Highly cooperative chimeric super-SOX induces naive pluripotency across species

(高协同性嵌合超级SOX诱导跨物种初始多能性)

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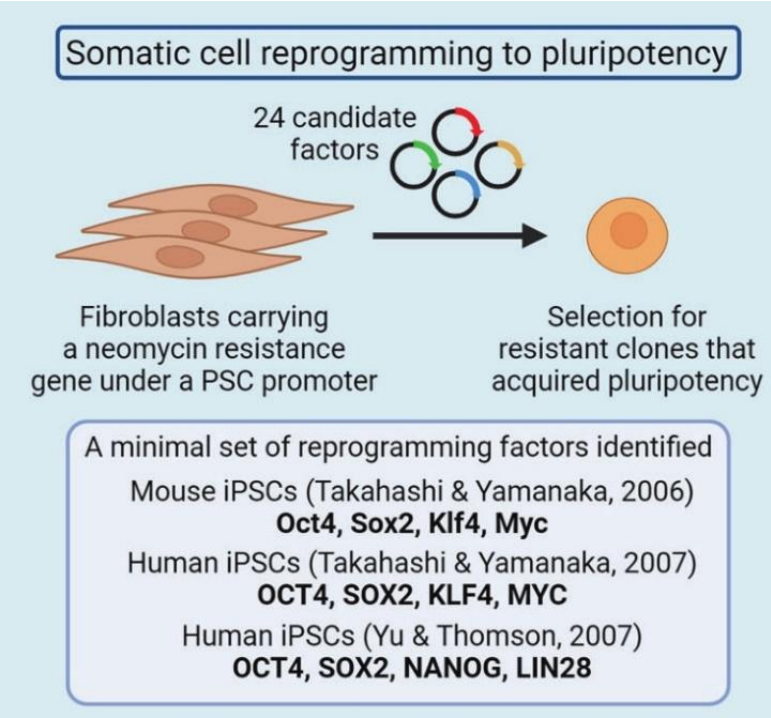
Introduction

Induced pluripotent stem cells (iPSCs) are a type of cell generated through the reprogramming of terminally differentiated somatic cells. These cells closely resemble embryonic stem cells (ESCs), possessing **self-renewal capacity** and **multilineage differentiation potential.**



The groundbreaking experiments of fibroblast reprogramming to pluripotaency were pioneered by Kazutoshi Takahashi and Shinya Yamanaka.

A minimal combination of **Oct4, Sox2, Klf4, and cMyc (OSKM)**

Introduction

Feature	Naive State	Primed State
Developmental Analog	Pre-implantation blastocyst (Inner Cell Mass)	Post-implantation embryo (Epiblast)
Pluripotency	Broadest potential, can contribute to embryonic & extra-embryonic lineages	Restricted, biased toward forming embryonic germ layers

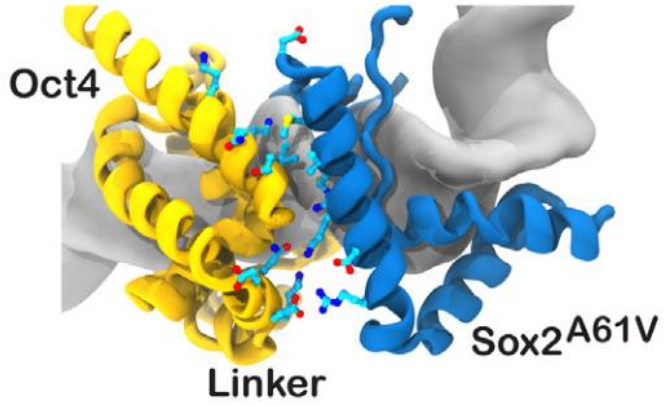
Traditional iPSC reprogramming methods typically yield primed-state cells. Obtaining naïve-state iPSCs is a significant goal in the field. But it often inefficient or even completely ineffective when applied traditional iPSC reprogramming methods to non-model organisms . Additional, the resulting iPSCs frequently exhibit poor quality, compromised pluripotency, or can only be maintained in a primed state.

Question

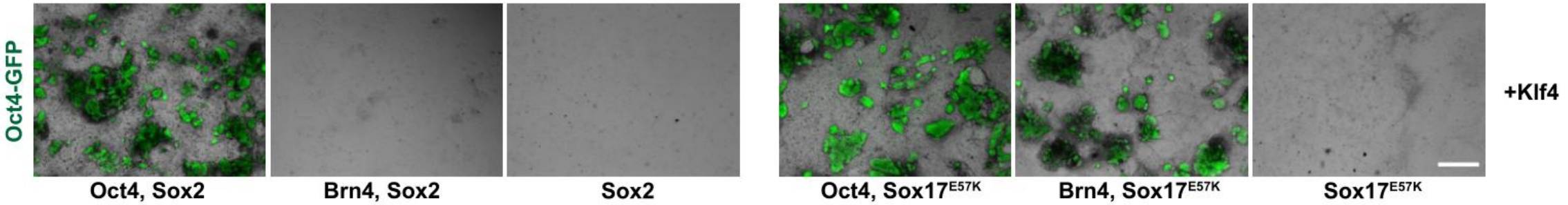
While iPSC reprogramming methods have achieved a series of significant breakthroughs in mice and humans, iPSC reprogramming in non-model organisms remains challenging.

Is there a universal molecular tool that can work across species to directly create and maintain high-quality naïve pluripotency in various mammals?

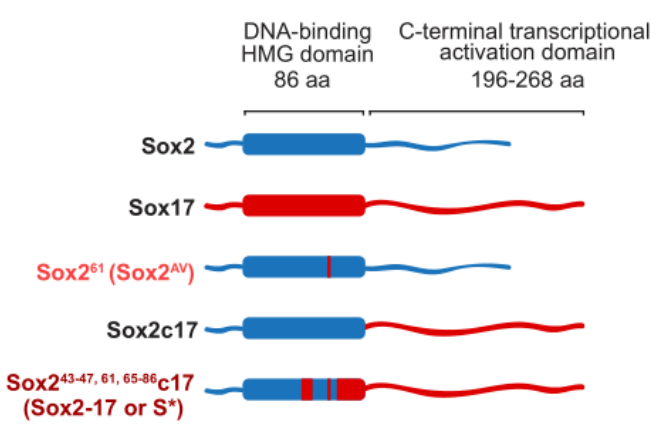
They built a chimeric super-SOX factor, Sox2-17, that enhanced iPSC generation and the developmental potential across Species.



