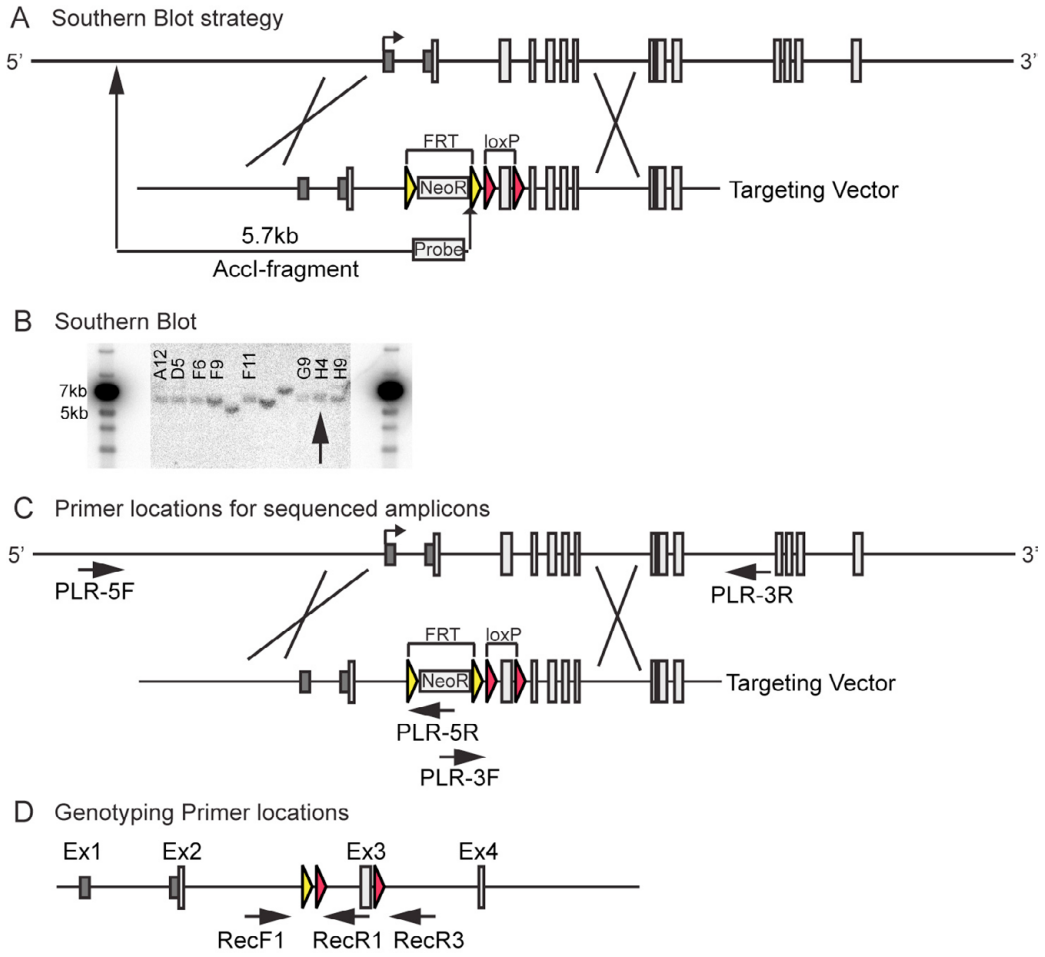


Fig. S1. Genetic manipulation of the *Porcn* locus. (**A**) Schematic of Southern blot strategy depicting the *Porcn* locus and targeting vector. A probe detecting the Neo^{R} cassette was used to detect genomic *Acc*I fragments of the correct size (5.7 kb). (**B**) Southern blot of candidate clones. Labeled clones display the correct fragment size. A subclone of cell line H4 (arrow) was used for the generation of animals. (**C**) Clones were further analyzed by locus-specific long-range PCR with complete sequencing of amplicons. Primer locations for long-range PCR are indicated. (**D**) Location of genotyping primers in the *Porcn* locus.

