

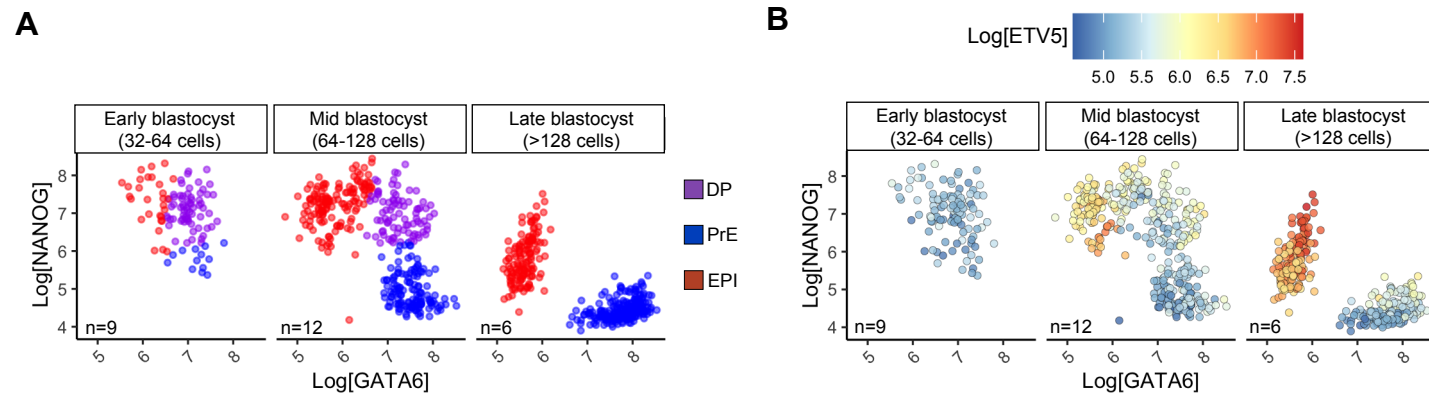


Fig. S1. *Etv4* and *Etv5* expression during mouse embryonic development (related to Figure 1)

- (A)** Quantification of NANOG and GATA6 levels in blastocyst (from Figure 1B). Clustering into double positive (DP; NANOG+;GATA6+), epiblast (EPI; NANOG+;GATA6-) and primitive endoderm (PrE; NANOG-;GATA6+)
- (B)** Heatmap of ETV5 levels in blastocyst compared to NANOG and GATA6 levels (from Figure 1B)