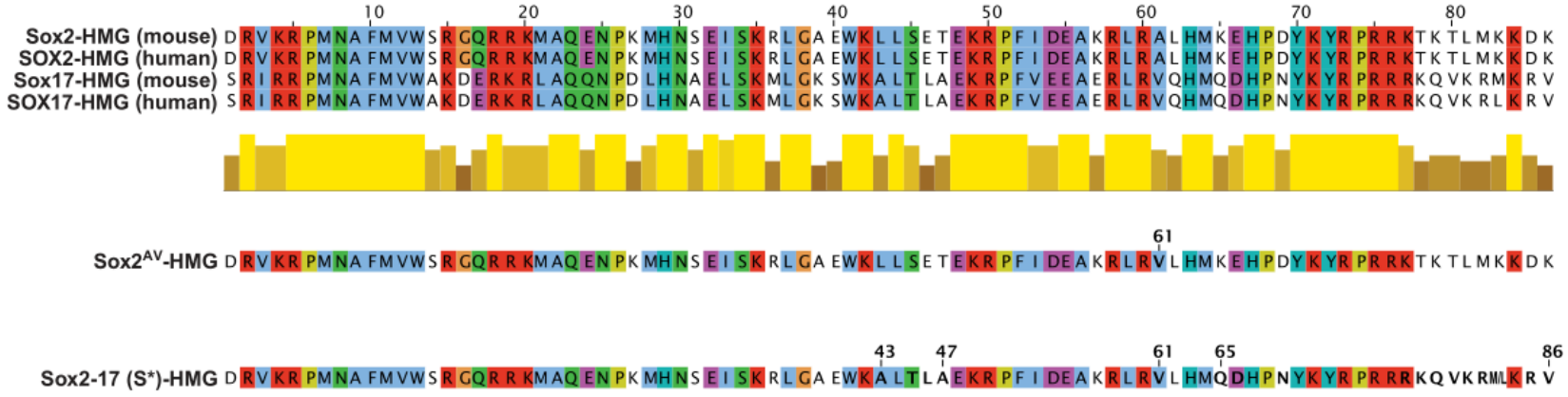
- Defining the structural elements of Sox17 that enable iPSC generation



Retroviral reprogramming of Oct4-GFP (OG2) MEFs on 21 days post infection

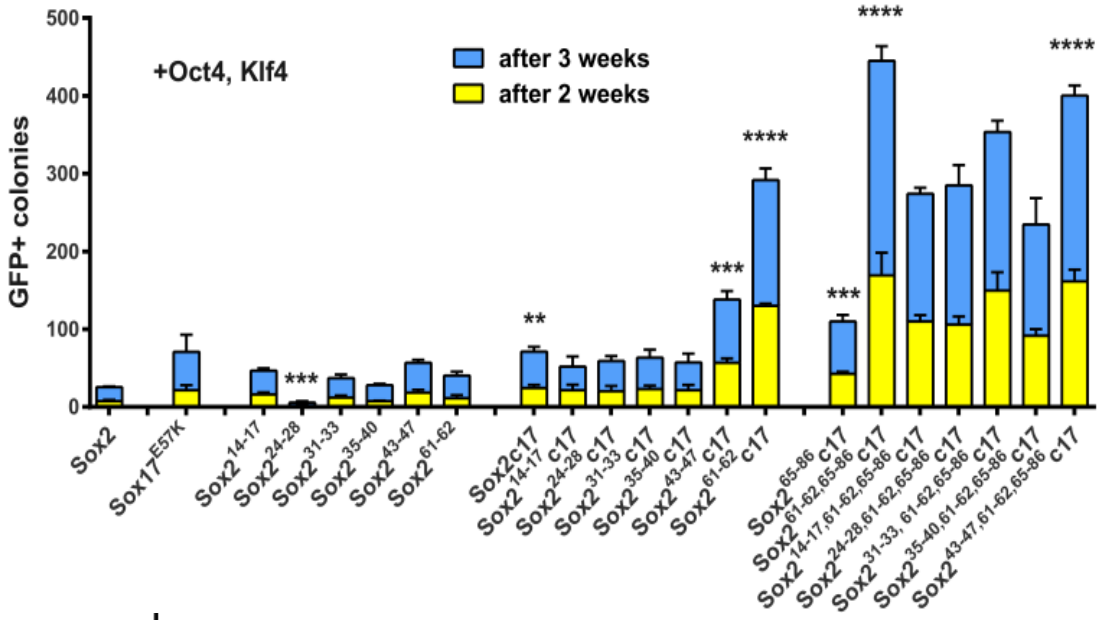


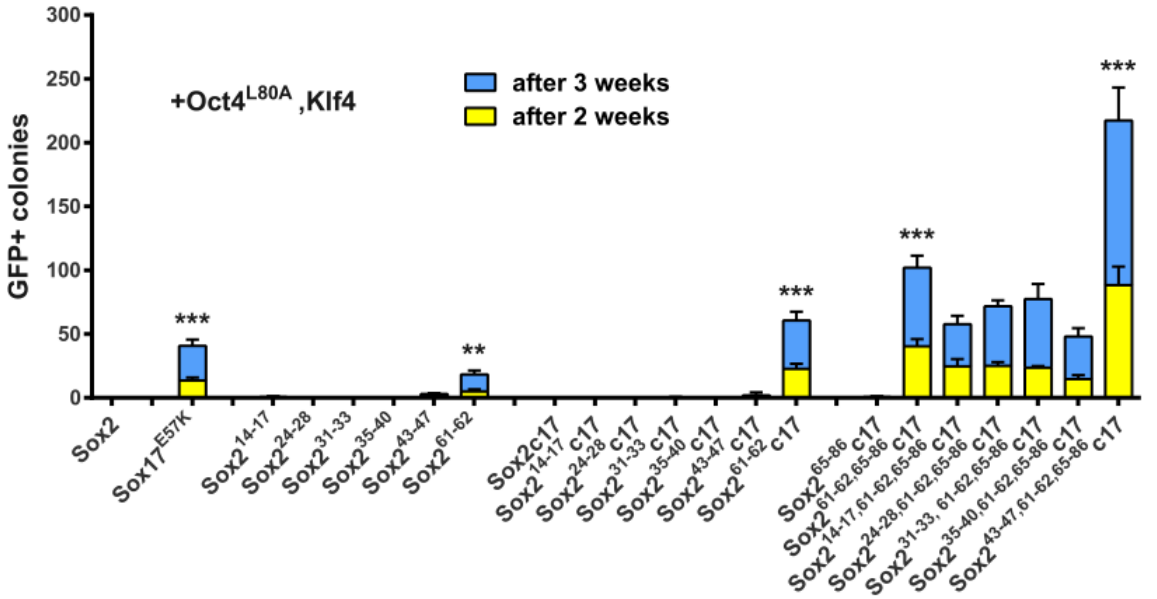
The structure of Sox2 and Sox17 and chimeric TFs

