

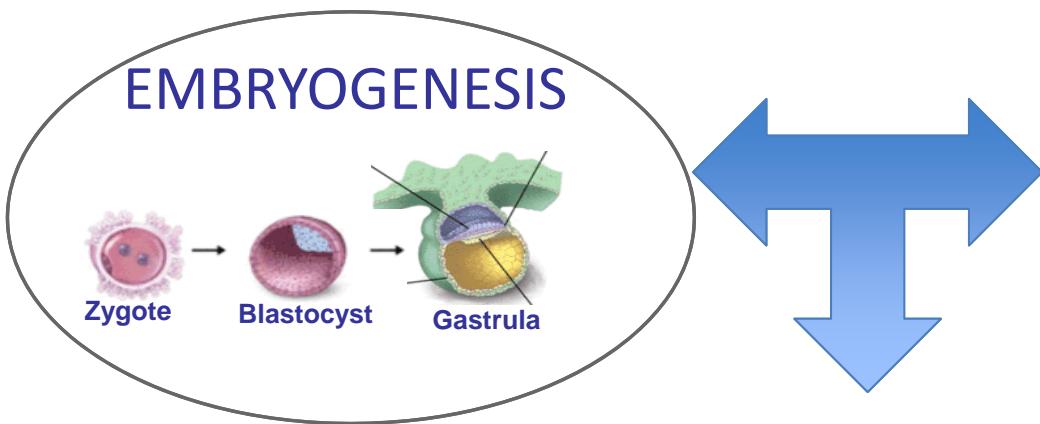
Molecular control of cell fate decision in pluripotent and adult stem cells: from basic science toward therapy

Gabriella Minchiotti

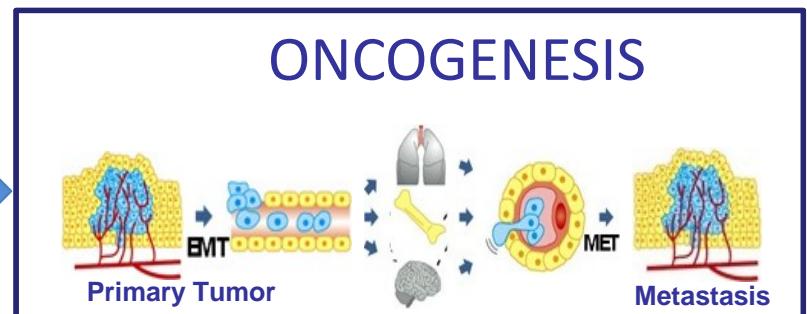
INBB, Roma 24 Ottobre 2014

Convergent pathways in embryogenesis, oncogenesis and tissue regeneration

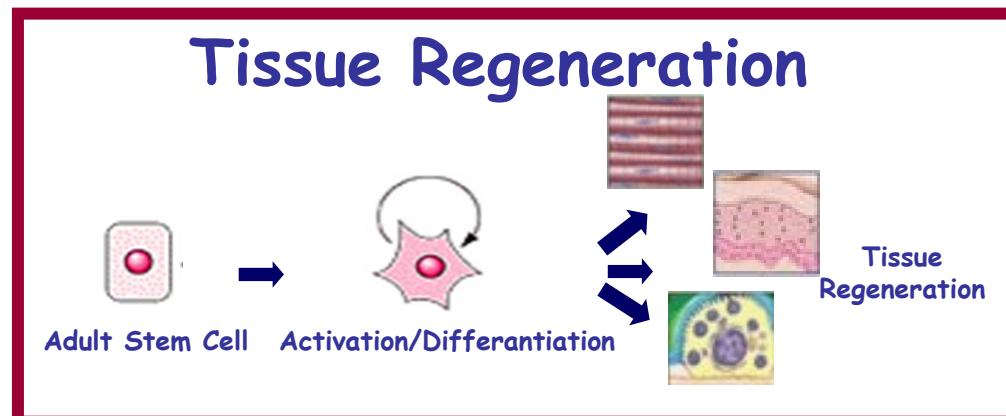
Embryonic Stem Cells



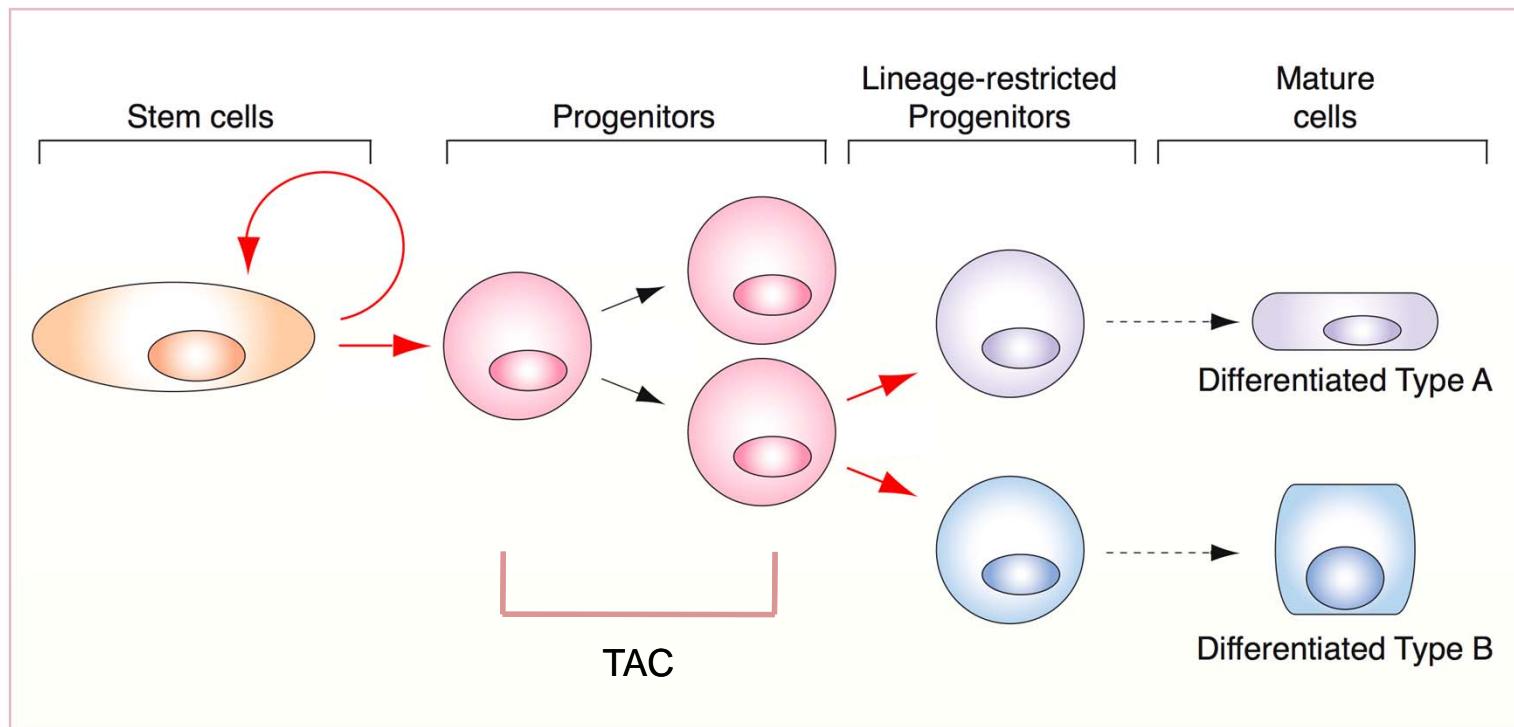
Cancer Stem Cells



Adult Stem Cells



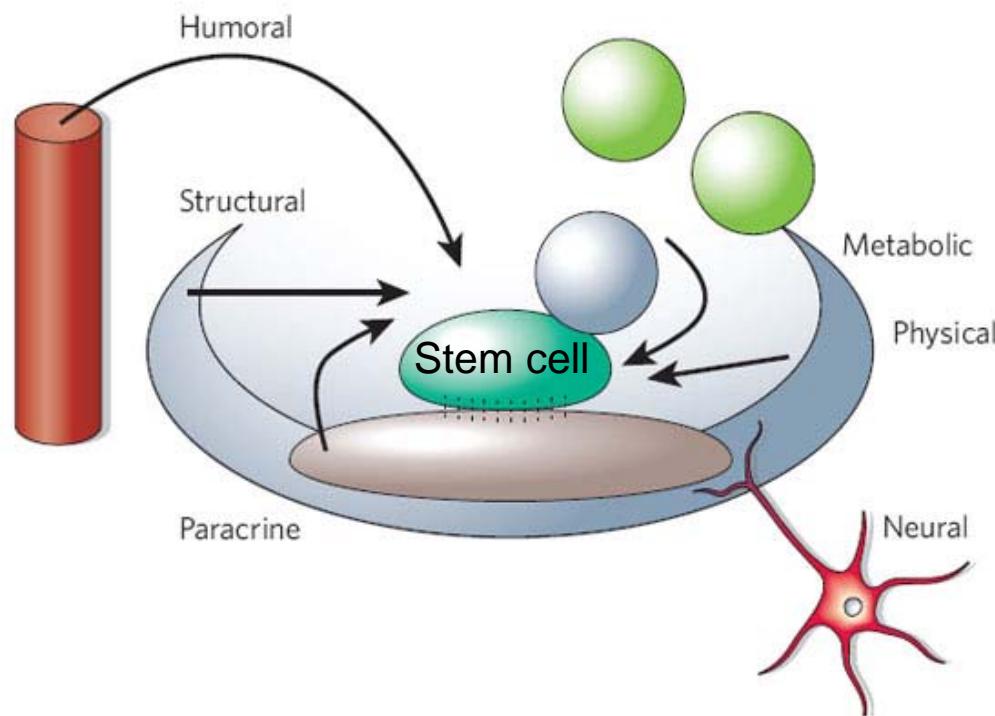
Mechanisms of cell lineage specification in mammals



