Online-only Methods

hESC lines and adherent differentiation. Four hESC lines were used in this study: HSF1(XY), HSF6(XX), H1(XY), and H9(XX). Undifferentiated cultures of hESCs were maintained on irradiated MEFs as previously described[5]. Briefly, all cultures were grown at 37°C with 5% CO_2 in KSR + bFGF medium [Knockout DMEM, supplemented with 20% Knockout Serum Replacer, 1 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM β-mercaptoethanol, and 4 ng/ml recombinant human basic fibroblast growth factor (bFGF, R&D systems)]. To adherently differentiate hESCs on matrigel, about 5×10^4 hESCs (less than 50% confluency of a six-well plate) were treated with collagenase type IV (1mg/ml) for 10 minutes, scraped with a 5 ml plastic pipette, and transferred with fresh KSR + bFGF media to an identical well coated with matrigel. Conditioned media (KSR + bFGF media collected after overnight incubation on irradiated MEFs) was used to maintain the undifferentiated cells whenever drug selection (geneticin at 200 μg/ml, and zeocin and blasticidin at 2 μg/ml) was required. Differentiation began upon aspiration of KSR + bFGF media or conditioned media, washed once with PBS w/o Ca^{++} and Mg^{++}, and replaced with differentiation media [Knockout DMEM supplemented with 20% fetal bovine serum, 1 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM β-mercaptoethanol, and 50 ng/ml recombinant human BMP4, BMP7, and BMP8b (R&D systems)]. Differentiation media was changed following 7 days of incubation if longer differentiation was necessary.

Western Blot analysis. To collect the adherently differentiated cells, first they were washed with 3 ml cold PBS w/o Ca^{++} and Mg^{++}, then scraped from the plate in 1 ml PBS plus 2x protease inhibitors (Complete Mini, Roche) and transferred immediately to a 1.5 ml tube on ice. The PBS cell suspension was then spun at 5000 rpm in a microcentrifuge for 3 minutes and the supernatant was discarded. The cell pellet was resuspended with 200 μl RIPA buffer (50mM Tris, 150 mM NaCl, 0.5% Sodium deoxycholate, 1% NP-40, 0.1% SDS, pH 8) plus 2x protease inhibitors (Complete Mini, Roche). The cell pellet suspension was pipetted rigorously at least 10 times, then vortexed for 30 seconds. The suspension was again spun down for 3 minutes at the same speed. The supernatant was measured for protein concentration and denatured at a 1:1 ratio with 2x Laemmli buffer at 95°C for 5 minutes, then loaded onto either a 10% or 12% SDS-PAGE gel. The SDS-PAGE gels were run at 150 volts for one hour and transferred to a PVDF

membrane for one hour at 100 volts in CAPS buffer (10mM CAPS, 10% methanol, pH 11). Transferred blots were blocked in 5% non-fat milk for one hour at room temperature. The blot was subjected to one hour of primary antibody incubation [1:500 for anti-VASA (Abcam), 1:500 anti-DAZL-150[28] , 1:1000 for anti-V5 (Abcam), 1:10,000 for anti-UBC9 (Abcam) and anti-GAPDH (Abcam)], followed by 2 quick rinses and 3 washes for 5 minutes in TBST (TBS, pH7.4 with 0.1% Tween 20). Secondary antibody [1:10,000 anti-rabbit-HRP conjugated (Amersham)] incubation had the same duration and washes. ECL+ (Amersham) was used to detect the HRP signal on film.

Quantitative PCR on mRNA expression and statistical analysis. Total RNA was collected using the RNeasy system (Qiagen) or PicoPure RNA isolation (Arcturus) and cDNA was prepared with SuperScript III Reverse Transcriptase (Invitrogen) according to manufacturer's protocols. Quantitative PCR was conducted with Taqman probes (Applied Biosystems) using the Fluidigm System (Biomark). Gene expression is normalized to a set of housekeeping genes (*GAPDH*, *RPLPO*, *CENTRIN*, *CTNNB1*, *ACTB*) and calculated according to geNorm program[29] for GFP- versus GFP+ cells. Gene expressions of the replated cells, fetal liver cDNA (Clontech), and overexpressed cells were normalized again to either the expression of GFP+ cells or control cells carrying empty p2k7, and the normalized expression value is therefore fold of change. Statistical analysis was calculated using student t-test or one way ANOVA by Prism 5.0 program.

