

Supplementary Materials for

Flexible adaptation of male germ cells from female iPSCs of endangered *Tokudaia osimensis*

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SUPPLEMENTARY MATERIALS

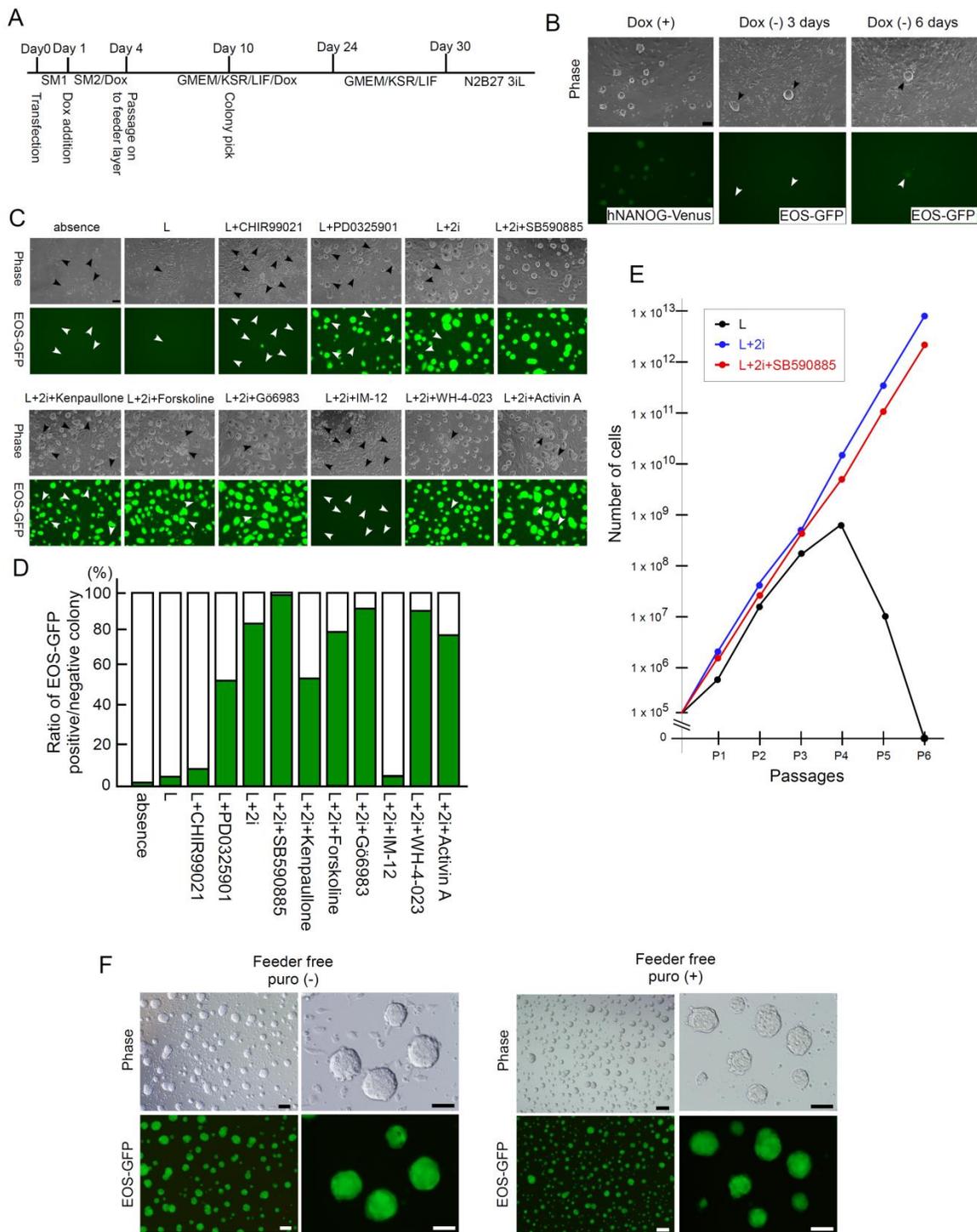


fig. S1. Determining appropriate culture conditions for *T. osimensis* iPSCs. (A)

