Human primordial germ cells (hPGCs), the precursors of sperm and eggs, originate during weeks 2–3 of early post-implantation development. Using in vitro models of hPGC induction, recent studies have suggested that there are marked mechanistic differences in the specification of human and mouse PGCs. This may be due in part to the divergence in their pluripotency networks and early post-implantation development. As early human embryos are not accessible for direct study, we considered alternatives including porcine embryos that, as in humans, develop as bilaminar embryonic discs. Here we show that porcine PGCs originate from the posterior pre-primitive-streak competent epiblast by sequential upregulation of SOX17 and BLIMP1 in response to WNT and BMP signalling. We use this model together with human and monkey in vitro models simulating peri-gastrulation development to show the conserved principles of epiblast development for competency for primordial germ cell fate. This process is followed by initiation of the epigenetic program and regulated by a balanced SOX17–BLIMP1 gene dosage. Our combinatorial approach using human, porcine and monkey in vivo in vitro models provides synthetic insights into early human development.

We first determined the origin of porcine PGCs (pPGCs) in peri-gastrulating embryos at embryonic days 9.5–16 (E9.5–E16). At around E9.5–E10, key pluripotency markers NANOG, OCT4 and SOX2 are detected in the epiblast of bilaminar embryos (Fig. 1a). In E11 pre-primitive-streak (pre-PS)-stage embryos with an incipient anterior–posterior axis (Extended Data Fig. 1a), BRACHYURY (also known as T) expression is evident in the posterior pseudo-stratified epiblast cells, together with NANOG and OCT4, but SOX2 is down-regulated (Fig. 1b).

In the midline of early-PS-stage embryos (~E11.5–E12), we detected the first cluster of SOX17+ cells in the posterior end of the nascent primitive streak (Fig. 1c, d, Extended Data Fig. 1b); most of these express BLIMP1, except for those at the anterior end (arrows in Fig.1c, d). Expression of SOX17 precedes BLIMP1, and NANOG is upregulated in SOX17+BLIMP1+ pPGCs (Fig. 1d, Extended Data Fig. 1b). In embryos at E12.5–E13.5, pPGCs exhibit co-expression of SOX17, BLIMP1, NANOG, TFAP2C, OCT4, and the pPGC cell surface marker Sda/GM2 (ref. 12), but have low levels of T expression (Fig. 1e, Extended Data Fig. 1c, d). This pPGC cluster of around 60 SOX17+BLIMP1+ cells, located at the border between embryonic and extra-embryonic tissues in early-PS-stage embryos (~E12), increases to more than 300 pPGCs by E15.5 (Extended Data Fig. 2a–c). A six-hour pulse of EdU labelling shows that DNA synthesis ceases soon after detection of the Sda/GM2 epitope (Fig. 1f, Extended Data Fig. 2d), indicating that the sharp increase in pPGCs is probably due to the additional recruitment from T+ competent progenitors. Thereafter, pPGCs enter quiescence and pause prior to migration, as in mice (Fig. 1f, Extended Data Fig. 2c). Notably, PRDM14 expression in pPGCs is weak and apparently cytoplasmic (Extended Data Fig. 1f), whereas SOX2 is undetectable (Extended Data Fig. 2e, f).

Initiation of the germline-specific epigenetic program is evident in nascent pPGCs with a global reduction in 5-methylcytosine (Extended Data Fig. 3b, c) and concomitant enrichment of 5-hydroxymethylcytosine (5hmC) (Fig. 1g, h). Consistently, UHRF1 is downregulated (Fig. 1i) and TET1 is upregulated (Extended Data Fig. 3a). Progressive reduction in H3K9me2 and G9A (also known as EHMT2) expression was also evident (Extended Data Fig. 3b, c) and global DNA demethylation followed as pPGCs migrate towards the gonads (Extended Data Fig. 3d). pPGCs continued to express SOX17, BLIMP1, TFAP2C, OCT4 and NANOG (arrowheads in Fig. 1e, Extended Data Fig. 2e, f), as seen in equivalent hPGCs in vivo. Thus, pPGCs originating from the peri-gastrulation porcine epiblast exhibited close similarities with hPGCs following sequential SOX17 and BLIMP1 expression, and the onset of the epigenetic program.

To determine when porcine epiblasts gain competency for pPGC fate, we isolated epiblast discs from the hypoblast and trophoderm at different stages of development (Fig. 2a, b, d, e). We exposed them to cytokines, including either BMP2 or BMP4 (refs 2–5) (hereafter referred to as cytokines), for 64 h to induce pPGCs ex vivo (Fig. 2f). Although no response was seen in E10.5–E11 early bilaminar disc epiblast, an efficient induction of pPGCs occurs in epiblasts from E11.5 pre-PS embryos, with co-expression of SOX17, BLIMP1, NANOG, OCT4 and TFAP2C (Fig. 2g, h, Extended Data Fig. 3e). Notably, we detected a strong BMP2/4 signal from the posterior pre-PS and early-PS pig embryos in vivo, but BMP inhibitor (BMPI) abrogates pPGC induction (Fig. 2g, Extended Data Fig. 3e). The competency for pPGC fate wanes during early-PS-stage concomitantly with the onset of gastrulation and mesendoderm differentiation (Fig. 1c). WNT signalling from the posterior pre-PS epiblast is also important (Fig. 2a), as evidenced by the high proportion of T+ cells, a downstream target of WNT7, during pPGC induction (Fig. 1b). A WNT inhibitor (WNTi) diminished pPGC induction, but not in early-PS-stage epiblasts when they were already competent for pPGC fate (Fig. 2g, Extended Data Fig. 3e). Expression of T in response to WNT is a common feature of competency for germ cell fate.

