Table S1. Components for knockout serum replacer medium (KSR medium) (500 ml)

Component	Supplier	Catalogue number	Stock Concentration	Final concentration	Volume used
Advanced DMEM/F12	Invitrogen	12634010	-	78%	400 ml
Knockout serum replacer	Invitrogen	10828028	-	20%	100 ml
L-glutamine	Sigma	25030024	200 mM	2 mM	5 ml
β mercaptoethanol	Sigma	M6250	14.3 M	0.1 mM	3.5 µl
Penicillin/streptomycin	Sigma	15140-122	100%	1%	5 ml

Table S2. Components for XEN cell maintenance medium

Component	Supplier	Catalogue number	Stock concentration	Final concentration	Volume used
RPMI 1640	Invitrogen	61870-010	-	85%	500 ml
Fetal bovine serum	Bioserum	S1818	-	13%	75 ml
L-glutamine	Sigma	25030024	200 mM	2 mM	5.75 ml
βmercaptoethanol	Sigma	M6250	14.3 M	0.1 mM	4 µl
Penicillin/streptomycin	Sigma	15140-122	100%	1%	5.75 ml

Modified from Kunath et al. (Kunath et al., 2005) (500 ml)

Table S3. Components for EpiSCs maintained in chemically defined medium with bovine serum albumin (CDM - BSA medium) (500 ml)

Component	Supplier	Catalogue number	Stock concentration	Final concentration	Volume used
Ham's F12	Invitrogen	31765027	-	49% (v/v)	250 ml
Iscove's modified Dulbecco's medium (IMDM)	Invitrogen	21980032	-	49% (v/v)	250 ml
BSA	Europa Bioproducts	Batch A	-	5 mg/ml	2.5 g
Chemically defined lipid	Invitrogen	11905031	100x	1×	5 ml
Insulin	Roche	1376497	10 mg/ml	7 µg/ml	350 µl
Transferrin	Roche	652202	30 mg/ml	15 µg/ml	250 µl
Monothioglycerol	Sigma	Sigma M6145	11.5 M	450 µM	20 µl
Penicillin/streptomycin	Sigma	15140-122	100%	1%	5 ml

Table S4. Primers used for quantitative RT-PCR analysis

Name	Forward primer	Reverse primer
Gata4	CATCAAATCGCAGCCT	AAGCAAGCTAGAGTCCT
Gata6	ACCATCACCCGACCTACTCG	CGACAGGTCCTCCAACAGGT
Sox7	CAAGGATGAGAGGAAACGTCTG	TCATCCACATAGGGTCTCTTCTG
Sox17	GGAATCCAACCAGCCCACTG	GGACACCACGGAGGAAATGG
Pdgfra	CCTCAGCGAGATAGTGGAGAAC	ACCGATGTACGCATTATCAGAGT
Sparc1	AGGGCCTGGATCTTCTTTCTC	CAAATTCTCCCATTTCCACCT
Lama1	AGGTCTGCGTTGAGTGTTCTG	CAGTACTATGCCGTCAGCGAT
Lamb1	CAGAATGCAGACGATGTTAAGAA	GGCATCTGCTGACTCTTCAGT
tPA(Plat)	CTGACTGGACAGAGTGTGAGC	ACAGATGCTGTGAGGTGCAG
Hnf4a	CGAACAGATCCAGTTCATCAAG	ATGTGTTCTTGCATCAGGTGAG
Dkk1	TACAATGATGGCTCTCTGCAGCCT	TGGTCAGAGGGCATGCATATTCCA
Afp	AGCTGACAACAAGGAGGAGTG	TTAATAATGGTTGTTGCCTGGA
Gapdh	AATGGAATACGGCTACAGC	GTGCAGCGAACTTTATTG
Dab2	GGCAACAGGCTGAACCATTAGT	TTGGTGTCGATTTCAGAGTTTAGAT
Nanog	AGCAGATGCAAGAACTCTCCTC	AAGTTGGGTTGGTCCAAGTCT
Oct4	AGAAGTCCCAGGACATCAAA	TGCTTTGCATATCTCCTGAA
Sox2	TAGAGCTAGACTCCGGGCGATGA	TTGCCTTAAACAAGACCACGAAA
Col4a1 *Pdx1*	GGTCCTGTCTGGAAGAGTTT AGTGGGCAGGAGGTGCTTAC	AAATACAATGGGAGGGAGAA ACCCTCAGACTGCTGTCCTC
Nkx2.5	CAGCAACTTCGTGAACTTTGGC	AATCTGAGGGACAGGGCATAGTGG
Sox1	Mm_Sox1_1_SG Quantitect primers assays*	
Olig3	CGTCTGAACTCGGTCTCCTC	GTTCAGGTCGTGCATCCTCT
Smmhc	ACATGGCTTCCAGTGTCTCC	CTTCTACTACCTGCTCGCCG
Sma	CGCTGTCAGGAACCCTGAGA	CGAAGCCGGCCTTACAGA
Sm22a	GCAGTTGGCTGTCTGTGAAG	AACGATGGAAACTACCGTGG
Apoe	GTGGGCCGTGCTGTTGGTCA	AGACAGCGTCTGCACCCAGC
Ihh	AGATCGCGCGCAGCTCTGAG	CTCCACTGCTAAGCGCGCCA
Cited1	CTGGCGGCATCAACTGCCAC	CAGTAGCCGCAGCCCCTTGG

