

COURS CELULES SOUCHES (D. Aberdam) 2010

Ma 2/3: cours 1: Définitions et généralités sur les CS

Ve 5/3: cours 2: CS somatiques et plasticité

Ma 8/3: cours 3: CS hématopoétiques (Dr. Rouleau)

Ve 12/3: cours 4: CS embryonnaires

Ma 16/3: Partiel 1

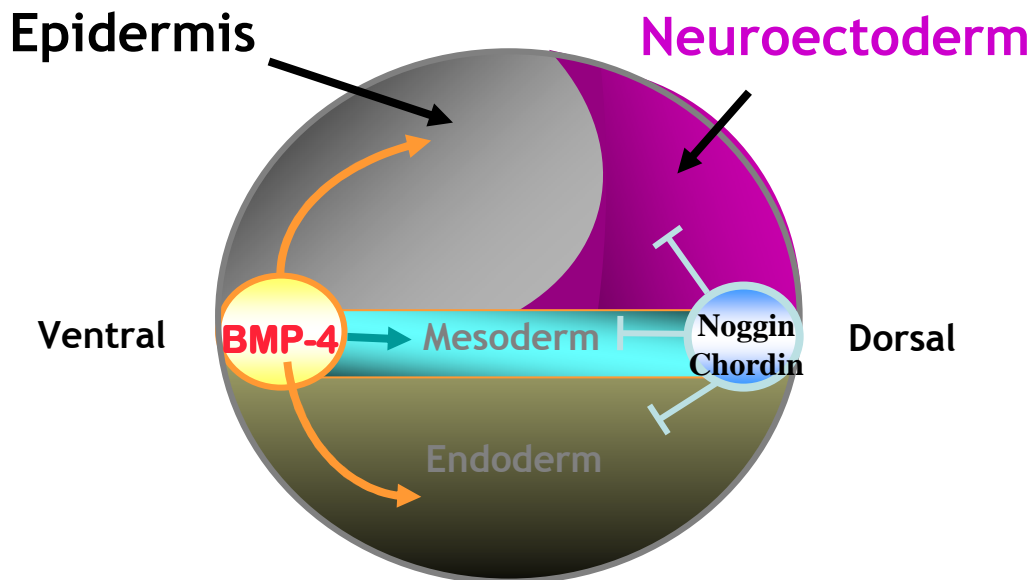
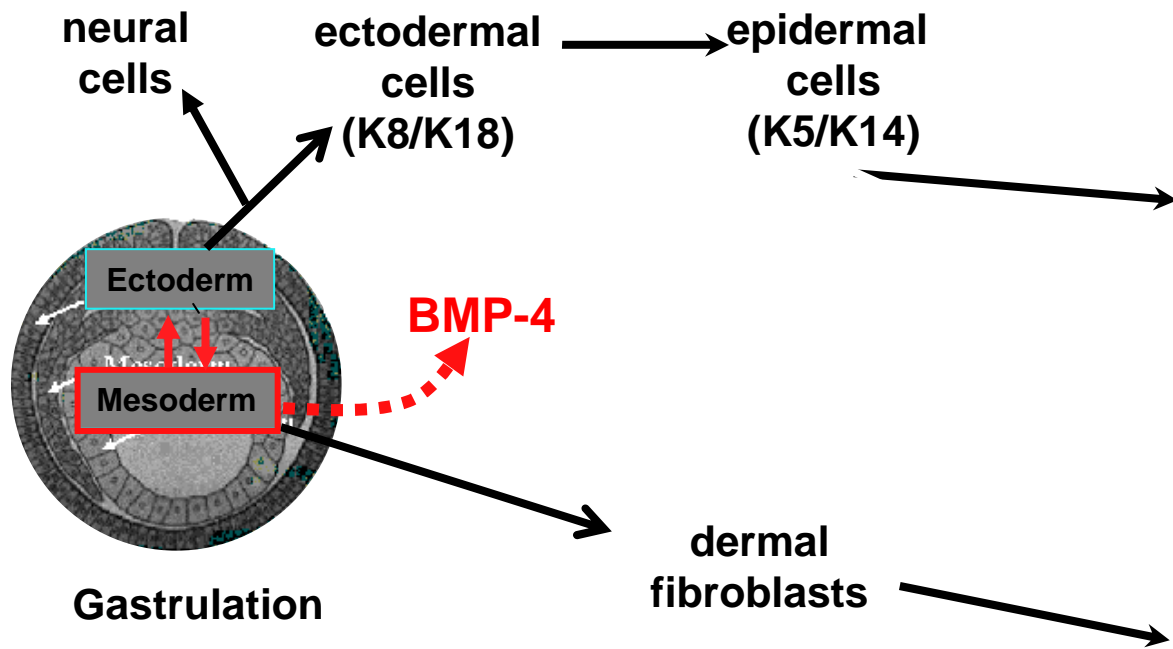
Ve 19/3: cours 5: CS adultes et physiopathologie

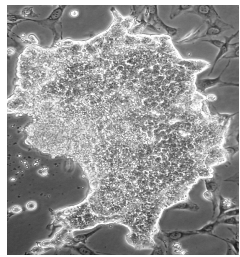
Ma 23/3: cours 6: CS reprogrammées (iPS) + labo

Ve 26/3: Cours 7: CS tumorales

Ma 30/3: Partiel 2

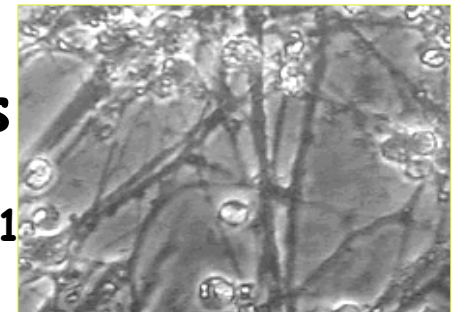
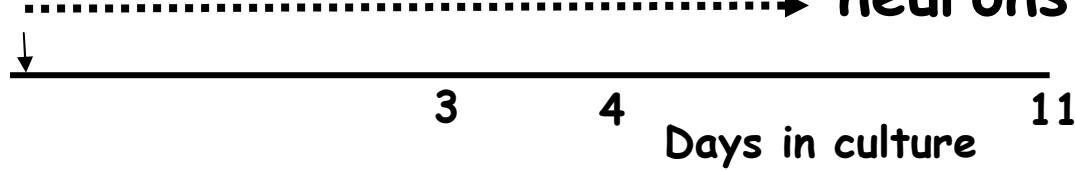
Examen: mi-mai





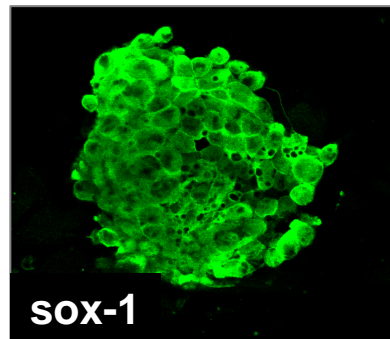
ES cells

stromal
fixed-feeders
(- serum)

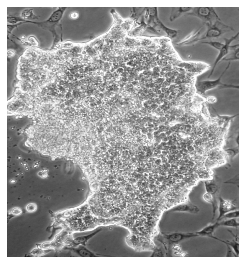


**Neural
precursors
(sox-1⁺)**

**Neural cells
(most TH⁺)**

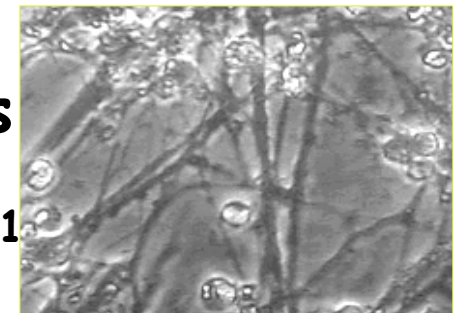
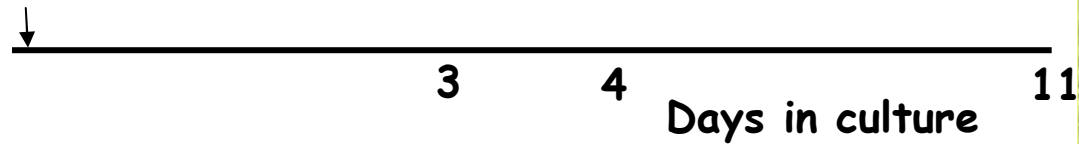


sox-1

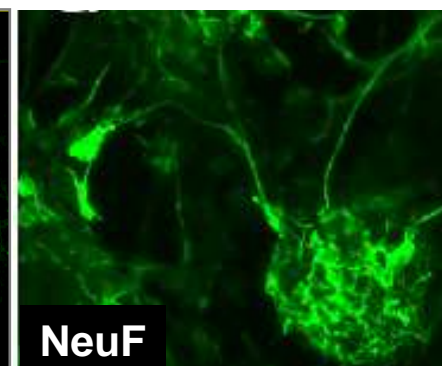
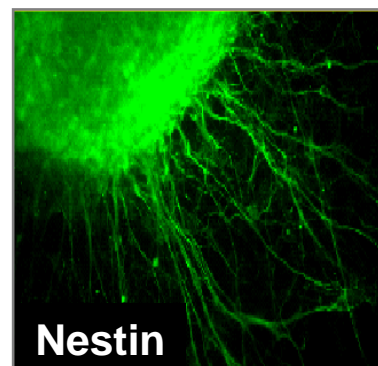
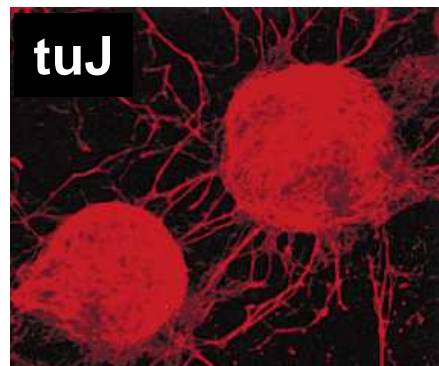


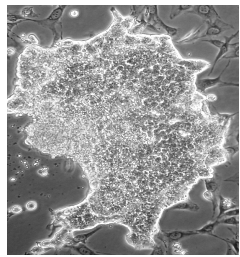
ES cells

stromal
fixed-feeders
(- serum)



**Neural cells
(most TH⁺)**



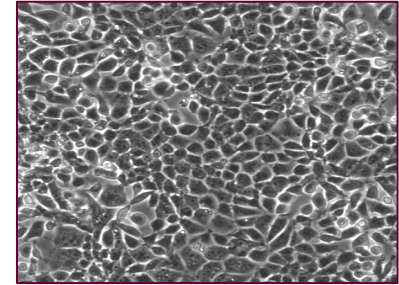


ES cells

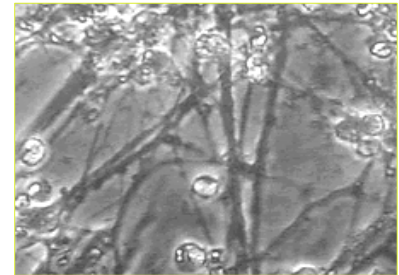
mesenchymal
fixed-feeders
(- serum)

BMP-4

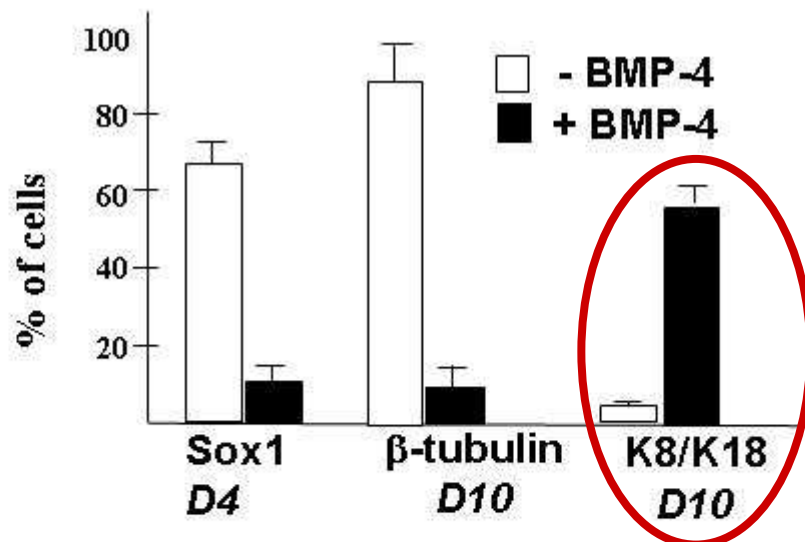
K18



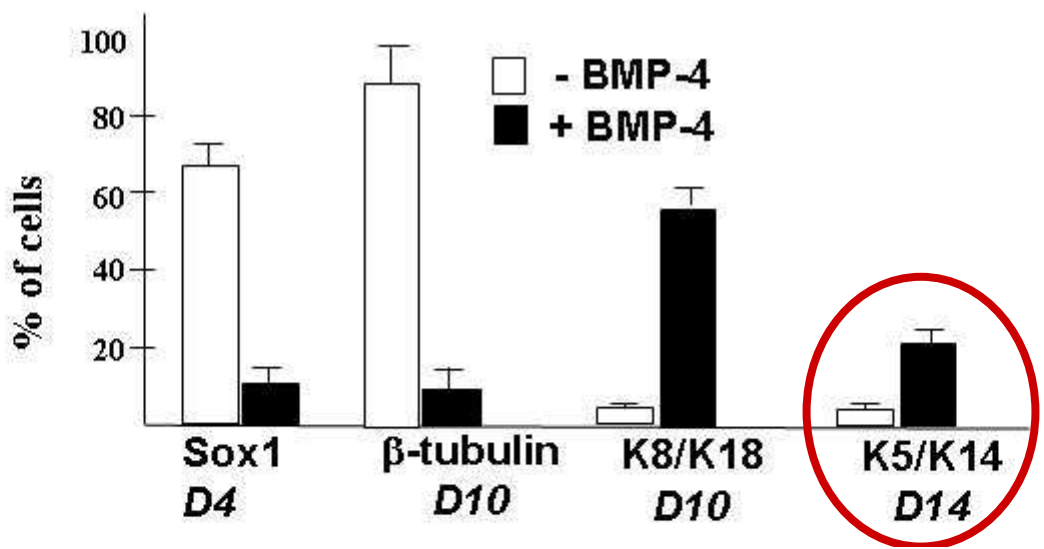
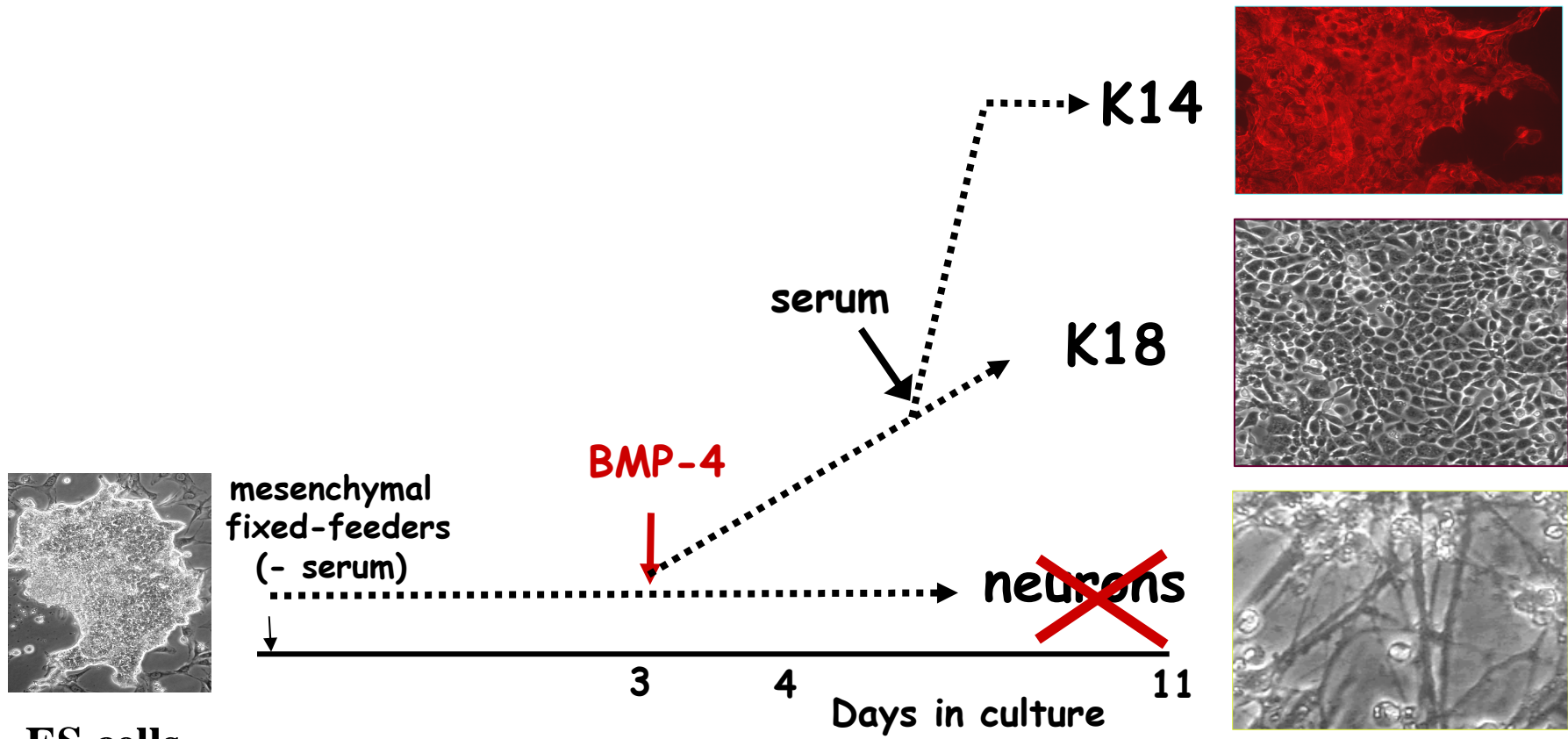
~~neurons~~



3 5 11
Days in culture



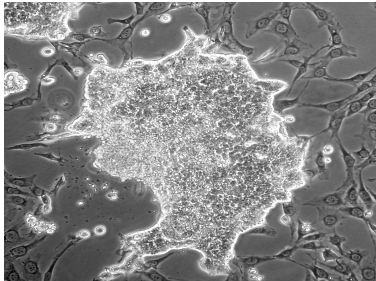
~~Neural cells
(NeuN, NeuroF
Nestin, β -III tubulin)~~