As we cannot examine early human embryos directly, we simulated human peri-gastrulation development and induced hPGC fate in vitro. We established an in vitro model with human pluripotent stem cells (hPSCs) for mesendoderm and peri-gastrulation development (Fig. 3a). These hPSCs in a defined conventional medium (hereafter denoted as conv-hPSCs, see Methods) are probably equivalent to the
reSeArCh

Dashed lines highlight SOX17 + streak with SOX17 (see Extended Data Fig. 4). By checking for induction of hPGCs by cytokines at 6 h intervals (Fig. 3a), we observed a peak of competency at around 12 h during mesendoderm differentiation, declining thereafter (Fig. 3b, d), illustrating a step-wise transition in cell states. PGC-competent cells showed moderate upregulation of primitive-streak pre-gastrulating porcine epiblast21. To follow gain and loss of competency for hPGC specification over the course of mesendoderm differentiation, we used cells with a sensitive NANOS3–tdTomato reporter (see Extended Data Fig. 4). By checking for induction of hPGCs by cytokines at 6 h intervals (Fig. 3a), we observed a peak of competency at around 12 h during mesendoderm differentiation, declining thereafter (Fig. 3b, d), illustrating a step-wise transition in cell states. PGC-competent cells showed moderate upregulation of primitive-streak
marks T, MIXL, GSC, EVX1 and EOMES, and a slight reduction of SOX2 (Fig. 3e, Extended Data Fig. 5a, b). From 18 h onwards, there was upregulation of late primitive-streak markers TBX6, MESP1/2, and SNAI1/2, which trigger epithelial–mesenchymal transition, occurring during gastrulation\(^{12}\) (Extended Data Fig. 5b). The response of cells to signalling also changes, as BMP2/4 now induces mesodermal cells (Extended Data Fig. 5b, e–h), whereas activin A and a BMP inhibitor efficiently induce definitive endoderm\(^{18}\) (Fig. 3c, d. Extended Data Fig. 5e–h). Thus, from around 12 h onwards, the mesodermal precursors (pre-ME) are competent for PGC fate, but from around 18 h (mesendoderm), competency for PGC declined, with concomitant gain of competency for definitive endoderm and/or mesoderm fates (Fig. 3a–c). Both WNT and ACTIVIN/NODAL signalling are necessary for competency for hPGCs in 12 h pre-ME (Extended Data Fig. 5c). Inhibition of BMP during mesendoderm differentiation markedly reduced the efficiency of hPGC induction, suggesting a role for endogenous BMP (Extended Data Fig. 5d). Addition of BMP during pre-ME (12 h), however, does not affect hPGC competency, but favours differentiation into lateral mesoderm at 24 h, which originates from the mid/posterior primitive streak (Extended Data Fig. 5e–h). By contrast, definitive endoderm differentiation occurs in the anterior primitive streak, as seen in primitive-streak-stage porcine embryos (arrowheads in Fig. 1d, Extended Data Fig. 1c), suggesting that BMP confers posterior characteristics to the primitive streak during gastrulation (Extended Data Fig. 5i). The combined evidence from porcine embryos, and simulations in vitro with conv-hPSCs, shows progressive and transient acquisition of competency for hPGC, followed by competency for definitive endoderm and/or mesoderm.

To test whether this model applies to non-human primates, we used cynomolgus monkey PSCs (cmPSCs) to induce mesendoderm differentiation (Extended Data Fig. 6a, b). Remarkably, cmPSCs also showed a temporal gain of competency for cmPGC fate at around 12 h during mesendoderm differentiation, and thereafter definitive endoderm and mesoderm at 24 h (Extended Data Fig. 6c–f). A recent study suggested...
that cmPGCs originate from the epiblast-derived nascent amnion, but also potentially from the posterior epiblast; a dual origin is, however, also possible. A detailed molecular analysis of amnion is important, as SOX17 and BLIMP1 also have roles elsewhere, including extraembryonic tissues, which exhibit great developmental diversity and less developmental constraint than epiblasts. Further traceable analysis of in vivo non-human primate embryos and potentially of human embryos in vitro is merited.

We next examined the combinatorial roles of key transcription factors, considering that SOX17 is the key regulator of both hPGCs and definitive endoderm\(^1\);\(^2\);\(^2\) (Fig. 3d). Ectopic expression of SOX17 in pre-ME at 12 h induces hPGCs, resulting in NANOS3–tdTomato\(^+\)OCT4\(^+\)BLIMP1\(^+\) cells, but in mesendoderm at 24 h it induces FOXA2\(^+\) (definitive endoderm) efficiently (Fig. 3f, g). During the induction of hPGCs in pre-ME by BMP (Extended Data Fig. 7a, b), we first detected SOX17 at 12 h, and around 30–40% of the cells also expressed BLIMP1 but not TFAP2C (Extended Data Fig. 1e). TFAP2C was detected at around 18 h as the number of SOX17\(^+\)BLIMP1\(^+\)TFAP2C\(^+\) cells increased progressively. These putative hPGCs eventually formed a cluster in the middle of the embryos by 48 h (Extended Data Fig. 7b, c, e). From 12 h onwards, NANOG expression increased progressively in SOX17\(^+\)BLIMP1\(^+\) cells (Extended Data Fig. 7e, f). However, unlike in mice,\(^3\);\(^4\) NANOG alone cannot induce hPGCs (Extended Data Fig. 8a–c) and PRDM14 expression appears low\(^4\);\(^5\), and might be cytoplasmic in E14 hPGCs (Extended Data Fig. 1f) and in in vivo gonadal hPGCs.\(^6\) PRDM14 is critical for PGC fate and epigenetic reprogramming in mice,\(^7\) but its role, if any, in hPGCs and pPGCs requires further studies.

