

Salk Institute For Biological Studies, La Jolla California

Medicina Regenerativa, Enfermedad y Envejecimiento

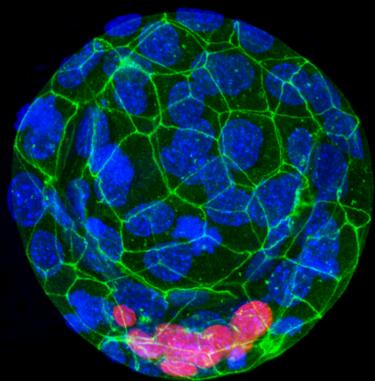


Juan Carlos Izpisua Belmonte

# Embryo Development



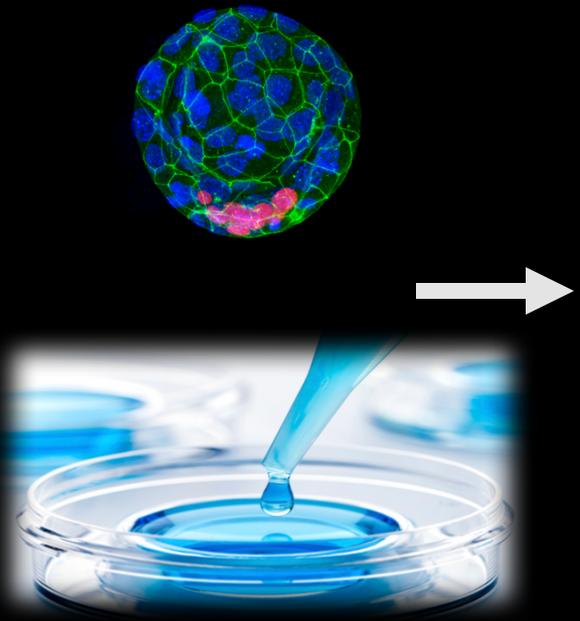
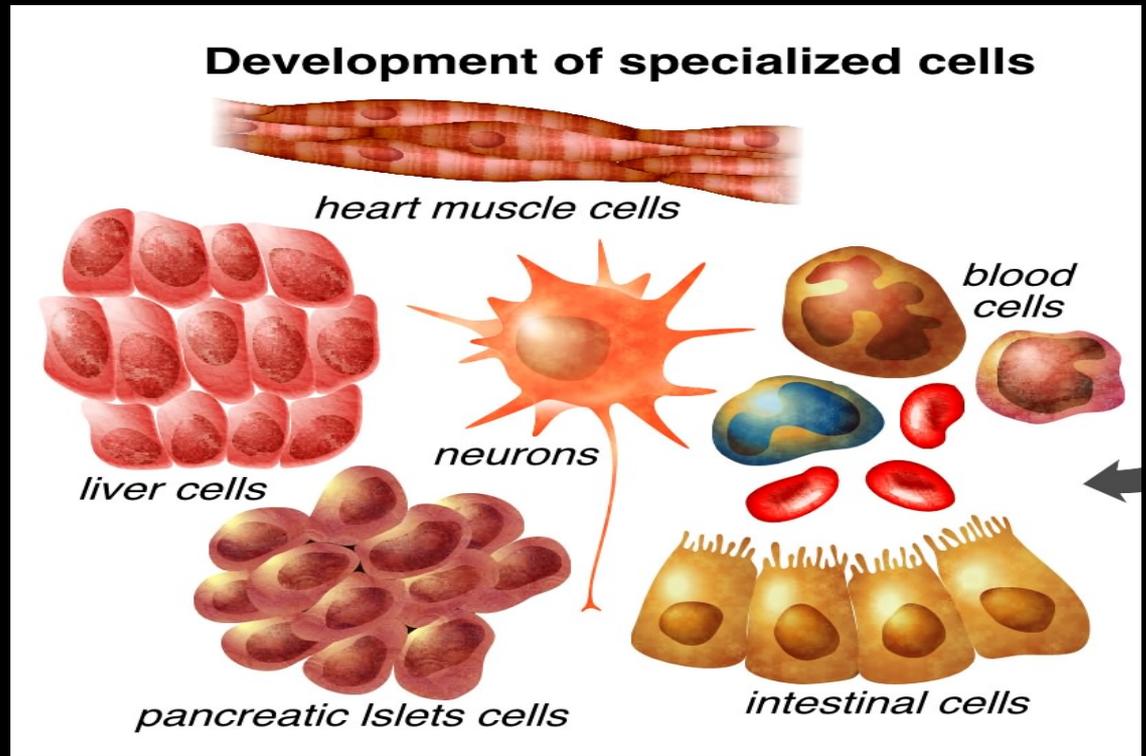




# Enfermedad y Envejecimiento



Embryonic Stem Cells or iPS cells → Adult Cells

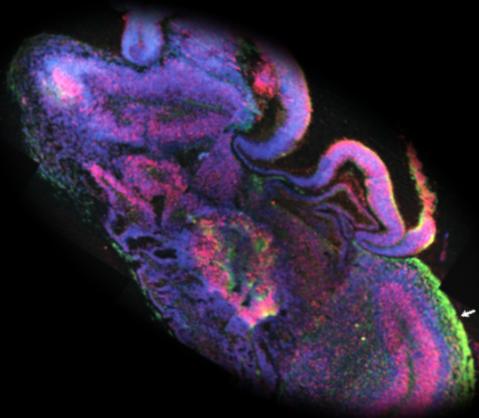


Cell, Tissue and Organ Generation *in Vitro*

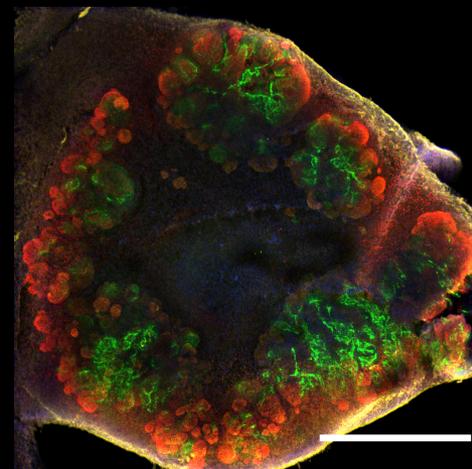
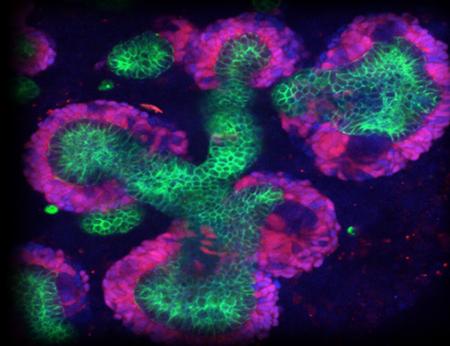
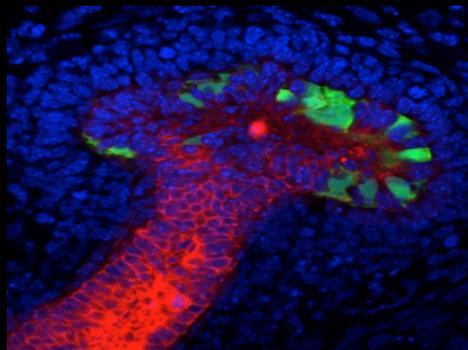
# Cardiomyocytes



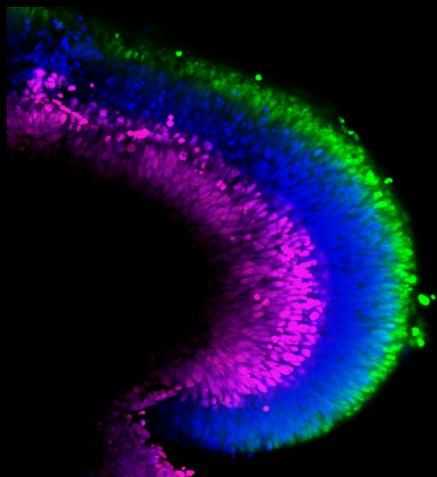
# Organoids



*Brain*

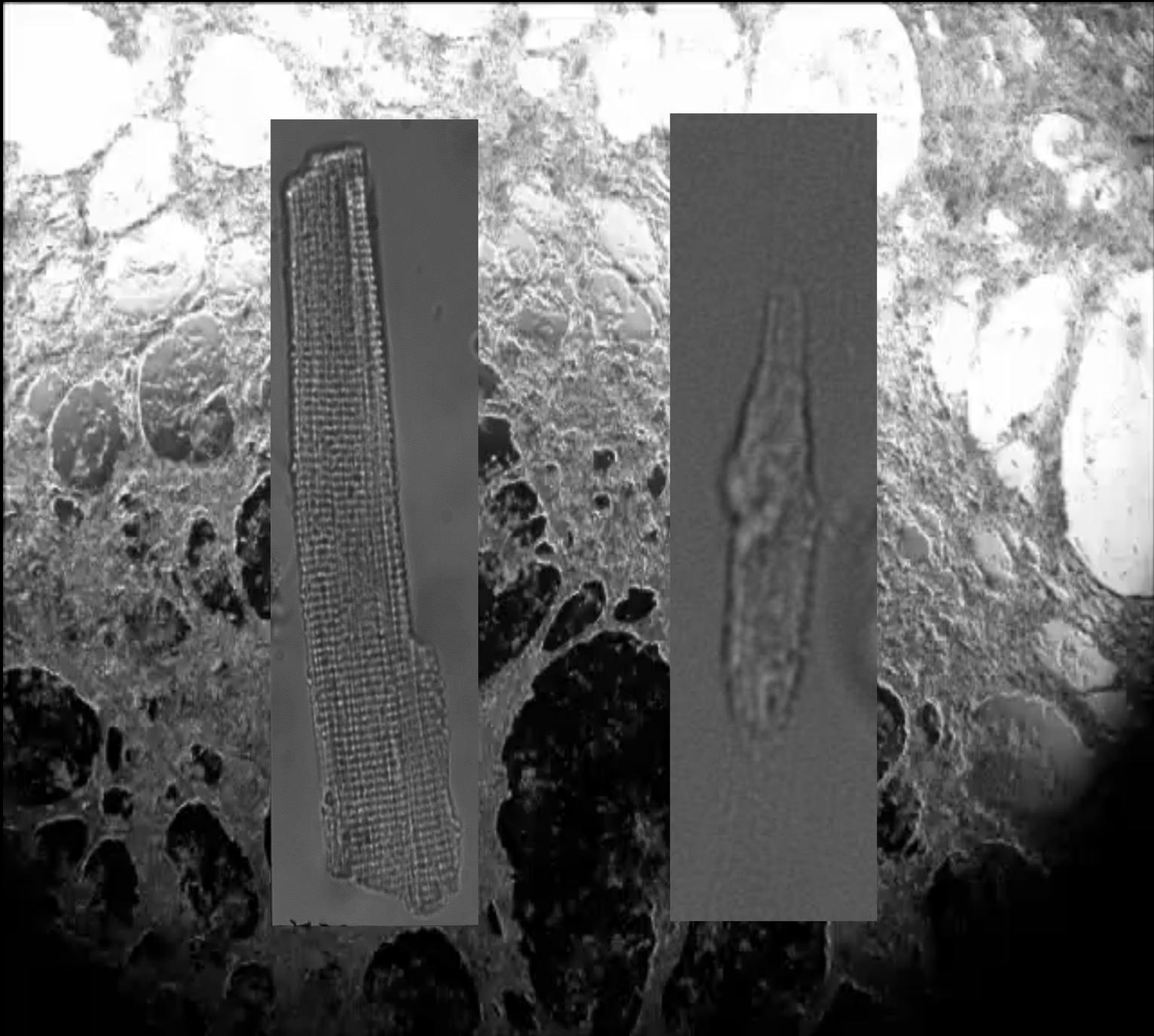


*Kidney*



*Eye*

Nuria Montserrat



# Size

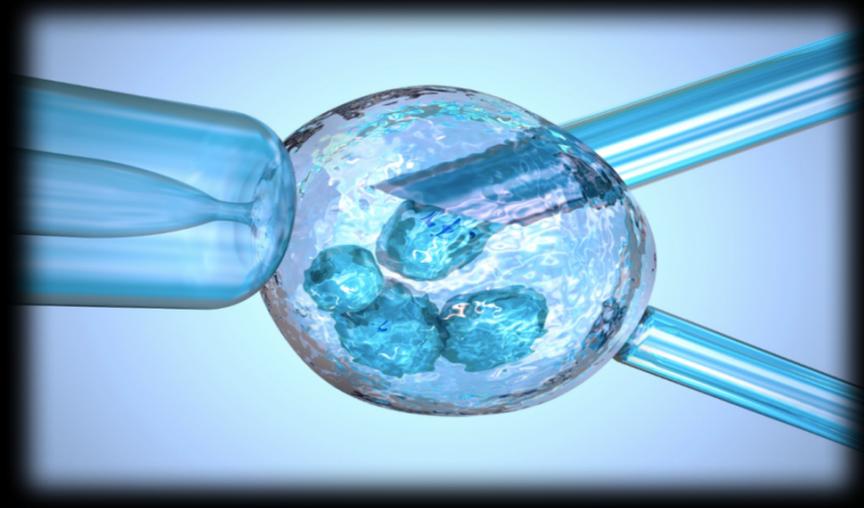


# Cell, Tissue and Organ Generation

In Vitro



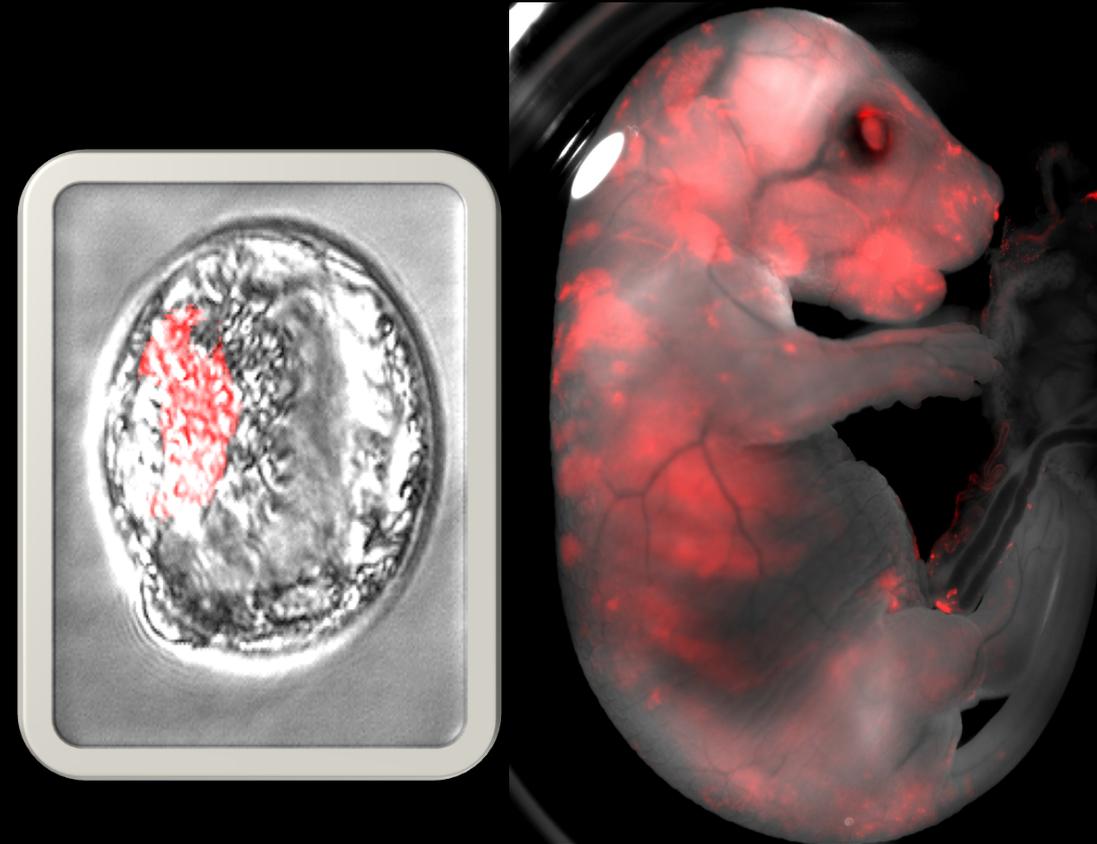
In Vivo



# Chimeras



# Interspecies Chimeras: Rat Mouse



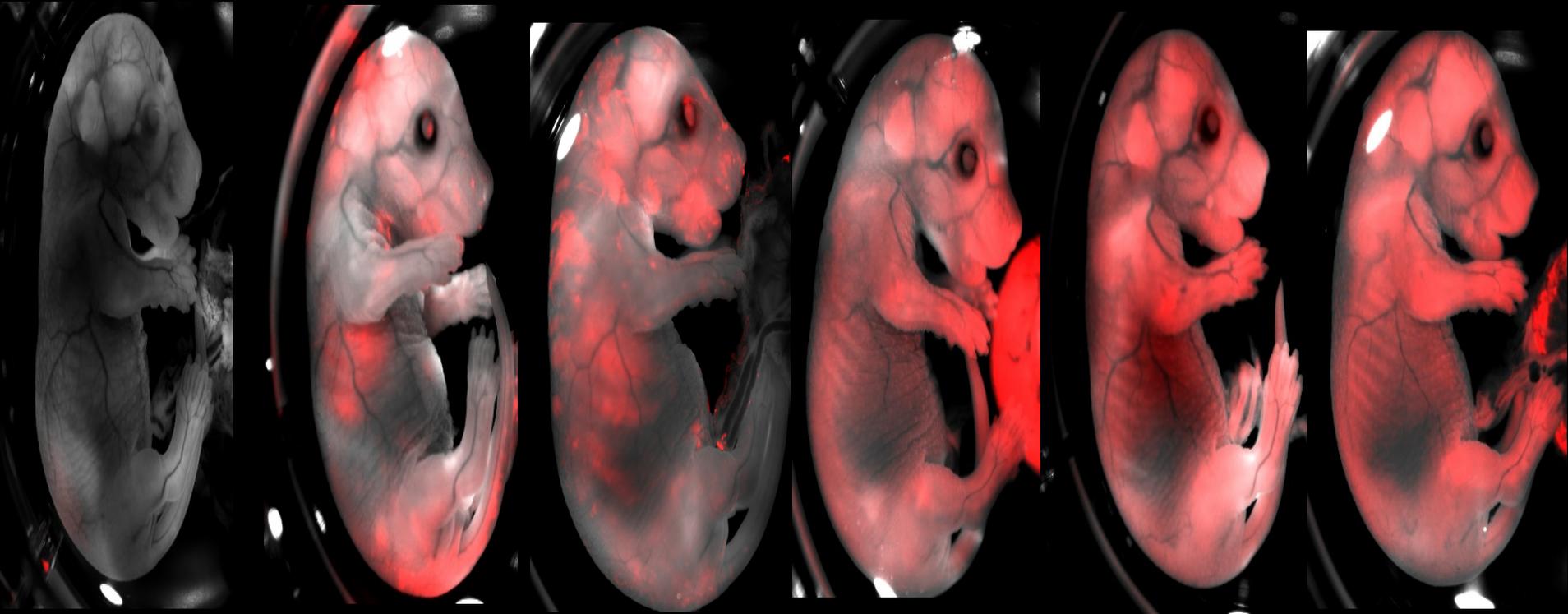
E18.5



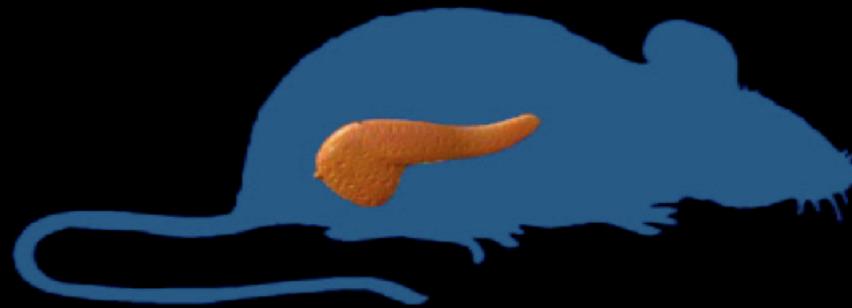
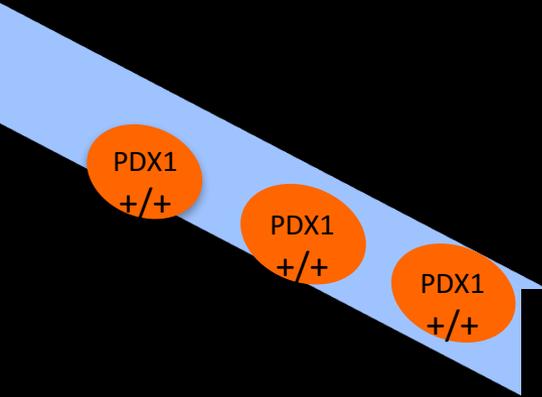
Mouse or Rat?

