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Supplemental Information

In Vitro Modeling of Human Germ Cell Development Using Pluripotent Stem Cells

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Supplemental Experimental Procedures

Crystal violet staining and MTT assay

Cell were fixed by 4% paraformaldehyde (PFA) and then incubated with 0.05% crystal violet buffer (Sigma-Aldrich) for 30 min before photograph. For MTT assay, cells were incubated with 0.5 mg/mL MTT solution (Sigma-Aldrich) at 37 ℃ for 2 h, washed with DMSO, and then measured for absorbance intensity at 490 nm and 650 nm.

Karyotyping

Karyotyping of the hESC and iPSC lines was carried out every 10 passages. Each cell line was analyzed by standard G-banding techniques and interpreted with the International System for Human Cytogenetic Nomenclature.

Western Blots

Western blots were performed according to standard protocols (Zhang et al., 2016) and the final fluorescent signals against targeted proteins were detected using the Li-COR Odyssey system (LI-COR Biosciences, Lincoln, NE). Primary antibodies were used: PLZF (R&D, MAB2944), Tubulin (Santa Cruz, sc-69966), MVH (Abcam, ab27591), DAZL (Abcam. ab34139), β-actin (Cell Signaling Technology, CST#3700).

Molecular diagnosis of Y-chromosomal microdeletions

The molecular diagnosis of Y-chromosomal was performed following the protocols by European Academy of Andrology and the European Molecular Genetics Quality Network (Simoni et al., 1999; Simoni et al., 2004). Primers are listed in Supplemental Table S5.

RNA sequencing and analyses

Biological duplicates were prepared from human PSCs and differentiated cells on day 12 (mixed population with more than 80% PLZF+ cells), differentiated population with enforced PLZF expression, and CD90+ cells isolated from human testes. *In vitro* derived haploid cells (pooled from two independent experiments) and spermatids pooled from testes of two OA patients were harnessed by flow cytometry through Hoechst 33342 staining. RNA-seq library was constructed using NuGen Ovation Ultralow System (NuGen) following the manufacture's instruction. The Resulting libraries were size selected by agarose gel electrophoresis, and subsequently sequenced using the Illumina Hiseq-2500 platform with a 2x50 bp modality. Raw sequencing reads mapped to the human reference genome hg19 using TopHat2 (Kim et al., 2013). FPKM (fragments per kilobase per million mapped reads) was computed using HTseq (Anders et al., 2015). Genes for RNA-seq analyses were obtained from http://www.ebi.ac.uk/QuickGO/. Genes/transcripts for germ cell related factors include: GO_0001541, GO_0007276, GO_0007281, GO_0007283, GO_0007286, GO_0007310, GO_0007530, GO_0007548, GO_0008354, GO_0008406, GO_0008584, GO_0009566, GO_0019953, GO_0036099, GO_0042078, GO_0046661, GO_0048232, GO_0048477, GO_0048515, GO_0072520; Databases for genes/transcripts in stem cell functions include: GO0019827, GO_0072089, GO_0048867, GO_0048866, GO_0048865, GO_0048864, GO_0048863, GO_0030718, GO_0017145; Genes/transcripts in three germ layers: GO_0007398，GO_0007492，GO_0007498.

References cited in Supplemental Information

Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq--a Python framework to work with high-throughput sequencing data. Bioinformatics *31*, 166-169.

Easley, C.A.t., Phillips, B.T., McGuire, M.M., Barringer, J.M., Valli, H., Hermann, B.P., Simerly, C.R., Rajkovic, A., Miki, T., Orwig, K.E., *et al.* (2012). Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell reports *2*, 440-446.
Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome biology *14*, R36.
Simoni, M., Bakker, E., Eurlings, M.C., Matthijs, G., Moro, E., Muller, C.R., and Vogt, P.H. (1999). Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int J Androl *22*, 292-299.
Simoni, M., Bakker, E., and Krausz, C. (2004). EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. Int J Androl *27*, 240-249.
Zhang, J., Wang, Q., Wang, M., Jiang, M., Wang, Y., Sun, Y., Wang, J., Xie, T., Tang, C., Tang, N., *et al.* (2016). GASZ and mitofusin-mediated mitochondrial functions are crucial for spermatogenesis. EMBO reports *17*, 220-234.
Zhang, X., Li, B., Li, W., Ma, L., Zheng, D., Li, L., Yang, W., Chu, M., Chen, W., Mailman, R.B., *et al.* (2014). Transcriptional repression by the BRG1-SWI/SNF complex affects the pluripotency of human embryonic stem cells. Stem cell reports *3*, 460-474.

Supplemental Figure Legends

Figure S1. Derivation of SLCs and haploid cells. (A) Apoptosis analyses using FITC-annexin V and propidium iodine were performed on different PSC lines at day 5 post differentiation using Easley's protocol (Easley et al., 2012) and the optimized method in this study. (B) Fold changes of percentage of PLZF+ cells in the presence or absence of vitamin C (Vc) during differentiation of H1 ESCs, measured by flow cytometry. A representative image of PLZF protein expression detected by Western Blots. (C) Flow cytometry of PLZF+ cells with different treatment of vitamin C and 2–mecaptoethanol (2ME) combination. (D) Percentage of PLZF+ cells (upper panel) and haploid population (lower panel) determined *via* flow cytometry using various concentration of xeno-free serum replacement in differentiation medium. (E) Western Blots were performed on H1 parental ESCs and the differentiated population on day 15 post ESC induction.

Figure S2. Characterization of SLCs and haploid cells. (A) FISH analyses on sex chromosomes

(right panel) and Chr 7 left panel) of H1 ESCs, sorted haploid cells derived from H1 ESCs, and diploid cells collected from the same batch of differentiation culture. Scale bar: 5 μm. (B) Sorting efficiency of PLZF+ SLCs from H1 ESCs used in imprinting analyses by flow cytometry.

