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Efficient generation of germline chimeras in a non-rodent species using rabbit induced pluripotent stem cells

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Efficient generation of germline chimeras in a non-rodent species using rabbit induced pluripotent stem cells

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浙江大学 生命演化研究中心

How to create a green fluorescent rabbit?

2012-01-01 ***Sherlock*** *S2E2*

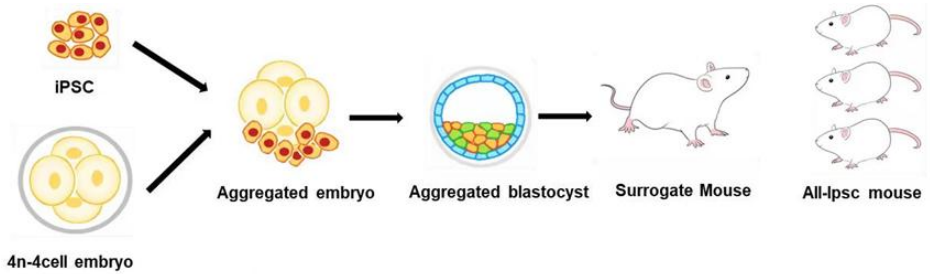
1.

2.

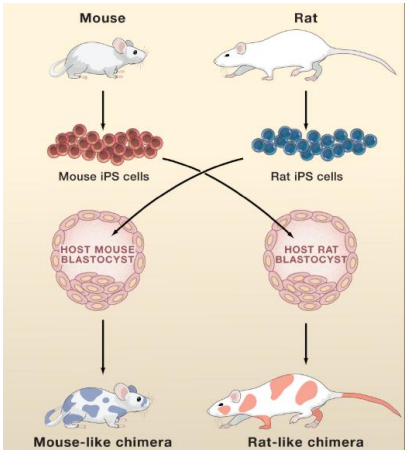
3.

??