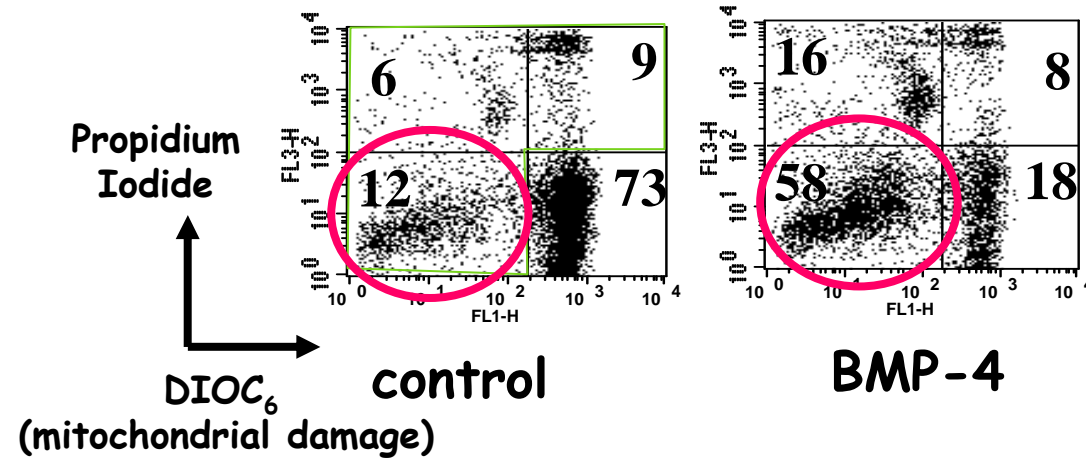
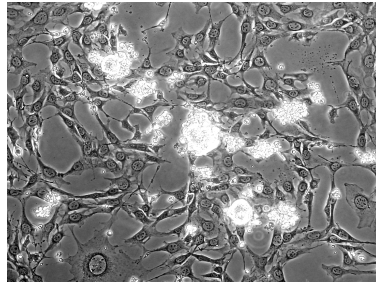
~~Neural cells~~
 (NeuN, NeuroF
 Nestin, β -III tubulin)

BMP-4 induces ES cell death by apoptosis

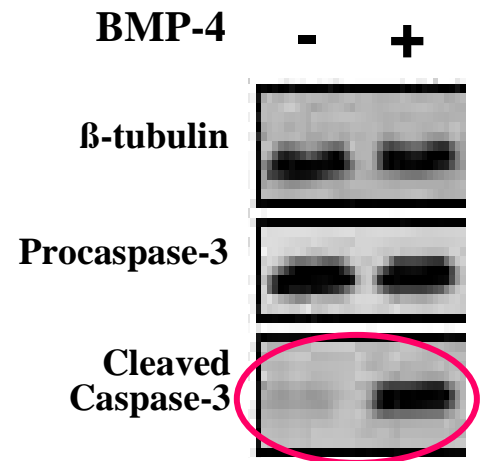
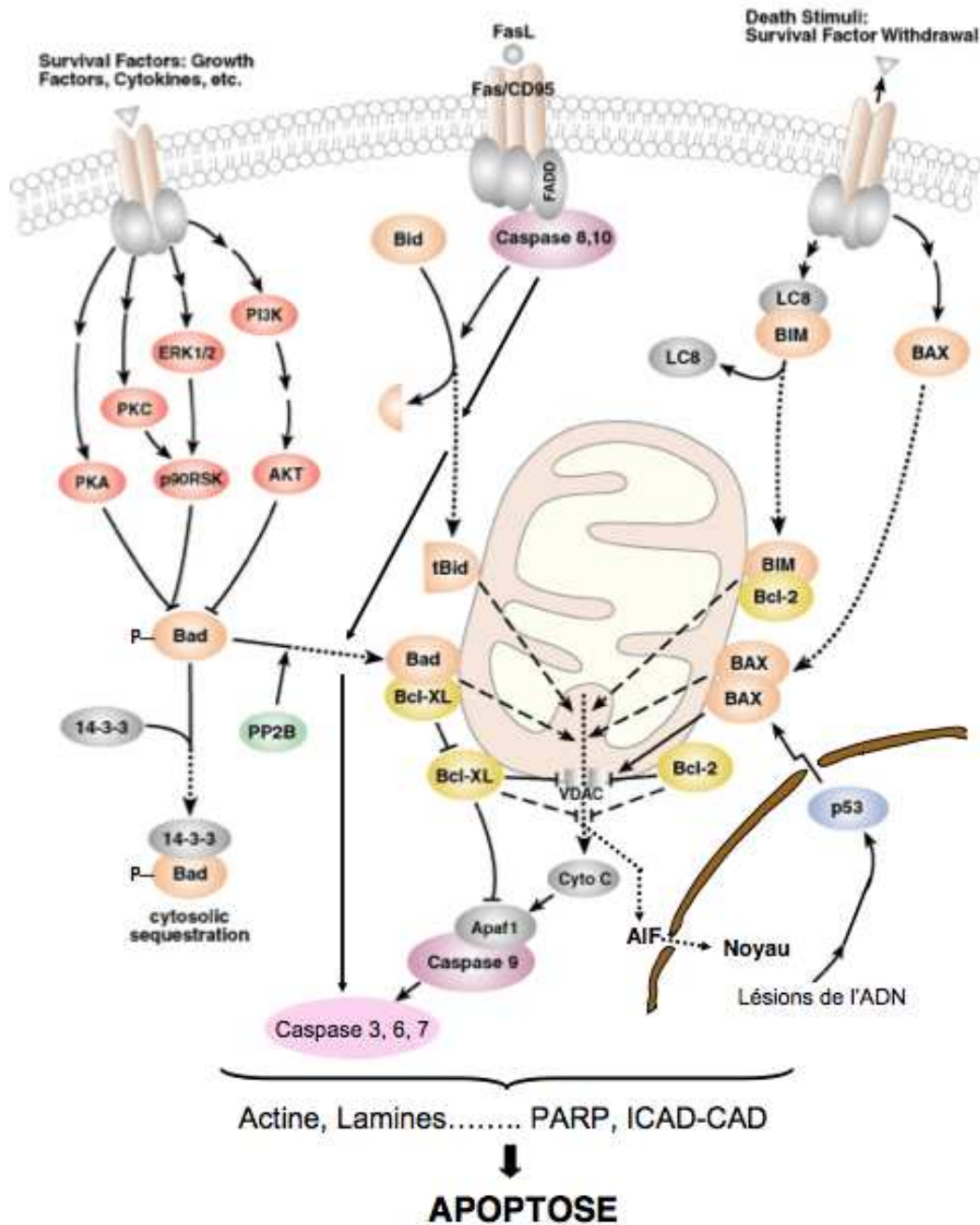
Control



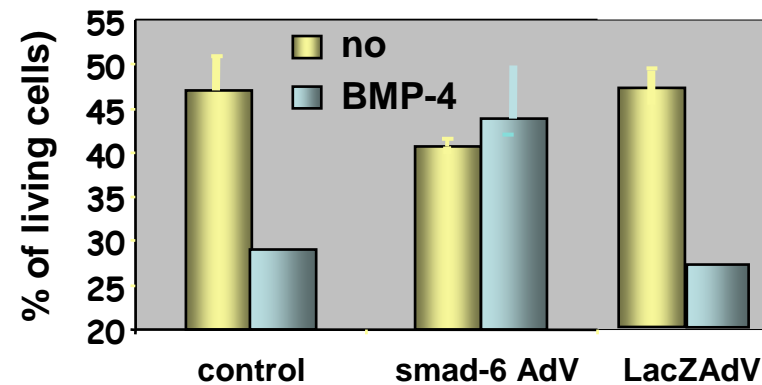
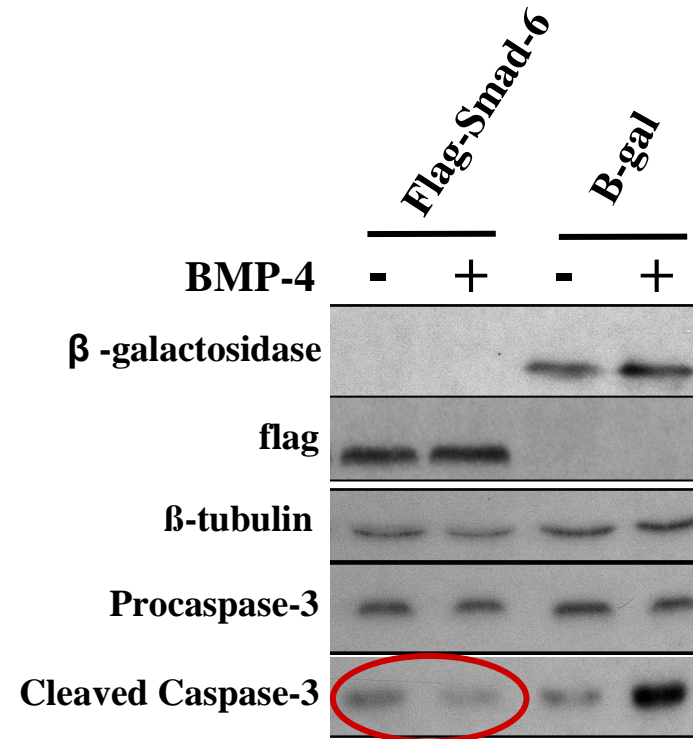
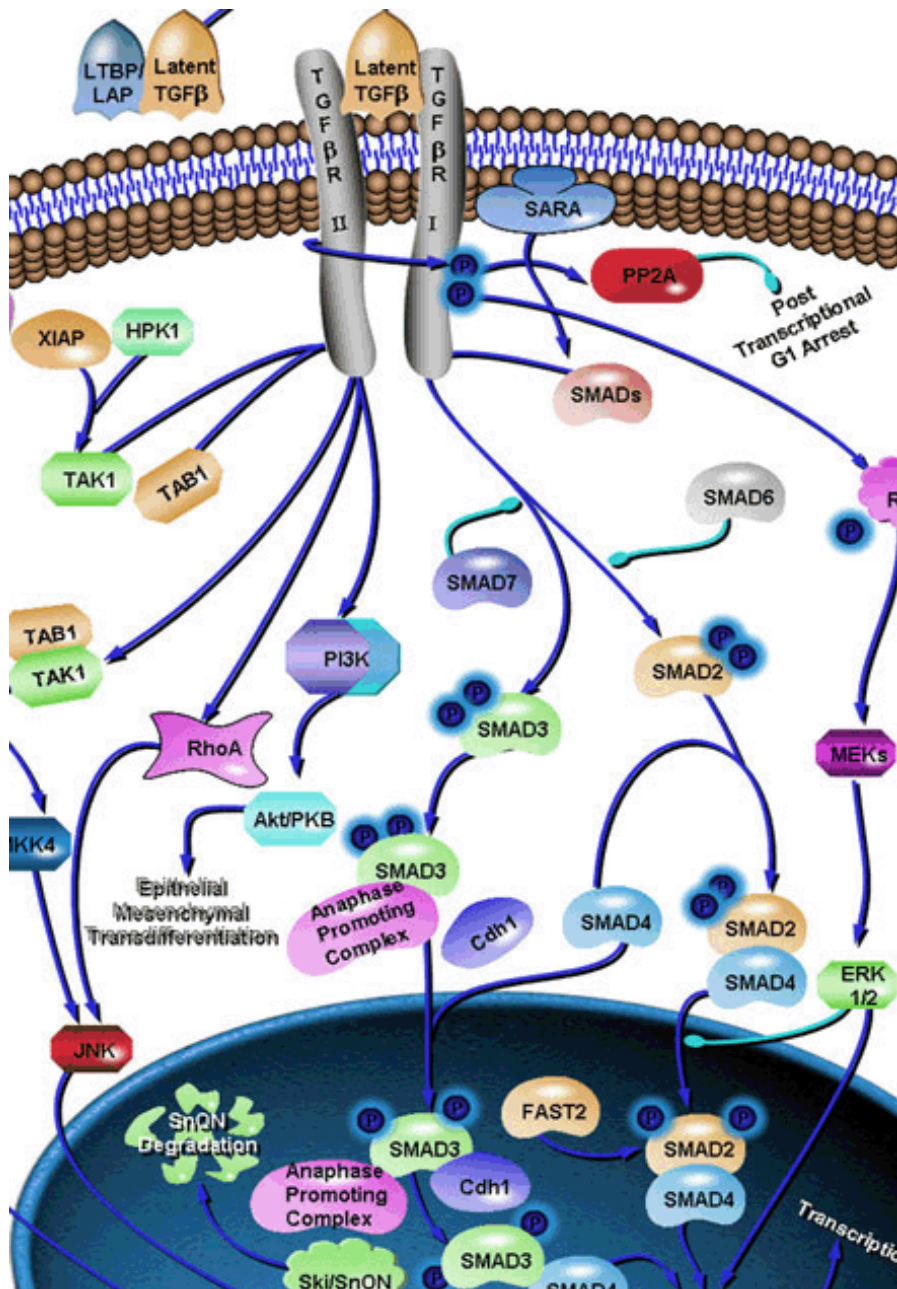
BMP-4

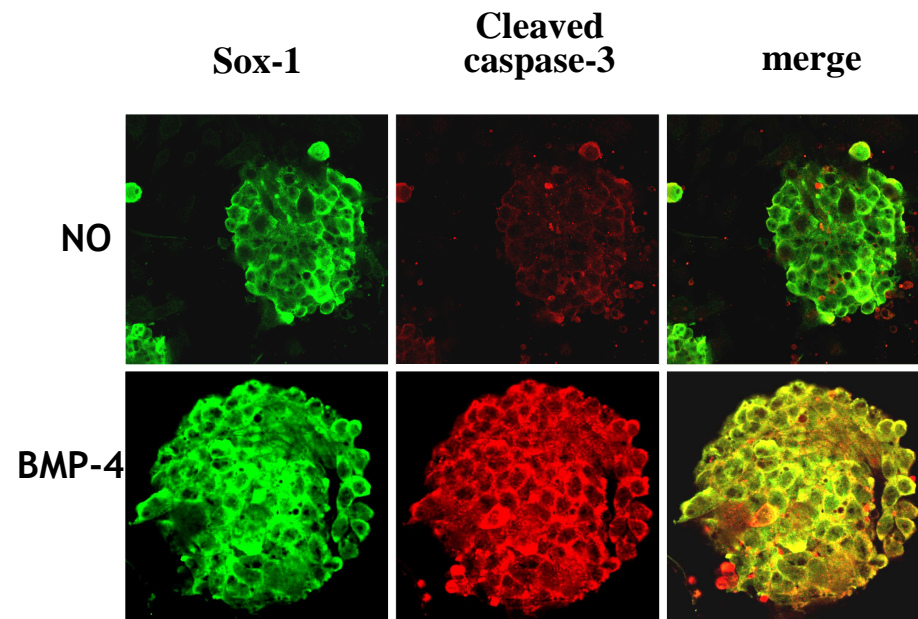
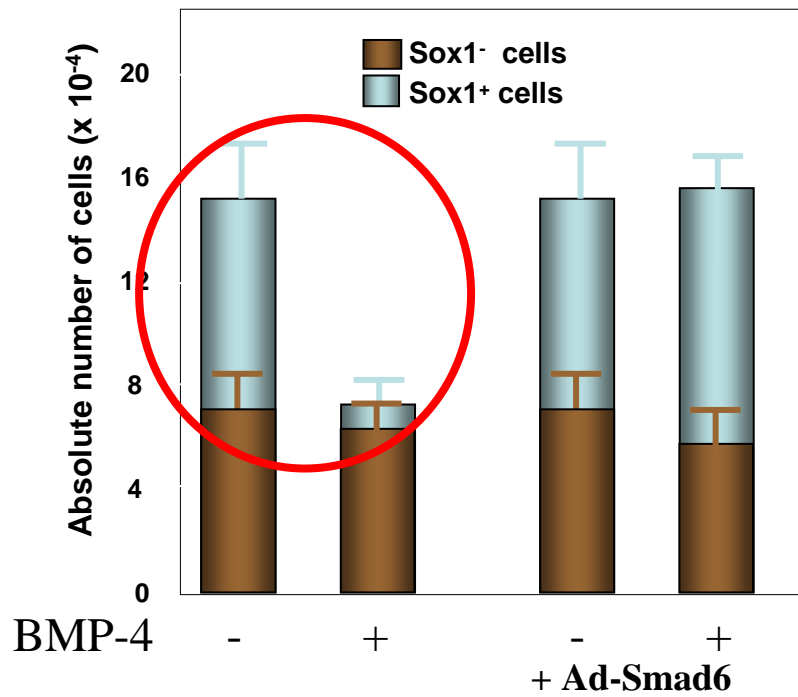
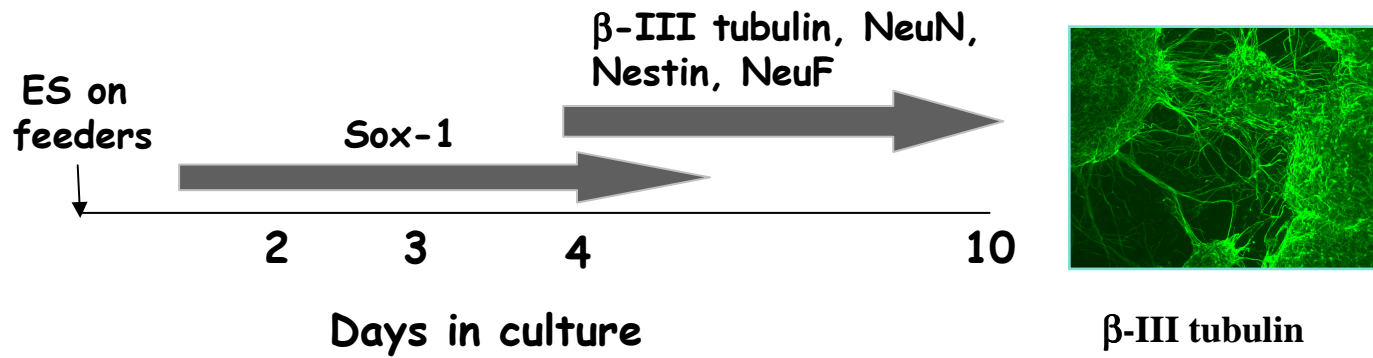


PI: intercalant se liant à l'ADN des cellules mortes
(membrane perméable)

