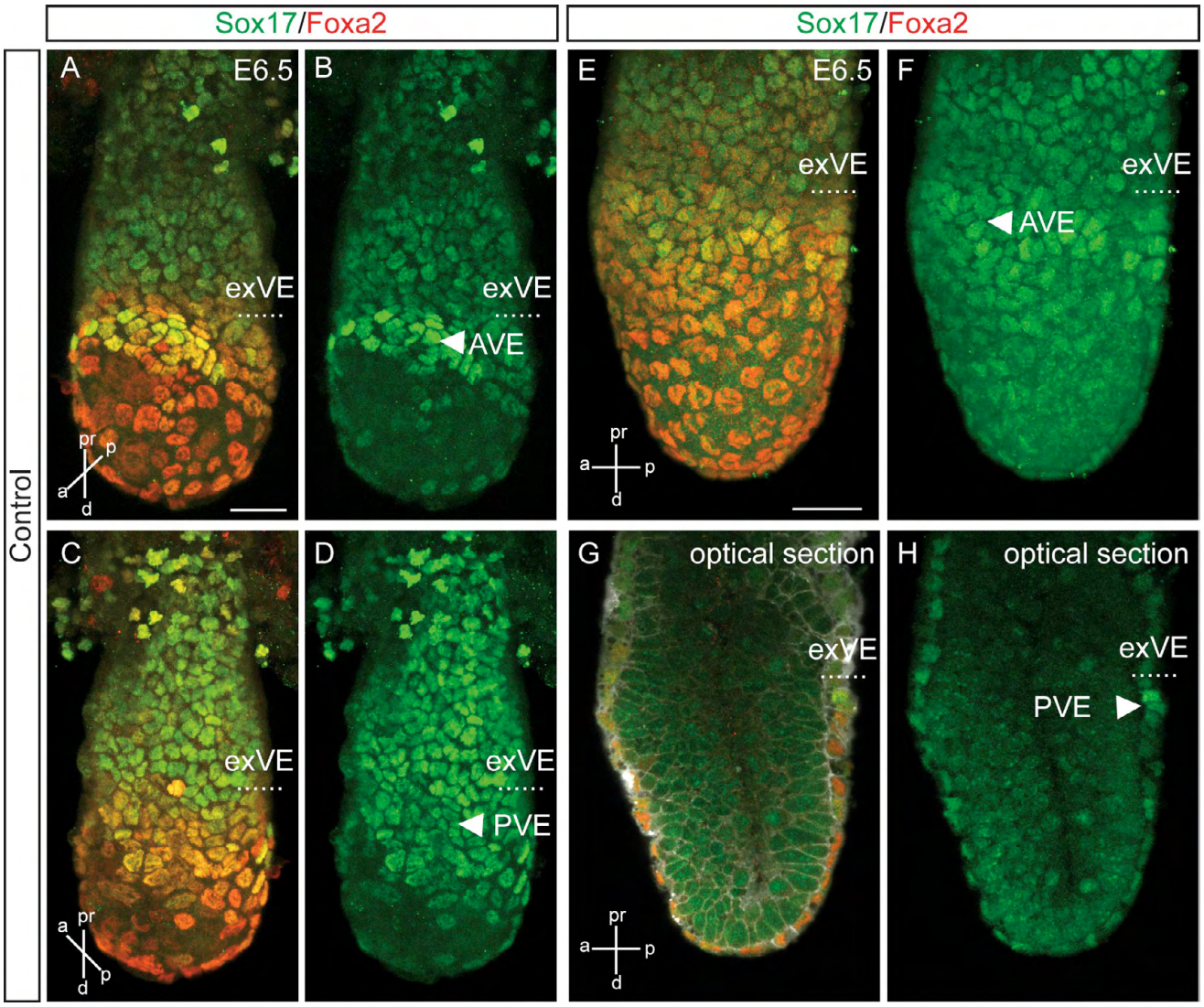


Fig. S1. Sox17 is localised to the AVE and PVE at E6.5. (**A-H**) Whole-mount antibody staining reveals that Sox17 is specifically localised to the extra-embryonic visceral endoderm (exVE), anterior visceral endoderm (AVE) (A,B,E,F) and posterior visceral endoderm (PVE) (C,D,G,H). Foxa2 shows a reciprocal proximal-distal localisation pattern in the embryonic visceral endoderm, AVE (A,E) and PVE (C,G). Scale bars: 25 μm.