TACs- Transit Amplifying Cells

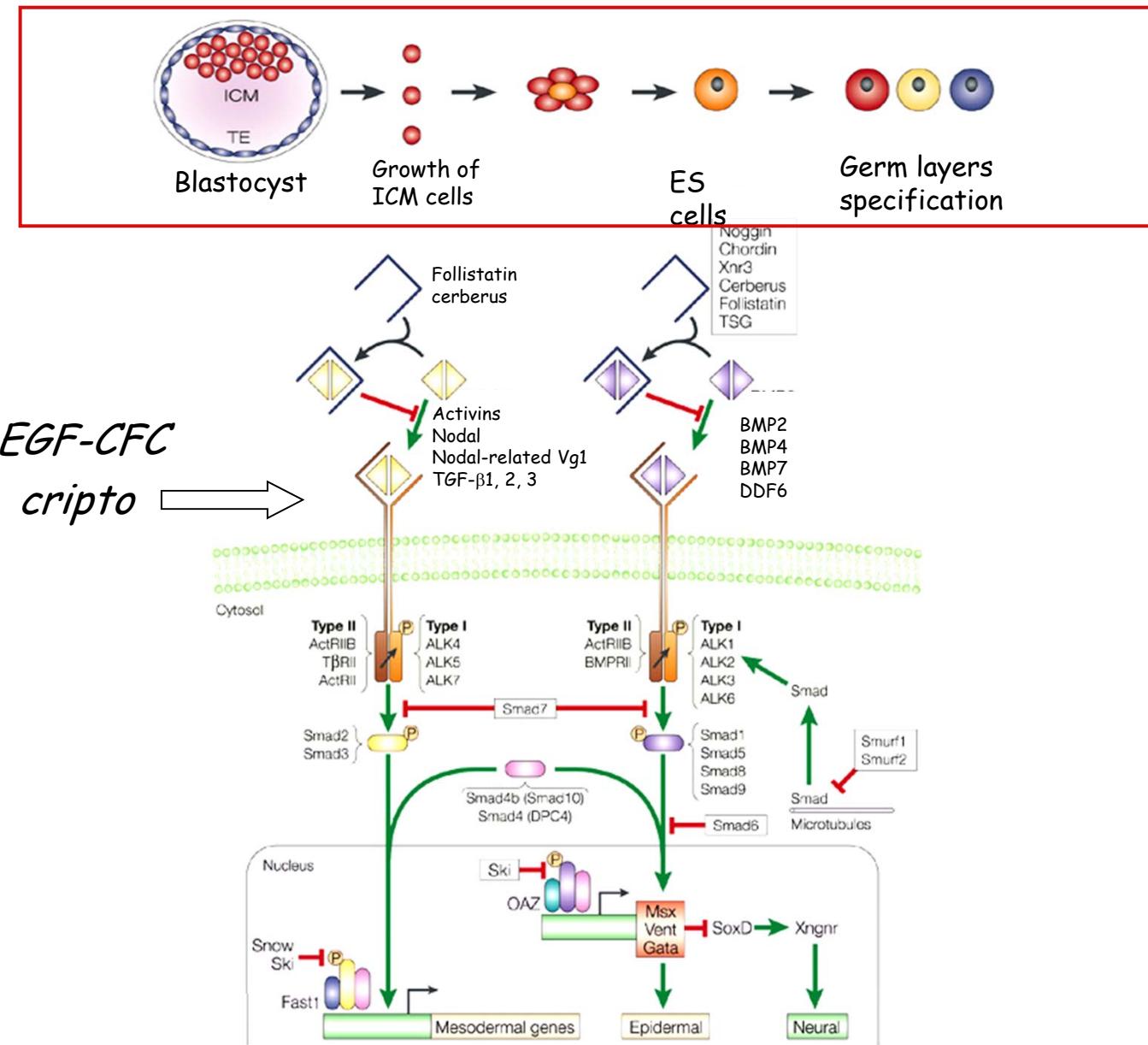
Adapted S. Pece et al., BBA 2011

Role of local environmental cues in defining cell-type identity

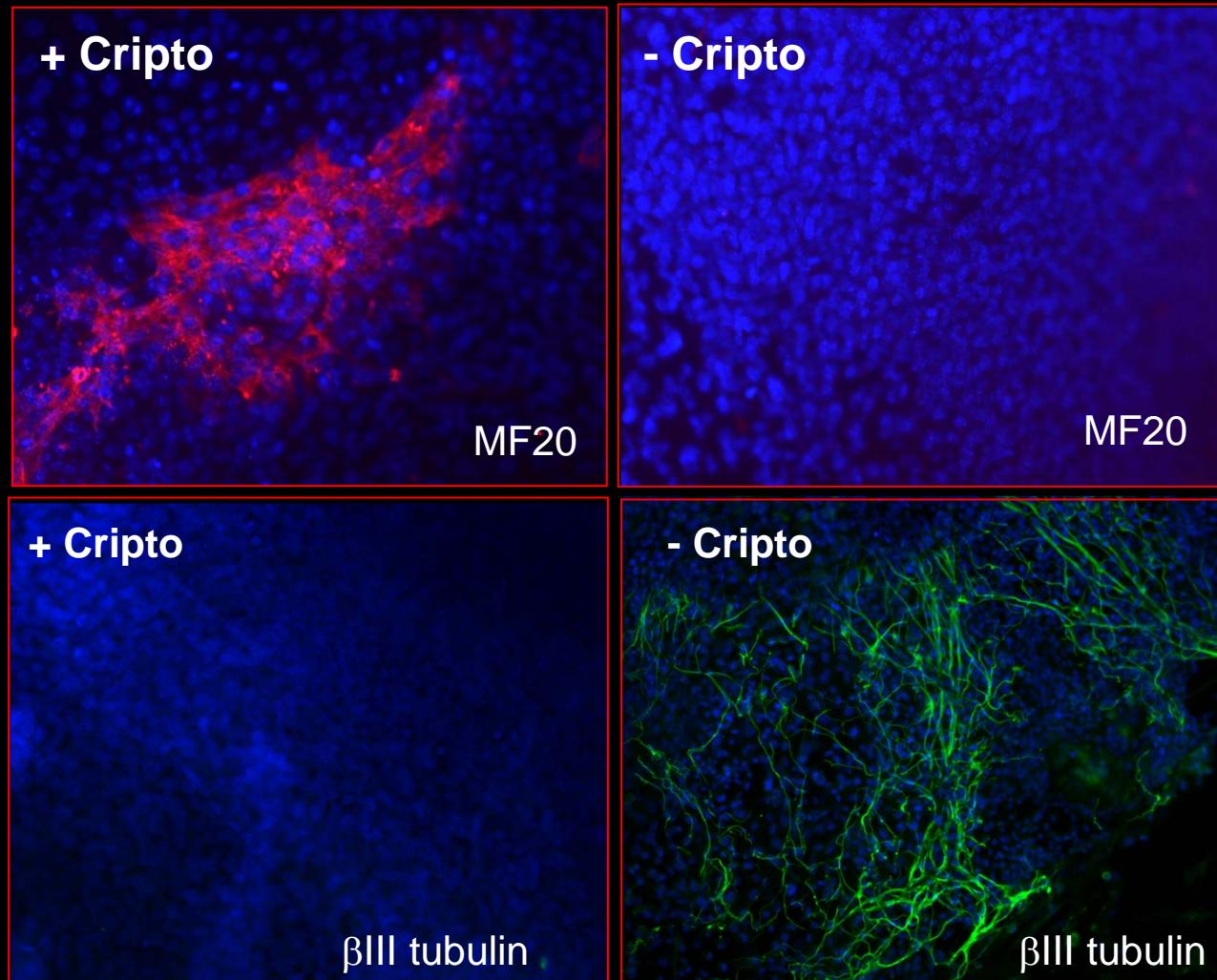


Modified from Scadden D., Nature 2006

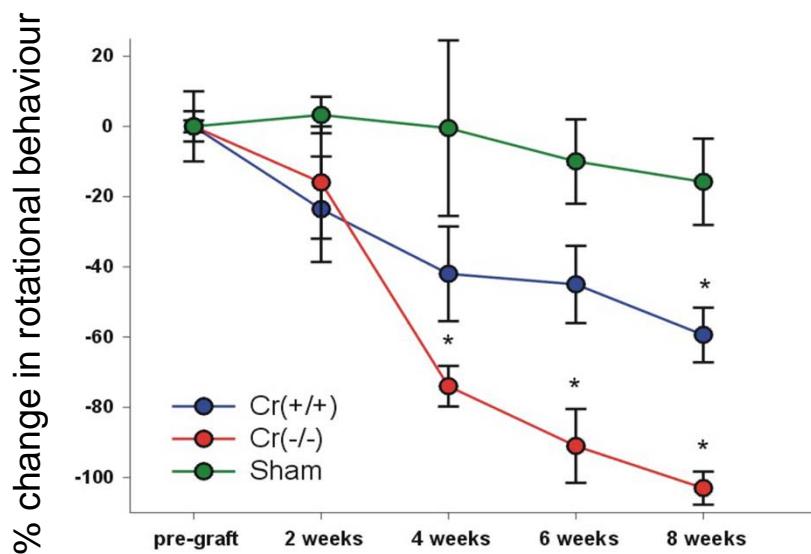
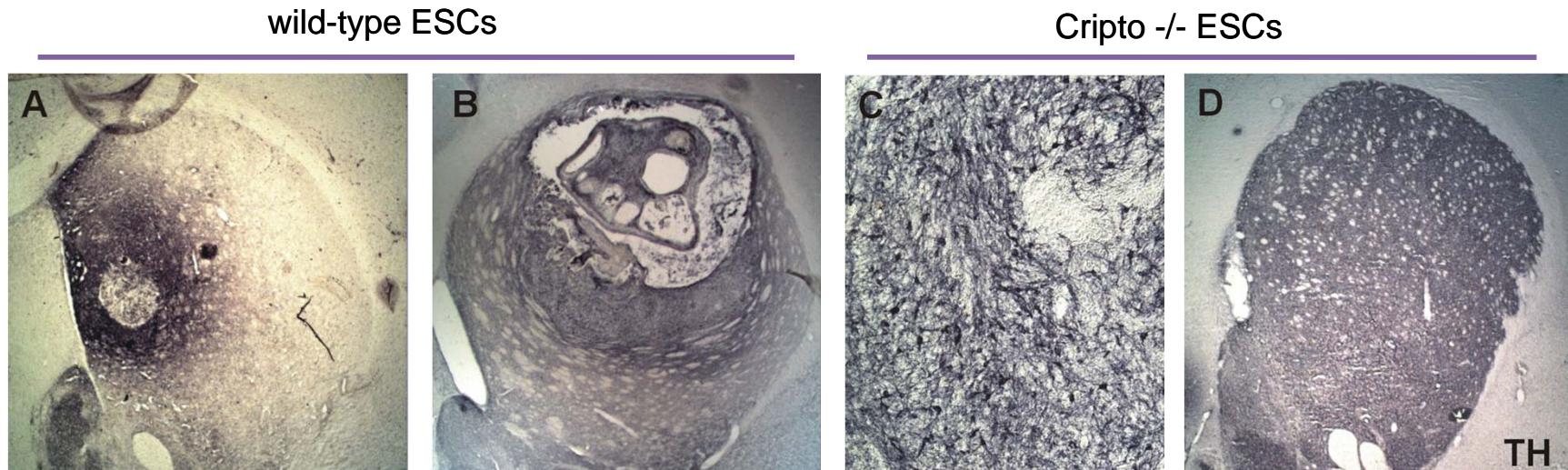
Signaling pathways involved in cell fate specification



Cripto redirects the neural fate of ESCs



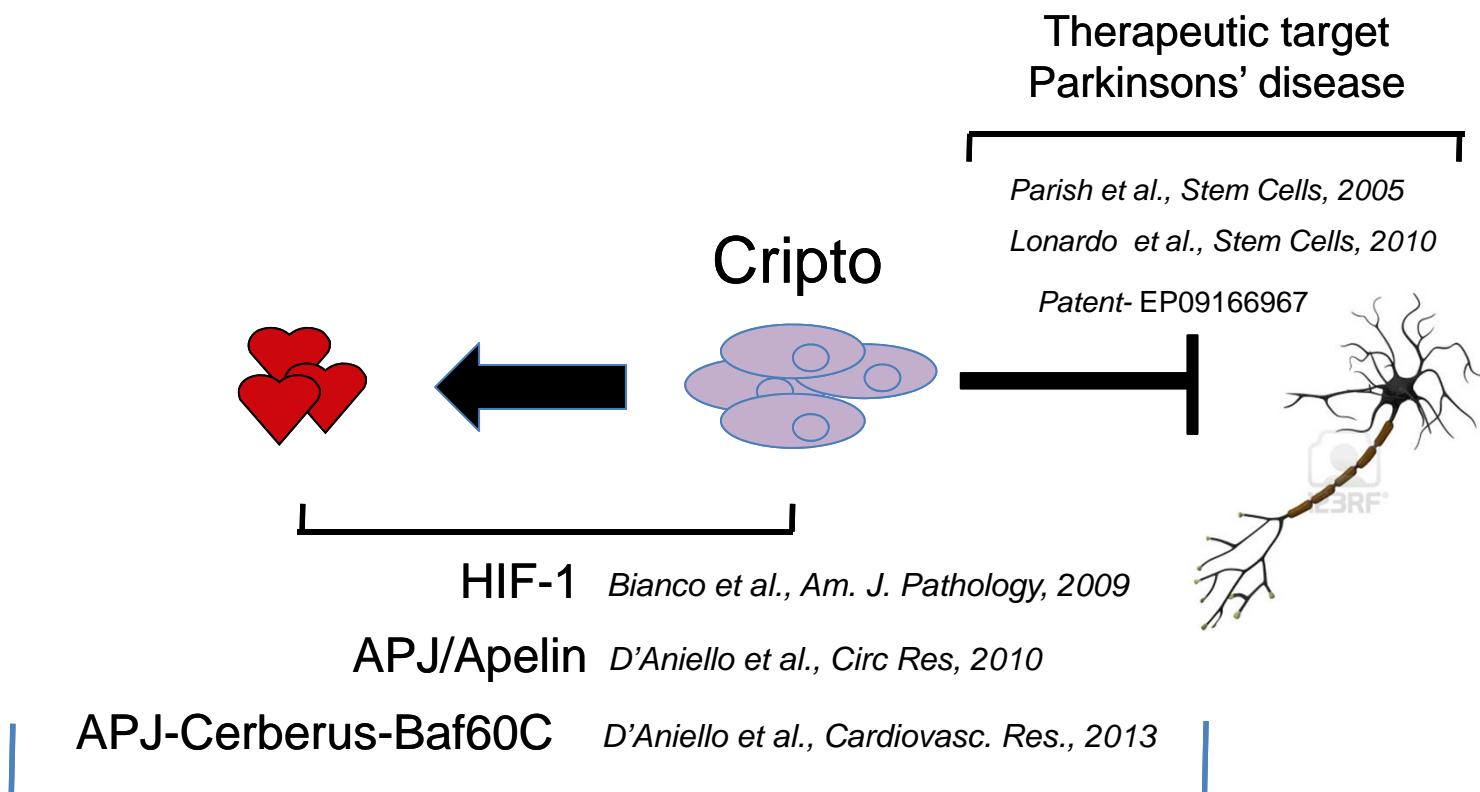
Genetic or pharmacological inhibition of Cripto in mouse ESCs cells improves functional integration of ESCs and blocks tumor formation in Parkinsonian rats