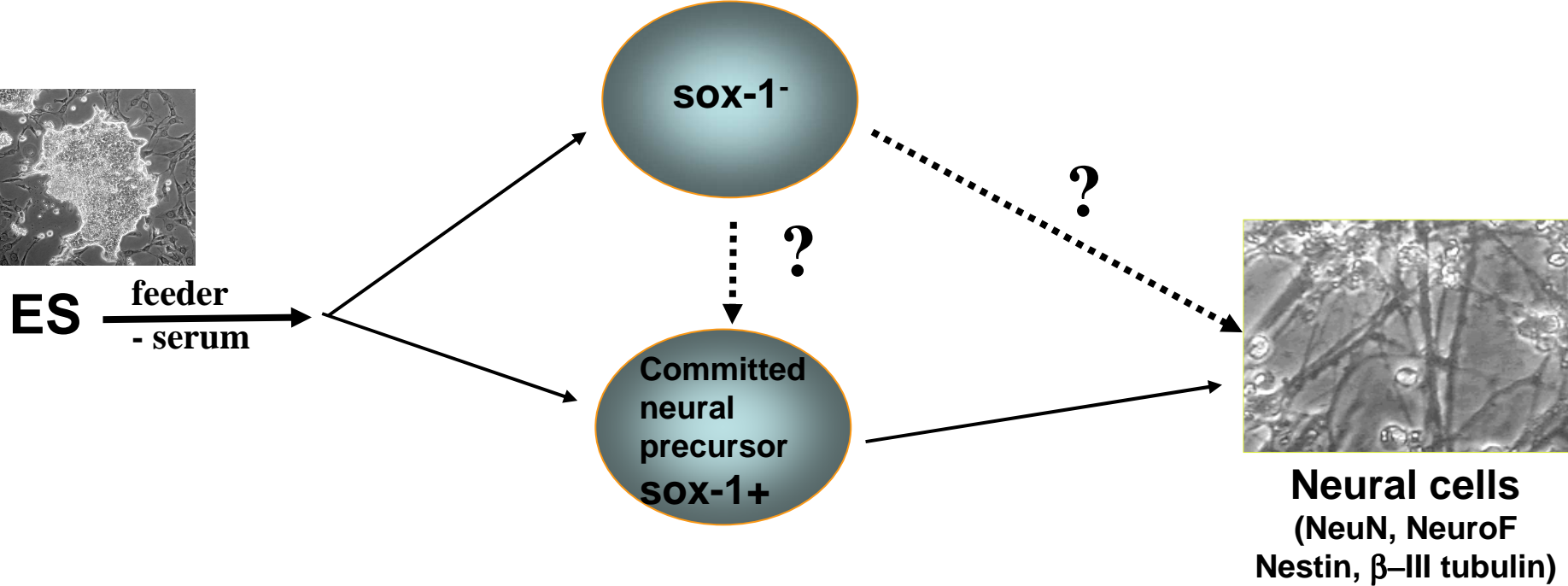


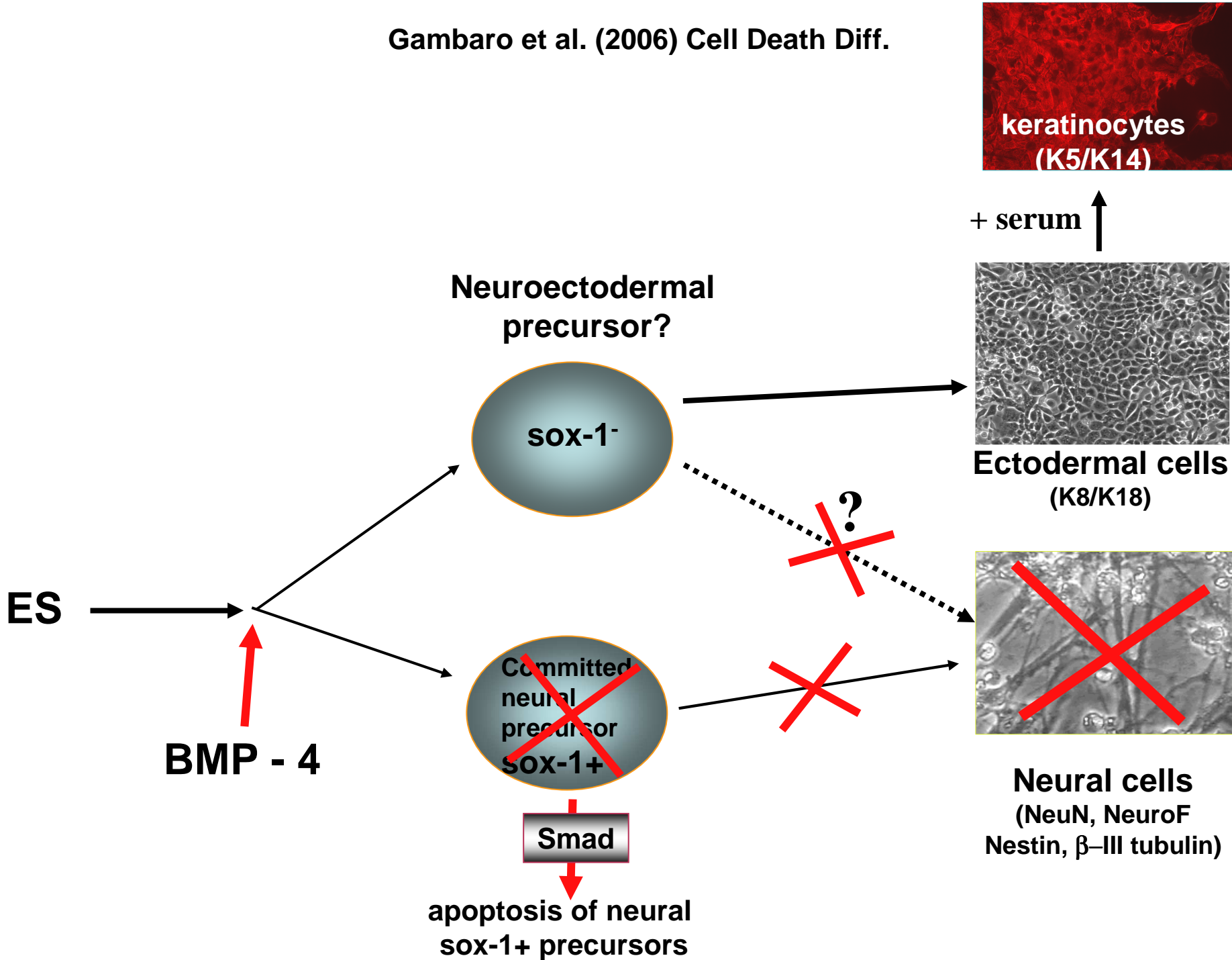
Apoptosis of BMP-4-induced ES cells occur through the Smad pathway



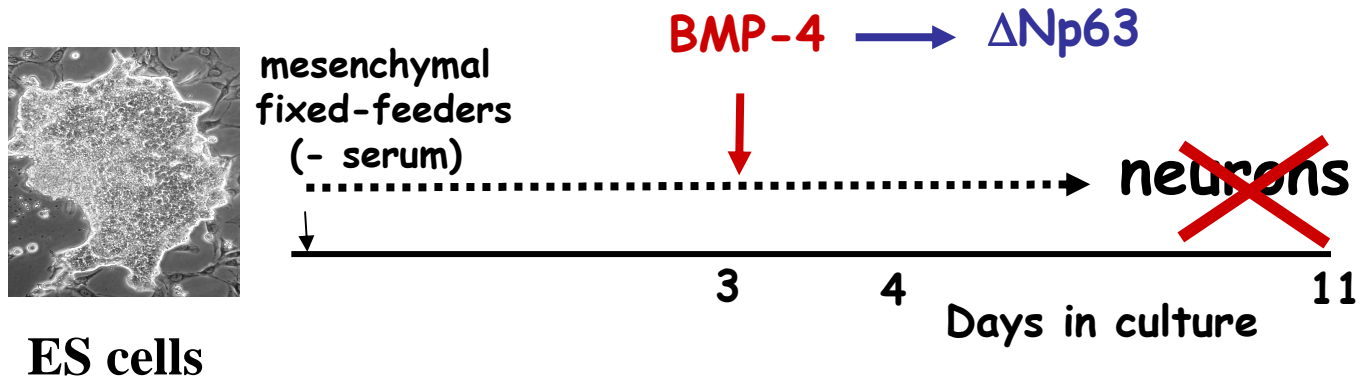


Gambaro et al. (2006) Cell Death Diff.

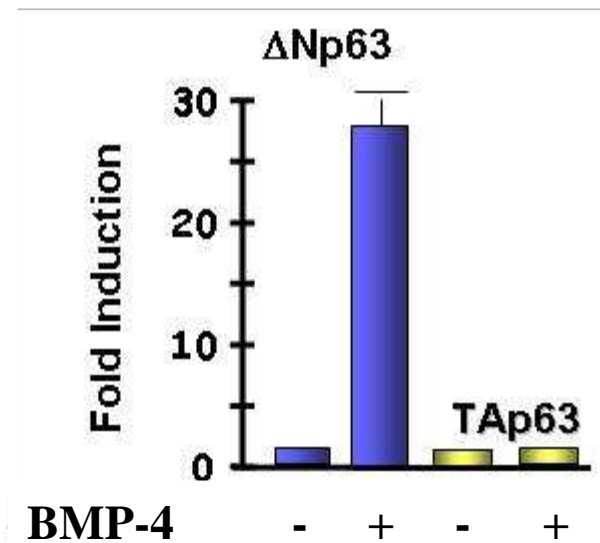




Δ Np63 is activated during BMP-4-induced ES cell commitment



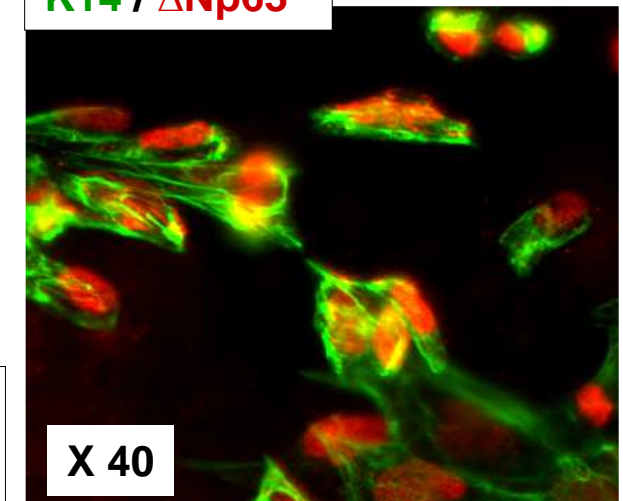
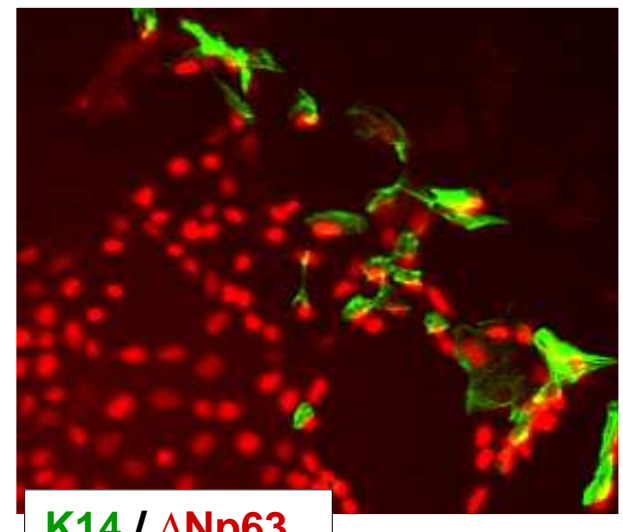
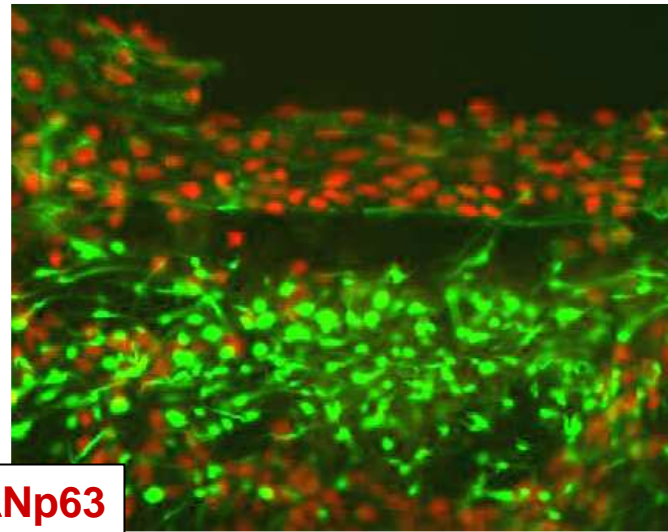
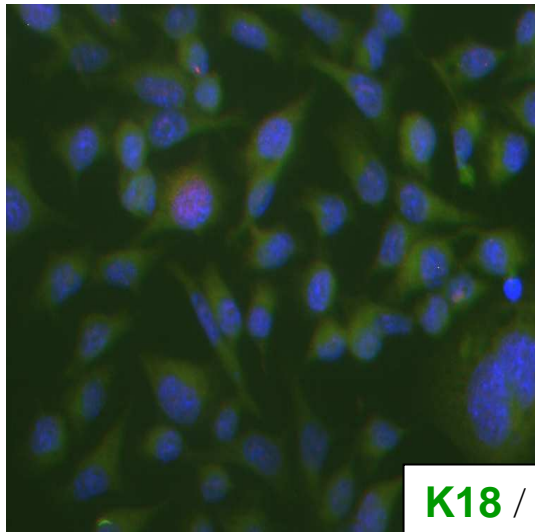
transcriptome analysis +/- BMP-4:



