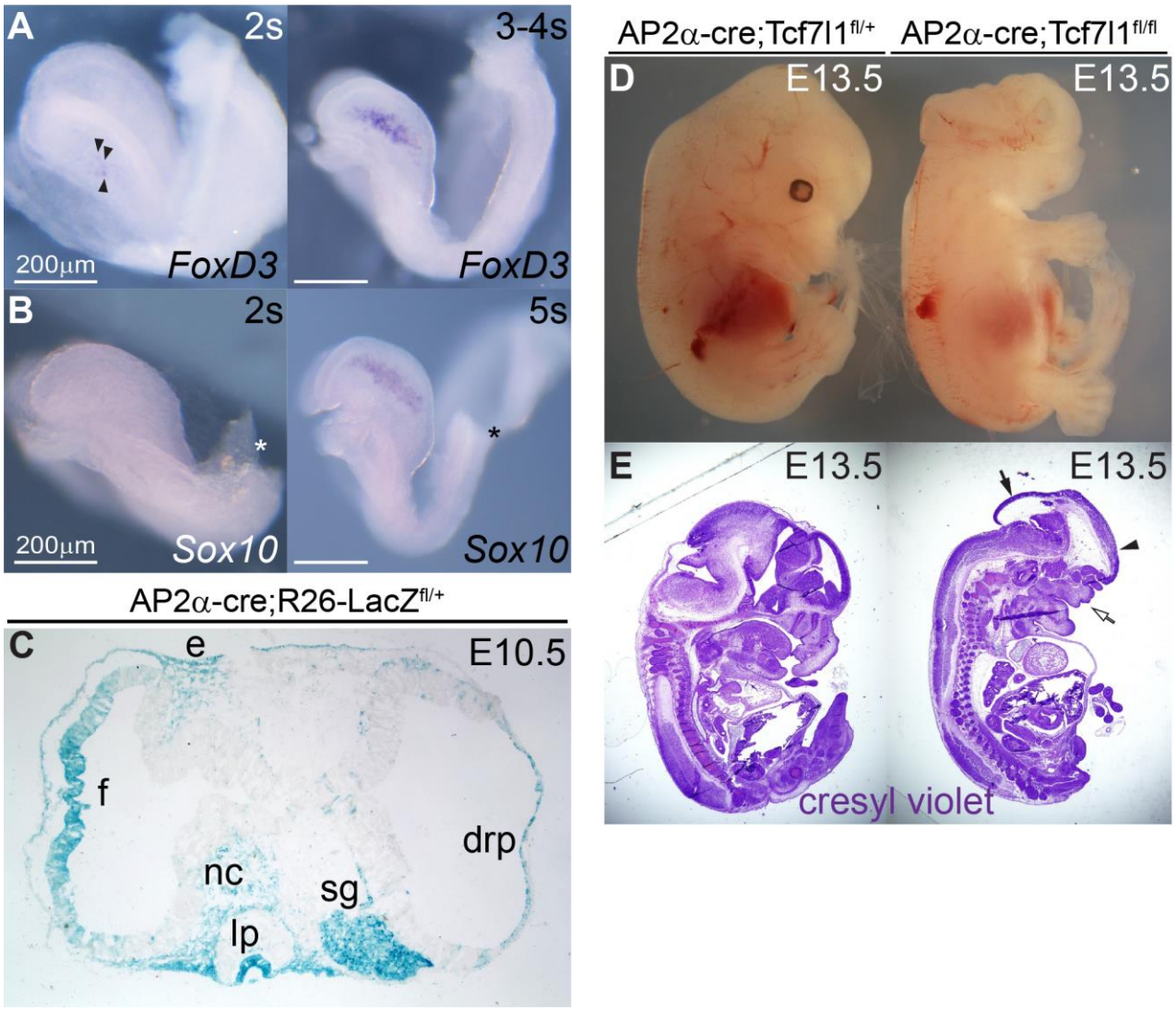

Fig. S1. Expression of NC genes at E8 and gross morphology of AP2α-Cre;Tcf7l1$^{fl/fl}$ mutants embryos. (A,B) Expression of NC genes during early mouse embryogenesis. RNA *in situ* hybridisation of *FoxD3* at stages 2s and 3-4s (A) and *Sox10* at stages 2s and 5s (B). * artefact caused by embryo dissection. **(C)** Mapping of AP2α-Cre recombination activity. A transverse section of AP2α-Cre;Rosa26$^{LacZfl/+}$ embryo at E10.5. Cre activity was detected using β-galactosidase enzyme assay. **(D)** Tcf7l1 deletion results in exencephaly. **(E)** Histological analysis of sagittal sections from the AP2α-Cre;Tcf7l1$^{fl/fl}$ "strong" mutant at E13.5 sections stained with cresyl violet show severe defects in the splanchnocranium, the forebrain (arrowhead) and midbrain (arrow).