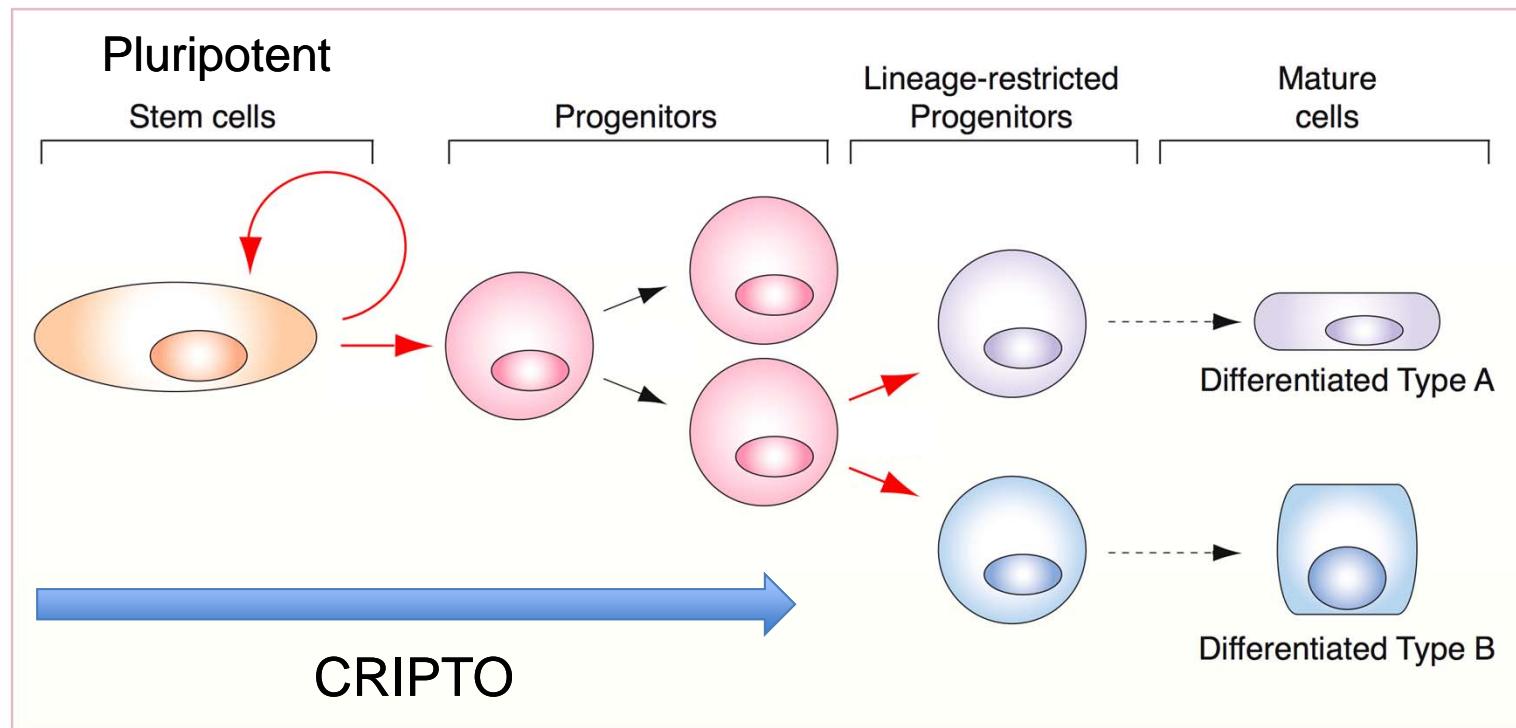
Parish, et al., *Stem Cells*, 2005

Lonardo, et al., *Stem Cells*, 2010

Cripto controls cell lineage specification in ESCs



Cripto regulates cell lineage commitment in pluripotent stem cells

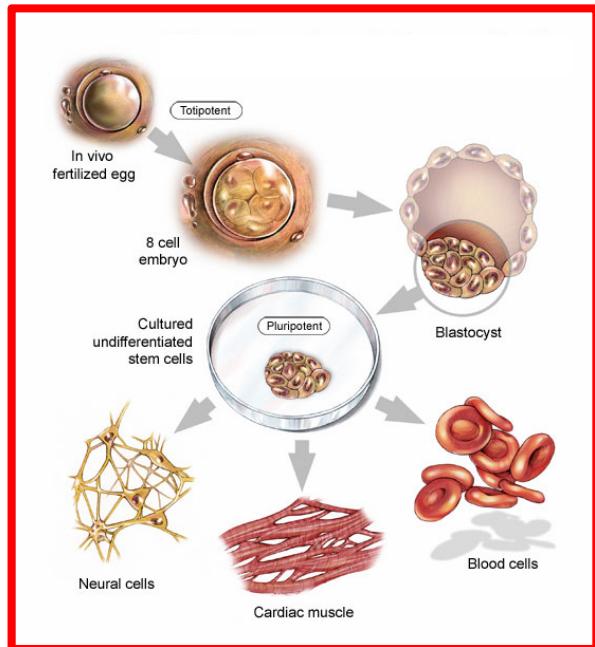


Adapted S. Pece et al., BBA 2011

ADULT STEM CELLS??

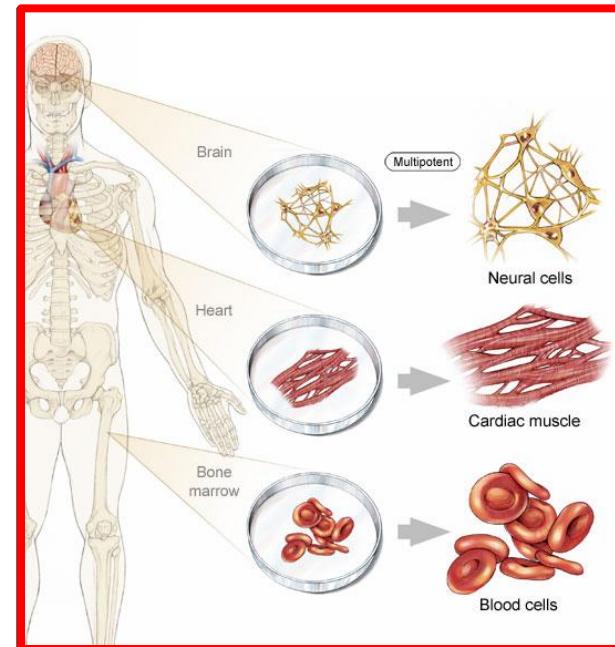
Common pathways in embryonic and adult stem cells

