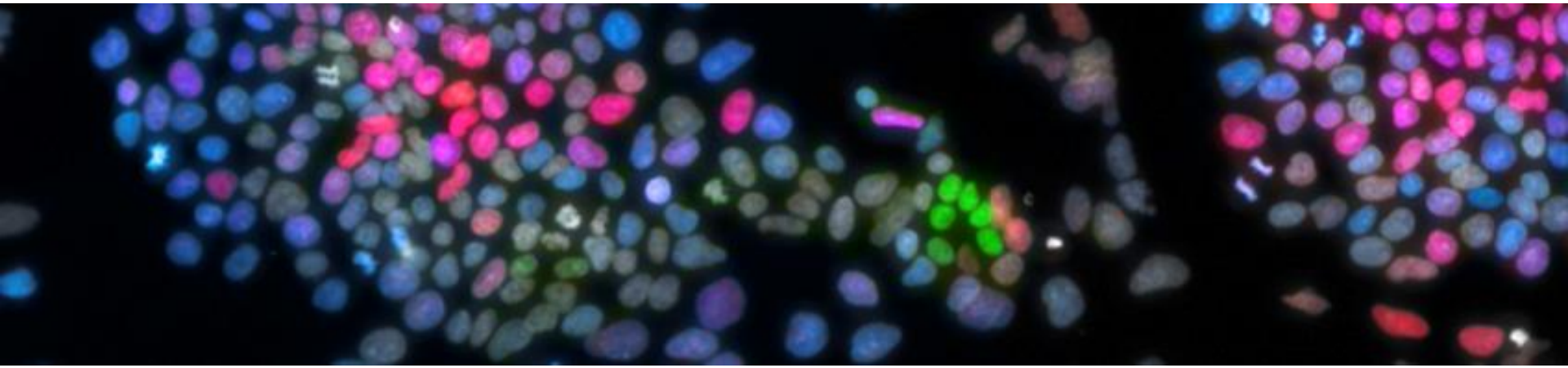
Examining the State of the Science of Mammalian Embryo Model Systems: A Workshop (NAS)



Jan 17th 2020

SESSION IV: COMPARATIVE EMBRYONIC DEVELOPMENT ACROSS SPECIES

Early Neural Crest Formation: from birds to humans

García-Castro Lab UCR, School of Medicine

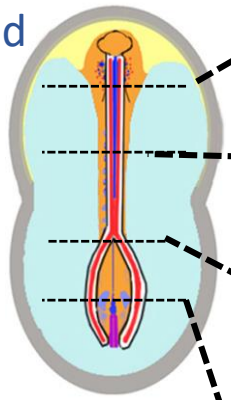
Wilhelm His, 1868

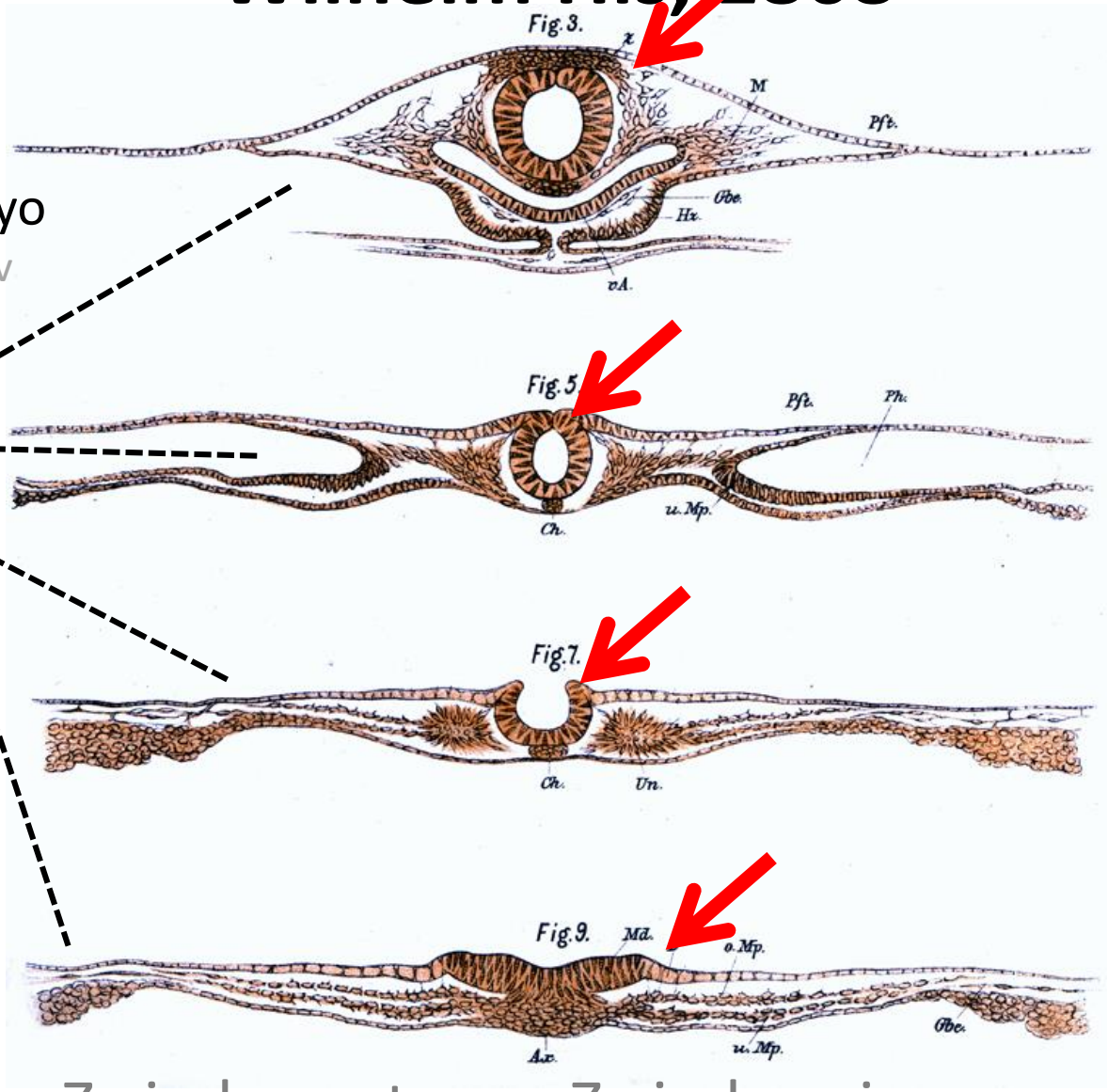
Chick embryo

36 hours dev

Head

Tail





Zwischengstrang, Zwischenrinne

Middle cord, Marshall 1879…

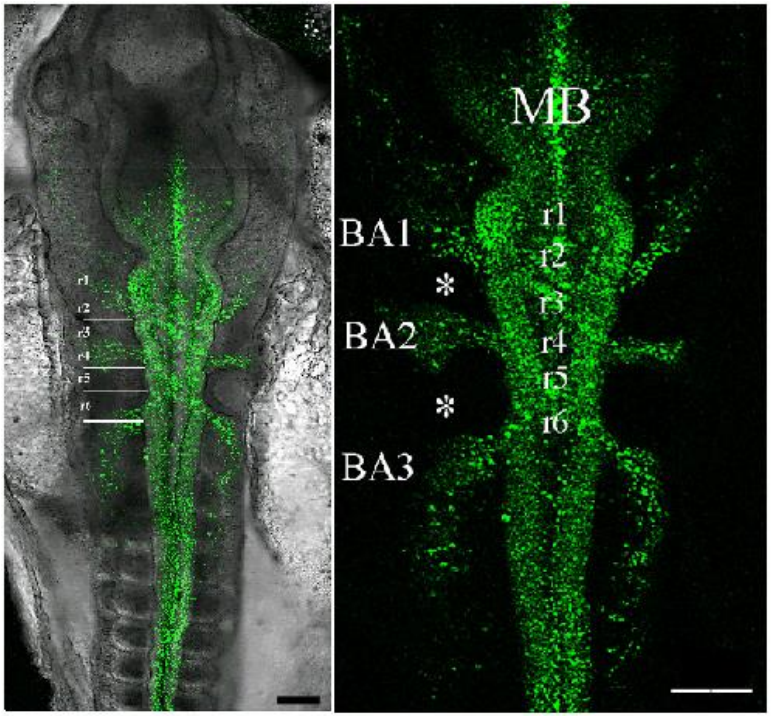
“NEURAL CREST”

Chick embryo

Head

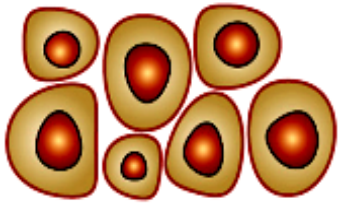
Tail

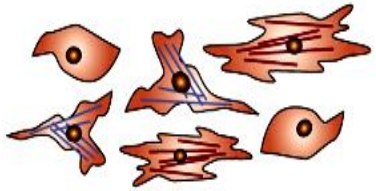
Neural Crest



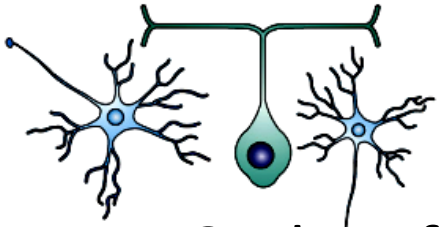
Migrate extensively through stereotypic patterns

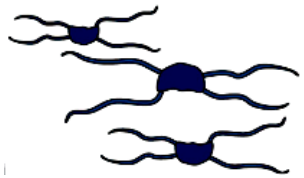
Neural Crest Derivatives



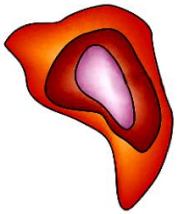


Cartilage, bone, and Connective tissue





neurons & glia of the PNS, and Melanocytes



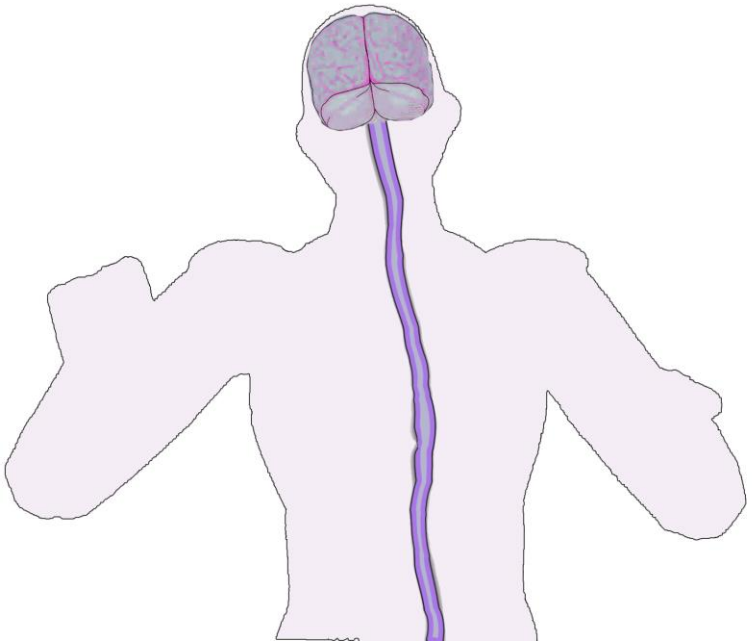
Sympathoadrenal cells

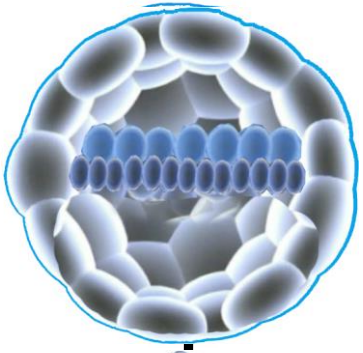
PATHOLOGIES: 1/3 of all birth defects.
Orofacial clefts, rare syndromes, and cancers

Differentiation Potential of Neural Crest

Neurons, Glia, melanocytes, Adipocytes, odontoblast, osteoblasts, chondroblasts, myocytes, Oxigen sensing cells of the Carotid Body, Thymus mesenchyme, etc....

How do NC cells Generate so Many Different Cell types?





