

ARTICLE

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OPEN

# A stably self-renewing adult blood-derived induced neural stem cell exhibiting patternability and epigenetic rejuvenation

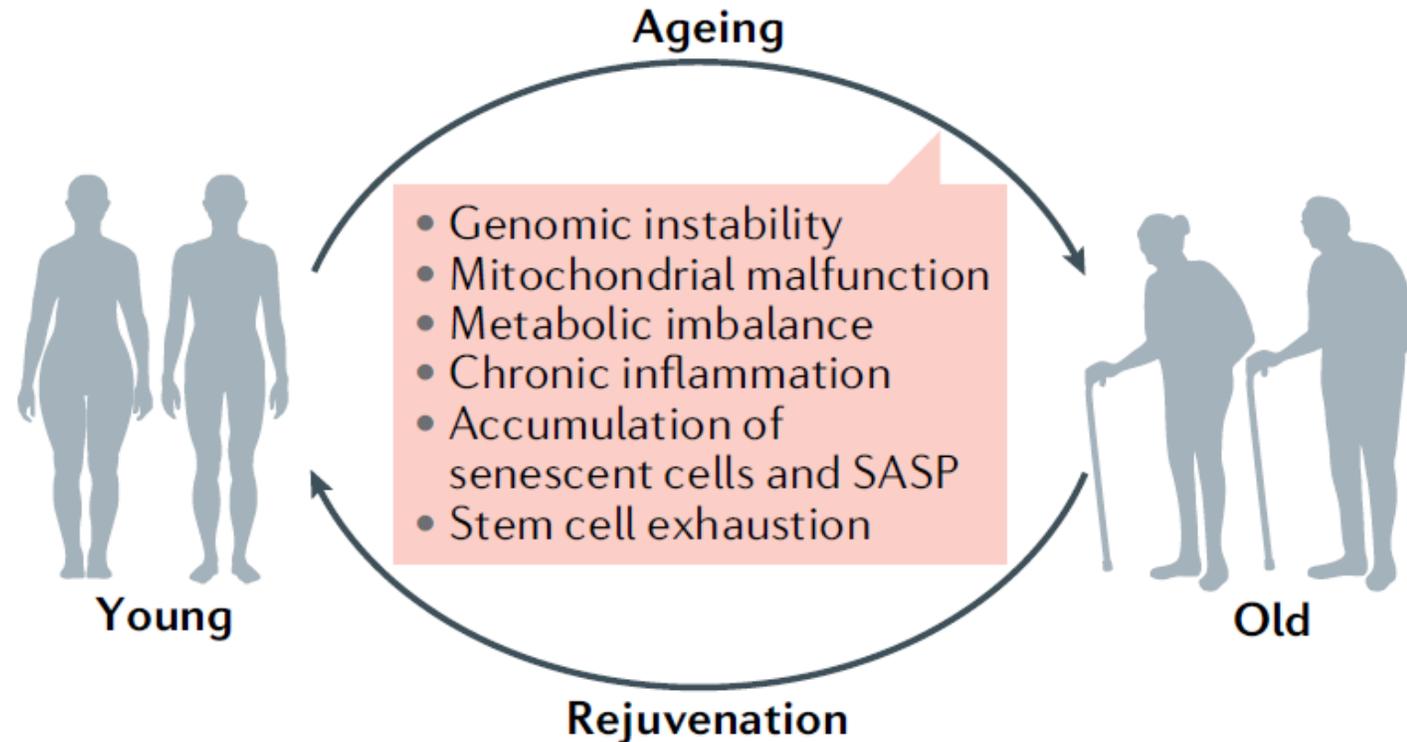
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Presented by Michael Springer

