

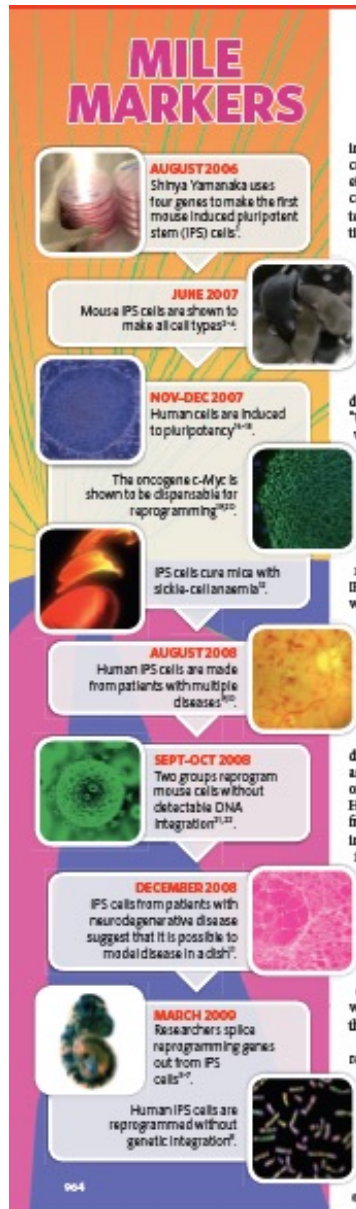
IPS-ES like



FAST AND FURIOUS

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

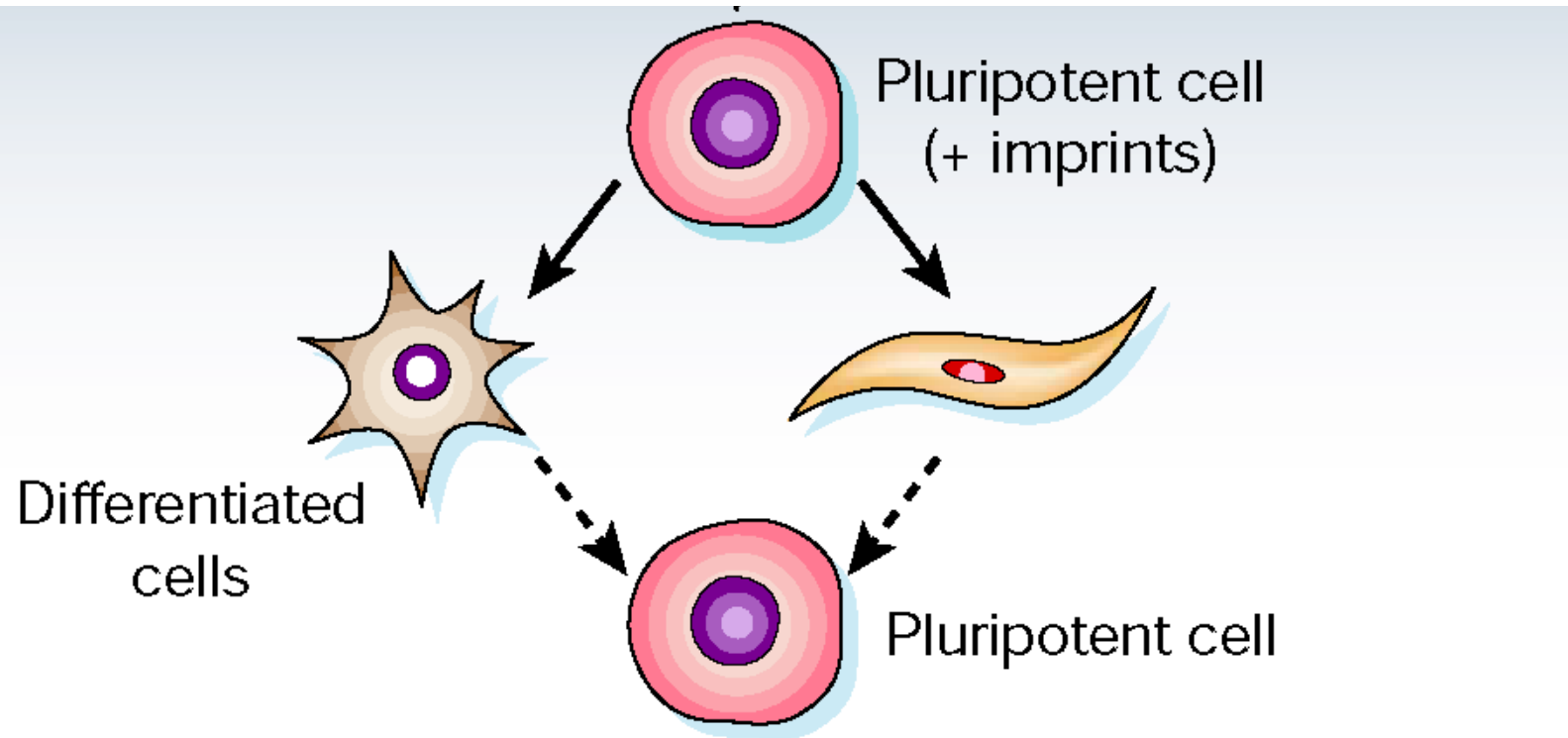
Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861

