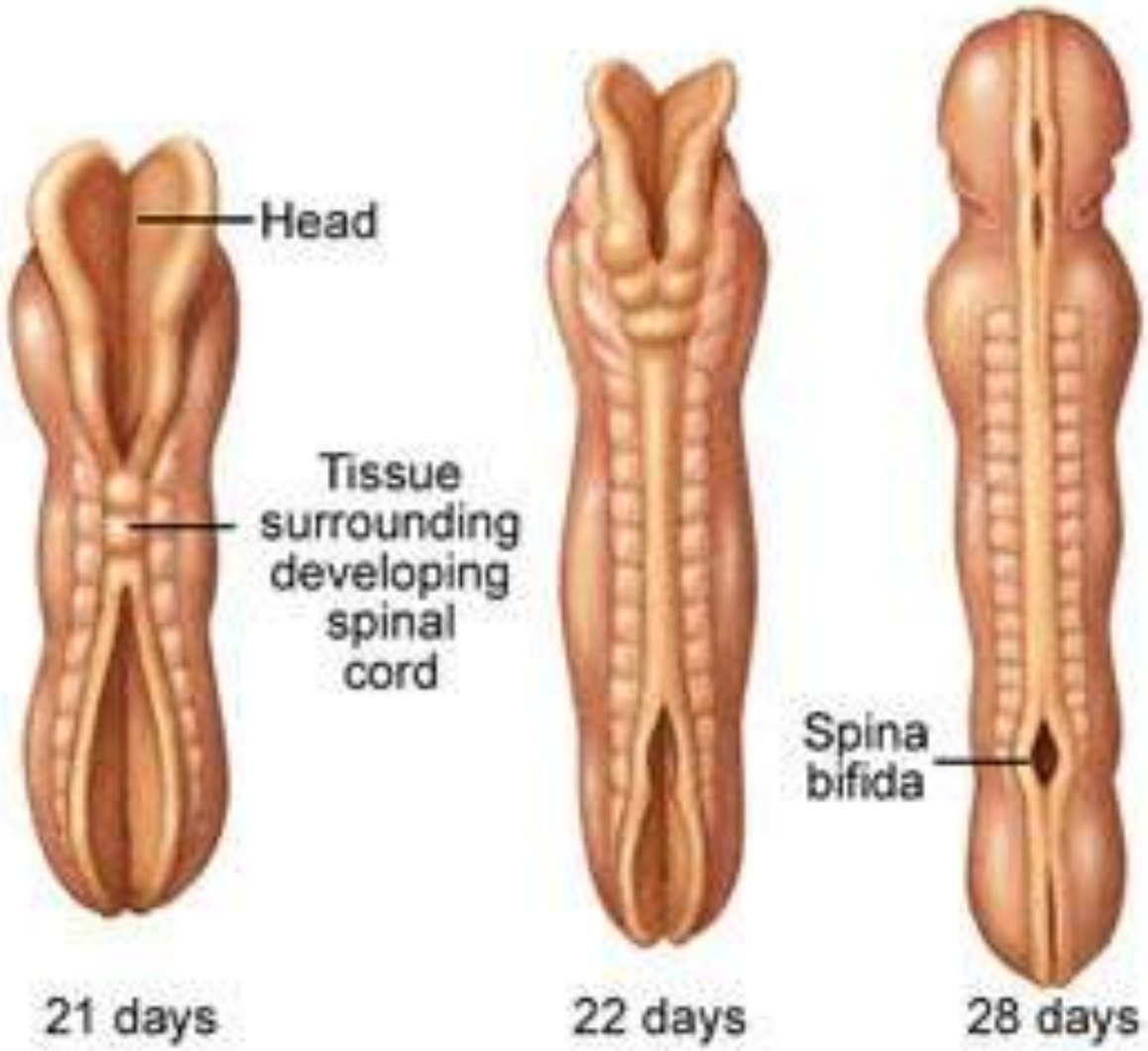
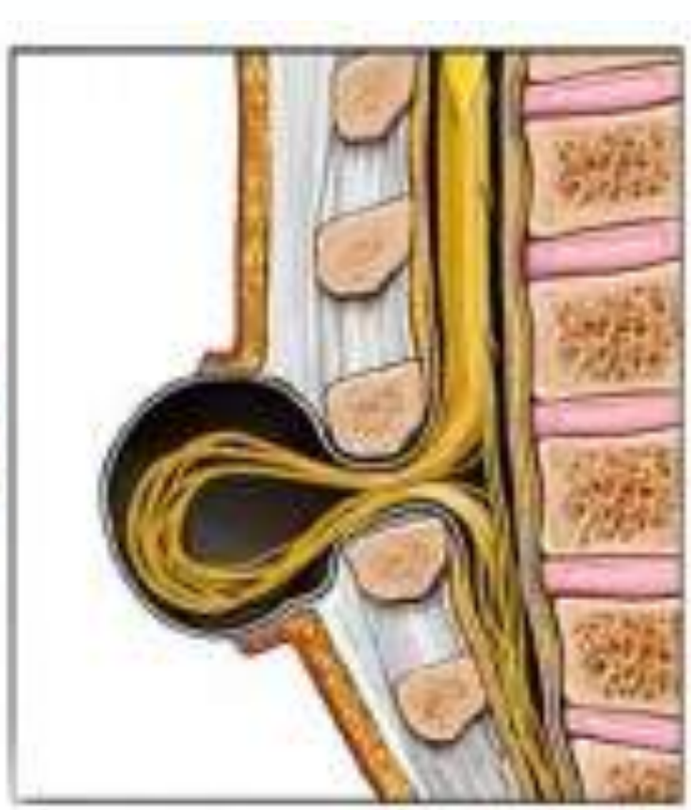
Spina bifida

1 in 1000 births





Defect in vertebrae allows spinal nerves to protrude

Identification of genes associated with human birth defects

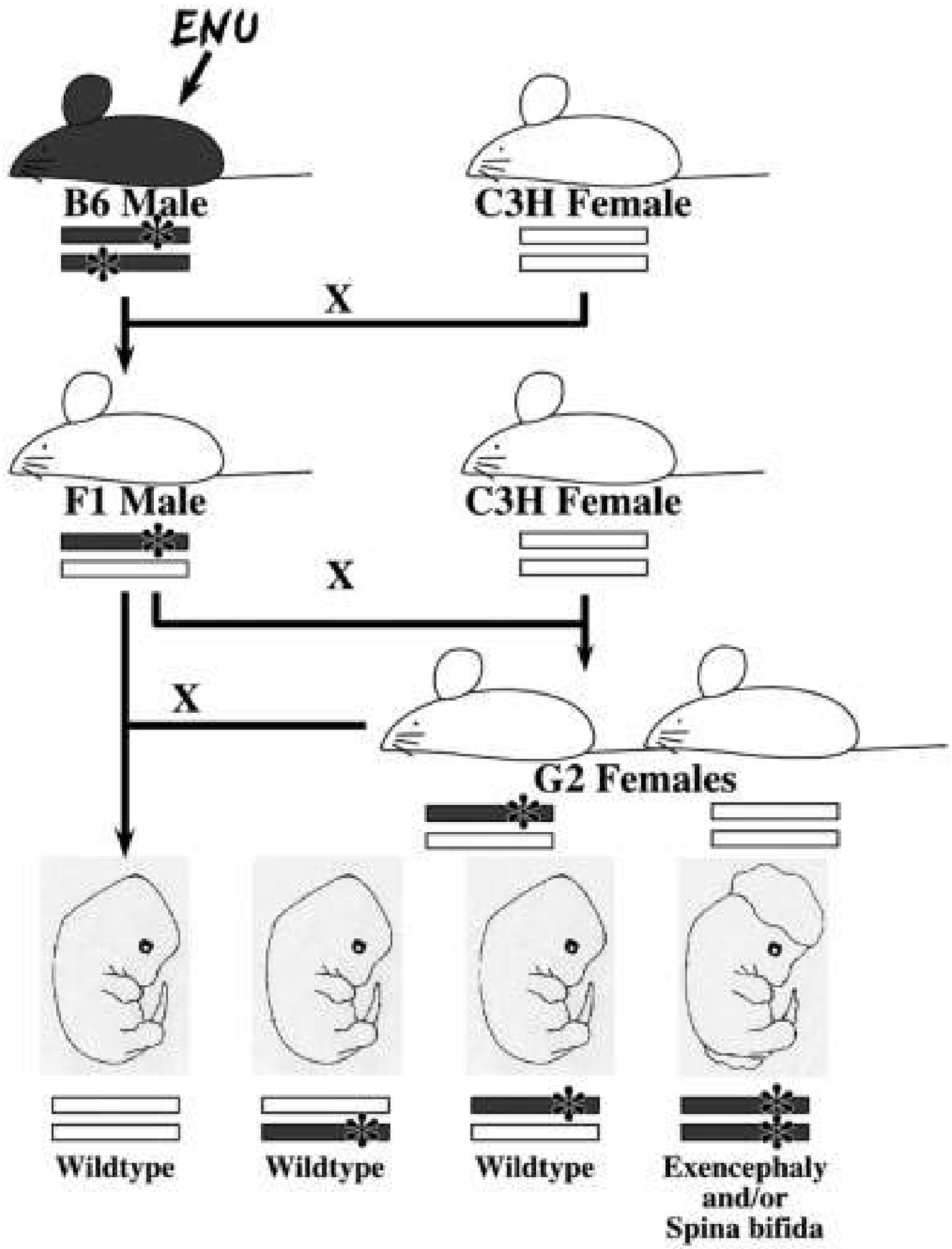
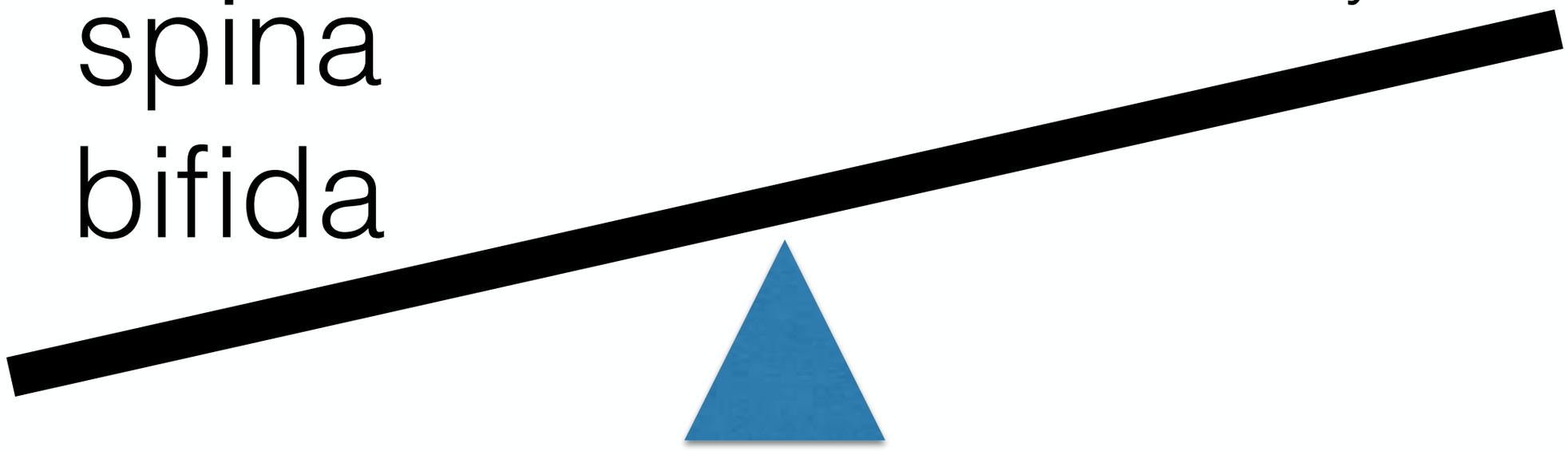


