The successful segregation of germ cells from somatic lineages is vital for sexual reproduction and species survival. In the mouse, primordial germ cells (PGCs), precursors of all germ cells, are induced from the post-implantation epiblast. Induction requires BMP4 signalling to prospective PGCs and the intrinsic action of PGC transcription factors. However, the molecular mechanisms that connect BMP4 to induction of the PGC transcription factors that are responsible for segregating PGCs from somatic lineages are unknown. Here we show that the transcription factor OTX2 is a key regulator of these processes. Downregulation of Otx2 precedes the initiation of the PGC programme both in vitro and in vivo. Deletion of Otx2 in vitro markedly increases the efficiency of PGC-like cell differentiation and prolongs the period of PGC competence. In the absence of Otx2 activity, differentiation of PGC-like cells becomes independent of the otherwise essential cytokine signals, with germline entry initiating even in the absence of the PGC transcription factor BLIMP1. Deletion of Otx2 in vivo increases PGC numbers. These data demonstrate that OTX2 functions repressively upstream of PGC transcription factors, acting as a roadblock to limit entry of epiblast cells to the germline to a small window in space and time, thereby ensuring correct numerical segregation of germline cells from the soma.

Different species form their germ cells by either of two general methods: segregation of preformed germplasm, or induction by signalling. In mammals, germ cell precursors arise by induction. In the mouse, competence to initiate germ cell development is restricted to a few cells within the E5.5–E6.25 epiblast. BMP4 from the extraembryonic ectoderm acts on these competent cells to specify germ cell identity. Specification also requires transcription factors, notably BLIMP1, AP2γ and PRDM14. However, the molecular mechanisms that connect the exposure of competent cells to BMP4 to the activation of PGC transcription factors are obscured by limited access to the peri-implantation embryo. Recently, a system for differentiation of PGC-like cells (PGCLCs) from embryonic stem cells (ESCs) via germline competent epiblast-like cells (EpiCs) has opened up investigation of molecular events segregating germline and soma.

During the ESC to EpiLC transition, the transcription factor OTX2 becomes expressed and redirects binding of OCT4 to genomic regulatory elements. OTX2 was previously characterized as a regulator of anterior patterning. Previous work has demonstrated that OTX2 and NANOGEN have antagonistic functions in ESCs. A positive role for NANOGEN in PGCLC differentiation has now also been added to the known requirements for Blimp1, Prdm14 and Tjp2c (also known as Ap2γ). We therefore assessed expression of the corresponding mRNAs following addition of PGCLC-inducing cytokines to EpiLCs. OTX2 activity, differentiation of PGC-like cells becomes independent of the otherwise essential cytokine signals, with germline entry initiating even in the absence of the PGC transcription factor BLIMP1. Deletion of Otx2 in vivo increases PGC numbers. These data demonstrate that OTX2 functions repressively upstream of PGC transcription factors, acting as a roadblock to limit entry of epiblast cells to the germline to a small window in space and time, thereby ensuring correct numerical segregation of germline cells from the soma.

To investigate at which stage of differentiation OTX2 influences entry to the mouse germline, we generated two cell lines, one with a loss of Otx2-expressing cells more slowly (Extended Data Fig. 2a, b). Moreover, while Otx2 mRNA declines upon FGF/Activin withdrawal, the kinetics of suppression are enhanced by PGCLC cytokine addition (Extended Data Fig. 2d). This suggests that PGCLC cytokines directly repress Otx2 transcription, a notion supported by the prompt decline in Otx2 pre-mRNA upon switching EpiLCs into PGCLC media (Extended Data Fig. 2e). BLIMP1 and AP2γ proteins were initially detectable at 24 h, but only in cultures treated with cytokines (Extended Data Fig. 2a, b) and only in cells with reduced Otx2 (Fig. 1c, d, Extended Data Fig. 2c). These results suggest that before the PGC gene regulatory network (GRN) becomes activated, the transcriptional circuitry of the formative pluripotent state characterized by OTX2 expression becomes extinguished.

The reciprocal relationship of OTX2 with BLIMP1 and AP2γ during PGCLC induction prompted determination of whether a similar spatio-temporal relationship between changes in expression of OTX2 and PGC transcription factors held in vivo. Whole-mount immunofluorescence of pre-streak stage embryos indicated that all epiblast cells express both OCT4 and OTX2 (Fig. 1e). At early-to mid-streak, OTX2 remains widely expressed in the epiblast except for cells showing incipient FRAGILIS expression (Fig. 1f, h). By late-streak to early-bud-stage, BLIMP1 is clearly detectable within the FRAGILIS field in cells lacking OTX2 (Fig. 1g, i). These results indicate that OTX2 is expressed ubiquitously in pre-streak epiblast cells but is specifically downregulated in the prospective PGC population before BLIMP1 expression.

Cytokine addition is required for PGCLC differentiation. To assess when cells were treated for one, two or six days with cytokines and analysed by FACS for surface expression of SSEA1 and CD61, which together act as a marker for PGCLCs. Cytokine treatment for the first day induces around half of the potentially responsive population to express both CD61 and SSEA1 (Extended Data Fig. 2f). Two or six days of cytokine treatment were equally effective at inducing CD61 and SSEA1 (Extended Data Fig. 2f). This suggests that cytokine exposure reaches maximum efficacy at two days, the time required to reduce OTX2 to minimal levels and initiate PGCLC transcription factor expression (Fig. 1b, d, Extended Data Fig. 2a–c).

To directly assess whether OTX2 downregulation influences entry of pluripotent cells into the germline, Otx2-null cells were examined. A transgenic Oct4ΔPE::GFP reporter activated upon germline entry was added to Otx2−/+ and Otx2−/− ESCs (Extended Data Fig. 1). Compared to Otx2 heterozygotes, Otx2−/− cell populations showed widespread activation of Oct4ΔPE, with essentially all cells activating GFP (Fig. 2a). Furthermore, the SSEA1+ CD61+ cell number is increased 5–10-fold in Otx2−/− versus Otx2−/+ cells (Fig. 2b). This was also the case in independently generated Otx2−/− ESCs (Extended Data Fig. 3b) and in additional independent Otx2−/− ESCs generated using CRISPR/Cas9 (Extended Data Fig. 1, 3c). Three new ESC lines lacking OTX2 protein (Extended Data Fig. 3d) showed enhanced CD61 and SSEA1 expression during PGCLC differentiation (Extended Data Fig. 3e). These results confirm that a lack of OTX2 promotes germline differentiation.