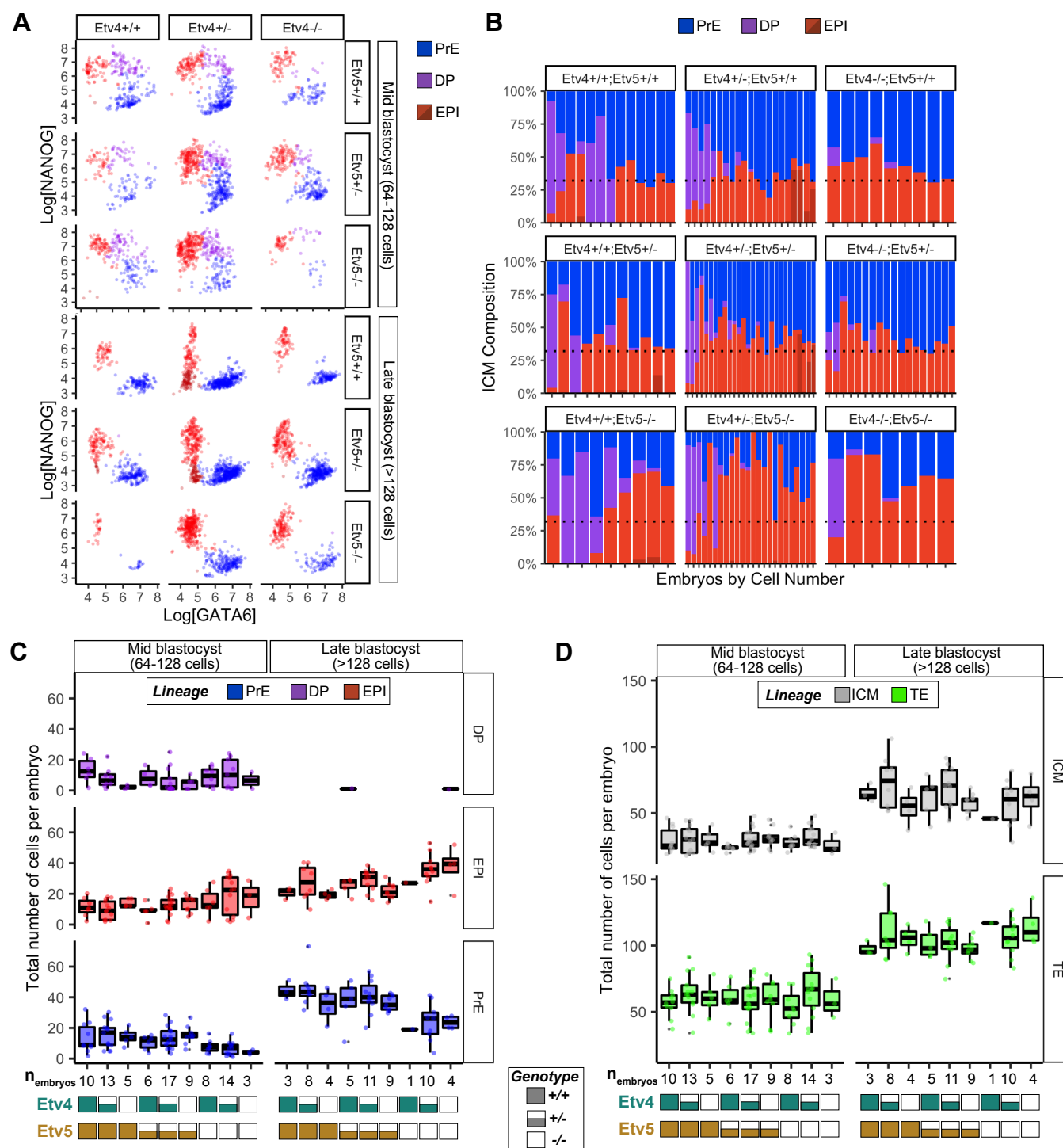


Fig. S2. Loss of *Etv5* compromises the formation of PrE (related to Fig. 2)

- (A)** Quantification of NANOG and GATA6 levels in an *Etv4*;*Etv5* allelic series of mutant embryos (from Figure 2A,B). Clustering into double positive (DP; NANOG+;GATA6+, purple), primitive endoderm (PrE; NANOG-;GATA6+, blue), epiblast (EPI; NANOG+;GATA6- light red, NANOG-;GATA6- dark red).
- (B)** Quantification of inner cell mass (ICM) lineage composition in an allelic series of *Etv4*;*Etv5* mutant embryos. Individual embryos shown and ordered by ascending cell number. Dotted line represents mean wild-type EPI:PrE composition by late blastocyst stage. (DP: purple, PrE: blue, EPI: red)
- (C)** Total number of cells per embryo in DP (purple), EPI (red) and PrE (blue) lineages
- (D)** Total number of cells per embryo in ICM (grey) and TE (green) lineages.