Background



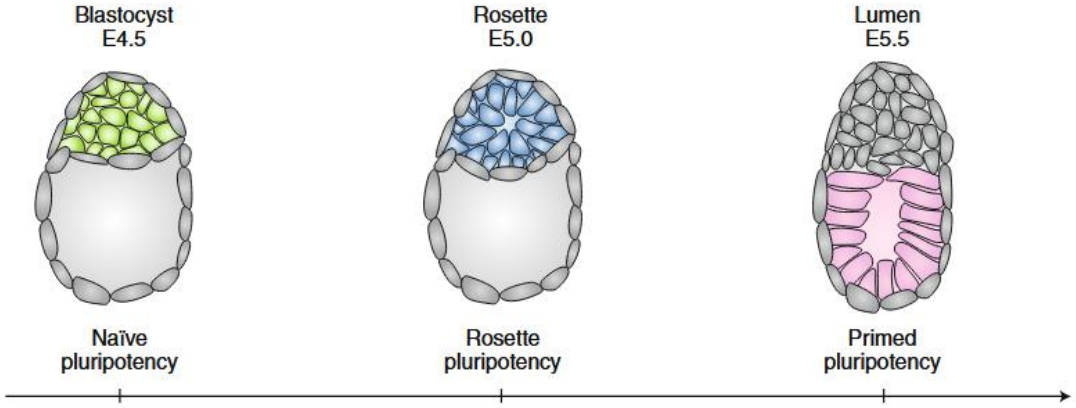
Tetraploid (4N) complementation assay



Chimeras

Naïve pluripotency

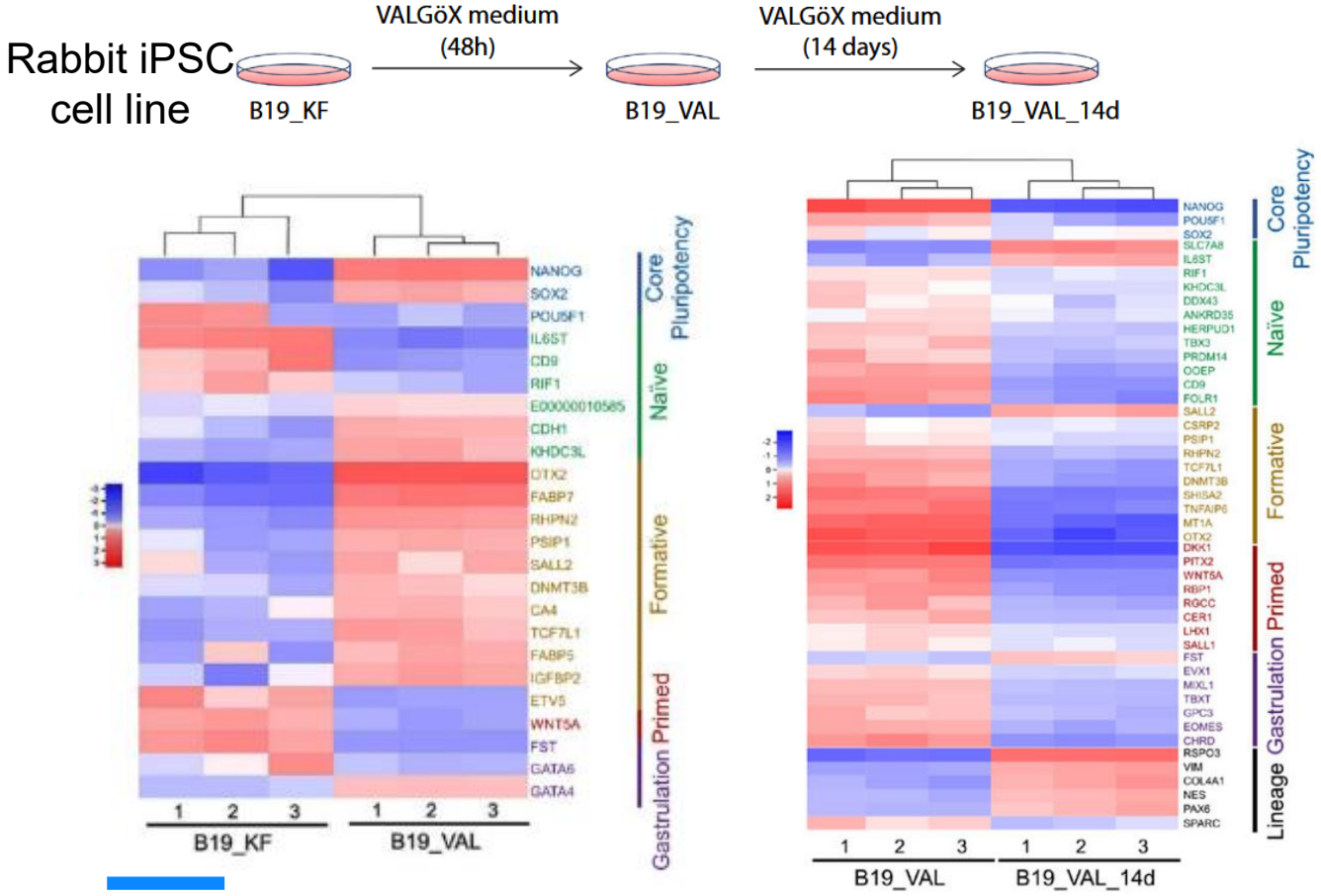
Germline chimeras

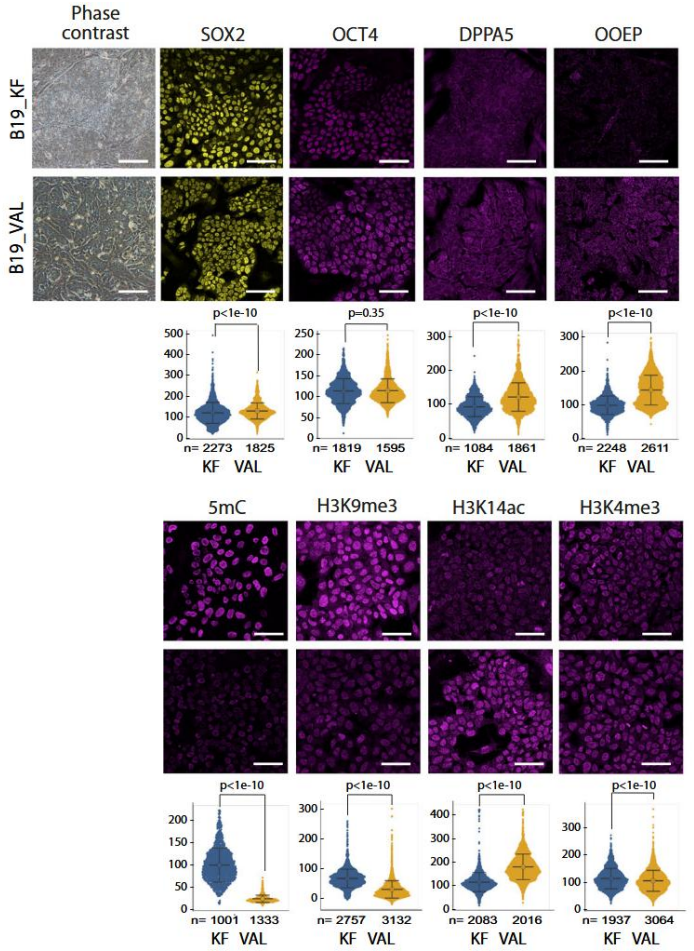


Dundes, C.E. and Loh, K.M. (2020)