Wu et al, Cell, 2017

# Interspecies Chimeras: Rat Mouse



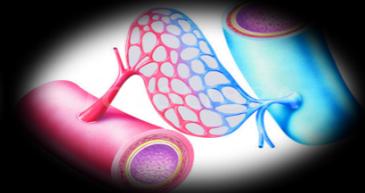
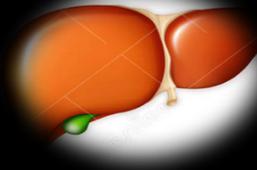
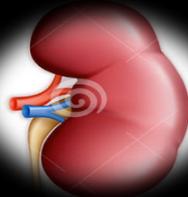
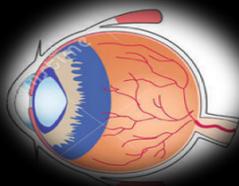
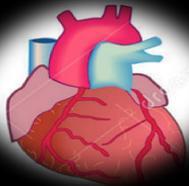
# Blastocyst complementation: Rat / Mouse



Rat Pancreas in a Mouse



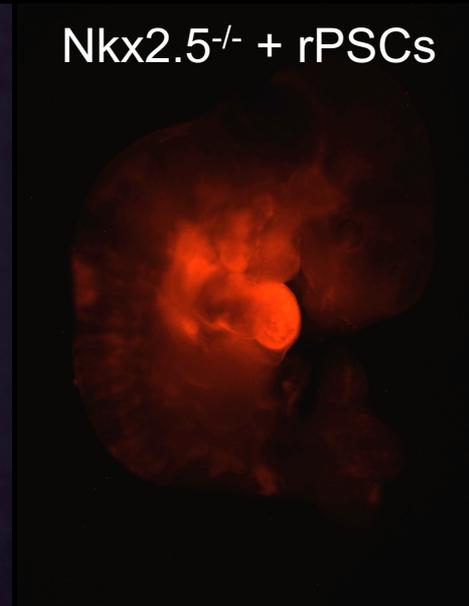
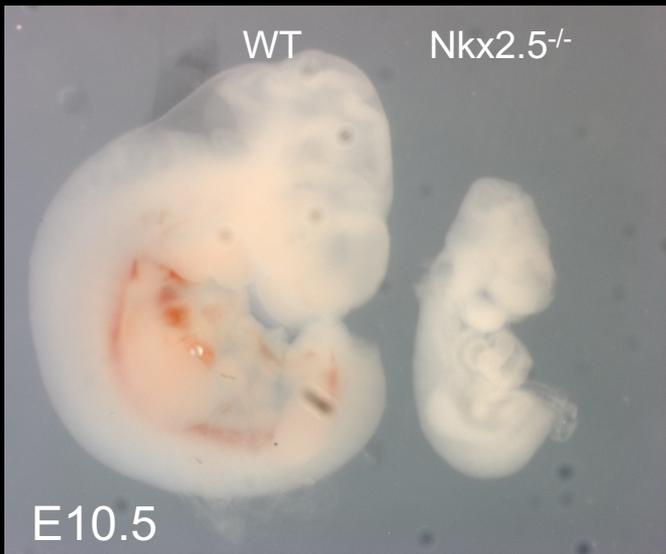
# Blastocyst complementation Screening



## Screening list

GENE NAME	LINEAGE	GENE NAME	LINEAGE	GENE NAME	LINEAGE
Osr1	Kidney	Foxa1	Liver	Foxc2	Endothelial
Lhx1	Kidney	Foxa2	Liver	Foxo1	Endothelial
Pax2	Kidney	Gata4	Liver	Ets1	Endothelial
Pax8	Kidney	Gata6	Liver	Fli1	Endothelial
Wt1	Kidney	Hnf1a	Liver	Etv2	Endothelial
Foxd1	Kidney	Hnf1b	Liver	etsrp	Endothelial
Hox11a	Kidney	Hnf4a	Liver	Rbpj	Endothelial
Hox11b	Kidney	Hhex	Liver	Hey1	Endothelial
Hox11c	Kidney	Prox1	Liver	Hey2	Endothelial
Eya1	Kidney	Tbx3	Liver	Prox1	Lymphatic
Six1	Kidney	HNF6	Liver	Mef2c	Heart
Six2	Kidney	Runx1	HSC	Nkx2.5	Heart
Wnt4	Kidney	Tal1	HSC	Tbx5	Heart
Fgf8	Kidney	Lmo2	HSC	Gata4	Heart
Bmp7	Kidney	Tel	HSC	Isl1	Heart
Notch2	Kidney	Gata2	HSC	Tbx1	Heart
Pod1	Kidney	Foxc1a	Endothelial	PAX6	Eye
Pdgfr	Kidney	Foxc1b	Endothelial	Foxf1	Lung
.....	.....	.....	.....	.....	.....

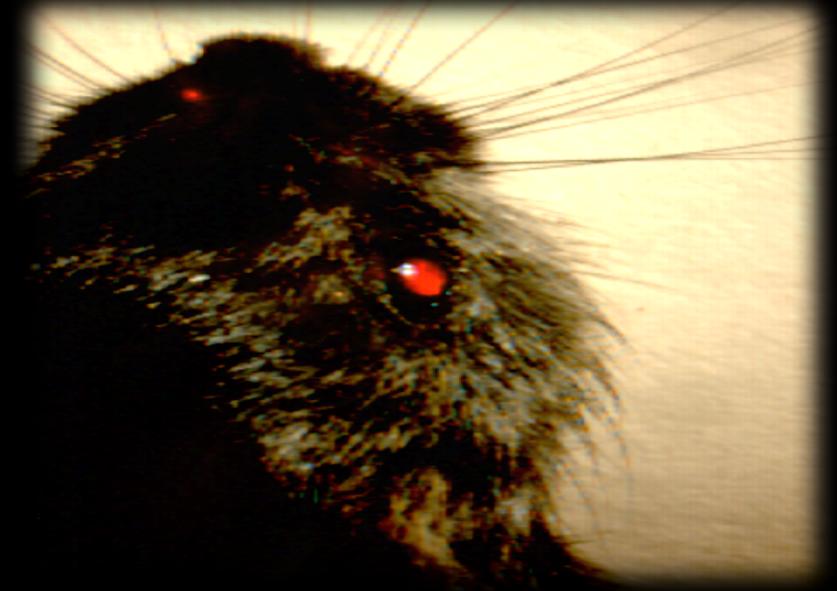
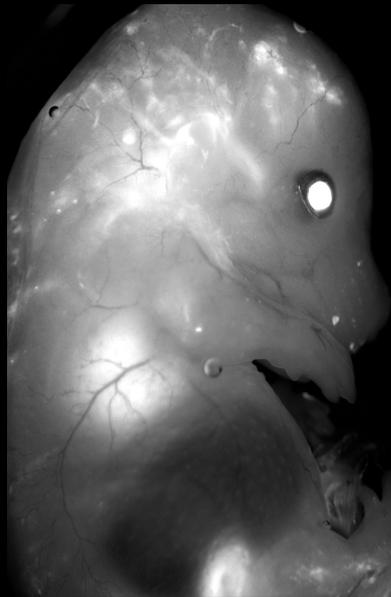
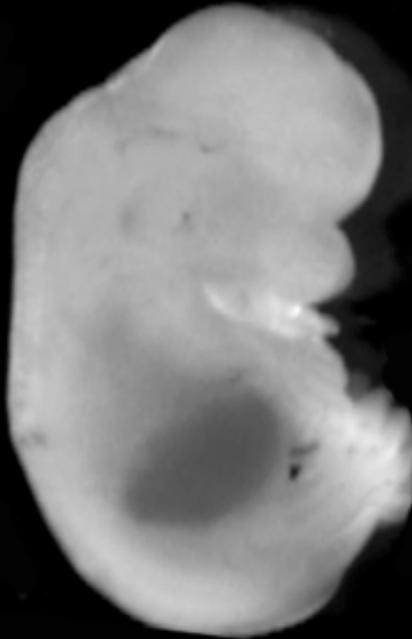
# Chimeric Heart



# Chimeric Eye

Knockout (KO)

KO+Rat iPSC



Wu et al. Cell 2017

# Rat-Mouse Chimeric Organs

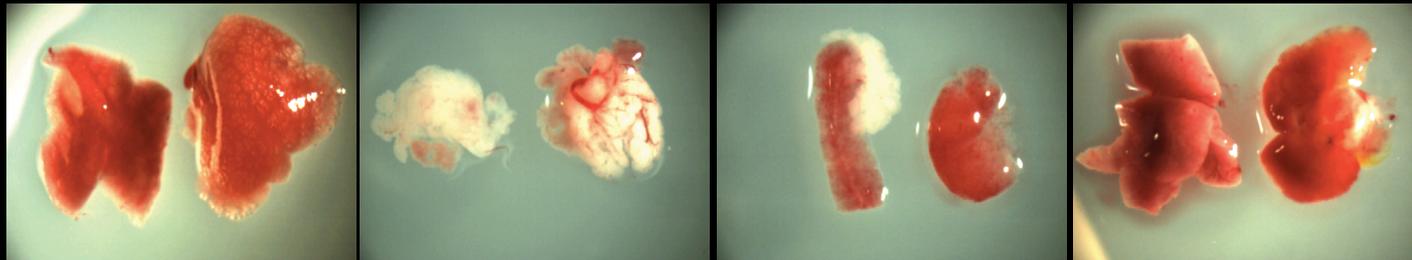


Brain

Heart

Intestine

Kidney



Lung

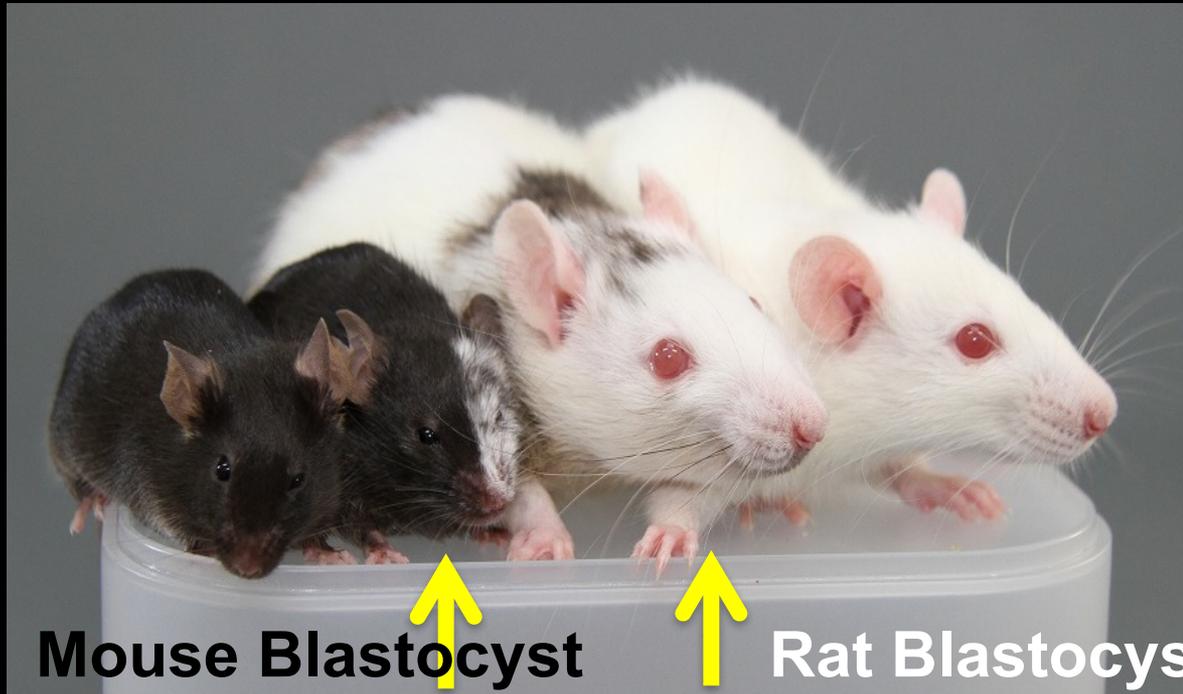
Pancreas

Spleen

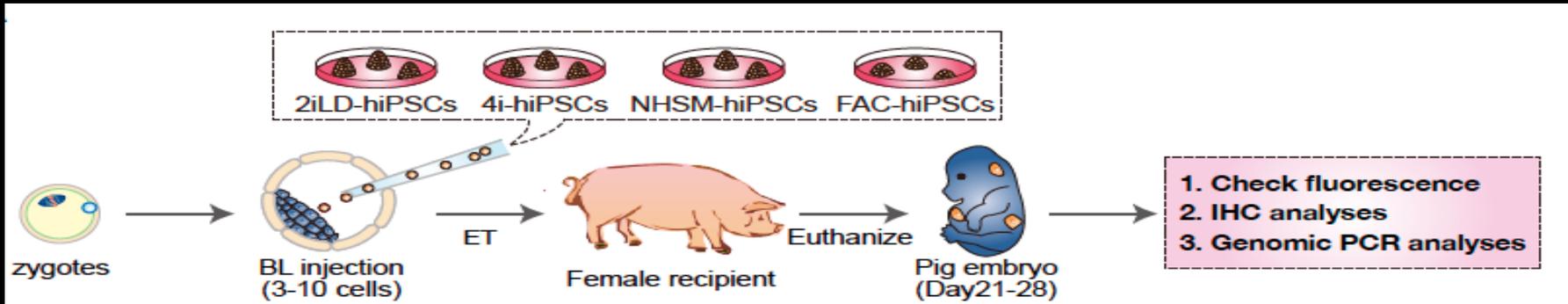
Liver



# Blastocyst / Foster Mother Determines Chimera Body Size



# Chimeric contribution of Human PSCs to pig embryos

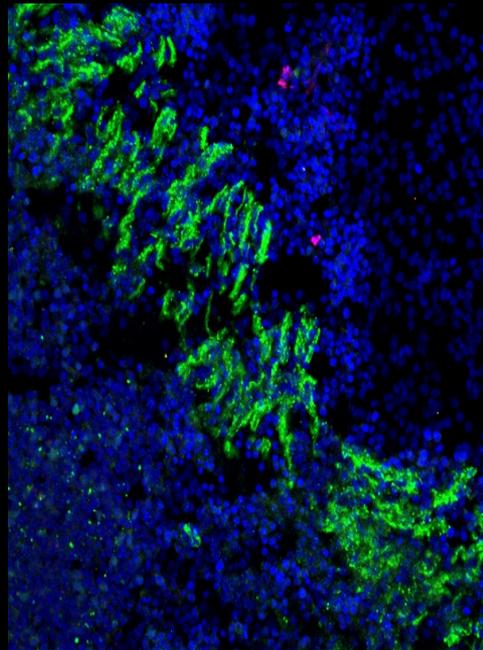
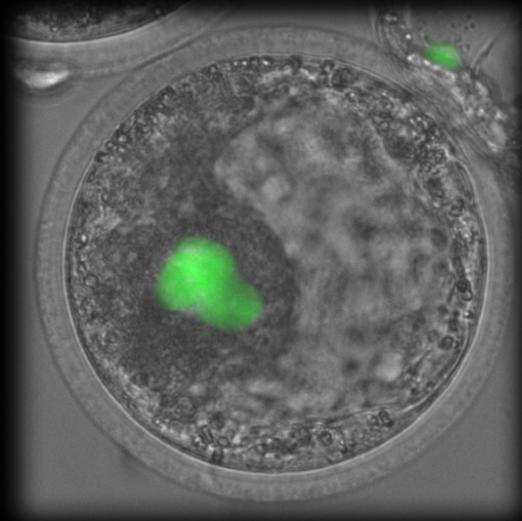
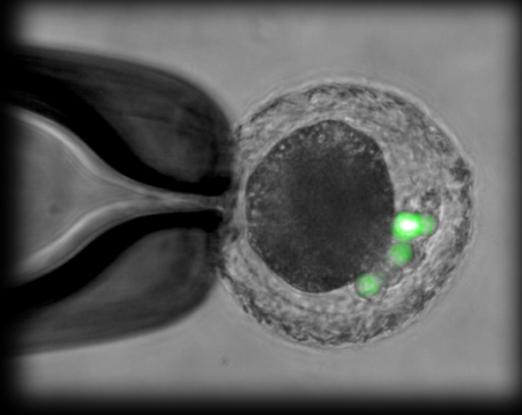


Experiment	# Donors	ZG/PT	BL from ZG/PT	2C	BL from 2C	MO-BL	#Injected BL	#Transferred BL
batch1	15	71	48	118	106		154	143
batch2	15	160	146	59	56		202	203
batch3	6					91	91	82
batch4	13	54	48				48	48
batch5	13	117	92	45	43		135	
batch6	14	66	50	60	58		108	237
batch7	10	42	37	62	62		99	
batch8	8	49	35	20	20		55	270
batch9	8	147	128	20	20		148	
batch10	6	14	10	100	94		104	
batch11	6	10	5	35	34		39	191
batch12	3	47	43				43	
batch13	6	46	40	46	42		82	82
batch14	4	60	48	6	2		50	50
batch15	16	202	171	142	89		260	220
batch16	24	213	151	291	271		422	408
<b>Subtotal</b>	<b>167</b>	<b>1298</b>	<b>1052</b>	<b>1004</b>	<b>897</b>	<b>91</b>	<b>2040</b>	<b>1924</b>
<b>Efficiency of IVC %</b>			<b>81</b>		<b>89</b>			<b>95</b>
batch17	PT	100	100				100	100
batch18	PT	41	41				41	41
<b>Total</b>		<b>1439</b>	<b>1193</b>				<b>2181</b>	<b>2075</b>

Wu et al., Cell  
2017

2C, 2-cell embryo; ZG, zygote; MO, morula; BL, blastocyst; PT, parthenote

# Chimeric contribution of **Human PSCs** to pig embryos



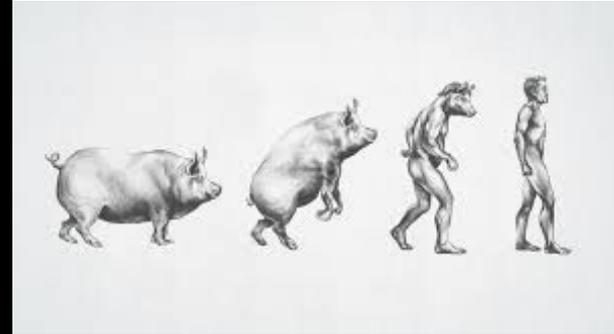
Low post-implantation  
interspecies chimerism

# Evolutionary Distance

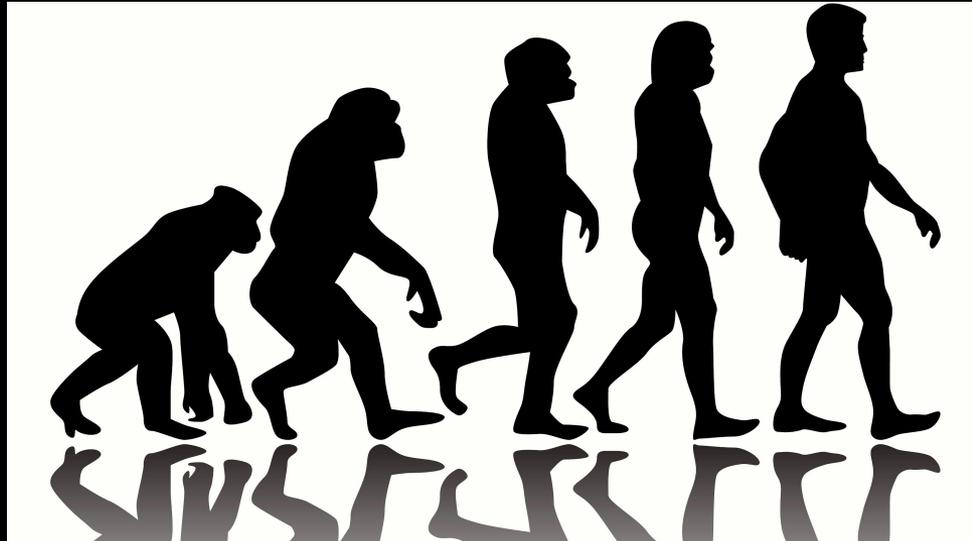
Mouse / Rat **21**



Pig / Human **100** MYA

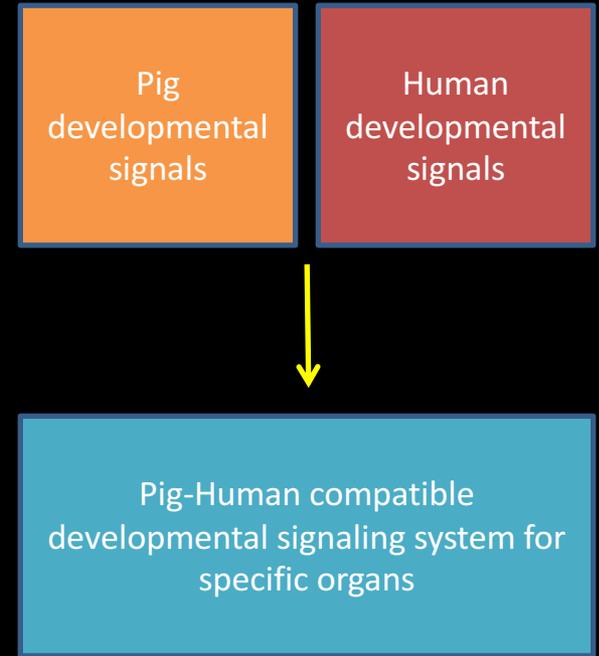
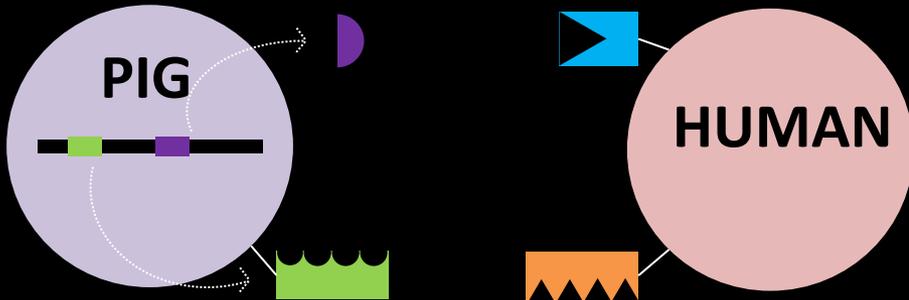


Monkey / Human **27** MYA

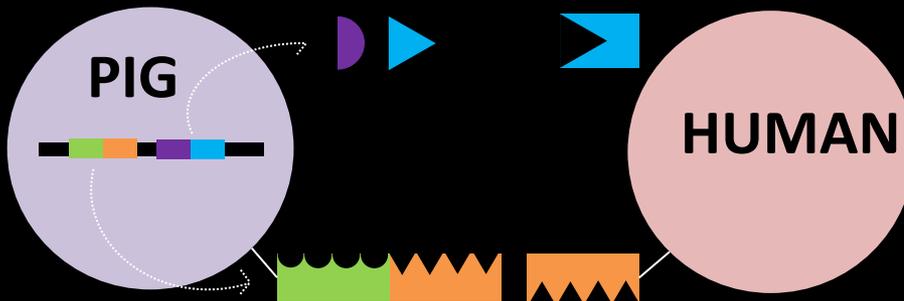


# Engineering developmental compatibility across species

## Compatible signaling system



## Compatible signaling system

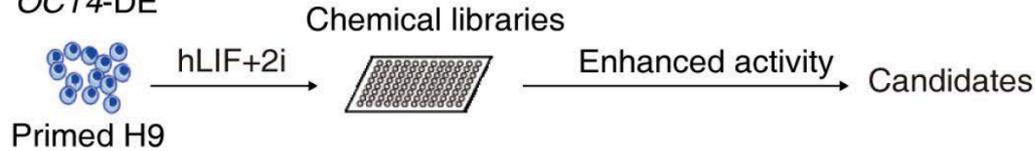


In frame fusion of human ligand homolog to the pig loci (TGFbeta, Hedgehog, Wnt, JAK/Stat, RTKs) or human receptor fusion (Notch).