Figure S3. Genome-wide transcription profiling of SLCs and haploid cells. (A) Sorting efficiency of CD90+ SSCs from biopsied testis samples used in RNA-seq by flow cytometry. (B) Genes analyzed in Figure 2B-C. We included 761 genes that were related to germ cell development from online database and as well displayed low expression in undifferentiated PSCs. In addition, we included 926 transcripts that were highly expressed in CD90+ cells and 350 transcripts that were associated with PSCs, thus totally the levels of 1,815 transcripts were analyzed on *in vitro* derived SLCs and *in vivo* CD90+ spermatogonia in Figure 2B. (C) Heat-map on 33 genes that were involved in germ cell development and genes that were expressed in stem cells were analyzed on PSCs, SLCs, and CD90+ cells collected from biopsied samples. (D) Real-time RT-PCR on transcript levels of genes that participated in germ cell development at various time points along H1 hESC differentiation. (E) Real-time RT-PCR assays on H1 hESCs, induced population at day 12 post hESC differentiation, and CD90+ cells isolated from biopsied human testes. (D-E) Data are shown as mean ± one s.e.m. from three independent experiments. (F) Percentage of transcripts upregulated in genes related to three germ layer specification. (G) Heat-map of RNA-seq on 98 transcripts (from 61 genes) in meiosis were analyzed on PSCs, *in vitro*-derived haploid cells pooled from two experiments, and pooled spermatids collected from two OA patients. Cluster analysis displayed that *in vitro* derived haploid cells closely resembled *in vivo* developed spermatids.

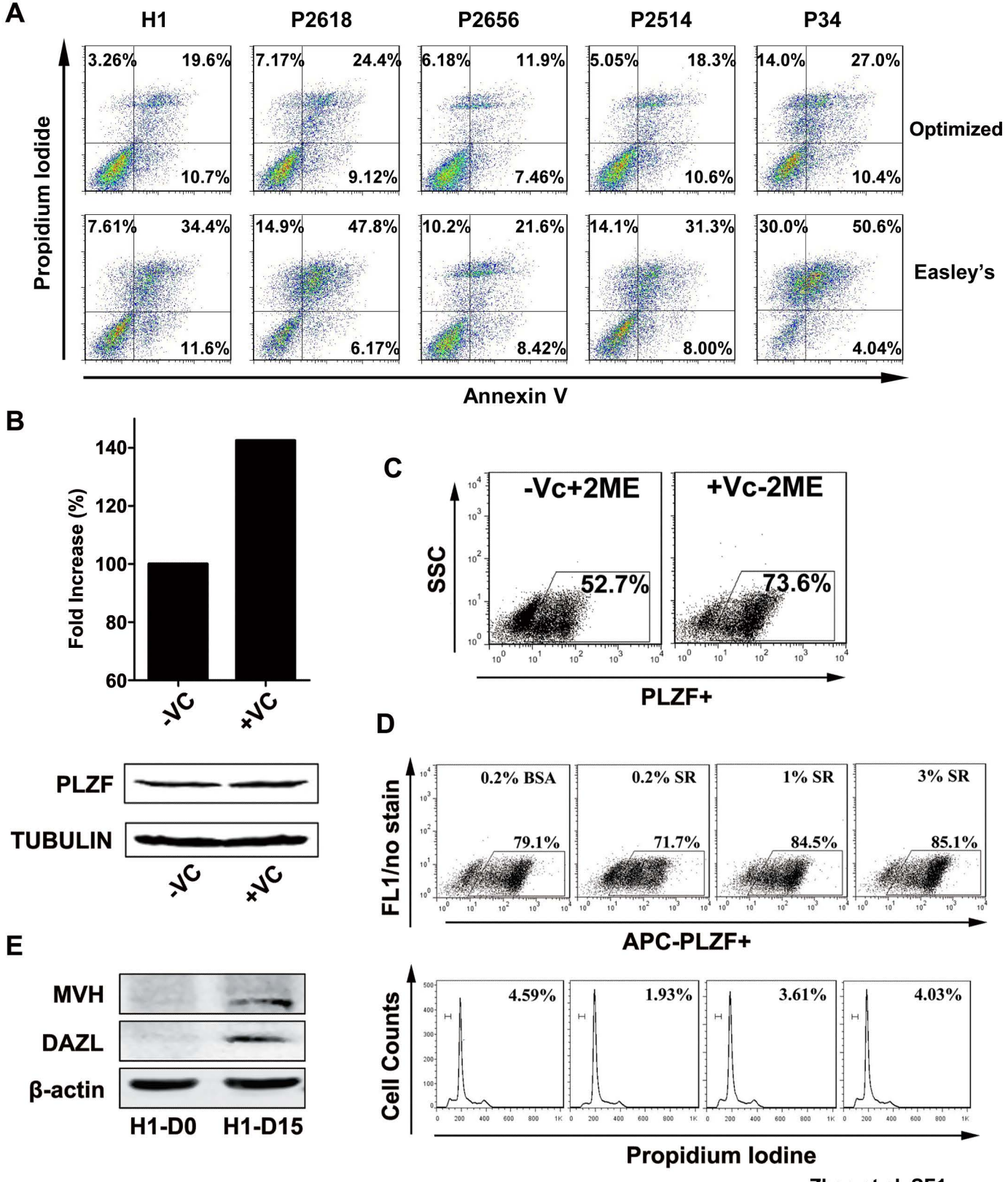
Figure S4. Targeting human *NANOS3* gene. (A) The start position of *NANOS3* deletion from

three PSC clones was labelled (with red arrow at the end of yellow highlighted sequences) in its cDNA sequences (NM_001098622). Sequences underlined: sgRNA targeting sequence. Sequences highlighted in blue: ending of first exon. (B) Sequencing of wildtype PSCs and PSCs clone #19, #23, and #24 with NANOS3 deletions. Please note, the NANOS3 deletion in #19 PSCs covers part of first exon, splicing donor site, and first intron, and in turn part of first intron is fused with first exon due to disrupted splicing. (C) Real-time RT-PCR analyses of relative *NANOS3* expression on wild-type H1 ESCs or ESCs with various *NANOS3* deletions at day 13 post differentiation. Data are shown as mean ± one s.e.m. from 6 independent experiments. ***: $p<0.001$

Figure S5. Establishing iPSC lines from NOA patients and normal controls. (A) PCR detection of Y-Chr microdeletion with STS in derived iPSC lines and control hESCs (H1). (B) Predicted copy numbers of genes at AZFc region in two NOA-iPSC lines. (C) Real-time PCR analyses of copy numbers of genes at AZFc region in control H1 ESCs and established iPSC lines. Data are shown as mean ± one s.e.m. from 3 independent experiments. (D) Origin of established iPSC lines examined by STR with PCR, compared to H1 and H9 ESCs used in the lab and their parental fibroblasts (Fib). (E) Representative karyotyping images from iPSC lines derived from NOA patients, P34 normal control, and H1 ESCs.