Embryonic Stem Cells



Cripto

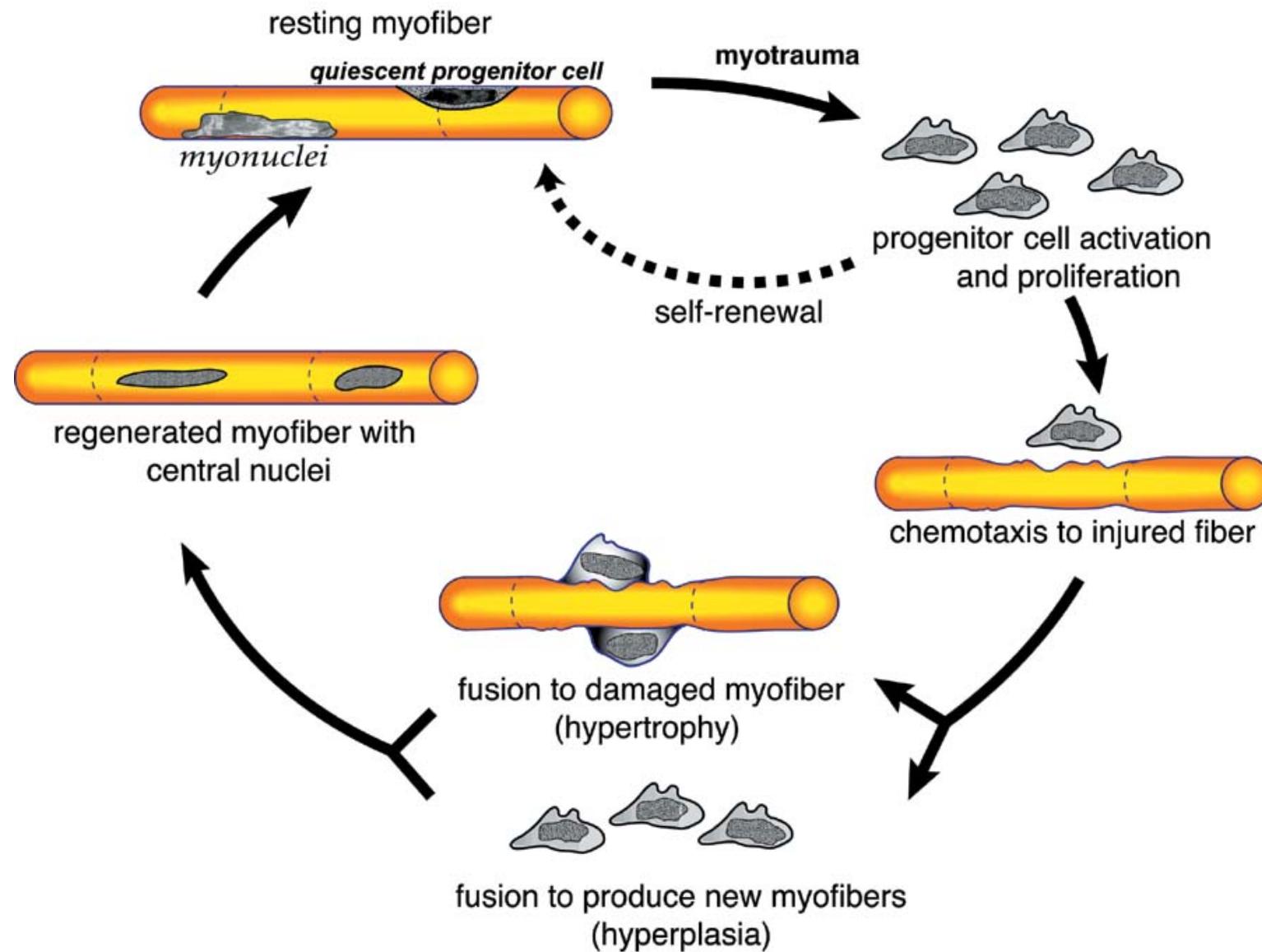
Adult Stem Cells



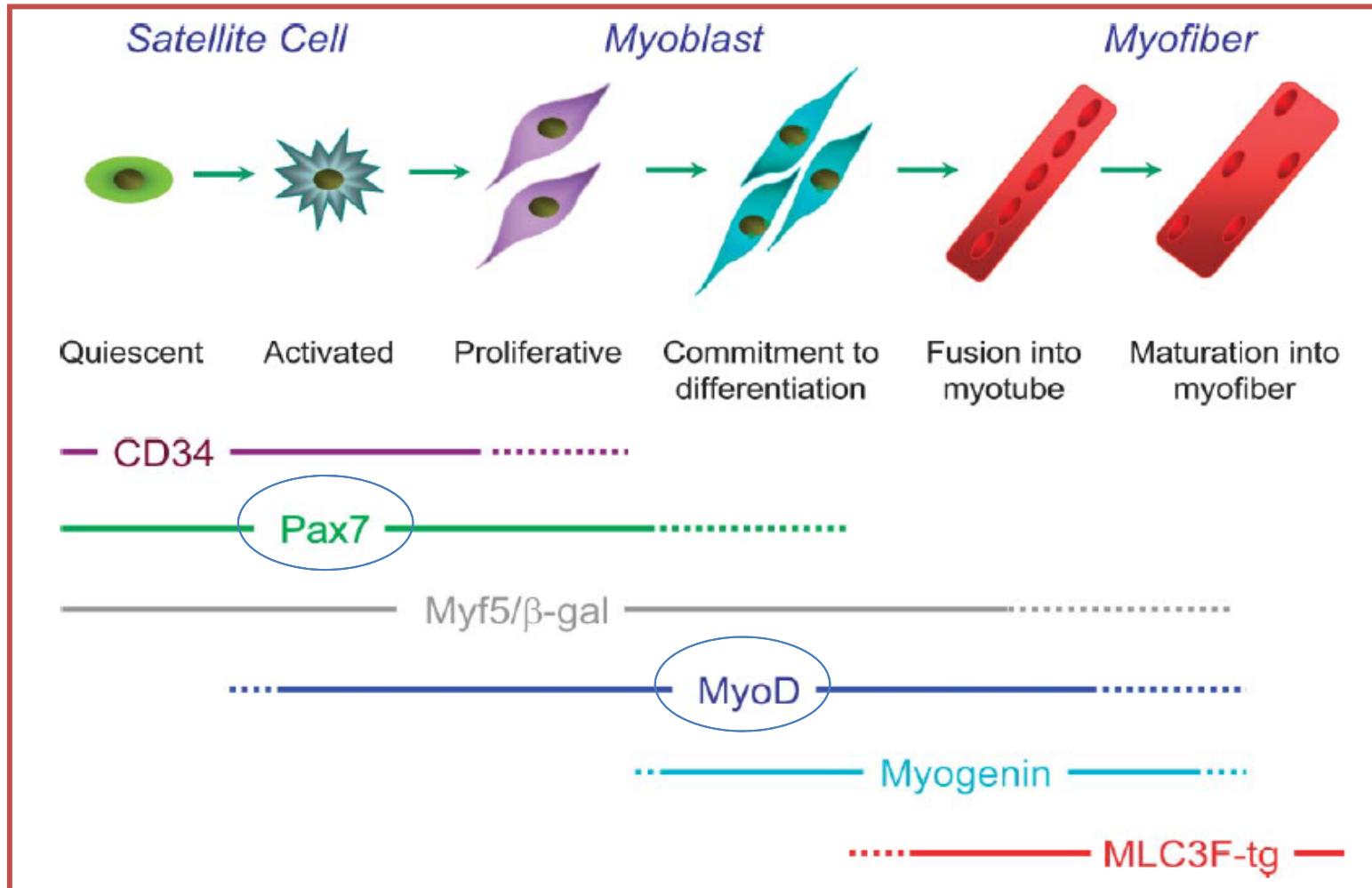
Cripto

Skeletal Muscle Regeneration

Skeletal muscle regeneration

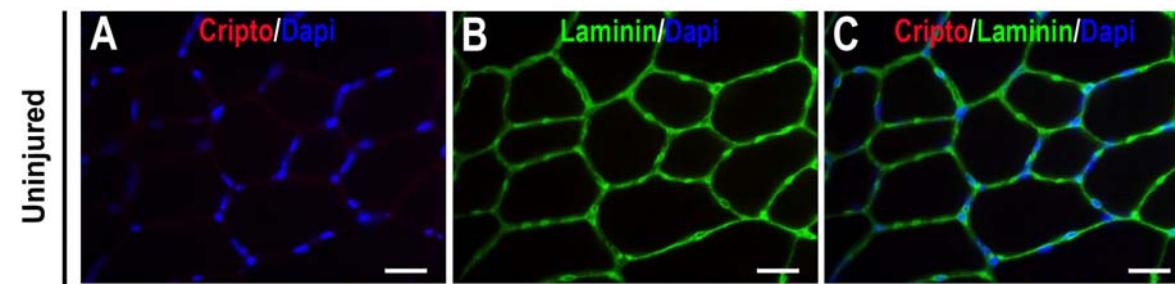


Sequential expression of myogenic transcription factors

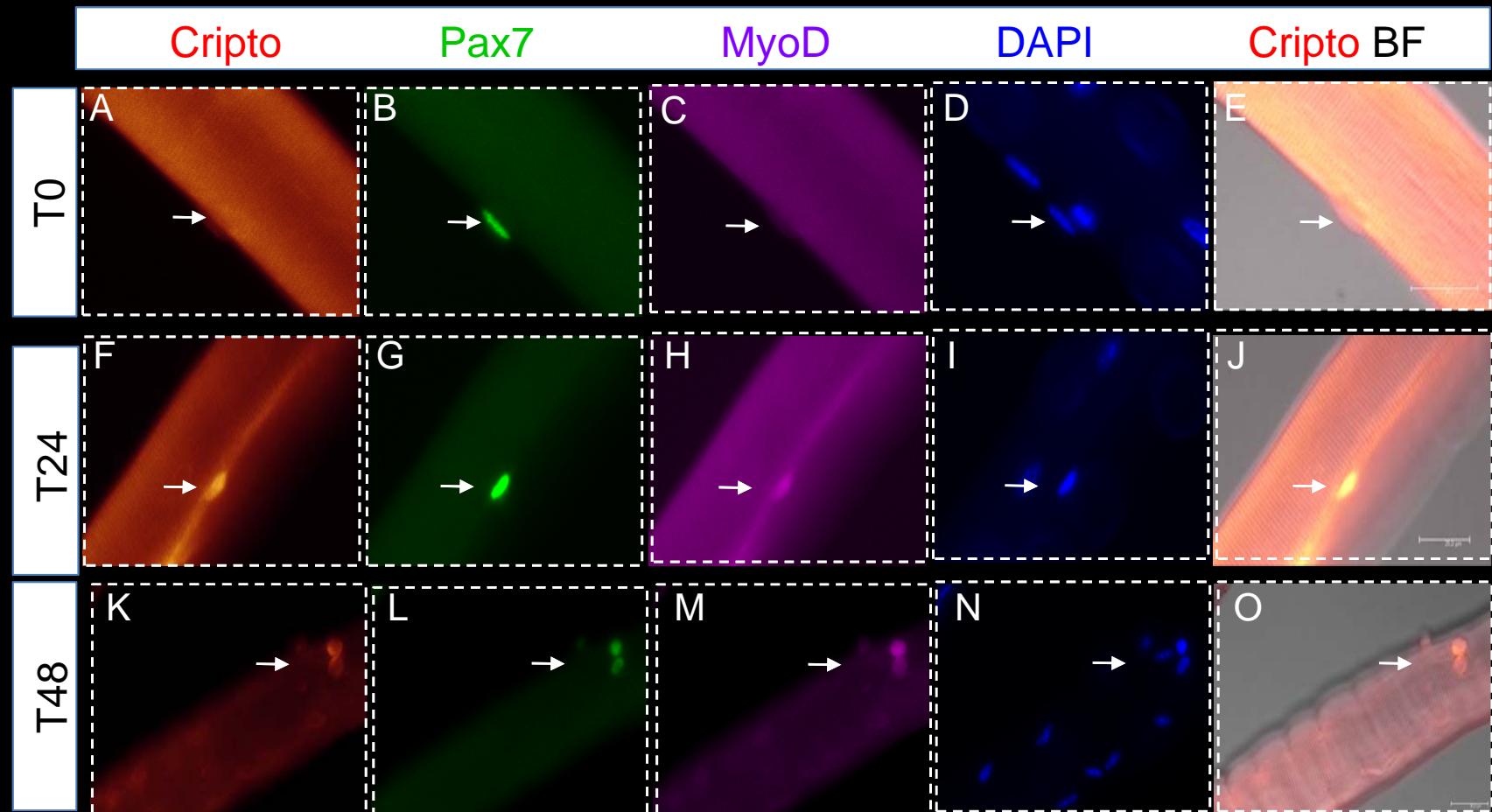


The Skeletal Muscle Satellite Cell: The Stem Cell That Came inFrom the Cold. Peter S. Zammit et al., 2006

Cripto is expressed in myoblasts and inflammatory cells in skeletal muscle regeneration

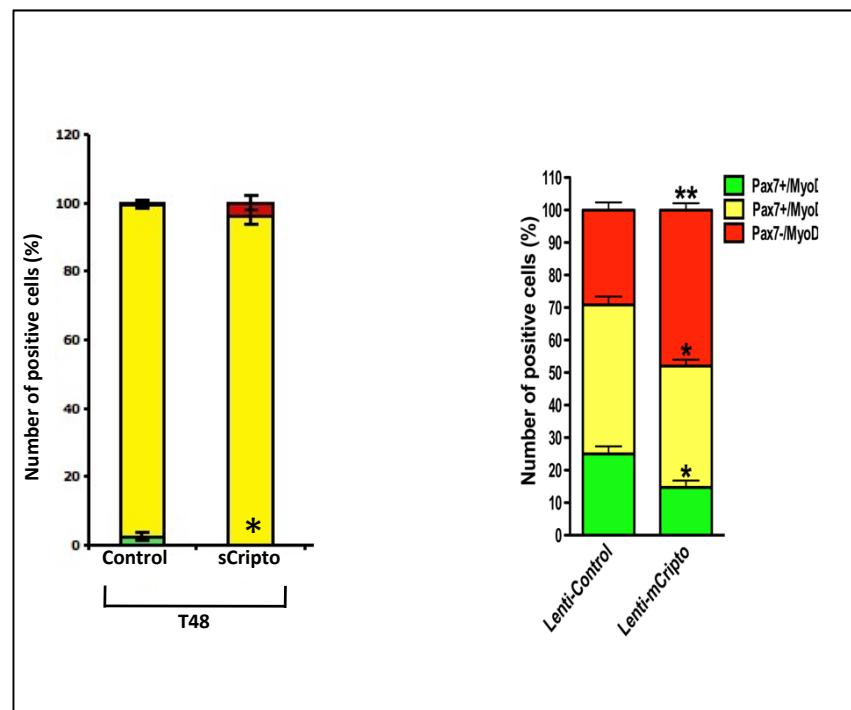
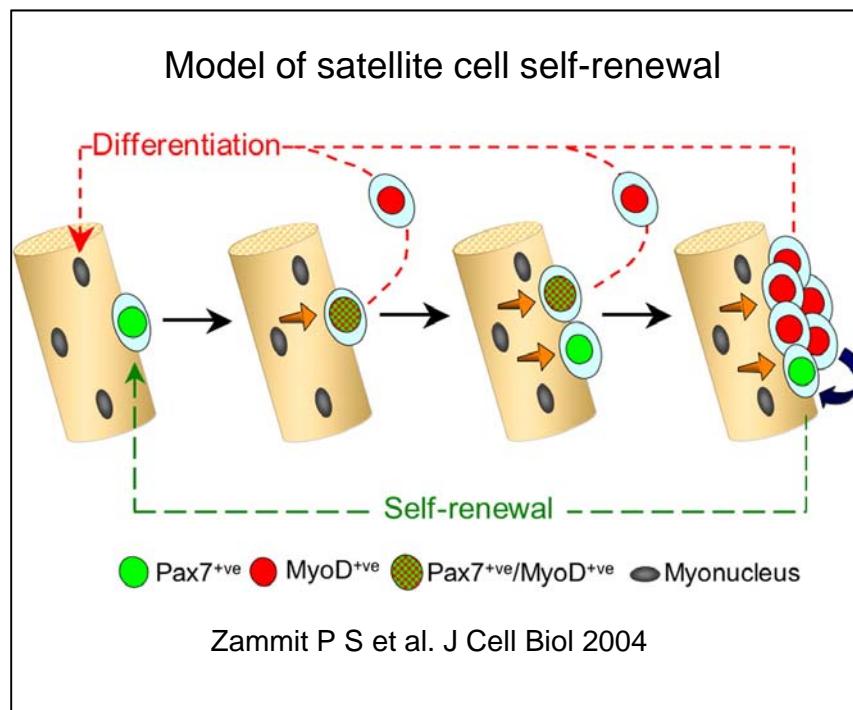


Cripto is expressed in myogenic precursor cells in isolated myofibers



Cripto promotes satellite cell lineage progression and proliferation

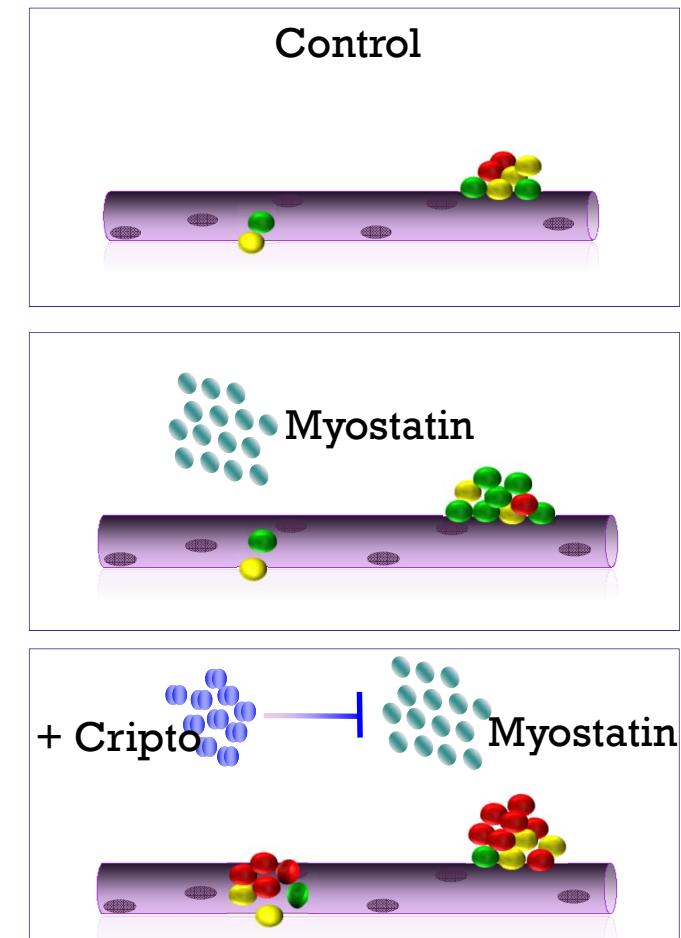
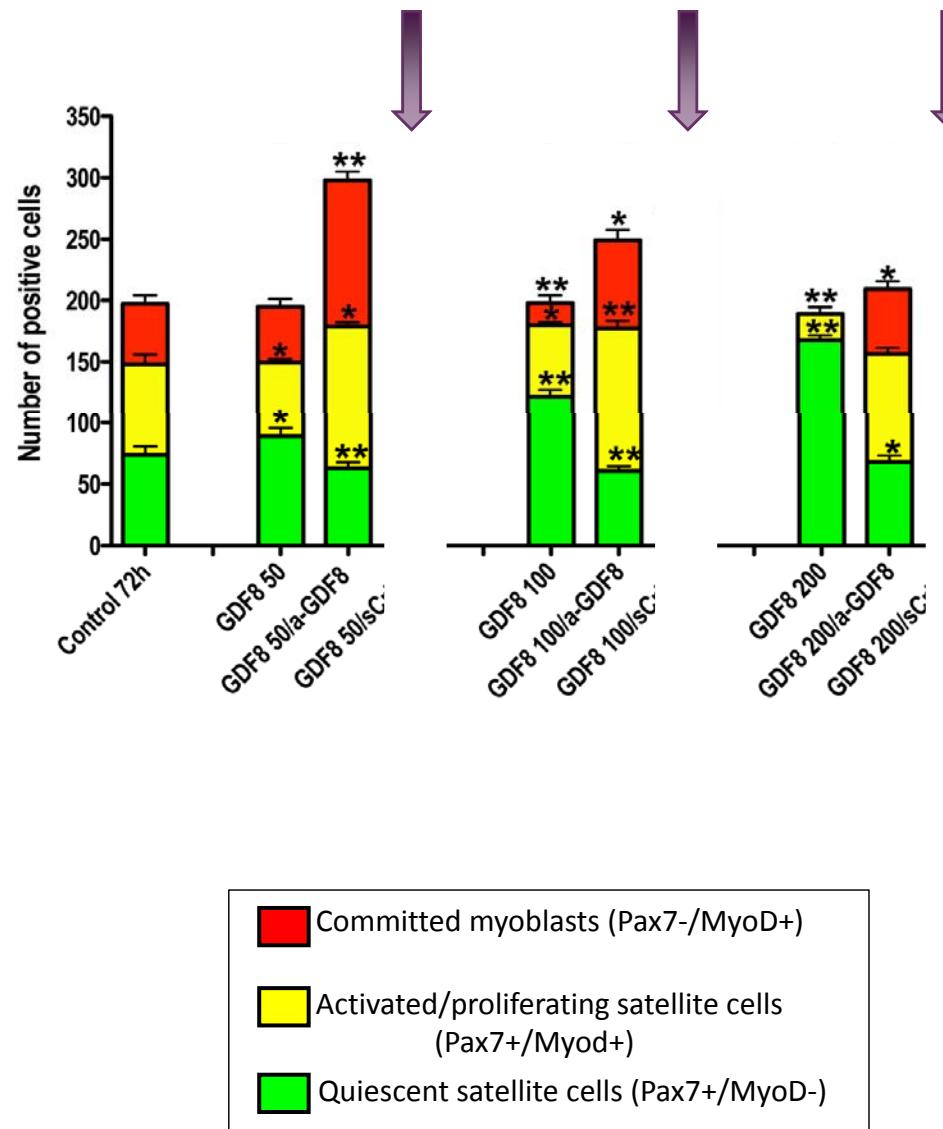
Isolated myofibers