We next tested the roles of SOX17, BLIMP1 and TFAP2C individually and in combination in the induction of hPGCs in the self-renewing PGC-competent hPSCs (comp-hPSCs),\(^8\) using a NANOS3–tdTomato reporter and inducible SOX17, BLIMP1 and TFAP2C transgenes (Extended Data Figs 4c–j, 6d–g, see Methods). BLIMP1 and TFAP2C individually and together elicit a negligible response after 2 days, whereas SOX17 alone produces a modest response, with or without...
Tfap2c (Fig. 4a). Notably, Sox17 and Blimp1 together produce a strong response and a large proportion of Nanos3–tdTomato+ cells (Fig. 4a), suggesting that they act synergistically and rapidly (within around 24 h), compared to the 96 h taken for cytokines to induce a similar response (Fig. 4b, c). This response is preceded by downregulation of Sox2 ( ref. 30 ) and upregulation of ‘naïve’ pluripotency genes including Klf4 and Tclf2p2l1 , as in hPGCs in vivo ( Fig. 4e, Extended Data Fig. 8h ). The response to Sox17–Blimp1 is otherwise similar to that induced by cytokines, as determined by global RNA-sequencing (RNA-seq) (Fig. 4d, Extended Data Fig. 8i,j). For further mechanistic insights, we induced ectopic Sox17 with or without Blimp1 expression in Sox17–null comp-hPSCs (Extended Data Figs 9a, 10a, b). Although Sox17 initiates HPGC fate, there is also a significant increase in expression of Foxa2, an endodermal gene (Fig. 4f). Accordingly, FACS-purified cells show Foxa1, Foxa2 and Hnf1a expression, as well as PGC markers Oct4, Nanog, Blimp1 and Tfpap2c (Extended Data Fig. 9b), and the cell surface marker Cxcr4 that is expressed by both endoderm19 and PGCs in humans (Extended Data Fig. 9c, d). This probably occurs owing to high ectopic Sox17 gene dosage exceeding the levels induced by BMP in wild-type comp-hPSCs (Extended Data Fig. 9b, e–h), without a proportionate increase in Blimp1 (Extended Data Fig. 9i). Notably, Blimp1 represses endodermal genes during hPGC specification9. Indeed, concomitantly high Blimp1 and Sox17 induce hPGCs robustly and rapidly, while suppressing endoderm genes (Fig. 4g, Extended Data Fig. 10c), and also initiate the germline-specific epigenetic program2, with downregulation of Dnmt3a, Dnmt3b and Uhrf1, and upregulation of Tet2 (Extended Data Fig. 8k), as in pPGCs (Fig. 1g–i, Extended Data Source, are available in the online version of the paper; references unique to Online Content provides insights into early human development and cell fate decisions. 

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** T.K. designed the experiments and performed cell culture, plasmid construction, immunofluorescence, qPCR, RNA-seq, western blots, data analysis and wrote the paper. W.W.C.T. designed experiments and analysed RNA-seq. N.I. performed preliminary work and designed experiments, and S.D. performed bioinformatics. A.S. helped with a hPSC reporter. H.Z., S.W., D.A.C. and C.A. designed and performed immunofluorescence and culture of pig embryos and epiblasts. D.A.C. and R.W. designed and performed in situ hybridization experiments and immunofluorescence. W.W.C.T., N.I. and S.W. made equal contributions. R.A. supervised the project, designed experiments, performed dissections and wrote the paper. M.A.S. supervised the project, designed experiments, and wrote the paper. All authors contributed to the manuscript.

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METHODS

Data reporting. No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded to allocation during experiments and outcome assessment.

Porcine embryo collection. All of the procedures involving animals have been approved by the School of Biosciences Ethics Review Committee, The University of Nottingham. Embryos were collected from crossbred Large White and Landrace sows (2–3 years old) between days 10 and 16 after artificial insemination. Embryos were selected from the surface horns with 30–40 mm in diameter supplemented with 1% FCS. Embryos older than E15 were retrieved by cutting the uterus longitudinally from the antimesometrial side and picked with forceps. Embryos at each stage were retrieved from multiple sows. All embryos were grouped by stages and washed with DMEM-F12 (Thermo Fisher Scientific) + 20% knockout serum replacement (KSR; Thermo Fisher Scientific) supplemented with 25 mM HEPS (wash medium) and transported to the laboratory in a portable incubator at 38.5°C.

Immunohistochemistry of porcine embryos and gonads. Embryos (embryonic day (E) 10–16; binactin (n = 4); pre-PS (n = 7); early-PS (n = 6); primitive streak (n = 10), late-PS (n = 13), E14 (n = 8), E16 (n = 10)) were processed as previously described15,31. Briefly, embryos and gonads were fixed in 2.5% paraformaldehyde (PFA) in PBS overnight at 4°C and transferred to methanol at −20°C for long-term storage. For less advanced embryos (E9.5–10.5) 2% agarose blocks were made before embedding in wax. Agarose blocks were then trimmed and dehydrated with increasing concentrations of ethanol (70%, 90% and 100%) for 2 h each step. After dehydration, blocks were processed in xylene followed by hot paraffin wax for 2 h and then cooled down in a metal mould. E14–15 embryos and gonads were dehydrated, embedded and processed as described above. All paraffin blocks were cut into 4-μm-thick sections and mounted onto SuperFrost plus glass slides. Sections were air-dried overnight and immunohistochemistry was conducted subsequently. For cryo-sections, fixed embryos were incubated in 30% sucrose/PBS overnight at 4°C prior to mounting in OCT compound. Cryo-sections were cut at 6 μm onto SuperFrost plus glass slides. Sections were left to air dry for 1–2 h before immunofluorescence. Unused slides were kept at −80°C in air-tight containers.

Immunofluorescence staining of porcine embryos. Sections were dewaxed with xylene for 3 min and rehydrated with decreasing concentrations of ethanol (100%, 90% and 70%) and transferred to PBS for 15 min. Antigen retrieval was then performed by boiling the slides in 0.01 M citrate buffer (pH 6.0) for 10 min. Sections were permeabilized with 1% Triton X-100 in PBS for 15 min. Triton X-100 was washed three times for 5 min each, and blocking solution (PBS supplemented with 5% BSA or 10% donkey serum/5% BSA/PBS) was added for 1–1.5 h. After blocking, sections were incubated with the desired primary antibody (see Supplementary Table 2) overnight at 4°C in a humidified chamber. Slides were then washed three times with 0.1% Tween-20/PBS. Finally slides were incubated with fluorescent (TRITC, Cy3 Alexa fluorophore 488, 555, 568 and/or 647; biotin, Abcam, Thermofisher Scientific (ERKi)-conjugated secondary antibodies for 40 min at room temperature. Slides were mounted with Fluoroshield with DAPI (Sigma) and sealed with nail varnish. Slides were kept at −20°C until observed.

