

## 1 **Supplementary Materials:**

2 Materials and Methods

3 Figs. S1 to S9

4 Tables S1 to S6

5 References (28–38)

6 Movie S1

## 1 **Methods**

### 2 **iPSC culture**

3 Human iPSC lines (ATCC-BXS0115, referred to as F2, ATCC-BXS0116, referred to as F3, and  
4 PGP1) were cultured in mTeSR Plus medium (Stemcell Technologies) on standard  
5 polystyrene plates coated with hESC-qualified Matrigel (Corning). hiPSCs were grown in a  
6 37 °C 5% CO<sub>2</sub> incubator. Passaging was performed by brief (3-5 minute) treatment with 0.5  
7 mM EDTA and 0.25X TRYPLE Express (Gibco) in phosphate-buffered saline (PBS), followed  
8 by pipetting to break the colonies into small clumps. Cells were treated with 10 μM Y-27632  
9 for 24 hours after each passage. Cells were tested every three months for mycoplasma  
10 using the ATCC Universal Mycoplasma Detection Kit. All cells tested negative. For  
11 experiments requiring single cell dissociation and counting, cells were harvested with  
12 Accutase and counted using trypan blue staining with a Countess II automated cell counter  
13 (Thermo Fisher).

### 14 **Generation and verification of reporter lines**

15 Knock-in donor plasmids targeting *REC8* and *SYCP3* were constructed by Gibson assembly  
16 of 5' and 3' homology arms, an insert containing a fluorescent marker joined to the gene of  
17 interest by a T2A linker, and a plasmid backbone containing an MC1-DTA marker to select  
18 against random integration. sgRNA oligos were cloned into pX330 (Addgene #42230), which  
19 also expresses Cas9. Oligo sequences are provided in Supplementary Table 4. All plasmids  
20 will be made available on Addgene following acceptance of this paper.

21 Knock-in electroporations were performed as previously described (25). In summary, 1 μg  
22 donor plasmid and 1 μg sgRNA/Cas9 plasmid were co-electroporated into 200,000 hiPSCs  
23 using a Lonza Nucleofector with 20 μL P3 solution and pulse setting CA-137. Colonies were  
24 picked after selection and genotyped by PCR. Successful knockin, absence of off-target  
25 edits, and euploidy of the reporter lines were all confirmed by whole genome sequencing  
26 (Novogene, 10X coverage) and SeqVerify computational analysis (18). Low-passage cells  
27 were cryopreserved using CryoStor CS10 (Stemcell Technologies) and banked for future  
28 use.

29 Functional validation of the reporter alleles was performed by CRISPRa. For each allele,  
30 three CRISPRa plasmids were constructed, each containing a sgRNA targeting the  
31 promoter as well as a doxycycline-inducible expression cassette for dCas9-VPR (28).  
32 Equimolar mixtures of plasmids (1 μg total) were electroporated into 200,000 hiPSCs using  
33 a Lonza Nucleofector with 20 μL P3 solution and pulse setting CA-137. After two days, cells  
34 were harvested with Accutase and analyzed by flow cytometry (Fig. S1).

## 1 **Identification of candidate meiosis-promoting factors**

2 We obtained a human fetal germ cell scRNAseq dataset from Garcia-Alonso *et al.* 2022  
3 (<https://www.reproductivecellatlas.org/gonads.html>) (16). We performed pySCENIC  
4 analysis (29), which involves inferring a gene regulatory network, finding transcription  
5 factor (TF) regulons based on known binding motifs, and ranking regulon activity in each  
6 cell type. We chose 21 TFs to screen based on high activity in STRA8+ and/or meiotic  
7 oogonia. Because regulon analysis ignores non-TF factors, including RNA-binding proteins,  
8 we selected 18 additional factors to screen based on a gene regulatory network analysis. In  
9 this analysis, we found differentially expressed genes between meiotic oogonia and all  
10 other cell types, then calculated which genes in the regulatory network were upstream of  
11 the differentially expressed genes, multiplying network edge weights by differential  
12 expression fold-changes to calculate a weighted score. We included a further 12 factors  
13 based on literature reports of pro-meiotic function. Finally, we included 27 factors from the  
14 Cancer Pathways ORFs library (30), which contains modulators of several common cellular  
15 signaling pathways. Our initial library for barcode enrichment screening contained 78  
16 factors. For scRNAseq screening, we included an additional 10 factors involved in  
17 epigenetics and signal transduction, bringing the total to 88. A full list of the factors in our  
18 library is provided in Supplementary Table 2.

## 19 **Plasmid library construction and PiggyBac transposon integration**

20 Expression plasmids for 88 candidate regulatory factors were constructed by MegaGate  
21 cloning (31) into a barcoded PiggyBac destination vector containing a doxycycline-  
22 inducible promoter (28), as well as a puromycin selection marker. Barcodes and transgene  
23 sequences were verified by Sanger sequencing. For later experiments, an alternative  
24 version of the plasmid lacking the barcode and containing a hygromycin selection marker  
25 was constructed for top candidate factors. For constitutive BCL2 expression, a version of  
26 the plasmid was constructed with an EF1a promoter instead of a doxycycline-inducible  
27 promoter. All plasmids will be made available on Addgene following acceptance of this  
28 paper.

29 Plasmids were pooled and co-electroporated into iPSCs along with a PiggyBac transposase  
30 expression plasmid (Systems Bioscience), using a Lonza Nucleofector with pulse setting  
31 CA-137. For medium-copy (3–5 per cell) integration, 5 fmol of pooled library and 500 ng of  
32 transposase plasmid were used per 200,000 hiPSCs and 20  $\mu$ L of P3. For high-copy (10–50  
33 per cell) integration, 50 fmol of pooled library was used instead. Average integration  
34 numbers were previously characterized for the same transposons used in this study (28),  
35 but not evaluated directly. Selection was performed with puromycin (400 ng/mL) and/or

1 hygromycin (50 µg/mL) beginning 2 days after nucleofection and continuing for at least 5  
2 additional days before subsequent experiments.

### 3 **Flow cytometry**

4 Flow cytometry was performed on a BD LSR Fortessa instrument. Cell sorting was  
5 performed on a Sony SH800S sorter using a 100 µm chip. Compensation controls were  
6 acquired using cells in which mGreenLantern and tdTomato reporters had been activated  
7 by CRISPRa. DAPI (100 ng/mL) was used to stain dead cells for exclusion. Data analysis  
8 was performed using the Cytoflow python package (v. 1.0.0) (32). Representative gating is  
9 shown in Fig. S3A.

### 10 **Immunofluorescence microscopy**

11 Cells were cultured on Matrigel-coated ibidi dishes (8-well, cat# 80826; or 96-well, cat#  
12 89626). For 8-well dishes, 100 µL of staining solutions and 200 µL of wash solutions were  
13 used per well. For 96-well dishes, 50 µL of staining solutions and 100 µL of wash solutions  
14 were used per well. All steps except primary antibody incubation were performed at room  
15 temperature.

16 Cells were washed with PBS and fixed with 4% PFA in PBS for 10 minutes, followed by one  
17 5-minute wash with PBS and one 5-minute wash with PBST (0.1% Triton X-100 in PBS). Cells  
18 were incubated with blocking solution (1% BSA and 5% normal donkey serum in PBST) for  
19 20-30 minutes, followed by an overnight incubation at 4 °C with primary antibodies in  
20 blocking solution. Three 5-minute PBST washes were performed, followed by a 1-hour  
21 incubation with secondary antibodies and DAPI (1 µg/mL) in blocking solution. Two 5-  
22 minute PBST washes were performed, and the cells were stored in the dark at 4°C in PBST  
23 prior to imaging on a Zeiss LSM980 confocal microscope using a LD C-Apochromat 40x/1.1  
24 water immersion objective. A list of antibodies used, and their dilutions, is provided in  
25 Supplementary Table 5.

26 Initial image processing was performed using FIJI (ImageJ version 2.14.0/1.54f) (33).  
27 Brightness and contrast were adjusted equally across all images from each experiment.  
28 Segmentation and quantification were performed using the cellpose Python package  
29 (version 2.2.3) (34). Thresholding was performed by using a negative control (typically  
30 hiPSCs) to determine the average background staining intensity, subtracting this average  
31 background intensity from all regions, then classifying any region that was >2x brighter than  
32 then 95<sup>th</sup> percentile of the negative control as positive. Analysis code is available on  
33 Github: <https://github.com/mpersonsmela/meiosis>

### 34 **Barcode enrichment screening**

1 Reporter iPSCs containing integrated expression transposon vectors were harvested with  
2 Accutase. Cells were seeded on Matrigel-coated 6-well plates (500,000 cells per plate) in  
3 mTeSR1 medium (Stemcell Technologies) with doxycycline (1  $\mu\text{g}/\text{mL}$ ) and Y-27632 (10  $\mu\text{M}$ ).  
4 A media change was performed after 24 hours. At this point, candidate differentiation  
5 media were tested, based on previous claims of meiosis induction in the literature. These  
6 included:

7 A-MEM with 1X Glutamax, 1X Insulin-Transferrin-Selenium-X supplement, 0.2% BSA, 0.2%  
8 chemically defined lipid concentrate, 200  $\mu\text{g}/\text{mL}$  ascorbic acid, 1  $\text{ng}/\text{mL}$  FGF2, and 20  
9  $\text{ng}/\text{mL}$  GDNF (35).

10 Nutrient restriction/retinoic acid: EBSS with 0.1X IMDM, 0.1X supplements (N2, glutamax,  
11 sodium pyruvate, MEM essential vitamins, non-essential amino acids), 0.1% FBS, 0.5%  
12 Knockout Serum Replacement, 0.6  $\text{mg}/\text{mL}$  glucose, 0.1  $\text{mg}/\text{mL}$  lactic acid, 0.5  $\text{mg}/\text{mL}$  BSA,  
13 5  $\mu\text{M}$  2-mercaptoethanol, 10  $\mu\text{M}$  ascorbic acid, 1  $\mu\text{g}/\text{mL}$  biotin, 3  $\text{ng}/\text{mL}$  beta-estradiol, 1  
14  $\text{ng}/\text{mL}$  FGF2, 1.5  $\text{ng}/\text{mL}$  GDNF, and 100 nM retinoic acid (7).

15 We additionally tested mTeSR1 (which maintains primed pluripotency), HENSM (which  
16 induces naïve pluripotency) (36), StemPro-34 based spermatogonial stem cell culture  
17 medium (37), and APEL2 (Stemcell Technologies; a “neutral” medium lacking growth  
18 factors).