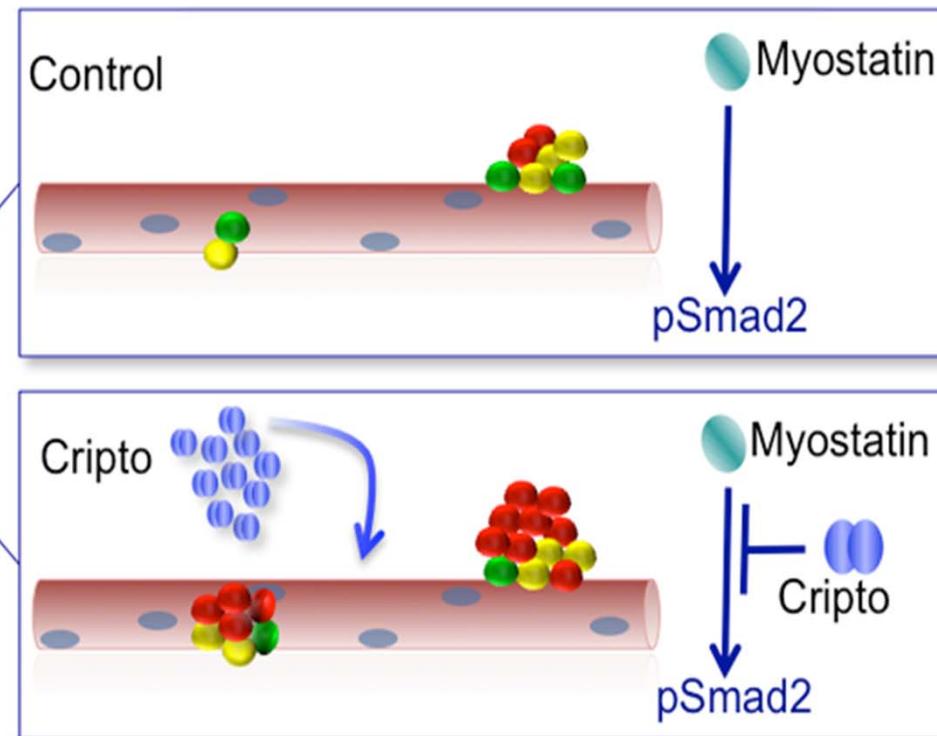
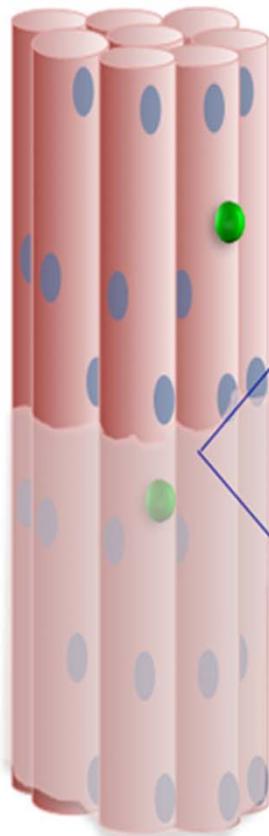


Legend:

- Red square: Committed myoblasts to differentiation (Pax7-/MyoD+)
- Yellow square: Activated/proliferating satellite cells (Pax7+/MyoD+)
- Green square: Quiescent satellite cells (Pax7+/MyoD-)

Cripto promotes satellite cell myogenic commitment antagonizing Myostatin





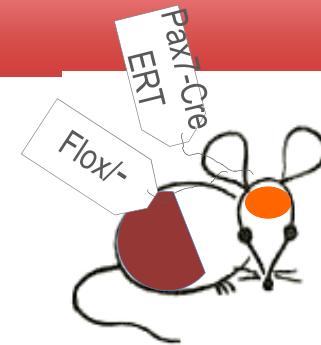
Myonuclei

Quiescent satellite cell

Activated/proliferating satellite cell

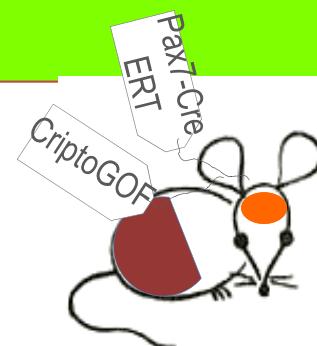
Committed to myogenesis satellite cell

CONDITIONAL SATELLITE CELL –SPECIFIC CRIPTO KO



Tg:Pax7-CreERT2::Cripto^{LoxP/-}

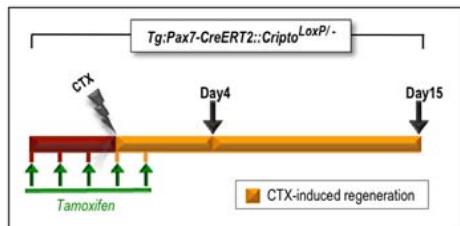
CONDITIONAL SATELLITE CELL –SPECIFIC CRIPTO GOF



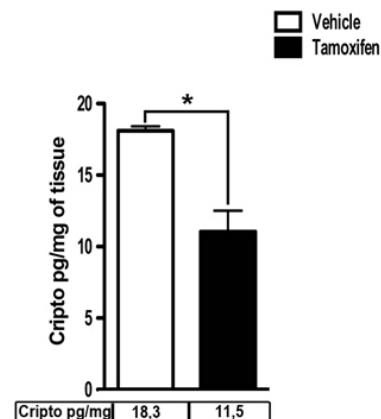
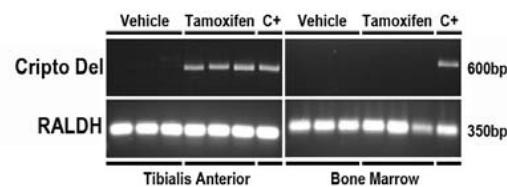
Tg:Pax7-CreERT2::CriptoGOF

Satellite cell-specific *cripto* ablation affects muscle regeneration

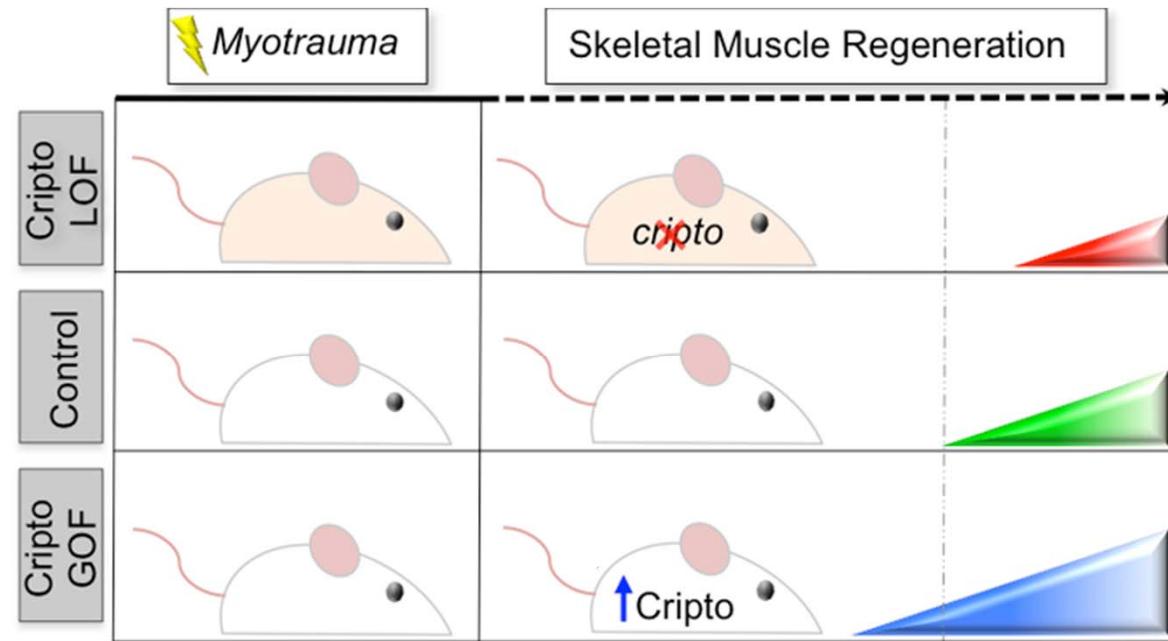
A *Tg:Pax7-CreERT2::Cripto^{LoxP/-}*



B



Impacts of Genetic modulation of Cripto signaling in the satellite cell compartment on skeletal muscle regeneration



Toward Pharmacological modulation of Cripto signaling for skeletal muscle regeneration

The *Cell maker*



The system executes

Protocols for targeted differentiation of ES cells

Screening of compounds libraries

Complete sterility

Advantage/benefits

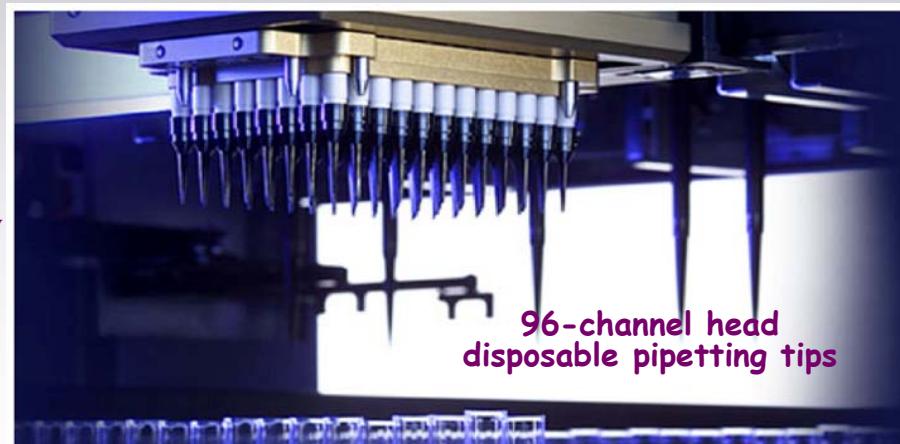
High standard reliability, performance and flexibility

Low user intervention

Contamination risk reduction

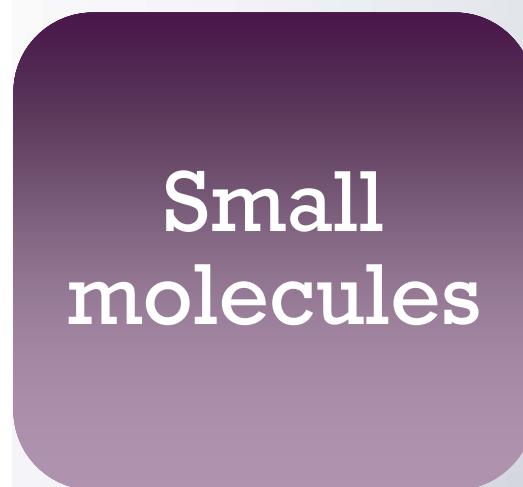
Method standardization

Up to 4000 single compounds simultaneously screened

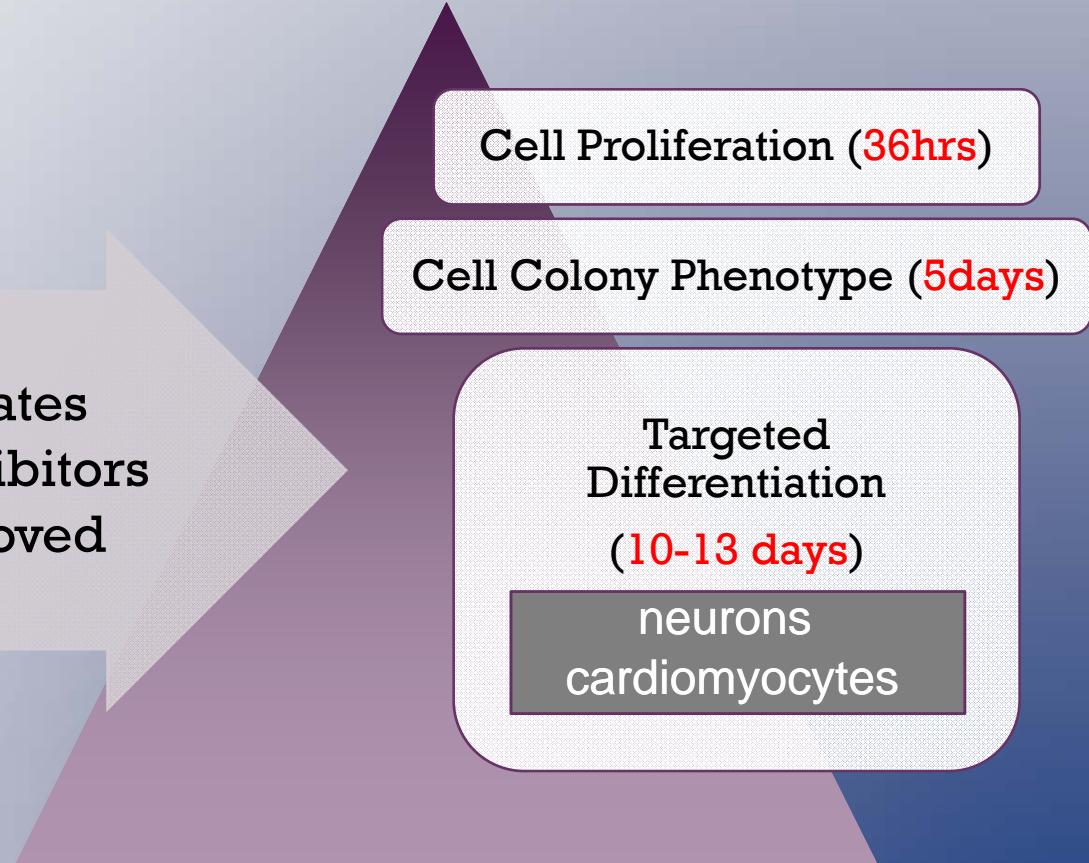


Co developed with Hamilton Robotics

Cell-based Phenotypic Screening:



- Metabolic Intermediates
- HDAC Inhibitors
- FDA-approved drugs



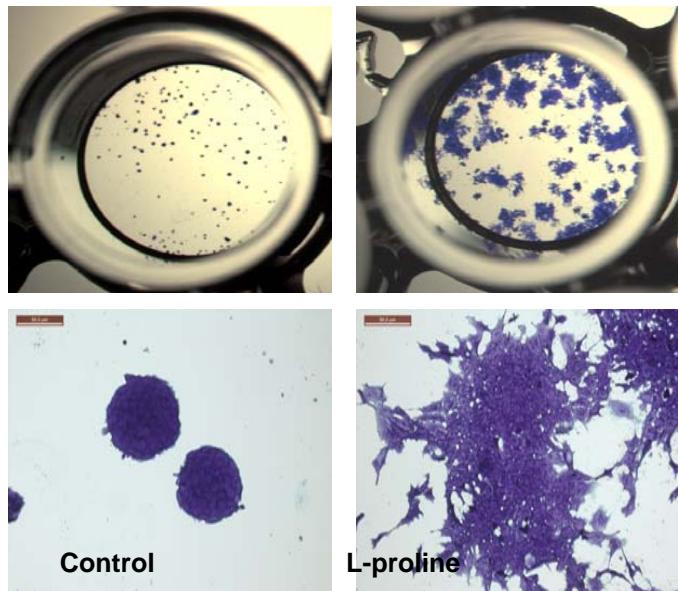
Casalino et al., JMCB, 2011

Casalino et al., Molecular Biotechnology, 2011

Franci et al., Biol. Open, 2013

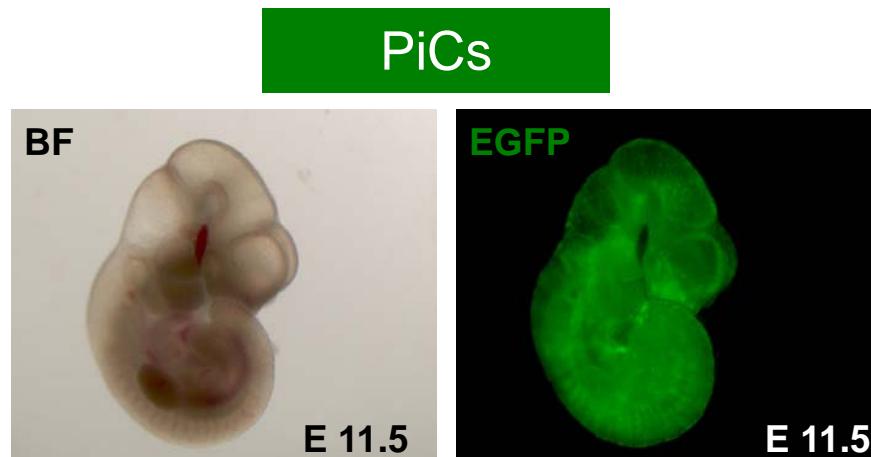
Comes et al., Stem Cell Reports, 2013

L Proline –induced cells (PiCs): a novel metastable state of pluripotent stem cells

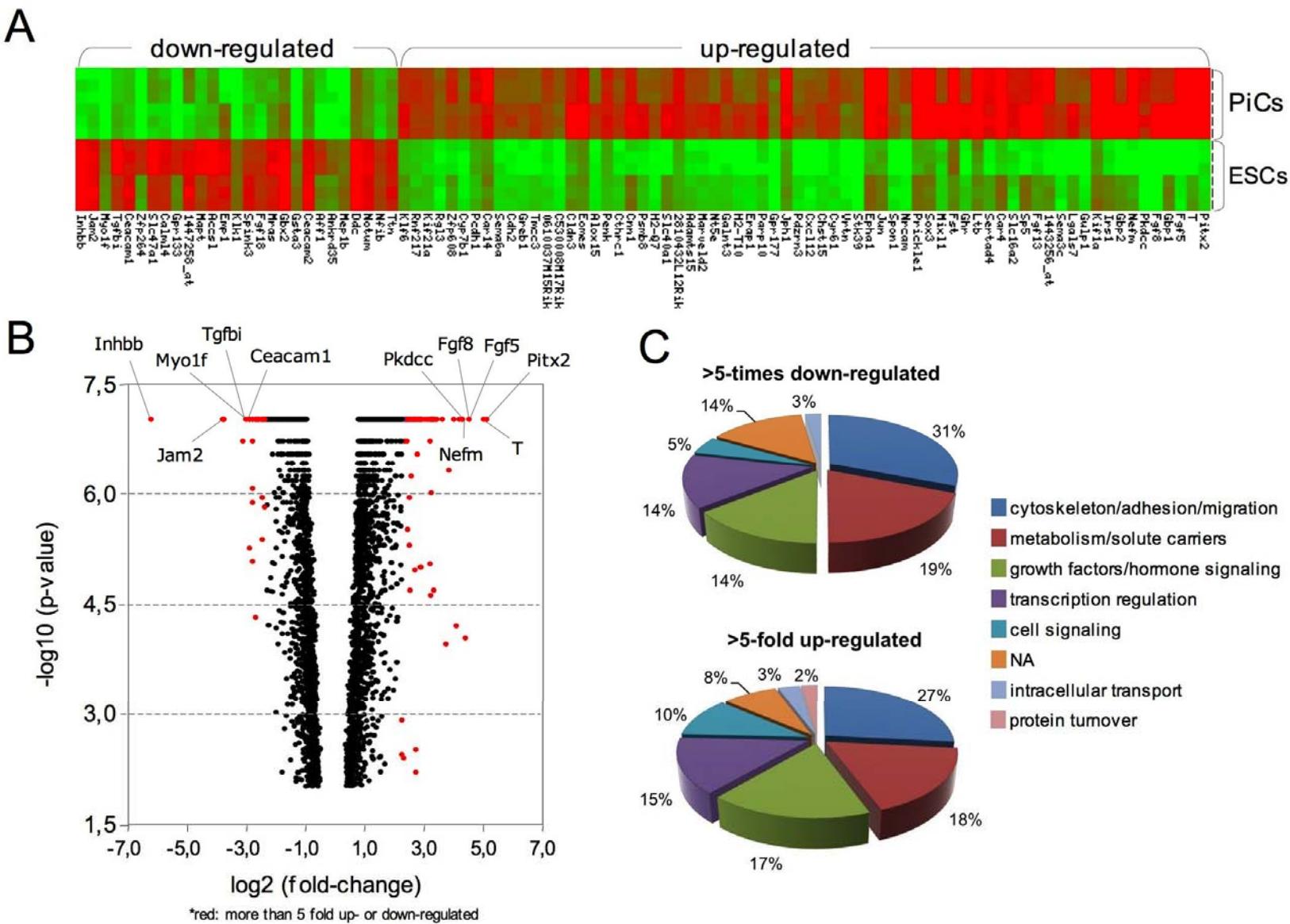


	ESCs	PiCs
Sensitivity to trypsin digestion	-	+
Alkaline phosphatase activity	+	-
<i>In vitro</i> cardiac and neural differentiation	+	+
Teratoma formation and blastocyst colonization	+	+

PiCs = Proline induced Cells



L-Pro induces remodeling of the ESC transcriptome



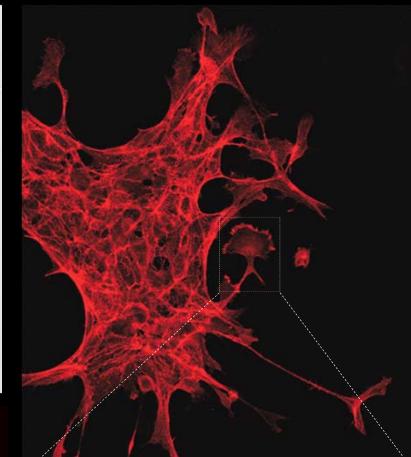
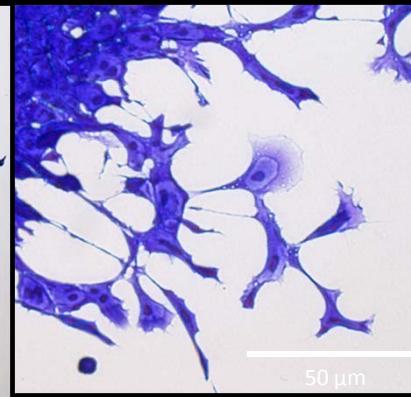
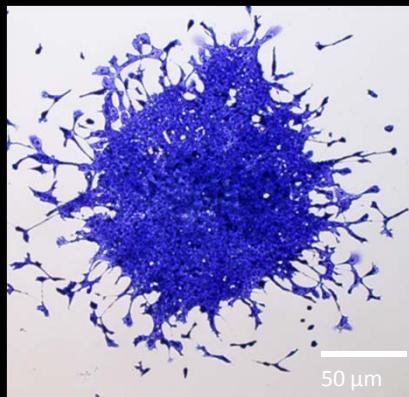
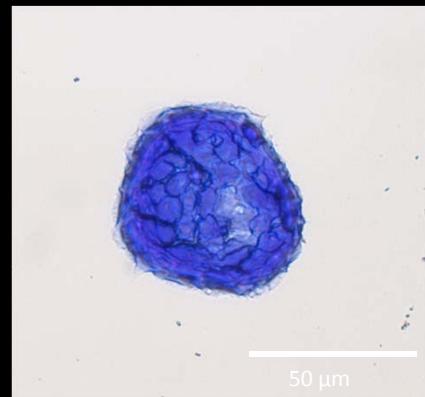
L-Proline -induced cytoskeletal rearrangements in ESCs

ESCs

PiCs

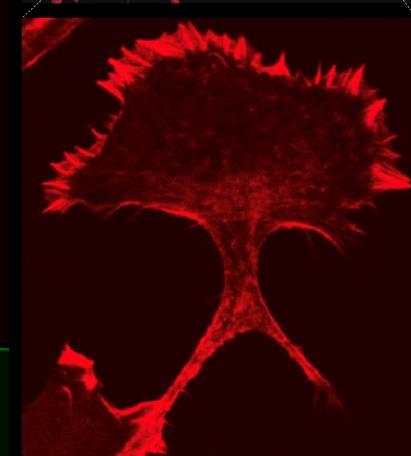
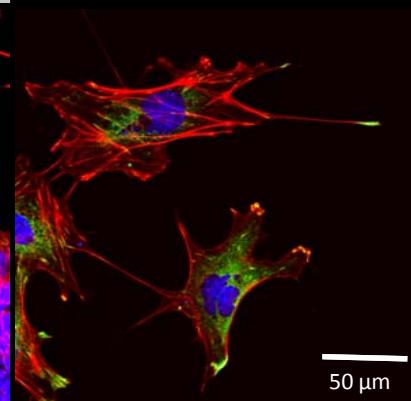
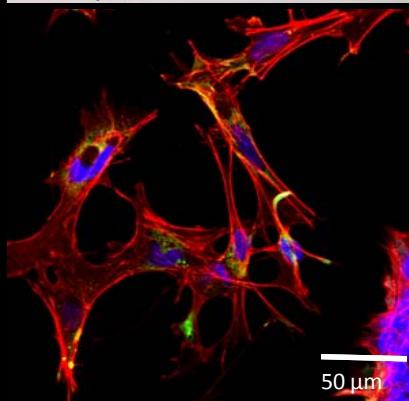
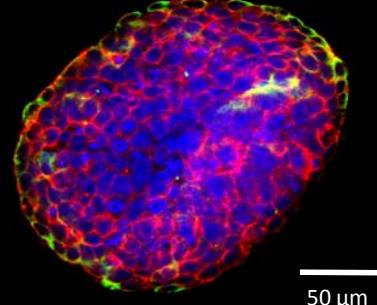
PiCs