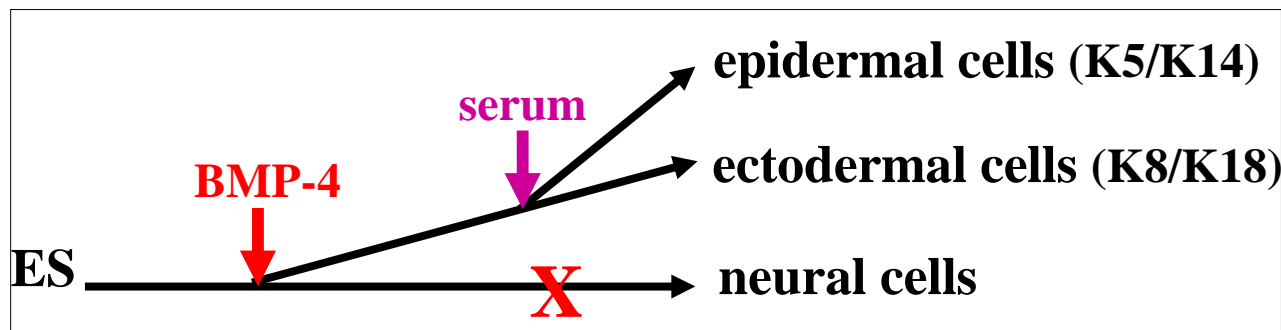
no

BMP4

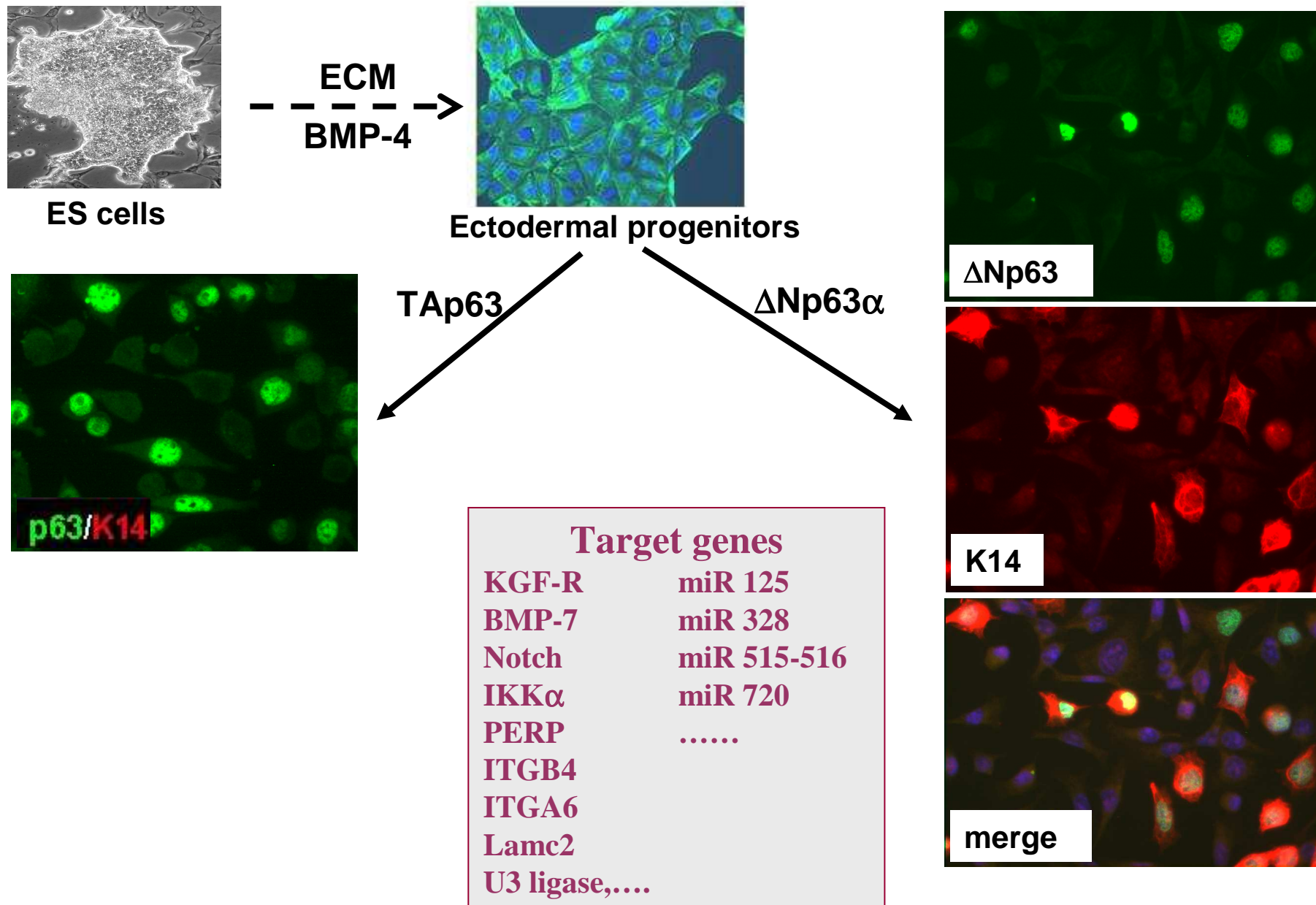
BMP4 + serum



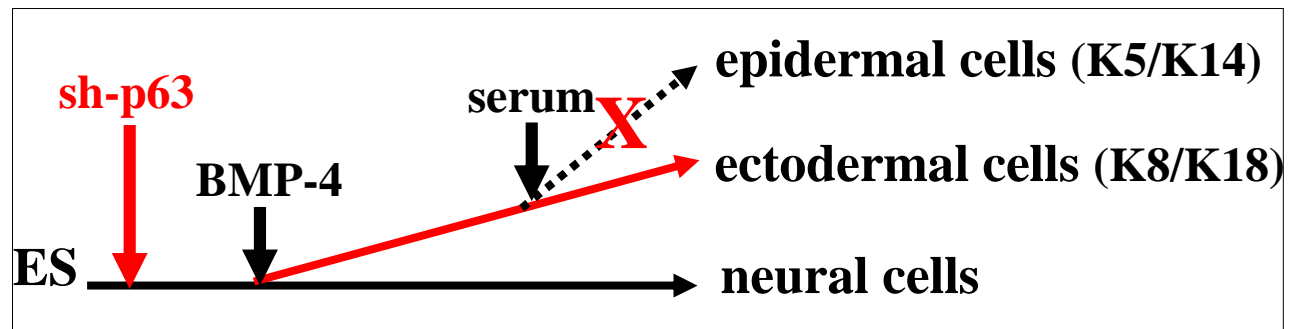
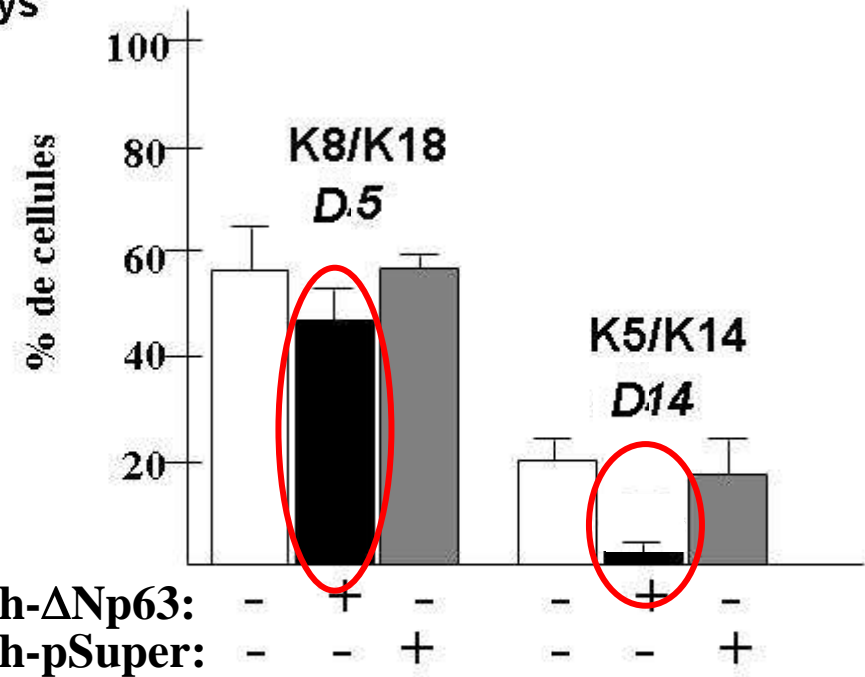
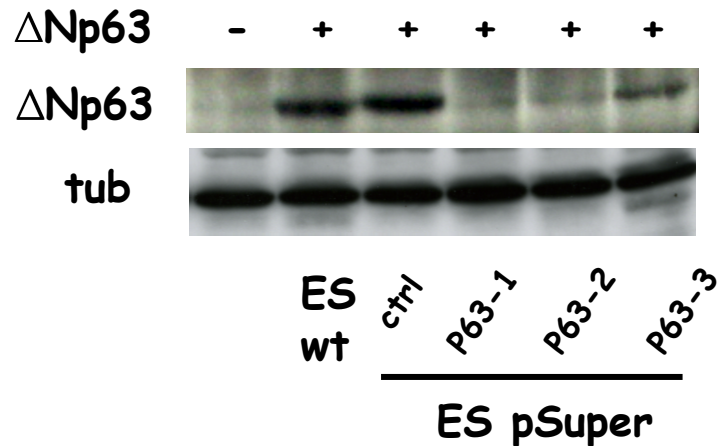
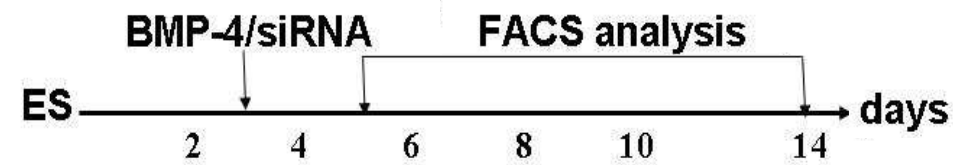
p63 is not necessary for ectodermal commitment
but may be required for epidermal fate



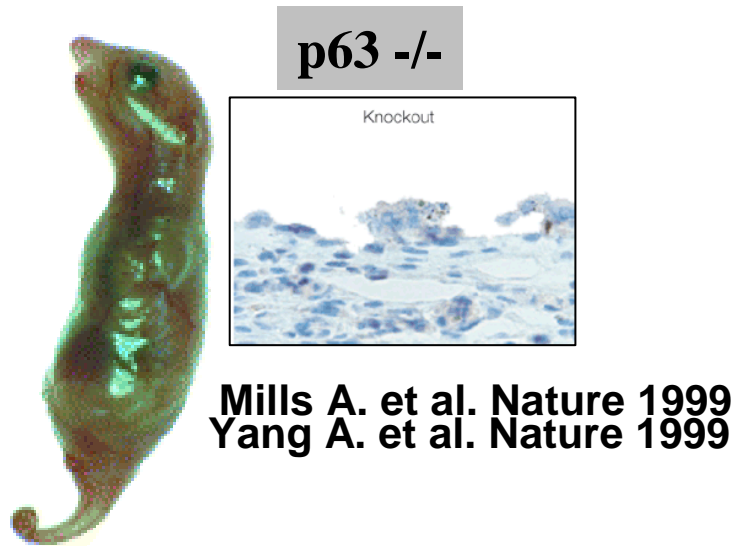
$\Delta Np63\alpha$ is necessary to induce epidermal differentiation of ES-derived ectodermal cells



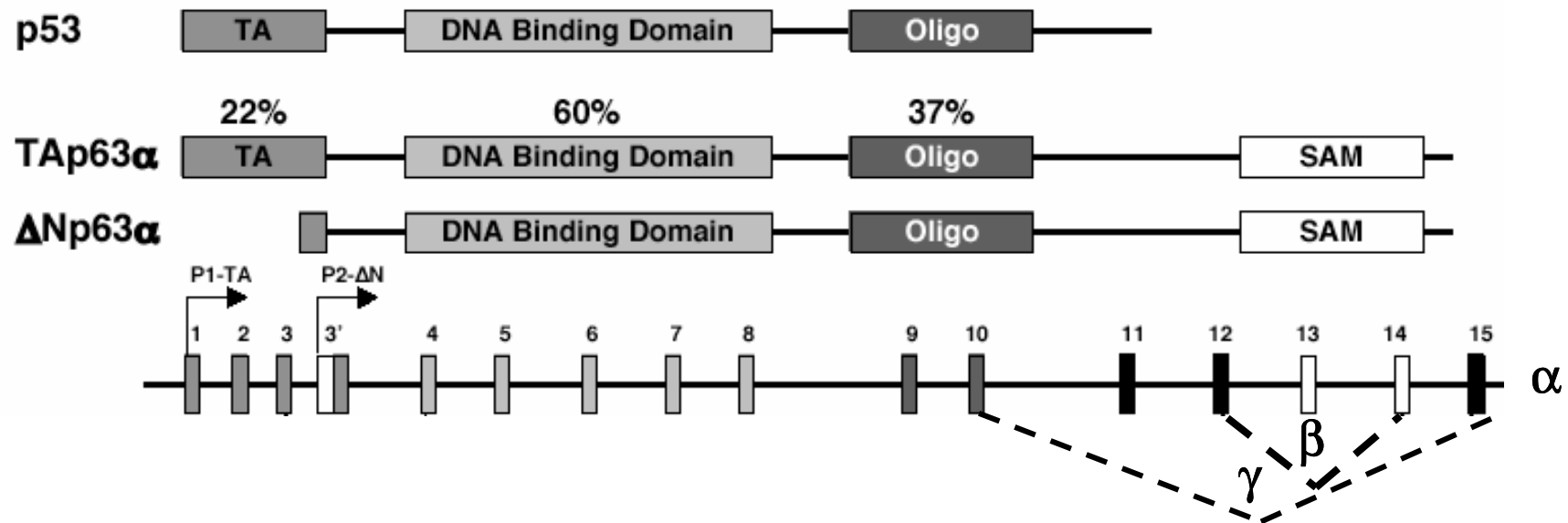
Δ Np63 is required for epidermal differentiation of ES cells



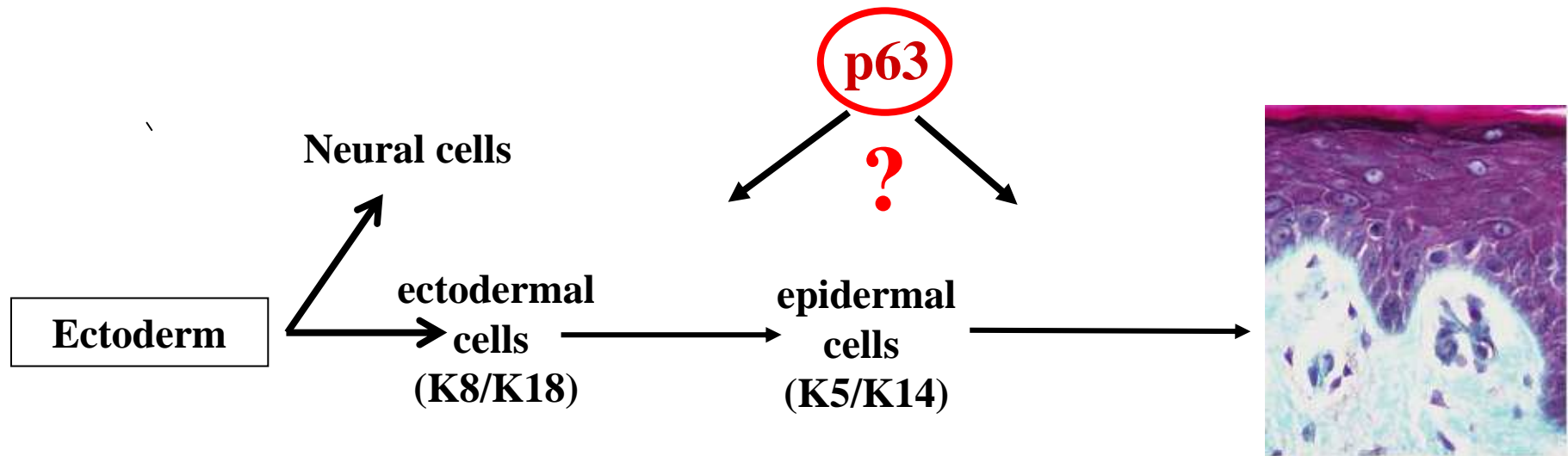
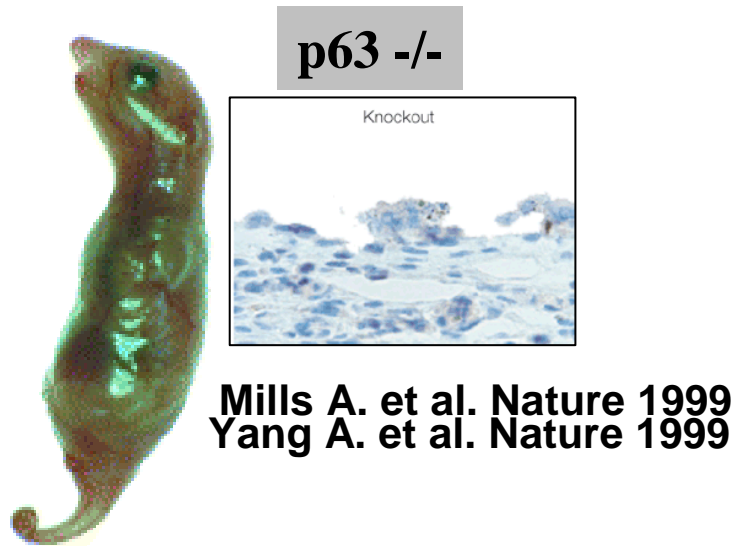
p63 is essential for mammalian epidermal morphogenesis



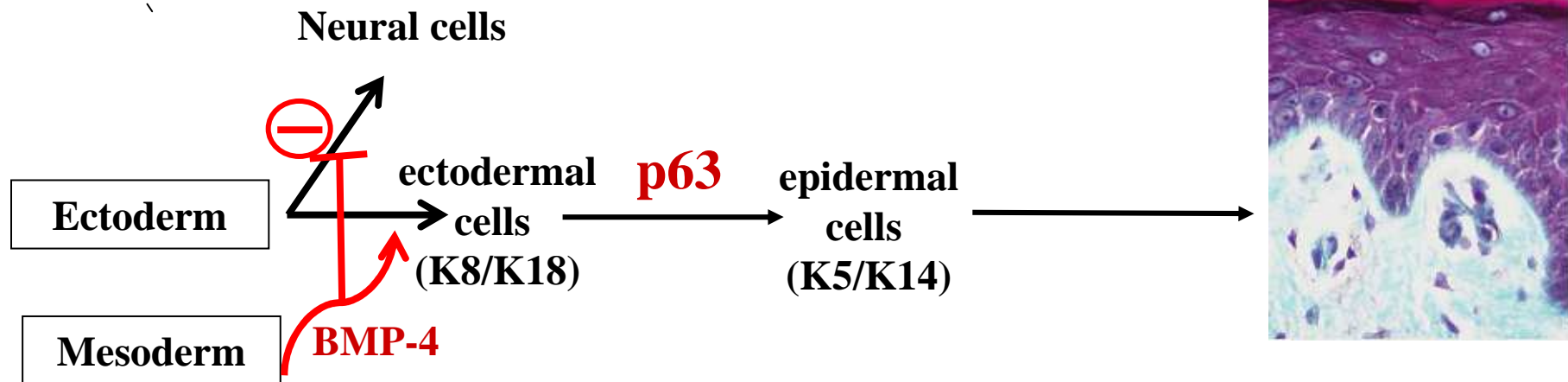
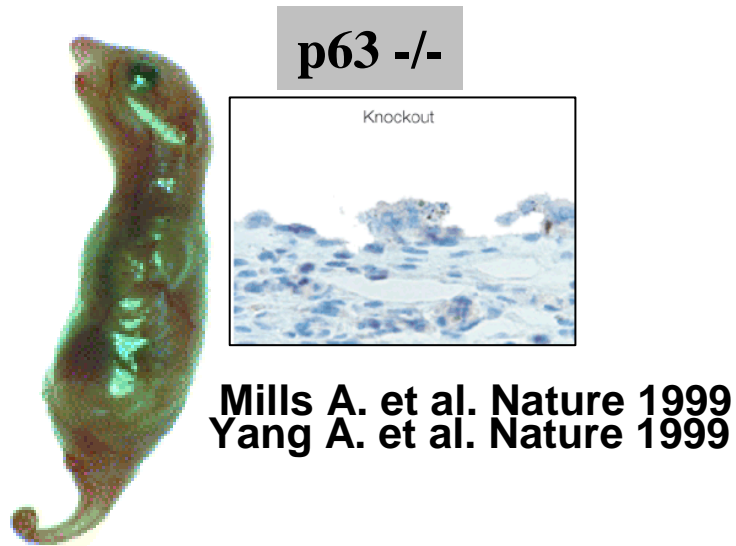
Celli J. et al. Cell 1999

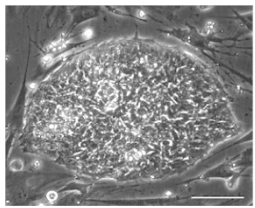


p63 is essential for mammalian epidermal morphogenesis



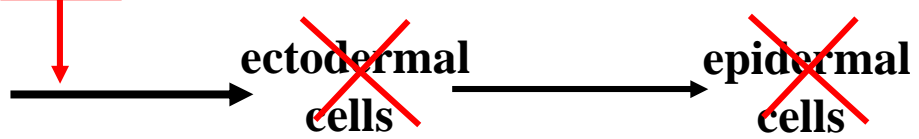
p63 is essential for mammalian epidermal morphogenesis