19 The cells were cultured in these six media, all supplemented with 1  $\mu\text{g}/\text{mL}$  doxycycline, for  
20 six additional days. A media change was performed each day. Cells were harvested with  
21 Accutase on day seven post-induction, and reporter-positive cells were isolated by FACS.  
22 DNA was extracted from REC8+, SYCP3+, and DDX4+ cells, as well as the pre-sorting  
23 population and pre-differentiation population. Barcodes were amplified by two rounds of  
24 PCR and sequenced on an Illumina MiSeq as previously described (25). Barcode  
25 enrichment was calculated by comparing the barcode frequencies in reporter-positive and  
26 pre-sorting cells. We also sequenced barcodes from the pre-differentiation cells, but did  
27 not use these for analysis since effects were dominated by changes in cell growth rate.

## 28 **CRISPRi of epigenetic factors**

29 Guide RNAs targeting the promoters of ten epigenetic factors (*MAX*, *MGA*, *E2F6*, *RNF2*,  
30 *PCGF6*, *SETDB1*, *DNMT1*, *DNMT3A*, *DNMT3B*, and *UHRF1*; three guides per gene) were  
31 cloned into a doxycycline-inducible dCas9-KRAB expression transposon plasmid (28).  
32 Each transposon plasmid was integrated into SYCP3 reporter iPSCs as described above.  
33 iPSCs were treated with 1  $\mu\text{g}/\text{mL}$  doxycycline in mTeSR1 for six days, harvested with  
34 Accutase, and analyzed by flow cytometry (Fig. S4). Additionally, knockdown efficiency was

1 evaluated by qPCR with PowerUp SYBR Green Master Mix (Thermo Fisher) using *GAPDH* as  
2 a control gene (Fig. S4C). Primers used are given in Supplementary Table 4.

### 3 **Screening of conditions for reporter activation**

4 Several flow cytometry experiments were performed in order to optimize conditions for  
5 activating REC8 and SYCP3 expression in male (PGP1) and female (F3) reporter lines.

6 First, expression vectors for sixteen promising factors (Fig. S3) chosen based on barcode  
7 enrichment data were individually integrated into REC8 and SYCP3 reporter iPSC lines, and  
8 expression was induced by treatment with 1  $\mu\text{g}/\text{mL}$  doxycycline in APEL2 medium following  
9 the protocol described above in the barcode enrichment section.

10 Second, a fractional factorial screen for REC8 activation was conducted in F3 and PGP1  
11 D4TR8G reporter lines, using 32 combinations of seven promising factors and following the  
12 same differentiation protocol. (Fig. 1C)

13 Third, REC8, SYCP3, and DDX4 activation was measured after performing differentiation  
14 using expression of STRA8, HOXB5, and BCL2 in the presence of different basal media and  
15 additives. Four basal media were tested: mTeSR1 (Stemcell Technologies); APEL2 (Stemcell  
16 Technologies); DMEM/F12 with 1X Glutamax and 10% KSR (Gibco); and Advanced RPMI  
17 with 1X nonessential amino acids, 1X Glutamax, and 0.5X B27 supplement minus Vitamin  
18 A (all Gibco). For each of the differentiation media, five additives were tested, as well as a  
19 doxycycline-only control. The additives and their concentrations were: 1  $\mu\text{M}$  retinoic acid, 1  
20  $\mu\text{M}$  AM580, 25  $\mu\text{M}$  PRT4165, 20  $\mu\text{M}$  RB3, and 1 mM sodium valproate. A media change was  
21 performed every day. At days 6, 7, and 8 post-induction, cells were harvested with  
22 Accutase and analyzed by flow cytometry (Fig. 1D)

23 Fourth, REC8, SYCP3, and DDX4 activation was measured in cells treated with or without  
24 DNMT1 inhibitor (5  $\mu\text{M}$  GSK3484862). Differentiation was performed using expression of  
25 STRA8, HOXB5, and BCL2 in APEL2 medium supplemented with 1  $\mu\text{M}$  AM580 and 1  $\mu\text{g}/\text{mL}$   
26 doxycycline. Cells were harvested with Accutase after 7 days and analyzed by flow  
27 cytometry (Fig. 1E).

### 28 **scRNAseq screening**

29 Prior to scRNAseq, the following six cell populations were generated by integration of  
30 transposon expression vectors:

- 31 • PGP1 D4TR8G, with BCL2, HOXB5, and STRA8 under hygromycin selection
- 32 • F3 D4TS3G, with BCL2, HOXB5, and STRA8 under hygromycin selection

- 1       • PGP1 D4TR8G, with BCL2, HOXB5, and STRA8 under hygromycin selection and the
- 2       full pool of 88 candidate factors under puromycin selection
- 3       • F3 D4TS3G, with BCL2, HOXB5, and STRA8 under hygromycin selection and the full
- 4       pool of 88 candidate factors under puromycin selection
- 5       • PGP1 D4TR8G, with the full pool of 88 candidate factors under puromycin selection
- 6       • F3 D4TS3G, with the full pool of 88 candidate factors under puromycin selection

7 The cells were differentiated according to the following method, which had been chosen  
8 based on its ability to activate REC8 and SYCP3 expression. Cells containing integrated  
9 expression vectors were seeded in mTeSR1 containing 10  $\mu$ M Y-27632, 5  $\mu$ M GSK3484862,  
10 and 1  $\mu$ g/mL doxycycline. After 24 hours, the medium was changed to APEL2 with 5  $\mu$ M  
11 GSK3484862, 1  $\mu$ M AM580, and 1  $\mu$ g/mL doxycycline. A media change was performed every  
12 other day. After a total of seven days of differentiation, cells were harvested with Accutase  
13 and sorted based on reporter expression. Cells were fixed using a Parse fixation kit, and  
14 scRNAseq library preparation was performed using a Parse WT Mega v2 kit. A list and  
15 description of samples is provided in Supplementary Table 6. Sequencing was performed  
16 on an Illumina Novaseq X Plus using three full 10B PE150 flowcells. Alignment and counts  
17 matrix generation was performed using the Parse Biosciences pipeline (v.0.9.6).

18 To enrich the library for barcode sequences, we first performed PCR with biotinylated  
19 primers to generate a dsDNA biotinylated bait containing the 120bp of sequence  
20 immediately 3' of the barcode sequence in our expression vector. We isolated the bait DNA  
21 from the PCR using a 3X volume of ProNex beads, and eluted in 10 mM Tris pH 8.0 buffer.  
22 Next, we used 200 fmol of bait DNA as a custom probe in the Parse Gene Capture kit,  
23 following the manufacturer's protocol aside from this substitution. After qPCR to verify the  
24 barcode enrichment, the resulting library was sequenced on one lane of a NovaSeq X Plus  
25 10B PE150 flowcell.

26 scRNAseq data were filtered by number of reads per cell (<100,000), number of genes  
27 detected (>1,000 and <14,000), and mitochondrial read percentage (<10%). Transgene  
28 barcode reads were merged into the dataset by matching cell barcodes. Analysis was  
29 performed using scanpy (38) for normalization, integration with the fetal germ cell  
30 reference atlas, and gene scoring (16).

### 31 **Refinement of factors for meiosis induction**

32 Several experiments were performed in order to optimize the protocol for meiosis  
33 induction. First, for each of the 23 candidate factors identified by the scRNAseq screen  
34 (excluding HOXB5, which was already integrated), an expression vector with a puromycin  
35 selection marker was integrated into PGP1 D4TR8G and F3 D4TS3G reporter hiPSCs which

1 already contained expression vectors for BCL2, HOXB5, and STRA8 with hygromycin  
2 selection markers. Two control conditions were additionally included: BCL2, HOXB5, and  
3 STRA8 only; and a no-factor control. Cells were differentiated using the same conditions as  
4 for the scRNAseq experiment (APEL2 with doxycycline, AM580, and GSK3484862). Cells  
5 were fixed and stained for SYCP3, HORMAD1, and DDX4 after 7 days of differentiation.

6 Second, expression vectors for the seven top factors identified in the previous experiment  
7 (BCL2, HOXB5, STRA8, myr-AKT1, BOLL, MEIOC, and MEIOSIN) were pooled and integrated  
8 into PGP1 D4TR8G and F2 D4TDZG hiPSCs. Cells were differentiated in the same manner,  
9 and fixation and staining for SYCP3, HORMAD1, and  $\gamma$ H2AX was performed after 7, 9, 13,  
10 and 16 days of differentiation. As a control, the same differentiation and staining was  
11 performed using only BCL2, HOXB5, and STRA8.

12 Third, a fractional factorial screen was performed in order to identify the contributions of  
13 the seven top factors. Sixteen combinations of the seven factors (including one control  
14 combination lacking all factors) were integrated into F2 D4TDZG, F3 D4TS3G, and PGP1  
15 D4TR8G reporter lines. Additionally, expression vectors for DAZL and BOLL were integrated  
16 in order to evaluate previous claims that those factors alone could induce meiosis (11, 14).  
17 Cells were fixed and stained for SYCP3, HORMAD1, and  $\gamma$ H2AX after 13 days of  
18 differentiation. Additional cells were analyzed by flow cytometry for reporter expression.

19 Fourth, a full factorial screen was performed in order to confirm the best factors for meiosis  
20 induction. A constitutive EF1a-driven BCL2 expression plasmid was integrated into F2  
21 D4TDZG, F3 D4TT2G, and PGP1 D4TR8G reporter hiPSCs under hygromycin selection.  
22 Then, all eight possible combinations of HOXB5, BOLL, and MEIOC expression vectors  
23 were integrated under puromycin selection. Cells were fixed and stained for after 13 days  
24 of differentiation, with the first 3 days at 37 °C and the remainder at 34 °C. Two stains were  
25 used: SYCP3, HORMAD1, and  $\gamma$ H2AX; and TEX12 and SYCP3.

#### 26 **Timing of media additives and factor expression:**

27 A Shield1-inducible HOXB5 PiggyBac transposon plasmid was constructed using an EF1a  
28 promoter driving HOXB5 with a C-terminal degradation domain, which could be stabilized  
29 by addition of Shield1. This plasmid, along with expression plasmids for constitutive EF1a-  
30 driven BCL2 and doxycycline-inducible BOLL, were integrated into F2 D4TDZG, F3 D4TS3G,  
31 and PGP1 D4TR8G reporter lines. Cells were seeded at a density of 50,000/cm<sup>2</sup> in Matrigel  
32 coated 96-well ibidi plates in mTESR1 + 10  $\mu$ M Y-27632. After 24 hours, the medium was  
33 changed to APEL2. Subsequently, a full media change was performed every 48 hours, and  
34 cells were fixed and stained after 11 days of differentiation. This experiment was performed  
35 at 37 °C.



