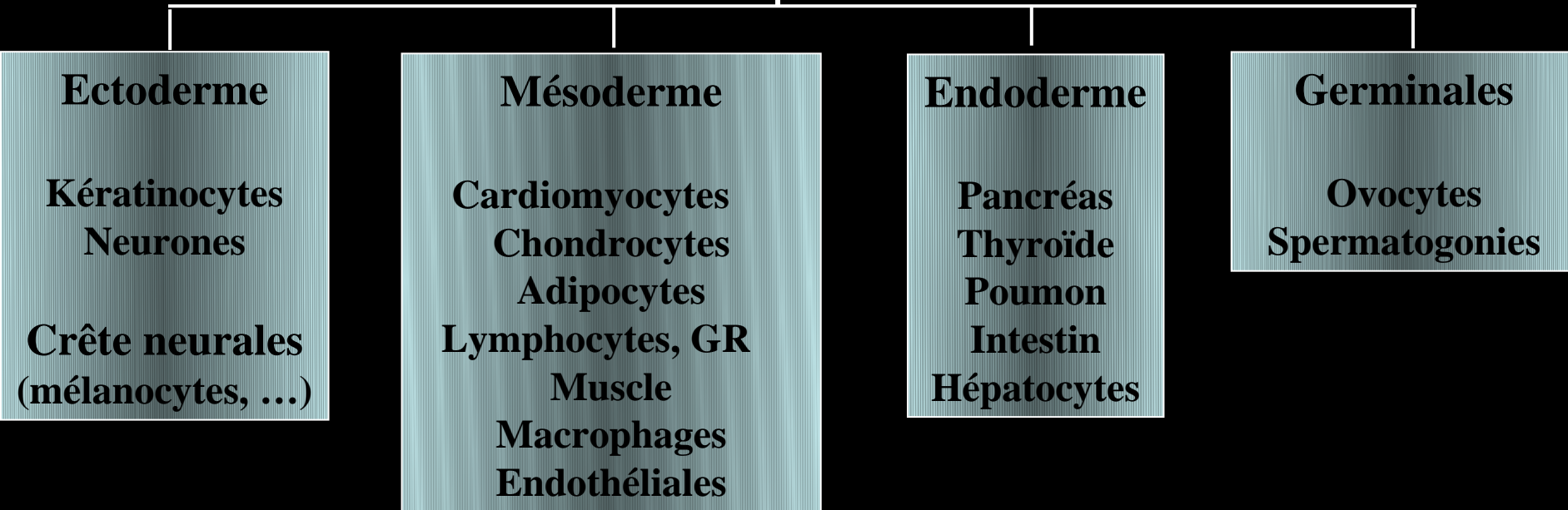


*auto-renouvellement*  
*activité télomérase élevée*

*pluripotence*



# Différentes possibilités d'induction de tolérance

**Transplantation  
dans des sites « immuno-  
privilégiés »**

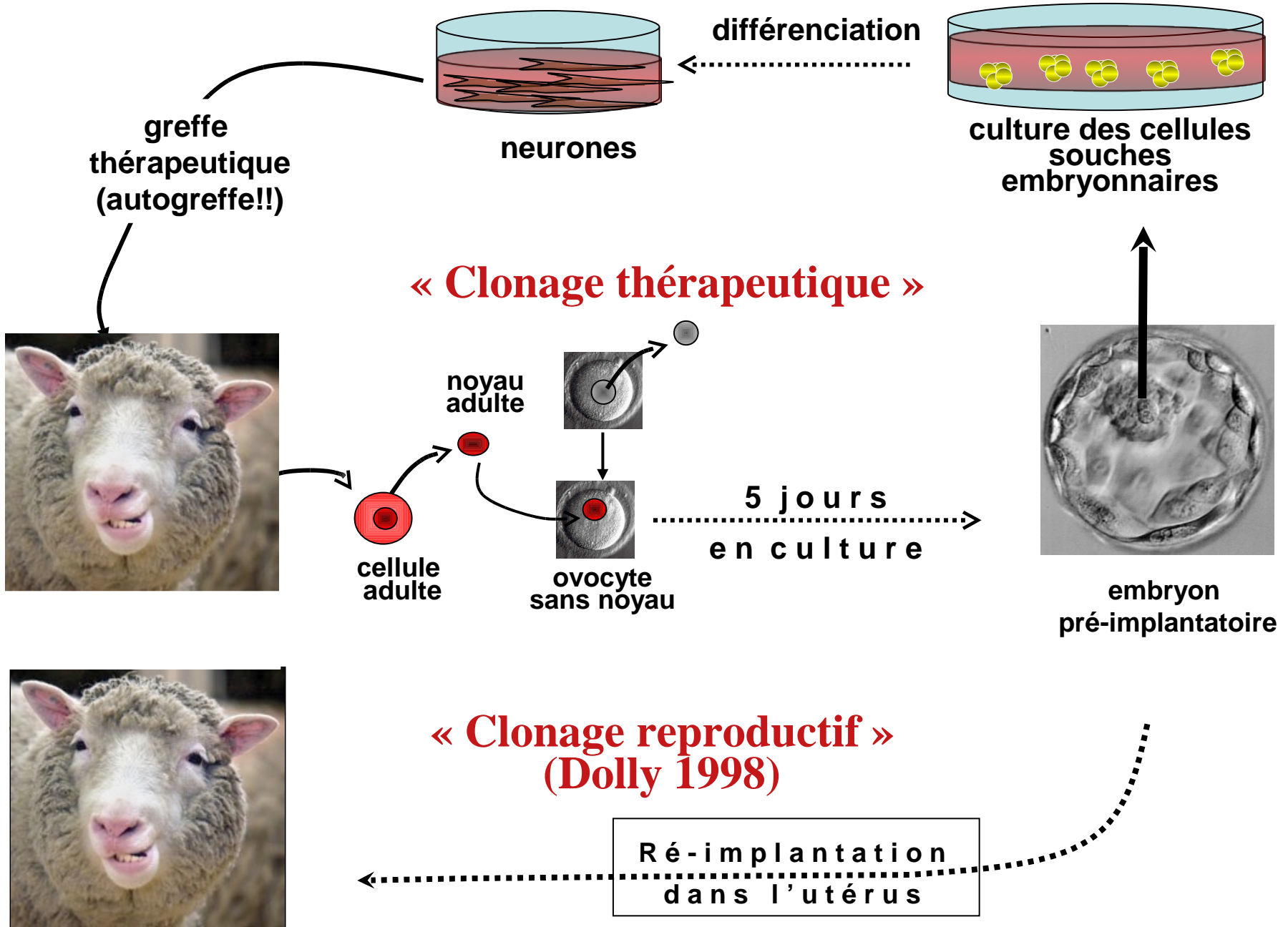
**Banque de lignées huES  
immunotypées  
(HLA)**

**Induction de tolérance par  
chimérisme hématopoïétique  
(MSC)**

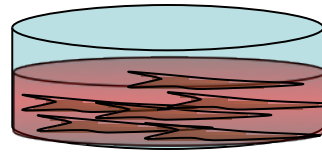


**transfert nucléaire  
(clonage thérapeutique)**

**Génération d'une  
lignée universelle  
(KO HLA classe 1/2)**

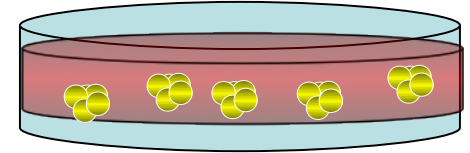


greffe  
thérapeutique  
(autogreffe!!)



neurones

différenciation



culture des cellules  
souches  
embryonnaires

## « Clonage thérapeutique »

- statut de l'embryon (âge, droit)
- dignité humaine (statut enfant issu du NT)
- marchandisation du corps de la femme
- espèces hybride (technique et problèmes)

Impossibilité sur l'homme mais: une barrière technique ne peut être une réponse à une question d'éthique.

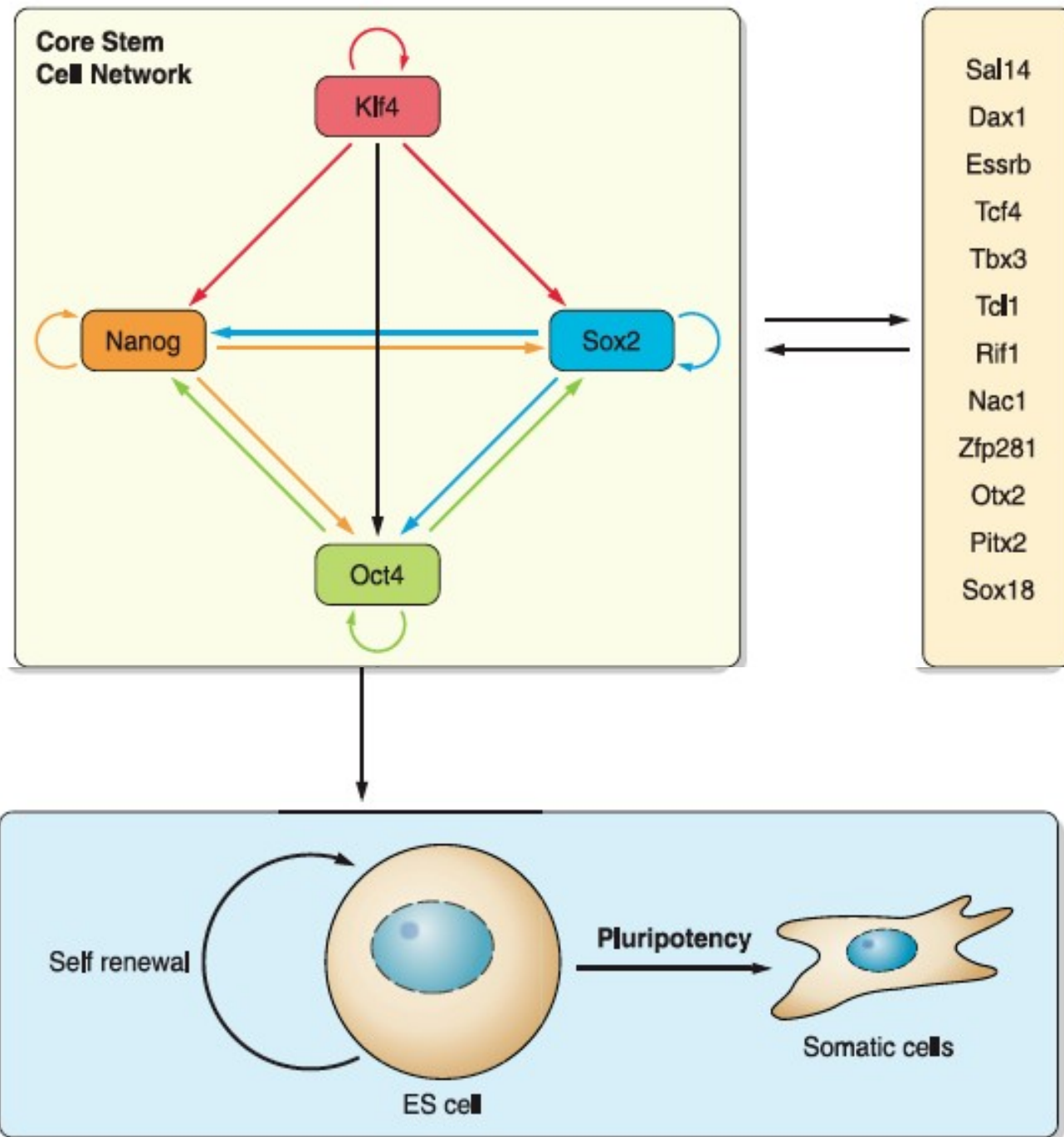


embryon  
pré-implantatoire

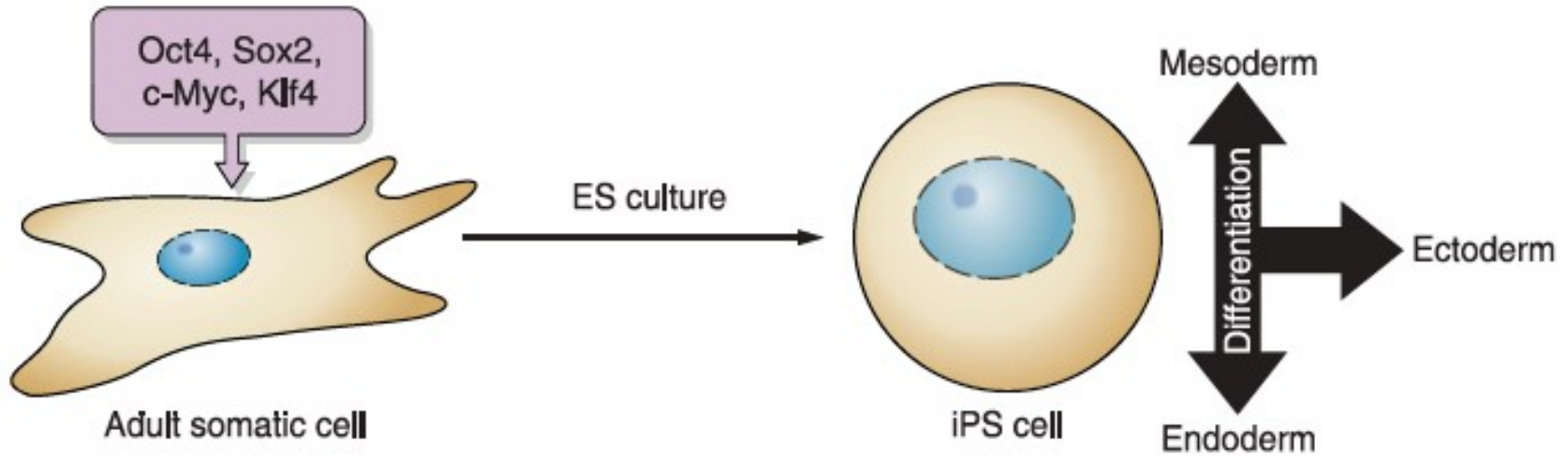
## « Clonage reproductif »

Ré-implantation  
dans l'utérus

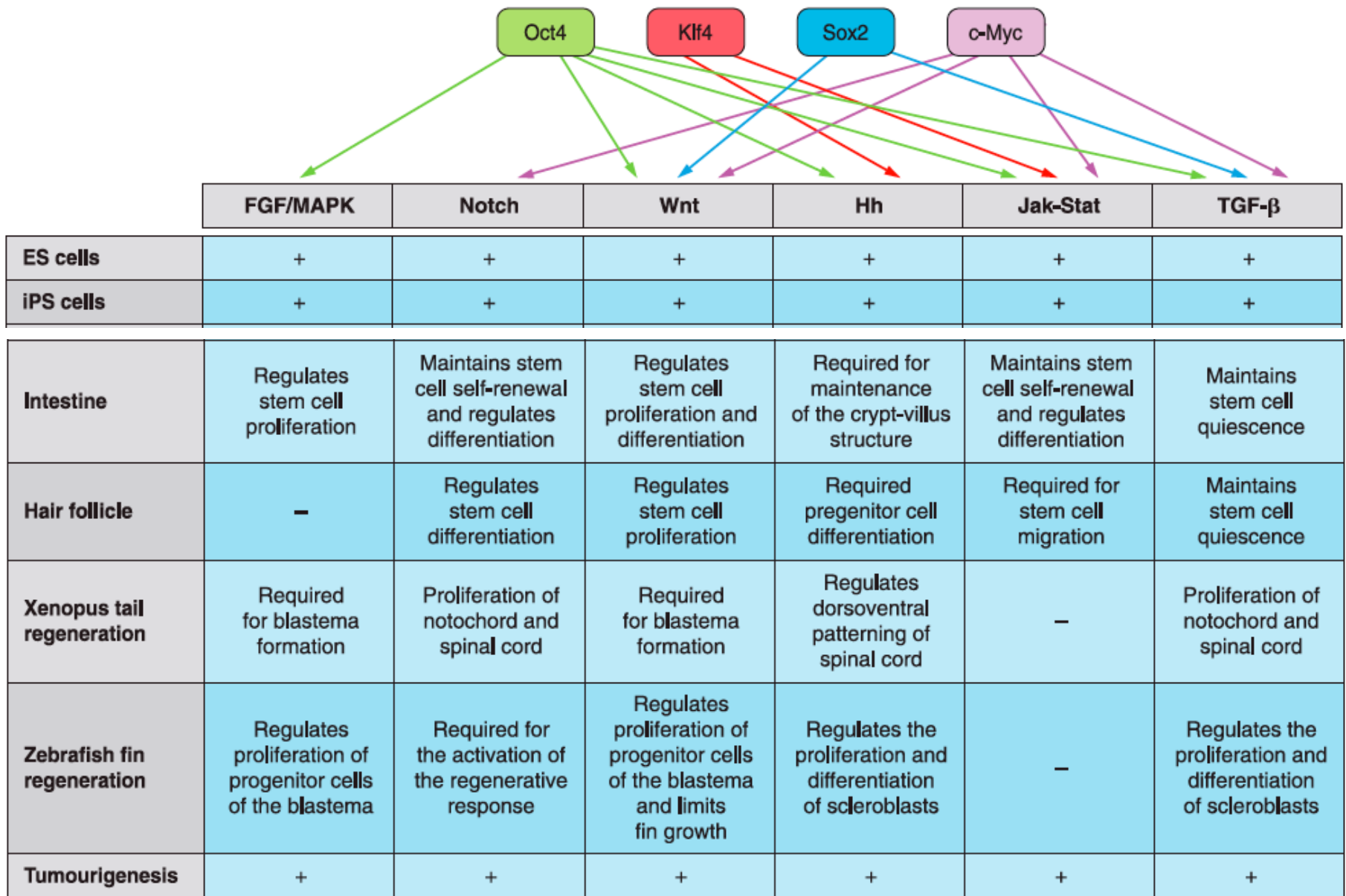




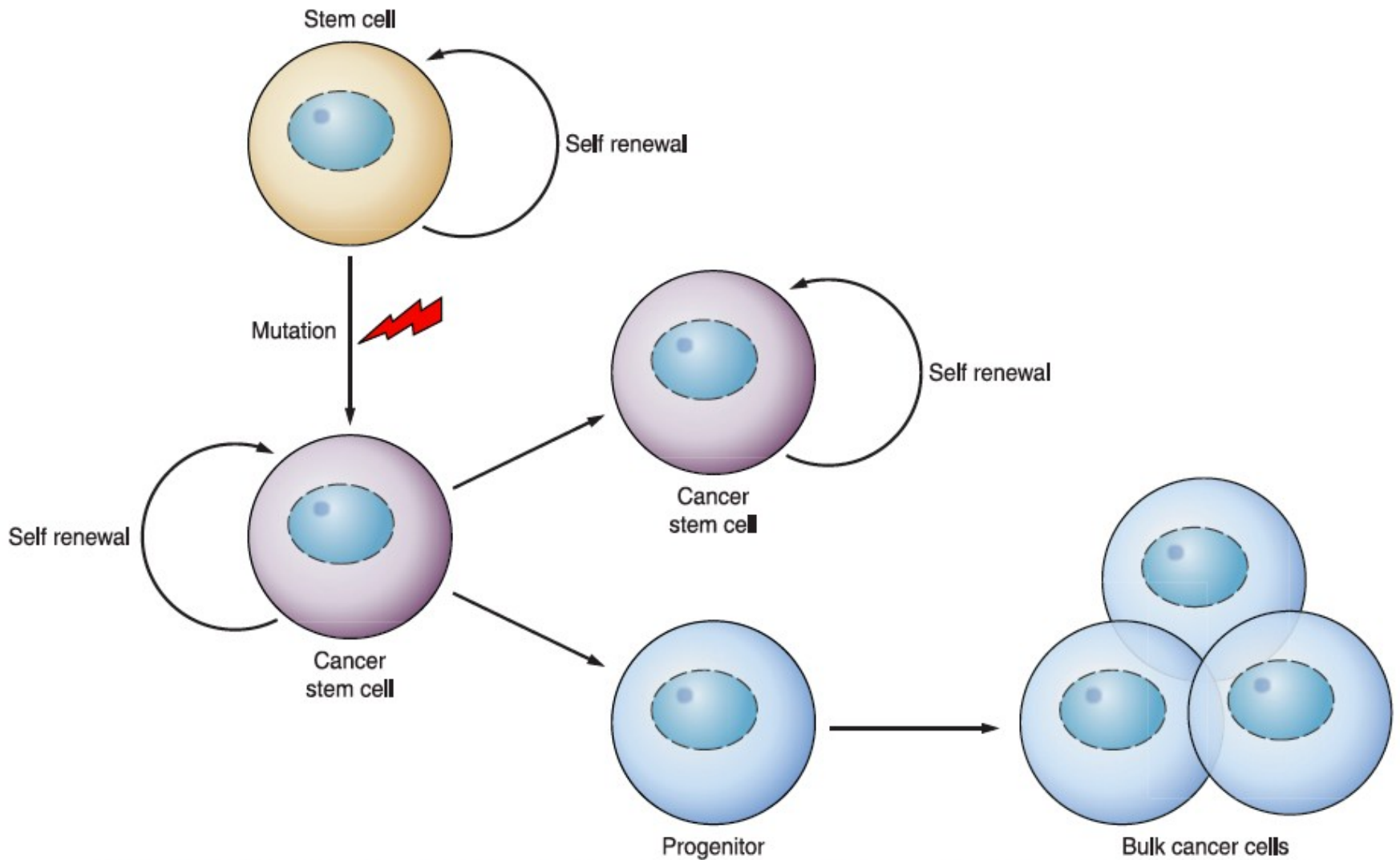
## Nuclear reprogramming



- **ES cell morphology**
- **Demethylation of pluripotency genes**
- **Reactivation of pluripotency genes**
- **X-chromosome reactivation**
- **Telomerase activity**
- **Loss of G1 checkpoint**



**FIGURE 4.** Key developmental signaling pathways regulated by the core stem cell factors. These pathways have been shown to have a role in both ES cells and iPS cells and also in tumorigenesis (+). These pathways have also been shown to be essential in somatic stem cell maintenance and vertebrate limb regeneration. Examples of the identified role of these various pathways in key cellular systems are shown in the table.



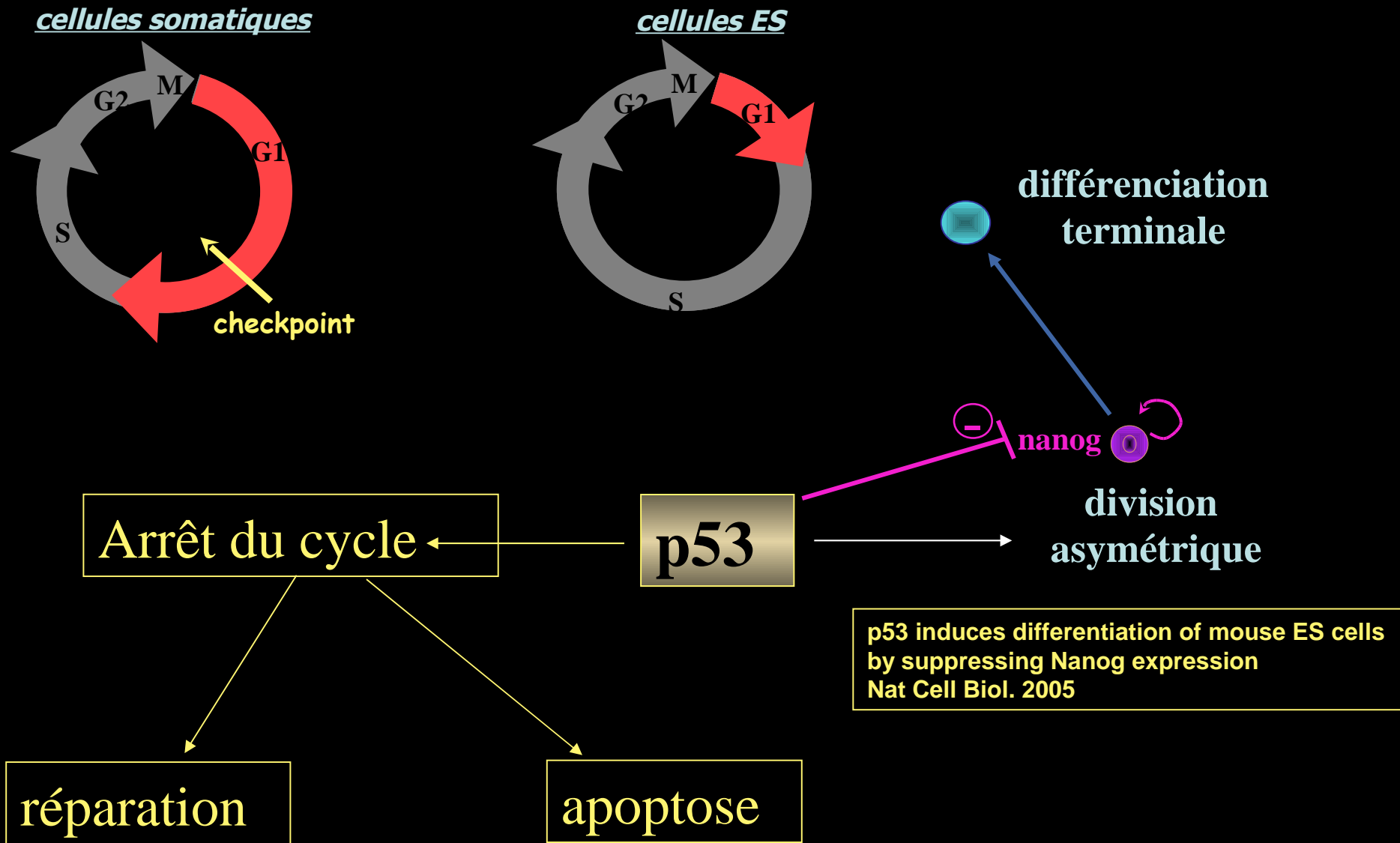
**FIGURE 5.** The cancer stem cell model. The cancer stem may occur as a result of acquired mutations in a somatic stem cell. The cancer stem cells retain the ability to self-renew and through multiple asymmetric cell divisions are able to produce daughter cells that may form the bulk tumor population.