To investigate at which stage of differentiation OTX2 influences germline entry, an Otx2−/ER transgene (enabling tamoxifen-induced
without OTX2 (Extended Data Fig. 3f), their expression was increased. Fgf5 addition, although
immunofluorescence of E14Tg2a aggregates after one day of PGCLC biologically independent replicates were performed. 
γ immunofluorescence for OTX2 and AP2 in cytospin preparations of e
expression, is sufficient to block cytokine-mediated PGCLC
OtX2 lacZ/GFP
To examine the mechanism by which OTX2 inhibits PGCLC differentiation, mRNAs were analysed. Expression of PGCLC transcription factor mRNAs occurred sooner and to a greater extent without OTX2, germline entry does not require cytokines. BLIMP1 is essential for wild-type cells to access the germline. re-localization of OTX2 was introduced into Otx2lacZ/GFP ESCs (Fig. 2c, Extended Data Fig. 4a). Tamoxifen treatment for the first two days suppressed emergence of SSEA1CD61 cells in the absence of cytokines (Fig. 3a). However, in Otx2−/− cells, cytokines were not essential for Oct△APE reporter activation (Fig. 3a), CD61 and SSEA1 surface expression (Fig. 3b, Extended Data Fig. 5a, b) or PGCLC transcription factor expression (Extended Data Fig. 5c, d). Indeed, mRNA profiling indicated that Otx2−/− and Otx2+/− EpiLCs were transcriptionally similar and that following differentiation, Otx2−/− cells resembled wild-type PGCLCs, irrespective of their exposure to cytokines (Extended Data Fig. 6a). Principal component and ternary analysis confirmed these assessments (Fig. 3c, Extended Data Fig. 6b). These results indicate that without OTX2, germline entry does not require cytokines. BLIMP1 is essential for wild-type cells to access the germline.3,4 To determine whether Otx2−/− cells retained this dependency, both Blimp1 alleles were deleted from ESCs of distinct Otx2 genotypes using CRISPR–Cas9 (Extended Data Figs. 1, 7a–c). PGCLC differentiation confirmed a BLIMP1 requirement for germline entry of OTX2-expressing cells (Fig. 3d, Extended Data Fig. 7d). However, deletion of Blimp1 from Otx2−/− cells did not affect the ability of Otx2−/− cells to induce CD61 and SSEA1 (Fig. 3d, Extended Data Fig. 7d). Although deletion of Blimp1 from wild-type cells increased expression of somatic transcripts during PGCLC differentiation, this did not occur in Otx2−/− Blimp1−/− PGCLCs (Extended Data Fig. 8a). Nor did deletion of Blimp1 impair the enhanced ability of Otx2−/− cells to activate expression of Prdm14, Ap2, Nanog or Oct4 mRNAs (Extended Data Fig. 8b) or DAZL protein (Extended Data Fig. 8c). During differentiation, PGCLCs that express OCT4 have higher H3K27me3 and lower H3K9me2 than OCT4-low cells. These relationships were maintained from wild-type cells increased expression of Blimp1 and Prdm14 mRNAs seen after 24 h (Extended Data Fig. 4e). This is consistent with a model in which BMP induction of Wnt enforces timely repression of Otx2 and full induction of Blimp1 and Prdm14. Finally, Otx2−/− cells did not activate T mRNA (Extended Data Fig. 4c) or protein (Extended Data Fig. 4f). Therefore, activation of T is dispensable for PGC induction, at least when OTX2 is absent. These observations suggest that the effects of Wnt signalling during PGC differentiation could be attributed to OTX2 downregulation. To assess whether OTX2 can interfere with the function of an established PGCLC GRN, OTX2 activity was restored at day 2, once the PGC GRN was already activated (Fig. 2e). This produced a similar proportion of SSEA1CD61 cells as cultures receiving no tamoxifen (Extended Data Fig. 4g, h). Therefore, OTX2 does not impair the function of an established PGC network, but rather restricts the efficiency with which EpiLCs enter the germline.

PGCLC induction is considered to strictly require cytokine addition.2 Consistent with this, Oct△APE::GFP was not expressed by aggregates of Otx2+/− cells cultured in the absence of cytokines (Fig. 3a). However, in Otx2−/− cells, cytokines were not essential for Oct△APE reporter activation (Fig. 3a), CD61 and SSEA1 surface expression (Fig. 3b, Extended Data Fig. 5a, b) or PGCLC transcription factor expression (Extended Data Fig. 5c, d). Indeed, mRNA profiling indicated that Otx2−/− and Otx2+/− EpiLCs were transcriptionally similar and that following differentiation, Otx2−/− cells resembled wild-type PGCLCs, irrespective of their exposure to cytokines (Extended Data Fig. 6a). Principal component and ternary analysis confirmed these assessments (Fig. 3c, Extended Data Fig. 6b). These results indicate that without OTX2, germline entry does not require cytokines. BLIMP1 is essential for wild-type cells to access the germline.3,4

These analyses suggest that OTX2 acts at the juncture between somatic and germline differentiation and inhibits PGCLC differentiation by preventing PGC transcription expression. A previous report has provided evidence for the involvement of T (Brachyury) in PGCLC induction by showing that BMP4 induced T expression via endogenous Wnt. We also found that T is activated robustly only when BMP4 is present (Extended Data Fig. 4b) with T and other somatic markers induced during PGCLC differentiation (Extended Data Fig. 4c). In vivo, BMP4 induces epiblast cells to secrete Wnt28. Therefore, to assess whether Wnt acts as an intermediary between BMP4 and activation of T and other somatic markers, Wnt signalling was mimicked by adding CHIR99021 to basal media (Extended Data Fig. 4d). T, Hoxa1 and Hoxb1 mRNAs were induced by CHIR99021 but Otx2 mRNA was repressed; effects that were reversed by addition of the Wnt antagonist XAV939 (Extended Data Fig. 4d). Therefore, Otx2 downregulation by BMP4 may occur via Wnt signalling. To further assess this, XAV939 was added during PGCLC differentiation. XAV939 did not affect Otx2 mRNA for 9 h, but dampened further reduction (Extended Data Fig. 4e). Moreover, XAV939 diminished induction of Blimp1 and Prdm14 mRNAs seen after 24 h (Extended Data Fig. 4e). This is consistent with a model in which BMP induction of Wnt enforces timely repression of Otx2 and full induction of Blimp1 and Prdm14. Finally, Otx2−/− cells did not activate T mRNA (Extended Data Fig. 4c) or protein (Extended Data Fig. 4f). Therefore, activation of T is dispensable for PGC induction, at least when OTX2 is absent. These observations suggest that the effects of Wnt signalling during PGC differentiation could be attributed to OTX2 downregulation.