- 1 The following media additive conditions were tested:
- 2 1. No additives (negative control)
- 3 2. Full, continuous dose of all additives (positive control): 5  $\mu$ M GSK3484862 (DNMT1i),
- 4 1  $\mu$ M AM580, 1  $\mu$ g/mL doxycycline, 500 nM Shield1
- 5 3. No doxycycline, 100 nM Shield1, full dose of DNMTi and AM580
- 6 4. 0.1  $\mu$ g/mL doxycycline, no Shield1, full dose of DNMTi and AM580
- 7 5. 0.1  $\mu$ g/mL doxycycline, 100 nM Shield1, full dose of DNMTi and AM580
- 8 6. 0.1  $\mu$ g/mL doxycycline, 500 nM Shield1, full dose of DNMTi, and AM580
- 9 7. 1  $\mu$ g/mL doxycycline, 100 nM Shield1, full dose of DNMTi and AM580
- 10 8. Full dose of all additives except doxycycline
- 11 9. Doxycycline added only after day 1. Other additives at full dose.
- 12 10. Doxycycline added only after day 3. Other additives at full dose.
- 13 11. Doxycycline added only after day 5. Other additives at full dose.
- 14 12. Doxycycline added only after day 7. Other additives at full dose.
- 15 13. Doxycycline added only after day 9. Other additives at full dose.
- 16 14. Full dose of all additives except Shield1
- 17 15. Shield1 added only after day 1. Other additives at full dose.
- 18 16. Shield1 added only after day 3. Other additives at full dose.
- 19 17. Shield1 added only after day 5. Other additives at full dose.
- 20 18. Shield1 added only after day 7. Other additives at full dose.
- 21 19. Shield1 added only after day 9. Other additives at full dose.
- 22 20. Full dose of all additives except AM580
- 23 21. AM580 added only after day 1. Other additives at full dose.
- 24 22. AM580 added only after day 3. Other additives at full dose.
- 25 23. AM580 added only after day 5. Other additives at full dose.
- 26 24. AM580 added only after day 7. Other additives at full dose.
- 27 25. AM580 added only after day 9. Other additives at full dose.
- 28 26. Full dose of all additives except DNMTi
- 29 27. DNMTi withdrawn after day 5. Other additives at full dose.
- 30 28. DNMTi withdrawn after day 7. Other additives at full dose.
- 31 29. DNMTi withdrawn after day 9. Other additives at full dose.
- 32 30. Shield1 added only from days 0–5, doxycycline added only from days 5–11, other
- 33 additives at full dose.
- 34 31. Shield1 added only from days 0–7, doxycycline added only from days 5–11, other
- 35 additives at full dose.
- 36 32. Shield1 added only from days 0–7, doxycycline added only from days 7–11, other
- 37 additives at full dose.

## 1 **Evaluation of temperatures and timings for meiosis induction**

2 As an initial experiment to evaluate the effects of lower temperature on male meiosis,  
3 PGP1 D4TR8G reporter hiPSCs containing integrated expression vectors for BCL2, HOXB5,  
4 BOLL, and MEIOC were seeded in 8-well ibidi dishes at 50,000 cells/cm<sup>2</sup> in mTeSR1 with 5  
5  $\mu$ M GSK3484862, 1  $\mu$ g/mL doxycycline, and 10  $\mu$ M Y-27632. After 24 hours, the media was  
6 replaced with APEL2 containing 5  $\mu$ M GSK3484862 and 1  $\mu$ g/mL doxycycline. A 50% media  
7 change was performed every 2 days, and GSK3484862 was withdrawn starting on day 7.  
8 Initially, all cells were cultured at 37 °C. One plate was moved to a 34 °C incubator after the  
9 first day. Cells were fixed on day 13, stained for HORMAD1, SYCP3, and  $\gamma$ H2AX, and  
10 imaged.

11 As a confirmatory experiment, F2 D4TDZG, F3 D4TS3G, and PGP1 D4TR8G reporter hiPSCs  
12 containing integrated expression vectors for BCL2, HOXB5, BOLL, and MEIOC were  
13 differentiated according to the same protocol. The following conditions were tested:

- 14 1. 34 °C starting on day 3, fixation at day 13
- 15 2. 34 °C starting on day 1, fixation at day 14
- 16 3. 34 °C starting on day 3, fixation at day 15
- 17 4. 34 °C starting on day 3, fixation at day 16
- 18 5. Continuous 37 °C, fixation at day 16
- 19 6. 34 °C starting on day 1, fixation at day 17
- 20 7. 34 °C starting on day 3, fixation at day 19
- 21 8. 34 °C starting on day 3, fixation at day 21

22 After fixation, cells were stained for HORMAD1, SYCP3,  $\gamma$ H2AX, and actin, and imaged.

## 23 **Final protocol for meiosis induction**

24 Based on the results of our screening and optimization experiments, which are described  
25 above, we have developed the following protocol for robust initiation of meiosis:

26 A constitutive or doxycycline-inducible expression vector for the anti-apoptotic factor  
27 BCL2, as well as doxycycline-inducible expression vectors for meiosis-promoting factors  
28 (HOXB5, BOLL, and/or MEIOC), are integrated into human iPSCs using PiggyBac  
29 transposase. The iPSCs are seeded at 50,000 cells/cm<sup>2</sup> on Matrigel-coated plates in  
30 mTeSR1 supplemented with 5  $\mu$ M GSK3484862, 1  $\mu$ g/mL doxycycline, and 10  $\mu$ M Y-27632.  
31 For 6-well plates, 1.5 mL of medium is used per well, and volumes for smaller plates are  
32 scaled down proportionally to their surface area. After one day, the media is replaced with  
33 APEL2 containing 5  $\mu$ M GSK3484862 and 1  $\mu$ g/mL doxycycline. A 50% media change is  
34 performed every 2 days, and GSK3484862 is withdrawn starting on day 7. Initiation of  
35 meiosis is complete by roughly day 13.

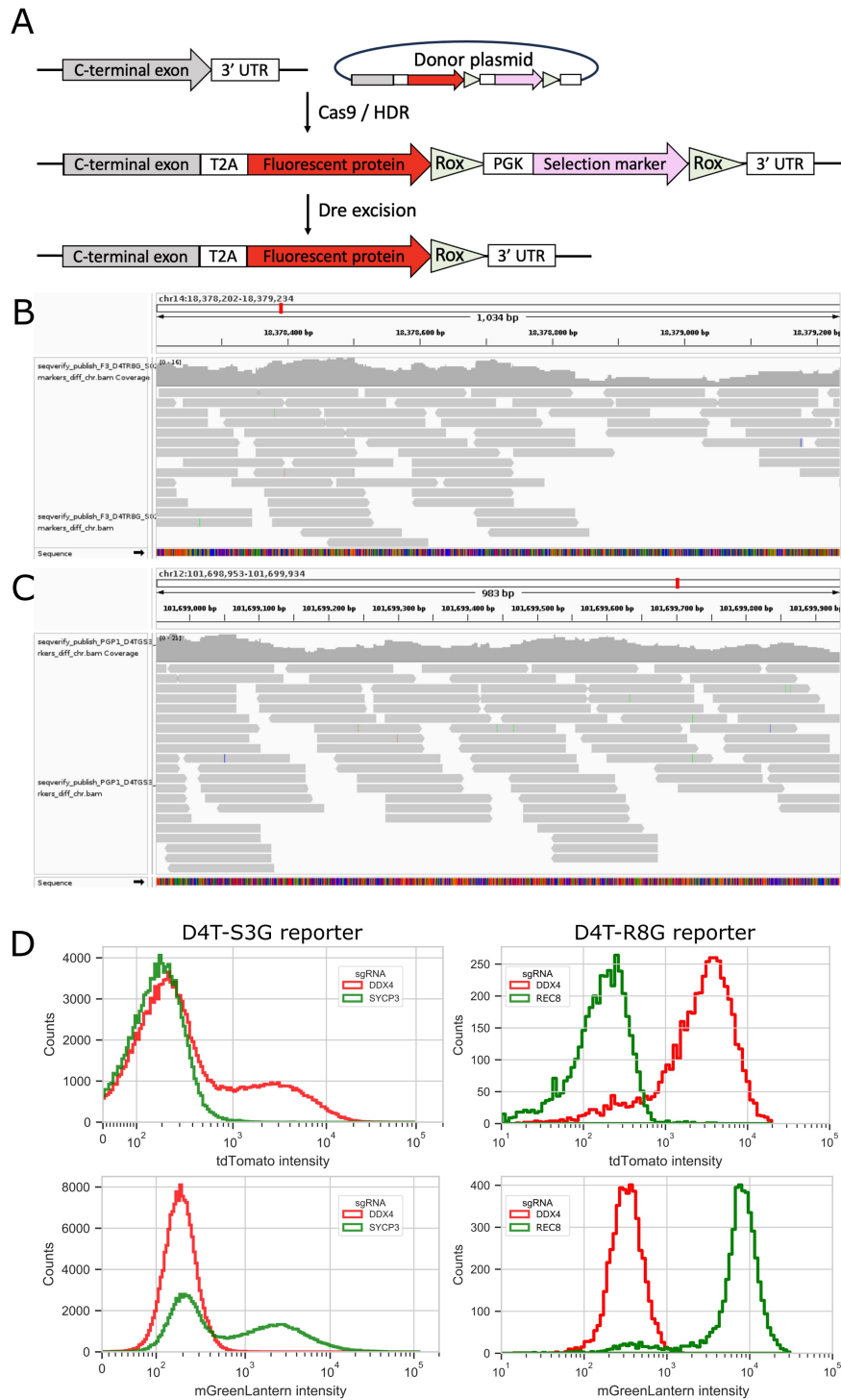
## 1 **Timecourse scRNAseq and imaging**

2 Plasmids for constitutive expression of BCL2 and doxycycline-inducible expression of  
3 HOXB5, BOLL, and MEIOC were integrated into F2 D4TDZG, F3 D4TT2G, and PGP1 D4TR8G  
4 reporter hiPSCs. Meiosis was initiated following the final optimized protocol, with cells  
5 cultured on 8-well ibidi dishes for immunofluorescence imaging and 12-well plates for  
6 scRNAseq. At each day from day 0 (hiPSC) to day 15, cells were fixed for imaging and  
7 harvested for scRNAseq. Stains used for imaging were: rabbit anti-HORMAD1, goat anti-  
8 SYCP3, mouse anti  $\gamma$ H2AX, and rat anti-T2A; and rabbit anti-TEX12, goat anti-SYCP3, mouse  
9 anti-RAD51, and rat anti-KI67. Samples for scRNAseq were counted and fixed using the  
10 Parse Biosciences fixation kit. Library preparation was performed using the Parse  
11 Biosciences WT v3 kit. Sequencing was performed on two lanes of a Novaseq X Plus 25B  
12 PE150 flowcell. Alignment and counts matrix generation was performed using the Parse  
13 Biosciences pipeline (v.1.2.1). Filtering, normalization, and cell type annotation were  
14 performed in scanpy as described above. To build a reference atlas, we combined the fetal  
15 gonad atlas and adult testis atlas (16, 22), removing somatic cell types from the testis atlas  
16 and only keeping genes that were expressed in both atlases.