**Table 3.** *Examples of the mutations that occur in the key self-renewal pathways and their incidence in various cancers*

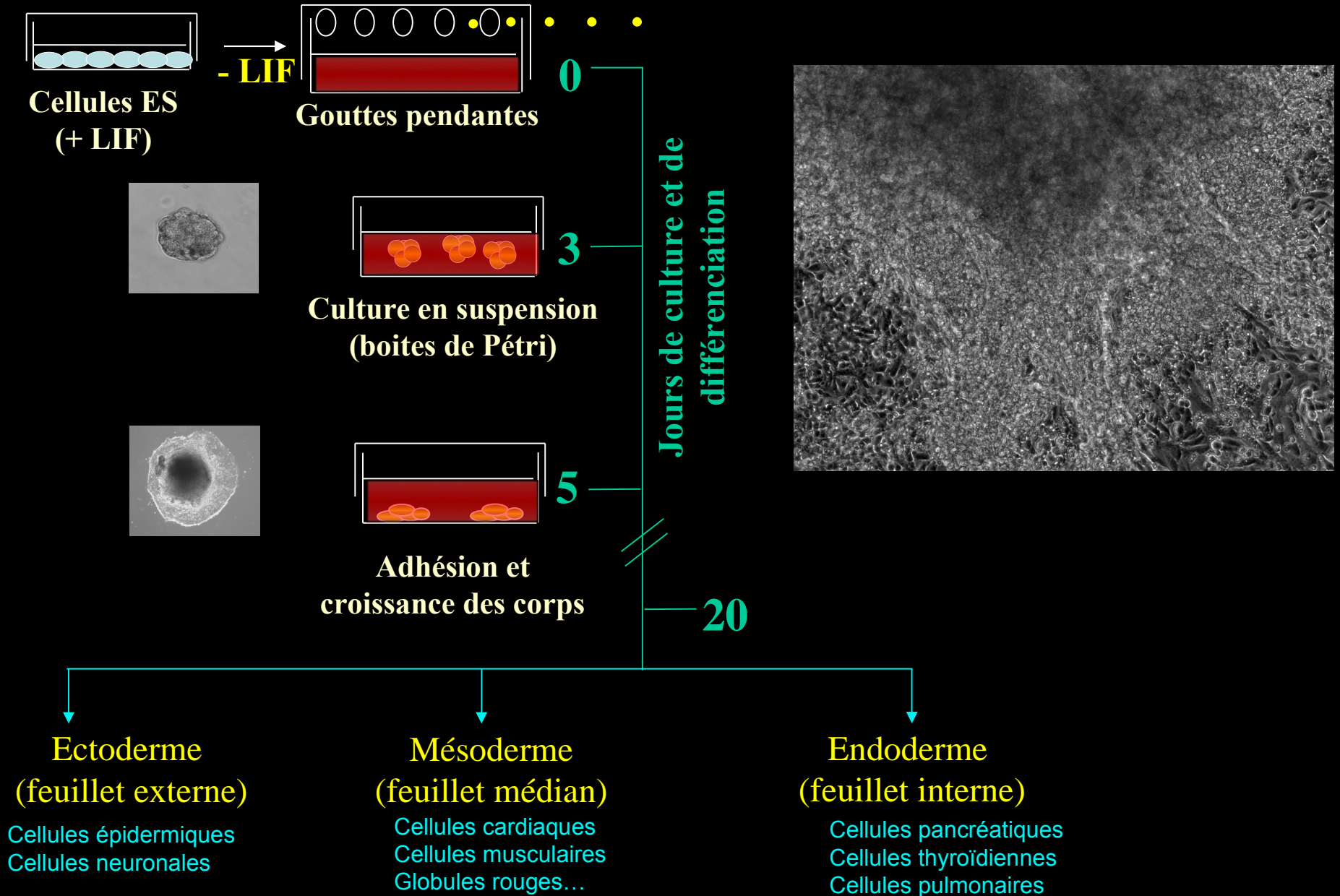
Pathway	Cancer/Tumor Type
TGF- $\beta$	↓ SMAD4 in 53% of pancreatic carcinomas
	↑ BMP2 in 98% of lung carcinomas
Wnt	↑ $\beta$ -Catenin in 48% of small intestinal carcinomas
	↑ $\beta$ -Catenin in 64% of gastric polyps
	↓ APC in 90% of colon cancers
	↓ APC in 76% of gastric adenomas
Hedgehog	↓ Ptch in 67% of basal cell carcinomas
	↑ Smo in 10% of basal cell carcinomas
	↓ Ptch in 10-20% of sporadic medulloblastomas
JAK-STAT	↑ STAT3 in 50% lung
	95% head and neck
Notch	↑ Notch in 55-60% of T-cell acute lymphoblastic leukemia
MAPK-ERK	↑ RAS in 45% of colon and 90% of pancreatic cancers
	↑ RAF in 60% of melanoma

[Adapted from Dreesen and Brivanlou (55).]

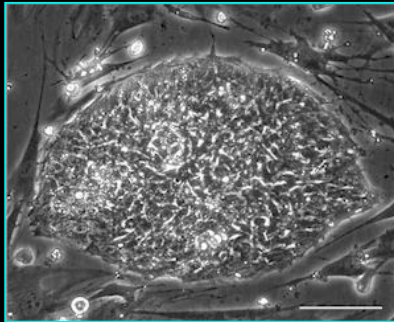
# p53, le gardien du génome



# Pluripotence des cellules couches embryonnaires (I)

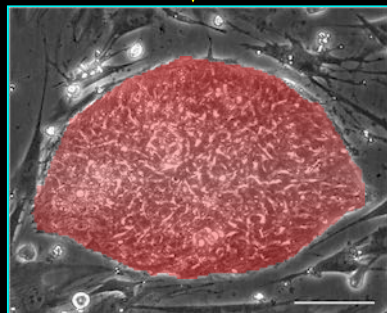


# Pluripotence des cellules couches embryonnaires (II)

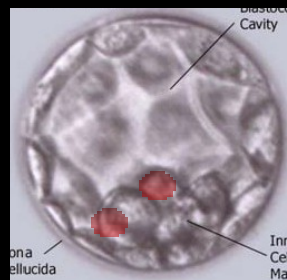


Cellules ES

Recombinaison  
homologue



Cellules ES  
dont un gène est modifié



Injection dans le  
blastocyste

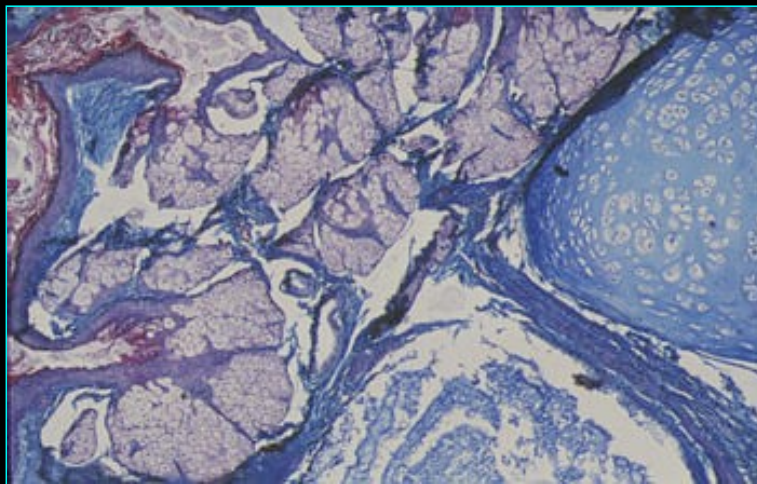
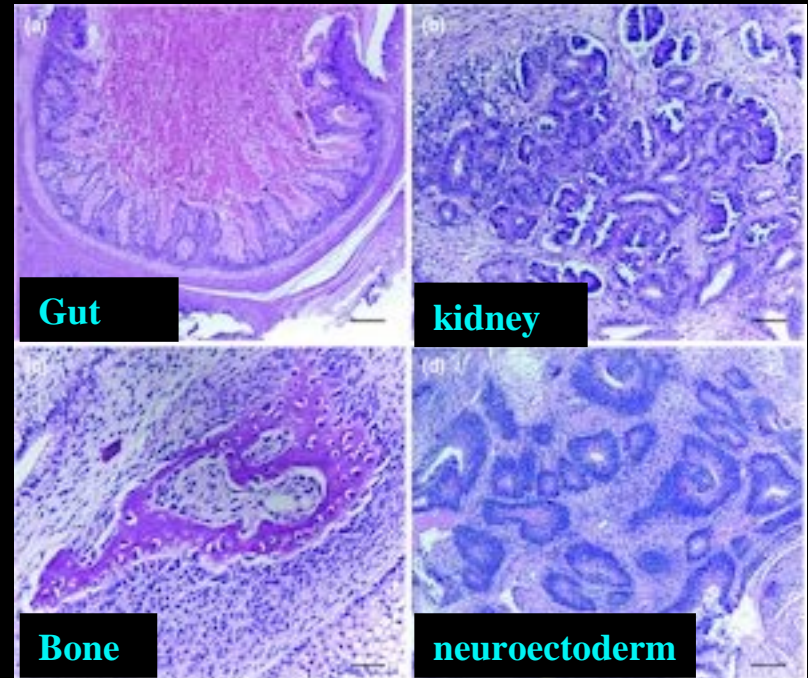
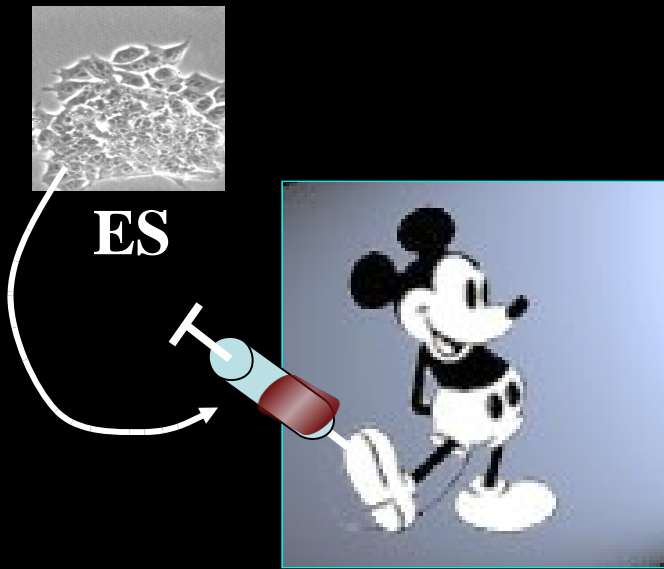


souris  
chimérique



Femelle  
porteuse

# Pluripotence des cellules couches embryonnaires (III)



**Tératome:** Tumeur bénigne ou maligne formée à partir de tissus multiples étrangers à la région qui l'entoure

# Utilité et perspectives d'utilisation des cellules ES

Etude des stades précoces du développement:

- Déterminer les conditions de différenciation
- Comprendre les pathologies congénitales.

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## Utilisation en thérapie cellulaire:

- **Neurodégénératives:** Parkinson, sclérose en plaque, Alzheimer, chorée de Huntington, para-tétraplégie.
- **Déficit cellulaire:** diabète de type I (HES produisent insuline in vivo); infarctus du myocarde; hépatite.
- **Manipulation génique des ES** avant transplantation pour réverter un phénotype pathologique.

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## Délivrance de médicaments

production massive de protéines recombinantes ou endogènes...

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## Délivrance de médicaments

production massive de protéines recombinantes ou endogènes...

## Regénération tissulaire contre le vieillissement

## Thérapie des cancers?

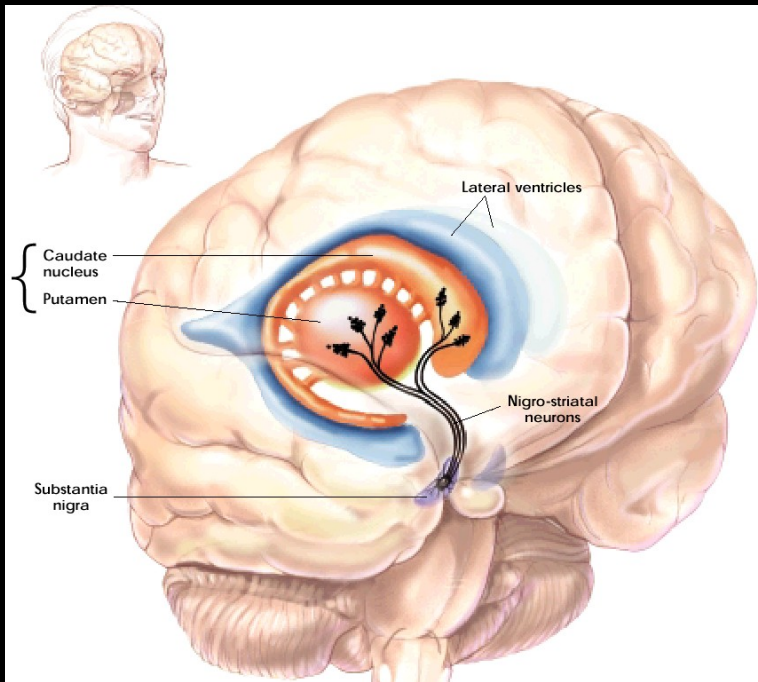
# Conditions d'utilisation des cellules ES en thérapie

- \* **Savoir et pouvoir cultiver durablement les cellules ES humaines**
- \* **Contrôler leur différenciation de manière sélective**
- **Pureté cellulaire: FACS, sélection génique, clones**
- **Contrôler le rejet de tissus**
- **Eviter les tératomes (indiff. cells?)**
- \* **Problème d'éthique lié à l'utilisation d'embryons surnuméraires**

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# La maladie de Parkinson



## *Prévalence:*

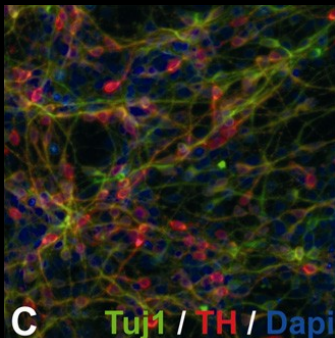
*~ 100 000 patients en France*

## *Symptômes:*

*Tremblements,  
Rigidité musculaire,  
Ralentissement des gestes (akinésie, bradykinésie)  
Instabilité posturale*

## *Pathologie:*

*Perte des neurones dopaminergiques de la  
substance noire (pars compacta)*



# Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease

J KIM\*, J M. AUERBACH\*†, J A. RODRÍGUEZ-GÓMEZ, I VELASCO, D GAVIN, N LUMELSKY, S LEE†, J NGUYEN†, R SÁNCHEZ-PERNAUTE†, K BANKIEWICZ† & R MCKAY NIH, Bethesda.

Modèle animal de la maladie de Parkinson:

Administration de 6-OH-DA dans le striatum détruit les neurones dopaminergiques.

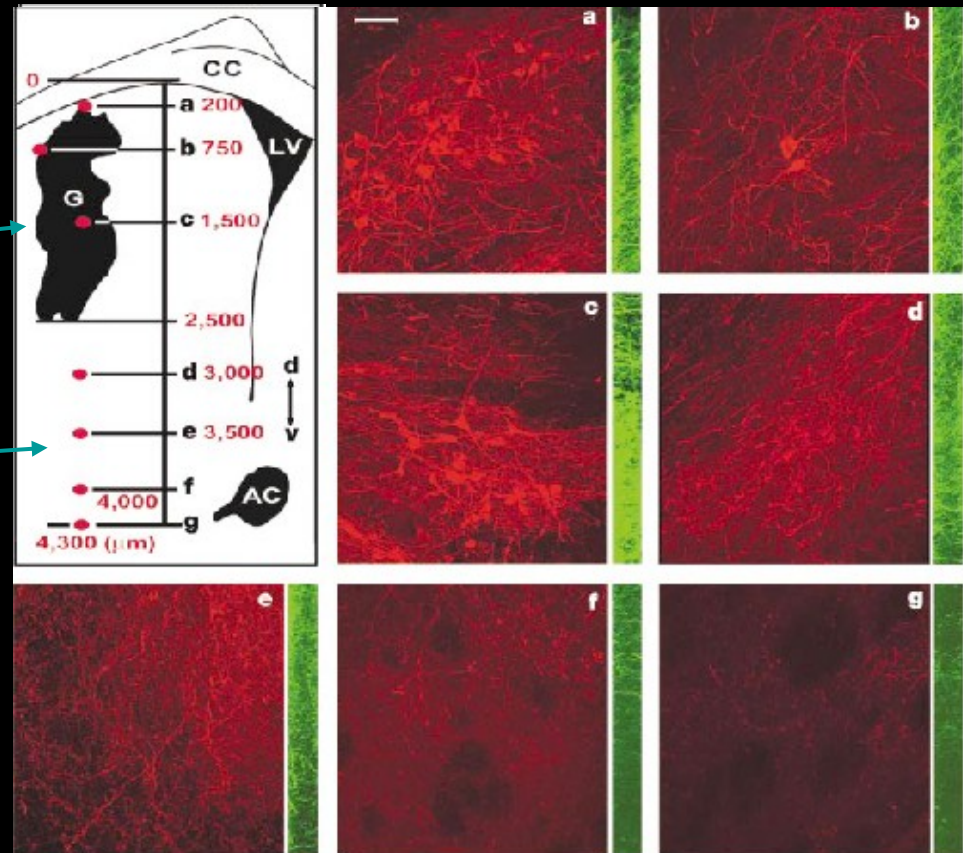
Commutateur de différenciation: promoteur *nurr-1* + SHH + FGF8:

- Isolement de neurones DOPA (78%)
- Injection des cellules dans le striatum.

Marquage anti-Tyrosine Hydroxylase (TH).

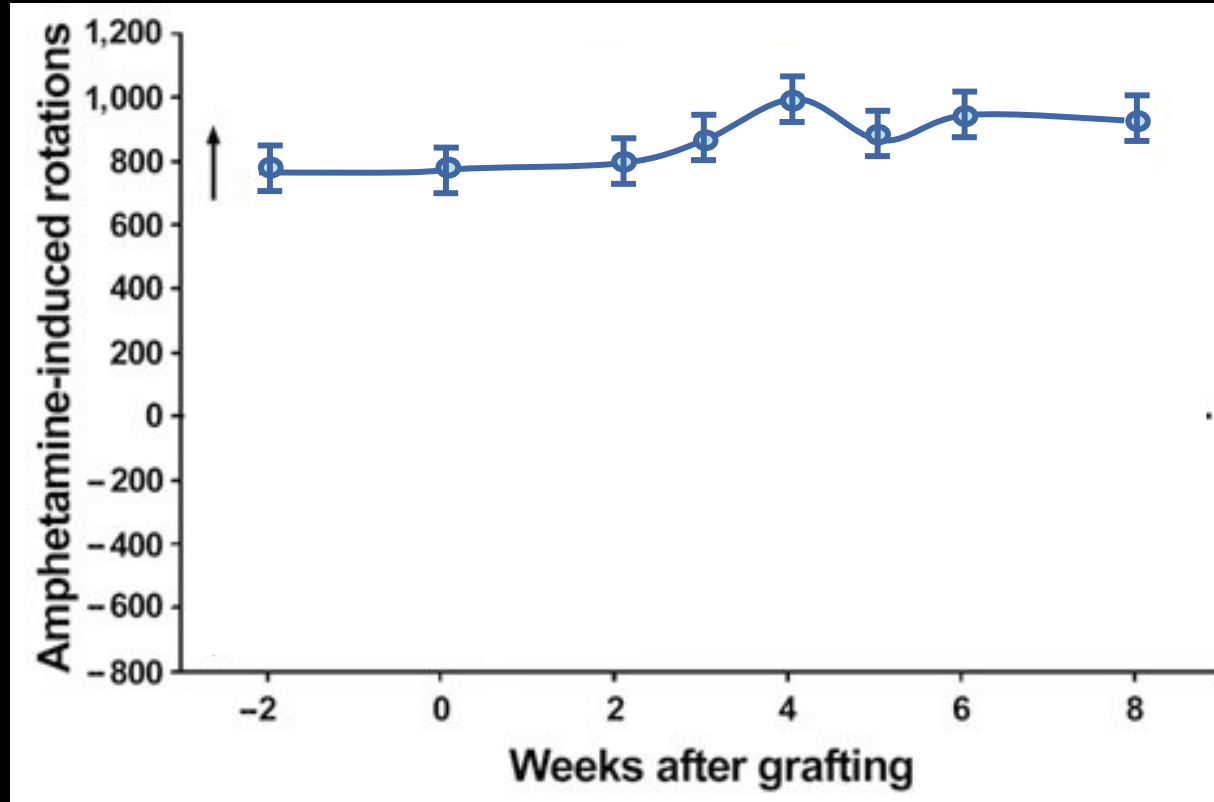
On retrouve les corps de cellules ES TH<sup>+</sup> dans la région de la greffe (a-c)

mais des prolongements dans le parenchyme du striatum hôte jusqu'à 2 mm de la greffe (d-g).



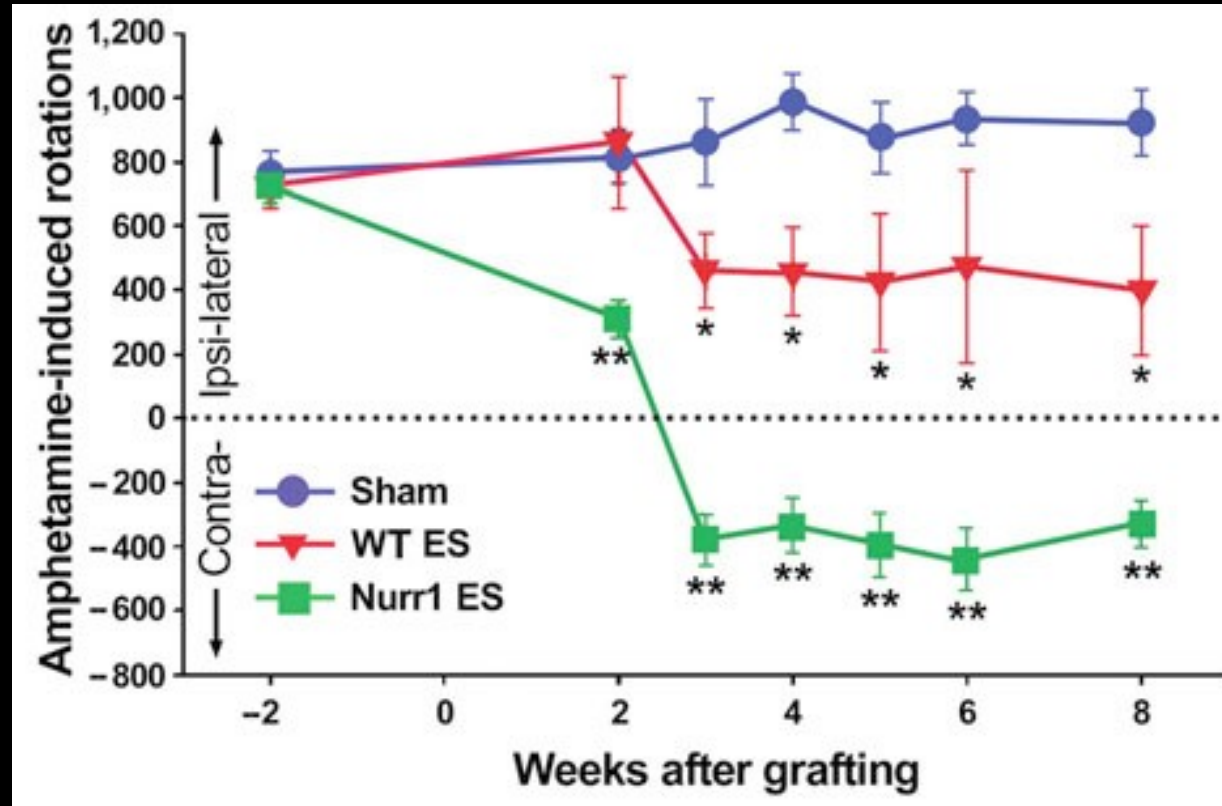
**Administration d'amphétamine aux animaux lésés par le 6-OH-DA induit une rotation autour du site d'injection.**

800 rotations/70 min  
1 rotation/5 sec



## Administration d'amphétamine aux animaux lésés par le 6-OH-DA induit une rotation autour du site d'injection.

800 rotations/70 min  
1 rotation/5 sec



By demonstrating efficacy while avoiding tumour formation, Kim *et al.* have achieved a [proof of principle](#), although ES cells that have been genetically modified in this way might not be desirable for use in people.

