

Table S1: Primers

Gene	Sequence	Rationale
<i>GAPDH</i>		
Forward	CTCCTCCACCTT TGACGCTG	Housekeeping
Reverse	TCCCTGTGTGCTCTTGCTG	
<i>TFAP2A</i>		
Forward	TAAAGCTGCCAACGTTACCC	Non-neural ectoderm
Reverse	GCACACGTACCCAAAGTCC	
<i>DLX3</i>		
Forward	AGCCTCCTACCGGCAATAC	Non-neural ectoderm
Reverse	TCCGGCTCCTCCTTCAC	
<i>DLX5</i>		
Forward	GCTAGCTCCTACCACCAGTAC	Non-neural ectoderm
Reverse	GGTTGCCATTACACATTCTCA	
<i>OCT4</i>		
Forward	ACCCCTGGTGCCGTGAA	Pluripotency
Reverse	GGCTGAATACCT TCCCAAATA	
<i>K18</i>		
Forward	CTCTGTCCAGGCGCCCAGCT	Surface ectoderm
Reverse	CCCCTGAAGCTGGTGGAG	
<i>SOX1</i>		
Forward	CAACTCGCAGGGCTACATGA	Neural ectoderm
Reverse	CTCCGACTTCACCAGAGAGC	
<i>SIX1</i>		
Forward	GGTTTAAGAACCGGAGGCAAA	Pan-placodal ectoderm
Reverse	TGCTTGTTGGAGGAGGAGTTA	
<i>Brachyury</i>		
Forward	ATGAGCCTCGAATCCACATAGT	Mesoderm
Reverse	TCCTCGTTCTGATAAGCAGTCA	
<i>FOXA2</i>		
Forward	GAGCAGCAGCGGGCGAGTTA	Endoderm
Reverse	CCCAGGCCGGCGTTCATGTT	
<i>EYA1</i>		
Forward	ACAGCCGACGGGTCTTTAA	Pan-placodal ectoderm
Reverse	TTGGTCGTGGGCTGAAACTA	
<i>PITX2</i>		
Forward	AGTCCGGGTTTGGTTCAAGA	Dental epithelial and Oral epithelial
Reverse	TGGATAGGGAGGCGGATGTA	
<i>PITX1</i>		
Forward	GTGGCGTAAGCGGAGCGTAA	Oral epithelial
Reverse	GACAGCGGGCTCATGGAGTTGAAG	
<i>AMBN</i>		
Forward	AGAGCACAGTGCATGTCAGC	Dental epithelial and Ameloblast
Reverse	AAGAACGGCACTGCAAACT	
<i>SP6</i>		
Forward	GGACTTCTCGCAGGGCTATG	Dental epithelial and Ameloblast
Reverse	GCTGCAGGAGCTTGAAAAG	

Table S2: Antibodies

Antibodies	Supplier	Identifier
TFAP2A	Santa Cruz	sc-12726
OCT4	Santa Cruz	sc-5279
SIX1	Proteintech	10709-1-AP
PITX2	Santa Cruz	sc-390457
PITX1	Santa Cruz	sc-271435
AMBN	Santa Cruz	sc-271012
AMELX	Santa Cruz	sc-365284
SOX2	Abclonal	A0561
Ki67	Abmart	TW0001

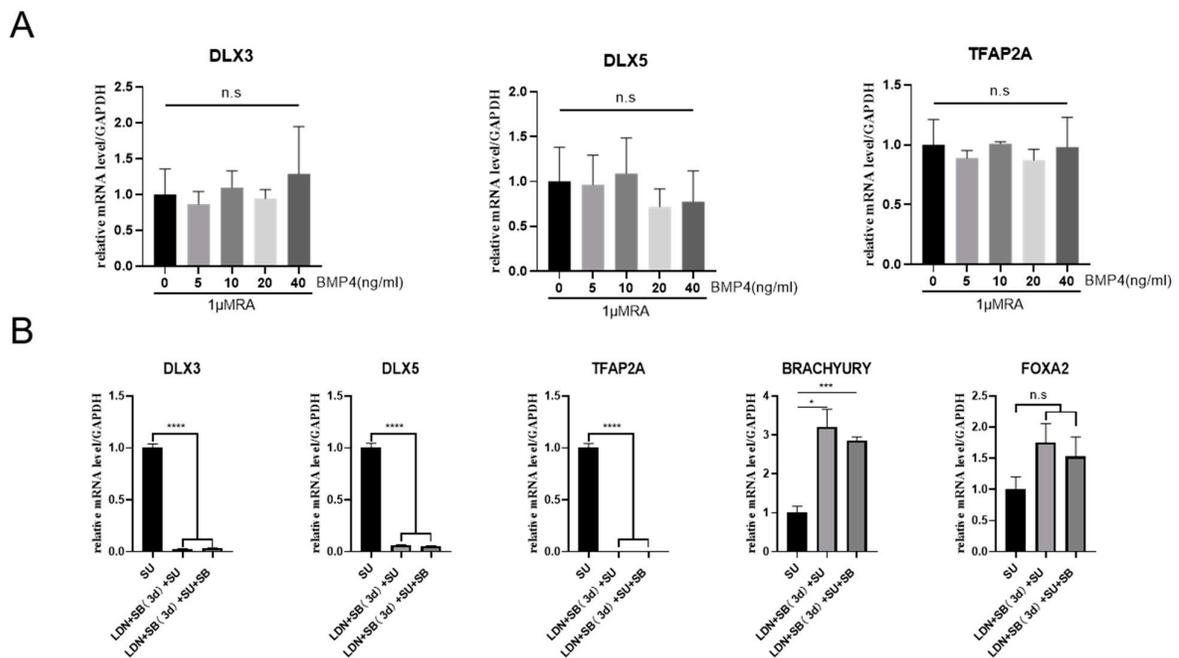


Figure S1. Selection of induction conditions for acquisition of NNE fate. **A.** qRT-PCR analysis revealed the expression of non-neural ectoderm (NNE) markers TFAP2A, DLX3, and DLX5 in response to the stimulation of 1 μ M RA in combination with BMP4 at various concentrations. **B.** qRT-PCR analysis revealed the expression of TFAP2A, DLX3, DLX5, Brachyury, and FOXA2 subsequent to the addition of LDN and SB during the early stages of differentiation. Data shown are mean \pm SEM of three independent experiments (three technical replicates per biological replicate). *, $p < 0.05$, ***, $p < 0.001$, ****, $p < 0.0001$ and n.s, not significant.