Supplemental Data

Radical acceleration of nuclear reprogramming by chromatin remodeling with the transactivation domain of MyoD

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Supplemental Materials and Methods

Immunoblotting

MEFs were transduced with MyoD-Oct4 fusion genes and analyzed with immunoblotting five days after transduction. All antibodies are listed in supplemental Table S1. SuperSignal West Dura (Thermo Scientific) was used to detect chemiluminescence signal.

Fluorescence microscopy

iPSCs were fixed with 4% formaldehyde for 10 min and permeabilized with 0.5% Triton X-100 for 3 min. Cells were then incubated with primary antibody and secondary antibody for 1 hr each at 25ºC. DNA was counterstained with Hoechst 33342. Used antibodies are listed in Table S1. Fluorescence signal was captured with a 10x A-Plan Ph1 Var1 objective (numerical aperture 0.25) and an AxioCam charge coupled device camera attached to an Axiovert 200M fluorescence microscope (all from Zeiss). Photoshop 7.0 (Adobe Systems) was used for image processing.

Alkaline phosphatase staining

Alkaline phosphatase was detected with an Alkaline Phosphatase Detection Kit (Millipore SCR004).

Flow cytometry

The percentage of GFP-positive or SSEA1-positive cells at each time point was determined with a FACSCalibur flow cytometer and analyzed using CellQuest Pro software (both BD Biosciences).

Quantitative RT-PCR (qRT-PCR)

cDNA for mRNA was prepared from iPSC colonies using a Cells-to-cDNA II kit (Ambion). qRT-PCR was performed with GoTaq qPCR Master mix (Promega) on a Realplex 2S system (Eppendorf). PCR primer sequences are listed in Table S2. Expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used to normalize the expression levels of mRNAs. The feeder-free ESC line CGR8.8 was used as a positive control.

DNA microarray analysis

RNA was prepared from CGR8.8 cells, MEFs, and a mouse iPSC clone prepared with the fusion gene between the M_3 domain of MyoD and Oct4 (M_3O-iPSC) on day 60 with the PureLink RNA total RNA purification system (Invitrogen). RNA was amplified and labeled using the Agilent Quick AmpLabeling Kit (Agilent Technologies) following the manufacturer's protocol. cRNA was hybridized overnight to Agilent Whole Murine Genome Oligo Microarray using the Agilent Gene Expression Hybridization Kit. The fluorescence signals of the hybridized microarrays were detected using Agilent's DNA Microarray Scanner. The Agilent Feature Extraction Software was used to read out and process the image files. Data were processed and visualized with Spotfire DecisionSite for Functional Genomics software. DNA microarray data have been deposited in the NCBI GEO database under the accession number GSE22327.

Karyotyping of human iPSCs

Adherent cells were arrested with colcemid, harvested, treated with 75mM KCl hypotonic solution, and fixed with methanol and acetic acid at 3:1. The cells were spread onto glass slides and stained with Wright-Giemsa stain. G-banded metaphases were evaluated using an Olympus BX61 microscope outfitted with 10x and 100x objectives. Metaphase cells were imaged and karyotyped using Applied Spectral Imaging (ASI) software.

Aggregation chimera and teratoma formation

Ten M_3O-iPSCs of a cloned line were transferred into a microdrop of KSOMaa solution (Millipore) with a zona-free 8-cell stage mouse embryo of the ICR strain (albino) after brief exposure to acidic Tyrode's solution (Millipore). Aggregated morula stage embryos at 2.5 days post coitum (dpc) that contained GFP-positive iPSCs were transferred into the uteri of 2.5 dpc pseudopregnant recipient mice. Embryos at 13.5 dpc were analyzed for chimera formation with X gal stain or for germline transmission with a fluorescence microscope. To prepare teratomas, one million cloned mouse or human M_3O-iPSCs were injected into the limb muscle of NOD/SCID mice. Teratomas were fixed with 10% formalin and embedded with paraffin after three weeks for mouse iPSCs and eight weeks for human iPSCs. Five-μm thick sections were stained with haematoxylin and eosin for histological analysis.