Figure S6. Characterization of iPSC lines from NOA patients and controls. (A) Immunofluorescence of pluripotency factors (including OCT4, NANOG, TRA-1-60 and TRA-1-81) on established iPSC lines. Scale bar: 100 μm. (B) Real-time RT-PCR analyses of pluripotency-related genes on NOA-iPSCs, compared to control H1 ESCs and their parental fibroblasts. Data

are represented as mean ± one s.e.m. from three independent experiments. (C) Images of embryoid bodies from differentiated iPSC lines and control H1 ESCs. Scale bar: 200 um.


A
H1
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P2656
P2514
P34
Optimized
Easley's
Propidium Iodide
Annexin V
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7.17%
24.4%
9.12%
6.18%
11.9%
7.46%
5.05%
18.3%
10.6%
14.0%
27.0%
10.4%
7.61%
34.4%
11.6%
14.9%
47.8%
6.17%
10.2%
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C
-Vc+2ME
+Vc-2ME
SSC
52.7%
73.6%
PLZF+
PLZF
TUBULIN
-VC
+VC
D
0.2% BSA
0.2% SR
1% SR
3% SR
79.1%
71.7%
84.5%
85.1%
FL1/no stain
APC-PLZF+
E
MVH
DAZL
β-actin
H1-D0
H1-D15
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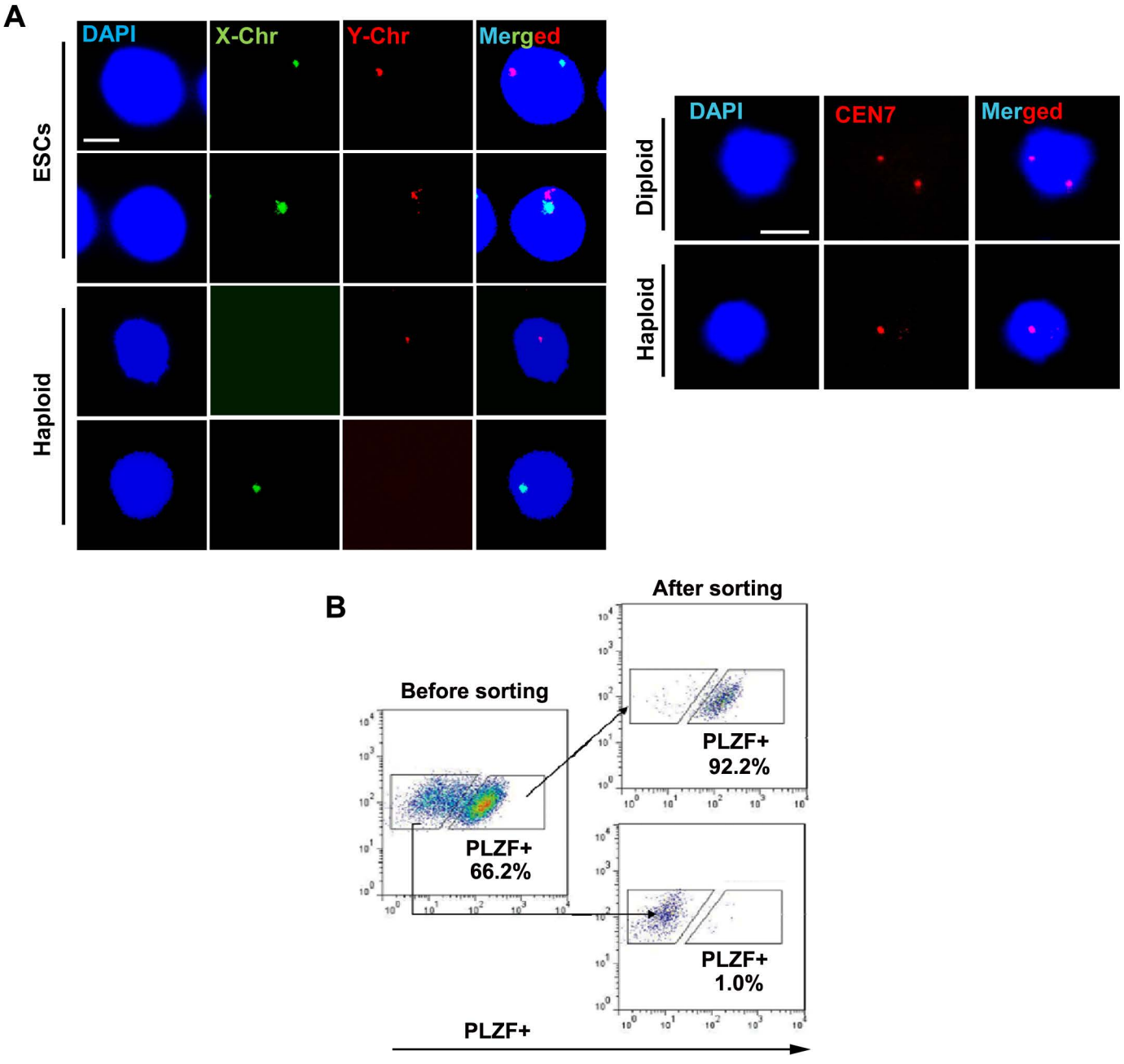
1.93%
3.61%
4.03%
Cell Counts
Propidium Iodine
Zhao et al. SF1