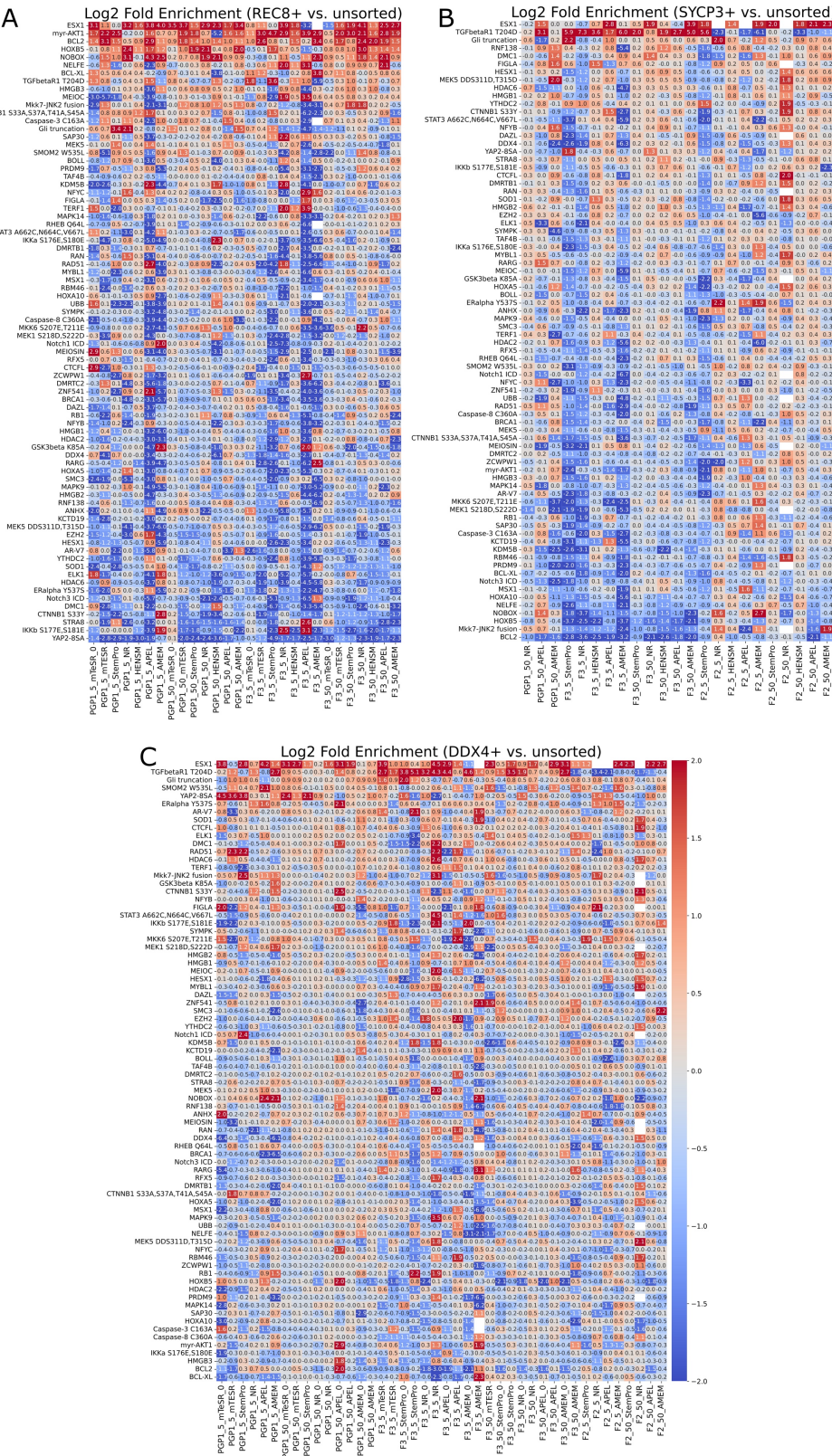
## 17 **Statistics and data analysis**

18 Results of fractional factorial screens were analyzed by fitting linear models using the lm  
19 function in R (version 4.3.2). For flow cytometry data, values expressed as a proportion (0 -  
20 100%) were logit-transformed before fitting the model. Significance calculations in bar  
21 plots were performed using two-tailed Mann-Whitney U tests.

22



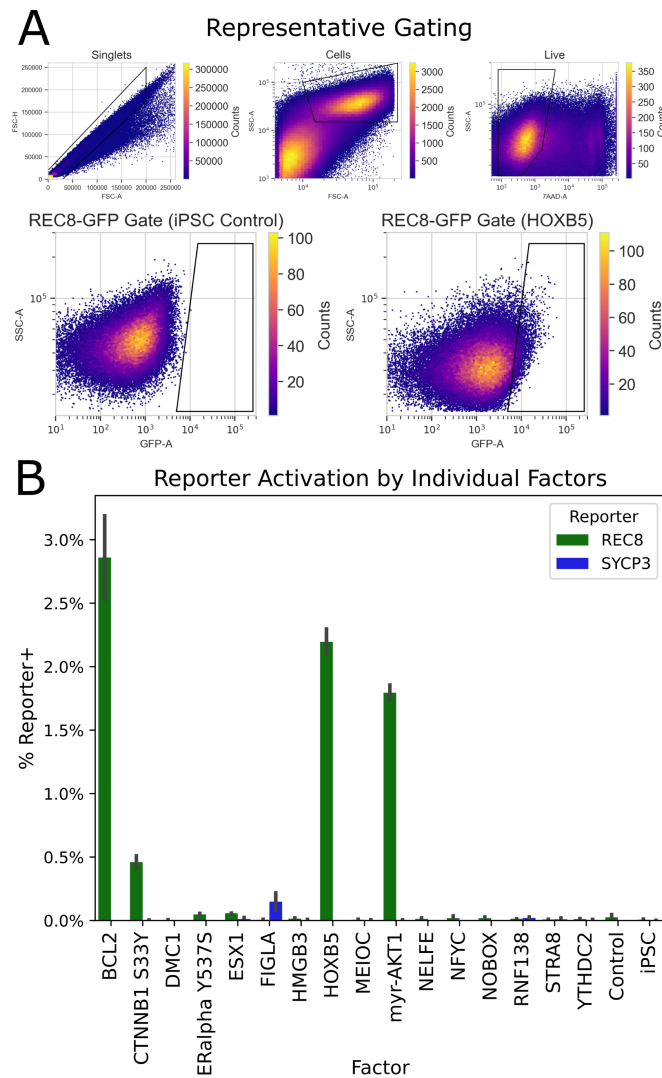
1  
 2 **Fig. S1. Construction and validation of hiPSC reporter lines.** (A) Knock-in editing  
 3 strategy. (B) SeqVerify validation of REC8 reporter allele using whole genome sequencing.  
 4 (C) SeqVerify validation of SYCP3 reporter allele using whole genome sequencing. (D)  
 5 Functional validation of reporter hiPSCs using flow cytometry and CRISPRa with gRNAs  
 6 targeting the promoters of *REC8*, *SYCP3*, and *DDX4*.



1 **Fig. S2. Barcode enrichment results** for (A) REC8, (B) SYCP3, and (C) DDX4. Reporter  
2 hiPSCs (F2, F3, and PGP1) were nucleofected with low (5 fmol) or high (50 fmol) doses of  
3 plasmid library pool, treated with doxycycline to induce expression, and differentiated in  
4 various media (mTeSR, StemPro, nutrient restriction (NR), HENSM, spermatogonial stem  
5 cell medium (AMEM), and APEL2). Reporter-positive cells were sorted after 7 days, and  
6 barcode frequencies were compared to unsorted cells.

7

1



2

3 **Fig. S3. Flow cytometry analysis of reporter expression induced by individual factors.**

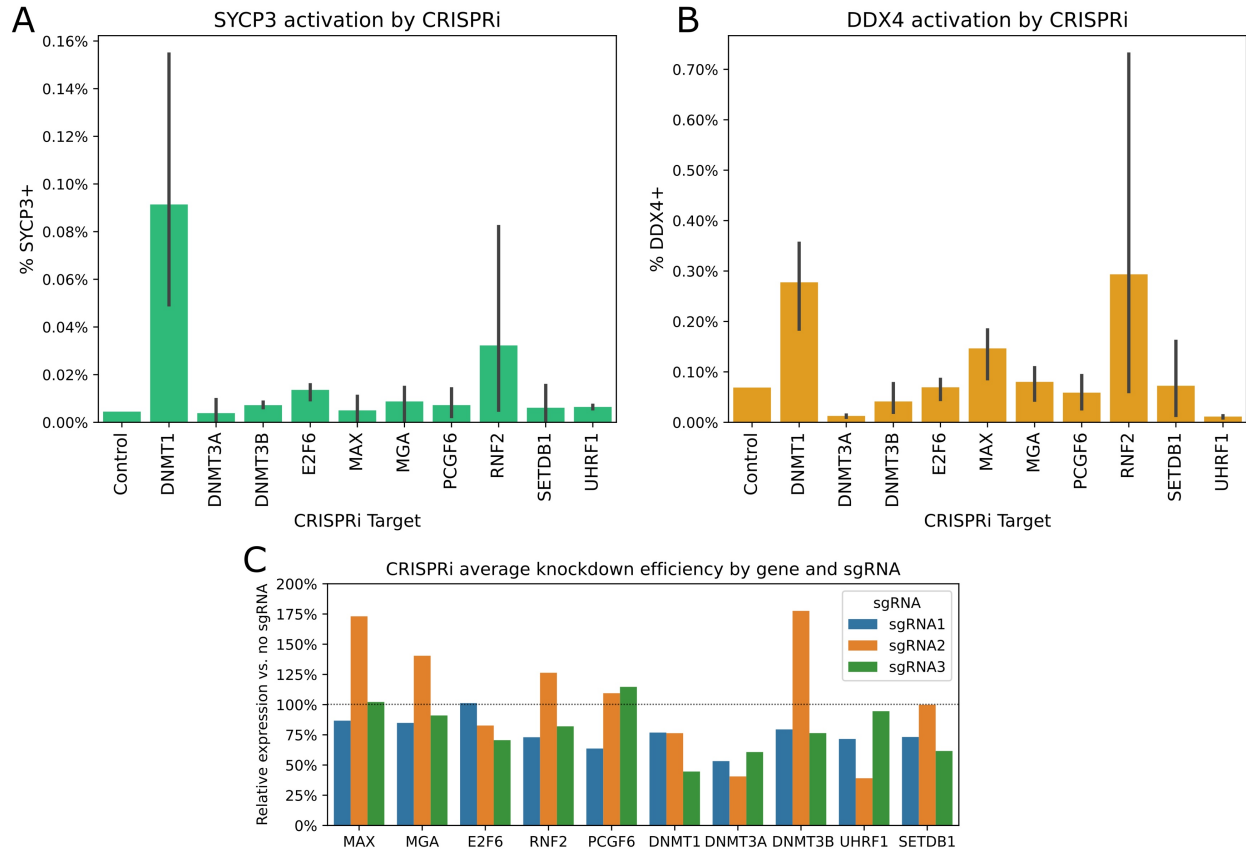
4 (A) Representative gating strategy for singlets, cells, live cells, and reporter-positive cells.