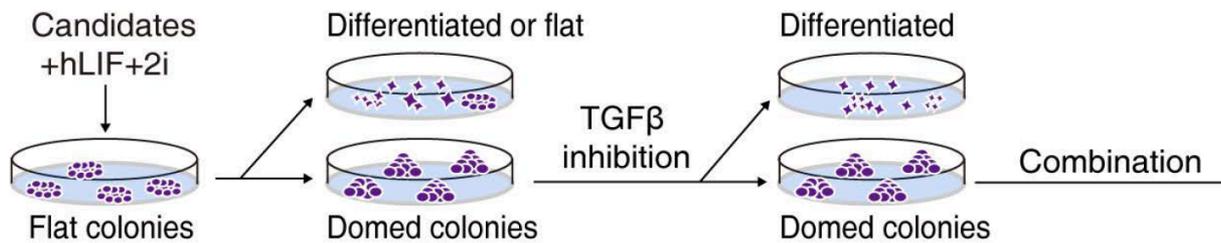
Engineering of porcine promoters of angiogenesis such as VEGF, FGF-1 or PKG receptors into human organoids

# Enhancing Chimeric Competency

1° Transfection  
*OCT4-DE*



2° Candidates  
+hLIF+2i



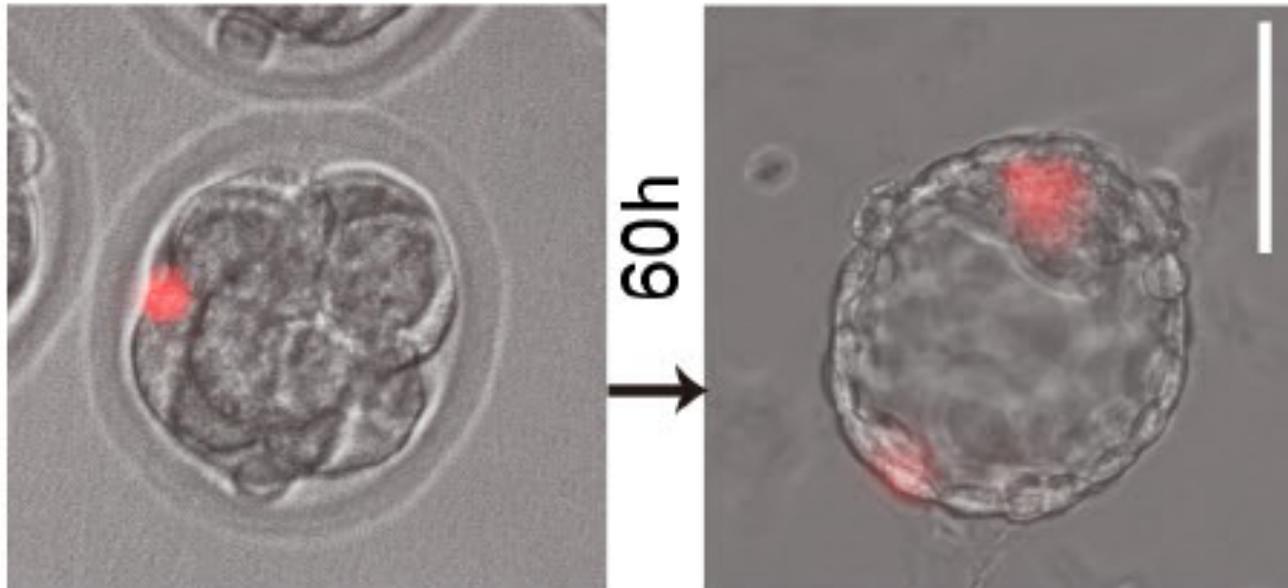
LCDM

L: Human LIF

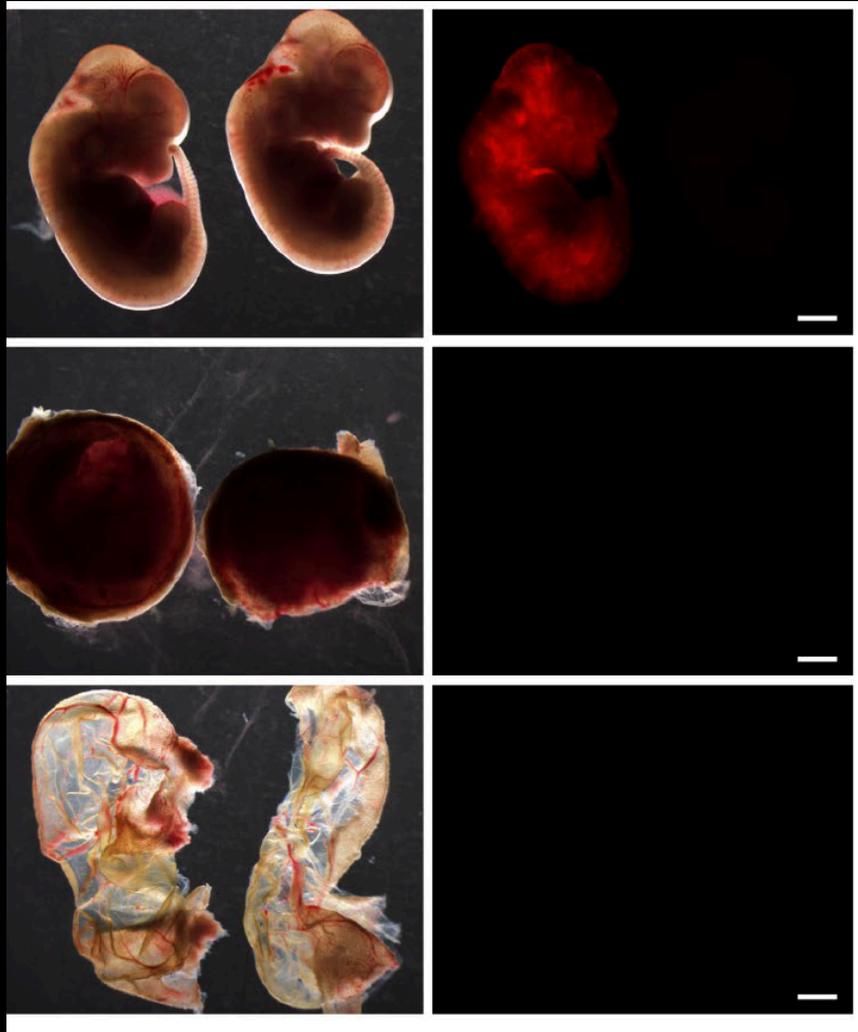
C: CHIR99021

D: (S)-(+)-Dimethindene maleate

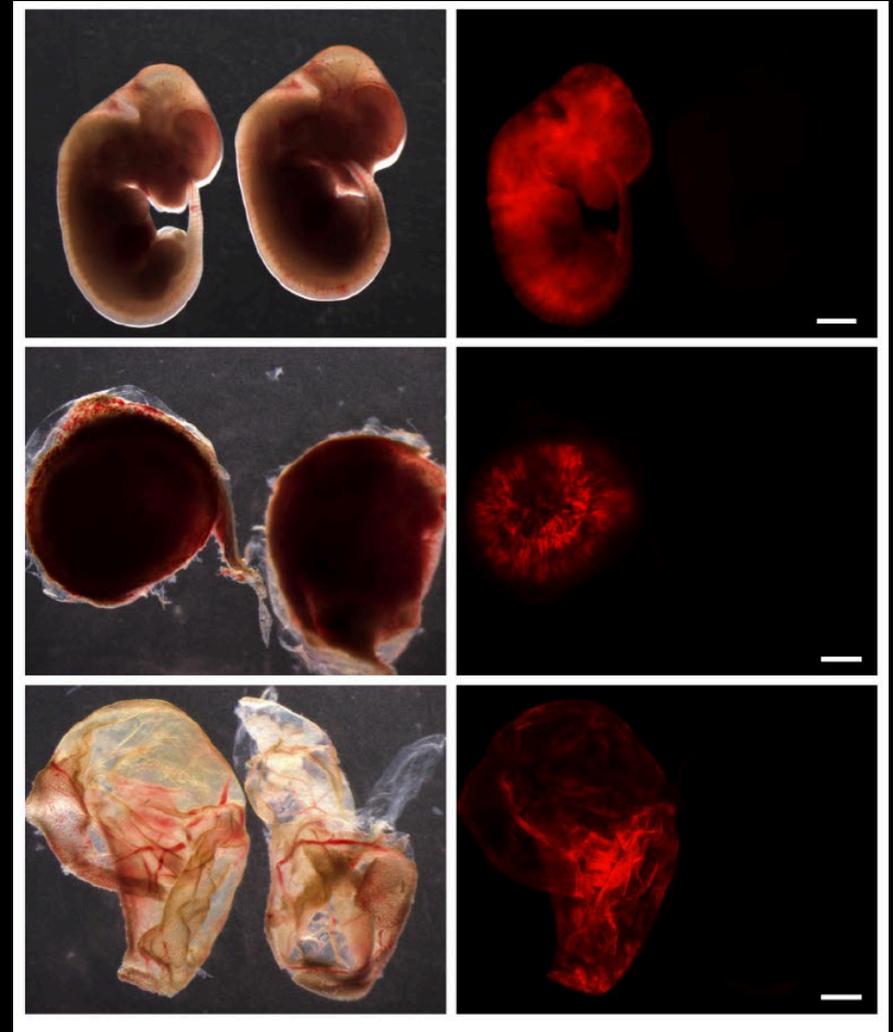
M: Minocycline hydrochloride



# Enhancing Chimeric Competency



Current Protocols



Yang et al. *Cell*

# Enhancing Chimeric Competency

F



Single mEPS  
-derived chimeras

G



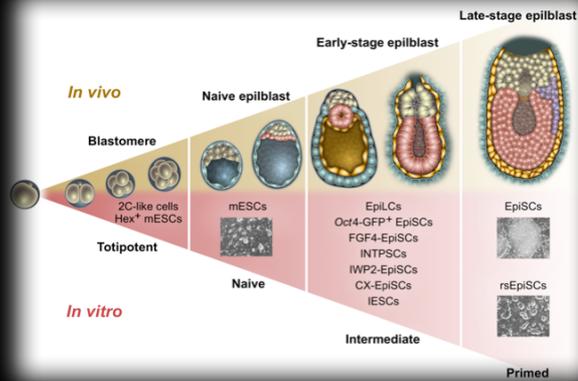
Offspring of single  
mEPS-derived chimeras

H

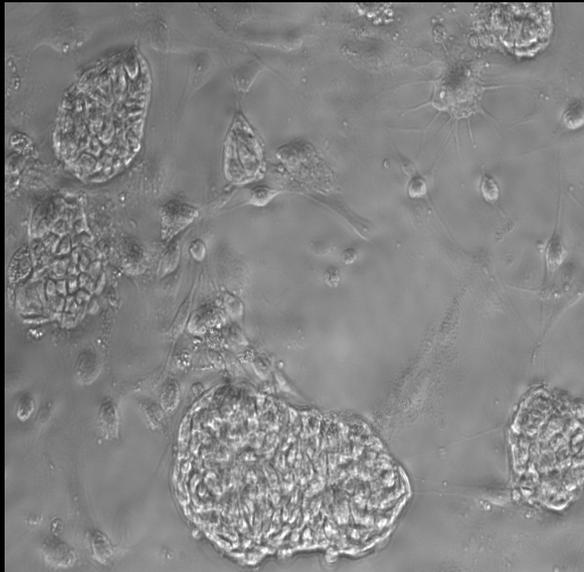


Single mEPS  
-derived mice by  
tetraploid complementation

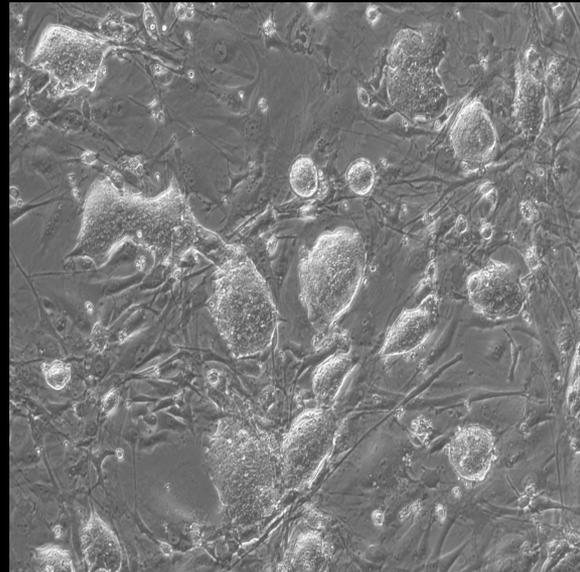
# EPS cells: Other species



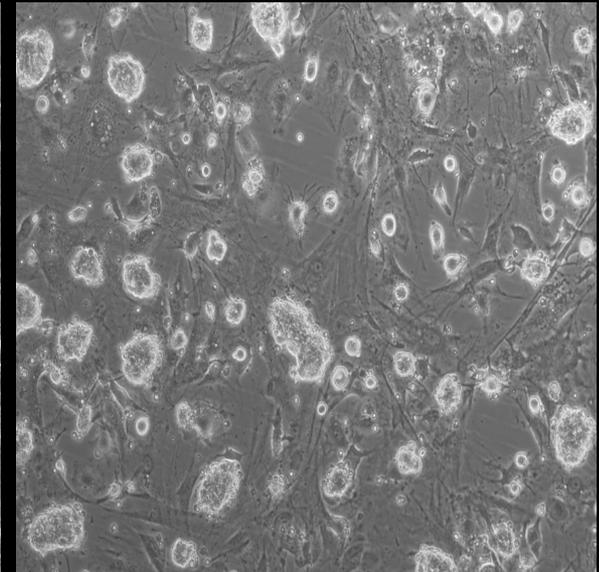
Sheep EPS cells



Pig EPS cells

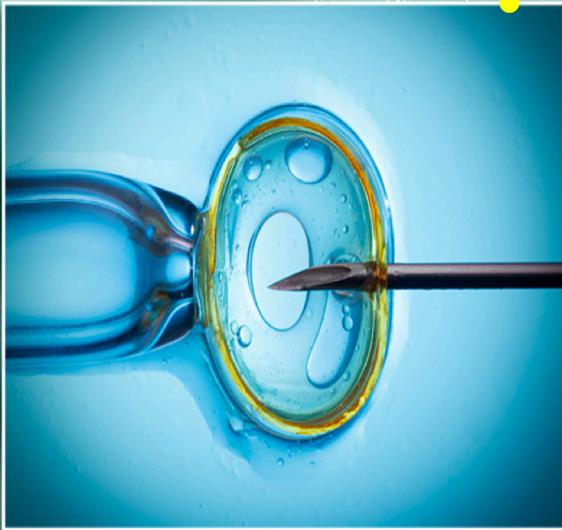


Rhesus EPS cells

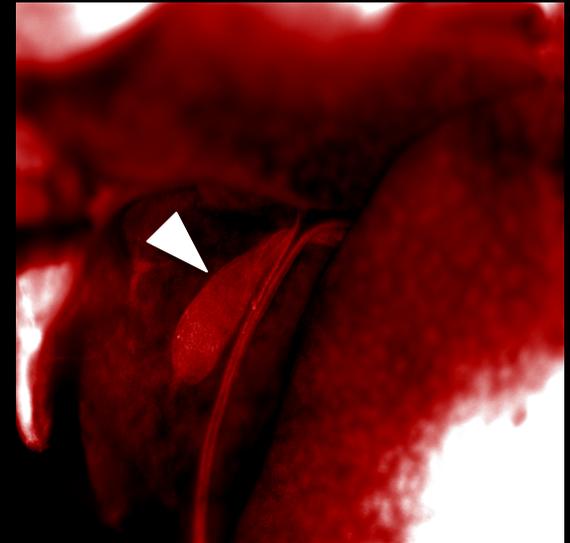
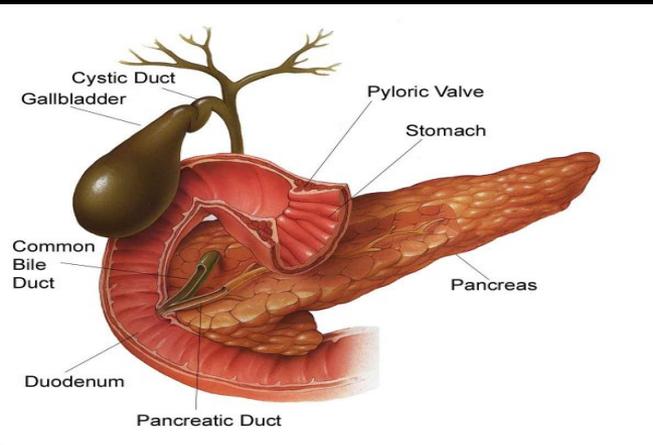


# Xenotransplantation: Immune compatible Pigs + Human Pig Chimeras

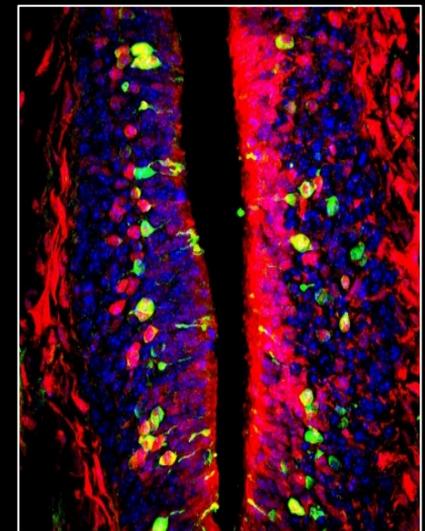
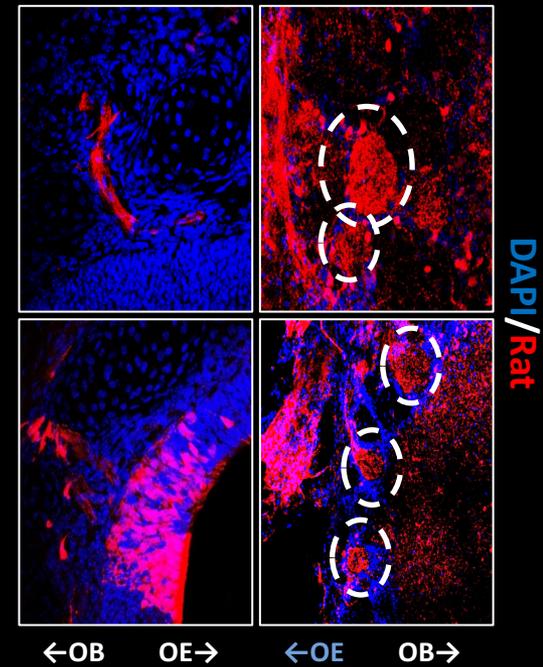
- Pig pluripotent stem cells
- Efficient gene editing tools



# Rat-Mouse chimeras: Gall bladder



# Rat - Mouse olfactory sensor neuron complementation



With Baldwin Lab

# The Lesser Egyptian Jerboa, *Jaculus jaculus*:

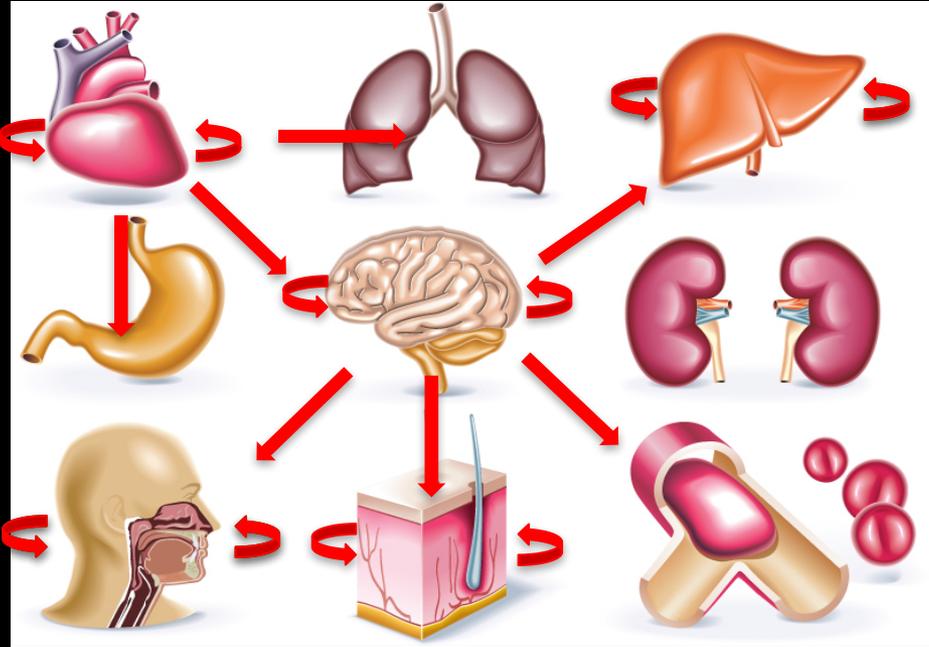
## Evolution and Organ Development



- Bipedal
- Three-toed
- Long hindleg
- Elongated and fused three central metatarsals

With Kim  
Cooper

# Organ Aging & Organismal Lifespan

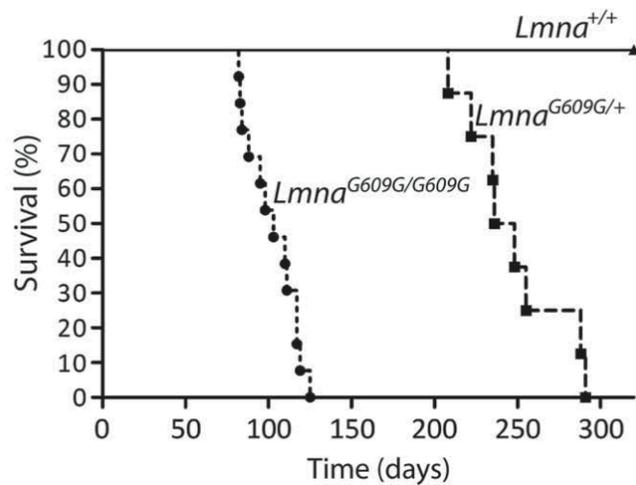


- Do specific organs drive organismal aging ?
- How aging in a specific organ interact with other organs to elicit organismal aging ?