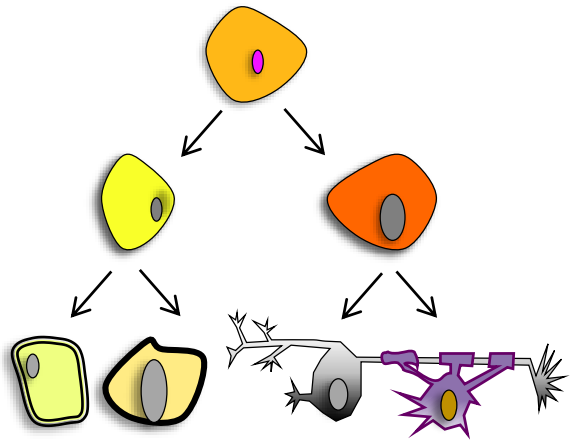
EPIBLAST
ESC

Gastrulation

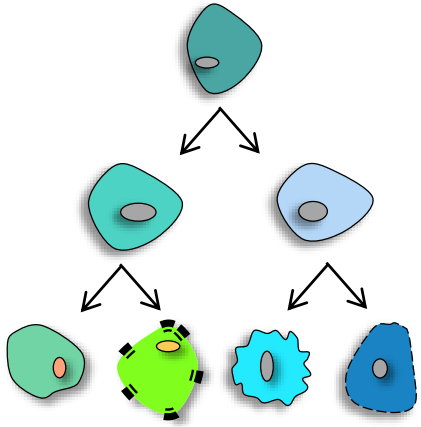
Ectoderm

Mesoderm

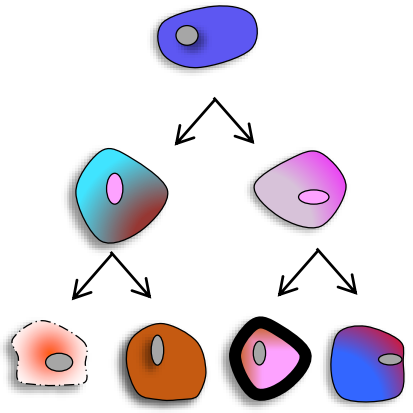
Endoderm



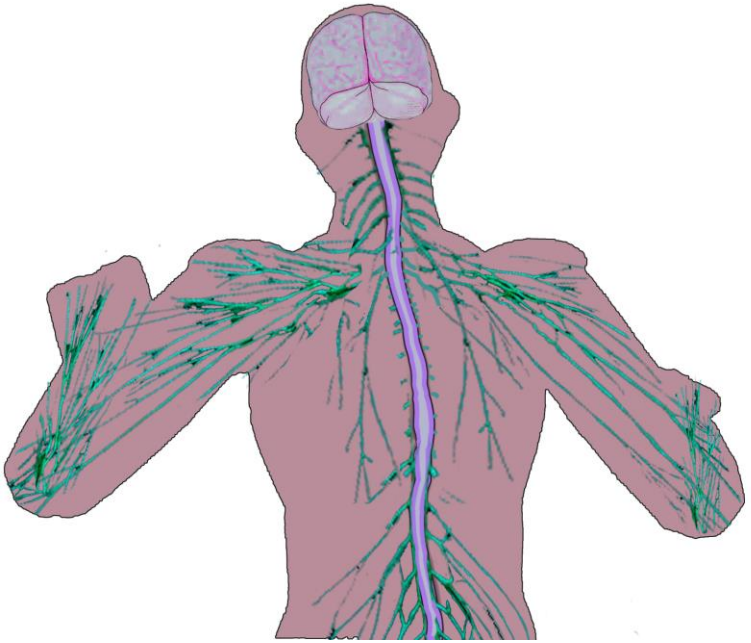
Skin & CNS



Muscle, Bone, Adipo, Blood



Gut, Thyroid, Lungs, Pancreas



EPIBLAST

Gastrulation

Melanocytes

PNS

Neural Crest Cells

Ectoderm

Mesoderm

Endoderm

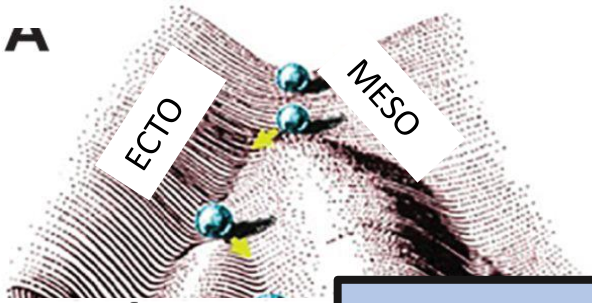
Skin & CNS

Muscle, Bone, Adipo, Blood

Gut, Thyroid, Lungs, Pancreas

Waddington Epigenetics

Sequential segregation of potential



EPIBLAST

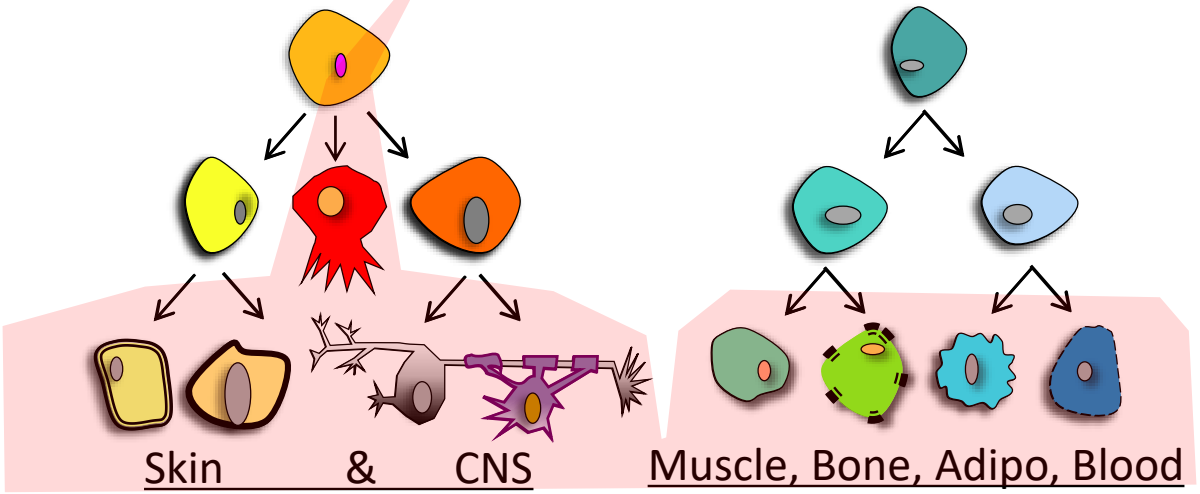
Melanocytes

Neural Crest

Revisit the Ontogeny of the Neural Crest

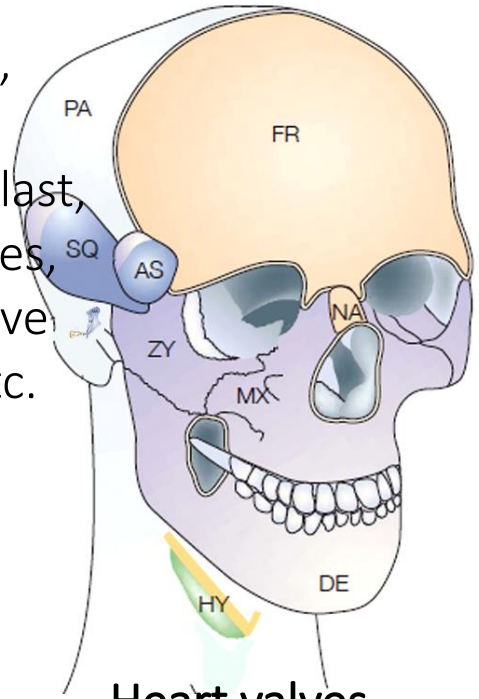
Ectoderm

Mesoderm



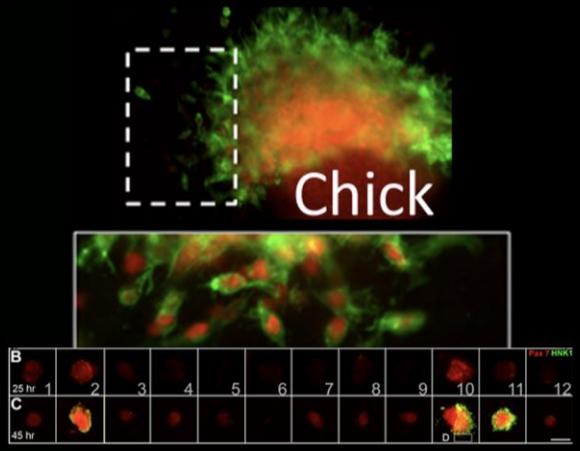
CRANIOFACIAL

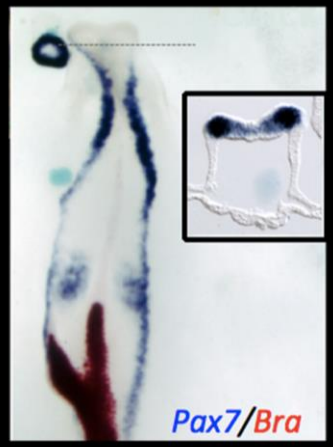
Bone ,
Cartilage,
Muscle,
blast,
tes,
tive
tc.

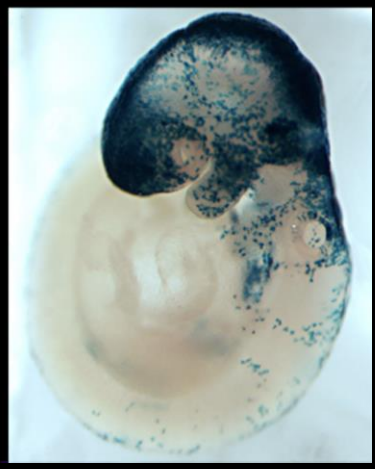


Heart valves

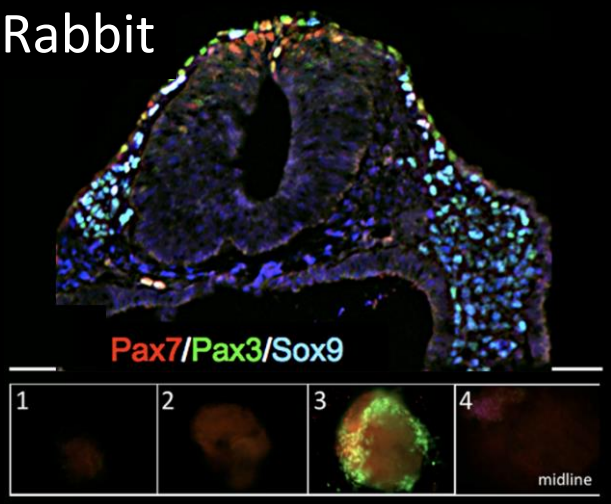
Garcia-Castro Lab: Neural Crest Ontogeny



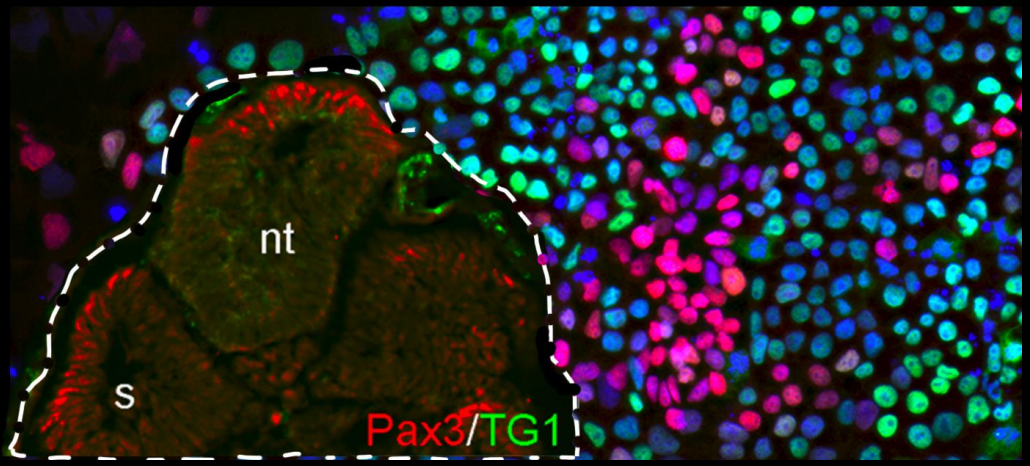




Rabbit



Human: embryonic & hES derived NC



”Ontogeny of Neural Crest "

Specification of chick NC before gastrulation.

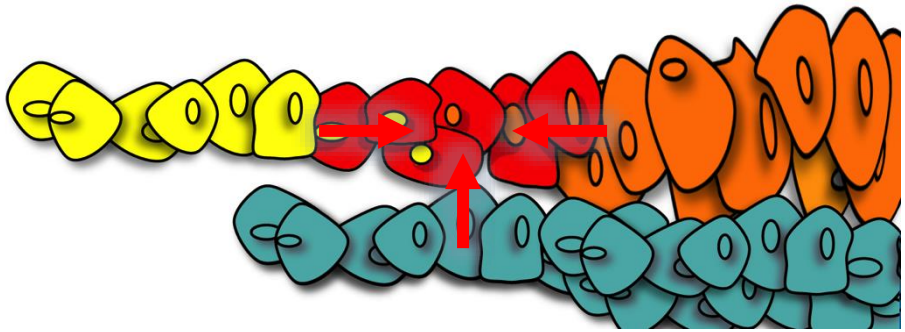
Human NC, Embryonic studies and PSC models suggest an early post epiblast differentiation.

Specification of mammalian NC in the Rabbit is evident during gastrulation

García-Castro Lab
UCR, School of Medicine

Neural Crest Formation

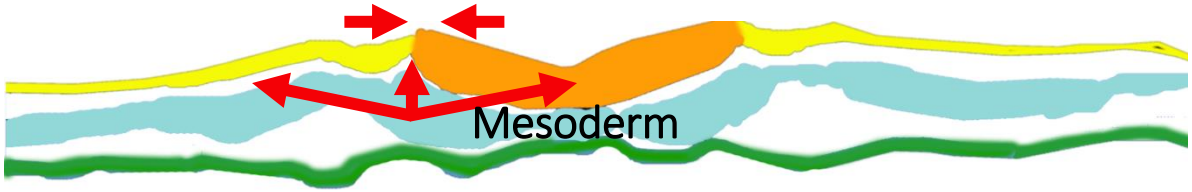
Classic Induction



Wnt
BMP
FGF

Cascade of Transcription Factors and other markers responsible for Neural Crest dev.