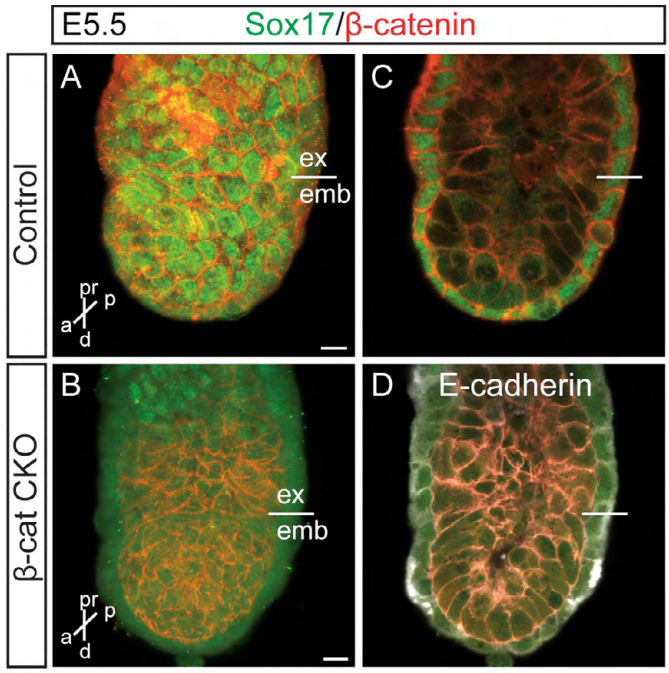


Fig. S2. Complete deletion of β-catenin and loss of Sox17 expression in the VE at E5.5. Sox17 and β-catenin antibody staining of control (**A**,**C**) and CKO (**B**,**D**) embryos confirms complete deletion of β-catenin in the VE at E5.5. E-cadherin staining is in white in D. The extra-embryonic (ex)-embryonic (emb) boundary is indicated. Scale bars: 10 μm.

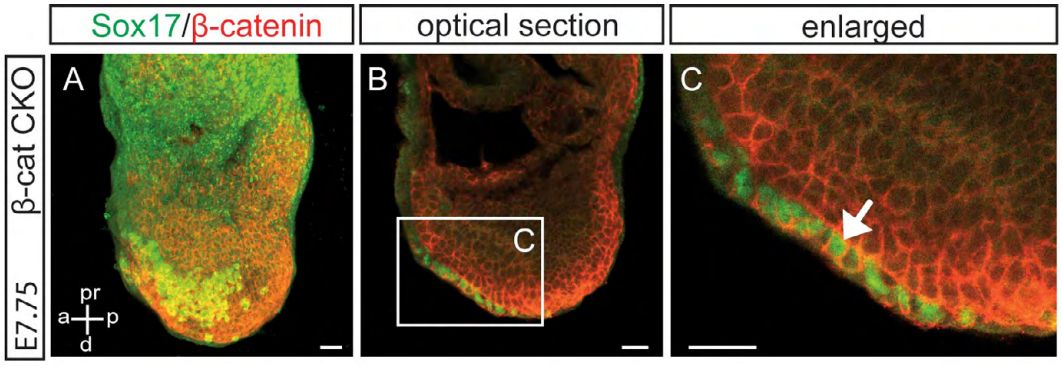


Fig. S3. ADE cells are double positive for Sox17 and β-catenin. IHC shows Sox17^{+} β-catenin^{+} cells (arrow) in the anterior endoderm of the mutant at E7.75 as *z*-projection (**A**), optical section (**B**) and higher magnification image (**C**). Scale bars: 25 μm.

