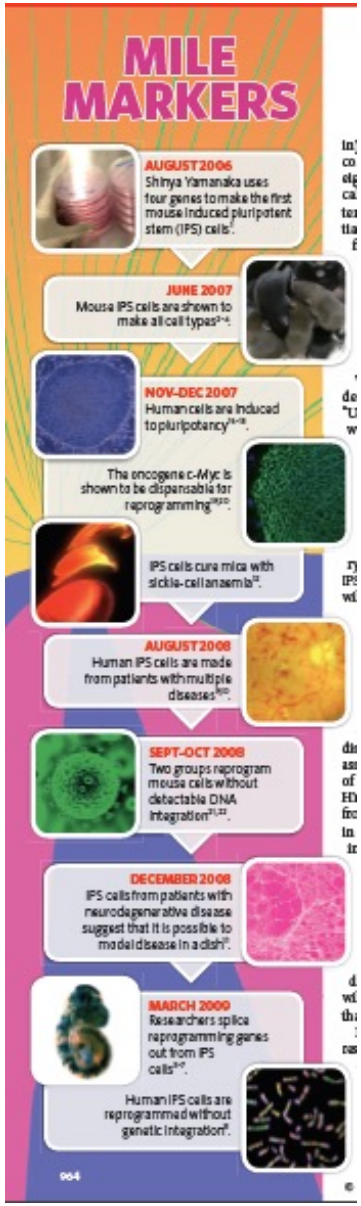
IPS-ES like

Baker Nature 2009

IPS mile markers



Baker Nature 2009

iPS history

Mouse

Generation of pluripotent stem cells from adult mouse liver and stomach cells. Science 2008; 321: 699

Man

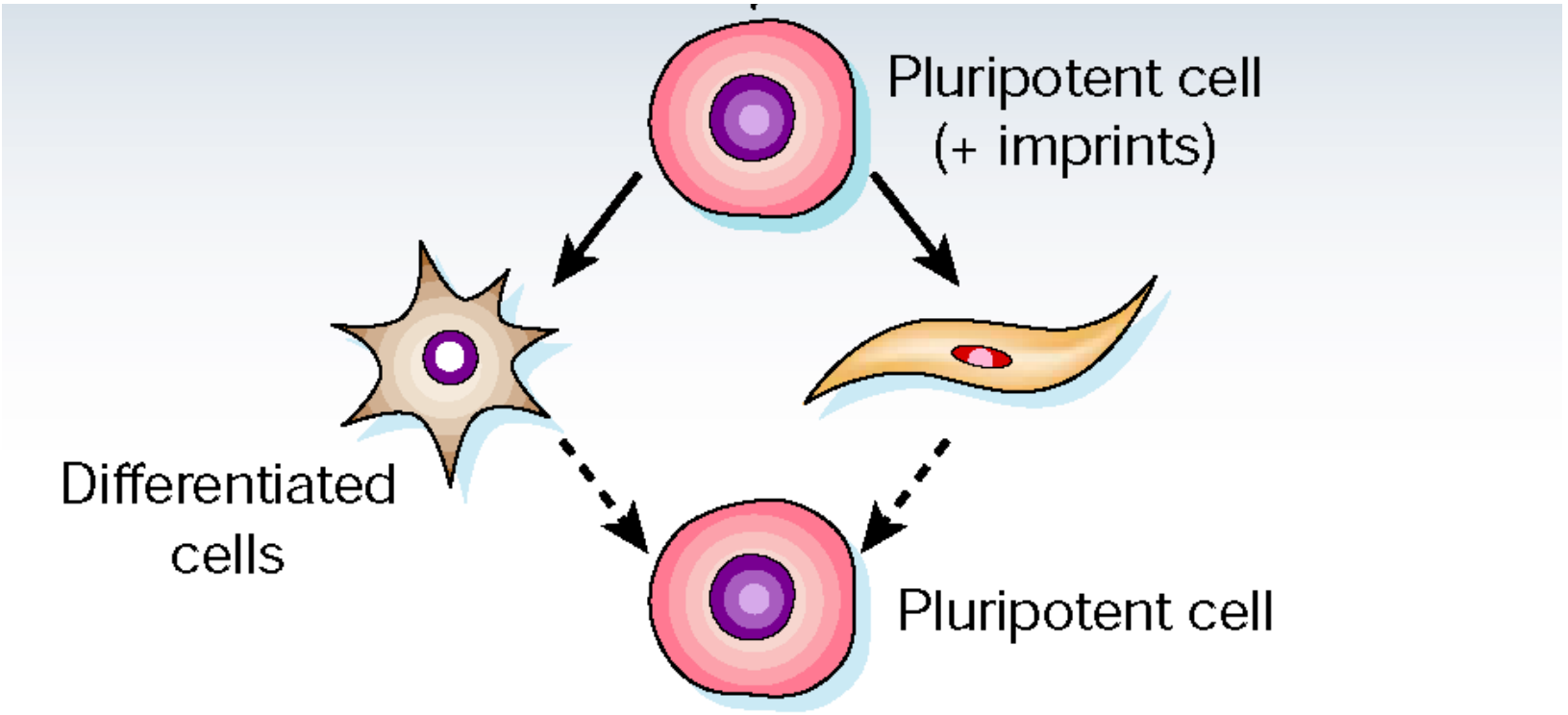
Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007; 131: 861

Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008; 451: 141

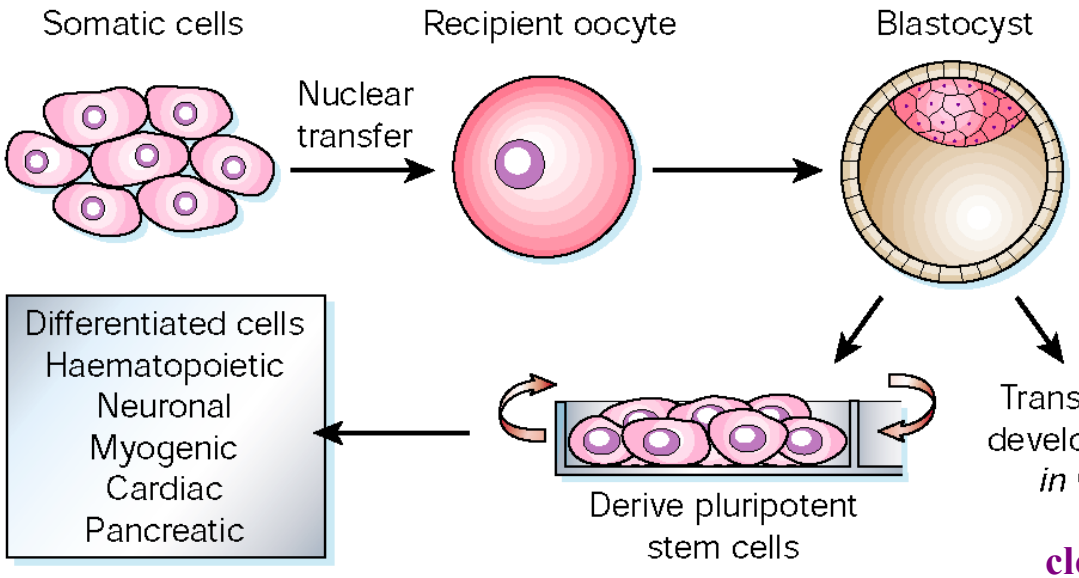
Functional cardiomyocytes derived from human induced pluripotent stem cells. Circ Res 2009; 104: e30

Disease-specific induced pluripotent stem cells. Cell 2008; 134: 877

Epigenetics reversibility



Somatic cell nuclear transfer (SCNT)



Dolly 1997-2003

Somatic cell nuclear transfer (SCNT)

Advantages
- no ethics
- histocompatibility

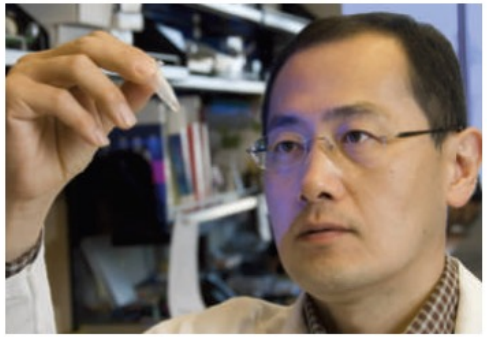
Disadvantages
- egg cells
- cost

IPS

Adult stem cells: multipotent and self-regenerating

Embryonic stem cells: pluripotent not self-regenerating

Embryonic stem like cells
Oct4 : transcription factor
Nanog: transcription factor
Sox2: transcription factor
c-Myc: transcription factor /proto-oncogene
Klf-4: transcription factor



Shinya Yamanaka made mouse iPS cells in 2006.