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

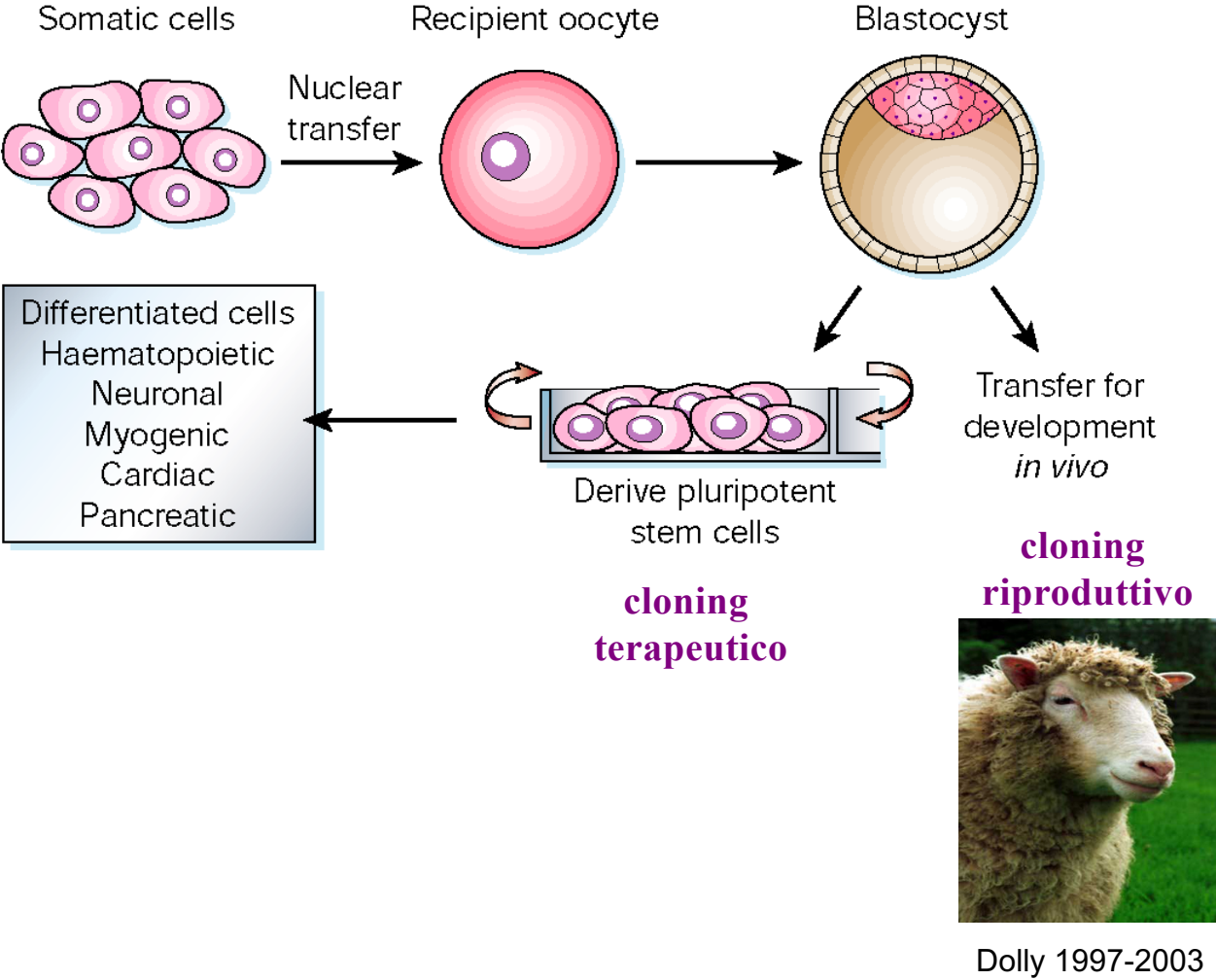
Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res* 2009; 104: e30