Porcine epiblast cultures and quantification of PGC induction. Embryos collected 11.5 days after artificial insemination were dissected under a dissecting microscope as described previously20. Trophectoderm and primitive endoderm collected 11.5 days after artificial insemination were dissected under a dissecting microscope and quantified using a microscope. Trophectoderm and primitive endoderm were incubated in 30% sucrose/PBS overnight at 4°C prior to mounting in OCT compound. Cryo-sections were cut at 60 μm onto SuperFrost plus glass slides. Sections were left to air dry for 1–2 h before immunofluorescence. Unused slides were kept at −80°C in air-tight containers.

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EdU labelling. Collected embryos (n = 7) were rinsed in wash medium before 6 h incubation in 10 μM EdU at 38.5°C. After rinsing, the embryos were fixed in 4% PFA for 2 h and then transferred to 1% PFA for long-term storage. Embryos were processed for immunohistochemistry as described above, except that the EdU staining protocol was performed following manufacturer instruction (Click-iT EdU, Invitrogen) prior to addition of primary antibodies used for co-labelling.

Cell culture. NANOG−mCherry hPSCs, SOX17−knockout hPSCs, SOX17−knockout + inducible SOX17 (IS) hPSCs lines were established previously4,5. NANOG−mCherry hPSC line was newly generated by gene targeting (see ‘Vector construction and gene introduction’). A cynomolgus monkey PSC (cmPSC) line (MF12 embryonic stem cells (ESCs)) was from E. Curnow, Washington National Primate Research Centre. All cell lines were confirmed as mycoplasma negative.

Undifferentiated comp-hPSCs were maintained on vitronectin-coated plates in Essential 8 medium2 (Thermo Fisher Scientific) according to manufacturer’s protocol. Cells were passed every 3–5 days using 0.5 mM EDTA/PBS without breaking cell clumps.

Undifferentiated comp-hPSCs were maintained on mitomycin-C treated or irradiated mouse embryonic fibroblast (MEF) (urchased from MitoGlobal (stem prepared in house) in knockout DMEM (Thermo Fisher Scientific) supplemented with 2% KSR, 0.1 mM NEAA, 0.1 mM 2-mercaptoethanol, 100 U ml−1 penicillin, 0.1 mg ml−1 streptomycin, 2 ml−1 t-glutamine, 20 ml−1 human LIF (Stem Cell Institute, University of Cambridge (SCI)), 8 ng ml−1 BFGF (SCI), 1 ng ml−1 TGF-3 (Peprotech), 3 μM GSK3 (CHIR99021, Miltenyi Biotec), 1 μM ERK1 (PD0325901, Miltenyi Biotec), 5 μM p38 (SB203580, Tocris biosciences), and 5 μM JNK (SP600125, Tocris biosciences), as previously reported4,35. Cells were passed every 2–4 days using TrypLE express or 0.25% trypsin/EDTA (Thermo Fisher Scientific). Before harvesting comp-hPSCs on MEF, 10 μM of ROCKI (Y-27632, Tocris biosciences) was added to the medium.

Undifferentiated cmPSC were maintained on mitomycin-C treated or irradiated MEF supplemented with 500 μg ml−1 (12T3) supplement with 20% KSR, 0.1 mM NEAA, 0.1 mM 2-mercaptoethanol, 100 U ml−1 penicillin, 0.1 mg ml−1 streptomycin, 2 ml−1 t-glutamine, 20 ml−1 BFGF (SCI). Before differentiation, cmPSC were maintained on MEF in Essential 8 medium supplemented with 5% KSR, 2.5 μM WNTI (IWR-1; Tocris) at least for 2 passages. Cells were passed every 3–4 days using 0.25% trypsin/EDTA (Thermo Fisher Scientific). Before harvesting cmPSC on MEF, 10 μM of ROCKI (Y-27632, Tocris biosciences) was added to the medium.

For mesendoderm induction and subsequent definitive endoderm or lateral mesoderm induction, we optimized published protocols with slight modifications. For the basic induction, we used a RPMI medium which is composed of Advanced RPMI 1640 Medium (Thermo Fisher Scientific) supplemented with 1% B27 supplement (Thermo Fisher Scientific), 0.1 mM NEAA, 100 μM l−1 penicillin, 0.1 mg ml−1 streptomycin, 2 ml−1 t-glutamine. For mesendoderm induction, trypsinized conv-hPSCs were seeded on vitronectin-coated dish at 200,000 cells

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per well in 12-well plate and cultured in mesendoderm induction medium for 6–36 h as indicated in the figures. Mesendoderm induction medium contained ARB27 medium supplemented with 100 ng ml⁻¹ activin A (SCI), 3 μM GS K31 and 10 μM of ROCKi. For definitive endoderm induction, mesendoderm induction medium was replaced with definitive endoderm induction medium after washing with PBS once and cells were cultured for further 2–3 days. Definitive endoderm induction medium was composed of ARB27 medium supplemented with 100 ng ml⁻¹ activin A and 0.5 μM BMPi (Sigma). For lateral mesendoderm induction, mesendoderm induction medium was replaced with lateral mesendoderm induction medium after washing with PBS once and cells were cultured for further 1 day. Lateral mesendoderm induction medium was composed of ARB27 medium supplemented with 100 ng ml⁻¹ BMP2 (SCI), 2.5 μM WNTi (IWR-1) and 10 μM TGfi3 (SB 431542; Tocris).