Drp, dorsal roof plate; e, surface ectoderm; f, forebrain; lp, lens pit; nc-neural crest; sg, sensory ganglion.

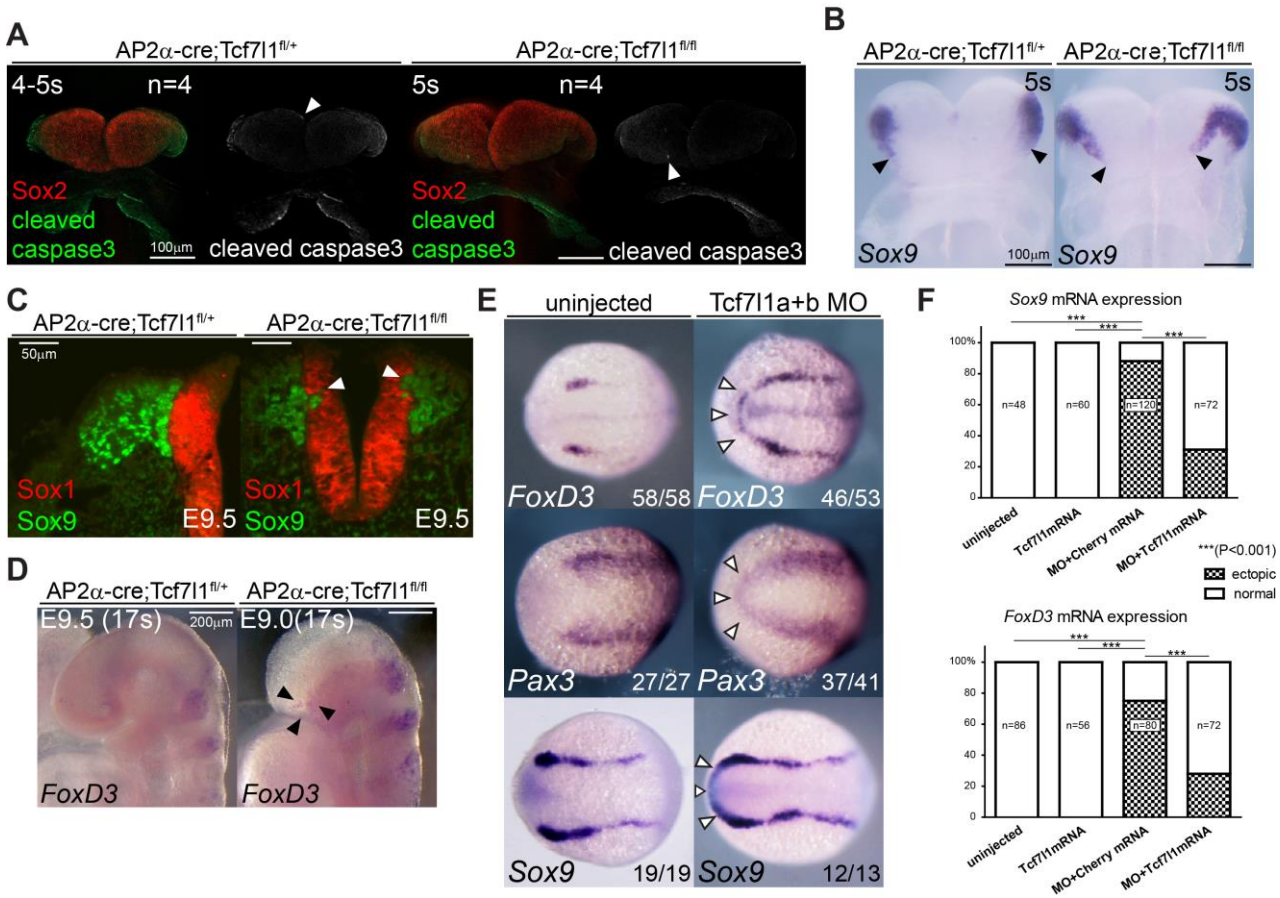

Fig. S2. Expansion of NC markers after ablation of Tcf7l1 in mouse and zebrafish. **(A)** Apoptosis is not altered in Tcf7l1 mouse mutant embryos at 5s stage as analyzed by immunofluorescence using anti-Caspase3 antibody, frontal view on the ANF, arrowheads points at one positive cell in each embryo. **(B)** *Sox9* mRNA expands rostrally in AP2α-Cre;Tcf7l1$^{fl/fl}$ mutant at 5s stage. Expansion of *Sox9* is marked by arrowheads. **(C)** Immunohistological staining of transverse sections from the hindbrain at E9.0 revealed ectopic Sox9-positive/Sox1-negative cells (arrowheads) in AP2α-Cre;Tcf7l1$^{fl/fl}$ mutants. **(D)** Aberrant *FoxD3* mRNA expression (arrowheads) in Tcf7l1 conditional mutants at E9.0. **(E)** Morpholino (MO) knock-down of Tcf7l1a and Tcf7l1b variants in zebrafish at 12hpf stage. Expression of *FoxD3*, *Pax3* and *Sox9* mRNA expands anteriorly along the anterior NPB in Tcf7l1 morphants (arrowheads). **(F)** Rescue of Tcf7l1 morphants using co-injection of mouse Tcf7l1 mRNA and control mRNA Cherry. Quantification of the ectopic expression shown in (E, arrowheads) and normal pattern of Sox9 and FoxD3 transcripts. Statistical significance was calculated using Fishers test.