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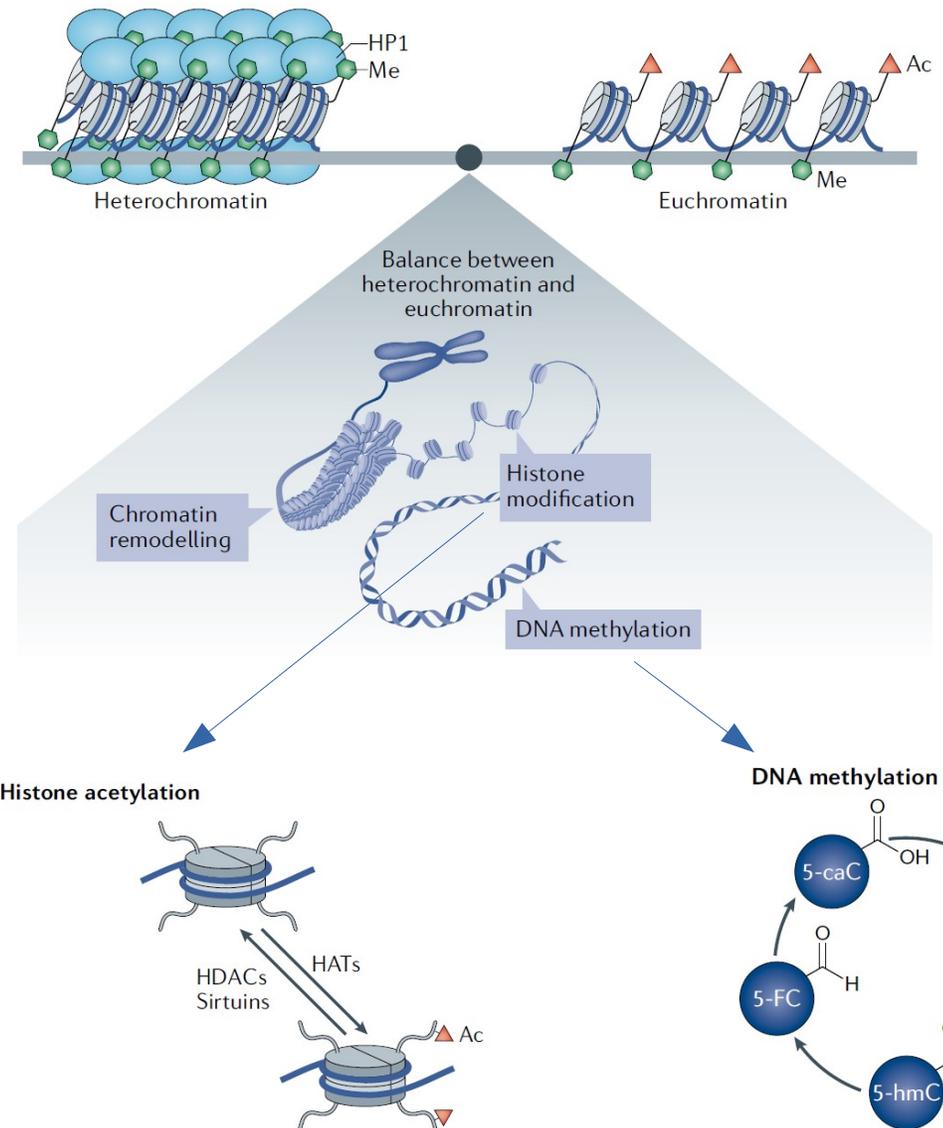
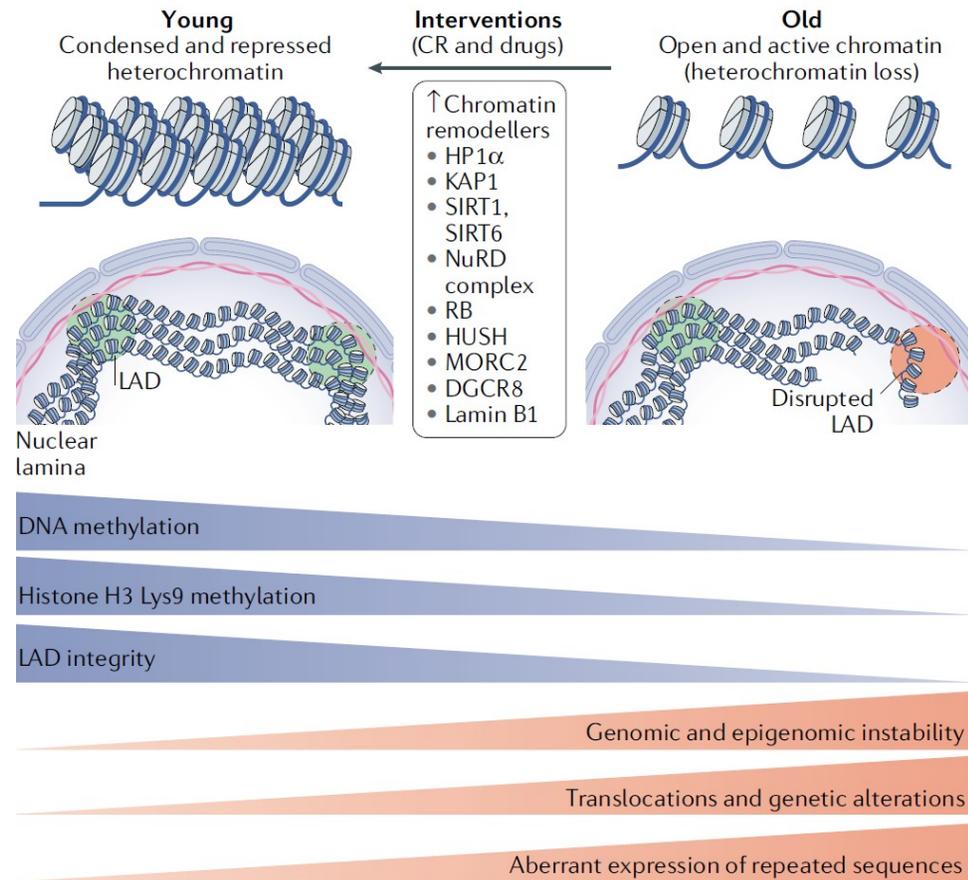
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# The many ways of ageing