5 (B) Activation of REC8 and SYCP3 reporters by sixteen individual factors chosen based on

6 barcode enrichment results (n = 2 biological replicates per factor per reporterX). Error bars

7 are standard error of the mean.

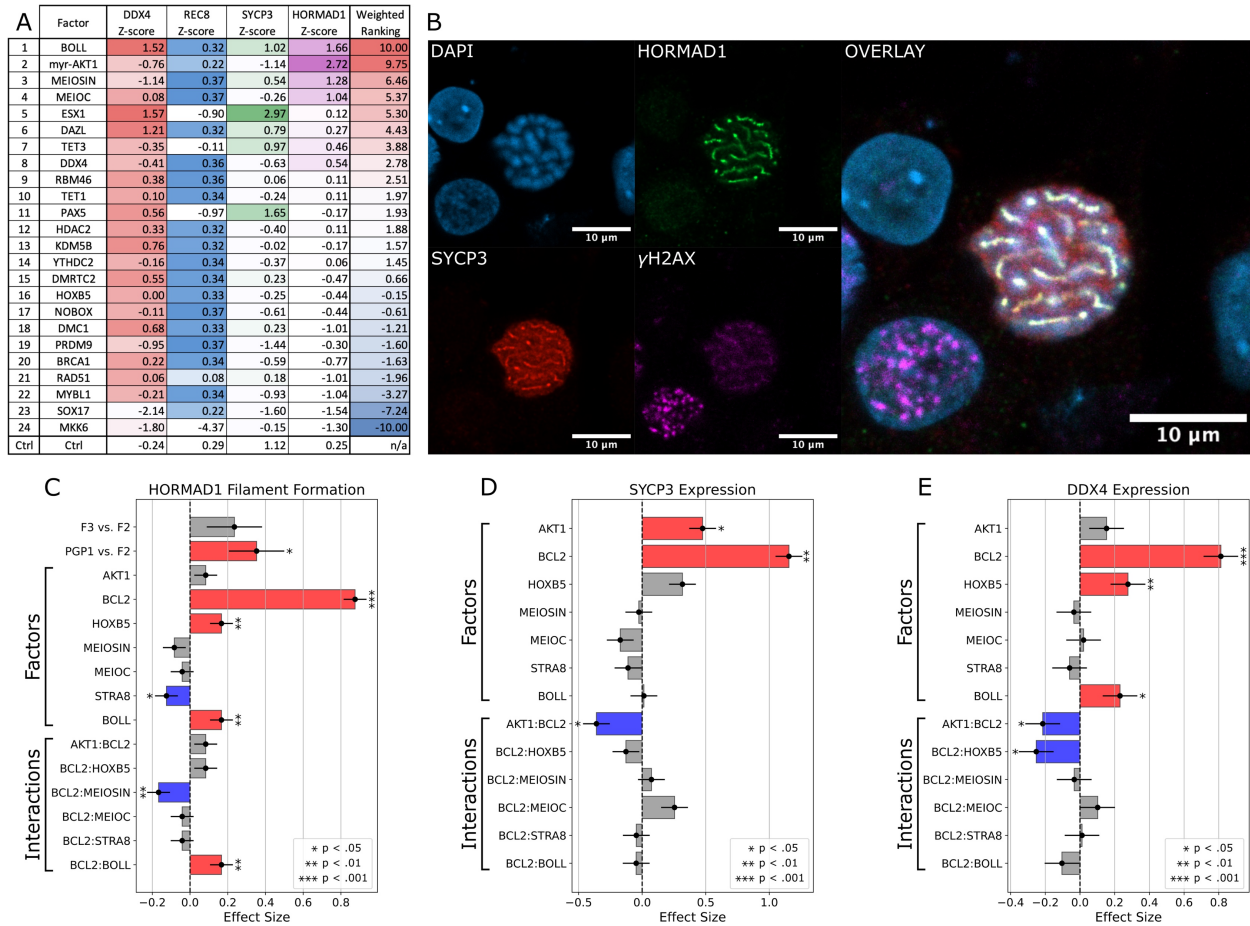
8



1  
2 **Fig. S4. Pilot screen for activation of SYCP3 and DDX4 expression upon CRISPRi**  
3 **knockdown of ten epigenetic modifiers. (A)** Activation of SYCP3 expression measured by  
4 flow cytometry (n = 3 sgRNAs per gene). **(B)** Activation of DDX4 expression measured by  
5 flow cytometry (n = 3 sgRNAs per gene). **(C)** qPCR measurement of average knockdown  
6 efficiency (n = 2 technical replicates per guide), calculated by  $2^{-\Delta\Delta Ct}$  with *GAPDH* as a  
7 reference gene. The bulk knockdown efficiency was poor for most guides, although it is  
8 possible that subpopulations of cells experienced a greater knockdown.