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

Epigenetics reversibility



Somatic cell nuclear transfer (SCNT)



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

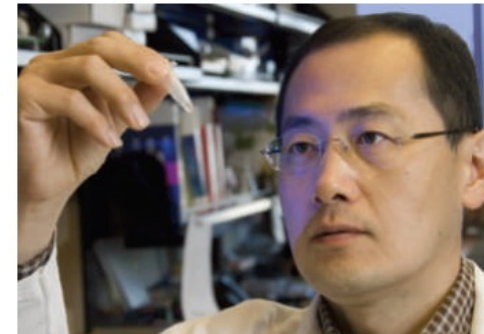
Oct4 : transcription factor

Nanog: transcription factor

Sox2: transcription factor

c-Myc: transcription factor /proto-oncogene

Klf-4: transcription factor



Shinya Yamanaka made mouse iPS cells in 2006.

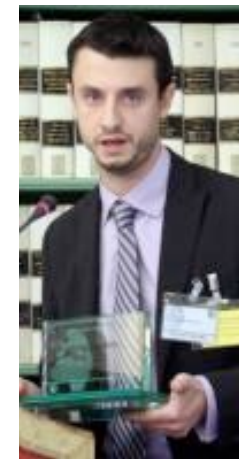
IPS

donor

Germ cells

transplant

....