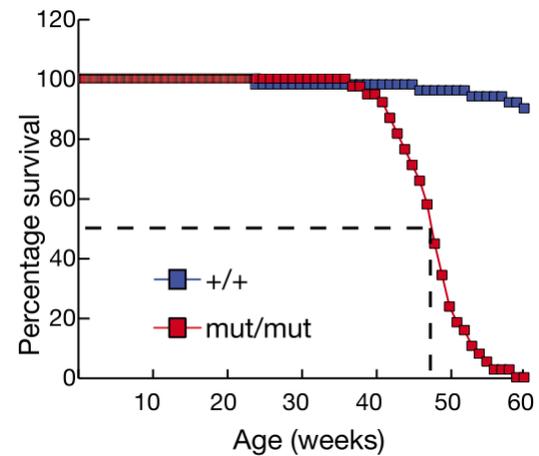
# Premature Aging Mouse Models

## Progeria

$Lmna^{+/+}$   $Lmna^{G609G/+}$   $Lmna^{G609G/G609G}$

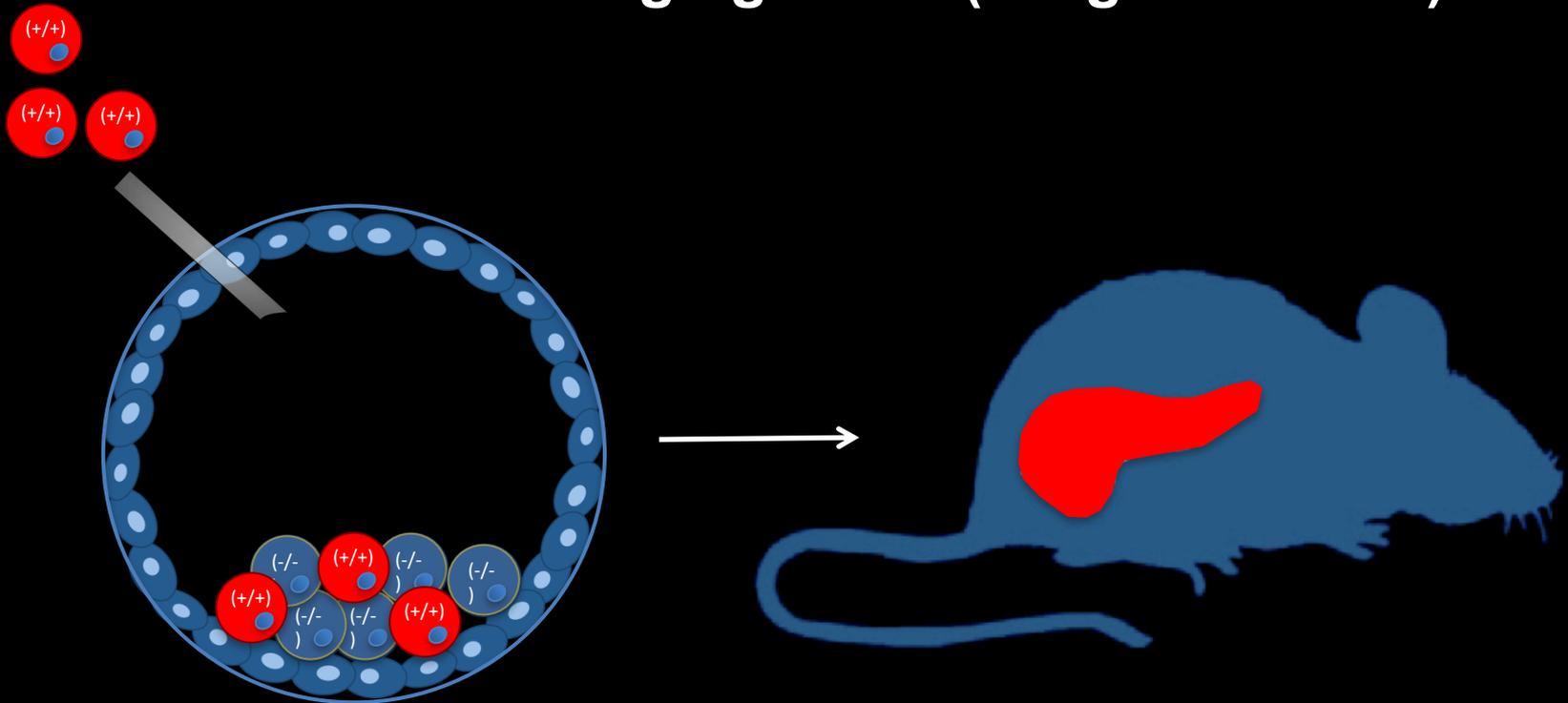


## PolG



# Organ Complementation

Premature Aging PSCs (Progeroid Mice)



Premature aging organ growing in a normal aging host

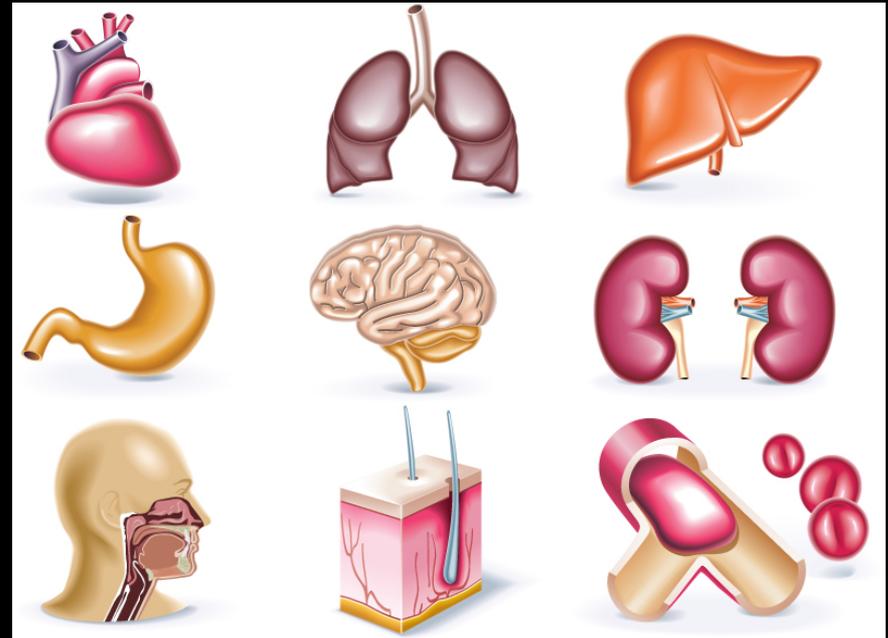
# Targets of Blastocyst Complementation

Premature Aging Germ Layer:

Ectoderm  
Endoderm  
Mesoderm

Premature Aging Organs:

Brain  
Skin  
Liver  
Pancreas  
Spleen  
Thymus  
Heart  
Muscle  
Bone Marrow  
...



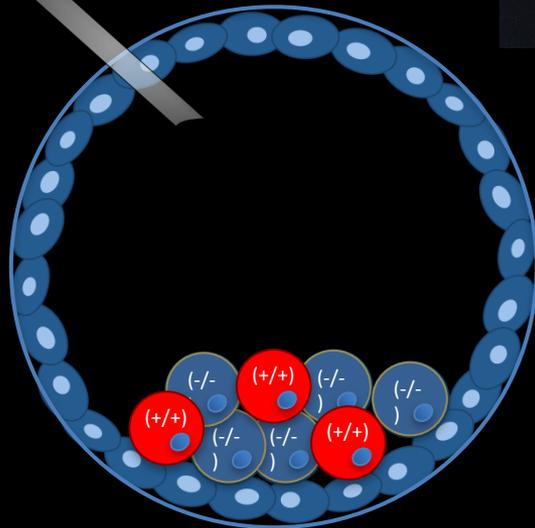
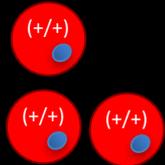
# Organ Complementation: Naked Mole Rat and Mice/Rats



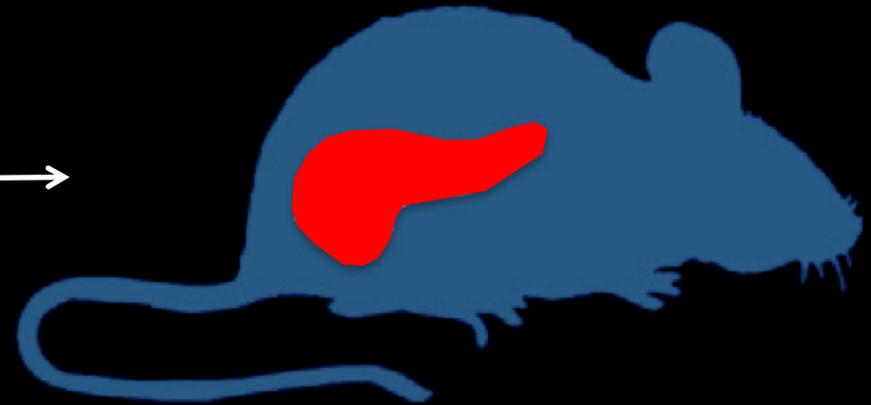
Naked Mole Rat Longevity:

**Up to 30 years!!!**

Naked Mole Rat's PSCs



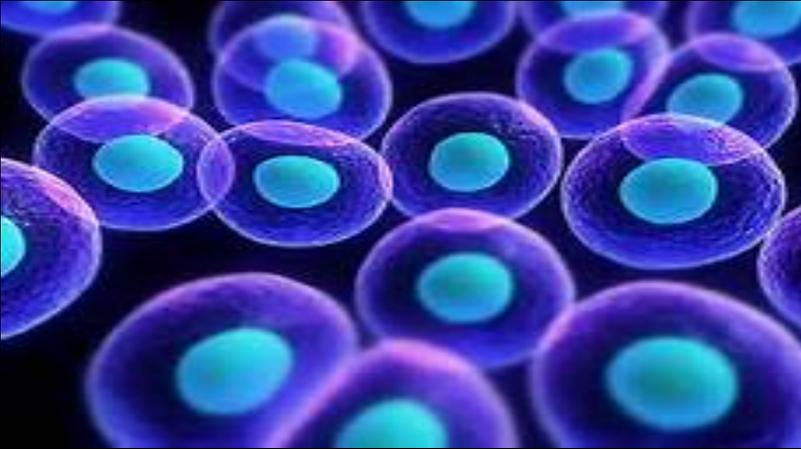
Rat Blastocyst



**Naked Mole Rat organ growing  
in a normal aging host**

# *Genetic and Epigenetic Strategies*

## *Cellular Strategies*



# Gene Editing in dividing and non dividing cells

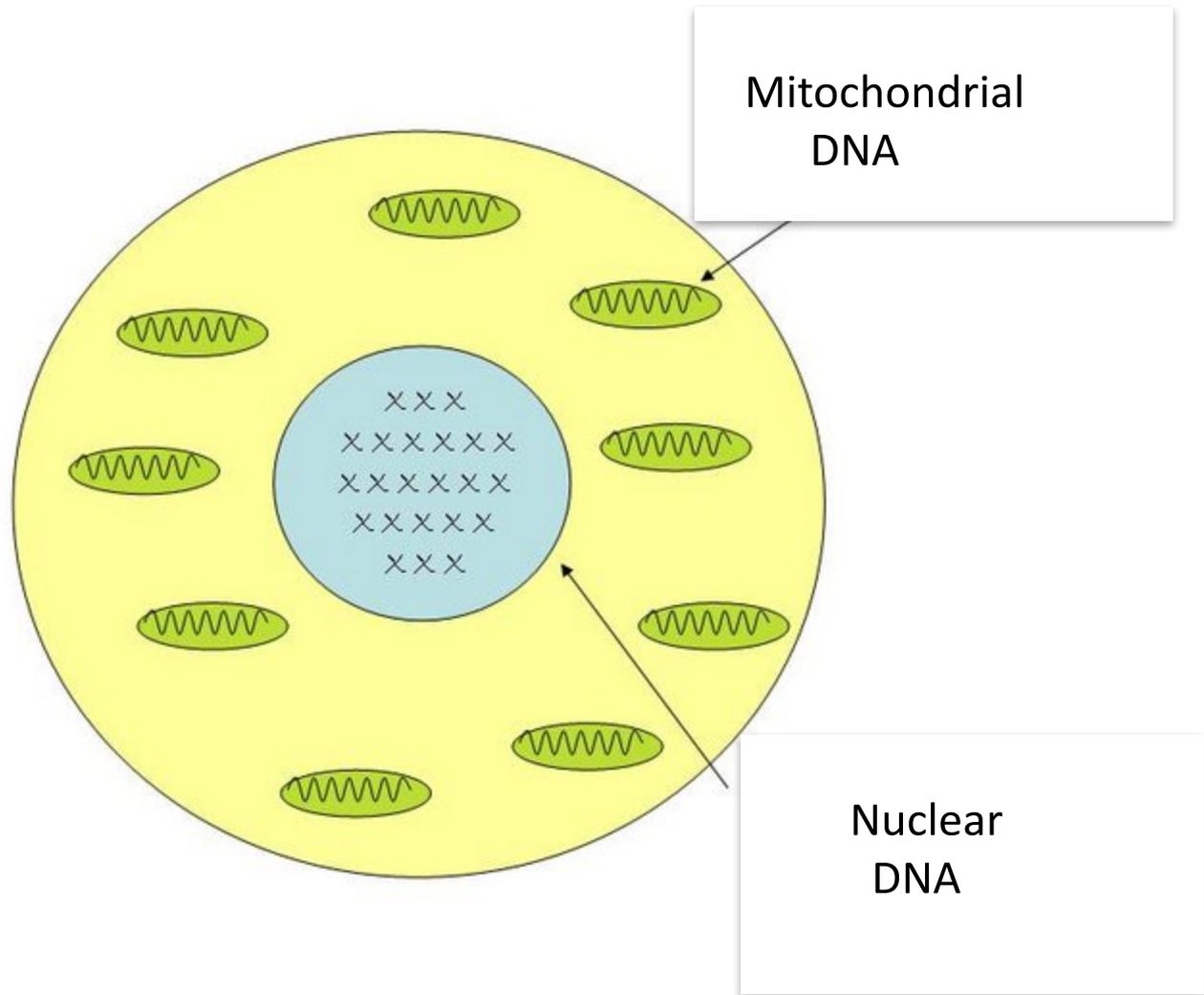


**Oocyte  
Embryo**



**Adult**

# DNA: Nuclear & Mitochondrial



# Clinical Disorders Caused by Mutations in mtDNA

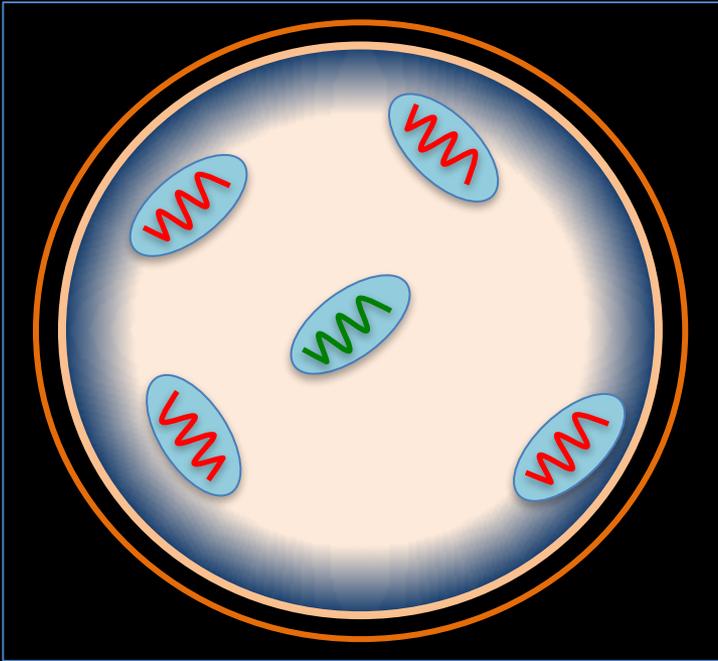
1:200 Female Carrier



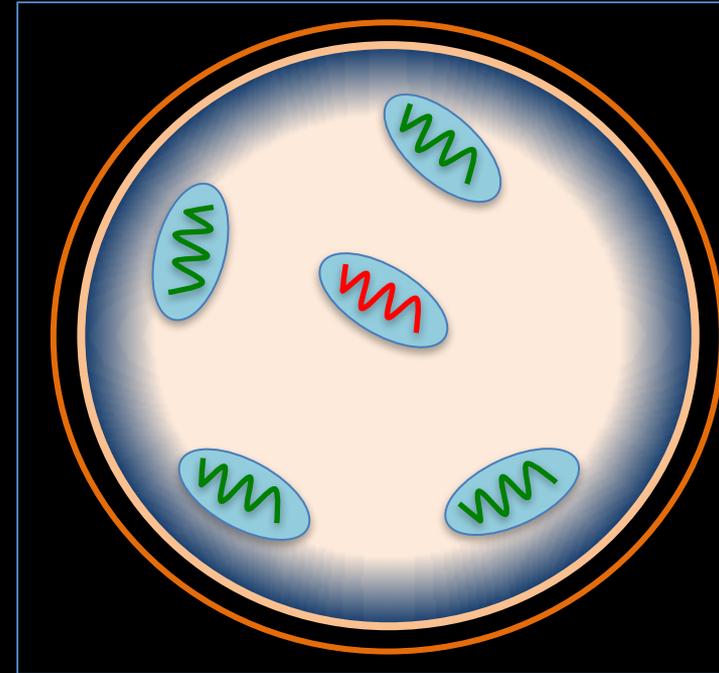
**Zygote**



# Heteroplasmic Shift



**Disease**



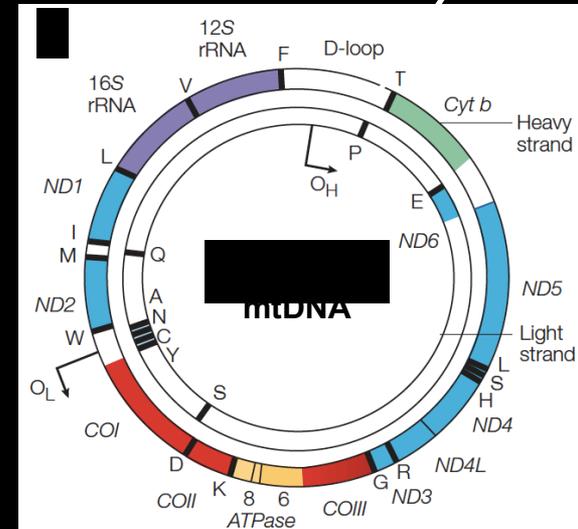
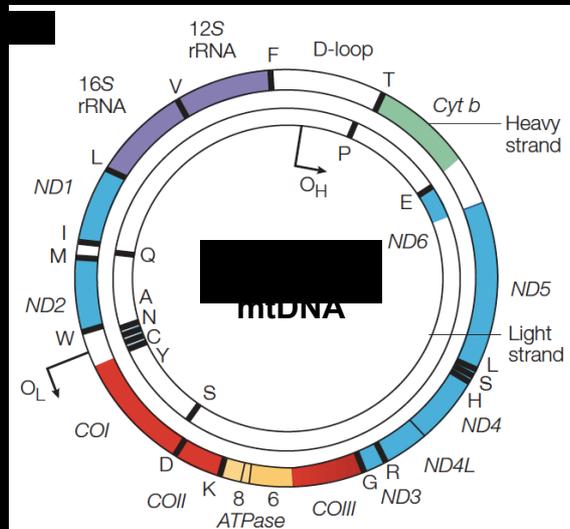
**Healthy**