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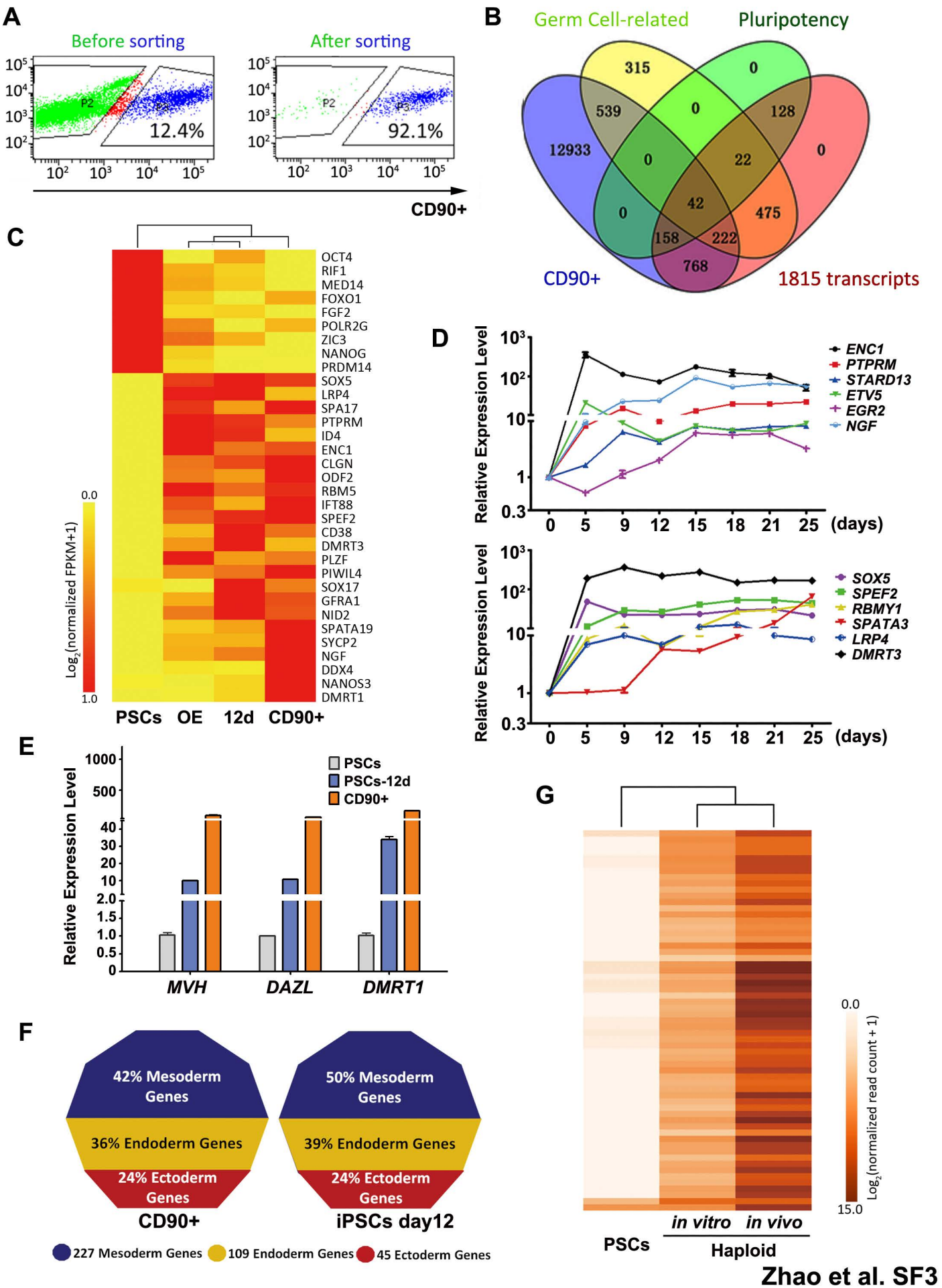
A
Before sorting
After sorting
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P3
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92.1%
CD90+
B
Germ Cell-related
Pluripotency
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0
539
0
128
12933
0
22
0
0
42
475
158
222
768
CD90+
1815 transcripts
C
OCT4
RIF1
MED14
FOXO1
FGF2
POLR2G
ZIC3
NANOG
PRDM14
SOX5
LRP4
SPA17
PTPRM
ID4
ENC1
CLGN
ODF2
RBM5
IFT88
SPEF2
CD38
DMRT3
PLZF
PIWIL4
SOX17
GFRA1
NID2
SPATA19
SYCP2
NGF
DDX4
NANOS3
DMRT1
Log2(normalized FPKM+1)
0.0
1.0
PSCs
OE
12d
CD90+
D
Relative Expression Level
ENC1
PTPRM
STARD13
ETV5
EGR2
NGF
(days)
SOX5
SPEF2
RBMY1
SPATA3
LRP4
DMRT3
E
Relative Expression Level
PSCs
PSCs-12d
CD90+
MVH
DAZL
DMRT1
F
42% Mesoderm Genes
36% Endoderm Genes
24% Ectoderm Genes
CD90+
50% Mesoderm Genes
39% Endoderm Genes
24% Ectoderm Genes
iPSCs day12
227 Mesoderm Genes
109 Endoderm Genes
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in vitro
in vivo
Haploid
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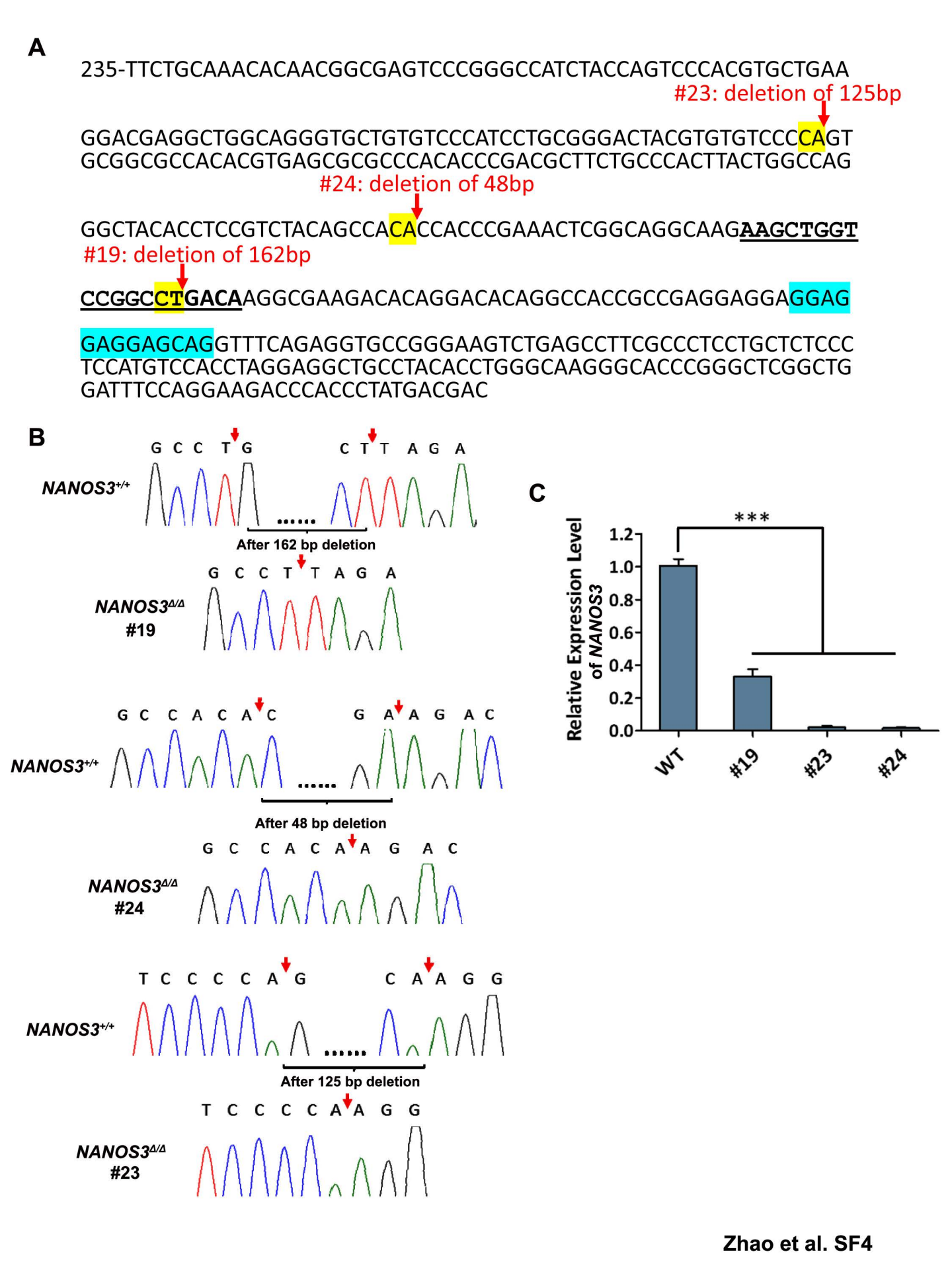
Zhao et al. SF3



