

## **Supplementary Materials for**

### **Determination of Trunk Neural Crest Cell Fate and Susceptibility to Splicing Perturbation by the DLC1-SF3B1-PHF5A Splicing Complex**

Zhengfan Zheng, Suisui Guo, Yanxia Rao, Man Ning Hui, May Pui Lai Cheung, Hoi Yau Tam, Kelvin K.W. Wong, Rakesh Sharma, Jessica Aijia Liu and Martin Cheung\*

\*Corresponding author. Email: M.C. [mcheung9@hku.hk](mailto:mcheung9@hku.hk)

The PDF file includes:

Figs. S1 to S8

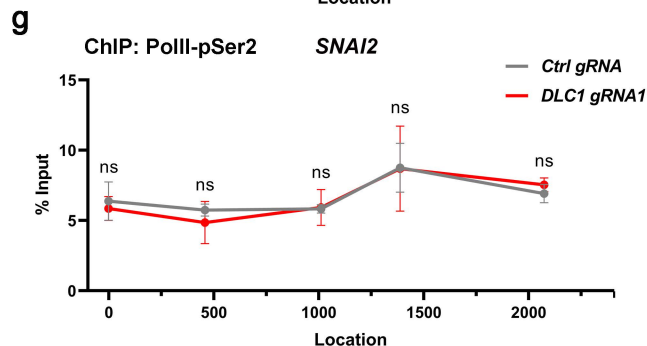
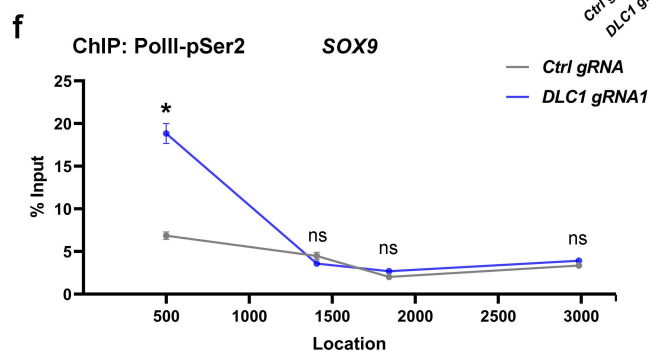
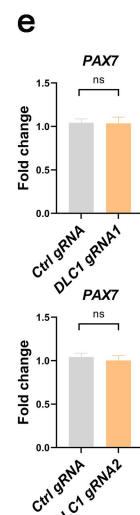
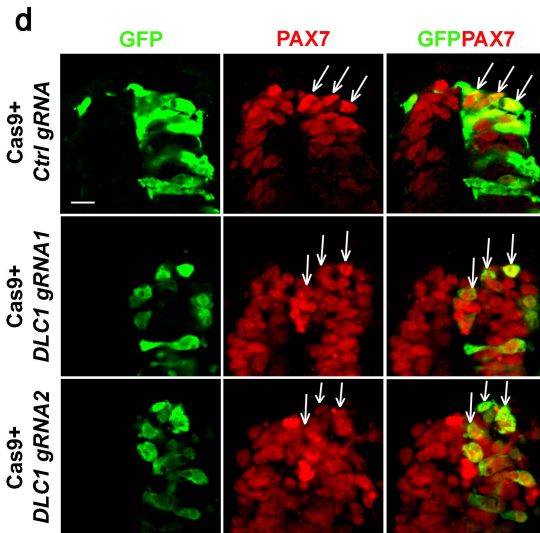
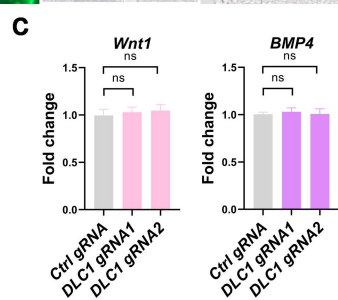
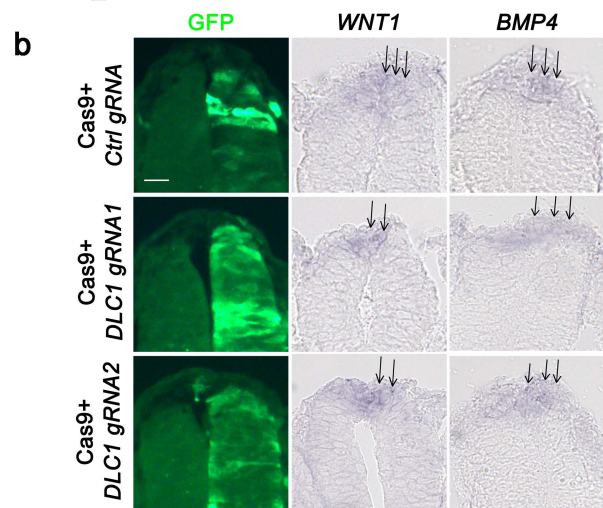
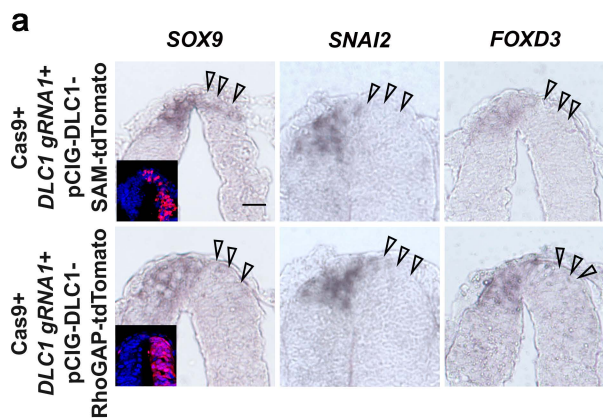
Tables S1 to S12

Other Supplementary Materials for this manuscript include the following:

Dataset 1

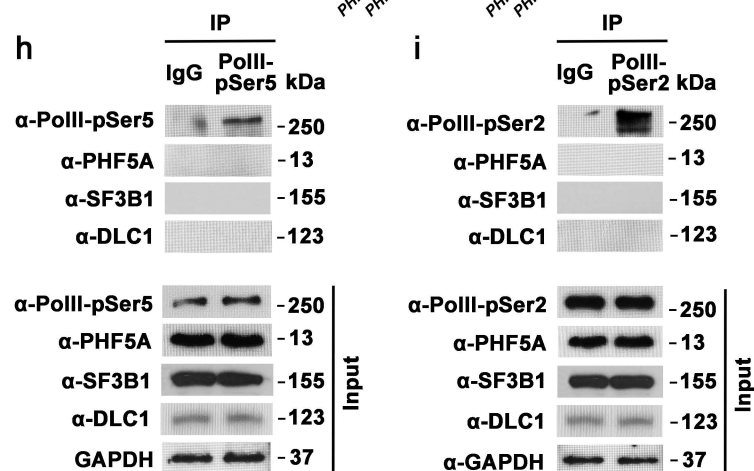
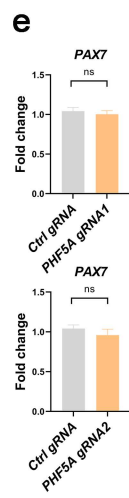
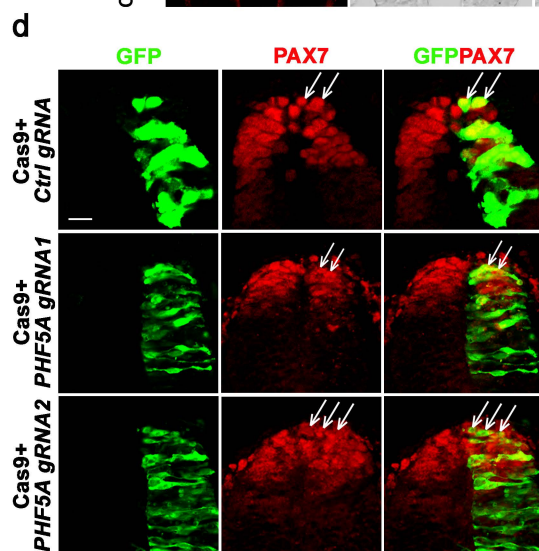
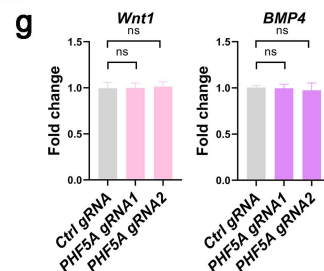
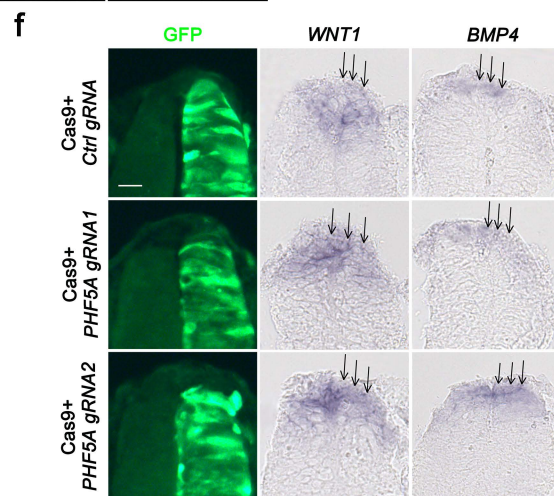
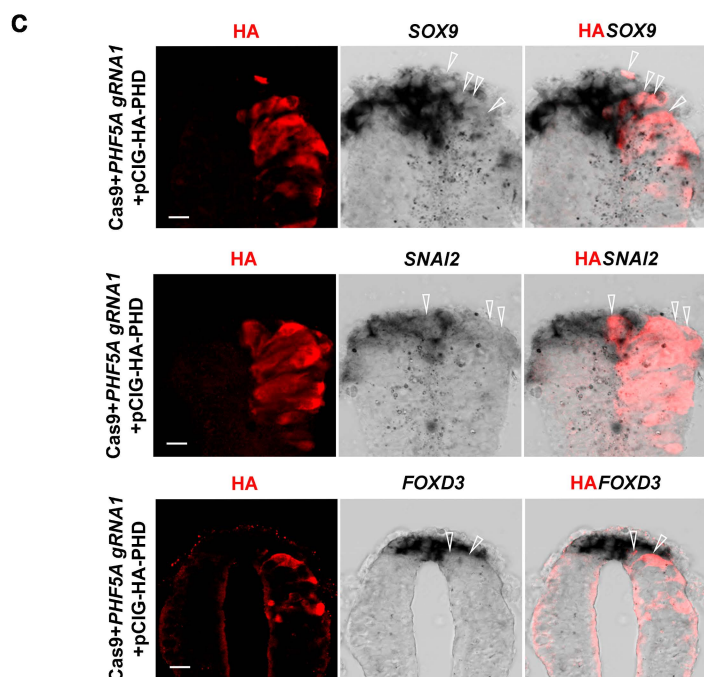
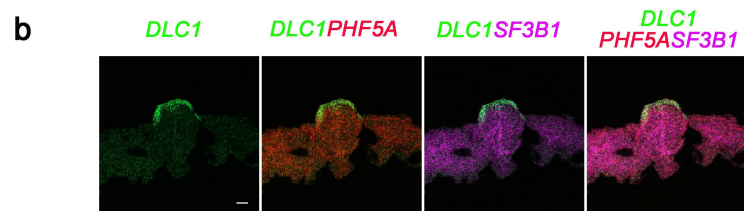
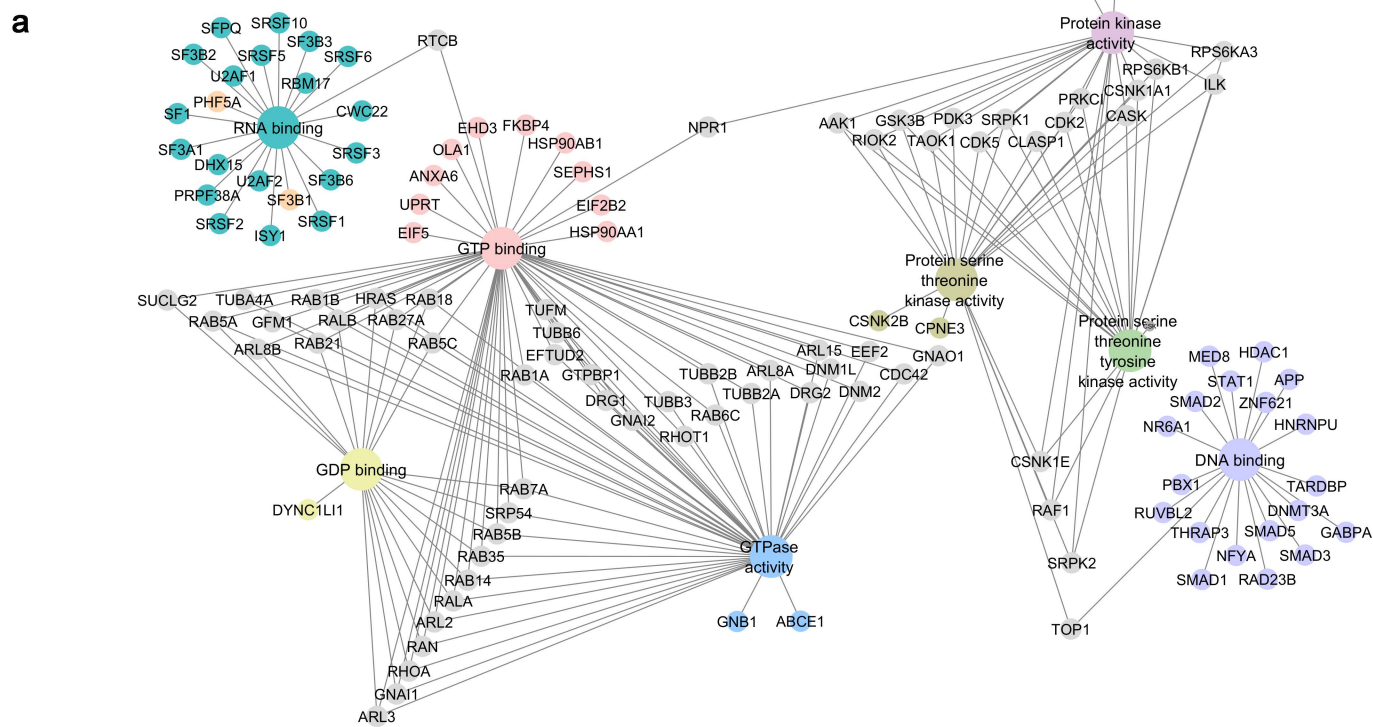
Dataset 2