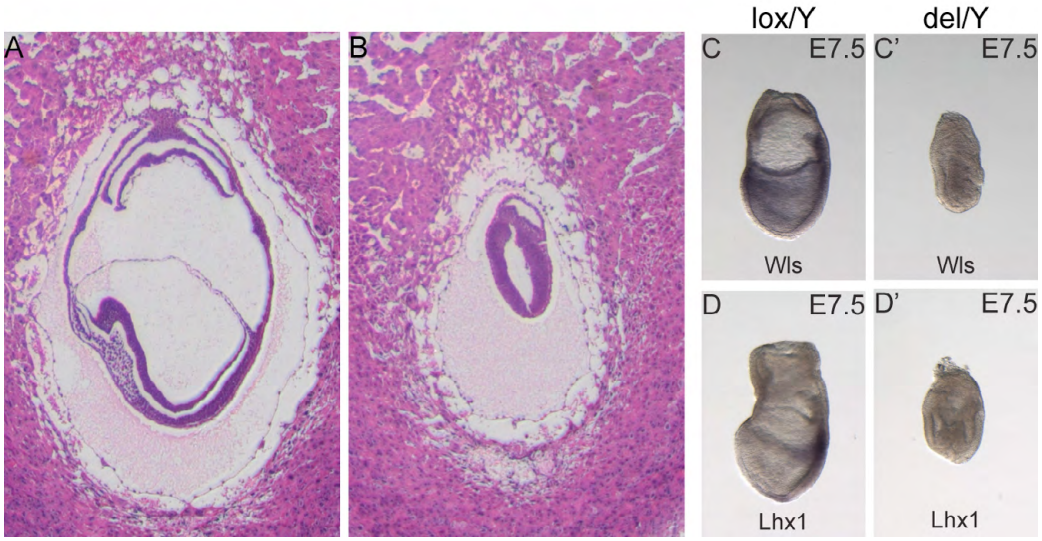


Fig. S2. *Porcn* zygotic hemizygous phenotype. (**A**,**B**) Sections of E7.5 decidua showing the reduction in size and absence of amnion and chorion in *Porcn*$^{del/Y}$ embryos (B) compared with control littermates (A). (**C-D′**) Representative images of *in situ* hybridization at E7.5. *Porcn* mutant embryos fail to express the canonical Wnt signaling target *Wls* (C′) and the migrating mesoderm marker *Lhx1* (D′).