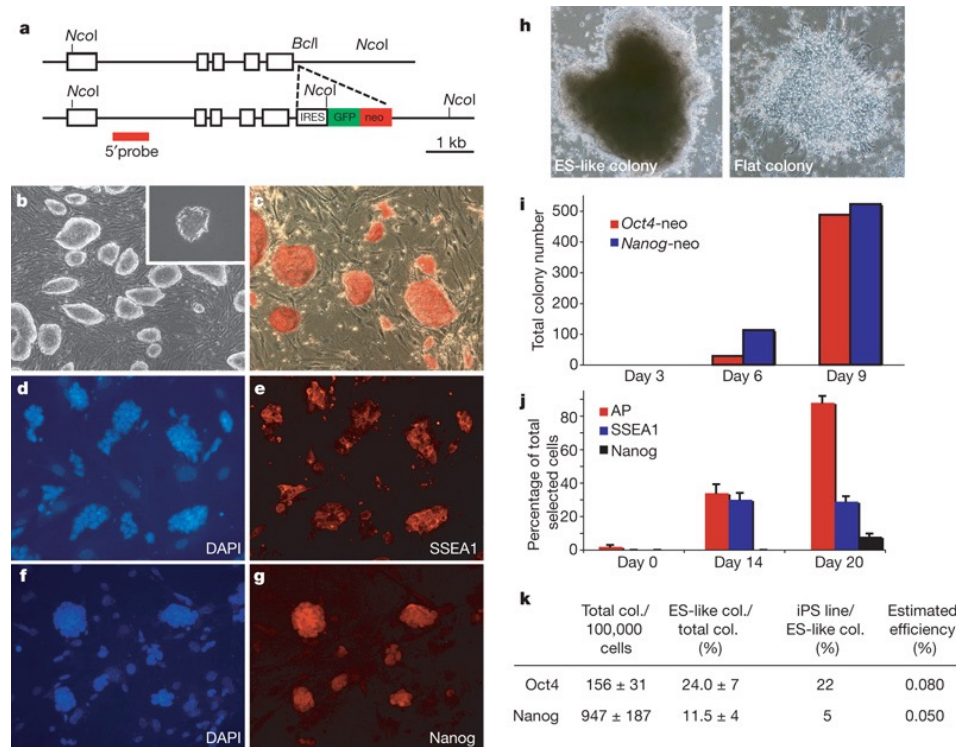


Alessandro Rosa, Erasmus Seminar

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



Colony G418 res

Time is needed for colony formation

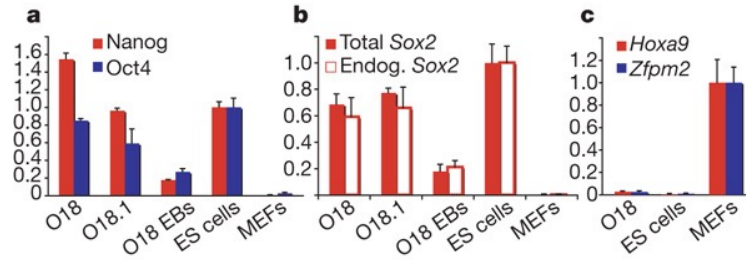
Different ES markers at different times

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

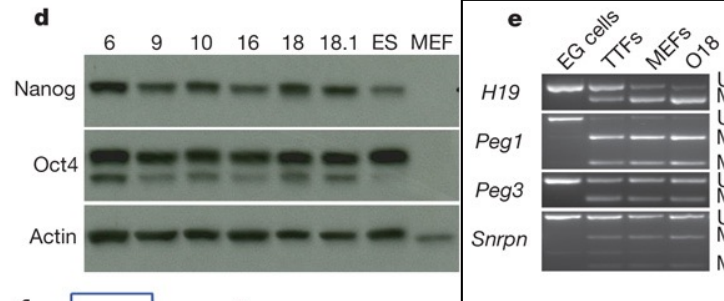
Wernig et al Nature 2007

Expression and DNA methylation

Measurement of markers of ES or MEF or embryoid bodies byrtQPCR

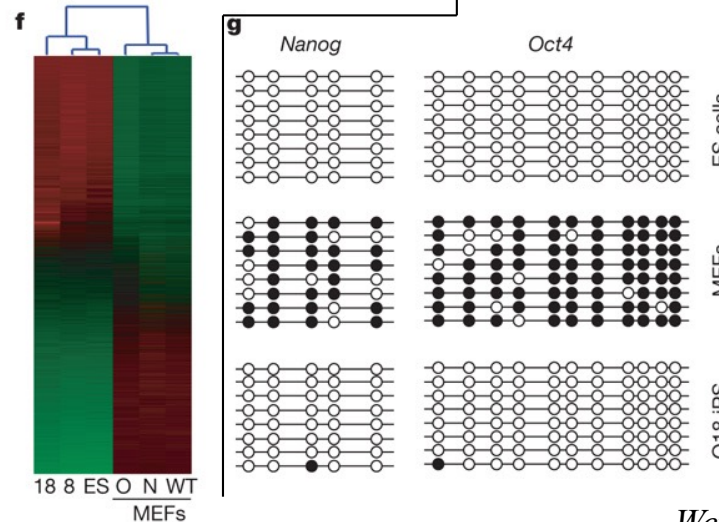


Measurement of markers of ES or MEF by western on Ips and controls



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

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



