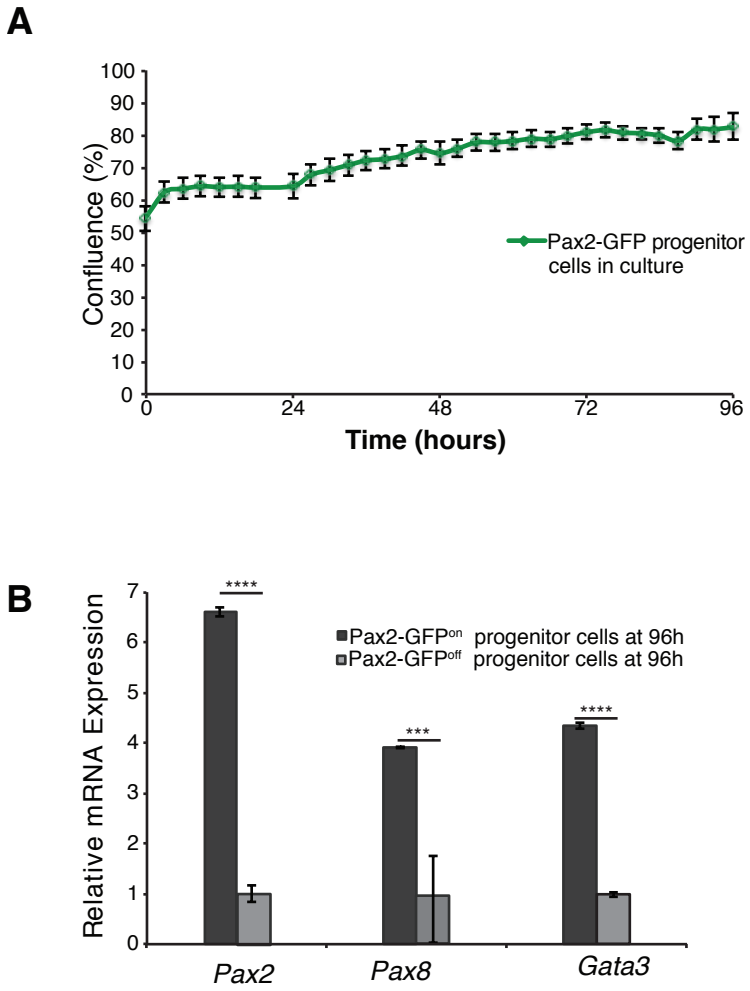