Fig. 1 | Ot2x expression is downregulated before expression of PGCLC transcription factors. a, Scheme for PGCLC differentiation. b, Top, scheme illustrating the time-points (hours) during PGCLC differentiation when mRNAs were analysed. Bottom, real-time PCR (RT-PCR) of Ot2x and PGCLC transcription factors in wild-type E14Tg2a ESCs. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates. c, Single-cell quantification of immunofluorescence for OTX2 and AP2 in cytopsin preparations of EpiLCs and cell aggregates at day 1 and day 2 of PGCLC induction. Two biologically independent replicates were performed. d, Whole-mount immunofluorescence of E14Tg2a aggregates after one day of PGCLC differentiation; n = 3; scale bars, 50 μm (top), 10 μm (bottom). e–g, Representative confocal images of whole mount staining of embryos at pre-streak (e, n = 4), early streak (f, n = 3) and late streak (g, n = 3) stages; scale bars, 40 μm (e), 100 μm (f, g). h, i, Magnified image of the regions highlighted in f and g, respectively. OTX2-negative cells expressing BLIMP1 and FRAGILIS are outlined (g, h); scale bar, 20 μm.

re-localization of OTX2 was introduced into Otx2lacZ/GFP ESCs (Fig. 2c, Extended Data Fig. 4a). Tamoxifen treatment for the first two days suppressed emergence of SSEA1CD61 cells (Fig. 2d). These results establish that enforcing OTX2 activity at a time when cells are competent to enter the germline2 and when cytokines act to decrease endogenous Ot2x expression, is sufficient to block cytokine-mediated PGCLC differentiation. To examine the mechanism by which OTX2 inhibits PGCLC differentiation, mRNAs were analysed. Expression of PGCLC transcription factor mRNAs occurred sooner and to a greater extent without OTX2 (Fig. 2e). In contrast, enforcing OTX2 activity inhibited induction of PGCLC transcription factor mRNAs (Fig. 2e). NANOG also directs PGCLC differentiation.20,26 Consistent with this, endogenous Nanog mRNA was induced precociously in Ot2+/− cells (Fig. 2e). In addition, although Fgf5, Foxd3 and Oct6 mRNAs were not induced without OTX2 (Extended Data Fig. 3f), their expression was increased above wild-type levels by OTX2 induction (Extended Data Fig. 3f).
in multiple Otx2−/− cell lines (Fig. 3e, Extended Data Fig. 7e, f). Nevertheless, at day 6 of PGCLC differentiation, Otx2−/− Blimp1+/− cells were unable to adopt the mature PGCLC transcriptome observed in Otx2+/- or Otx2+/+ Blimp1+/- cells (Fig. 3c, Extended Data Fig. 6). These results indicate that without OTX2, BLIMP1 is not required for phenotypic aspects of germline entry, but is required for a fully mature PGC phenotype.

Although EpilCs respond to cytokine induction by PGCLC differentiation, this is not maintained upon continued passaging in medium containing Activin/FGF12, suggesting that in these conditions, cells lose germline competence. To assess whether OTX2 affects the period during which pluripotent cells remain competent for germline entry, EpilCs were passaged in EpilC medium for a further two days (Fig. 4a). At this point the Oct4ΔPE::GFP reporter is inactive in both Otx2+/- and Otx2−/− cells (Extended Data Fig. 9a). Cells were then transferred to PGCLC differentiation medium for 6 days. Notably, Oct4ΔPE::GFP was reactivated robustly in Otx2−/− but not Otx2+/- cells (Fig. 4b). Moreover, whereas all cell lines expressed Blimp1 and Prdm14 mRNAs, CD61/SSEA1 surface expression and Prdm14 expression were observed only in Otx2−/− and not in Otx2+/- or Otx2+/+ cells (Extended Data Fig. 9b–d). This indicates that in the absence of Otx2 the period of competence to enter the germline is extended.

To determine whether Otx2−/− cells exhibit an increased propensity to enter the germline in vivo, Otx2+/-+ or Otx2−/− ESCs constitutively expressing GFP were compared in chimaeras following morula aggregation (Fig. 4c). Otx2+/- and Otx2−/− cells had a comparable capacity to produce chimaeras (Extended Data Fig. 9e, g). However, an enhanced proportion of Otx2−/− cells expressed BLIMP1 or SOX2 (Fig. 4d, Extended Data Fig. 9f), indicating that enhanced germline entry is a cell autonomous property of Otx2−/− cells.

To determine whether Otx2−/− embryos also showed enhanced PGC numbers, Otx2−/− mice15 were inter-crossed and Otx2−/− embryos analysed at E7.5. These embryos show strong developmental defects15,16 but also showed increased PGC numbers (Fig. 4e, Extended Data Fig. 10a, b), confirming that in vivo, OTX2 acts as a negative regulator of the PGC programme.

Previous studies have shown that, in mice, competence to enter the germline exists transiently in embryos from E5.5–E6.251. In wild-type cells, germline entry requires BMP4 signalling from the extraembryonic ectoderm2 and is critically dependent on the downstream action of BLIMP13. Our work identifies OTX2 as an intermediary fulcrum in these processes (Fig. 4f). During the period of PGC competence, BMP4 represses Otx2 expression, partly by endogenous Wnt activation (Fig. 4g). This reduction in OTX2 is necessary for expression of the PGC transcription factors BLIMP1, PRDM14, AP2γ and NANOG as enforcing Otx2 activity prevents their expression. Moreover, the rate of Otx2 decline appears important as without cytokines, germline entry is diminished. We propose that without cytokines, the window for germline entry closes before Otx2 is reduced below a threshold necessary for PGC transcription factor expression. Furthermore, in the absence of Otx2, PGC transcription factor expression does not require BMP4, indicating that BMP4 functions by repressing Otx2. Otx2−/− cells also exhibit an extended competence period, suggesting that Otx2 starts a process that defines the extent of the competence
Fig. 4 | Otx2−/− ESCs contribute to the germine at an enhanced rate in vivo. a, Scheme for PGCLC differentiation, initiated from day 4 EpilCs obtained after one passage from EpilCs. b, Representative morphologies and Oct4ΔPE:GFP expression from aggregates at day 6 of PGCLC differentiation from EpilCs day 4; n = 3 for 1 clone of each genotype; scale bar, 200 μm. c, Scheme for generating chimaeras of GFP-labelled Otx2+/+ or Otx2−/− ESCs with wild-type host embryos. d, Comparison of the percentage contribution of GFP-labelled wild-type (n = 6) or Otx2-null ESCs (genotypes indicated, n = 9 for each) to the PGC population in E7.5 chimaeric embryos. Each dot represents the percentage from one chimaera, centre lines and error bars represent means ± s.d. P value (two-sided unpaired t-test, 0.95 confidence intervals) is indicated. GFP-positive cells were counted within the PGC population marked with BLIMP1 or SOX2 in each embryo. e, Comparison of PGCs number in wild-type (n = 4) and Otx2−/− (n = 3) E7.5 embryos. PGCs were identified with BLIMP1, AP2+, and FRAGILIS. Values are means ± s.d. f, Model indicating the point of operation of OTX2 during germline and soma segregation. g, A scheme illustrating the regulatory relationships upstream and downstream of Otx2 during germline segregation.

