SUPPLEMENTAL FIGURES

Figure S1

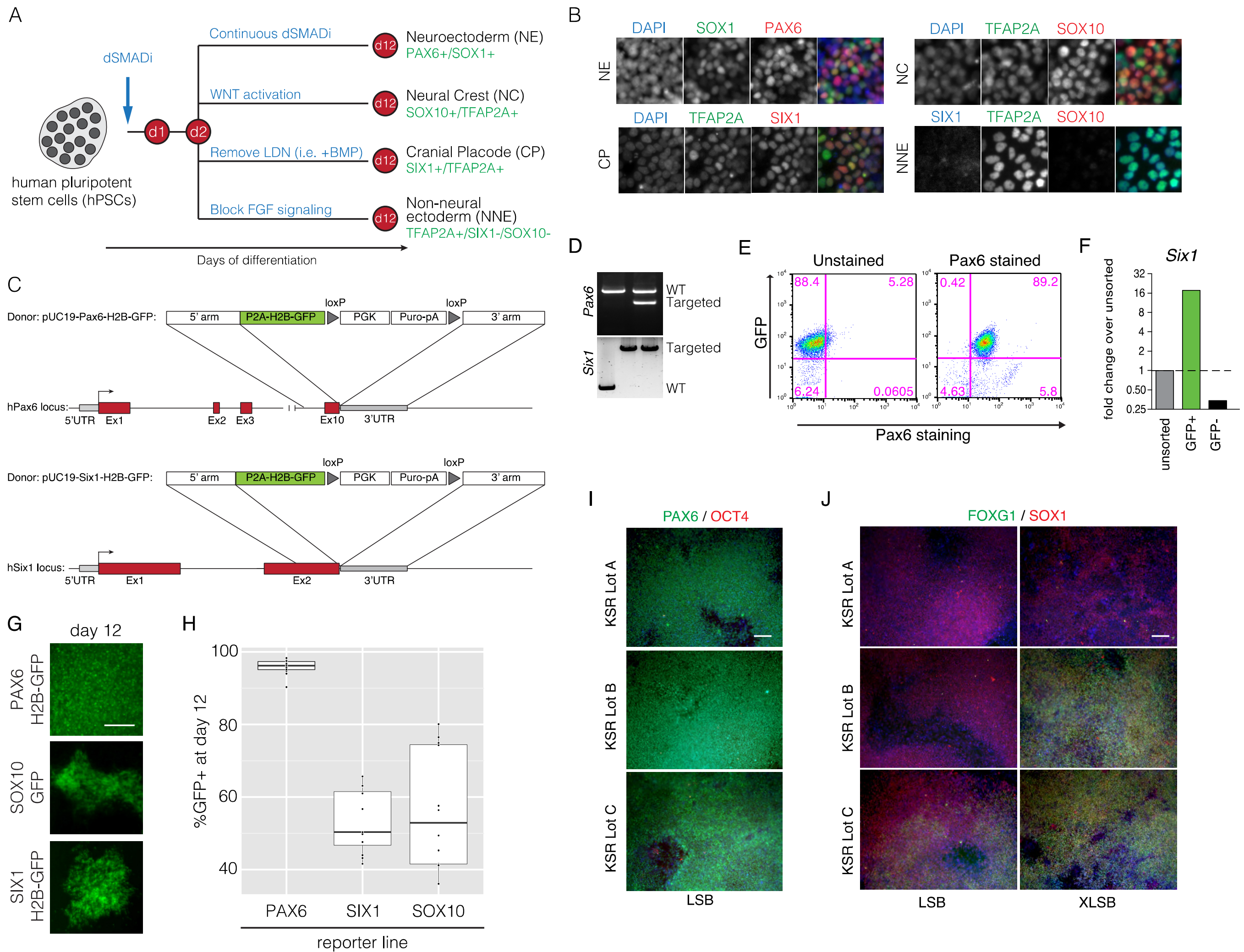


Figure S1. Related to Figure 1. Differentiation of hPSCs towards the four ectodermal lineages in serum replacement conditions affects regional patterning. A. Schematic of the general strategies for differentiation into the four ectodermal lineages using knockout serum replacement. B. Transcription factor expression combinations that distinguish cell identity at the end of the differentiation (i.e. day 12). C. Schematic of targeting the donor plasmid into the Pax6 locus. D. PCR of genomic DNA to identify clones that carry the reporter transgenes. E. Intracellular FACS for Pax6 and GFP during neuroectoderm formation. F. Quantitative PCR for Six1 in