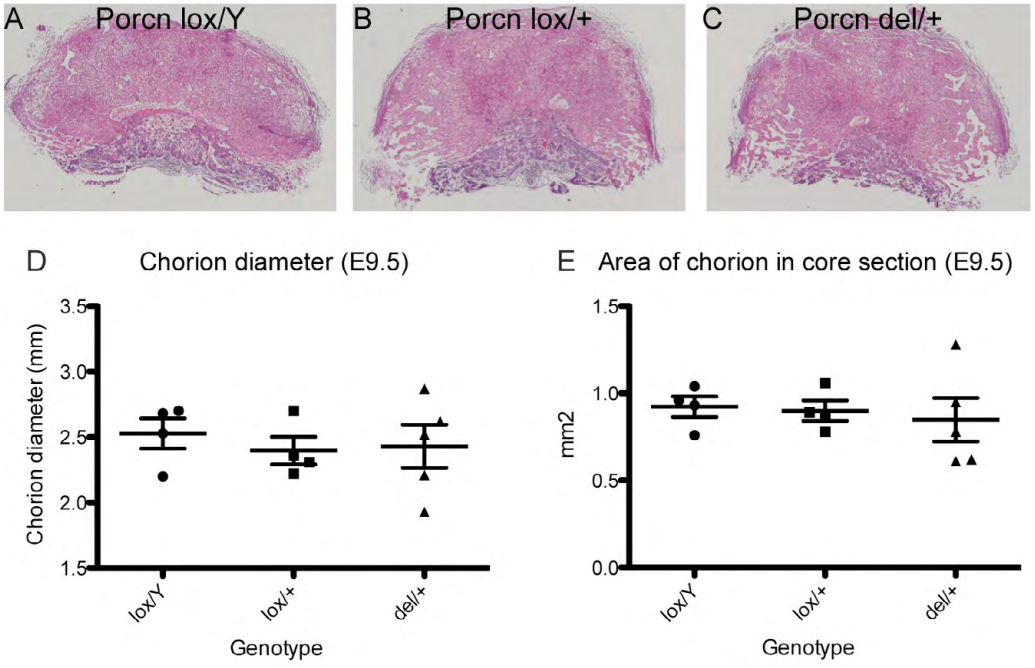
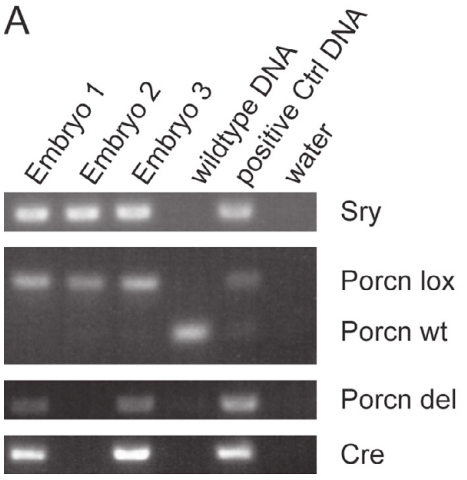


Fig. S3. *Porcn* zygotic heterozygous phenotype. (**A-C**) Representative images of H&E-stained placental core sections at E9.5 of *Porcn*$^{lox/Y}$, *Porcn*$^{lox/+}$ and *Porcn*$^{del/+}$. (**D**,**E**) Quantification of the width (D) and area (E) of the chorionic disc in core sections revealed no statistically significant differences (Student's *t*-test).



B

Genotypes observed (expected)	*Ttr::Cre*$^{+/tg}$	No *Cre*	Σ
Porcn$^{lox/Y}$	7 (6.5)	7 (6.5)	14 (13)
Porcn$^{lox/+}$	8 (6.5)	4 (6.5)	12 (13)
Σ	15 (13)	11 (13)	26

Fig. S4. Visceral endoderm-specific deletion of *Porcn*. *Porcn*$^{lox/lox}$ females were crossed to *Ttr::Cre*$^{tg/+}$ males to generate embryos with VE-specific *Porcn* deletion. (**A**) PCR analysis shows Cre-mediated deletion in E7.5 embryos carrying Cre recombinase (embryos 1 and 3). *Sry*-specific primers were used to determine the sex of embryos. (**B**) All possible genotypes were observed at the expected Mendelian ratios at weaning age.