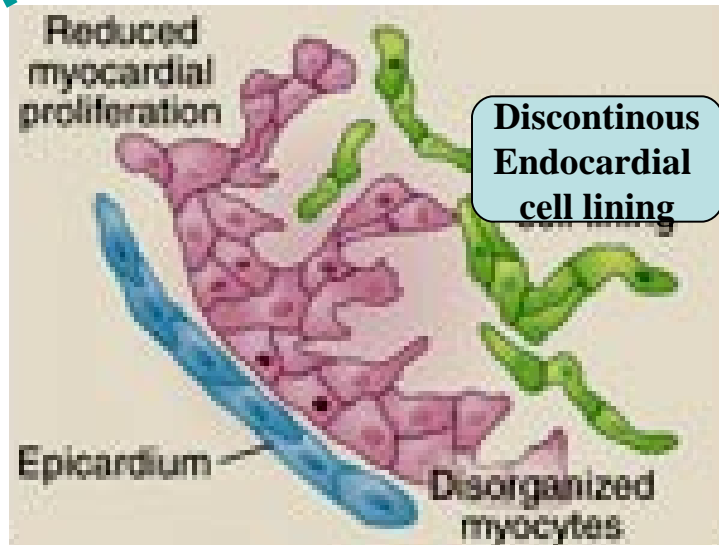
# Rescue of Cardiac Defects in *Id* Knockout Embryos by Injection of Embryonic Stem Cells

Diego Fraidenraich,<sup>1</sup> Elizabeth Stillwell,<sup>1</sup> Elizabeth Romero,<sup>1</sup> David Wilkes,<sup>3</sup> Katia Manova,<sup>2</sup> Craig T. Basson,<sup>3</sup> Robert Benezra<sup>1\*</sup>

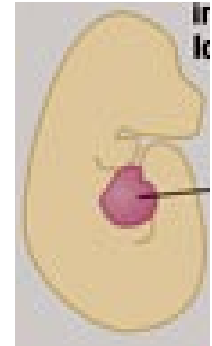
**Embryos lacking the *Id1*, *Id2*, and *Id3* genes display multiple cardiac abnormalities and die around E13.5.**

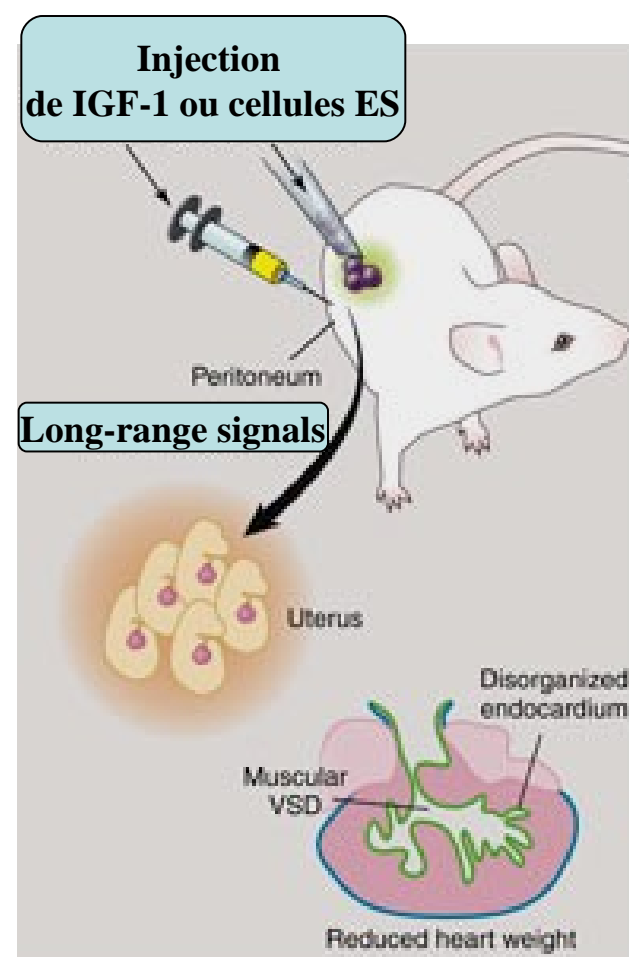
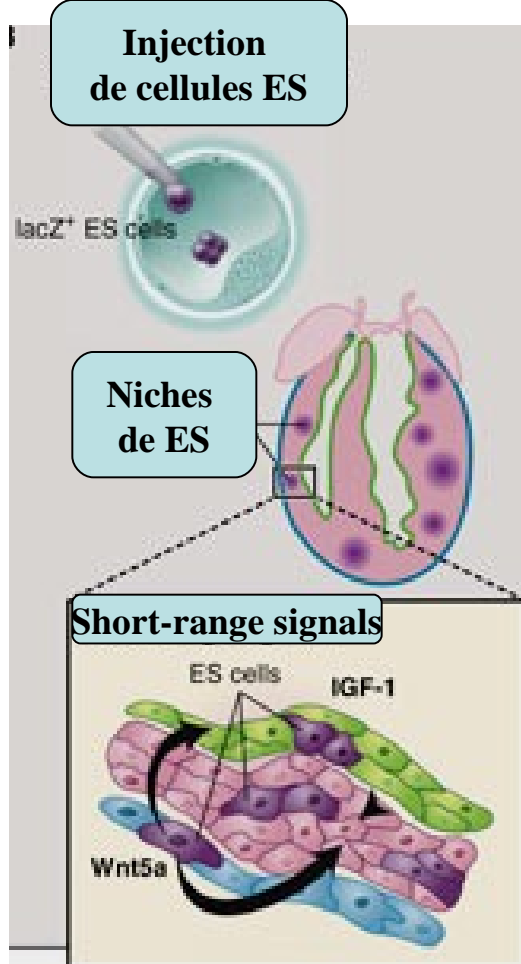


Reduced myocardial proliferation



Discontinuous Endocardial cell lining





**B. Injection of a small number of wild-type ES cells carrying a lacZ marker into Id mutant blastocysts resulted in about 20% chimerism of heart tissue and complete rescue of the Id mutant phenotype.**

**C. Wnt5a, a locally secreted factor, and IGF-1, a bloodstream factor that promotes myocyte proliferation--were identified as potential candidates involved in the rescue process.**

# 166 genodermatosis (139 genes)

## DISORDERS OF KERATINIZATION

- Ichthyosiform dermatoses
- Ichthyosis vulgaris
- X-linked ichthyosis
- Lamellar ichthyosis
- Congenital ichthyosiform erythroderma
- Epidermolytic hyperkeratosis
- Harlequin fetus
- Palmoplantar keratodermas
- Darier disease

## DISORDERS OF COHESION

### Epidermolysis bullosa

- Kindler syndrome

## PIGMENTATION DISORDERS

- Oculocutaneous albinism
- Tyrosinase-negative
- Tyrosinase-positive
- Ocular albinism
- Piebaldism
- Waardenburg syndrome
- Incontinentia pigmenti
- Neurofibromatosis type 1
- Neurofibromatosis type 2
- Tuberous sclerosis

## VASCULAR DISORDERS

- Ataxia-telangiectasia
- Hereditary hemorrhagic telangiectasia

## ECTODERMAL DYSPLASIA SYNDROMES

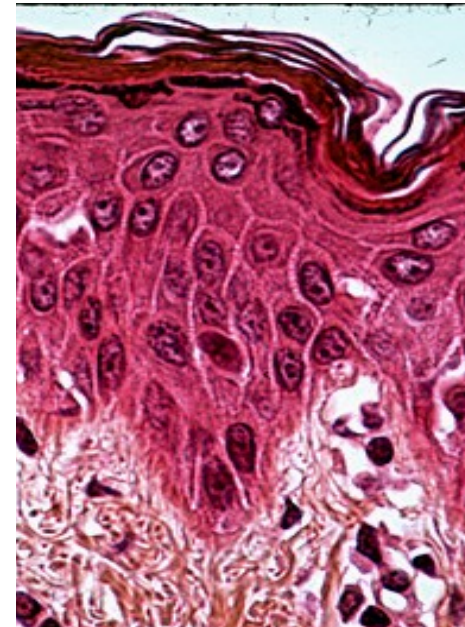
- Anhidrotic ectodermal dysplasia
- Hypohidrotic ectodermal dysplasia with immunodeficiency
- Hidrotic ectodermal dysplasia

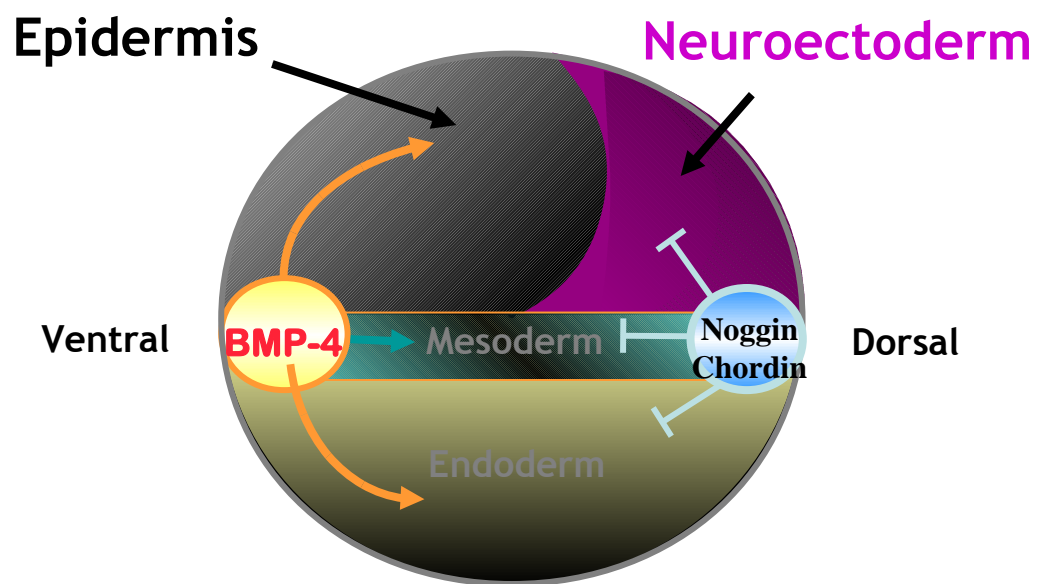
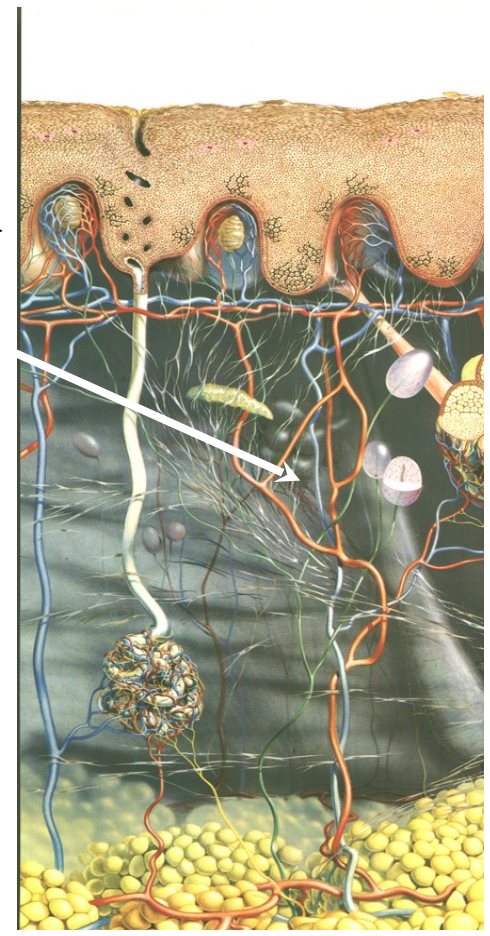
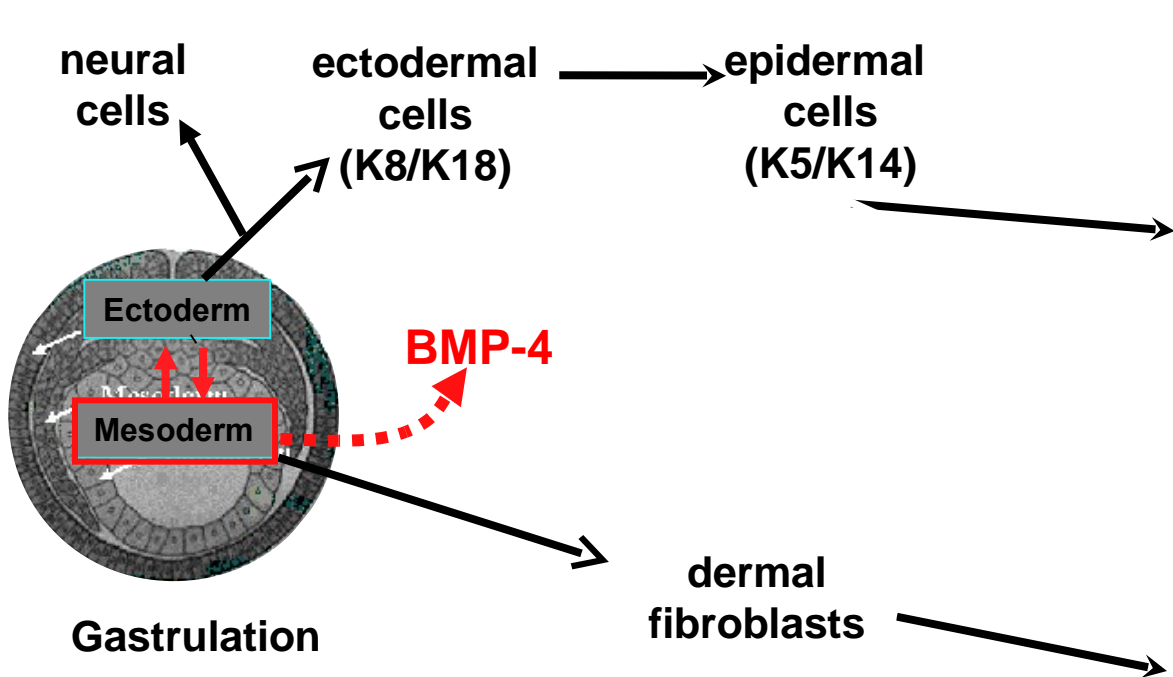
### p63-related ectodermal dysplasia

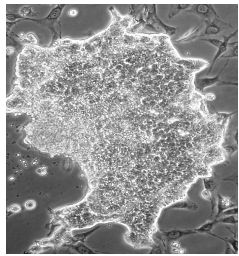
- Pachyonychia congenita

## DISORDERS WITH MALIGNANT POTENTIAL

- Basal cell nevus syndrome
- Gardner's syndrome
- Peutz-Jegher syndrome
- Xeroderma pigmentosum
- Epidermolysis bullosa syndromes

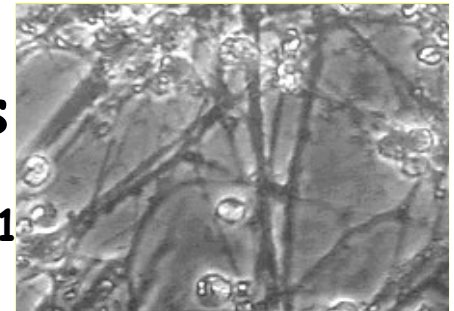
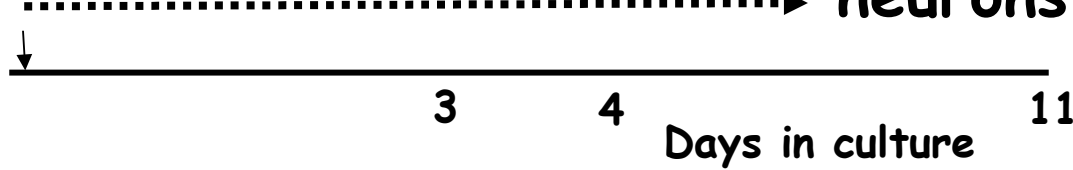






**ES cells**

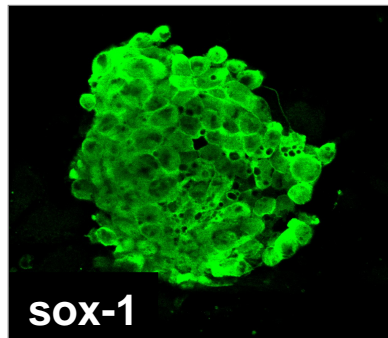
stromal  
fixed-feeders  
(- serum)



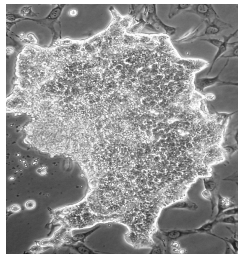
**neurons**

**Neural precursors**  
(sox-1<sup>+</sup>)

**Neural cells**  
(most TH<sup>+</sup>)

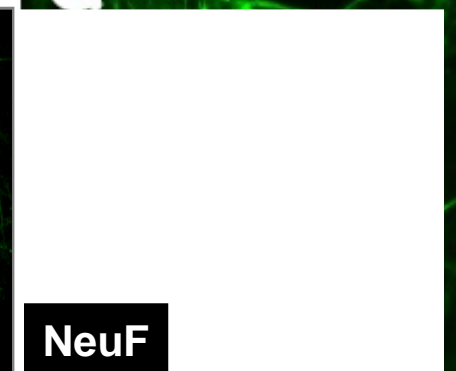
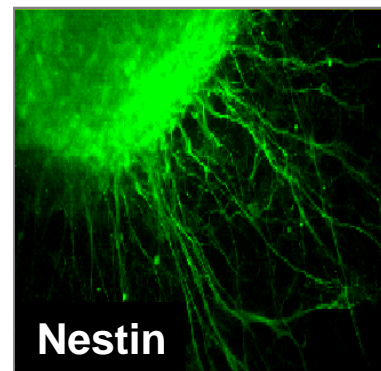
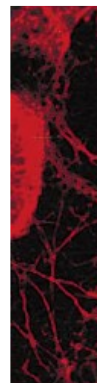
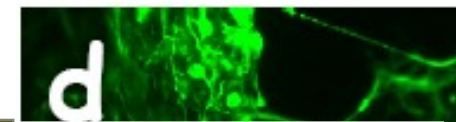
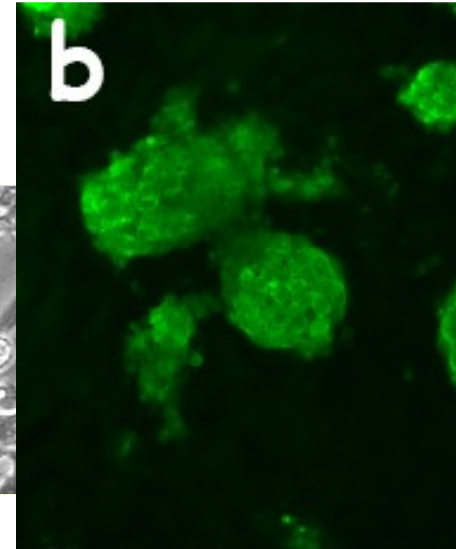
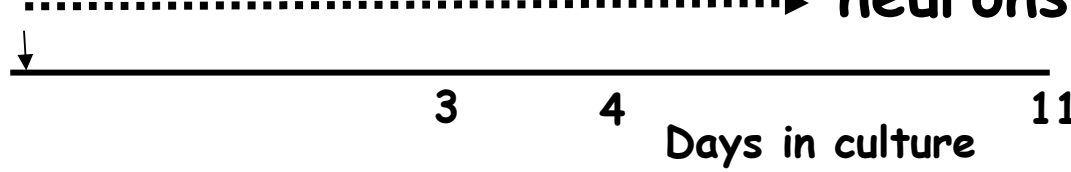


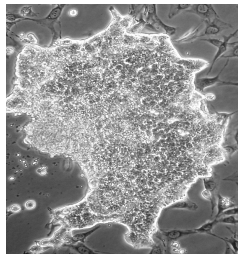
**sox-1**



ES cells

stromal  
fixed-feeders  
(- serum)





ES cells

mesenchymal  
fixed-feeders  
(- serum)

**BMP-4**

**K18**

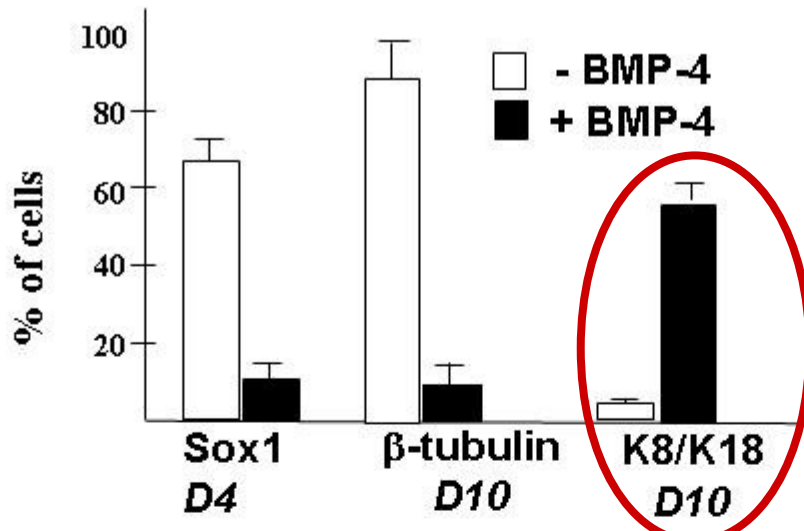
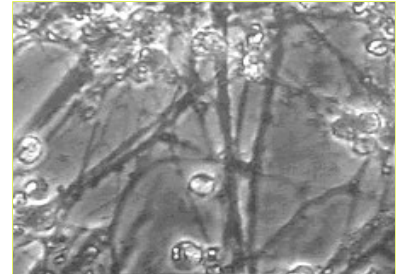
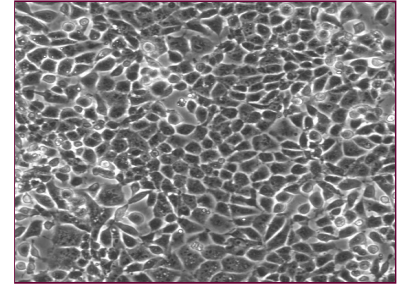
~~neurons~~