unsorted, Six1-GFP positive and Six1-GFP negative cells. G. PAX6, SIX1 and SOX10 GFP reporter line expression of GFP at day 12 of differentiation. H. Quantification of the percentage of GFP undergoing differentiation towards specific lineages. I. Several knockout serum replacement lots were tested for the ability to generate the neuroectoderm (three are presented), determined for the expression of PAX6 and downregulation of the stem cell factor OCT4. J. Similar to I, the expression of SOX1 is

consistent between lots of KSR, however the anterior marker FOXG1 is variable even in the presence of the WNT inhibitor, XAV-939. Scale bars 50μm.

Figure S2

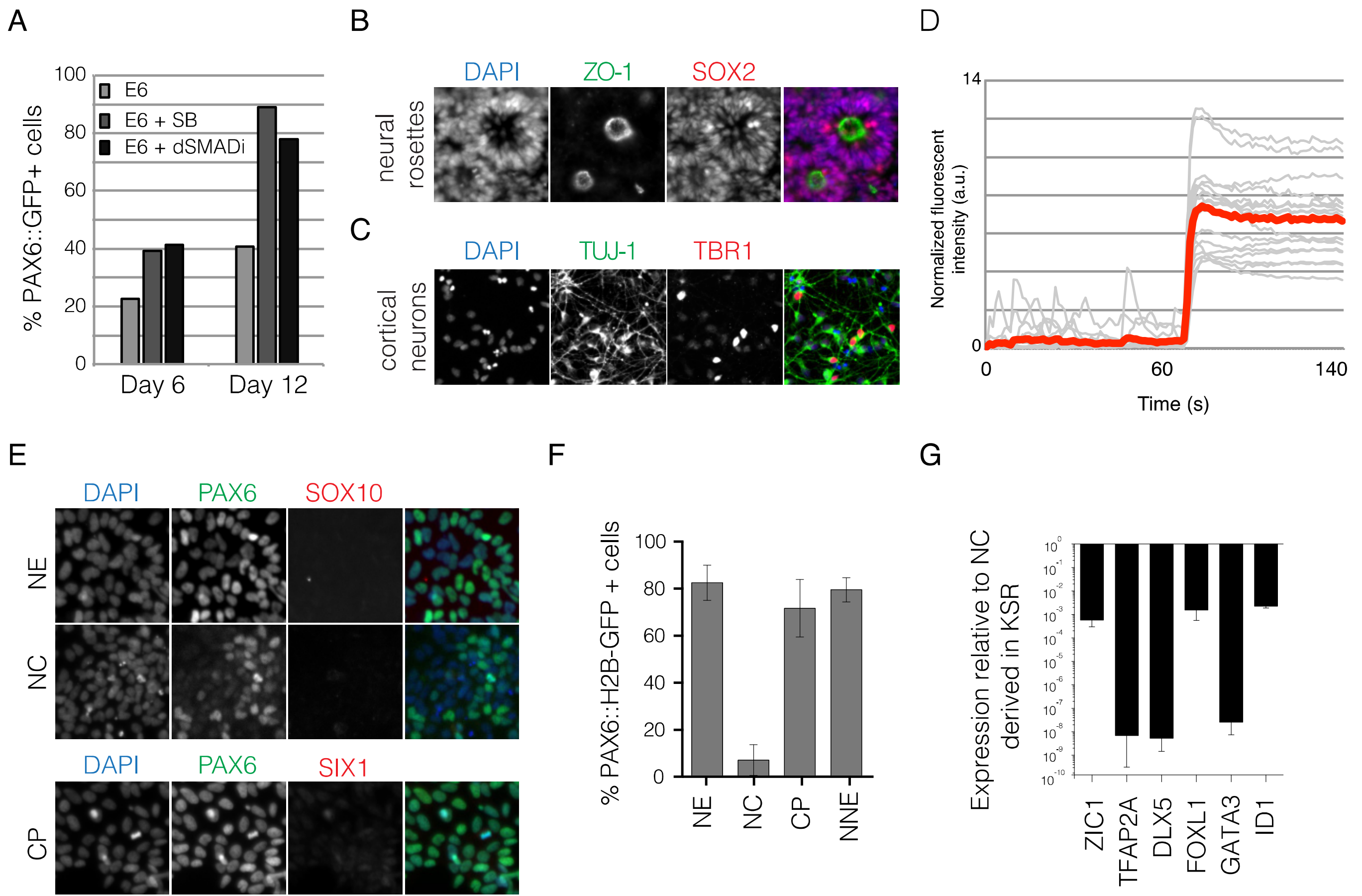
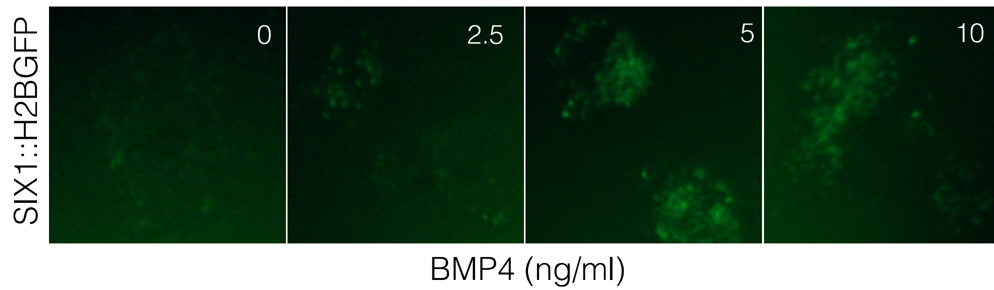