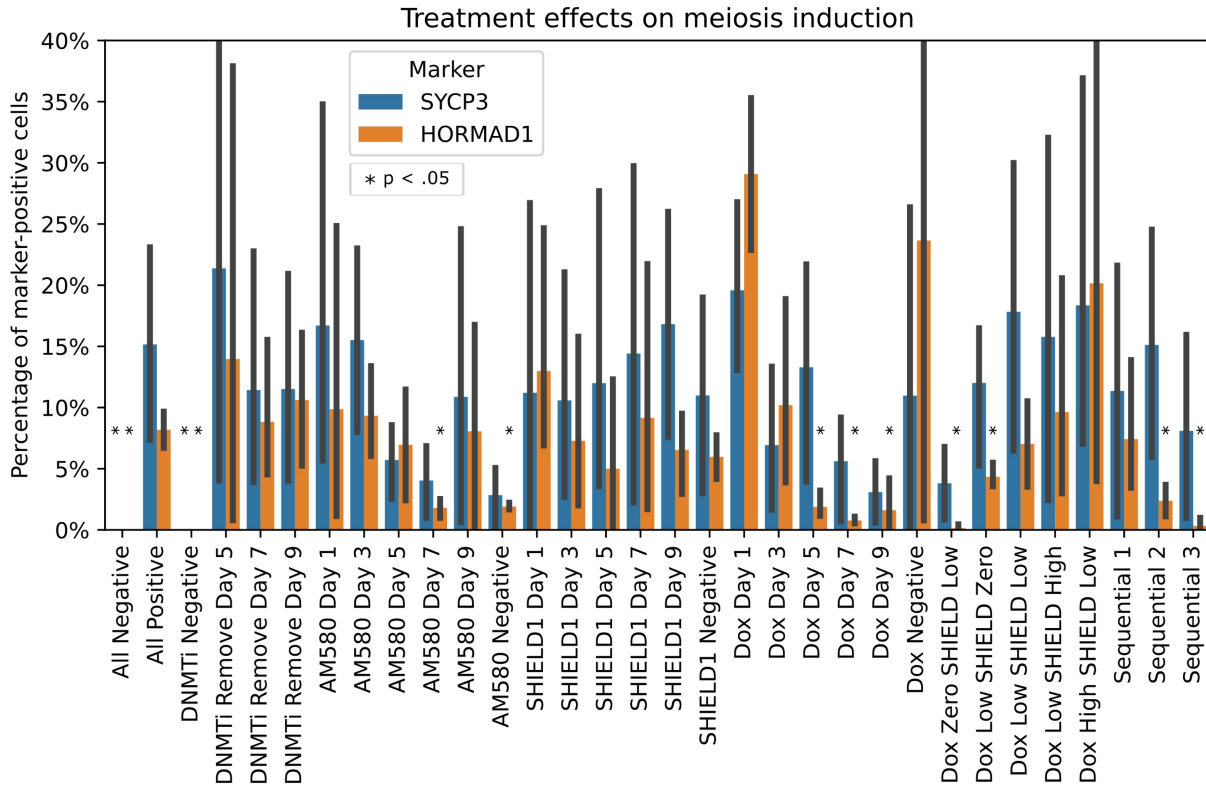
9



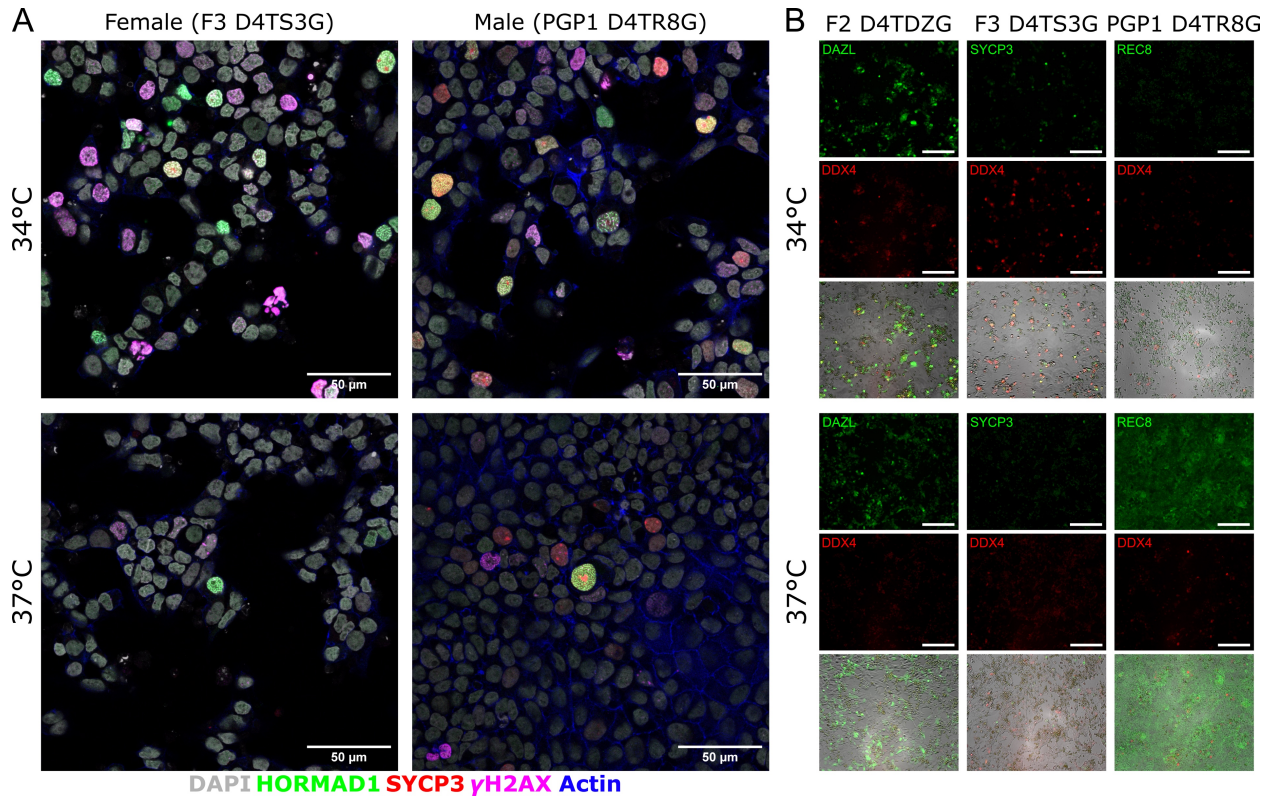


1  
 2 **Fig. S5. Optimization of factors for meiosis induction.** (A) 24 candidate factors identified  
 3 by an scRNAseq screen (Fig. 2D) were each co-expressed with STRA8, BCL2, and HOXB5.  
 4 Reporter expression was analyzed by flow cytometry, and HORMAD1 expression was  
 5 analyzed by immunofluorescence microscopy. Factors were ranked according to the  
 6 results. (B) HORMAD1 and SYCP3 filament formation observed by immunofluorescence  
 7 microscopy after twelve days of expression of seven top factors (STRA8, BCL2, HOXB5,  
 8 BOLL, AKT1, MEIOSIN, and MEIOC). (C) Results of a fractional factorial screen of the seven  
 9 top factors, for HORMAD1 filament formation. (D) Results for SYCP3 expression. (E) Results  
 10 for DDX4 expression.

11

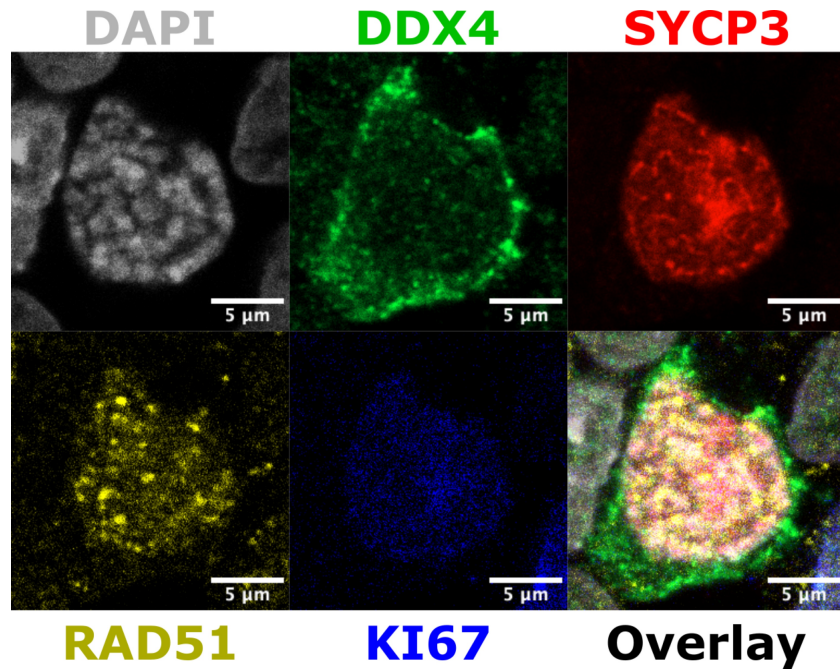


1  
 2 **Fig. S6. Quantification of the effects of omitting various components of the meiosis**  
 3 **induction protocol, or reducing their doses.** Significance test comparisons are to the “all  
 4 positive” control.



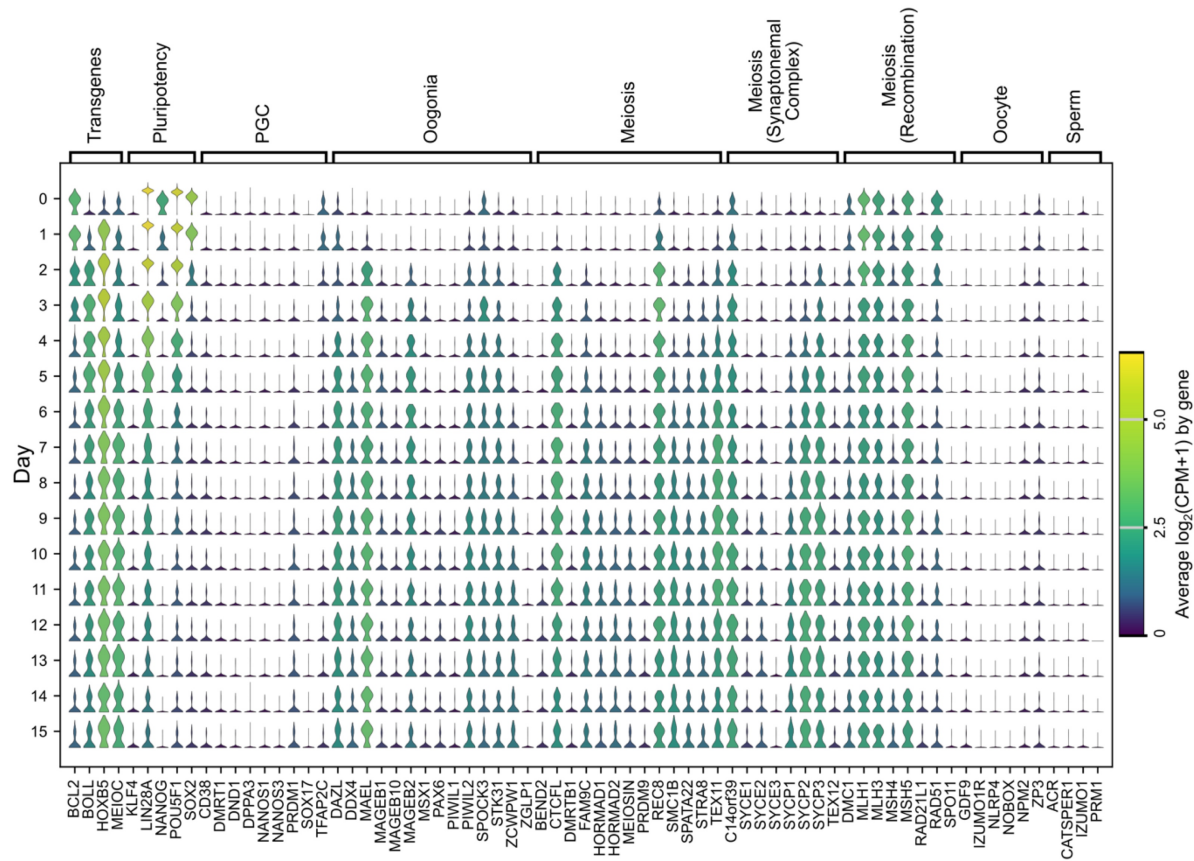
1  
2 **Fig. S7. Microscope images of meiosis induction from male and female hiPSCs at 34 °C**  
3 **vs. 37 °C. (A)** Immunofluorescence microscopy, staining for DNA (DAPI; gray), HORMAD1  
4 (green), SYCP3 (red), γH2AX (magenta), and actin (phalloidin; blue). Scale bar is 50 μm. **(B)**  
5 Live imaging of fluorescent reporter expression of DAZL, SYCP3, REC8, and DDX4. Scale  
6 bar is 200 μm.

7



2 **Fig. S8. Immuno-staining for DDX4, SYCP3, RAD51, and KI67 in a day 15 female meiotic**  
3 **cell.** Cytoplasmic DDX4 staining, filamentous SYCP3 staining, and nuclear RAD51 foci are  
4 observed.

5



1  
 2 **Fig. S9. Violin plots showing expression of marker genes from days 0 to 15 of meiosis**  
 3 **induction.** Units for the color scale are  $\log_2(\text{CPM}+1)$ . Categories include: exogenous  
 4 transgenes, pluripotency markers, primordial germ cell markers, oogonia markers, meiosis  
 5 markers, synaptonemal complex components, recombination markers, oocyte markers,  
 6 and sperm markers.  
 7

<b>Study</b>	<b>Summary of method</b>	<b>Outcome</b>
Kee <i>et al.</i> 2009	Differentiation of hESCs in BMP-containing medium for 7 days, with overexpression of DAZL and BOLL.	Weak expression of SYCP3 by immuno-staining; REC8 and HORMAD1 expression not observed.
Medrano <i>et al.</i> 2011	Differentiation of hESCs by FGF withdrawal from culture medium for 14 days, with overexpression of DAZL and DDX4.	Cells differentiated without upregulating SYCP3 or REC8.
Eguizabal <i>et al.</i> 2011	Differentiation of hESCs by FGF withdrawal from culture medium for 21 days, followed by retinoic acid treatment for 21 days, followed by harvesting cells and replating in medium with forskolin, LIF, FGF, and CYP26 inhibitor for 14-28 days.	Cells overgrew during the initial 21-day differentiation. When the protocol was modified to start at the retinoic acid step, REC8 was upregulated but other markers were not expressed.
Easley <i>et al.</i> 2012	Culturing male hiPSCs in mouse spermatogonial stem cell maintenance medium for 10 days.	Cells differentiated without upregulating SYCP3 or REC8.
Jung <i>et al.</i> 2017	Treatment of hESCs with BMP4 for 1 hour, followed by overexpression of DAZL and BOLL for 7 days.	Weak expression of SYCP3 by immuno-staining; REC8 and HORMAD1 expression not observed.