# mtDNA Heteroplasmic Mouse: NZB/BALB

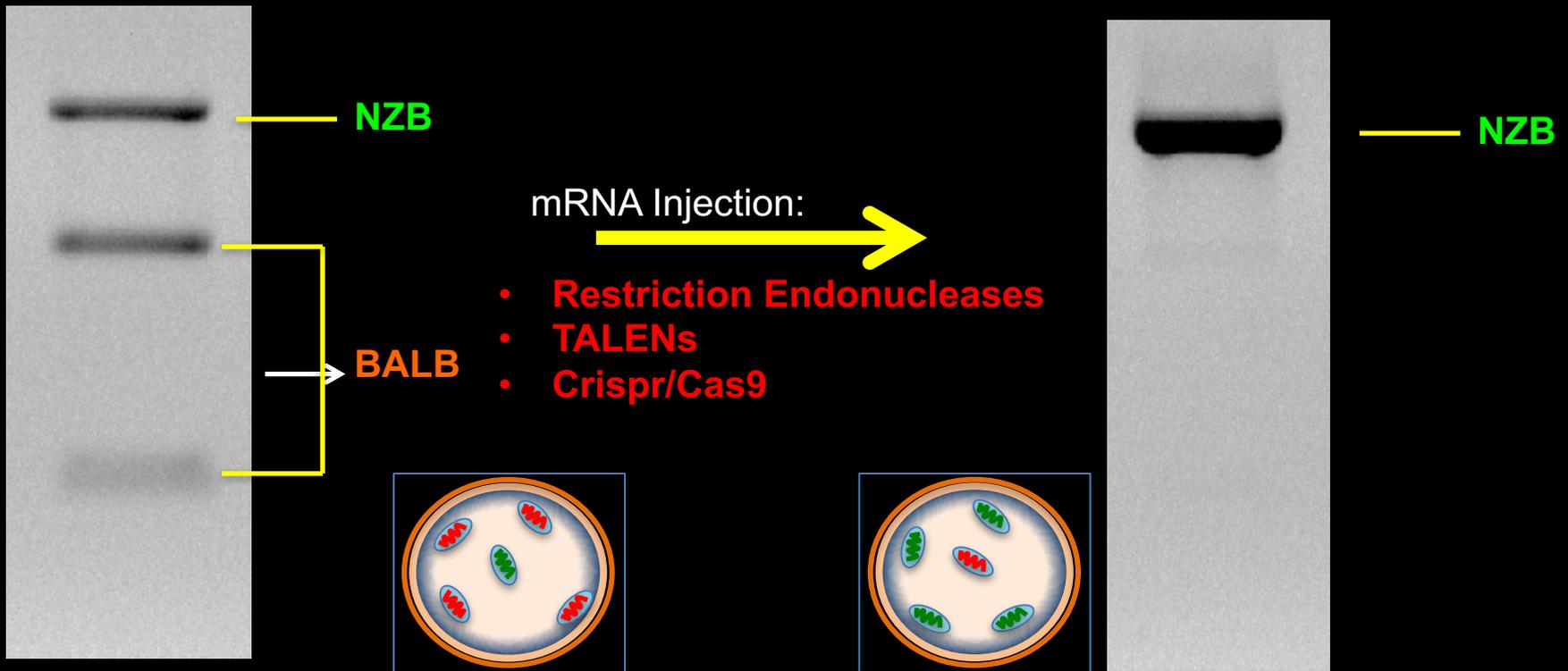


NZB/BALB mice

Different  
Nucleotide



# Heteroplasmic shift in NZB/BALB mice



Reddy et al. Cell

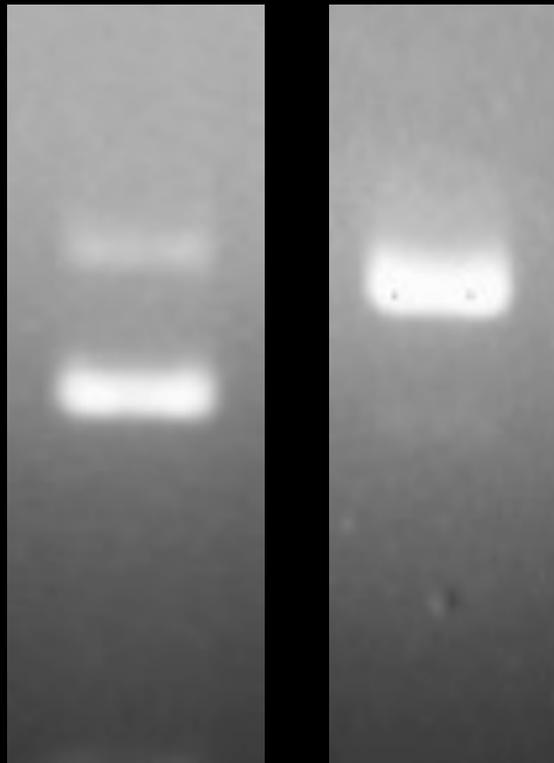
# Analysis Mitochondrial Disease Patient Oocytes

- Female carrier m.8993T>C mutation – NARP / MILS
- 34 years old
- Family history of mitochondrial disease

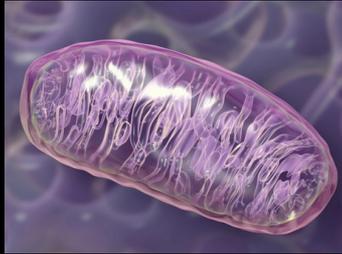
Oocytes from 8993T>C patient

Wild Type ----

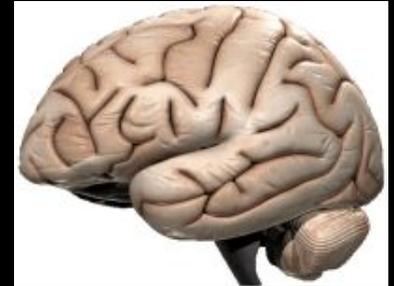
Mutant ----



# Clinical Disorders Caused by Mutations in mtDNA



1:200 Female Carrier



Diabetes mellitus and deafness

Myoclonic Epilepsy

Mitochondrial myopathy

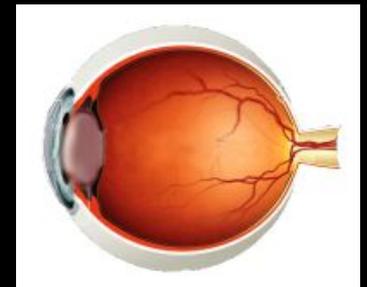
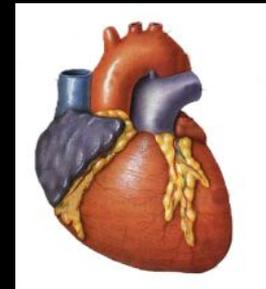
Leber's optic neuropathy

Leigh disease

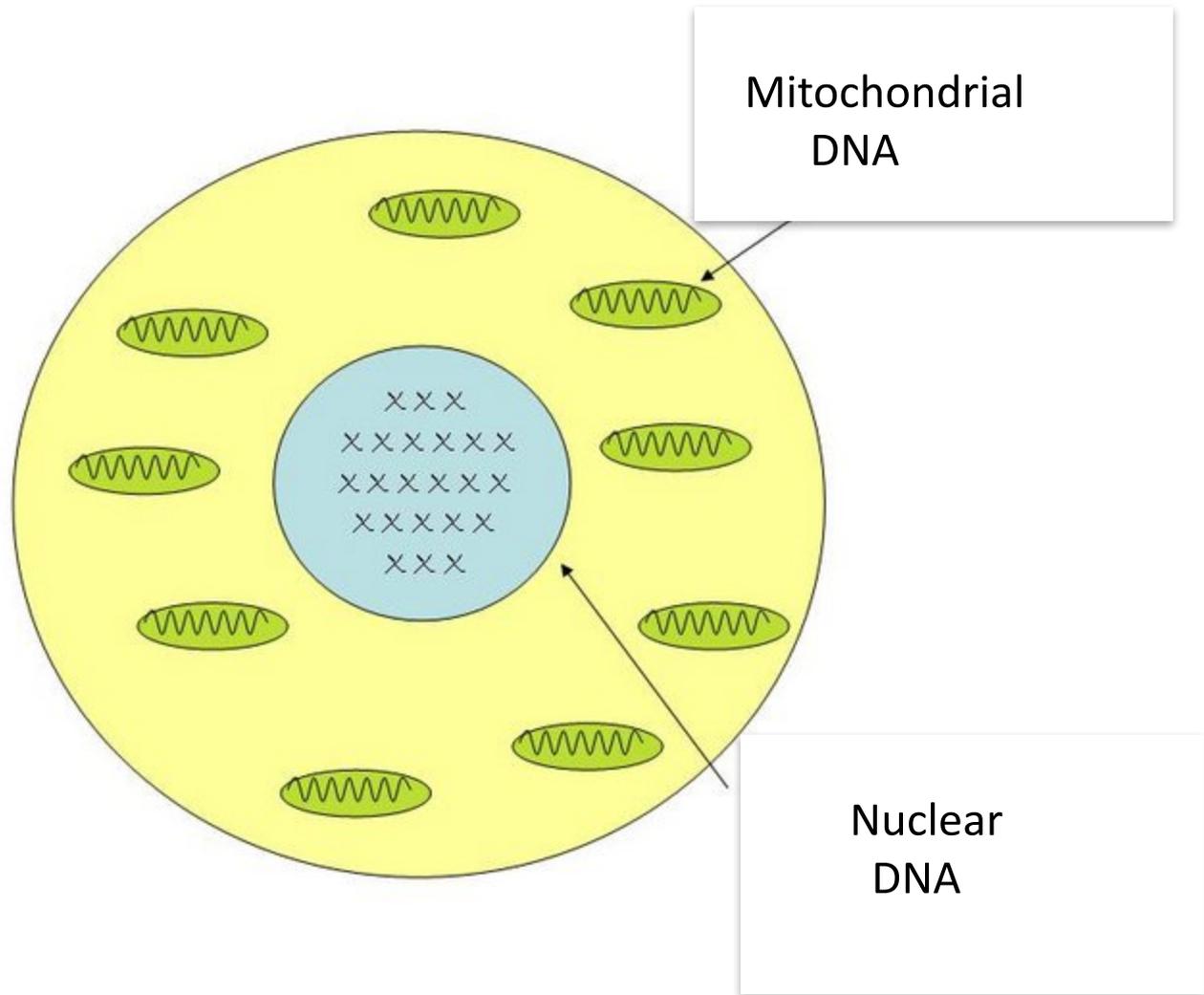
Mitochondrial encephalopathy

...

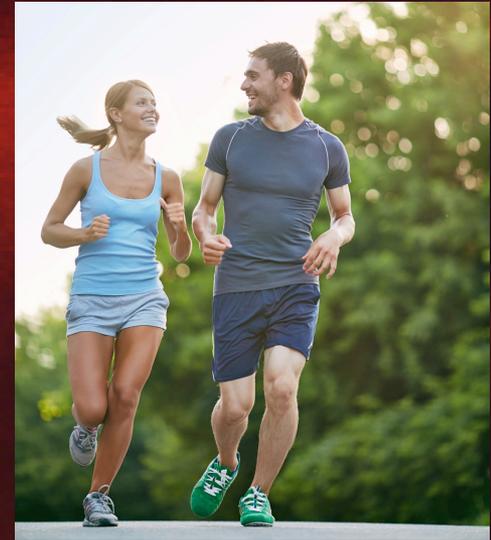
↓↓ ATP



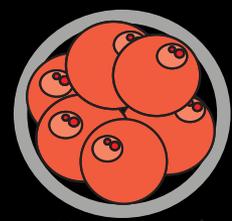
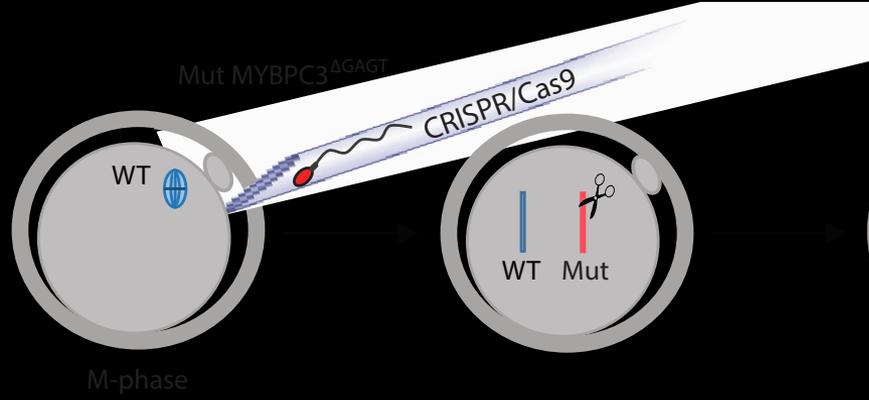
# DNA: Nuclear & Mitochondrial



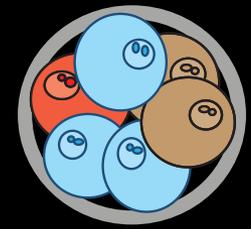
# MYBPC3 Mutation: Sudden Arrhythmic Death Syndrome



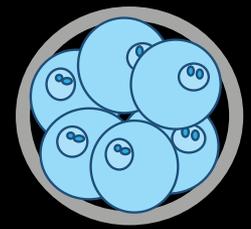
# MYBPC3 Correction



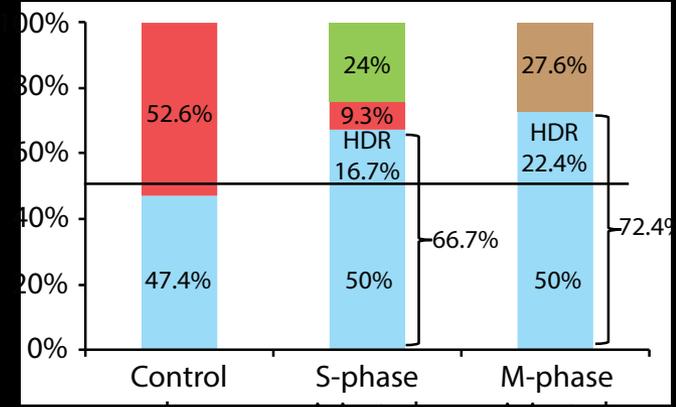
Untargeted mutant embryo



Mosaic embryo



HDR repaired embryo



- Low Efficiency
- Mosaicism
- Off-Target Mutations



Zygotes

8-cell embryos

Blastocysts

# ➤ 7000 Monogenic Diseases

Correction in Human Embryos by CRISPR-CAS9

Heterozygous mutations:



Hereditary Cancers

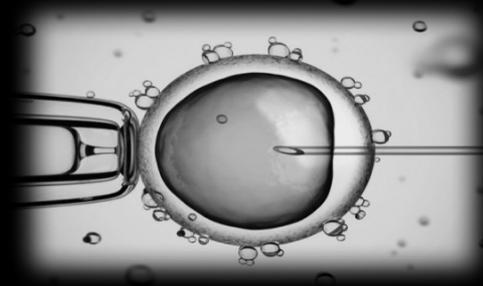
Marfan syndrome,

Huntington's disease,

Tuberous sclerosis

etc.

# Gene Editing in dividing and non dividing cells



**Oocyte  
Embryo**



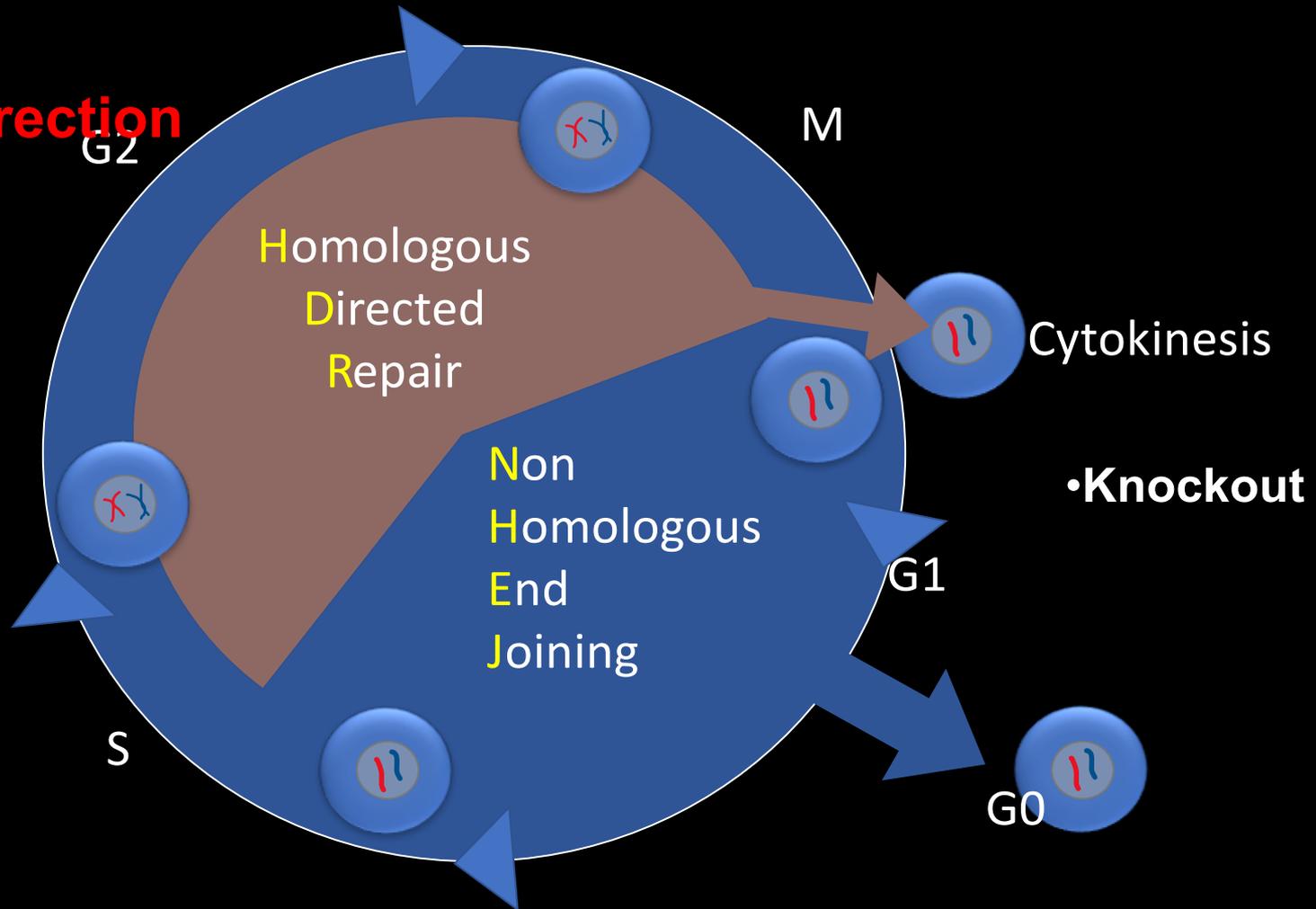
**Adult**

# DSB repair: HR vs. NHEJ

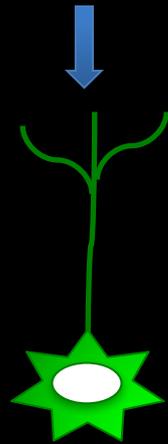
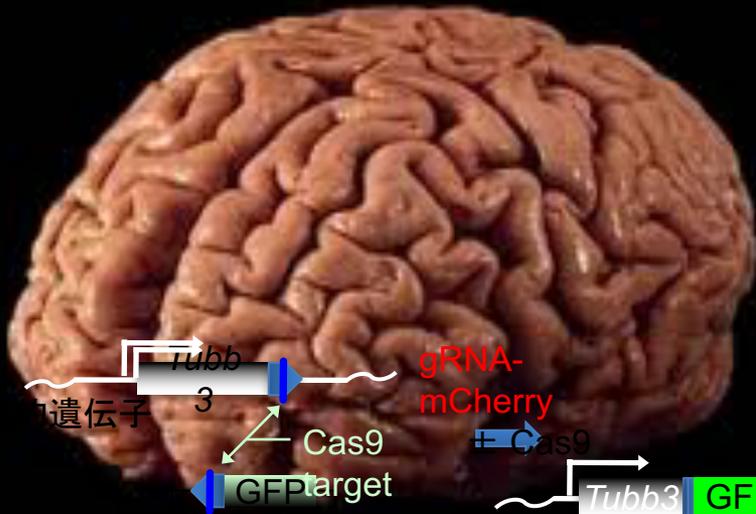
## Re-Writing the Genome

Site-specific nuclease (ZFN, TALEN, CRISPR/Cas9)

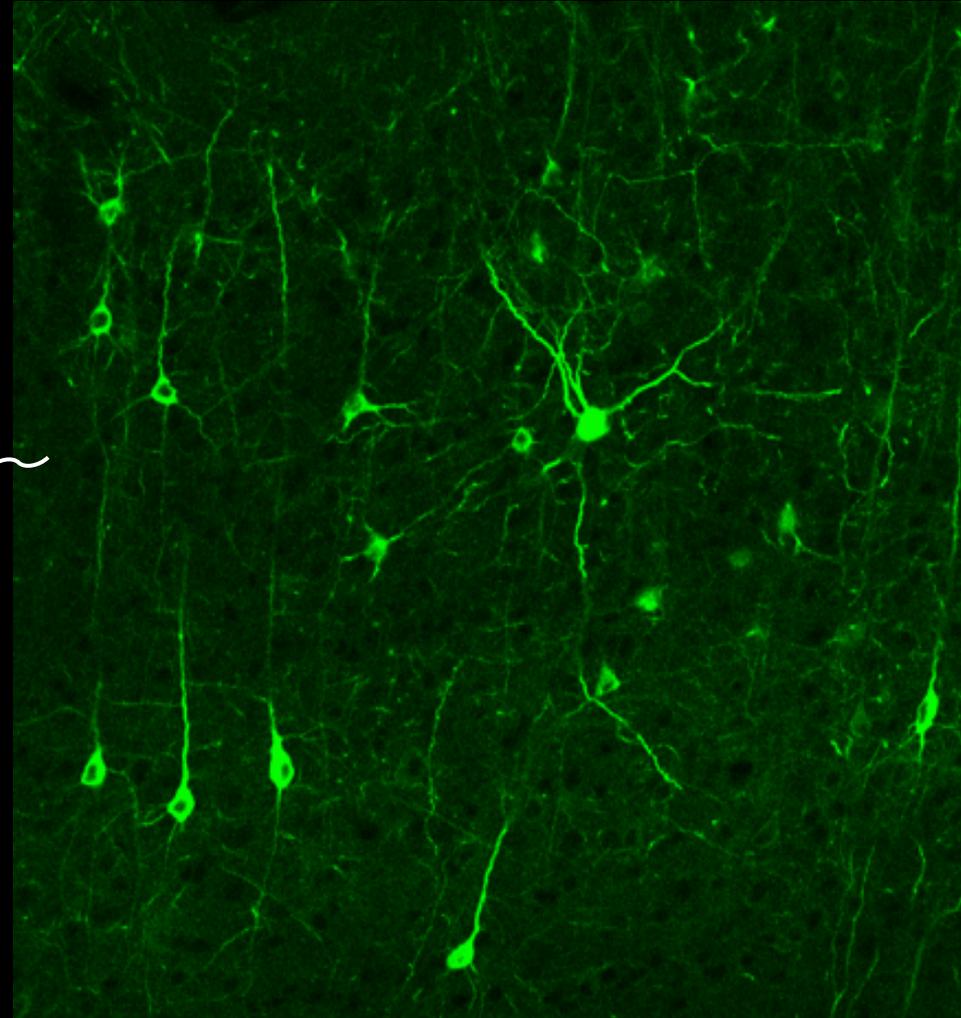
- Knockout
- Knock-in
- Gene correction



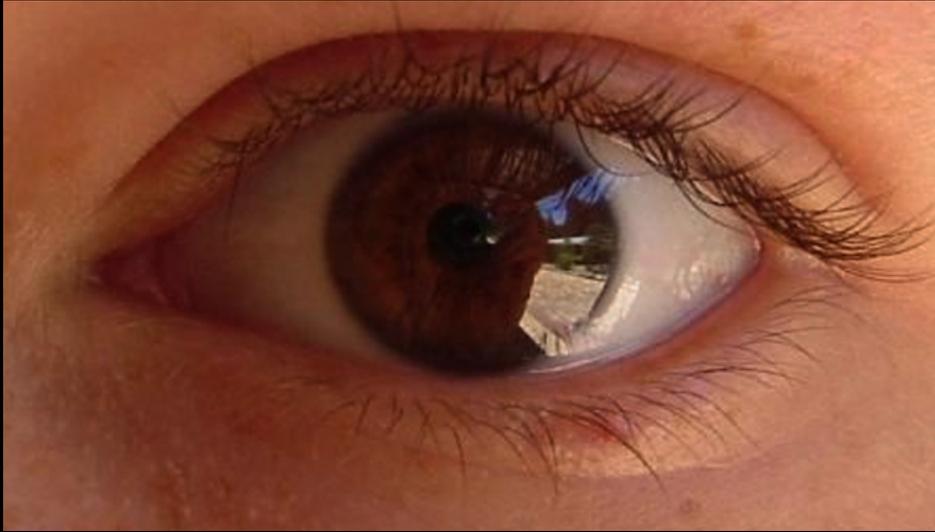
# HITI (NHEJ): Brain (*in vivo*)