Table 1
Mouse Lines with NTDs That Have Been Identified in the Sloan-Kettering Mouse Mutagenesis Screen

Line	Phenotype	Mapped to chromosome	Gene
2	Exencephaly, craniofacial defects and omphalocele	15	?
11A	Exencephaly, cardiovascular defect, polydactyly	7	?
12A	Exencephaly	4	?
12D	Exencephaly	16	?
16C	Exencephaly, eye defect	8	?
20	Exencephaly, curly tail, fused digits kidney and lung defects	2	Laminin α5
22C	Exencephaly, small forebrain and eye defect	1	?
26	Exencephaly	12	?
27E	Exencephaly	12	?
34B	Exencephaly	1	?
Dey	Exencephaly, spina bifida, gastrulation and eye defect	3	Novel
C2	Exencephaly, spina bifida	7	?
F11	Exencephaly and vascular defects	3	Novel
Opm	Exencephaly and eye defect	12	Novel
Z4	Exencephaly	18	?
G2E	Exencephaly and eye defect	4	Novel
7A5	Exencephaly and small forebrain	5	?
31B	Exencephaly and small forebrain	2	
1B	Exencephaly, spina bifida, branchial arch and cardiovascular defect	6	?
F19	Exencephaly	?	?
33C	Exencephaly	19	?
12	Exencephaly	1	?
lilR3	Exencephaly, neural patterning	16	?
Kif3a	Exencephaly, neural patterning, left-right patterning	11	Kif3a
Opb2	Exencephaly, spina bifida, neural patterning, left-right patterning	1	Rab23
2A	Exencephaly, neural patterning	11	?
Wimple	Exencephaly, neural patterning, left-right patterning	5	IFT172
Ling-ling	Exencephaly, neural patterning, left-right patterning	9	Novel
10	Exencephaly, neural patterning, eye defect and cardiovascular defect	10	?
Flexo	Exencephaly, neural patterning, left-right patterning	14	IFT88/polaris
Hennin	Exencephaly, neural patterning, left-right patterning	16	Novel
20D	Exencephaly, neural patterning, left-right patterning	?	?

Understanding spina bifida

Ethical cost
+
Monetary cost



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Organoids are providing alternative approaches for designing genetic model systems.