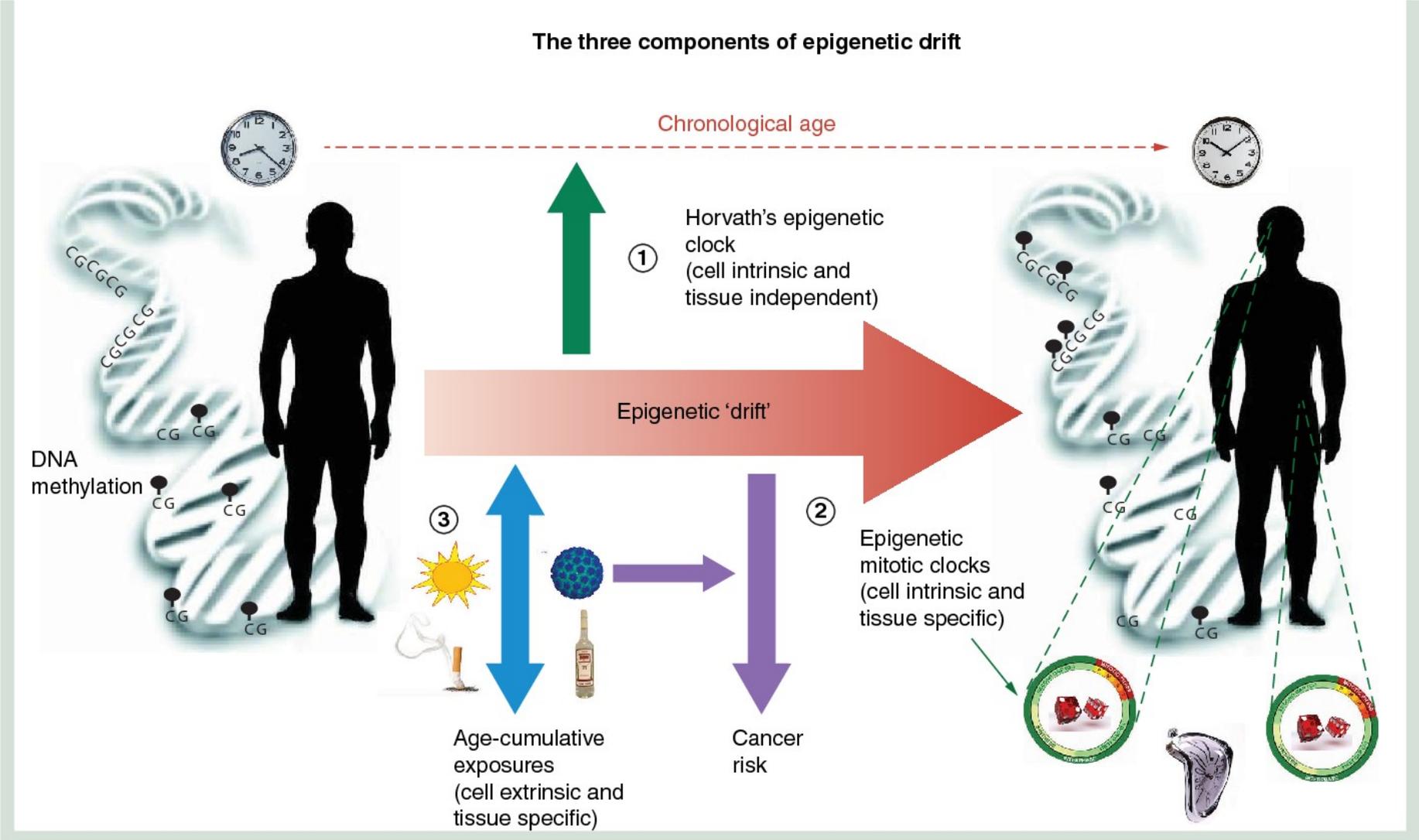


In many aspects even the „WHY“ of ageing is not well understood

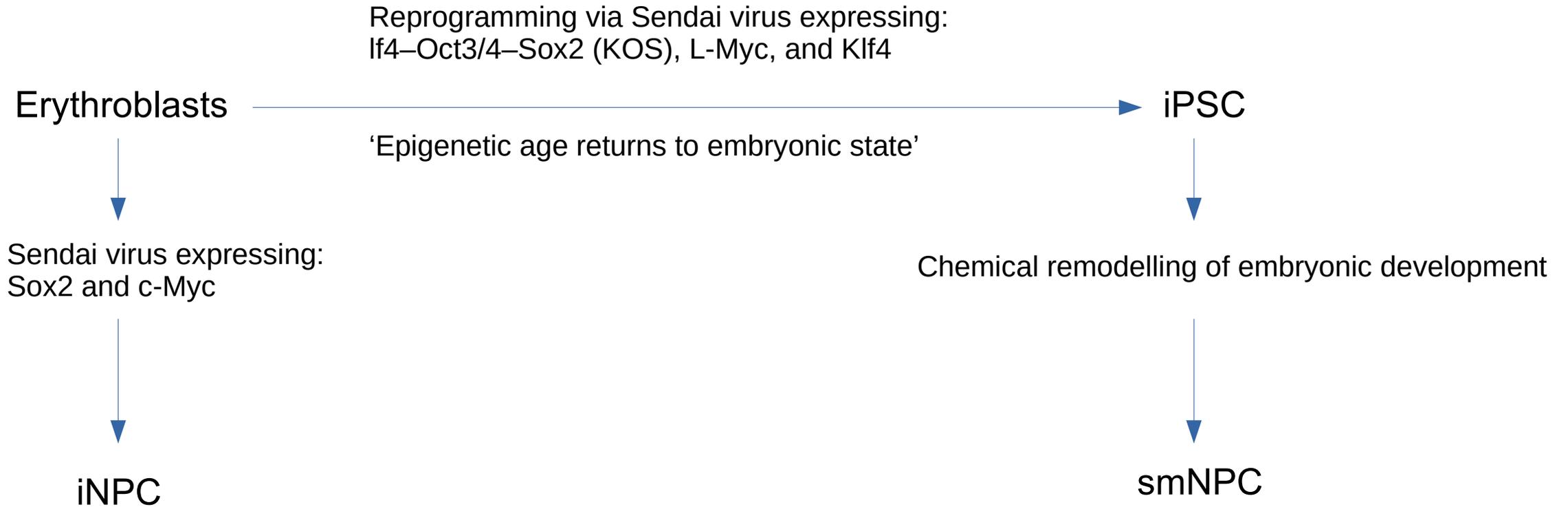
# Epigenetic regulations



# Horvath's clock



# Generation of stem cell types



# Viral vectors

## Classic versions

### Retrovirus

Oct4  
Sox2  
Klf4  
cMyc

### Lentivirus

Oct4  
Sox2  
Nanog  
Lin28

### Sendai virus

Oct3/4  
Sox2  