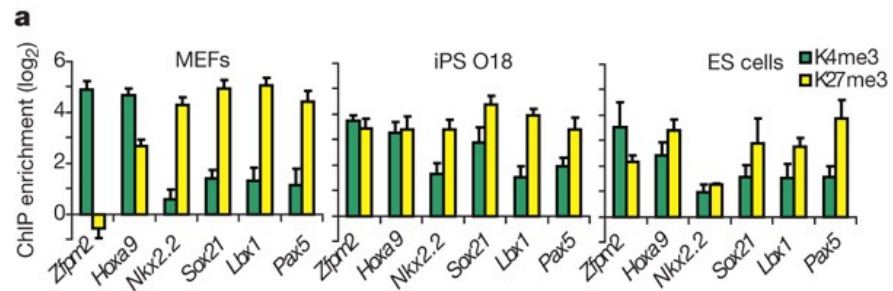
Measurement of promoter methylation

COBRA

Chromatin modifications

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

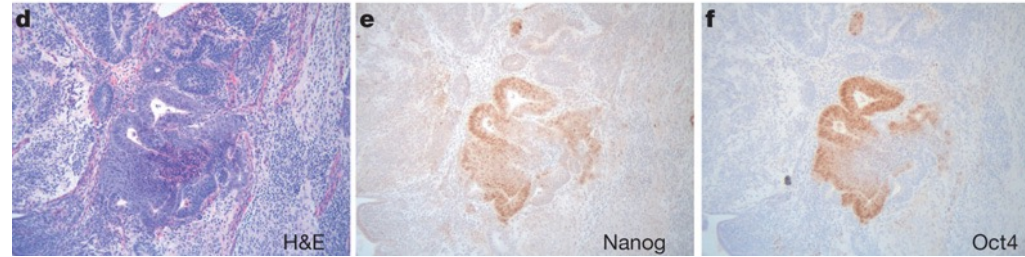
ChIP and Q PCR to measure H3 methylation state in association with specific genes



ES = Ips # MEF

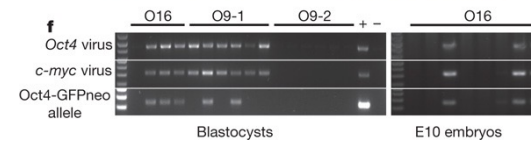
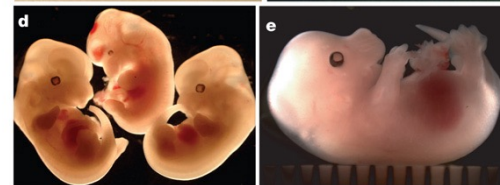
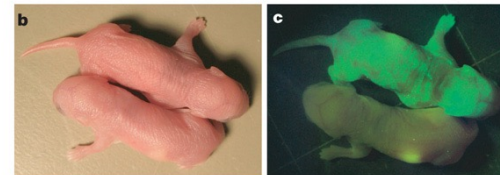
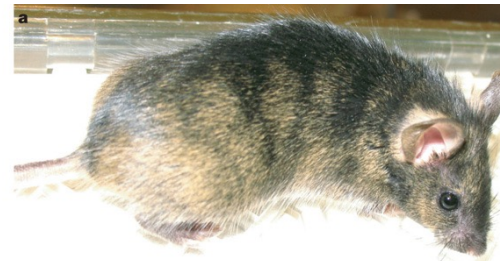
Developmental potential

Teratoma from
Ips-three germ
layers



Ips injected in 2N or 4N blastocysts
for chimeras

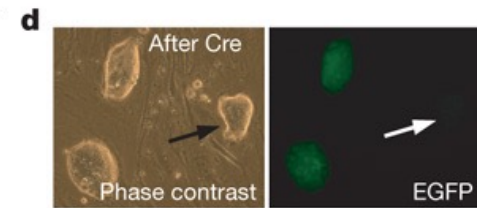
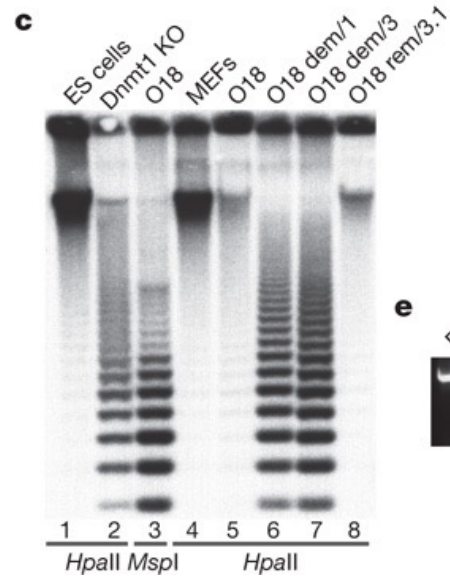
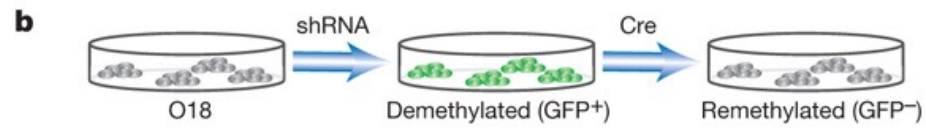
F0 and F1