period. Also, as Otx2−/− cells can initiate PGC differentiation without BLIMP1, this suggests a reciprocal relationship between BLIMP1 and Otx2, in which BLIMP1 represses21,29,30 and Otx2 activates somatic gene expression17. Supporting this, during PGCLC differentiation, Otx2−/− cells do not activate mesendoderm genes17 (Extended Data Fig. 4c) that are otherwise repressed by BLIMP130. This may explain why some aspects of the PGCLC differentiation phenotype can be divorced from a BLIMP1 requirement in Otx2−/− cells. This places Otx2 at a developmental crossroads where it acts to control excessive access to the germline (Fig. 4f).

Finally, our findings are noteworthy in light of the hypothesis that the neural lineage is the default developmental pathway for vertebrate cells3. Interestingly, neural induction requires inhibition of BMP signalling28. BMP is a known facilitator of germine entry and is identified here as a key Otx2 repressor. The default neural induction hypothesis is based principally on studies in chicks and frogs, species in which PGCs are formed by germplasm segregation. Yet, induced germine segregation is considered the ancestral mechanism that predates the recurrent evolution of germplasm-2. The highly efficient entry of pluripotent cells into the germine in the absence of OTX2 reported here suggests that the germine may be the ancient default option that must be overcome in order to elaborate the ancillary structures of the soma.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0581-5.

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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. All mouse studies were performed in accordance with UK Home Office regulations under project licence PPL 60/4435 and carried out in a Home Office-designated facility.

Cell culture and differentiation. ESCs were Routinely cultured in GMEM with supplements that include 100 μM LIF and 10% FCS at 3–10 × 10^6 cells per cm^2 density on tissue culture flasks coated with 0.1% gelatin. Cells were routinely tested for mycoplasma contamination. EpLc differentiation and PGC/LC induction were carried out according to protocol previously described. For cytokine-free differentiation, EpLc were dissociated and resuspended at 8 × 10^4 cells ml^(-1) in GKM15 medium (GMEM supplemented with 15% KOSR). 25 μl drops containing 2,000 cells were plated on the lids of tissue culture dishes and incubated over a reservoir of PBS for 2 days. Hanging drops were then collected and transferred to untreated culture dishes supplemented with GKM15 medium and rotated at 72 r.p.m. Fresh medium was replenished every other day. CHIR (3 μM) and XAV939 (2 μM) were used to induce and repress Wnt signalling.

Generation of knock-out cell lines. For a summary of cell lines used in this report and a history of their derivation, see Extended Data Figure 1.

Otx2-knockout (Otx2loxP/loxP) and Otx2 conditional knockout (Otx2loxP/; Rosa26creER^T2) cell lines and targeting strategies have been described previously. To derive Otx2-knockout (Otx2loxP/loxP) cell lines from Otx2loxP/; Rosa26creER^T2 cells, embryos were isolated at E7.5, washed in PBS, fixed overnight in 4% PFA/PBS, dehydrated and transferred to untreated culture dishes supplemented with GKM15 medium and rotated at 72 r.p.m. Fresh medium was replenished every other day. CHIR (3 μM) and XAV939 (2 μM) were used to induce and repress Wnt signalling.

Immunohistochemistry. For whole-mount staining of embryos and PGC/LC aggregates, embryos and PGC/LC aggregates were fixed with 4% paraformaldehyde (PFA) in PBS (room temperature, 30 min). For Fraggil staining, embryos were washed three times in PBS/0.1%BSA and blocked overnight at 4 °C in 3% donkey serum (Sigma)/1% BSA/PBS solution. For other antibodies, samples were washed three times with PBS/0.1% TritonX100 (PBST), permeabilized in 0.5% Triton X100/PBS solution for 15 min and incubated in 1% glycine in PBST for 20 min. After three washes, samples were blocked overnight at 4 °C using 3% donkey serum, 1% BSA (Sigma) in PBST (blocking buffer). Samples were then incubated for 2 h with diluted primary antibodies, rinsed four times for 20 min each in PBST and incubated in 1% glycine in PBST with diluted secondary antibody. Samples were then rinsed four times for 20 min each in PBST. DAPI (Molecular Probes, D1306) was used for nuclear staining (same for other staining). For imaging, embryos were treated with 10%, 25%, 50%, 97% thioldiolen (Sigma, 166782) for 5 min in each gradient and were imaged using the TCS SP8 inverted confocal microscopy (Leica).

For staining of frozen sections, aggregations were fixed in 4% PFA (room temperature, 20 min). After washing in PBS, samples were embedded in Tissue-Freeze medium (Thermo Fisher scientific, 6502Y) and sectioned at a thickness of 30 μm. Sections were then incubated in blocking buffer (room temperature, 1 h). After antigen retrieval, sections were incubated in blocking buffer (room temperature, 1 h), followed by incubation with primary antibodies for 2 h at 4 °C. After three washes in PBS, membranes were incubated with IRDye conjugated secondary antibody, followed by three washes in PBST before being visualized in LI-COR Odyssey Imaging Systems. Primary and secondary antibodies are listed in Supplementary Table 2.

RNA analysis. Total RNA was extracted using either Trizol (Invitrogen,15590626) or RNeasy micro (Qiagen,740040) and treated with DNase (Qiagen, 109740) and transferred to untreated culture dishes supplemented with 1/500, respectively, and incubated (30 min, 4 °C). After three washes in PBST, membranes were incubated with IRDye conjugated secondary antibody, followed by three washes in PBST before being visualized in LI-COR Odyssey Imaging Systems. Primary and secondary antibodies are listed in Supplementary Table 2.