organoid = Miniature organ-like structure made in cell culture

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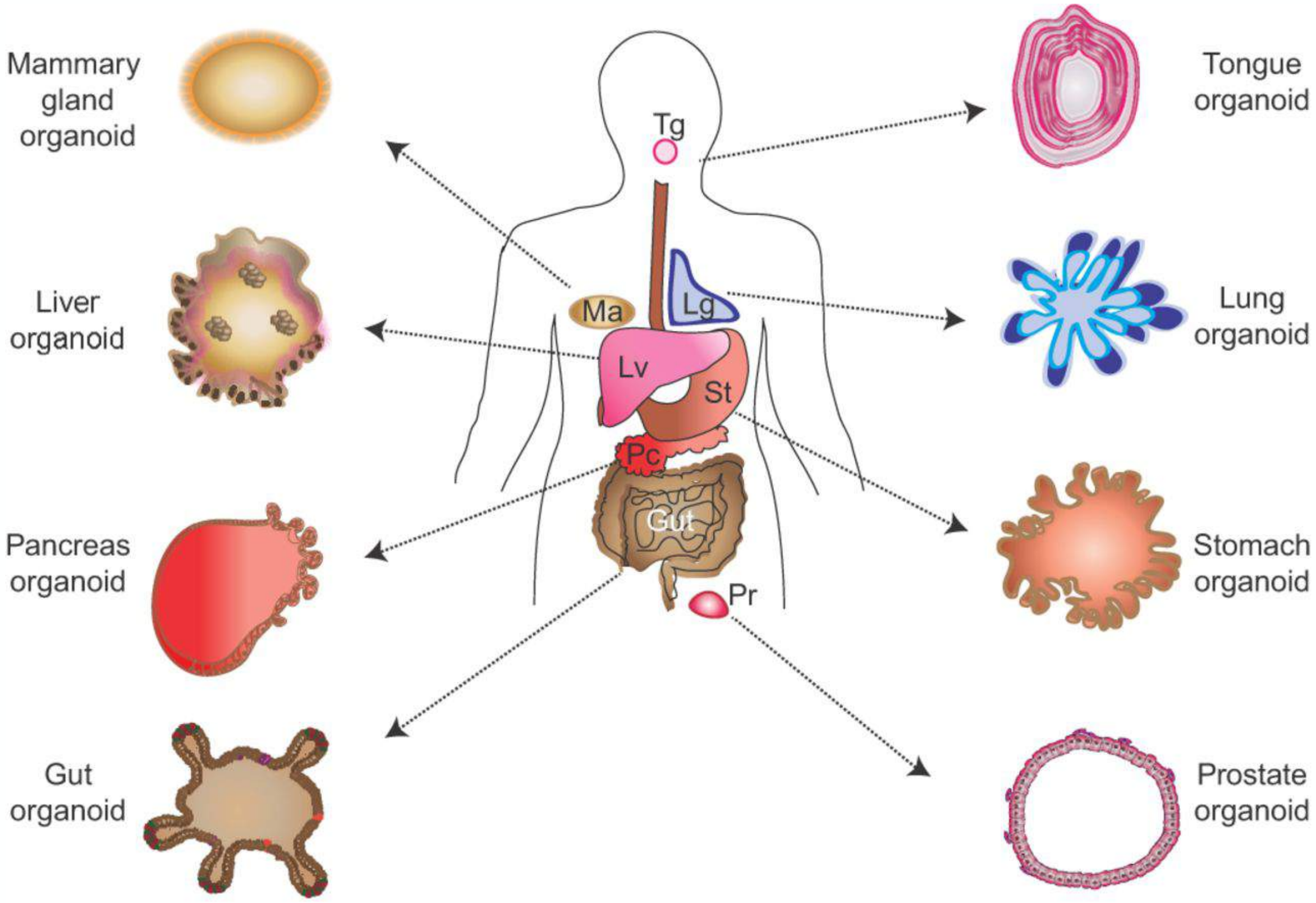
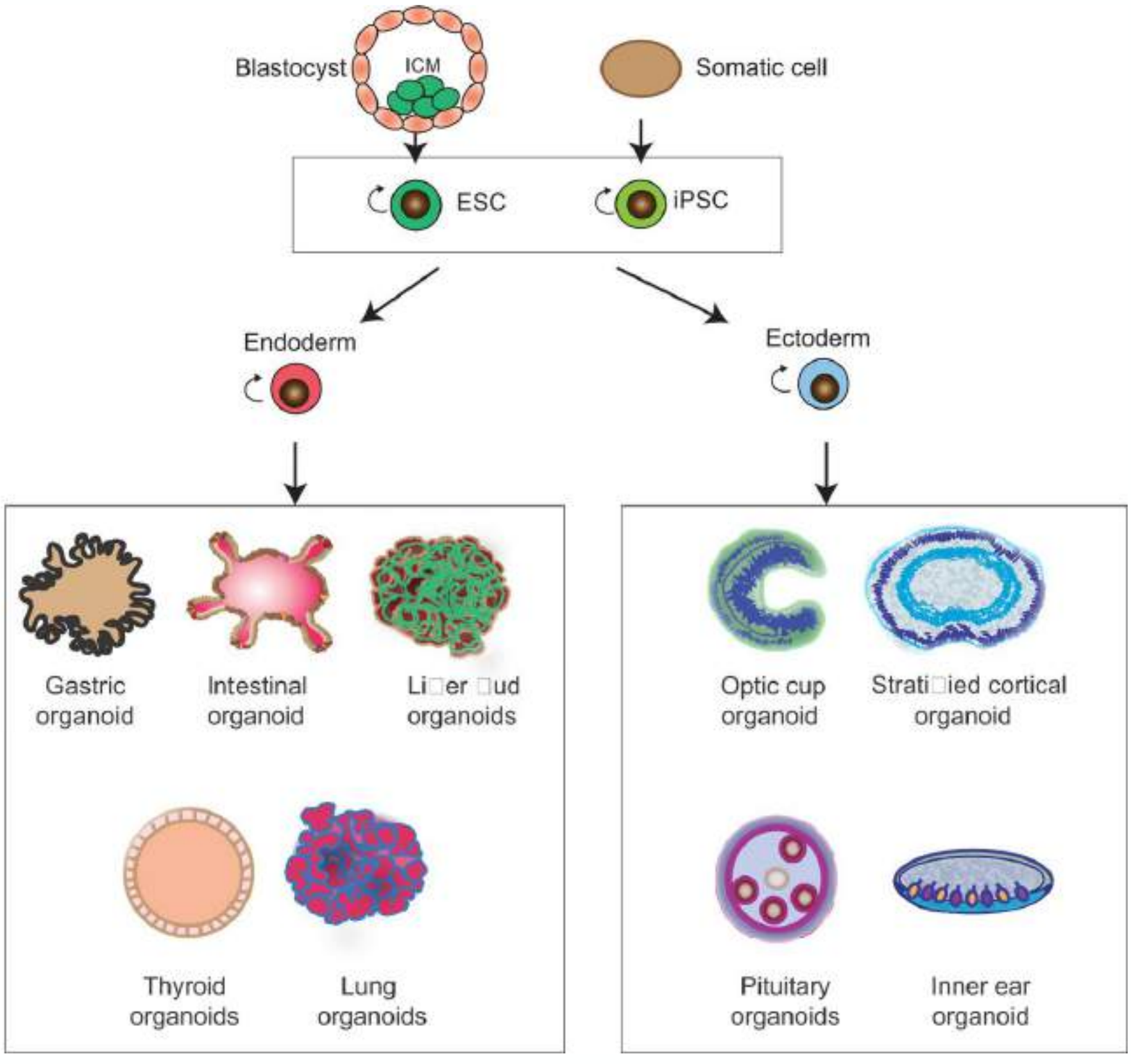
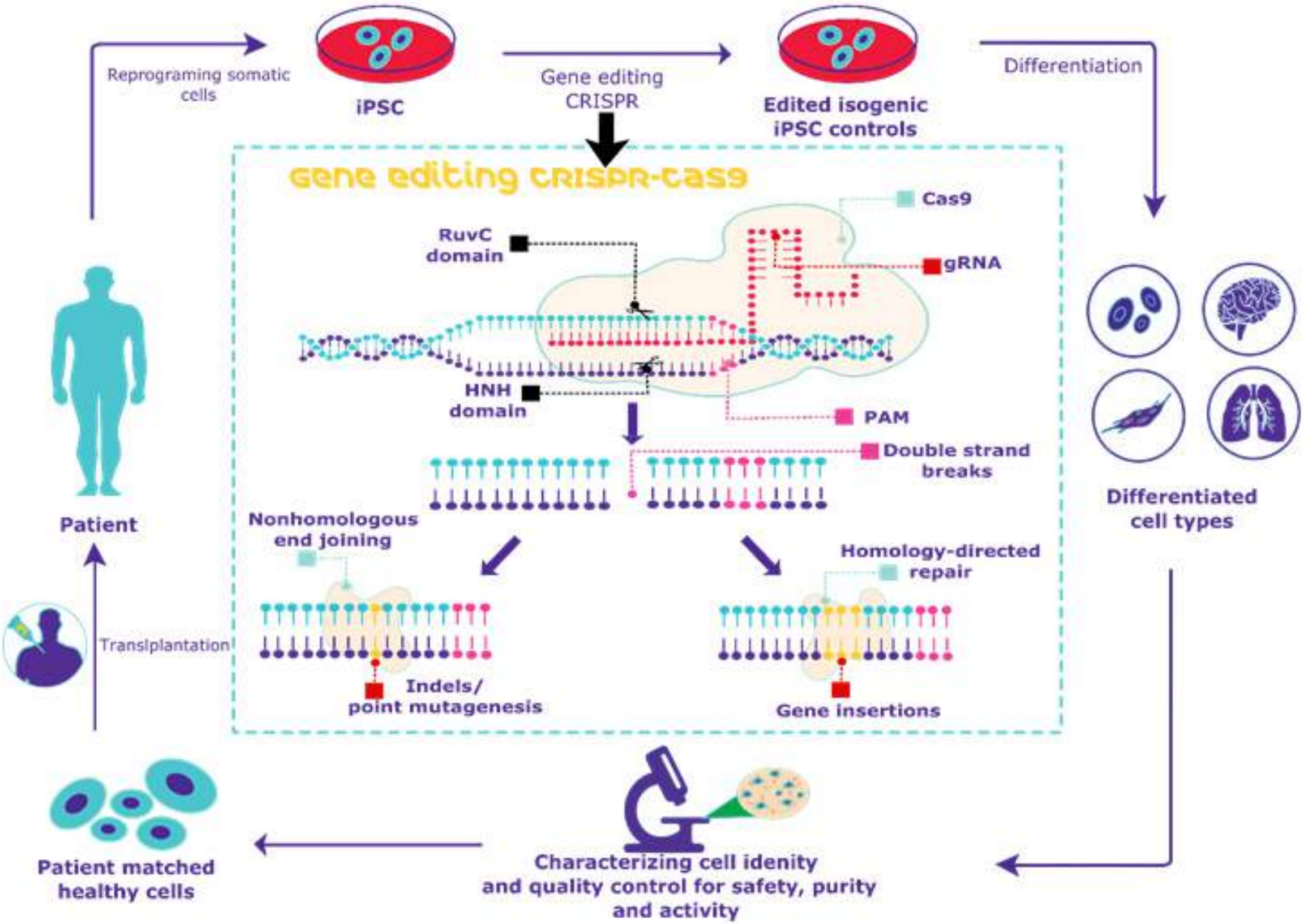