Results 1. VALGöX culture regimen steers iPSCs toward the naïve state

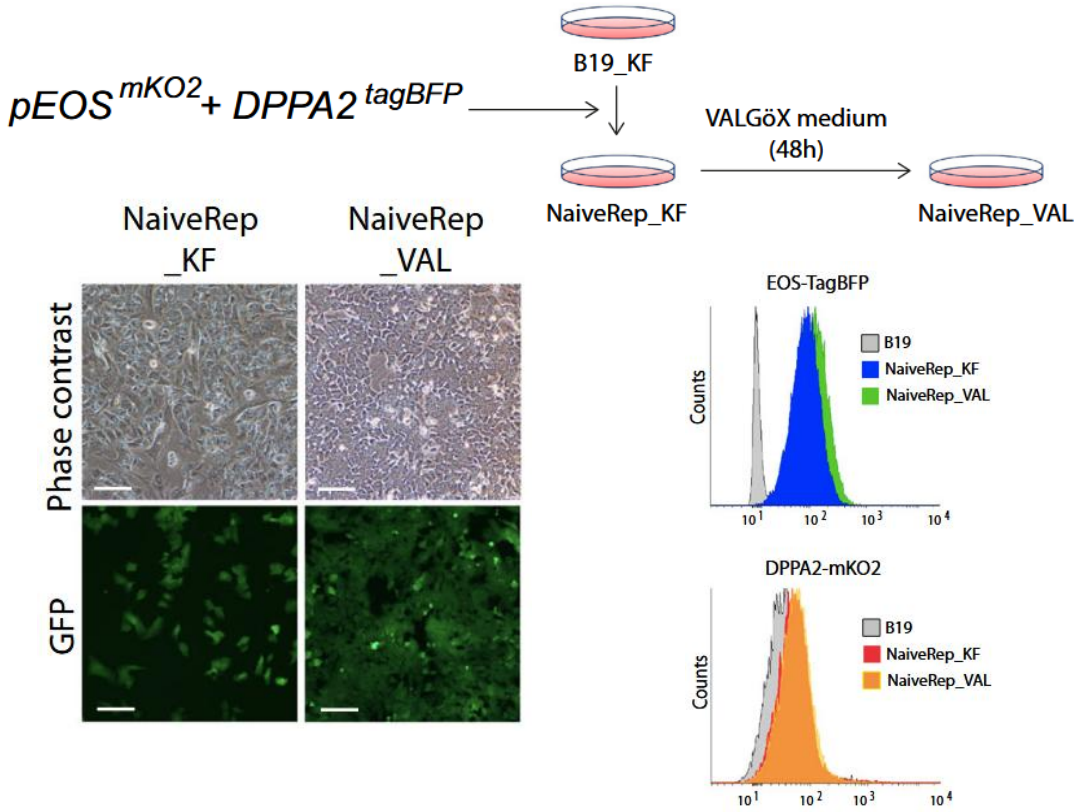
- A new chemical cocktail medium called **VALGÖX** (VAL, Gö6983, XAV939).
- Initiated the naïve pluripotency transition but couldn't maintain it long-term.

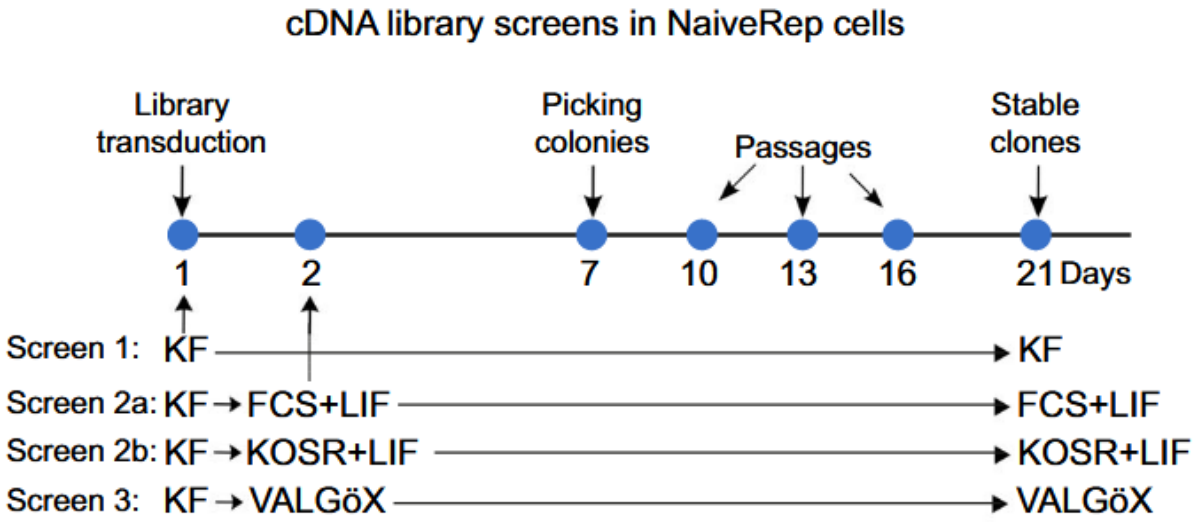




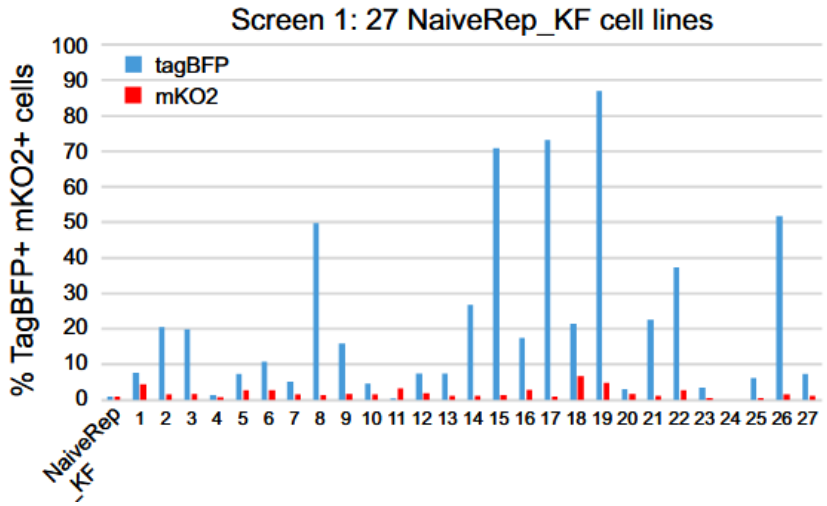
Results 2. cDNA screening identifies genes supporting the naïve state

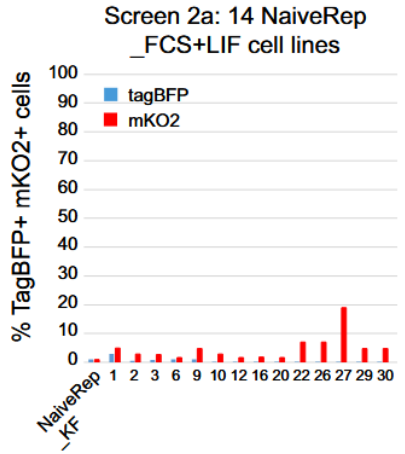
- Established a reporting system and conduct gene screening under different cultivation conditions