Klf4  
lf4  
L-Myc

Problem: Integrate into DNA

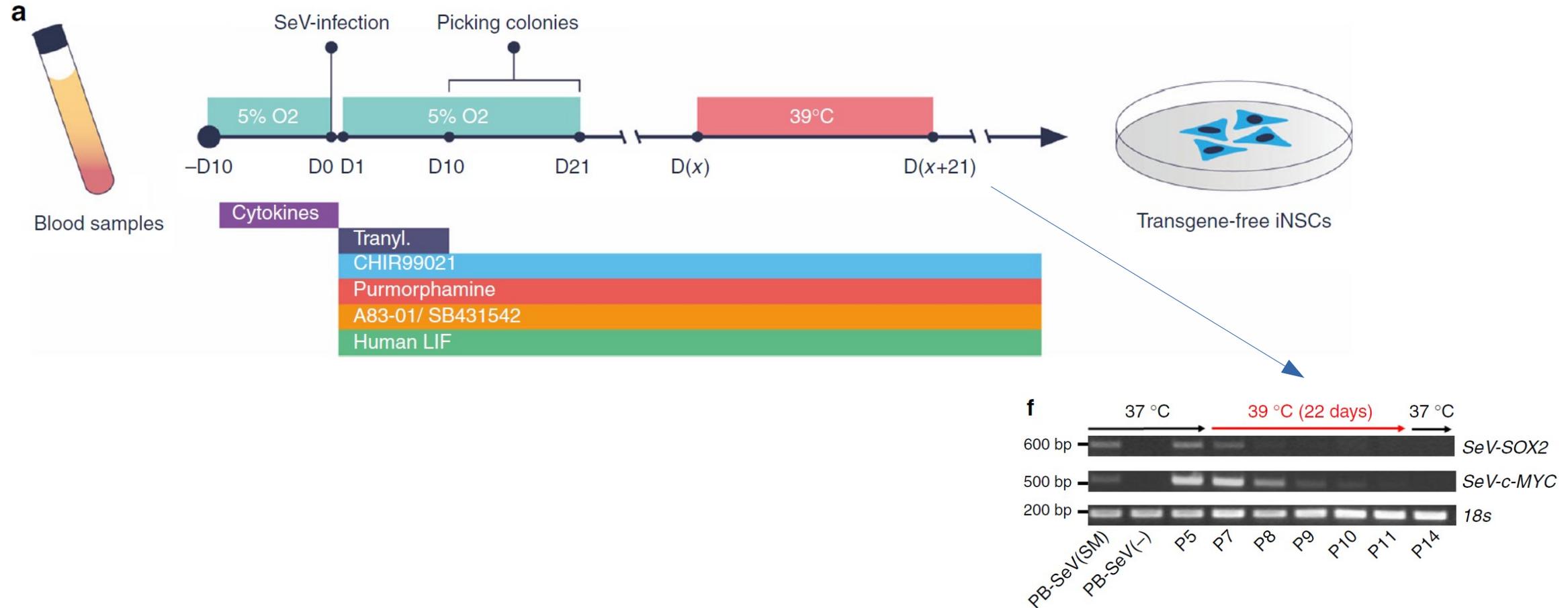
Does not integrate into DNA

New version degenerates at high temperatures

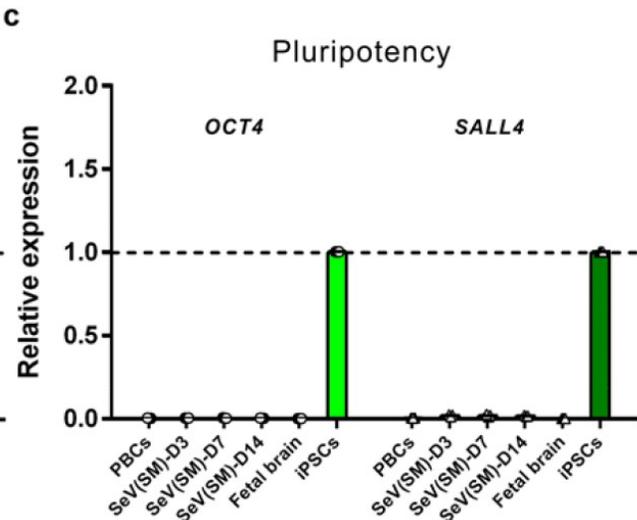
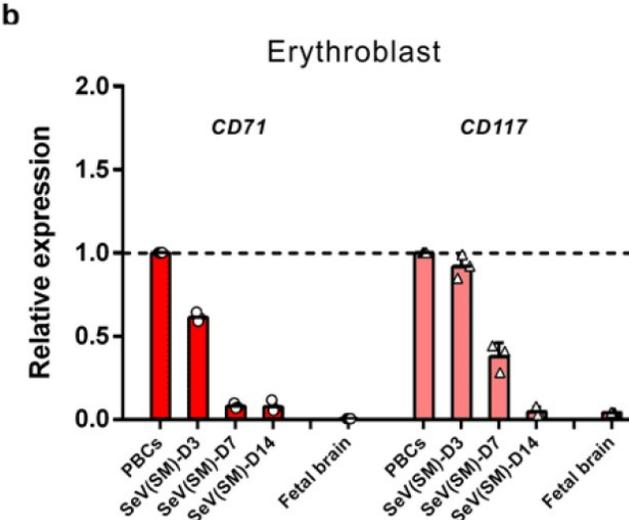
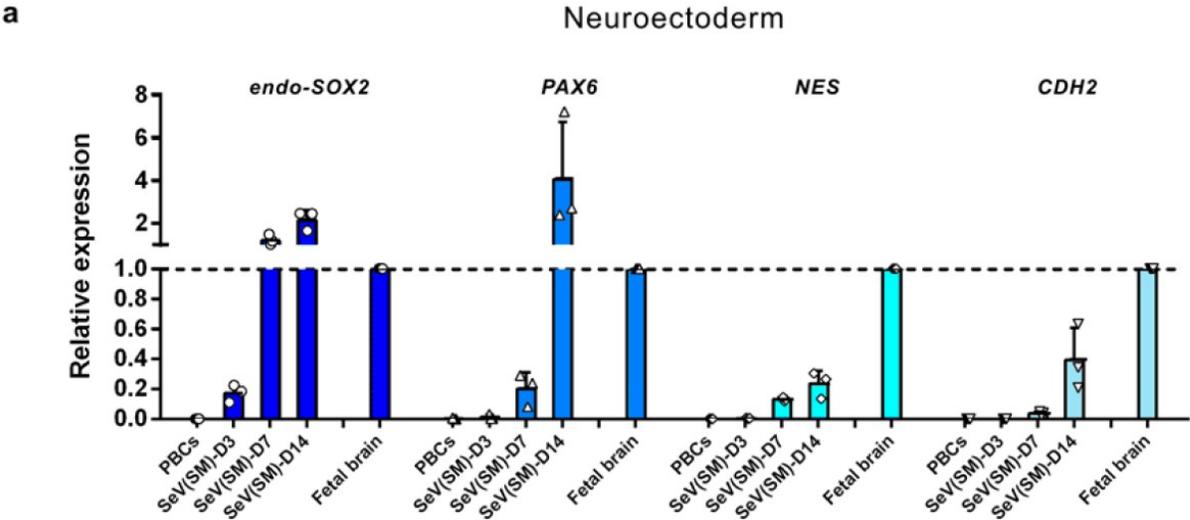
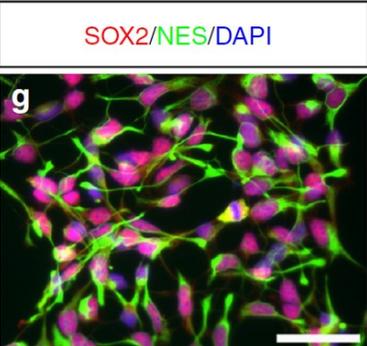
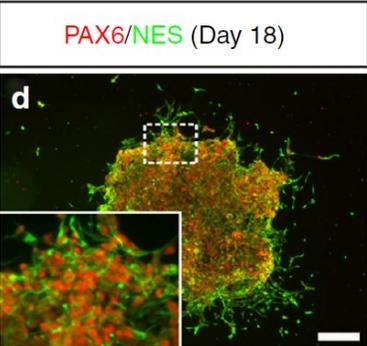
# Neural stem cell wish list