*This primer mix is purchased from Qiagen.

Table S5. Antibodies used for immunohistochemistry and flow cytometry

Primary antibody	Supplier	Catalogue number	Species	Dilution used
Dab2	Santa Cruz Biotech	SC-13982	Rabbit	1:500
Gata4	Santa Cruz Biotech	SC-9053	Rabbit	1:500
Gata6	R&D	AF1700	Goat	1:500
Laminin	Abcam	Ab16048	Rabbit	1:500
Nanog	Cosmo Bio	REC-RCAB0002P-F	Rabbit	1:500
Oct4	Santa Cruz Biotech	SC-5279	Mouse	1:500
Sox7	R&D	MAB2766	Goat	1:500
Sox17	R&D	AF1924	Goat	1:500
Sparc1	Santa Cruz Biotech	SC-25574	Goat	1:500
SSEA-1	BD Pharma	560079	Mouse	1:500
Secondary antibody	**Supplier**	**Catalogue number**	**Species**	**Dilution used**
Alexa Fluor 568 anti - mouse IgG	Invitrogen	A10037	Donkey	1:300
Alexa Fluor 568 anti - rabbit IgG	Invitrogen	A10042	Donkey	1:300
Alexa Fluor 568 anti - goat IgG	Invitrogen	A10057	Donkey	1:300
APC-AffiniPure Anti-Mouse IgM	Jackson Immuno Research	115-136-075	Goat	1:500

Table S6. Testing the requirement and dose of RA and activin for cXEN derivation

	No activin		5 ng/ml activin		10 ng/ml activin		20 ng/ml activin	
	+F/H	–F/H	+F/H	–F/H	+F/H	–F/H	+F/H	–F/H
0 μM RA	9.6±0.0	9.3±1.4	9.0±0.7	9.9±0.3	7.5±1.8	6.9±0.7	8.4±1.5	8.9±0.6
0.001 μM RA	10.1±4.1	14.1±2.2	10.3±2.7	15.7±3.7	10.6±1.9	13.4±4.7	8.3±1.7	13.5±1.9
0.01 μM RA	18.5±6.0	27.2±2.8	19.0±9.9	22.8±18.8	27.6±12.7	40.3±8.9	28.1±14.2	34.67±11.7
0.1 μM RA	19.2±13.0	26.3±7.0	20.8±14.3	31.2±3.5	29.1±14.8	37.0±9.1	24.1±15.9	33.9±7.4
1 μM RA	20.9±14.1	27.1±6.7	24.2±12.1	32.8±2.5	30.6±15.0	38.1±10.0	28.2±10.2	37.9±7.8
10 μM RA	22.7±13.7	27.8±8.9	23.2±15.0	33.7±8.9	24.3±13.7	32.3±8.4	22.4±13.2	33.3±5.9
100 μM RA	23.9±3.1	14.4±4.0	28.0±8.5	26.4±9.7	22.1±6.2	27.6±6.4	25.3±7.3	21.5±8.5

Sox17::GFP reporter cell lines were used to assay for the presence or absence of XEN-like cells. Percentage expression was determined by flow cytometry and the data are the mean percent of *Sox17::GFP*-high expressing cells ± s.e.m. of technical replicates (*n*=3). ±24 ng/ml of FGF2 (F) and 1 μg/mL heparin (H) in standard XEN media.