Crystal violet

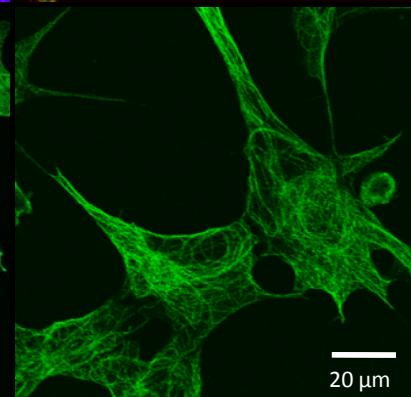
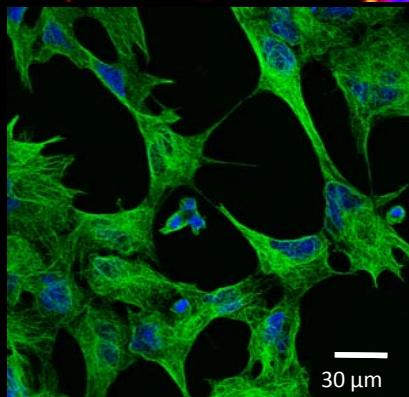
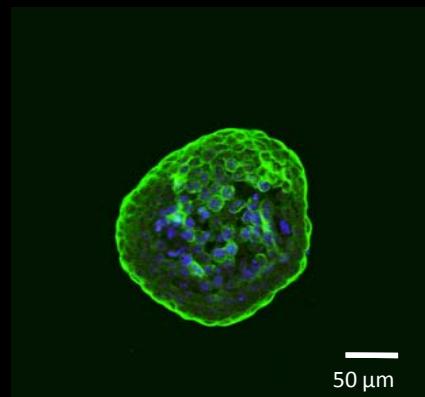


Phalloidin

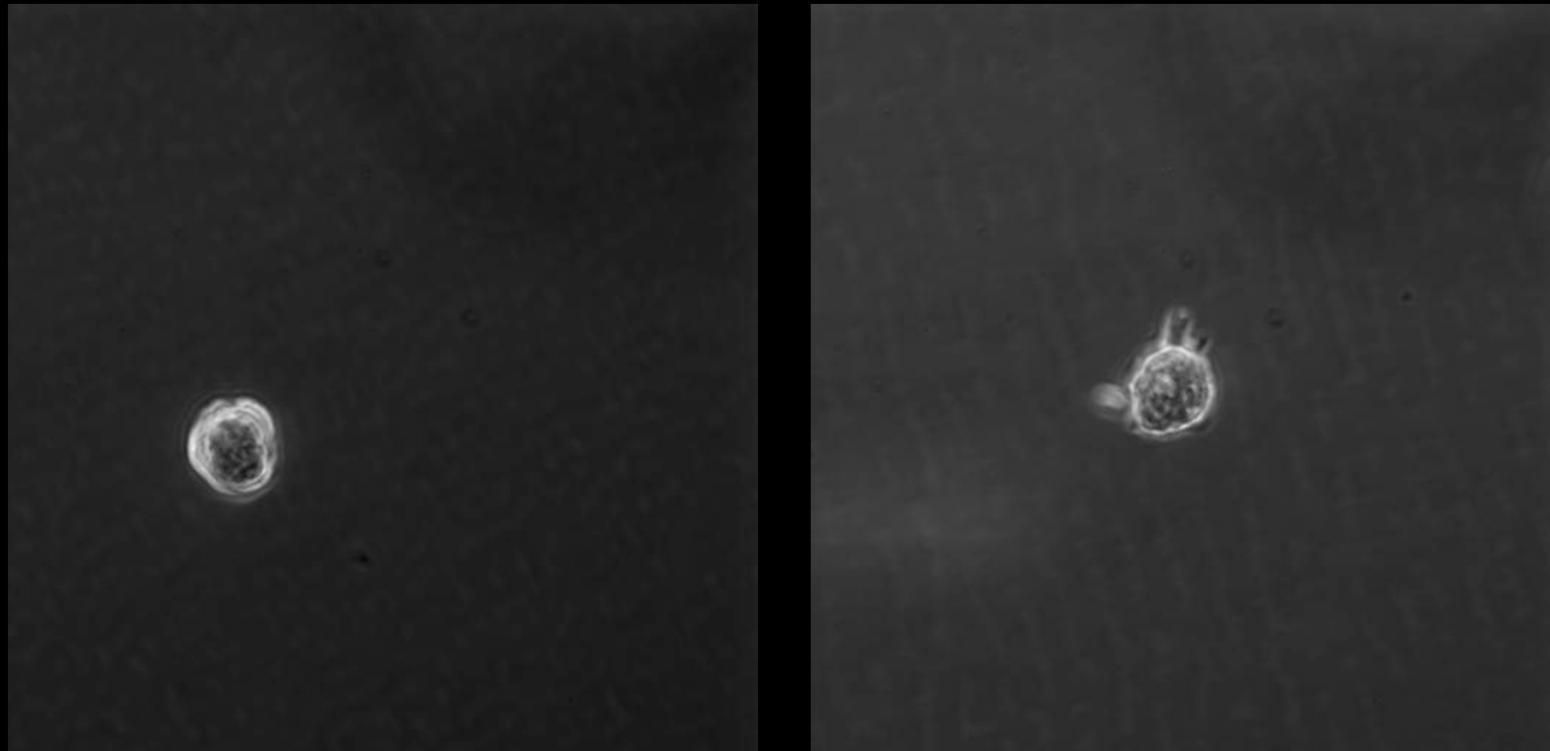
Phalloidin Vinculin TO-
P3



β -tubulin TO-P3



L-Pro induces a motile phenotype in ESCs

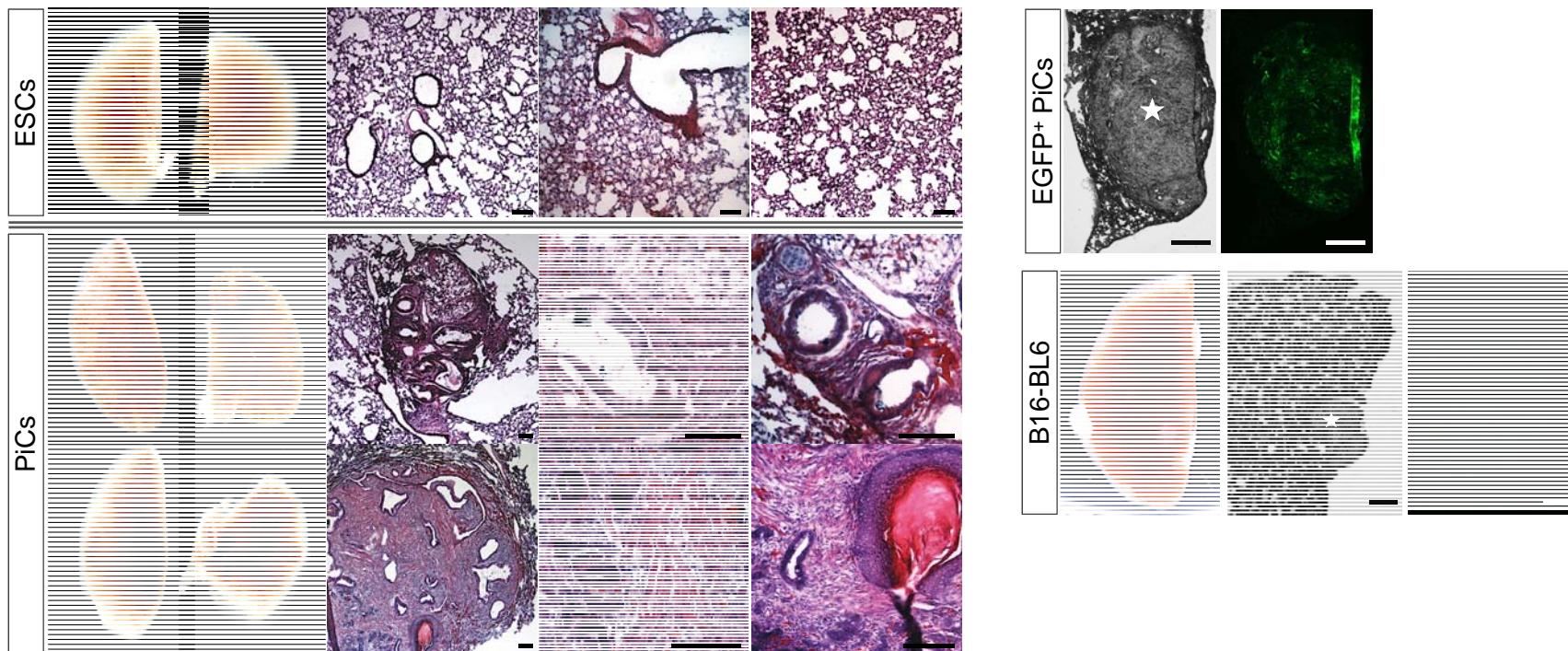
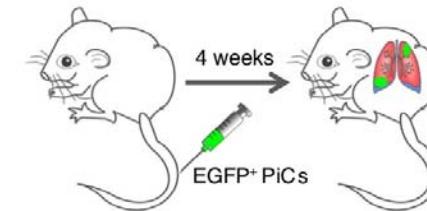
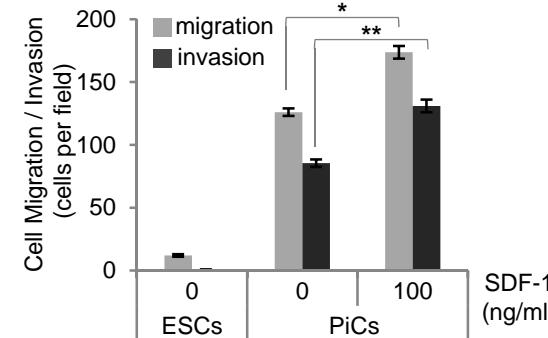
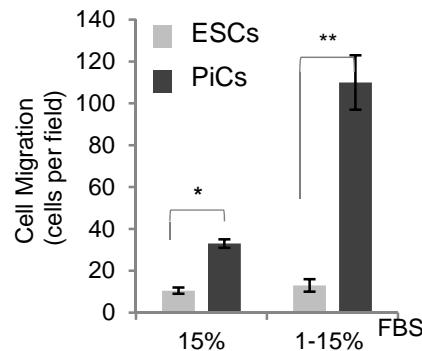


Control ESCs

L-proline -treated ESCs

From Day 3 to Day 4 (images were collected every 5 minutes/ 20x objective)

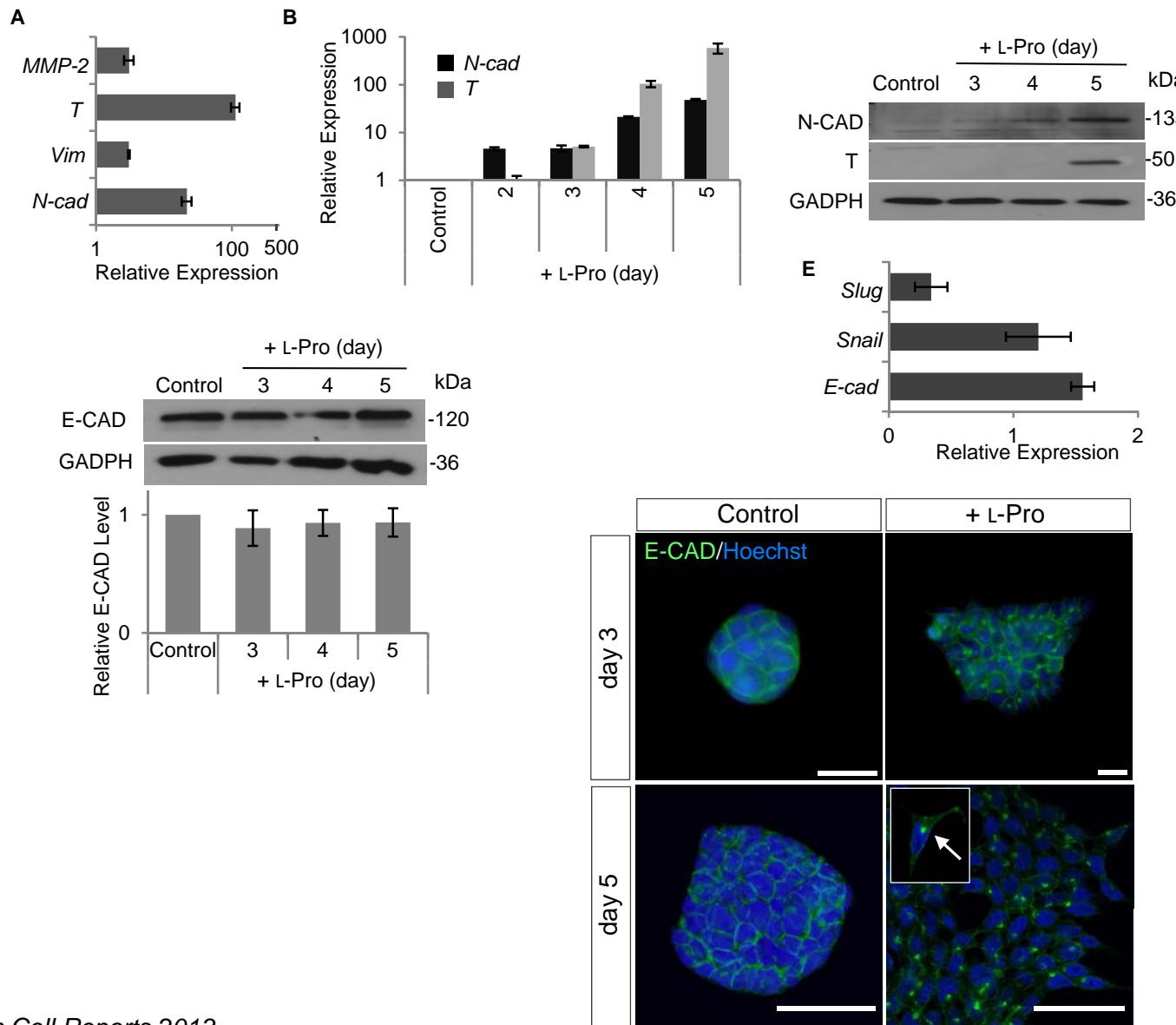
PiCs are invasive and metastatic pluripotent stem cells