3

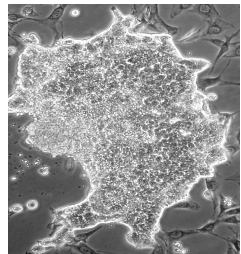
5

11

Days in culture

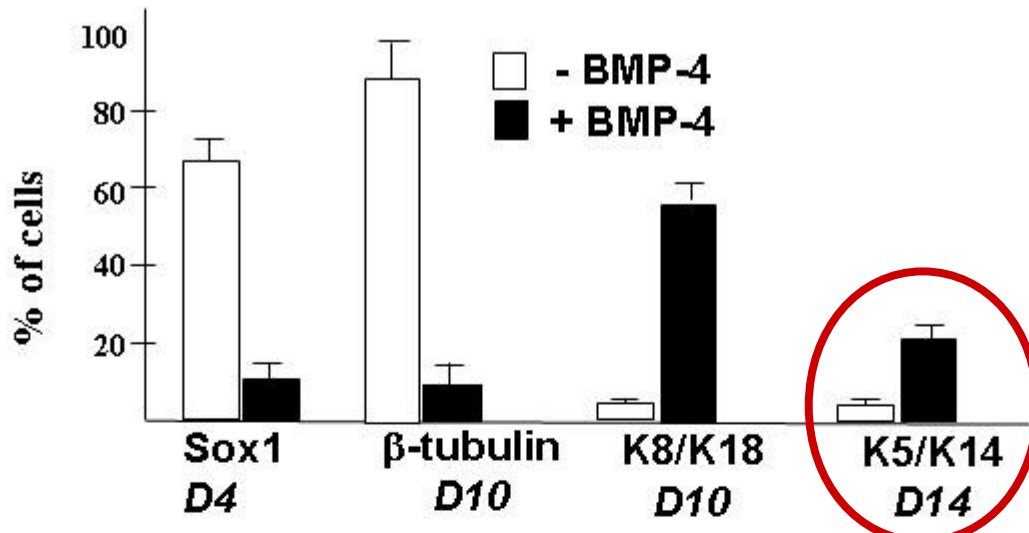
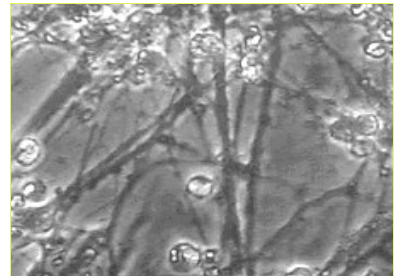
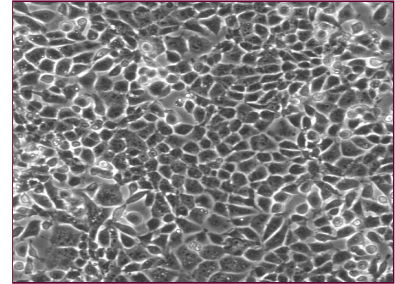
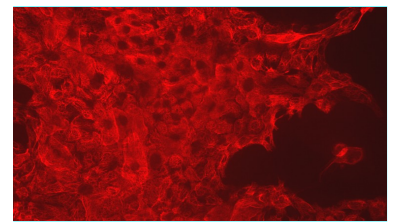
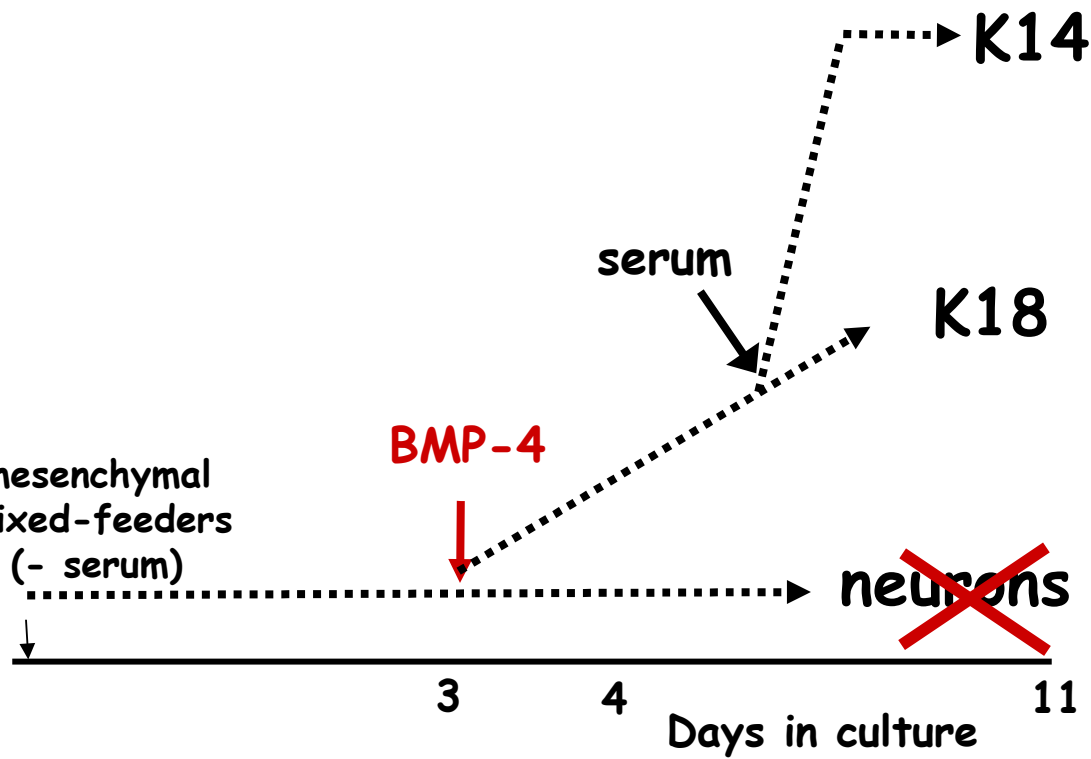


~~Neural cells  
(NeuN, NeuroF  
Nestin,  $\beta$ -III tubulin)~~



ES cells

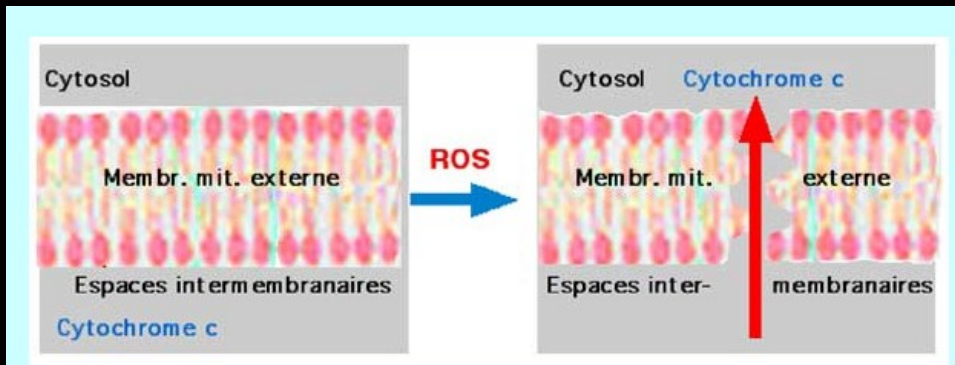
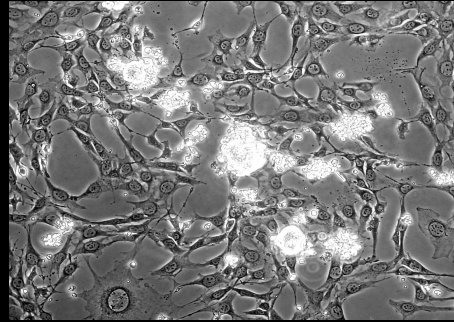
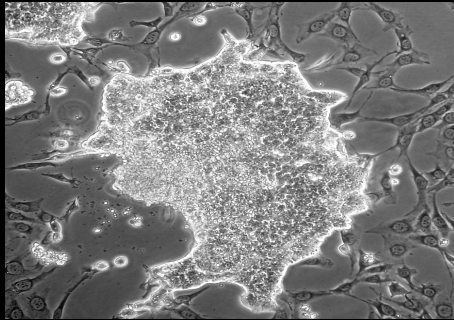
mesenchymal  
fixed-feeders  
(- serum)



~~Neural cells  
(NeuN, NeuroF  
Nestin,  $\beta$ -III tubulin)~~

# Contrôle

# BMP-4



## DIOC6:

- retains in intact cells
- excluded in apoptotic cells

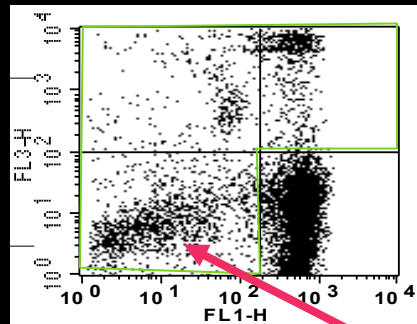
## Iodure de Propidium:

- excluded in living cells
- retains in death cells

dead cells

living cells

Iodure de Propidium



DIOC<sub>6</sub>

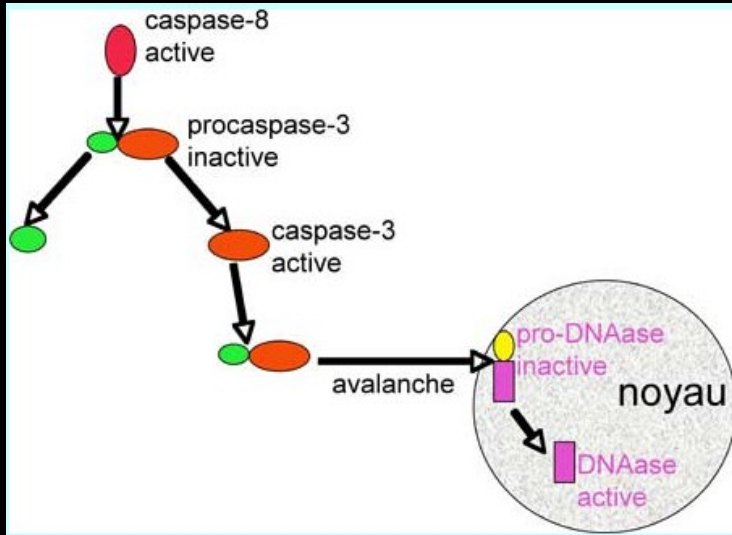
contrôle

(dommage mitochondrial)

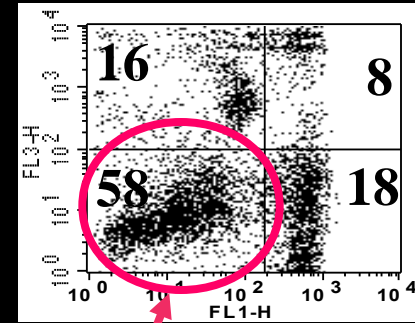
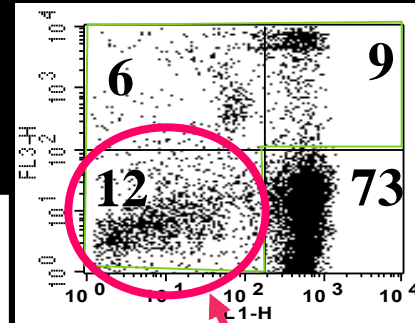
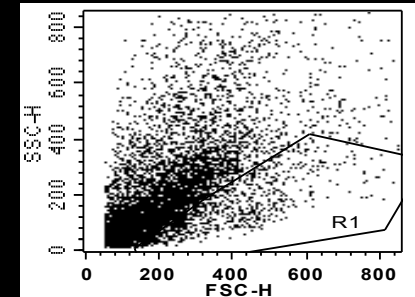
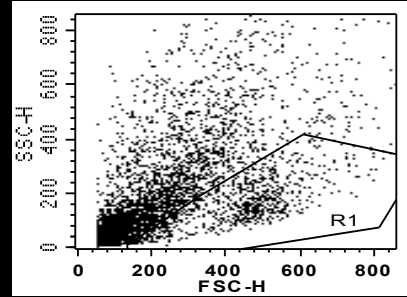
apoptotic cells

# BMP-4 induit l'apoptose des cellules ES

Gambara et al. (2006) Cell Death Diff. 13. 1075-1087



Iodure de Propidium

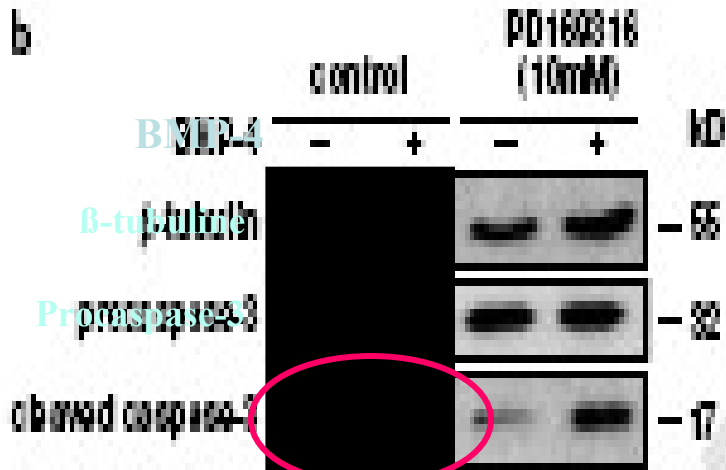


contrôle

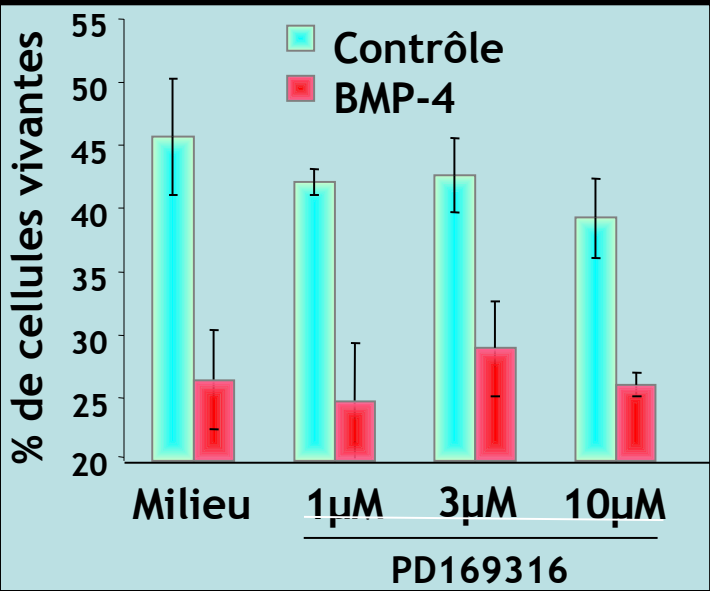
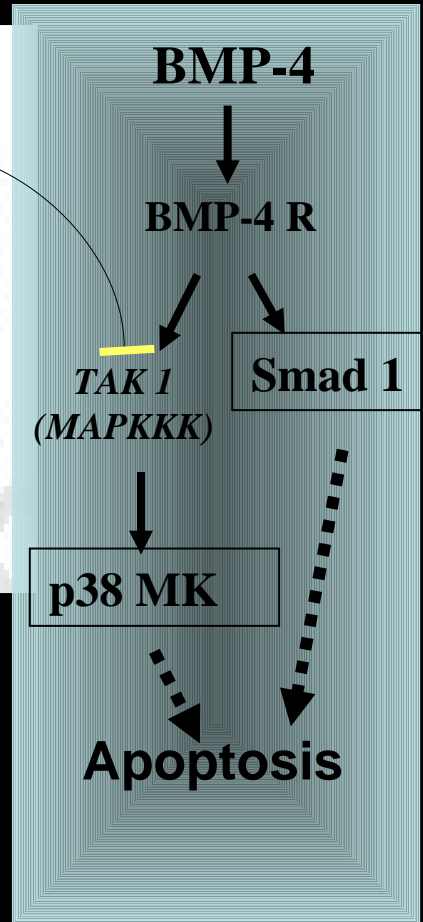
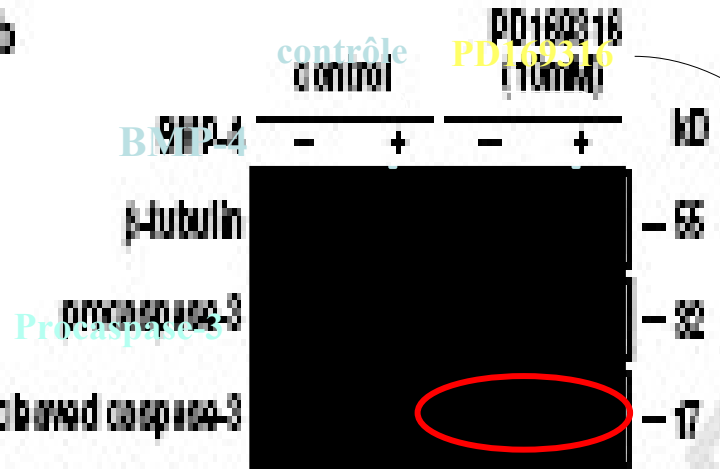
BMP-4

apoptose spontanée

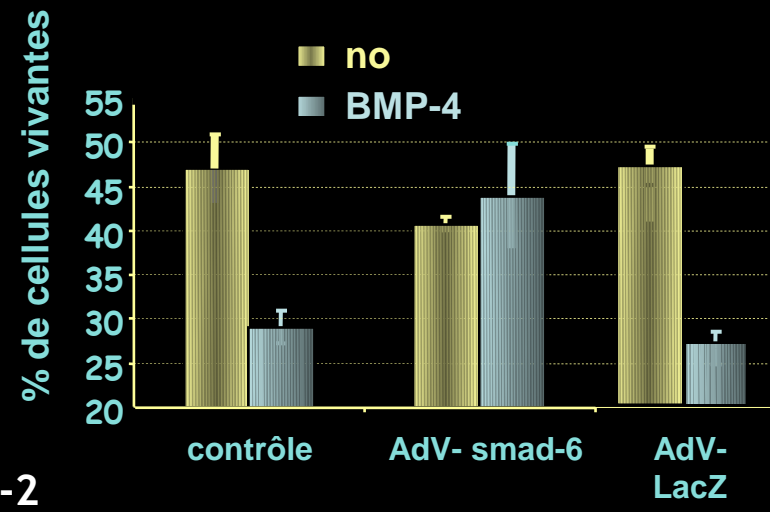
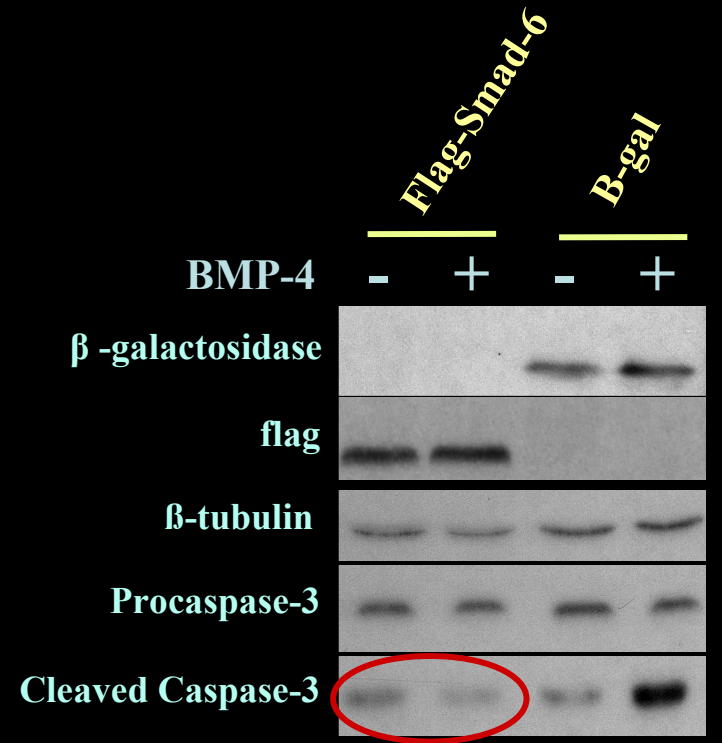
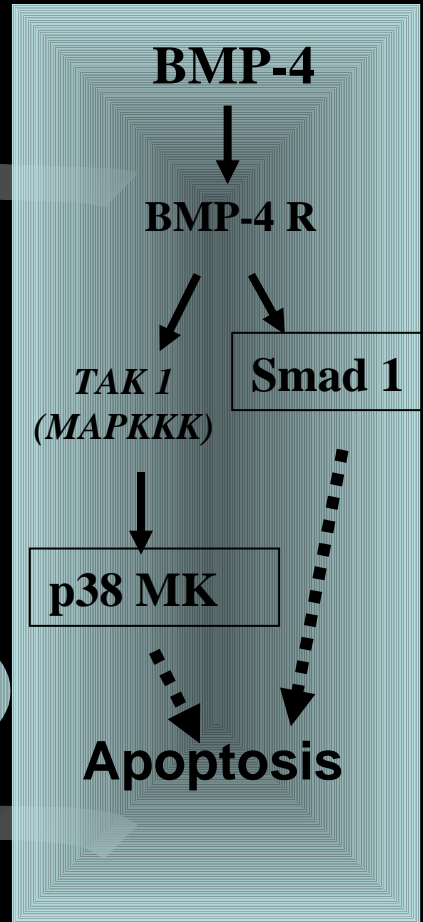
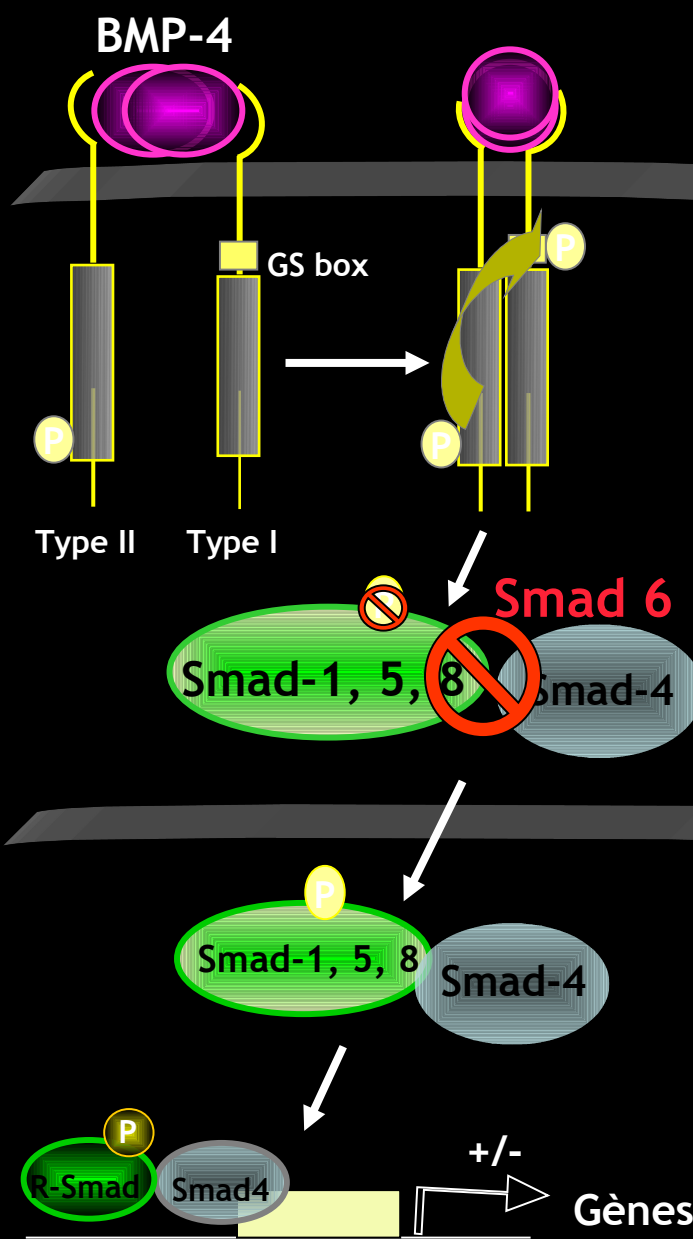
apoptose induite