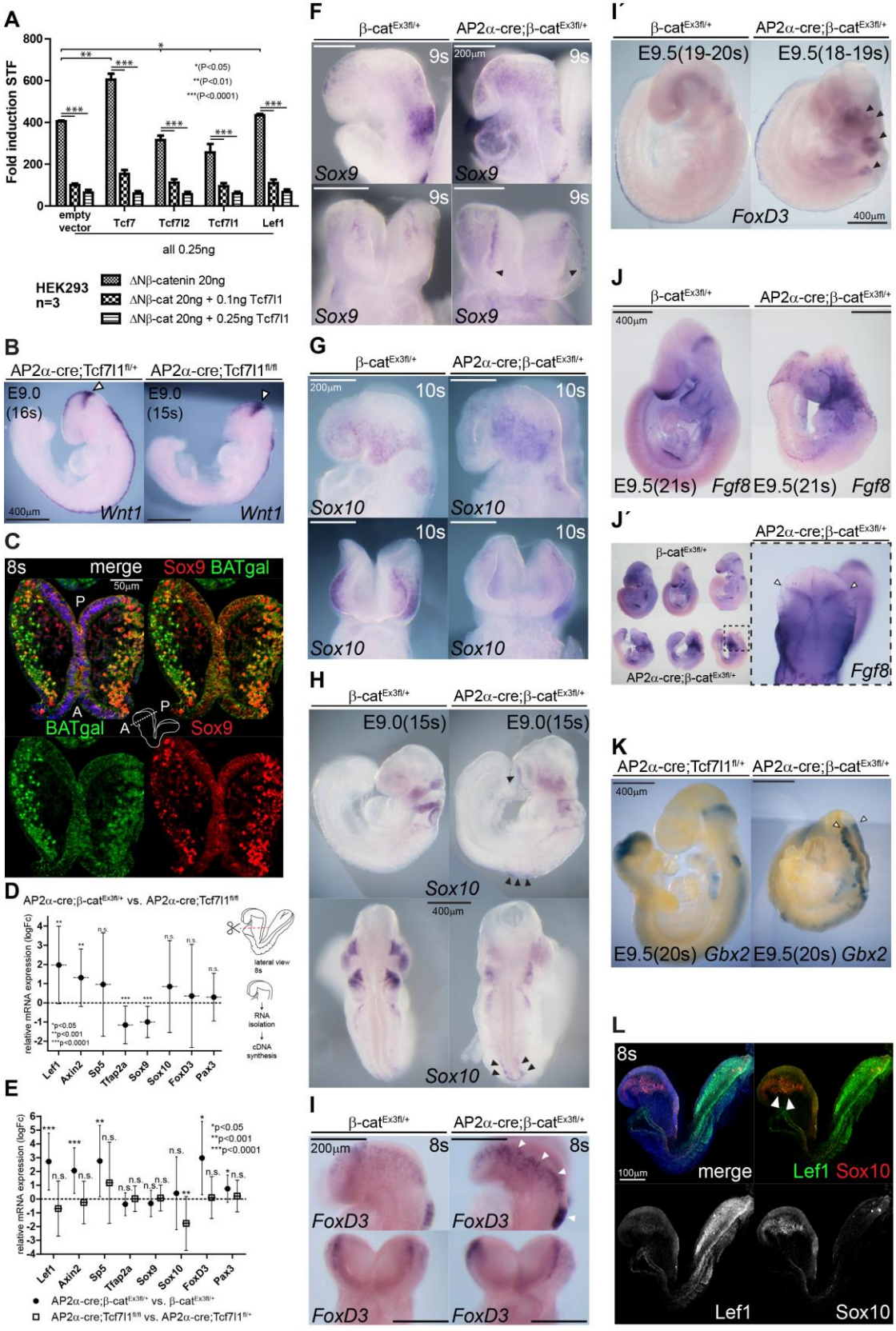

Fig. S3. Analysis of NC cells in AP2α-Cre;β-cat$^{Ex3fl/+}$ embryos. (A) SuperTopFlash luciferase reporter assay in HEK293 cells documents Tcf7l1 ability to repress Tcf/Lef driven gene expression. Activation of the pathway was achieved by co-transfection with non-degradable form of β-catenin (ΔNβ-catenin). Statistical significance was calculated using Student´s *t*-test. Error bars indicate ± SD. **(B)** The expression of *Wnt1* transcripts is unchanged in the MHB (arrowhead) of AP2α-Cre;Tcf7l1$^{fl/fl}$ mutants at E9.0. **(C)** Sox9 and BAT-gal immunostaining of transverse sections of 8s wild-type embryos. Please note the large overlap between the Sox9-positive and BAT-gal-positive delaminating cells. Nuclei were counterstained with DAPI. **(D)** qRT-PCR graph showing differential expression of Wnt target genes and genes involved in the NC induction and specification. Samples from dissected heads of the AP2α-Cre;β-cat$^{Ex3fl/+}$ vs. AP2α-Cre;Tcf7l1$^{fl/fl}$ mutants at 6-12s stage, n=6 for each genotype. Values are in log2 scale, Statistical significance was calculated by a two-tailed Student's *t*-test. Error bars indicate ± SD. **(E)** qRT-PCR graph showing differential expression of Wnt target genes and genes involved in the NC induction and specification. Samples from dissected heads of the AP2α-Cre;β-cat$^{Ex3fl/+}$ vs. β-cat$^{Ex3fl/+}$ (full circles) and AP2α-Cre;Tcf7l1$^{fl/fl}$ vs. AP2α-Cre;Tcf7l1$^{fl/+}$ (empty squares) at 6-12s stage, n=5 for each genotype. Values are in log2 scale, Statistical significance was calculated by a two-tailed Student's *t*-test. Error bars indicate ± SD. **(F-G)** RNA *in situ* hybridisation of *Sox9* (F) and *Sox10* (G) in AP2α-Cre;β-cat$^{Ex3fl/+}$ mutants showing anterior expansion of *Sox9* (arrowheads) but reduced and more dispersed expression of *Sox10* at 8s stage. Side (top) and frontal view (bottom). **(H)** *Sox10* is expressed in higher levels in the anterior head and caudal trunk (arrowheads) of the AP2α-Cre;β-cat$^{Ex3fl/+}$ mutants than in the controls at E9.5. Side (top) and frontal view (bottom). **(I,I´)** *Foxd3* expression is more abundant in AP2α-Cre;β-cat$^{Ex3fl/+}$ mutants than in the controls at 8s (white arrowheads) (I) and E9.5 stage (black arrowheads). Side (top) and frontal view (middle) are shown at 8s stage, and side (bottom) view is shown for E9.5 (I´). **(J-J')** *Fgf8* mRNA is abnormally spread in large areas around branchial arches but it is detected in the MHB (arrowheads) of AP2α-Cre;β-cat$^{Ex3fl/+}$ mutants. **(K)** MHB marker *Gbx2* is ectopically expressed caudally from MHB (marked by arrowheads) in the AP2α-Cre;β-cat$^{Ex3fl/+}$ mutants at E9.5. **(L)** Whole mount immunofluorescence showing overlapping expression of Lef1 and Sox10 in NC cells (arrowheads) in wild-type embryo at 8s stage. Nuclei were stained with Hoechst.
A, anterior; P, posterior.