Organoids can be derived from adult somatic cells of individual patients



CRISPR/Cas9 gene editing of patient specific samples

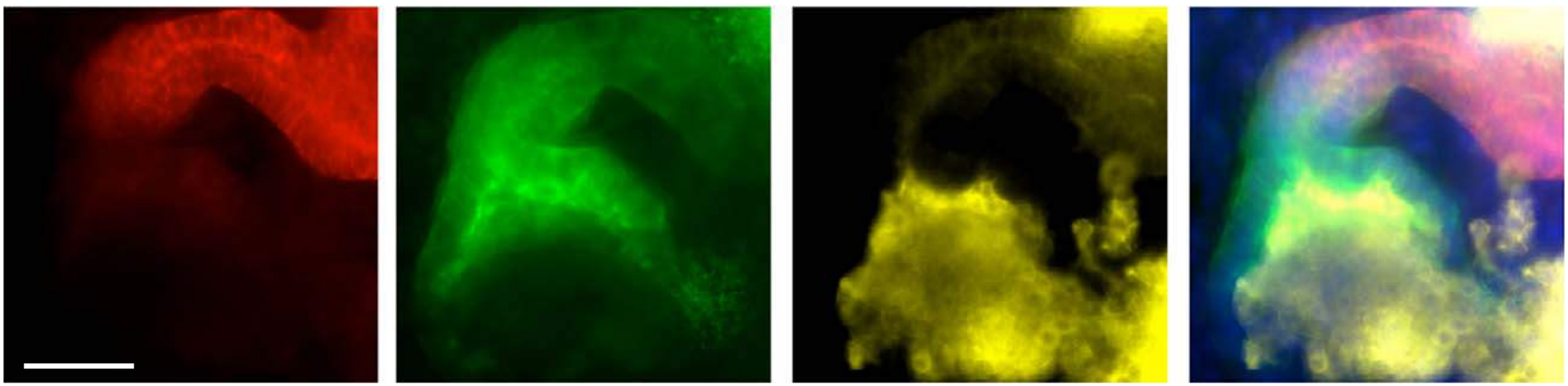


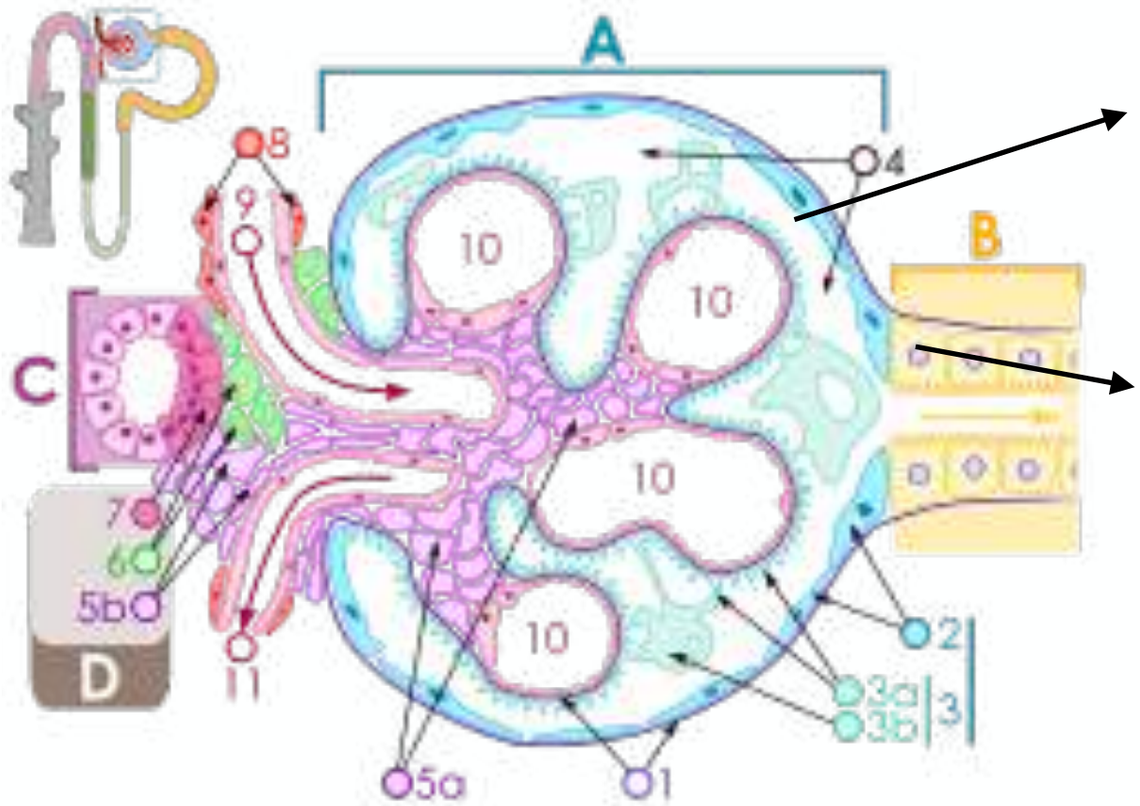
Sigma-Aldrich Website

“mature” kidney-like organoid

e

ECAD | LTL | PODXL | Merge/DNA





Glomerulus

capsule = PODXL+ Podocytes wrapping and filtering blood

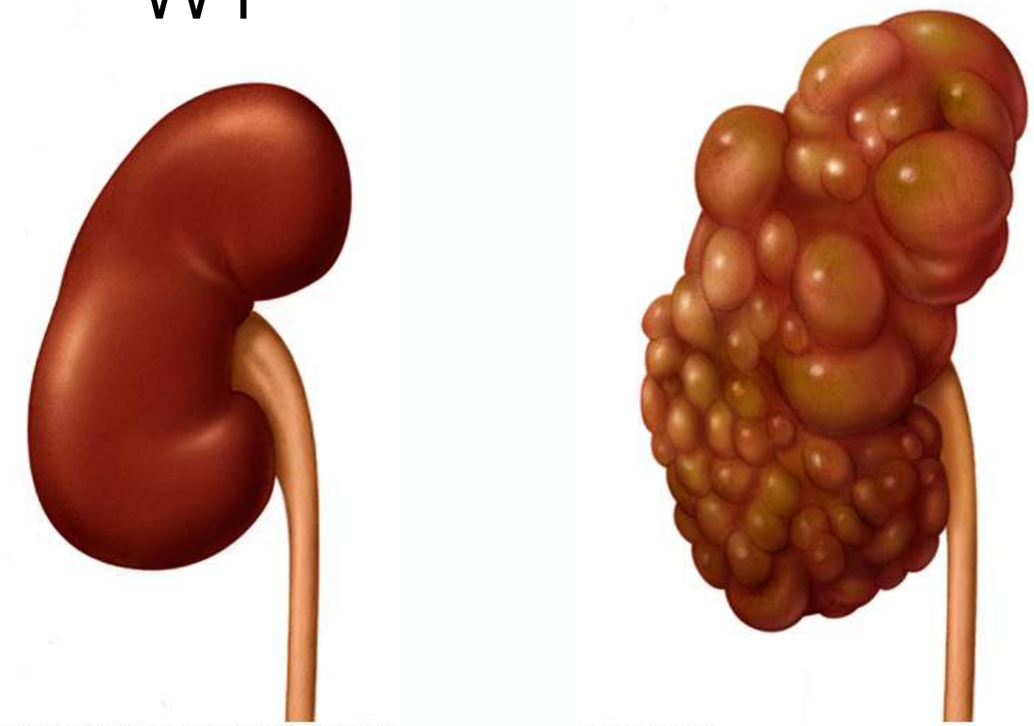
proximal tube = LTL+ epithelium

Distal tube = E-Cad+ epithelium

Adapted from Freedman et al., *Nature Communications* 2016

Differentiated organoids with Pkd (polycystic kidney disease) mutated form cysts

WT



Polycystic kidney

1 in 500 to 1 in 1000 people world wide
No cure - fatal

Adapted from Freedman et al., *Nature Communications* 2016

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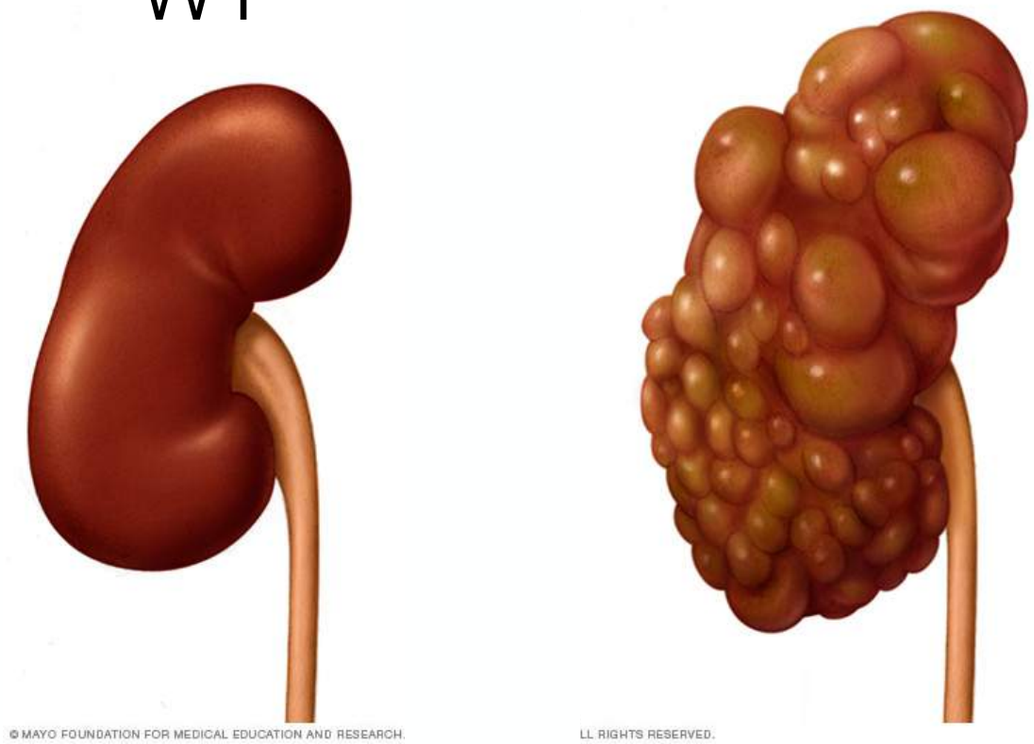