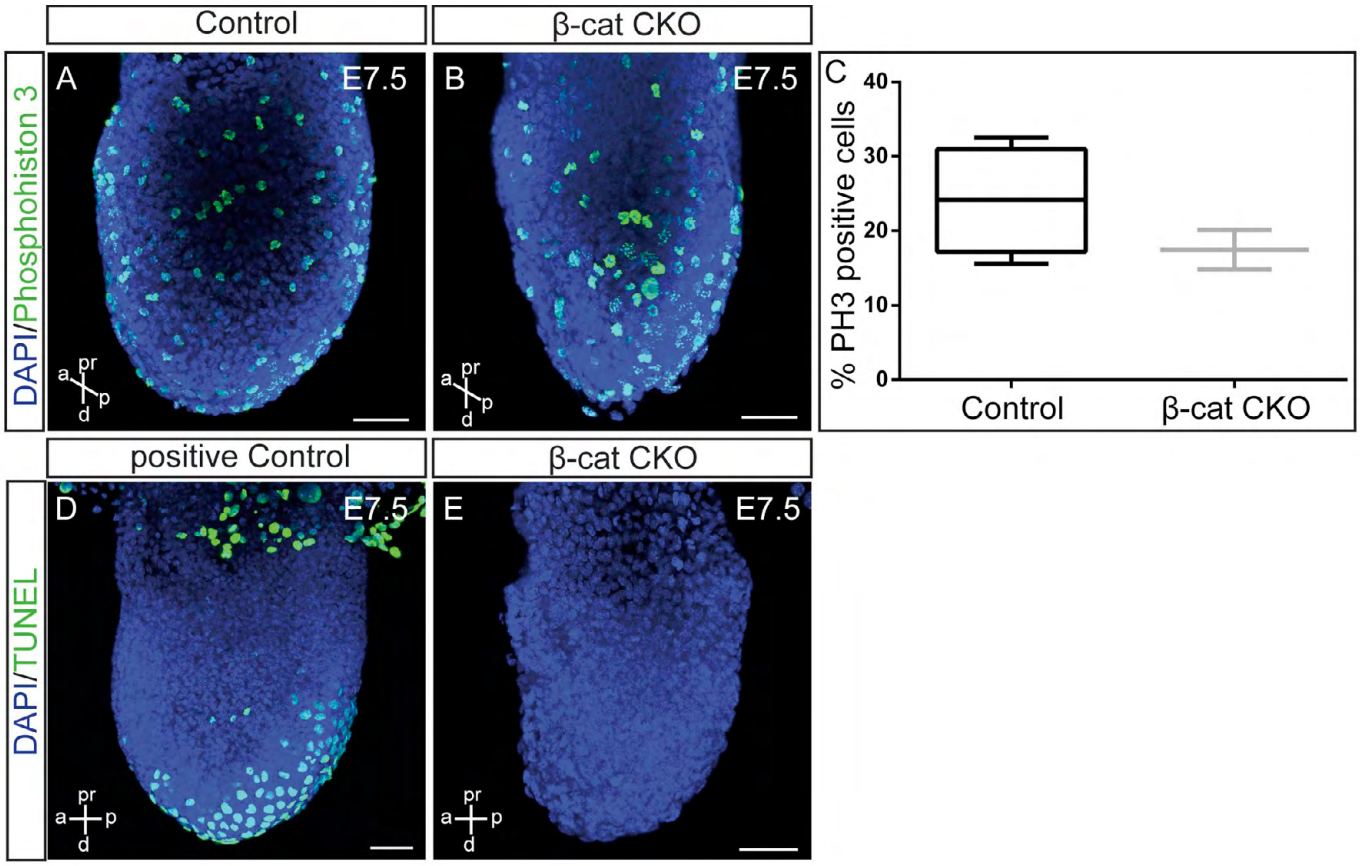


Fig. S4. Proliferation and apoptosis rates are unchanged in CKO embryos. (**A**,**B**) Cell proliferation indicated by phospho-histone H3 (PH3) antibody staining in control (A; *n*=4) and CKO (B; *n*=2) at E7.5. (**C**) PH3+ cells were quantified with IMARIS. Unpaired *t*-test revealed no significant change in proliferation. (**D**,**E**) No TUNEL-positive cells are detected in CKO (E; *n*=8) at E7.5. A positive control embryo is shown in D. Scale bars: 50 µm.