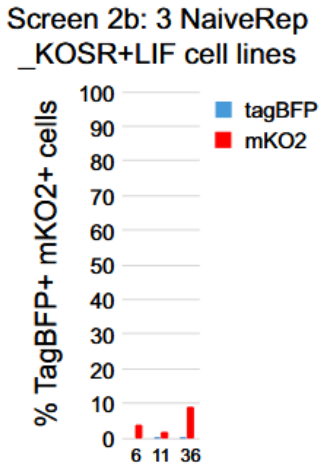




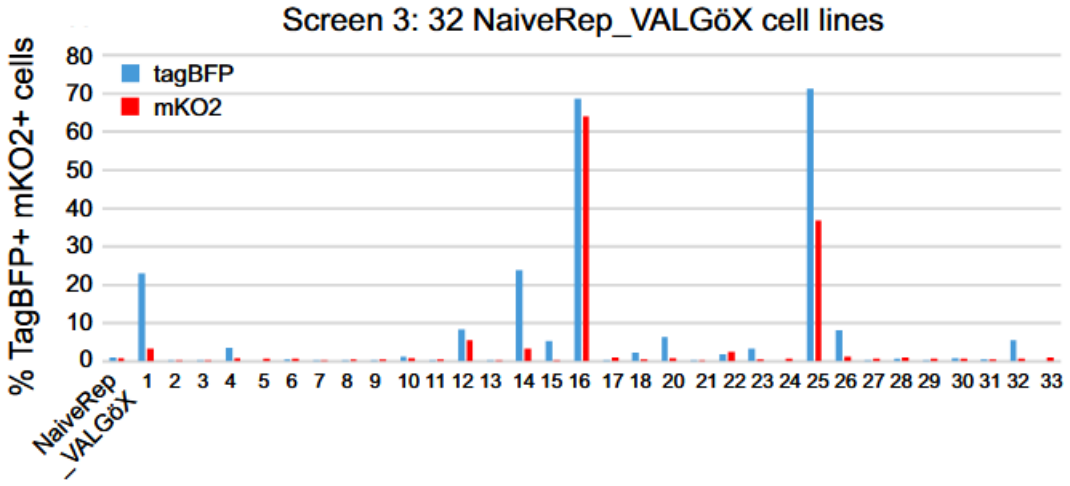
Results 2. cDNA screening identifies genes supporting the naïve state







- The colonies selected under the VALGÖX cultivation conditions were superior to those obtained under other conditions.



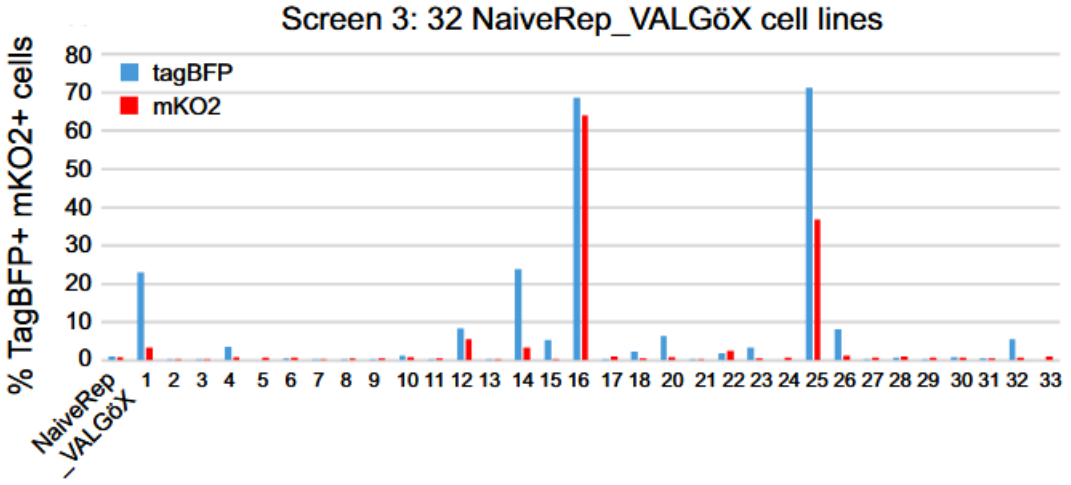
Results 2. cDNA screening identifies genes supporting the naïve state

Screen 3: Genomic PCR of 21 NaiveRep_VALGöX cell lines

Clones	1	3	4	6	7	8	10	12	13	14	16	17	20	21	22	25	26	27	30	31	32	Total
ERAS	X	X	X		X	X	X	X	X	X			X		X	X	X	X	X		X	16
PRMT6	X	X	X		X			X	X	X			X	X	X	X	X	X		X	X	15
BMI1			X		X					X			X		X		X	X				7
KLF2	X							X		X	X					X						5
TFCP2L1	X		X							X	X			X						X		5
USP21						X				X		X					X		X			5
SUV39H1													X			X		X			X	4
TRIM8				X					X	X				X								4
NANOG									X					X						X		3
CCNE1						X								X							X	3
MYC											X						X		X			3
KDM4d		X					X														X	3
DAX1											X					X				X		3
DPPA4						X										X	X					3
KLF4	X															X						2
SOX2							X		X													2
KAT2B		X		X																		2
KLF5											X				X							2
ESRRB		X								X												2
NR5A2											X										X	2
ESRRG					X				X													2
TBX3										X				X								2
KLF12									X													1
GBX2																X						1
GASC1										X												1
TFAP2C																X						1
DPPA2														X								1
OOEP																			X			1