IPS

donor

Germ cells

transplant

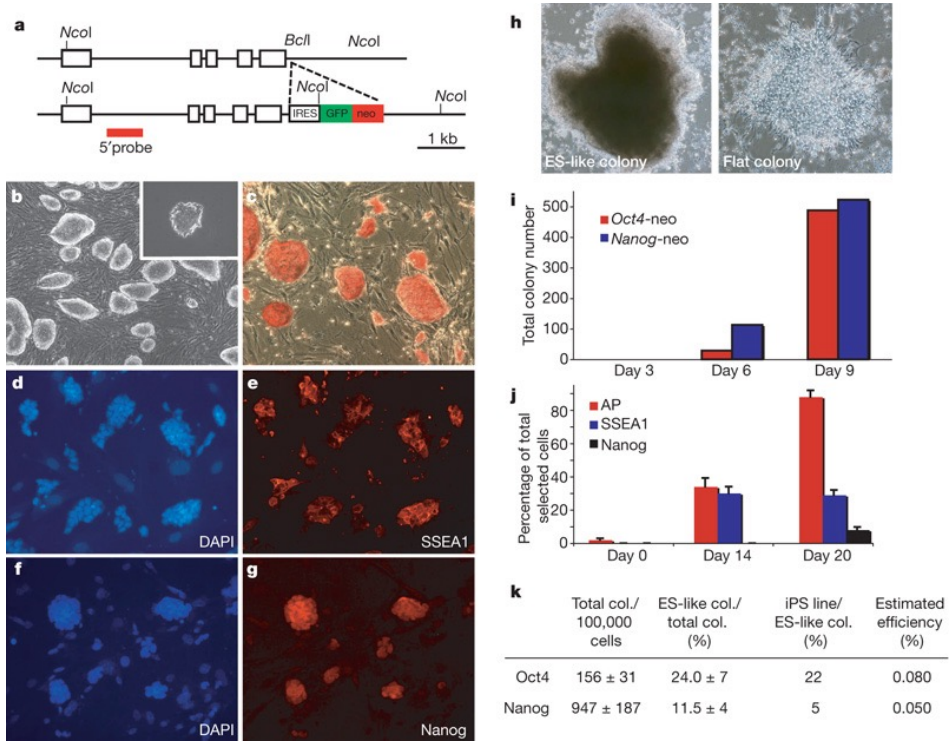
….

Alessandro Rosa, Erasmus Seminar

Selection of **mouse** fibroblasts for Oct4 or Nanog activation

Homologous recombination in MEF to obtain Oct4-neo or Nanog-neo. Neo selection kills the cells because in differentiated cells these genes are silenced.
Then addition of retro-Oct4, Sox2, c-Myc, or Klf4

Colony derived cell line -iPS



k

	Total col./ 100,000 cells	ES-like col./ total col. (%)	iPS line/ ES-like col. (%)	Estimated efficiency (%)
Oct4	156 ± 31	24.0 ± 7	22	0.080
Nanog	947 ± 187	11.5 ± 4	5	0.050

Colony G418 res

Time is needed for colony formation

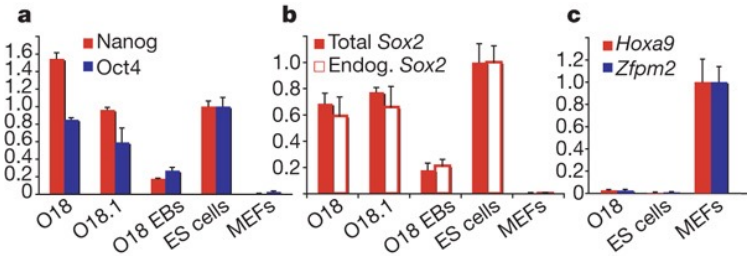
Different ES markers at different times

Oct4 less colonies more iPS
Nanog easier to activate oct4 more important for the pluripotent state