DNA methylation analysis. VASA:GFP+, VASA:GFP-, and 1N populations were collected by FACS; adult skin fibroblast, H9 and HSF1 were collected from trypsinized cell suspensions; replated GFP+ cells were collected by colony picking under dissection microscope (pooled of 5 colonies). Genomic DNA was extracted using the ZR Genomic DNA II kit (Zymo Research) or QIAamp DNA Mini kit (Qiagen). For bisulfite sequencing analysis of *H19* locus, 100 ng of genomic DNA was processed using the Qiagen Epitect Bisulfite Kit (Qiagen) according to protocol. 1µl of bisulfite-treated genomic DNA was PCR amplified at the *H19* locus using primers as previously described[4] . The resultant product was gel-extracted using the Qiaquick gel extraction kit (Qiagen) and cloned into the TOPO TA vector (Invitrogen). At least 20 clones were sequenced using ABI BigDye v3.1 dye terminator sequencing chemistry (Applied

Biosystems) and ABI PRISM 3730xl capillary DNA analyzer for sequence analysis. Quantitative DNA methylation analysis using methylation-sensitive/dependent restriction assay was carried out according to the previous study[5]. Briefly, ~50-200ng of each genomic DNA sample was divided equally for control (with buffers), methylation-sensitive (NotI, HhaI, and HpaII; New England Biolabs), and methylation dependent (McrBC; New England Biolabs) restriction digestions. 1/30 of the digested genomic DNA was then used as the input for qPCR reaction (ABI Power SYB master mix) using specific primers for the DMRs of *H19*[30], *PEG1*[30], *SNRPN*[23], and *KCNQ*[23] respectively, and triplicates were ran for each reaction. Percent of hypermethylation was calculated as previously reported[31], and the average percentages with lower standard deviation were chosen between methylation sensitive and methylation dependent restriction reactions.

Alkaline phosphatase assay. Cells grown on MEFs were subjected to alkaline phosphatase staining using Vector Red Alkaline Phosphatase Substrate Kit I (Vector Laboratories) according to manufacturer's protocols.

shRNA vectors and overexpression vectors. All shRNAs targeting human *DAZL*, *DAZ*, and *BOULE* were constructed using the Block-iT inducible H1 lentiviral system (Invitrogen). All shRNAs were first introduced into pENTR/H1/TO vectors and transferred into pLenti4/BLOCK-iT-DEST destination vectors (please see the table below for sequence information). When shRNA was tested in 293FT cells for its efficacy of silencing, transient transfection without selection was carried out and cell lysate was collected after 24 hours. After specific shRNA was chosen, viral supernatant was prepared as described for pLVGV. Subsequently, the viral supernatant was used to transduce hESCs on matrigel as described above, except with zeocin selection (2μg/ml) for 3 days for stable integration of the shRNA vector into hESCs. Overexpression vectors were constructed by inserting the EF1α promoter, and *DAZL*, *DAZ2*, or *BOULE* cDNA into the $p2k7_{blas}$ vectors, selected with blasticidin (2μg/ml) in culture for 3 days to ensure stable integration and subjected for each differentiation experiment.

shRNA	DNA sequence
shDAZL1	GGATGGATGAAACTGAGATTA
shDAZL2	GCATATCCTACTTACCCAAAT
shDAZL3	GCCAAATGAATGTTCAGTTCA
shDAZL4	GCAGAAGATAGTAGAATCACA
shDAZL5	GGATATCAGTTGCCTGTATAT
shDAZL6	GGTGGTATCTTGTCTGTTTAA
shDAZL7	GGTATCTTGTCTGTTTAATCC
shDAZ1	GCAAATCCTGAGACTCCAAAC
shDAZ2	GGAAGCTGCTTTGGTAGATAC
shDAZ3	GCCACGTCCTTTGGTAGTTAA
shDAZ4	GCATTTCCTGCTTATCCAAAT
shDAZ5	GCATTTCCTGCTTATCCAAGT
shBOULE1	GCATCTTTGTAGGAGGAATTG
shBOULE2	GGATCCCTCGTTCTAGTATAA
shBOULE3	GCTGGAACAATGTATCTAACA
shBOULE4	GCAACCTTCTGAGGTTATTTA
shBOULE5	GCTCCAAGTGCCATCACTATG

VASA, OCT4 whole cell staining and 5-Methylcytosine nuclei staining. Cell suspensions from FACS were collected onto a glass slide with a Cytospin (Thermo Scientific) at 800rpm for 5 min followed by a wash in PBS, and fixed with 4% paraformaldehyde/PBS for 15 minutes. For VASA and OCT4 staining, slides were blocked in 1% BSA/0.1% Tween-20/PBS for 1 hour, followed by overnight incubation with primary antibody [1:500 for anti-VASA (Abcam), 1:200 anti-OCT4 (Santa Cruz)]. Slides were then rinsed twice and washed 3 times, 5 min with 0.1% Tween-20/PBS (PBST). Secondary antibody, anti-rabbit-594 (Invitrogen) was incubated for 1 hour at room temperature on the slides followed by the same washes. The slides were then mounted with Prolong Antifade with DAPI (Molecular Probes). For 5MC staining, fixed cells were incubated with 1% Triton X-100 for 1 hour, washed with PBST twice for 5 min. Denaturation of DNA was carried out by treating cells with 4 M HCl/0.1% Triton X-100 for10 minutes and immediately neutralized with 100 mM Tris/HCl (pH 8.5) for 30 minutes. Slides were blocked in 1% BSA/0.1% Tween-20/PBS at 4ºC overnight. This was then stained with antibody against 5-Methylcytosine (Genway) at a concentration of 1:100 in blocking buffer for 4

hrs at room temperature. The slides were washed, incubated with secondary, and mounted the same as described above.