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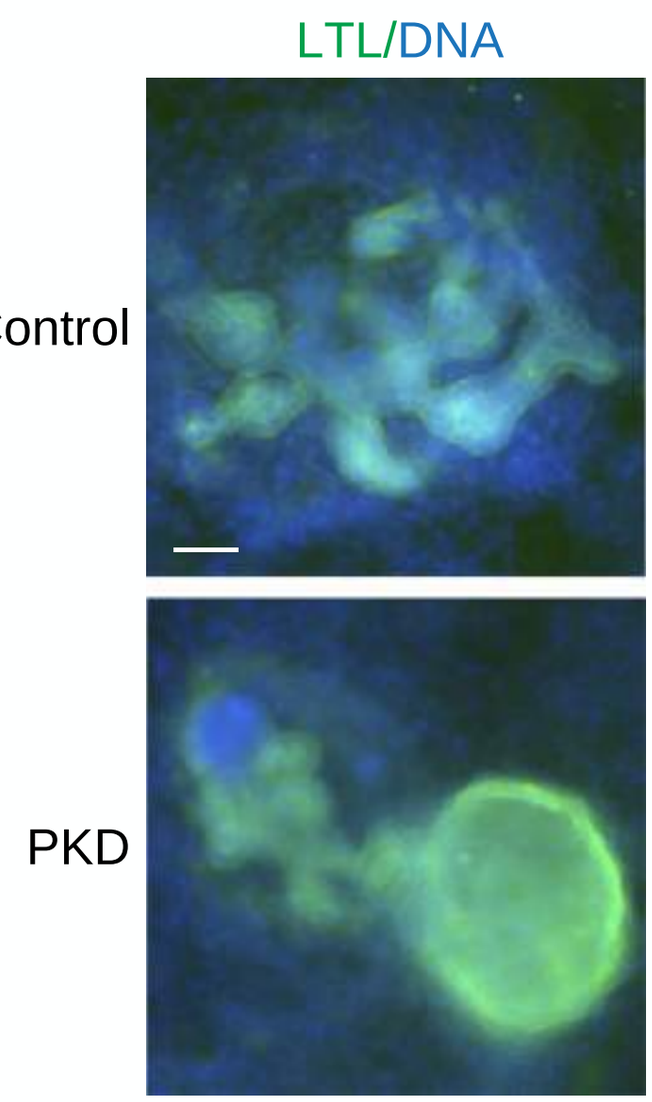

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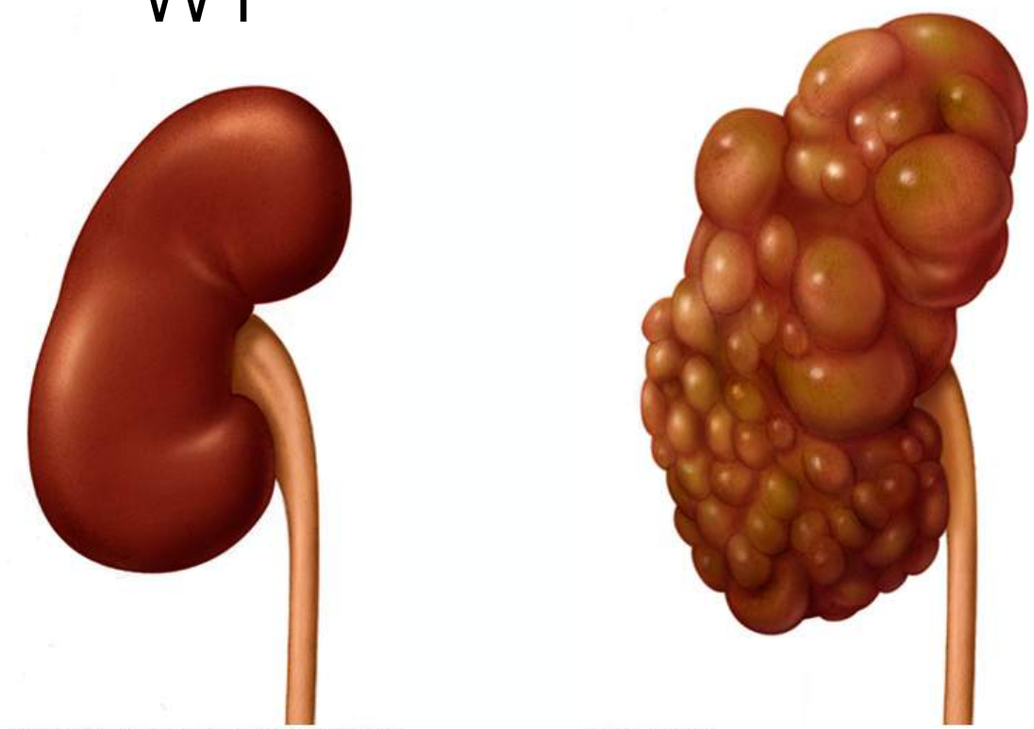

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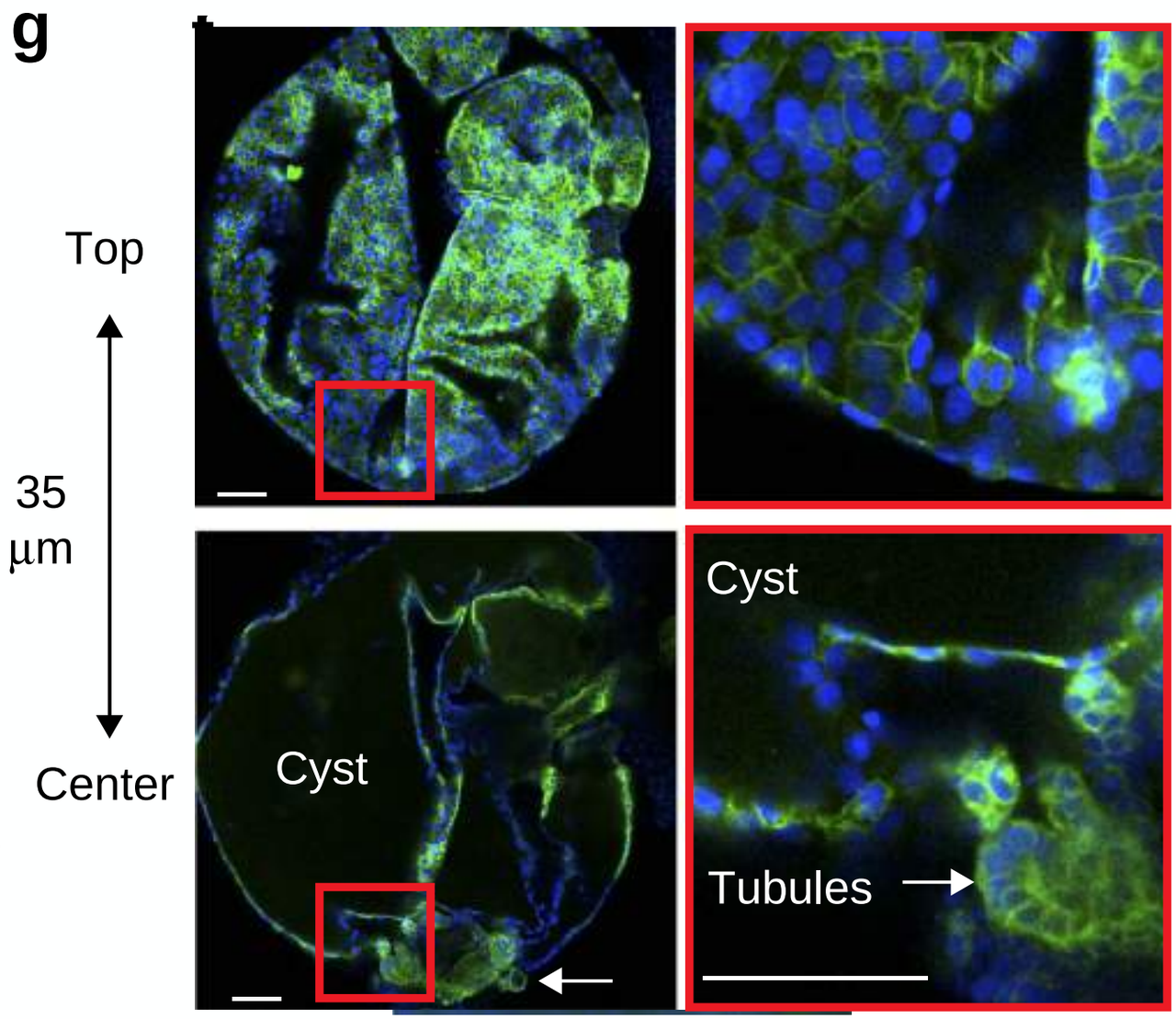

