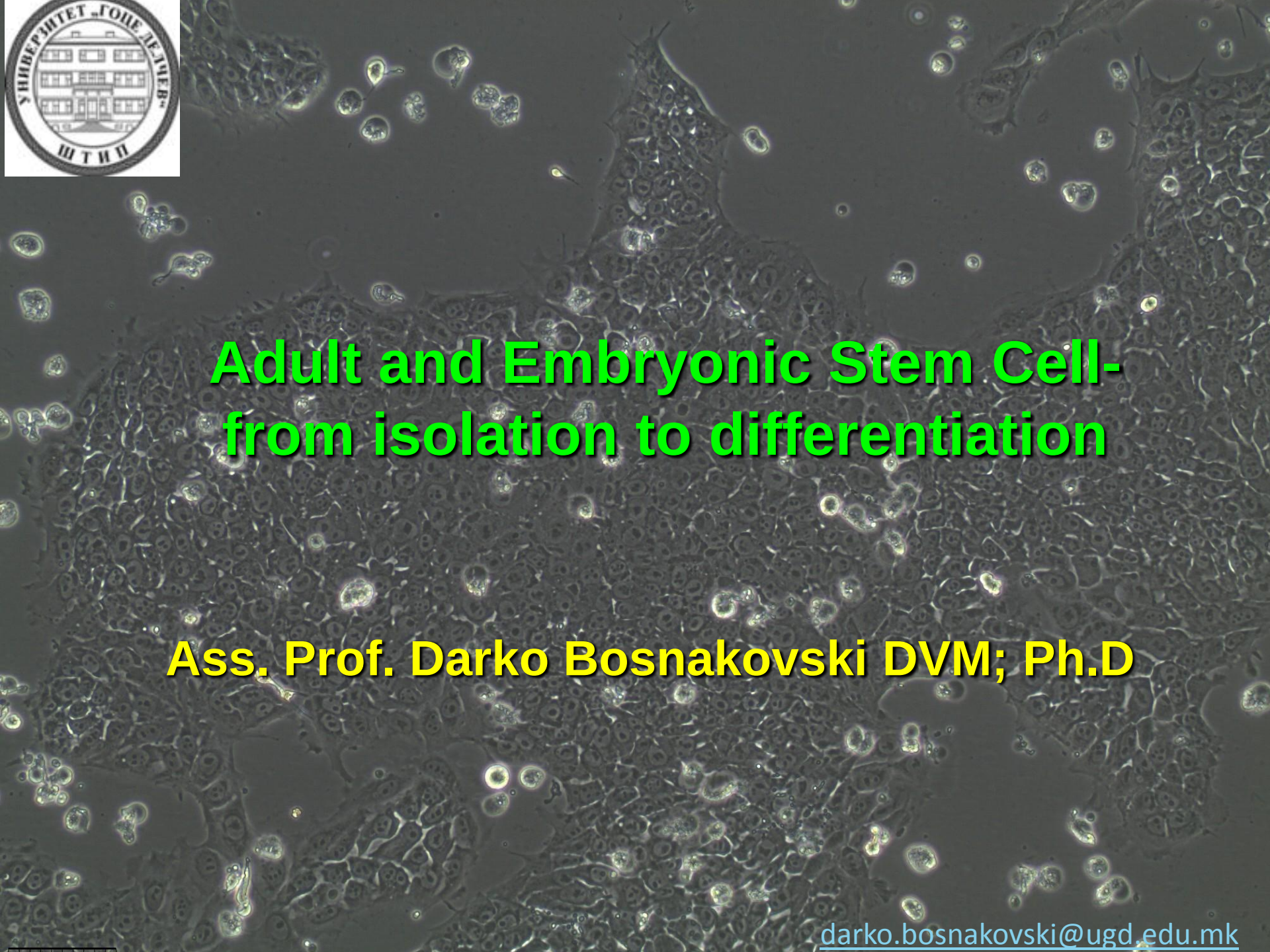
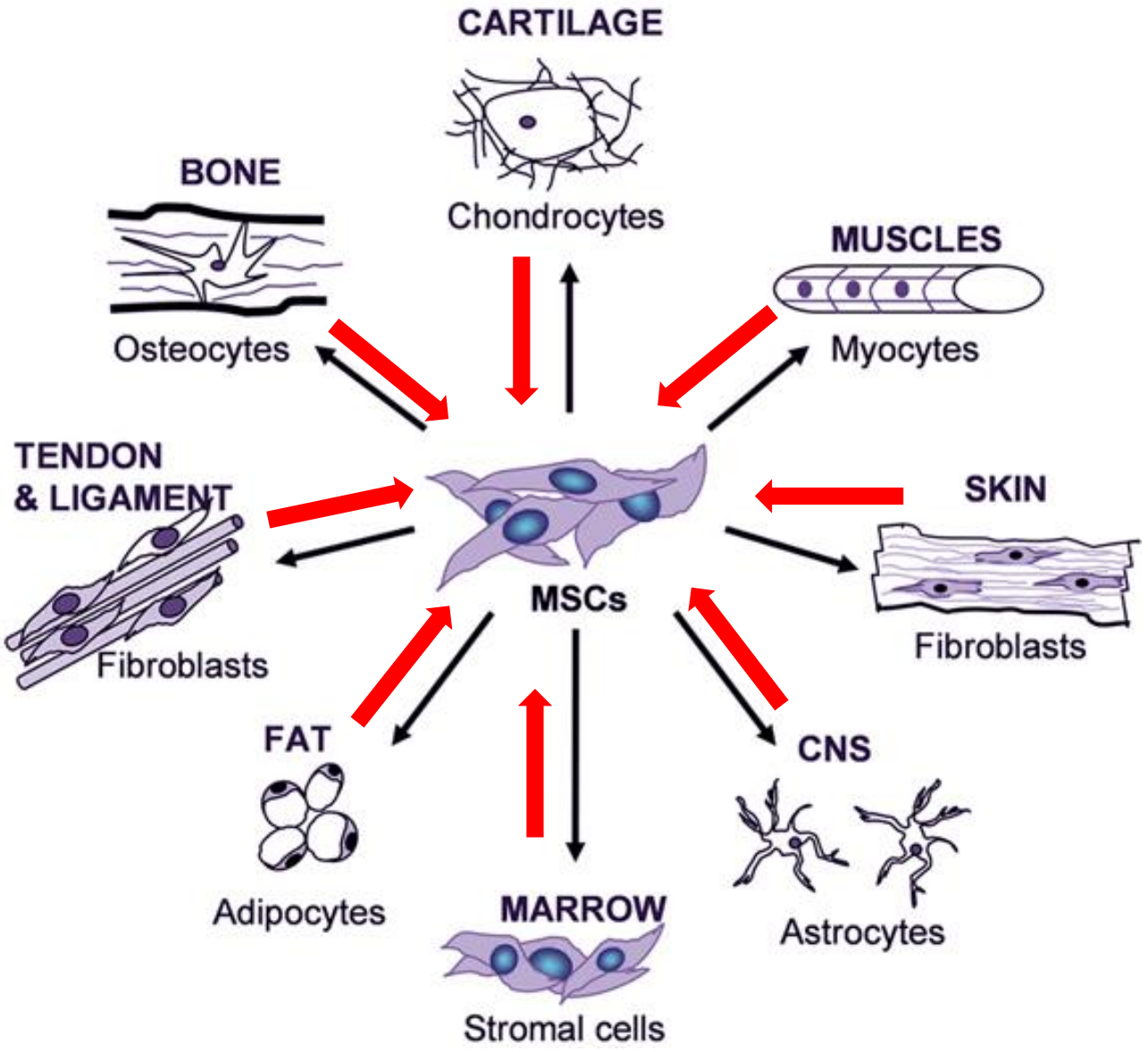


Adult and Embryonic Stem Cell- from isolation to differentiation

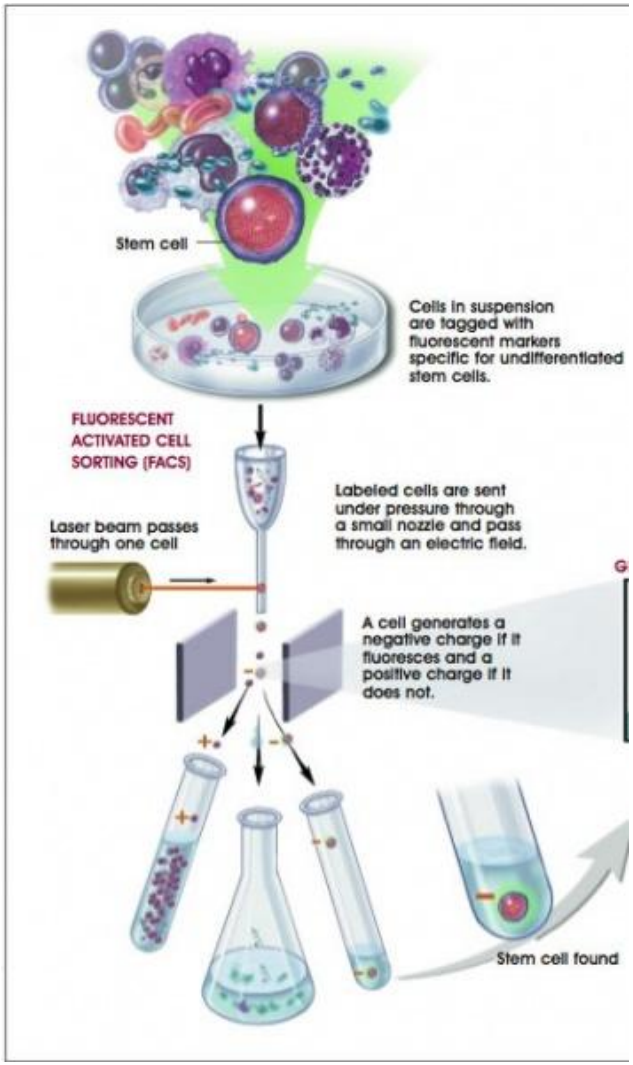
Ass. Prof. Darko Bosnakovski DVM; Ph.D

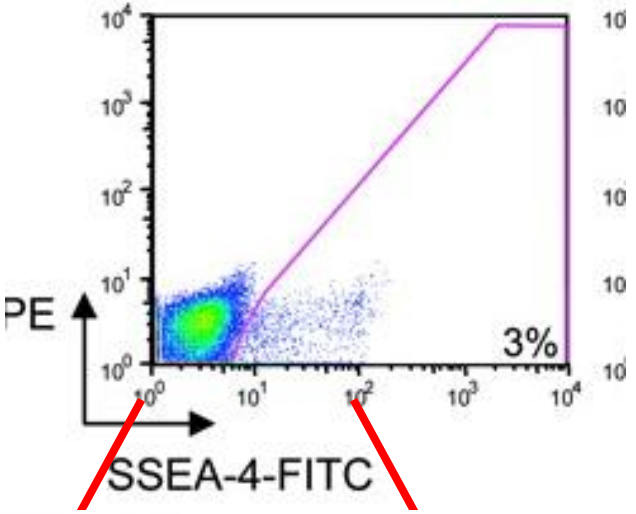
darko.bosnakovski@ugd.edu.mk

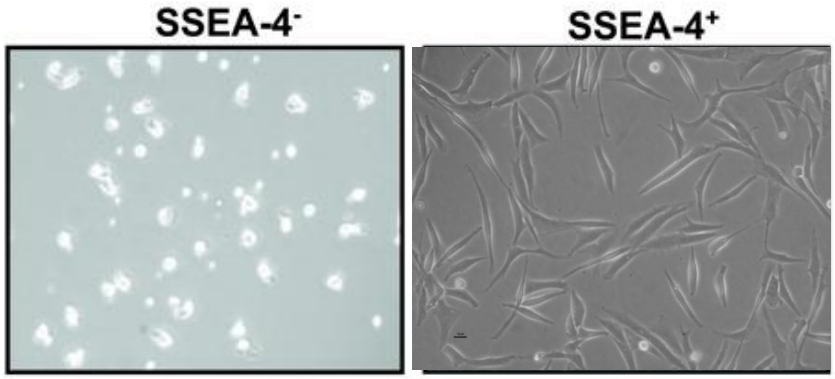
From where and how to get stem cells?