Embryoids were formed according to a previous report. Briefly, conv.- or comp-hPSCs, pre-ME or mesendoderm were trypsinized into single cells and harvested into Corning Costar Ultra-Low attachment multwell 96-well plate (Sigma) or PrimeSurface 96v plate (S-Bio) at 4,000 cells per well in GK15 medium or ARB27 medium. To improve the cell aggregation, in some experiments, we added 0.25% (v/v) poly-vinyl alcohol (Sigma) in the basal medium, according to published protocol. For hPGE induction with cytokies, 500 ng ml⁻¹ BMP4 or BMP2 (SCI), 10 ng ml⁻¹ human LIF (SCI), 100 ng ml⁻¹ SCF; 50 ng ml⁻¹ EGF (R&D Systems), and 10 μM ROCKi were added to GK15 medium or ARB27 medium. Notably, use of ARB27 medium for the basal medium improved the efficiency of hPGE induction, especially, when we used pre-ME cells (see Extended Data Fig. 4i). A detailed protocol of the in vitro differentiation system described above is also available at Protocol Exchange.

For induction of exogenous transgenes, 100 μM dexamethasone (Sigma), 0.5 μg ml⁻¹ doxycycline (Sigma) and/or 0.5 μM Shiled-1 (Clontech) were added to the medium.

Vector construction and gene introduction. For construction of NANOS3–tdTomato knock-in targeting vector, 5′ and 3′ arms amplified from NANOS3–mCherry knock-in targeting vector, 5′-ROX-PGK-Puro-3′ was cloned into pBluescript KS(+). For construction of control pCAG-Dre-IRES-Hygromycin, puromycin resistance gene was cloned into pCAGGS-Dre-IRES-Puro which was replaced by hygromycin resistance gene.

For construction of dox/Shield-1 inducible system, SOX17 cDNA without stop codon amplified from cDNA of HPGC and desribdomed-stabilized domain amplified from pBMN YFP-FKBp (Addgene) were cloned into PiggyBAC vector used previously.

For construction of dox-inducible system, TetG amplified from pCMV-TetG3 (Clonetech) was cloned into PiggyBAC vector which contains CAG-promoter and IRES–neomycin-resistance gene. TReg3 cassette amplified from pTReg3 (Clonetech), human BLIMP1 mRNA amplified from TRE-Tight BLIMP1-EFGR(60%)-1 human NANO G mRNA amplified from pEF-Bos-CMV-EN2L (Addgene) and human TdT2P mRNA amplified from hPGE were cloned into PiggyBAC vector. All fragments were amplified by PCR using PrimeSTAR MAX or PrimeStar GXL DNA polymerase (Takara Bio) according to the manufacturer's protocol.

For gene targeting of NANOS3–tdTomato reporter, W12 male hESC and H9 female hESC lines were used. For the gene targeting method, electroporation or lipofection was carried out as described before. In brief, for electroporation, ~1–5 × 10⁶ comp-hPSCs suspended in PBS were mixed with targeting vector and CRISPR–Cas9 plasmid and transfected to a Gene Pulser cuvette (Bio-Rad). Electroporation was carried out using Pulse Controller equipment (Bio-Rad). For lipofec- tion, reverse transfection was carried out. 2 × 10⁵ comp-hPSCs were suspended in 100–200 μl of Opti-MEM containing plasmids and lipofectamine 2000 (Thermo Scientific) complex, and left for 5 min at room temperature. After electroporation or lipofection, PSCs were seeded onto 4 drug resistance (DR4) MEF (GlobalStem or SCI) and left for 5 min at room temperature. After electroporation or lipofection, reverse transfection was carried out. 2 μg of pROX4-E02S-CK2M-EN2L (a gift from S.C.I.), human BLIMP1-EFGR(60%)-1 human NANO G mRNA amplified from pEF-Bos-CMV-EN2L (Addgene) and human TdT2P mRNA amplified from hPGE were cloned into PiggyBAC vector. All fragments were amplified by PCR using PrimeSTAR MAX or PrimeStar GXL DNA polymerase (Takara Bio) according to the manufacturer's protocol.

For gene introduction using PiggyBAC system, reverse transfection was carried out. 2 × 10⁵ SOX17-knockout comp-hPSCs, NANOS3–tdTomato comp-hPSCs were suspended in 100–200 μl of Opti-MEM containing plasmids and lipofectamine 2000 (Thermo Scientific) complex, and left for 5 min at room temperature. The cells were seeded on DR4 MEF at several different concentrations, and then 48 h later, drugs for selection were added to the culture medium. Quantitative reverse transcription PCR. Total RNA was extracted using PicoPure RNA Isolation Kit (Thermo Fisher Scientific) and cDNA was synthesized using QuantiTect Reverse Transcription Kit (QIAGEN) according to manufacturer's protocols. RT–qPCR was performed and analysed as described previously and the primers sequences used in the paper are listed in Supplementary Table 3. Values normalized to GAPDH and relative to control samples (comp-hPSCs or hPSCs) are shown in the figures. Error bars are mean ± s.d. from technical triplicates with two experiments.

Immunofluorescence staining of cells and cyro-section of embryos. Cells were cultured on μ-Slide 8 well (ibidi) and fixed in 4% PFA for 10 min at room temperature. Embryos were fixed in 4% PFA for 2–4 h or overnight at 4°C and embedded in OCT compound for frozen sections. Each sample was incubated with primary antibodies for 1–2 h at room temperature or overnight in a cold room and with fluorescent-conjugated secondary antibodies for 1 h at room temperature. Antibodies used here are listed on Supplementary Table 2. After antibody treatment, samples were stained with DAPI (Sigma) to mark nuclei and were observed under confocal laser scanning microscopy.

Flow cytometry analysis. PSCs, embryos, definitive endoderm and lateral mesendoderm were trypsinized with 0.25% trypsin/EDTA at 37 °C for 5–15 min and stained with Alexa-488 or 647 conjugated anti-TNAP antibody (BD Bioscience), PerCP-Cy5.5 conjugated anti-CXCR4 antibody (Biolegend), PE-Cy7 conjugated anti-PDGFRA antibody (Biolegend) and/or Alexa-647 conjugated anti-CD38 antibody and subjected to FACS LSR Frosketa cytometry (BD Bioscience). FACS data were analysed by Flowjo software.