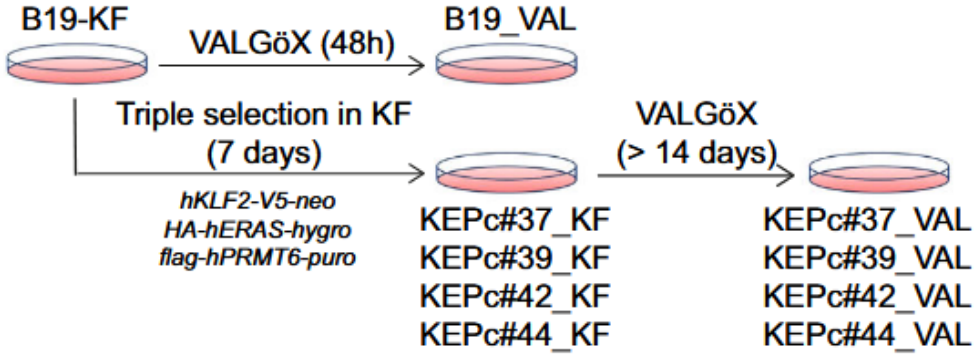
- Three key genes: ERAS, PRMT6, and KLF2 (KEP), were identified as the key naïve pluripotent genes from the results of screen3.

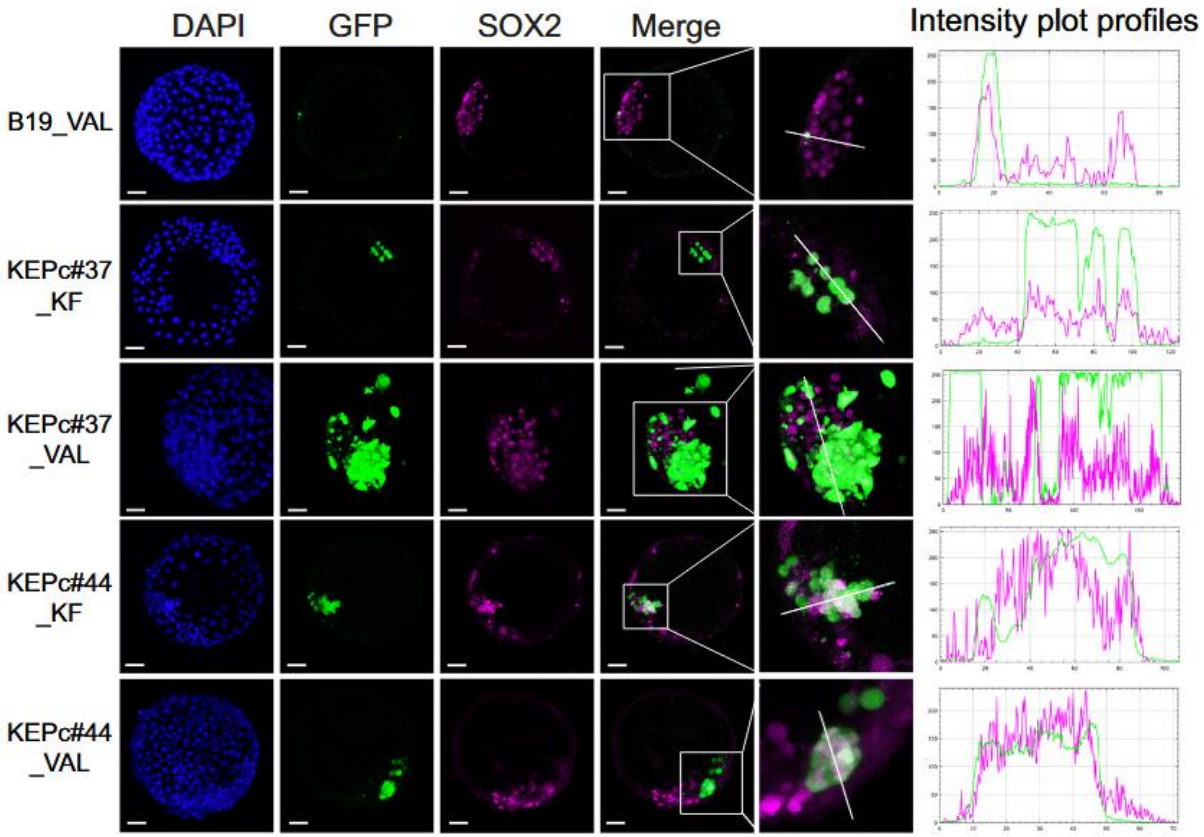
No occurence for:
LargeT
E1A-12S
GFI1
SALL4
KHDC1
SMAD7
KLF17
DPPA5



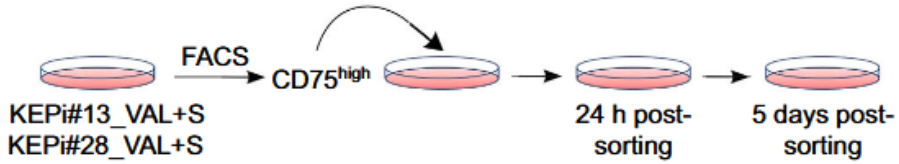
Results 3. KLF2, ERAS, and PRMT6 stabilize iPSCs in a naïve-like state

