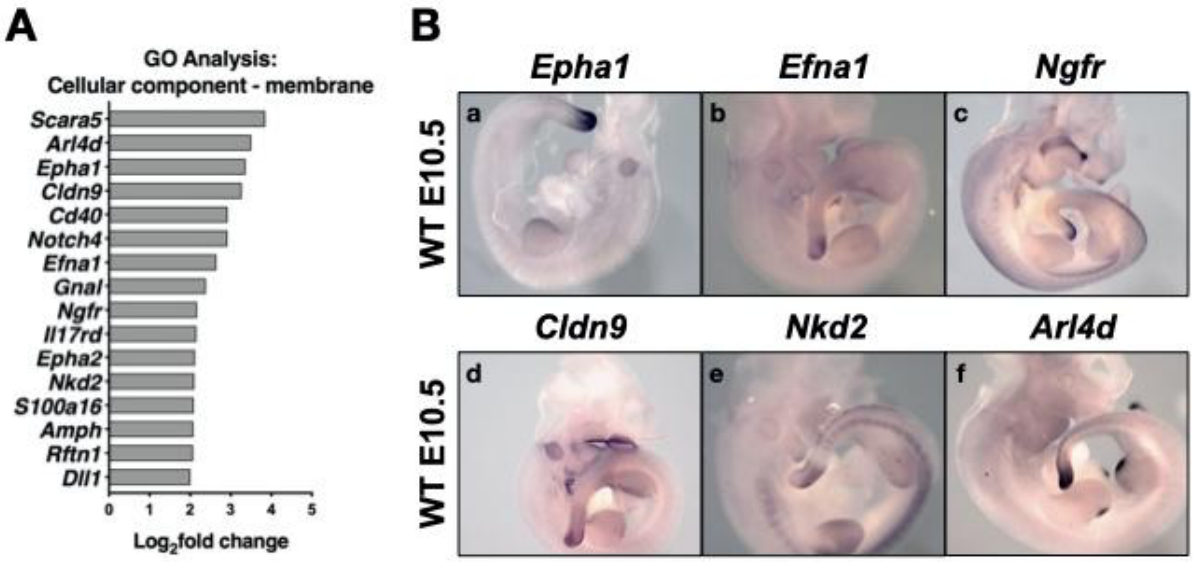

Fig. S1. Identification of potential cell-surface markers for axial progenitors. A. Differentially expressed genes encoding for plasma membrane proteins according to gene ontology classification. **B.** Whole mount ISH of E10.5 embryos using probes for *Epha1* (a), *Efna1* (b), *Ngfr* (c), *Cldn9* (d), *Nkd2* (e) and *Arl4d* (f).