Chromatin immunoprecipitation (ChIP)

ChIP was performed as described in the instruction of the EZ Magna ChIP G kit (Millipore). All antibodies are listed in Table S1. PCR primer sequences are listed in Table S2. PCR amplification levels were first normalized against the value obtained with control IgG. The normalized values with ESCs or MEFs were then defined as 1.0 depending on antibodies to obtain relative expression levels in other cells.

DNA methylation analysis

Genomic DNA from mouse iPSCs was treated with bisulfite with an EZ DNA Methylation-Gold kit (Zymo Research). The DNA sequence at the *Oct4* proximal promoter region was amplified with PCR using the primers listed in Table S2 and cloned into the pCR2.1-TOPO vector (Invitrogen) for sequencing.

Table S1. Antibodies used in immunoblotting, immunofluorescence staining and ChIP

Immunoblotting (primary antibodies)

Antigen	Manufacturer	Catalog #
Oct4	Santa Cruz Biotechnology	sc-9081
Histone H2A	IMAGENEX	IMG-358

Immunoblotting (secondary antibodies)

Name	Manufacturer	Catalog #
Peroxidase-conjugated anti-rabbit IgG	Jackson ImmunoResearch	211-032-171
Peroxidase-conjugated anti-mouse IgG	Jackson ImmunoResearch	115-035-174

Immunofluorescence staining (primary antibodies)

Antigen	Manufacturer	Catalog #
Oct4	Santa Cruz Biotechnology	sc-8628
Nanog	Abcam	ab21624
SSEA1	R&D Systems	FAB2155P
SSEA4, Alexa Fluor 488-labeled	BD Biosciences	560308
TRA-1-60, Alexa Fluor 555-labeled	BD Biosciences	560121
TRA-1-81, phycoerythrin-labeled	BD Biosciences	560161

Immunofluorescence staining (secondary antibodies)

Name	Manufacturer	Catalo g#
PE-labeled anti-mouse Ig(M+G)	BD Biosciences	550589
Alexa Fluor 555-labeled anti-rabbit IgG	Invitrogen	A21429
Alexa Fluor 488-labeled anti-goat IgG	Invitrogen	A11055

ChIP

Antigen	Manufacturer	Catalog #
Oct4	Santa Cruz Biotechnology	sc-9081
Sox2	Santa Cruz Biotechnology	sc-17320
Parafibromin	Bethyl Laboratories	A300-170A
Paf1	Abcam	ab-20662
Leo1	Abcam	ab-70630
H3K4me3	Abcam	ab-1012
H3K9ac	Abcam	ab-4441
H3K14ac	Millipore	07-353
H3K9me3	Millipore	07-523
H3K27me3	Millipore	07-449
Control IgG	Santa Cruz Biotechnology	sc-2027

Table S2. Primers used for quantitative RT-PCR, bisulfite sequencing and ChIP

Quantitative RT-PCR (mouse)

Gene	Forward	Reverse
Oct4 endogenous	TCTTTCCACCAGGCCCCCGGCTC	TGCGGGCGGACATGGGGAGATCC
Sox2 endogenous	AAAGGAGAGAAGTTTGGAGCCCGA	GGGCGAAGTGCAATTGGGATGAAA
Nanog	AGCAGAAGATGCGGACTGTGTTCT	CCGCTTGCACTTCATCCTTTGGTT
Thy1	GCCTGACCCGAGAGAAGAAGAAG	TGGTGGTGAAGTTCGCTAGAGTAAG
Col6a2	CCACCACTGAAAGGAACAACAA	TCCAACACGAAATACACGTTGAC
Fgf7	CCATGAACAAGGAAGGGAAA	TCCGCTGTGTGTCCATTTAG
GAPDH	TGCACCACCAACTGCTTAG	GATGCAGGGATGATGTTC

Quantitative RT-PCR (human)