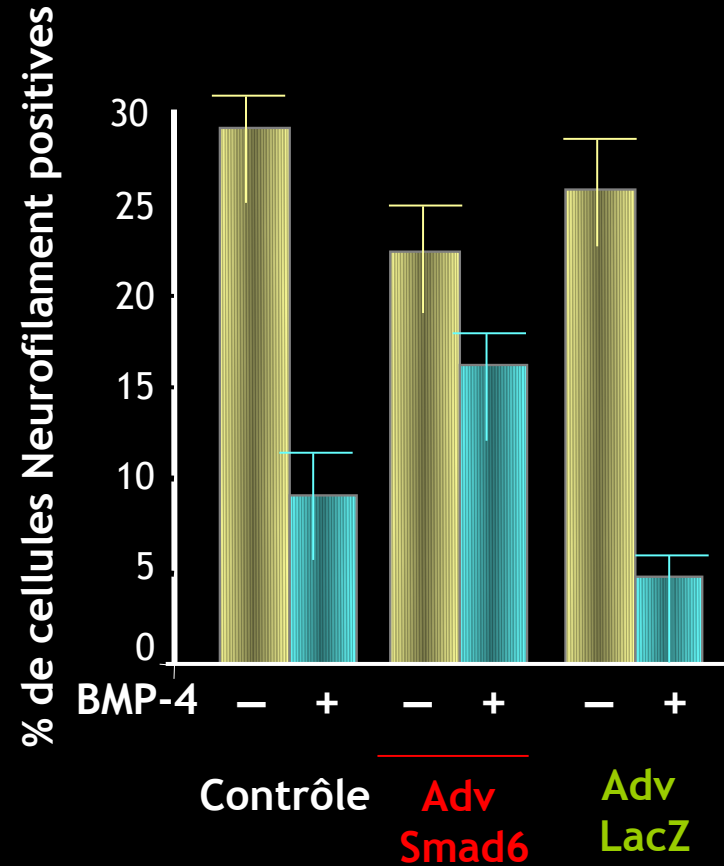
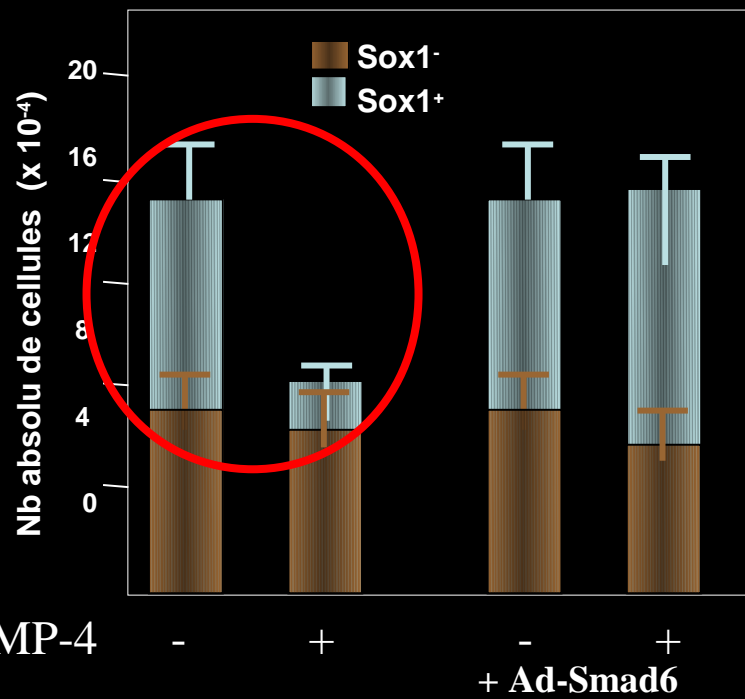
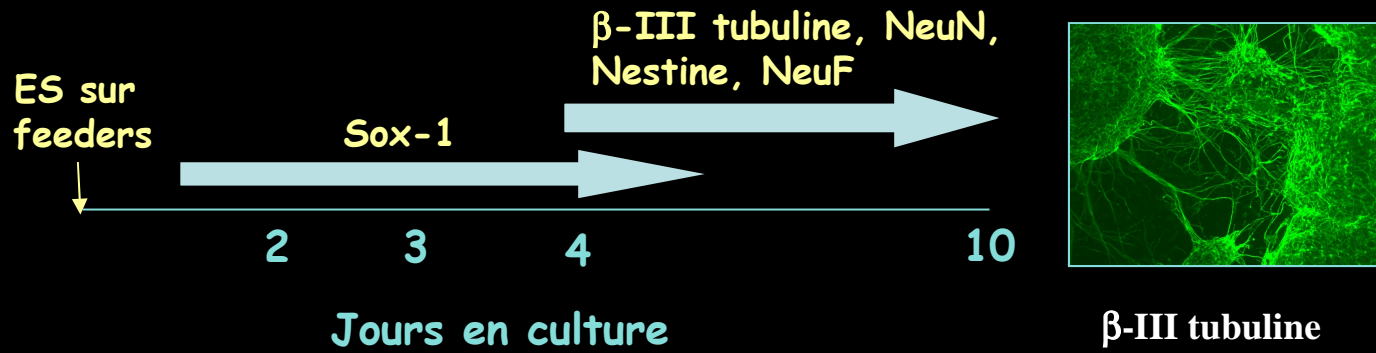
chondrial)



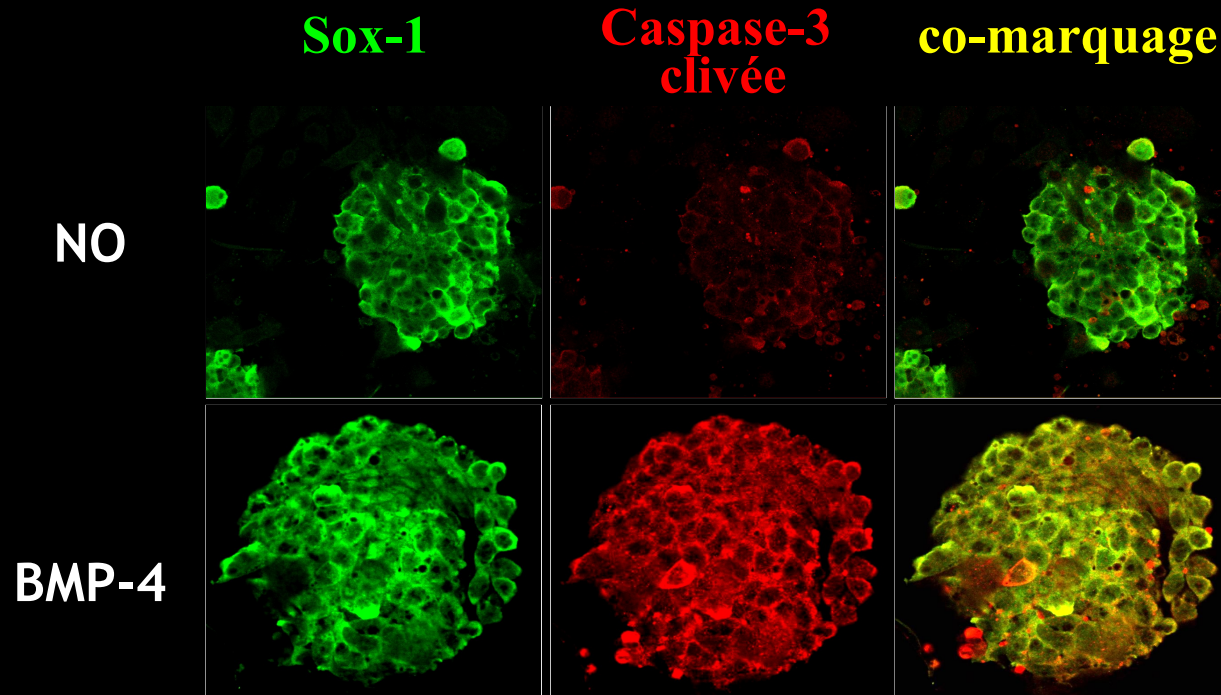
# BMP-4 induit l'apoptose des ES cells via la voie des Smad



Gènes cibles: msx-1 et msx-2



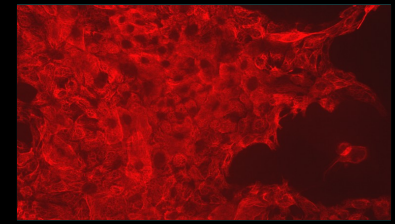
# BMP-4 induit l'apoptose des précurseurs neuronaux sox-1<sup>+</sup>



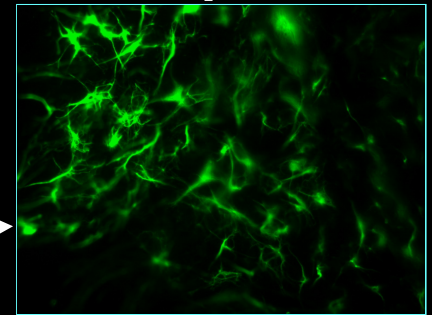
# BMP-4 induit l'apoptose des progéniteurs neuronaux issus de cellules ES

Gambaro et al. (2006) Cell Death Diff. 13. 1075-1087

K14

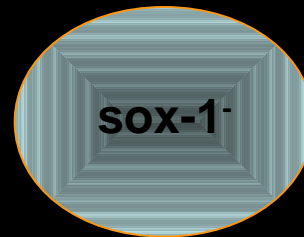


+ serum



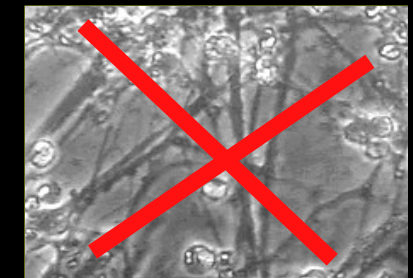
cellules ectodermales  
(K8/K18)

précurseur  
neuroectodermale ?

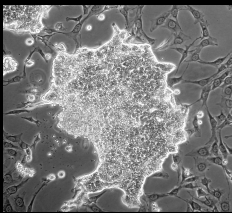


Smad

apoptosis of neural  
sox-1+ precursors



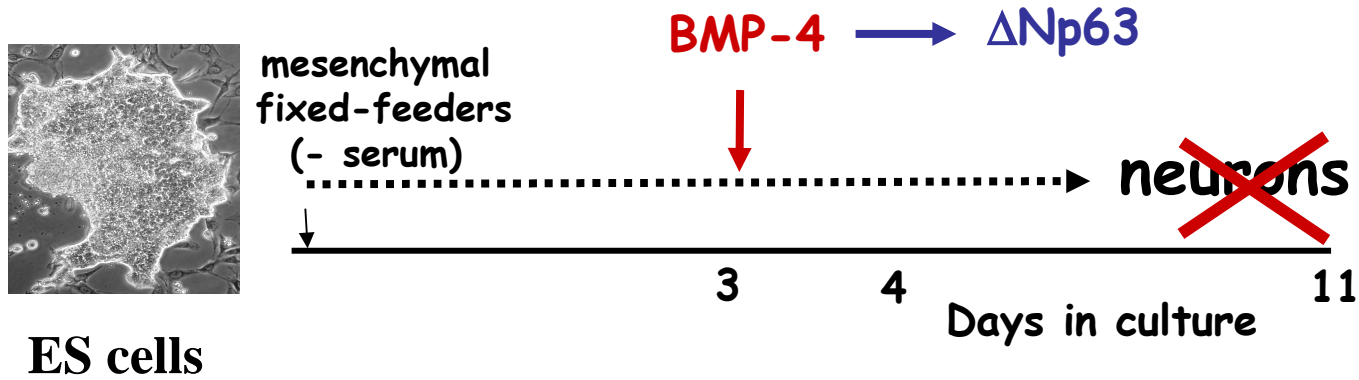
neurones  
(NeuN, NeuroF  
Nestine,  $\beta$ -III tubuline)



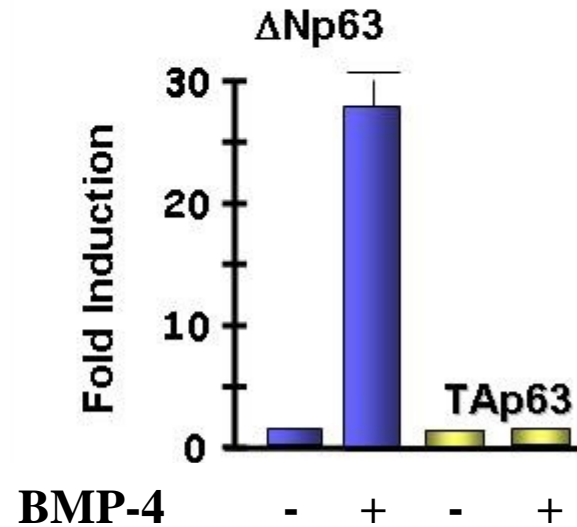
ES

BMP - 4

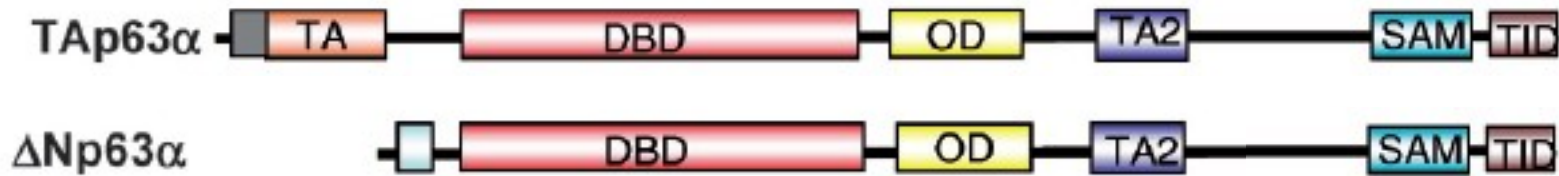
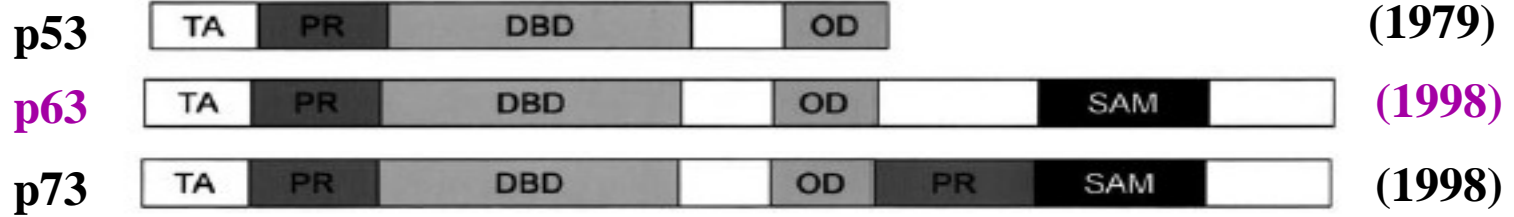
# $\Delta$ Np63 is activated during BMP-4-induced ES cell commitment



transcriptome analysis +/- BMP-4:



## p63 belongs to the p53 gene family



*p21, Notch*

cell proliferation

cell adhesion

*lama3, Itgb4, Perp*

**$\Delta$ Np63**



**TAp63**

proapoptotic

cellular senescence  
genomic stability  
(SKPs)

# p63-related ectodermal dysplasia syndromes

(Celli J. et al. Cell 1999)



*ectrodactyly*



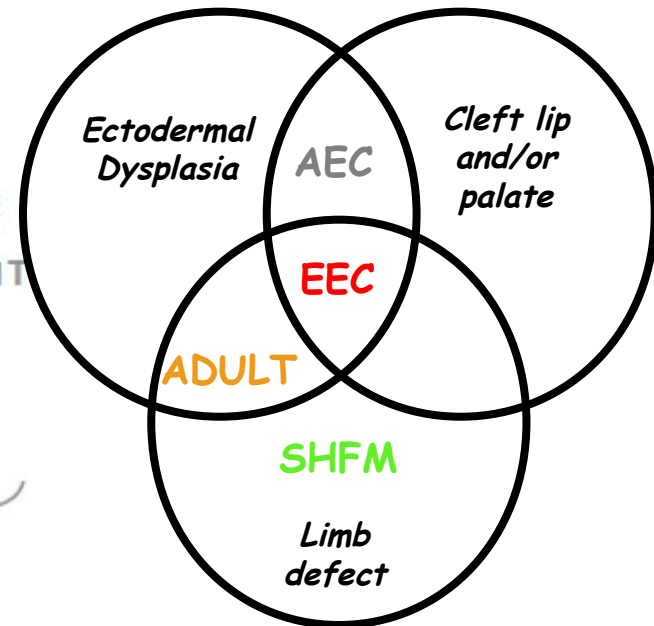
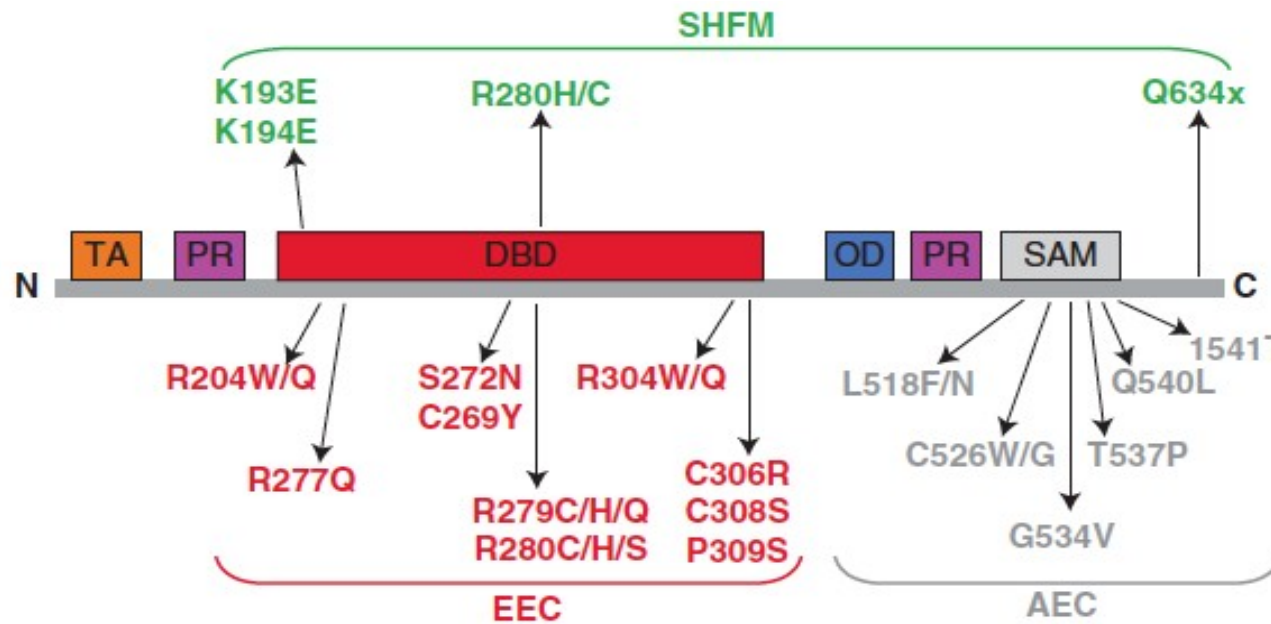
*ectodermal dysplasia*



*Cleft lip/palate*

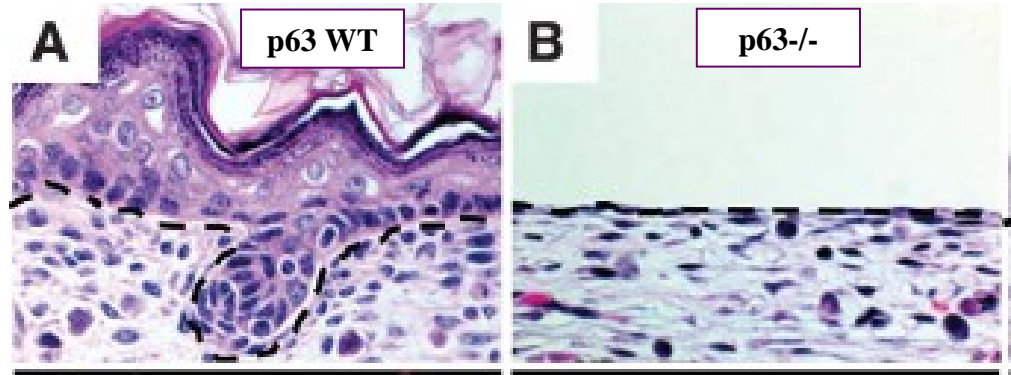
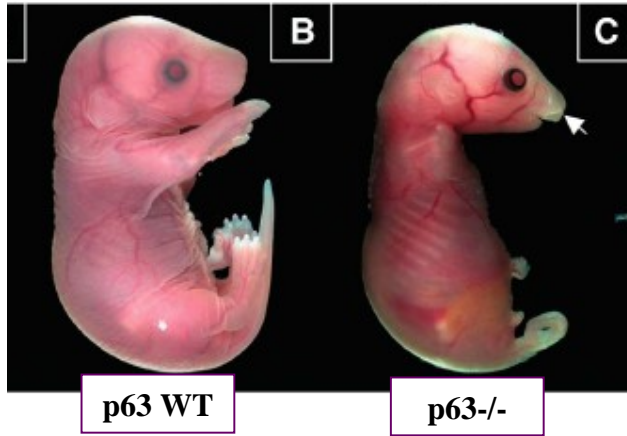


*Ankyblepharon*



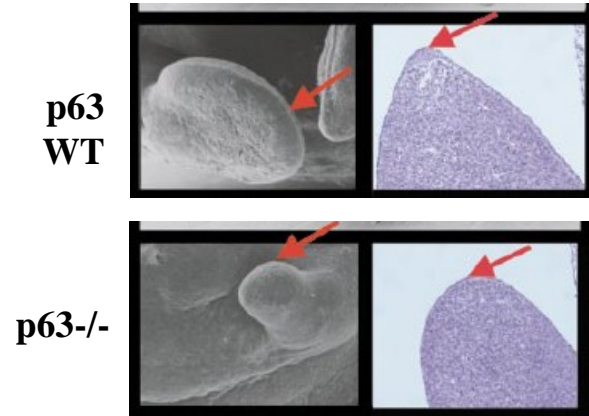
# p63 is required for normal ectodermal development

(Mills, Nature 1999; Yang, Nature 1999)



→ No stratified epithelia: skin remains single ectodermal layer.

→ No limbs: no AER (apical ectodermal ridge) formation and cranio-facial defects

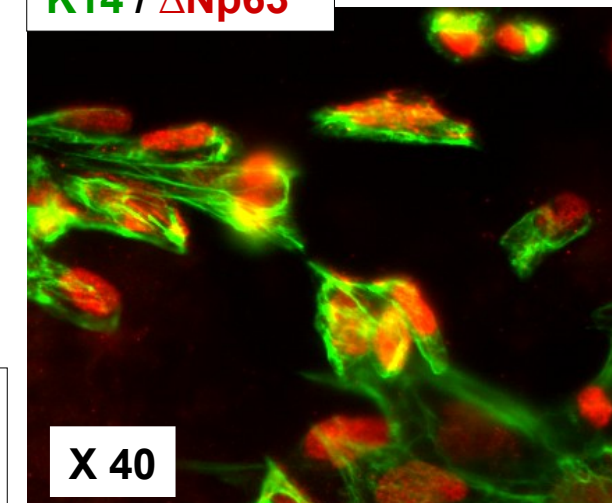
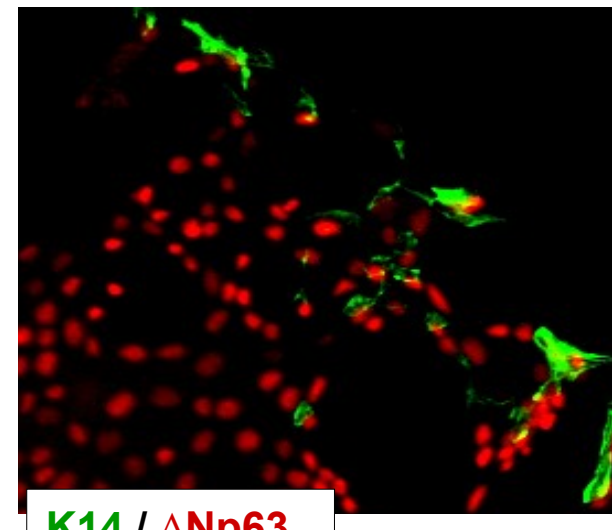
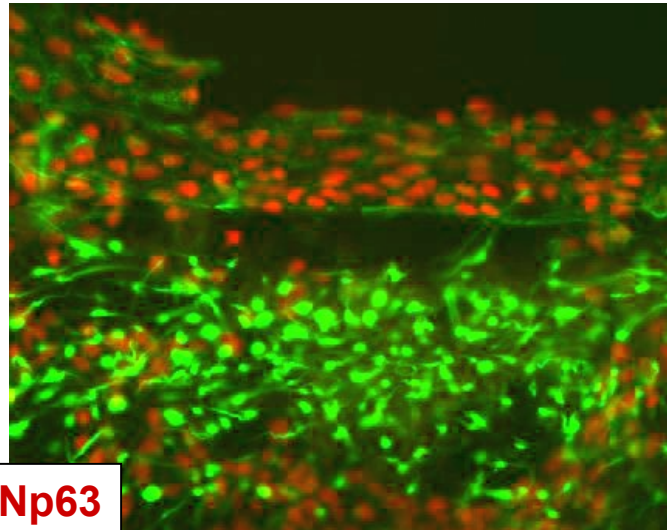
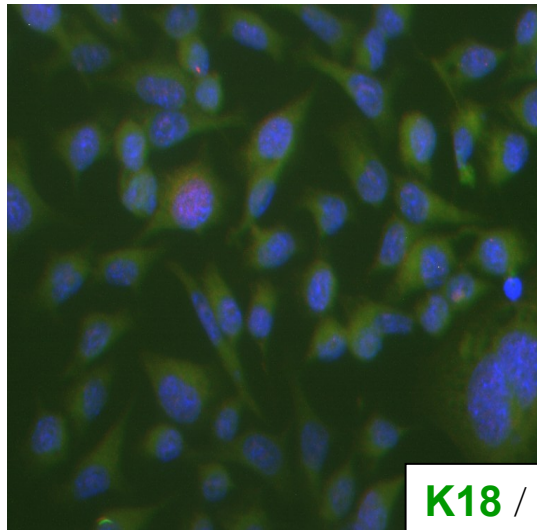


What is the function and regulation of p63 during development and in pathology ?