SSEA4 Identifies Mesenchymal Stem Cells in Bone Marrow

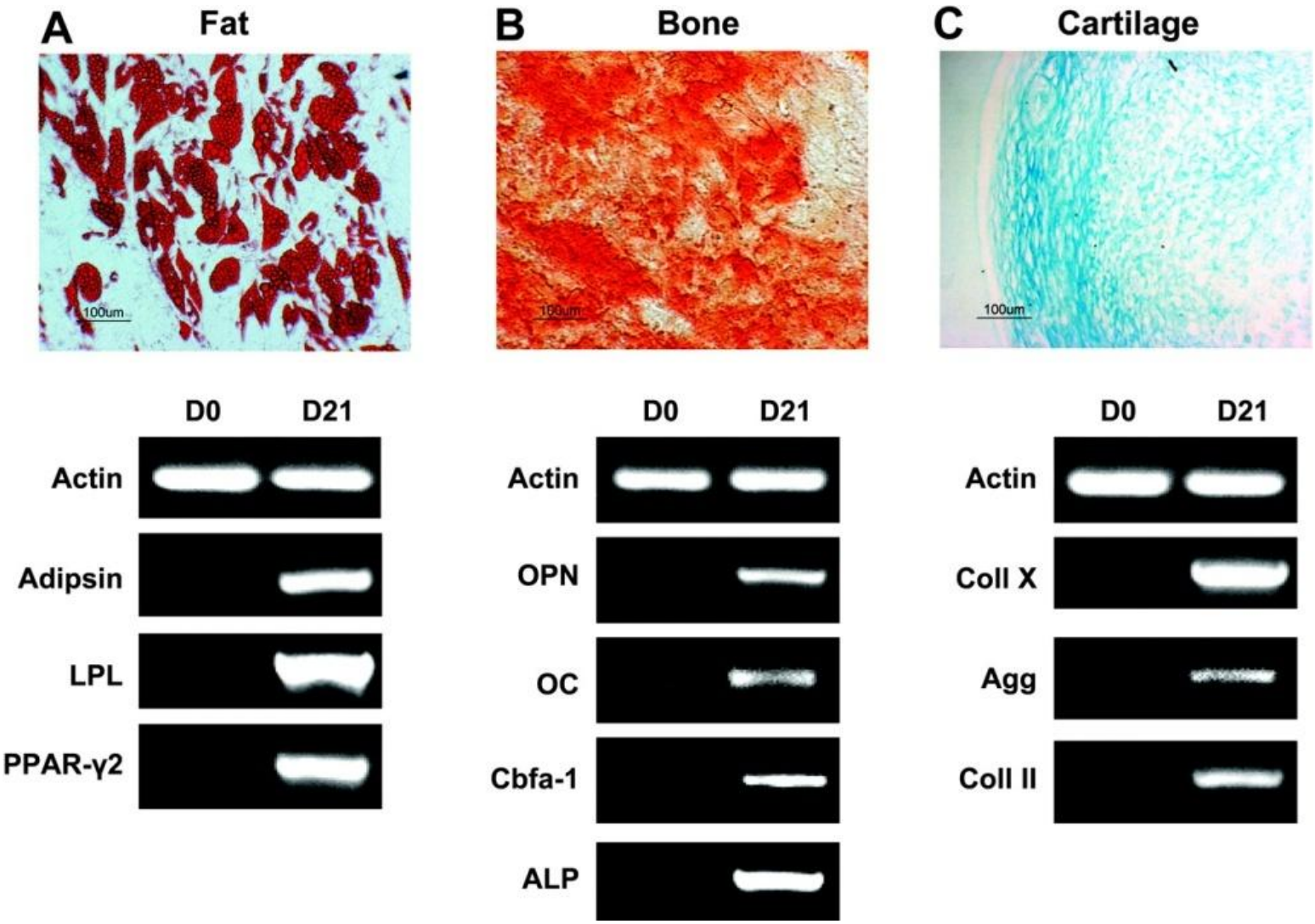






Gang and Bosnakovski; Blood 2007;109:1743-1751

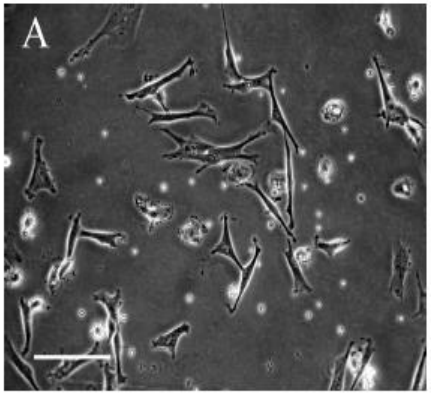
In vitro multilineage differentiation of SSEA4^{+} cells



Gang and Bosnakovski et al; Blood 2007

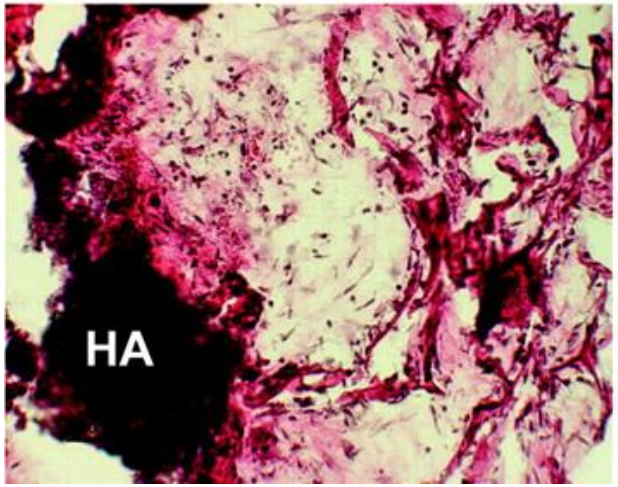
Osteogenic differentiation of SSEA4^{+} stem cells on hydroxyapatite-in vivo