Transcriptomic profiling and data analysis. Total RNAs were extracted using RNA Plus Micro Kit (Qiagen, cat.74034) following manufacturer’s instructions. RNAs were labelled using Illumina TotalPrep RNA Amplification Kit (Illumina, cat. AM11102) following manufacturer’s instructions. Briefly, 100 to 300 ng total RNA were reverse transcribed using oligo(dT) primers, followed by second-strand cDNA synthesis. The dsDNAs were then in vitro transcribed using SuperScript First Strand Synthesis Kit (Invitrogen, 1809107) and quantitative PCR performed using SYBR Green Kits (Santa Cruz Biotechnology, 623815) on Roche LightCycler 480 cDNA equivalents of 25 ng total RNA per reaction. Values for each gene were normalized to expression of TATA-box Binding Protein (TBP) according to 2^-DeltaCt formula. Oligonucleotide sequences are shown in Supplementary Table 3.

Embryo microarrays. E2.5 embryos were collected from superovulated F1 (CBA B6) females with M2 buffer (Sigma, M7677). After being cultured in KSM (Millipore, MR-020P-5F) for 15 min, zona pellucidae were removed by Tyrode’s solution (Sigma, T1788) and embryos cultured in KSM in aggregation plates prepared by aggregation needle (BLS, DN-10). Wild-type coronal- or sagittal sections and processed for immunohistochemistry (IHC) with antibodies against BLIMP1, AP2γ, Otx2 (Santa Cruz Biotechnology, SC-53162) and Fragilis (R&D Systems, AF3377). All sections were analysed and those including PGCs captured for cell-counting analysis.

If not specified, primary and secondary antibodies used are listed in Supplementary Table 2.

Methylation quantification. Cytosinase slides were stained at the same time and imaged using a Zeiss Observer microscope (Zeiss). Plan-Apo 20 × NA, 0.8 objective (Zeiss), a Hamamatsu ORCA-Flash4.0 V3 camera (Hamamatsu), a Colibri 7 (Zeiss) light source and the following filter cubes (name, excitation LED, beam splitter, band pass emission filter; 49 DAP, 395, 480/40; 38 HE GFP, 495, 525/50; 43 HE dsRed, 570, 605/70; Cy5, 660, 700/775). Images were analysed using CellProfiler software (version 2.2.0). DAPI staining was first used to segment individual nuclei based on the diameter and intensity of the objects (25–90 pixel units and intensity threshold >0.05 respectively), then the intensity of OTX2 and AP2γ of the segmented nuclei were measured and the mean intensity of each channel were reported. Over 8,000 segmented nuclei of each samples were analysed. The data were analysed in R and plots were generated using the ggplot2 package. The Otx2-knockout EpLc are negative for both OTX2 and AP2γ; staining and therefore were used to set up the threshold for gating OTX2 and AP2γ for all samples (negative gate >99.5%). Flow cytometry. FACS analysis was performed as described. Cells grown in monolayer or embryoid bodies in suspension were dissociated into single cells with trypsin and neutralized in PBS/10%FCS. A maximum of 5 × 10^6 cells were collected by centrifugation and the pellet resuspended in 100 μl PBS/10%FCS supplemented with Alexa 488 anti-mouse/human IgG4 (Invitrogen, cat. A-11008); blocked in 10% milk (w/v) (room temperature, 1 h), followed by incubation with primary antibodies (4 °C, overnight). After three washes in PBST, membranes were incubated with IRDye conjugated secondary antibody, followed by three washes in PBST before being visualized in LecyT CUSTOM Imaging Systems. Primary and secondary antibodies are listed in Supplementary Table 2.

SDS–PAGE electrophoresis and immunoblotting. Immunoblot analysis was performed as described. Briefly, protein samples from cells lysates, along with protein ladder (Novex, cat. LC5925) were loaded on 10% Bis-Tris Gels (Novex, cat. BG00102BOX) and electrophoresis performed at 200 V for 60–80 min. Proteins were then transferred onto Nitrocellulose membrane (Capitol Scientific, cat. 10401396), blocked in 10% milk (w/v) (room temperature, 1 h), followed by incubation with primary antibodies (4 °C, overnight). After three washes in PBST, membranes were incubated with IRDye conjugated secondary antibody, followed by three washes in PBST before being visualized in LI-COR Odyssey Imaging Systems. Primary and secondary antibodies are listed in Supplementary Table 2.