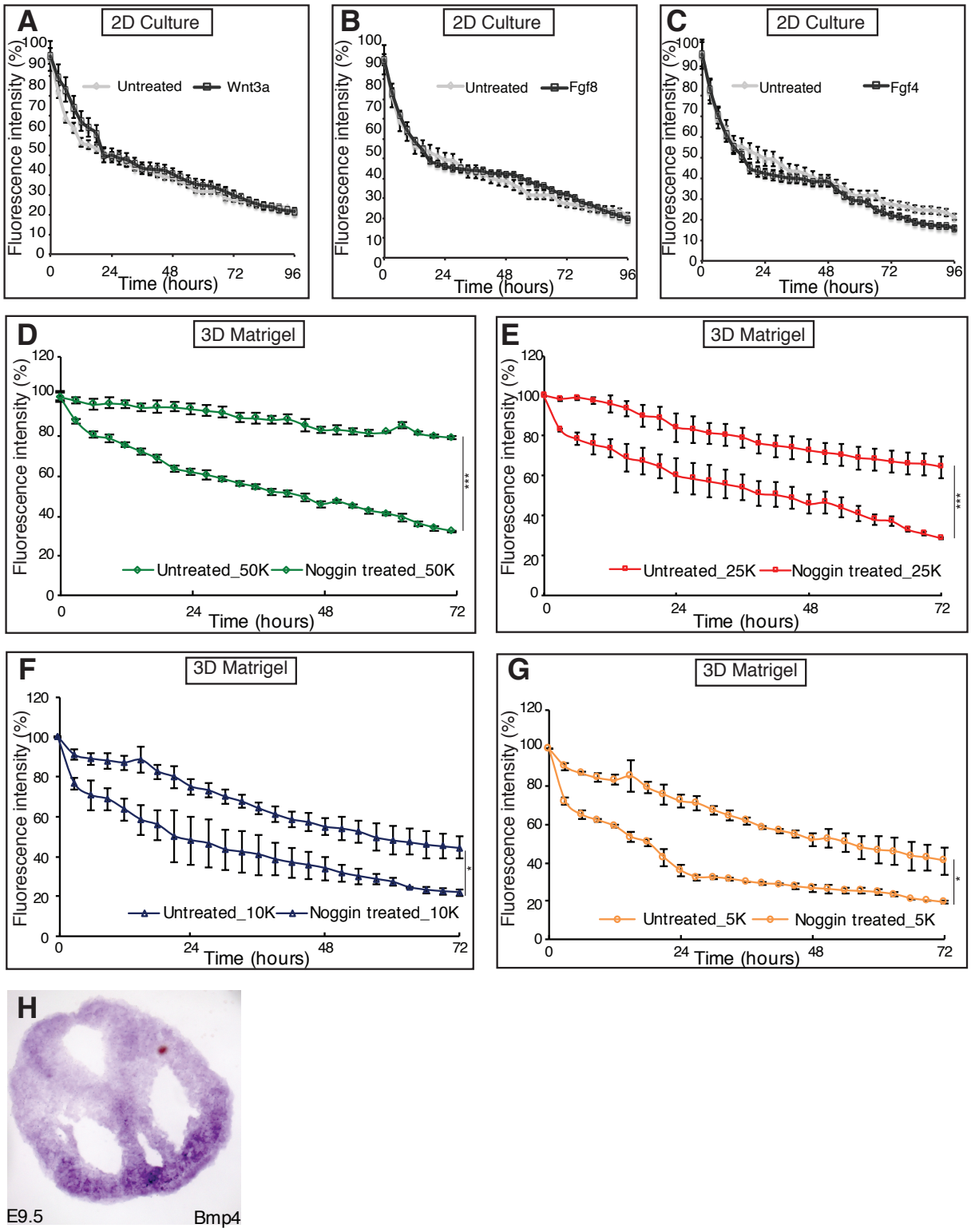
Supplementary Figures and Tables