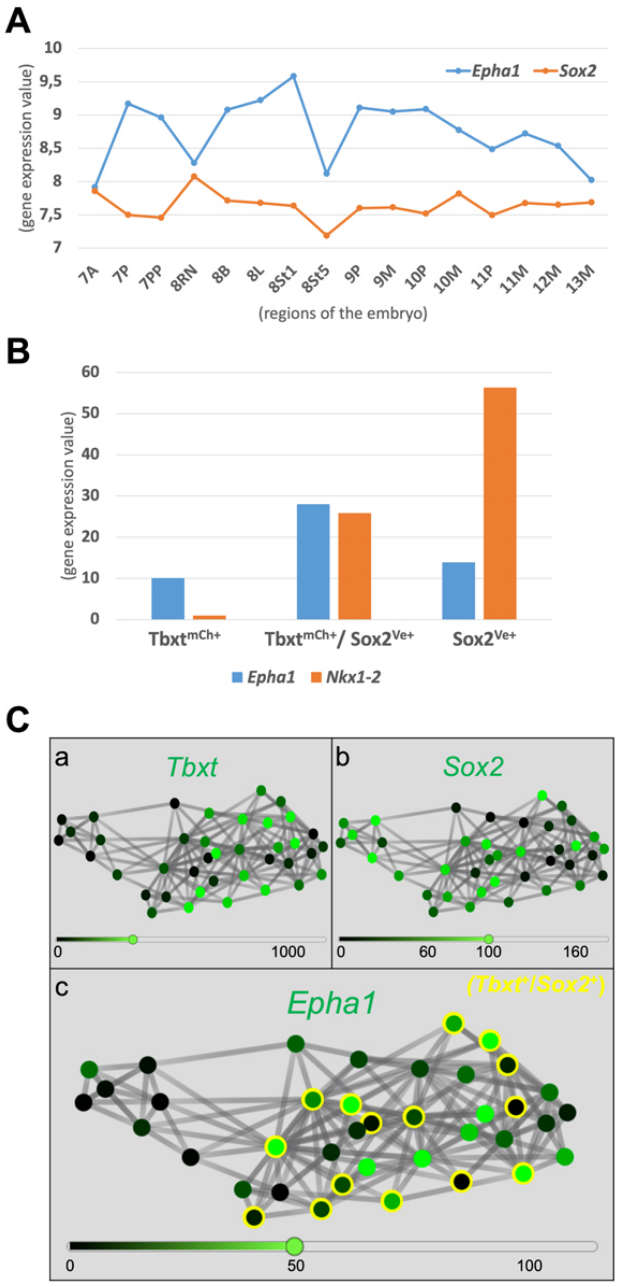

Fig. S2. *Epha1* expression in cells of NMC-populations. **A**. Microarray data from (Wymeersch et al., 2019) indicates that *Epha1* is expressed at the posterior region of the mouse embryo during the emergence of NMC cells ("7P" - E7.5 posterior epiblast) and by E8.5 is highly expressed in NMC-regions ("8B" = NSB and "8L" = CLE). During tailbud stages, *Epha1* is highly expressed in the CNH ("9M = E9.5 CNH; "10M" = E10.5 CNH; "11M" = E11.5 CNH and "12M" = E12.5 CNH) and its expression decays with the end of axis elongation ("13M" = E13.5 CNH). Contrasting with regions that contain lateral and paraxial mesoderm progenitors (LPMPs) (Wymeersch et al., 2016) ("7PP" = posterior-most part of the epiblast at E7.5 and "8St5" = posterior-most part of the primitive streak at E8.5), the regions with early mesoderm progenitors are also high *Epha1* positive ("8St1" = anterior part of the primitive streak at E8.5; "9P", "10P" and "11P" = region posterior to the CNH at E9.5, E10.5 and E11.5, respectively).

Epha1 is not expressed in the anterior part of the embryo at E7.5 ("7A") and also in the rostral node region at E8.5 ("8RN"), where notochord progenitors are present. **B**. Comparison between *Epha1* and *Nkx1-2* expression in sorted Tbxt$^+$, Sox2$^+$ and Tbxt$^+$/Sox2$^+$ cells at E8.5 (from Koch et al., 2017). *Epha1* is among the 154 genes that are highly expressed in Tbxt$^+$/Sox2$^+$ cells (group1 in Koch et al., 2017) and its expression in Tbxt-mCh$^+$/ Sox2-Ve$^+$ cells is slightly higher than *Nkx1-2*. **C**. *Epha1* is expressed in *Tbxt$^+$/Sox2$^+$* single-cells from E8.5 CLE (from Gouti et al., 2017). *Tbxt* (C**a**) and *Sox2* (**Cb**) expression in microdissected single cells from the E8.5 CLE. *Epha1* is expressed in the majority (2/3) of *Tbxt* and *Sox2* double positive cells (circled in yellow) (**Cc**).