1 **Supplementary Table 1.** Replication attempts of previous reported methods for inducing  
2 meiosis from human pluripotent stem cells. The methods were carried out as described in  
3 their respective papers (see References), although PiggyBac transposon plasmids were  
4 used instead of lentivirus for transgene overexpression.  
5

id	Factor	Reason for inclusion	Barcodes
1	MEK1 S218D,S222D	CPO library	TGACATTAAGCAATTGGTGA
2	myr-AKT1	CPO library	GACTGTCTCCTTGTTAAAT
3	RHEB Q64L	CPO library	CTGTTTGGCTCCATTGGCCA
4	IKKa S176E,S180E	CPO library	GAACGCGTTAAATTATTGCA
5	IKKb S177E,S181E	CPO library	GAGTCCGCACCAGGGCCGGC
6	STAT3 A662C,N664C,V667L	CPO library	TTCATATCGATCTTTAGCGC
7	CTNNB1 S33A,S37A,T41A,S45A	CPO library	AAATATTTATGACACCTGTA
8	GSK3beta K85A	CPO library	TCCGCTGATCGCAGTCCCT
9	CTNNB1 S33Y	CPO library	GAGACATGCTGCTAAGCGGT
10	MAPK9	CPO library	ATATCCGTCGCAGAACAGTT
11	Mkk7-JNK2 fusion	CPO library	TTAAAAACAATCAGTAAAGA
12	MEK5 DDS311D,T315D	CPO library	ATCGTAGCTGTCGTTGTTCCG
13	MEK5	CPO library	TGATGAGACTCTCATCATCA
14	Notch1 ICD	CPO library	GTAGTCACTTTTCCTTTTTG
15	Notch3 ICD	CPO library	TTATCATGTGCGTCCAAGAA
16	MAPK14	CPO library	GAATTTGATCGTGGTATTA
17	MKK6 S207E,T211E	CPO library	TTCTCTAGAGTATTGTTGAA
18	Gli truncation	CPO library	CTGTAAATTGTGTTGTGCCG
19	SMOM2 W535L	CPO library	GAAATATTTTCCTTGTTCCA
20	TGFbetaR1 T204D	CPO library	TCAAACGATCAAGTGAATGA
21	BCL2	CPO library	TTAATATCTGCTAAAAAAG
22	BCL-XL	CPO library	TCGAGTGATTGTACGCGATA
23	Caspase-8 C360A	CPO library	AAACAACAACGATCGAGTTA
24	Caspase-3 C163A	CPO library	GTTATTCCAAAGTTACAAGT
25	ERalpha Y537S	CPO library	GGGAGCTTTGGTAATCAAAC
26	AR-V7	CPO library	TAGACCCTACGGTCAAACCTA
27	YAP2-8SA	CPO library	GGCAAGGCTTACGACAGTGA
28	EZH2	Regulon analysis (oogonia_meiotic)	CCATGTGACAAATCCCATC
29	KCTD19	Known from literature	AGAAGACCTTGATAAGCGCT
30	BRCA1	Regulon analysis (oogonia_meiotic)	CAAGTTTGACAGACCTTCGA
31	DMRTB1	GRN analysis	TTGTAAGGCACACCGAGTCT
32	HMGB2	GRN analysis	GAACGGTCAACAACATTTAA
33	RAN	GRN analysis	GGCAGTGGTGAACGCAGATA
34	HMGB1	Regulon analysis (oogonia_STRA8)	CGAACAGTACACATCACGTC
35	HESX1	Regulon analysis (oogonia_meiotic)	TACCGAATGTAATGCCGCAC

36	RNF138	GRN analysis	TACAAAAAAATCATTTTTGA
37	HOXB5	Regulon analysis (oogonia_STRAB)	GATGAGCGCTGCATTTTAC
38	HOXA5	Regulon analysis (oogonia_STRAB)	TTTTCTGGGGTGGCTCCCAA
39	HOXA10	Regulon analysis (oogonia_STRAB)	TTTATCGTTTGTACAATGAA
40	HMGB3	Regulon analysis (oogonia_STRAB)	GCCGGTAACGTCGGTGACGA
41	DMC1	GRN analysis	TGACGTTGGTTTGATAGCG
42	TERF1	GRN analysis	AGCAGGTTTATCTGATGCAG
43	ZCWPW1	Known from literature	TGACTAAGTATTCATTGGC
44	RBM46	Known from literature	AATGACTTTGAGCCAACAGA
45	RFX5	Regulon analysis (oogonia_STRAB)	AAGAGGCACTGCACTGTCA
46	CTCFL	Known from literature	TCTTTGTCGTATGGTGTCAA
47	MYBL1	Regulon analysis (oogonia_meiotic)	TTTGTATATTCGGCAATGT
48	DMRTC2	GRN analysis	TTATGACTTTGACTTGACAA
49	ANHX	GRN analysis	GGCAACGCACTTGACGCCAG
50	RB1	Regulon analysis (oogonia_meiotic)	ACCTTACTTAAAATCGTTTC
51	MEIOSIN	Known from literature	CACACTCATTGAACTTGA
52	SAP30	Regulon analysis (oogonia_meiotic)	TAAATGTGTGGGTAAAAGTT
53	HDAC2	Regulon analysis (oogonia_meiotic)	ATGCAATGCTAAGTTGGAGA
54	SMC3	GRN analysis	TGCTTGTGAGTTTGGTGGAT
55	KDM5B	Regulon analysis (oogonia_meiotic)	ACTGCGCTGTCTTTTCAA
56	SYMPK	Known from literature	GGTGCAGAAGCTAACTTCC
57	MSX1	Regulon analysis (oogonia_STRAB)	CATATTTAGTTCTCCTTCTT
58	YTHDC2	Known from literature	TGCCTATTATAGGGGTGCCG
59	NELFE	Regulon analysis (oogonia_STRAB)	TGAGCAATATACAAAAGACA
60	ZNF541	Known from literature	CTTCATAAACCCATCGTTGA
61	NFYB	Regulon analysis (oogonia_meiotic)	GACTGTAAGTGCATGCAA
62	MEIOC	Known from literature	GATTTGAGTACGATTACTC
63	ESX1	GRN analysis	GAACCAGAAACAAAATAAAA
64	STRAB	Known from literature	GGGCAGGTAACGAGGGGGG
65	PRDM9	GRN analysis	CCAGGAAGTATCTATTCTTC
66	HDAC6	Regulon analysis (oogonia_meiotic)	GCCTTCCCTTCCAGATCCTA
67	RAD51	GRN analysis	AAGACCATCTGACTCAAACA
68	SOD1	GRN analysis	ATTGGCGGTGCGCTCGCCGA
69	UBB	GRN analysis	TGAATCACAGAAGAATACTC



70	RARG	Regulon analysis (oogonia_STRA8)	TAAGGAAGTGAGCCAGATCT
71	NFYC	Regulon analysis (oogonia_meiotic)	ATATTGCATGAAACCCTGTT; CACCGTTTCCCTGACTAAAA; GCTGCAATCTGTAGACCA
72	NOBOX	GRN analysis	AAATTCAAAGAAGATAAAAAG; AAGATGTTAGAATCAAGAAG; GCCAACTACAATAAATATTC
73	FIGLA	GRN analysis	ATTGACTATGACACCGATGA; TTATTGGCGAGGTTAACAAC; GTAAATGTCCTTAATAACGT
74	DAZL	Known from literature	ACAGCGAACGAACATGATCT; GATATATTTAGTTGCTTCGC; AGTTTGTCTGTAAGCGAAAC
75	BOLL	GRN analysis and known from literature	ATTGGGGGTAAGTCTATCTT; ATGATCATTGTATATACCGT; ATGGGGCTCGGAGCAGGACA
76	DDX4	GRN analysis and known from literature	CATAAGCTTCTTGTCTCTAC; GGTGCAGGAGCTAACTTTCC; TAAAACCGGGCTTTTTCAC
77	ELK1	Regulon analysis (oogonia_STRA8)	GCAGGAAGCGTTGTGTGCA; ATCCCTTCTACCAACCACC
78	TAF4B	Known from literature	ACTAACAATCCAGAACTTGC; TTCTCAATGCTGGGTGATA
79	DNMT1 CRISPRi	DNA demethylation	ATAGAGAGACCATTAATGGGTCTCCTAGG
80	DPPA3	DNA demethylation	GATAGCATGACTTATGAAAT
81	TET1	DNA demethylation	GGTATTTTGCCCGTGATTCT
82	TET3	DNA demethylation	AGATCTCTAGAAATCTAAAA
83	SMAD1-active	BMP signaling	AATACCTATCACTATAGCTT
84	SMAD9-active	BMP signaling	CACAGAGGTCGGACGAAGTT
85	DMRT1	Known from literature	TTACTTCGGAAACTGAAGAG; TTATTGGAGACAACAACGCA
86	PAX5	Known from literature	GCTGCCTCTCAGATTTCTGA
87	PAX6 isoform a	GRN analysis	CTTTTCAAATAAGACGTCTA
88	PAX6 isoform b	GRN analysis	ATTGAAGGACTTTTCAGTTA

1 **Supplementary Table 2.** Factors screened for meiosis induction. #1-78 were included in  
2 barcode enrichment screening, and #79-88 were added for scRNAseq screening.

3

DMC1
HORMAD1
HORMAD2
INCA1
MEIOB
PRDM9
RAD51AP2
SCML1
SHCBP1L
SMC1B
SPATA22
SPDYA
SPO11
SYCE2
SYCE3
SYCP1
SYCP2
SYCP3
TEX12

1 **Supplementary Table 3.** Genes used for meiosis gene score calculation.