Tubb3(Tuj1)-GFP



# Retinitis pigmentosa



Retinal degeneration  
model rat

## LETTER

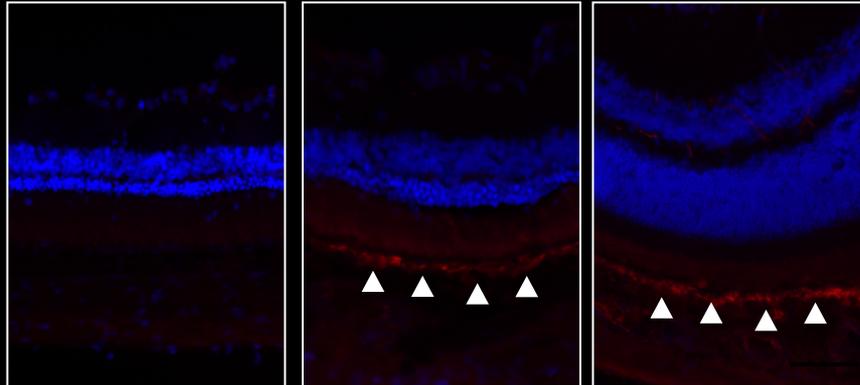
doi:10.1038/nature20565

### *In vivo* genome editing via CRISPR/Cas9 mediated homology-independent targeted integration

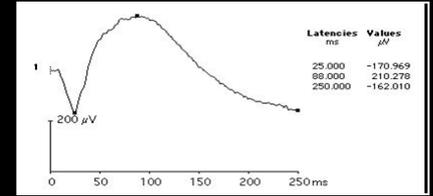
Keiichiro Suzuki<sup>1\*</sup>, Yuji Tsunekawa<sup>2\*</sup>, Reyna Hernandez-Benitez<sup>1,3\*</sup>, Jun Wu<sup>1,4\*</sup>, Jie Zhu<sup>5,6</sup>, Euseok J. Kim<sup>7</sup>, Fumiuyuki Hatanaka<sup>1</sup>, Mako Yamamoto<sup>1</sup>, Toshikazu Araoka<sup>1,4</sup>, Zhe Li<sup>8</sup>, Masakazu Kurita<sup>1</sup>, Tomoaki Hishida<sup>1</sup>, Mo Li<sup>1</sup>, Emi Aizawa<sup>1</sup>, Shicheng Guo<sup>8</sup>, Song Chen<sup>8</sup>, April Goebel<sup>1</sup>, Rupa Devi Soligalla<sup>1</sup>, Jing Qu<sup>9,10</sup>, Tingshuai Jiang<sup>6,11</sup>, Xin Fu<sup>5,6</sup>, Maryam Jafari<sup>6</sup>, Concepcion Rodriguez Esteban<sup>1</sup>, W. Travis Berggren<sup>12</sup>, Jeronimo Lajara<sup>4</sup>, Estrella Nuñez-Delgado<sup>4</sup>, Pedro Guillen<sup>4,13</sup>, Josep M. Campistol<sup>14</sup>, Fumio Matsuzaki<sup>2</sup>, Guang-Hui Liu<sup>10,15,16,17</sup>, Pierre Magistretti<sup>3</sup>, Kun Zhang<sup>8</sup>, Edward M. Callaway<sup>7</sup>, Kang Zhang<sup>5,6,18,19</sup> & Juan Carlos Izpisua Belmonte<sup>1</sup>



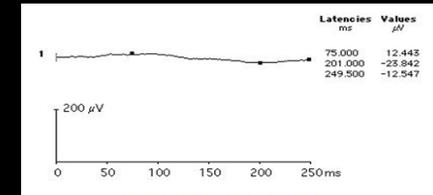
Mutant      Treated      Wild Type



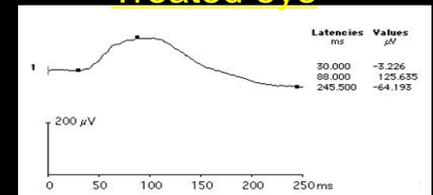
### Wild Type



### Mutant eye



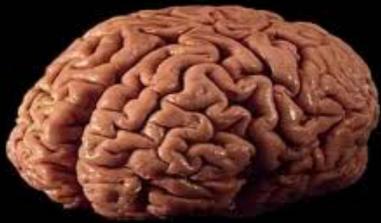
### Treated eye



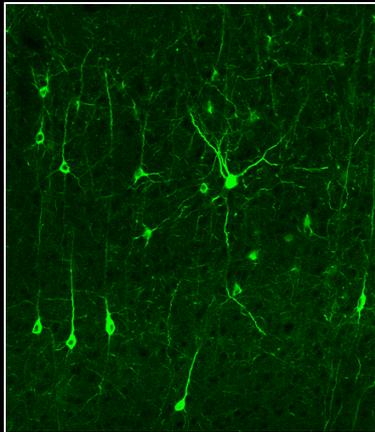
Suzuki et al. Nature 2016



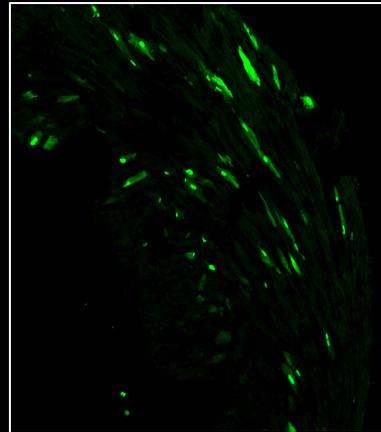
# Homology-independent targeted integration (HITI) *in vivo*



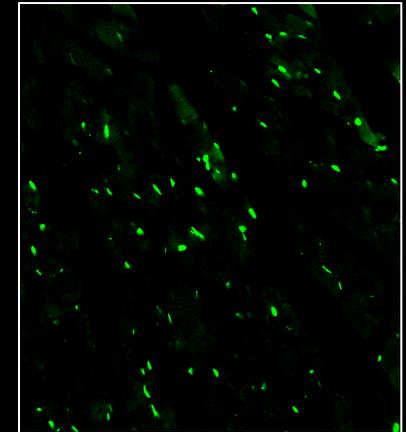
**Brain**



**Heart**



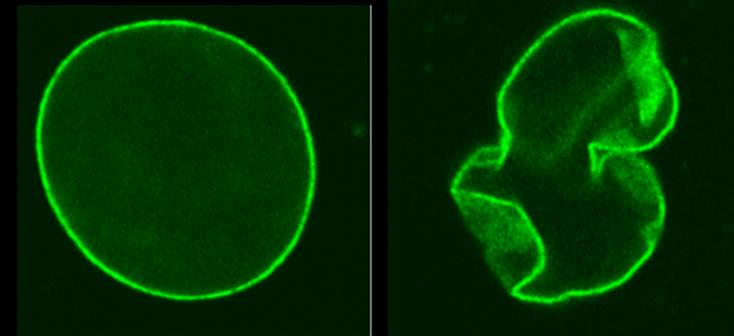
**Muscle**





# Progeroid Syndromes

## Hutchinson-Gilford progeria syndrome



Progeroid Mouse HG



Lopez Otin et al.

**G608G (GGC→GGT)**

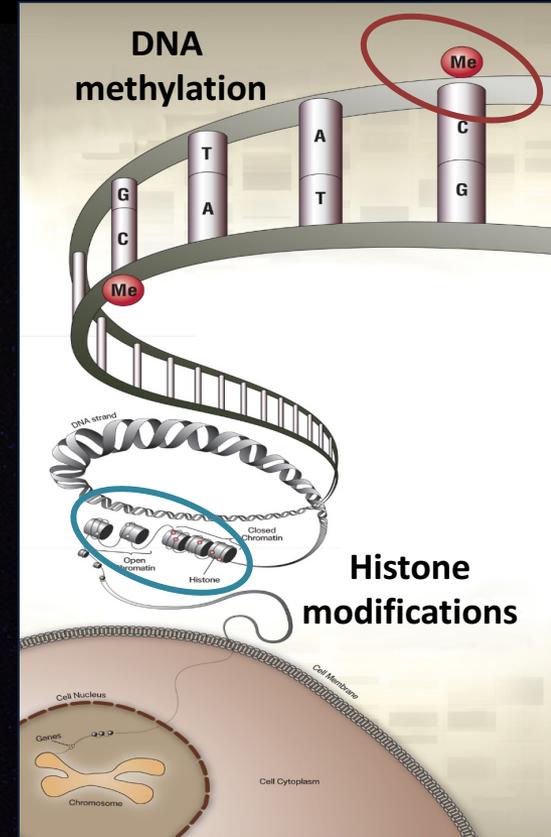
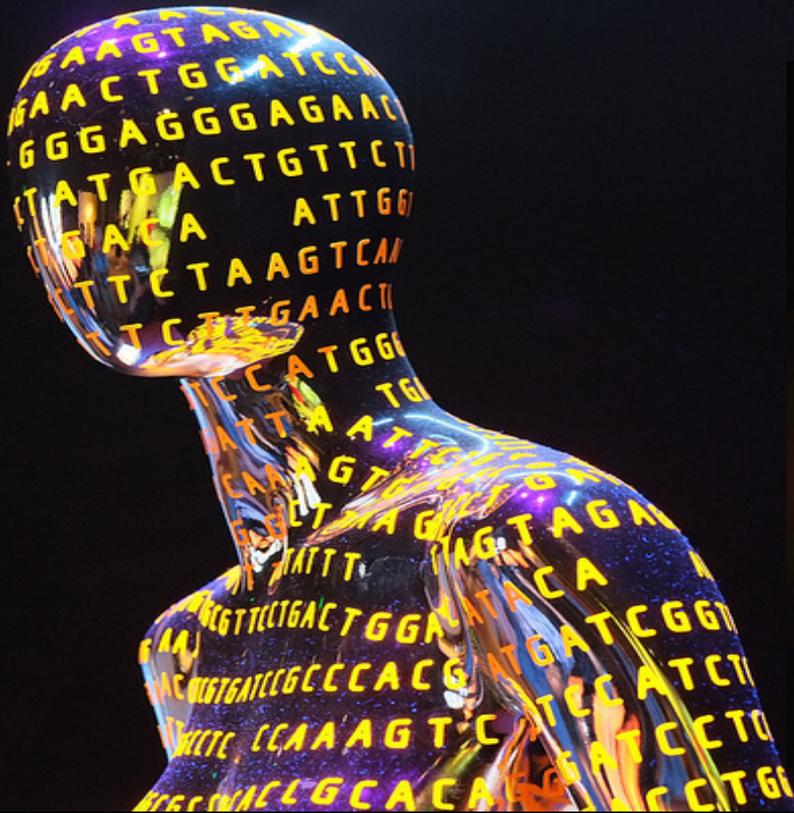
**MUTATION in LMNA GENE: PROGERIN**

# HITI Ameliorates Progeria In Vivo



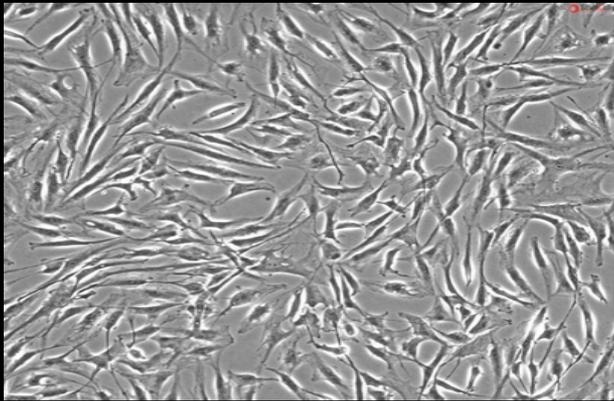
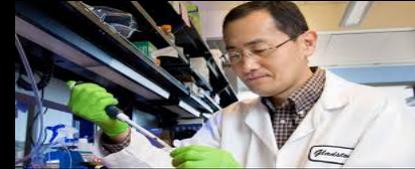
# Our Genome: Our Identity, Our Destiny ?

## The Epi-Genome



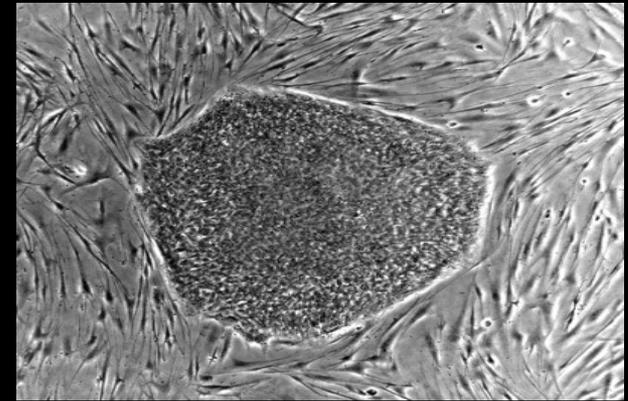
**Cellular reprogramming:**

**An Epigenetic Experiment**



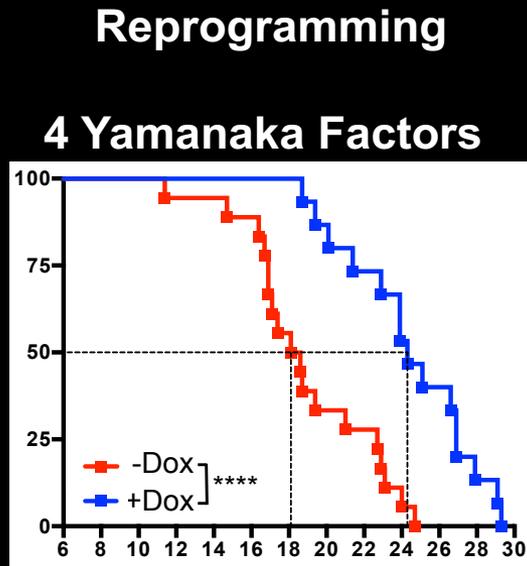
Adult Cell

OSKM



Embryo Like Cell

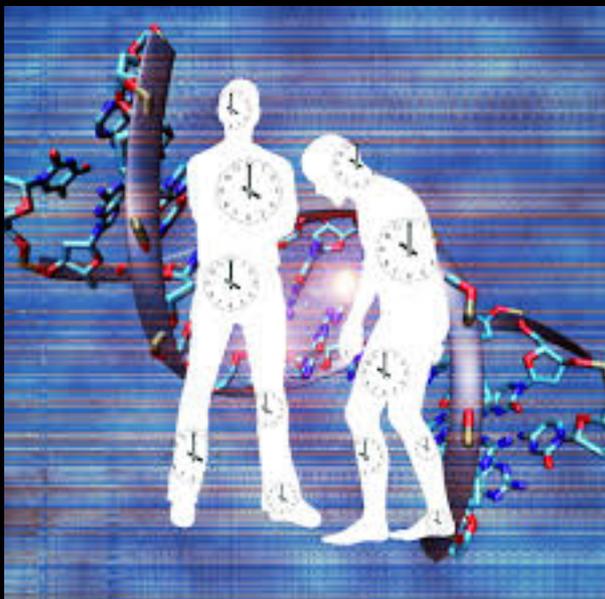
# Reprogramming of progeroid mice



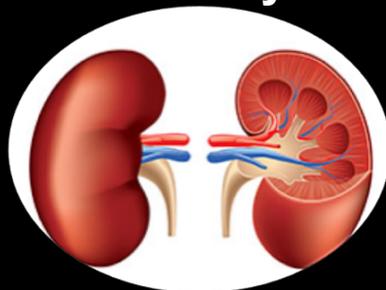
**30-40 % Increase  
in Life Span**



# Tissue Specific Rejuvenation by Reprogramming

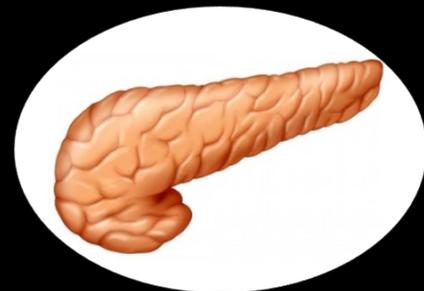


## Kidney



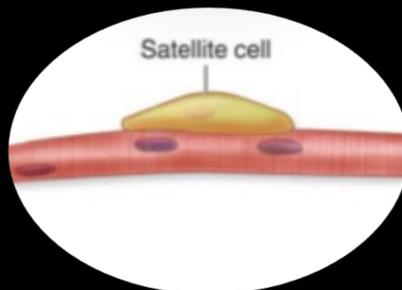
Six2-Cre  
Podocin-Cre  
Pax2-Cre

## Pancreas



Pdx1-Cre

## Muscle



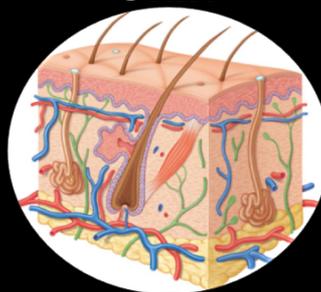
Pax7-Cre

## Heart



Myh6-Cre

## Skin

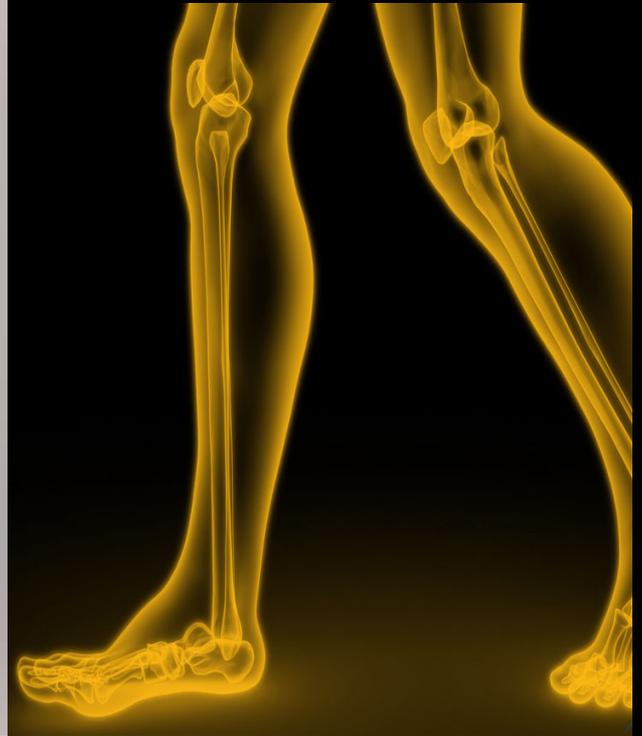


K14-Cre



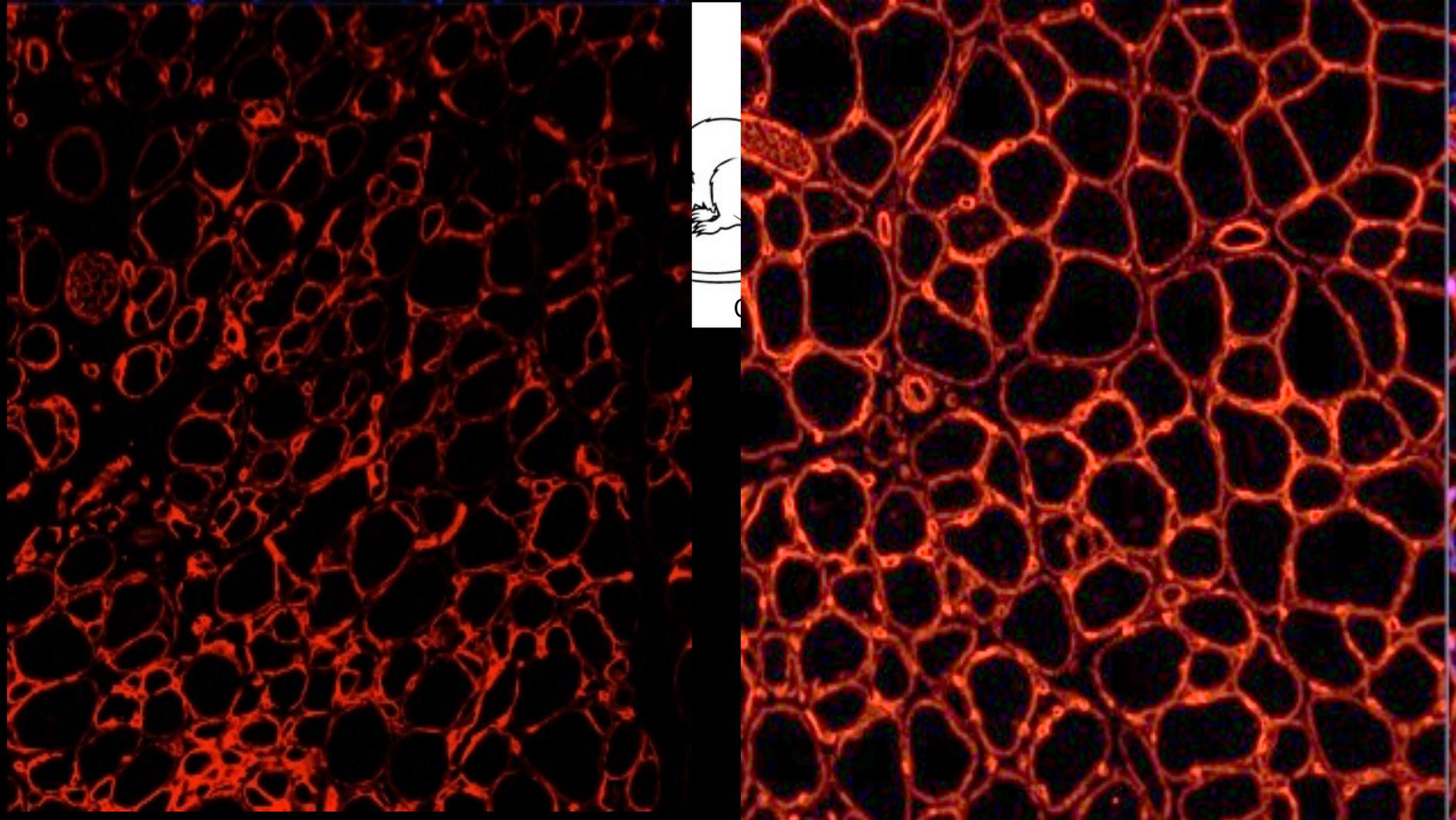
Camk2-Cre,  
Glast-Cre,  
Thy1-Cre,  
Th-Cre

# Rejuvenation of the Locomotor System



Dr. Pedro Guillen, Clinica Cemtro.