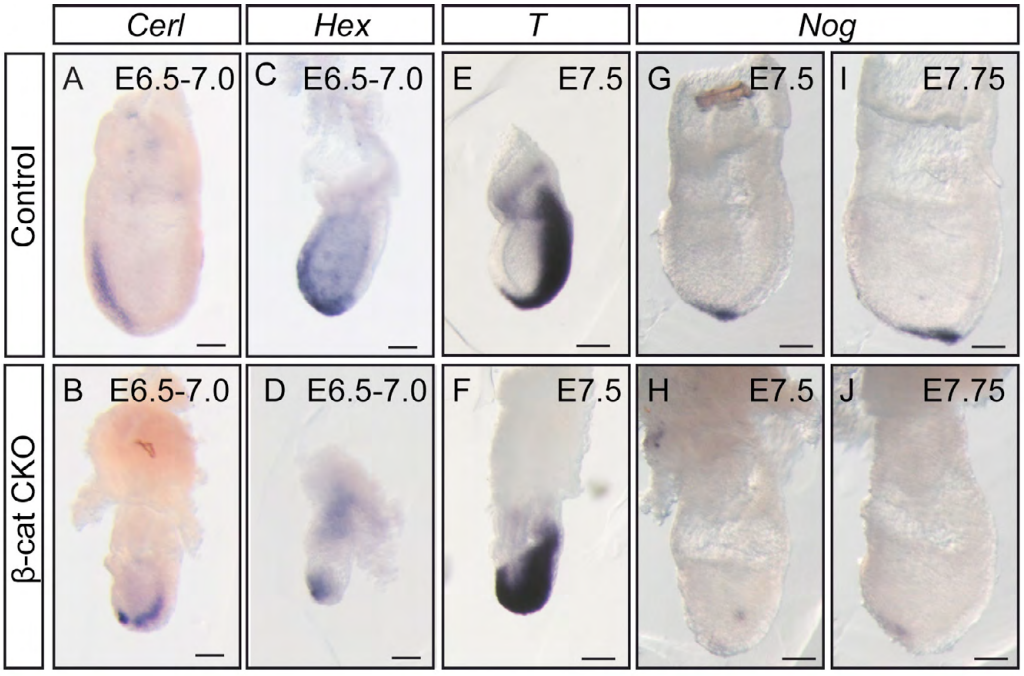


Fig. S5. Defective AVE migration and failure of organizer gene induction in CKO embryos. (**A-J**) WISH with the indicated antisense probes and at the indicated embryonic stages. In CKOs *Cerl* (B) and *Hex* (D) expression remain distally compared with control (A,C). *T* levels are comparable in CKO (F) and control (E) embryos at E7.5. Mutants show reduced expression of *Nog* in the node region (H,J) compared with controls (G,I) at E7.5. Anterior to the left. Scale bars: 100 µm.