Dataset 3



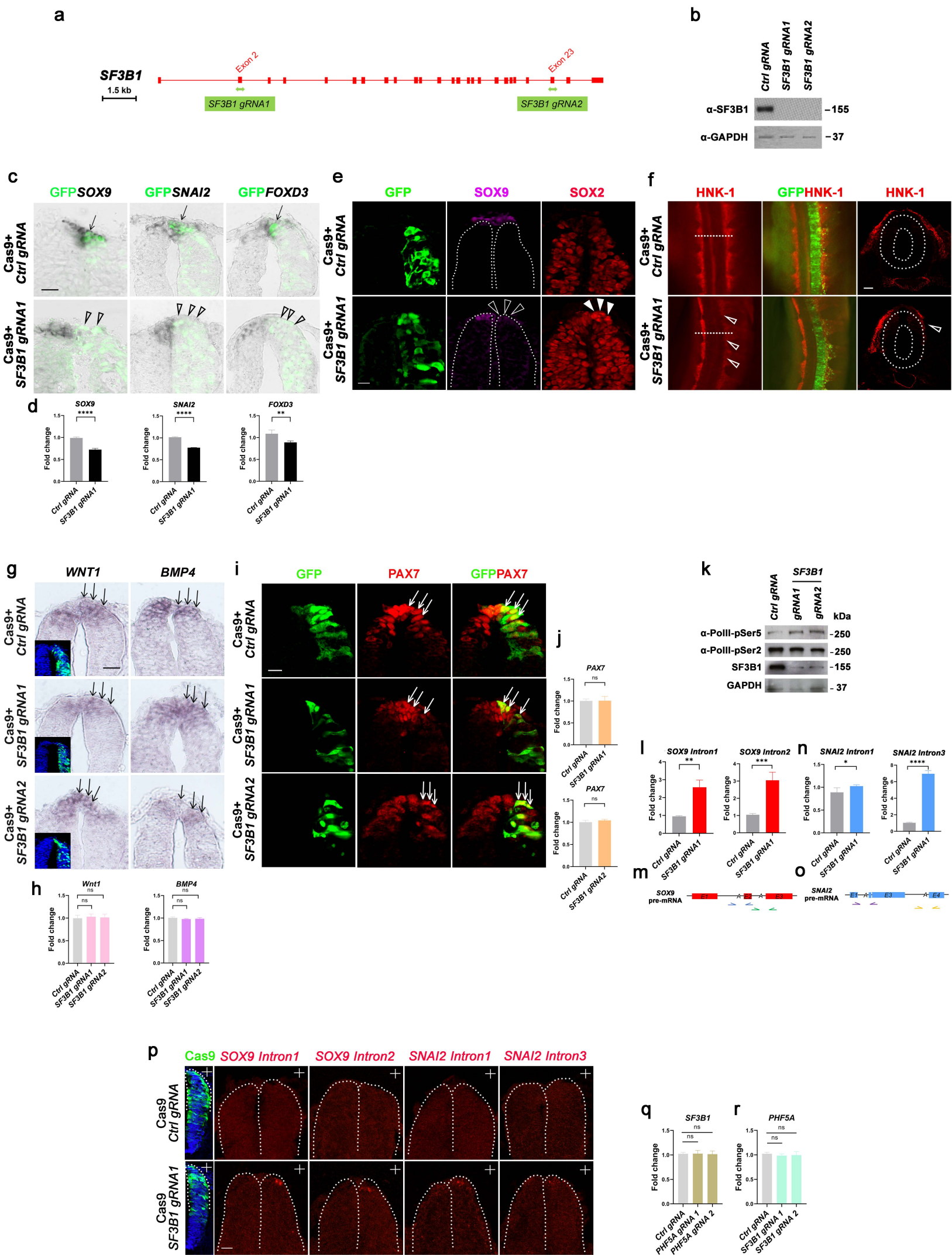
**Figure S1. *DLC1* knockout does not affect the expression levels of WNT1, BMP4, PAX7, and the transcriptional elongation activity of RNA PolII-pSer2.**

**a** ISH shows that overexpression of SAM domain alone or RhoGAP domain alone was not able to restore the expression of *SOX9*, *SNAI2*, and *FOXD3* (open arrowheads) in *DLC1 gRNA1* transfected cells (n=5 per treatment). Insets show transfection of the indicated constructs (red) on the right side of the neural tube. **b** Overexpression of *DLC1 gRNA1* and *gRNA2* did not alter the expression patterns of *WNT1* and *BMP4* (black arrows) in *DLC1*-depleted cells compared to Ctrl gRNA (n=5 per treatment). **c** RT-qPCR analysis shows that the expression levels of *WNT1* and *BMP4* remained unaltered in *DLC1 gRNA* transfected cells compared to *Ctrl gRNA*. **d** Immunofluorescence (IF) reveals no alteration in the expression pattern of PAX7 (white arrows) in *DLC1*-ablated cells compared to Ctrl gRNA (n=3 per treatment). **e** The expression level of PAX7 remained unaltered in cells transfected with *DLC1 gRNA 1* or *gRNA2* (n=5 per treatment). **f** and **g** Chromatin immunoprecipitation-qPCR analysis revealed a higher enrichment of PolII-pSer2 at the 500 bp downstream of the *SOX9* transcriptional start site in cells transfected with *DLC1 gRNA*, compared to those transfected with Ctrl gRNA. However, the enrichment was reduced to a level similar to the Ctrl gRNA for the rest of the *SOX9* gene body. The transcriptional elongation activity for PolII-pSer2 at multiple sites along the *SNAI2* gene remained unchanged in the absence of *DLC1*. Scale bars apply to all images, 50µm. \*:  $p < 0.05$ , ns: no significant difference. Ten embryos per treatment were used for the RT-qPCR and ChIP-qPCR analysis.