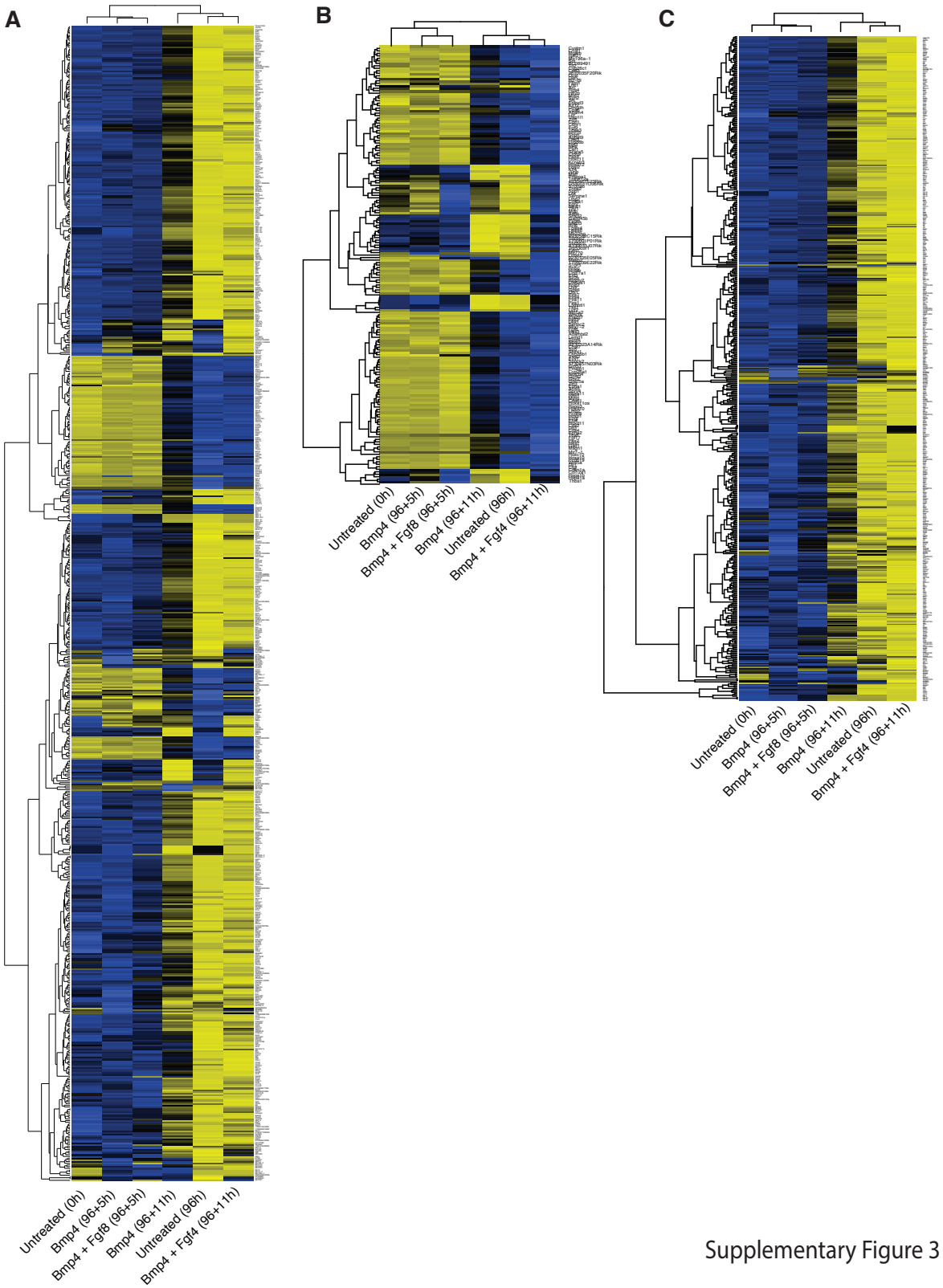
Supplementary Figure 1

A) Measurement of Pax2-GFP^{+ve} tailbud progenitor cell confluence over 96h in culture. Loss of fluorescence is not accompanied by a loss Pax2-GFP tailbud progenitors over 96h. B) qRT-PCR of renal markers in sorted Pax2-GFP^{on} cells which remain after 96h of co-culture with MEFs. mRNA levels were normalized to B2m and to Pax2-GFP^{off} cells at 96h (Mean +/- SD, n=3). Student's t-test was used to determine statistical differences.