Immunofluorescence quantification. Cytosinase slides were stained at the same time and imaged using a Zeiss Observer microscope (Zeiss). Plan-Apo 20 × NA, 0.8 objective (Zeiss), a Hamamatsu ORCA-Flash4.0 V3 camera (Hamamatsu), a Colibri 7 (Zeiss) light source and the following filter cubes (name, excitation LED, beam splitter, band pass emission filter; 49 DAP, 395, 480/40; 38 HE GFP, 495, 525/50; 43 HE dsRed, 570, 605/70; Cy5, 660, 700/775). Images were analysed using CellProfiler software (version 2.2.0). DAPI staining was first used to segment individual nuclei based on the diameter and intensity of the objects (25–90 pixel units and intensity threshold >0.05 respectively), then the intensity of OTX2 and AP2γ of the segmented nuclei were measured and the mean intensity of each channel were reported. Over 8,000 segmented nuclei of each samples were analysed. The data were analysed in R and plots were generated using the ggplot2 package. The Otx2-knockout EpLc are negative for both OTX2 and AP2γ; staining and therefore were used to set up the threshold for gating OTX2 and AP2γ for all samples (negative gate >99.5%).
E14Tg2a ESCs and two independent Otx2-knockout cell lines (labelled with Flag-GFP) were used for aggregation. Cells were trypsinized (37 °C, 1 min), washed and re-suspended with G MEM/FCS medium. Each embryo was aggregated with 6–8-cell clumps and cultured in the incubator. The next day, good-quality blastocysts were picked and transferred to E0.5 CD1 recipient38.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability**
All the data sets generated or analysed during the current study are available from the corresponding author on reasonable request.
Extended Data Fig. 1 | Summary of cell lines used in this report. Otx2lacZ/GFP and Otx2lacZ/fl ESCs have been described previously\(^1\). Summarized below are further modifications to Otx2 or Blimp1, or transgene additions in the above or wild-type backgrounds. Further schematic details illustrating the points of Cas9 modification of Otx2 or Blimp1 and genotype verification of derived cell lines are shown in Extended Data Fig. 3 and Extended Data Fig. 7, respectively.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Competence for germline entry is preceded by downregulation of OTX2 protein. a, Representative cytospin images of OTX2, BLIMP1 and AP2γ staining using E14Tg2a aggregates after 1 day or 2 days of PGCLC differentiation; n = 2; scale bar, 100 μm. b, Whole-mount immunofluorescence of E14Tg2a aggregates after 1 (D1) or 2 days (D2) of differentiation of EpiLCs in the presence or absence of cytokines. Representative images of EpiLCs and BLIMP1 are shown; n = 3; scale bar, 50 μm. c, Magnified image of the region highlighted in b; scale bar, 10 μm. d, Quantitative transcript analysis of Otx2 in E14Tg2a cultures with (n = 4) or without cytokines (n = 7) at indicated time point. Schematic illustration is shown in Fig. 1b. Expression levels are normalized to TBP; values are means ± s.d. e, Top, primers used for Otx2 pre-mRNA transcript analysis are shown relative to the primary transcript structure. Bottom, quantitative transcript analysis of Otx2 pre-mRNA at the indicated times (minutes) after changing E14Tg2a EpiLCs into PGCLC medium. Expression levels are normalized to TBP and shown relative to expression at t = 0; values are means ± s.d.; n = 3 biologically independent replicates. f, Assessing the temporal requirement of cytokine treatment for efficient PGCLC induction. Aggregates of E14Tg2a EpiLCs treated with cytokines for 1 (d0–d1), 2 (d0–d2) or 6 days (d0–d6) were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLCs differentiation; n = 3.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Independent Otx2<sup>−/−</sup> clones show enhanced PGCLC induction efficiency. a, The gating strategies for analysing PGCLCs by flow cytometry. Cells were first gated based on the FSC (size) and SSC (complexities) scatter plot, followed by selection for singlets based on linear correlations between FSC-area and FSC-height. Live cells were then gated based on exclusion of DAPI to indicate cell membrane integrity. Live cells were then analysed for SSEA1 and CD61. Cells stained for fluorescence minus one (FMO) were used to set gates; stained and non-stained cells are also shown. b, Otx2<sup>lacZ/</sup>fl and Otx2<sup>lacZ/−</sup> cells with the Oct4ΔPE::GFP reporter (two independent clones each) were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation. For clone 5 and clone 1, n = 2; for clone 11 and clone 2, n = 9. c, Diagram showing the gRNA sequence (in red) and targeting strategy for generating Otx2-knockout cell lines. Red arrows represent genotyping primers used for screening clones. d, Immunoblot analysis of OTX2 protein expression in EpiLCs of E14Tg2a and three Otx2<sup>−/−</sup> clones. Experiment preformed once. e, E14Tg2a and three independent Otx2<sup>−/−</sup> clones generated by CRISPR/Cas9 were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation. Two biologically independent experiments for clone c11, one for clone c17 and c19. f, Q-RT–PCR of epiblast markers during the time-course outlined in Fig. 1b. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | OTX2 restricts PGC specification during the first two days of induction. a, OTX2 immunofluorescence of Otx2lacZ/GFP::Otx2ERT2 ESCs before or after treatment with tamoxifen for 20 min; n = 2 biologically independent experiments; scale bar, 20 μm. b, Quantitative transcript analysis of T (Brachyury) during the time-course outlined in Fig. 1b in basal GK15 medium supplemented with the indicated cytokines. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates. c, Quantitative transcript analysis of T (Brachyury), Hoxa1 and Hoxb1 during the time-course outlined in Fig. 1b in indicated cell lines. Expression levels are normalized to TBP; values are means ± s.d.; n = 2 biologically independent replicates. d, Left, scheme illustrating the strategy for induction or repression of Wnt signalling. E14Tg2a EpiLCs were aggregated in the indicated media and transcripts analysed at 0, 9 and 18 h. Right, quantitative transcript analysis of T (Brachyury), Hoxa1, Hoxb1 and Otx2 during the time-courses outlined on the left. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates. e, Quantitative transcript analysis of T (Brachyury), Otx2, Blimp1 and Prdm14 during E14Tg2a differentiation in three different media conditions (GK15, without cytokines; PGCLC, GK15 with cytokines; PGCLC + XAV, GK15 with cytokines and with XAV939) at the indicated time point. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates. f, Whole-mount immunofluorescence analysis of AP2γ and T (Brachyury) in E14Tg2a and Otx2lacZ/GFP day 2 (D2) PGCLC aggregates; n = 2 biological replicates; scale bar, 50 μm. g, Scheme illustrating tamoxifen administration schemes. h, Otx2lacZ/GFP::Otx2ERT2 cells were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation following the tamoxifen treatment regime outlined (g); n = 2 biological replicates.
Extended Data Fig. 5 | PGCLC differentiation of Otx2-null cells in the absence of cytokines. a, Otx2<sup>fl/fl</sup> and Otx2<sup>−/−</sup> cells carrying the Oct4ΔPE::GFP reporter (aggregates shown in Fig. 3a) were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation in the absence of cytokines; n = 7. b, E14Tg2a and three independent Otx2<sup>−/−</sup> clones generated by CRISPR/Cas9 were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation in the absence of cytokines. Two biologically independent experiments for clone c11, one for clone c17 and c19. c, Quantitative transcript analysis of mRNAs encoding PGC transcription factors during differentiation without PGCLC cytokines at indicated time point. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates. d, Whole-mount immunostaining of aggregates of Otx2<sup>fl/fl</sup>GFP cells at day 2 in the absence of cytokines for OTX2, BLIMP1 and AP2γ; scale bar; 40 μm; n = 3.
Extended Data Fig. 6 | Transcriptome analysis of EpiLcs and day 6 PGCLCs. 

**a**, Heat map of the normalized gene expression and principal component analysis of microarray data (from three biologically independent replicates under seven different conditions) ordered by unsupervised hierarchical clustering; rows correspond to transcripts and columns to cells. Differentiations performed in the presence (+Cyt) or absence (-Cyt) of cytokines are indicated. WT, E14Tg2a; O−/-, Otx2lacZ/GFP; O−/- B−/-, Otx2lacZ/GFP; Blimp1−/−.

**b**
Extended Data Fig. 7 | PGCLC induction of independent Blimp1-null cell lines. 

**a.** Scheme showing the strategy used to generate Blimp1-null cell lines. A pair of gRNAs flanking Blimp1 exon5 were co-expressed to ensure complete deletion of Blimp1 exon5. Red arrows represent genotyping primer pairs used to screen clones.

**b.** Blimp1-null clones used in Fig. 3 (b) or Extended Data Fig. 8d (c) were genotyped using primers indicated in a; n = 2 biologically independent replicates for both, all clones have been sequenced.