Scheme of the derivation of *T. osimensis* iPSCs. SM1: somatic medium 1, SM2: somatic medium 2. **(B)** Dox-independent appearance of iPSC (5f1-1) colonies and their EOS-GFP

fluorescence. Arrowheads indicate colonies observed after the withdrawal of Dox. Scale bar indicates 100 μm . **(C)** Phase-contrast and fluorescent images of iPSCs (4f1-1) cultured using several chemicals and cytokines. Arrowheads indicate differentiated colonies. Scale bar indicates 100 μm . L: LIF, 2i: CHIR99021 and PD0325901. **(D)** Ratio of EOS-GFP-positive and -negative colonies cultured using several chemicals and cytokines. Open bar: EOS-GFP-negative colonies, closed bar: EOS-GFP-positive colonies. **(E)** Proliferation of cells in N2B27 medium including LIF (black), LIF+2i (blue), and LIF+2i+SB590885 (red). **(F)** Puromycin treatment concentrated undifferentiated *T. osimensis* iPSCs in feeder-free culture. Scale bar, 100 μm .

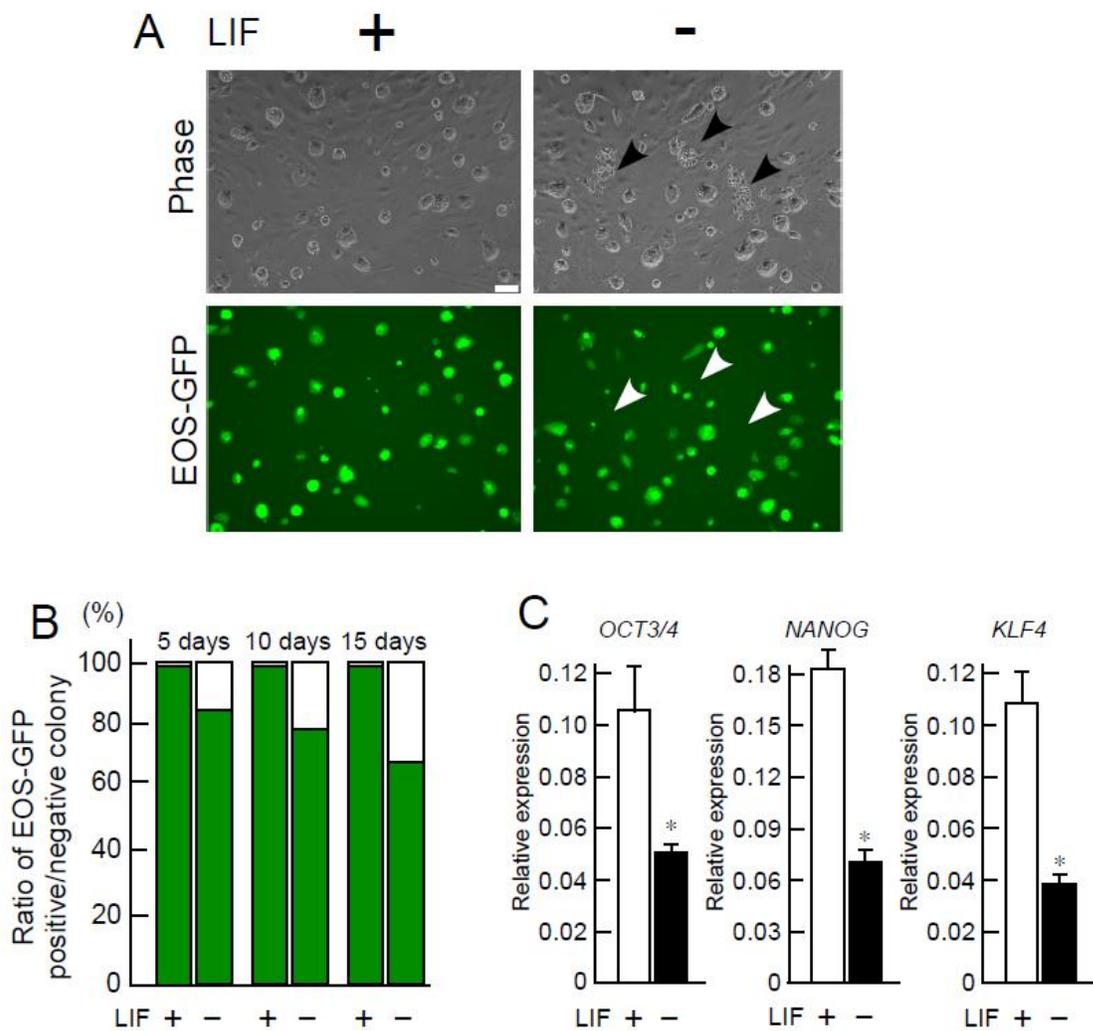


fig. S2. LIF-dependent maintenance of naïve pluripotency of *T. osimensis* iPSCs. (A) *T. osimensis* iPSCs (4f1-1) that had been cultured using N2B27 3i medium in the presence (+) or absence (-) of LIF. Despite the presence of 3i, iPSCs differentiated according to the withdrawal of LIF. Arrowheads indicate differentiated iPSCs. Scale bar indicates 100 μ m. (B) Ratio of EOS-GFP-positive and -negative colonies cultured in the presence (+) or absence (-) of LIF. The number of EOS-GFP-negative cells increased with the culture period (5, 10, and 15 days) after the withdrawal of LIF (LIF-) from N2B27 3iL culture medium. (C) qRT-PCR of pluripotency-related genes, *Oct3/4*, *hNANOG*, and *Klf4*, showed reduced expression after withdrawal of LIF from N2B27 3iL culture medium. The means

and \pm SDs from at least three independent experiments are shown. Values were compared using *t* tests. **P* < 0.05.