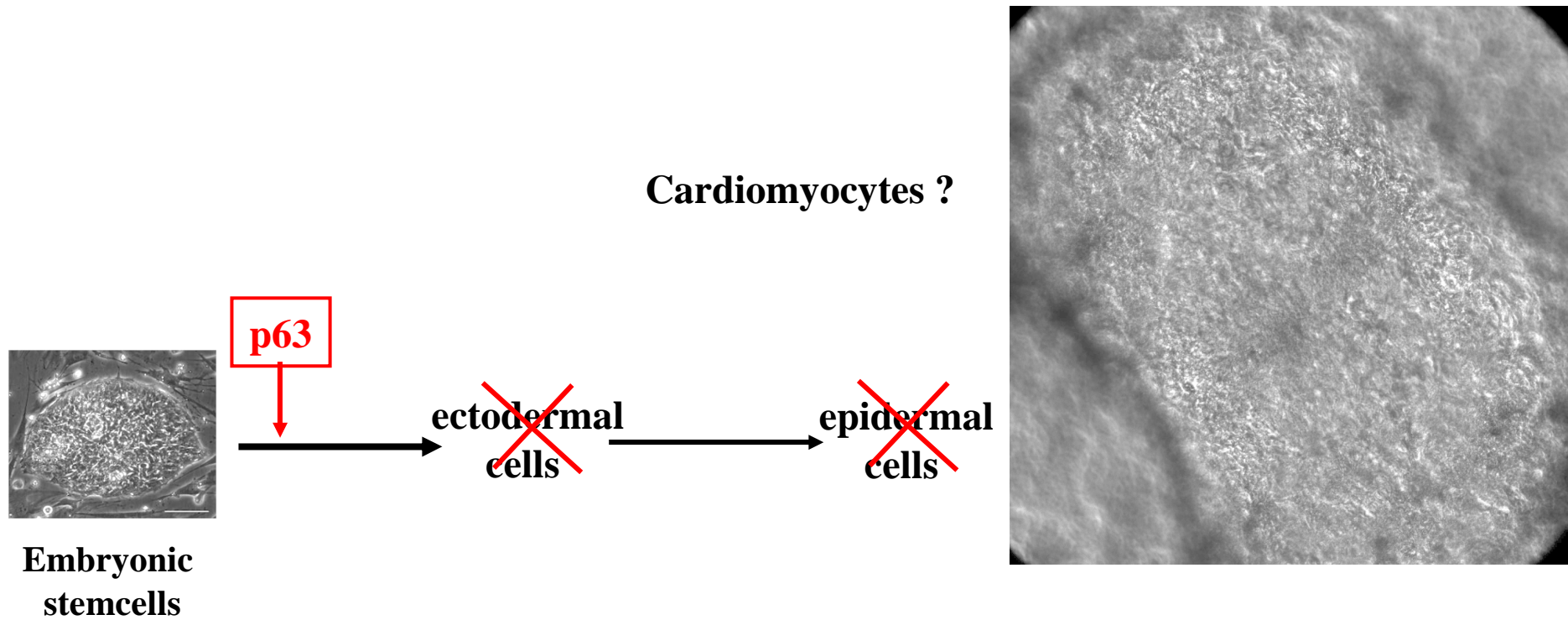


Embryonic
stemcells

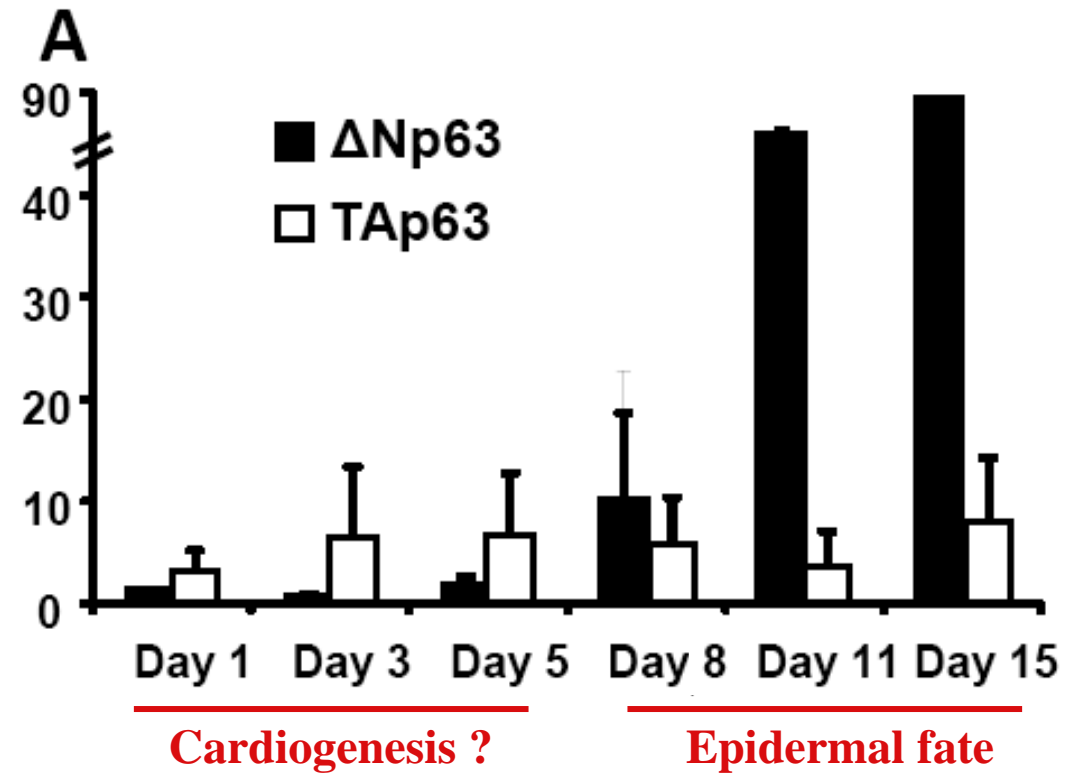
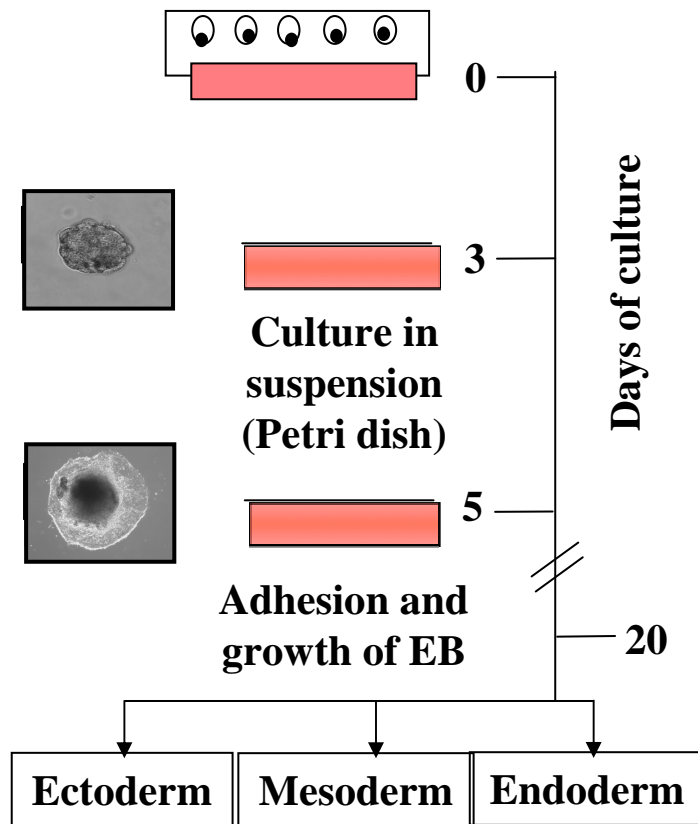
p63



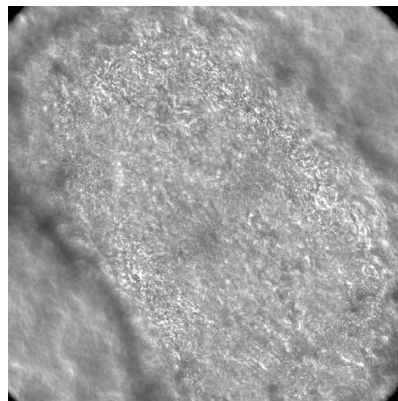
How an epithelial-specific TF (p63) induce cardiogenesis of ES cells ?



Embryoid bodies (EB) from ES cells

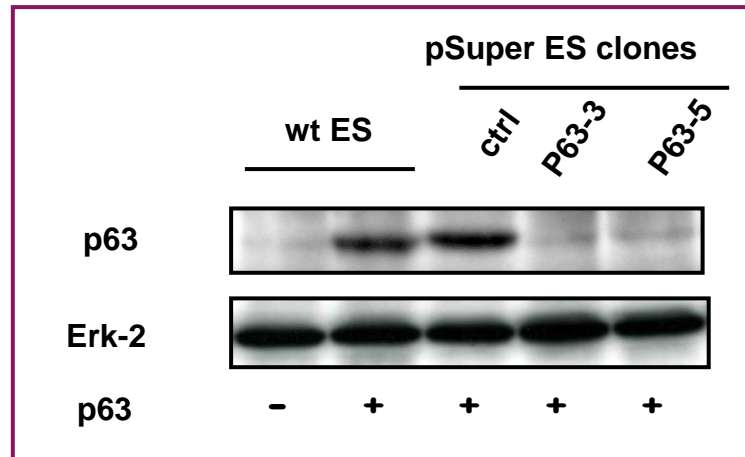


spontaneous
cardiomyocytes



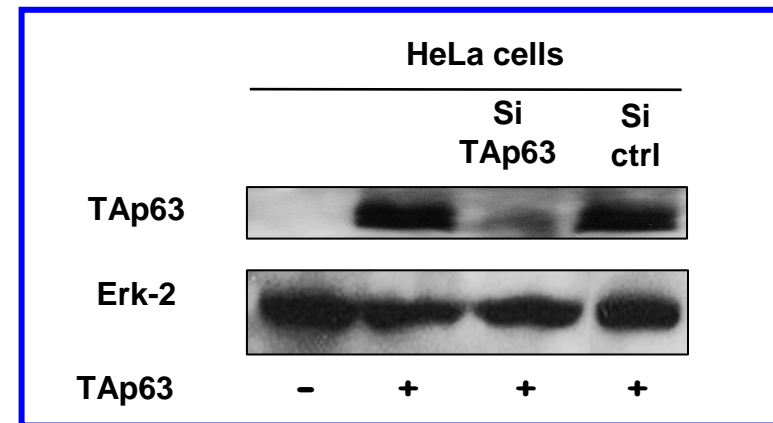
Alain Medawar, Matthieu Rouleau

Inactivation of p63 by shRNA (*)



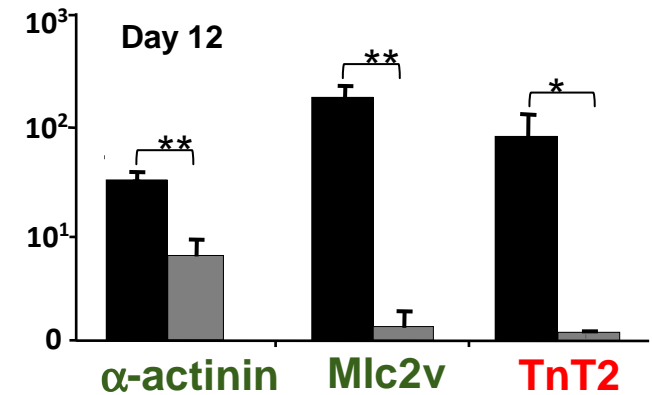
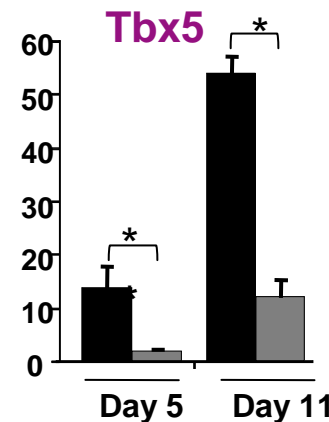
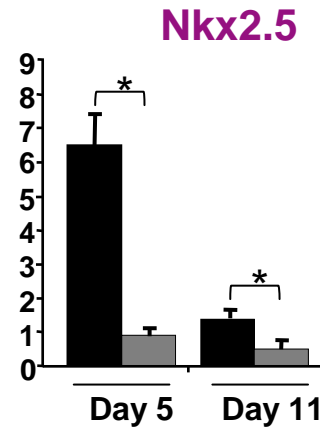
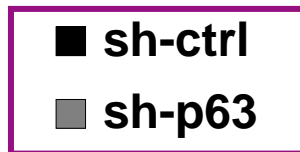
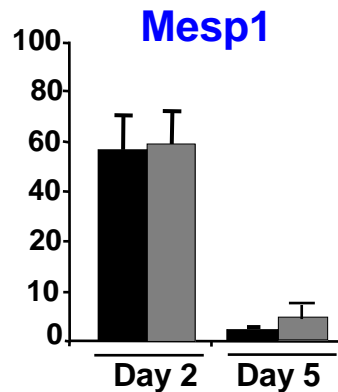
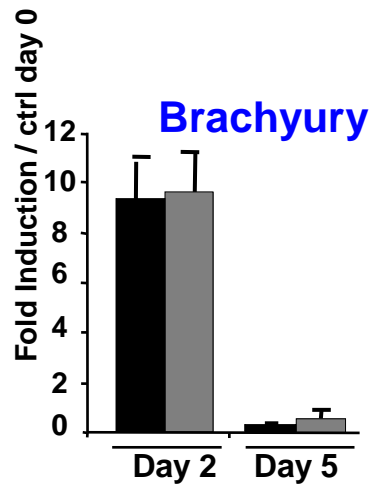
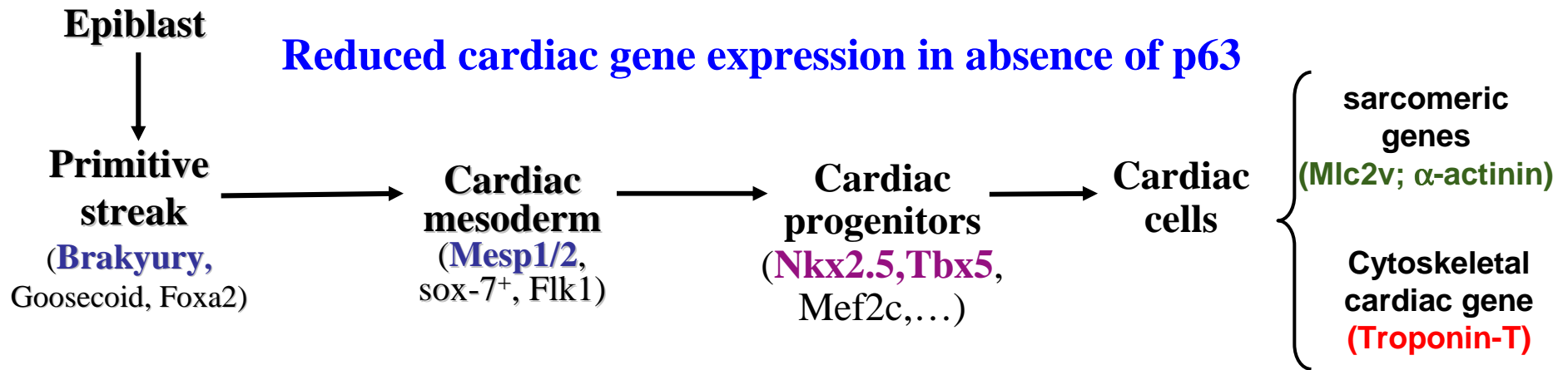
(*) Two independent clones

Inactivation of TAp63 by siRNA



Ratio	sh-control	sh-p63	si-control	si-TAp63
Beating area/EB (mean+/-sd)	1.6+/-0.1	0.1+/-0.1	1.5+/-0	0.3+/-0.2
% Troponin-T ⁺ cells	14.8+/-0.4	1.2+/-0.15	12.7+/-1	5.1+/-0.5

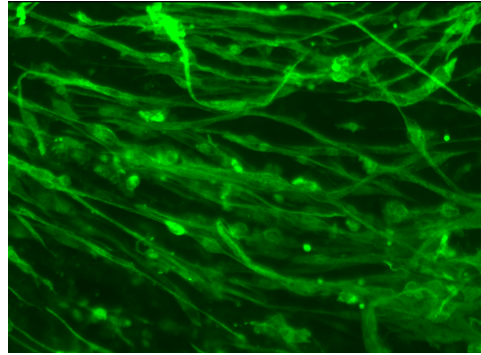
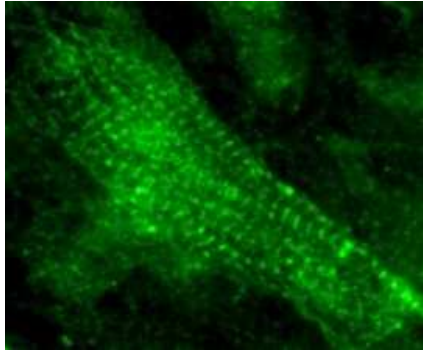
Inactivation of Δ Np63 by siRNA does not repress cardiogenesis



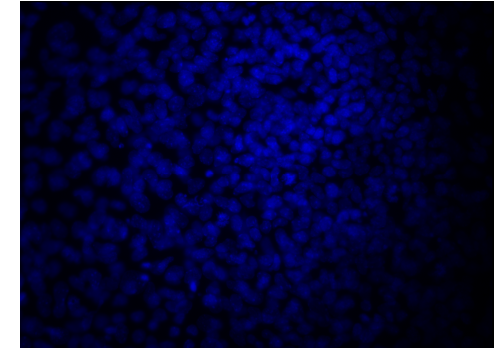
Alain Medawar

sh-control ES cells

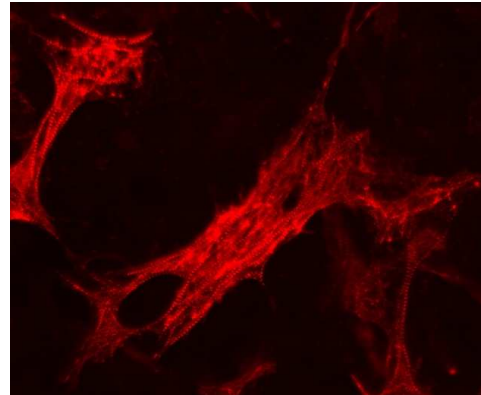
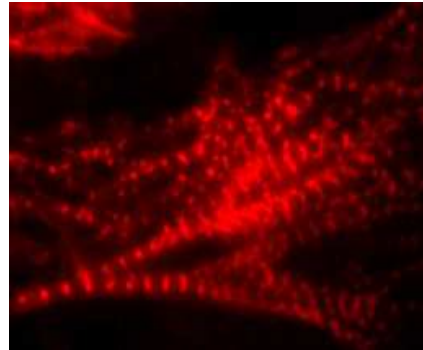
shp63 ES cells



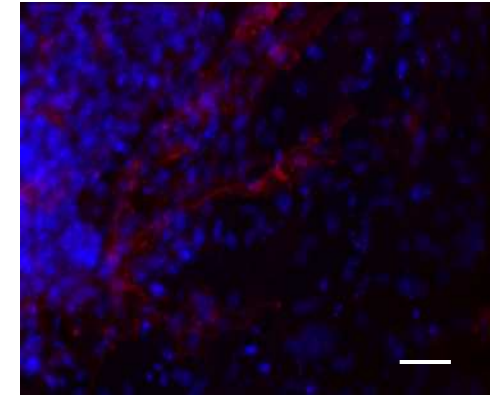
Mlc2v



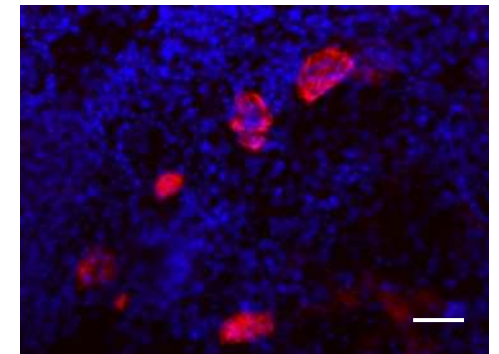
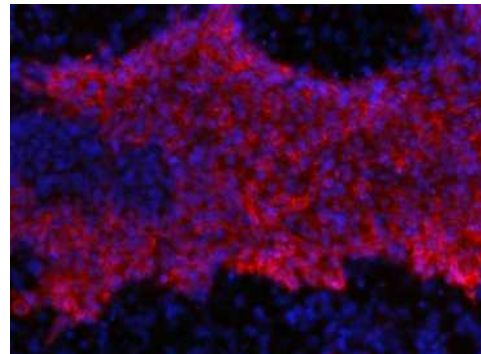
sarcomeric