Western blot analysis. Western blot analysis was carried out as described before. Briefly, whole-cell extracts were prepared from day 4 embryos in lysis buffer composed of 50 mM Tris-HCl (pH 7.5), 0.15M NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate and complete mini EDTA free protease inhibitor cocktail (Roche Applied Science). After electrophoresis, proteins were transferred to nitrocellulose membranes. Membranes were incubated in Western Blocking Reagent (Roche Applied Science) and treated with antibodies. Primary antibodies against SOX17 (goat IgG; R&D systems, see Supplementary Table 2), and H3 (rabbit IgG; Abcam ab1791) were used. Horseradish peroxidase-conjugated secondary antibodies against goat or mouse IgG were added (Dako, Life Technologies). After antibody treatment, blots were developed using ECL Western Blotting Detection System (GE Healthcare).

Preparation of RNA-sequencing libraries. Total RNA (5 ng) was reverse-transcribed and amplified into cDNA using Ovation RNA-Seq System V2 (Nugen). Amplified cDNA was sonicated to 250 bp by Covaris S220 Focused Ultrasound (Covaris). Subsequent library preparation was carried out using Ovation Rapid DR Multiplex System (Nugen). Fragmented cDNA using Ovation Rapid DR Multiplex System (Nugen). Libraries were quantified by qPCR using KAPA Library Quantification Kit (Kapa Biosystems). Libraries were subjected to single-end 50 bp sequencing on HiSeq 2500 sequencing system (Illumina). Every four indexed libraries were multiplexed to one lane of a flowcell, resulting in >40 million single-end reads per sample.

Bioinformatics analysis. Adapter- and quality-trimmed RNA-seq reads were mapped to the human reference genomes (UCSC GRCb37/hg19) using TopHat2 (http://ccb.jhu.edu/software/tophat/version-2.0.13) guided by ENSEMBL 83 gene models. Raw counts per transcripts were obtained using featureCounts, only the transcript per gene was kept. Replicates were evaluated, raw counts were normalized, and the differential expression of transcripts was statistically evaluated by the R Bioconductor DESeq2 package (http://www.bioconductor.org). Expression-normalized transcript counts were further normalized by transcript length (per kb). Transcript annotations in all bioinformatics analyses were based on ENSEMBL (Release 83) considering protein coding, long-noncoding RNA and
processed transcripts. Hierarchical clustering was performed with the R hclust function using the Ward's method. Principal components were computed by singular value decomposition with the R princomp function on scaled DESeq-normalized expression levels. Only the 80% most highly expressed transcripts were used for clustering and principal component analysis. t-statistic stochastic neighbour embedding (t-SNE) analysis was performed with the R Rtsne package with default parameters and perplexity = 3. Gene set enrichment analysis (GSEA) was performed with the R phenoTest package on RNAseq data (log2(normalized counts)). hPGC-specific genes were defined as follows: (1) upregulated in developmental week 7 male hPGCs over gonadal somatic cells (log2(fold change) > 3 and adjusted \( P < 0.05 \)); and (2) upregulated in day 4 hPGCs induced by cytokines over comp-hPSCs in our previous study; and (3) upregulated in developmental week 7 male hPGCs over comp-hPSCs.

Data availability. RNA-seq data have been deposited in Gene Expression Omnibus (GEO) under accession number GSE85378. All other data are included within the paper, Source Data and Supplementary Information.

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Extended Data Figure 1 | Expression of key germ cell genes in early pPGCs

**a**, Representation of mouse, pig and human embryos before gastrulation.

**b**, Sections of early-PS and primitive-streak-stage embryo showing SOX17, BLIMP1, NANOG and OCT4. Yellow dashed insets show cells at high magnification and white dashed lines mark SOX17$^+$ and/or BLIMP1$^-$ cells. Scale bar, 20μm.

**c**, Primitive-streak-stage embryo (~E12) with a cluster of pPGCs (arrow) with multiple combinations of PGC gene expression (SOX17, BLIMP1, NANOG, TFAP2C, OCT-4, Sda/GM2 and mesoderm gene, T). Arrowheads at the anterior streak point to primitive endoderm (SOX17$^+$ BLIMP1$^+$ and NANOG$^-$ cells). Scale bar, 20μm.

**d**, Late-PS (~E12.5–E13.5) embryo with a pPGC cluster (arrow) showing NANOG, SOX17 (split colour image of e), BLIMP1, and T expression. Arrowheads mark early migratory PGCs in the primitive endoderm. Scale bar, 25μm.

**e**, E14 embryo stained for SOX17, BLIMP1 and TFAP2C. Yellow dashed insets show cells at high magnification and white dashed lines mark SOX17$^+$ BLIMP1$^+$ and TFAP2C$^-$ cells. Scale bar, 20μm.

**f**, Immunostaining for PRDM14 co-stained with Sda/GM2 and SOX17 in E14 (pPGC cluster) embryos and E26 gonads. Arrows point to pPGCs in the gonad. Scale bar, 20μm.
Extended Data Figure 2 | Proliferation and development of early pPGCs. a, OCT4 RNA in situ hybridization identifies the pPGC cluster (arrow) in the posterior end of E13.5–E15.5 embryos. Insets show whole embryos. b, Whole-mount OCT4 immunohistochemistry of a porcine embryo. Dashed square marks the area shown at higher magnification on the top right. Arrow points to the pPGC cluster. Bottom right: cross section of the embryo (line in the wholemount image) shows migratory pPGCs (red cells). c, Number of pPGCs at different stages as indicated.