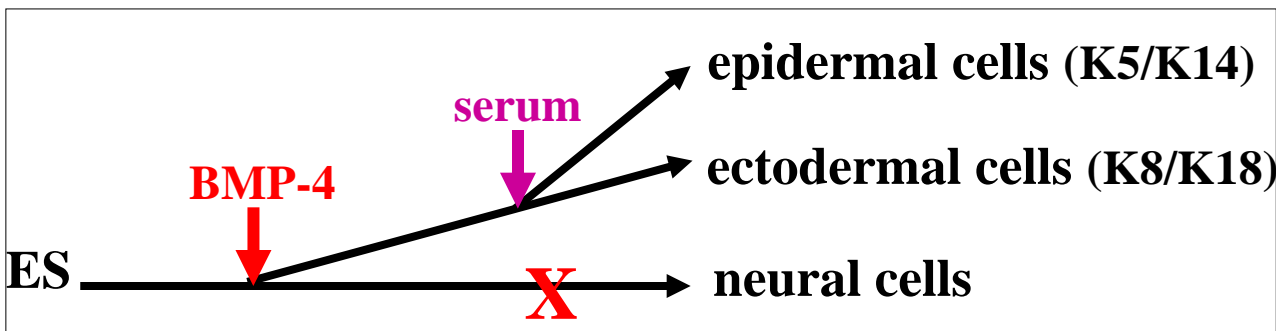
no

BMP4

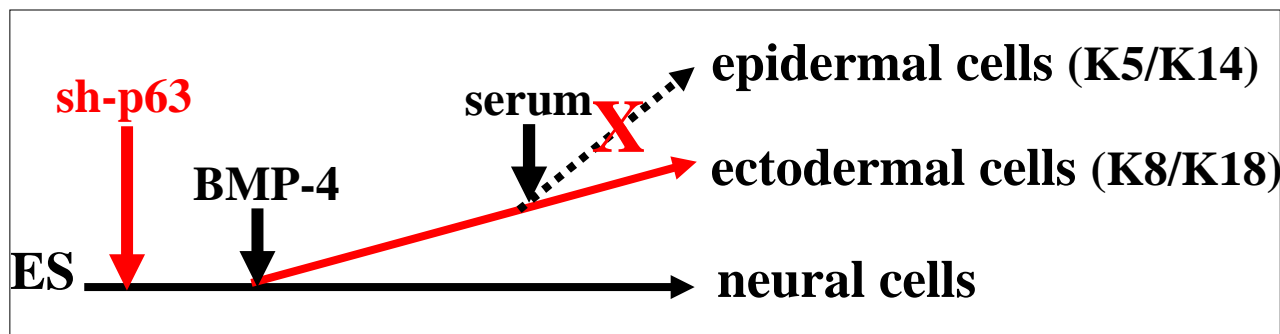
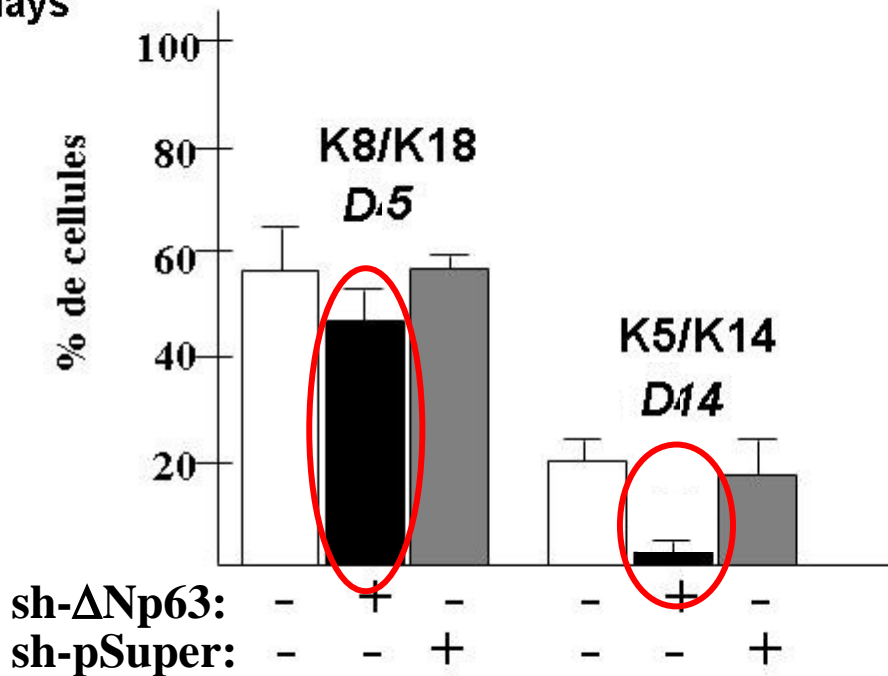
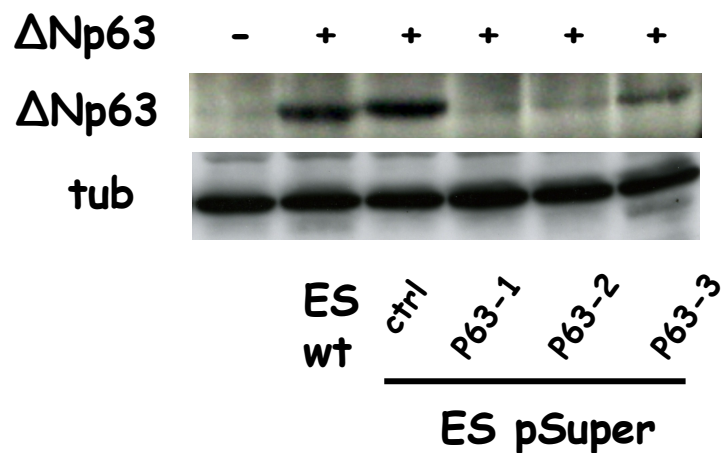
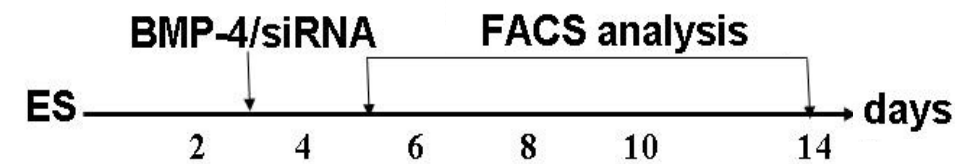
BMP4 + serum



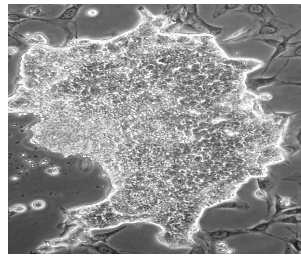
p63 is not necessary for ectodermal commitment but may be required for epidermal fate



# $\Delta$ Np63 is required for epidermal differentiation of ES cells

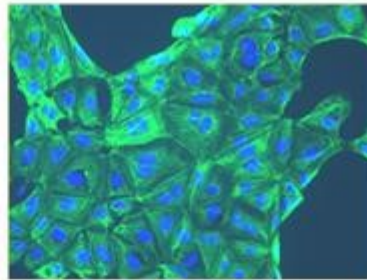


# $\Delta Np63\alpha$ is necessary to induce epidermal differentiation of ES-derived ectodermal cells



ES cells

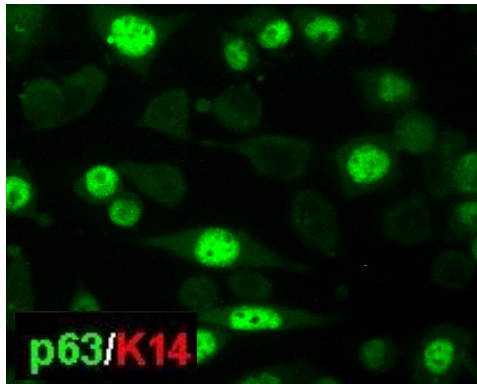
ECM  
BMP-4



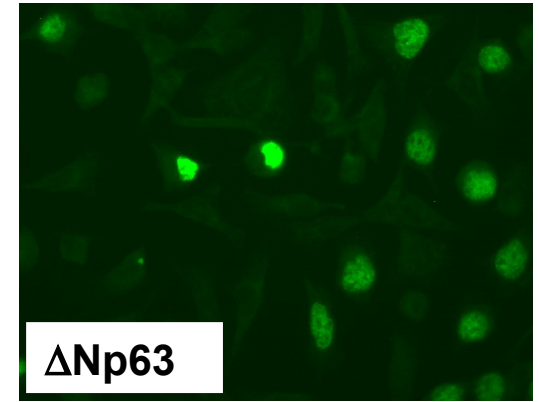
Ectodermal progenitors

TAp63

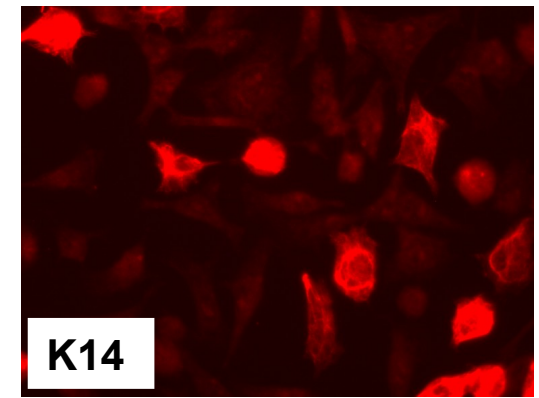
$\Delta Np63\alpha$



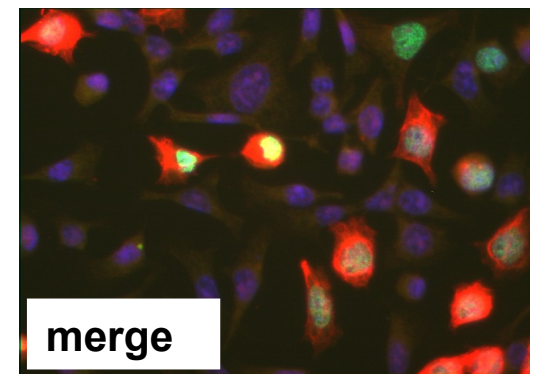
p63/K14



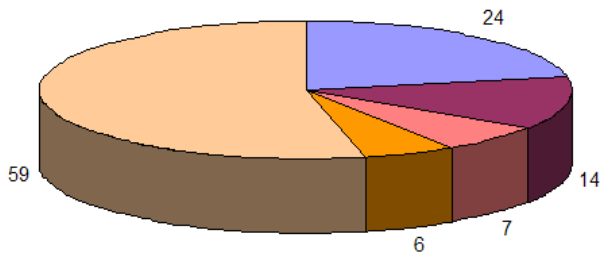
$\Delta Np63$



K14



merge

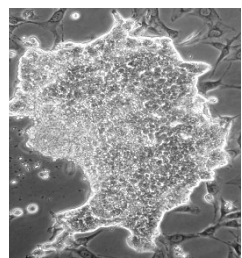


## Target genes

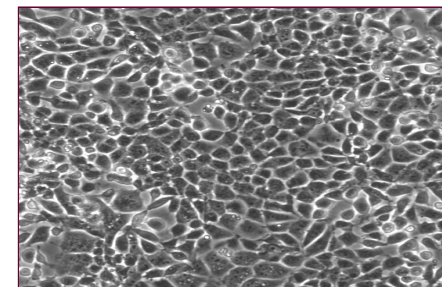
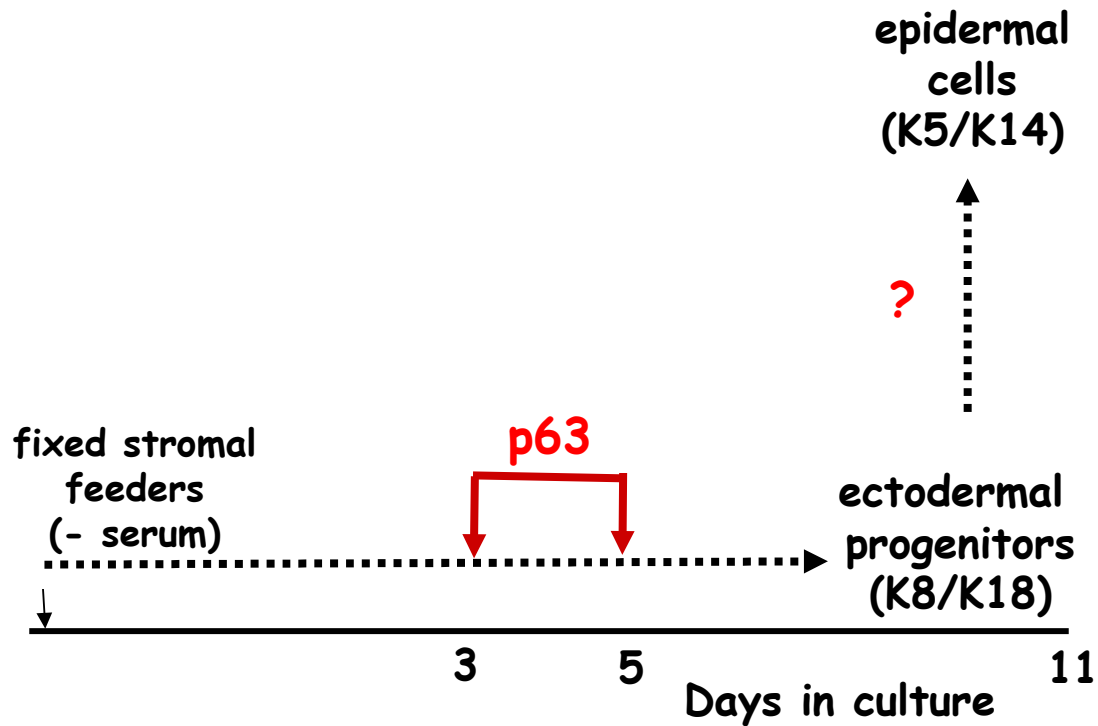
- |                |             |
|----------------|-------------|
| KGF-R          | miR 125     |
| BMP-7          | miR 328     |
| Notch          | miR 515-516 |
| IKK $\alpha$   | miR 720     |
| PERP           | .....       |
| ITGB4          |             |
| ITGA6          |             |
| Lamc2          |             |
| U3 ligase,.... |             |

**New function for p63 during embryonic development ?**

# Could p63 replace BMP-4 to induce epidermal fate?

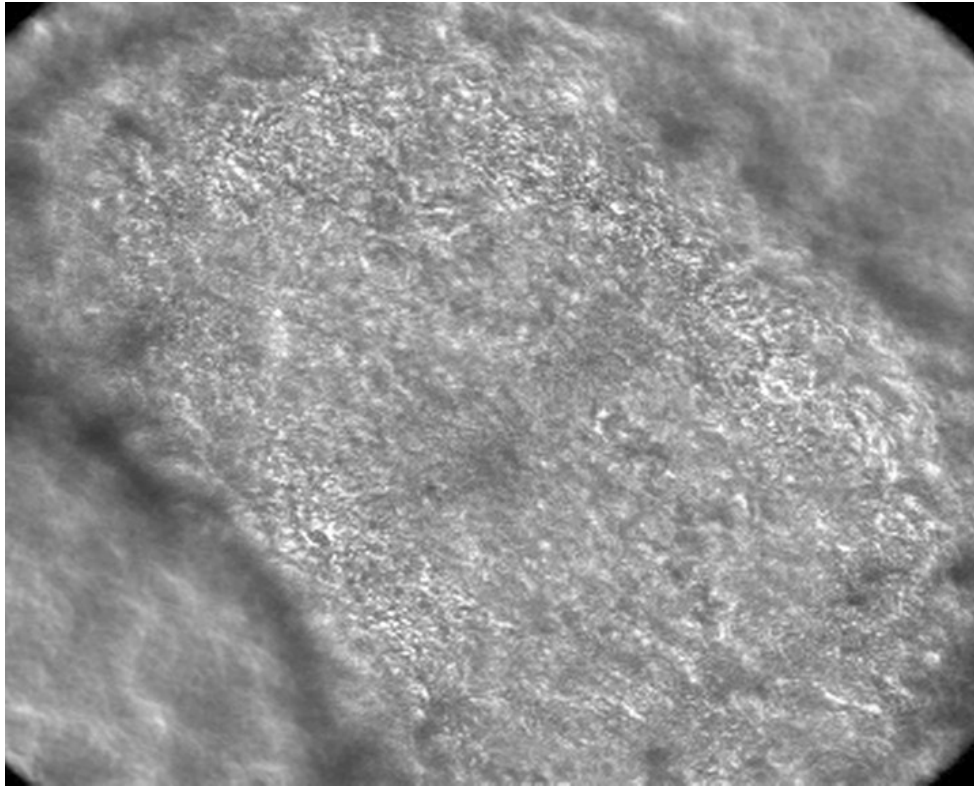


ES cells

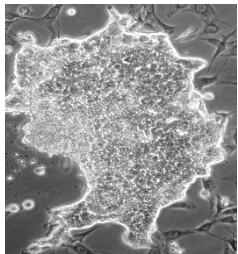


# p63 cannot replace BMP-4 to induce epidermal fate

## Link to cardiogenesis?



~~epidermal cells (K5/K14)~~



ES cells

fixed stromal feeders (- serum)

p63

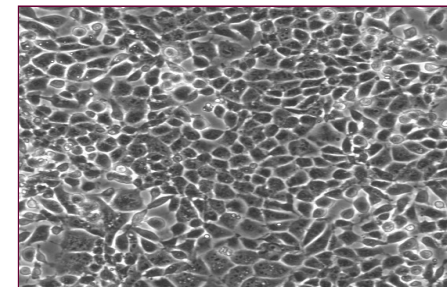
3

5

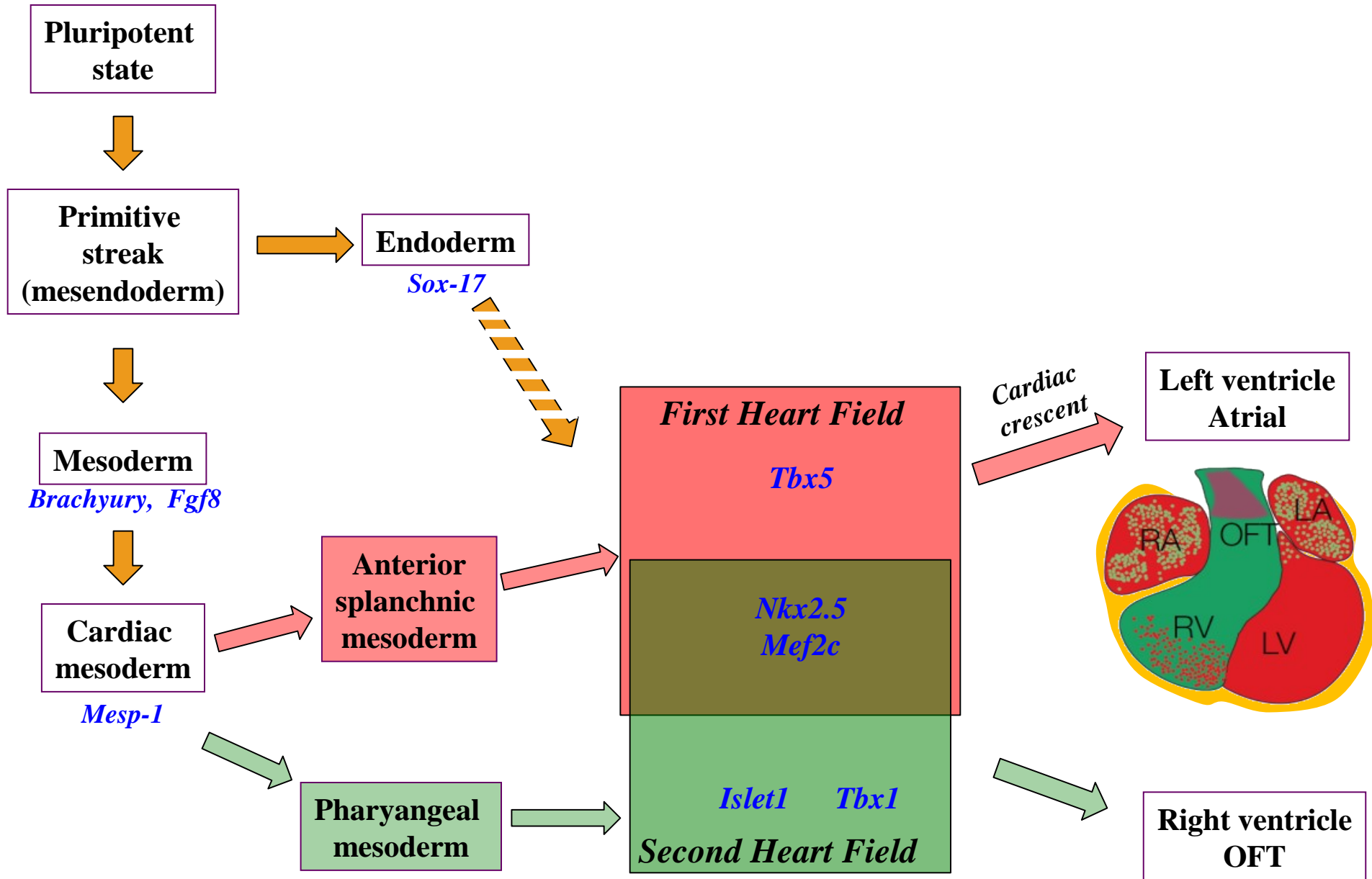
ectodermal progenitors (K8/K18)

11

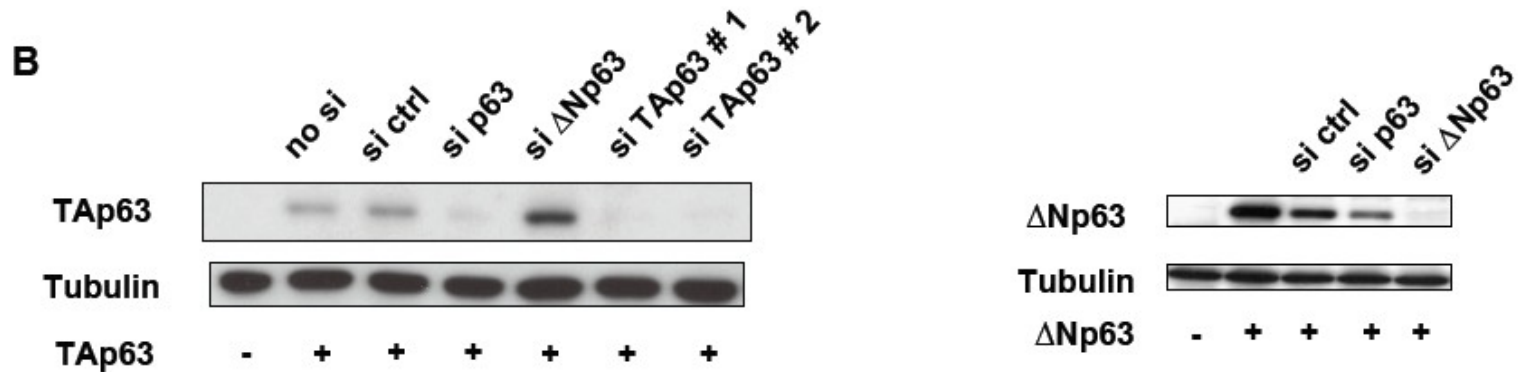
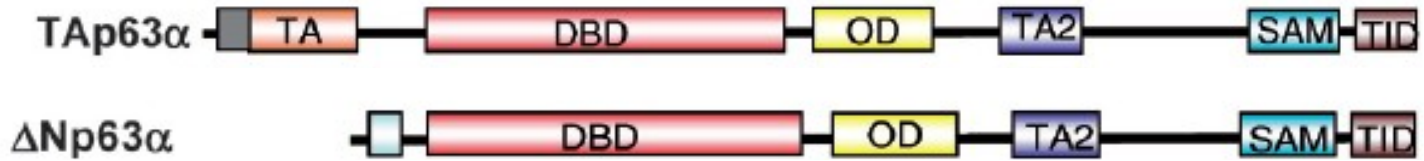
Days in culture