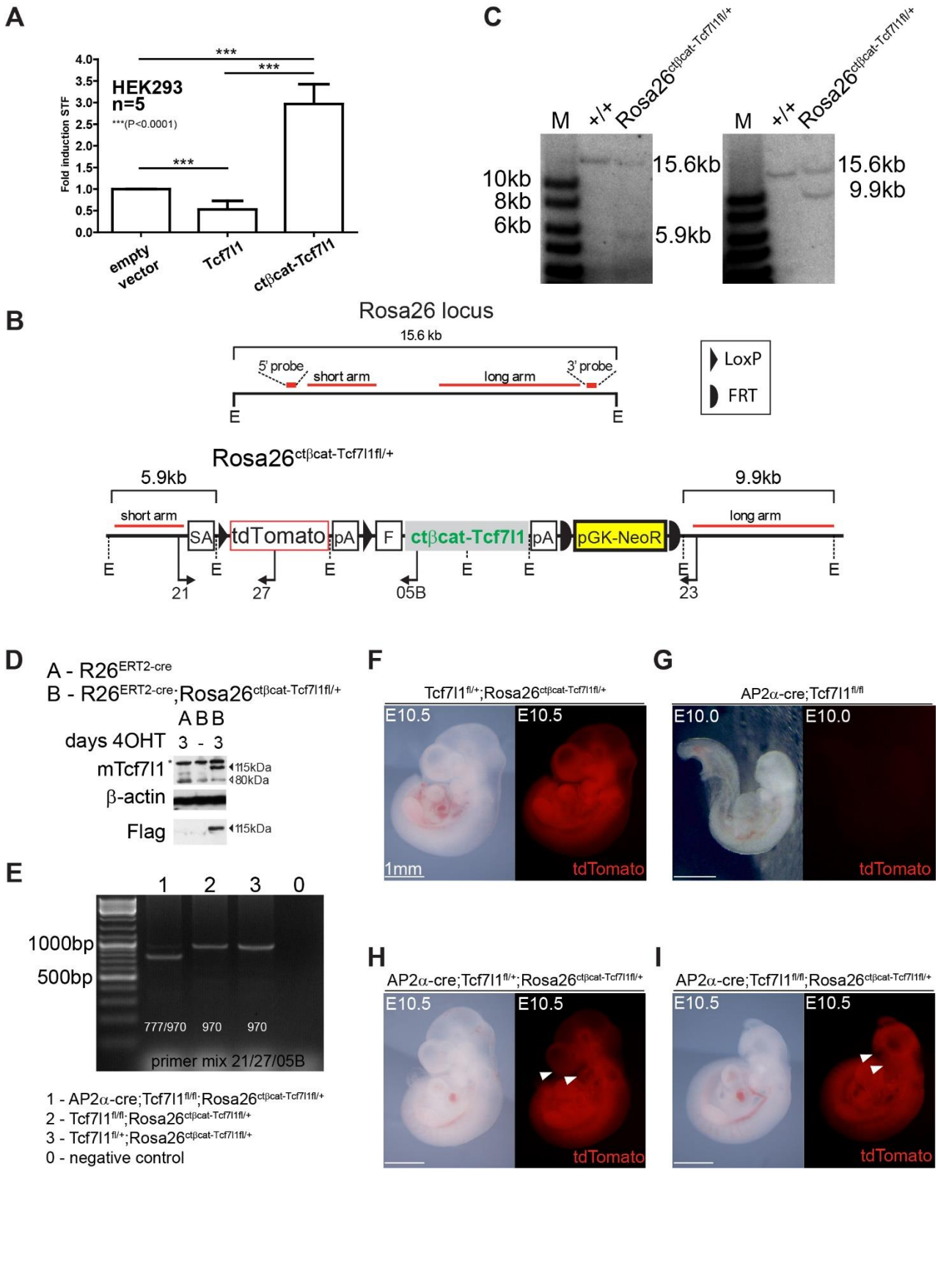

Fig. S4. Generation and validation of the mouse strain Rosa26$^{ctβcat\text{-}Tcf7l1fl/+}$. (A) SuperTopFlash assay in HEK293 cells shows ctβcat-Tcf7l1 mediated activation and Tcf7l1 mediated repression of the Tcf/Lef reporter. Statistical significance was calculated using Student´s *t*-test. Error bars indicate ± SD. **(B)** Scheme of the Rosa26 locus and the targeting vector, homology arms are depicted in red. **(C)** Southern blotting analysis confirms correct homologous recombination in ES cells, short arm is on the left. **(D)** Western blotting of lysates from mouse embryonic fibroblasts (MEFs) isolated from the R26$^{ERT2\text{-}cre}$;Rosa26$^{ctbcat\text{-}Tcf7l1fl/+}$ embryos revealed the presence of the ctβcat-Tcf7l1 fusion protein 3 days after administration of 4-OHT (4-*hydroxitamoxifen*). Size of the endogenous Tcf7l1 is approximately 80kDa, ctβcat-Tcf7l1 protein size is around 115kDa. **(E)** PCR confirmation of Cre recombination in AP2α-Cre;Tcf7l1$^{fl/fl}$; Rosa26$^{ctβcat\text{-}Tcf7l1fl/+}$ compound mutants, resulting size of the PCR products was 970bp prior and 777bp after recombination**. (F-I)** Rosa26$^{ctbcat\text{-}Tcf7l1fl/+}$ mice ubiquitously express fluorescent protein tdTomato. The loss of its expression upon Cre recombination is marked by arrowheads in the AP2α-Cre;Tcf7l1$^{fl/fl}$;Rosa26$^{ctβcat\text{-}Tcf7l1fl/+}$ embryos (H) and the AP2α-Cre;Tcf7l1$^{fl/fl}$;Rosa26$^{ctβcat\text{-}Tcf7l1fl/+}$ compound mutants (I). AP2α-Cre;Tcf7l1$^{fl/fl}$ serves as a negative control (G).