2

Name	Sequence	Purpose
MPS542_Barcode pulldown_3'_F	/5BiosG/GTTTAGAGCTAGAAATAGC	Barcode capture (scRNAseq)
MPS543_Barcode pulldown_3'_R	/5BiosG/TCCAAACTCATCAATGTATC	Barcode capture (scRNAseq)
MPS546_Illumina_p5	AATGATACGGCGACCACCGAGATCT	Barcode capture (scRNAseq)
MPS547_Illumina_p7	CAAGCAGAAGACGGCATAACGAGAT	Barcode capture (scRNAseq)
oAMP123_barcode_F	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNATACTCAGAAG ATGTCACCTACC	Barcode enrichment
oAMP124_barcode_R	GAGTTCAGACGTGTGCTCTTCCGATCTNNNCATTTCATAGTTCTTGC TCAGTGG	Barcode enrichment
MPS017_REC8_5'arm_fwd	TGGAGCCCAGTGTTCCTCCCTTATG	Cloning
MPS018_REC8_seq_fwd	CAAGAAAAGCCATATGGTCCG	Cloning
MPS019_REC8_5'_rev	GTGGAATCTGGGCCCGGCT	Cloning
MPS020_REC8_3'fwd	GGTTAGAGTCCATTTACAAAGCTGC	Cloning
MPS021_REC8_rev	TAGAAGTGGCCGGTTTCCTG	Cloning
MPS022_REC8_3'rev	TGCTCTCTGAAGAGATTTTGCC	Cloning
MPS458_BB_SYCP3 Gibson	caaataggggttccgcgcacatttccccgTCCGAGACCTAAATTGC GGG	Cloning
MPS459_SYCP3_mGL Gibson	GTTTTTTAAAGTCAGGAAGCATCTAAATATGGTGTCaagggcgag gagctgttcac	Cloning
MPS460_SYCP3_5'_rev	GGACACCATATTTAGATGCT	Cloning
MPS461_T2A-SYCP3-Gibson	CCTGGAATACTTTTTCCGGAGGACACCATtgggccaggattctcc tcga	Cloning
MPS462_SYCP3_3'_fwd	ATGGTGTCTCCGGAAAAAAG	Cloning
MPS463_SYCP3_BB Gibson	GAAAACCACACTGACAAGATCTGGAGGTTCTTCTATGTGAGAACA GGCAT	Cloning
MPS166_REC8_1F_CRISPRa	caccgCCTGGCAACAGGGTCTCCCG	CRISPRa sgRNA
MPS167_REC8_1R_CRISPRa	aaacCGGGAGACCTGTGCCAGGc	CRISPRa sgRNA
MPS168_REC8_2F_CRISPRa	caccgTGACAGCCAATGGGGAACGG	CRISPRa sgRNA
MPS169_REC8_2R_CRISPRa	aaacCCGTTCCTCCATGGCTGTCAc	CRISPRa sgRNA
MPS170_REC8_3F_CRISPRa	caccGTCGGGAACGCCAAGTATCC	CRISPRa sgRNA
MPS171_REC8_3R_CRISPRa	aaacGGATACTTGGCGTTCCCGAC	CRISPRa sgRNA
MPS172_REC8_4F_CRISPRa	caccgCCTCGGGAGACCTGTGTC	CRISPRa sgRNA
MPS173_REC8_4R_CRISPRa	aaacGGCAACAGGGTCTCCCGAGGc	CRISPRa sgRNA
MPS479_SYCP3_F_CRISPRa	accGCGCCCAATAGCTGGCCCA	CRISPRa sgRNA
MPS480_SYCP3_R_CRISPRa	aacTGGGCCAGCTATTTGGGCGC	CRISPRa sgRNA
MPS023_REC8_outer_5'	ACTTCTCCATCCCCAGGTC	Genotyping
MPS024_REC8_outer_3'	GCCACCACACCATACATTTCAATC	Genotyping
MPS489_SYCP3_outer_5'	TGTCGAAATCTTCTGCTGTGT	Genotyping
MPS490_SYCP3_outer_3'	ACCATTCACTCTTAACATCATGGA	Genotyping
MPS064_REC8_cterm sgRNA_1 FWD	caccGCCGGGGCCAGATTCCACTG	Knock-in sgRNA
MPS065_REC8_cterm sgRNA_1 REV	aaacCAGTGGAACTCTGGGCCCGGC	Knock-in sgRNA
MPS066_REC8_cterm sgRNA_2 FWD	caccGTAATGGACTTAACCTCAG	Knock-in sgRNA
MPS067_REC8_cterm sgRNA_2 REV	aaacCTGAGGTTAGAGTCCATTTAC	Knock-in sgRNA
MPS068_REC8_cterm sgRNA_4 REV	aaacGGGCCACAGATTCCACTGAGGC	Knock-in sgRNA
MPS069_REC8_cterm sgRNA_4 FWD	caccGCCTCAGTGGAACTTGGGCC	Knock-in sgRNA
MPS070_REC8_cterm sgRNA_5 REV	aaacGATTCCACTGAGGTTAGAGTC	Knock-in sgRNA
MPS071_REC8_cterm sgRNA_5 FWD	caccGACTCTAACCTCAGTGGAAATC	Knock-in sgRNA
MPS072_REC8_cterm sgRNA_3 REV	aaacAGATTCCACTGAGGTTAGAGC	Knock-in sgRNA
MPS073_REC8_cterm sgRNA_3 FWD	caccGTCTAACCTCAGTGGAAATCT	Knock-in sgRNA
MPS464_SYCP3_sgRNA_fwd	caccGCATCTAAATATGGTGTCTC	Knock-in sgRNA
MPS465_SYCP3_sgRNA_rev	aaacGAGGACACCATATTTAGATGC	Knock-in sgRNA
qMPS001F_GAPDH	GGTGACCAGGCGCCCAATACGA	qPCR
qMPS001R_GAPDH	CGCTTCGCTCTCTGCTCCTCTGT	qPCR
qMPS070F_DPPA3	ACGCCGATGGACCCATCACAGTTT	qPCR
qMPS070R_DPPA3	TCTCGGAGGAGATTTGAGAGGCC	qPCR
qMPS071F_REC8	TACCTGCTCCTGGTGTCTC	qPCR
qMPS071R_REC8	TGGATCAGGAGGCCACATA	qPCR
qMPS072F_SYCP3	TCTACTTACTGTGTCACAAATGA	qPCR
qMPS072R_SYCP3	TCTCTTGCTGTGAGTTTCCA	qPCR

qMPS073F_MAX	GGACTCGGCTTGTGTGTC	qPCR
qMPS073R_MAX	GGTTGCTCTTCGTCGCTCT	qPCR
qMPS074F_MGA	ACCGAACAGAATAACCCGCC	qPCR
qMPS074R_MGA	AATGGCCTTCCCATCCGTG	qPCR
qMPS075F_E2F6	ACCCAGTCTCCTCCTGGAC	qPCR
qMPS075R_E2F6	TATTTTTGATGGCAGCAGGC	qPCR
qMPS076F_RNF2	GCAGGAGCCGCAATGTCT	qPCR
qMPS076R_RNF2	ATTGCCTCCTGAGGTGTTG	qPCR
qMPS077F_PCGF6	GAGGACGAGGACGAGGAGTT	qPCR
qMPS077R_PCGF6	GATTAATCAGGCGCTCCTCC	qPCR
qMPS078F_DNMT1	CCCCAAAGAACCAACGGAGA	qPCR
qMPS078R_DNMT1	CTGAATGCACTTGGGAGGGT	qPCR
qMPS079F_DNMT3A	CACCGGCCATACGGTGGAG	qPCR
qMPS079R_DNMT3A	TGTTGAGCCCTCTGGTGAAC	qPCR
qMPS080F_DNMT3B	GGAGATTCGCGAGCCAG	qPCR
qMPS080R_DNMT3B	CTCCCTTCATGCTTTCCTGC	qPCR
qMPS081F_UHRF1	ACAGGGGCAAACAGATGGAG	qPCR
qMPS081R_UHRF1	TGGATGGTGTCAATCAGGCG	qPCR
qMPS082F_SETDB1	GTTGTGAGTCTGGGGTCTGG	qPCR
qMPS082R_SETDB1	ATGCTTTTGTCTCTCCCGT	qPCR

1 **Supplementary Table 4. Oligos used in this study.**

2

<b>Primary Antibodies</b>					
<b>Target</b>	<b>Antibody type</b>	<b>Supplier</b>	<b>Catalog#</b>	<b>Dilution</b>	<b>RRID</b>
SYCP3	Rabbit IgG, polyclonal	Abcam	ab15093	1:250	AB_301639
DDX4	Rabbit IgG, polyclonal	Abcam	ab13840	1:250	AB_443012
KI67	Rat IgG, monoclonal	Thermo	14-5698-37	1:250	AB_2865119
HORMAD1	Rabbit IgG, polyclonal	Proteintech	13917-1-AP	1:250	AB_2120844
$\gamma$ H2AX	Mouse IgG, monoclonal	<a href="#">Millipore</a> <a href="#">Sigma</a>	05-636	1:500	AB_309864
Centromere	Human IgG, polyclonal, FITC-labeled	Antibodies Inc	<a href="#">15-235-F</a>	1:200	AB_2797147
SYCP3	Goat IgG, polyclonal	R&D Systems	AF3750	1:250	AB_2197194
T2A	Rat IgG, monoclonal	Millipore Sigma	<a href="#">MABE1923</a>	1:250	AB_3097817
TEX12	Rabbit IgG, polyclonal	Abcam	ab122455	1:250	AB_11128111
RAD51	Mouse IgG, monoclonal	Novus	NB100-148	1:250	AB_10002131
<b>Secondary antibodies (all donkey polyclonal)</b>					
<b>Target</b>	<b>Fluorophore</b>	<b>Supplier</b>	<b>Supplier Number</b>	<b>Dilution</b>	<b>RRID</b>
Goat IgG	AF568	Thermo Fisher	<a href="#">A11057</a>	1:500	AB_2534104
Mouse IgG	AF647	Thermo Fisher	<a href="#">A31571</a>	1:500	AB_162542
Rabbit IgG	AF488	Jackson	711-545-152	1:500	AB_2313584
Rat IgG	CF568	Sigma	SAB4600077	1:500	AB_2827516
Rabbit IgG	AF568	Thermo Fisher	A10042	1:500	AB_2534017
Rat IgG	Dylight755	Thermo Fisher	SA5-10031	3:500	AB_2556611

1 **Supplementary Table 5.** Antibodies used in this study.

2

#	Cell line	Factors	Sorting	Total Cells	Germ cell	Germ cell mitotic	PGC	Oogonia STRA8	Oogonia meiotic	Pre-oocyte	Pre-spermatogonia
1	PGP1 D4TR8G	BCL2, HOXB5, and STRA8	unsorted	3553	1323	350	257	1605	0	0	18
2	F3 D4TS3G	BCL2, HOXB5, and STRA8	unsorted	5308	1187	320	299	3464	4	0	34
3	PGP1 D4TR8G	BCL2, HOXB5, STRA8, and the pool of 88 factors	unsorted	90809	32279	10728	9246	37839	27	1	689
4	PGP1 D4TR8G	BCL2, HOXB5, STRA8, and the pool of 88 factors	sorted DDX4+	59668	20629	6630	6399	24732	65	6	1207
5	PGP1 D4TR8G	BCL2, HOXB5, STRA8, and the pool of 88 factors	sorted REC8+	76522	26398	11405	9701	28595	14	0	409
6	F3 D4TS3G	BCL2, HOXB5, STRA8, and the pool of 88 factors	unsorted	50837	14834	4481	3267	27916	38	1	300
7	F3 D4TS3G	BCL2, HOXB5, STRA8, and the pool of 88 factors	sorted DDX4+	59742	14949	6030	5233	32593	100	4	833
8	F3 D4TS3G	BCL2, HOXB5, STRA8, and the pool of 88 factors	sorted SYCP3+	88342	23339	6991	5549	51541	238	6	678
9	PGP1 D4TR8G	Pool of 88 factors	unsorted	10069	3607	2197	2213	2019	1	0	32
10	PGP1 D4TR8G	Pool of 88 factors	sorted DDX4+	28038	9798	6119	5401	6617	2	0	101
11	PGP1 D4TR8G	Pool of 88 factors	sorted REC8+	27982	10776	5316	5497	6114	6	1	272
12	F3 D4TS3G	Pool of 88 factors	unsorted	35747	9359	9648	9883	6772	1	0	84
13	F3 D4TS3G	Pool of 88 factors	sorted DDX4+	25380	10364	4287	4396	5996	11	1	325
14	F3 D4TS3G	Pool of 88 factors	sorted SYCP3+	32779	12425	5816	5202	8966	34	0	336
15	Mix of PGP1 D4TR8G and F3 D4TS3G	No factors	unsorted	5312	1227	1880	2016	189	0	0	0

1 **Supplementary Table 6.** List of samples in the scRNAseq screening experiment, and  
2 numbers of annotated cell types.