hydroxyapatite

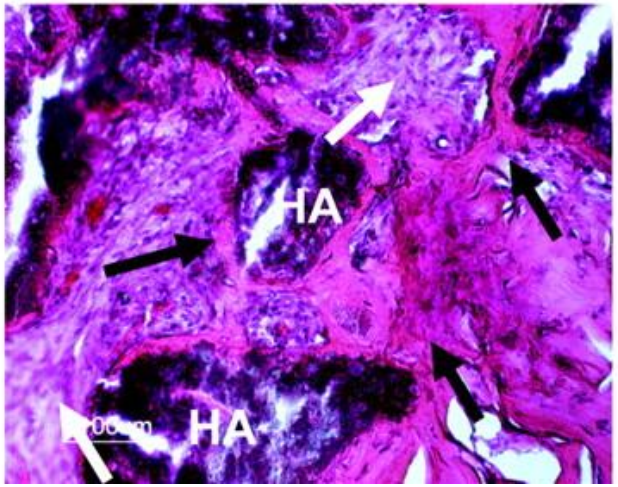


hMSC

control



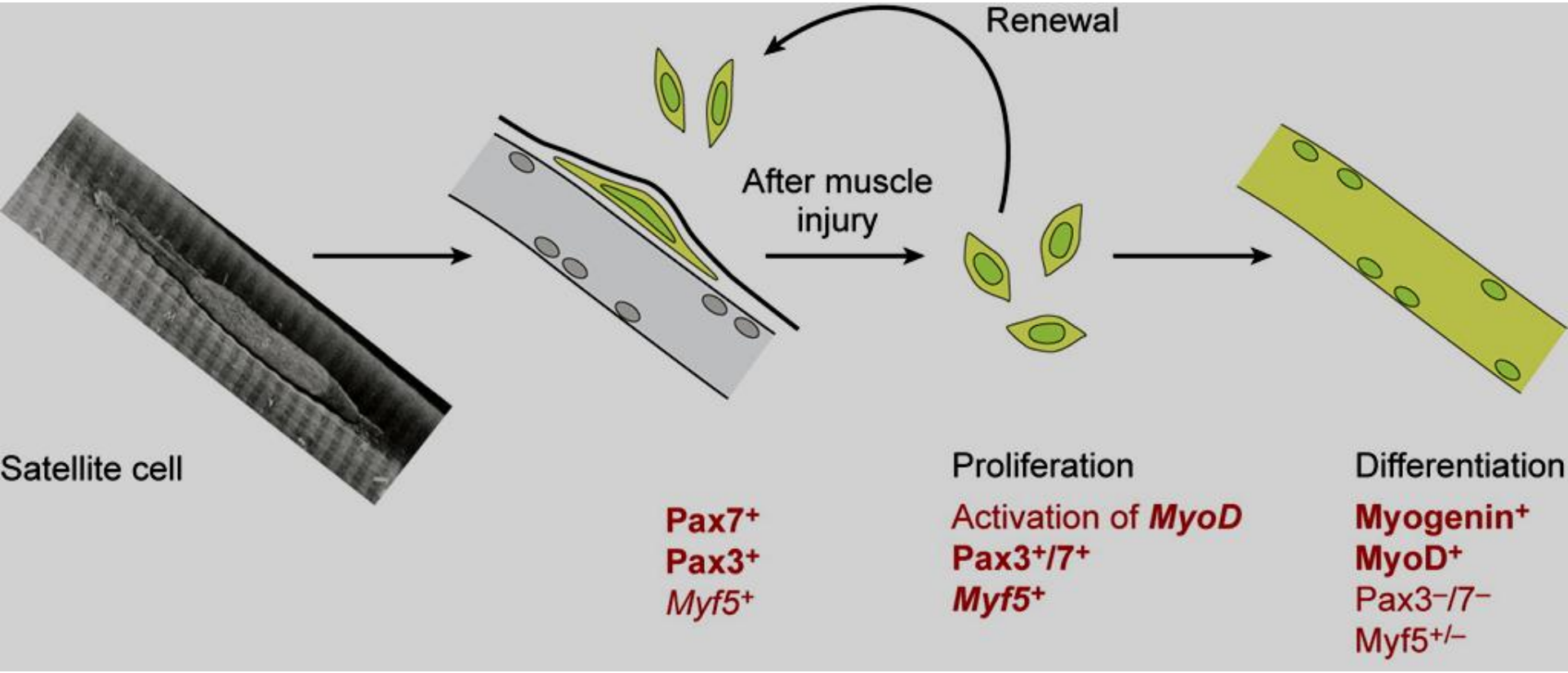
SSEA-4^{+} cells



Gang and Bosnakovski et al; Blood 2007

Tissue specific stem cells

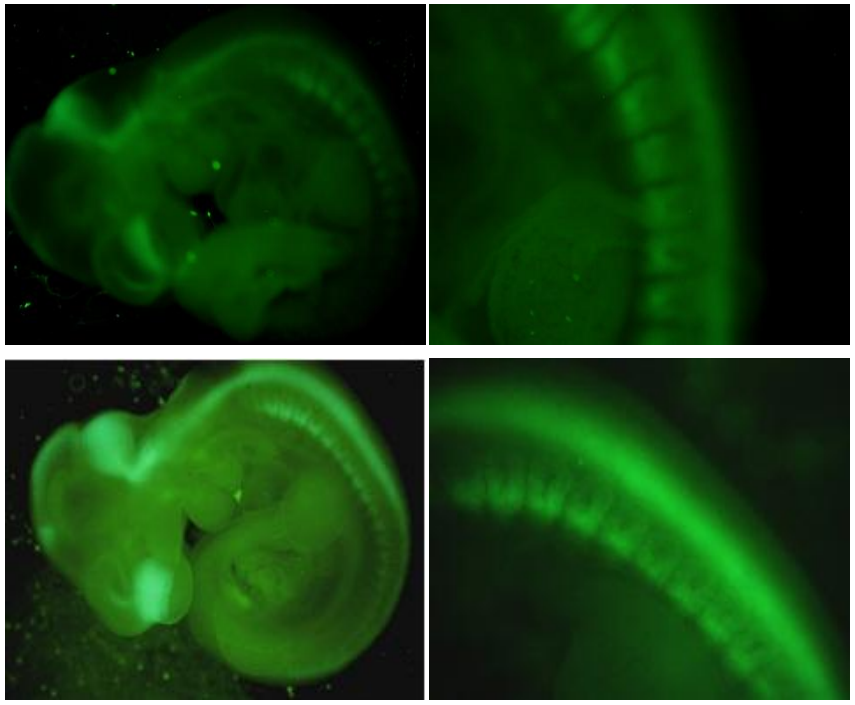
Myogenesis



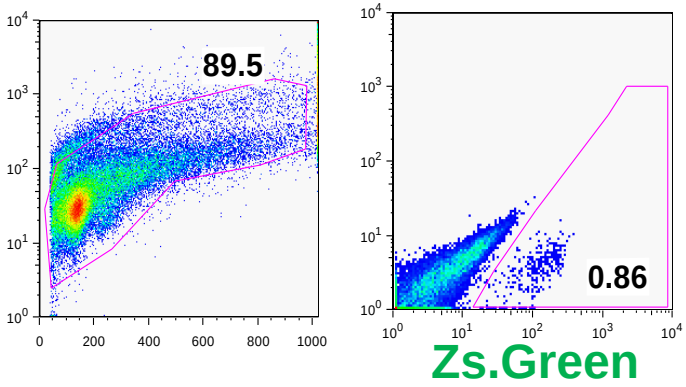
Buckingham & Relaix. Cell Dev Biol 2007

Transgenic animals –
Model for isolation of tissue specific stem cells

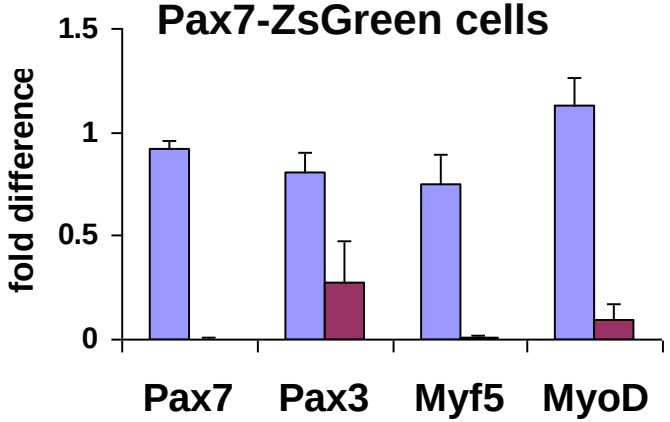
Embryos from Pax7-ZsGreen mouse



FACS profile of Pax7-ZsGreen mouse muscle



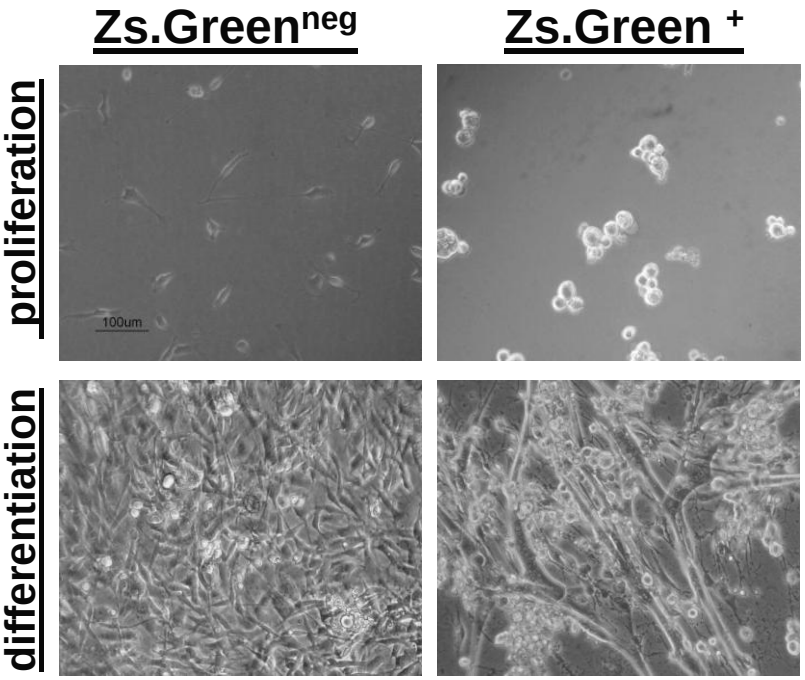
Transcriptional profile of Pax7-ZsGreen cells



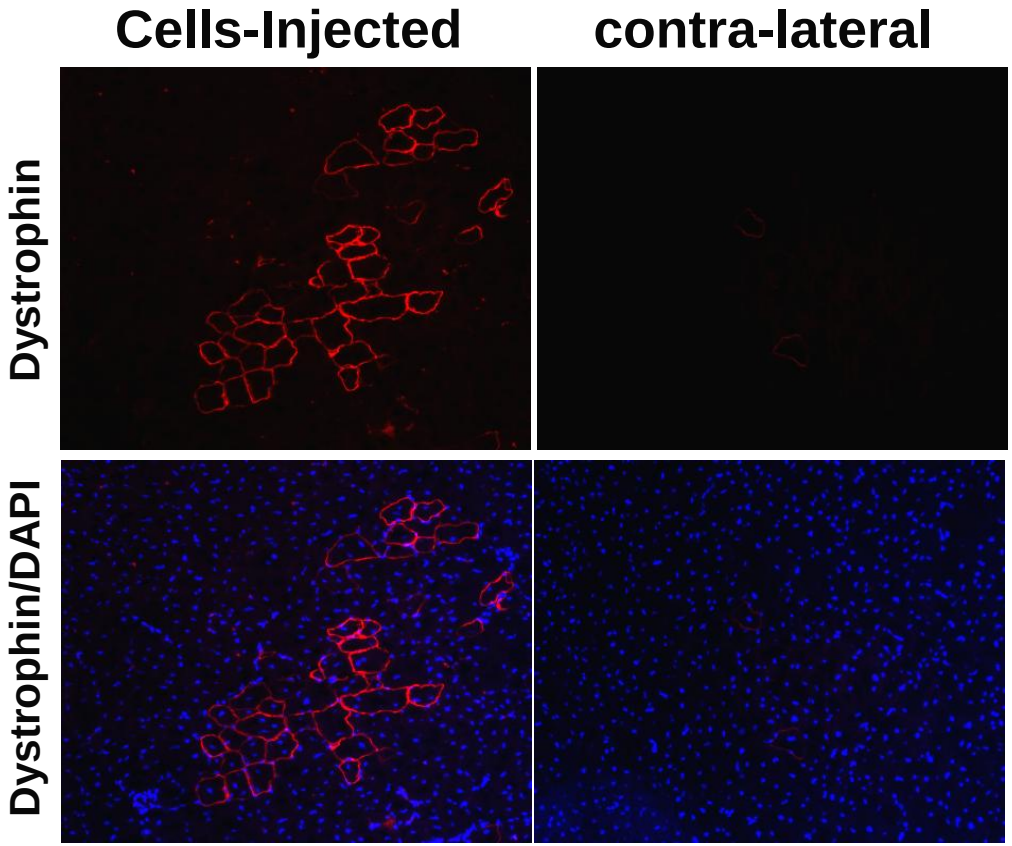
Bosnakovski et al.; Stem Cells 2008
Tang W, Zeve D, Bosnakovski et al.; Science 2008