Supplementary Figure 2

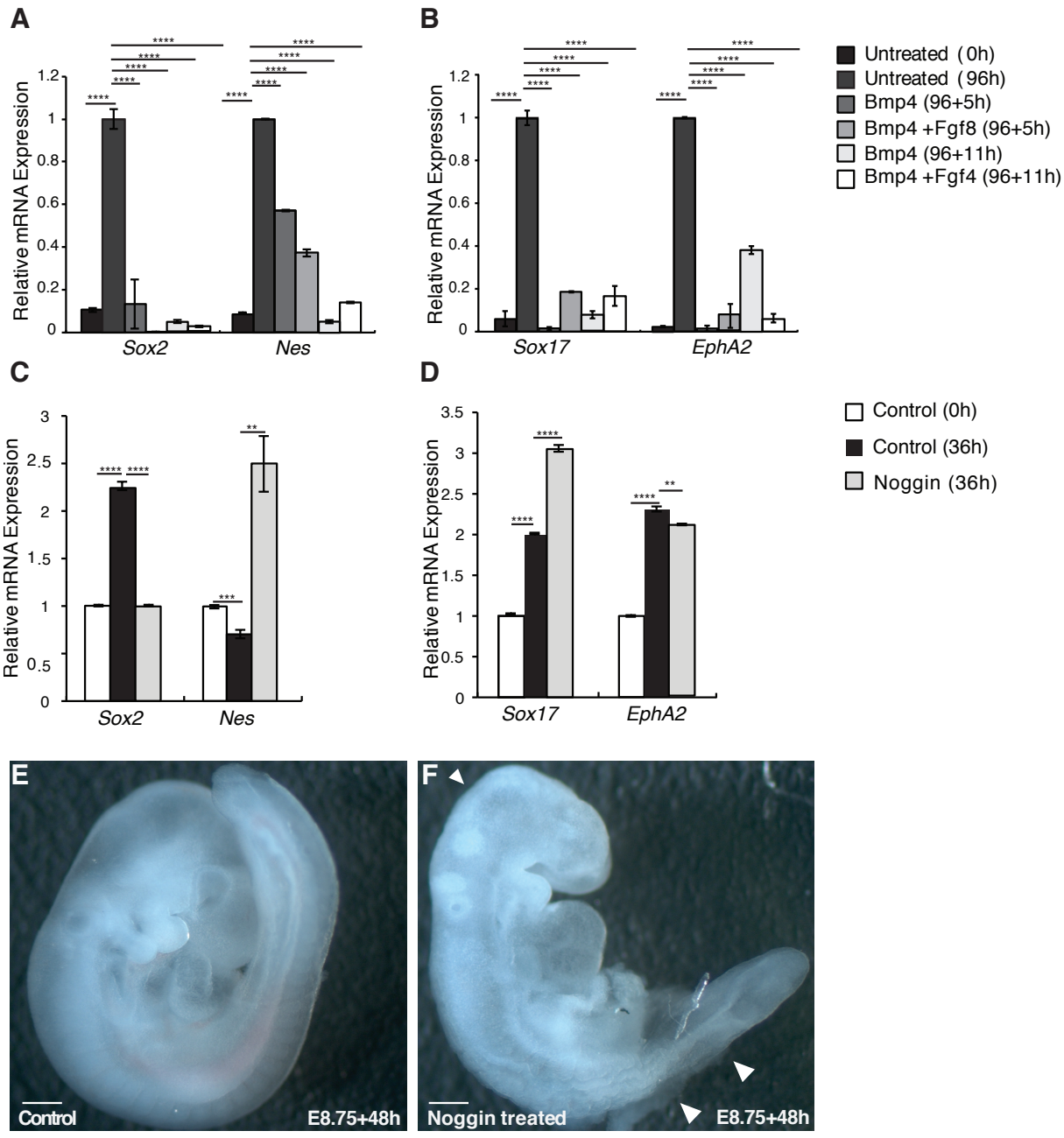
Measurement and quantification of Pax2-GFP expression over 96h in culture. A-C) Fluorescence intensity (%) of Pax2-GFP tailbud mesoderm progenitors treated with Wnt3A (A) Fgf8 (B) or Fgf4 (C). (Mean +/- SD, n=3). Mesoderm progenitors were co-cultured with MEFs. (D-G) Pax2-GFP^{+ve} tailbud mesoderm progenitor cells were plated in 3D matrigel cultures and GFP fluorescence was measured over 72 hours when treated with Bmp inhibitor Noggin at different densities. D) 50,000 cells (50K) E) 25,000 cells (25K) F) 10,000 cells (10K) G) 5,000 cells (5K). The statistical differences were calculated using Student's t-test. H) In situ hybridization of *Bmp4* shows the expression domain in the ventral mesoderm region of transversal sections of E9.5 embryo tailbud (20X).



Supplementary Figure 3

Supplementary Figure 3

A) Heatmap of RNA read counts of differentially regulated genes identified by pairwise comparisons performed between conditions, which were used to identify gene signatures. B) Heatmap of genes upregulated in Pax2-GFP^{on} cells (Pax2-GFP^{on} signature). C) Heatmap of genes upregulated in Pax2-GFP^{off} cells (Pax2-GFP^{off} signature).



Supplementary Figure 4

A-B) qRT-PCR of neural and hindgut lineage markers from cells untreated at 0h and 96h or treated with Bmp4 alone, with Fgf8 or with Fgf4 at 96h post initial plating for 5h or 11h respectively. Cells used for RNA extraction were sorted out from MEFs. mRNA levels are normalized to *B2m* and to untreated cells at 96h. (Mean +/- SD, n=3). One-way Anova was used to calculate statistical significance and all samples were compared to untreated at 96h.