Figure S2. Related to Figure 1. Differentiation toward the ectoderm is skewed toward the CNS in the chemically defined system. A. Quantification of Pax6::GFP positive cells in the absence of small molecules, addition of SB, and the addition of LDN plus SB. B. Immunofluorescence staining of ZO-1 and SOX2 representing rosette stage cells. C. Differentiation of the rosettes into neurons stained with TUJ-1 and TBR1. D. Cortical neurons (day 50 of differentiation) exhibit response to glutamate. E. Immunofluorescence of SOX10 and SIX1 with PAX6 in the differentiation towards neural crest and placode, respectively. F. Quantification of PAX6::H2B-GFP expression in E. G. Quantitative PCR of general immediate early genes during the differentiation of neural crest in KSR and E6.

Figure S3

A



B

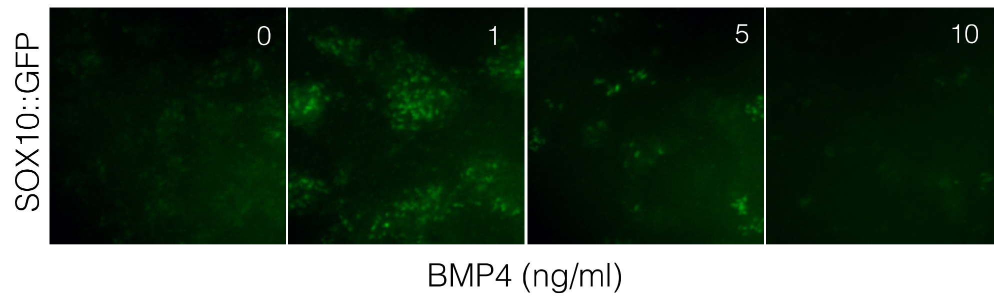
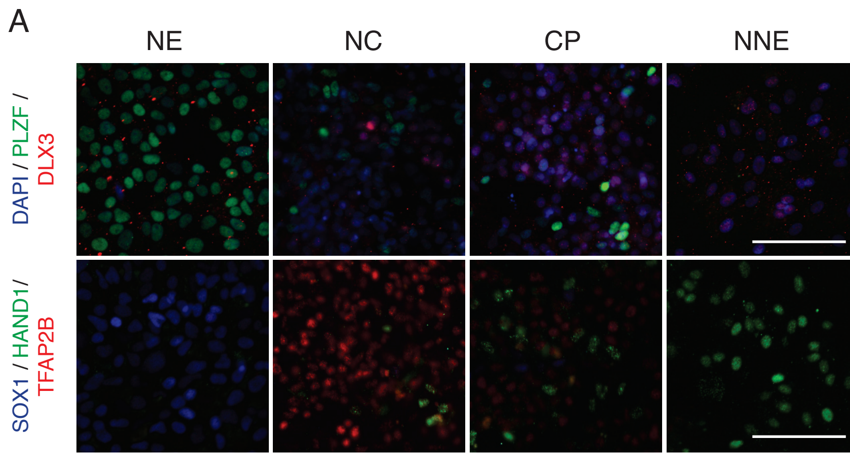