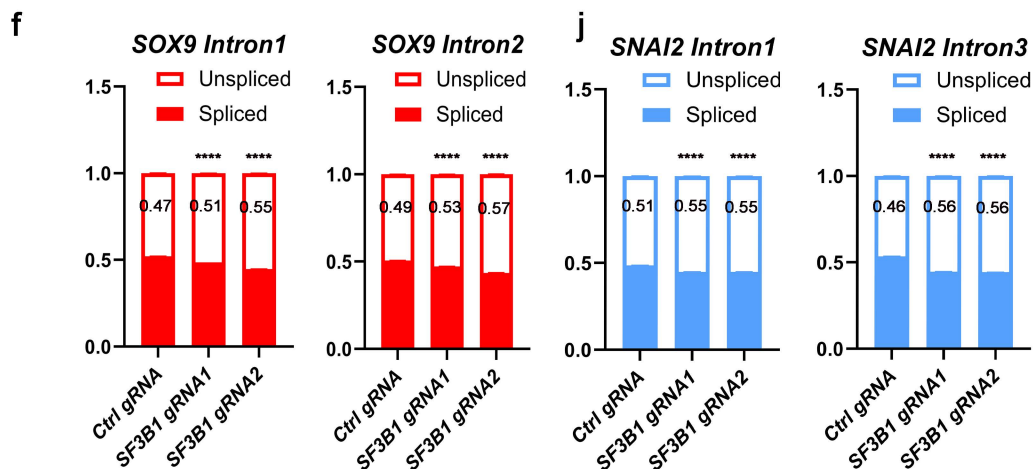
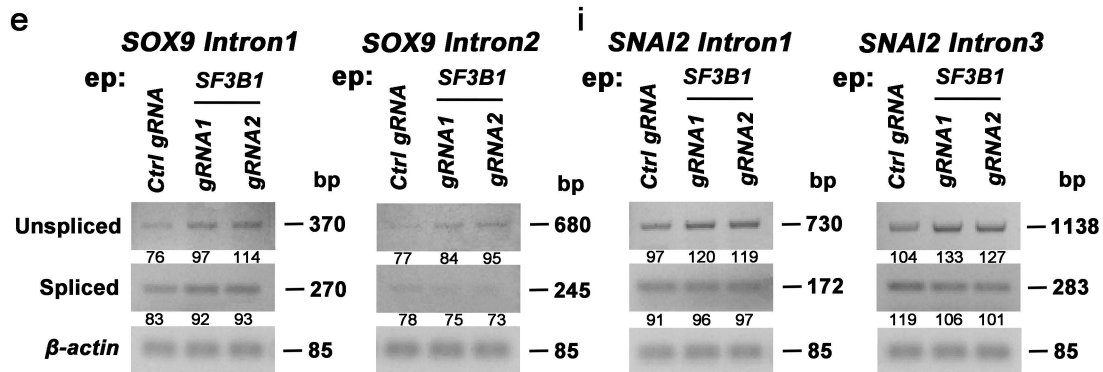
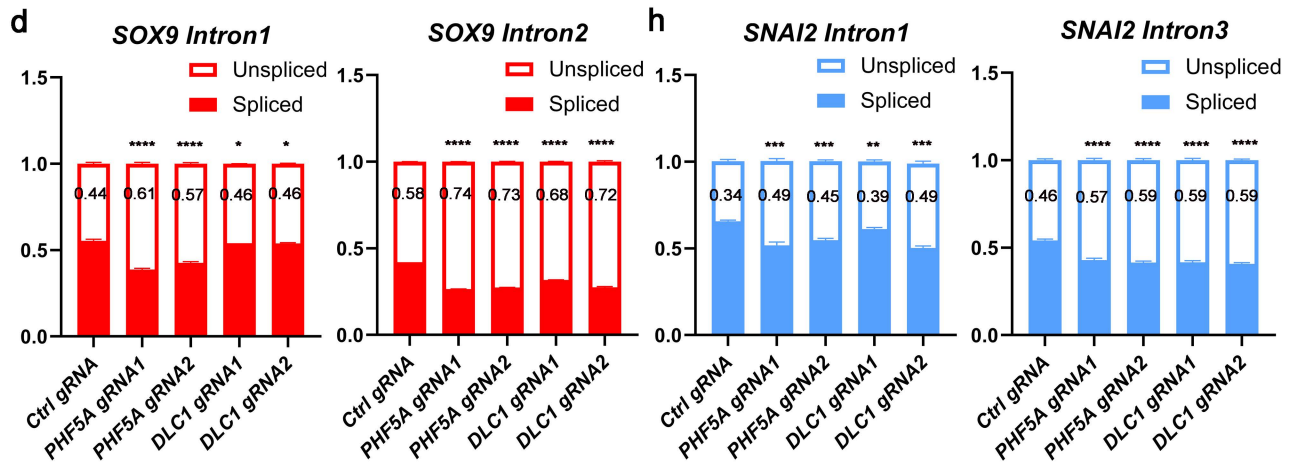
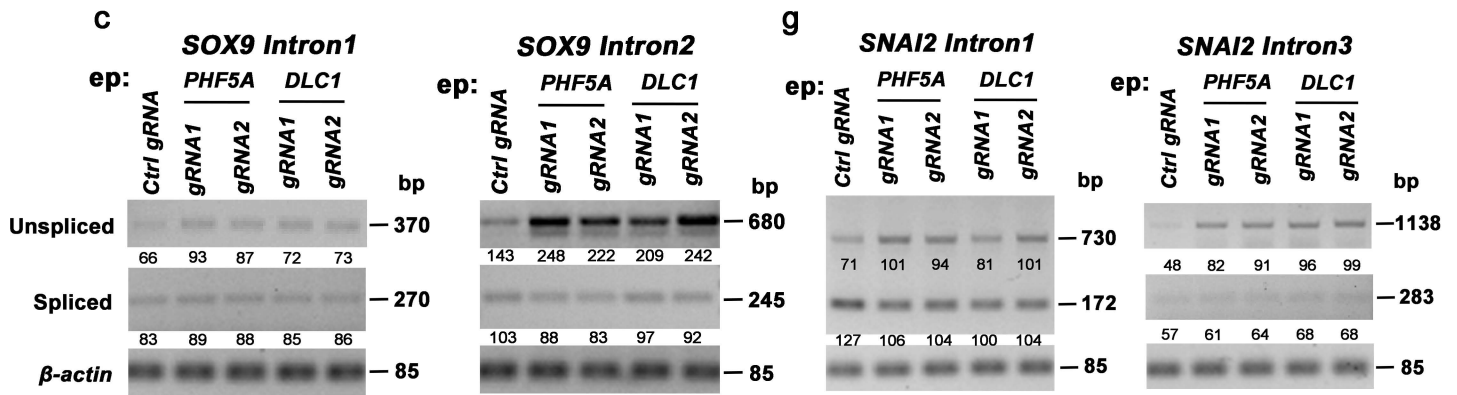
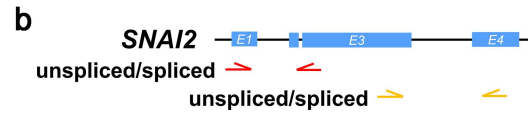
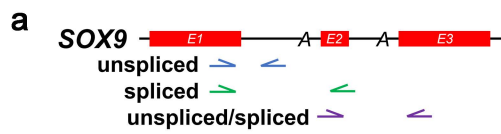
**Figure S2. *PHF5A* knockout does not affect the expression levels of WNT1, BMP4, and PAX7.**

**a** Interactome of DLC1 showing different functional categories of partner factors. **b** HCR analysis showed the co-expression of endogenous DLC1, PHF5A, and SF3B1 in emigrating NCCs. **c** IF and ISH showed that overexpression of the HA-PHD domain alone was not able to rescue the loss of *SOX9*, *SNAI2*, and *FOXD3* (open arrowheads) in *PHF5A gRNA1* transfected embryos. **d** Electroporation of *PHF5A gRNA1* and *gRNA2* did not alter the PAX7 expression pattern in the dorsal neural tubes (white arrows). **e** Expression level of *PAX7* remained unchanged in *PHF5A*-ablated cells compared to Ctrl gRNA. **f** Expression patterns of *WNT1* and *BMP4* remained unchanged in cells transfected with *PHF5A gRNA1* and *gRNA2* (black arrows). **g** RT-qPCR confirmed unaltered expression levels of *WNT1* and *BMP4* in *PHF5A*-depleted cells. **h** and **i** Immunoprecipitation (IP) showed no interaction between PolIII-pSer5 (**h**) or PolIII-pSer2 (**i**) and PHF5A, SF3B1 or DLC1. Scale bars apply to all images: 50  $\mu$ m, ns: no significant difference. 6 embryos per treatment were used for imaging. 10 embryos per treatment were used for the RT-qPCR analysis. 30 embryos per treatment were used for the IP.



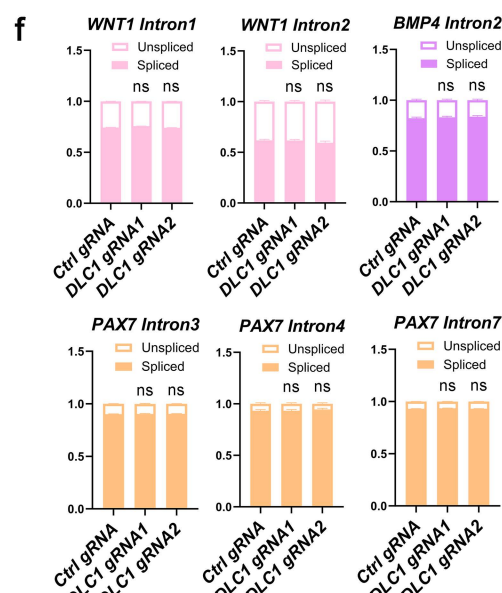
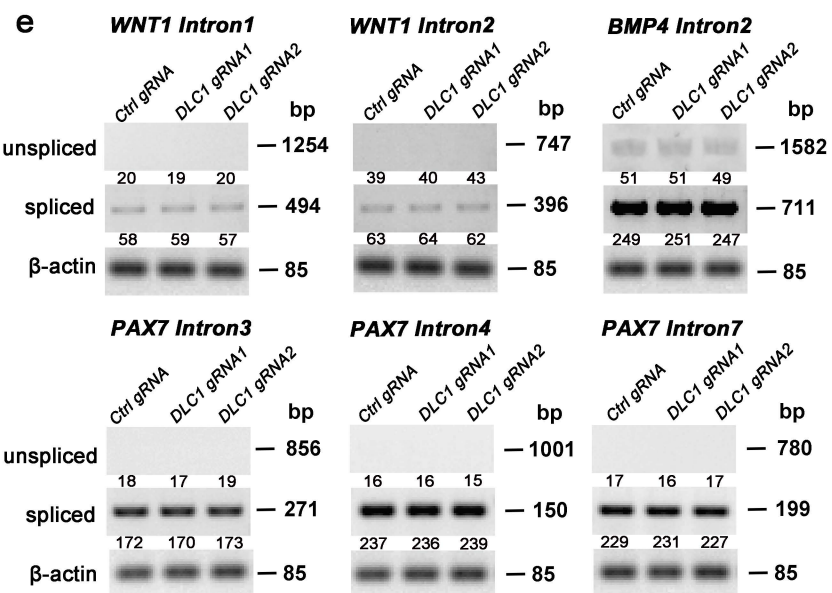
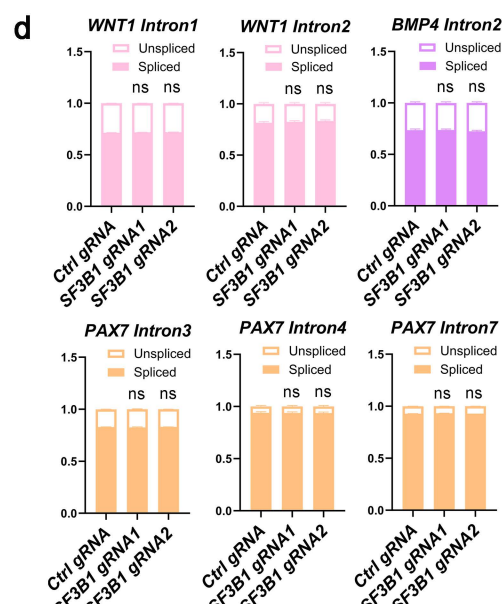
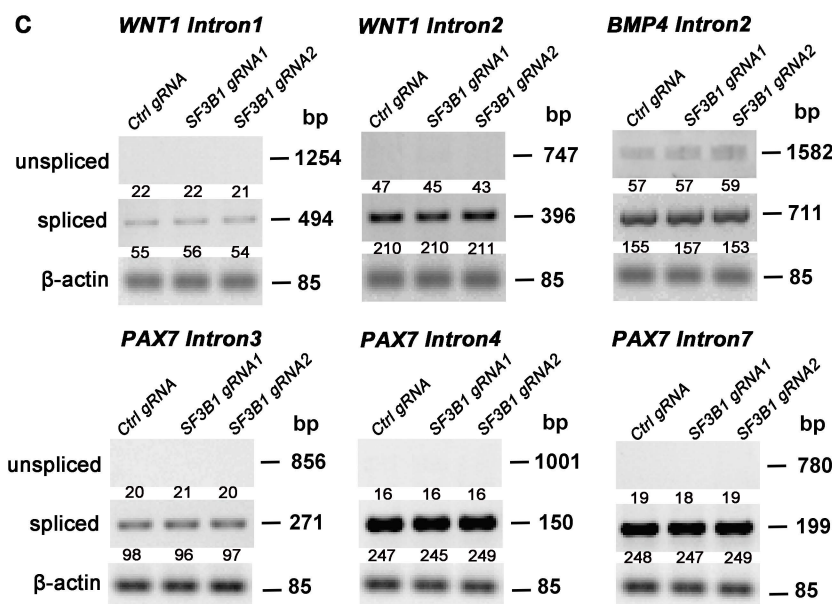
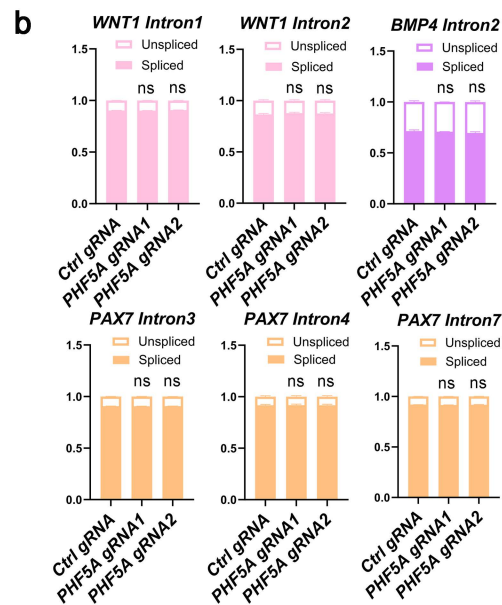
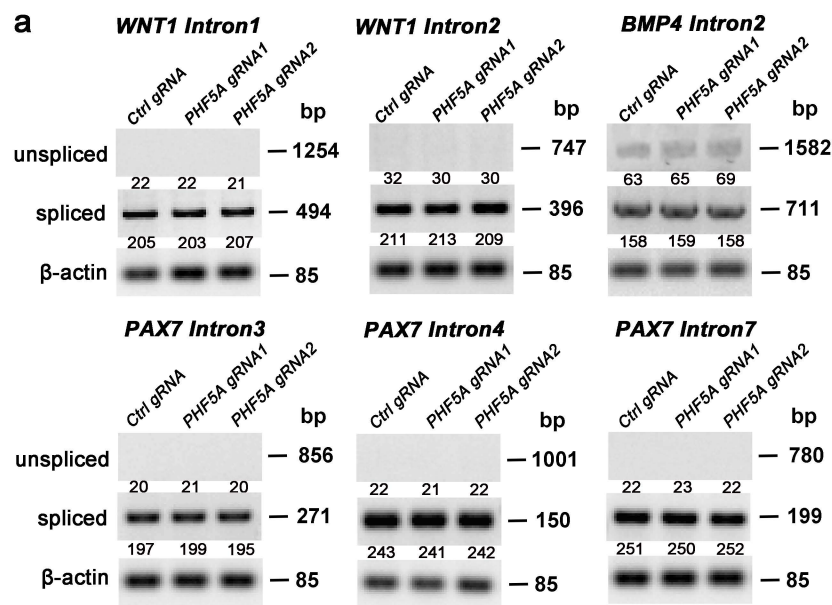
**Figure S3. *SF3B1* knockout does not affect the expression levels of WNT1, BMP4, PAX7, and transcriptional machinery.**