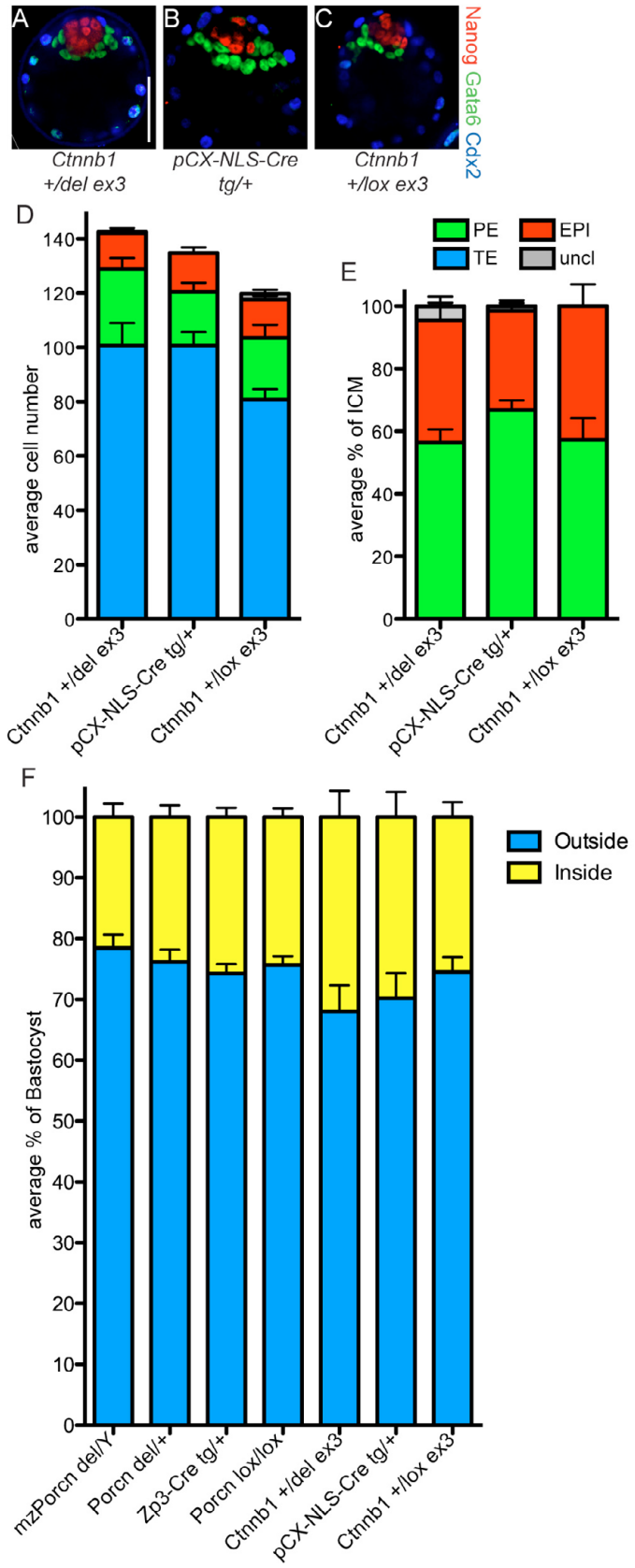


Fig. S5. Preimplantation development is unperturbed in embryos with ectopic canonical Wnt signaling. (**A-C**) Representative confocal sections of E4.5 blastocysts immunostained for Nanog, Gata6 and Cdx2. (**D**) Differences approaching significance can be observed in the quantification of cell fates between embryos carrying an allele encoding stabilized β-catenin (*Ctnnb1*$^{+/del\ ex3}$) and control embryos (chi-square test, P=0.05118). Graph displays average cell number/embryo for each cell fate (n=5 embryos/genotype). (**E**) Normalized cell fate distributions within the inner cell mass (ICM). (**F**) Normalized distribution of inner cells (ICM, yellow) and outer cells (TE, blue) of all genotypes assessed at E4.5. Error bars indicate s.e.m. TE, trophectoderm; PE, primitive endoderm; EPI, epiblast; uncl, unclassified cells.

