

Supplementary Material

mE1

TTTGACGCACATGTATTCATTAAGTGGATGAATGAATGAATGGGCCGTGCTCTCTGAGTCTGAACTGGA
TGTCCAAGCATTATGTGCACTGGCTGTATACTCAGTGCTATGTACTCTTTGCTGATTTTCCAGACACTCC
TGGGAGGTCAGGGCTGCTTGGGGCTACCAAGTCCGTGACTATGCTGAGGTCACACAGTTAGCCTAGAC
CAAGCCACCACTTCTGACTGAACAGCATCCGACTCTGTAGTGTAACCCACTGACCACATTTCTCAGCCTC
CAACCCACATGGACCCGACCTGCCTTGGCCAGACCTCCTTACTCTCCTGGTCCCATAGTCTCCTTGTA
CTTCTCCCCCAGCCAAAGGCATCTGCCCCAACCCCTCAGCCGGCACAGGCCAAGACCAAGGATGGAAGC
CATCATTTCCATACAGCTGTCCCTGGGATCTGAGCCTGGGGAGCCCCCTCATTTTTATTGAAAAGGCTG
AATTGTCTTGGCATTGTTGGGGGCTGCCTGGGCGGGGCTGGGGAGGCTATTATCCTACATTATCTCACT
CCCCTGCCCTGAAAAGAGCCTTGAAGTCCCTCCCTCCCTCCGAGCCTTTTACACTTAATTACCTCAC
TCCTCACCAGCAGCCTTCAGAGGGACCACAACCTGGGGCGTGAGTAATCCCCCCCCCACACCCAGCCTG
TCTGCTAGGAGGGAATCACCTGTGGCCTGGAAAGAAAGGAACAGACTGTGTATGTAGGCCCTTCCAGC
CCGAGGACTCTGGGGCTGCTGCCACAAGGGGCAGGCGCTGACCCAGACTCCCCGATGGCCTTTGCCA
GTCCTGGTGTGAGGCTCTCCACCTTCTGGTCCCAGATTGTGAGCTGGGACTTTCCAGGCTGCAGAAGA
CATGTATACACGTGTGCGCGCGCGCATGCATGTGCACACACACACACACACACACACACACACACAC
GTCTTACTGCCTTGCTTTCAAAACAGGAGGCAGGCTGAACTCCATCCCCAGAGCAGCCTGCATCACGCT
GTGCAAACATGGCTTCCAGGGTTGAAGCTCCCTCATCTTGACAGCAAATGCCTTGTGATCCTTTCTTTCCC
CTGGGCTCTACTCTTTAGTGTGCTGGAAGGGTCACTGGGCCCTGAGAGCCCTTTCTTATTCTCCAGCTGC
TAGACATAGACACTGTCTGTCAAAGACAGGGAAAGAGTGAGGGAGGGAGGGAGTCTGAATGCATCT
TCCTTTGCTAGTGTTGGGCCAGGTCTACTGGGGAGCCATTGCTGGAATATAGTAAGTGGGAGTGAA
GAGCCCATGGTGTAGGAATGGACCTGCTGGGAAGGTCTGCTCTGGCAAAGTGGATCCTTGGATCAGCC
TCCCCTAAGCCATCCTATCCCAGGCTGTAGGCTCCTGGCTTTGGTGCTCAGGAGCCCTGATGACCTGGA
GCATCTCAGGTCAAAGCTGCTTCTTTGCCCCAGTGGCCAGGTTAGGGAGACCTCCATCACTGAGAGT
CACTAATGACACTCCCATGTACCATGGGTACCACTGTACCCCCGCCCCCAGTAAGATGGTGCAG
CTGGGGCCGAGAAGCAGCTGT

cE1

GGTACCGGCACACGGGTCCCCAAAGTCCCTCGCCAGCCCCTCTCTCCCAGCACGGCACGGGGGAACA
ATCGCCTCCGAGCCCCGCGAGCGAGCAGGAATTATTTCCATACAGCTGCTCGGGAAGGCAGGGATGG
GGATGCAGCCATACCCAACCGCGCTCCGGGAGAACGCCCCGGGCCTTTCTTCTGGCTCTCCCCCTCCC
CGCCGTGGTGGGGGCAGGTTTGGGGGGGCCAGCCGCCCTGCAGTCAGCAATATTAGCACAGCTCCG
CAGGACTGATTGCAGGTTATTAACATACATTATCTCAGTCCCCTGCCTCTGAAAGGAGCCAGTTCCTATT
CCCCCTCTCCAGCCCTCCCTCCCCCTGTCTTTTACACTTAATTACCTCCCTTATTACACCACAGCCTGGAG
AGAGAAAAAATCACAGGGCAGCGGGCAGGCAGGCAAGCTCGGCCCACTAGGGCGTAAGTAATCGCC
CGTTCCTCGAG