**a** Schematic diagram shows *gRNA1* and *gRNA2* oligos targeting *exon 2* and *exon 23* of *SF3B1* respectively. **b** Western blot shows depletion of SF3B1 in lysates extracted from embryos electroporated with *SF3B1 gRNA1* and *gRNA2* compared to *Ctrl gRNA*. **c** Expression of *SOX9*, *SNAI2* and *FOXD3* was reduced in *SF3B1*-depleted cells (open arrowheads) compared to *Ctrl gRNA*. Black arrows indicate unaltered expression of NC specifier genes in cells transfected with *Ctrl gRNA*. **d** RT-qPCR confirmed reduced expression levels of *SOX9*, *SNAI2*, and *FOXD3* in cells transfected with *SF3B1 gRNA1* compared to cells transfected with *Ctrl gRNA*. **e** Electroporation of *SF3B1 gRNA1* led to a reduction of SOX9 expression (open arrowheads) in the dorsal neural tube where SOX2 expression was upregulated (white arrowheads). Dotted lines outline the neural tube. **f** Whole Mount IF and the transverse section revealed reduced expression of HNK-1 (open arrowheads) in the *SF3B1 gRNA1* transfected side. White dotted lines in the whole mount IF indicate the level of cross-sectioned. **g** Expression patterns of *WNT1* and *BMP4* remained unchanged (black arrows) in embryos treated with *SF3B1 gRNA1* and *gRNA2*. **h** RT-qPCR confirmed no change in the expression levels of *WNT1* and *BMP4* in *SF3B1*-depleted cells. **i** Electroporation of *SF3B1 gRNA1* and *gRNA 2* did not alter the expression pattern (white arrows) and levels of PAX7 (**j**). **k** Western blot shows unaltered expression levels of PolIII-pSer5 and PolIII-pSer2 in embryos treated with *SF3B1 gRNA1* and *gRNA2*. **l** Expression levels of *SOX9 introns 1/2* and (**n**) *SNAI2 introns 1/3* were increased in embryos treated with *SF3B1 gRNA1* compared to *Ctrl gRNA*. **m**, **o** Schematic diagram shows intron-retained *SOX9* and *SNAI2* pre-mRNA in *SF3B1*-depleted cells. Arrows indicate primer pairs flanking the branch point adenosine (A) in each intron for qPCR analysis. **p** HCR showing the detection of retained introns 1 and 2 of *SOX9* and introns 1 and 3 of *SNAI2* in the dorsal neural tubes transfected with *SF3B1 gRNA*, while no signal was detected in *Ctrl gRNA*. Dotted lines outline the transverse neural tubes where the “+” indicates the transfected side. **q** and **r** RT-qPCR shows that *PHF5A gRNA* and *SF3B1 gRNA* did not affect the expression level of *SF3B1* and *PHF5A*, respectively. Scale bars apply to all images: 50  $\mu$ m. Mean  $\pm$  SD. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ , ns: no significant difference. 6 embryos per treatment were used for ISH and IF analyses. Ten embryos per treatment were used for the RT-qPCR, IR-qPCR, and Western blot analysis.



**Figure S4. Perturbation in the expression of *DLC1*, *PHF5A*, or *SF3B1* augments the ratio of unspliced transcripts of *SOX9* and *SNAI2*.**

**a** and **b** Schematic diagram showing primer pairs used for detection of unspliced and spliced transcripts of *SOX9* (**a**) and *SNAI2* (**b**). RT-PCR (**c**, **e**) and stacking bar graphs (**d**, **f**) show increased levels of unspliced *SOX9* intron 1 and intron 2 in all gRNA-mediated *PHF5A*, *DLC1*, and *SF3B1* KO compared to *Ctrl* gRNA. RT-PCR (**g**, **i**) and stacking bar graphs (**h**, **j**) show increased levels of unspliced *SNAI2* intron 1 and intron 3 all gRNA-mediated *PHF5A*, *DLC1*, and *SF3B1* KO compared to *Ctrl* gRNA. The band intensity for each transcript is shown below the gel images. Mean  $\pm$  SD. \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ . 10 embryos per treatment were used for the RT-PCR analysis.

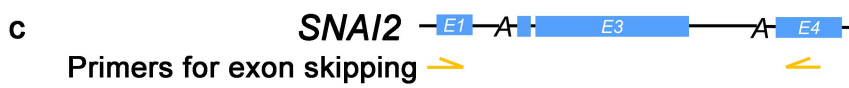
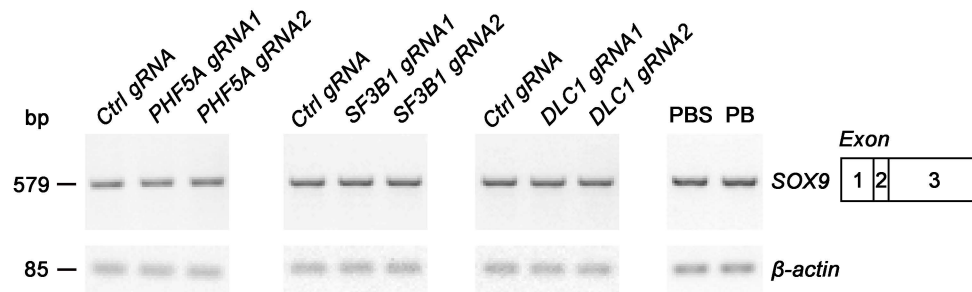


**Figure S5. Perturbation in the expression of DLC1, PHF5A, or SF3B1 does not affect the splicing of *WNT1*, *BMP4*, and *PAX7* pre-mRNA.**

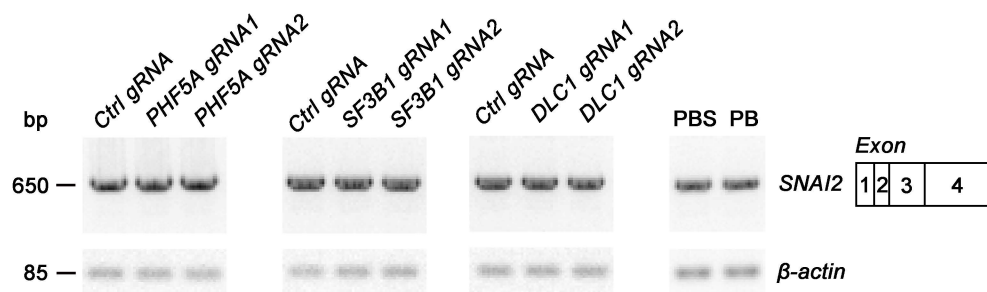
**a**, **c**, and **e** RT-PCR analysis revealed minimal levels of unspliced *WNT1* intron 1, *WNT1* intron 2, *PAX7* intron 3, *PAX7* intron 4, and *PAX7* intron 7 in the absence of *PHF5A* (**a**), *SF3B1* (**c**) or *DLC1* (**e**). However, there was no significant difference in the levels of unspliced *BMP2* intron 2 between gRNA treatment for each gene and *Ctrl* gRNA. **b**, **d**, and **f** Stacking bar graphs indicated that the ratio of unspliced transcripts of *WNT1* intron 1, *WNT* intron 3, *PAX7* intron 3, *PAX7* intron 4, *PAX7* intron 7, and *BMP4* intron 2 remained unchanged compared to *Ctrl* gRNA. The band intensity for each transcript is displayed below the gel images. Mean  $\pm$  SD. ns: no significant difference. 10 embryos per treatment were used for the RT-PCR analysis.