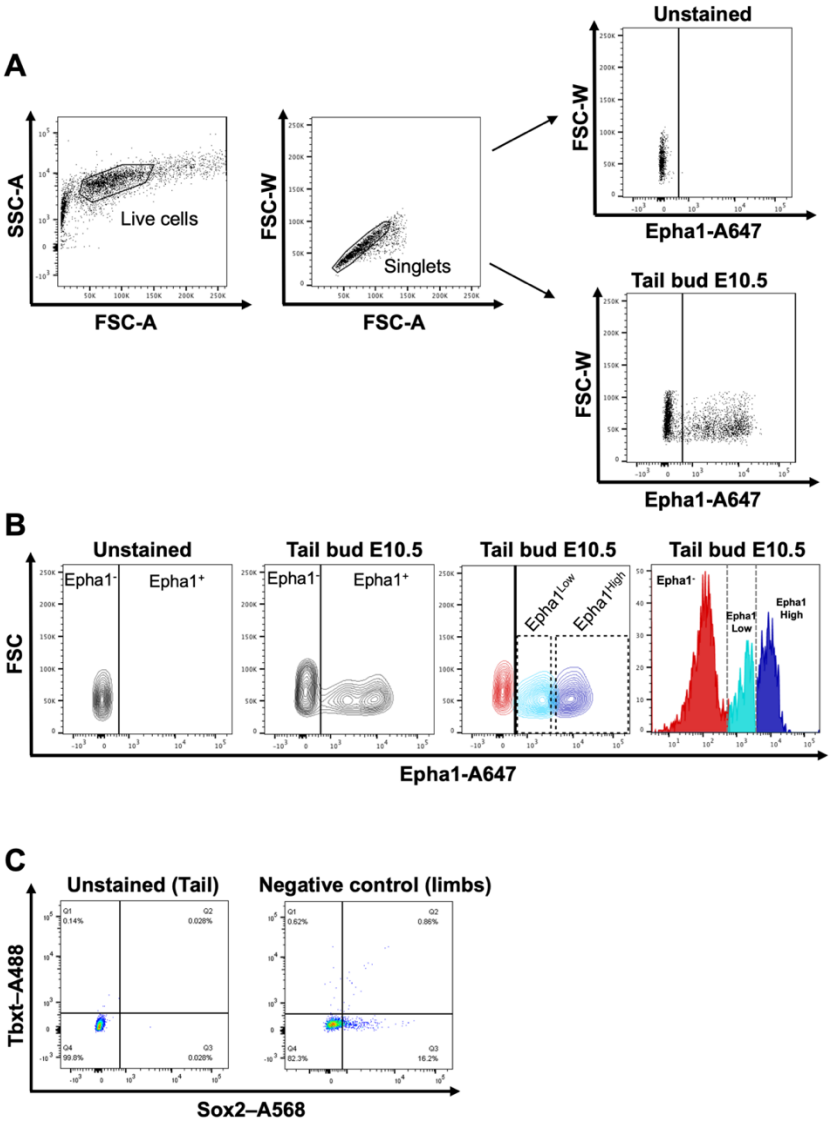

Fig. S3. Gating strategy for FACS analysis used to define Epha1 positive cells and Sox2/Tbxt quadrants. **A.** Representative example of exclusion of debris and doublets cells (using forward scatter and side scatter gates) and definition of Epha1 gating. **B.** Representative gating scheme illustrating the two subpopulations for Epha1-positive cells: Epha1^{Low} (light blue) and Epha1^{High} (dark blue), and a representative histogram showing two peaks with different fluorescent intensity in the Epha1-A647 channel, corresponding to the defined Epha1^{Low} and Epha1^{High} populations. All gates were firstly set using unstained control. **C.** Gating strategy for defining intracellular staining of Sox2-A568 and Tbxt-A488 on unstained cells from tail and stained cells from forelimbs (negative control) of E10.5 mouse embryos.