- Fast and easy
  - Direct route without stopping at iPSC
- No chromosomal/genomic damage
  - Avoid OCT4
- No remains of genetical modification
  - Use of heat sensitive Sendai virus
- Easily accessible precursors
  - Blood derived erythroblasts
- In many cases no age related behaviour
  - Indicated by DNAm or transcriptional signatures
- Responsive an tripotent
  - Verifiable by differentiation

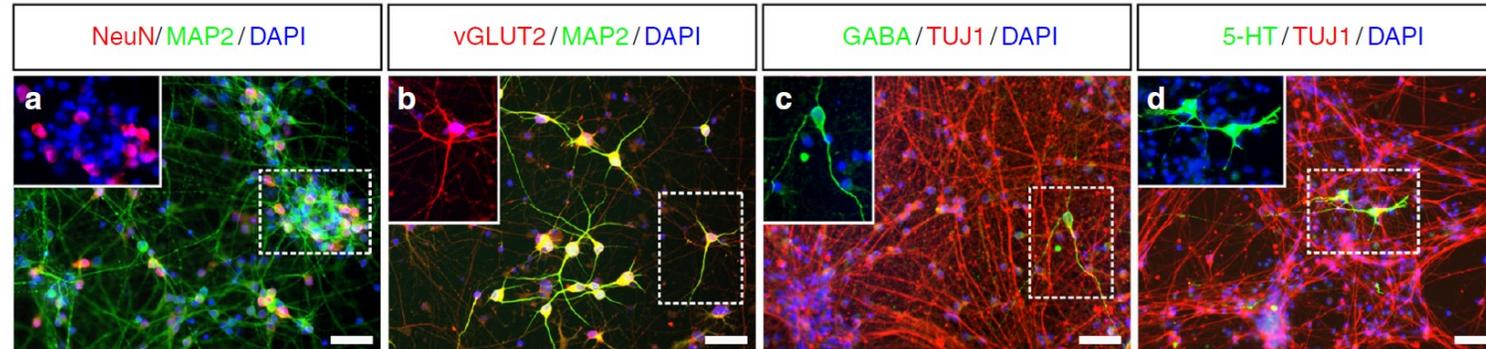
# Applied protocol



# Direct conversion of adult human PBCs into iNSCs

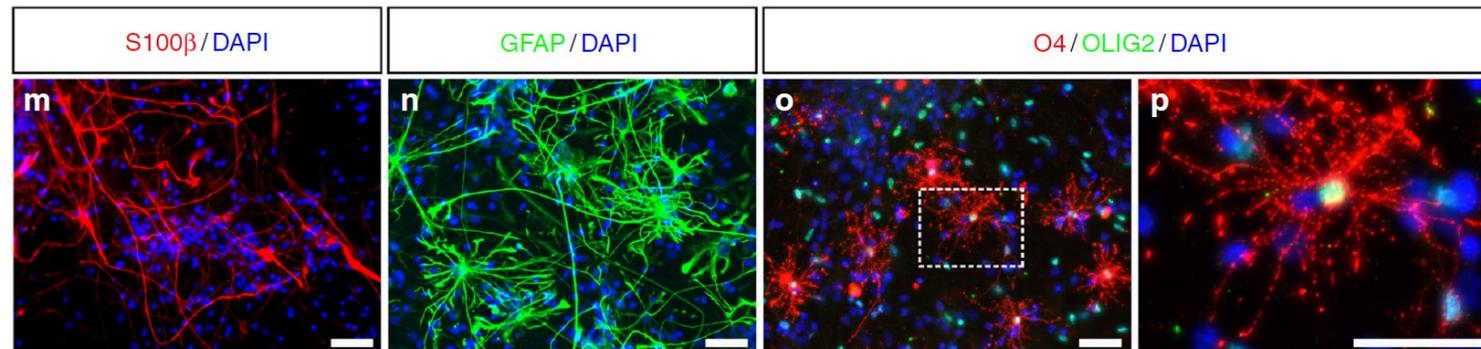