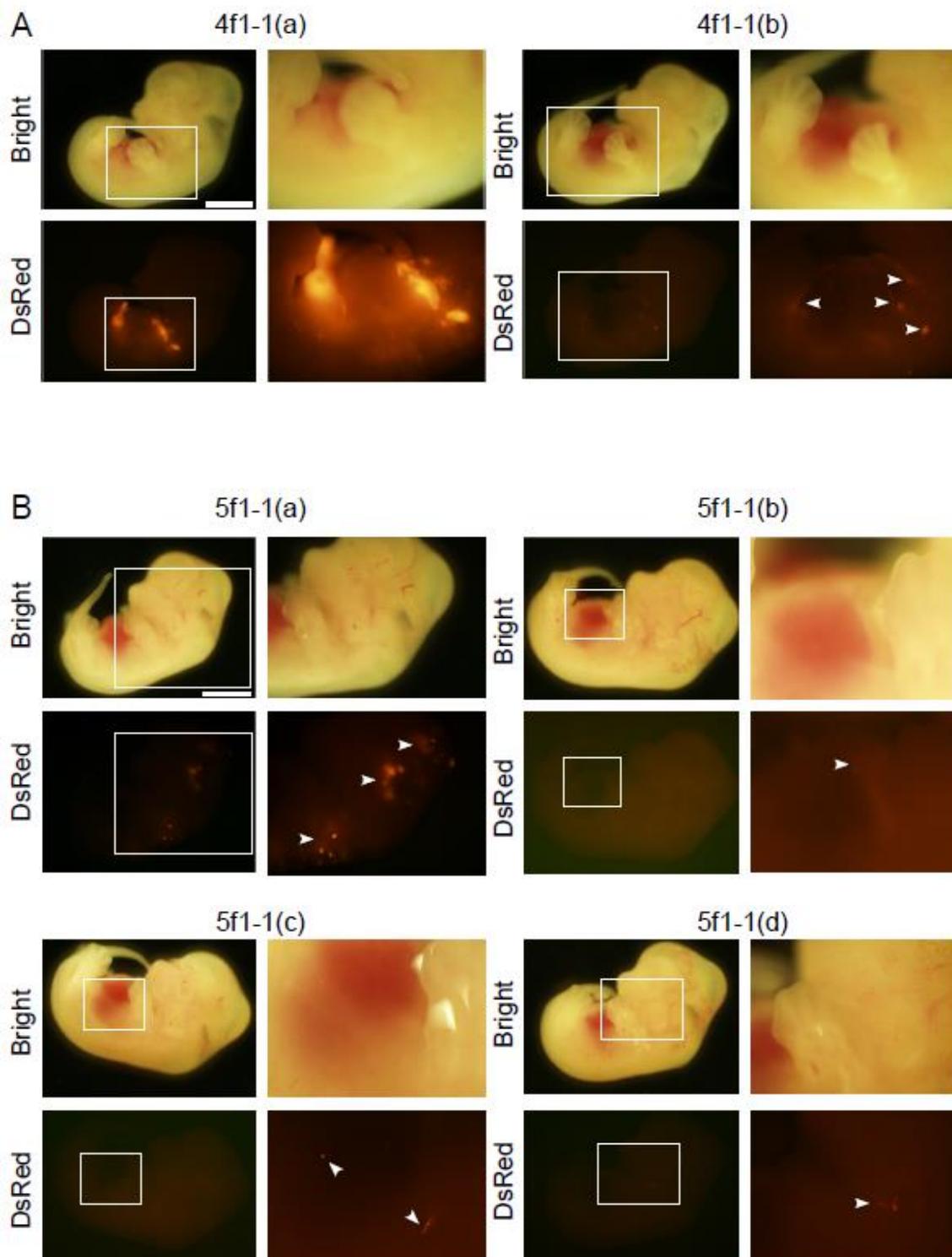


fig. S3. Chimeric contribution of *T. osimensis* iPSCs in interspecific embryos. (A and B) Chimeric distribution of 4f1-1 cells (A: two embryos) and 5f1-1 cells (B: four embryos) in 12.5 dpc interspecific chimera are shown by the DsRed signals. The boxed region is enlarged at the right. Arrowheads indicate DsRed signals. Scale bars indicate 500 μm .

table S1. Characterization of *T. osimensis* iPSC lines.

Cell lines	ECC	AP	RT-PCR	Karyotype	Teratoma	LIF withdraw	Chimera
4f1-1	+	+	+	+	+	+	+
5f1-1	+	+	+	+	+	n.d.	+
5f2-7	n.d.	+	+	+	+	n.d.	n.d.
5f2-11	n.d.	+	+	+	+	n.d.	n.d.
5f2-14	n.d.	+	+	+	+	n.d.	n.d.
5f2-18	n.d.	+	+	+	+	n.d.	n.d.

ECC: evaluation of culture condition, AP: alkaline phosphatase, +: examined, n.d.: not determined.

table S2. PCR primers.

Gene	Forward (5' to 3')	Reverse (5' to 3')
Degenerate primers		