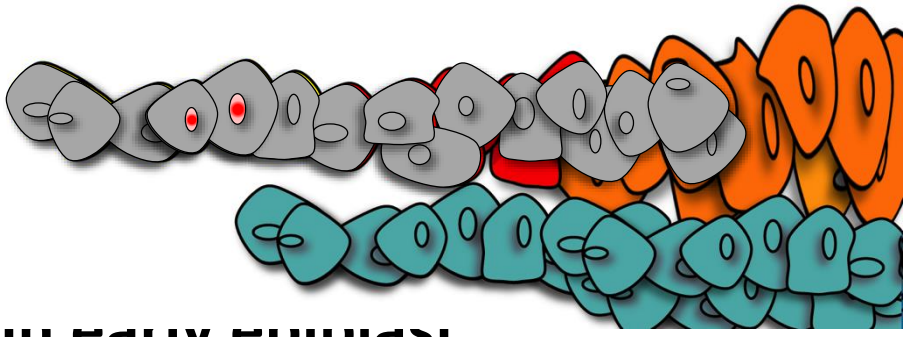
Non Neural Ecto **Neural Ectoderm**



NCC Induction

Neural Crest Formation

Our work suggest **earlier** NC induction
(prior to definitive mesoderm and neural tissues)



NCC

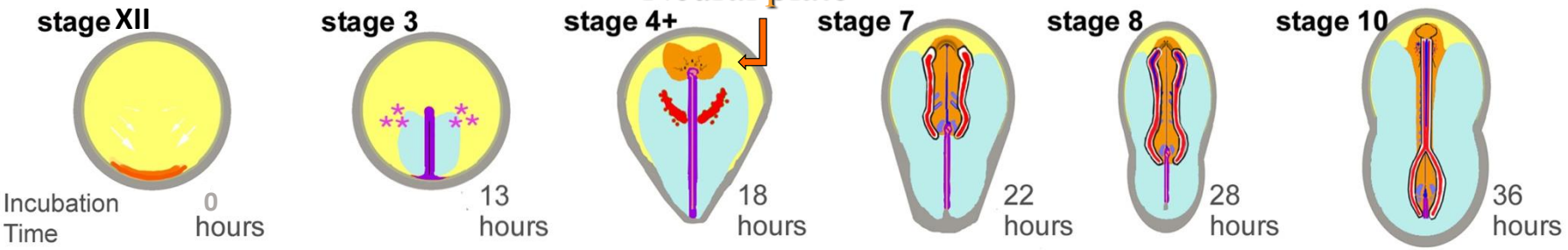
in early epiblast

Specification: initiation of a program, the NC fate program. The specified cell does not display known NC markers yet, but it is able to do so under "undisruptive conditions". Gain of expression of NC markers used to **assess** NC fate. **NOT COMMITED**, could be persuaded to do otherwise

Early avian NC development

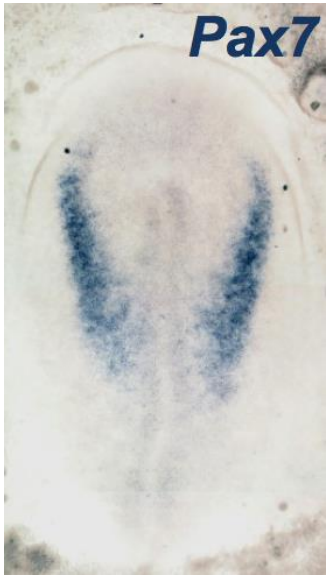
Snail2, Sox9, FoxD3 exp

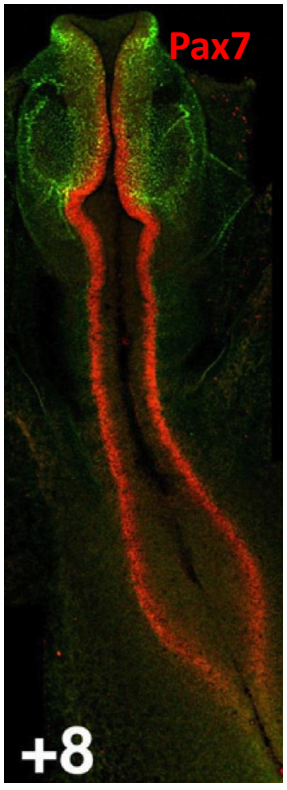
Neural plate



Specified

Pax7 expression & requirement

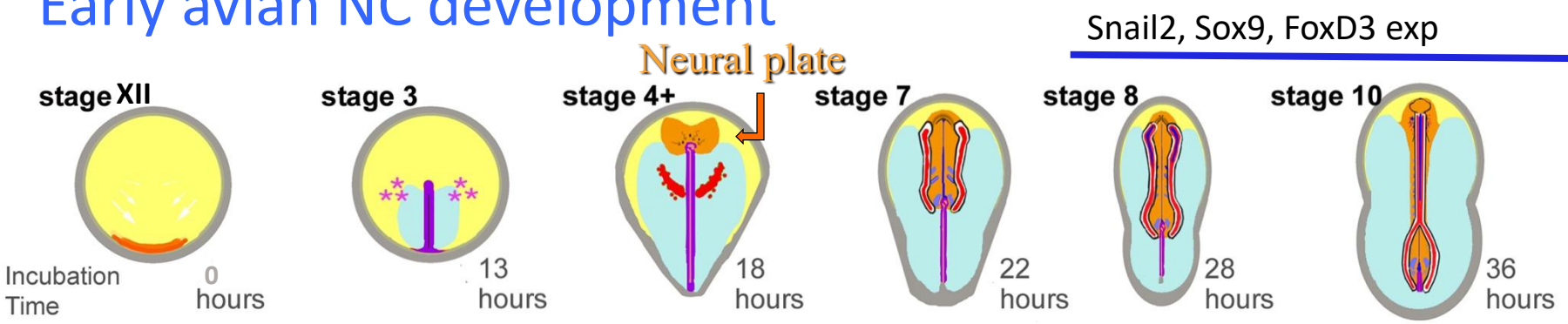




Pax3
Sox9
Ets1
Sox10
FoxD3

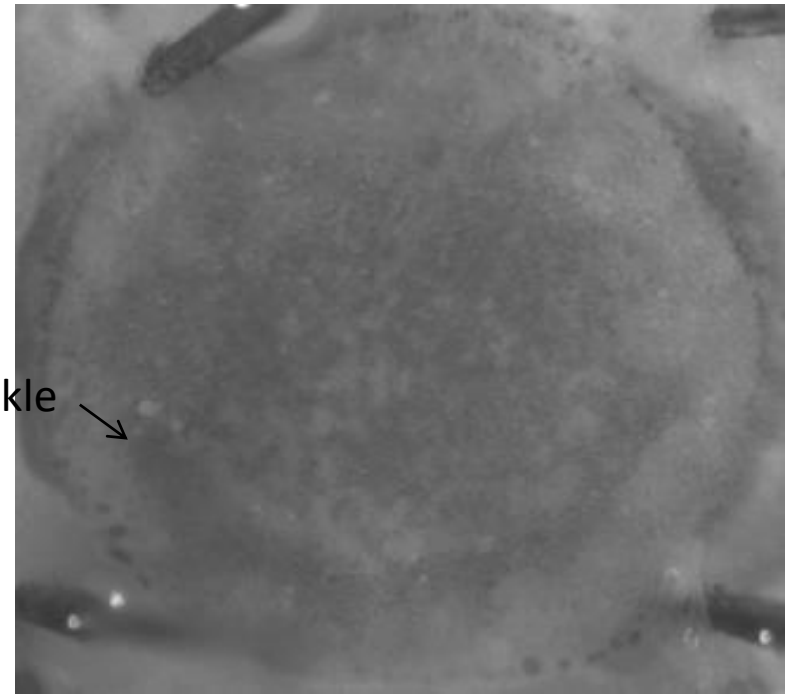
Basch *et al.* 2006
Nature 441:218-222

Early avian NC development



Earlier specification?

Basch *et al.* **Nature** 441:218-222



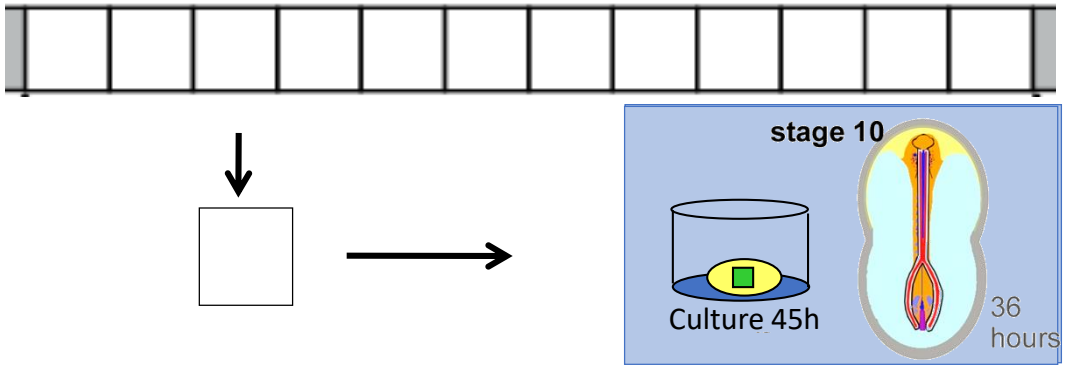
Eileen Uribe-Querol

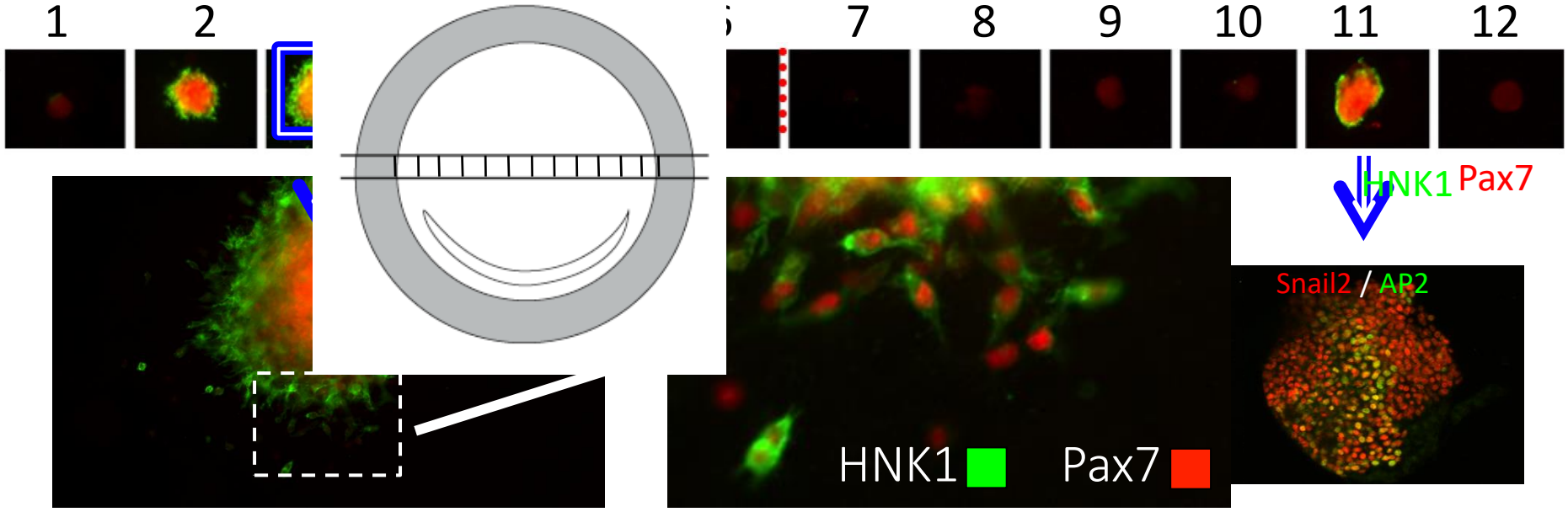
(Assistant Professor, UNAM, Mexico)

Blastula studies of NCC specification

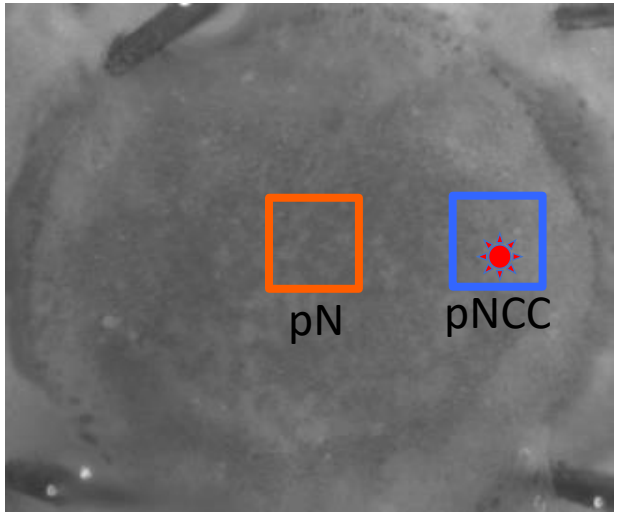
Dissect epiblast tissues, explant into collagen gels

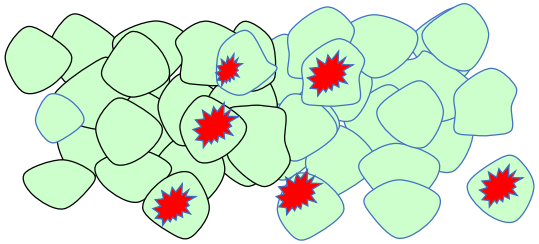
Culture in isolation /non inducing conditions



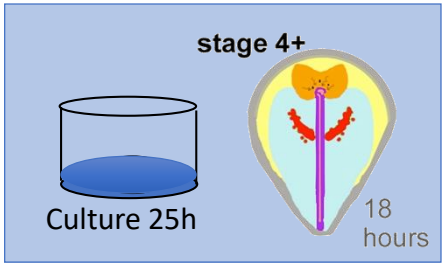


Are NC markers **induced** during culture?