Myogenic potential of Pax7-ZsGreen^{+} cells

In vitro



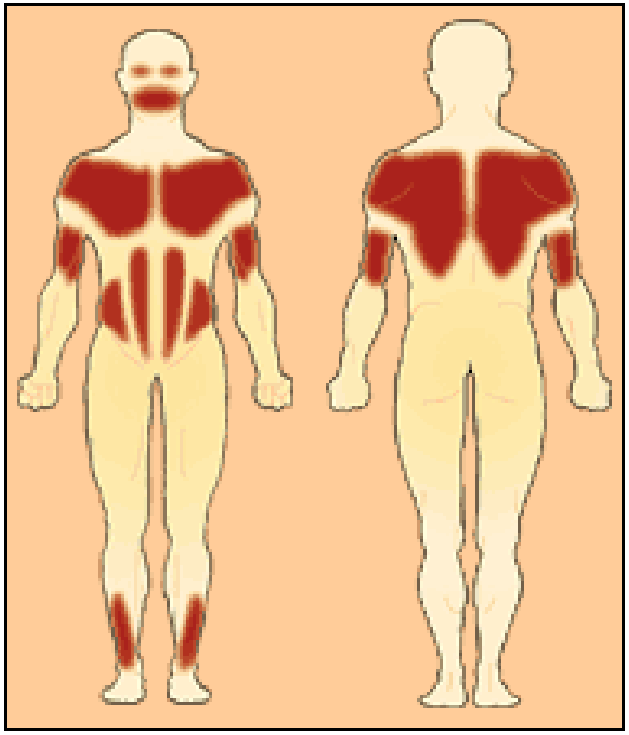
In vivo

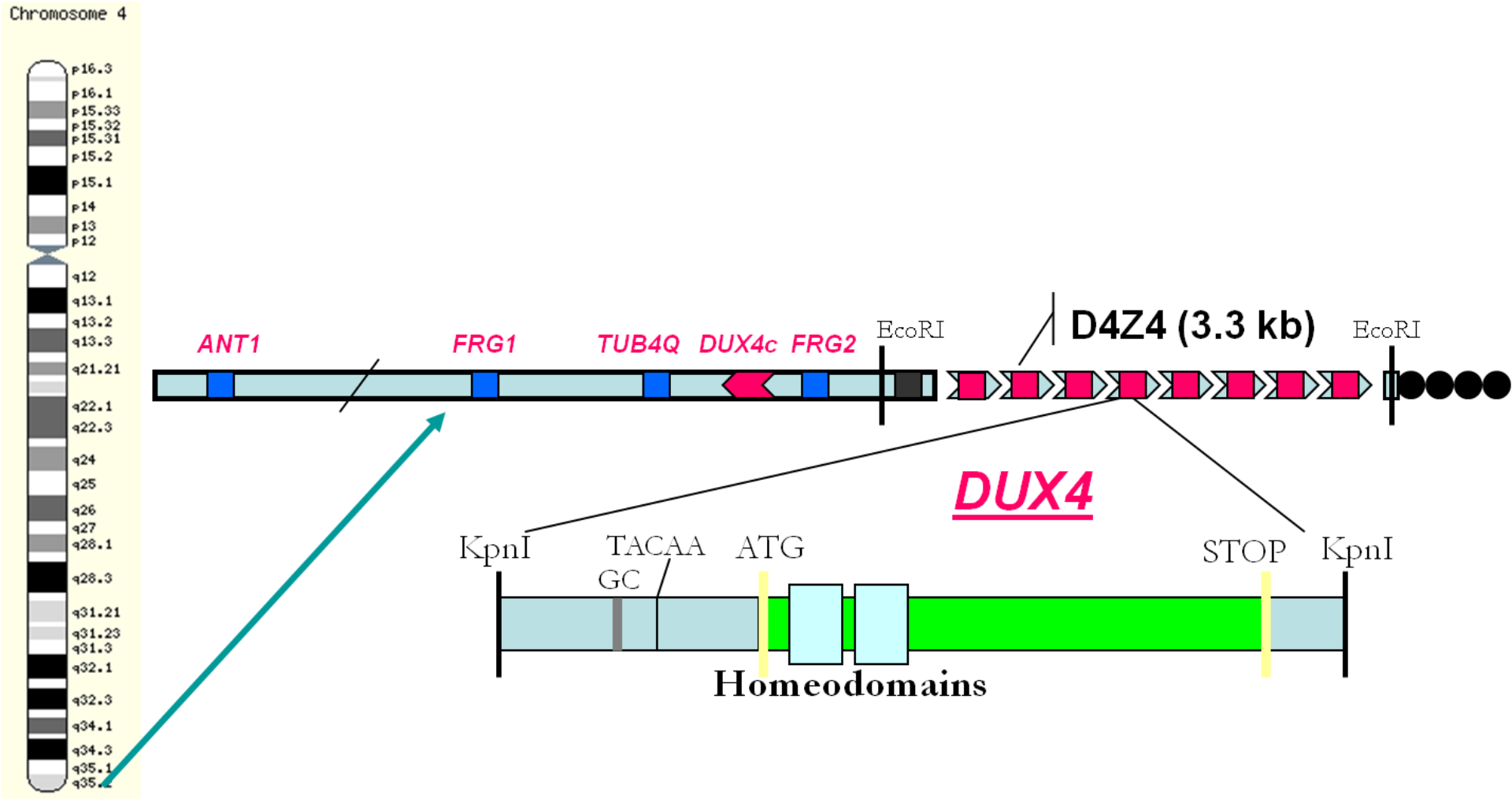


***Bosnakovski* et al.; Stem Cells 2008**

Facioscapulohumeral Muscular Dystrophy (FSHD)

- **FSHD is an autosomal dominant inherited disorder;**
- **Third most common inherited neuromuscular condition (1:14,000);**
- **Muscles on the face are first affected;**
- **There is no specific treatment.**

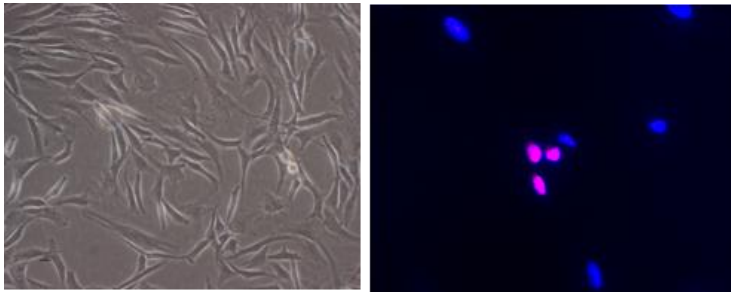


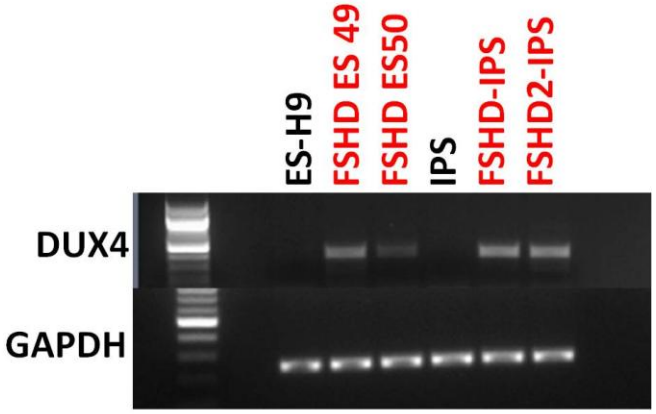

Chromosome 4
p16.3
p16.1
p15.33
p15.32
p15.31
p15.2
p15.1
p14
p13
p12
q12
q13.1
q13.2
q13.3
q21.21
q22.1
q22.3
q24
q25
q26
q27
q28.1
q28.3
q31.21
q31.23
q31.3
q32.1
q32.3
q34.1
q34.3
q35.1
ANT1
FRG1
TUB4Q
DUX4c
FRG2
EcoRI
D4Z4 (3.3 kb)
EcoRI
DUX4
KpnI
TACAA
GC
ATG
STOP
KpnI
Homeodomains

DUX4 and FSHD

DUX4 is expressed in FSHD cells

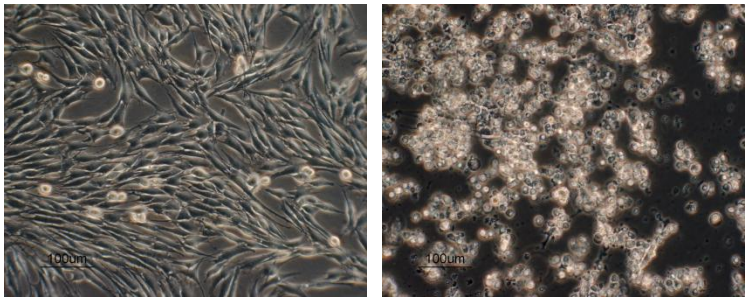
DUX4/DAPI



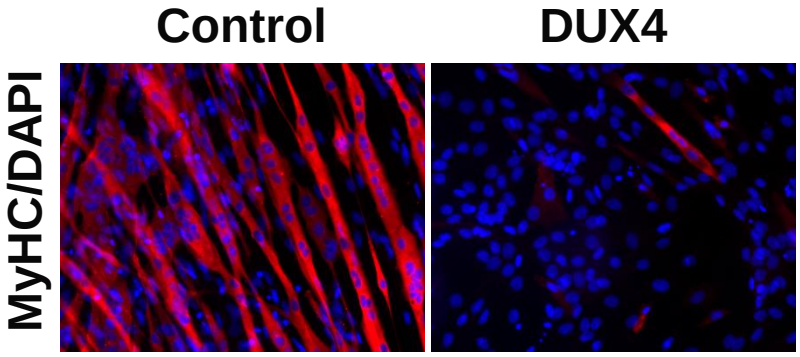


DUX4 induces cell death

Control + DUX4



DUX4 affects myogenesis

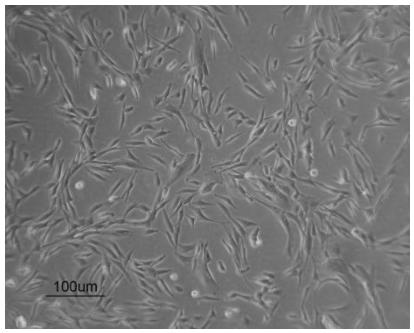


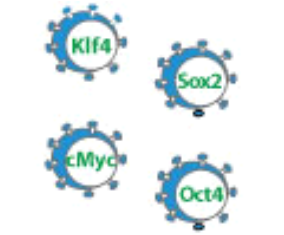
***Bosnakovski* et al.; EMBO J 2008**
***Bosnakovski* et al.; Exp Neurol 2008**
***Bosnakovski* et al.; PlosOne 2009**

Inducible DUX4 mouse-Animal model for FSHD

***Bosnakovski* et al.; in submission**

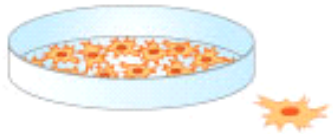
Generation of IPS cells from patient with muscular dystrophy (FSHD)



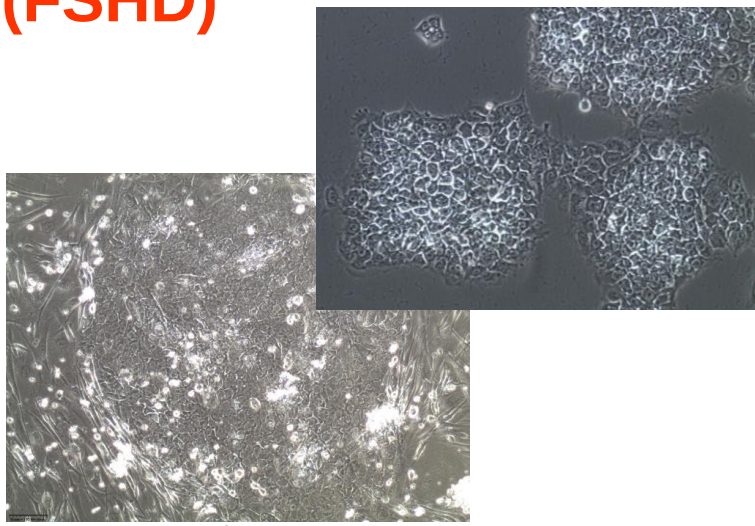


iPSC Factor Viruses

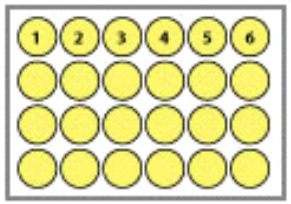
Transduce with iPSC virus and **wait 2 days**



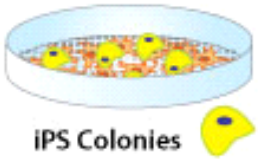
Myoblasts from FSHD patients



Expand the best iPS Colonies (4 weeks)

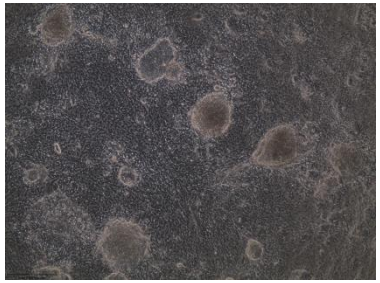


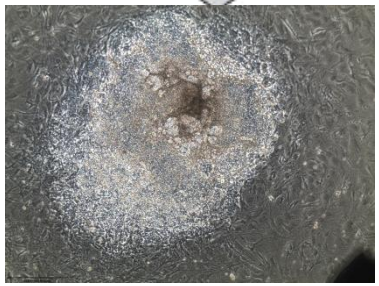
Re-plate cells on feeder cell layers + ES Medium