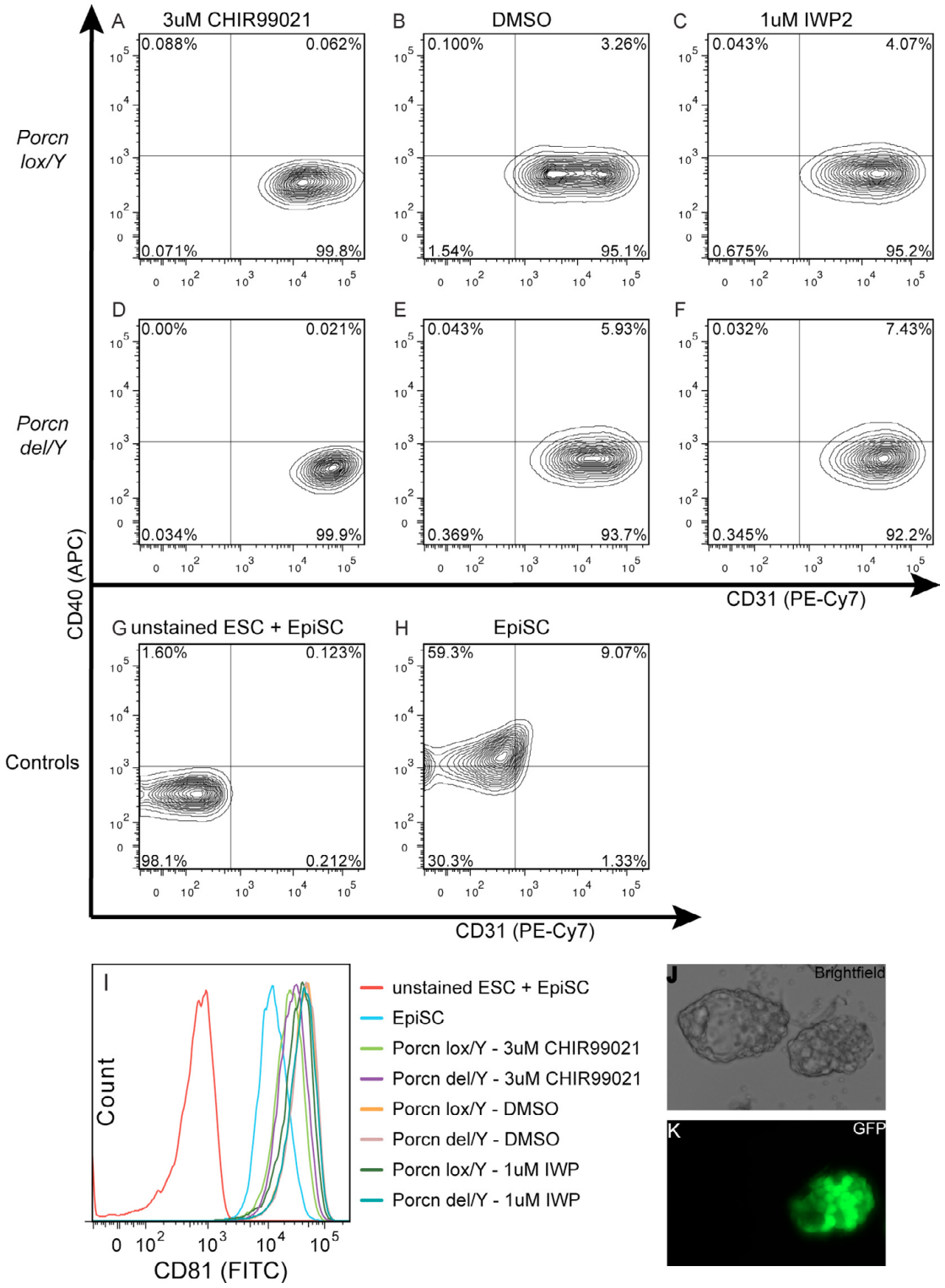


Fig. S6. *Porcn* is not required for the maintenance of pluripotency of ESCs *in vitro* or for embryonic diapause *in vivo*. (**A-H**) Flow cytometry plots of ESCs displaying expression of the ESC marker CD31 and the EpiSC marker CD40. *Porcn* wild-type (lox/Y, A-C) and mutant (del/Y, D-F) ESCs after three passages in the absence of serum and feeder cells, and in the presence of Mek inhibitor and LIF. Wnt signaling levels were manipulated by Gsk3 inhibition (CHIR99021, A,D) or Porcn inhibition (IWP2, C,F). DMSO treatment (B,E) served as a control. Cells in all conditions (A-F) maintained high expression of CD31 and low levels of CD40, and thus displayed a distinct profile compared with EpiSCs (H). (G) Unstained mixture of ESCs and EpiSCs as a negative control. (**I**) Histograms of CD81 obtained by flow cytometric analysis of *Porcn* wild-type (lox/Y) and mutant (del/Y) ESCs cultured as in A-F. Consistent with CD31 expression (A-F), CD81 expression levels remained high in all conditions tested when compared with EpiSCs and unstained cells. (**J**,**K**) Representative diapause embryos at EDG10. Brightfield (J) and GFP fluorescence (K) image of diapause blastocysts recovered from $Porcn^{lox/lox}$, $Zp3\text{-}Cre^{+/tg}$ females crossed to $XEGFP^{tg/Y}$ males. The GFP^{+} embryo is heterozygous for *Porcn*, whereas the GFP^{-} embryo is a maternal and zygotic *Porcn* mutant ($Porcn^{del/Y}$). When transferred into surrogates, 90% of $Porcn^{del/Y}$ embryos implanted and developed to gastrulation stages (n=10).