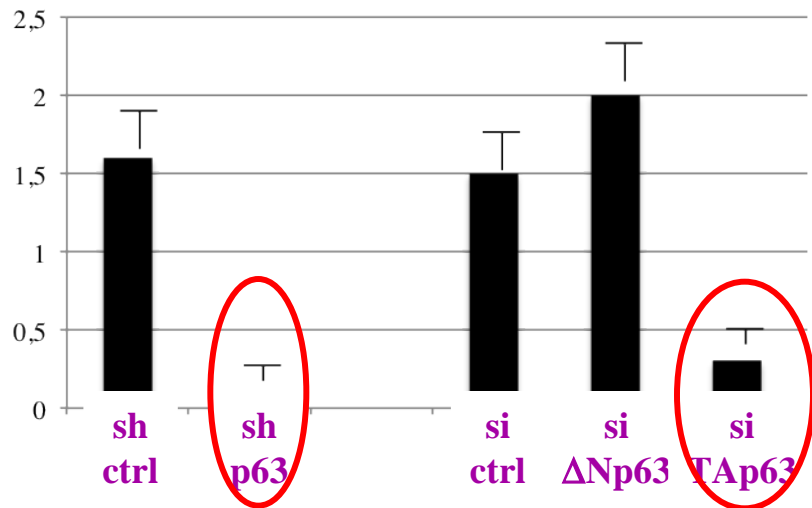
# The cardiac developmental pathways



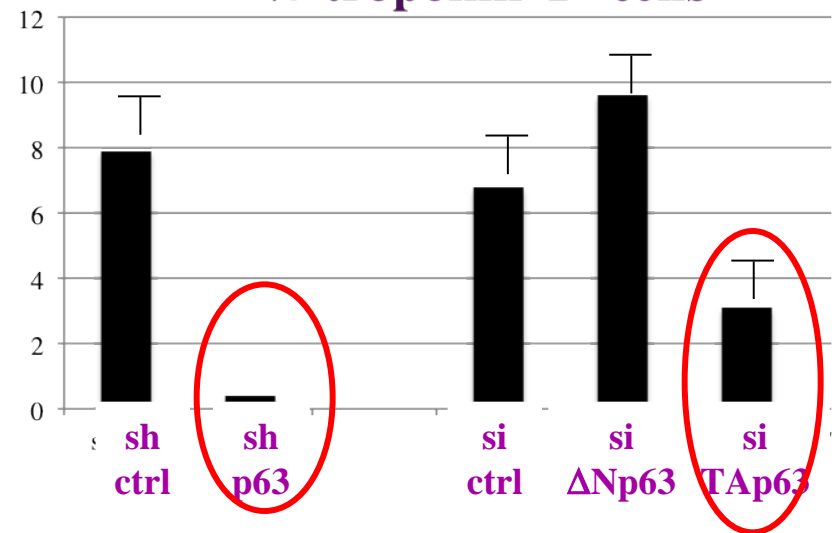
# TAp63 is required for *in vitro* cardiogenesis



Ratio beating area/EB



% troponin-T<sup>+</sup> cells



**Multipotent Cardiovascular progenitors**  
(Mesp1/2, sox-7<sup>+</sup>, Flk1)

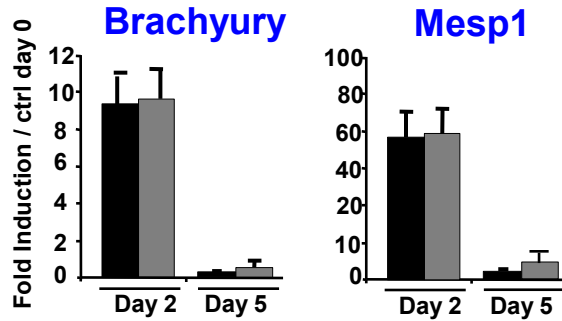
**Cardiac progenitors**  
(Nkx2.5, Tbx5, Mef2c, Isl1)

**Cardiac cells**  
(TpnT,  $\alpha$ -actinin)

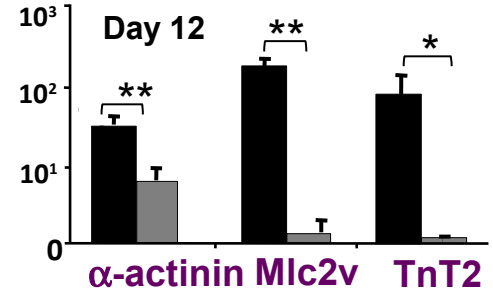
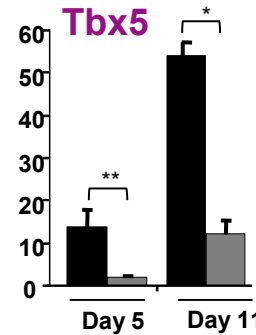
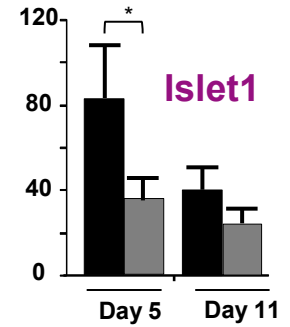
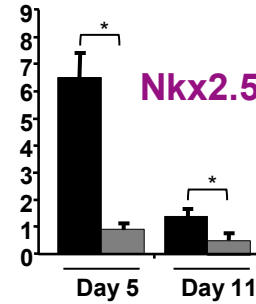
**Primitive Mesoderm**  
(Brachyury T)

*Primitive streak (EMT)*

**Epiblast**



■ sh-ctrl  
■ sh-p63

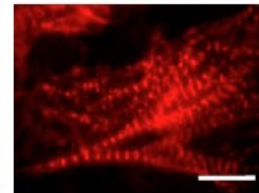
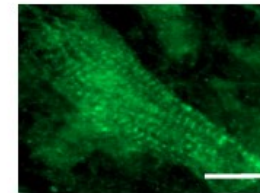


**Which cells are producing p63?**

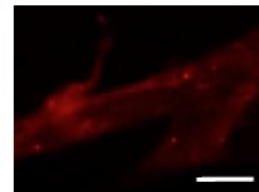
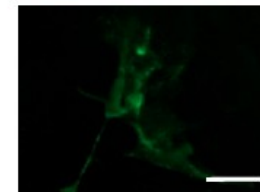
sh-ctrl

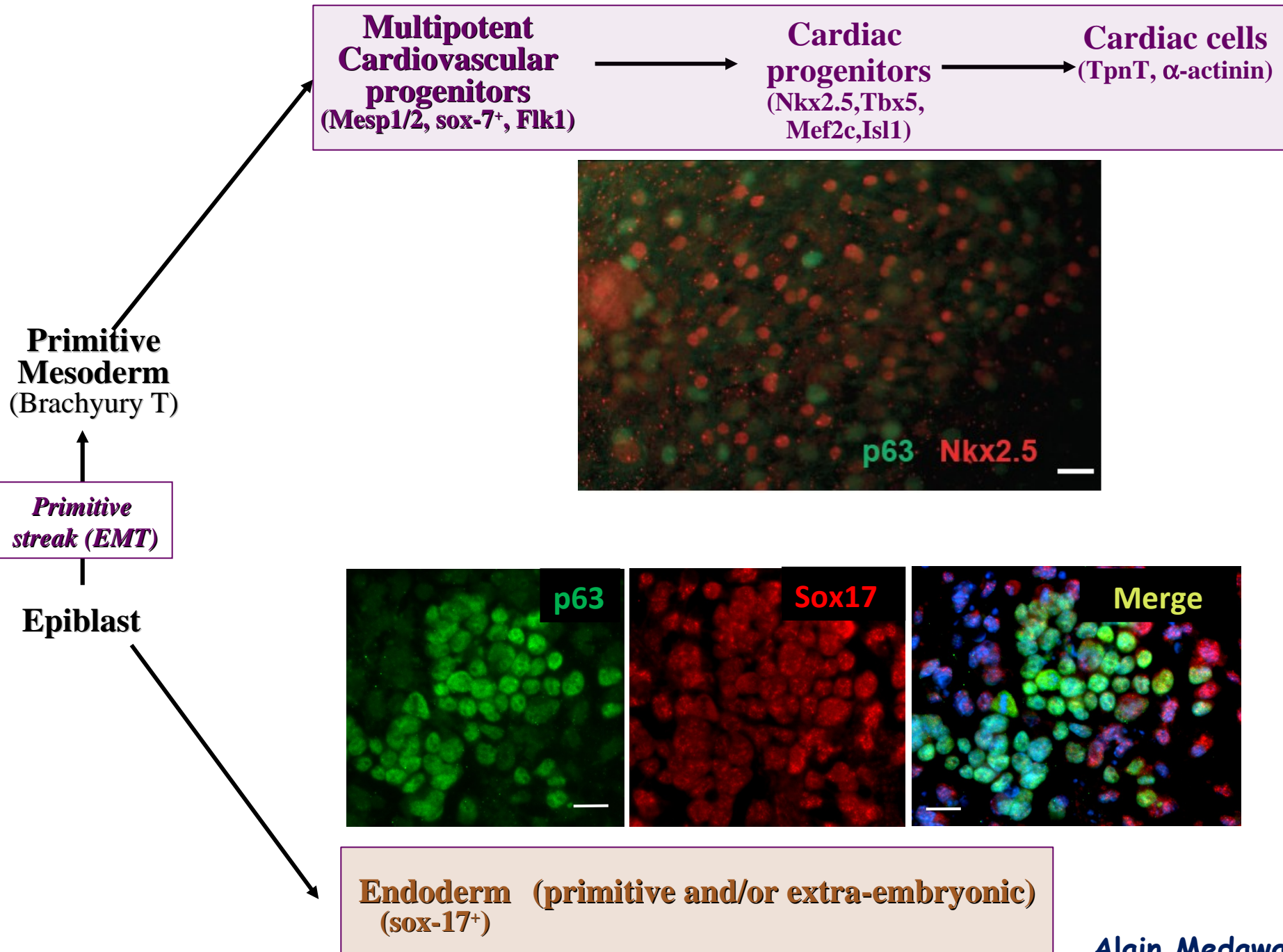
MLC2v

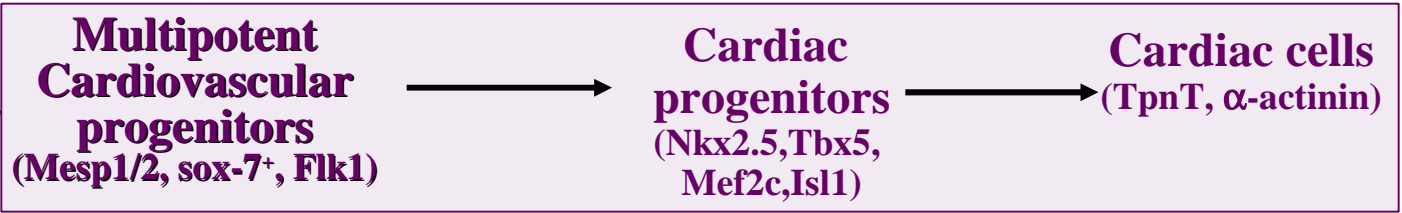
$\alpha$ -actinin



sh-p63



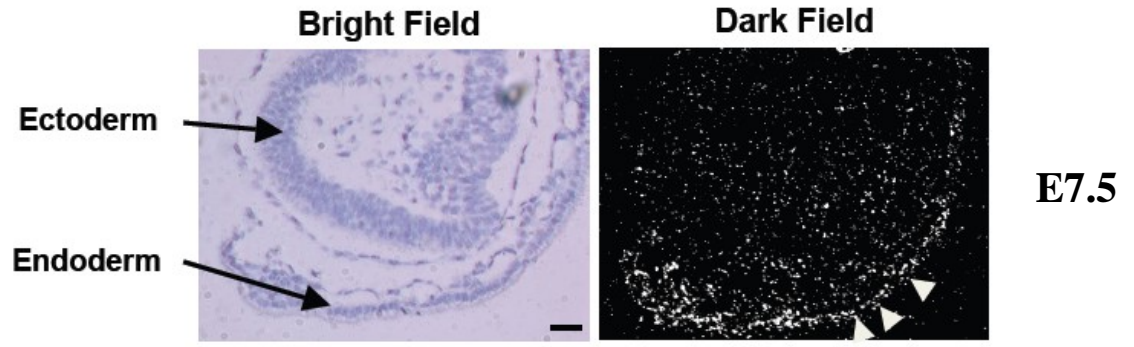




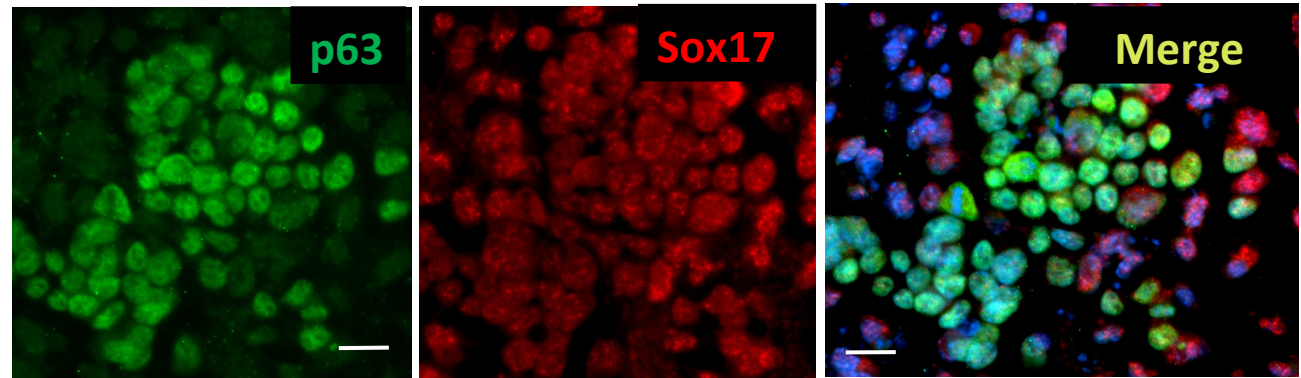
**Primitive Mesoderm**  
(Brachyury T)

*Primitive streak (EMT)*

**Epiblast**

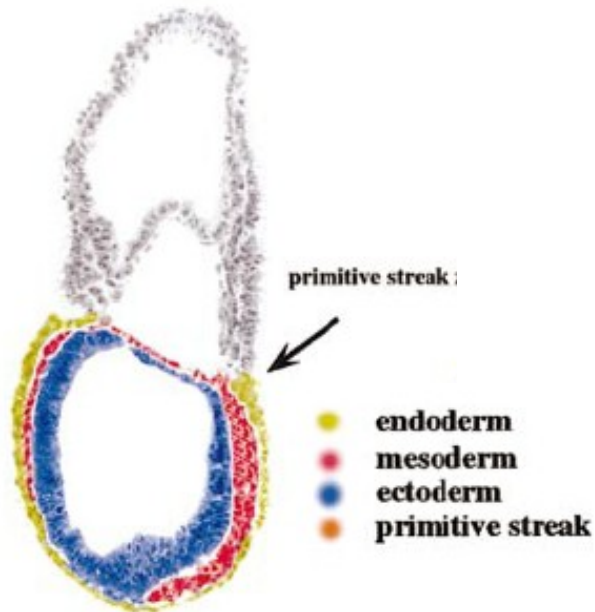
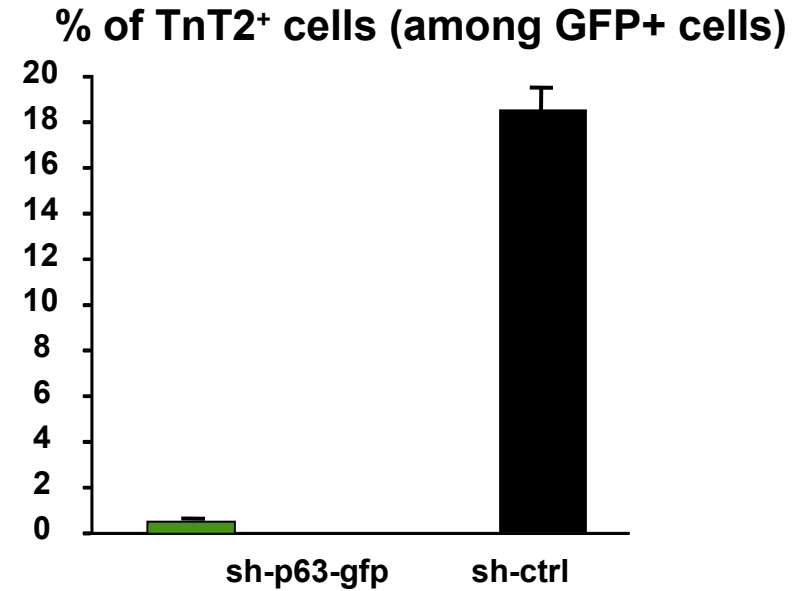
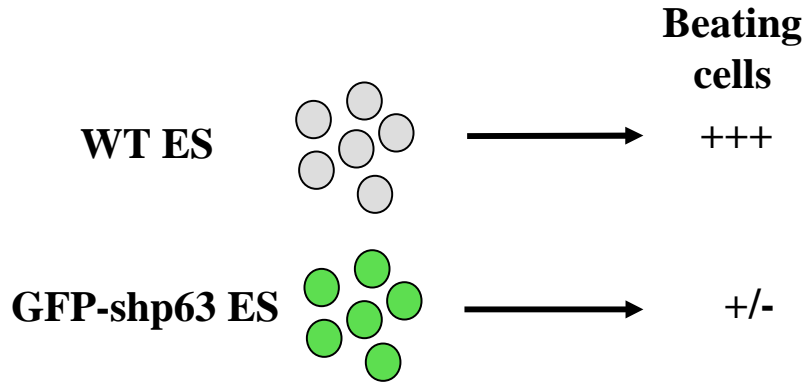


Marja L Mikkola, Stockholm)

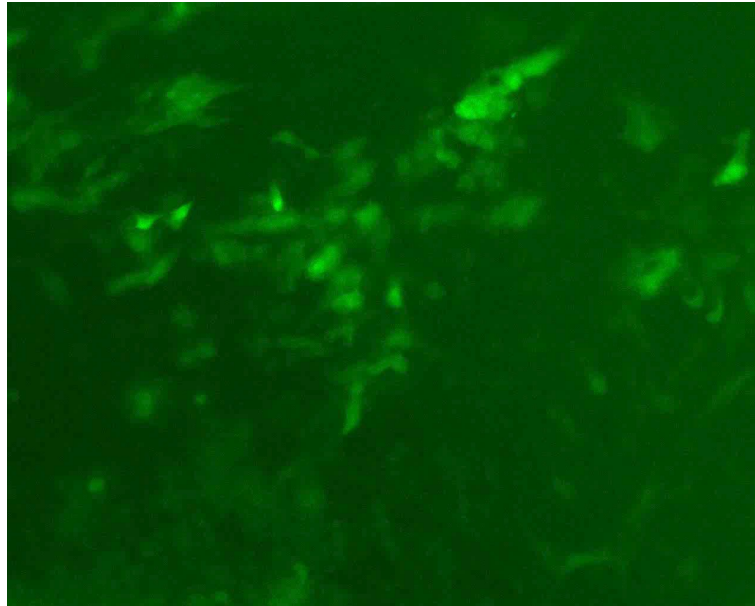
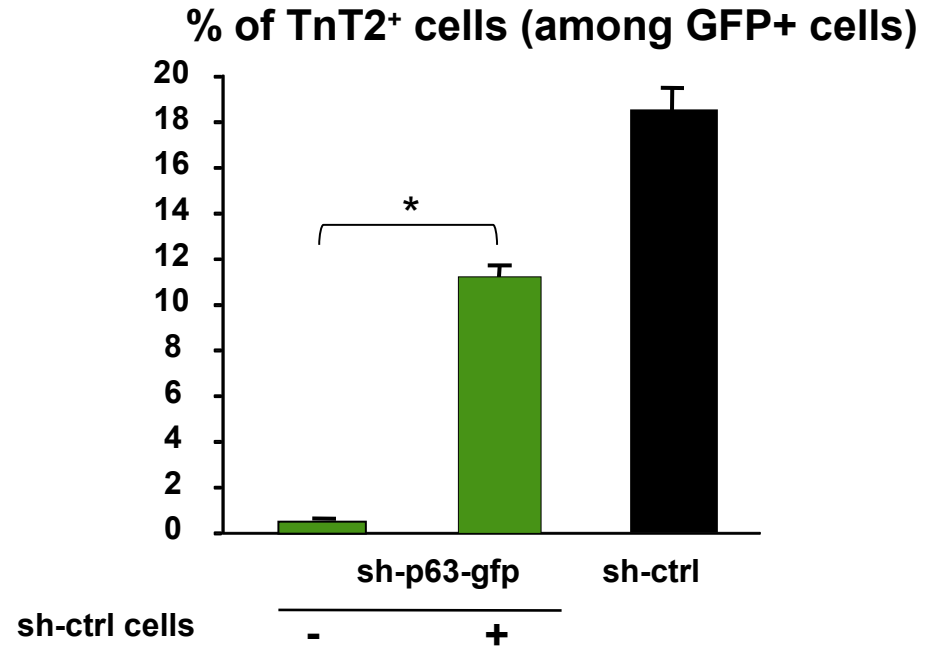
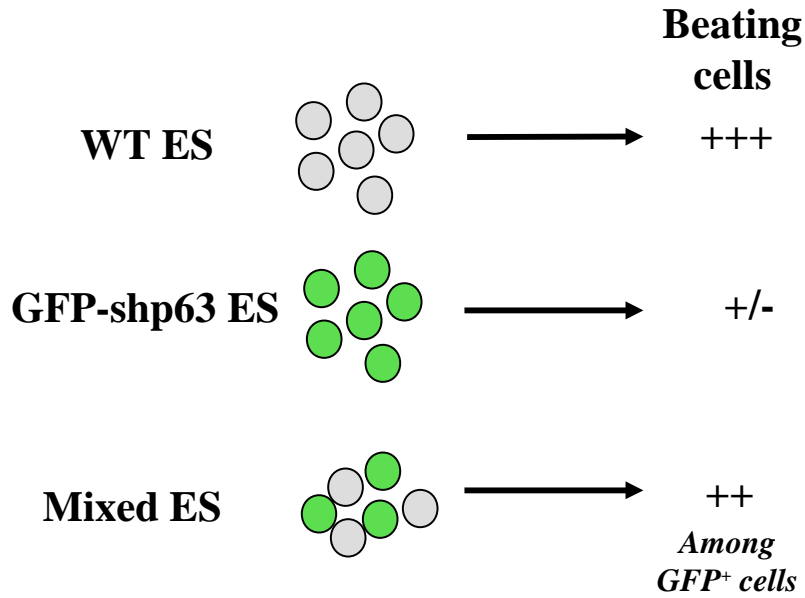