M1 – The underlined indicates the mutated motifs

GGTACCGGCACACGGGTCCCCAAAGTCCCTCGCCAGCCCCTCTCTCCCAGCACGGCACGGGGGAACA
ATCGCCTCCGAGCCCCGCGAGCGAGCAGGAATTcacacacacacacacCTCGGGAAGGCAGGGATGGGA
TGCAGCCATACCCAACCGCGCTCCGGGAGAACGCCCCGGGCCTTTCTTCTGGCTCTCCCCCTCCCCGCC
GTGGTGGGGGCAGGTTTGGGGGGGCCAGCCGCCCTGCAGTCAGCAcacacacacacacacCGCAGGACT
GATTGCAGGTTATTAACATACATTATCTCAGTCCCCTGCCTCTGAAAGGAGCCAGTTCCTATTCCCCCTCT
CCCAGCCCTCCCTCCCCCTGTCTTTTACACTTAATTACCTCCCTTATTACACCACAGCCTGGAGAGAGAAA
AAATCACAGGGCAGCGGGCAGGCAGGCAAGCTCGGCCCACTAGGGCGTAAGTAATCGCCGTTCTCT
CGAG

M2 - The underlined indicates the mutations in Ascl1 sites.

GGTACCGGCACACGGGTCCCCAAAGTCCCT~~caaagagtc~~cctaCTCCCCAGC~~caaagagtc~~cctaACAATCGCCT
CCGAGCCCCGCGAGCGAGCAGGAAT~~aaaTTC~~~~caaagagtc~~cctaGGGAAGGCAGGGATGGGGATGCAGCC
ATCACCCAACCGCGCTCCGGGAGAACGCCCGGGCCTTTCTTCTGGCTCTCCCCCTCCCCGCCGTGGTG~~c~~
~~aaagagtc~~cctaGGG~~caaagagtc~~cctaTGCAGTCAGCA~~AgggT~~AgcacaggaccctaGGACTGATTGCAGGTTATT
AACATACATTATCT~~C~~~~caaagagtc~~cctaTGAAAGG~~caaagagtc~~cctaTCCCCCTCTCCCAGCCCTCCCTCCCCCT
GTCTTTTACACTTAATTACCTCCCTTATTACACCACAGCCTGGAGAGAGAAAAATCAC~~caaagagtc~~cctaG
GCAGGCAAGCTCGGCCAACTAGGGCGTAAGTAATCGCCCGTTCCTCGAG

Table S1: Primers used for PCR amplification of control fragments spanning the 62 kb Scrt2 locus for 3C assay.

Primer	Sequence
FRAG1-F	5' TAGAACAGGAGGGGACACAG
FRAG1-R	5' GCTGCGTGTGAGAAATGGGG
FRAG2-F	5' CACTGTAGGGGCTGCAAAGAG
FRAG2-R	5' AGAGGAGGAAAGTGAAGGAGAG
FRAG3-F	5' GTTCCAGCACCAAACCTCCC
FRAG3-R	5' CATCCCAATGGCAGAGAAAC
FRAG4-F	5' TAATTAAAGCGGCTCAGAACG
FRAG4-R	5' ACGAAGGTGTAGCTGCAGTG
FRAG5-F	5' CACTGCAGCTACACCTTCGT
FRAG5-R	5' GTCCCCACTGCATCTCCATG
FRAG6-F	5' GCAATAAGGCTGAAGGATTTTC
FRAG6-R	5' TTCAGCAGAAGGGACAGAAT
FRAG7-F	5' ATTCTGTCCCTTCTGCTGAA
FRAG7-R	5' CTTCTCCCCTTCCATTCTC
FRAG8-F	5' GCCTAACACAGCCTGGTATG
FRAG8-R	5' CTTTGGCTCACGGTCTCAT
FRAG9-F	5' GTTTATCCCATCCCAAGAGC
FRAG9-R	5' GAAGACGGAGCAGGAAGACT
FRAG10-F	5' CAGCGATTCTATGCAACCCC
FRAG10-R	5' GCATATCACCATGTCCCTCC
FRAG11-F	5' CCTGCAGTGTGATTTACGGG
FRAG11-R	5' CAAGGTAAACTGCGGAGAG

Table S2: Primers used for 3C-qPCR.