Table S1. Antibodies used for imaging of preimplantation embryos

Antibody/stain	Source, catalog number	Dilution
Primary antibody		
Rabbit anti-Nanog	Cosmo Bio, REC-RCAB0002	1:200
Goat anti-Gata6	R&D Systems, AF1700	1:200
Mouse anti-Cdx2	BioGenex, MU392-UC	1:200
Secondary antibody		
Donkey anti-rabbit Dylight 549	Jackson ImmunoResearch	1:400
Donkey anti-goat Alexa Fluor 488	Molecular Probes	1:400
Donkey anti-mouse Alexa Fluor 633	Molecular Probes	1:400
Nucleus visualization		
Hoechst 33342	Molecular Probes	

Table S2. TaqMan gene expression assays used for single-cell gene expression analysis

Probe	Function	Lineage
Actb	Housekeeping gene	n/a
Axin2	Wnt target	n/a
brachyury (*T*)	Wnt target/lineage marker	Meso/endoderm
c-Myc	Wnt target	n/a
cyclin D1 (*Ccnd1*)	Wnt target	n.a
Ctnnb1	Wnt component	n/a
Cripto	Lineage marker	Epiblast
Ddx3y	Sexing/genotyping	Male specific
Dnmt3b	Lineage marker	Epiblast
Eomes	Lineage marker	Trophectoderm
Fgf4	Lineage marker	Epiblast
Fgf5	Lineage marker	Epiblast
Fgfr2	Lineage marker	Trophectoderm
Gapdh	Housekeeping gene	n/a
Gata3	Lineage marker	Trophectoderm
Gata4	Lineage marker	Primitive endoderm
Gata6	Lineage marker	Primitive endoderm
Gbx2	Wnt target/lineage marker	n/a, neural
Klf2	Lineage marker	Epiblast
Klf4	Lineage marker	Epiblast
Lef1	Wnt target	n/a
Nanog	Lineage marker	Epiblast
Oct3/4	Lineage marker	Epiblast
Otx2	Lineage marker	Epiblast/neural
Pdgfra	Lineage marker	Primitive endoderm
Pecam1	Lineage marker	Epiblast
Porcn	Wnt component	n/a
Rex1	Lineage marker	Epiblast
Sox2	Lineage marker	Epiblast
Sox7	Lineage marker	Primitive endoderm
Sox17	Lineage marker	Primitive endoderm
Stella	Lineage marker	Epiblast
Tcf1	Wnt target	n/a
Uty	Sexing/Genotyping	Male specific
Wls	Wnt target	n/a
Xist	Sexing/genotyping	Female specific

Table S3. Antibodies used for FACS analysis

Antibody	Source, catalog number	Dilution
Primary		
Rat anti-CD31–PECy7	Biolegend, 102418	1:200
Rat anti-CD81–FITC	R&D Systems, FAB4865F	1:20
Goat anti-CD40	R&D Systems, AF440	1:50
Secondary		
Donkey anti-goat DyLight 649	Jackson ImmunoResearch, 705-495-147	1:800

Table S4. Raw data from Fluidigm analysis

Download Table S4