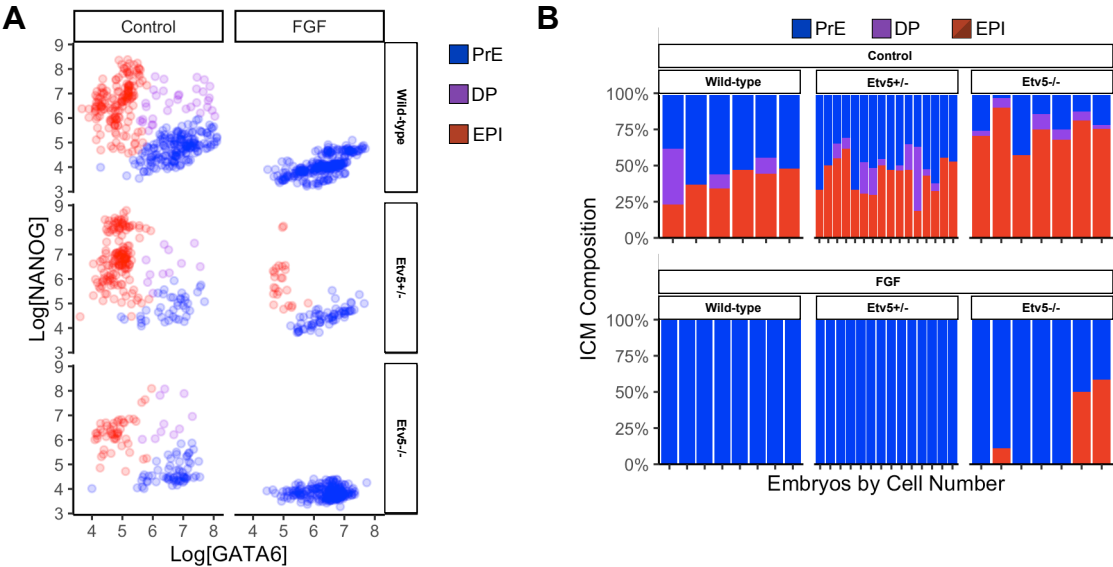


Fig. S3. Mechanism of *Etv5* action on ICM cell fate decision (related to Fig. 3)

- (A)** Quantification of NANOG and GATA6 levels in control and FGF treated wild-type, *Etv5*^{+/-} and *Etv5*^{-/-} embryos (related to Figure 3B). Clustering into primitive endoderm (PrE; blue), epiblast (EPI; red), and double positive (DP; purple).
- (B)** Quantification of inner cell mass (ICM) lineage composition in control and FGF treated wild-type, *Etv5*^{+/-} and *Etv5*^{-/-} embryos (PrE: blue, EPI: red, DP: purple). Individual embryos shown and ordered by ascending cell number.