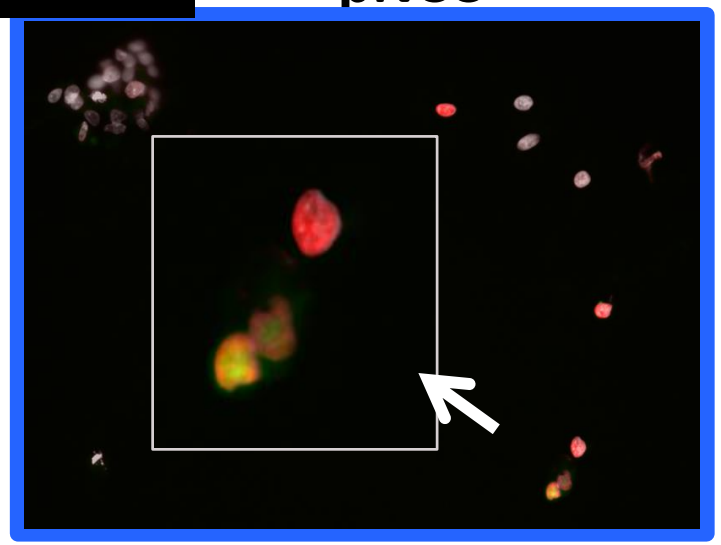
Prospective NCC



pN

Dapi Pax7 Sox9

pNCC

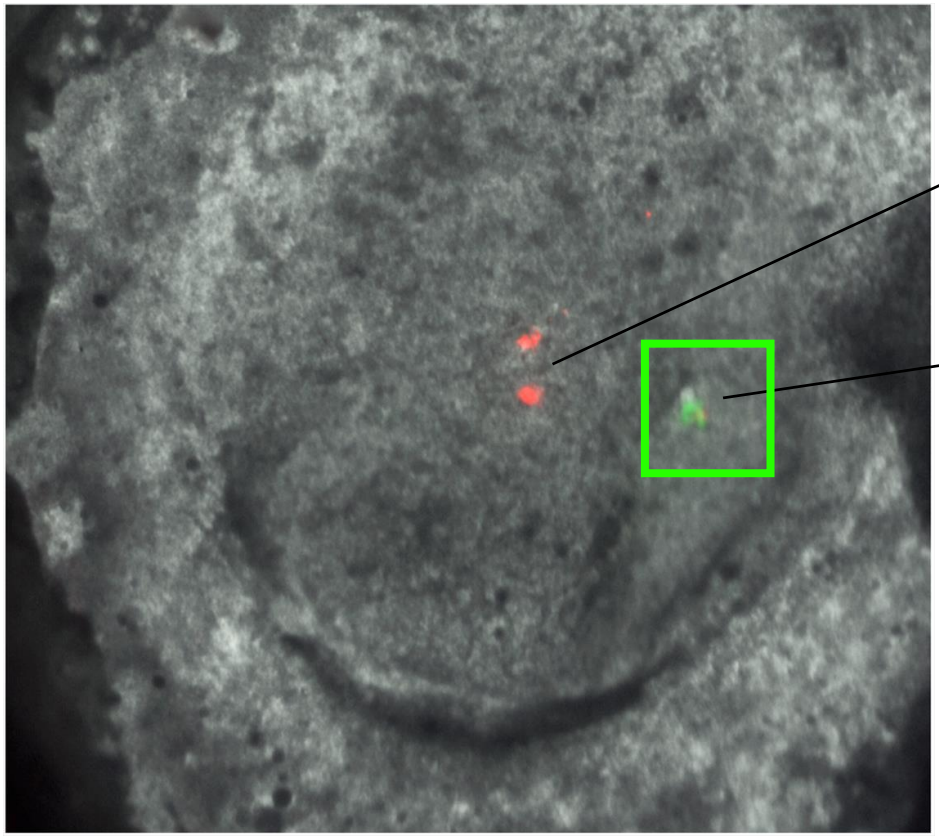


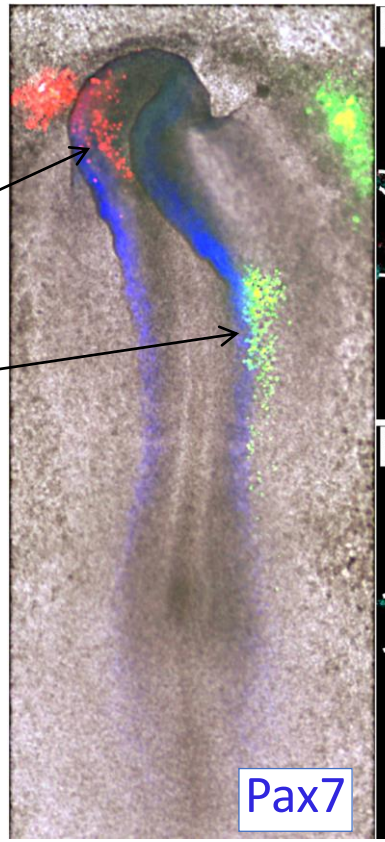
pN: 6% Pax7, 5% Sox9.

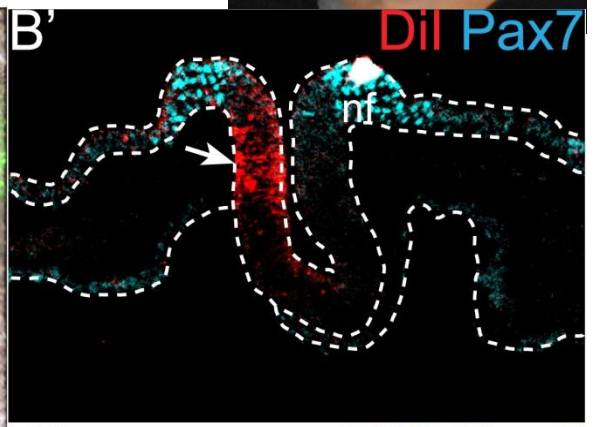
pNCC: 45% Pax7, 23% Sox9.

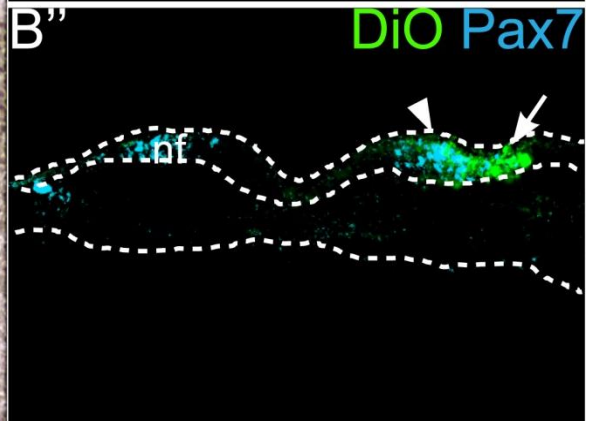
Epiblast Fate Mapping

Stephanie Vadasz









Cell tracing summary

Are human Neural Crest also specified before gastrulation?

Can we translate the gains from Chick NC studies?

Neural ectoderm

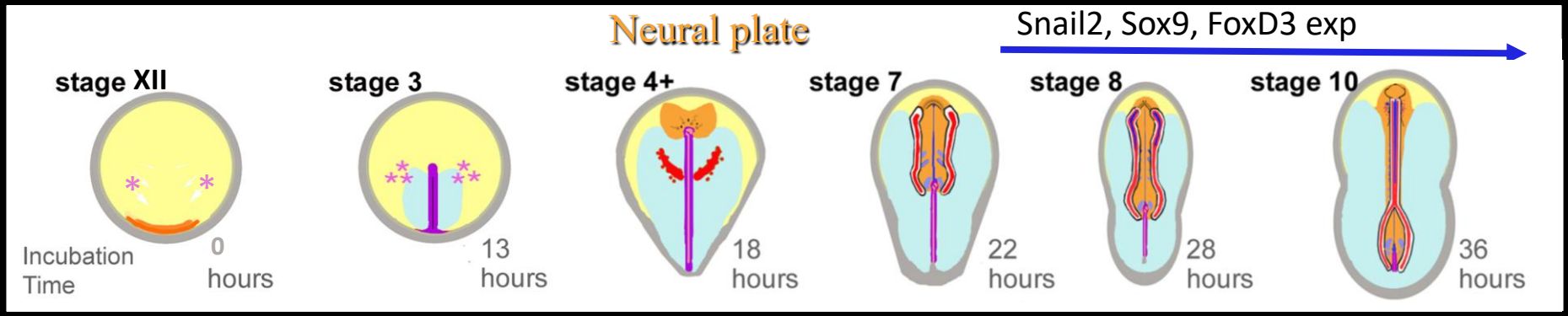
Non-neural ectoderm

Mesoderm

Medial	0/3→ NC
Intermediate	7/9→ NC

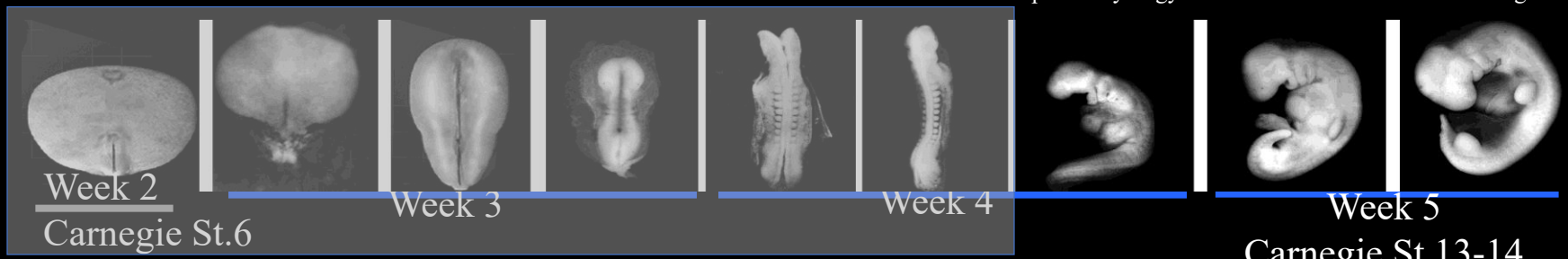
$\chi^2 = 5.600$, $df = 1$, $\chi^2/df = 5.60$, $P(\chi^2 > 5.600) = 0.0180$

Analysis of early human NCC development



Specified → **Pax7 expression & requirement**

http://embryology.med.unsw.edu.au/wwwhuman/Stages/



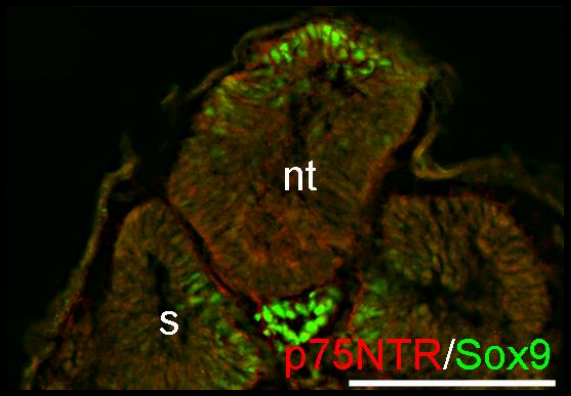
Ronan O'Rahilly

Histological analysis — Carnegie St.12-18

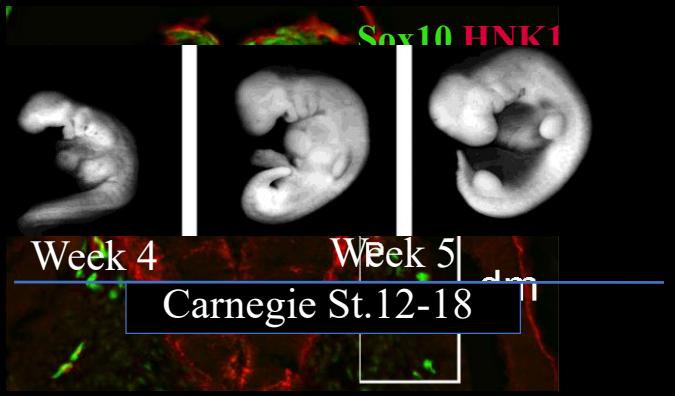
Dr. Erik Sundström
(Karolinska Instituet, Sweden)

Analysis of early human NCC development

Primagratory NCC



Migratory NCC



Pax3, Pax7, Sox9, Sox10,
Msx1/2, AP2, HNK1, P75^{NTR}

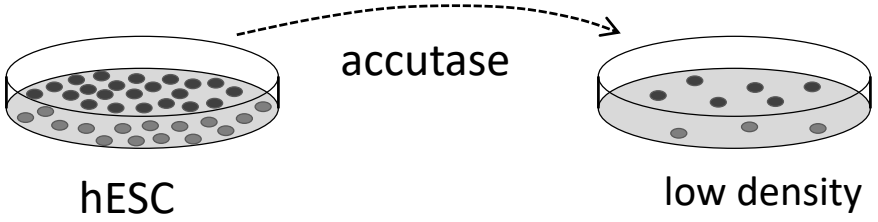
Erin Betters

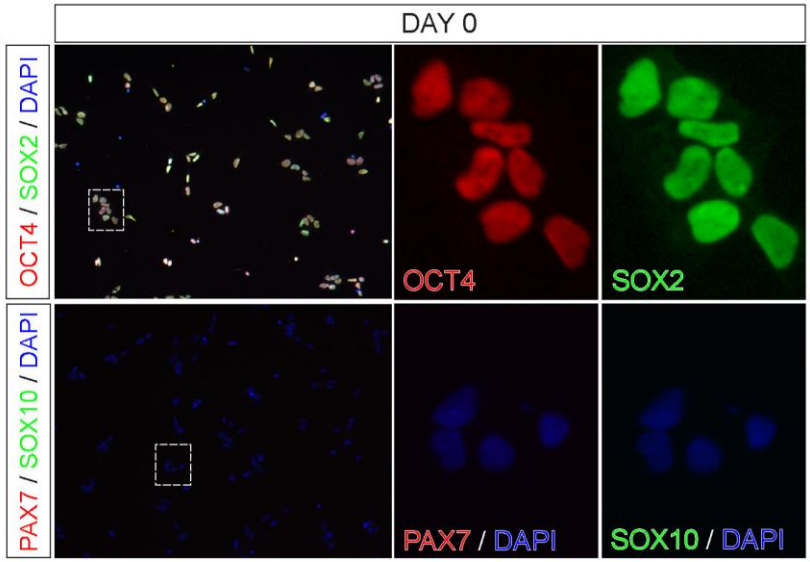
Betters et al, Dev. Biol. 2010

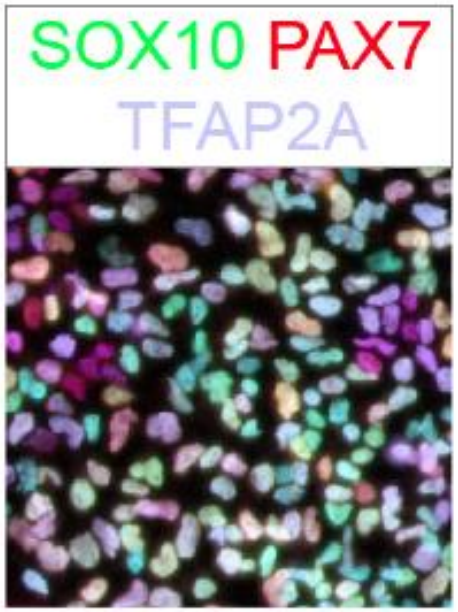
Modeling hNC development with PSC

Pomp et al 2005 (R. Goldstein lab→ Studer, Wysocka, Dalton, Bajpai, Toguchida, Andrews, etc)