**b** SOX9 Exon skipping :

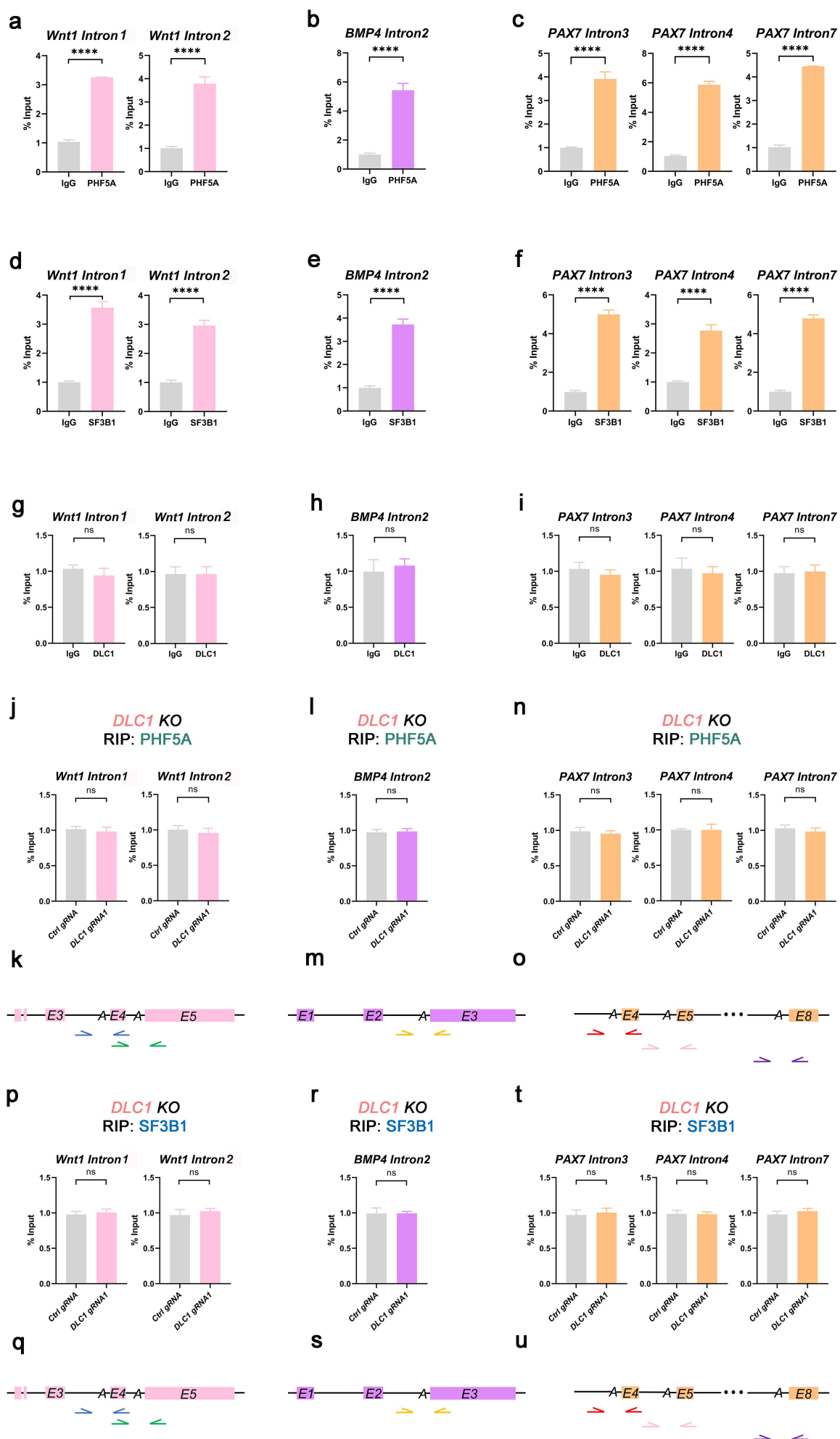


**d** SNAI2 Exon skipping :



**Figure S6. Perturbation in the expression of *DLC1*, *PHF5A*, or *SF3B1* does not lead to exon skipping of *SOX9* and *SNAI2*.**

**a** and **c** Schematic diagrams illustrate the primer pairs utilized for detecting exon skipping in *SOX9* (**a**) and *SNAI2* (**c**). **b** and **d** RT-PCR analysis demonstrated no evidence of exon skipping in *SOX9* and *SNAI2* in the absence of *PHF5A*, *SF3B1*, or *DLC1* or after PB treatment when compared to the Ctrl gRNA or PBS Ctrl respectively. Ten embryos per treatment were used for the RT-PCR analysis.

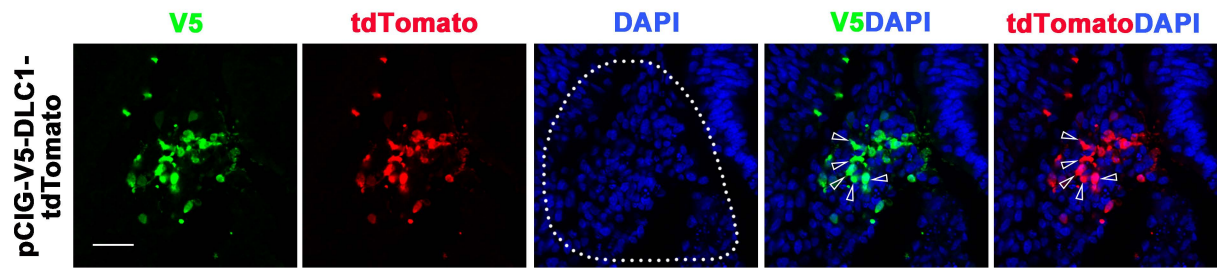


**Figure S7. PHF5A and SF3B1 splicing factors but not DLC1 bind to introns of *WNT1*, *BMP4*, and *PAX7*.**

**a to f** RNA immunoprecipitation-qPCR (RIP-qPCR) analysis indicated that PHF5A and SF3B1 exhibited the capability to bind to the introns of *WNT1* (**a, d**), *BMP4* (**b, e**) and *PAX7* (**c, f**). **g to i** However, DLC1 did not show any binding affinity towards any of the introns. The knockout of *DLC1* did not affect the binding capacity of PHF5A (**j, l, n**) and SF3B1 (**p, r, t**) to the introns of *WNT1*, *BMP4*, and *PAX7*. **k, m, o, q, s, u** Schematic diagram illustrated the primer pairs used for the RIP-qPCR analysis. Mean  $\pm$  SD. \*\*\*\*:  $p < 0.0001$ , ns: no significant difference. 30 embryos per treatment were used for the RIP-qPCR analysis.

a

ep in somite:



**Figure S8. Nuclear localization of ectopic DLC1 in somite.**

a IF staining for V5 revealed that the ectopic DLC1 fused with a V5-tagged exhibited nuclear localization in somites (n=3). Dotted ring outlines the somite. Open arrowheads indicate co-localization of ectopic DLC1 with tdTomato and DAPI, which stained the nuclei. Scale bar: 50  $\mu\text{m}$ .

| No. | <i>gRNA</i> oligos | Orientation of oligos | Sequence of oligos          |
|-----|--------------------|-----------------------|-----------------------------|
| 1   | <i>PHF5A gRNA1</i> | Forward               | GGATGGGTGCACTCCTTGCAGTAGT   |
| 2   |                    | Reverse               | AAACACTACTGCAAGGAGTGCACCC   |
| 3   | <i>PHF5A gRNA2</i> | Forward               | GGATGGATCTGTCTTGGA ACTGCCA  |
| 4   |                    | Reverse               | AAACTGGCAGTTCCA AGA CAGATCC |
| 5   | <i>SF3B1 gRNA1</i> | Forward               | GGATGGGAGTAGGCCTCGACTCCAC   |
| 6   |                    | Reverse               | AAACGTGGAGTCGAGGCCTACTCCC   |
| 7   | <i>SF3B1 gRNA2</i> | Forward               | GGATGGTCTTCAAGCAATGGCGTAA   |
| 8   |                    | Reverse               | AAACTTACGCCATTGCTTGAAGACC   |
| 9   | <i>DLC1 gRNA1</i>  | Forward               | GCCTGGAGACGCTGATCCTT        |
| 10  |                    | Reverse               | CAGCCCTGTCACGCGCACAC        |
| 11  | <i>DLC1 gRNA2</i>  | Forward               | GATGACACCAACAAACCTGG        |
| 12  |                    | Reverse               | GGCACAGGCTGAGGTGGCAC        |

**Table S1.**

*gRNA* oligos used for CRISPR/Cas9 KO

| No. | Primers               | Orientation of primers | Sequence of primers  |
|-----|-----------------------|------------------------|----------------------|
| 1   | <i>SOX9 Intron 1</i>  | Forward                | GCACTCAGGTAGGTGTCACA |
| 2   | <i>SOX9 Intron 1</i>  | Reverse                | TCTCCAACATCGAAACCTTC |
| 3   | <i>SOX9 Intron 2</i>  | Forward                | CCACGAGAAACGCAAAGCTG |
| 4   | <i>SOX9 Intron 2</i>  | Reverse                | CTGCGACTGCCCTGAAAG   |
| 5   | <i>SNAI2 Intron 1</i> | Forward                | GTCTTTAGCCATCAGTATGT |
| 6   | <i>SNAI2 Intron 1</i> | Reverse                | GGTATGGGGATATAATCACT |
| 7   | <i>SNAI2 Intron 3</i> | Forward                | CTGAAAGCTGTTGTATCTGT |
| 8   | <i>SNAI2 Intron 3</i> | Reverse                | CGGTTTCTCTCCTAAAAAGG |

**Table S2.**

Primers used for RIP-qPCR and intron retention analysis

| No. | Primers                         | Orientation of primers | Sequence of primers         |
|-----|---------------------------------|------------------------|-----------------------------|
| 1   | <i>SOX9 unspliced Intron1_1</i> | Forward                | GGTGCTCAAGGGCTACGACT        |
| 2   | <i>SOX9 unspliced Intron1_1</i> | Reverse                | TAT TCC GCC GAA CAA CTT TG  |
| 3   | <i>SOX9 spliced Intron1_2</i>   | Forward                | GGTGCTCAAGGGCTACGACT        |
| 4   | <i>SOX9 spliced Intron1_2</i>   | Reverse                | TGGTCCTTCTTGTGCTGCAC        |
| 5   | <i>SOX9 unspliced Intron2</i>   | Forward                | AAGAAGGACCACCCCGACTACAA     |
| 6   | <i>SOX9 unspliced Intron2</i>   | Reverse                | TGCTGAGCGTCCGTTTTG          |
| 7   | <i>SNAI2 unspliced Intron1</i>  | Forward                | GAAGTGGACACTCATAACAG        |
| 8   | <i>SNAI2 unspliced Intron1</i>  | Reverse                | CAGAAAGCGGAGAGAGGTC         |
| 9   | <i>SNAI2 unspliced Intron2</i>  | Forward                | GACCTATTCCACTTTCTCTG        |
| 10  | <i>SNAI2 unspliced Intron2</i>  | Reverse                | CCTCAGATTGGATCTGTCT         |
| 11  | <i>WNT1 unspliced Intron3</i>   | Forward                | CAT CAT CAA CGT GGC CTC ATC |

|    |                               |         |                               |
|----|-------------------------------|---------|-------------------------------|
| 12 | <i>WNT1 unspliced Intron3</i> | Reverse | GTA GGT TCA TCA GGA AGC GCA   |
| 13 | <i>WNT1 unspliced Intron4</i> | Forward | TCG ATT TCG GGA GGC TCT TC    |
| 14 | <i>WNT1 unspliced Intron4</i> | Reverse | CCA TCT TCC CGC TGT AGG TG    |
| 15 | <i>BMP4 unspliced Intron2</i> | Forward | CCA AGT CCT GCT GGG AGG TA    |
| 16 | <i>BMP4 unspliced Intron2</i> | Reverse | ACG TGT TTG CCC TGA TGA GT    |
| 17 | <i>PAX7 unspliced Intron3</i> | Forward | GGT GAA CCA ACT CGG AGG G     |
| 18 | <i>PAX7 unspliced Intron3</i> | Reverse | CCC AGG GTT CTC CCT CTT GTA   |
| 19 | <i>PAX7 unspliced Intron4</i> | Forward | ACT CCC GAC GTG GAG AAG AAA   |
| 20 | <i>PAX7 unspliced Intron4</i> | Reverse | GAT GCG TAG CAC ACG GCT AAT   |
| 21 | <i>PAX7 unspliced Intron7</i> | Forward | TAC CCA ACC ACC ACC ATT TCC   |
| 22 | <i>PAX7 unspliced Intron7</i> | Reverse | GAT TCA TGT GGT TGG CAG GAG   |
| 23 | <i>SOX9 Exon2 skipping</i>    | Forward | ATC CAG CAA GAA CAA ACC CC    |
| 24 | <i>SOX9 Exon2 skipping</i>    | Reverse | GGT TTC GAT GTT GGA GAT GAC G |
| 25 | <i>SNAI2 Exon2/3 skipping</i> | Forward | GCC TTC AAA ATG CCA CGC TC    |
| 26 | <i>SNAI2 Exon2/3 skipping</i> | Reverse | GAA AGC CCT GTT GCA GTG AG    |