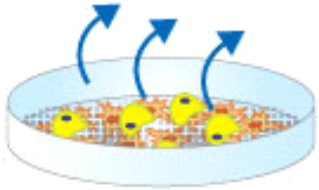


iPS Colonies

Wait 2 weeks to visualize iPS colonies change medium every day





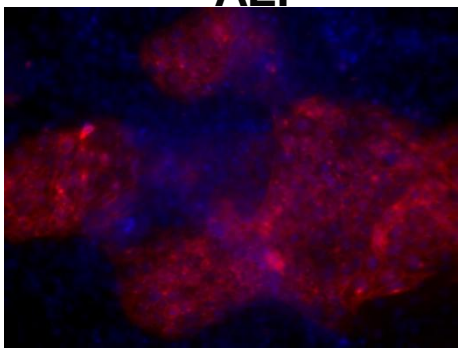


Pick individual iPS Colonies

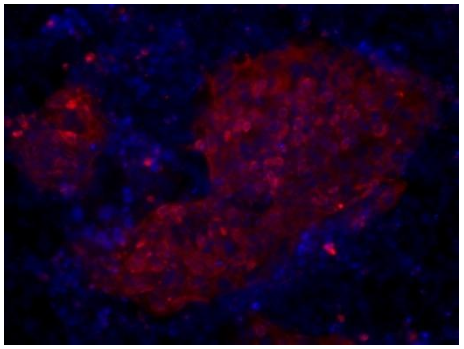
***Bosnakovski* et. al. in submission**

Characterization of FSHD-IPS cells

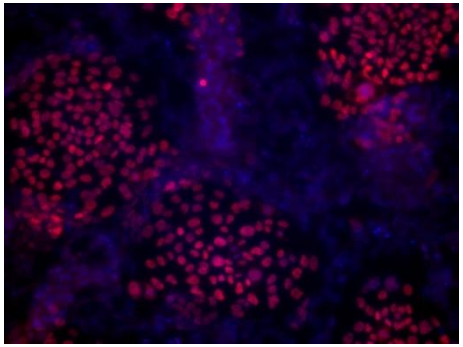
ALP



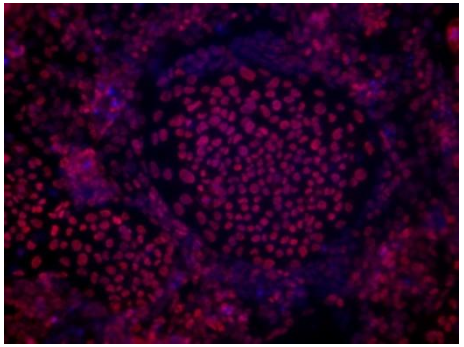
TRA1-60



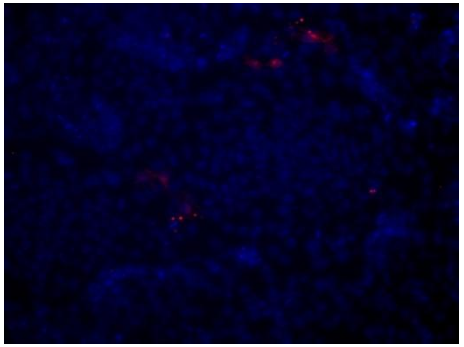
NANOG



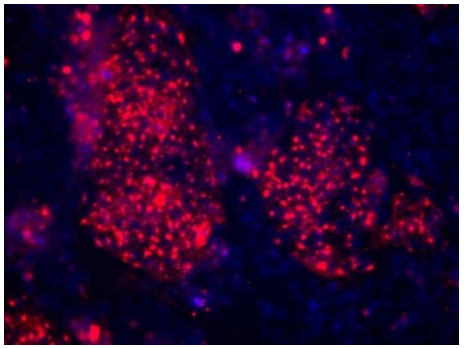
OCT4



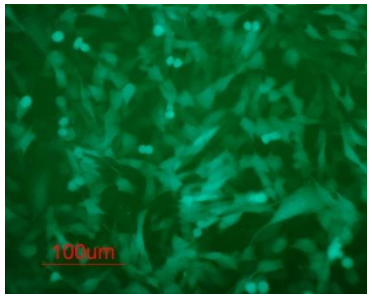
SSEA1



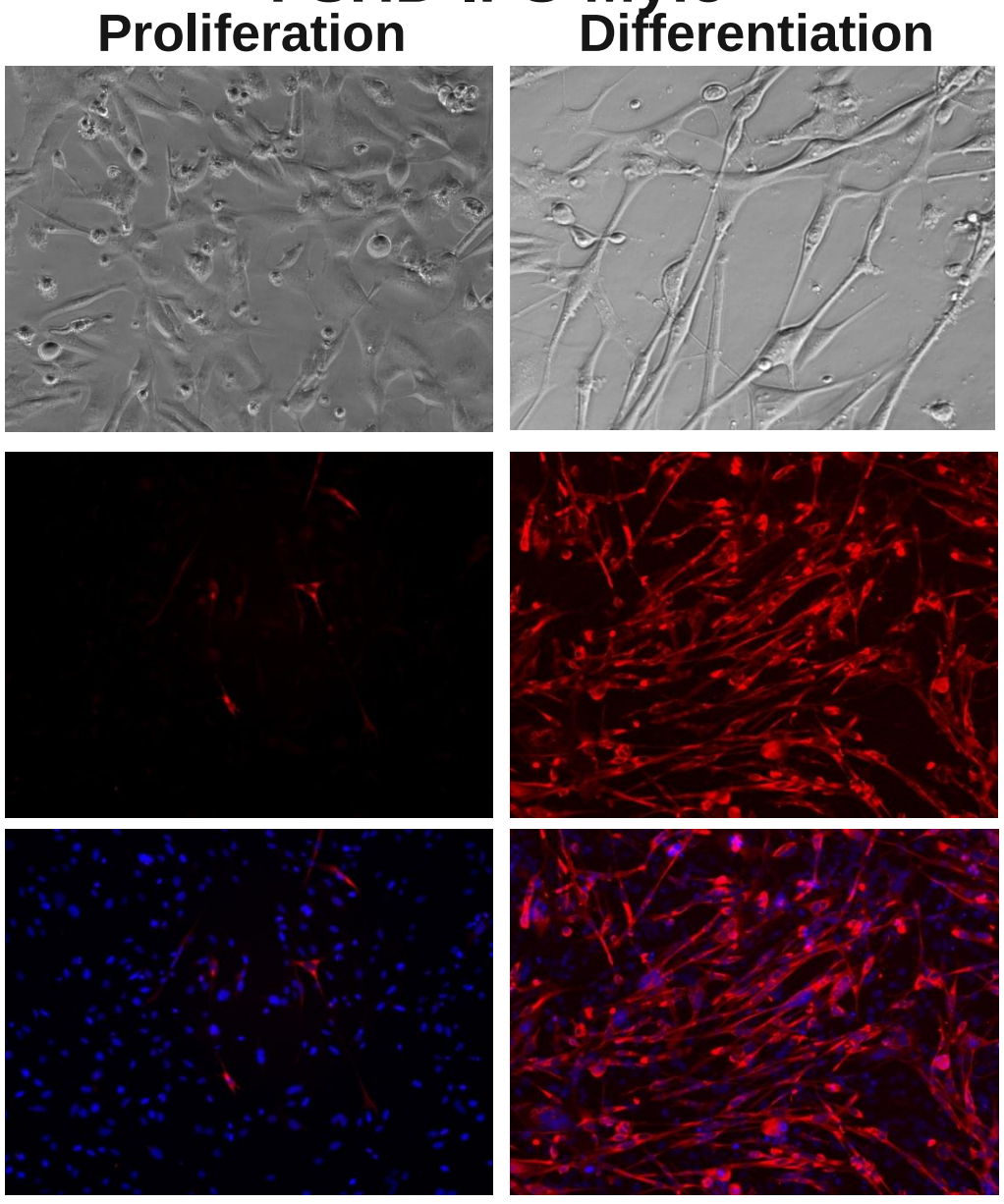
SSEA4



Myogenesis of ES/IPS cells

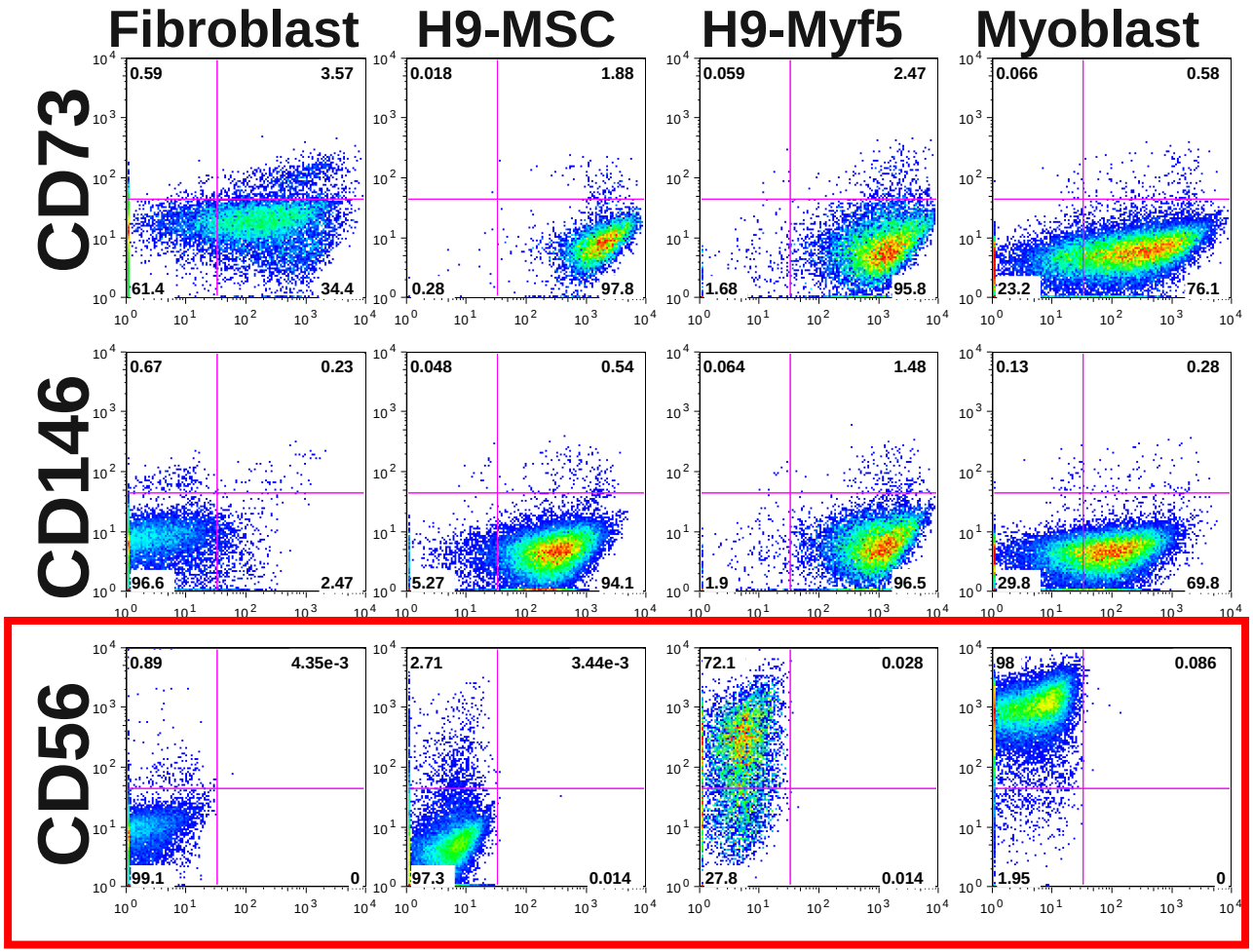


FSHD-IPS-Myf5