Meiotic spreads, SCP3 and γH2AX staining. Meiotic spreads were carried out as described previously[32] with some modifications. Briefly, differentiated hESCs were prepared as single cells as for FACS analysis. Cells were lysed by a hypotonic solution and dropped on glass slides freshly submerged in 1% PFA and 0.15% TritonX-100. Slides were incubated overnight at 4°C until the suspension was semi-dry. Slides were treated with 0.04% photoflo for 5 min and blocked with 4% goat serum. Rabbit anti-SCP3 (1:1000, Novus) and mouse-anti-γH2AX (1:200, Abcam) were incubated for 3 hours at room temperature. Washes, secondary antibodies incubation [1:1000, anti-rabbit594 and anti-mouse488 (Molecular Probe)], and mounting are the same as described above.

FACS analysis for DNA content. Single cell suspensions were prepared as described above. Fixing and staining of the DNA was conducted as described previously[33]. Briefly, cells were fixed in 70% ethanol for 1 hour at room temperature, followed by incubation in 0.5ml staining solution (0.1% Triton in PBS, 0.2mg/ml RNaseA, 0.02mg/ml propidium iodide) then transferred to a FACS tube and incubated at 37°C for >15min. The cell suspension in the staining solution was subjected to FACS analysis.

FISH of FACS cells. 1N, 2N, and 4N Cells were collected by cytospin as described above after the DNA content FACS. Slides were fixed with Carnoy's fixative (1:3 of acetic acid:methnol), 5 min, air dried in chemical hood, followed by dehydration in ice cold 70%, 80%, 100% ethanol, 2min, and air dried for 5 min. FISH probe (against chromosome 16, Vysis) was directly denatured on slides at 85°C and hybridized at 37°C overnight. Slides were washed with 2×SSC once, followed by 0.1%SDS in 2×SSC (prewarm) at 50°C, 5 min, and mounted as described above.

Acrosin staining. The FACS collected 1N population was cytospun onto glass slides, washed once with PBS, and fixed with 4% paraformaldehyde/PBS for 15 minutes. Fixed cells were then incubated with 1% Triton X-100 for 15 min, washed twice with 0.1% Tween-20/PBS for 5 min.

Slides were blocked with 4% goat serum for 1 hour at room temperature, then stained with antibody against Acrosin (Santa Cruz) diluted 1:50 in blocking buffer for 4 hrs at room temperature. Slides were rinsed twice and washed 3 times with PBST, for 5 min. The secondary antibody used was anti-rabbit-Cy5 (Molecular Probes) at 1:1000 for 1 hr at room temperature, followed by the same washes, mounting as described above.

Cloning and silencing of DAZ1-4. cDNA clones of DAZ1-4 [NCBI accession numbers: DAZ1:BC114927, DAZ2: NM_020363, DAZ3: BC113005, DAZ4: BC047480] were obtained from ATCC, PCR amplified, cloned into pENTR-D-TOPO vector, and subsequently cloned into pLenti4/TO/V5-DEST (Invitrogen). Expression of these cDNAs resulted in ~42kD, 63kD, 50kD, 44kD proteins respectively on Western blot detected by mouse anti-DAZ1 antibody (Abcam). Cotransfection of the overexpression vector and shDAZ4 at the ratio of 1:1 and 1:2 into 293FT cells was carried out and all the lysates were collected after 24 hour and loaded equally onto 10% SDS-PAGE gel.

Replating of VASA:GFP+ cells. VASA:GFP- and GFP+ cells were collected in ice cold KSR media without bFGF [Knockout DMEM, supplemented with 20% Knockout Serum Replacer, 1 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM β-mercaptoethanol,]. Immediately after FACS collection, cells were distributed into wells plated with inactivated MEFs containing KSR media. ~ 20,000 cells was distributed into each well of 6-well plate. Culture was then grew at 37°C with 5% CO_2. Media was changed every 3 days. Colonies usually appeared after 7 days and at 1/5000 survival rate. Colonies expanded much slower than hESC colonies and harvested when colonies size were about 200 µm for various assays.

Supplementary References

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