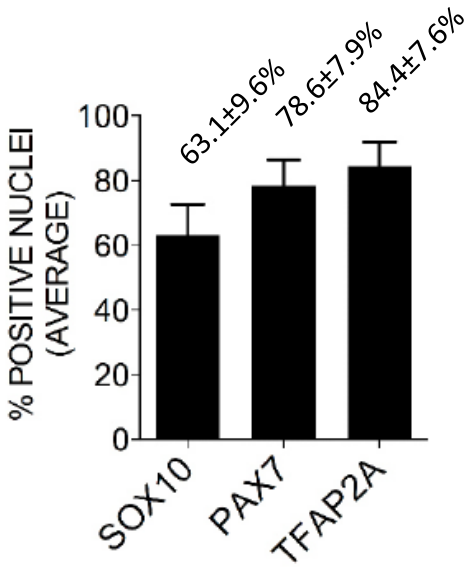
Fast derivation of hNC from hESC via WNT in defined media





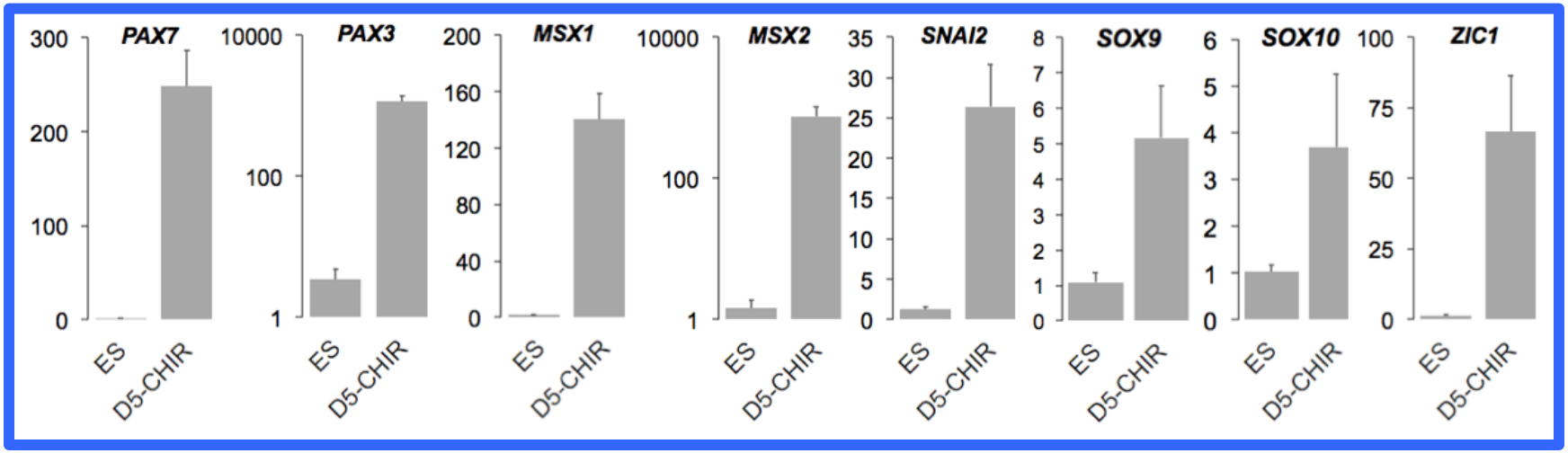


~95% of SOX10⁺ cells co-expressed PAX7 & TFAP2A

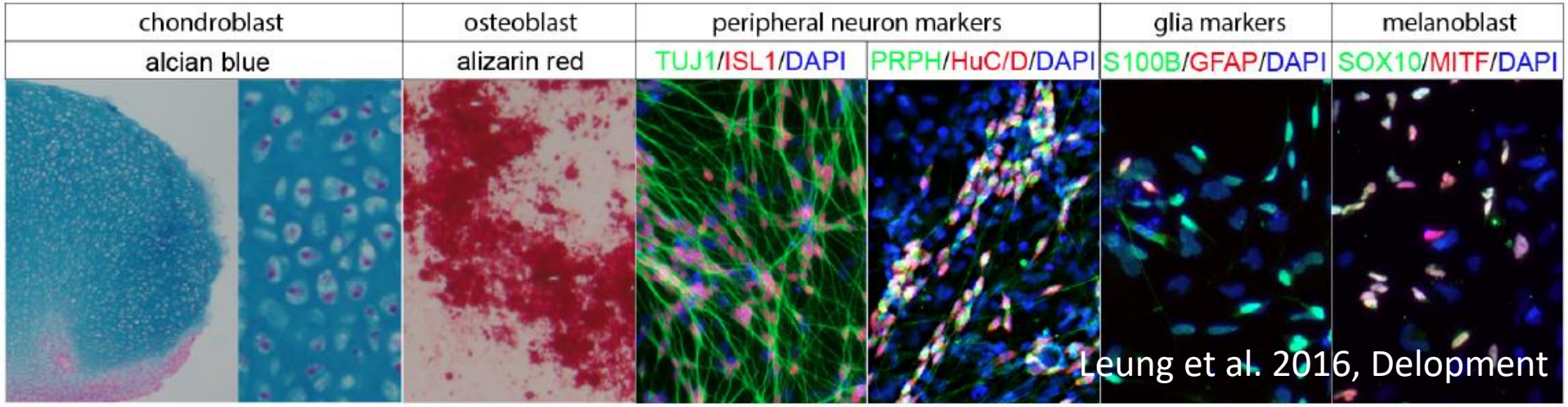


Validation of NC status

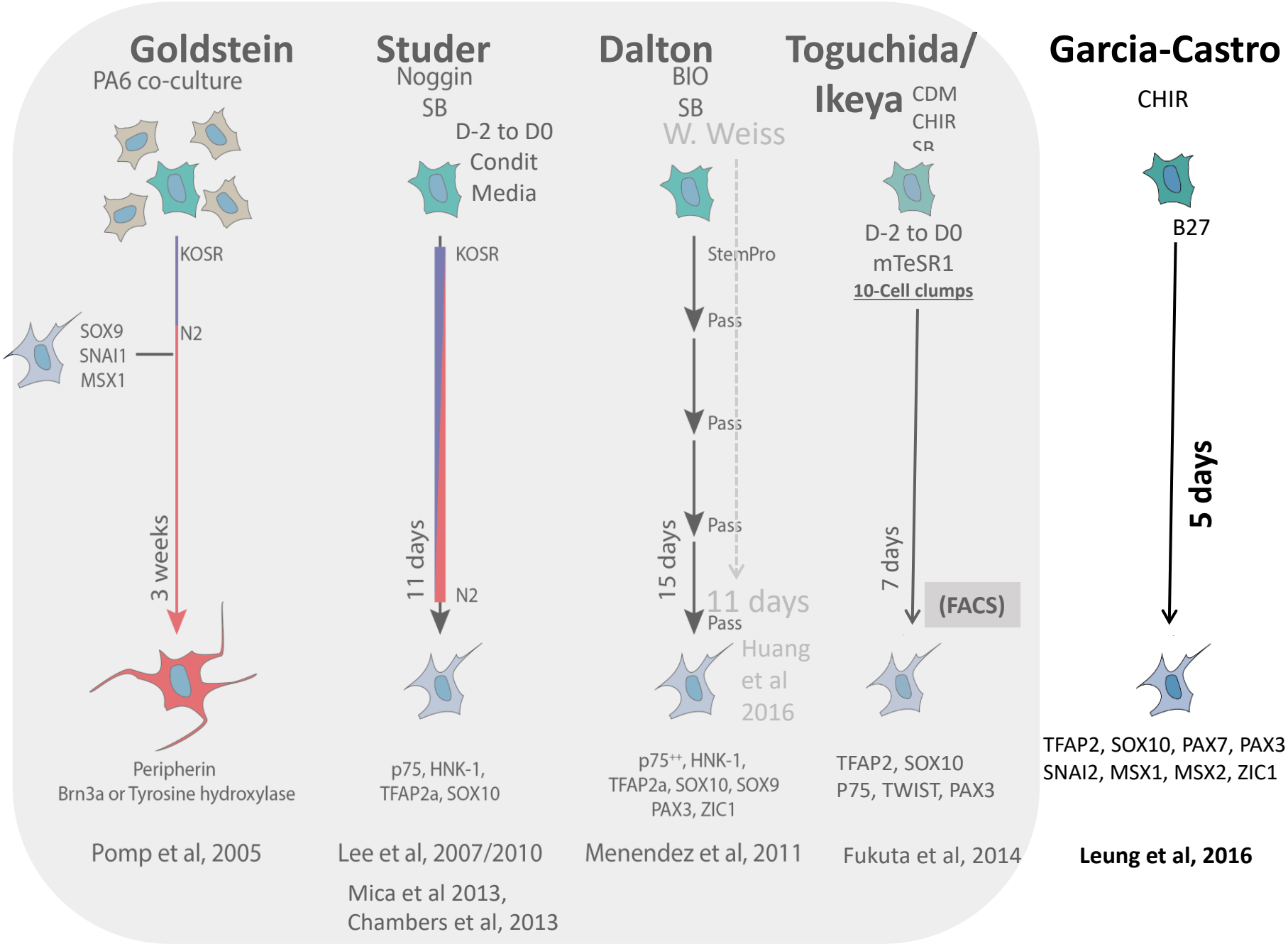
Expression of NCC markers



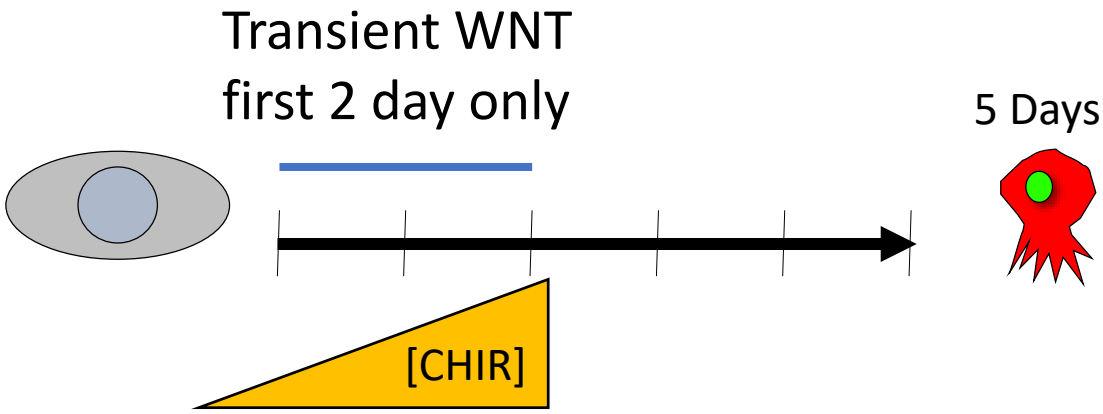
Wnt-induced hNCC generate genuine NCC-derivatives



HISTORY OF NEURAL CREST INDUCTION FROM hPSCS



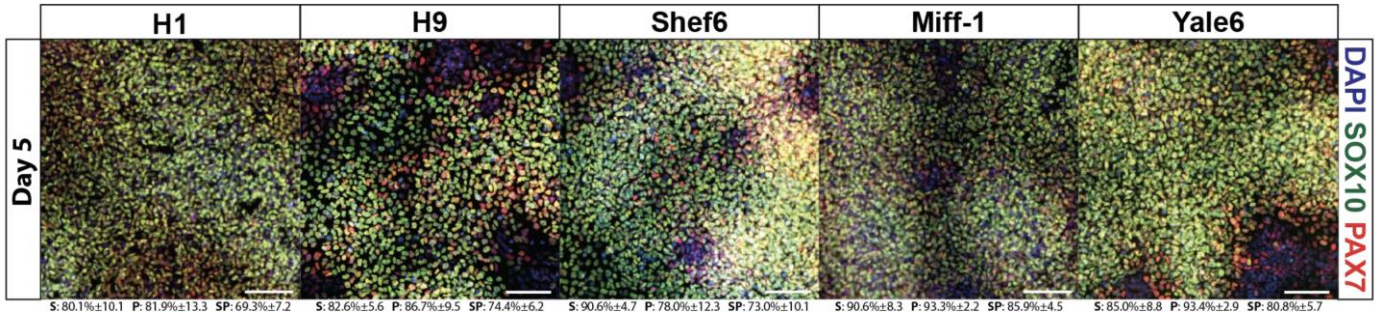
Characterizing the signaling parameters



Low CHIR (WNT)
Anterior NC
No Hox-(cartilage)

High CHIR (WNT)
Posterior NC
Hox+ (adrenal)