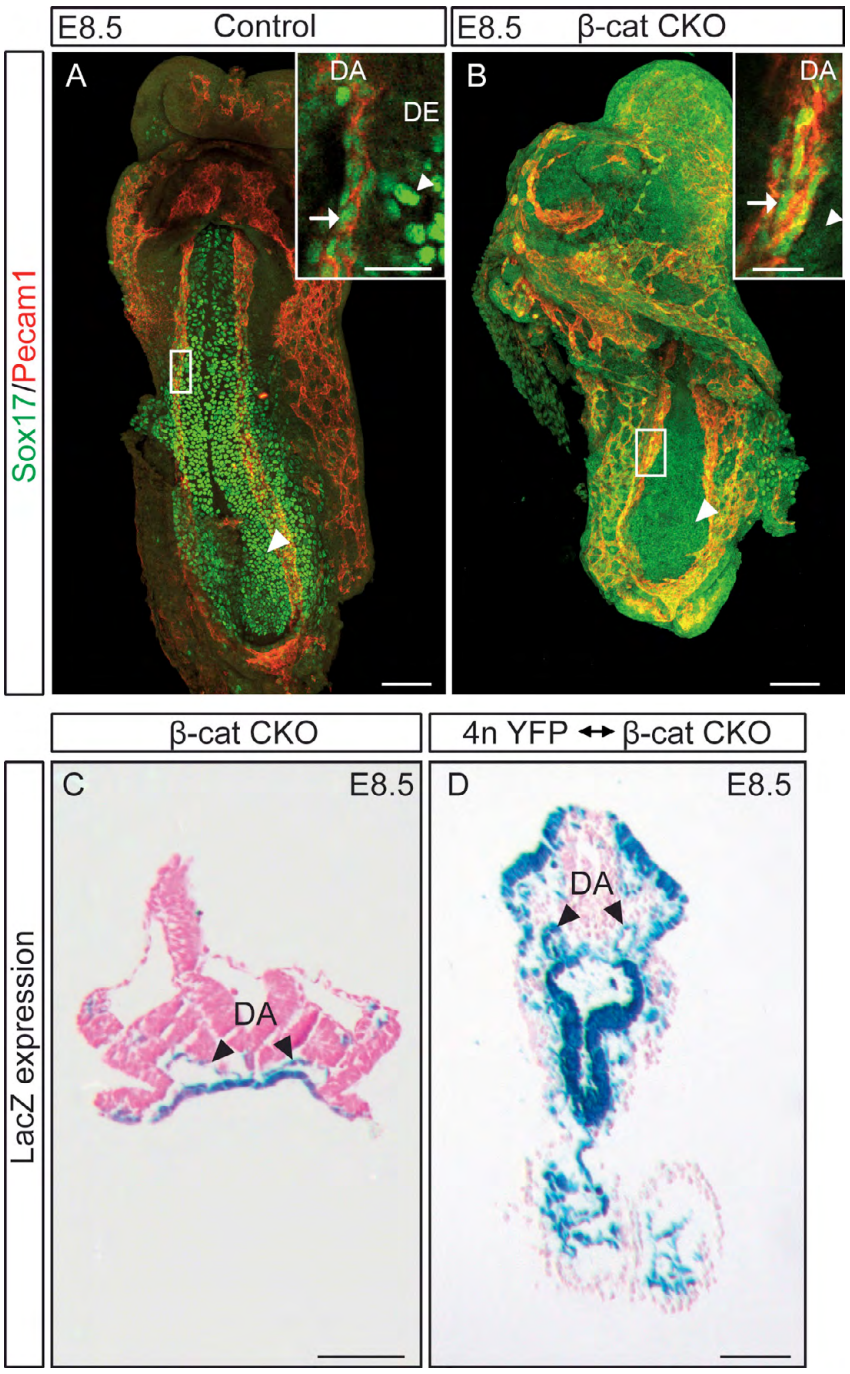
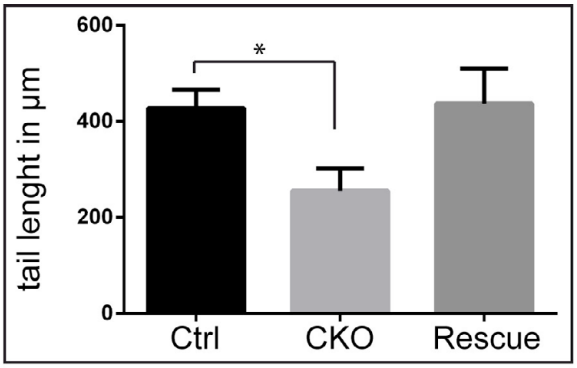


Fig. S6. Sox17 is expressed in the dorsal aorta and vascular endothelial cells in β-catenin CKO embryos. (**A**) Sox17 localisation to the gut endoderm (arrowhead) and colocalisation with Pecam1 in the dorsal aorta (DA, arrow) and blood vessels in control embryos as revealed by whole-mount antibody staining at E8.5 (inset). (**B**) Loss of Sox17 synthesis in the gut endoderm (arrowhead) but not in the endothelial cells of the DA (arrow) and blood vessels in β-catenin CKOs (inset). (**C**,**D**) Paraffin sections of β-catenin CKO embryos and aggregation chimeras at E8.5. Arrowheads point to the epithelial tube-like structure of the DA. Scale bars: 100 μm, except 25 μm in insets.