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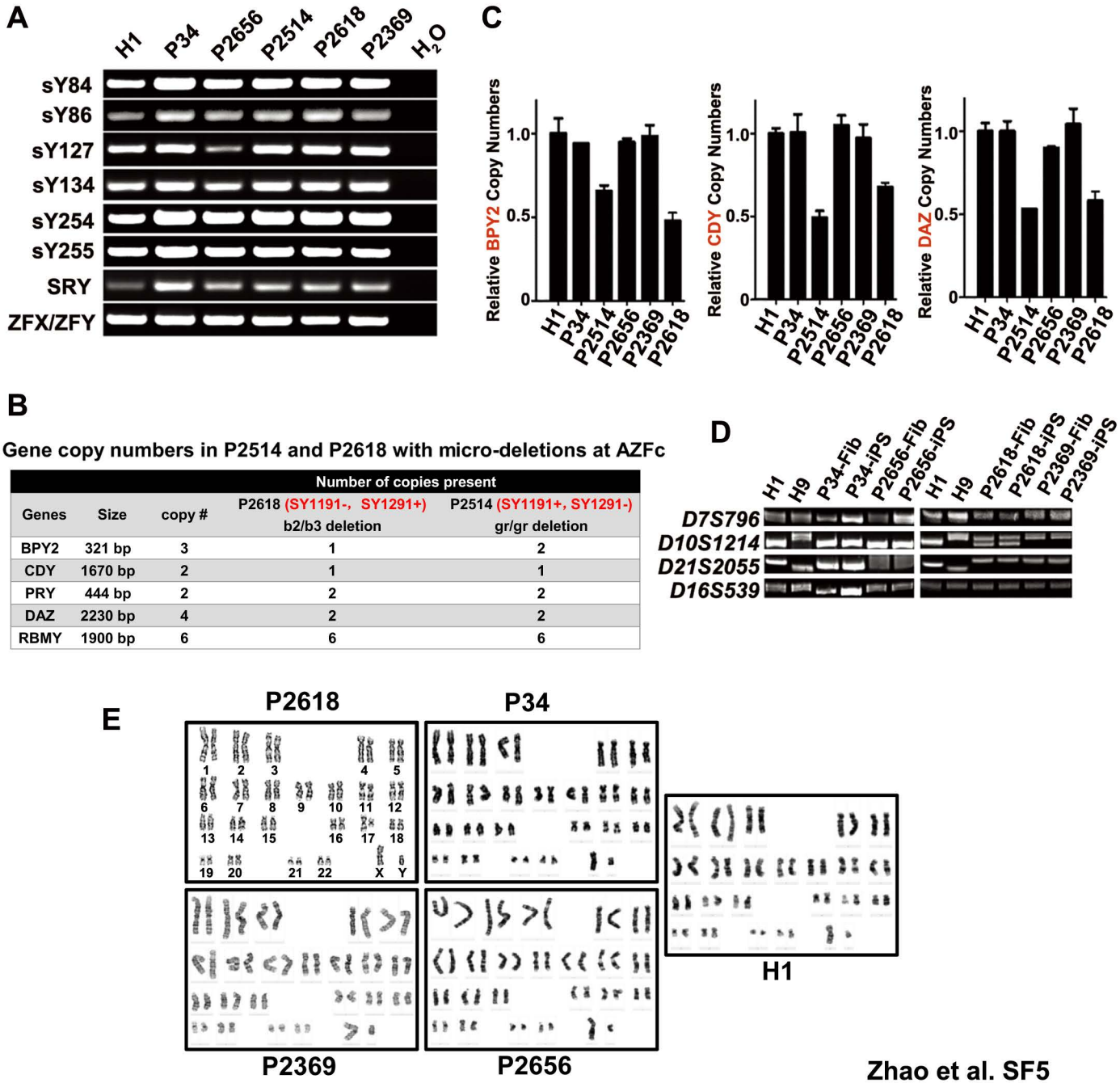
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P2618
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H2O
sY84
sY86
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sY255
SRY
ZFX/ZFY
C
Relative BPY2 Copy Numbers
Relative CDY Copy Numbers
Relative DAZ Copy Numbers
1.0
0.5
0
H1
P34
P2514
P2656
P2369
P2618
B
Gene copy numbers in P2514 and P2618 with micro-deletions at AZFc
Number of copies present
Genes
Size
copy #
P2618 (SY1191-, SY1291+) b2/b3 deletion
P2514 (SY1191+, SY1291-) gr/gr deletion
BPY2 321 bp 3 1 2
CDY 1670 bp 2 1 1
PRY 444 bp 2 2 2
DAZ 2230 bp 4 2 2
RBMY 1900 bp 6 6 6
D
H1
H9
P34-Fib
P34-iPS
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H1
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P2369-iPS
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D16S539
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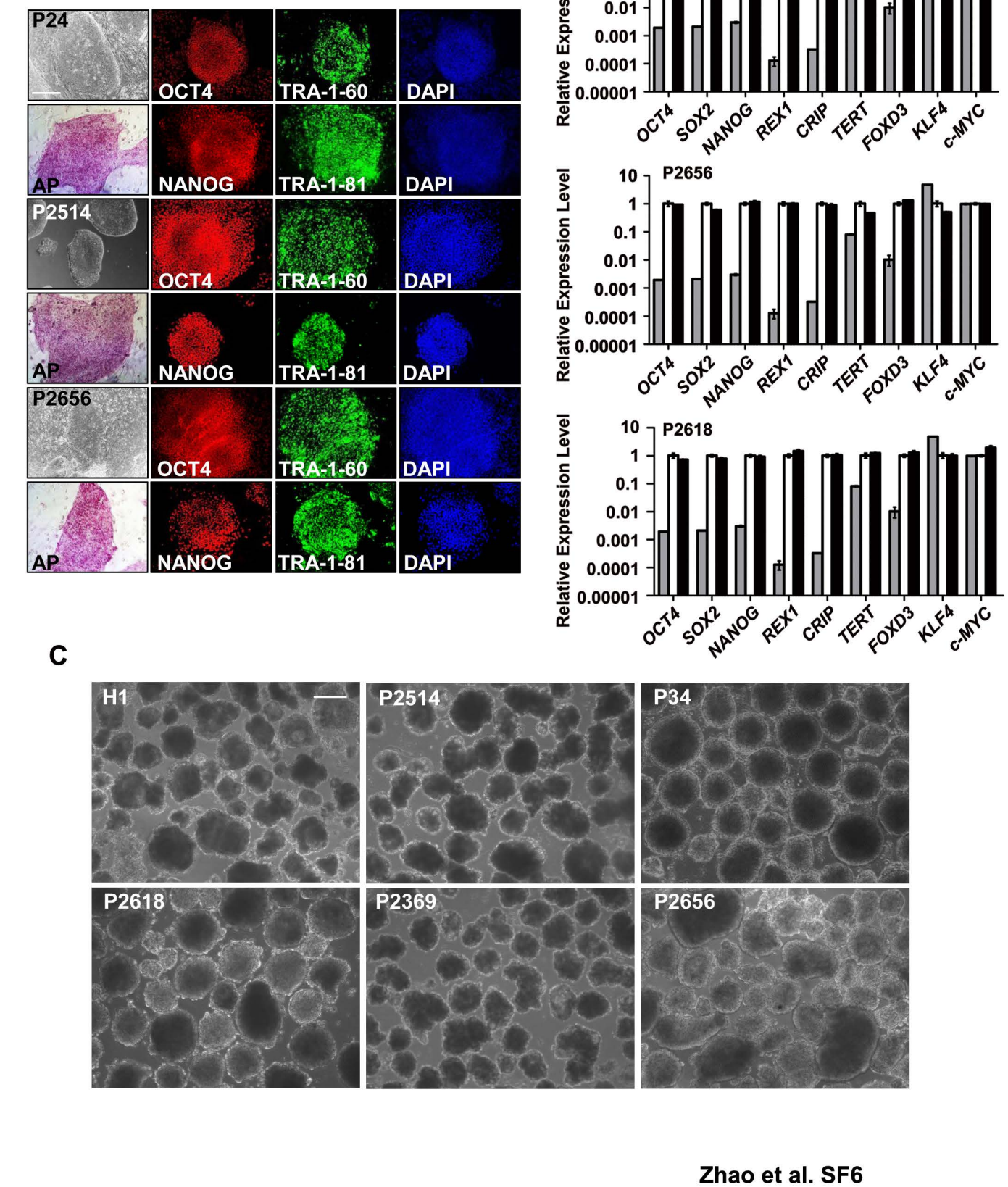
P34
H1
P2369
P2656
Zhao et al. SF5