Primer	Sequence
Constant-F	5' TGACGCAGGTCAAGAGTCAA
Peak1-F	5' TGGATCCCGCAAACCAATG
Peak2-F	5' GGGCTGGCTCTGTCTGTG
Peak3-F	5' CTGGCAGAGAGCAGGATGT
Peak4-F	5' GGCTTCAGCTGGAAAAACAG
Peak5-F	5' ACAGGTGACCTTGGACTTGG
Peak6-F	5' ATCCTCATCCCTCCCTGTG

Table S3: Primers used to clone the fragments used in this work.

Name	Sequence
mE1-F	5' GGTACCCACATGTATTCATTAAGTGGATGA
mE1-R	5' CTCGAGCAGCTGCTTCTCGGCCCCAGCTGCAC
cE1-F	5' GGTACCGGCACACGGGTCCCCAAAGTC
cE1-R	5' CTCGAGGAACGGGCGATTACTTACGCC
USE-F	5' TCTTACGCGTGCTAGCCCCTCAGCACCTTGGGAGC
USE-R	5' ATCCGAGATCTCGAGCCCACTCCCGTTCACGCCTG
USE1-F	5' TCTTACGCGTGCTAGCCCGACCTGCTGCTTTCCACCTC
USE1-R	5' ATCCGAGATCTCGAGCCCTGGGACGGGCAAGGAGCA
USE3-F	5' TCTTACGCGTGCTAGCCCCTGCAAACGTGCCTTCAAGA
USE3-R	5' ATCCGAGATCTCGAGCCCACTCCCGTTCACGCCTGGGGC
Ep2-F	5' TCTTACGCGTGCTAGCCCAGGAGCGCATCATGCAGGCACT
Ep2-R	5' ATCCGAGATCTCGAGCCCGCTCACAGCCCTGCTTCCCCTC
Ep4-F	5' GTACCGAGCTCTTACGCGTGACCCAAACCTCCAGGAC
Ep4-R	5' ATCTCGAGCCCGGGTCGACGCACCAAGACACCCTGGGAC

Table S4: oligos used as guides in CRISPR/Cas9 or dCas9 assays.

Name	Sequence
cE1 sgRNA1-F	5' AGTCGCCTGCCCCGTCCCAGGCACAC
cE1 sgRNA1-R	5' AAACGTGTGCCTGGGACGGGCAGGC
cE1 sgRNA2-F	5' AGTCGGCCGTGGTGGGGGCAGTTT
cE1 sgRNA2-R	5' AAACAAACCTGCCCCACCACGGCC
cE1 sgRNA3-F	5' AGTCGAGGCAAGCTCGGCCCACTA
cE1 sgRNA3-R	5' AAAGTAGTTGGGCCGAGCTTGCCTC
cE1 sgRNA1 Scrambled-F	5' AGTCGGCCAACGCCTCGTCCGCACC
cE1 sgRNA1 Scrambled-R	5' AAACGGTGCGGACGAGGCGTTGGCC
cE1 sgRNA2 Scrambled-F	5' AGTCGGGTTGTGGTGCGCGGTAGGC
cE1 sgRNA2 Scrambled-R	5' AAACGCCTACCGCGCACCACAACCC
cE1 sgRNA3 Scrambled-F	5' AGTCGGCAAGCCGAACCTAACGTGC
cE1 sgRNA3 Scrambled-R	5' AAACGCACGTTAGGTTTCGGCTTGCC

Data availability

Data	Repository	Accession	Reference
H3K27ac and IgG of HH23 neural tubes	Github	https://github.com/goescp/Scrt2 data	Current work
Epigenetic profile of E11.5 mouse neural tube	ENCODE Project https://www.encodeproject.org/	ATAC-seq ENCSR215ZYV; H3K4me3 ENCSR215ZYV; H3K27ac ENCSR215ZYV; H3K4me1 ENCSR215ZYV; H3K9ac ENCSR215ZYV; H3K4me2 ENCSR215ZYV; H3K27me3 ENCSR215ZYV	Luo et al., 2020