Gene	Forward	Reverse
OCT4 endogenous	CCTCACTTCACTGCACTGTA	CAGGTTTTCTTTCCCTAGCT
SOX2 endogenous	CCCAGCAGACTTCACATGT	CCTCCCATTTCCCTCGTTTT
KLF4 endogenous	GATGAACTGACCAGGCACTA	GTGGGTCATATCCACTGTCT
c-MYC endo.	TGCCTCAAATTGGACTTTGG	GATTGAAATTCTGTGTAACTGC
NANOG	TGAACCTCAGCTACAAACAG	TGGTGGTAGGAAGAGTAAAG
LIN28	GAGCATGCAGAAGCGCAGATCAAA	TATGGCTGATGCTCTGGCAGAAGT
DPPA2	AGGCTTCATAGGCATGCTTACCCT	TGAAGCCTTGCTCTCTTGGTCACT
DPPA4	AGACACAGATGGTTGGGTTCACCT	TGCACTCACTCTCCCTTCTTGCTT
GDF3	ACACCTGTGCCAGACTAAGATGCT	TGACGGTGGCAGAGGTTCTTACAA
REX1	TGAATAGCTGACCACCAGCACACT	ACAGGCTCCAGCCTCAGTACATTT
TERT	TGTGCACCAACATCTACAAG	GCGTTCTTGGCTTTCAGGAT
TDGF1	TGCCCAAGAAGTGTTCCCTGTGTA	AAAGTGGTAGTACGTGCAGACGGT
GAPDH	AACAGCGACACCCACTCCTC	CATACCAGGAAATGAGCTTGACAA

Bisulfite sequencing

Gene	Forward	Reverse
Oct4	AGGTTGAAAATGAAGGTTTTTT	TCCAACCCTACTAACCCATCACC

ChIP

Region	Forward	Reverse
Oct4 Region 1	GGAACTGGGTGTGGGGAGGTTGTA	AGCAGATTAAGGAAGGGCTAGGACGAGAG
Oct4 Region 2	AGGTCAAGGGGCTAGAGGGTGGGATT	TGAGAAGGCGAAGTCTGAAGCCA
Oct4 Region 3	TAGGAGCTCTTGTTTGGGCCATGT	ACAAGGGTCTGCTCGTGTAAAGGT
Sox2 Region 1	TTTTGGTTTTTAGGGTAAGGTACTGGGAAG	CCACGTGAATAATCCTATATGCATCACAAT
Sox2 Region 2	CACATGAAGGAGCACCCGGATTAT	TCCGGGAAGCGTGTACTTATCCTT

Supplemental Figure Legends

Figure S1. Immunoblotting of MyoD-Oct4 fusion proteins.

Expression of transduced MyoD-Oct4 fusion genes was evaluated with an antibody against Oct4 (top). Expression of histone H2A was examined as a loading control (bottom). Bands correspond to the predicted molecular mass of each protein. Identities of extra bands marked with asterisks are unknown.

Figure S2. ChIP analyses of the *Sox2* gene.

(A) Binding of Oct4 and Sox2 at the enhancer.

(B) Binding of parafibromin and the levels of histone modifications associated with active genes on day 9.

(C) Levels of histone modifications associated with suppressive genes on day 9.

Figure S3. ChIP analyses on day 9 of the *Oct4* gene comparing transduction of one (1F), two (2F), three (3F) and four (4F) transcription factor genes.

(A) Transcription factor binding.

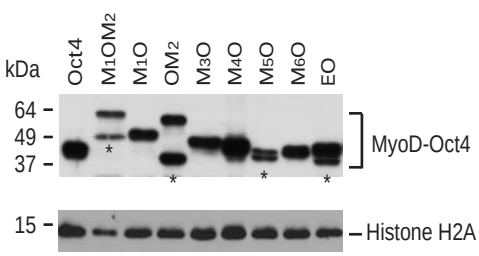
(B) Histone modifications associated with gene activation.

Figure S4. ChIP analyses on day 9 of the *Sox2* gene comparing transduction of one (1F), two (2F), three (3F) and four (4F) transcription factor genes.

(A) Transcription factor binding at the enhancer.