Zhao et al. SF6

Primers used for PCR analyses	
Name	**sequence (5'-3')**
hMVH-F	TCAGACTTTTGAAGAAGCTAATCTC
hMVH-R	CAAGCCATCAAATCTCGTCC
hDAZL-F	GCCCACAACCACGATGAATC
hDAZl-R	CGGAGGTACAACATAGCTCCTTT
hDKKL1-F	CTCTACCCTGGTGATCCCCTC
hDKKL1-R	CGAAGCAGGTTACCTTTCAGGA
hENC1-F	GCTGCTGTCTGATGCACAC
hENC1-R	AGAGTTGCACTACCATGTCCT
hPTPRM-F	TCCAGCAAGAGTAATTCTCCTCC
hPTPRM-R	CATGTACGTGTTGGGTCTCCA
hRBMY1-F	CCATCACAGAGAGCGATATTCG
hRBMY-R	CGGCTTACACCTGTTTTCCTC
hSPATA3-F	GCAGCCTAGCCCTGAATCC
hSPATA3-R	CCTTCACGTTAGCATCTGGAG
hSTARD13-F	CGAGGAGACAGAAATGGGTCA
hSTARD13-R	TCCACTGCTTTCGCTGTGAAT
human Stra8-F	ACTCTCAGTCTGATCTCATAGCC
human Stra8-R	TACCAAGGGGAGGAACCATTC
hTDRD9-F	AAAGGTTGCAGGTCTATCCACT
hTDRD9-R	CAACTCAATCGCAGACTCTGAT
hBLIMP1-F	TAAAGCAACCGAGCACTGAGA
hBLIMP1-R	ACGGTAGAGGTCCTTTCCTTTG
hCXCR4-F	CTTCTGGGCAGTTGATGCCGT
hCXCR4-R	CTGTTGGTGGCGTGGACGAT
hNANOS3-F	AAGGCGAAGACACAGGACAC
hNANOS3-R	CGAAGGCTCAGACTTCCCG
hPLZF-F	GAGATCCTCTTCCACCGCAAT
hPLZF-R	CCGCATACAGCAGGTCATC
hGFRα1-F	CCAAGCACAGCTACGGAATG
hGFRα1-R	CAGGCACGATGGTCTGTCG
hTKTL1-F	TGGACAATCTTGTGGCAATCTT
hTKTL1-R	CAGCGCCTCTGATAGATGTTTAT
hDMRT3-F	ATGTGGCAAAGAGTAAGGGCT
hDMRT3-R	GCGGTCTGTTGGCTTTCAAG
hSYCP3-F	TTTGTTTCAGCAGTGGGATT
hSYCP3-R	TTCCGAACACTTGCTATCTC
hTNP1-F	GACCAGCCGCAAATTAAAGAGT
hTNP1-R	GGTTGCCCTTACGGTATTTTCT
hSPA17-F	AGAGAGCAACCGGACAATATACC
hSPA17-R	CTGCTGGATCAAAGTTGGTTTTC
hNGF-F	GGCAGACCCGCAACATTACT
hNGF-R	CACCACCGACCTCGAAGTC
hLRP4-F	GTGAGGAGGACGAGTTTCCCT
hLRP4-R	TCACCGTCGCAGTACCAATG
hNID2-F	CCGGTGCTGTCGTCGTTAC
hNID2-R	GGCTTCGTAGAAGTGCAGGG