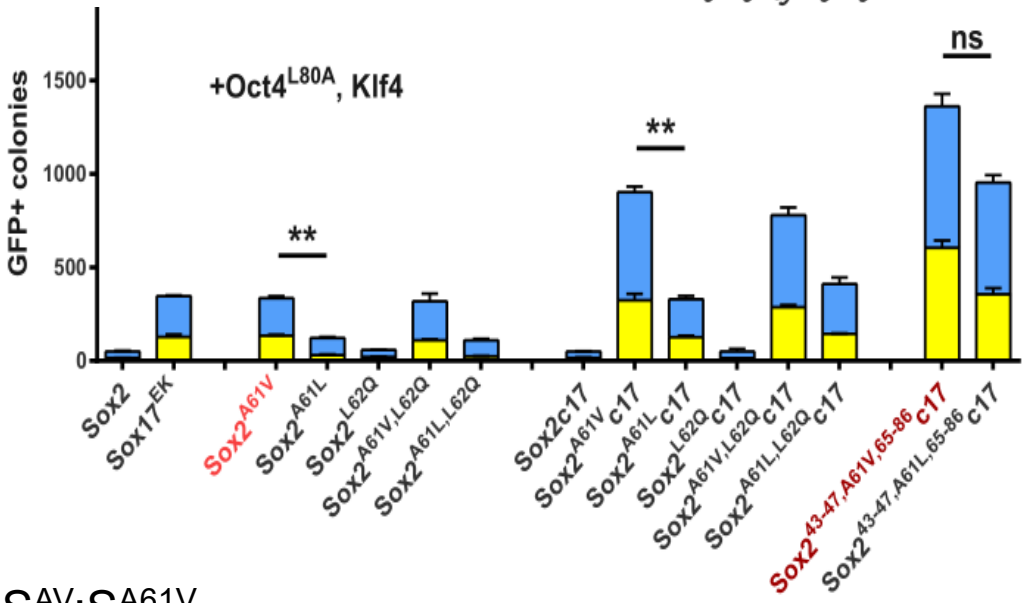


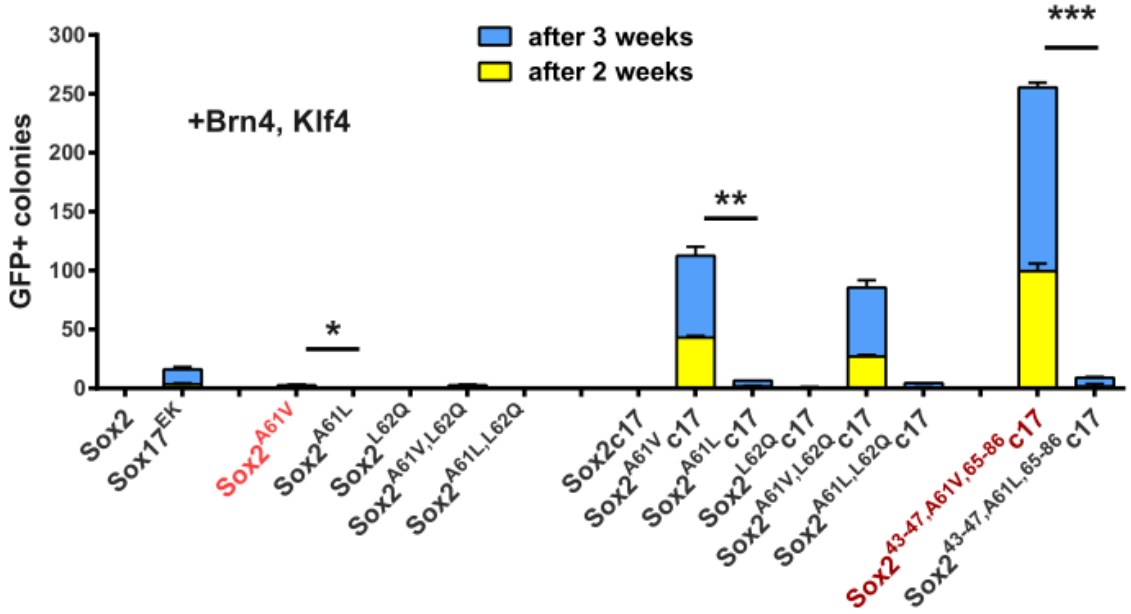
Protein sequence alignment of DNA binding domains of mouse and human Sox2, Sox17, and the most crucial chimeric Soxes