Gomez *et al.* 2019a Dev Biology
Gomez *et al.* 2019b Development



XENO-Free model high efficiency across multiple ES/iPSC

Hackland *et al.* 2019 Stem Cell Reports

Garcia-Castro

CHIR

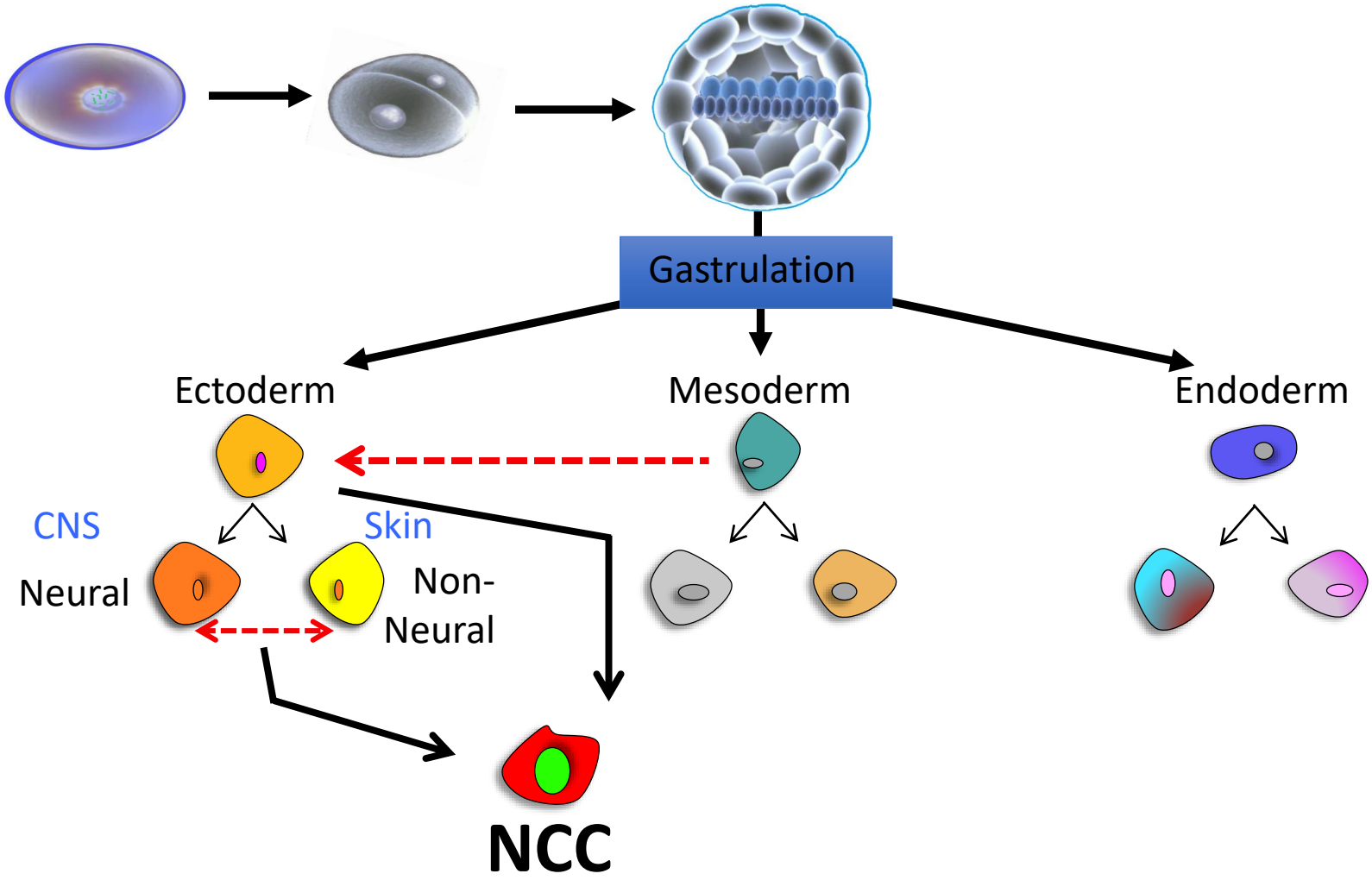
B27

5 days

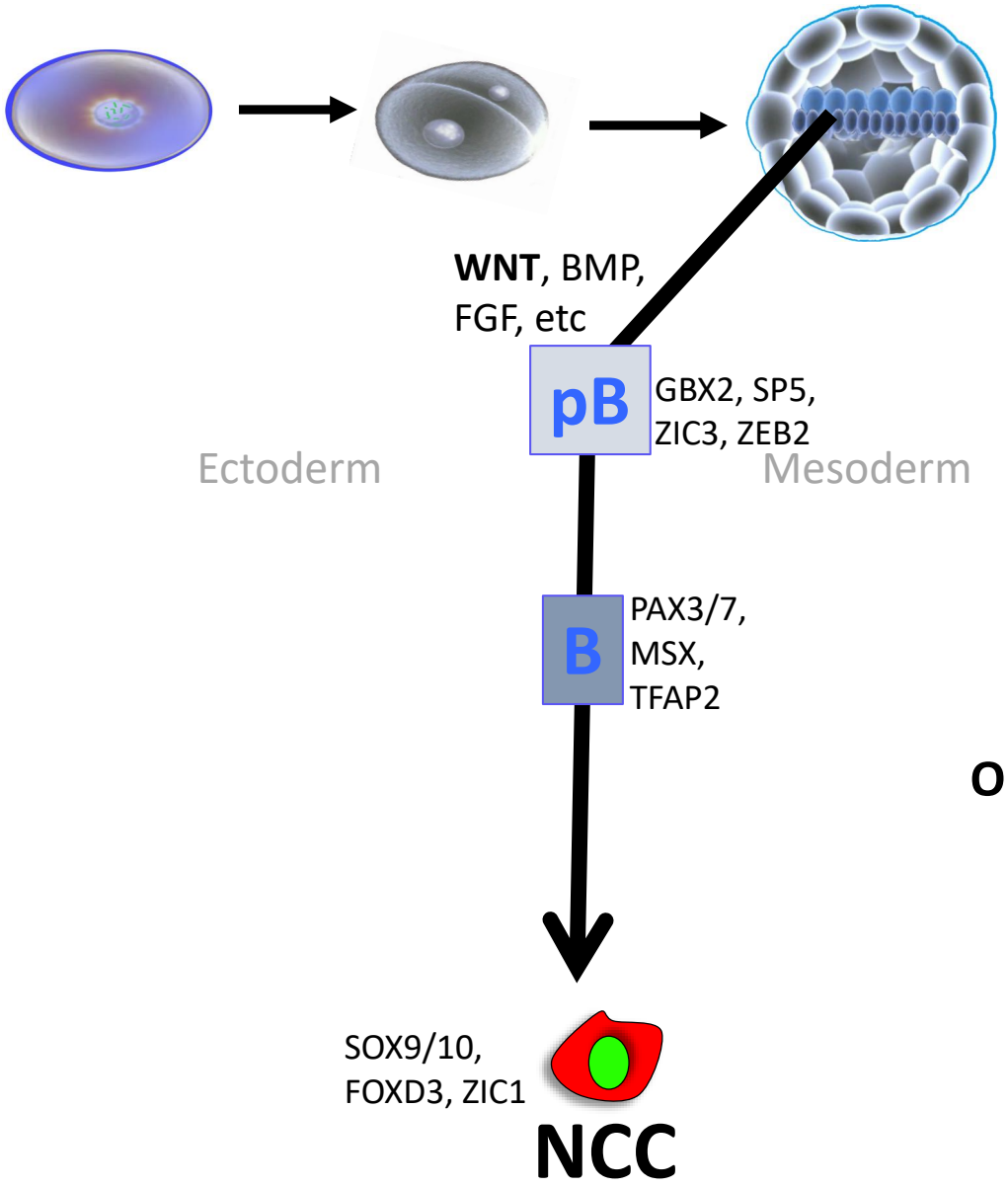
TFAP2, SOX10, PAX7, PAX3
SNAI2, MSX1, MSX2, ZIC1

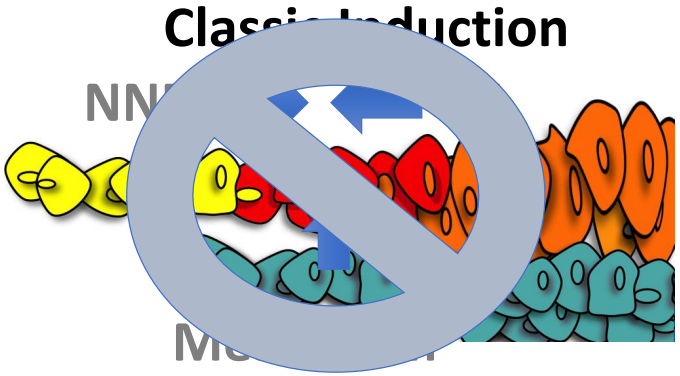
Leung et al, 2016

Ontogeny of Wnt induced hNC



Ontogeny of Wnt induced hNC





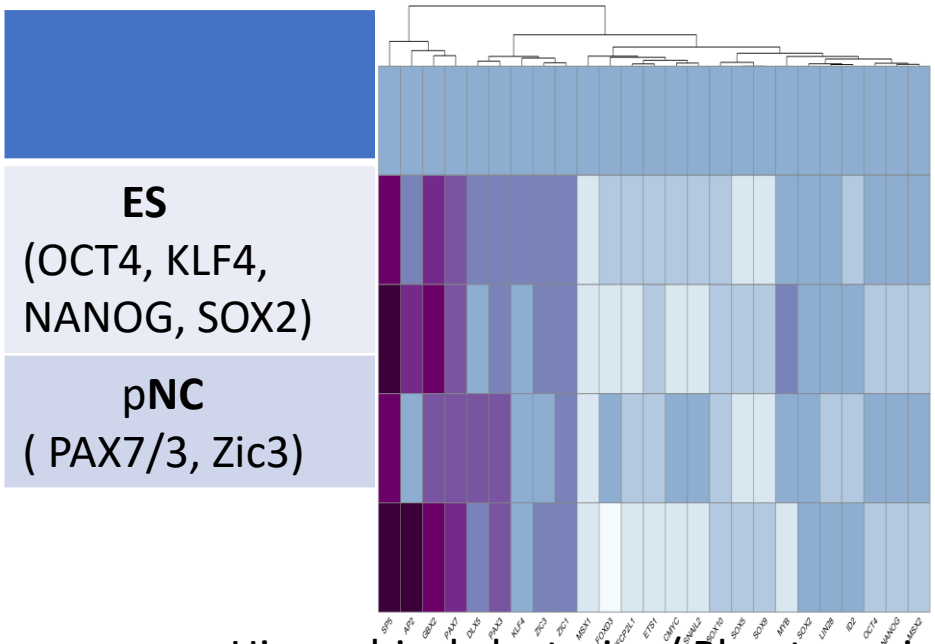
Ontogeny and signaling contributions

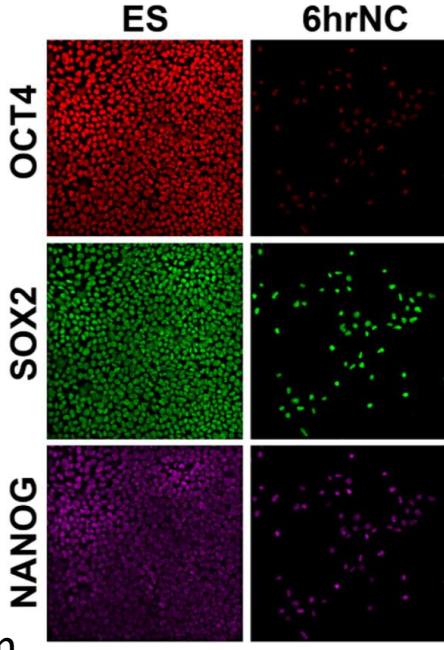
Leung *et al.* 2016, Development

Further analyses of hNC Ontogeny

EPIGENETIC, TRANSCRIPTOMIC, AND FUNCTIONAL ASSAYS

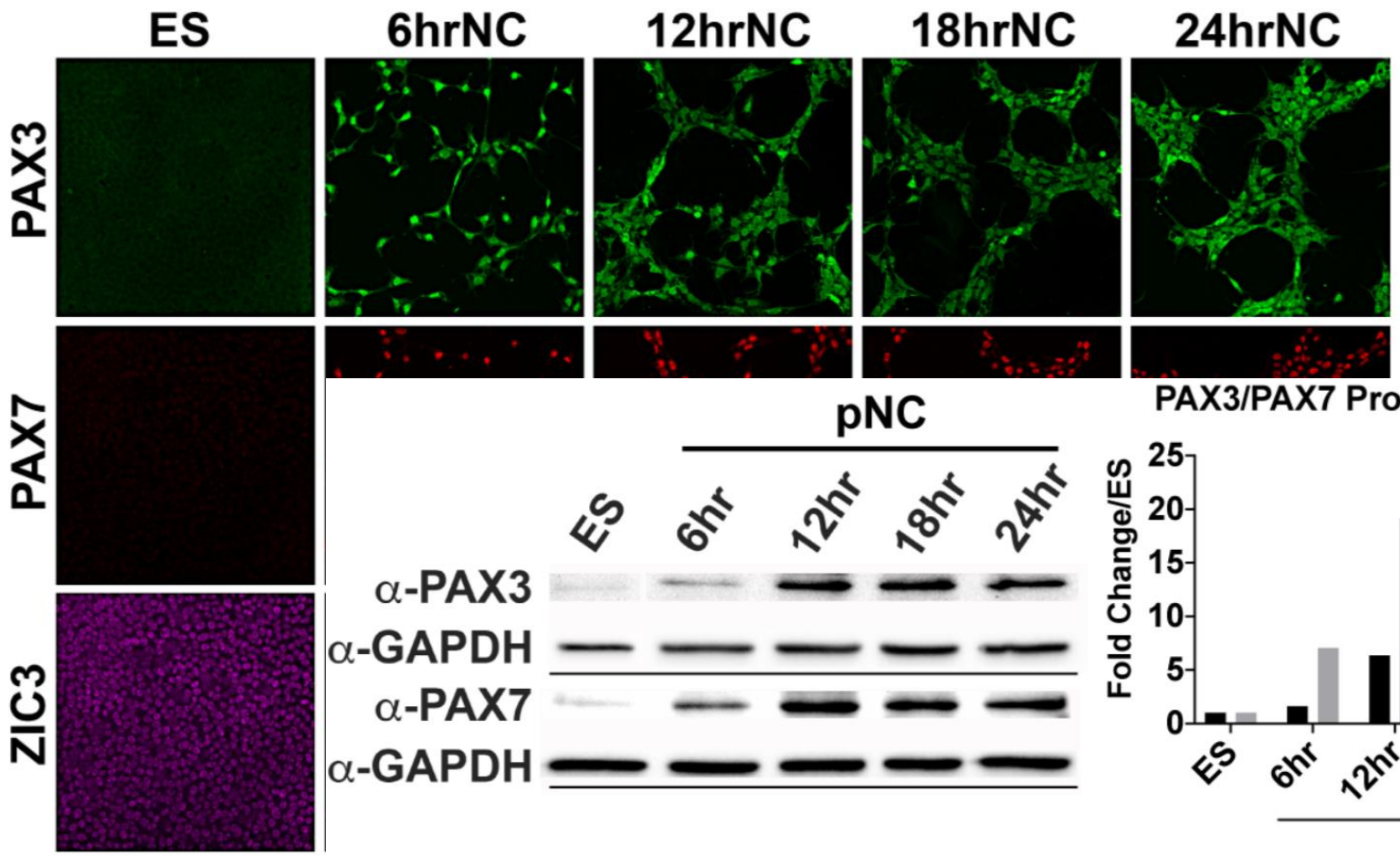
Compare epigenetic regulation and expression of ES and NC markers after WNT induction





Hierarchical clustering/ Pheatmap in R-studio program.