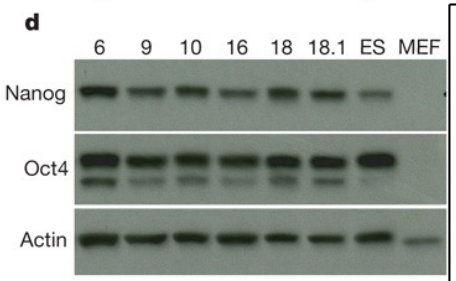
Wernig et al Nature 2007

Expression and DNA methylation

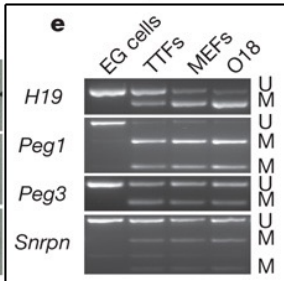
Measurement of markers of ES or MEF or embryoid bodies byrtQPCR



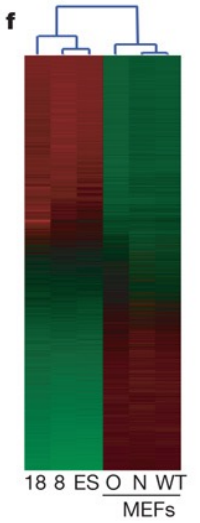
Measurement of markers of ES or MEF by western on Ips and controls

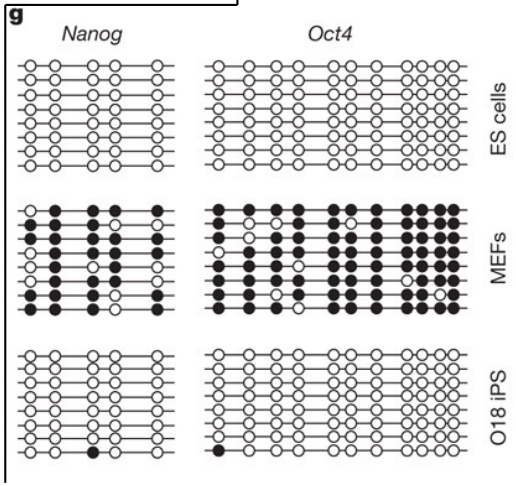


Measurement of promoter methylation (in germ cells mprinting is erased)



Measurement of markers of ES or MEF by gene chip on Ips and controls





Measurement of promoter methylation

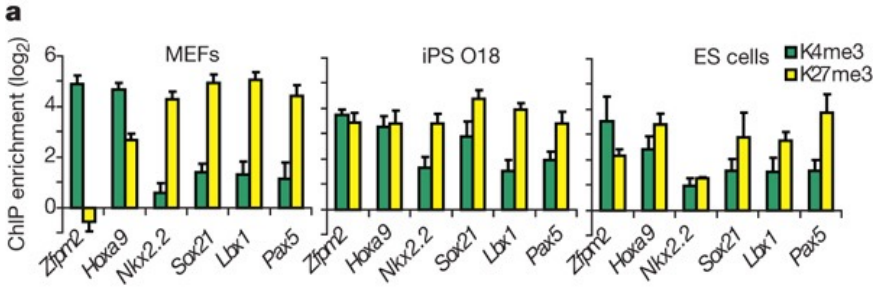
COBRA

Wernig et al Nature 2007

Chromatin modifications

Histone H3 lysine4 and 27 are active or repressive marks. Down stream targets of oct4, nanog, sox2

ChIP and Q PCR to measure H3 methylation state in association with specific genes