- Defining the structural elements of Sox17 that enable iPSC generation





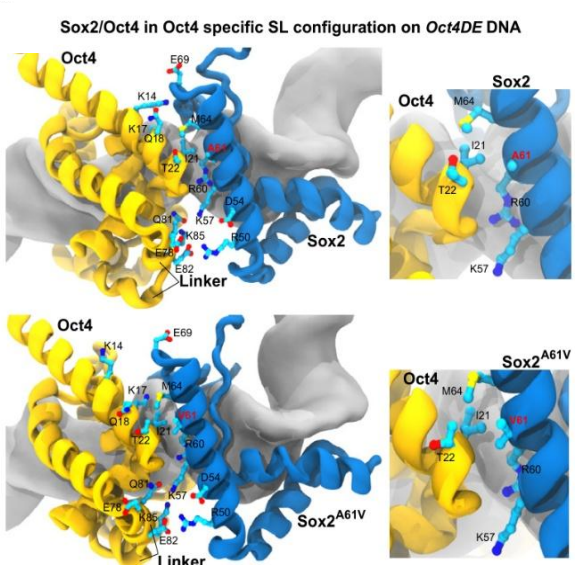


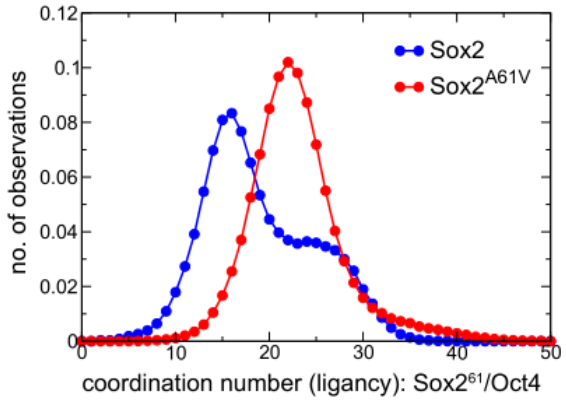


S^{AV}:S^{A61V}

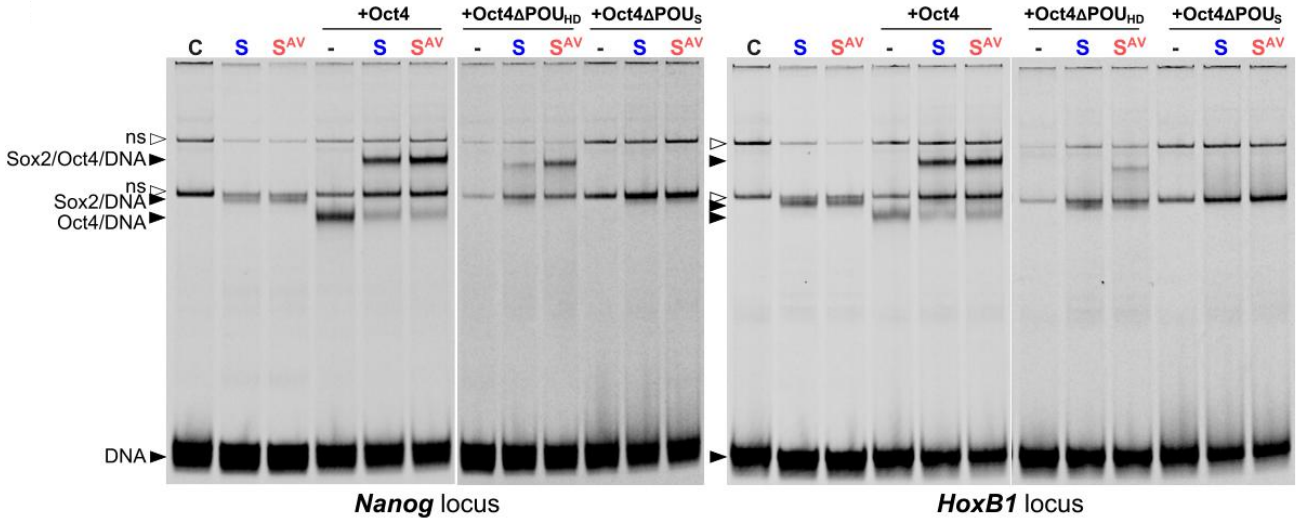
S^{*}:Sox2 $^{43–47,61,65–86\ c17}$ (Sox2-17)

- Enhanced Sox/Oct cooperativity rescues non-functional POU factors in reprogramming

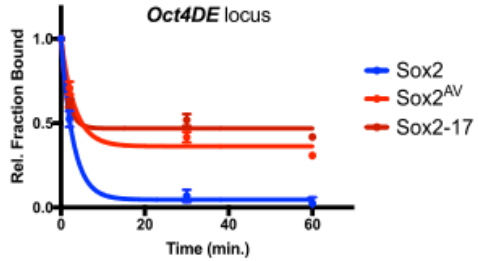


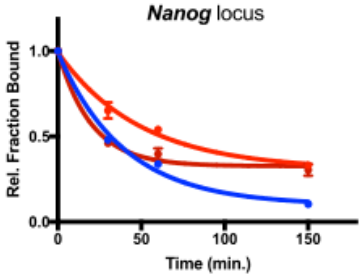


Models of Sox2/Oct4 and Sox2AV/Oct4 heterodimers on Oct4DE DNA in Oct4-specific SL configuration.



EMSAs with HEK293 lysates on the Nanog promoter and HoxB1 enhancer DNA

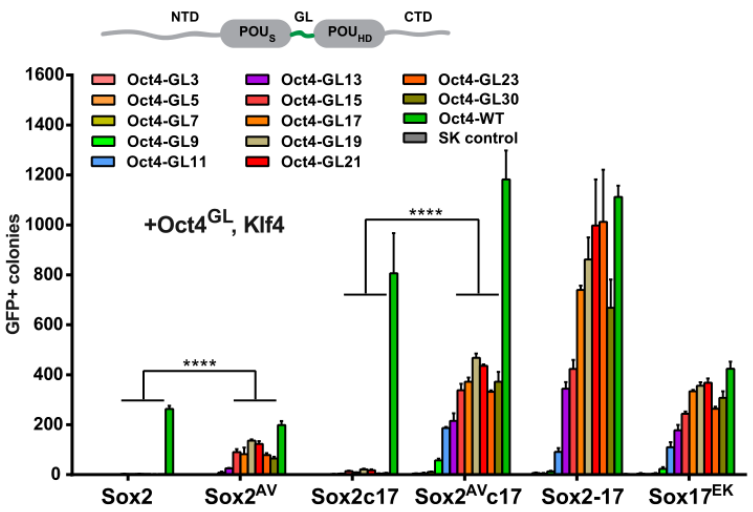


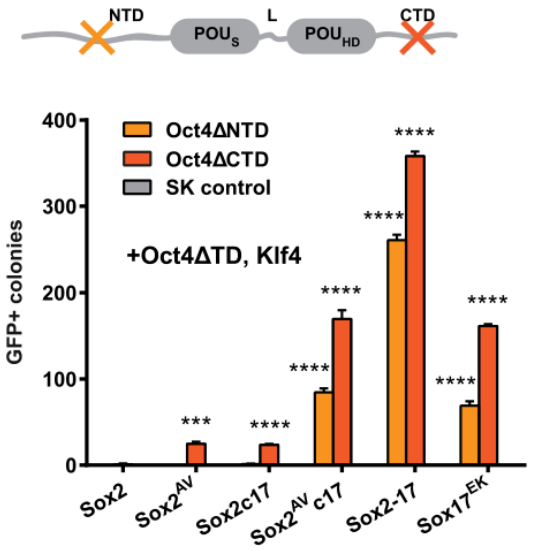


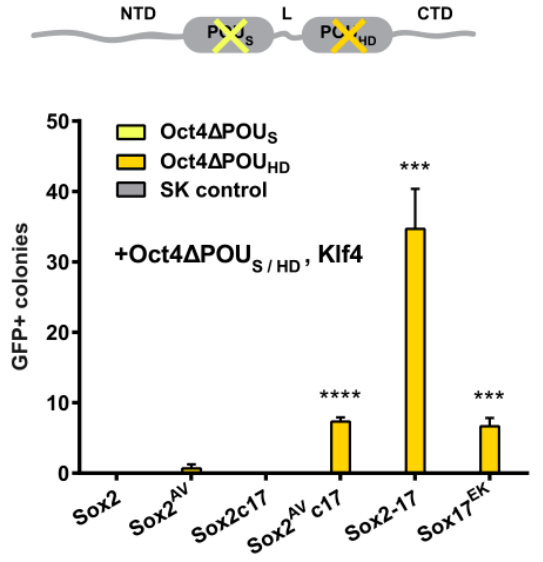
Representative kinetic off-rate EMSAs with HEK293 lysates on Oct4DE, Nanog promoter,

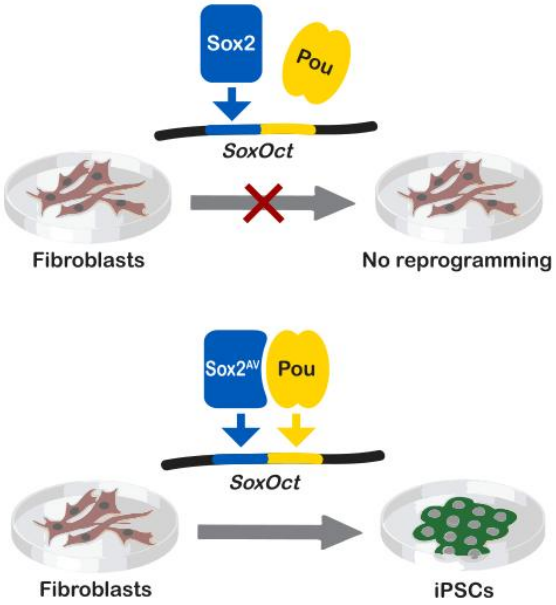
- Enhanced Sox/Oct cooperativity rescues non-functional POU factors in reprogramming