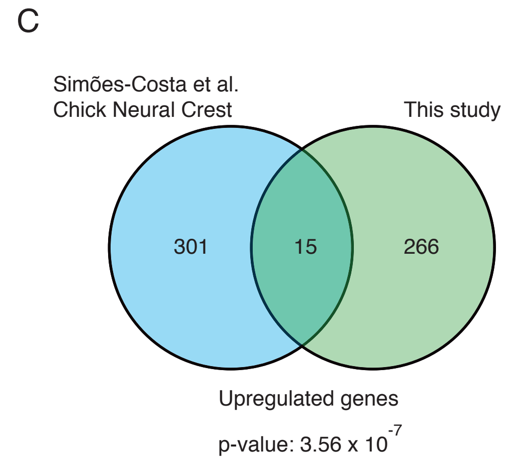
Figure S3. Related to Figure 2. Expression of non-CNS fates are induced by a gradient of BMP. A. Live images of the CP marker SIX1::GFP reporter line during the differentiation towards placode on day 12. B. Similar to A, using the NC marker SOX10::GFP reporter during differentiation towards neural crest on day 12.

Figure S4

A



C



B

NE NC CP NNE

%SOX1 (0–100)

%PLZF (0–100)

%DLX3 (0–100)

%TFAP2B (0–100)

%HAND1 (0–100)

BJ1 (iPSC) | HUES8 | H9 | MRC5 (iPSC)
H1 | MEL1 | HUES6 | SeV6 (iPSC)

D

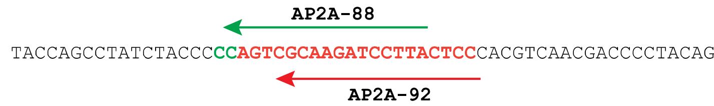
List of common known genes

ACSL6
EBF1
HCFC2
ITGA4
KAL1
LIMCH1
LSAMP
NRP2
PAX3
RFTN2
RXRG
SOX10
SOX5
TFAP2A
TFAP2B

Figure S4. Related to Figure 3. Differentiation strategies towards the ectodermal lineages are applicable to other pluripotent stem cell lines. A. Representative images of immunofluorescence staining on differentiations of the ectoderm. B. Quantification on the percentage of cells positive for particular markers during the differentiation. C. Comparative analysis of a dataset of purified chick neural crest compared to the purified human neural crest cells in this study. The overlap significance was tested using the hypergeometric distribution. D. The list of genes in common from the Venn diagram in C. Scale bars 50μm.