**Table S3.**

Primers used for semi-quantitative RT-PCR.

| No. | Primers        | Orientation of primers | Sequence of primers           |
|-----|----------------|------------------------|-------------------------------|
| 1   | SOX9           | Forward                | CGG ATC CAG CAA GAA CAA A     |
| 2   | SOX9           | Reverse                | GGT ACT GGT CAG CCA GCT TC    |
| 3   | SNAI2          | Forward                | CCTGGCTACTTCAAGGGCA           |
| 4   | SNAI2          | Reverse                | AGATTGGATCTGTCTGCGAAAG        |
| 5   | FOXD3          | Forward                | GCGAGTTCATCAGCAACCG           |
| 6   | FOXD3          | Reverse                | GGGGGATCTTCACGAAGCAG          |
| 7   | $\beta$ -actin | Forward                | GAGTCCTGTGGTATCCATGAACT       |
| 8   | $\beta$ -actin | Reverse                | GGCTGTGATCTCCTTCTGCA          |
| 9   | BMP4           | Forward                | TTT TGT GTC TCA CCT CTC AGG G |
| 10  | BMP4           | Reverse                | AGC AGC TGA CTC TCC AGT GA    |
| 11  | WNT1           | Forward                | TGT TTG GGG GTC TCT TTG CC    |
| 12  | WNT1           | Reverse                | TGC AGC GAA GCT TTG TTC AT    |
| 13  | PAX7           | Forward                | GAATCCTGTTAGCAATGGC           |
| 14  | PAX7           | Reverse                | AGAGATGGAGTTGGTGGTG           |

**Table S4.**

Primers used for RT-qPCR.

| No. | Primers                 | Orientation of primers | Sequence of primers  |
|-----|-------------------------|------------------------|----------------------|
| 1   | <i>SOX9 gene body_1</i> | Forward                | CGCTTTCTCGCATGAATCT  |
| 2   | <i>SOX9 gene body_1</i> | Reverse                | TTCTCTTGAGGTCGGGTGTT |
| 3   | <i>SOX9 gene body_2</i> | Forward                | AAACCAGGAGGCAAGTGAGT |
| 4   | <i>SOX9 gene body_2</i> | Reverse                | CCAAACGAGAGGTGTGAGAG |

|           |                          |         |                                   |
|-----------|--------------------------|---------|-----------------------------------|
| <b>5</b>  | <i>SOX9 gene body_3</i>  | Forward | AAGTCGGTGAAGAACGGG                |
| <b>6</b>  | <i>SOX9 gene body_3</i>  | Reverse | TCGCTGATGCTGGAGGA                 |
| <b>7</b>  | <i>SOX9 gene body_4</i>  | Forward | CCGCTCGCAGTACGATTA                |
| <b>8</b>  | <i>SOX9 gene body_4</i>  | Reverse | GGGTTTCATGTAGGTGAAGGTG            |
| <b>9</b>  | <i>SNAI2 gene body_1</i> | Forward | AAA TGC CAC GCT CCT TCC           |
| <b>10</b> | <i>SNAI2 gene body_1</i> | Reverse | CCT ACC GCC AAG CCA CAA A         |
| <b>11</b> | <i>SNAI2 gene body_2</i> | Forward | TGT TCC AGG GCA CTG TCT TTA       |
| <b>12</b> | <i>SNAI2 gene body_2</i> | Reverse | CCA AAC GCT CAG AAT GTT CAT C     |
| <b>13</b> | <i>SNAI2 gene body_3</i> | Forward | TGA TGC CCA GTC TAG GAA ATC G     |
| <b>14</b> | <i>SNAI2 gene body_3</i> | Reverse | GCA GAC GCA AGG TAG CGT GT        |
| <b>15</b> | <i>SNAI2 gene body_4</i> | Forward | GAA GGC GTT GCT TTA AGG ATT       |
| <b>16</b> | <i>SNAI2 gene body_4</i> | Reverse | GCT TTG GTG ATG AAG TGT TTC TAA C |
| <b>17</b> | <i>SNAI2 gene body_5</i> | Forward | GGC TTT CGC AGA CAG ATC CA        |
| <b>18</b> | <i>SNAI2 gene body_5</i> | Reverse | CTC ATG TTT GTG CAG AAG AGA CAT T |

**Table S5.**

Primers used for ChIP-qPCR.

| No. | Sequences of <i>DLC1</i> probes                |
|-----|------------------------------------------------|
| 1   | CCTCGTAAATCCTCATCAaaCAGCAGCTCGCAGCCAGTCACAAGC  |
| 2   | CATAGAGCTGTGCATACTGCGGGAAaaATCATCCAGTAAACCGCC  |
| 3   | CCTCGTAAATCCTCATCAaaTTCATCACTGCACATTTGTTTAAAG  |
| 4   | CTTTTCCTGTGGGGACTGATTTCTAaaATCATCCAGTAAACCGCC  |
| 5   | CCTCGTAAATCCTCATCAaaTGGATCTGCTTTGGGGAGGAAGGAC  |
| 6   | GTTGTTTCGATCATTATCTACATCAaaATCATCCAGTAAACCGCC  |
| 7   | CCTCGTAAATCCTCATCAaaTCACTCGTACTGGAATACAGGATTG  |
| 8   | ATGTCTTCATTTTCAAGATCAGCCAaaATCATCCAGTAAACCGCC  |
| 9   | CCTCGTAAATCCTCATCAaaCGGCTACTCAATGAGCTAGCGGAAT  |
| 10  | CCTGGCACATTGTCGTAAATGCTCAaaATCATCCAGTAAACCGCC  |
| 11  | CCTCGTAAATCCTCATCAaaCTGTCCTCCAGTTCACAGAAGCATT  |
| 12  | TGAGATGGCCATGTCCATGGAACTaaATCATCCAGTAAACCGCC   |
| 13  | CCTCGTAAATCCTCATCAaaAAGGCTGATGTGATTGCCATTGAGG  |
| 14  | CCTCGTAAATCCTCATCAaaAAGGCTGATGTGATTGCCATTGAGG  |
| 15  | CCTCGTAAATCCTCATCAaaAAGCTTTGAAGGAGCTTTGTTCTTG  |
| 16  | CAGGATGGGACCACTAATAATAAGGaaATCATCCAGTAAACCGCC  |
| 17  | CCTCGTAAATCCTCATCAaaGAGAATAGAGGCCACCTCCTGTGCGC |
| 18  | CTGGTGGCTGCTGGTGCTGCTGGTAaaATCATCCAGTAAACCGCC  |
| 19  | CCTCGTAAATCCTCATCAaaGCTGGCTGCATTTGGGAGGAGAGGA  |
| 20  | GCTGAGGTCGGTGAGCACGCTCTCGaaATCATCCAGTAAACCGCC  |
| 21  | CCTCGTAAATCCTCATCAaaTGGCTTTGGAGGGAACACATCGAAC  |
| 22  | CTCTGGGGAGGAGGAGGTGTTAACAaaATCATCCAGTAAACCGCC  |
| 23  | CCTCGTAAATCCTCATCAaaGGCCGGTGAGAGCTCTGGAAGCTGT  |
| 24  | CCTCGTAAATCCTCATCAaaGGCCGGTGAGAGCTCTGGAAGCTGT  |
| 25  | CCTCGTAAATCCTCATCAaaGTTTAAATGGAAGAGGGAAGGTGCT  |
| 26  | TGGGGAGGAATTCTCTCTTTTGAGAaaATCATCCAGTAAACCGCC  |
| 27  | CCTCGTAAATCCTCATCAaaAGGACCTCCCTGTTCTCGTCAGGTA  |

|    |                                                |
|----|------------------------------------------------|
| 28 | TCACTCAGGAAATAGAGAAGAATCTaaATCATCCAGTAAACCGCC  |
| 29 | CCTCGTAAATCCTCATCAaaTCAATGGTAGTTTTCCATAACCGGA  |
| 30 | AAAAC TTCTTCTGGTGCAGCAGCAAaaATCATCCAGTAAACCGCC |
| 31 | CCTCGTAAATCCTCATCAaaAGTTTAGATTTTCCTGACCCGCAGG  |
| 32 | CTTAAATCAATTCTGCACATGTAGGaaATCATCCAGTAAACCGCC  |
| 33 | CCTCGTAAATCCTCATCAaaCCACCCTTCTCTCTGCCCCCAACCA  |
| 34 | AAAGCAAATAAAACCCAAGACAAAAaaATCATCCAGTAAACCGCC  |
| 35 | CCTCGTAAATCCTCATCAaaTGGATAAGGAGTGAACAGTAGCCAG  |
| 36 | AATTGCTTTCACTCCCAAAAGATCAaaATCATCCAGTAAACCGCC  |
| 37 | CCTCGTAAATCCTCATCAaaTAATCTCTGTGCAGACCTTTCTCTG  |
| 38 | CAACCATCAATACACTCATTCACTCaaATCATCCAGTAAACCGCC  |
| 39 | CCTCGTAAATCCTCATCAaaAACTATTCCAGTGGATACATAAGTC  |
| 40 | CTCAGTGAGAACACTTTGAAGAGTAaaATCATCCAGTAAACCGCC  |

**Table S6.**

*DLCI* probes used for HCR.

| No. | Sequences of <i>PHF5A</i> probes                |
|-----|-------------------------------------------------|
| 1   | GAGGAGGGCAGCAAACGGaaCTTCTACCCCGGGAACACCCGTACC   |
| 2   | TTCAAAC TCA GTCTGTCTCGCCGCTtaGAAGAGTCTTCCTTTACG |
| 3   | GAGGAGGGCAGCAAACGGaaGGGTGGTGCTTCGCCATGGCGCCGT   |
| 4   | GCCTGCTTGCGGCAGAAGATGAGATtaGAAGAGTCTTCCTTTACG   |
| 5   | GAGGAGGGCAGCAAACGGaaAGTAGTAGGCATCAGACACCCCTGG   |
| 6   | CTTTTTCCTGGATGGTGCACCTCTtaGAAGAGTCTTCCTTTACG    |
| 7   | GAGGAGGGCAGCAAACGGaaACGAGCCGTAGTTACACTCATCACA   |
| 8   | CCCCGCAGATGACGCAGCGCCCTTGtaGAAGAGTCTTCCTTTACG   |
| 9   | GAGGAGGGCAGCAAACGGaaACGAGTCACAGATCACACATTTGCC   |
| 10  | TGCGCACGAGGGTGCAGGGCCGCACtaGAAGAGTCTTCCTTTACG   |
| 11  | GAGGAGGGCAGCAAACGGaaATCCTACAGGAGTGGTTACAGAAAG   |
| 12  | ACAAGAGAAGAAGCTTTATTGGTATtaGAAGAGTCTTCCTTTACG   |
| 13  | GAGGAGGGCAGCAAACGGaaAACAGCTGAGCTCTCCCTAGGAAAG   |