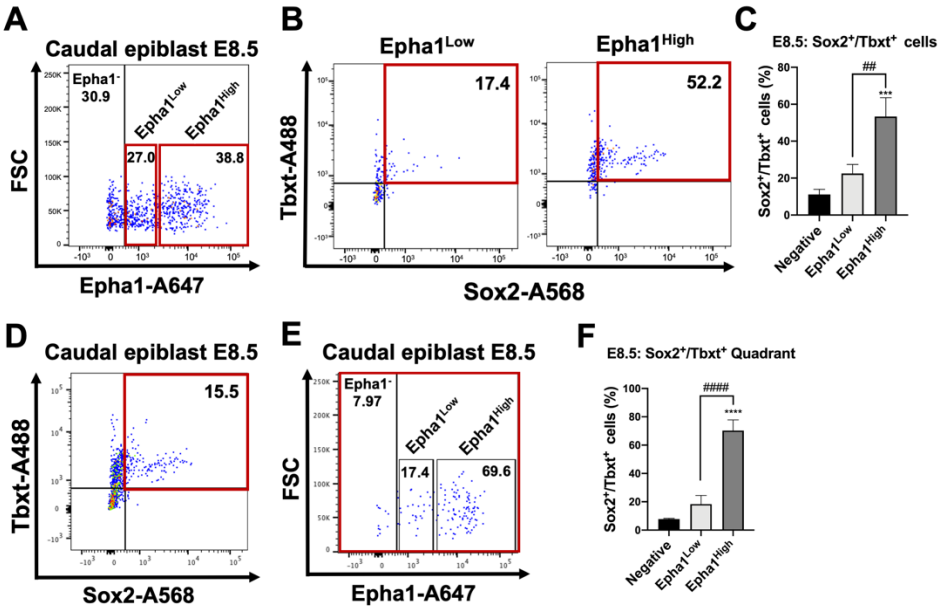

Fig. S4. Flow cytometry analysis of Epha1 subpopulations that co-express Sox2 and Tbxt in E8.5 embryos. A. FACS dot-plot displaying Epha1 subpopulations of E8.5 posterior ends. **B**. FACS profiles of Sox2 and Tbxt expression in cells from the Epha1^{Low} and Epha1^{High} compartments indicated in A. **C.** Quantification of Sox2^{+}/Tbxt^{+} cells within the different Epha1 subpopulations from posterior ends of E8.5 (from Table 1). **D.** FACS dot-plot displaying Sox2 and Tbxt expression in cells from the posterior regions of E8.5 embryos. **E.** FACS profile showing distribution of Sox2^{+}/Tbxt^{+} cells from the red window in D among the Epha1 compartments. **F.** Quantification of the distribution of Sox2^{+}/Tbxt^{+} cells from the posterior region of E8.5 embryos between the different Epha1 subpopulations (from Table 2).

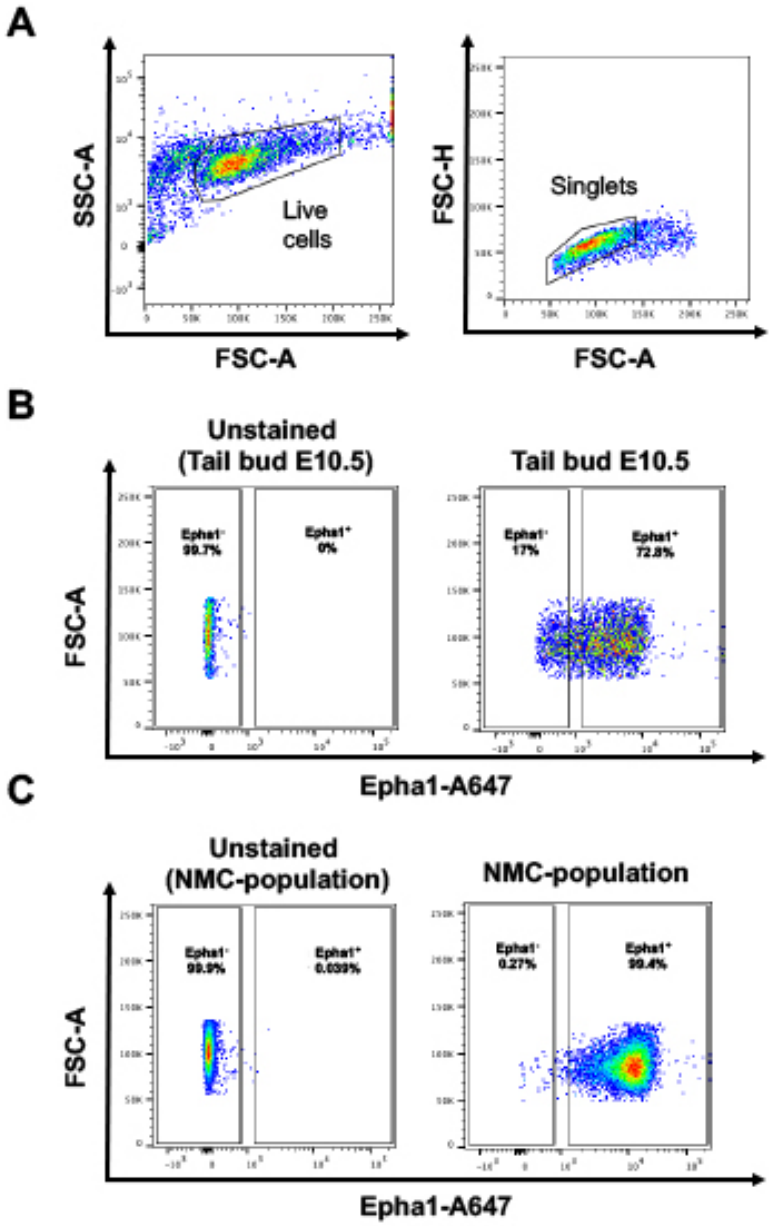

Fig. S5. Gating strategy for FACS-sorting of Epha1^{+} and Epha1^{-} cells from E10.5 tail buds and *in vitro* derived NMC-populations for in vitro culture experiments. A. Cells were first gated for singlets discrimination using side and forward scatter detectors. **B**. Representative sorting gates for Epha1 positive and negative populations from E10.5 Tail buds. **C**. Representative sorting gates for Epha1 positive and negative populations from in vitro derived NMC-populations.