**d.** Cells of the indicated genotypes (c) were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of aggregation in the presence of PGC induction cytokines; n = 2.

**e.** Cells of the indicated genotypes (c) were assessed by flow cytometry for surface expression of SSEA1 and CD61 at day 6 of aggregation in the absence of PGC induction cytokines; n = 2.
Extended Data Fig. 8 | *Otx2*−/−*Blimp1*−/− PGCLCs activate PGC markers. **a**, Quantitative analysis of somatic transcripts at day 2 of PGCLC induction in the indicated cell lines. Expression levels are normalized to TBP; values are means ± s.d.; *n* = 4 biological replicates, each dot represents the value from one experiment. **b**, Quantitative analysis of PGC transcription factor transcripts at day 2 of PGCLC induction in the indicated cell lines. Expression levels are normalized to TBP; values are means ± s.d.; *n* = 6 biological replicates for E14Tg2A and Otx2lacZ/GFP, and 4 for Blimp1-knockout cell lines each dot represents the value from one experiment. **c**, Immunofluorescence staining for OCT4 and DAZL of cryo-sections of Otx2lacZ/GFP and Otx2lacZ/GFP*Blimp1*−/− aggregates at day 6 of PGCLC induction; scale bar, 50 μm and 20 μm; *n* = 2 biologically independent replicates. **d**, OCT4, H3K27me3 and H3K9me2 immunofluorescence analysis of cryo-sections of E14Tg2A, Otx2lacZ/GFP and Otx2lacZ/GFP*Blimp1*−/− aggregates at day 6 of PGCLC induction; scale bar, 50 μm; *n* = 2 biologically independent replicates.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | OTX2 safeguards somatic lineages.

a. Representative morphologies and Oct4ΔPE::GFP expression of EpiSCs after one passage from EpiLCs (n = 3 for 1 clone of each genotype); scale bar, 200 μm. b, c. Flow cytometry analysis for surface expression of SSEA1 and CD61 at day 6 of PGCLC differentiation, initiated from EpiSCs after one passage from EpiLCs. One experiment for c5 and c1 and 6 biologically independent replicates for C11 and C2 (b); n = 6 biologically independent replicates (c). d. Quantitative transcript analysis of PGC transcription factors in the indicated cell lines. Expression levels are normalized to TBP; values are means ± s.d.; n = 3 biologically independent replicates, each dot represents the value from one experiment. e. Comparison of the frequency of degree of chimaerism (top) and the germ cell numbers (bottom, centre lines and error bars represents means ± s.d.) in E7.5 chimaeric embryos formed using wild-type or Otx2-null ESCs. P value (two-tailed unpaired t-test, 0.95 confidence intervals) is indicated. High, >70%; moderate, 30–70%; low, <30%. f, Bright-field and representative images of E7.5 chimaeric embryos formed by wild-type host embryos and GFP-labelled Otx2+/− (n = 6), Otx2−− (n = 9) or Otx2lacZ/GFP (n = 9) ESCs assessed for GFP and BLIMP1/SOX2 expression, with magnified images of the proximal posterior regions. The proportion of BLIMP1-positive cells expressing GFP in the embryos is indicated; scale bar, 100 μm (left), 20 μm. g, Summary of embryo aggregations.
Extended Data Fig. 10 | Otx2-null embryos exhibit increased number of PGCs. a, b, Frontal-coronal (a) and sagittal (b) sections of wild-type and Otx2\(^{-/-}\) E7.5 embryos stained with BLIMP1, AP2\(^{y}\) and Fragilis to detect PGCs. All sequential sections spanning the PGCLCs niche are shown; scale bar, 50μm. The experiments were repeated in four wild-type and three Otx2-knockout embryos.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☑   | An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | The statistical test(s) used AND whether they are one- or two-sided |
| ☑   | Only common tests should be described solely by name; describe more complex techniques in the Methods section. |
| ☑   | A description of all covariates tested |
| ☑   | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☑   | A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☑   | For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted |
| ☑   | Give P values as exact values whenever suitable. |
| ☑   | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☑   | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☑   | Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated |
| ☑   | Clearly defined error bars |
| ☑   | State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

BD FACSDIVA for FACS data collection;

Data analysis

FlowJo V10 was used for FACS data analysis
CellProfiler software (version 2.2.0) was used for cytospin immunostaining quantification (Figure 1C), see detailed in methods
ImageJ was used for confocal images
Graphpad prism 7 was used for qPCR data and t test analysis
Illumina GenomeStudio software was used to obtain the signal intensity value and detection p-value for each probe on the microarray with standard settings. Quantile-normalization was performed by R (https://www.R-project.org) using the limma package with the default settings.
The genefilter package was used to perform a variance-based filtering of gene expressions in R with variance cutoff of 0.75. Unsupervised hierarchy clustering, generation of heatmap and PCA and ternary plot were carried out by means of R using the following packages: cluster, pheatmap, FactoMineR, ggtern and RColorBrewer.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The microarray data has been deposited in the GEO database under accession number GSE116640. Source data for the graphs (Fig1c, Fig4d, e; ED Fig9e) and gel image (ED Fig 3d) are provided. All the other datasets generated or analysed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences

Sample size
Sample size was decided based on previous experience with the variance of the measured data. For microarray, sample size was calculated by the cell lines and cell types were used, each of them have three biological replicate. For animal work, the minimum number of animals necessary to achieve the scientific objectives was used because of the ethical reason. For E14Tg2a cell line, 6 chimeras were analyzed; for Otx2KO cell lines, 9 chimeras were analyzed for each of them.

Data exclusions
In Figure4 d-f, to assess the germ cells contribution in chimeras, some of chimeras were exclude because of antibodies staining failed.

Replication
The details of replication for each experiment are specified in figure legend. All replication attempts were successful with similar results.

Randomization
For wholemont embryos staining in Fig1 e-i and embryo aggregation, random C57BL/6J or CBA/C57B6 F1 females were used to setup for plugs. And a random TG2A Otx2-/- clone was picked up from 3 of them for embryo aggregation experiment.

Blinding
germ cells contribution in chimeras were checked by two individuals and one of them didn’t know the genotype of cell lines which was used in the chimeras.