Figure S5

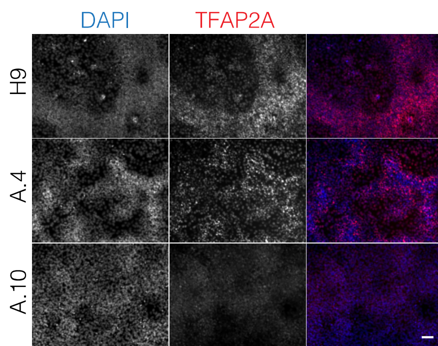
A



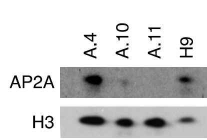
B

	DNA mutation	Protein mutation
wildtype allele 1	CCCTACCAGCCTATCTACCCCCAGTCGC------AAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	S69fsX
AP2A.4 allele 1	CCCTACCAGCCTATCTACCCCCAGTAGATAGGGTAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	
wildtype allele 2	CCCTACCAGCCTATCTACCCCCAGTCGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	S69_D71del
AP2A.4 allele 2	CCCTACCAGCCTATCTACCCCCAG---------CCTTGCTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	
wildtype allele 1	CCCTACCAGCCTATCTACCCCCAGTCGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	Q68fsX
AP2A.10 allele 1	CCCTACCAGCCTATCTACCCCC-------------------CCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCANCACCC	
wildtype allele 2	CCCTACCAGCCTATCTACCCCCAGTCGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	Q68_Y73del
AP2A.10 allele 2	CCCTACCAGCCTATCTACCCC------------------TCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	
wildtype allele 1	CCCTACCAGCCTATCTACCCCCAGTCGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	Q68fsX
AP2A.11 allele 1	CCCTACCAGCCTATCTACCCCCA--CGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	
wildtype allele 2	CCCTACCAGCCTATCTACCCCCAGTCGCAAGATCCTTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	S69fsX
AP2A.11 allele 2	CCCTACCAGCCTATCTACCCCCAGT----------TTACTCCCACGTCAACGACCCCTACAGCCTGAACCCCCTGCACGCCCAGCCGCAGCCGCAGCACCC	

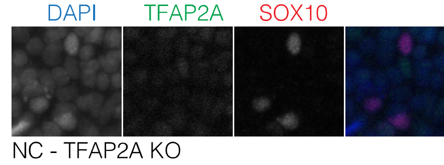
C



D

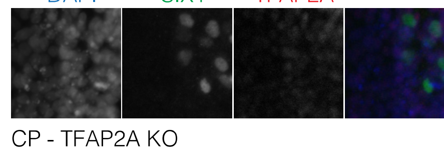


E



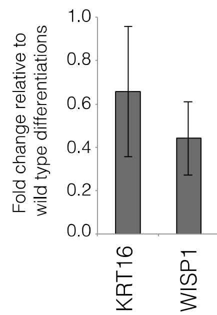
NC - TFAP2A KO

F



CP - TFAP2A KO

G



H

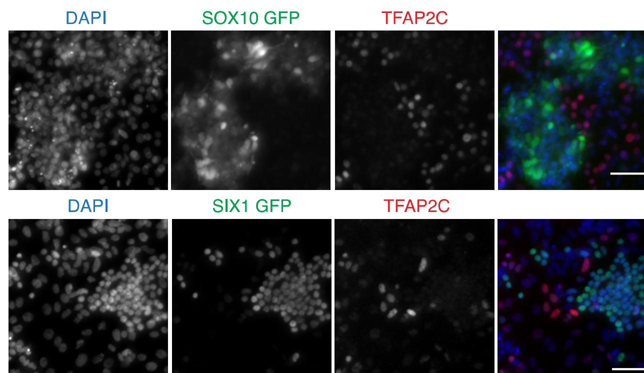


Figure S5. Related to Figure 5. Validation and characterization of TFAP2A knockout cells. A. Schematic of the guide RNAs targeting TFAP2A. B. Sequencing results of three potential clones indicate two had frameshift mutations (AP2A.10 and AP2A.11) and the other clone (AP2A.4) harbors a frameshift mutation on one allele and a 9bp on the other allele. C. Immunofluorescence of TFAP2A in different ES clones treated with 20ng/ml BMP4 for 3 days. D. A western blot was performed to validate the loss of protein expression in the mutant lines after 3 days of BMP4 treatment. E. Immunofluorescence of SOX10 positive cells lacking TFAP2A expression on day 12. F. Immunofluorescence of SIX1 positive cells lacking TFAP2A expression on day 12. G. Quantitative PCR analysis of the expression of *KRT16* and *WISP1* during the derivation of NNE from the TFAP2A KO cells compared to wildtype. H. Immunofluorescence staining of TFAP2C in combination with Sox10 GFP and Six1-H2B GFP demonstrating little overlap. Scale bars 50μm.