The structure of OCT4 protein



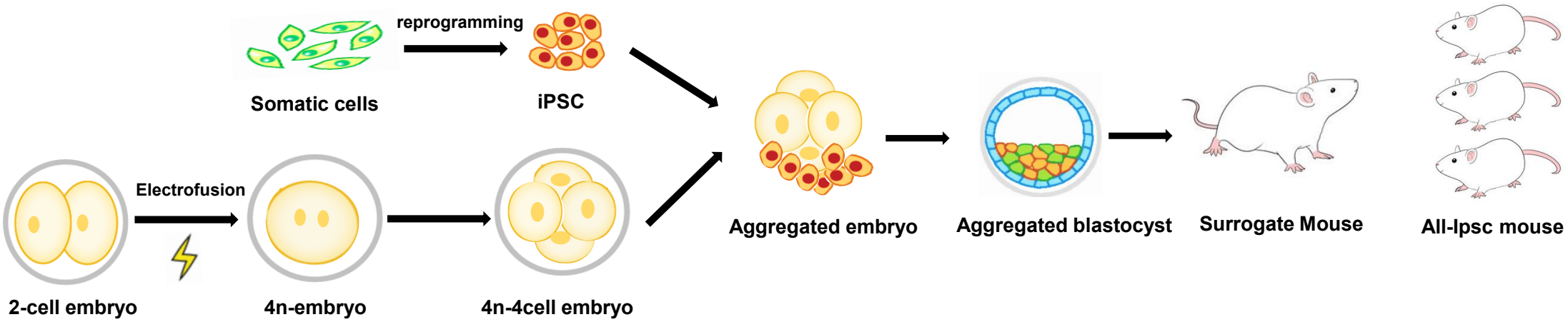




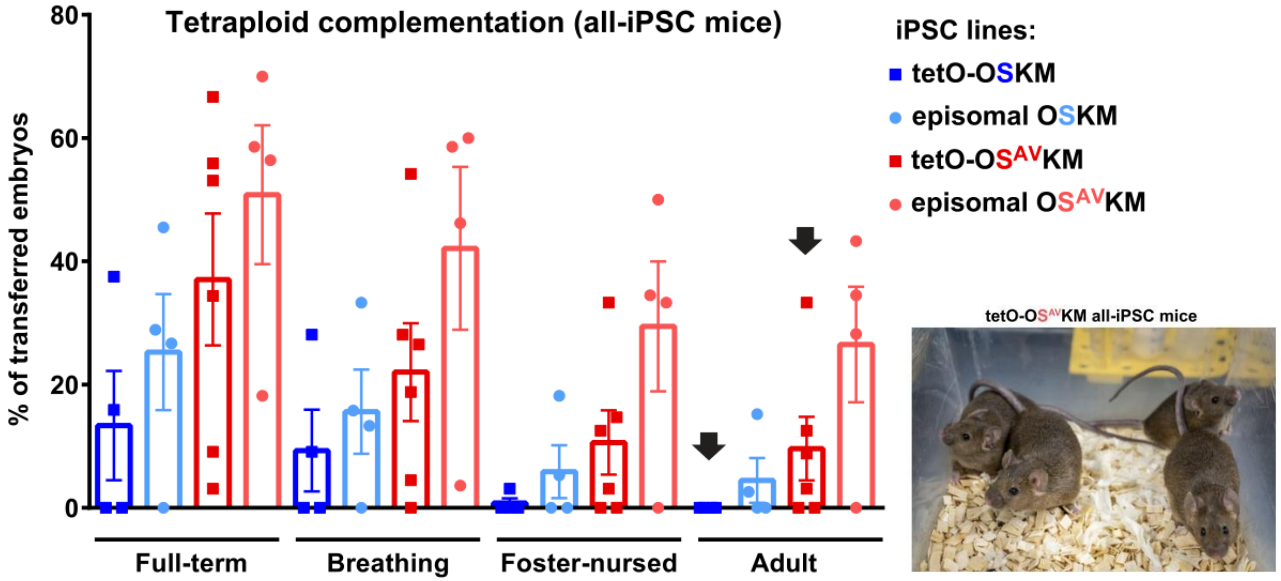


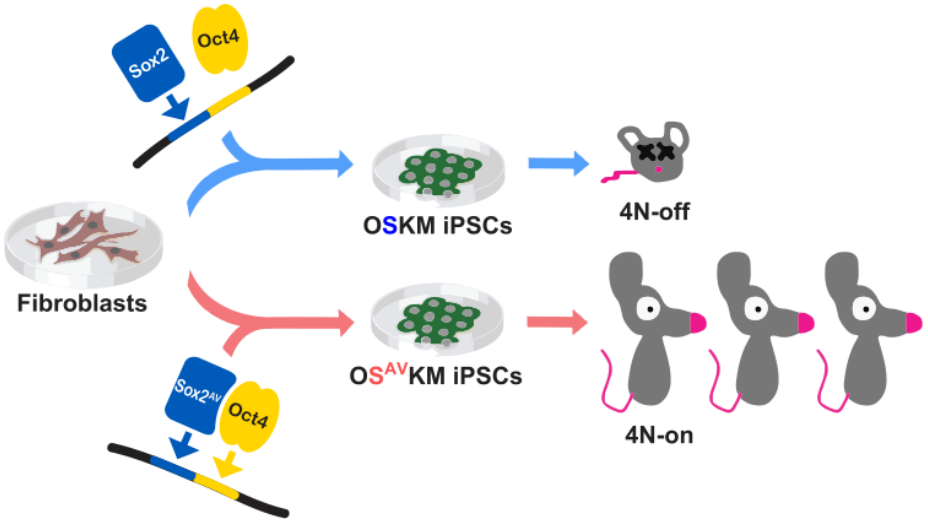
OSK reprogramming of OG2 MEFs with monocistronic retroviral vectors carrying Oct4 domain deletion of linker (A), NTD or CTD (B), and POUS or POUHD (C).