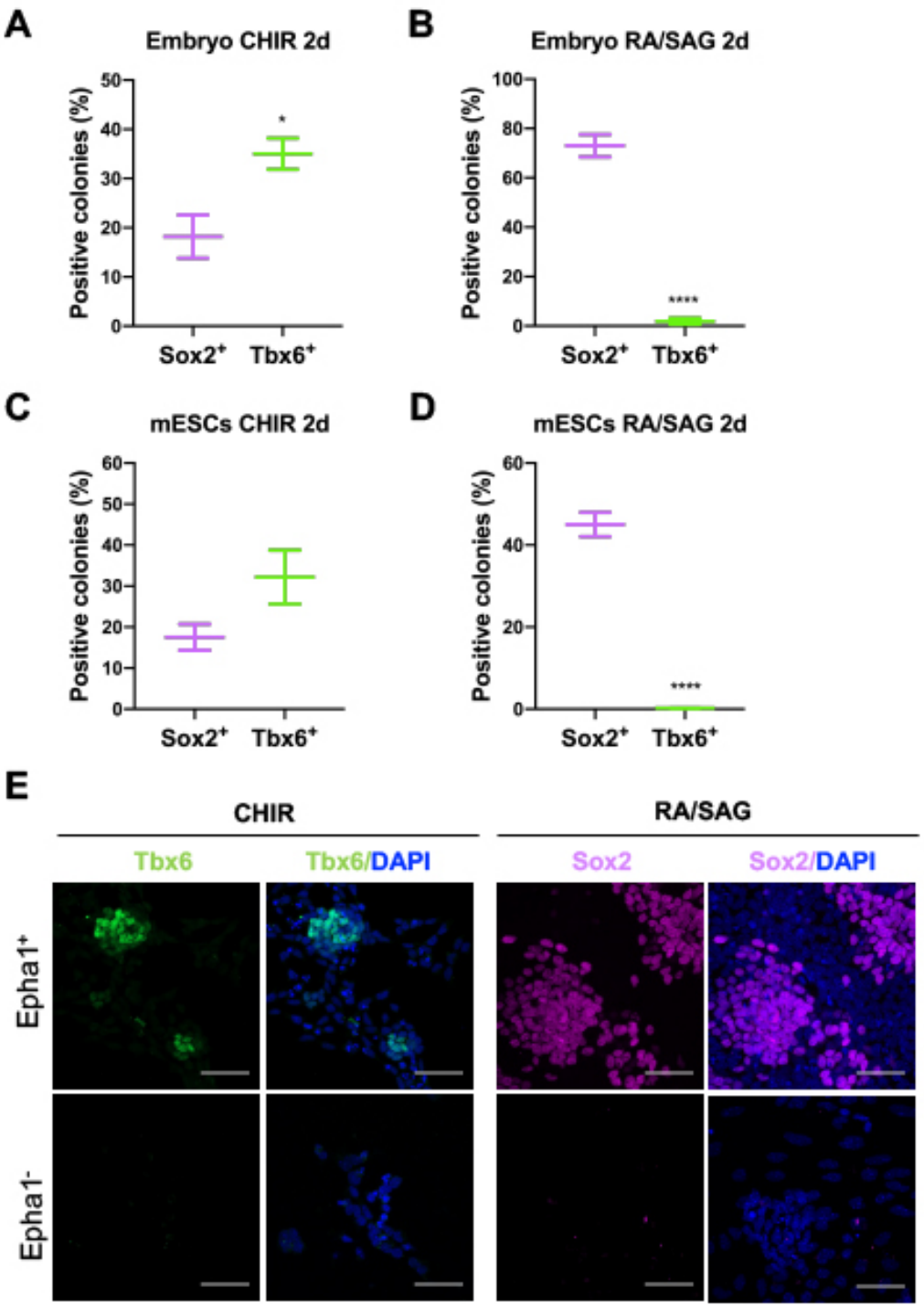

Fig. S6. Differentiation potential of sorted populations. A-D. Quantification of neural (Sox2$^+$) and mesodermal (Tbx6$^+$) derivatives over DAPI of Epha1$^+$ sorted cells from Embryo (E10.5) and mESCs differentiated with CHIR or RA/SAG. Five fields for each condition were counted and the results are presented as mean±SEM. Statistical analysis was assessed using Student's t-test. *p<0.05 and ****p<0.0001. **E.** Immunofluorescence staining of Epha1$^+$ and Epha1$^-$ sorted cells from E10.5 embryo tail buds, differentiated towards mesodermal (CHIR) or neural (RA/SAG) derivatives. Scale bar: 50 µm. "mESCs" means mouse embryonic stem cells.