Wernig et al Nature 2007

Ips tolerate genomic demethylation

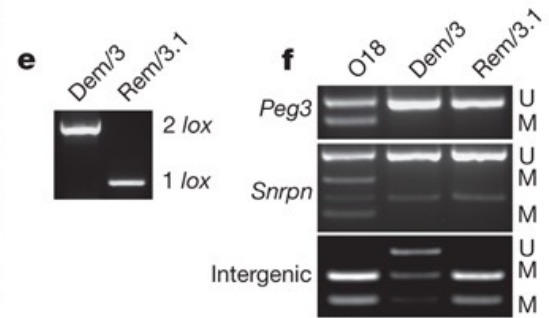
LV-siDnmt1/GFP/loxP



Morphology

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

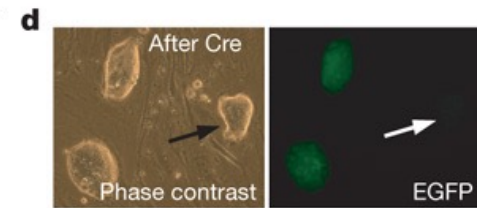
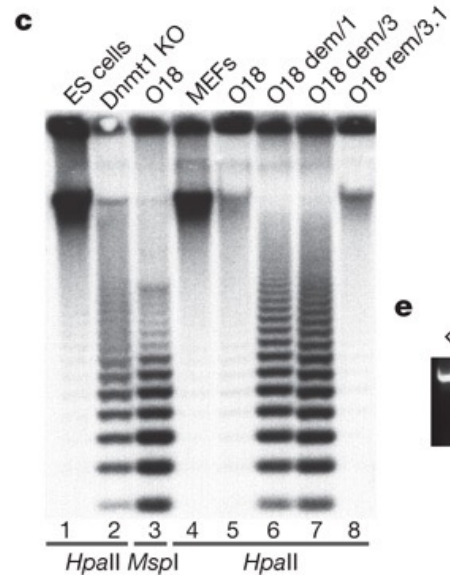
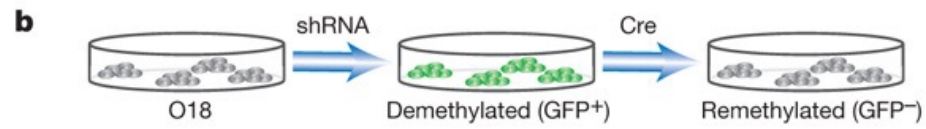
Southern with methylation sensitive enzyme (HpaII)
And methylation insensitive (MspI)