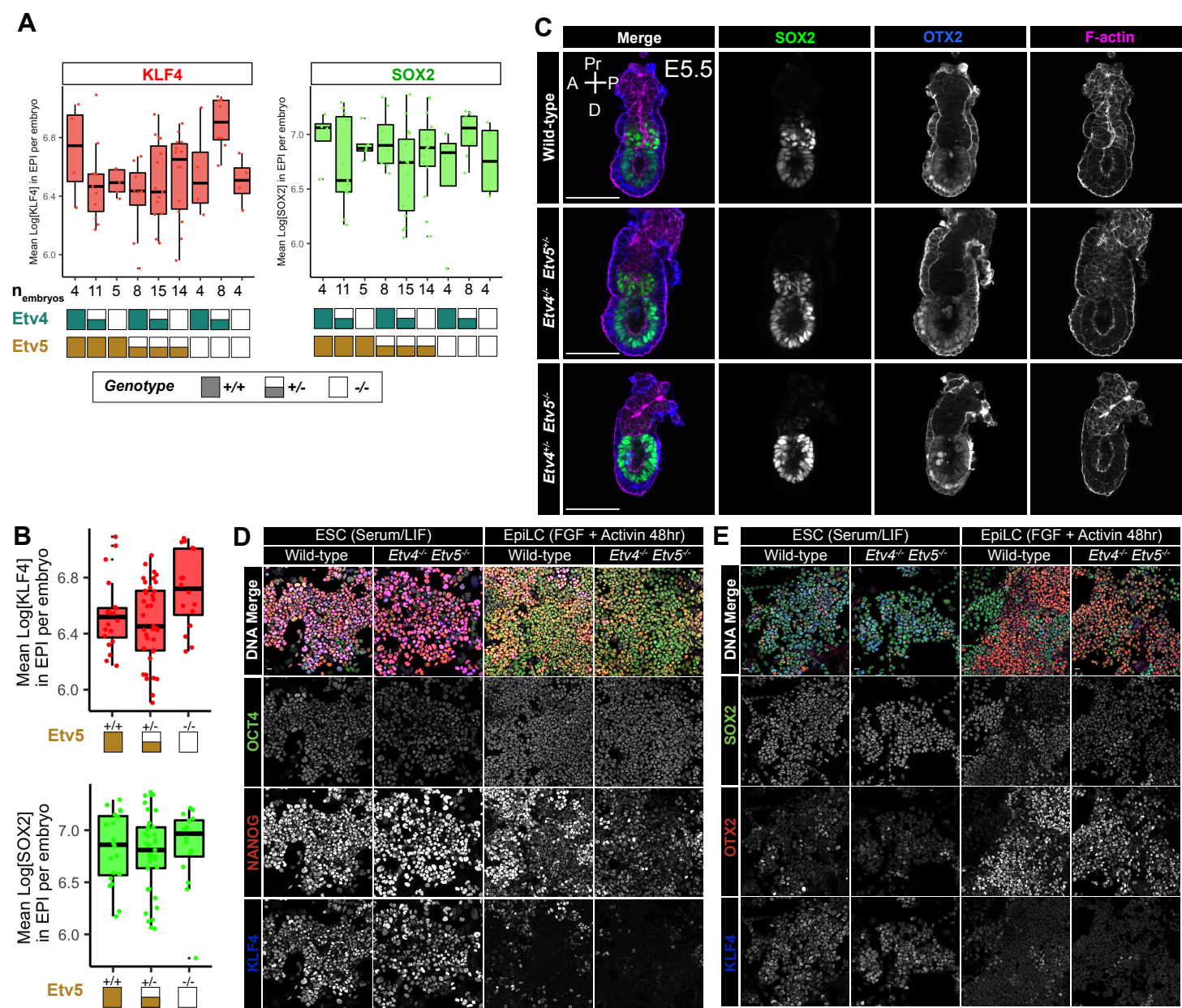


Fig. S4. Loss of *Etv4/5* causes a delay in the progression of pluripotency

(A) Quantification of KLF4 and SOX2 levels in epiblast cells per embryo in an allelic *Etv4;Etv5* series of late-stage blastocysts (from Figure 4A).

(B) Quantification of KLF4 and SOX2 levels in epiblast cells per embryo in an allelic *Etv4;Etv5* series of late-stage blastocysts from (A) grouped by *Etv5* genotype.

(C) Confocal images of immunofluorescence staining of SOX2, OTX2 and F-actin in post-implantation *Etv4;Etv5* day E5.5. embryos. Pr = Proximal, D = Distal, A = Anterior, P = Posterior. Scale bar 100µm

(D-E) Confocal images of immunostaining for OCT4, NANOG, KLF4 (D) and SOX2, OTX2, KLF4 (E) in wild-type and *Etv4^{-/-};Etv5^{-/-}* mESC in naïve conditions (Serum/LIF) and differentiated to epi-like cells (EpiLC, FGF+Activin 48h). Scale bar 20µm