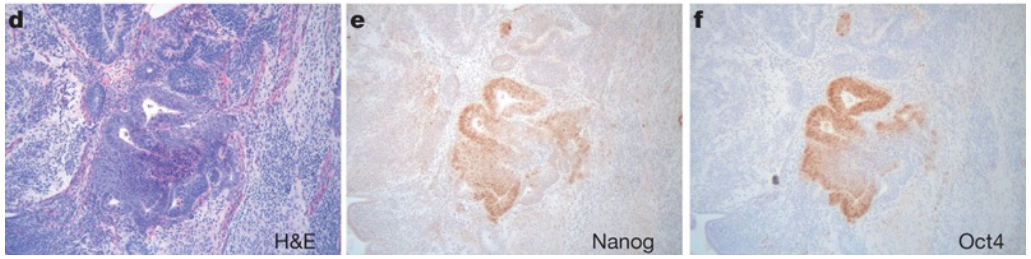


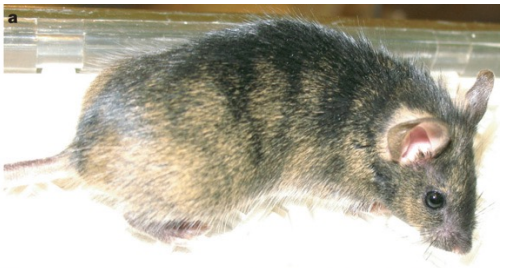
ES = Ips # MEF

Wernig et al Nature 2007

Developmental potential

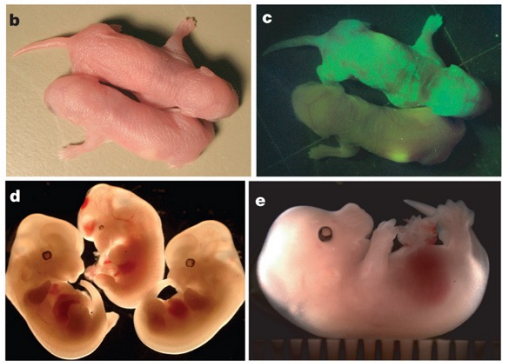
Teratoma from Ips-three germ layers



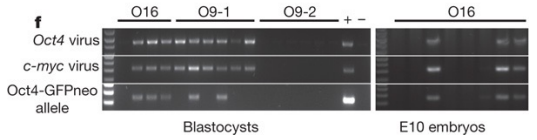


Ips injected in 2N or 4N blastocysts

for chimeras



F0 and F1

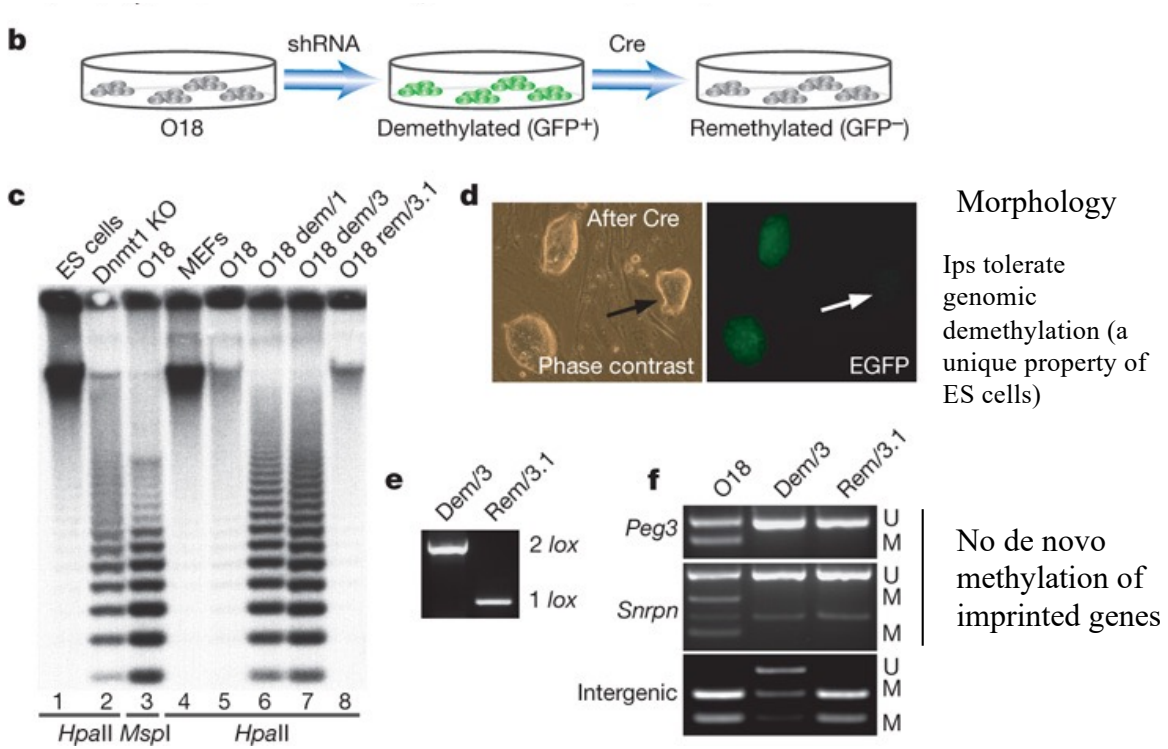


Wernig et al Nature 2007