hODF2-F	GCACAGCTTCGGTCCAAAGA
hODF2-R	TCTGCCTTATGCTGATTCCCG
hCLGN-F	GTTCCTCCTATCAAACCTCCCA
hCLGN-R	TCCGTGTCTTCATTCCAGTCAT
hIFT88-F	TCCTGAAACTTCACGCAATCC
hIFT88-R	GACCACCTGCATTAGCCATTC
hSTK31-F	GTATGGCAGTGTGGATATAGGGG
hSTK31-R	CCTGCTGAAGGTTGACTGGT
hSOX5-F	GAACAACAGGTGCTTGATGGG
hSOX5-R	GCCCTCGGGATTCCCTATAAAT
hSOX9-F	AGCGAACGCACATCAAGAC
hSOX9-R	CTGTAGGCGATCTGTTGGGG
hACROSIN-F	TTCGTGTGGGCGCTTCATT
hACROSIN-R	AGTCCAGGTCGATGAGATCCA
hPRM1-F	AGAGCCGGAGCAGATATTACC
hPRM1-R	TCTACATCGCGGTCTGTACCT
hSPATA19-F	CCCTTCCTACCAATAACCAGTTC
hSPATA19-R	ACATCTTCTCCCTTACACCCTG
hSPEF2-F	CTTGGAGCCAACACTTAACCTT
hSPEF2-R	GACGTTGCATGGTTTGCATCT
hACRBP-F	GAAAACCACGGCTTAGTGCC
hACRBP-R	GCAACGGTAGTGAGTGAACTG
hPGK2-F	TTGACGAGAACGCTCAGGTTG
hPGK2-R	ACGGCCCATTCCAAACAATTAG
hSOX17-F	GTGGACCGCACGGAATTTG
hSOX17-R	GGAGATTCACACCGGAGTCA
hNESTIN-F	CTGCTACCCTTGAGACACCTG
hNESTIN-R	GGGCTCTGATCTCTGCATCTAC
hT-F	CTGGGTACTCCCAATGGGG
hT-R	GGTTGGAGAATTGTTCCGATGA
hAFP-F	AGACTGAAAACCCTCTTGAATGC
hAFP-R	GTCCTCACTGAGTTGGCAACA
hCDX2-F	GACGTGAGCATGTACCCTAGC
hCDX2-R	GCGTAGCCATTCCAGTCCT
hGAPDH-RNA-F	ATTGCCCTCAACGACCACTTTG
hGAPDH-RNA-R	TTGATGGTACATGACAAGGTGCGG
hPAX6-F	TGGGCAGGTATTACGAGACTG
hPAX6-R	ACTCCCGCTTATACTGGGCTA
hITGA6-F	ATGCACGCGGATCGAGTTT
hITGA6-R	TTCCTGCTTCGTATTAACATGCT
hSTX2-F	TGAGAGTGGGAACCGGACTT
hSTX2-R	TTCTAGCTCGTCGTCTGTGGT
hGATA6-F	CTCAGTTCCTACGCTTCGCAT
hGATA6-R	GTCGAGGTCAGTGAACAGCA
hTNRC6C-F	CGTCCAAAGCCCTTCTAATCAG
hTNRC6C-R	TCTCCCATCTCCAATCATAGTGT
hPAF1-F	CCTGACACCTACCGCATCG
hPAF1-R	TGTACTCTGTCTTTCGCATCCA