|    |                                                |
|----|------------------------------------------------|
| 14 | TGGATTAATCTGTCAAAGAAAACATtaGAAGAGTCTTCCTTTACG  |
| 15 | GAGGAGGGCAGCAAACGGaaGCAGTATGGAAATATACTGGAGGAA  |
| 16 | ATGGTGCTAGCTTTTTTCACAGAACAtaGAAGAGTCTTCCTTTACG |
| 17 | GAGGAGGGCAGCAAACGGaaCCTCTGCGCTTCGGTACCAACGTGC  |
| 18 | GACAGGGAGCAGACACACAAGACTCtaGAAGAGTCTTCCTTTACG  |
| 19 | GAGGAGGGCAGCAAACGGaaTGGTAAGAAGGGAAGCACAGCTTTT  |
| 20 | GTGATTTCTAACAGCTGAGAGGAGAtaGAAGAGTCTTCCTTTACG  |
| 21 | GAGGAGGGCAGCAAACGGaaGAGCCAGTCTCTCCTCCATATGCAA  |
| 22 | CGCCAGCAACGGCACTTGGTTTTTAtaGAAGAGTCTTCCTTTACG  |
| 23 | GAGGAGGGCAGCAAACGGaaAGTTGAGACAGCTACTAGCAAAGCT  |
| 24 | GATTGCCCACCAGGATATTTCTGAGtaGAAGAGTCTTCCTTTACG  |
| 25 | GAGGAGGGCAGCAAACGGaaCATTCTGATAGTCAGTTCAGTCAG   |
| 26 | AAAAACATGAGCCAGTCCACATTTAtaGAAGAGTCTTCCTTTACG  |
| 27 | GAGGAGGGCAGCAAACGGaaCAAGTCCAGAGATGTTCCAAGACCC  |
| 28 | CATAGTCATCTGGTTTTTCAGTAATAtaGAAGAGTCTTCCTTTACG |
| 29 | GAGGAGGGCAGCAAACGGaaGAACAGATAGGATCACCTCTTCTTG  |
| 30 | TGCCAACCTGACAGGAGGTGTAGCTtaGAAGAGTCTTCCTTTACG  |

**Table S7.**

*PHF5A* probes used for HCR.

| No. | Sequences of <i>SF3B1</i> probes              |
|-----|-----------------------------------------------|
| 1   | GTCCCTGCCTCTATATCTtAGTTACGTATCCAGCAAACCTGCTG  |
| 2   | GTCTTCCAATTCAGTTGCTGCAATGtCCACTCAACTTTAACCCG  |
| 3   | GTCCCTGCCTCTATATCTtTATCCGGTGGAGTCGAGGCCTACTC  |
| 4   | CTGCCGCCATAAATCTCTTGATCATtCCACTCAACTTTAACCCG  |
| 5   | GTCCCTGCCTCTATATCTtCTTGAATTTCTCGGATCTGTGCTTC  |
| 6   | GTGCTTCATCGAGGGCAGGCTTTTTtCCACTCAACTTTAACCCG  |
| 7   | GTCCCTGCCTCTATATCTtGCAGTGCCACTGGAGCATGGTACCC  |
| 8   | GTTCAAGTTGACTGCGGTATGTCATTtCCACTCAACTTTAACCCG |
| 9   | GTCCCTGCCTCTATATCTtGGGAAATTATCATCATCCGCCTGTG  |

|    |                                                |
|----|------------------------------------------------|
| 10 | CATCTGCAAAAGGATCAAGACGCTCtCCACTCAACTTTAACCCG   |
| 11 | GTCCCTGCCTCTATATCTtTCTTTTGAGGACGGTGTCTGCAA     |
| 12 | TTTTGTA CT CATCTTCCCGATCTGCtCCACTCAACTTTAACCCG |
| 13 | GTCCCTGCCTCTATATCTtTTTTGTCCAGCGCGAACAACGCCGA   |
| 14 | CTTCGTGGGTCTTGGCAATCTTCGCtCCACTCAACTTTAACCCG   |
| 15 | GTCCCTGCCTCTATATCTtCATTCA TTTTAGGGTCTGGTGTCTT  |
| 16 | CACGCATAACATCCATGTATGTTCTtCCACTCAACTTTAACCCG   |
| 17 | GTCCCTGCCTCTATATCTtGTGTTGCACCAGGAGTCTGATCAGC   |
| 18 | CTTGATCCCAACTGGATAACTTTTTtCCACTCAACTTTAACCCG   |
| 19 | GTCCCTGCCTCTATATCTtTGGTGGCTGAGATGCGGCTCCATTG   |
| 20 | TTGATCCCAACGCCGCTTGCGTTTTtCCACTCAACTTTAACCCG   |
| 21 | GTCCCTGCCTCTATATCTtATCGATTTTACGTGCGCTGGAAGT    |
| 22 | CTCTTTCTGTTTTAGGAGTTTCATCtCCACTCAACTTTAACCCG   |
| 23 | GTCCCTGCCTCTATATCTtCAGGTGTGTCCCGTCCAGGTGTTGC   |
| 24 | CACCTCCATGGCCTGGTGTGTCATGtCCACTCAACTTTAACCCG   |
| 25 | GTCCCTGCCTCTATATCTtGATCCCATATCTTTGAGCCAGGGGT   |
| 26 | CTCCTGCTGGCGTGTGACTGGGAGTtCCACTCAACTTTAACCCG   |
| 27 | GTCCCTGCCTCTATATCTtGGCCTGGAGTTTCATCCCATCGCAG   |
| 28 | CACCGGGAGTTTCGCTACCCTTTGCTtCCACTCAACTTTAACCCG  |
| 29 | GTCCCTGCCTCTATATCTtCTGGTGTACCAATGGGTGTTTTGCC   |
| 30 | CTGGCGTAGGAGTTGCCATGTTCA TtCCACTCAACTTTAACCCG  |
| 31 | GTCCCTGCCTCTATATCTtTCTGGCTAGCAGGTGTTTCATCCCA   |
| 32 | GTGTCAAACAGGAGTGCTGCCACCTtCCACTCAACTTTAACCCG   |
| 33 | GTCCCTGCCTCTATATCTtTTGGTGTCTACCAATGGAATCACC    |
| 34 | GGGACTTTCTTTTACTTGCTCCTGGtCCACTCAACTTTAACCCG   |
| 35 | GTCCCTGCCTCTATATCTtTCAGAAAGTGGTCTGTTTCTCTCAT   |
| 36 | TCTGGGAACATAGCATCCAATTCTTtCCACTCAACTTTAACCCG   |

**Table S8.**

*SF3B1* probes used for HCR.

| No. | Sequences of SOX9 probes                       |
|-----|------------------------------------------------|
| 1   | GAGGAGGGCAGCAAACGGaaTCGGCGTTGTGCAGATGCGGGTACT  |
| 2   | CACAGCTTGCCCAGCGTCTTGCTGAtaGAAGAGTCTTCCTTTACG  |
| 3   | GAGGAGGGCAGCAAACGGaaATCCGTTACCCGCACGGGCATGGG   |
| 4   | GCTTCACGTGGGGTTTGTTCCTTGCTtaGAAGAGTCTTCCTTTACG |
| 5   | GAGGAGGGCAGCAAACGGaaTGGCTCACGGCCTCGCGGATGCACA  |
| 6   | AGGGTCCAGTCGTAGCCCTTGAGCAtaGAAGAGTCTTCCTTTACG  |
| 7   | GAGGAGGGCAGCAAACGGaaTCAGGTCCGGGTCGCCTTTGGGGAA  |
| 8   | GGAATTTGTCCTCGTCGCTCTCCTTtaGAAGAGTCTTCCTTTACG  |
| 9   | GAGGAGGGCAGCAAACGGaaTGTCCGAGCCGGATCCGGAGGGGGCA |
| 10  | TGTTCTCTTGAGGTCGGGTGTTCTCtaGAAGAGTCTTCCTTTACG  |
| 11  | GAGGAGGGCAGCAAACGGaaGGGCGGCTGAGGGGCGGGGAAGGGT  |
| 12  | GAGAAAACGGAGAGAGAAAAAAACAAtaGAAGAGTCTTCCTTTACG |
| 13  | GAGGAGGGCAGCAAACGGaaTCCTCCTGCTCCGACTGCCCGTTCT  |
| 14  | GGGGAGATGTGGGTCTGCTCGGAGCtaGAAGAGTCTTCCTTTACG  |
| 15  | GAGGAGGGCAGCAAACGGaaAAGGGACGCTTCTCGCTCTCATTCA  |
| 16  | ACCCGCAGCCGCTCGGCCTCCTCCAAtaGAAGAGTCTTCCTTTACG |
| 17  | GAGGAGGGCAGCAAACGGaaGAAGAGCGCGAGTTTGTACAAACCC  |
| 18  | GTTTCTCCTTAAGAAAGAAAAATAAtaGAAGAGTCTTCCTTTACG  |
| 19  | GAGGAGGGCAGCAAACGGaaCACCGTTACCTTCCCAACAACGCTC  |
| 20  | TTACAGTTCTGACCATAATTTAACAtaGAAGAGTCTTCCTTTACG  |
| 21  | GAGGAGGGCAGCAAACGGaaAGAGCGTCGGTTATTTTTTTTTTTTG |
| 22  | AGCGCAGGGCTGCTTTAAGGCTTTGtaGAAGAGTCTTCCTTTACG  |
| 23  | GAGGAGGGCAGCAAACGGaaACTGAGCTTTCTTTTTTCTCCCCC   |
| 24  | AAAATAGAGAATTTTTCCTCACAGAtaGAAGAGTCTTCCTTTACG  |
| 25  | GAGGAGGGCAGCAAACGGaaAACTTGTAACCAAAGTCCAGCAAAC  |
| 26  | AAACCCAAACAACCCCTTAAGAAACtaGAAGAGTCTTCCTTTACG  |
| 27  | GAGGAGGGCAGCAAACGGaaCTGACAGAAGTCCTCCACTCTCACT  |
| 28  | TCATTTGATTTGTAAGAAAGGAAAtaGAAGAGTCTTCCTTTACG   |

|    |                                                |
|----|------------------------------------------------|
| 29 | GAGGAGGGCAGCAAACGGaaCTTCACGTGGCTTTAAGGCCGGGTG  |
| 30 | GATTGGTTTGTtTTATTTATTTATTTtaGAAGAGTCTTCCTTTACG |
| 31 | GAGGAGGGCAGCAAACGGaaAGGAGCCGGAGTTCTGGTGCTCGGT  |
| 32 | CGCTCTGCCCCGGCGGCGTGGCTGTAtaGAAGAGTCTTCCTTTACG |
| 33 | GAGGAGGGCAGCAAACGGaaGCTGCTGCTGCTCGCTGTAGTGGCT  |
| 34 | GCTGCTGCTGCTGCTGCTGCGGGGAtaGAAGAGTCTTCCTTTACG  |
| 35 | GAGGAGGGCAGCAAACGGaaGCCGCTGCTGTGCCGGACCCTGCTC  |
| 36 | GGCTCAGCTGCTCTGTtTTTGATGTGtaGAAGAGTCTTCCTTTACG |
| 37 | GAGGAGGGCAGCAAACGGaaTGATGCCGTAGGTACCGCTGTAGGT  |
| 38 | CGCCCGCCGGAGAGCTGGCCGAGCTtaGAAGAGTCTTCCTTTACG  |
| 39 | GAGGAGGGCAGCAAACGGaaTCGAAGGTTTCGATGTTGGAGATGA  |
| 40 | GGCAGGTATTGGTCGAACCTCGTTGAtaGAAGAGTCTTCCTTTACG |