α -actinin

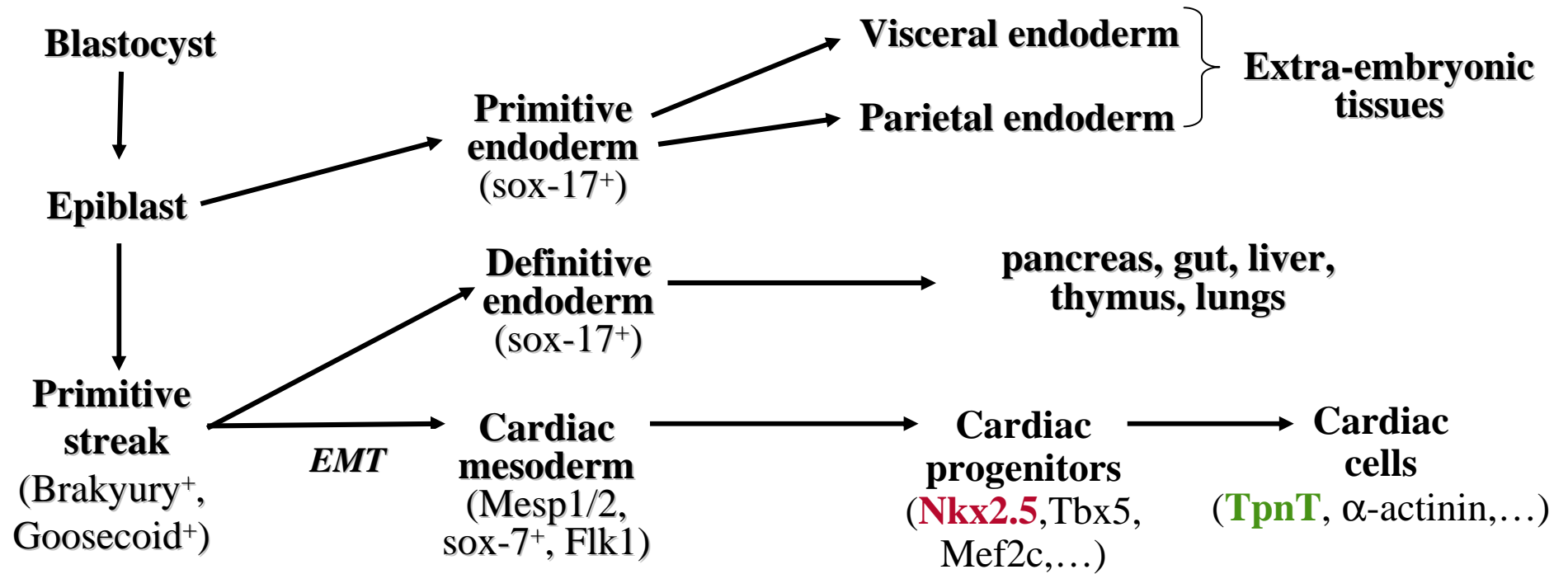


Troponin-T

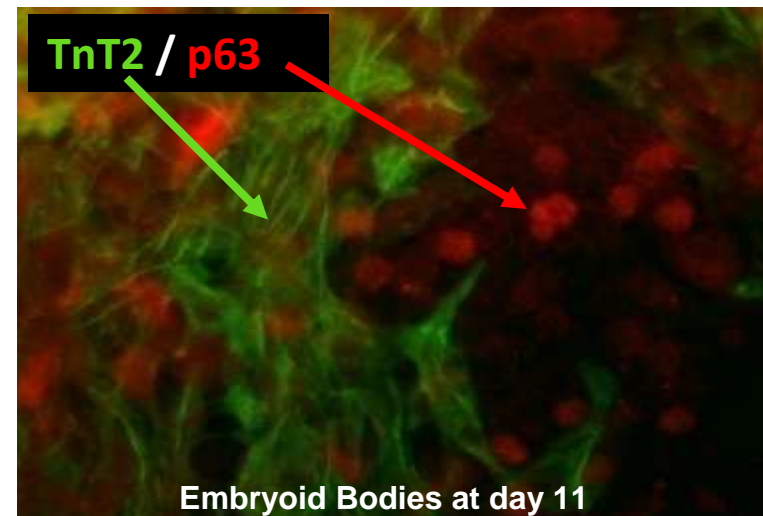
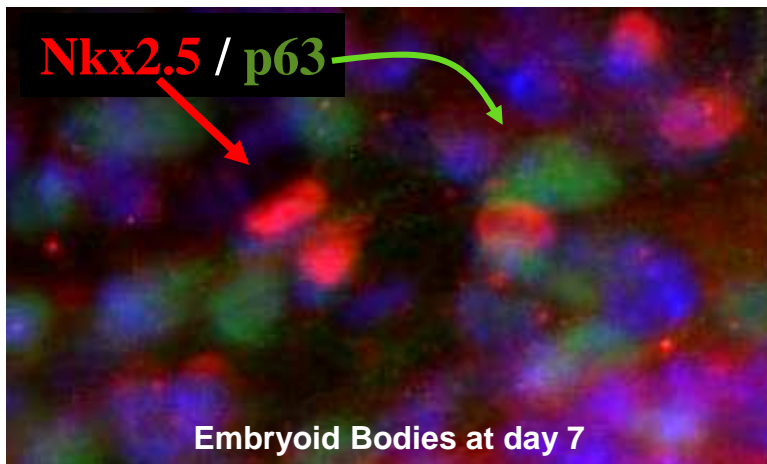
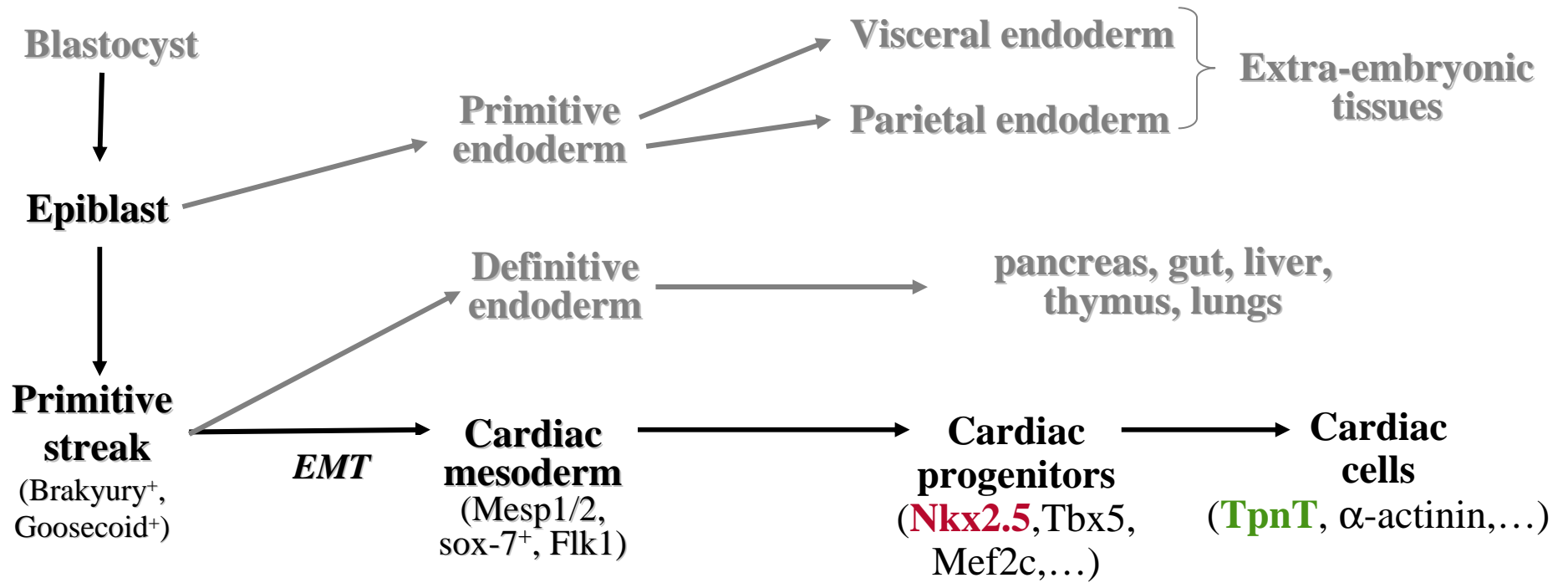


Alain Medawar

Which cells are expressing p63 during cardiogenesis ?

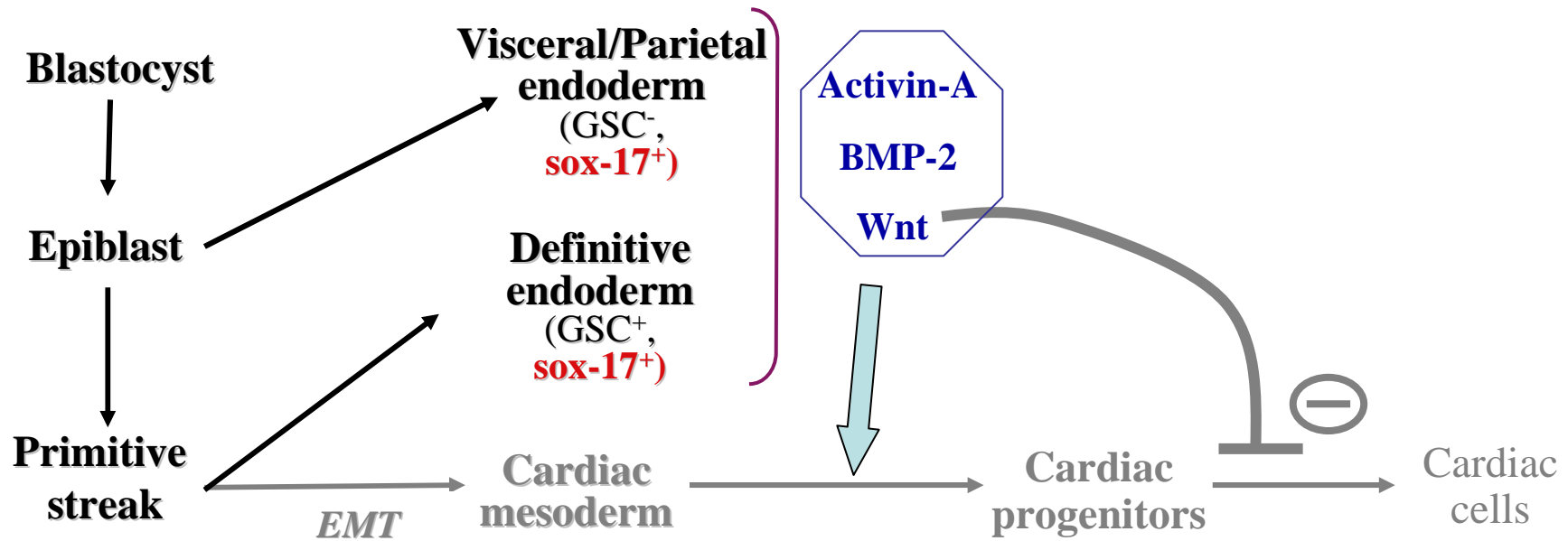


Which cells are expressing p63 during cardiogenesis ?

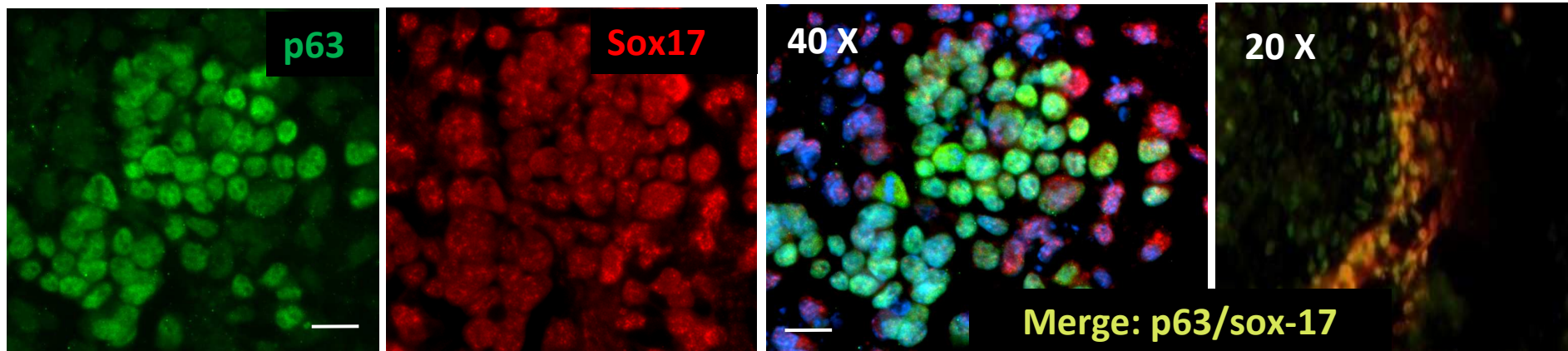


Alain Medawar

Which cells are expressing p63 during cardiogenesis ?

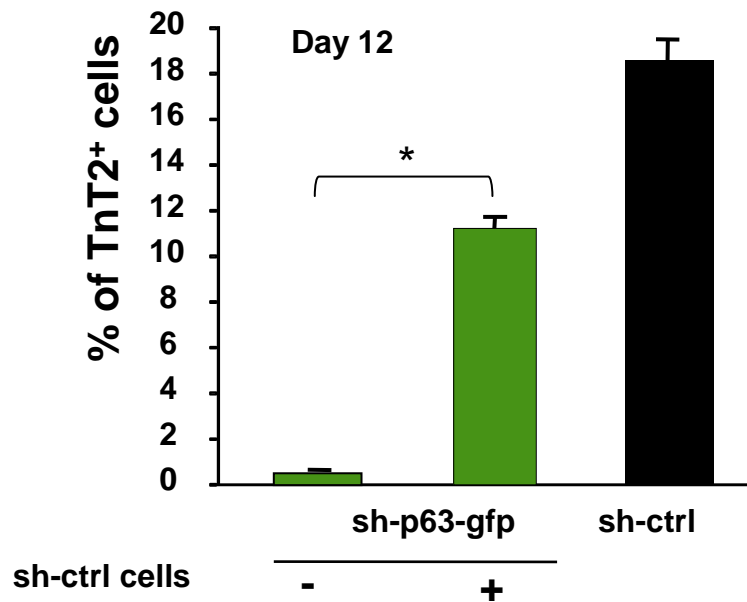
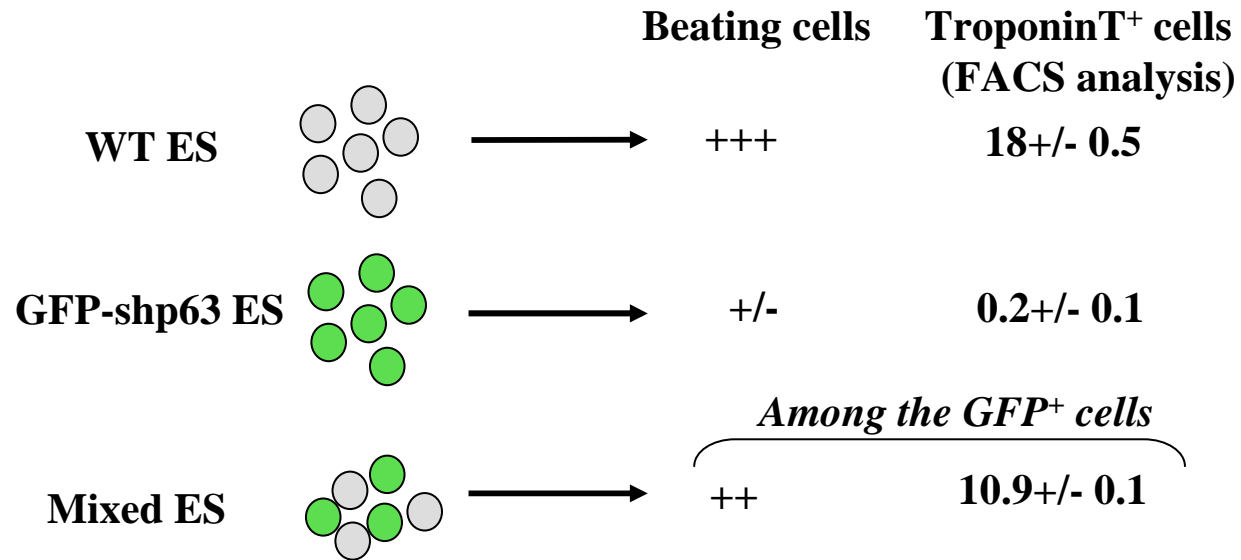


p63 is expressed by Sox-17⁺ endodermal cells

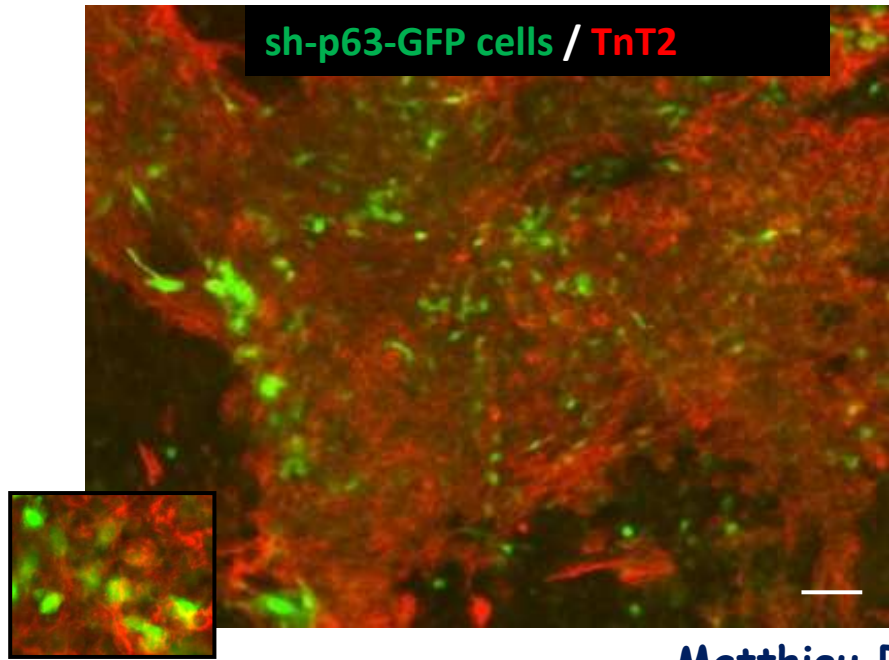
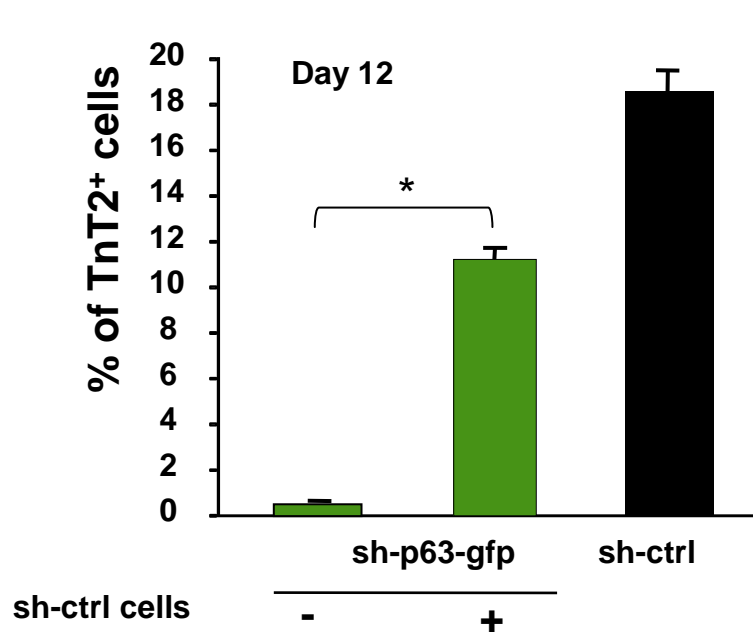
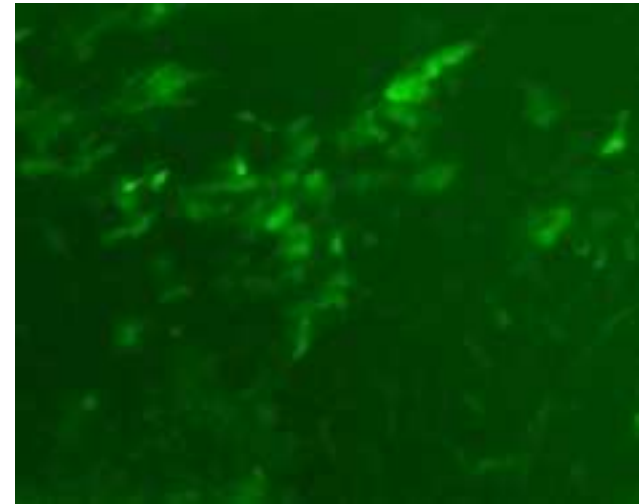
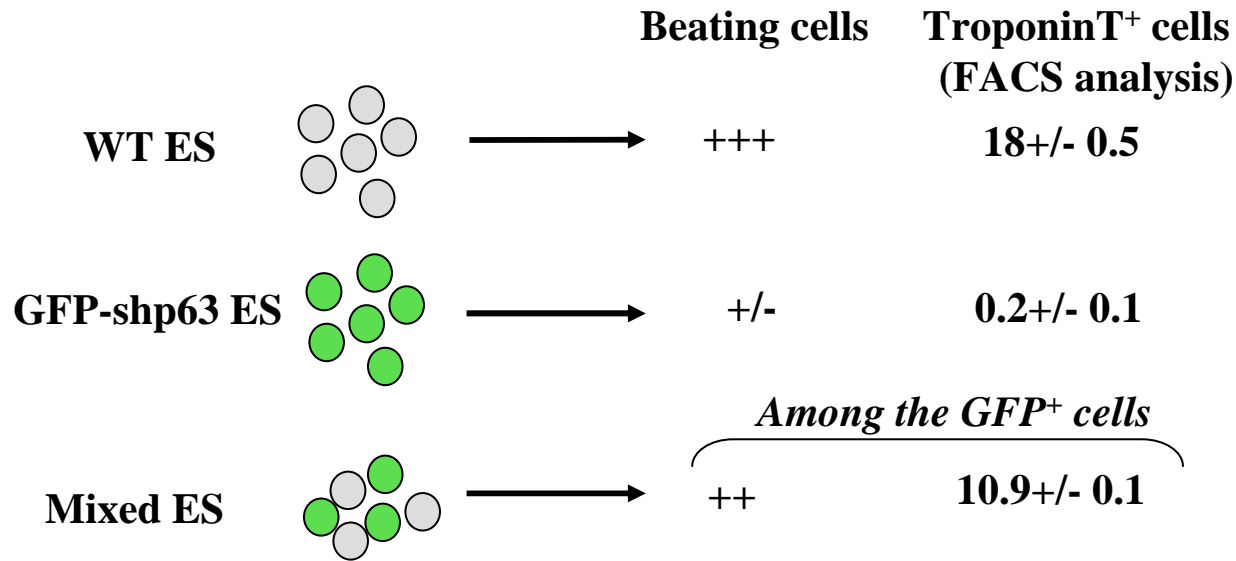


Embryoid bodies at day 6

Non-cell autonomous effect of p63 on cardiogenesis ?