C-D) qRT-PCR of neural and hindgut marker genes *Sox2, Nestin, Sox17 and EphA2* in *ex utero* cultured embryos treated or not with Noggin. mRNA levels were normalized to *B2m*. One-way Anova was used to determine statistical differences and all samples were compared to control at 36h. (Mean +/- SD, n=3)

E-F) Embryos dissected at E8.75 and cultured *ex utero* for 48 hours in the presence or absence of recombinant Noggin show abnormalities in somites, neural tube and tailbud development (arrowhead points to defects of somites, neural tube and tailbud); Scale bars-1000μm, n=3

Tables S1-S12. Pairwise comparisons of mesoderm progenitors in all conditions distinguishes a Pax2-GFPon (green) and a Pax2-GFPoff (black) signature

Click here to Download Tables S1 - S12

Tables S13-S16. Genes differentially regulated in the Pax2-GFPon (Table S13) or Pax2-GFPoff (Table S14) signatures, including those in the Pax2-GFPon signature identified as presomitic mesoderm associated (Table S15) and those in the Pax2-GFPoff signature distinctly associated with muscle, heart, blood, bone and endothelial cell signatures (Table S16).

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Supplementary Table S17

The primer sequences used for genotyping and qPCR analyses

Primers	Forward Primer	Reverse Primer
Pax2_geno	ACTGGTGTGAGAGGCGGGTTCTT	AAACAGGCAGAGTGGCTGAGTAT
Pax2GFP_geno	GCTGGCGAAAGGGGGATGTGCTG	AAACAGGCAGAGTGGCTGAGTAT
mPax2_qPCR	CGGTGGCACTGGGACGCAAGG	GAGGAAGCACAGGAGGGAAAG
mPax8_qPCR	GGGCTCTACCTACTCTATCAA	GGACCACTGCTGCTGCTCTGT
mGata3_qPCR	ACAGAAGGCAGGGAGTGTGTGAAC	ATGGTAGAGTCCGCAGGCATTG
mMesogenin1_qPCR	CCAGCTCCTTCTCTGGAGTC	AGCCAACATGCTGTAATCCA
mCdx4_qPCR	TTCTTCACCACTCCATCTGC	GAAGGATGCTTCCGTTTCTC
mBrachyury_qPCR	GCGGGAAAGAGCCTGCAGTA	TTCCCCGTTCACGTACTTCC
mNkx1-2_qPCR	TTCTGGACCTCATTCTTCCC	CTGGGAACCCTTATTAGCCA
mMyh6_qPCR	GAGGACCAGGCCAATGAGTA	GCTGGGTGTAGGAGAGCTTG
mCol3a1_qPCR	GGAGCCCCTGGACTAATAGG	ATCCATCTTTGCCATCTTCG
mGata6_qPCR	GAGCTGGTGCTACCAAGAGG	TGCAAAAGCCCATCTCTTCT
mActa2_qPCR	TGTGCTGGACTCTGGAGATG	GAAGGAATAGCCACGCTCAG
mHba-x_qPCR	CTGTCTGCTGGTCACAATGG	GGGAGGAGAGGGATCATAGC
mHbb-y_qPCR	CTTGGGTAATGTGCTGGTGA	GTGCAGAAAGGAGGCATAGC
mCdh5_qPCR	GTTGCCACATCTCAGGGAAT	CCTTCCTCCAGCTGTCACTC
mPax1_qPCR	CAGCAAACCTCGAGTTACCA	GGGCACGTTGTACTTGTCAC
mSox2_qPCR	AAGGGTTCTTGCTGGGTTTT	AGACCACGAAAACGGTCTTG
mNestin_qPCR	GGAACCCAGAGACTGTGGAA	CACATCCTCCCACCTCTGTT
mSox17_qPCR	GCCCTCATGAACTTGCTCTC	TACAGCGAGCCTCAGAGTGA
mEphA2_qpcr	ACTATGGCGGCTGTATGTCC	TCTAGCGCTCCATTCTCCAT

Supplementary Table S18

Antibodies used for immunocytochemistry and immunofluorescence staining

Antibody	Source	Dilution
GFP (Rabbit)	Invitrogen, a6455	1:200
Ter119 (Biotin)	Stemcell Technologies, 60033AD	1:100
Gata4 (Mouse)	Santa Cruz, sc-25310	1:50
Pax7 (Chicken)	Supernatant, DHSB	1:10