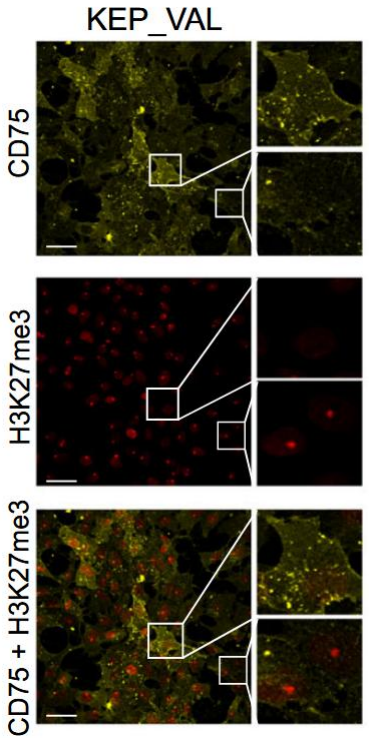
- The transduction of KEP enhanced the naïve plulotency as well as embryo colonization capability of B19.

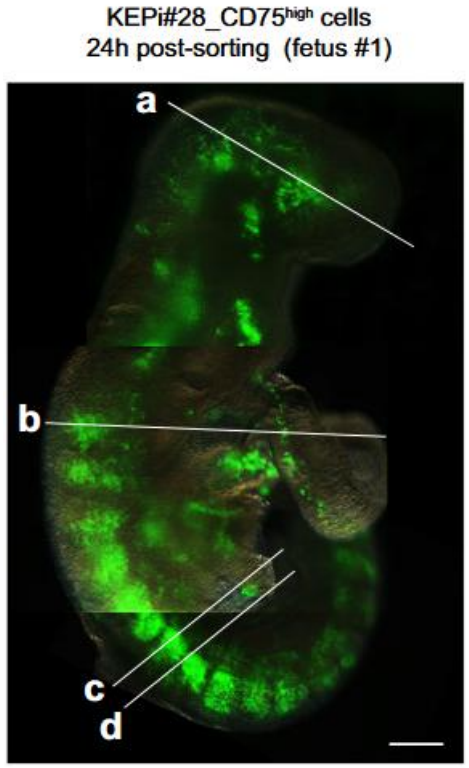


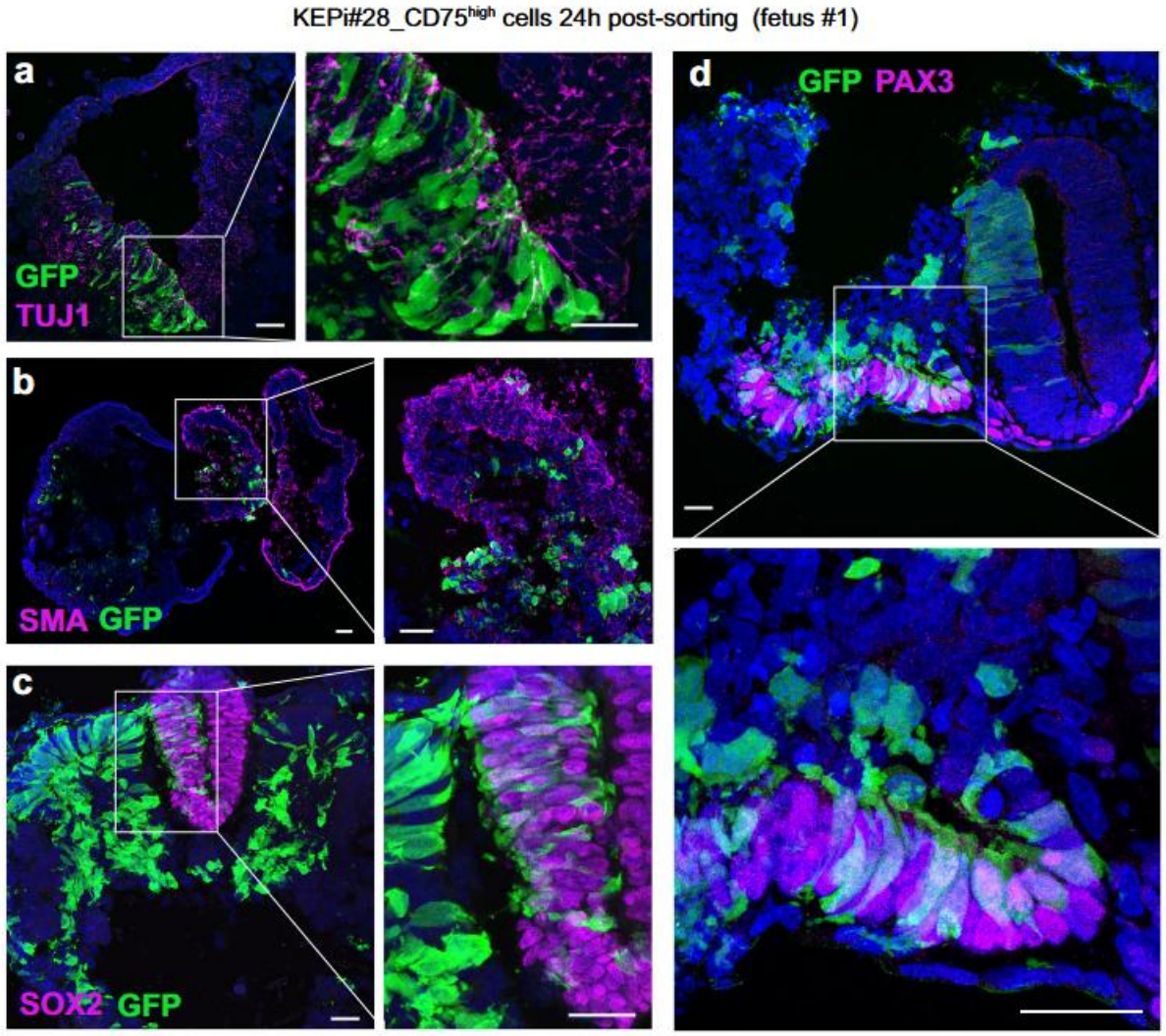


Results 4. High-CD75 KEP cells generate germline chimeras



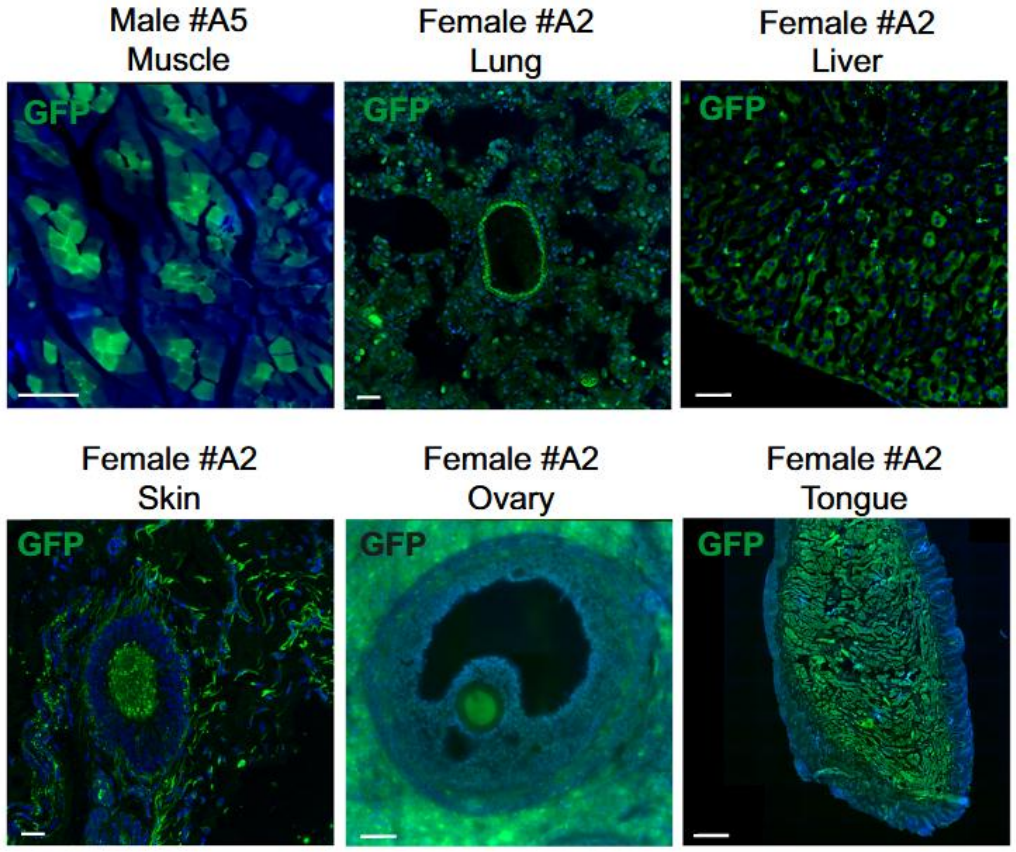