Ips tolerate genomic demethylation

LV-siDnmt1/GFP/loxP

Southern with methylation sensitive enzyme (HpaII) And methylation insensitive (MspI)



Morphology

Ips tolerate genomic demethylation (a unique property of ES cells)

No de novo methylation of imprinted genes

Wernig et al Nature 2007

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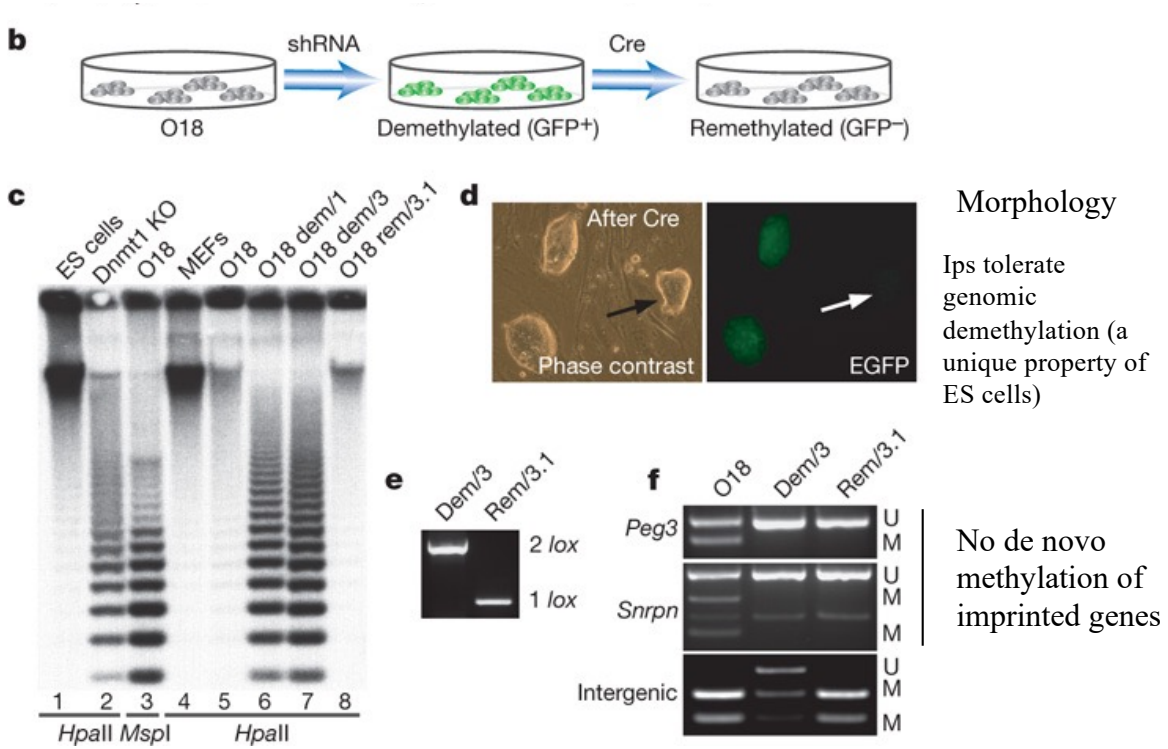