# Spontaneous differentiation of iNSCs



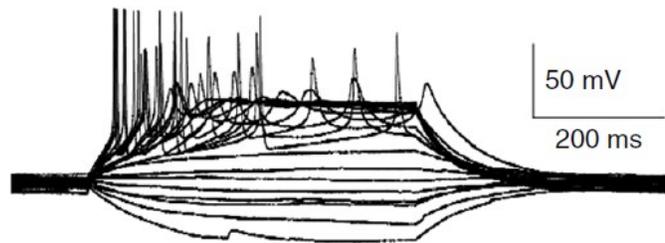
Astrocytes

Oligodendrocytes

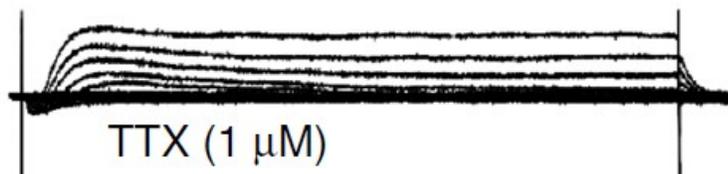


# Test of functionality via electrophysiology

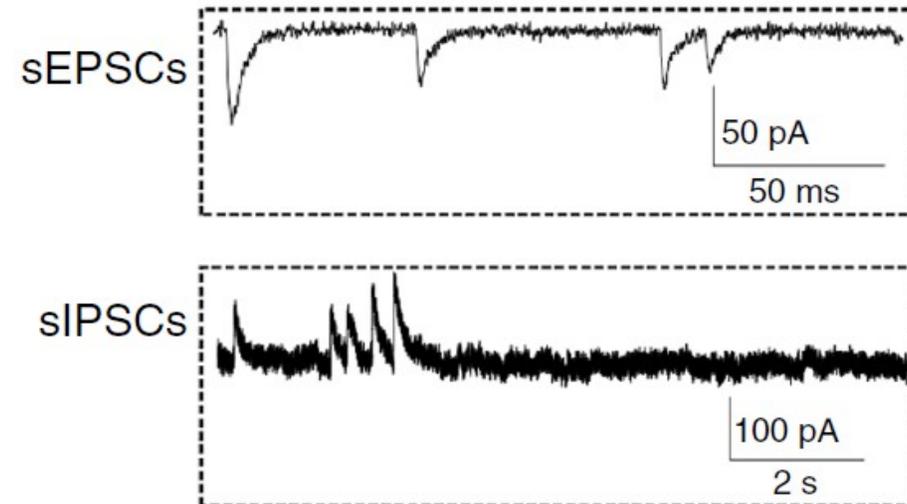
## Depolarization



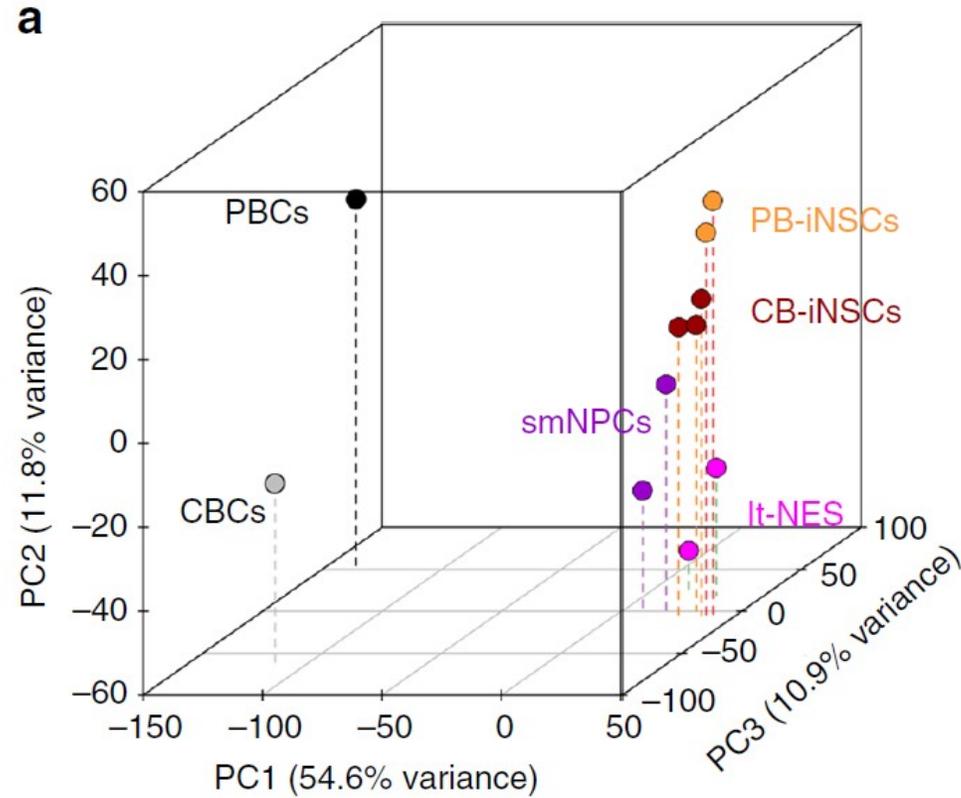
## Effect of Tetrodotoxin



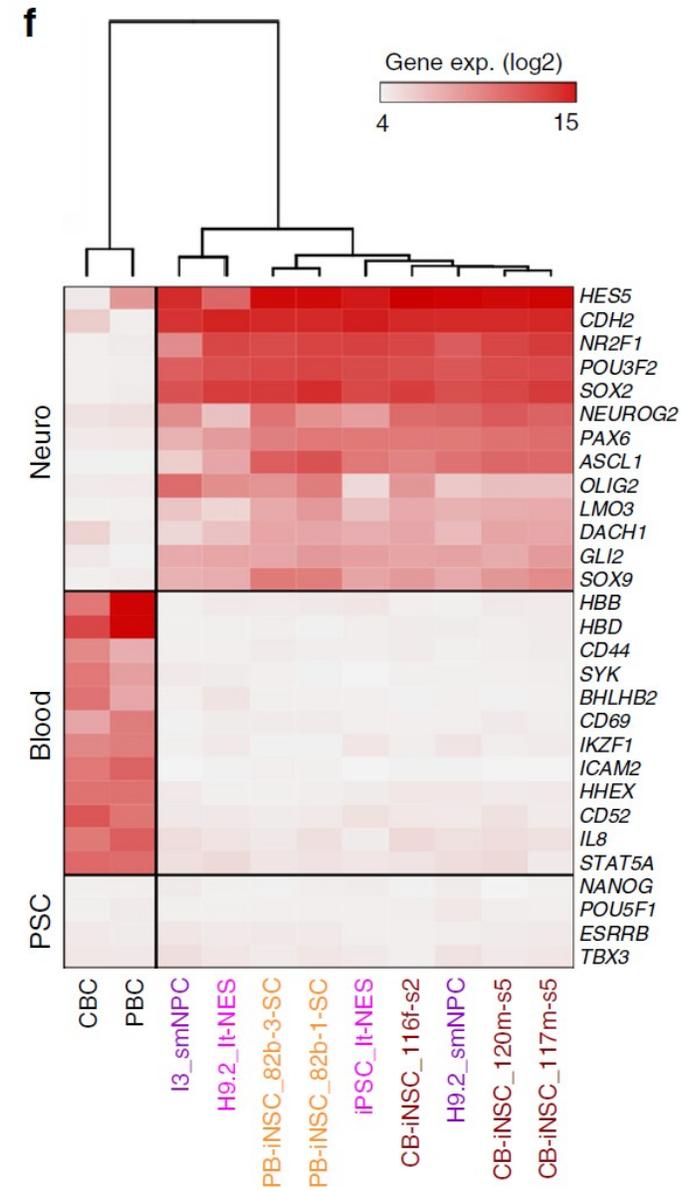
## Functional excitation and inhibition



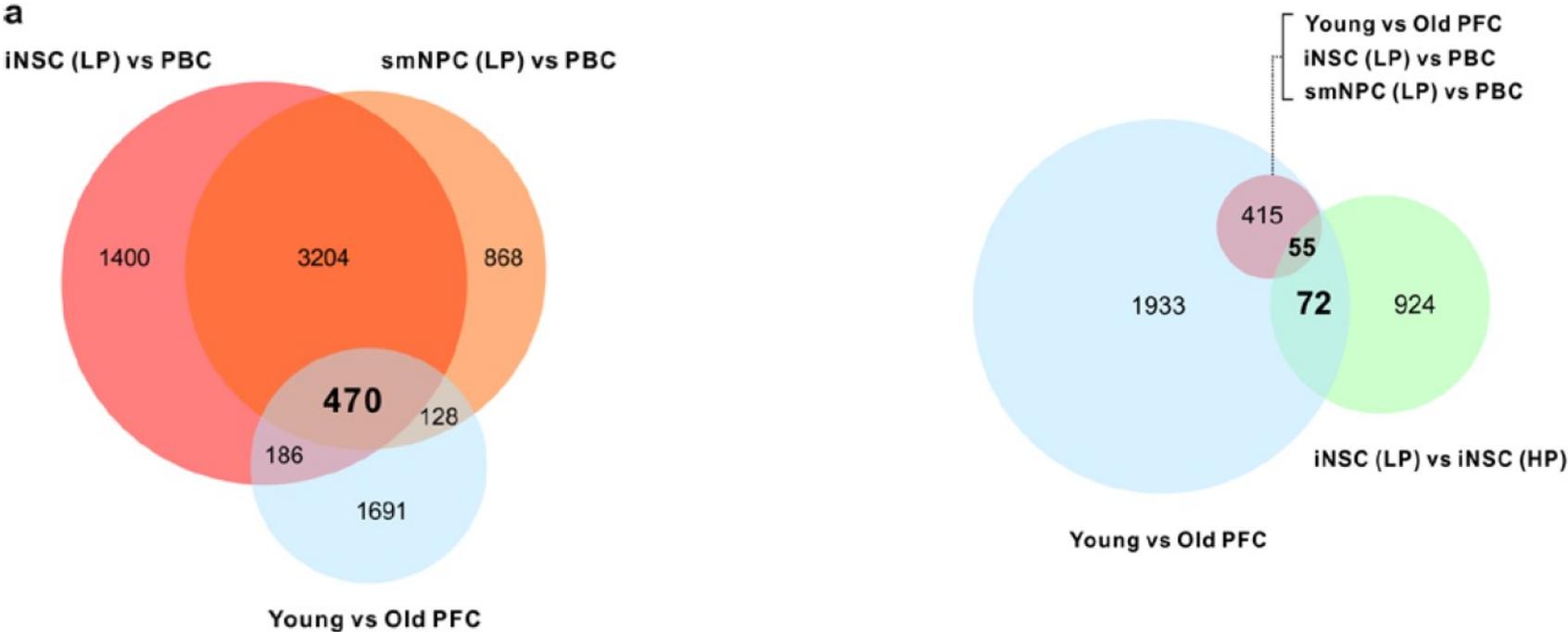