No de novo methylation of imprinted genes

Ips tolerate genomic demethylation

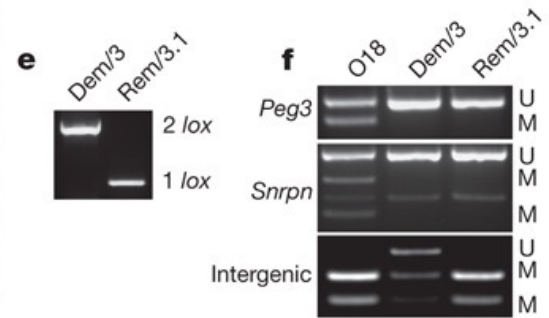
LV-siDnmt1/GFP/loxP



Morphology

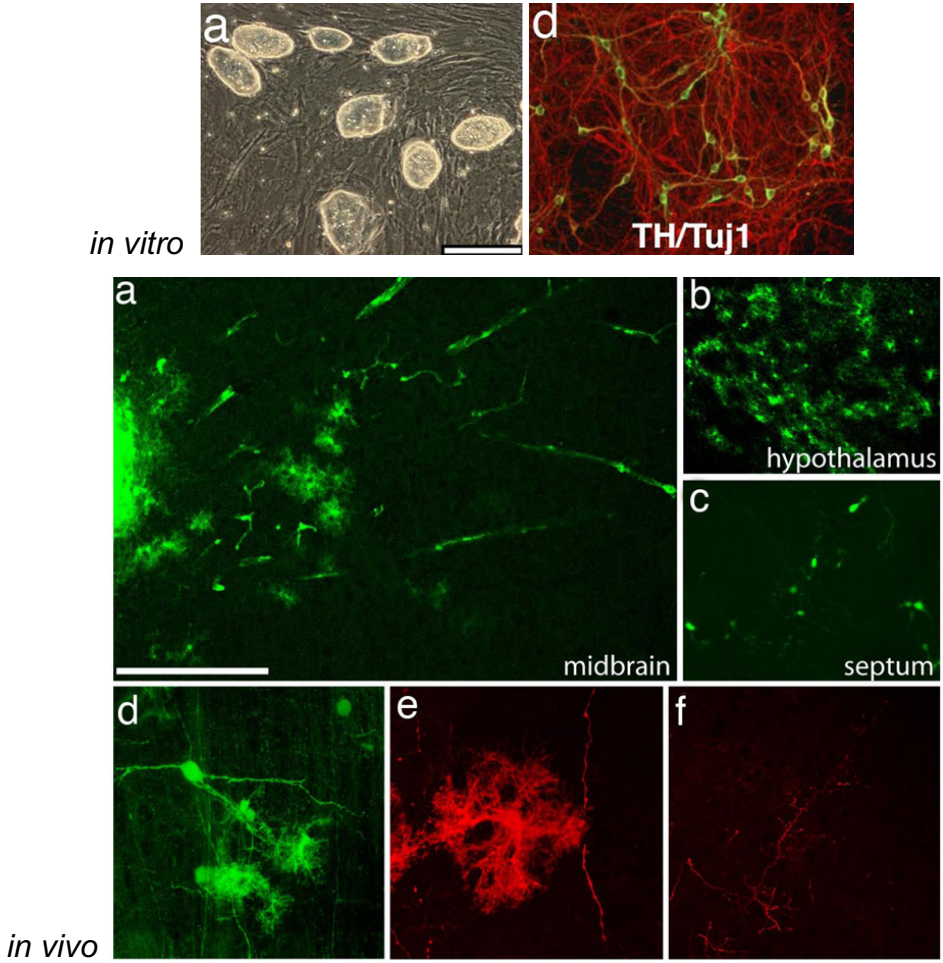
Ips tolerate genomic demethylation (a unique property of ES cells)