d, Immunostaining of EdU-labelled embryos at the indicated stages with different antibody combinations identifying the PGCs. The pPGC cluster is highlighted with dashed white line. Arrows show SOX17+EdU+ and SOX17+BLIMP1+EdU+ cells. Scale bar, 20 μm. e, Immunostained migratory pPGCs (arrows); inset show cells at higher magnification. f, Immunostained gonadal pPGCs. Inset shows SOX2+ neural tissue.
Extended Data Figure 3 | Epigenetic reprogramming in pre- and early migratory pPGCs, and key germ cell markers in migratory pPGC and cultured porcine epiblast. a, A cluster of pPGCs (dashed line) at E13 stained for TET1 and OCT4. Scale bar, 20 μm. b, Serial sections of E14 embryos immunostained for different epigenetic markers combined with BLIMP1, NANOG and SOX17. Dashed lines highlight pPGC clusters. Scale bar, 20 μm. c, Quantification of 5-methylcytosine and H3K9me2 in embryos of different stages. Numbers of cells analysed are indicated (boxes, mean and interquartile ranges; whiskers, maximum and minimum; *P < 0.01; Mann–Whitney U-test). d, Serial sections of E16 embryos showing migratory pPGCs (arrows) immunostained for the indicated epigenetic marks. Scale bar, 20 μm. e, Triple immunostaining of epiblasts cultured under different conditions. Scale bar, 10 μm.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | Characterization of NANOS3–tdTomato reporter hPSC. a, Targeting strategy for making NANOS3–tdTomato (NT) reporter. b, Representative genotyping of targeted clones using genomic DNA. c, Conventional (conv) and PGC-competent (comp) hPSCs states are reversible; the latter is equivalent to pre-ME (12 h at mesendoderm induction). Conv-hPSCs are cultured in Essential 8 medium on vitronectin coated dishes (see Methods). Comp-hPSCs are cultured in the hPSC medium containing inhibitors (i) (GSK3i, ERKi, p38i, JNKi) on MEF (see Methods). d, NANOS3–tdTomato reporter conv-hPSCs and comp-hPSCs, and day 1–4 embryoids induced with or without cytokines. e, FACS pattern and percentage of NANOS3–tdTomato+AP+ cells shown in Extended Data Fig. 4d. f, FACS pattern and percentage of NANOS3–tdTomato+AP+ cells in multiple clones derived from Wis2 or H9 hESC lines. g, Immunostaining of day 4 embryoids induced from pre-ME (12 h) or comp-hPSCs by BMP containing cytokines. Scale bar, 50 μm. h, Comparison of sensitivity of two NANOS3 reporter cell lines. FACS patterns of day 4 embryoids induced from comp-hPSCs (harbouring NANOS3–mCherry reporter or NANOS3–tdTomato reporter) with or without cytokines. i, Comparison of hPGC induction efficiency derived from pre-ME (12 h) or comp-hPSCs. Representative images and FACS patterns are shown. j, Scatter plot (mean ± s.d.) shows percentage of NANOS3–tdTomato+AP+ cells in indicated condition (n = 6). Paired t-test was used to test for statistical significance (*P < 0.05).
Extended Data Figure 5 | Characterization of pre-ME and mesendoderm induced from Conv-hPSC. a, Immunostaining of conv-hPSCs during 12–24 h mesendoderm induction. Scale bar, 50 μm. b, Gene expression (RT-qPCR) change during mesendoderm induction. c, FACS patterns of day 4 embryoids induced from pre-ME (12 h) with cytokines. Pre-ME was induced with or without GSK3i or activin A. d, FACS patterns of day 4 embryoids induced from pre-ME (12 h) with cytokines. Pre-ME was induced with or without BMP2 or the inhibitor. e, Schematics of definitive endoderm or lateral mesoderm (LM) differentiation from mesendoderm. f, Relative induction efficiency of definitive endoderm or lateral mesoderm from mesendoderm induced with or without BMP2 (definitive endoderm, n = 5; lateral mesoderm, n = 6). Paired t-test was used to test for statistical significance (⁎ P < 0.05). h, Immunostaining of definitive endoderm and lateral mesoderm in Extended Data Fig. 5f. Scale bar, 50 μm.

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Extended Data Figure 6 | Robust induction of cmPGCs from cells during mesendoderm differentiation. a, Schematic of in vitro differentiation of cmPSCs. The same system was adopted as shown for conv-hPSCs differentiation in Fig. 3. b, Bright field image of undifferentiated cmPSCs. c, Immunostaining of day 2 embryoids induced with cytokines from cells at 0 h (cmPSCs), 12 h and 24 h during mesendoderm differentiation. Dashed lines highlight SOX17^{+} BLIMP1^{+} TFAP2C^{+} cmPGCs. Scale bar, 50 μm. d, Immunostaining of day 2 pre-ME-derived embryoids (12 h) in