(B) Histone modifications associated with gene activation and suppression.

Fig. S1 Kikyo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Oct4
M1OM2
M1O
OM2
M3O
M4O
M5O
M6O
EO
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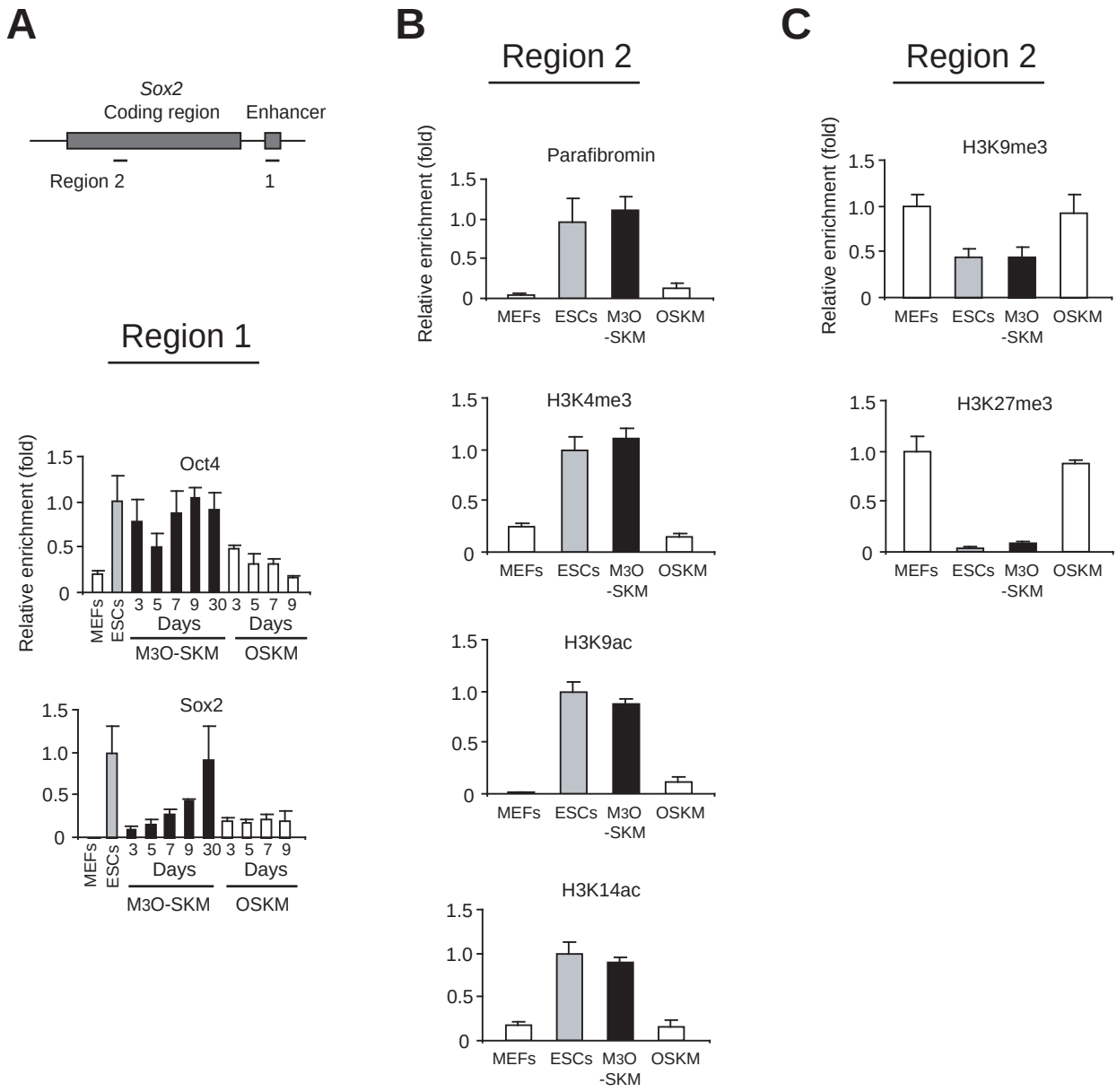
MyoD-Oct4
Histone H2A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Sox2
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Enhancer
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Region 1
Oct4
Relative enrichment (fold)
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1.0
0.5
0
MEFs
ESCs