hKIF16B-F	CTGGCTTAATACCTCGGATCTGT
hKIF16B-R	GCTCACGGACTCTCAAATTGAAG
hMEOX1-F	GGGGGTTCCAAGGAAATGGG
hMEOX1-R	CGAGTCAGGTAGTTATGATGGGC
hFOXF1-F	CCCAGCATGTGTGACCGAAA
hFOXF1-R	ATCACGCAAGGCTTGATGTCT
hBMP4-F	TGAGCCTTTCCAGCAAGTTT
hBMP4-R	CTTCCCCGTCTCAGGTATCA
hEGR2-F	GACACGGCACATCCGAATC
hEGR2-R	GCACTGCTTTTCCGCTCTTT
hETV5-F	CAGTCAACTTCAAGAGGCTTGG
hETV5-R	TGCTCATGGCTACAAGACGAC
hID4-F	TCCCGCCCAACAAGAAAGTC
hID4-R	TGTCGCCCTGCTTGTTCAC
hHAND1-F	GAGAGCATTAACAGCGCATTCG
hHAND1-R	CGCAGAGTCTTGATCTTGGAGAG
Primers to amplify STS and coding genes at AZF region	
Name	**sequence (5'-3')**
hAZFA-SY84-F	AGAAGGGTCTGAAAGCAGGT
hAZFA-SY84-R	GCCTACTACCTGGAGGCTTC
hAZFA-SY86-F	GTGACACACAGACTATGCTTC
hAZFA-SY86-R	ACACACAGAGGGACAACCCT
hAZFB-SY127-F	GGCTCACAAACGAAAAGAAA
hAZFB-SY127-R	CTGCAGGCAGTAATAAGGGA
hAZFB-SY134-F	GTCTGCCTCACCATAAAACG
hAZFB-SY134-R	ACCACTGCCAAAACTTTCAA
hAZFC-SY1191-F	CCAGACGTTCTACCCTTTCG
hAZFC-SY1191-R	GAGCCGAGATCCAGTTACCA
hAZFC-SY1291-F	TAAAAGGCAGAACTGCCAGG
hAZFC-SY1291-R	GGGAGAAAAGTTCTGCAACG
hAZFC-SY254-F	GGGTGTTACCAGAAGGCAAA
hAZFC-SY254-R	GAACCGTATCTACCAAAGCAGC
hAZFC-SY255/DAZ-F	GTTACAGGATTCGGCGTGAT
hAZFC-SY255/DAZ-R	CTCGTCATGTGCAGCCAC
hZFX-F	ACCGCTGTACTGACTGTGATTACAC
hZFX-R	GCACCTCTTTGGTATCCGAGAAAGT
hZFY-F	ACCACTGTACTGACTGTGATTACAC
hZFY-R	GCACTTCTTTGGTATCTGAGAAAGT
hSRY-SY14-F	GAATATTCCCGCTCTCCGGA
hSRY-SY14-R	GCTGGTGCTCCATTCTTGAG
hBPY2-DNA-F	CCAGGACCATGTGATATGG
hBPY2-DNA-R	CTAATTCCCTCTTTACGCATGACC
hCDY-DNA-F	GCTGCCAGCAAGAACGTTAG
hCDY-DNA-R	TTTGTGGTCAAAGGGGCTGT
hGAPDH-DNA-F	GGGAAGCTCAAGGGAGATAAAATTC
hGAPDH-DNA-R	GTAGTTGAGGTCAATGAAGGGGTC

Primers for bisulfate-sequencing	
Name	**sequence (5'-3')**
H19 DMR-F-1	AGGTGTTTTAGTTTTATGGATGATGG
H19 DMR-R-1	TCCTATAAATATCCTATTCCCAAATAACC
H19 DMR-F-2	TGTATAGTATATGGGTATTTTTGGAGGTTT
H19 DMR-R-2	TCCTATAAATATCCTATTCCCAAATAACC
PEG10 DMR -F	GGTGTAATTTATATAAGGTTTATAGTTTG
PEG10 DMR -R	AACAAAAAAAAATAAAATCCCACAC
SNRPN DMR-F-1	GGTTTTTTTTTATTGTAATAGTGTTGTGGGG
SNRPN DMR-R-1	CTCCAAAACAAAAAACTTTAAAACCCAAATTC
SNRPN DMR-F-2	CAATACTCCAAATCCTAAAAACTTAAAATATC
SNRPN DMR-R-2	GGTTTTAGGGGTTTAGTAGTTTTTTTTTTTTGG
Primers to amplify STR	
Name	**sequence (5'-3')**
D7S796-F	TTTTGGTATTGGCCATCCTA
D7S796-R	GAAAGGAACAGAGAGACAGGG
D10S1214-F	ATTGCCCCAAAACTTTTTTG
D10S1214-R	TTGAAGACCAGTCTGGGAAG
D21S2055-F	AACAGAACCAATAGGCTATCTATC
D21S2055-R	TACAGTAAATCACTTGGTAGGAGA
D16S539-F	GGGGGTCTAAGAGCTTGTAAAAAG
D16S539-R	GTTTGTGTGTGCATCTGTAAGCATGTATC
Primers to amplify endogenous or exogenous genes	
Name	**sequence (5'-3')**
Exo-hOCT4-F	CCAGTATCGAGAACCGAGTGAG
Exo-hOCT4-R	AAGACAGGGCCAGGTTTCC
Exo-hSOX2-IRES-F	GCAGATGCAGCCCATGCA
Exo-hSOX2-IRES-R	AAGACAGGGCCAGGTTTCC
Exo-hKLF4-IRES-F	GGCTGCGGCAAAACCTA
Exo-hKLF4-IRES-R	AAGACAGGGCCAGGTTTCC
Exo-hC-MYC-IRES-F	TGAACAGCTACGGAACTCTTGTG
Exo-hC-MYC-IRES-R	CCAAAAGACGGCAATATGGTG
hOCT4-F	CAAAGCAGAAACCCTCGTGC
hOCT4-R	TCTCACTCGGTTCTCGATACTG
hSOX2-F	TACAGCATGTCCTACTCGCAG
hSOX2-R	GAGGAAGAGGTAACCACAGGG
hNANOG-F	ATGAACATGCAACCTGAAGACG
hNANOG-R	GCTGATTAGGCTCCAACCATAC
hREX1-F	AAAGCATCTCCTCATTCATGGT
hREX1-R	TGGGCTTTCAGGTTATTTGACT
hCRIPTO/TDGF1-F	TACCTGGCCTTCAGAGATGACA
hCRIPTO/TDGF1-R	CCAGCATTTACACAGGGAACAC
hTERT-F	AGCATTCCTGCTCAAGCTGACT
hTERT-R	ACTCACTCAGGCCTCAGACTCC
hFOXD3-F	AAGCCCAAGAACAGCCTAGTGA

hFOXD3-R	GGGTCCAGGGTCCAGTAGTTG
hKLF4-F	TGACCAGGCACTACCGTAAA
hKLF4-R	GACTCAGTTGGGAACTTGACC
hMYC-F	GGGCTTTATCTAACTCGCTGTA
hMYC-R	GTCCTTGCTCGGGTGTTGTA
Primers for sgRNA in NANOS3 knockout	
Name	**Sequence (5'-3')**
F (5'-3')	CGAAGCTGGTCCGGCCTGACAGTTTTAGAG
R (5'-3')	CTAGCTCTAAAACTGTCAGGCCGGACCAGCTTCG
Primers for sequencing NANOS3 knockout locus	
Name	**Sequence (5'-3')**
F (5'-3')	TTCTGCAAACACAACGGCGAG
R (5'-3')	TCCAGCCTGAGCAACAGAAAGAG