**Table S9.**

*SOX9* probes used for HCR.

| No. | Sequences of <i>SNAI2</i> probes              |
|-----|-----------------------------------------------|
| 1   | GTCCCTGCCTCTATATCTtGTAGCCAGGGTCTGGAGAAAGCCTT  |
| 2   | CAGTGTGGGTTCTGATGTGCCCTTGtCCACTCAACTTTAACCCG  |
| 3   | GTCCCTGCCTCTATATCTtTGTGGGTCCTGATGTGCATCTTAAG  |
| 4   | CGCAGATCTTGCAGACGCAAGGTAGtCCACTCAACTTTAACCCG  |
| 5   | GTCCCTGCCTCTATATCTtAGTACTTGCAGCTGAACGATTTCTT  |
| 6   | CTCCCAGGCTGACATACTCCTTGTCtCCACTCAACTTTAACCCG  |
| 7   | GTCCCTGCCTCTATATCTtCCAACCCAGAGAAAGTGGAAATAGGT |
| 8   | CATCACAGTGCAGCTGCTTGTGTTTtCCACTCAACTTTAACCCG  |
| 9   | GTCCCTGCCTCTATATCTtGCTTCGATGGCATGAGGGTCTGAAA  |
| 10  | TTGCATAAACCGCACTGAAACTTTTTtCCACTCAACTTTAACCCG |
| 11  | GTCCCTGCCTCTATATCTtGGTGGGCTGACCCTTCCCAAAGATG  |
| 12  | TCTTTGGAGGAGGTGTCGGATGGTGtCCACTCAACTTTAACCCG  |
| 13  | GTCCCTGCCTCTATATCTtTTGGGTAGAGGAGATGGTAGCAGCC  |
| 14  | GGGTATCCAGAAAGCGGAGAGAGGTtCCACTCAACTTTAACCCG  |

|    |                                                |
|----|------------------------------------------------|
| 15 | GTCCCTGCCTCTATATCTttGCTACAGAGCTCAGGATCTCTGGCT  |
| 16 | GTAGTCCACACAGTAATGGGGTTGTttCCACTCAACTTTAACCCG  |
| 17 | GTCCCTGCCTCTATATCTttGTCCCCTCTCTTGCACTTATTCCCG  |
| 18 | AAAAAAATAATTGTCAAACCTCCCATttCCACTCAACTTTAACCCG |
| 19 | GTCCCTGCCTCTATATCTttATGTTAGAAGATGGTCAGGCATGCA  |
| 20 | AATAGCAAAGTTTAGAGGAACGATTttCCACTCAACTTTAACCCG  |
| 21 | GTCCCTGCCTCTATATCTttAGGAATGTATTAGTAACTTTGCAGC  |
| 22 | CTGTTTCTTTGGTTGAAATGGCTTGttCCACTCAACTTTAACCCG  |
| 23 | GTCCCTGCCTCTATATCTttATCTACTTGAAATGTCATGCATGGC  |
| 24 | GAAAGCTGATAACGCTTCTTCGTTTttCCACTCAACTTTAACCCG  |
| 25 | GTCCCTGCCTCTATATCTttGAACGTGAATGTGTGACTAAACAGA  |
| 26 | TCCCCATATCTCAGAGTTCACACTTTttCCACTCAACTTTAACCCG |
| 27 | GTCCCTGCCTCTATATCTttGCAGCCAGATTCCTCATGTTTGTGC  |
| 28 | TGACTGCATGACTCAGTGTGCTACGttCCACTCAACTTTAACCCG  |
| 29 | GTCCCTGCCTCTATATCTttGAAAGCCCTGTTGCAGTGAGGACAG  |
| 30 | ATGAGCCCTCAGATTGGATCTGTCTttCCACTCAACTTTAACCCG  |
| 31 | GTCCCTGCCTCTATATCTttGGCAGGCTTTCTCCTTCTTGCAGAT  |
| 32 | GACCAGGAAGGAGCGTGGCATTtTTGttCCACTCAACTTTAACCCG |
| 33 | GTCCCTGCCTCTATATCTttCACACGCGCACACTATCACTGCGAG  |
| 34 | TCCTTTTGGGTCGGCGCAGCGGTGCTttCCACTCAACTTTAACCCG |
| 35 | GTCCCTGCCTCTATATCTttATGCACTCCGCTGTGTAGTGGCACT  |
| 36 | ATCGGTCCCTATCGCATTGTCTGCTttCCACTCAACTTTAACCCG  |
| 37 | GTCCCTGCCTCTATATCTttCTTCTGGATGTTCTCAAGCACTTTT  |
| 38 | GGGAAGGGTTTTCTGCTCCTCCAATttCCACTCAACTTTAACCCG  |
| 39 | GTCCCTGCCTCTATATCTttTCGCAGGACGGACCCAATCCCTGCT  |
| 40 | AACAGGCTGTTTTTCTGGGAGGGCTttCCACTCAACTTTAACCCG  |

**Table S10.**  
*SNAI2* probes used for HCR.

| No. | Sequences of <i>SNAI2</i> Intron1 probes       |
|-----|------------------------------------------------|
| 1   | GAGGAGGGCAGCAAACGGaaTGAGTTCTCTTTTTTTGGCTTCCCG  |
| 2   | GATAAGAGGTCAGTTTACTGATTCAtaGAAGAGTCTTCCTTTACG  |
| 3   | GAGGAGGGCAGCAAACGGaaATGGGACACACTGCCCCGCTAAGGGC |
| 4   | TTCCAGATGTTTGATAAAAGAATTaGAAGAGTCTTCCTTTACG    |
| 5   | GAGGAGGGCAGCAAACGGaaGGCAGAGCGAGGCTCGGAGCAGGCT  |
| 6   | GGGGACATTGCAAGAGAGATGTTATaGAAGAGTCTTCCTTTACG   |
| 7   | GAGGAGGGCAGCAAACGGaaGCGCTCAGGAAATGCTGCGGACGAG  |
| 8   | TCTGCCTGGGAGCTGCCTTTGAAATaGAAGAGTCTTCCTTTACG   |
| 9   | GAGGAGGGCAGCAAACGGaaTGCTGGCGAGCCCAGGCTGAGGGAG  |
| 10  | TTCCAGGCACAGCGGCCAGCGAGATaGAAGAGTCTTCCTTTACG   |
| 11  | GAGGAGGGCAGCAAACGGaaATACTGGTTCAGACAAGCTGTTCTT  |
| 12  | GATAGTTTTGATGTGCCGTACAAATaGAAGAGTCTTCCTTTACG   |
| 13  | GAGGAGGGCAGCAAACGGaaTTTCCCTCCTTGGAATTACTGTGT   |
| 14  | AATCTTACAAAATGCAAAACTCCGAtaGAAGAGTCTTCCTTTACG  |
| 15  | GAGGAGGGCAGCAAACGGaaGCCAAGGGAATTTTCCTAATTACCC  |
| 16  | CTCTTTTGCCTGTCCTCTTACAGCAtaGAAGAGTCTTCCTTTACG  |
| 17  | GAGGAGGGCAGCAAACGGaaTCCCCCCCCCAAATTACAGACTGAG  |
| 18  | AGTTCAAAGATGCACAGATAAACAAtaGAAGAGTCTTCCTTTACG  |
| 19  | GAGGAGGGCAGCAAACGGaaGCCACAGAACGTTCTGGTTATCTAG  |
| 20  | CATTTAGGATGAAAGTTTCTCAAATaGAAGAGTCTTCCTTTACG   |
| 21  | GAGGAGGGCAGCAAACGGaaAGACAGCTGAGCGCTGCTGGATTTC  |
| 22  | TTTCAAACGTTTGCATTTTGAAAATaGAAGAGTCTTCCTTTACG   |
| 23  | GAGGAGGGCAGCAAACGGaaTCCCTCCCCACCGCTCTCCCCCCTT  |
| 24  | CGGACCACACTGACTGTCTCCCATTaGAAGAGTCTTCCTTTACG   |
| 25  | GAGGAGGGCAGCAAACGGaaTATGCTGTGCGGTGTGCCGCTAGGG  |
| 26  | AACTCCACCTGCCCAGGCACATATTaGAAGAGTCTTCCTTTACG   |
| 27  | GAGGAGGGCAGCAAACGGaaCAGCCCATTTTTTTTTTGCAGCAGG  |
| 28  | GTGTCCTGTGTTCTACAGAGCCCACTaGAAGAGTCTTCCTTTACG  |

|    |                                                |
|----|------------------------------------------------|
| 29 | GAGGAGGGCAGCAAACGGaaGTTAAGACACGTCAGAACAAAGTGC  |
| 30 | AGAAAAAGAAAAAGCCCCCTCTGGTTtaGAAGAGTCTTCCTTTACG |
| 31 | GAGGAGGGCAGCAAACGGaaGGGATGCCAGAACAAGTGCAGAGAA  |
| 32 | GTCAAGCTGCATGGCTTCTTAATCTtaGAAGAGTCTTCCTTTACG  |
| 33 | GAGGAGGGCAGCAAACGGaaTTTAGCCGCTCAATGTCTCCCTTGT  |
| 34 | GCCATTTTCAAGAACTGATTAATAAaGAAGAGTCTTCCTTTACG   |

**Table S11.**

*SNAI2 Intron1* probes used for HCR.

| No. | Sequences of <i>SNAI2 Intron3</i> probes      |
|-----|-----------------------------------------------|
| 1   | GTCCCTGCCTCTATATCTttGGCCACAGATACAACAGCTTTCAGC |
| 2   | AAAACATGCACATCACAAAGTTAATttCCACTCAACTTTAACCCG |
| 3   | GTCCCTGCCTCTATATCTttTTTGCAGCATCTGCTAATAAGTGCA |
| 4   | TGTGGATTGCACTTGCAGAACTCAGttCCACTCAACTTTAACCCG |
| 5   | GTCCCTGCCTCTATATCTttATGTACAGGTGCAGACAGTATTCCT |
| 6   | TGAAGACAAGGTGCTGACACCTTCTttCCACTCAACTTTAACCCG |
| 7   | GTCCCTGCCTCTATATCTttCAGAGTGTCTTCCTGCTTAGCTCCG |
| 8   | TCCTCCCAATATTTTACATATTTTttCCACTCAACTTTAACCCG  |
| 9   | GTCCCTGCCTCTATATCTttTTAGAAGTGGCAAACAGCTGTACAG |
| 10  | TGAATGACTTGGGACGAGGGCTTTGttCCACTCAACTTTAACCCG |
| 11  | GTCCCTGCCTCTATATCTttCTCTTGAGGACTTGAGAGAGTGCAT |
| 12  | AATGCTTTGGTGATGAAGTGTTTCTttCCACTCAACTTTAACCCG |
| 13  | GTCCCTGCCTCTATATCTttGAGATAAGCCTCTCATGCATTGCCT |
| 14  | AAAACCTGCCAATTGCTTAAGGGGTttCCACTCAACTTTAACCCG |
| 15  | GTCCCTGCCTCTATATCTttTCCTTAAAGCAACGCCTTCAGCTAG |
| 16  | TTTCCAAGCCTGAAAGTTAACGTAAttCCACTCAACTTTAACCCG |
| 17  | GTCCCTGCCTCTATATCTttCCCACAGCTATGCTCCCCTCCCACC |
| 18  | GAAAGAGGTGATCAGAACAGAATCTttCCACTCAACTTTAACCCG |

**Table S12.**

*SNAI2 Intron3* probes used for HCR.