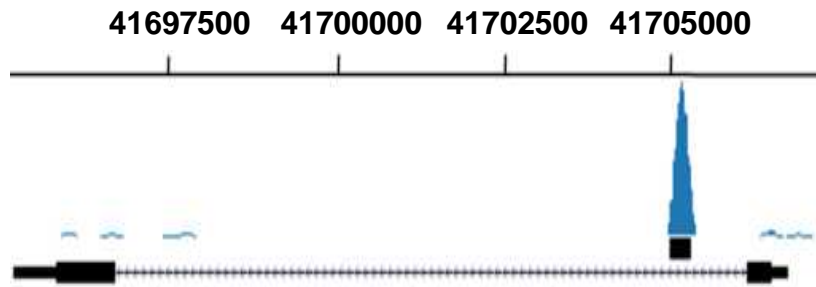
Non-cell autonomous effect of p63 on cardiogenesis ?



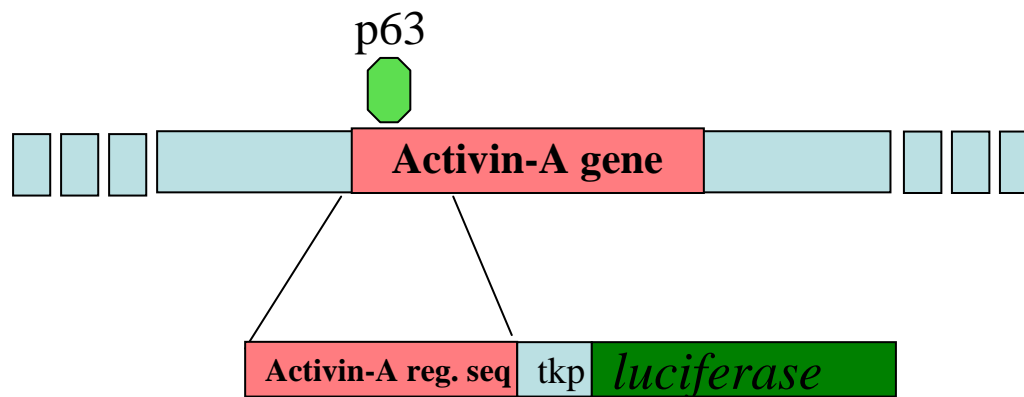
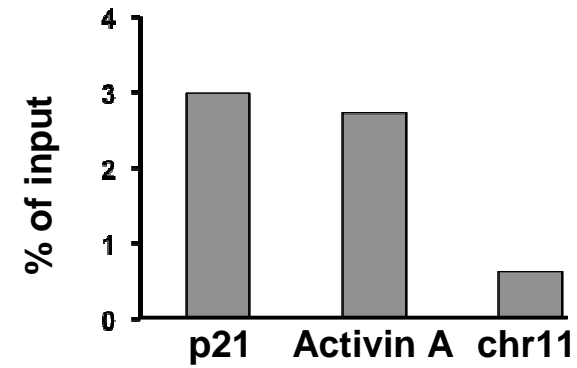
Matthieu Rouleau

Activin-A gene expression is reduced in absence of p63

chip-seq

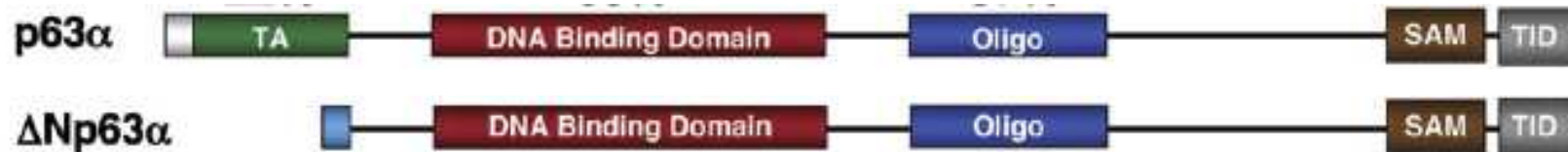
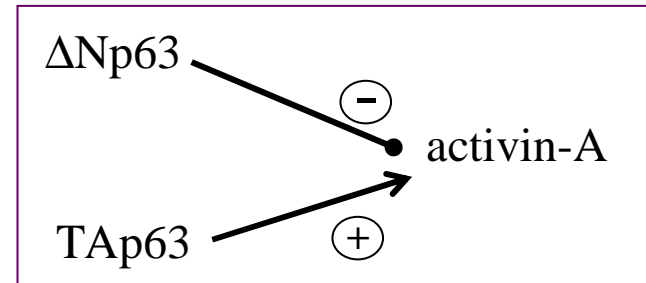
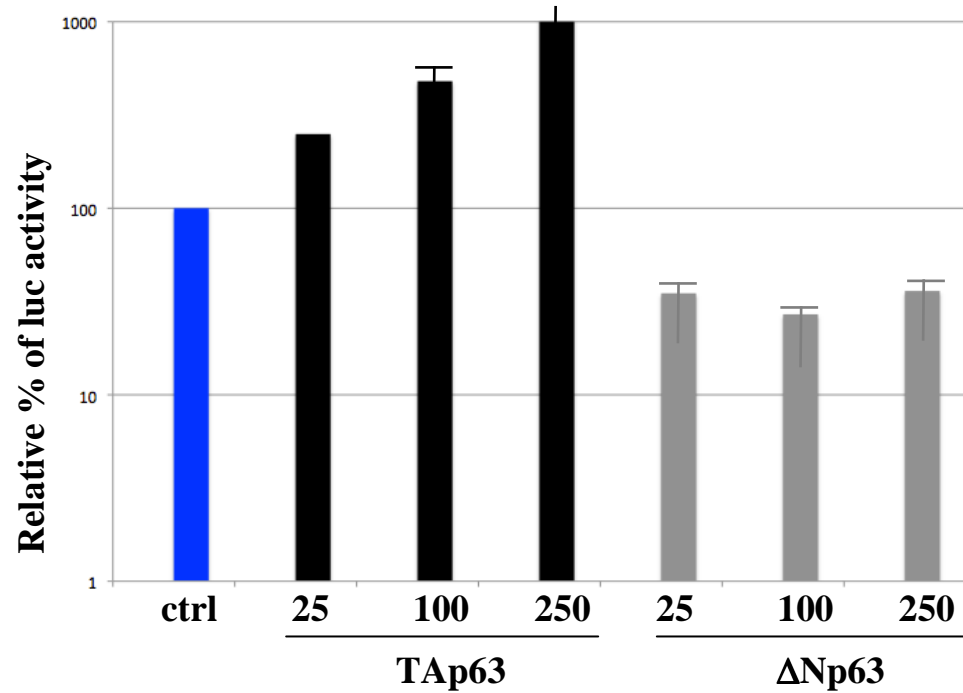


J. Zhou/H. van Bokhoven

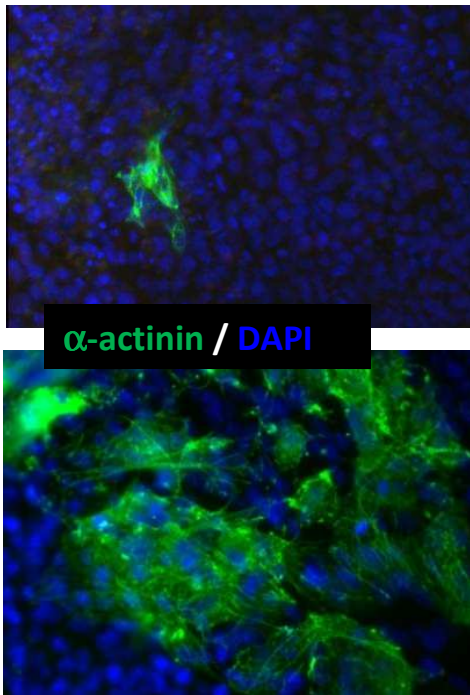
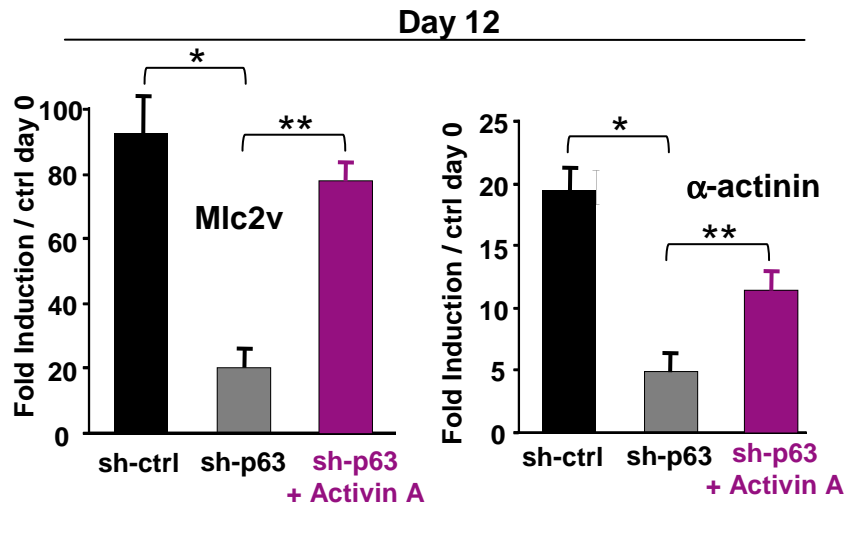
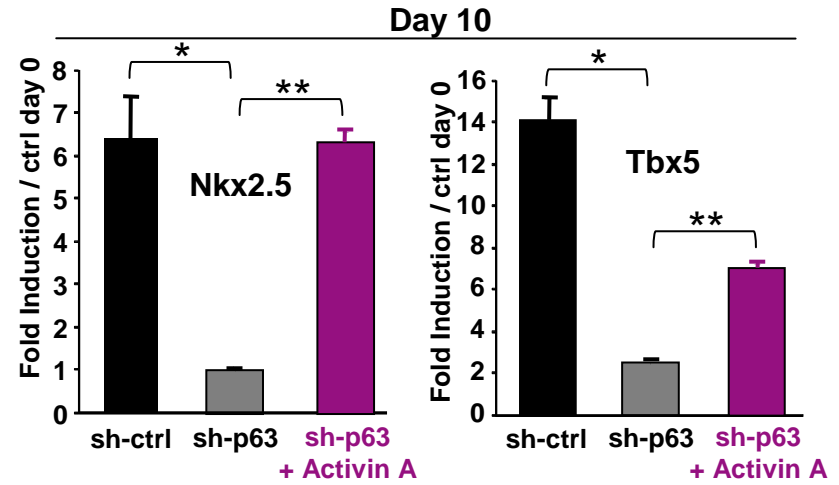
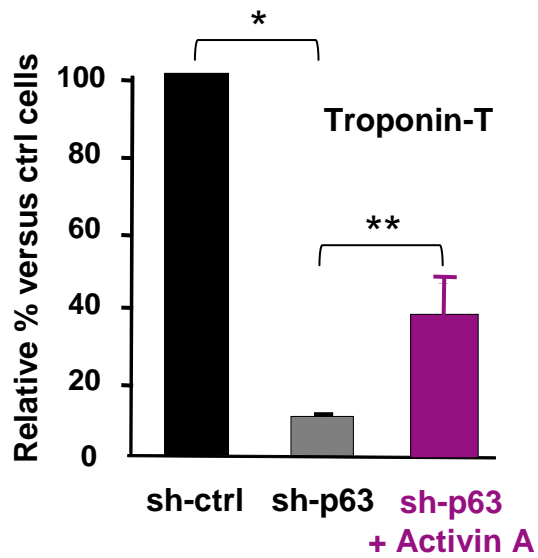


Matthieu Rouleau

p63 regulates activin-A promoter



Cardiac rescue by exogenous Activin-A



sh-p63

α -actinin / DAPI

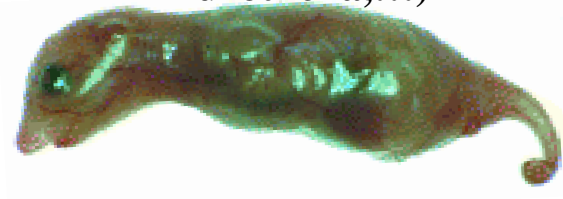
sh-p63
+
Activin A

Day 12

p63 is a master epithelial TF gene

p63 KO mice

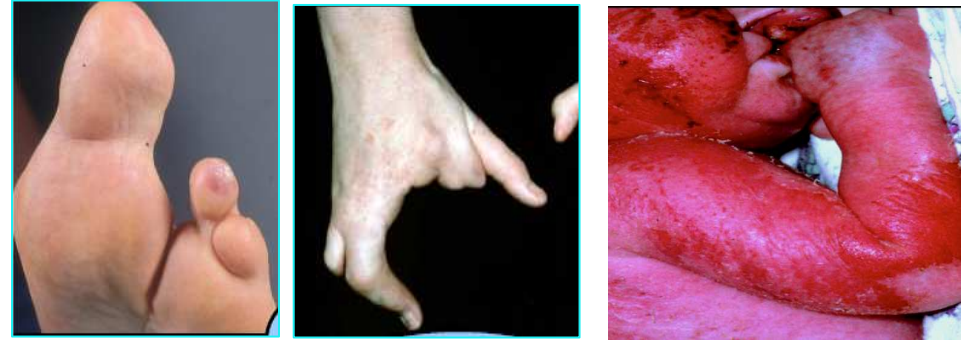
(limbs, epidermis, breast, prostate, urothelia,...)



(Yang, Nature 1999; Mills, Nature 1999)

Die in utero or at birth

Human ectodermal dysplasia syndromes



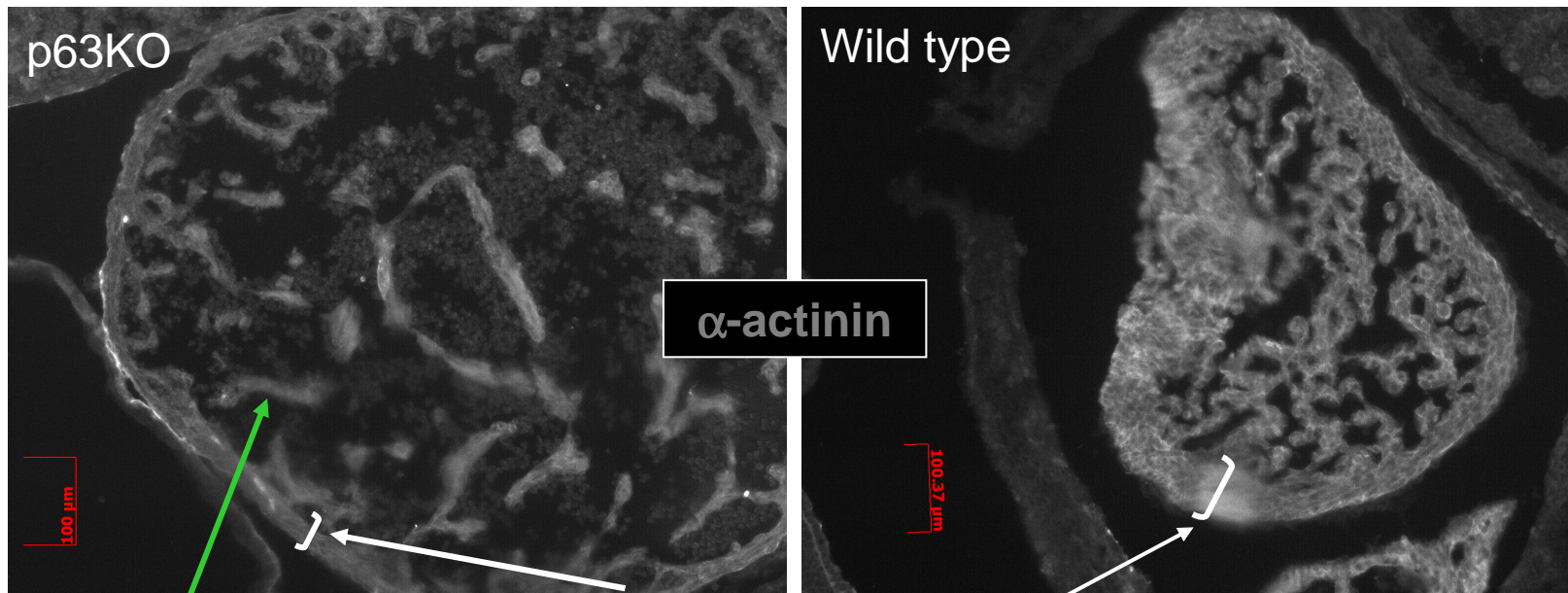
(Celli J. et al. Cell 1999)

?