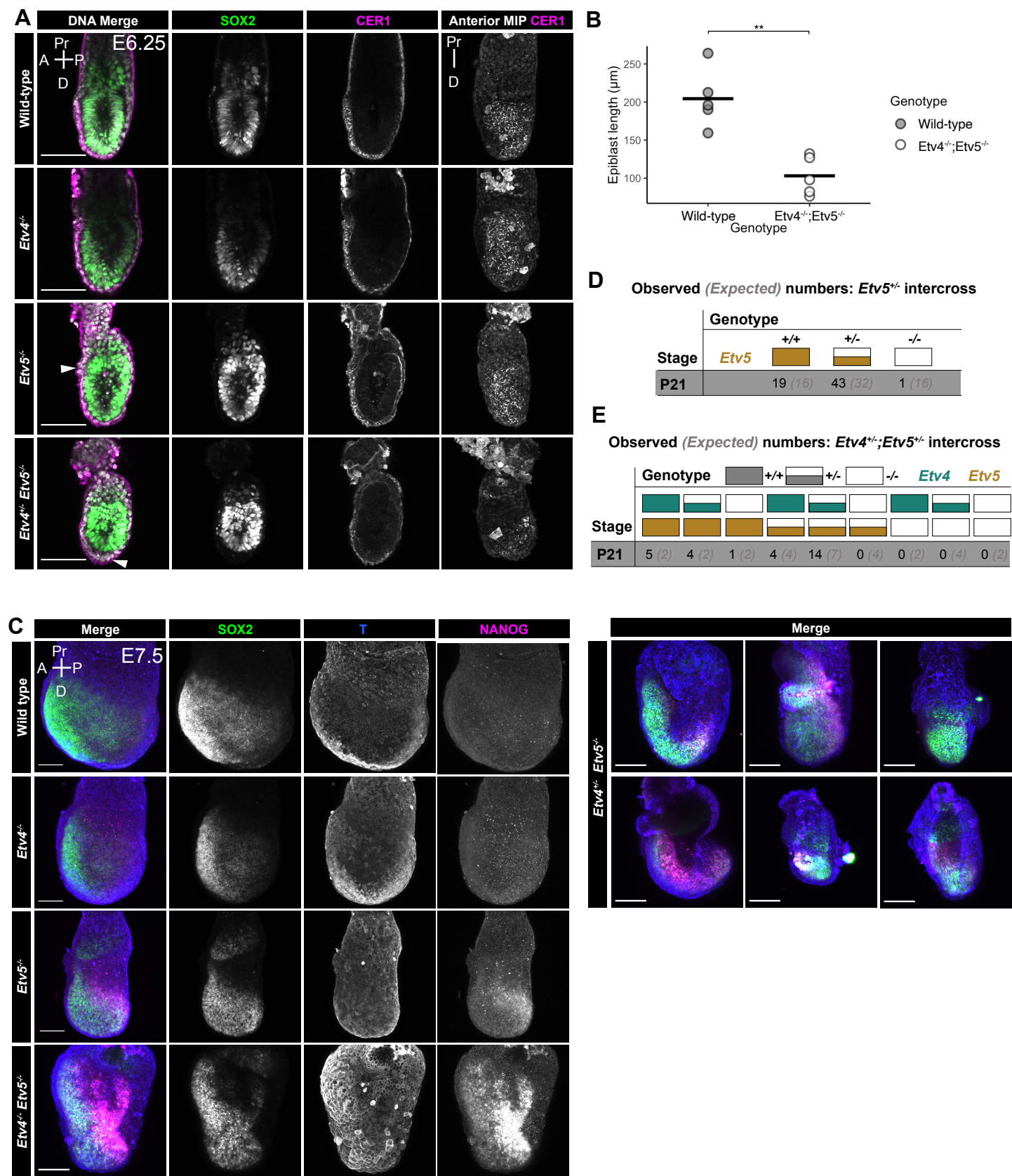


Fig. S5. Compound *Etv4/5* mutants have developmental delay and anterior visceral endoderm migration defects

- (A)** Confocal images of an allelic series of *Etv4;Etv5* embryos at pre-streak stages, embryonic day (E) 6.25 immunostained for SOX2, and CER1. Anterior max intensity projection (right) and z-slice (left). Arrow heads show abnormal anterior/distal visceral endoderm migration and/or morphology. A = Anterior, P= Posterior. Pr = Proximal. D = Distal. Scale bar 100μm
- (B)** Confocal maximum intensity projection images of an allelic series of *Etv4;Etv5* embryos at late-bug gastrulation stages, embryonic day (E) 7.5 immunostained for SOX2, T and NANOG. Arrow head shows abnormal anterior/distal visceral endoderm migration and/or morphology. A = Anterior, P= Posterior. Pr = Proximal. D = Distal. Scale bar 100μm. Range of abnormal morphologies amongst *Etv4*^{+/-}; *Etv5*^{-/-} embryos (right).
- (C-D)** Observed and expected numbers from *Etv5*^{+/-} **(C)** and *Etv4*^{+/-}; *Etv5*^{+/-} **(D)** heterozygous intercrosses at weaning, postnatal day (P) 21.

Table S1. Antibodies used in this study

Antibody	Species	Company	Catalogue #	RRID	Dilution
Anti-Etv5	Rabbit	Proteintech	13011-1-AP	AB_2278092	1:100
Anti-Gata6	Goat	R&D	AF1700	AB_2108901	1:100
Anti-Nanog	Rabbit	Reprocell	REC-RCAB0002PF	AB_567471	1:500
Anti-Nanog	Rat	e-biosciences	14-5761	AB_2865129	1:100
Anti-Sox2	Rat	e-biosciences	14-9811-82	AB_11219471	1:200
Anti-Klf4	Rabbit	Cell Signalling	4038	AB_2265207	1:100
Anti-Otx2	Goat	R&D	AF1979	AB_2157172	1:500
Anti-Cer1	Goat	R&D	AF1986	AB_2077219	1:300
Anti-T	Goat	R&D	AF2085	AB_2200235	1:100
Anti-T	Rabbit	Abcam	ab209665	AB_2750925	1:100

Table S2. TaqMan Gene Expression Assays used in this study

Gene	Assay ID
<i>Actb</i>	Mm00607939_s1
<i>Fgf4</i>	Mm00438916_g1
<i>Gapdh</i>	Mm99999915_g1