Figure S6

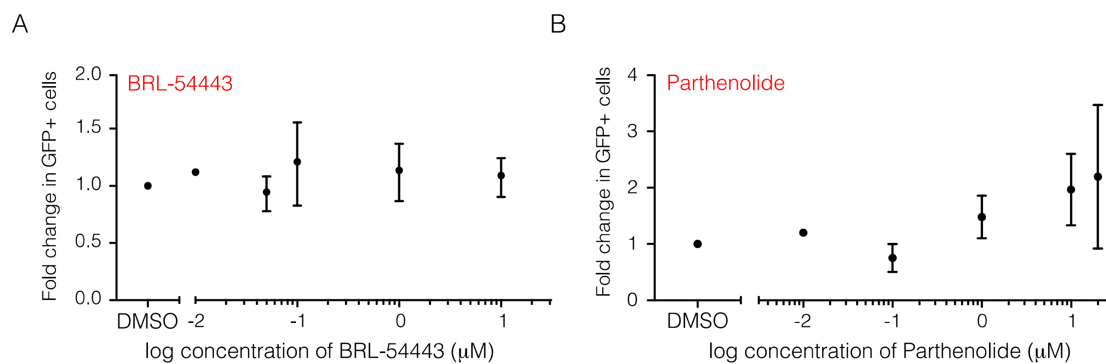


Figure S6. Related to Figure 6. Other hit validation from the chemical screen did not produce a significant difference in SIX1::GFP expression. A. Quantification of SIX1 expression after the treatment of differentiating placode cells with BRL-54443. B. As in A, but with Parthenolide.

Figure S7

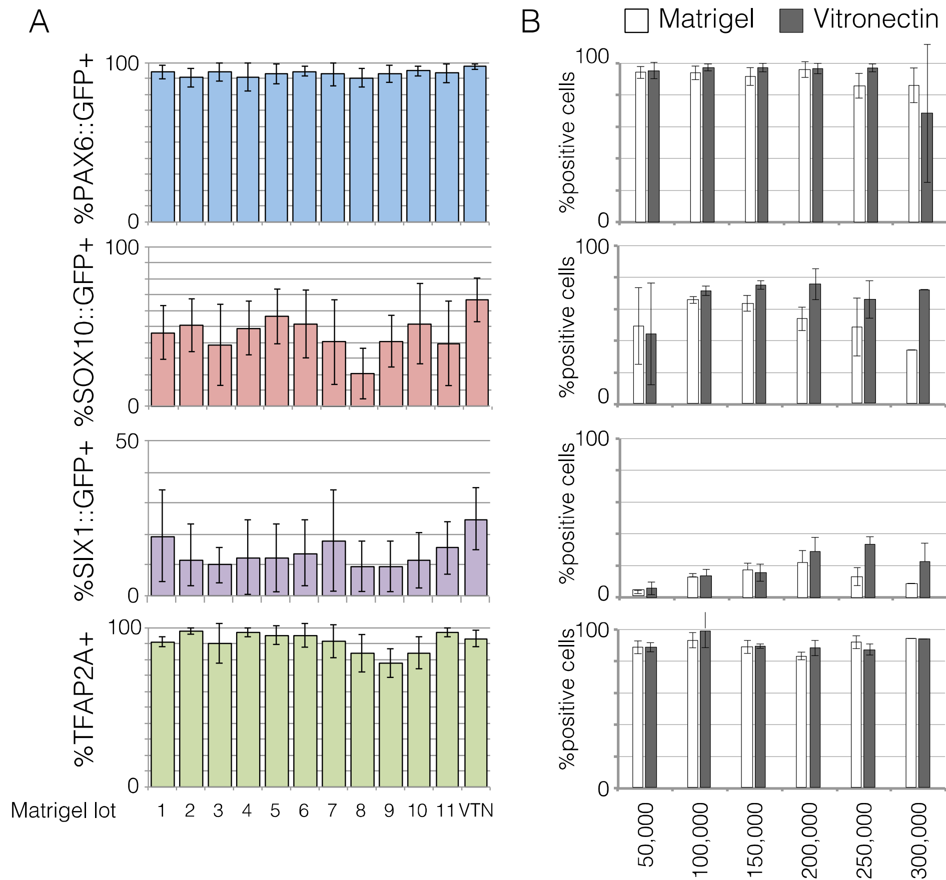


Figure S7. Related to Figure 7. Cell density and plating substrate have minimal impact on the derivation of the ectodermal lineages. A. Quantification of reporter GFP lines and staining for TFAP2A for NNE during the differentiation of the four ectodermal lineages was performed on Vitronectin and eleven independent lots of Matrigel. B. Analysis of GFP positive cells or staining for TFAP2A during the ectodermal differentiations plated with either Matrigel (white bars) or Vitronectin (grey bars) with variable starting cell numbers (per cm^2).