Table S7. Testing the requirement for exogenous FGF2 and heparin or MEFs for cXEN derivation from *Fgf4*+/+, *Fgf4*+/- and *Fgf4*-/- mESCs

	Day 6											
	+ Exogenous FGF2/H, +MEFs			**+ Exogenous FGF2/H, no MEFs**			**No exogenous FGF2/H, + MEFs**			**No exogenous FGF2/ MEFS**		
	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	
Sox7-high	43.3±4.25	32.3±22.6	27.6±13.4	41.4±17.1	20.0±17.5	25.7±11.2	47.1±14.8	24.7±23.9	13.5±21.8	34.0±13.6	23.5±23.8	0
Nanog-high	0.0± 0.0	4.3± 7.4	0.0± 0.0	0.0± 0.0	4.1± 8.0	3.5± 7.8	0.0± 0.0	11.7±14.8	10.5±11.5	0.0± 0.0	8.9± 12.5	3

Percentage of Sox7- or Nanog-high-expressing cells observed as a proportion of total DAPI nuclear staining following immunofluorescence analysis 6 days after initiation of cXEN derivation. +/+, +/– and –/– represent $Fgf4^{+/+}$, $Fgf4^{+/-}$ and $Fgf4^{-/-}$ cell lines, respectively. Data are mean±s.e.m. of n=5 separate counts for $Fgf4^{+/+}$ and $Fgf4^{-/-}$ mESCs, and 10 separate counts for $Fgf4^{+/-}$ mESCs. Cells cultured in standard XEN media +/– 24 ng/ml of FGF2 and 1 μg/ml heparin (H) in the presence or absence of mouse embryonic fibroblasts (MEFs) as indicated.

Table S8. FGF/ERK signalling inhibition during cXEN derivation

	DMSO	SU5402	PD0325901	PD173074
Gata6-high	18.2 ± 7.1	12.3 ± 3.5	3.1 ± 2.6	6.8 ± 5.2
Sox7-high	13.1 ± 7.0	11.3 ± 11.4	1.9 ± 1.7	5.9 ± 7.7
Nanog-high	0	2.7 ± 3.1	25.2 ± 0.4	9.0 ± 9.1
Oct4-high	2.0 ± 3.3	2.4 ± 1.9	37.6 ± 1.9	13.7 ± 12.1

Percentage of Sox7-, Gata6-, Oct4- or Nanog-high-expressing cells observed as a proportion of total DAPI nuclear staining following immunofluorescence analysis 3 days after initiation of cXEN derivation in the absence of FGF and heparin. Wild-type cells were grown in standard XEN media supplemented with 10 µL DMSO (control), 10 µM SU5402, 5 µM PD0325901 or 1 µM PD173074. Data are mean±s.e.m. of *n*=3 biological replicates and *n*=5 technical replicates.

Table S9. mESCs (from serum or 2i and LIF) converted to cXEN cells

Genotype of the cell line:	cXEN cells?	Fluorescent cXEN?
Wild-type line 1	+	n/a
Wild-type line 2	+	n/a
Wild-type line 3	+	n/a
CAG::H2B-GFP	+	-
CAG::YFP	+	-
Sox17$^{GFP/+}$ UbiC::dTomato	+	+
Sox17$^{GFP/+}$	+	+
AFP::GFP	+	+ (in BMP4 conditions)
Pdgfra$^{H2B-GFP/+}$	+	+
Pdgfra$^{H2B-GFP/ H2B-GFP}$	-	n/a
***Fgf4$^{+/-}$* line 1**	+	n/a
***Fgf4$^{+/-}$* line 2**	+	n/a
***Fgf4$^{-/-}$* line 1**	+	n/a
***Fgf4$^{-/-}$* line 2**	+	n/a
Sox17$^{-/-}$	-	n/a
Gata6$^{-/-}$	-	n/a
Gata4$^{-/-}$	-	n/a
Gata4$^{-/-}$* and *Gata6$^{-/-}$	-	n/a
***Nanog* overexpression**	-	n/a
Wild-type (from 2i and LIF) line 1	+	n/a
Wild-type (from 2i and LIF) line 2	+	n/a
Wild-type (from 2i and LIF) line 3	+	n/a

Table S10. Embryoid body chimeras

	UbiC::dTomato **mESC**	***UbiC::dTomato*** **cXEN cell**	***CAG::GFP*** **eXEN cell**
EBs generated	42	84	42
Incorporation	20	23	11
Interior	20	n/a	1
Periphery	18	23	10

Single *UbiC::dTomato* mESCs, *UbiC::dTomato* cXEN or *CAG::GFP* embryo-derived XEN cells were aggregated together with 200 wild-type mESCs to form embryoid bodies using the hanging drop technique. After 5 days, embryoid bodies were collected, stained with Hoechst nuclear dye and imaged on a confocal microscope. Contribution of the fluorescently labelled cells to the interior and/or periphery of chimera embryoid bodies was quantified.