- Highly cooperative Sox2AV improves the developmental potential of mouse OSKM iPSCs

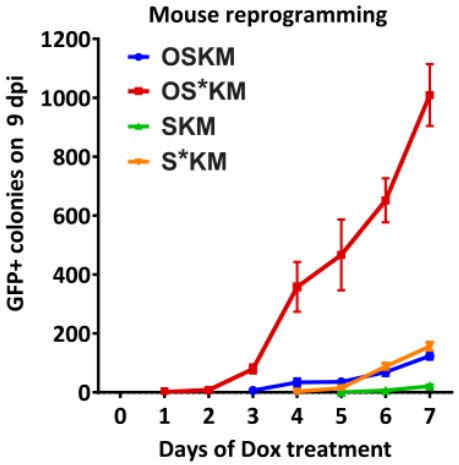


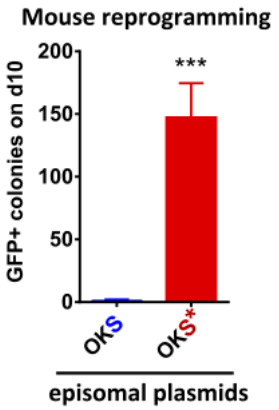
Tetraploid (4N) complementation assay

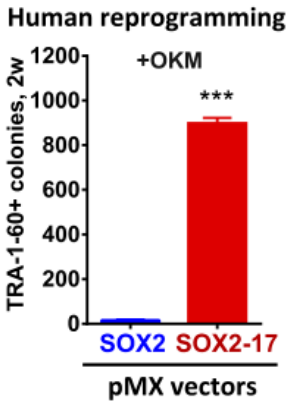


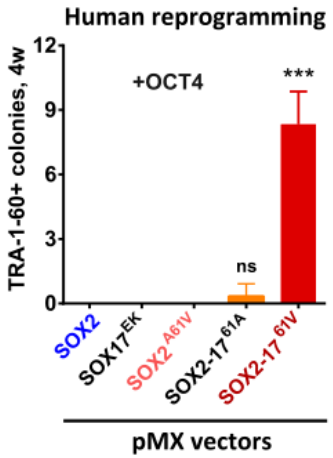


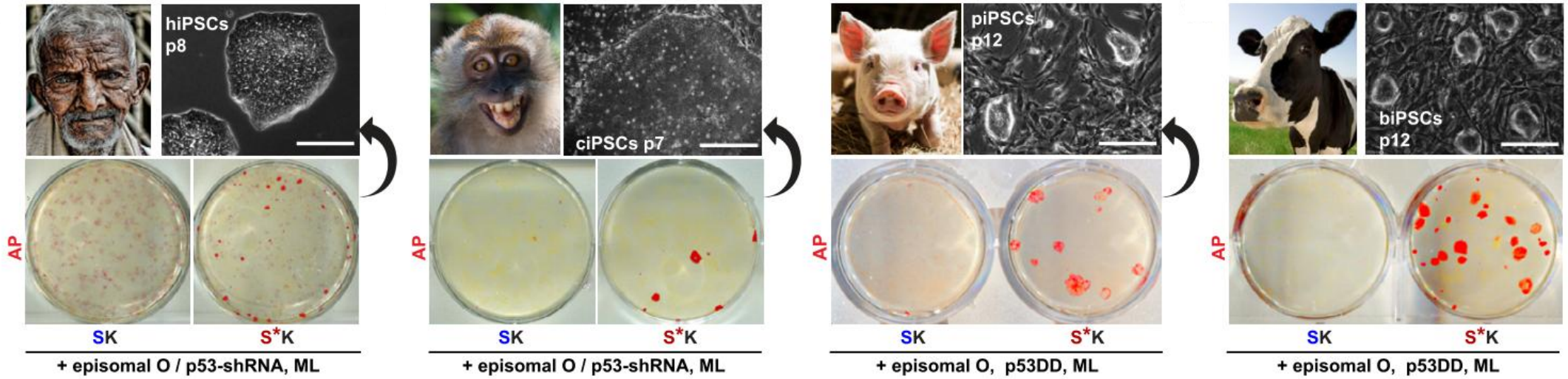
- Chimeric super-SOX enhances iPSC generation in five species