Morphology

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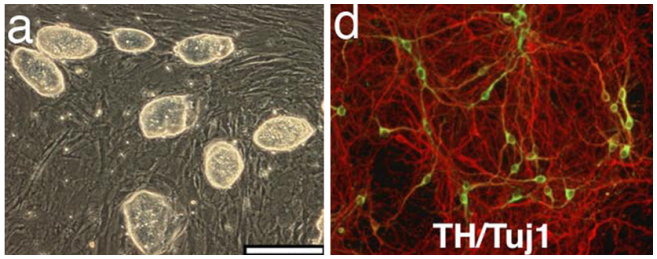
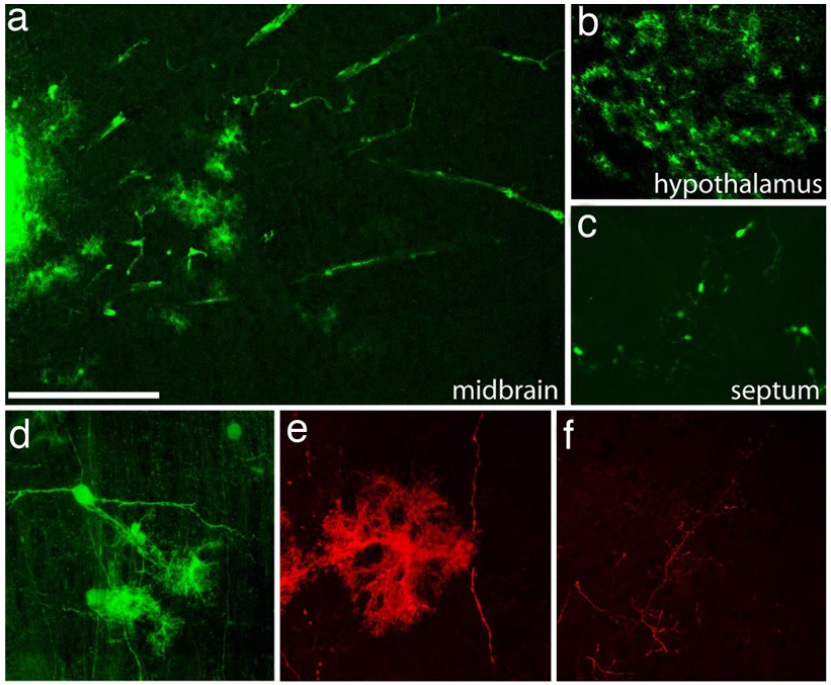
Wernig et al Nature 2007