* p= 0.002499 (mann-whitney test)

Fig. S7. Significantly reduced tail size in CKO embryos. Quantification of posterior tail length by measuring the distance from the last somite to the end of the tail in control (*n*=6), CKO (*n*=6) and rescued CKO chimera (*n*=6) at the 6- to 7-somite stage, with Leica and AxioVision. Tail length is significantly reduced in CKO embryos compared with control embryos and rescued chimera. Error bars indicate s.d. *P*-value was calculated using the wilcox.test function of the R statistics package.

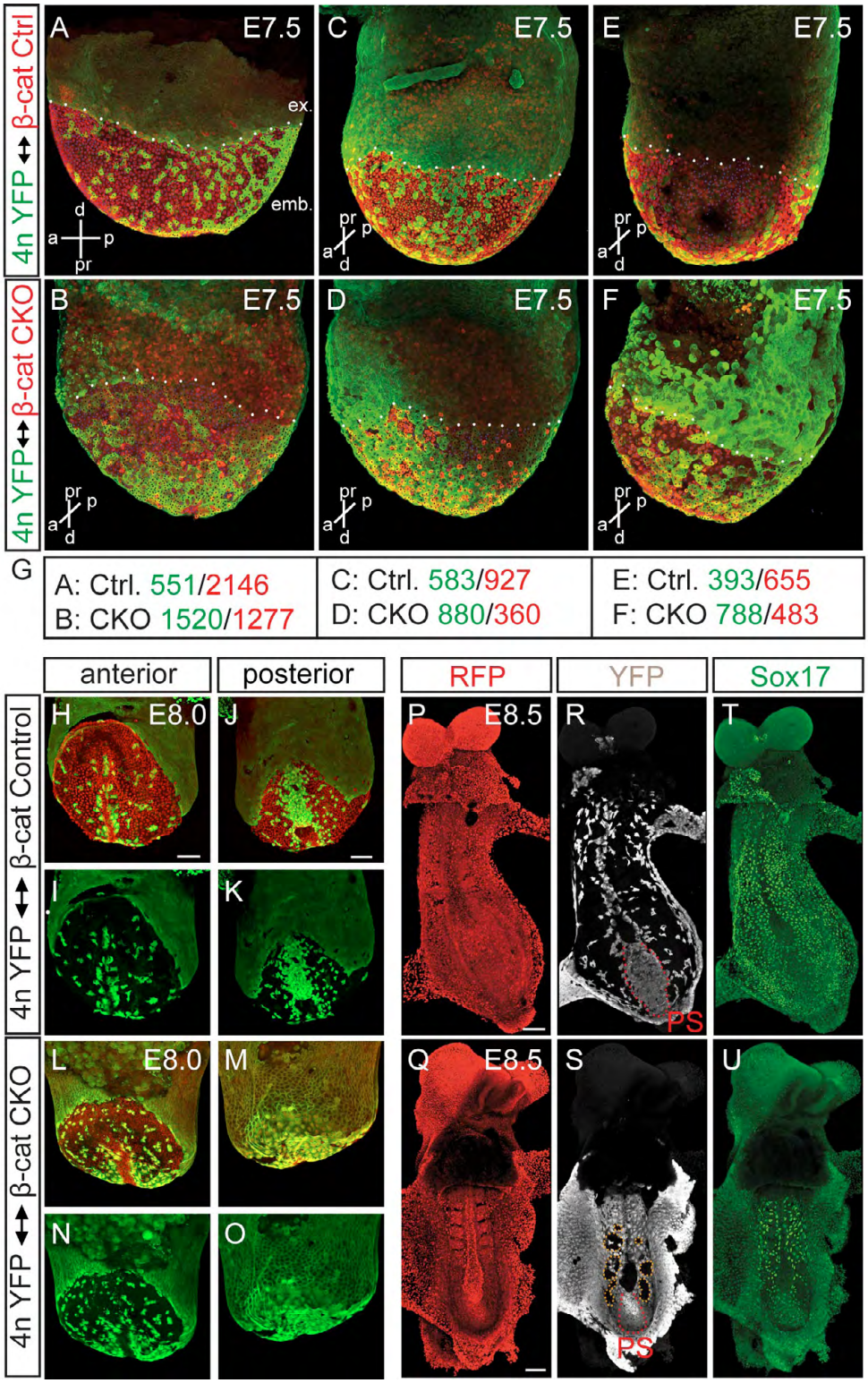


Fig. S8. High contribution of YFP⁺ VE cells to CKO aggregation chimera. (**A-F**) Representative β-catenin CKO and control aggregation chimeras at comparable stages stained with antibodies to GFP (YFP⁺ VE cells) and RFP (β-catenin control or CKO ESCs). White dotted line indicates ex/emb boundary. (**G**) Higher contribution of 4n YFP⁺ VE cells (green) to aggregation chimera (*n*=3) compared with control (*n*=3) at E7.5. (**H-O**) Comparable numbers of CKO or control ESCs in the anterior (H,I,L,N) but not in the posterior region (J,K,M,O) of chimeric embryos at E8.0. (**P-S**) Contribution of CKO or control ESCs (P,Q) and YFP⁺ VE cells (R,S) to aggregation chimera at E8.5. (**T**,**U**) Sox17 expression in the endoderm. Scale bars: 100 μm.

Movie 1. 4n YFP$^+$ ↔ 2n β-catenin CKO at E8.5. Transverse sections of the 4n YFP$^+$ ↔ 2n β-catenin CKO embryo in Fig. 5M, from caudal to rostral. β-catenin CKO ESCs are shown in red, VE cells in white and Sox17-expressing cells in green.