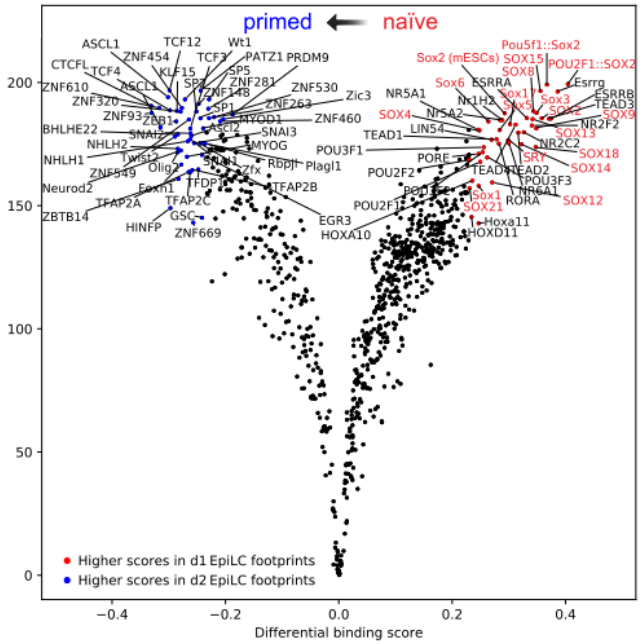


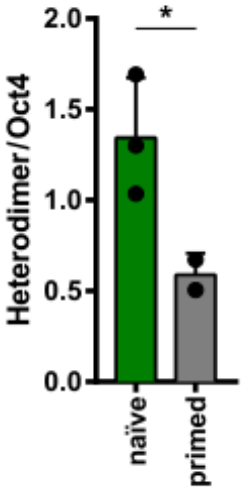


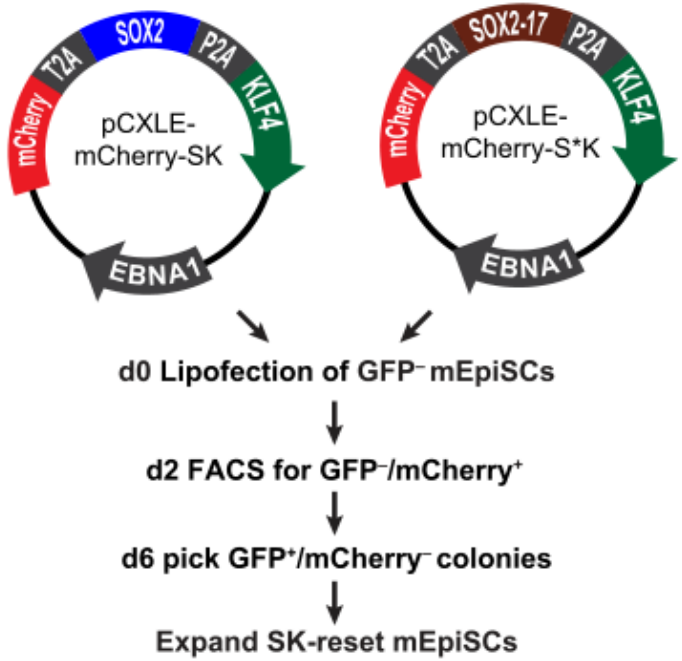


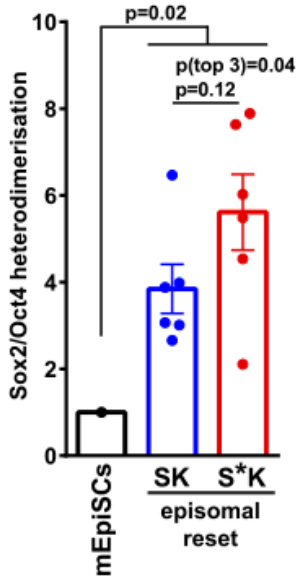
Representative whole-well AP stainings for episomal reprogramming of (N) 56-year-old human male dermal fibroblasts on day 25, (O) Cynomolgus macaque fibroblasts on day 25, (P) porcine fetal fibroblasts on day 21, (Q) bovine fetal fibroblasts on day 21 after nucleofection.

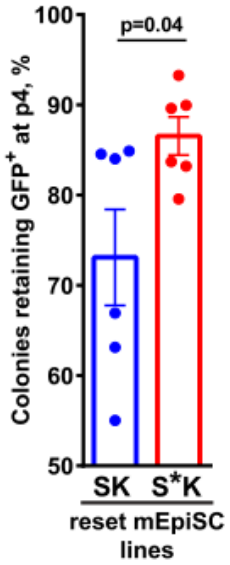
- Sox2/Oct4 dimerization is at the core of naïve pluripotency

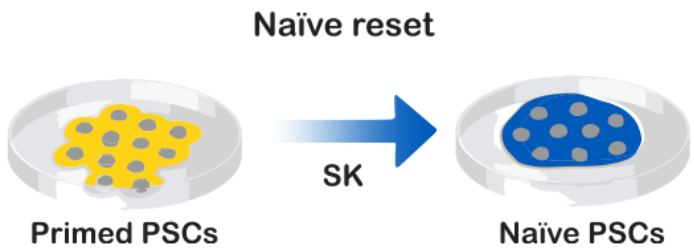




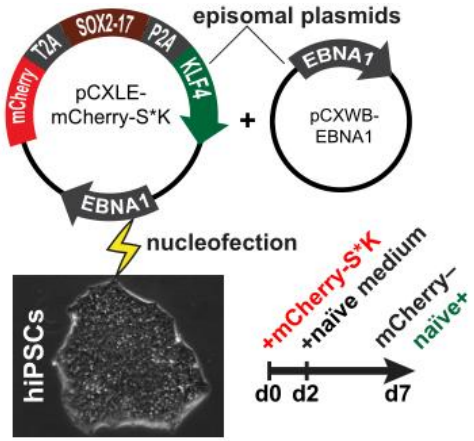


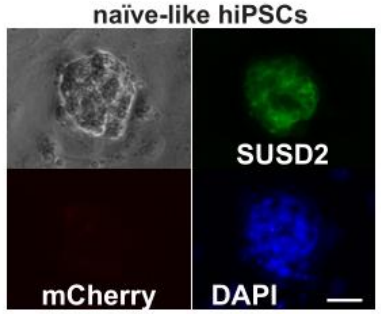


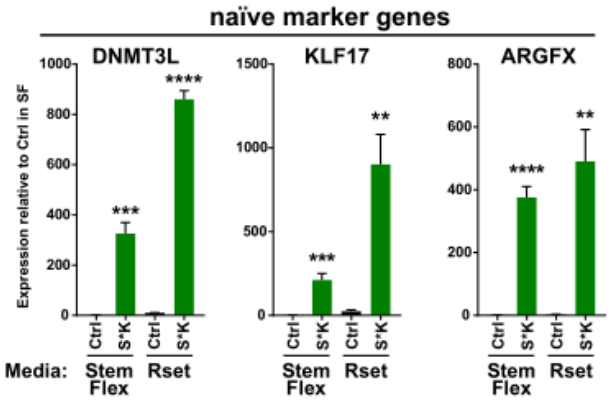


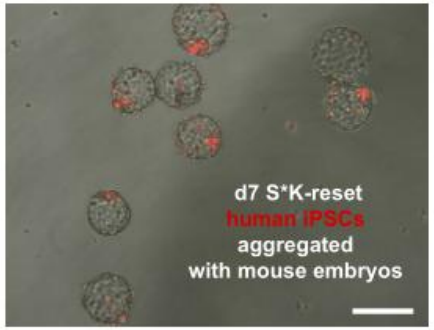


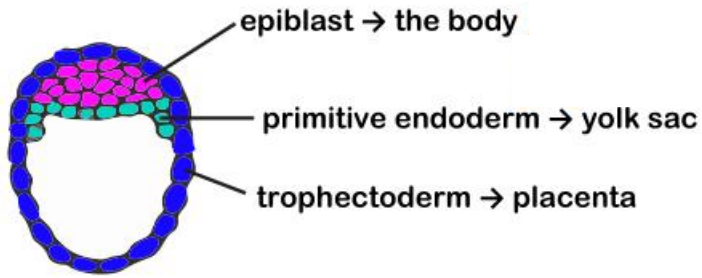
- Episomal SK reset enhances the developmental potential of PSCs in three species