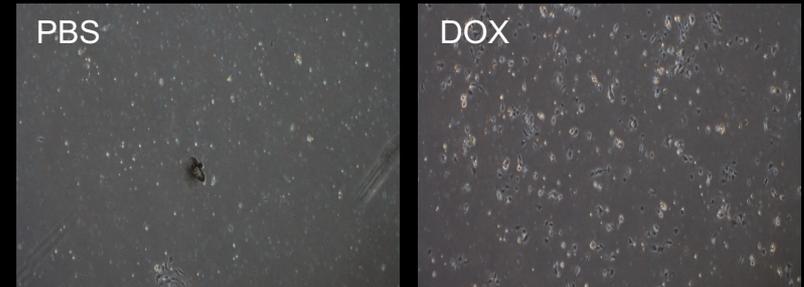
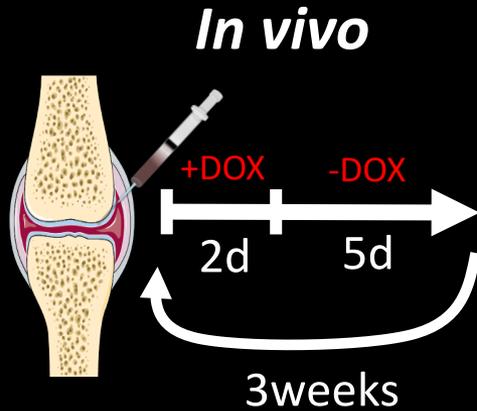
# Improved regeneration of muscle in WT animals by Yamanaka Factors



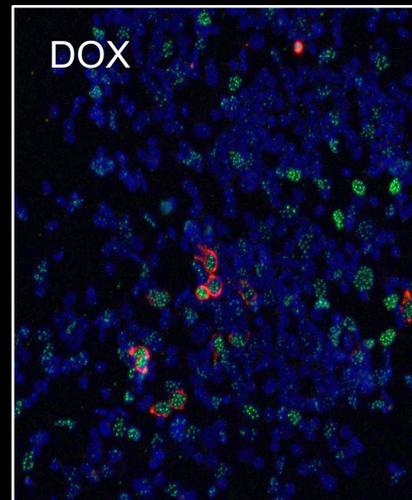
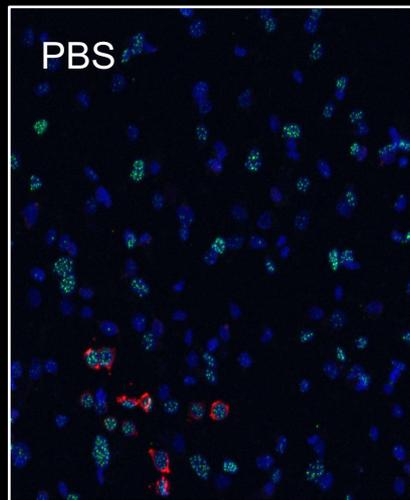
Not treated

Treated

# Partial Reprogramming of chondrocytes *in vivo*

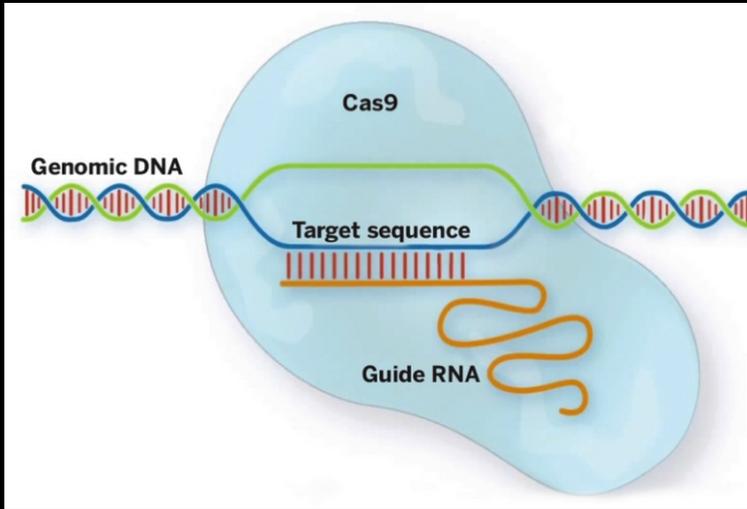


Cyclic 4F expression *in vivo* increased chondrocyte cell number

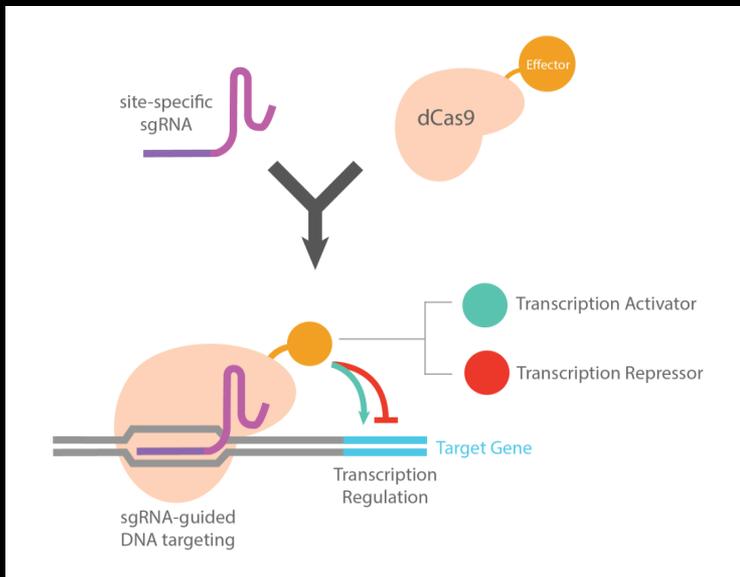


Sox9  
Col2a  
DAPI

4F expression *in vivo* increases chondrocyte markers maintenance

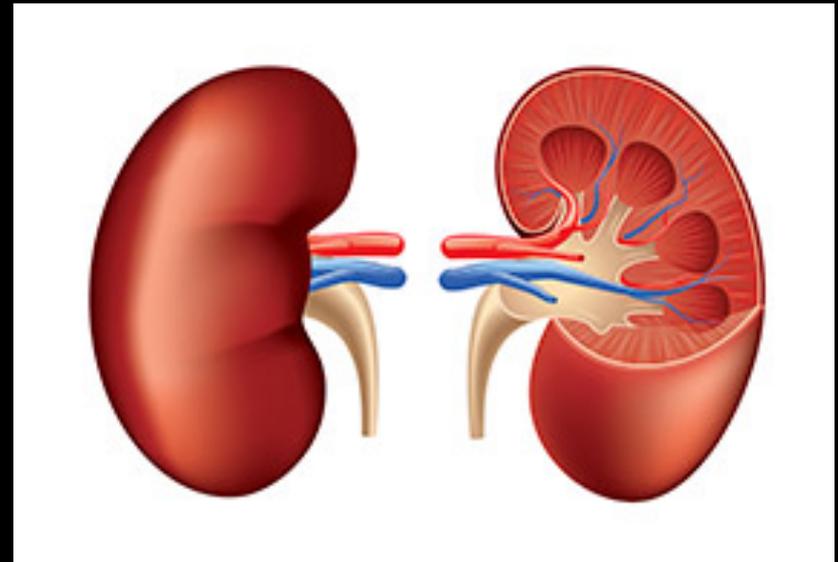
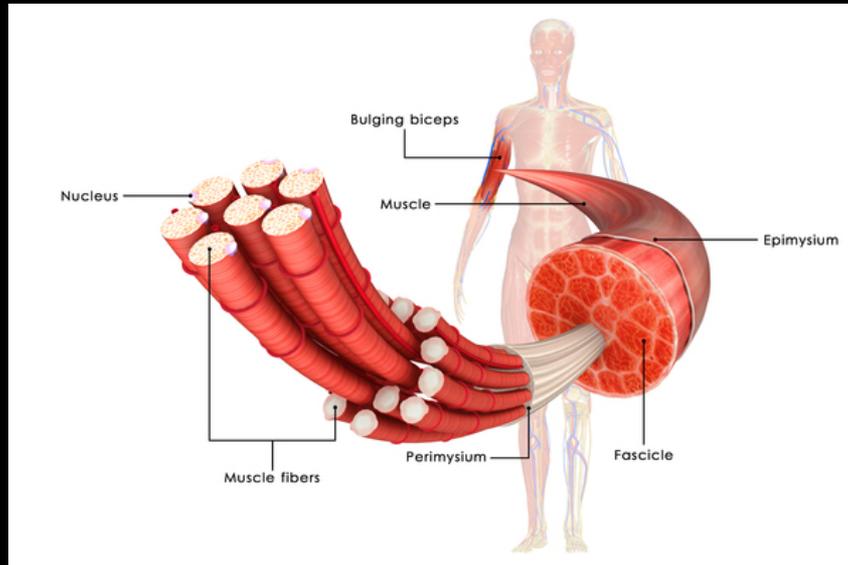


## CRISPR-Cas9 and Genome Editing

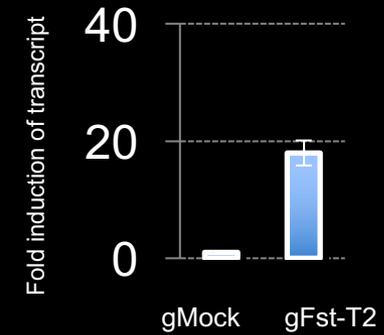
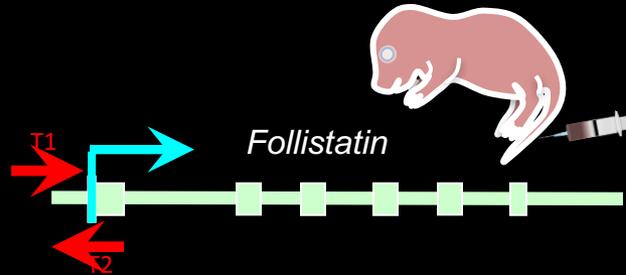
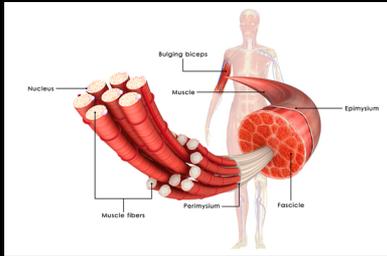


## Repurposing CRISPR-Cas9 for Epigenome Editing : **TGA**

# TGA in Vivo

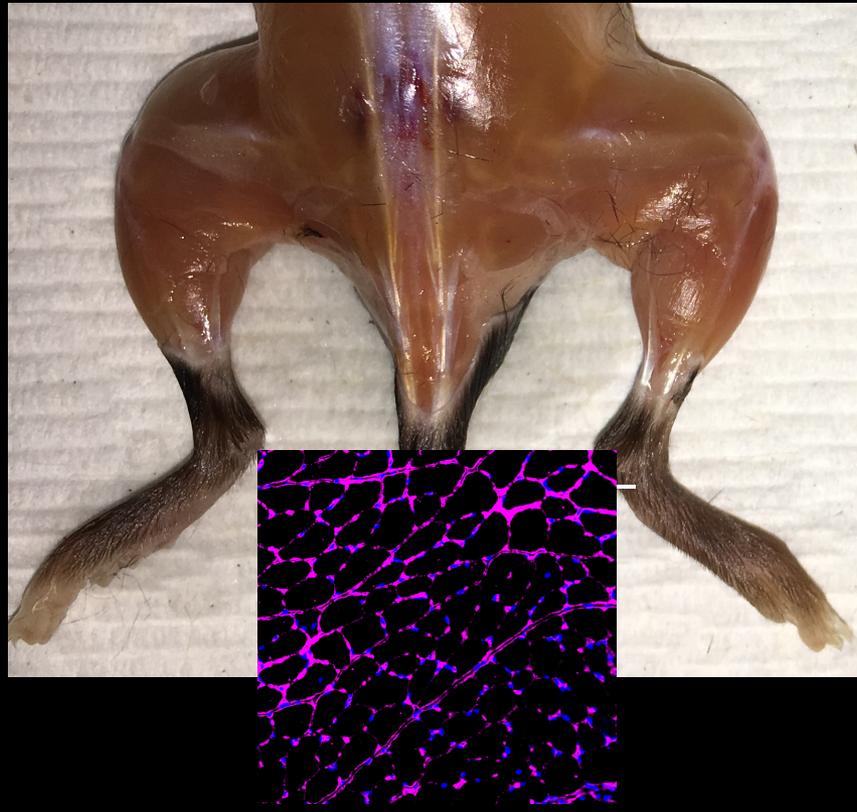


# TGA enables phenotypic transformation in a *mdx* mouse *in vivo*

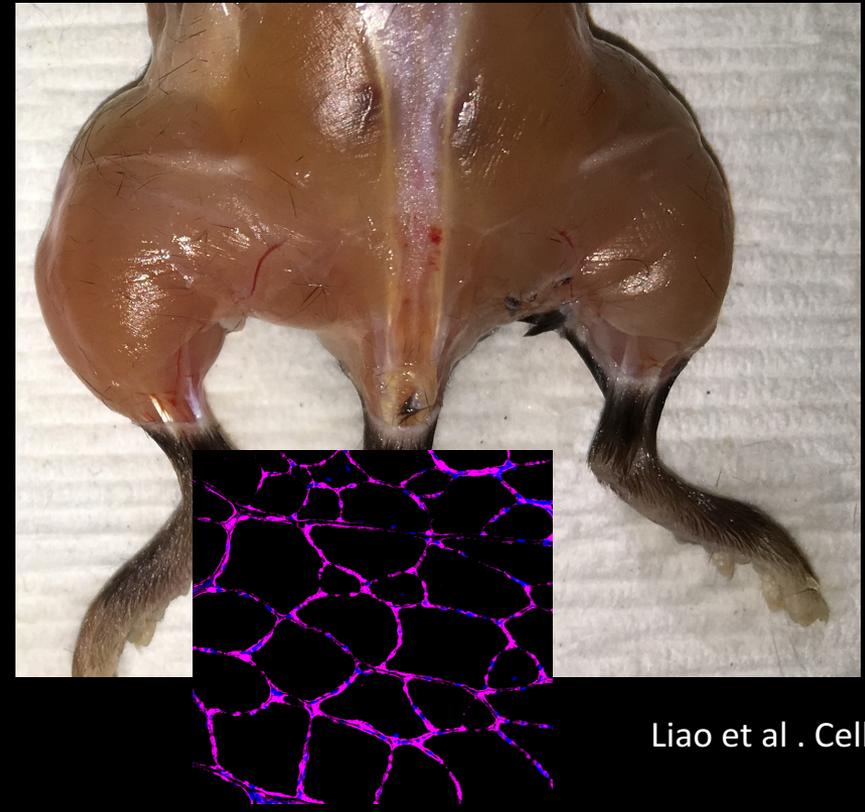


Muscular Dystrophies

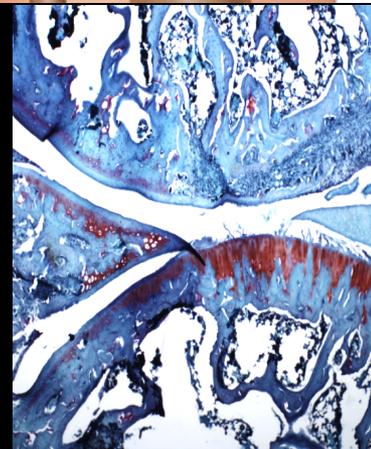
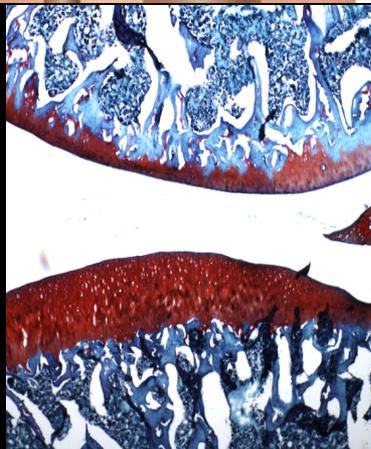
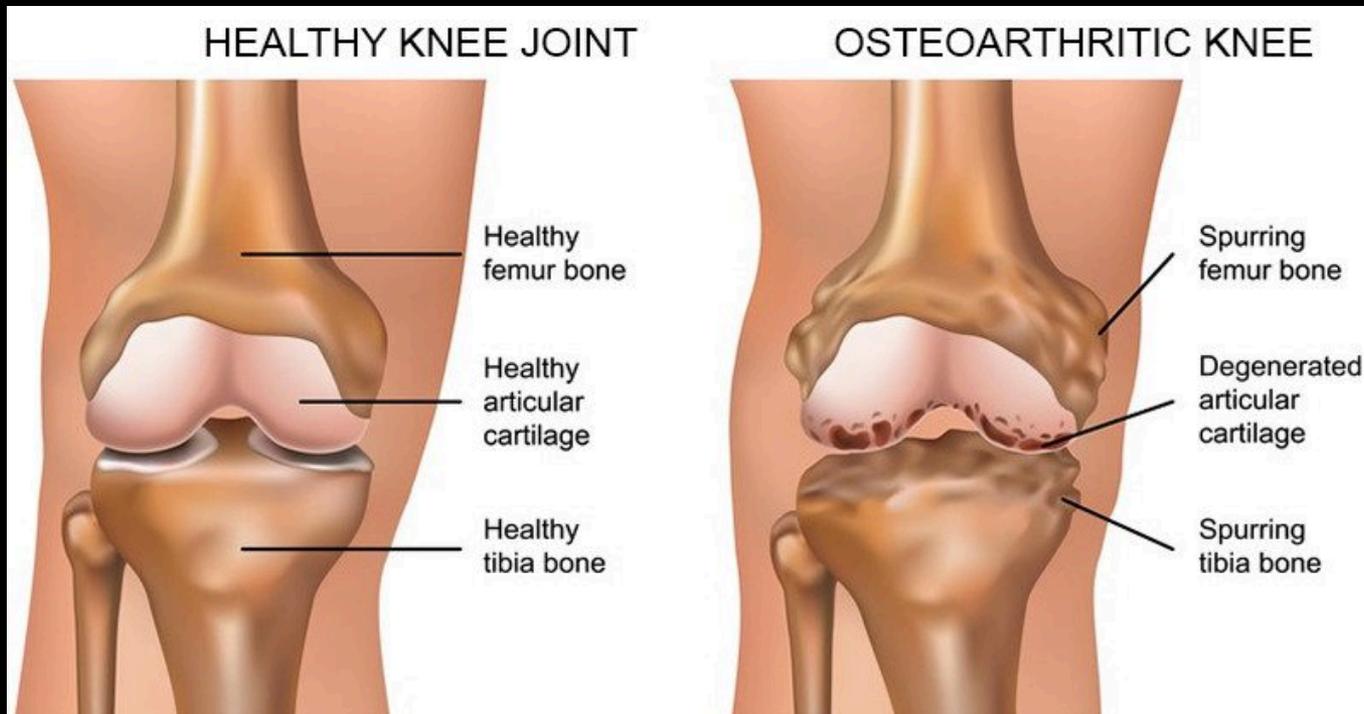
dgMock



dgFst



# Cartilage and Bone Aging disorder: Osteoarthritis

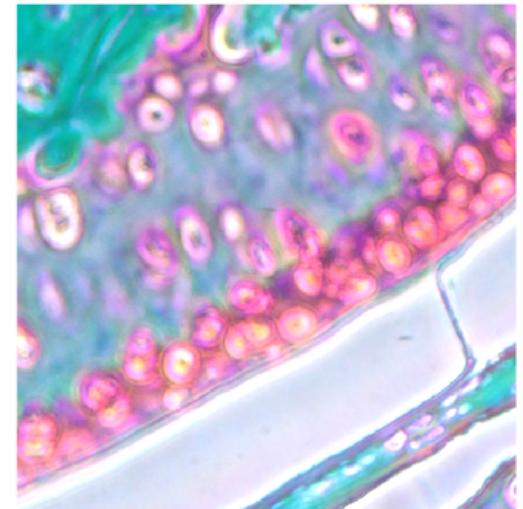
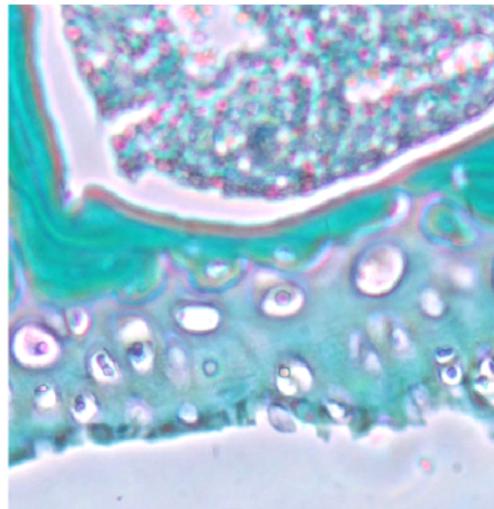
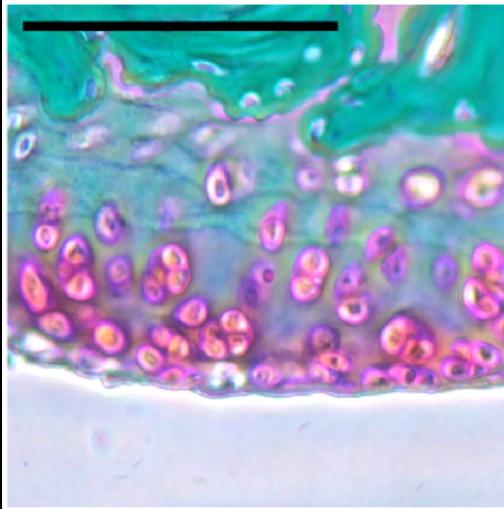
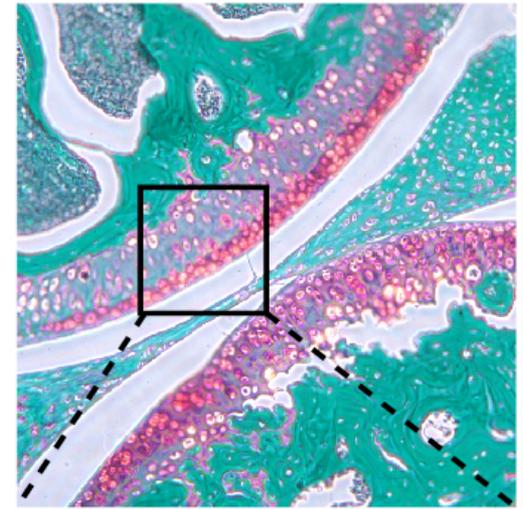
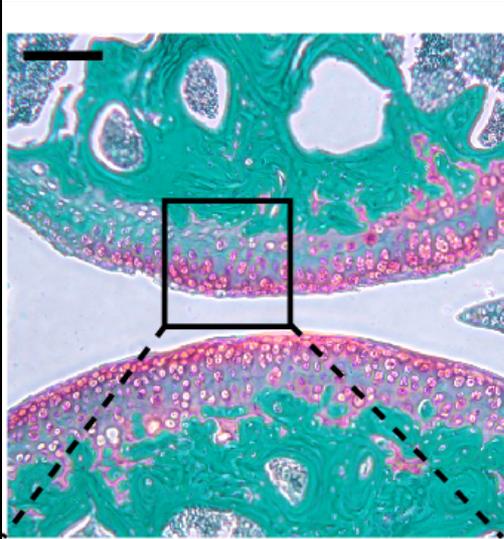