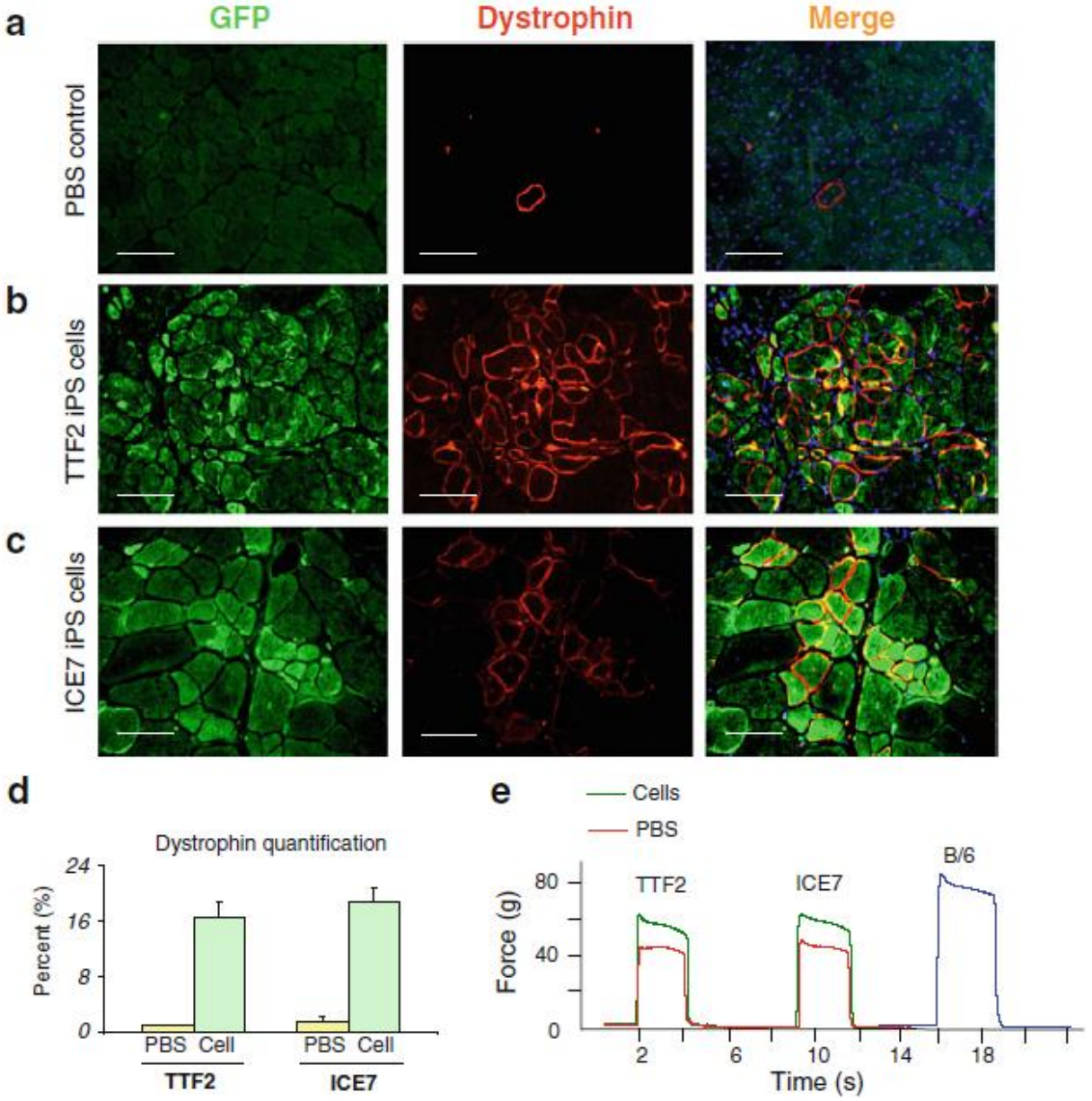


***Bosnakovski* et. al. in submition**

Myoblast from IPS cells has similar surface profile as myoblast



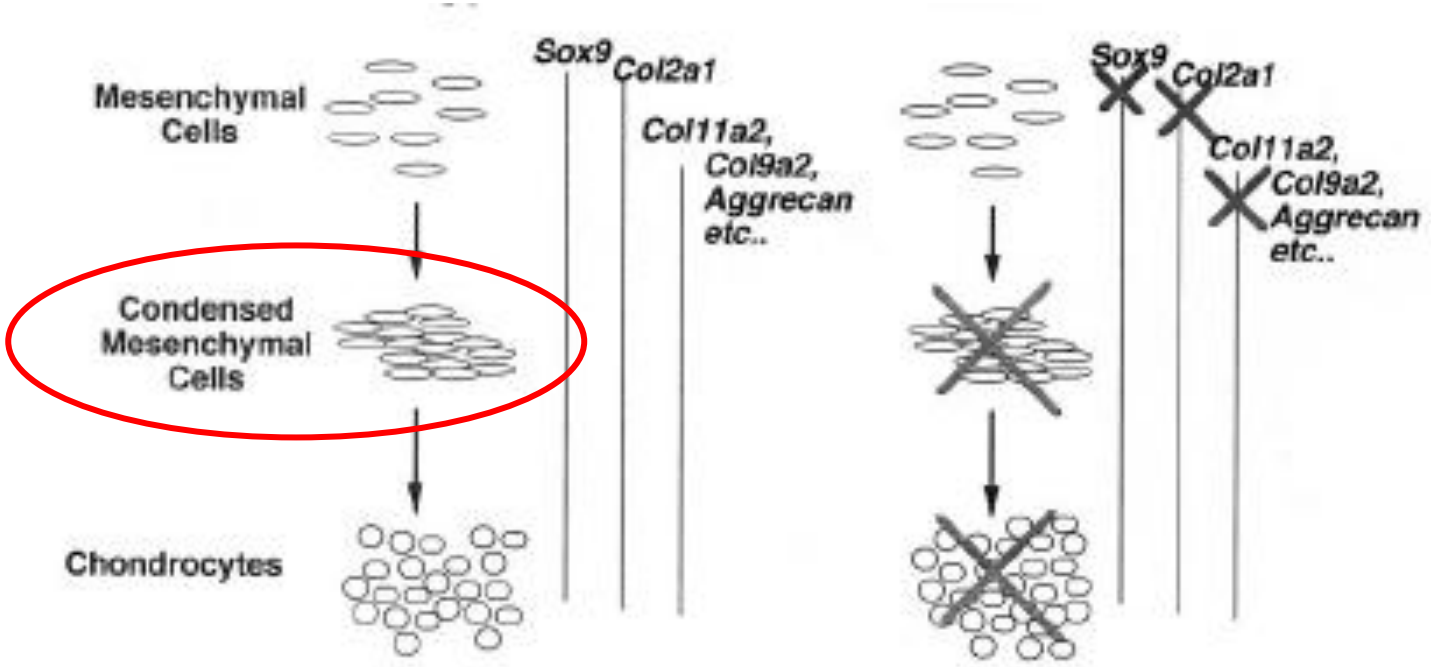
Functional regeneration of damage muscle with IPS cells



Darabi R, Pan W, Bosnakovski D et. al. Stem Cell Rev. 2011 Apr 2

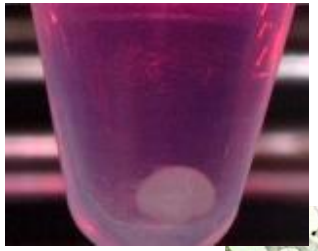
CHONDROGENIC DIFFERENTIATION

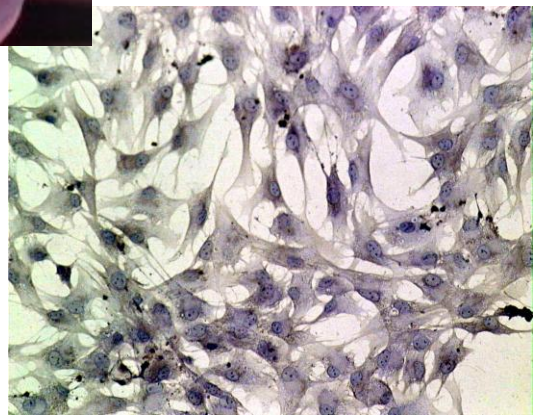
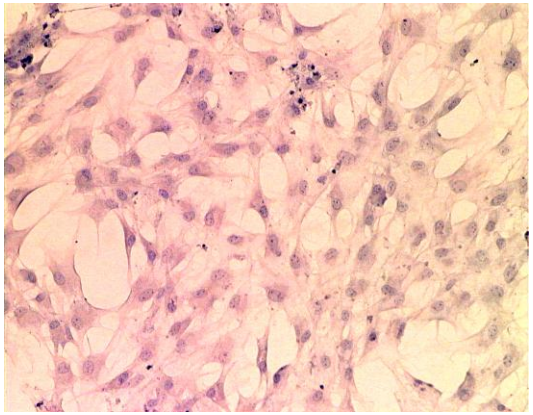
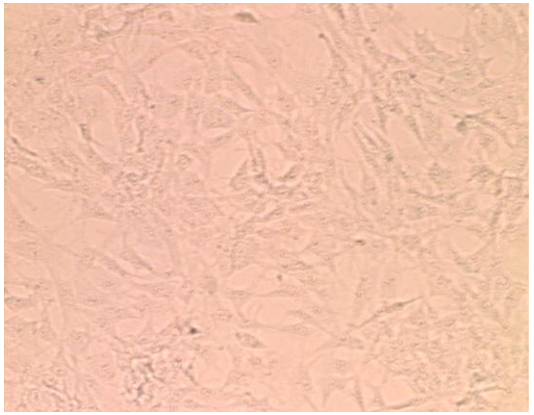
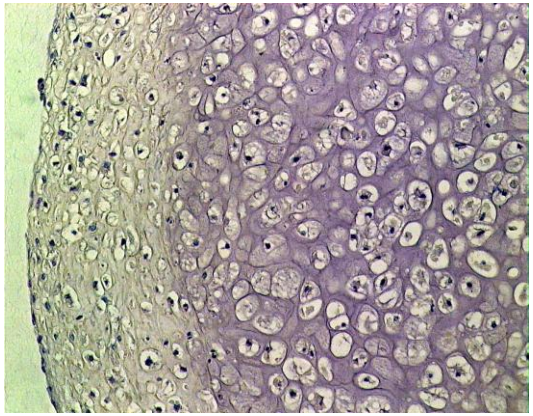
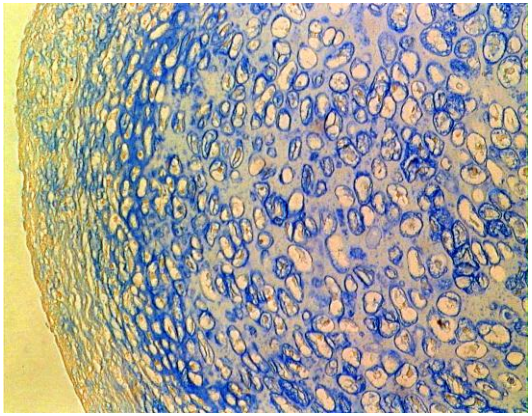
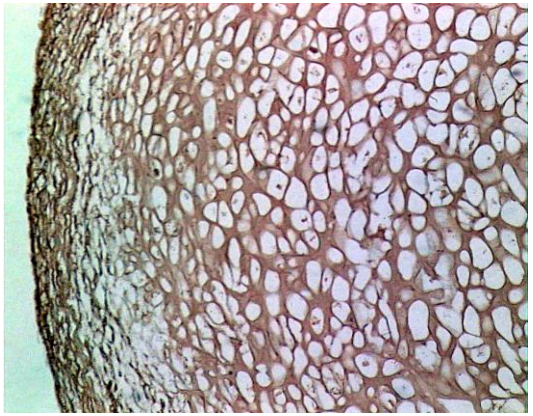
- **Strong cell to cell interaction;**
- **Extracelluar matrix and bioactive factors**