**Endoderm (primitive and/or extra-embryonic)**  
(sox-17<sup>+</sup>)

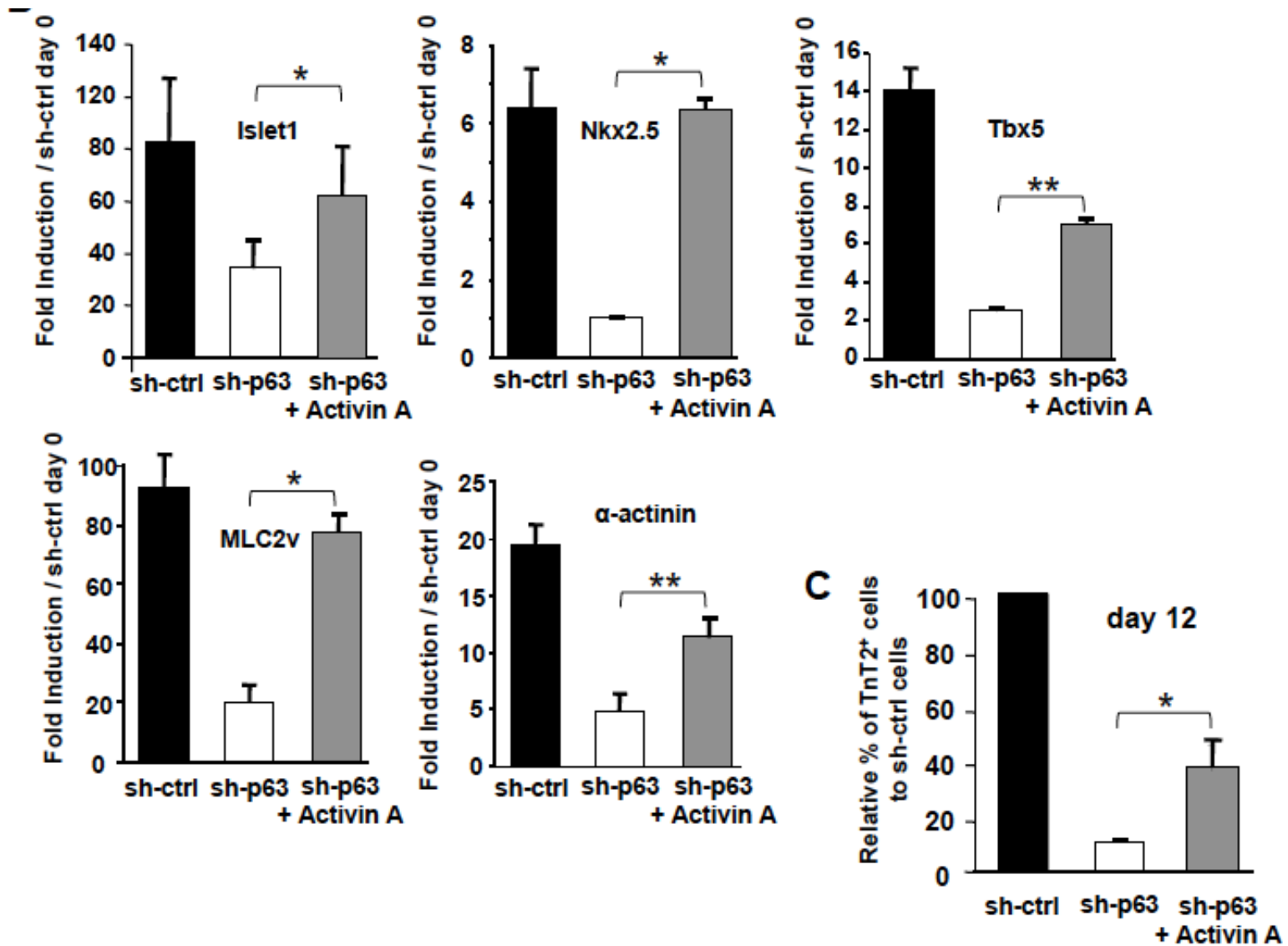
# Cell non-autonomous effect of p63 on cardiogenesis



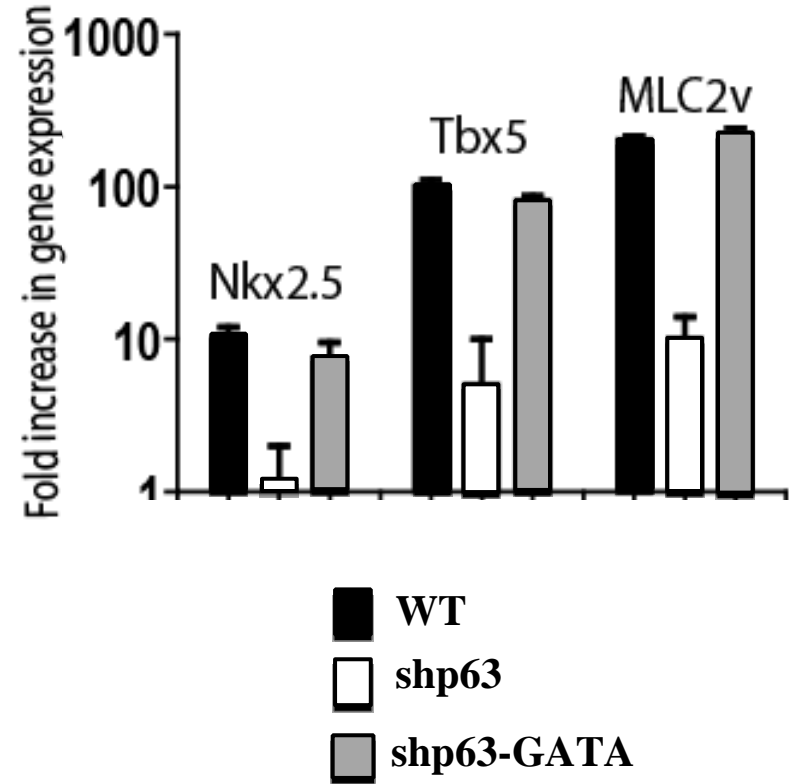
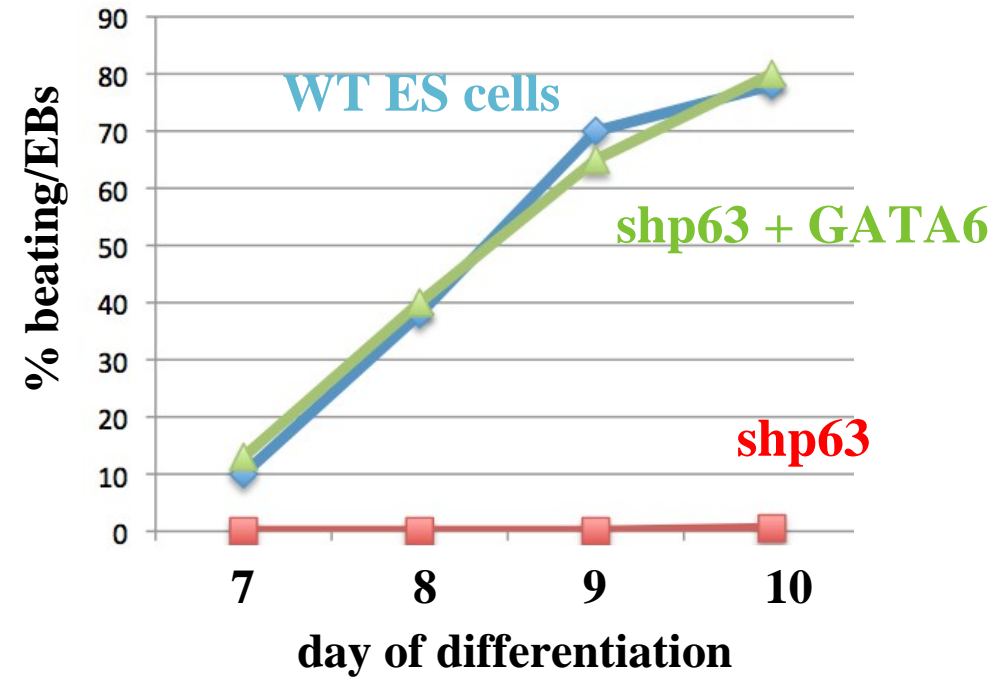
# Non-cell autonomous effect of p63 on cardiogenesis



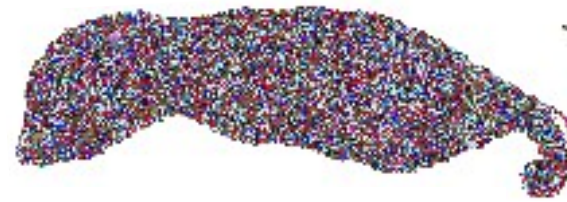
# Cardiac rescue by exogenous Activin-A



# Cardiac rescue by ectopic GATA6

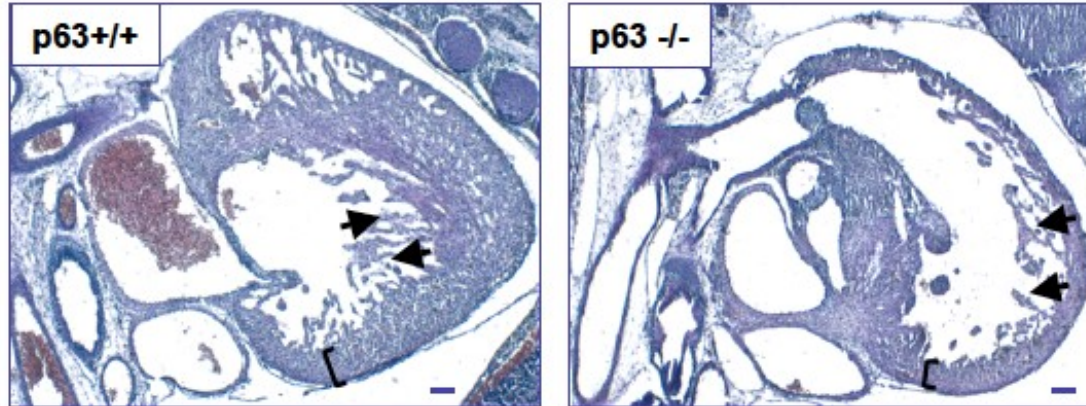


# p63 KO mice

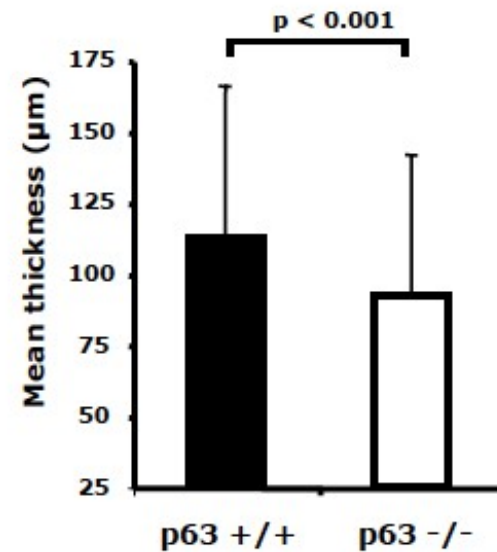
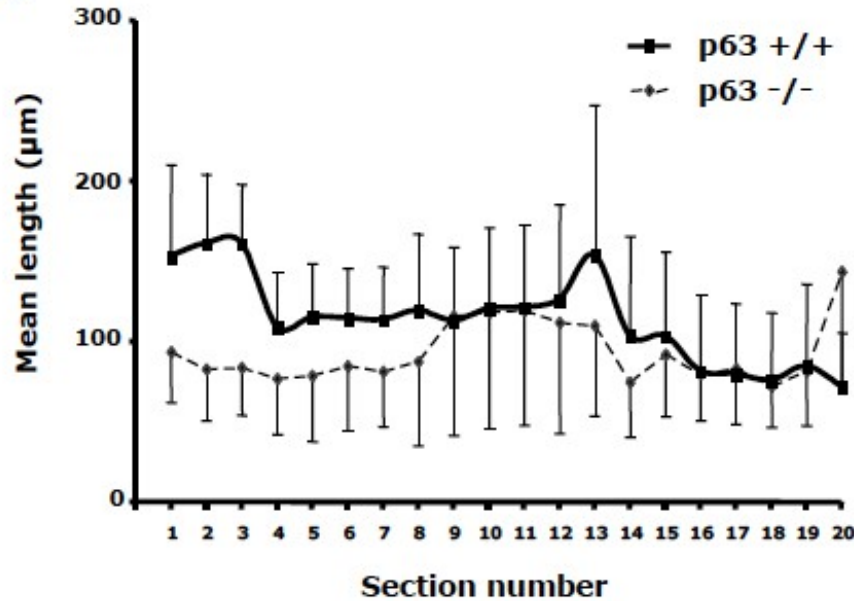


(Yang, Nature 1999; Mills, Nature 1999)

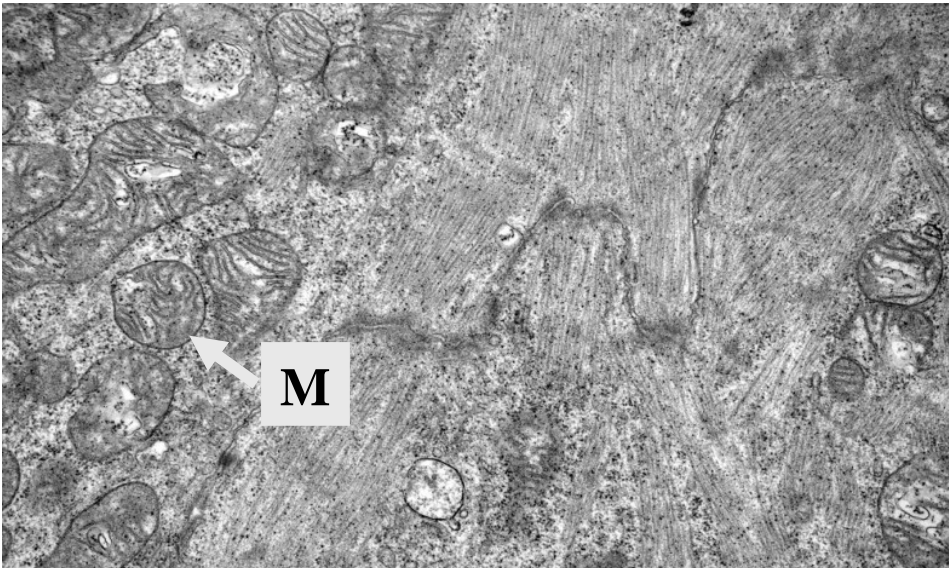
### A



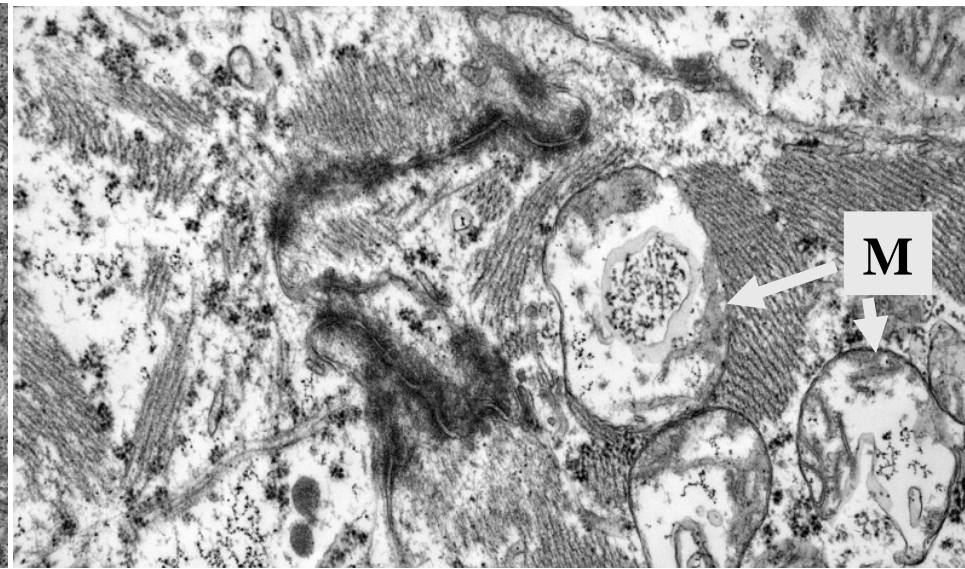
### B



wild type newborn heart tissue

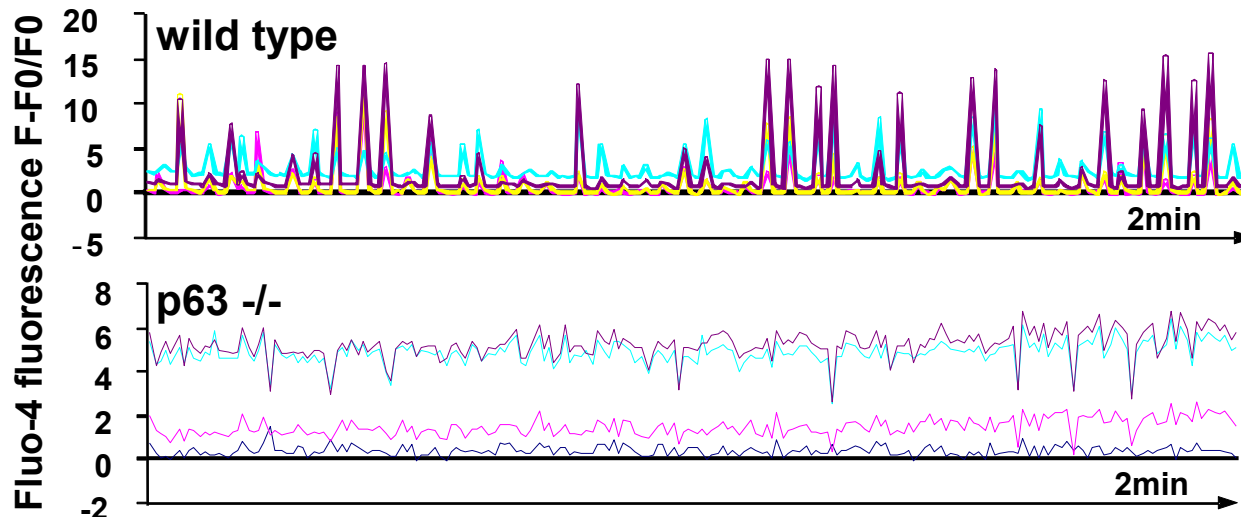


p63<sup>-/-</sup> newborn heart tissue



Myofibrillar fragmentation and disorganization.  
Mitochondria were randomly distributed, large and swollen

Intracellular Ca<sup>2+</sup> transient in embryonic hearts (E10.5)

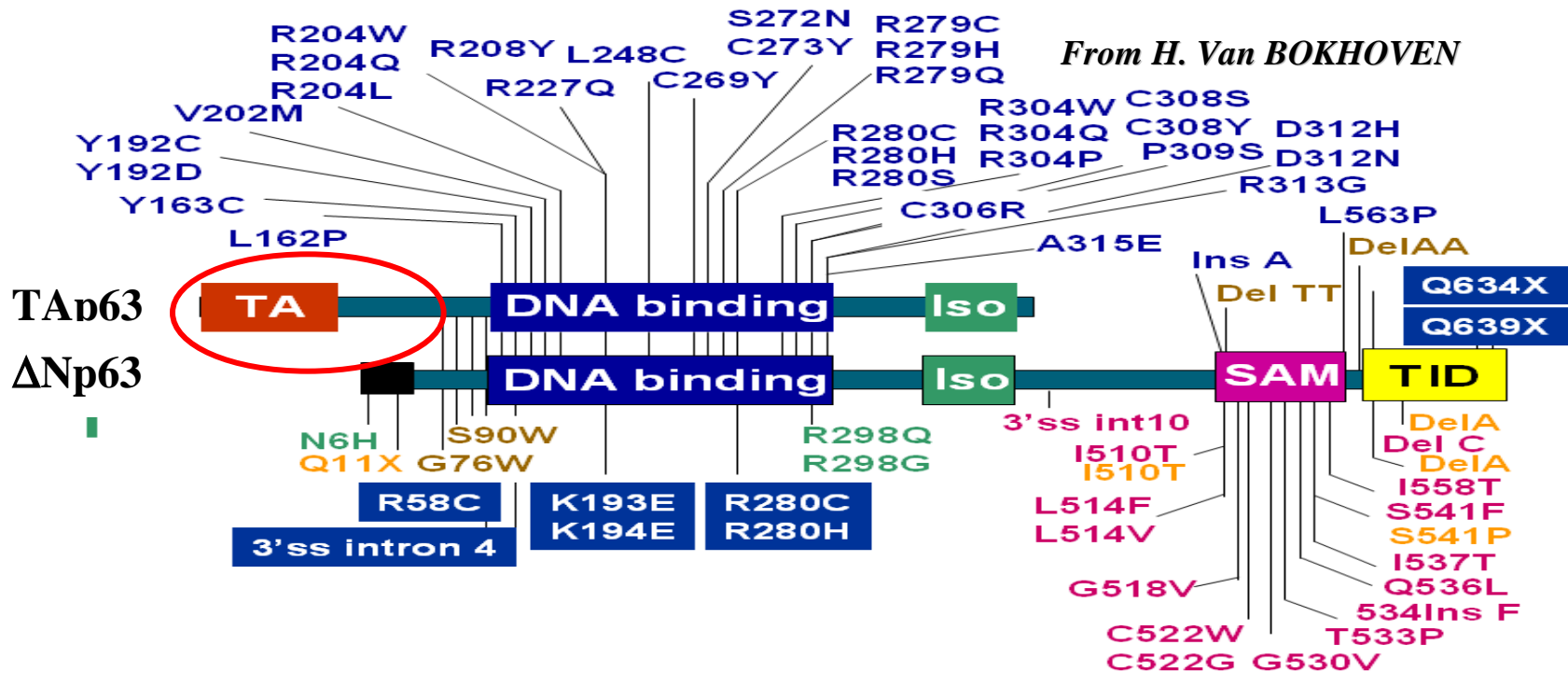


# Could p63-related ED patients suffer from unexpected heart defect?

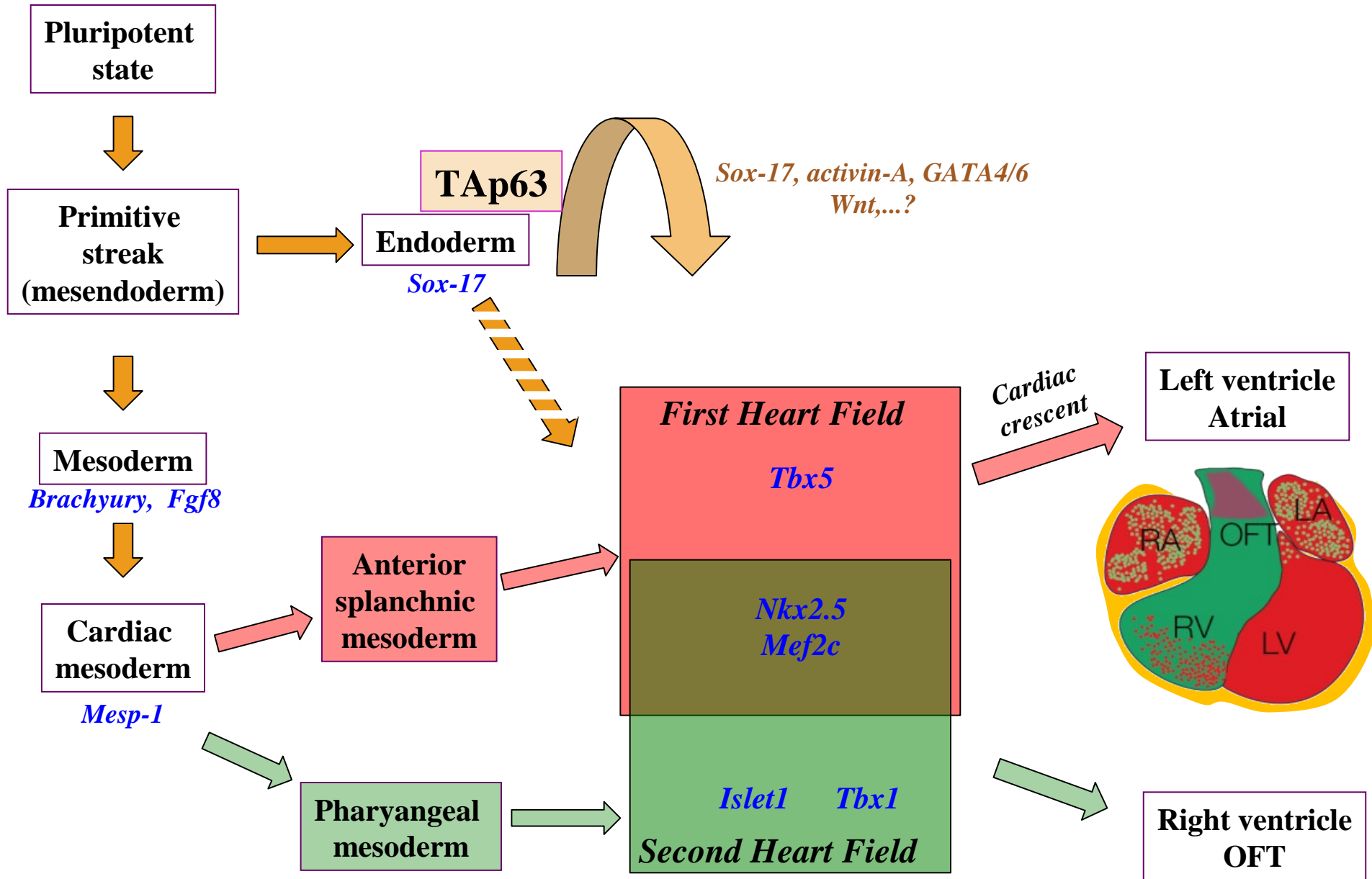
European Journal of Medical Genetics xx (2008) 1–4

R298Q mutation of *p63* gene in autosomal dominant ectodermal dysplasia associated with arrhythmogenic right ventricular cardiomyopathy

Rinne T. et al. (2008) Human Mol Genet. 17: 1968-77:  
 Patient 1 with a systolic murmur  
 Patient 3 with an atrial septal defect



# The cardiac developmental pathways



# The cardiac developmental pathways