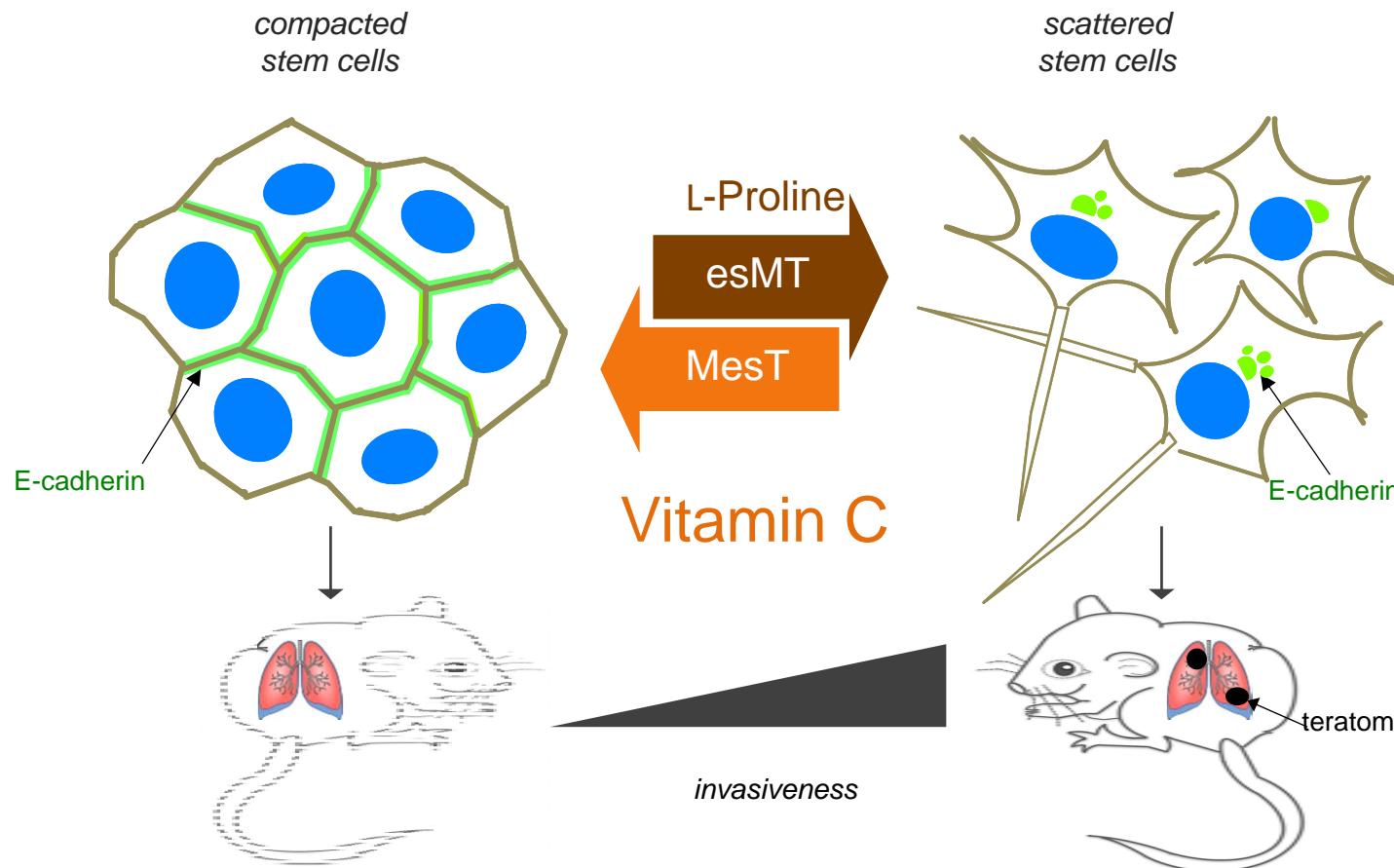


1×10^6 ESCs or PiCs injected into tail veins; mice were sacrificed 4 weeks after injection

Comes et al., Stem Cell Reports 2013

L-Proline induces a fully reversible EMT –like transition in embryonic stem cells: embryonic stem to Mesenchymal Transition (esMT)





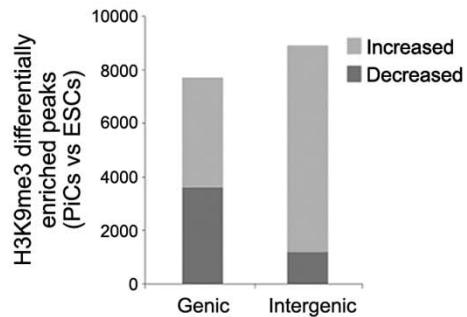
Vitamin C is a key cofactor of the reactions driven by histone demethylases of the JMJ family

Vitamin C improves cell reprogramming (*Shi et al., Cell Stem Cell 2009*) by modulating H3K9 and H3K36 methylation (*Chen J. et al Nature Genet., 2012*)

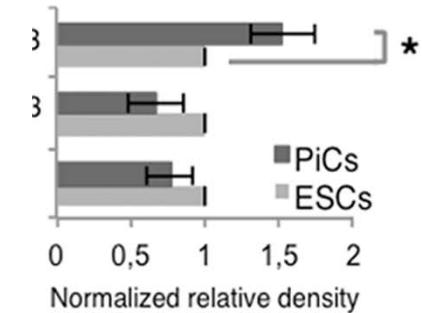
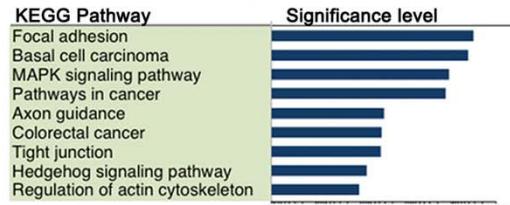
L-Proline is a genome-wide inducer of H3K9 and H3K36 methylation

H3K9me3 peak distribution

ESCs (18,464 peaks) PiCs (25,867 peaks)

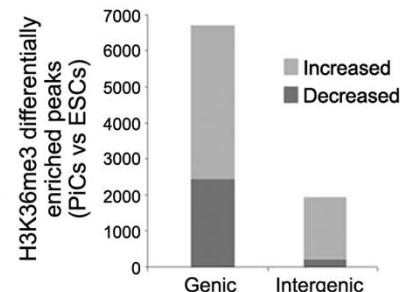


Differentially H3K9me3 enriched genes
5542 439 1082 Differentially expressed genes

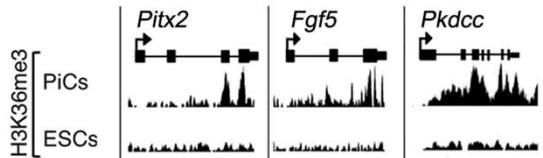
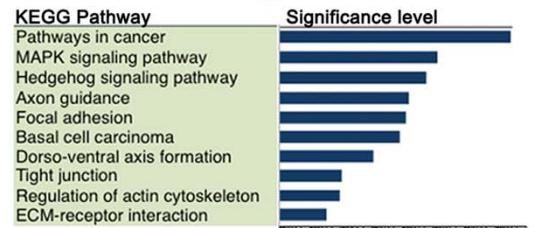


H3K36me3 peak distribution

ESCs (11,150 peaks) PiCs (12,559 peaks)

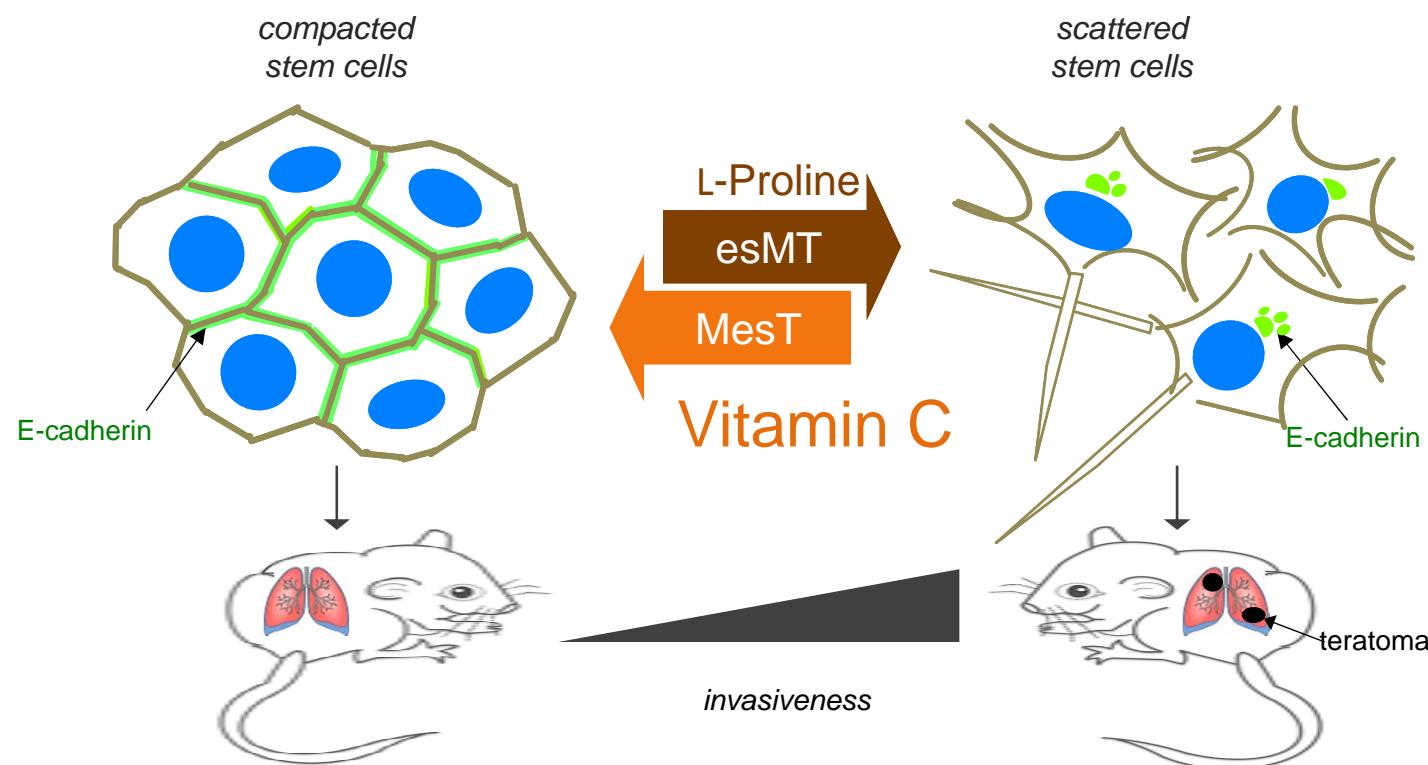


Differentially H3K36me3 enriched genes
3697 628 893 Differentially expressed genes



L-Pro triggers an esMT reminiscent of the EMT that occurs at the invasive front of the tumor, and contributes to the acquisition of cell plasticity and invasiveness.

L-Pro–induced esMT is fully reversible (MesT) and is accompanied by a global remodeling of H3K9 and H3K36 methylation status





Past members

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Nicola Laprano

External collaborations

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Silvia Brunelli, HSR, Milan
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Sharhaghim Tajbakhsh, Pasteur Institute, Paris
Ann Zeuner, Ruggero de Maria, ISS, Rome
TIGEM Bioinformatic Core Facility, Naples
Dror Seliktar, Technion, Haifa, Israel

Thanks to



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Cristina D'Aniello
Gennaro Andolfi

IGB collaborations

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Maria Matarazzo
Dario De Cesare
Laura Casalino

IGB Facilities

Integrated Microscopy
Animal House
FACS
NGS

EMBO WORKSHOP ON

Stem Cell Mechanobiology in
Development and Disease
October 18-21, 2015
Capri, Naples



Invited Speakers

Yohanns Bellaïche, France
Françoise Brochard-Wyart, France
Chen Christofer S., USA
Giulio Cossu, UK
George Q. Daley, USA
Carl-Philipp Heisenberg, Austria
Donald E. Ingber, USA
Benoît Ladoux, France
David A. Lee, UK
Chad Medalla, Switzerland
Christine Mummery, The Netherlands

Paolo Netti, Italy
Graziella Pellegrini, Italy
Stefano Piccolo, Italy
Mattieu Piel, France
Giorgio Scita, Italy
Dror Sellktar, Israel
GV Shivashankar, Italy
Craig A. Simons, Canada
Molly Stevens, UK
Viola Vogel, Switzerland
Fiona Watt, UK

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Paolo A. Netti, Italian Institute of Technology (IIT), Naples, Italy

Viola Vogel, Laboratory of Applied Mechanobiology, ETH, Zürich, Switzerland



The IGB Meeting Coordinators

Maria R. Matarazzo, Maria G. Miano

Workshop Secretariat

Anna Maria Aliperti, Federica Staempfli