E12.5 KO embryos suffer from dilated cardiomyopathy

E12.5 embryo Brdm2 mice (129Sv/B57BL6)

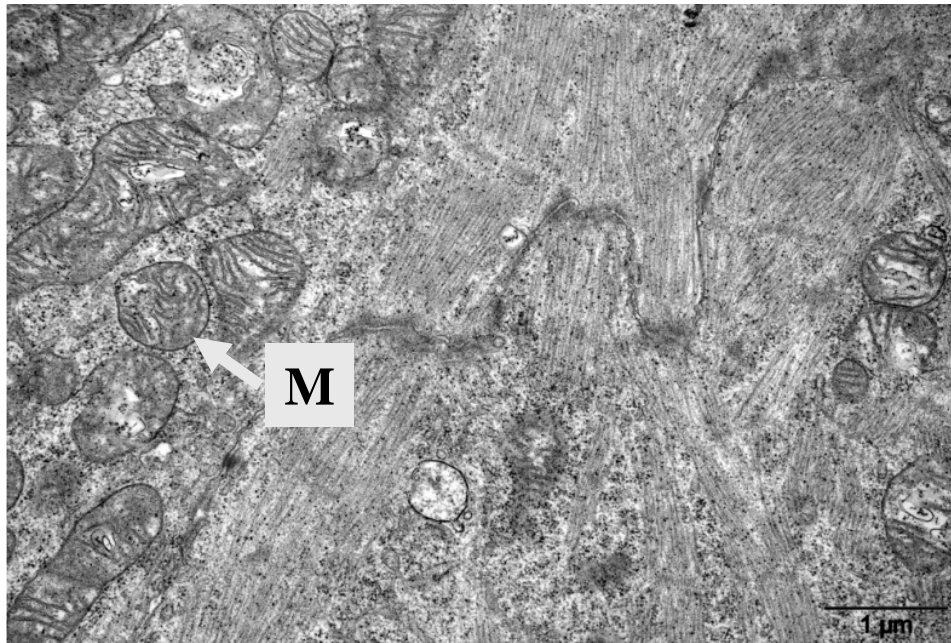
n= 6 (from 3 pregnancies)



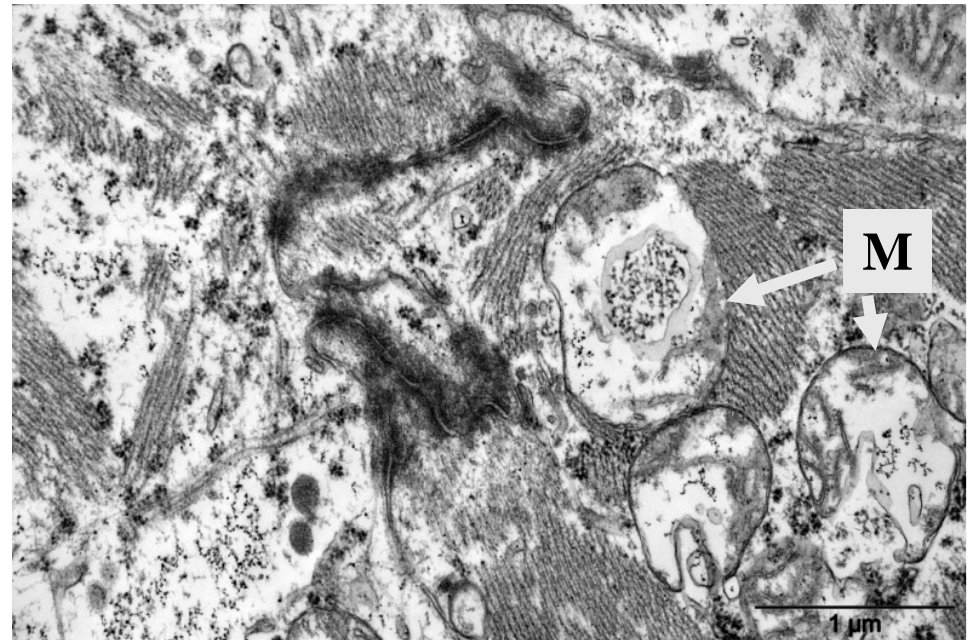
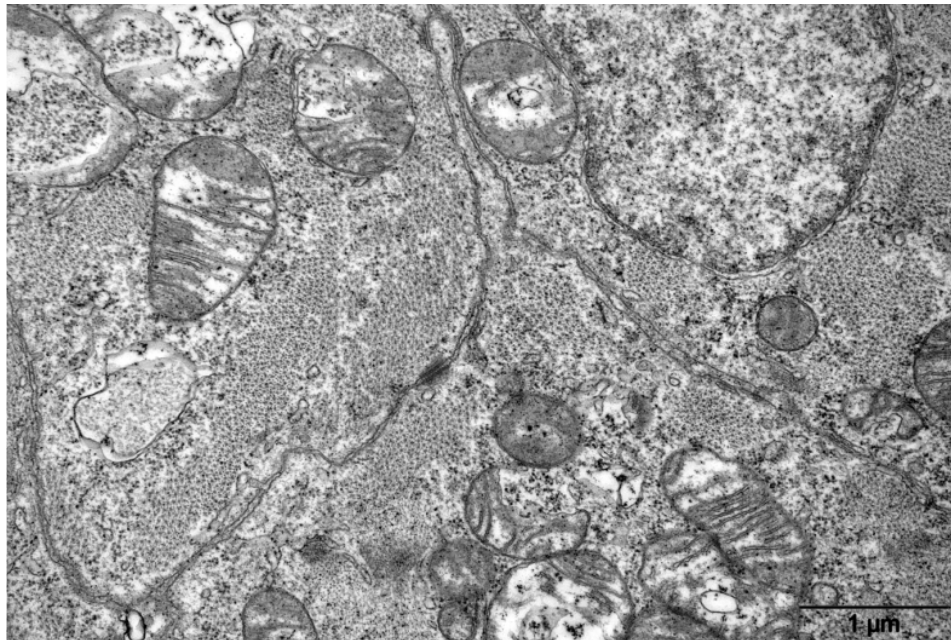
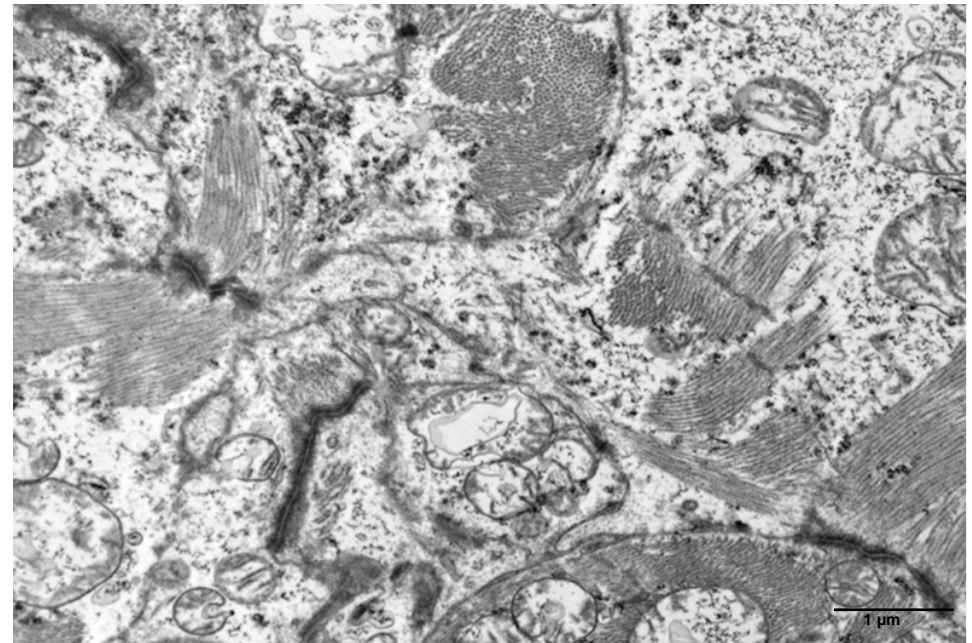
Deficient trabeculation

Thin ventricular wall

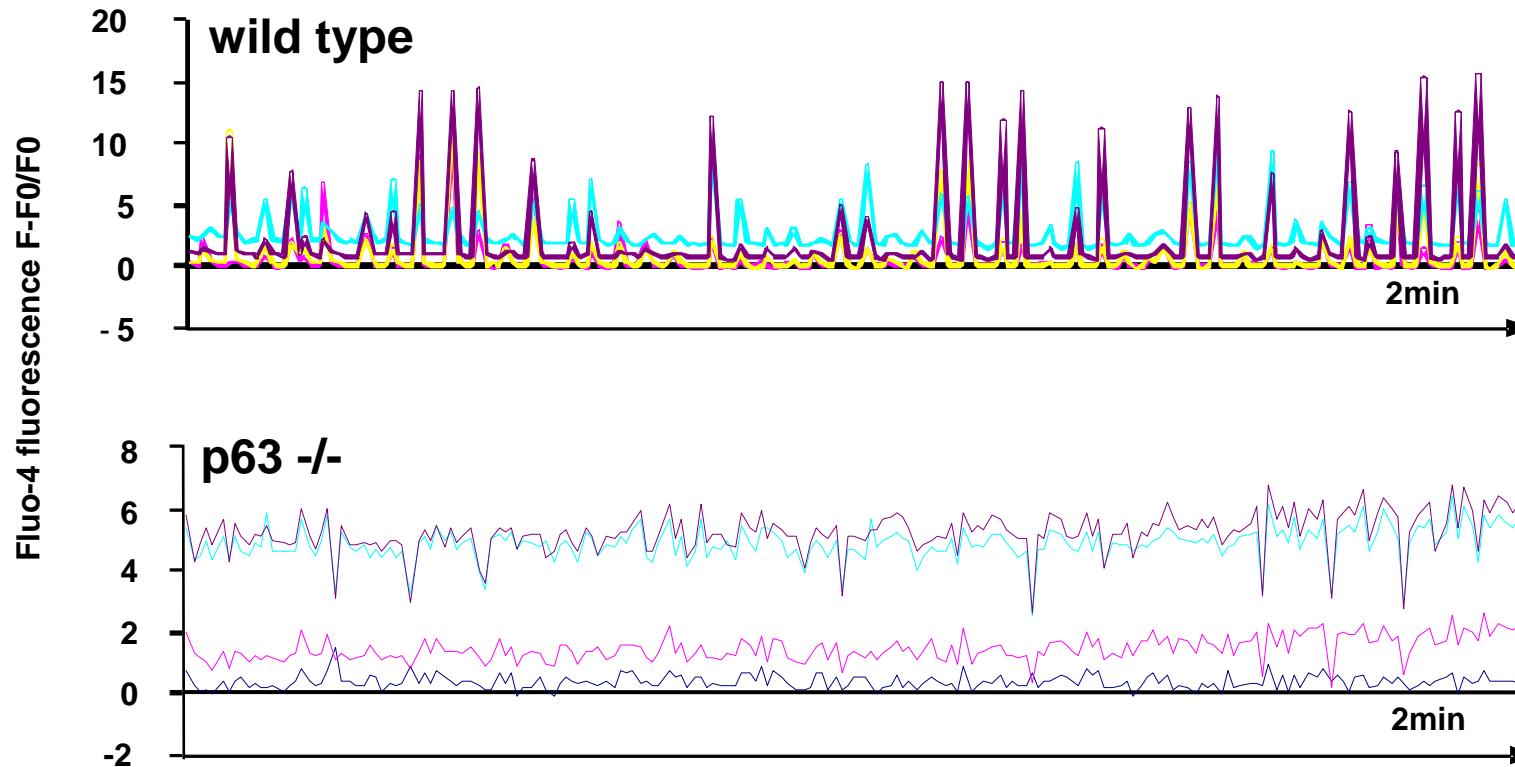
wild type newborn heart tissue



p63^{-/-} newborn heart tissue



Intracellular Ca²⁺ transient in embryonic hearts (E12.5)



In utero, E12.5 embryos were loaded with fluo-4 to monitor spontaneous Ca²⁺ spiking in the myocardium, as an index of excitation-contraction coupling.

Rhythmic Ca²⁺ oscillations were observed in WT heart embryos (n=3).

However, weak Ca²⁺ spiking were observed in p63-deficient embryos (n=3).

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

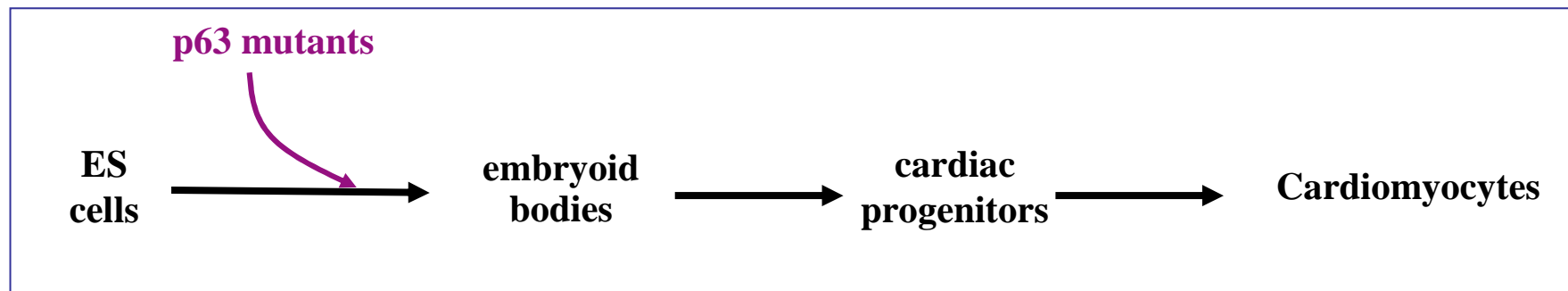
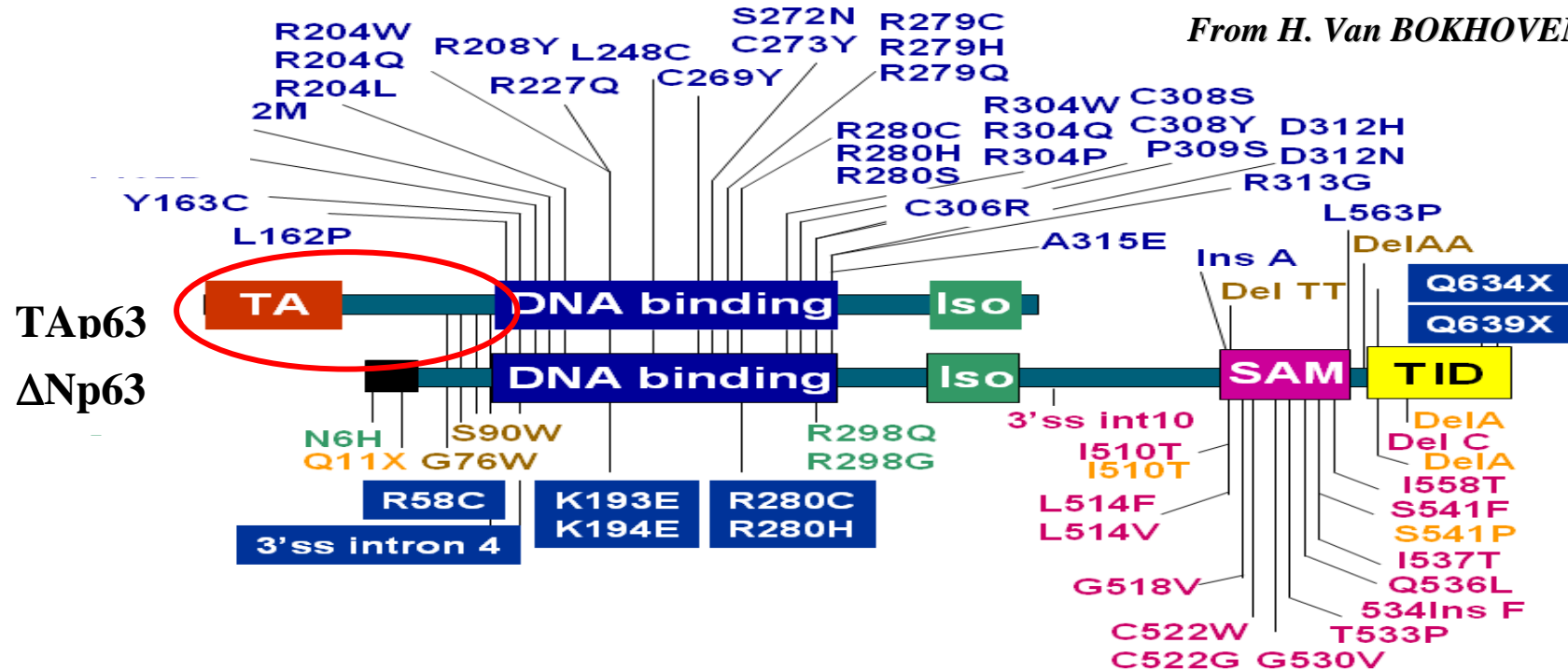
Patient 3 with an atrial septal defect

17. Chiodini BD, Lewis CM. Meta-analysis of 4 coronary heart disease genome-wide linkage studies confirms a susceptibility locus on chromosome 3q. Arterioscler Thromb
18. Senzaki H, Inui M, Ban S, Masutani S, Morsy M, Kobayashi T, Nagasaka H, Sasaki N, Kyo S, Yokote Y. Dilated cardiomyopathy in a 3-year-old girl with a terminal deletion, 46,XX,del(3)(q27-qter), of the long arm of chromosome 3. Eur J Pediatr. 2003; 162(6):403-

Coincidence or linkage?

Are TAp63 mutants responsible for heart failure?

From H. Van BOKHOVEN



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Roni SHAKED

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Alain MEDAWAR

Matthieu ROULEAU

Shoam ARAD

Zohar WOLCHISKY

Cancer
stem cells

Thierry VIROLLE

Mohamed FAREH

Virginie de THILLOT

Laurent TURCHI

David DEBUYRNE

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H. ZHU

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Inserm

Institut national
de la santé et de la recherche médicale

ANR AGENCE
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DE LA
RECHERCHE



ISRAEL SCIENCE FOUNDATION

	Nom	Prénom						Partiel 1				Partiel 2	EXAMEN
			02/03/10	05/03/10	09/03/10	12/03/10	16/03/10	19/03/10	23/03/10	26/03/10	30/03/10	28/05/2010 (10-12h)	
1	ABGRALL	KEVIN	absent	absent	OK		X				X		
2	BARRES	CELINE	OK	OK	OK								
3	BENZAQUEN	JONATHAN	OK	absent	absent		X				X		
4	BELLON -SERRE	GABRIELLE	OK	OK	OK								
5	BOUSKINE	ADIL	absent	OK	OK								
6	CODJIA	PÉKÈS	OK	absent	OK								
7	GRANDJACQUO	DELPHINE	OK	OK	absente								
8	IMBO	ALEXIA	OK	OK	OK								
9	ITURZAETA	HENRI	OK	absent	OK								
10	LERAY	LOIC	absent	absent	absent		X				X	X	
11	MERELLO	MARION	OK	OK	OK								
12	MUNYEJABO	JEAN VICTOR	absent	absent	absent		X				X	X	
13	PARDET	NOEMIE	absent	absent	OK		X				X		
14	PERRON	LAËTITIA	OK	OK	OK								
15	TOUBLANC	CHRYSTELLE	OK	OK	OK								

labo le Mardi 23 (au lieu du Vendredi 26)

Correction cours CSH: multipotente au lieu de pluripotente)