Table S1. CuffDiff2 differential gene expression analysis between Tail^{Desc} and Tail^{Prog} cell populations. (attached exel file).

Click here to download Table S1

Table S2. CuffDiff2 differential gene expression analysis between Tail^{Desc} and Tail^{Tot} cell populations. (attached exel file).

Click here to download Table S2

Table S3. DEseq2 differential expression analysis between Epha1^{High} and Epha1^{Low} cell populations. (attached exel file).

Click here to download Table S3

Table S4. DEseq2 normalized counts from Tail^{Desc}, Tail^{Prog}, Epha1^{High} and Epha1^{Low} cell populations (attached exel file)

Click here to download Table S4

Table S5. List of primers used in this work.

Primers for genotyping (sequence 5' to 3')		
cre	Forward	CGAGTGATGAGGTTCGCAAG
	Reverse	CACCAGCTTGCATGATCT
***YFP* wild type Allele**	Forward	CTGGCTTCTGAGGACCG
	Reverse	CAGGACAACGCCCACACA
***YFP* mutant Allele**	Forward	AGGGCGAGGAGCTGTTCA
	Reverse	TGAAGTCGATGCCCTTCAG

Primers for RT-qPCR (sequence 5' to 3')		
β-Actin	Forward	ATGAAGATCCTGACCGAGCG
	Reverse	TACTTGCGCTCAGGAGGAGC
Arl4d	Forward	GCCTCGAGGGCTGAAGACACCCCAGCTT
	Reverse	CTGAATTCGCCTTGCTGATCCGGTGTAA
Cdx2	Forward	GCGAAACCTGTGCGAGTGGATG
	Reverse	TTTCCTCTCCTTGGCTCTGCG
Cldn9	Forward	GCCTCGAGGGCTGGCTAGGAACTTTGGT
	Reverse	CTGAATTCGGACACGTACAGCAGAGGAG
Efna1	Forward	GCCTCGAGCTCTCTTGGGTCTGTGCTGC
	Reverse	CTGAATTCGTACTTCCGGGTCATCTGCTT
Epha1	Forward	GCCTCGAGCAAGATTGCAAGACTGTGGC
	Reverse	CTGAATTCCCTCCCACATTACAATCCCA
Mesp2	Forward	GCCATGAGTAGTGGGGTGTC
	Reverse	GTCAGCGGCTCTTTCTAGGG
Ngfr	Forward	GCCTCGAGTGCCTGGACAGTGTTACGTT
	Reverse	CTGAATTCAGGAATGAGGTTGTCAGCGG
Nkd2	Forward	GCCTCGAGGGAGAGAGAGTCCCGAAGGG
	Reverse	CTGAATTCACATGTCCTCTCTGGTGACTT
Olig2	Forward	TTACAGACCGAGCCAACACC
	Reverse	TCAACCTTCCGAATGTGAATTAGA
Sox2	Forward	TTTGTCCGAGACCGAGAAGC
	Reverse	CTCCGGGAAGCGTGTACTTA
Tbxt	Forward	ACCCAGCTCTAAGGAACCAC
	Reverse	GCTGGCGTTATGACTCACAG
Wnt3a	Forward	ATTGAATTTGGAGGAATGGT
	Reverse	CTTGAAGTACGTGTAACGTG
Primers for in situ hybridization probes (sequence 5' to 3')		
Arl4d	Forward	GCCTCGAGGGCTGAAGACACCCCAGCTT
	Reverse	CTGAATTCGCCTTGCTGATCCGGTGTAA
Cldn9	Forward	GCCTCGAGGGCTGGCTAGGAACTTTGGT
	Reverse	CTGAATTCGGACACGTACAGCAGAGGAG
Efna1	Forward	GCCTCGAGCTCTCTTGGGTCTGTGCTGC
	Reverse	CTGAATTCGTACTTCCGGGTCATCTGCTT
Epha1	Forward	GCCTCGAGCAAGATTGCAAGACTGTGGC
	Reverse	CTGAATTCCCTCCCACATTACAATCCCA
Ngfr	Forward	GCCTCGAGTGCCTGGACAGTGTTACGTT
	Reverse	CTGAATTCAGGAATGAGGTTGTCAGCGG
Nkd2	Forward	GCCTCGAGGGAGAGAGAGTCCCGAAGGG
	Reverse	CTGAATTCACATGTCCTCTCTGGTGACTT