into neurons

in vitro



in vivo



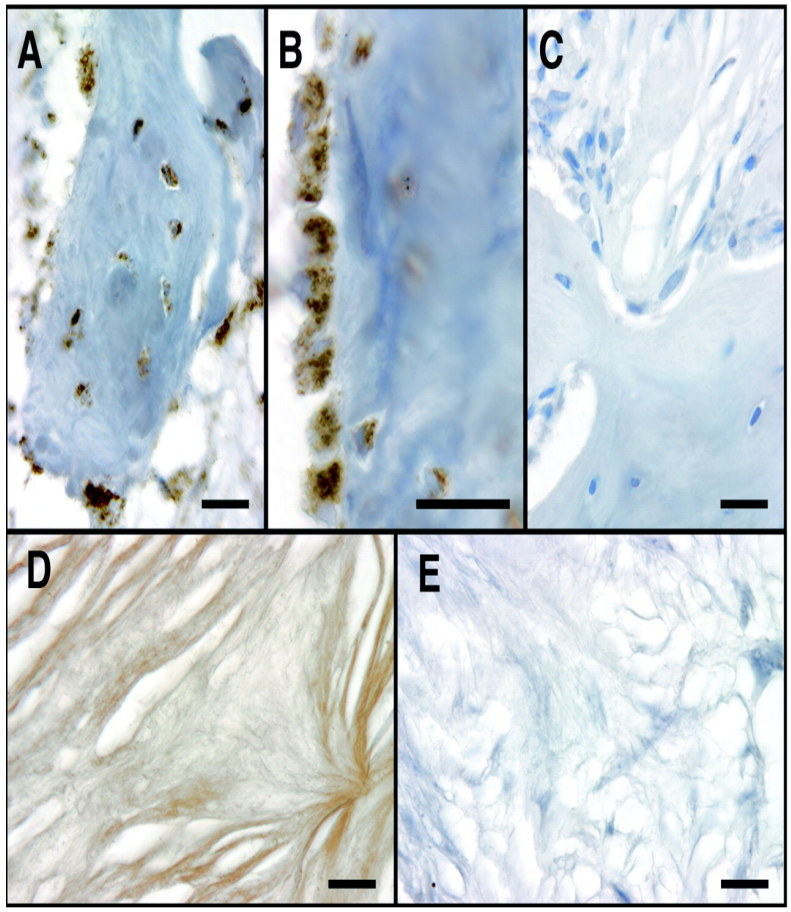
Wernig et al, 2008

QUESTIONS?

In vivo bone formation by targeted MSC (clones and polyclonal).
Demonstration of human bone

A, Band C AAV MSC

C and murine MSC



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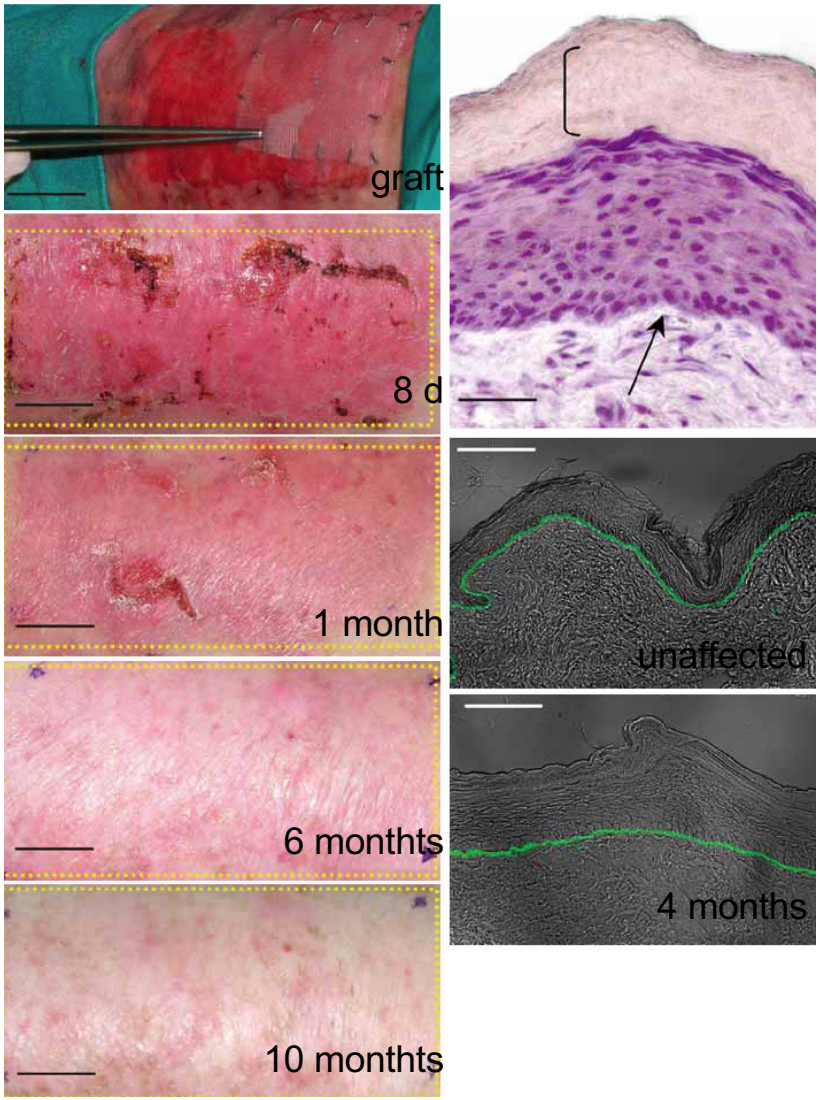
Hum collage n

Post natal epithelial cells



"Correction of junctional epidermolysis bullosa by transplantation of genetically modified epidermal stem cells"

Mavilio et al, 2006