Upregulation NC proteins in prospective NC soon after WNT activation



Leung *et al.* 2019, BioRxive

Prasad *et al.* 2020, Submitted

EPIGENETIC, TRANSCRIPTOMIC, AND FUNCTIONAL ASSAYS

INDICATE THAT 6 HOURS AFTER INDUCTION, THE NC PROGRAM IS INITIATED

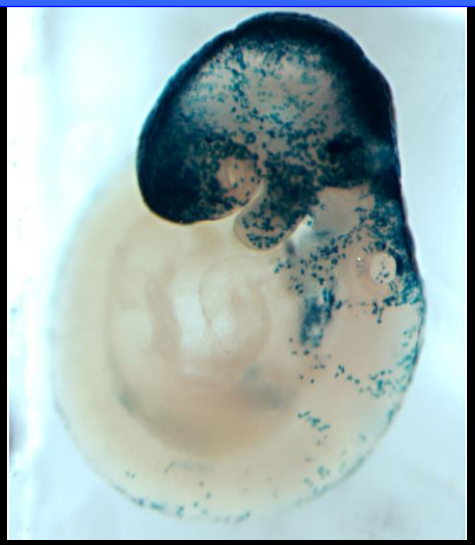
Can we translate the gains from Chick and model human NC studies to mammalian embryos?

Early NCC development in Mammals

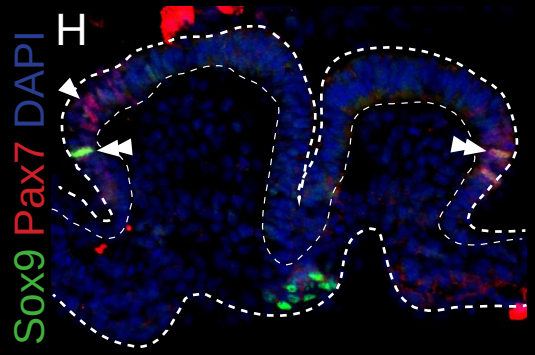
Casey DelConte

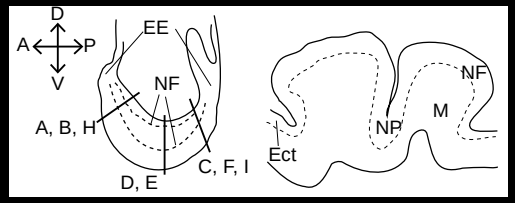
Barbara Murdoch

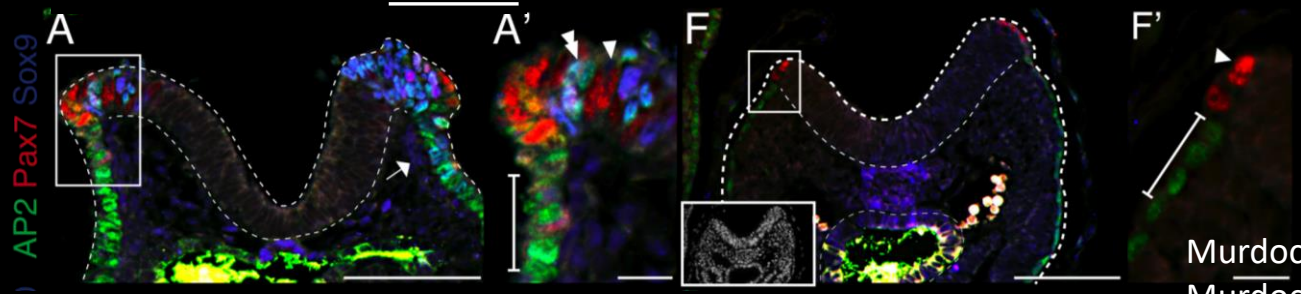
Pax7-cre
Pax7 LacZ
Pax7 EGFP
ROSA26



Mouse E7-E9.5





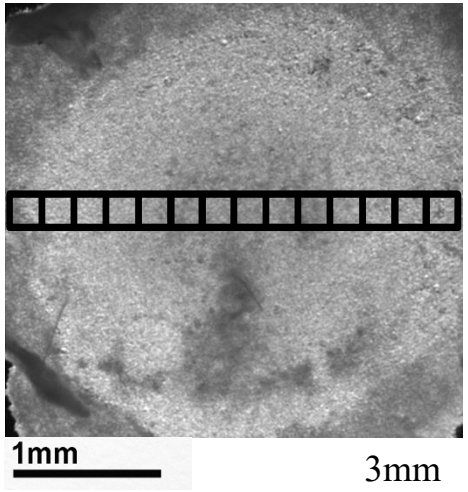


Pax7KO & Pax3KO

Murdoch *et al*. J Neuroscience 2010
Murdoch *et al.* PLoS ONE 2012
DelConte & García-Castro Submitted

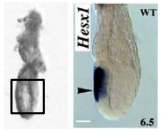
Rodent specific pre-gastrula development

Chick

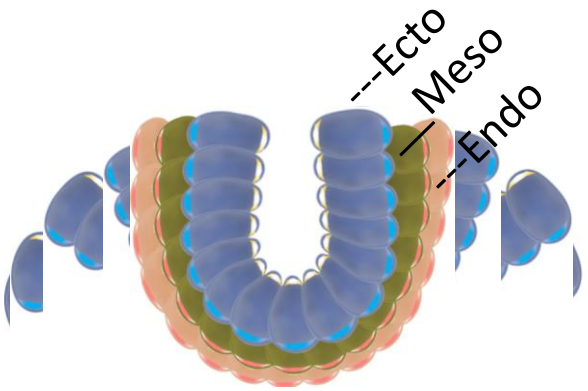


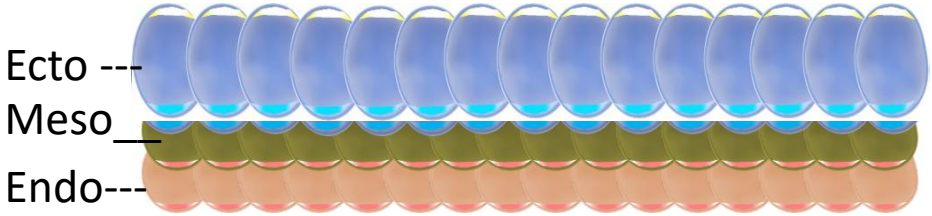
3mm

Mouse



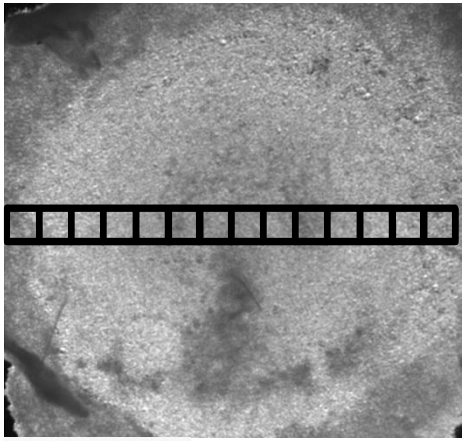
150µm





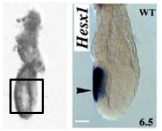
Rabbit development as a mammalian alternative

Chick



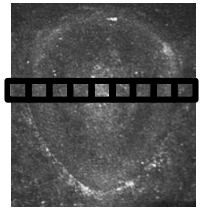
3mm

Mouse



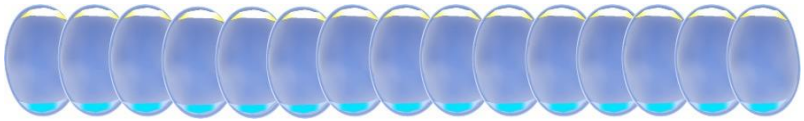
150µm

Rabbit



700µm

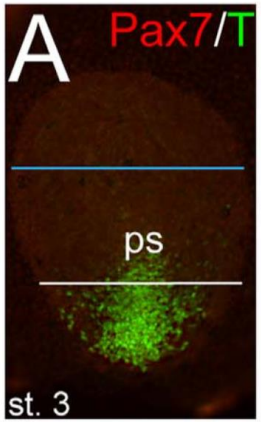
CHICK

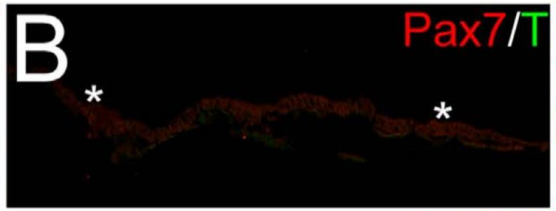


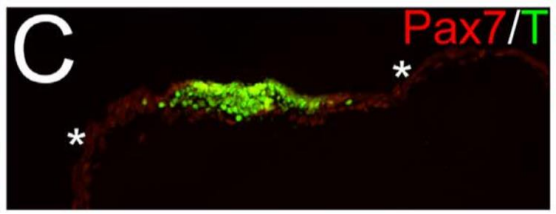
MOUSE

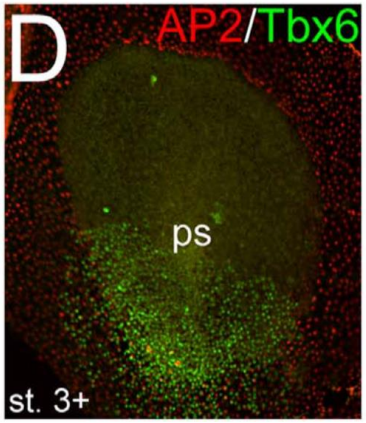
RABBIT

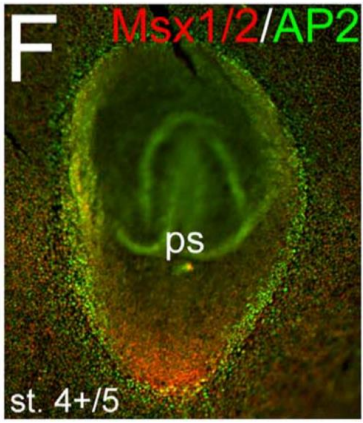
Expression of mesoderm, Neural, and NC markers in the rabbit

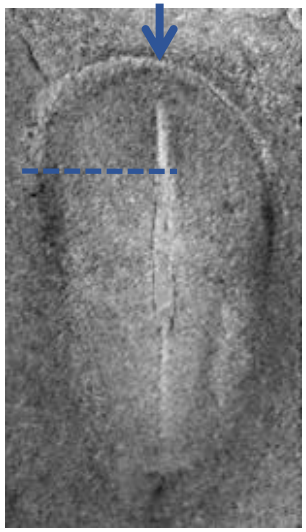




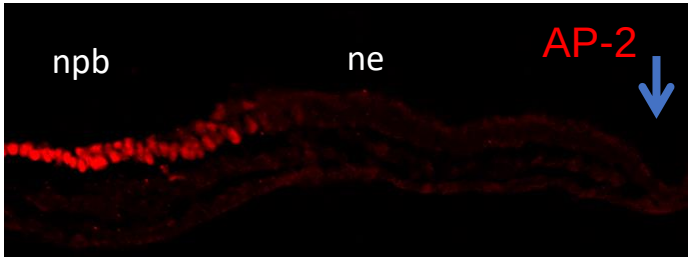


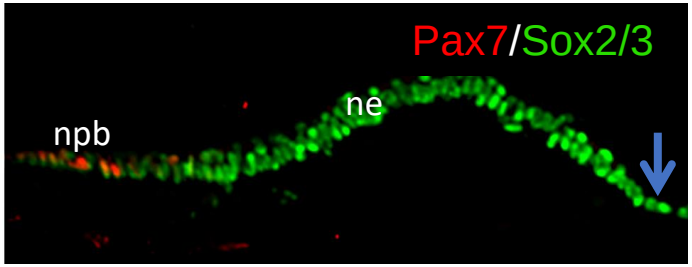






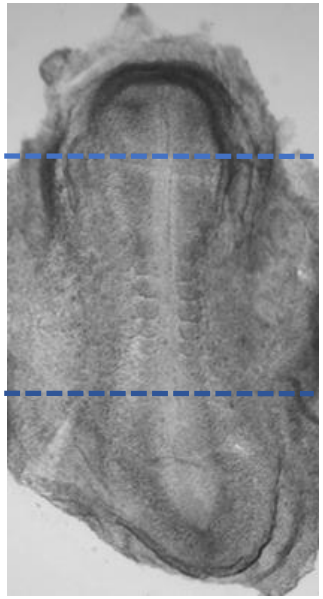
St. 6 Rabbit



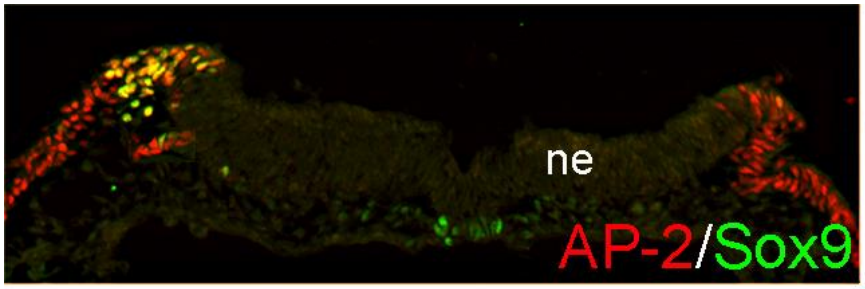


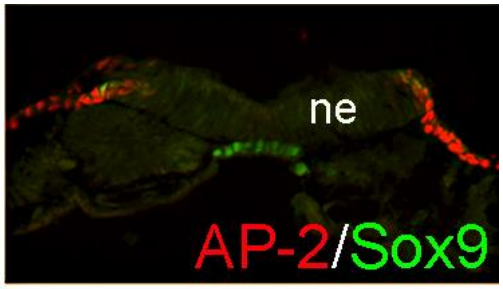
NCC Marker Expression in the Rabbit

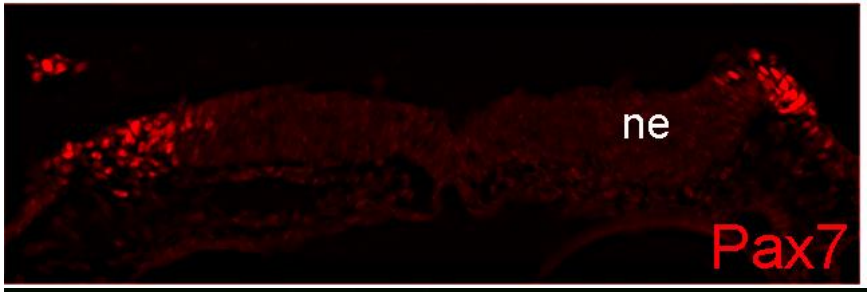
Expression assessed in embryos from St. 3 to St.11/12 (13ss).