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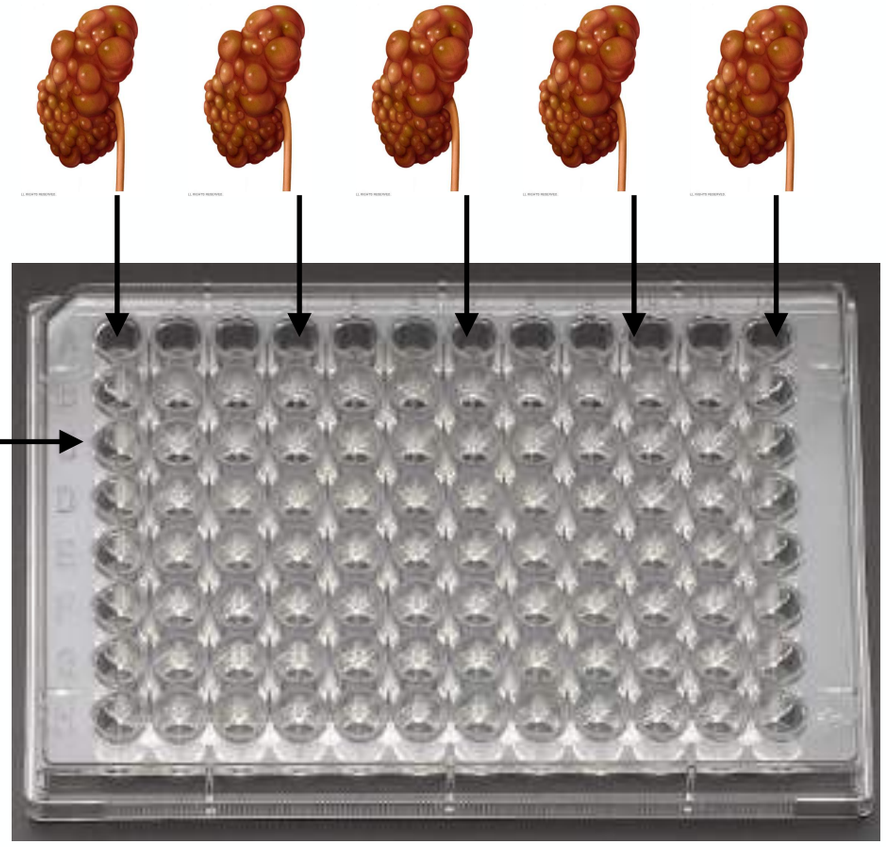
Organoids can be used for drug screens

Different small molecule in each well



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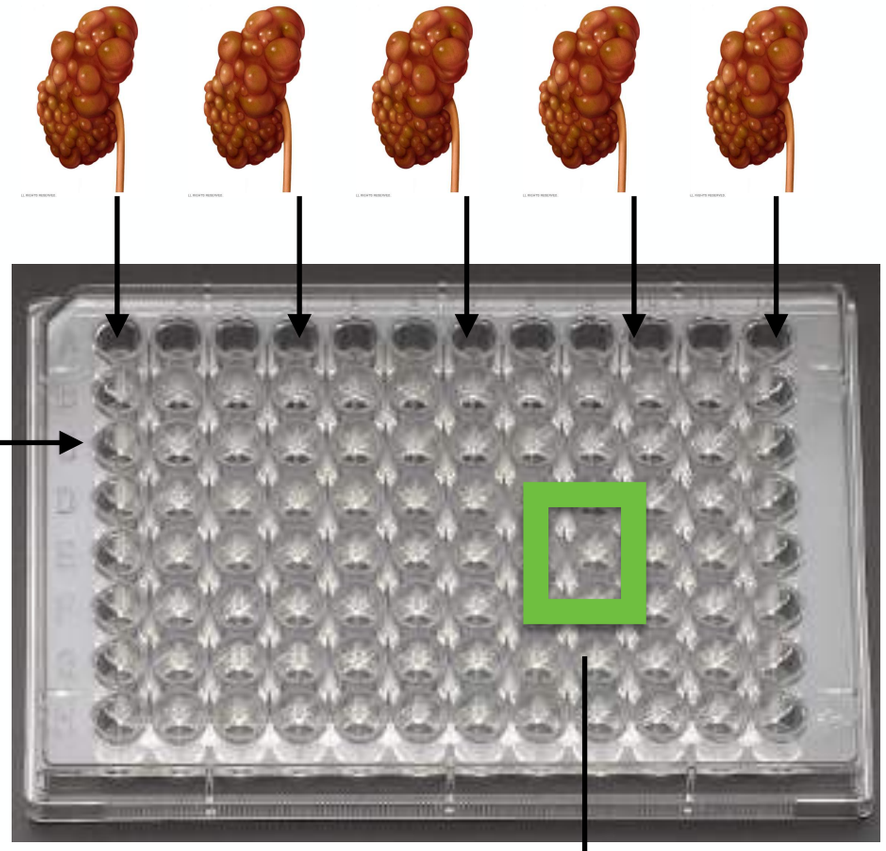
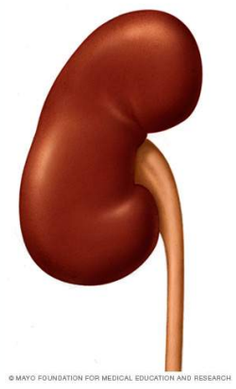
Different small molecule in each well





Animals **2013**, *3*, 238-273; doi:10.3390/ani3010238

OPEN ACCESS

animals

ISSN 2076-2615

www.mdpi.com/journal/animals

Review

Animal Experiments in Biomedical Research: A Historical Perspective

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Received: 15 February 2013; in revised form: 11 March 2013 / Accepted: 11 March 2013 / Published: 19 March 2013

Outline

- Discuss the benefits of and how to choose a model system.
- Go through a case study of using a zebrafish model to understand how melanomas form

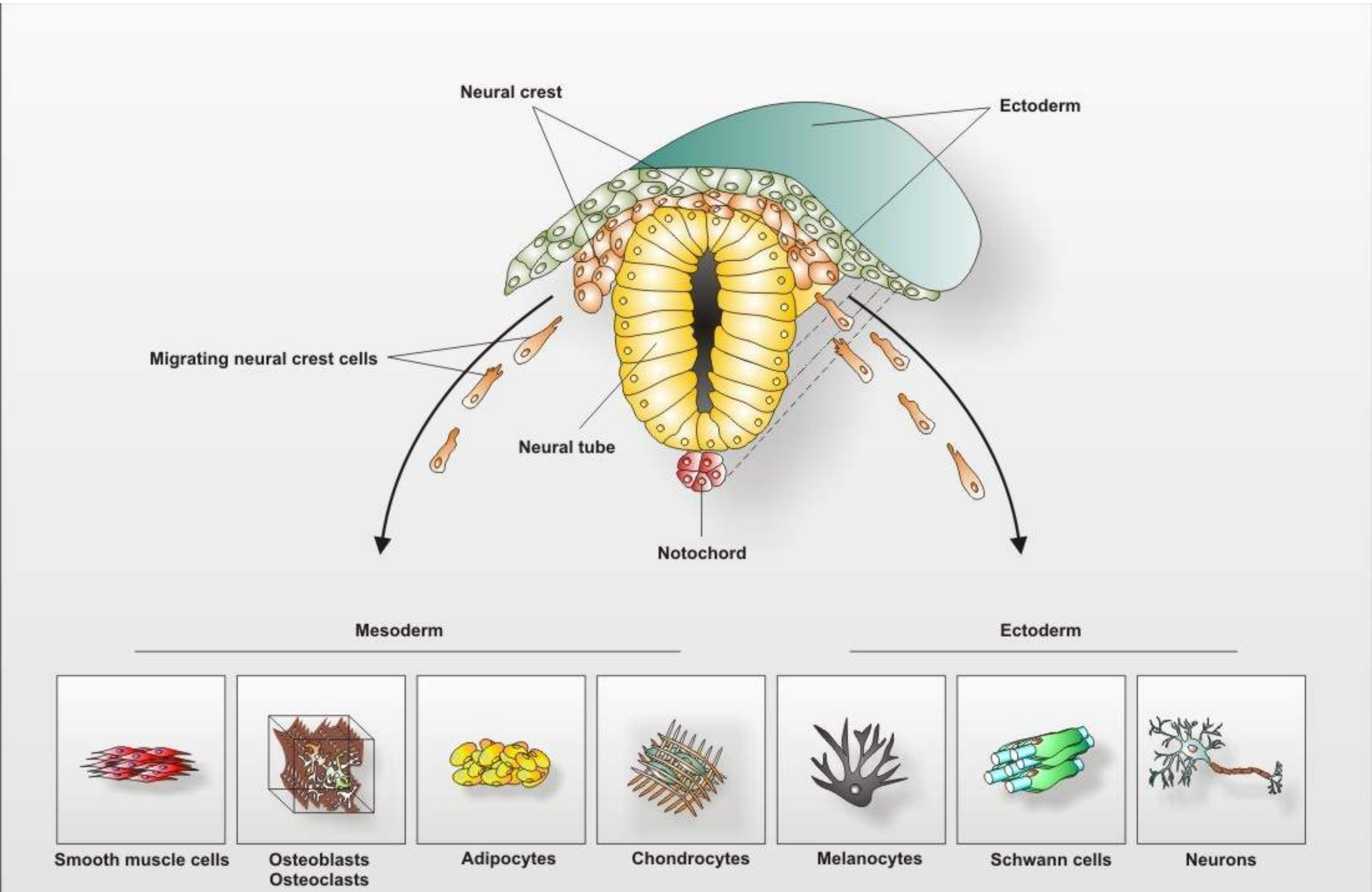
Testing the hypothesis that some cancers arise by reviving populations of embryonic stem cells