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Region 2
Parafibromin
H3K4me3
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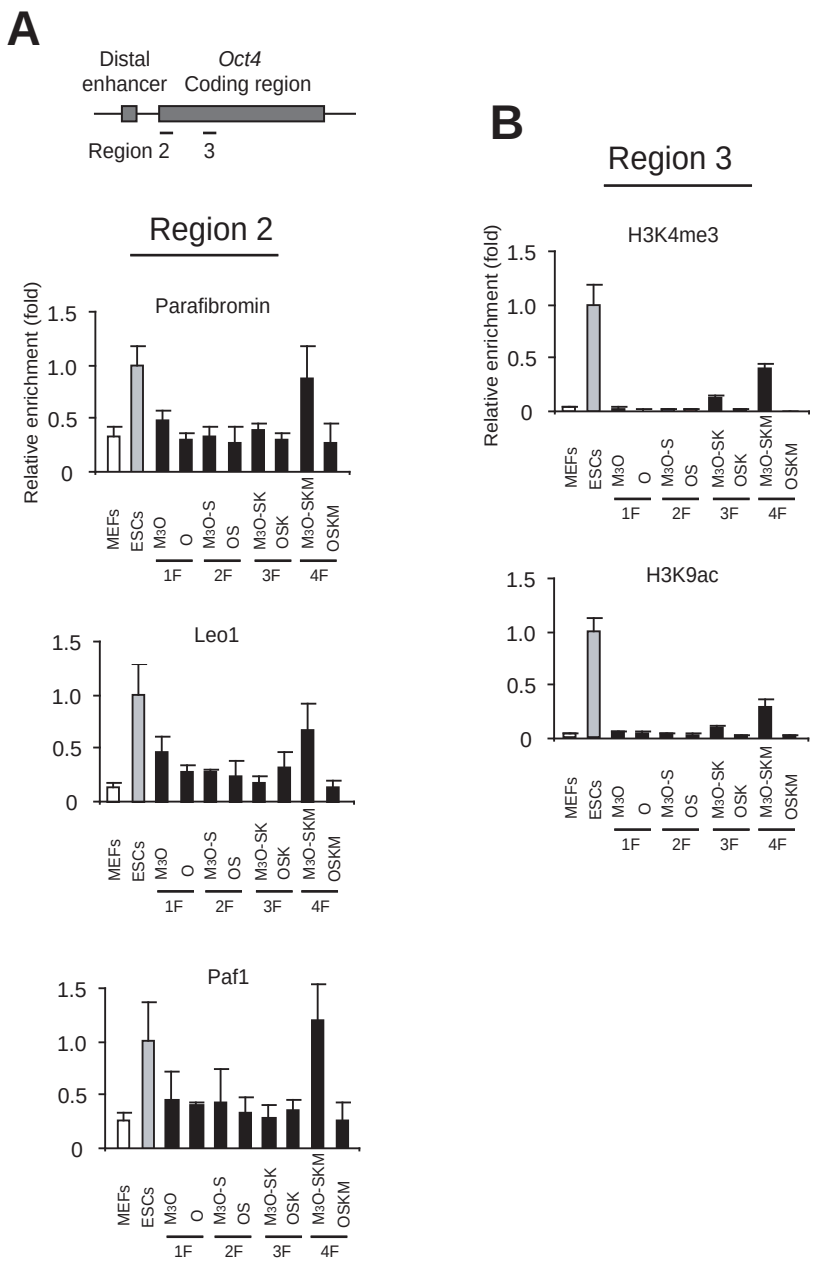
A
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Region 2
Parafibromin
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Paf1
Relative enrichment (fold)
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1.0
0.5
0
MEFs
ESCs
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O
M3O-S
OS
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3F