Table S1. Oligonucleotides for ChIP analysis

Name	Forward primer	Reverse primer	Genomic position mm9
TBE 1	CCCCACATCTCAAGTGCTG	TGGTGATCGAGCTCAGTTTG	chr1:4484862-4484967
TBE 2	CCGCTACTGTTTTCAATCGTC	CCCTCACCTCCACAGTGAC	chr1:4485823-4485971
TBE 3	GGCTTTGATAACGTCGTGAG	GTGAGTGGGCCATATTTCAG	chr1:4486458-4486528
Negative	GGATGGAAAGGCACCTATTG	ACAGTGGGTCAAGCACATTG	chr1:4488145-4488223
TBE 4-7	TAACTTCCAGGGCAGTTGTG	GTCTGTCTTTAGGGCATTTGG	chr1:4488882-4488998
TBE 4-7	TGTGGCGTTAAGTCACTGAGTC	ACTGCCCTGGAAGTTACTGAAG	chr1:4488983-4489067
TBE 8-10	AGTCTGAGAGAACATGGCACAC	GGCAAATTCTAATTCATCTGAGC	chr1:4489550-4489658
TBE 11	GTAGTGCACACCTTCAAAGAGG	AATGGCAGCTCACAATCATC	chr1:4490552-4490631
TBE 12-13	CTTCAGCAAAGGACTGTGAGTG	GTGTGTGGCCATGTAACCAG	chr1:4492173+4492320

Table S2. Tcf/Lef binding elements (TBEs) in the *Sox17* upstream and downstream regulatory regions

TBE	**Target sequence (5′-3′)**	**Genomic position mm9**
TBE 1	CATTCCTTTGAGTTTTC	chr1:4484854
TBE 2	GAGATCTTTGAAAAGAT	chr1:4485876
TBE 3	GCTGGCTTTGATAACGT	chr1:4486521
TBE 4	TGAATTATTGAAGGAGA	chr1:4488741
TBE 5	CTTCGATAAAAGCATTA	chr1:4488812
TBE 6	TTAATTTCAAAGATAAC	chr1:4488911
TBE 7	AGAACCTTTGTTATGTG	chr1:4489071
TBE 8	CAGATATCAAAATAAAT	chr1:4489533
TBE 9	TCCATCTTTGAACTCTT	chr1:4489588
TBE 10	AGTTCATCAAAGCTAAC	chr1:4489676
TBE 11	ACACCTTCAAAGAGGTG	chr1:4490617
TBE 12	TAGAAAACAAAGAAACA	chr1:4492229
TBE 13	CCTTCAGCAAAGGACTG	chr1:4492314