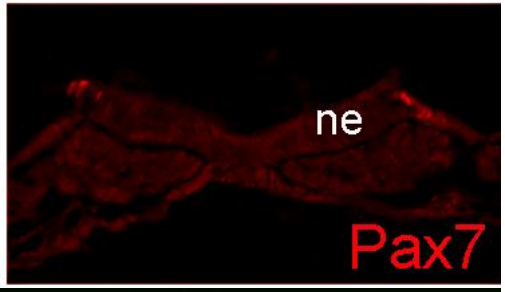


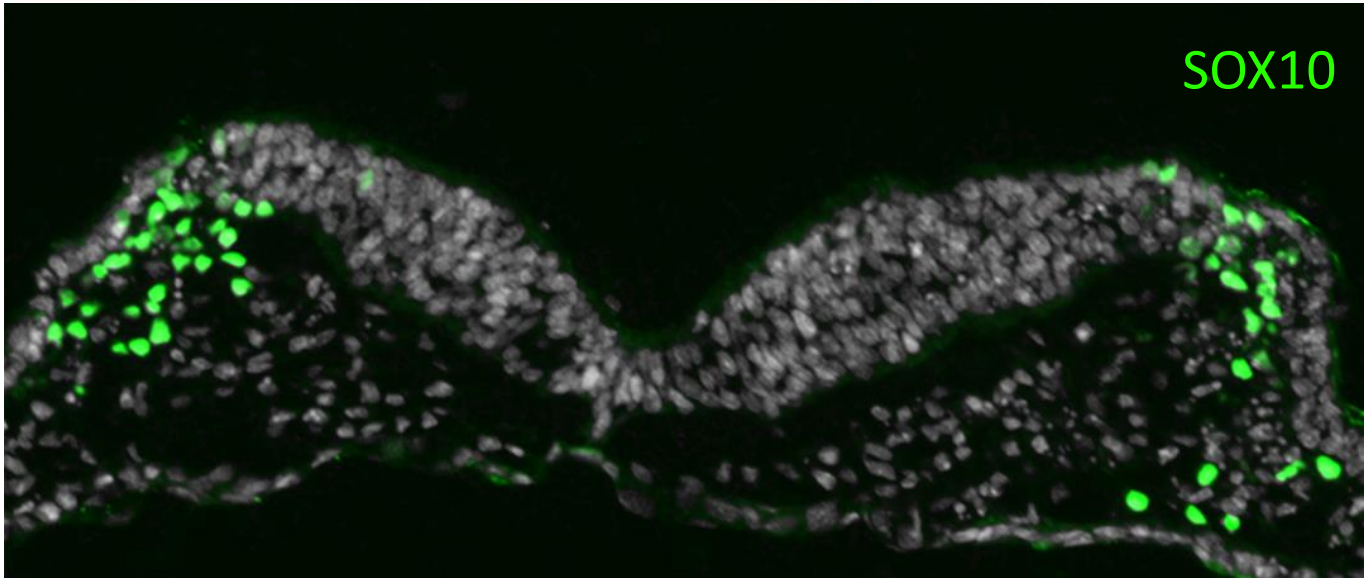
Rabbit 5-6ss/St.8



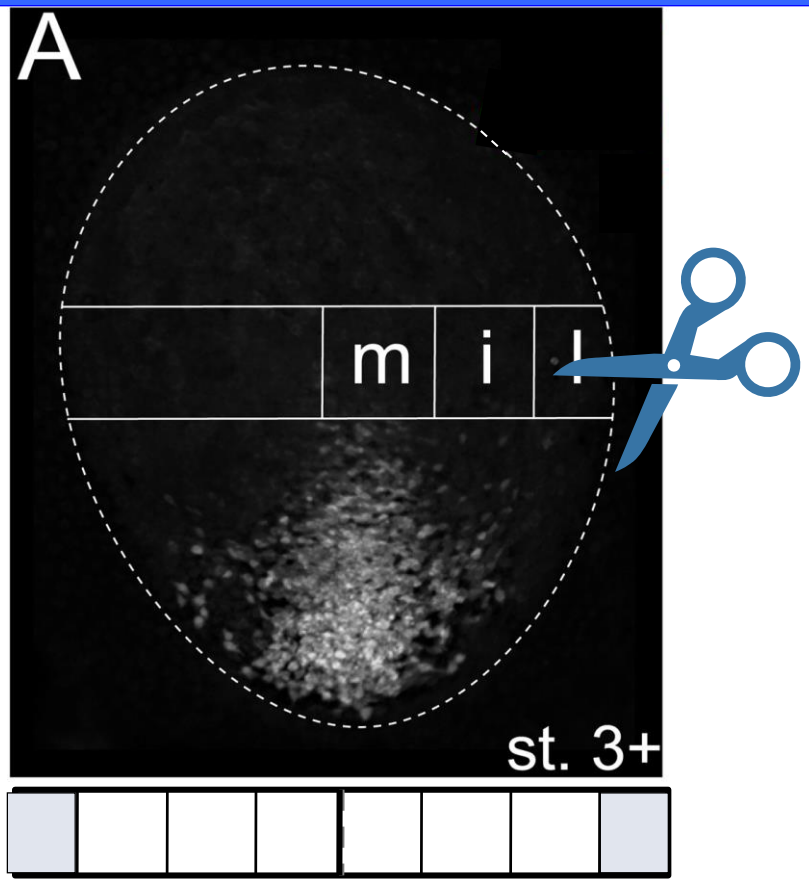




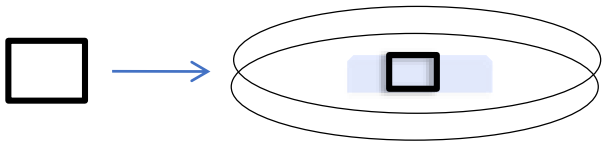




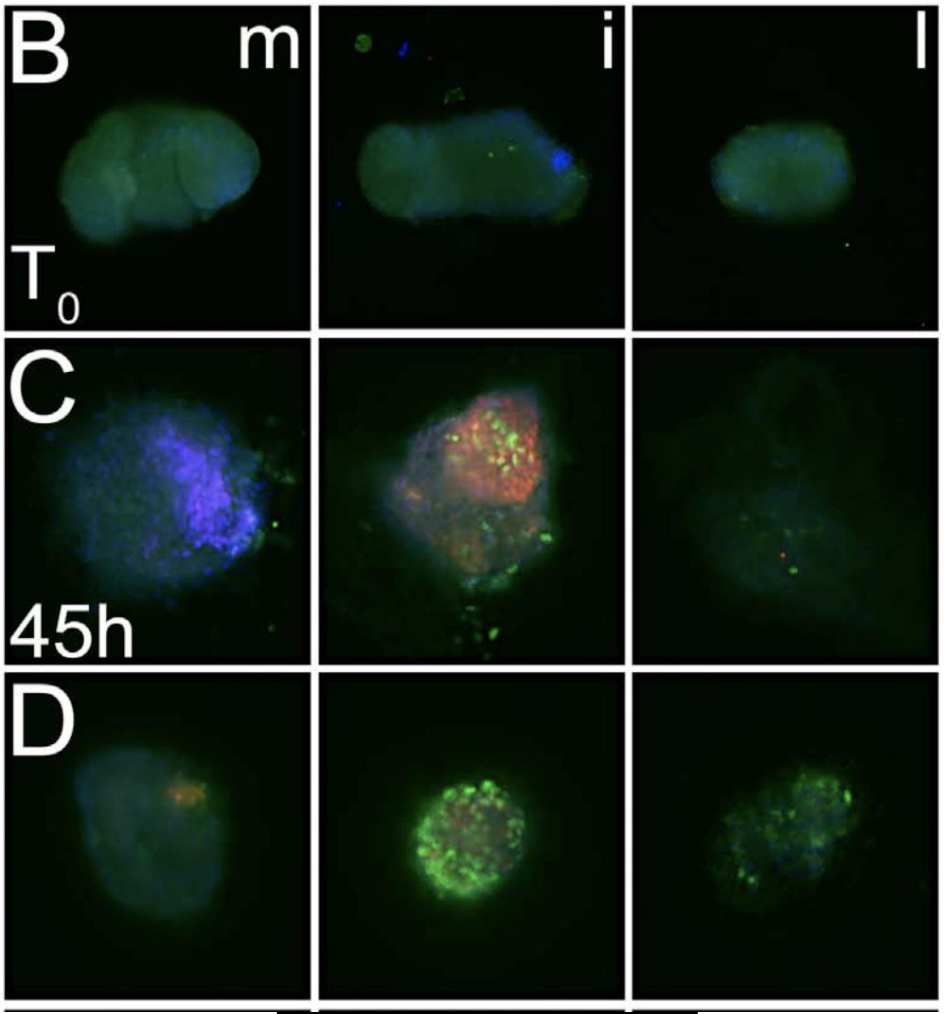
Are Rabbit NC specified during gastrulation?



midle-inter-lat

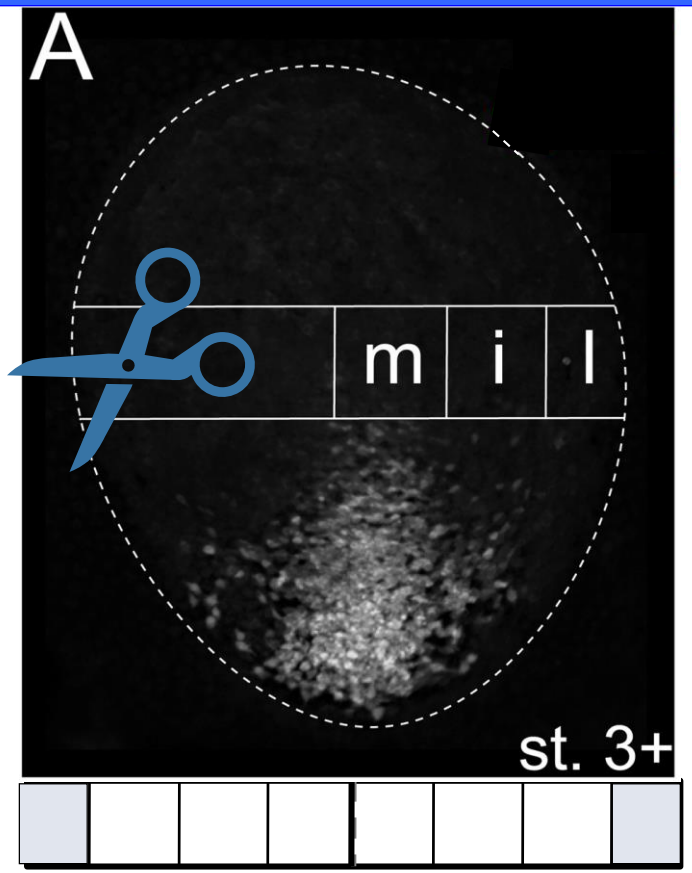


Culture, Fix and immunostain

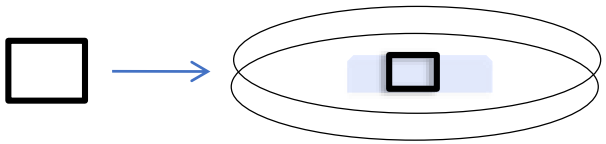


Sox10 Pax7 Bra

Are Rabbit NC specified during gastrulation?

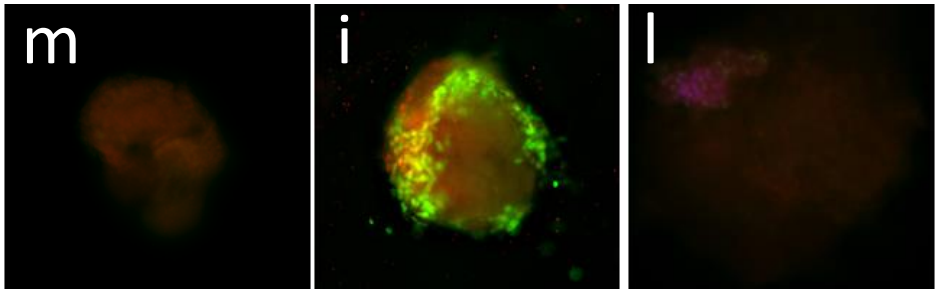


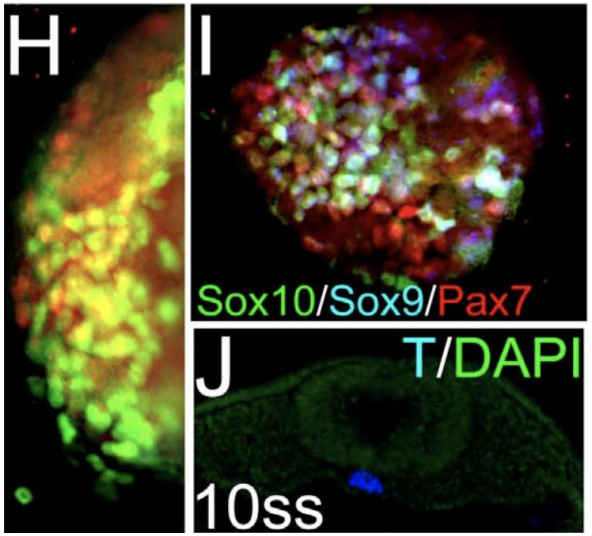
midle-inter-lat



Culture, Fix and immunostain

45 hr

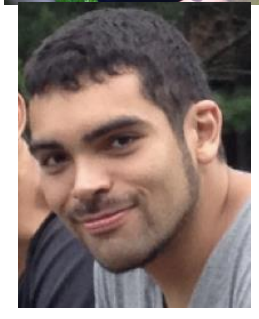




Betters et al., 2018
Developmental Biology

Conclusions

- Chick NC are specified at the blastula stage
- Human NC models based on ES/PSC suggest meso-neural independent and fast initiation of the NC fate
- Rabbit embryos reveal for the first time the specification of NC during gastrulation in mammals.



Alan Leung, Barbara Murdoch, Erin Betters, and many more…

Funding from NIH & CTSC