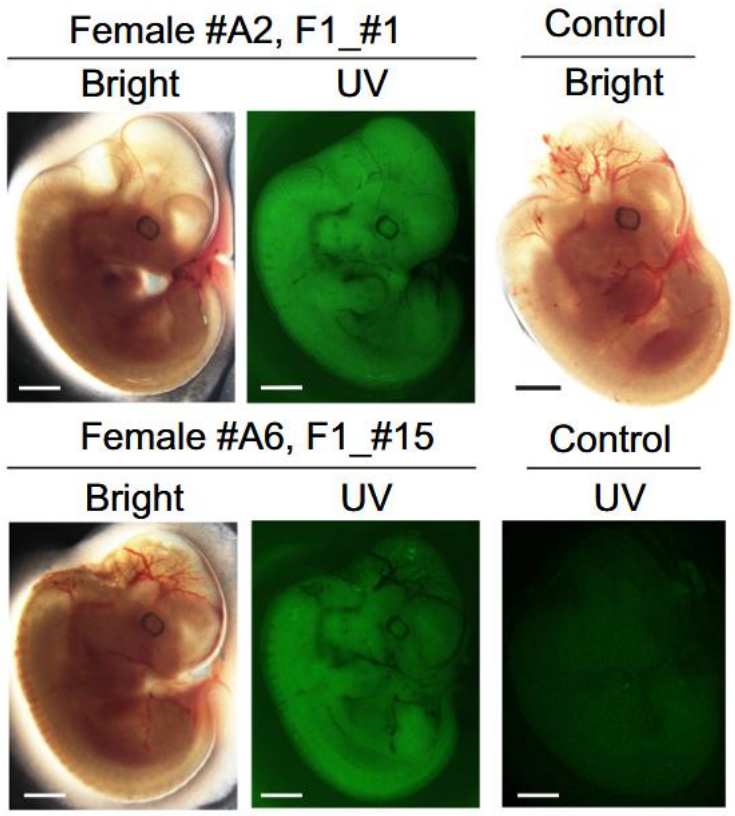




Results 4. High-CD75 KEP cells generate germline chimeras

- Virtually all analyzed organs were positive for GFP, with chimerism rates ranging from 0.01 to 100%
- The stem cells successfully integrated into the developing embryo and contributed to the animal's tissues