Crombrugghe et al. 2000

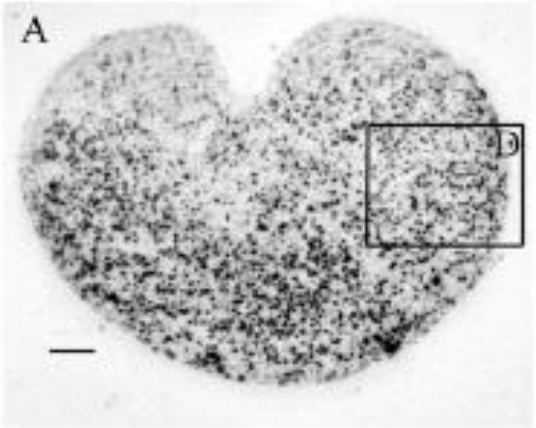
CHONDROGENIC DIFFERENTIATION OF BOVINE MSCs IN SYSTEM PELLET CULTURE

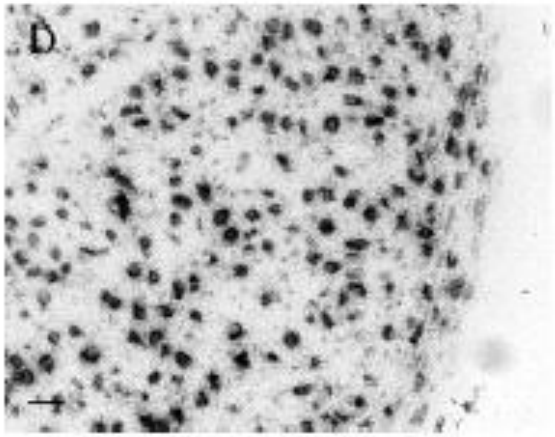


	H&E	Alcian blue	Collagen type II
Monolayer culture			
Pellet culture			

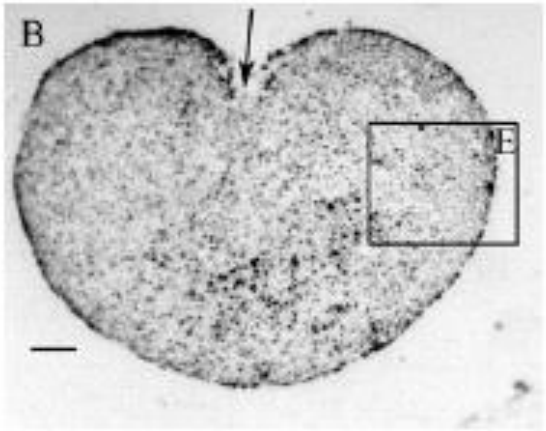
Bosnakovski et al. Exp Hematol , 2004

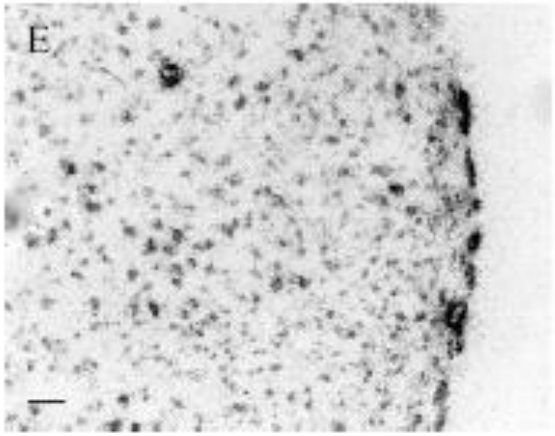
Collagen type II



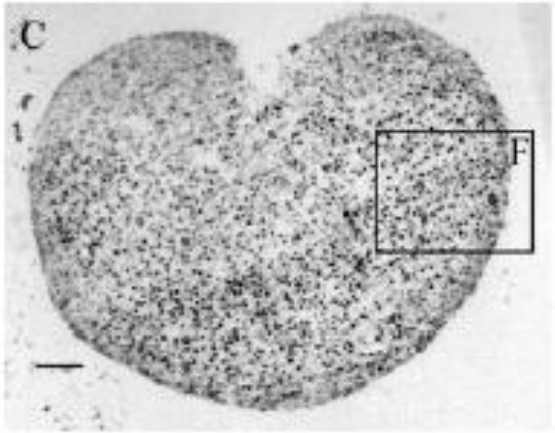


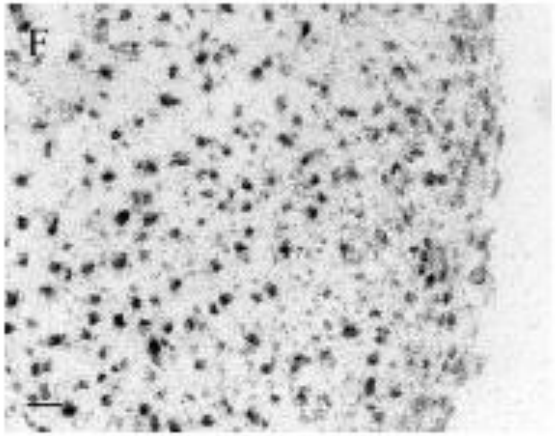
Collagen type I





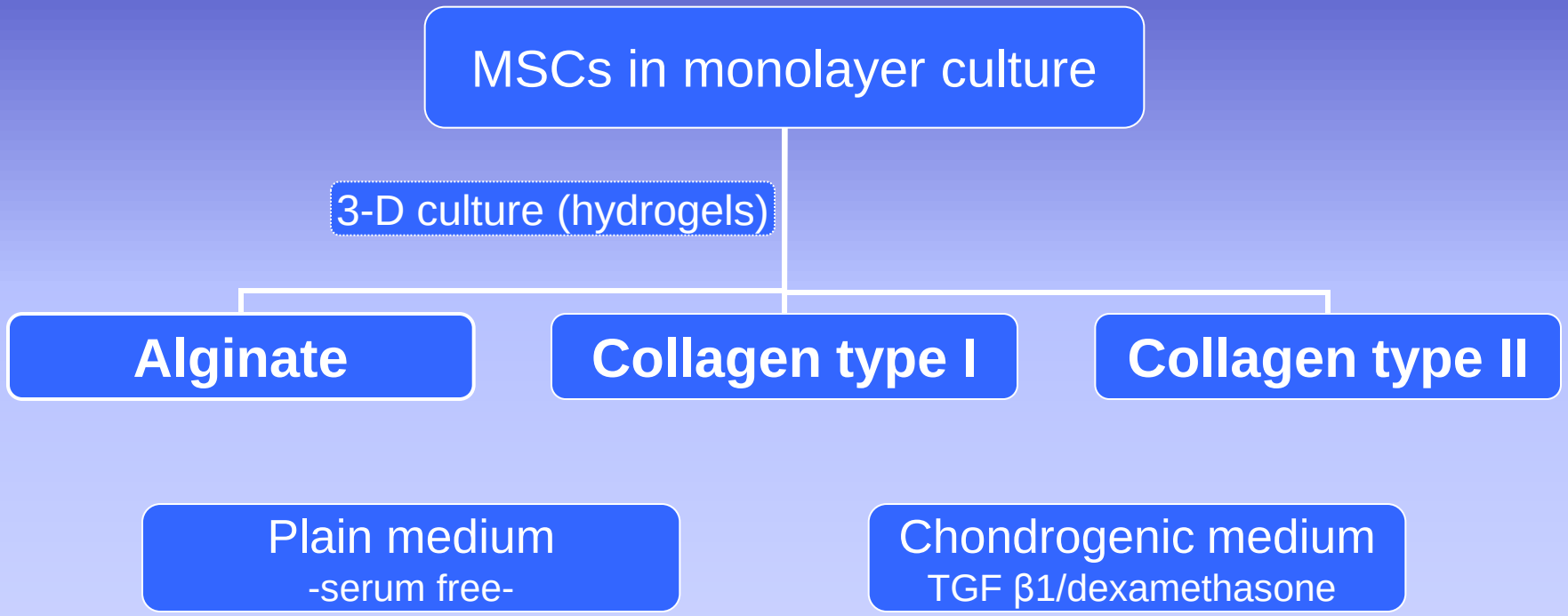
Aggrecan

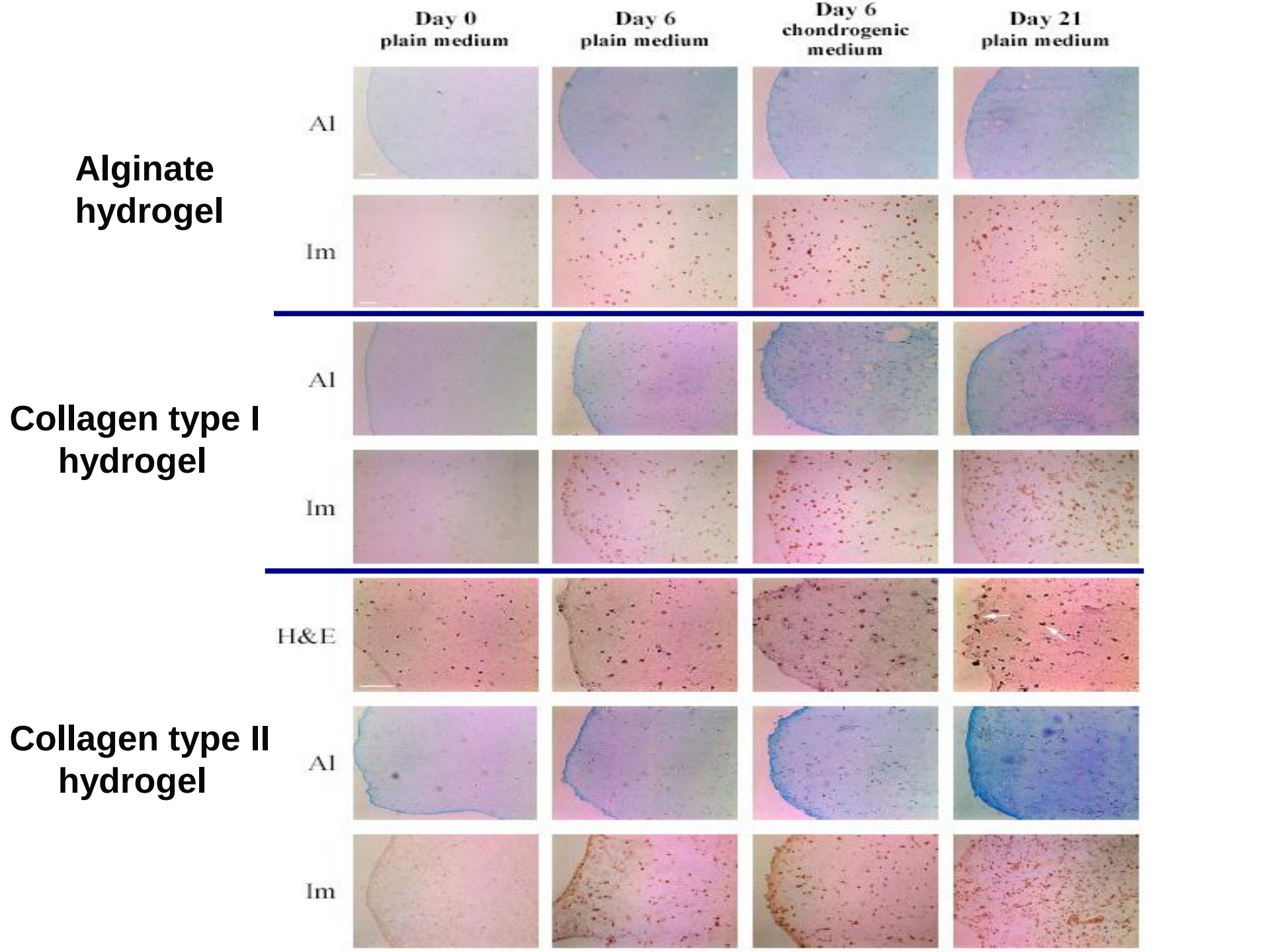




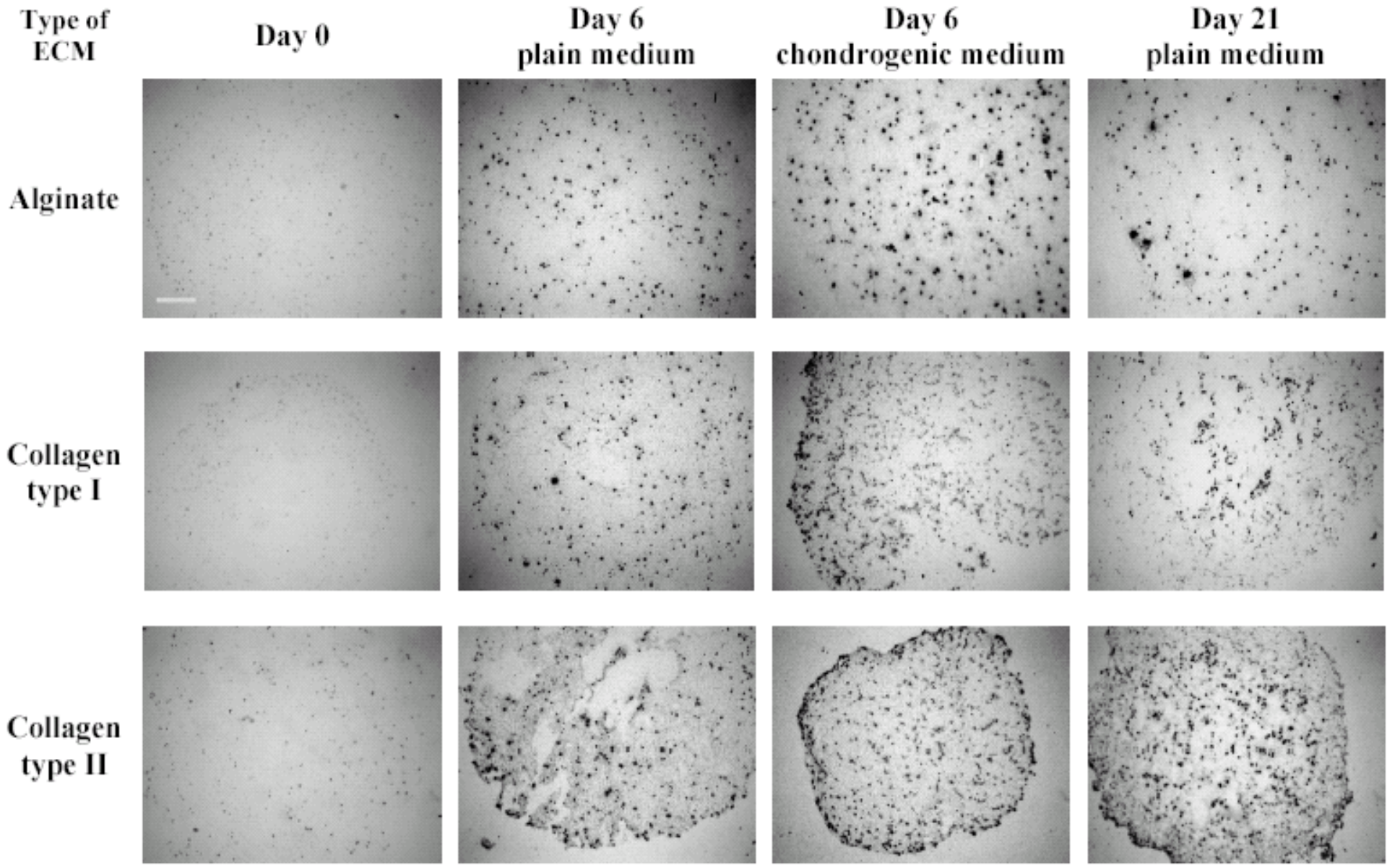
Bosnakovski et al. Exp Hematol , 2004

CHONDROGENIC DIFFERENTIATION OF MSCs IN DIFFERENT HYDROGELS: INFLUENCE OF COLLAGEN TYPE II ON MSC CHONDROGENESIS