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



No de novo methylation of imprinted genes

into neurons



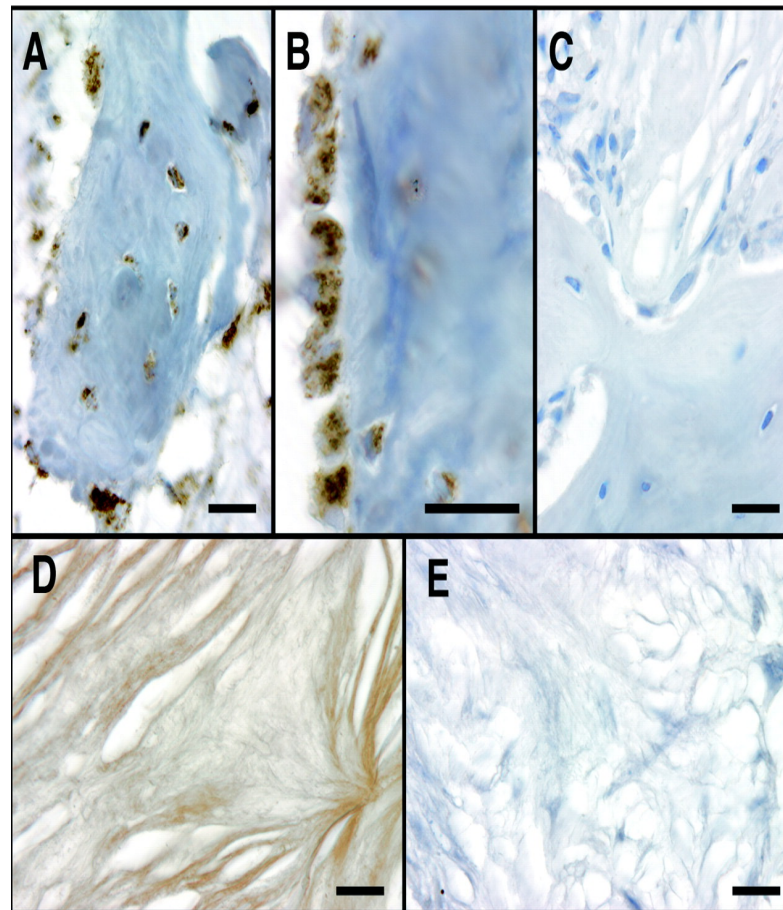
Wernig et al, 2008

QUESTIONS?

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

A, Band C AAV MSC

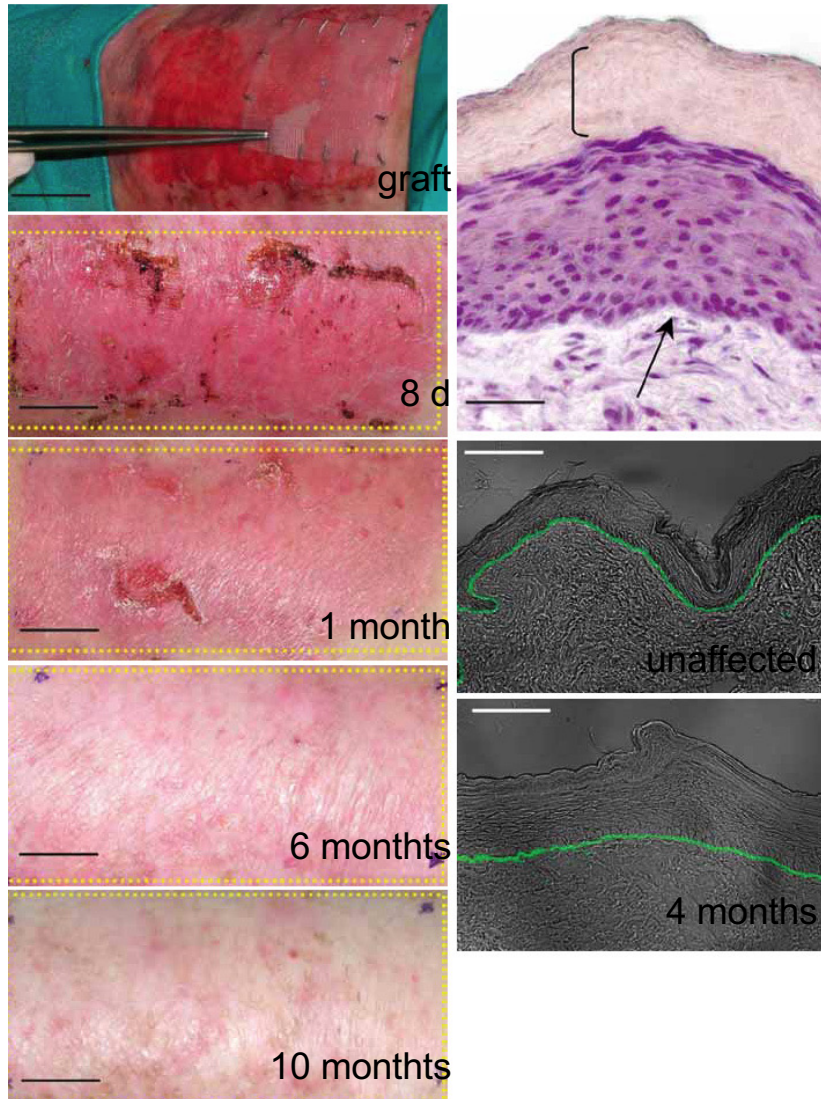
C and murine MSC



Hum
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Hum
collage
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Post natal epithelial cells



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

Mavilio et al, 2006