<i>OCT3/4</i>	GACACCTGGCTTCRGAYTT	CAGGGTGAGCCCCACRTC
<i>KLF4</i>	CACCTGGCGAGTCTGACAT	MGACGCCTTCAGCACRAACT
<i>SOX2</i>	AAYGCCTTCATGGTRTGGTC	ATGTAGGTCTGCGAGCTGGT
<i>cMYC</i>	ATGCCCCCTCAACGKAGCTT	AGGTACARGCTGGAGGTGGA
<i>NANOG</i>	AAGCAGAAGATGCGGACTGTGTTT	CTTCCAGATGCGTTCACCAGATAGC
<i>G3PDH</i>	GWGTSAAACGGATTTGGYCGT	CTAAGCAGTTGGTGGTGCAG
Endo-primers		
<i>OCT3/4</i>	AGACTTTGCAGCCTGAGGC	CCTGGGACTCCTCGGCAC
<i>KLF4</i>	GTGCAGCTTGCAGCAGTAAC	CAGGGCCGCTGCTCGCT
<i>SOX2</i>	AATGCCTTCATGGTGTGGTC	ATGTAGGTCTGCGAGCTGGT
<i>NANOG</i>	AAGCAGAAGATGCGGACTGTGTTT	CTTCCAGATGCGTTCACCAGATAGC
<i>G3PDH</i>	TGTTCCAGTATGACTCCACC	CATTGCTGACAATCTTGAGA
Tg-primers		
<i>Oct3/4</i>	AGCACGAGTGGAGAGCAACT	CCAAGGTGATCCTCCTCTTCTGC
<i>cMyc</i>	ATGCCCCCTCAAGAA	GGCTCGGGGACAGGGGC
<i>NANOG</i>	GACCAGAACTGTGTTCTCTTC	CACACCATTGCTATTCTTCGG

R:A or G, Y: C or T, M: A or C, K: G or T, W: A or T, S: C or G