A zebrafish melanoma model reveals emergence of neural crest identity during melanoma initiation

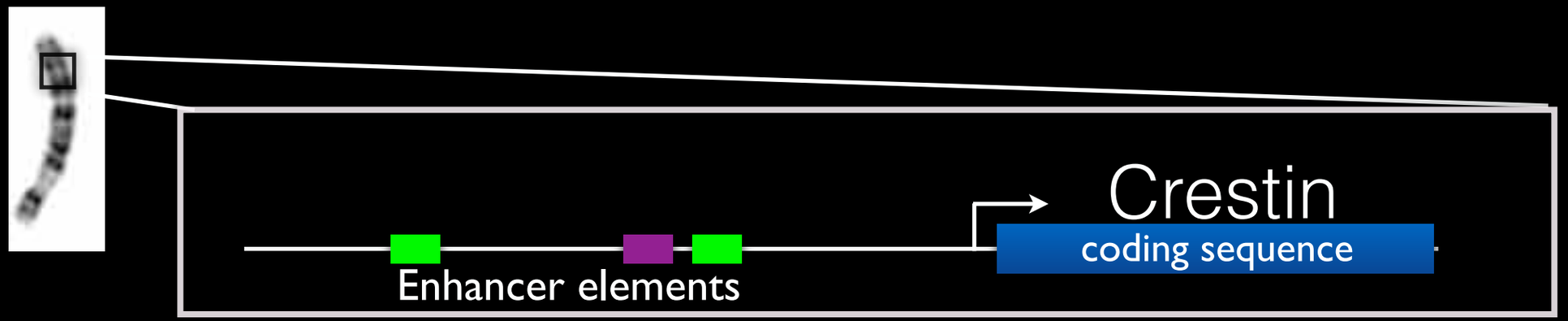
**Charles K. Kaufman,[1,2,3,4] Christian Mosimann,[5] Zi Peng Fan,[6,7] Song Yang,[1,2] Andrew J. Thomas,[1] Julien Ablain,[1,2,4] Justin L. Tan,[1] Rachel D. Fogley,[1] Ellen van Rooijen,[1,2,4] Elliott J. Hagedorn,[1,2,4] Christie Ciarlo,[1,4] Richard M. White,[8] Dominick A. Matos,[9] Ann-Christin Puller,[10] Cristina Santoriello,[1,11] Eric C. Liao,[2,4,12] Richard A. Young,[6,13] Leonard I. Zon[1,2,3,4,11]*