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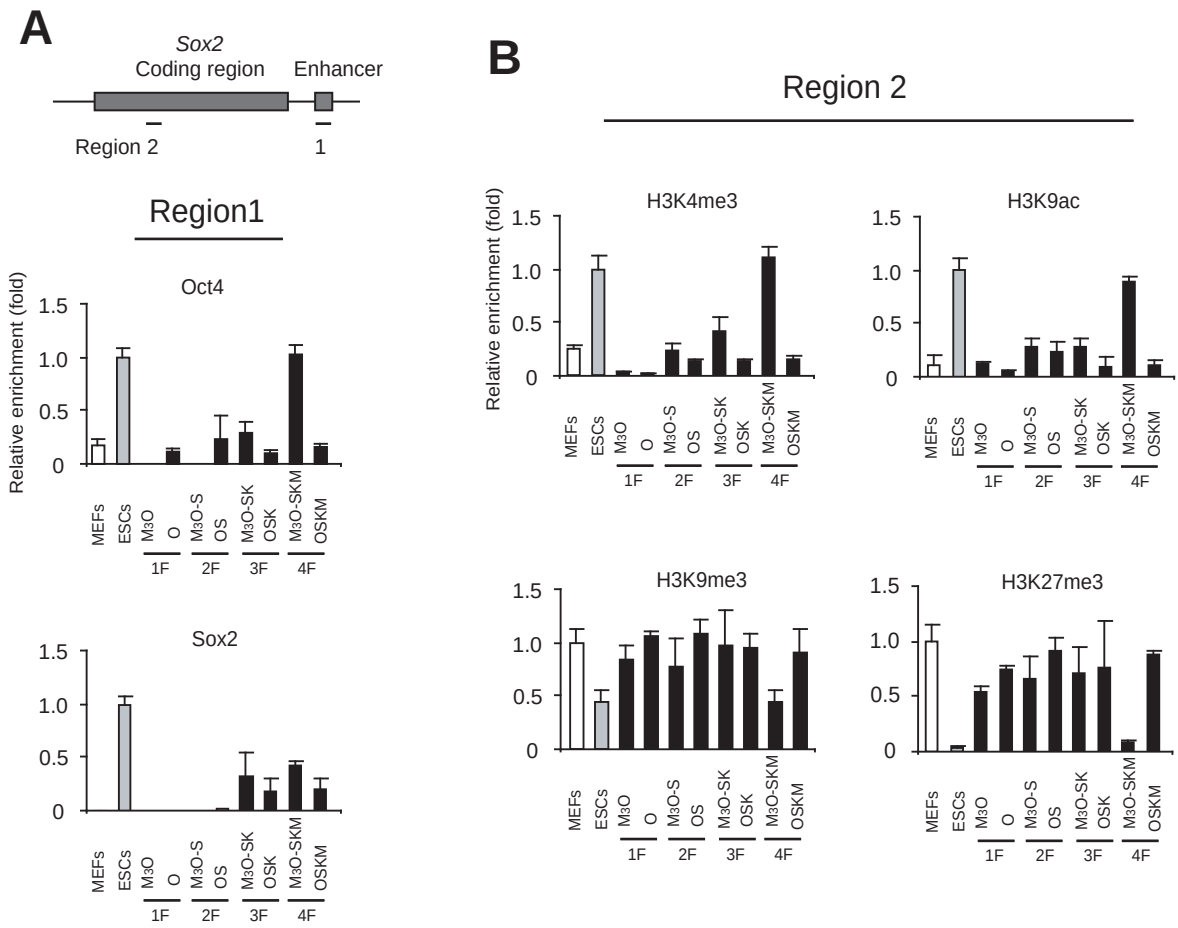
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Coding region
Enhancer
Region 2
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Region1
Oct4
Relative enrichment (fold)
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1.0
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MEFs
ESCs
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M3O-S
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OSK
M3O-SKM
OSKM
1F
2F
3F
4F
Sox2
B
Region 2
H3K4me3
H3K9ac
H3K9me3
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