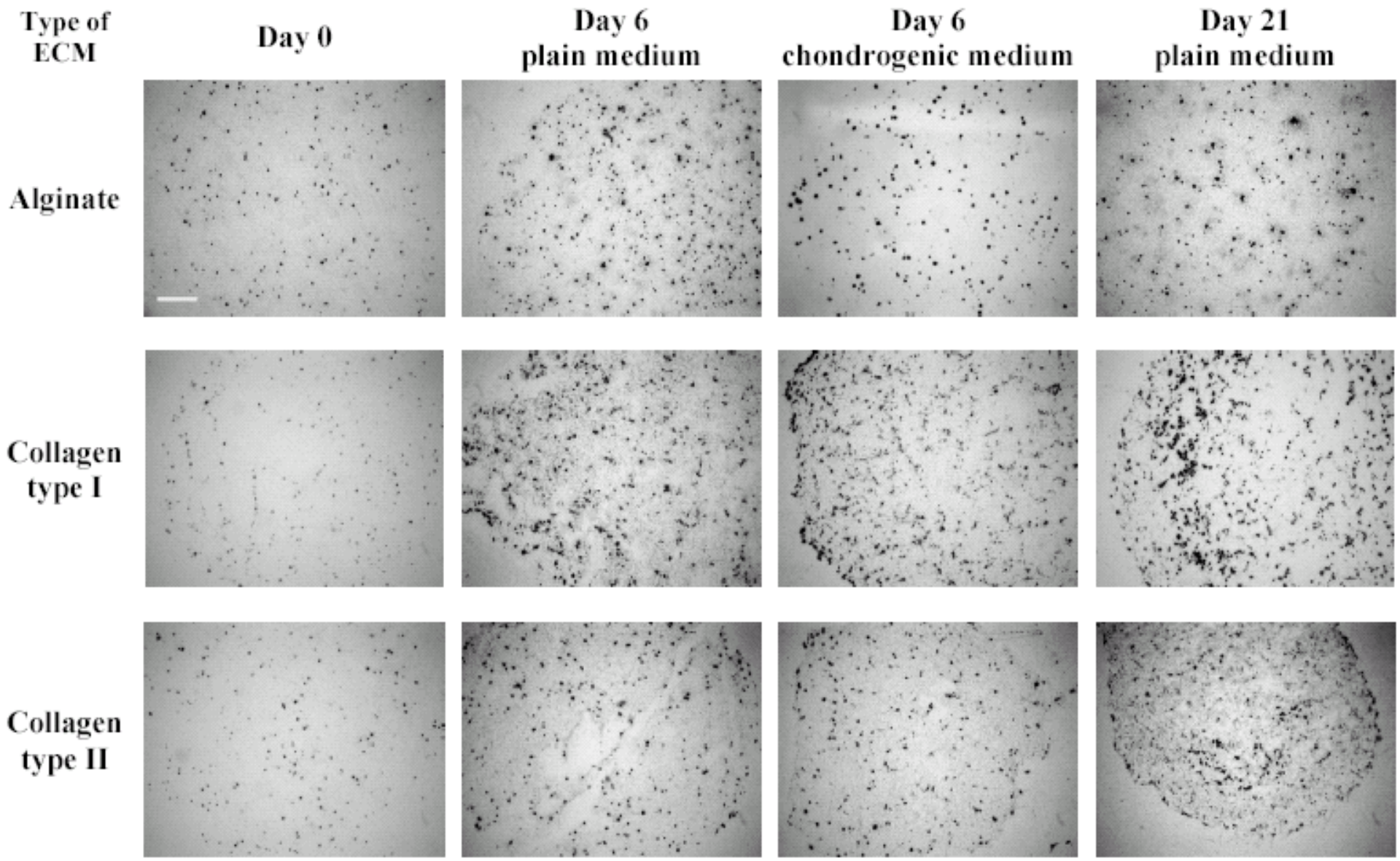

Day 0
plain medium
Day 6
plain medium
Day 6
chondrogenic
medium
Day 21
plain medium
Alginate hydrogel
Al
Im
Collagen type I hydrogel
Al
Im
H&E
Collagen type II hydrogel
Al
Im

In situ hybridization for collagen type II mRNA



Bosnakovski et al. Biotechnology and Bioengineering, 2006 20;93(6):1152-63

In situ hybridization for collagen type I mRNA



Bosnakovski et al. Biotechnology and Bioengineering, 2006 20;93(6):1152-63

Conclusion

- Collagen type II, which acts as a physiological articular cartilage matrix, can **initiate and maintain MSC chondrogenesis** and prior interaction with TGF β1 dramatically to enhance the differentiation

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