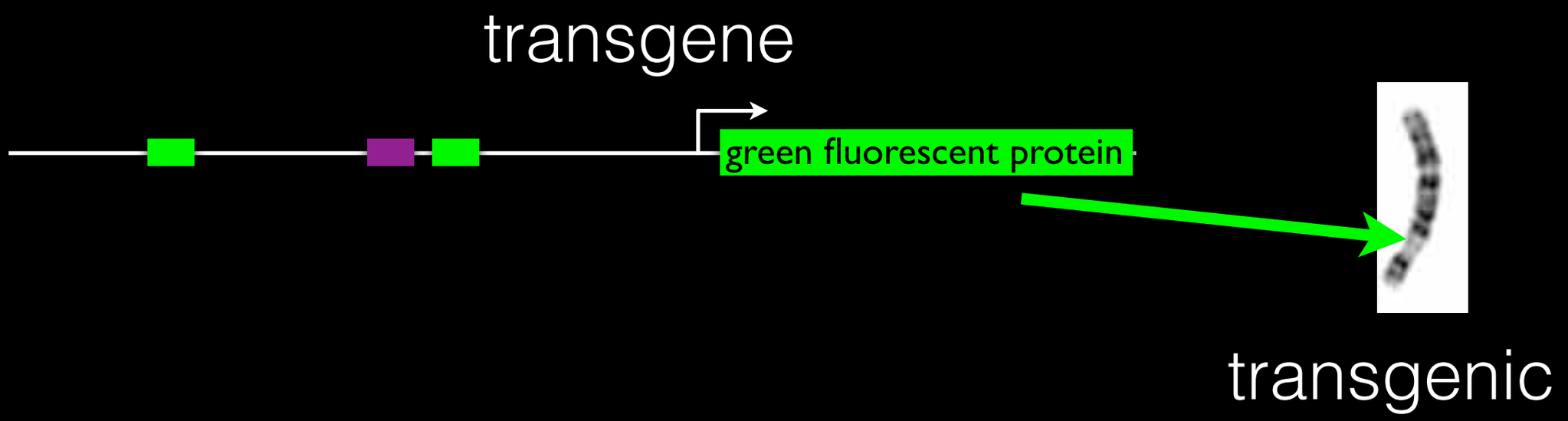
Science Jan 29th 2016

Neural crest cells

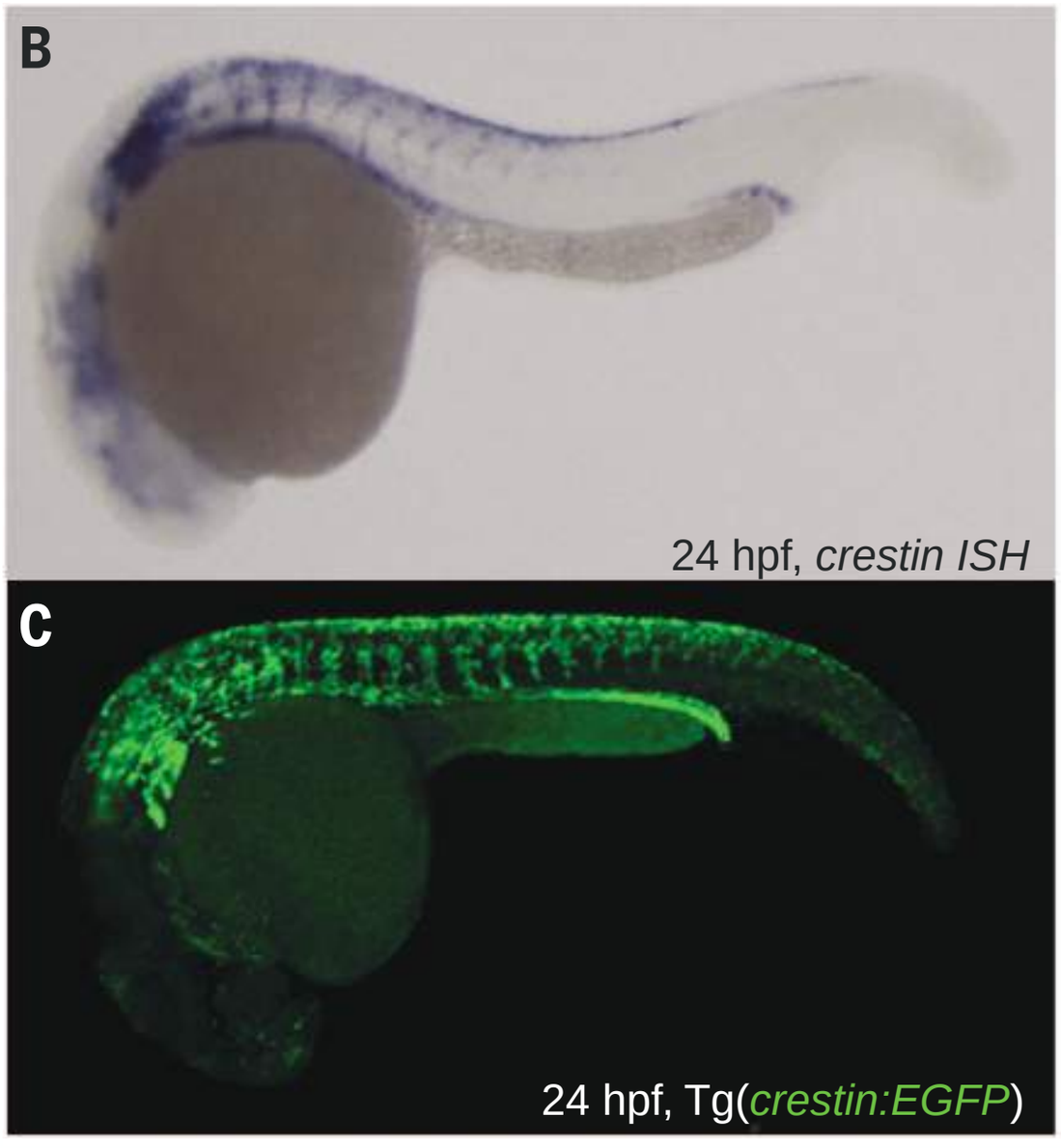


Transgenes and transgenics

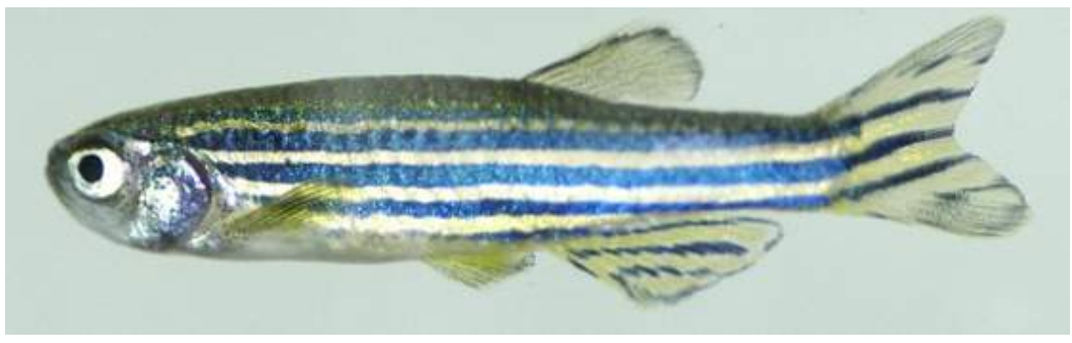




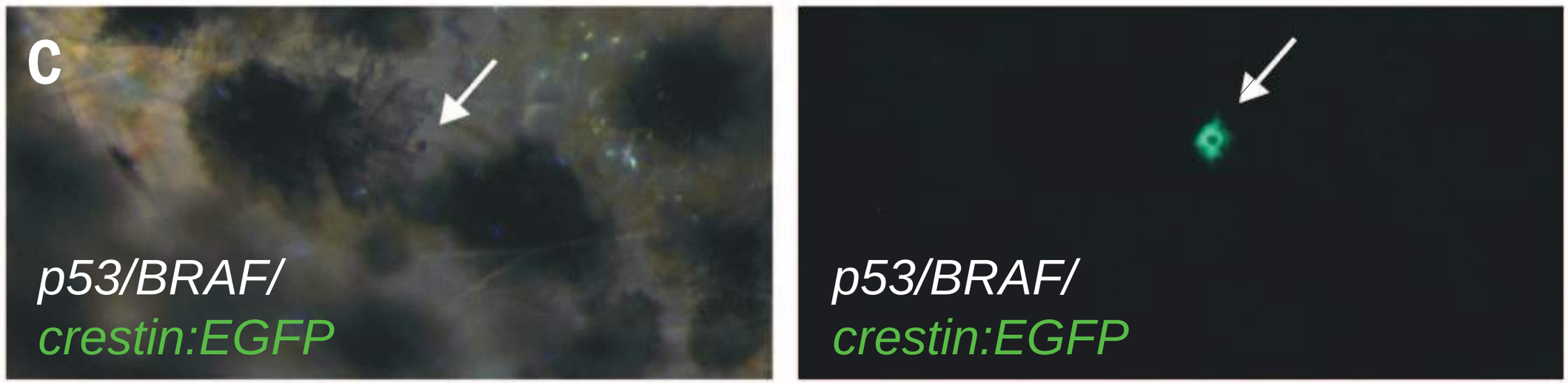
Control experiment shows that *crestin:EGFP* transgene expressed where *crestin* mRNA is

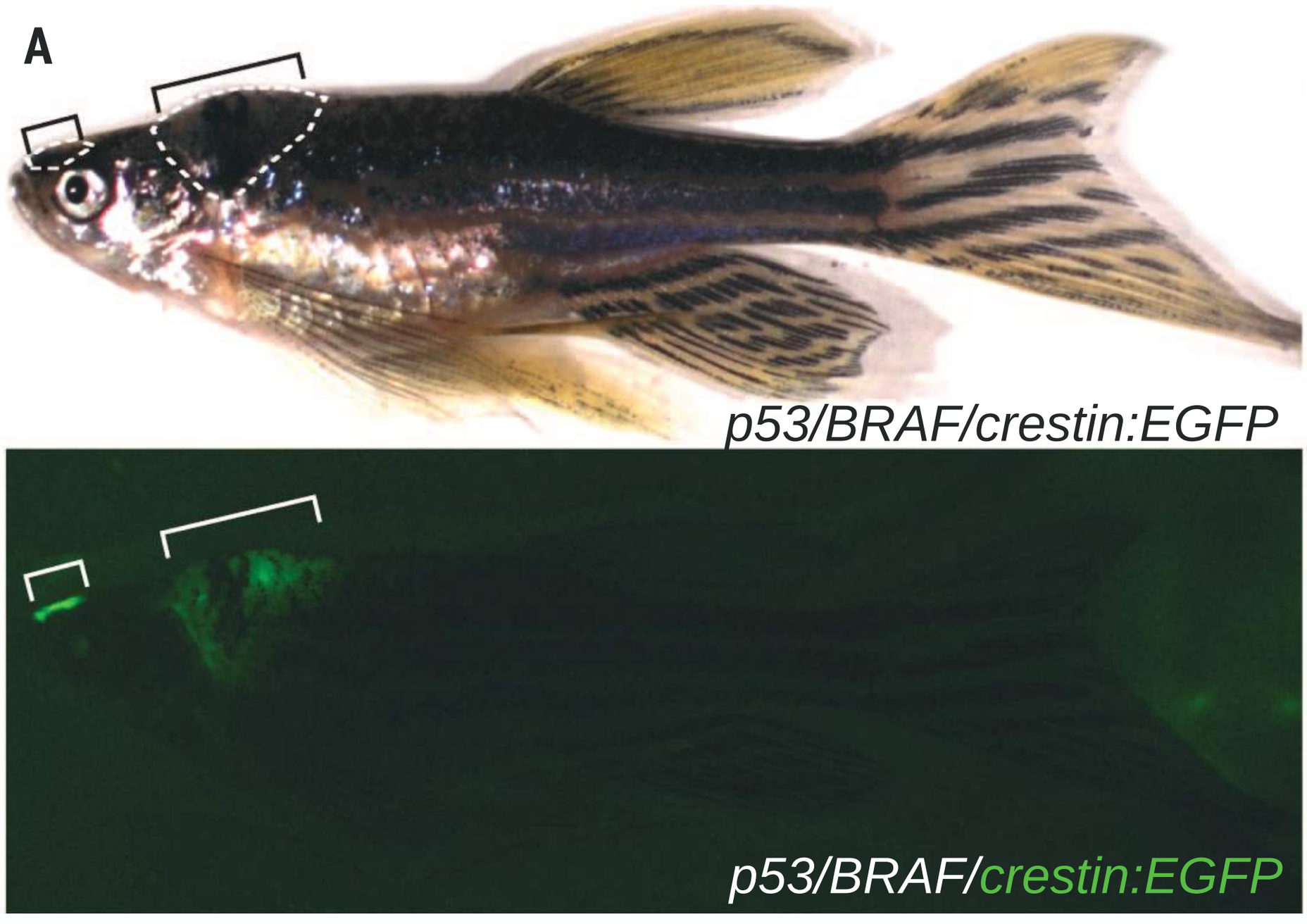


Crestin Not expressed in adults

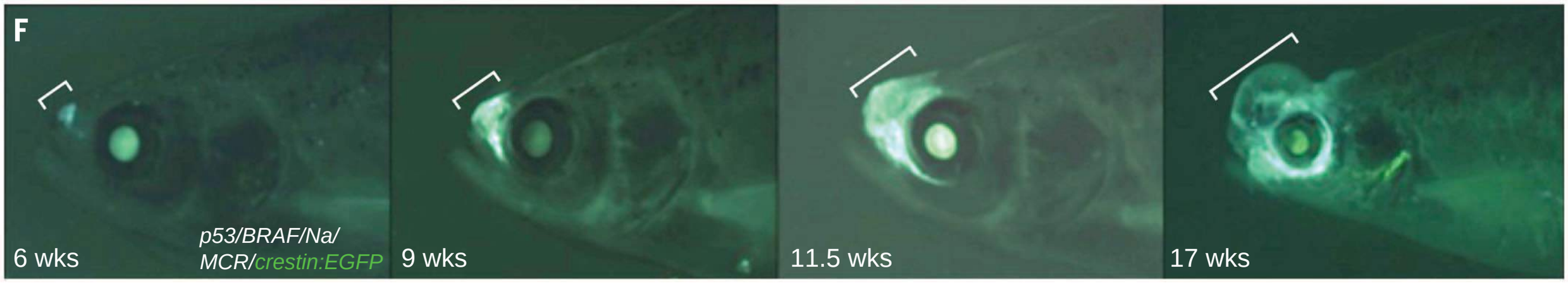


The authors next put their *crestin:EGFP* reporter into a p53 and BRAF mutant background and waited!




A
p53/BRAF/crestin:EGFP
p53/BRAF/crestin:EGFP

crestin positive melanomas grow



Melanomas are derived in part from a reactivation of developmental programs in adults.

Profiling the melanomas demonstrated that this cancer is due to transforming adult cells back to an embryonic **stem cell state**

On a side note transgenic animals also make adorable pets

Not discussed today

Computational approaches as models

Take home

Model systems are necessary to understand humankind biology

Choosing the right model system requires weighing the benefit vs the ethical and financial costs

The answer isn’t always going to be easy