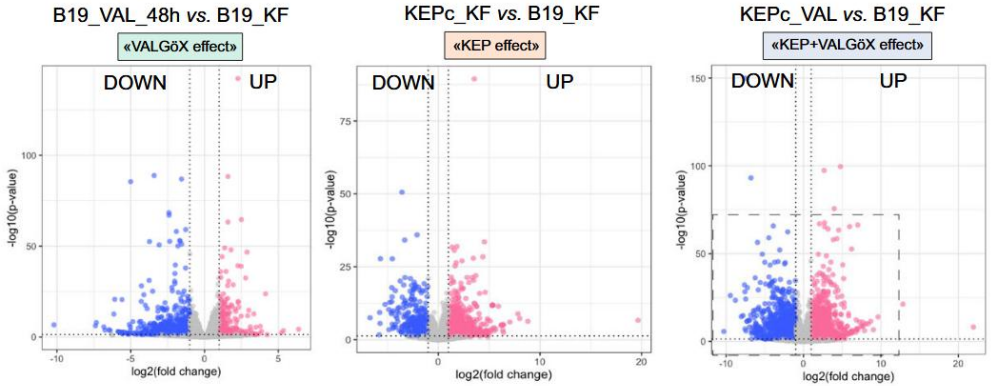


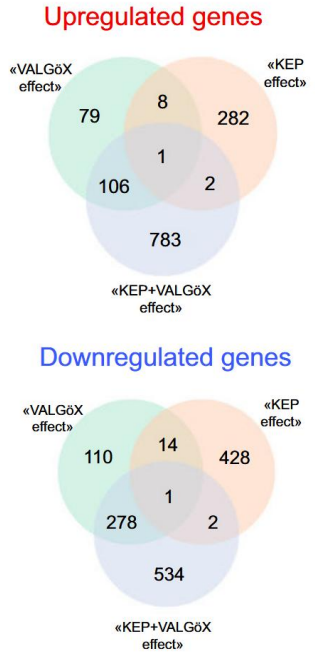


Results 5. Transcriptomic signature of enhanced embryonic colonization

- Transcriptome results emphasized the role of inhibiting the MAPK, WNT, HIPPO pathway in capturing and preserving naïve pluripotency.

«KEP+VALGöX effect» −(«VALGöX effect» + «KEP effect») = «Synergistic effect»





KEGG pathway enrichment analysis

Upregulated genes in «Synergistic effect»

Glycine, serine and threonine metabolism (p-value = 8.27E-04)
SDS, GLDC, PSAT1, CTH, AMT, ALDH7A1, GNMT

Cysteine and methionine metabolism (p-value = 0.013)
SDS, PSAT1, GSS, CTH, DNMT3A, SMS

Arginine and proline metabolism (p-value = 0.045)
SMS, CKMT1B, PYCR2, LDH7A1, CNDP1

NF-kappa B signaling pathway (p-value = 0.051)
EDARADD, ZAP70, DDX58, BTK, LTA, CD14, LTBR, GADD45G

Downregulated genes in «Synergistic effect»

Axon guidance (p-value = 8.14E-06)
EPHA7, FZD3, WNT5B, TRPC3, CXCR4, UNC5C, SSH3, NTN1, GNAI1, EFNB2, PPP3CA, EFNB3, DPYSL5, PLXNA2, LRRC4C, MYL9, EPHB3

Hippo signaling pathway (p-value = 3.27E-05)
FZD3, TGFB2, WNT5B, FZD4, WWC1, WNT8A, WNT8B, ID1, AXIN2, GDF6, TGFBR2, RASSF2, LATS2, WNT11, PPP2R2B

Wnt signaling pathway (p-value = 4.05E-05)
TLE4, FZD3, WNT5B, FZD4, PRICKLE2, WNT8A, WNT8B, AXIN2, SFRP4, PPP3CA, PLCB4, WNT11, DAAM1, ROR1, GPC4

MAPK signaling pathway (p-value = 4.59E-05)
PDGFRB, DUSP2, TGFB2, ANGPT1, BDNF, ARAF, PDGFB, TGFA, VEGFC, EGFR, NFKB1, CDC25B, TGFBR2, ELK4, PPP3CA, DUSP10, MAPKAPK2, MAP3K8, FLNC, CACNG4, FGF10

Summary & Discussion

- Building upon an optimized **VALGÖX culture medium** and a **naive pluripotency reporter system**, a cDNA library screen identified a critical combinatorial factor cocktail comprising KLF2, ERAS, and PRMT6.

- Establishing an inducible expression system and demonstrating the robust in vivo **chimeric competence** of naive pluripotent cells.

- **CD75 was identified** as an effective surface marker for enriching cells with high chimeric potential, enabling the efficient generation of chimeras through the enrichment of this naive pluripotent subpopulation.

- The transcriptomic results indicate a **synergistic effect** between the VALGöX culture regimen and KEP transgenes that enables rabbit iPSCs to enter a functional naive state.

Q & A

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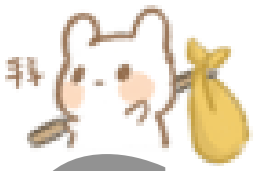
THANKS



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