# DGCR8 alleviates osteoarthritis

Control

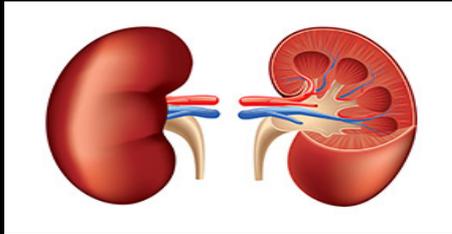
OA

OA + DGCR8

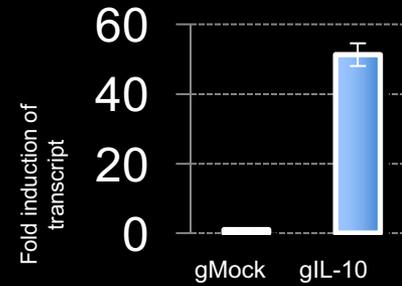


# Prophylactic Interventions of disease pathogenesis in a mouse model of acute kidney injury: IL-10 and Klotho

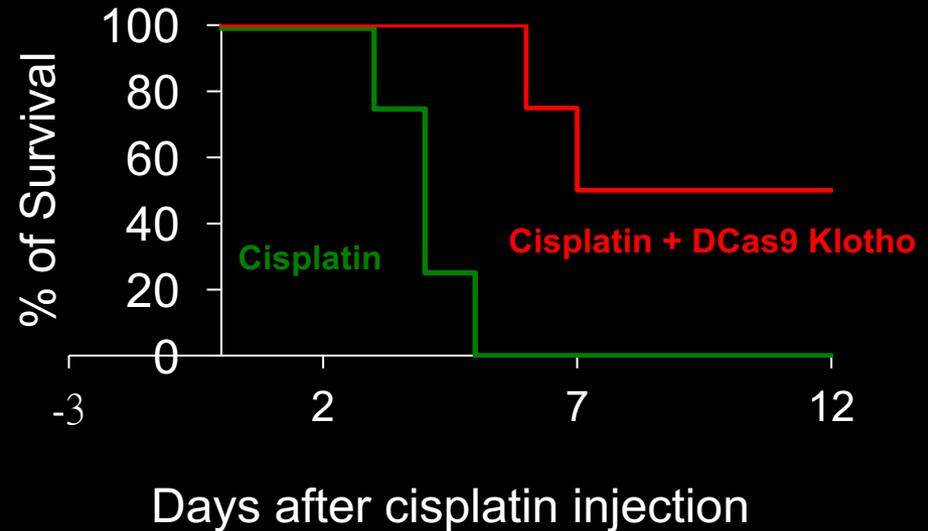
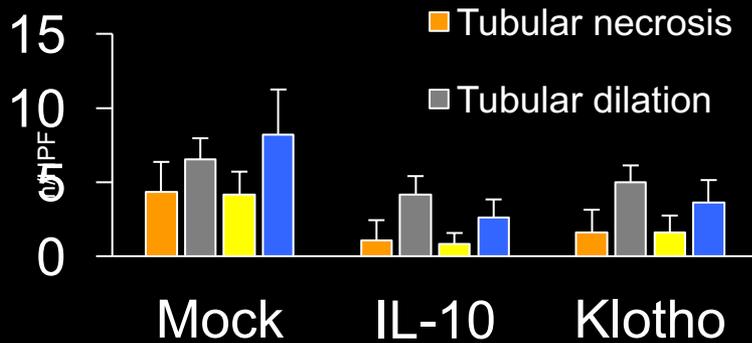
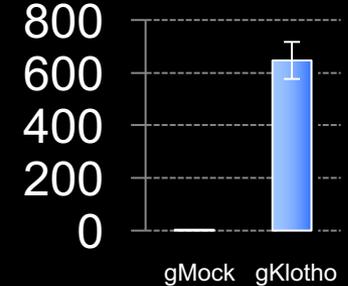
Cisplatin nephrotoxicity



***Il10***



***klotho***



# Epigenetic reprogramming of Aging by TGA approaches

Young



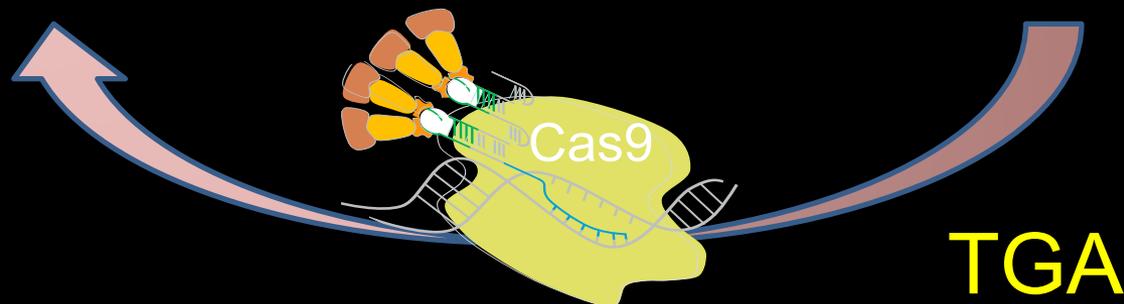
Old



**Yamanaka Factors**

**Transdifferentiation factors**

**Dedifferentiation factors**



# Advantages of TGA system

No DSB

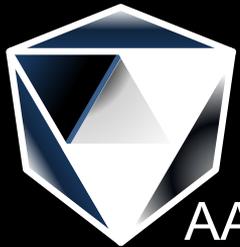


Not permanent



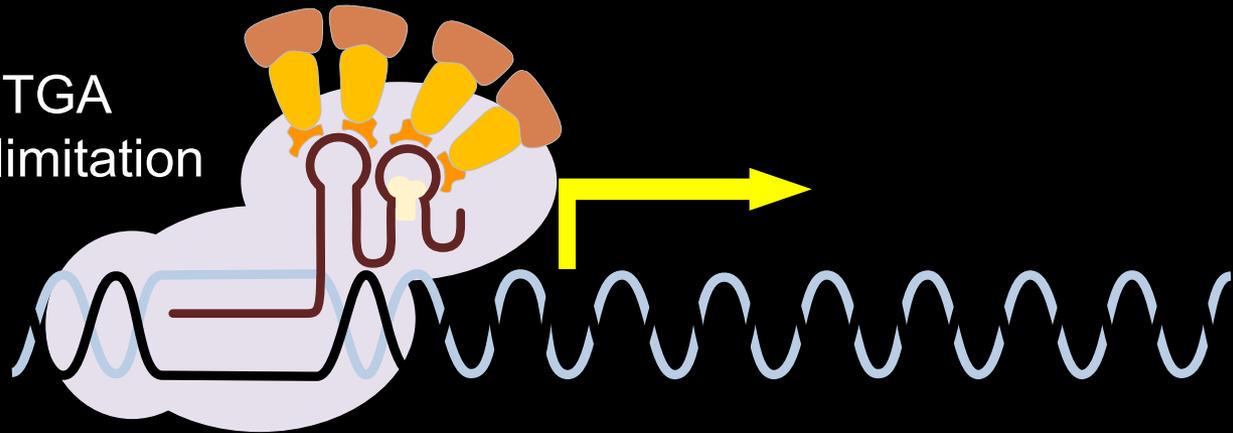
Size limitation

4.7 Kb

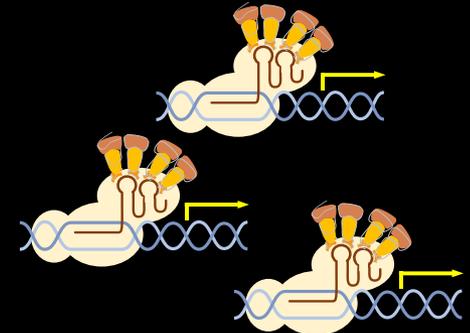
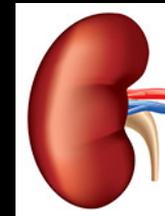
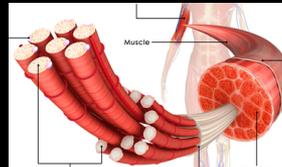
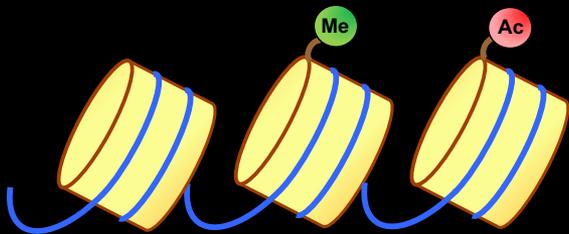


AAV

TGA  
No limitation



Closer to native epigenetic environment Target multiple genes and cell types

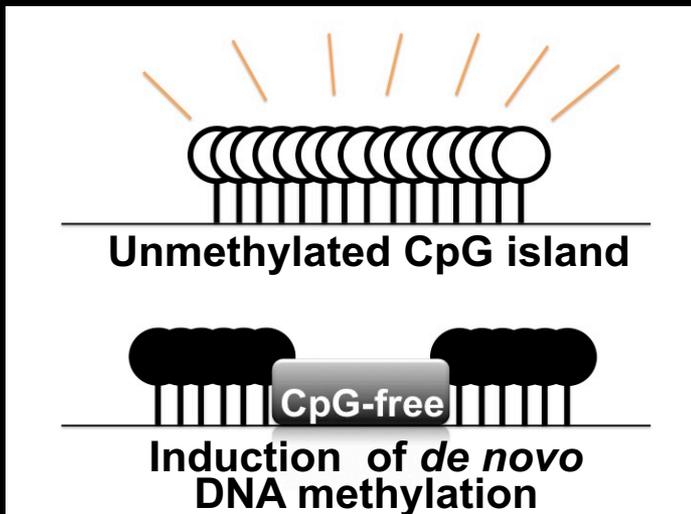
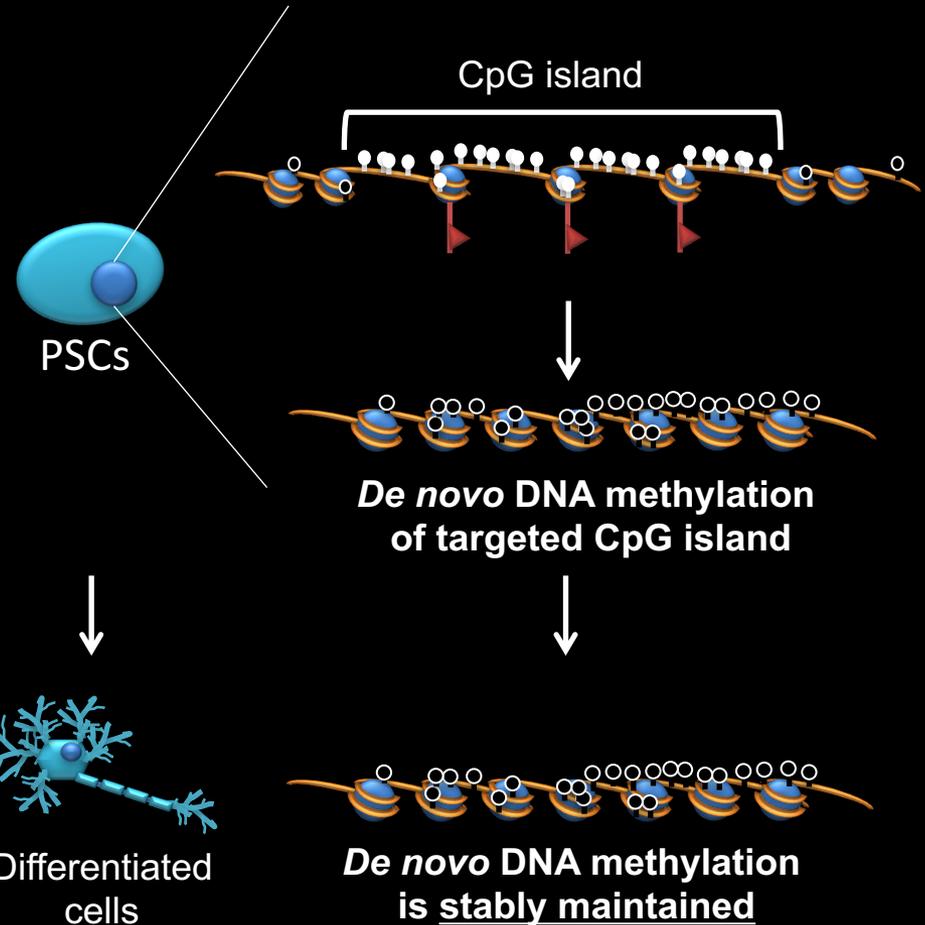


# Epigenome editing through induction of DNA methylation

## Integration of CpG-free DNA induces de novo methylation of CpG islands in pluripotent stem cells

Yuta Takahashi,<sup>1,2</sup> Jun Wu,<sup>1,3</sup> Keiichiro Suzuki,<sup>1</sup> Paloma Martinez-Redondo,<sup>1</sup> Mo Li,<sup>1,4</sup> Hsin-Kai Liao,<sup>1</sup> Min-Zu Wu,<sup>1,3</sup> Reyna Hernández-Benítez,<sup>1,4</sup> Tomoaki Hishida,<sup>1</sup> Maxim Nikolaievich Shokhirev,<sup>5</sup> Concepcion Rodriguez Esteban,<sup>1</sup> Ignacio Sancho-Martinez,<sup>1\*</sup> Juan Carlos Izpisua Belmonte<sup>1†</sup>

Takahashi *et al.*, *Science*, 2017



**Establishment of cancer-related epimutation**  
**Correction of aberrant imprinting syndromes**

## Stable, Induced and Transmissible Methylation



**Targeted Changes in Methylation in Embryonic, Neonatal, Adult, Aged and Diseased cells:** Differentiation, Self-renewal, Senescence, Neurodegeneration...etc

**Targeted Changes in Methylation in Anti-aging Regimes:** Calorie Restriction, IGF, Rapamycin...etc

# Reversibility of Aging and Disease



Chimeras

Genetics

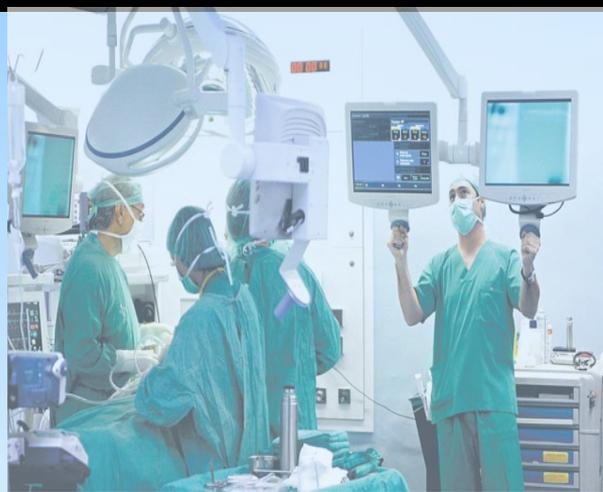
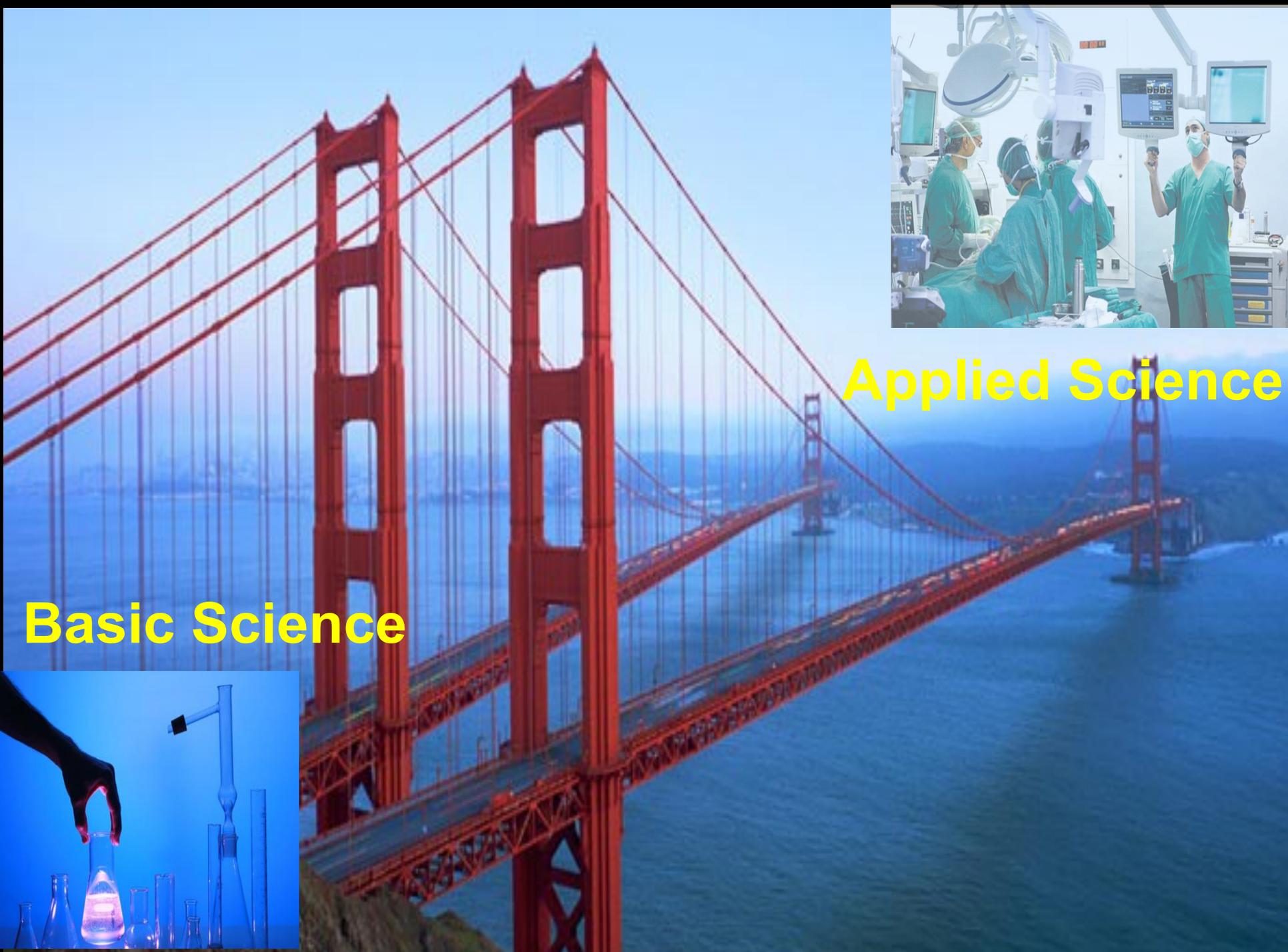


TGA

>Oct4  
>Sox2  
>Klf4  
>cMyc

HITI

Epi-Genetics



**Applied Science**

**Basic Science**





**Mutación al Azar**

**Selección Natural**

*“La especie que sobrevive no es la mas fuerte,  
ni tampoco la mas inteligente,  
sino aquella que mejor se adapta al cambio”*

*Charles Darwin*

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