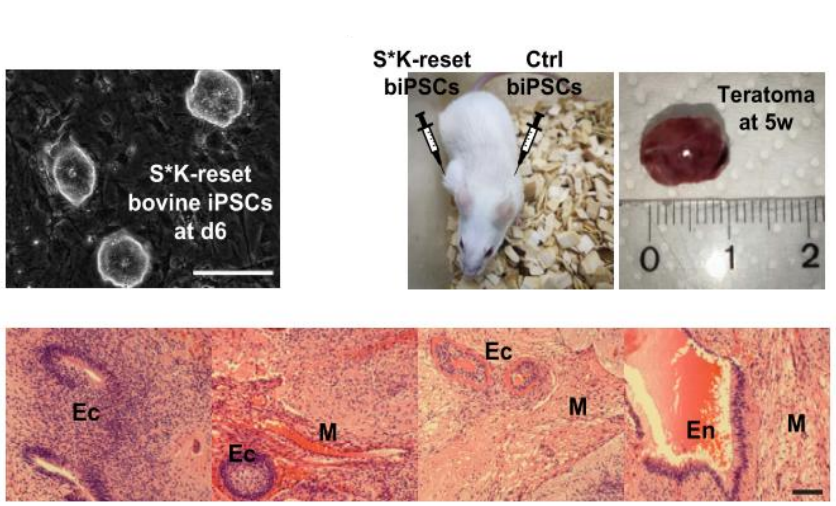




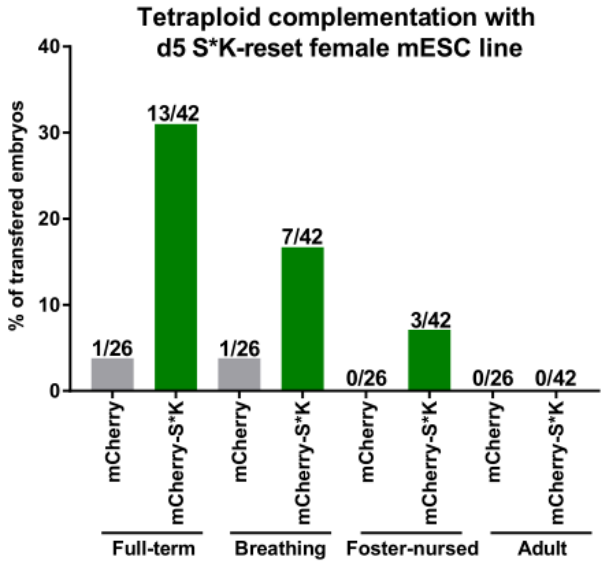


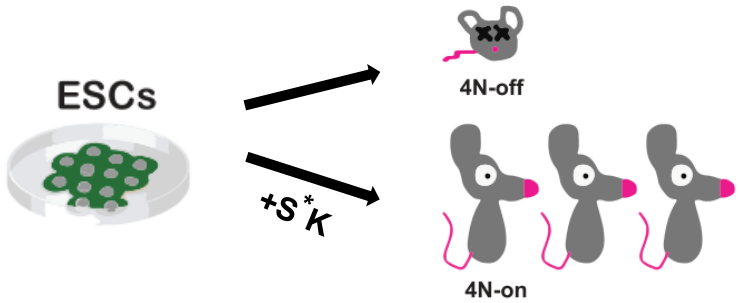
Cross-species human/mouse morula aggregation with day 7 SUSD2+ hiPSCs marked by constitutive RFP, reset with pCXLE-S*K (no mCherry)

- Episomal S*K reset enhances the developmental potential of PSCs in three species

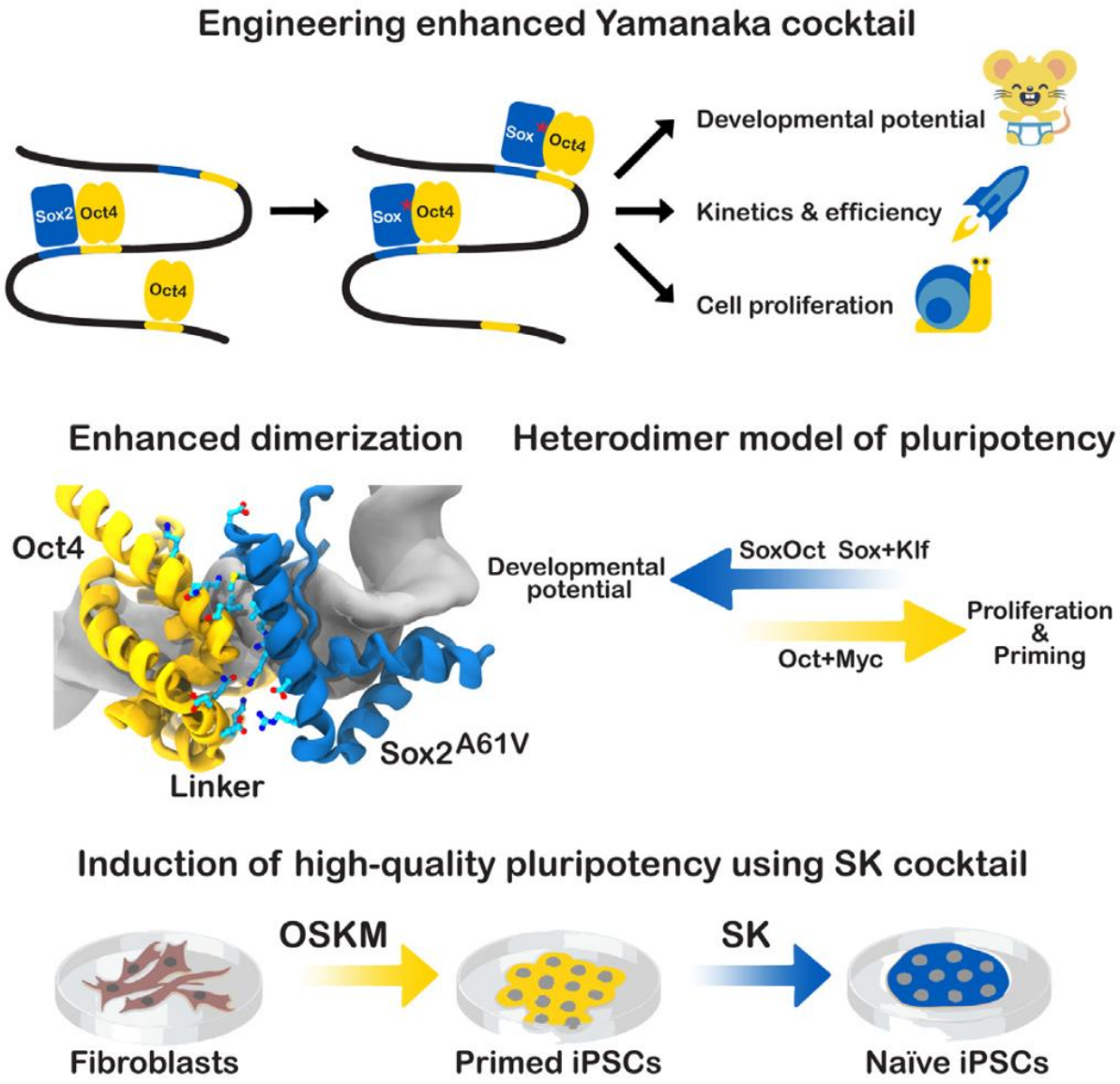


Teratoma generated by S*K-reset biPSCs





Summary



- Yamanaka cocktail upgraded with Sox2-17 enhanced iPSC generation in 5 species
- Sox2A61/V swap at Sox2/Oct4 interface improves the quality of OSKM iPSCs
- Sox2/Oct4 dimer is the master regulator of high-quality naïve pluripotency
- Naive reset using episomal SK boosts the developmental potential of PSCs across species

Limitation

- Their current protocol generates naïve stem cells with a transient 4-7 days window for use, as a culture media for their long-term, transgene-free maintenance is not yet available.
- They cannot exclude that a highly cooperative Sox or excess of Sox2 may participate in the developmental reset in ways beyond enhancing Sox/Oct dimerization.

Thank you