E, EcoRI cleavage site; SA, splice acceptor; pA, poly-adenylation signal; pGK-NeoR, neomycin expressing cassette; F, Flag tag sequence;*, non-specific binding of the antibody; primers for genotyping 21-JM21F, 23-JM23R, 27-JM27R, 05-JM05B.

Supplemental Table S1 (Related to Materials and methods)

ISH probes		
Gene	forward	reverse
FoxD3	GGACCGCAAGAGTTCGCGGA	TCCGGAGCTCCCGTGTCGTT
Sox9	GAGCACTCTGGGCAATCTCAG	CTCAGGGTCTGGTGAGCTGTG
Gbx2	Gift from Peter Rathjen	Adelaide University
Sp5	CGTGAAGACGCACCAAAATA	TATTTTCACGCTGCCAACTG
Fgf8	CAGGTCCTGGCCAACAAG	GAGCTCCCGCTGGATTCCT
Sox10	Gift from Anthony Firulli	Indiana University, USA
Sox2	Open Biosystems	BC057574
FoxG1	Gift from Stefan Krauss	OUS, Oslo, Norway
Six3	Gift from Guillermo Oliver	St. Jude Hospital, Memphis, USA
Wnt1	Gift from Andy McMahon	USC, USA
Tcf7l1	Open Biosystems	BC128306
Tcf7l2	Open Biosystems	BC052022
Pax3	ACTGTCTGTGATCGGAACACT	CTAGAACGTCCAAGGCTTACT

Antibodies		
Gene	Company	Dilution
Sox1	RαD AF3369	1:1000
Tfap2α	Sanαta Cruz Biotech SC-184	1:1000
Sox10	Santa Cruz Biotech SC-17342	1:1000
Sox9	Millipore AB5535	1:2000
N-cadherin	BD Transduction Lab. 610920	1:2000
GFP	Life Technologies A11122	1:1000
β-galactosidase	Abcam ab9361-250	1:1000
Lef1	Cell Siganling C12A5	1:1000
cleaved caspase-3	Cell Signaling 9664	1:2000
qPCR Primers		
Axin2	AGCTTCCGCGAGGATGCTCC	TGCACCAATCCTGGTCACCCA
Tfap2	CACGAGGACCTCTTGCACGG	GTTGGACTTGGACAGGGACA
Sox9	AGGAAGCTGGCAGACCAGT	TCCACGAAGGGTCTCTTCTC
Sox10	ATGTCAGATGGGAACCCAGA	CACGTTGCCGAAGTCGATGT
Pax3	AGGAGGCGGATCTAGAAAG	TCAGCGGTAAATCAGGTTCA
Sp5	AAGCAACACGTGTGCCACGT	GTCTTCACGTGCTTGGCGAG
FoxD3	ATCCTGGTCCATCTGTCCTG	GTAATCCGGGTGTTCCTTCA
Lef1	CCTTTCTCCACCCATCCCGA	ACAGGCTGACCTTGCCAGCC