Extended Data Fig. 6c for pluripotency markers. Notably, cmPGCs express SOX17, but not SOX2. By contrast, cmPSC colonies express SOX2 but not SOX17. e, Immunostaining of day 2 monkey definitive endoderm induced from pre-ME (12 h) and mesendoderm (24 h). Scale bar, 50 μm. f, Immunostaining of day 1 monkey lateral mesoderm induced from mesendoderm (24 h). Mesendoderm were induced with or without BMP. Notably, adding BMP during mesendoderm differentiation increased the efficiency for FOXF1^{+} HAND1^{+} lateral mesoderm cells, as shown in conv-hPSCs (Extended Data Fig. 5e–i). Scale bar, 50 μm.
Extended Data Figure 7 | Chronology of transcription factors expression during hPGC induction. a, Schematic of hPGC induction from pre-ME (12 h). b, Images of day 2 and 4 embryoids in response to BMP2 alone or BMP2 with LIF, SCF and EGF (cytokines). Notably, BMP2 alone can induce hPGC at almost the same efficiency as the full cytokines, but do not survive during extended culture, as shown previously. c, Immunostaining of embryoids induced with BMP2 alone or BMP2 with LIF, SCF and EGF showing expression of SOX17, BLIMP1 and TFAP2C. Scale bar, 50 μm. d, Proportion of SOX17^+ cells indicated in Extended Data Fig. 7c. e, Immunostaining of embryoids induced with BMP2 alone or BMP2 with LIF, SCF and EGF showing expression of SOX17, BLIMP1 and NANOG. Scale bar, 50 μm. f, Proportion of SOX17^+ cells in Extended Data Fig. 7e.
Extended Data Figure 8 | See next page for caption.
Extended Data Figure 8 | Effect of NANOG on hPGC induction, characterization of NANOS3–tdTomato reporter hPSCs containing inducible SOX17, BLIMP1 with or without TFAP2C, and similarity between cytokine- and SOX17–BLIMP1-induced hPGCs. a, Represents overexpression of dex-inducible NANOG transgenes in NANOS3–tdTomato reporter comp-hPSCs. b, Day 4 embryos following induction of NANOG (by dex), with or without cytokines as indicated. c, FACS patterns after induction of hPGCs: NANOS3–tdTomato + AP + cells (%) shown in Extended Data Fig. 8b. d, Represents overexpression of dex-inducible SOX17, dox-inducible BLIMP1, Shield1(S1)-inducible TFAP2C transgenes in NANOS3–tdTomato reporter comp-hPSCs. e, Immunostaining of NANOS3–tdTomato reporter comp-hPSCs + inducible SOX17/BLIMP1/TFAP2C (iSBT) 1 day after induction of SOX17, BLIMP1 and TFAP2C by addition of dex, dox or S1. Scale bar, 50 μm. f, Immunostaining of NANOS3–tdTomato reporter comp-hPSCs + iSB 1 day after induction of SOX17 or BLIMP1 by addition of dex or dox. Scale bar, 50 μm. g, Immunostaining of day 2 embryoid induced with or without dex to induce nuclear localization of SOX17. Notably, accumulation of SOX17 signal is observed in +dex condition. h, Changes in gene expression (RT-qPCR) during hPGC induction: comp-hPSCs control (AP + cells); NANOS3–tdTomato + hPGCs induced by SOX17–BLIMP1 or cytokines; NANOS3–tdTomato − cells in cells exposed to cytokines (error bars, mean ± s.d.). i, Unsupervised hierarchical clustering (UHC) of gene expression. j, Gene set enrichment analysis (GSEA) of 123 hPGC-specific genes (Supplementary Table 1) on the transcriptome of cytokine- and SOX17–BLIMP1-induced hPGCs. k, Heat map showing expression of epigenetic modifiers related to global DNA demethylation. Same datasets as shown in Fig. 4e were used for analysis.
Extended Data Figure 9 | See next page for caption.
Extended Data Figure 9  |  Response of SOX17-knockout comp-hPSC to SOX17. a, Overexpression of dex-inducible SOX17 (iS) in SOX17-knockout comp-hPSCs. b, Gene expression (RT–qPCR) on day 4 of FACS-sorted NANOS3–mCherry (NC)–alkaline phosphatase (AP)– hPGCs by RT–qPCR. c, FACS analysis of day 2 embryos induced from wild-type comp-hPSCs, SOX17-knockout comp-hPSCs and SOX17-knockout comp-hPSCs rescued with SOX17GR transgene (iS) (percentage of CXCR4–AP– cells). d, FACS pattern of day 2 embryo induced from NANO53–tdTomato reporter comp-hPSCs showing AP–CXCR4– cells expressing NANO53–tdTomato. e, Represents SOX17-inducible system (iSdd). Expression of SOX17 fused with destabilized domain (DD) can be induced by doxycycline (dox); addition of Shield1 (S1) can stabilize SOX17–DD protein. f, Western blots showing SOX17 expression level in day 5 embryoids from SOX17-knockout + inducible SOX17–DD (iSdd) comp-hPSCs. Embryoids were induced with cytokines. To induce SOX17, different concentration of dox and S1 were added. As controls, NANO53–mCherry–AP– hPGCs and NANO53–mCherry–AP– cells from wild-type comp-hPSC-derived embryoids induced with cytokines were used. Histone H3 (H3) was used for internal control. g, Immunostaining of day 4 embryoids from SOX17-knockout + iSdd comp-hPSCs. Embryoids from SOX17-knockout and wild-type comp-hPSC-derived with cytokines were used as controls. Scale bar, 50 μm. h, Quantification of immunostaining data in Extended Data Fig. 9g. The numbers of OCT4–BLIMP1– hPGCs, FOXA2–endodermal cells and OCT4–BLIMP1– hPGCs expressing FOXA2 were counted from 3 different embryoids. The proportions of the 3 populations are shown. i, Expression of SOX17 and BLIMP1 (RT–qPCR) in day 4 embryoids in response to different SOX17 dosage.
Extended Data Figure 10 | Response of SOX17-knockout comp-hPSCs to SOX17 and BLIMP1, and changes in epigenetic modifier expression after overexpression of SOX17 and BLIMP1. a, Overexpression of dex-inducible SOX17 (iS) and dox-inducible BLIMP1 (iB) in SOX17-knockout comp-hPSCs. b, Immunostaining of comp-hPSC 1 day after induction of SOX17 (dex) or BLIMP1 (dox), or both, and day 2 embryoids following dex-induced SOX17 with endogenous or dox-induced BLIMP1. Scale bar, 50 μm. c, Gene expression (RT–qPCR) in day 4 embryoids following induction by dex (+SOX17), dox (+BLIMP1), or dex + dox (+SOX17 and BLIMP1). Bulk cells of embryoids induced from SOX17-knockout and wild-type comp-hPSCs with cytokines were used as controls. d, Upon specification, hPGCs become refractory to activin or Wnt signalling. Left schematic shows the experimental design. The embryoids were transferred to the medium with or without GSK3i (3 μM) or activin A (100 ng ml⁻¹) at day 0, 1 or 2 to see the effect on PGC induction. FACS patterns (right) show the induction efficiency of hPGCs (percentage of NANOS3–tdTomato⁺AP⁺) at day 4. e, Day 4 embryoids induced by cytokines with or without activin A or GSK3i.