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
|     | Unique biological materials |
|     | Antibodies |
|     | Eukaryotic cell lines |
|     | Palaeontology |
|     | Animals and other organisms |
|     | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     | ChIP-seq |
|     | Flow cytometry |
|     | MRI-based neuroimaging |

Antibodies

Primary antibodies:
Rat anti Blimp1 antibody, from eBioscience (Monoclonal 6D3, Cat. 14-5963-82, Lot. 4310963) or from Santa cruz (monoclonal 6D3, Cat. SC47732, Lot. K2216, only this Lot. batch was worked in our hand), 1:100 diluted;
Goat anti human Otx2 antibody (immunogen Met1-Leu289, R&D system, AF1979), 1:200 diluted for IF and whole mount;
CHIP grade Rabbit anti Otx1+Otx2 antibody (Abcam, ab21990), 1:1000 for western blot;
Rabbit anti fragilis antibody (Abcam, ab15592), 1:200 diluted for embryo whole mount staining, no permeabilization before adding the primary antibody;
Mouse anti Flag antibody (monoclonal M2, Sigma F1804), 1:200 diluted for whole mount staining
Rabbit anti Sox2 antibody (Abcam, ab97959), 1:200 diluted for whole mount staining;
Mouse anti AP2r antibody (monoclonal 6E4/4, Santa Cruz, SC-12762, Lot. G2817), 1:200 diluted for whole mount staining and IF;
CHIP grade Rabbit anti Oct4 antibody (Abcam, ab19857, Lot. GR200357-2), 1:200 diluted for whole mount staining;
Goat anti Oct4(N19) antibody (Santa Cruz, SC-8628, Lot. A2115/21115), 1:200 diluted for IHC;
Goat anti Dazl(C20) antibody (Santa Cruz, SC-27333, Lot. K1513), 1:200/1:100 for IHC;
Goat anti T(Brachyury) antibody (R&D system, AF2085, Lot.K2P061612), 1:200 for IF;
CHIP grade Mouse anti H3K9me2 antibody (Abcam, ab1220, Lot.GR325223-3), 1:1000 for IHC;
Rabbit anti H3K27me3 antibody (Millipore, 07-449, Lot. JBC1873447), 1:1000 for IHC;
Goat anti fragilis antibody (R&D system, AF3377), 1:100 for IHC;
Anti-beta Actin HRP (Abcam, ab20272), 1:10000 for WB;
Secondary antibodies:
AlexaFluor 488 Donkey Anti-Mouse IgG(H+L) (Thermo Fisher scientific, A21202), 1:500 diluted;
Alexa Fluor 488 Donkey Anti-Rat IgG(H+L) (Thermo Fisher scientific, A21208), 1:500 diluted, 1:1000 for cytoplasm;
Alexa Fluor 568 Donkey Anti-Mouse IgG (Thermo Fisher scientific, A10037), 1:1000 diluted;
Alexa Fluor 647 Donkey Anti-Rabbit IgG (Thermo Fisher scientific, A11022), 1:1000 diluted;
Alexa Fluor 647 Donkey Anti-Goat IgG(H+L) (Thermo Fisher scientific, A11057), 1:1000 diluted;
CF568Donkey Anti-Rat IgG(H+L) (Sigma, SAB4600077), 1:1000 diluted
Alexa Fluor 647 Donkey Anti-Goat IgG(H+L) (Thermo Fisher scientific, A21447), 1:1000 diluted;
Donkey anti-rabbit secondary antibody 800CW (LI-COR, 926-32213), 1:1000 diluted;
Donkey anti-goat secondary antibody 680RD (LI-COR, 925-68074), 1:1000 diluted;
For FACS analysis:
Alexa Fluor® 647 anti-mouse/human CD15 (SSEA-1) (Biolegend, 125608), 1:200 diluted;
PE anti-mouse/rat CD61 (Biolegend, 104307), 1:500 diluted

Validation
If not specified, all the primary antibody were validated by the manufacturers. And also, we performed the staining with the secondary antibody only negative control and known expression patterns for the relevant antigen in positive control. FACS antibodies were validated with Isotype and FMOs before used. Gradient concentration were tested at the beginning.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | WT ES cells (E14Tg2A), Otx2lacZ/GFP, Otx2fl/lacZ are kind gift from Dr. Simeone (Acampora, 2013), Otx2lacZ/- , dP::EGFP C1 and C2 were derived from Otx2fl/lacZ, dPE::GFP by transfected with Cre. Otx2ERT2 cell line was derived by random integrate with Otx2ERT2 plasmid in the Otx2lacZ/GFP cells. Otx2-/- C11, C17 and C19 were derived from E14Tg2a by Crisper/Cas9. E14Tg2A,Blmp1KO C1 and C8; Otx2lacZ/GFP,Blm1KO C1 and C5; Otx2fl/lacZ and Otx2lacZ/-, Blmp1 KO were derived by Crisper/Cas9 to cut Blmp1 Exon5 with 2 Blmp1 gRNA. All the details are summarized in the Extended Data Figure 1. |
| Authentication | Genotyping and western blotting or immunostaining was used to authenticate new cell lines. Details are provided in 'Methods' |
| Mycoplasma contamination | All cell lines were tested negative for mycoplasma. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | For whole mount staining, 8 weeks C57BL/6J females were used to set up plugs with C57BL/6J males. And embryos were dissected at E5.5 and E6.5. For embryo aggregations, 3-4 weeks F1 females (C57BL/6J females cross with CBA males) were superovulated and embryos were collected at 2-cell state (E0.5). Aggregation embryos were transferred into MF1 recipients. E7.5 Otx2KO embryos generated from Otx2 heterozygous mice (Acampora, 1995). |
| Wild animals | This study did not involve wild animals |
| Field-collected samples | This study did not involve samples collected from the field |
Flow Cytometry

**Plots**
- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

| Sample preparation | Cells or aggregations from mouse ES cells were dissociated and filtered into single cells with trypsin. See details in the methods, Flow cytometry section. |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument         | BD LSR Fortessa analyser (5 laser) and BD FACS Aria III Fusion                                                                                                                                  |
| Software           | BD FACSDIVA™, Version 8.0.1 for data collection; FlowJo V10 for data analysis                                                                                                                    |
| Cell population abundance | After cell sorting, sorted samples were re-checked with FACS. And the purity is over 95%                                                                                                      |
| Gating strategy    | In all samples, cell population is initially identified on a FSC/SSC pseudocolor plot. Using a polygon gate to separate the distinct cell population from debris (over 80%). Doublets were excluded using FSC-A (Area)/FSC-H (Height) and dead cells were excluded by dapi. To defined the boundaries, FMOs (Fluorescence Minus One) controls or nonstaining samples were used to set the gate. And negative population in controls is over 99.5%. A representative gating strategy is shown in Extended data figure 3a. |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.