# Gene expression



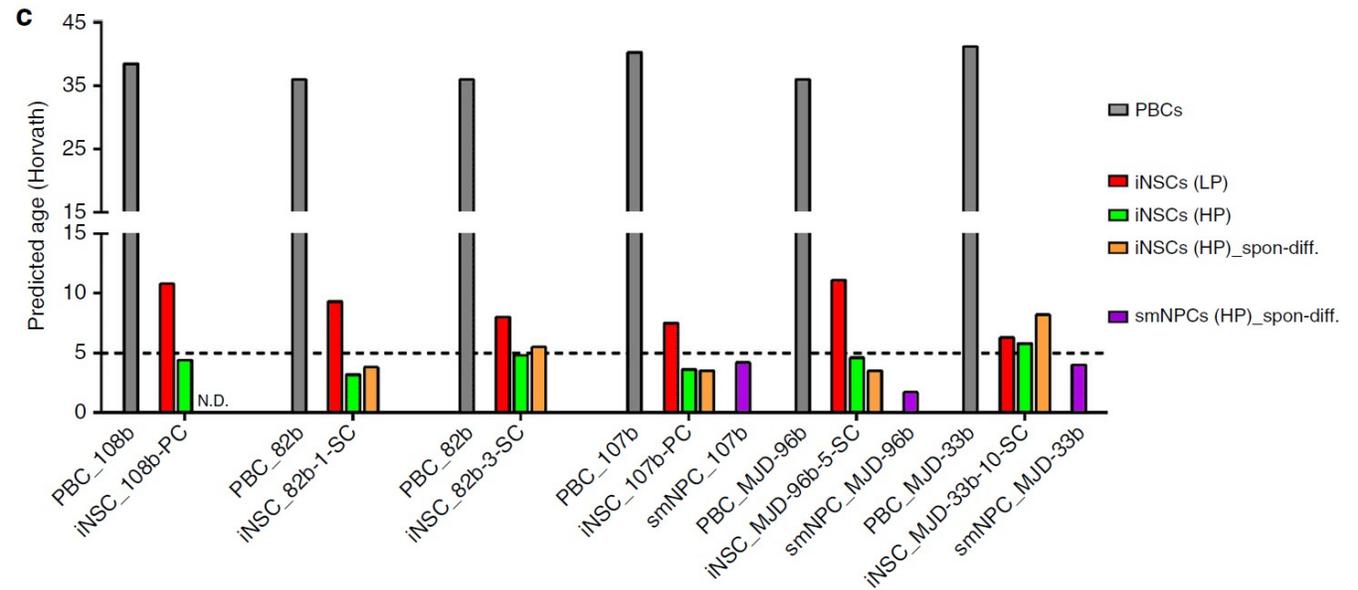
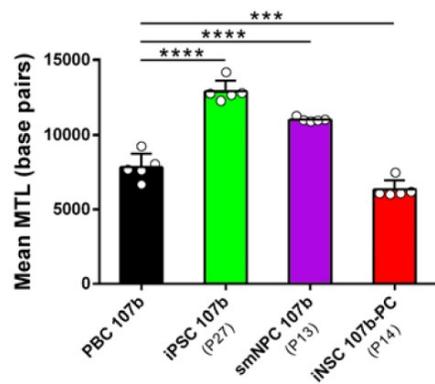
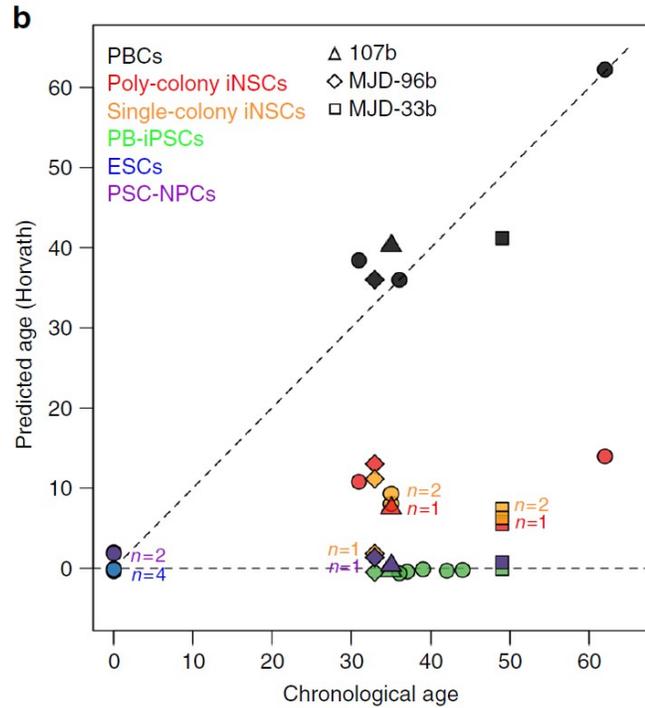
As to expect



# Gene expression

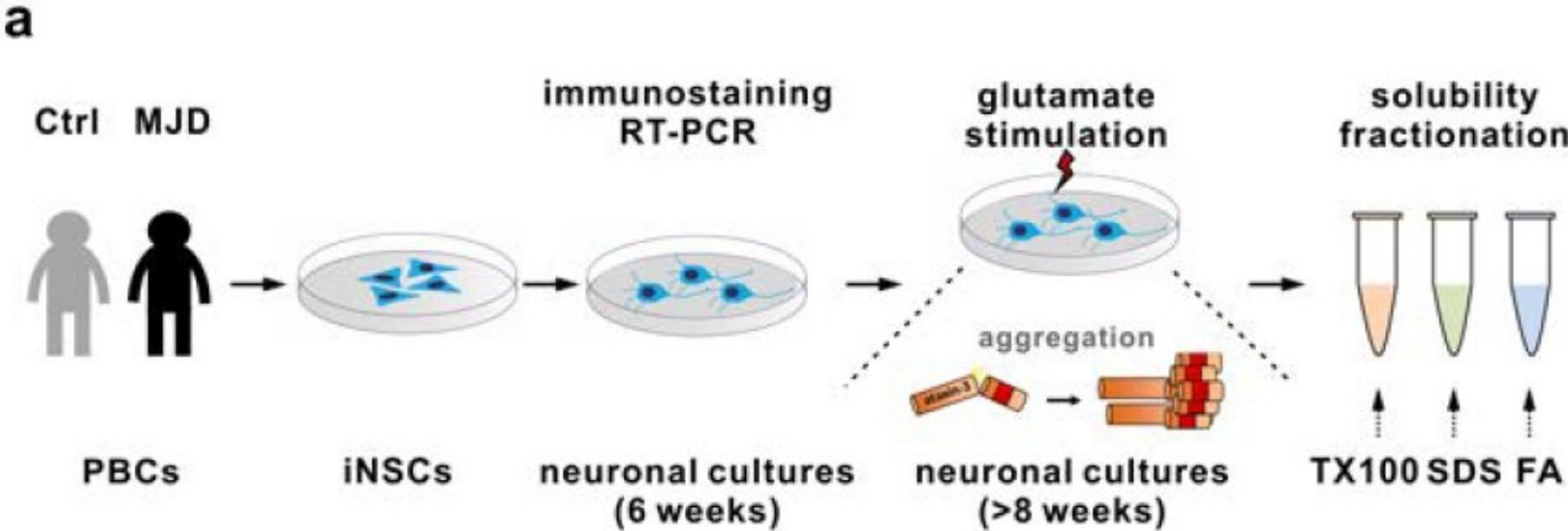


# Age-related epigenetic signatures



Cell populations	Gender	Back-ground	Passage	Clonal state	Chronological age	Age prediction		
						Horvath	Hannum	99CpGs
PBC_109b	M	Ctrl			62	62.2	72.3	50.8
PBC_MJD-33b	F	MJD			49	41.2	58.6	34.4
PBC_82b*	F	Ctrl			36	36.0	51.3	22.3
PBC_107b	M	Ctrl			35	40.2	55.5	27.6
PBC_MJD-96b	M	MJD			33	36.0	51.9	17.5
PBC_108b	F	Ctrl			31	38.4	48.8	26.5

# Prove of principle for disease modelling



# Summary

A direct conversion from PBCs into iNSC in absence of OCT4 was archived featuring following advantages:

- PBCs as easily accessible source
- No transgene integration into the genome
- Robust self-renewal and multipotency
- Ability to differentiate into glia and neuronal subtypes
- Skipping pluripotency by avoiding OCT4

# Discussion